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# REDUCTION BY SONICATION OF EXCESS SLUDGE PRODUCTION IN A CONVENTIONAL ACTIVATED SLUDGE SYSTEM CONTINUOUS FLOW AND LAB-SCALE REACTOR

S.VAXELAIRE, E.GONZE\*, G.MERLIN, Y.GONTHIER

Laboratoire Optimisation de la Construction et Ingénierie de l'Environnement, Polytech'Savoie,  
Université de Savoie, 73376 Le Bourget du Lac, France

\*evelyne.gonze@univ-savoie.fr

## Abstract

Conventional activated sludge wastewater treatment plants currently produce a large quantity of excess sludge. To reduce this sludge production and to improve sludge characteristics in view of their subsequent elimination, an ultrasonic cell disintegration process was studied. In a lab-scale continuous flow pilot plant, part of the return sludge was sonicated by low-frequency and high-powered ultrasound and then recycled to the aeration tank. Two parallel lines were used, one as a control, and the other as an assay with ultrasonic treatment. Reactors were continuously fed with synthetic domestic wastewater with a COD of approximately  $0.5 \text{ g l}^{-1}$  corresponding to a daily load of  $0.35\text{--}0.50 \text{ kg COD kg}^{-1} \text{ TS d}^{-1}$ . Removal efficiencies (carbon, particles), excess sludge production and sludge characteristics (particle size distribution, mineralization, respiration rate, biological component) were measured every day during the 56-day experiment. This study showed that while organic removal efficiency was not deteriorated, excess sludge production was decreased by about 25–30% by an ultrasonic treatment. Several hypotheses are advanced: (i) the treatment made a part of the organic matter soluble as a consequence of the floc disintegration and optimized the conversion of the carbonaceous pollutants into carbon dioxide and (ii) the treatment modified the physical characteristics of sludge by a mechanical effect: floc size was reduced, increasing the exchange surface and sludge activity. The originality of this study is that experiments were conducted in a continuous-flow activated sludge reactor rather than in a batch reactor.

**Keywords:** excess sludge reduction; wastewater treatment; ultrasound, activated sludge.

## INTRODUCTION

There are currently approximately 15,000 wastewater treatment plants in France, which produce approximately 1.3 MT of per year of sludge (dry matter). The production of urban activated sludge in the European Convention countries and Switzerland totals 7.7 MT, on the same order as North American countries (8 MT for the United States and Canada). The current means to eliminate this sludge are agricultural field disposal (60%), deposit in a technical centre (25%) and incineration or co-incineration (15%). These means are subjected to technological, social, legal and economic pressures and constraints as well as geographical availability [1]). Moreover, they will not be able to absorb the growing volume of sludge produced as a result of the new standards and obligations. It is therefore necessary to diversify the methods of sludge treatment and to control sludge quality (traceability). This step will involve additional cost. Currently, the total cost of sludge treatment and elimination is estimated at half of the cost of wastewater treatment. Consequently, the primary objectives in biological wastewater and sludge treatment processes are to produce a minimum quantity of solid, stable residues and to optimize the conversion of the carbonaceous pollutants into carbon dioxide. In order to respond to these problems, various sludge treatment processes have been developed. Whatever the technology used, the reduction of excess sludge is promoted by the modification of biological activity (maintenance metabolism, uncoupling metabolism, cryptic growth and predation of bacteria), the increase in the biodegradability of inert fractions of wastewater and/or the improvement in mass transfer [2, 3, 4, 5].

Several authors [6, 7, 8] have reviewed current processes to reduce sludge production, including physical, chemical, microbiological and combined technologies: membrane bioreactor,

thermal treatment, combination of thermal and alkaline or acid treatment, ozonation, chlorination, the high dissolved oxygen process, metabolic uncouplers, etc. Mechanical disintegration (mills, homogenizers, ultrasound) offers certain advantages: no rubbish, no chemical products, and no toxic by-products (chlorinated compounds, chemical uncouplers) in the effluent. Among these physical processes, sonication is a particularly attractive treatment because it requires no moving parts. It is the extreme shear forces developed during bubble collapse which locally produce mechanical effects resulting in the breakdown of bioflocs. The literature contains a great number of studies concerning the impact of ultrasonic treatment on biological sludge, with different applications reported. Ultrasonic disintegration was first studied as a pretreatment before anaerobic digestion to enhance efficiency: increased biogas production and decreased retention time in the digester [9 to 20]. Currently this process has been successfully implemented in municipal wastewater treatment plants under real-life conditions [10, 21]. NA *et al.* [22] plan to position the ultrasonic treatment after anaerobic digestion and before dewatering to reduce the water content of the sludge. YOON *et al.* [23] incorporated a sonicator into a membrane bioreactor and observed that excess sludge production was prevented by ultrasonic treatment, but unfortunately the filterability of the sludge was severely damaged. ZHANG *et al.* [24] used ultrasound to reduce the excess sludge from the sequential batch reactor system. With optimal experimental conditions, the sludge was reduced by 91%.

This paper examines the effects achieved with sonication as disintegration technology in the wastewater process rather than in sludge post-treatment. Our study was conducted in a continuous flow-activated sludge reactor versus a batch reactor because batch tests are inadequate to determine the ultimate effects of ultrasound. Results can be considerably different. For example, NICKEL *et al.* [10] noted that cell disintegration achieved with a continuously operated plug-flow process was significantly better as compared to a batch reactor with the same energy input. It is interesting to note that many experiments have been carried out in batch reactors, whereas the data in the literature on continuous pilot plants are very scarce. Removal efficiencies (carbon, nitrogen, particles) and excess sludge production were measured in a conventional activated sludge system incorporating an ultrasonic probe. Physical and biological sludge characteristics were also investigated.

## MATERIALS AND METHODS

### Activated sludge systems, ultrasonic device and operating conditions

Two lab-scale conventional activated sludge systems were used (Figure 1) and fed with the same synthetic influent. The first line (REF-line) without ultrasonic treatment was maintained as a control while the second line (US-line) with a sonicator was used to evaluate the utility of ultrasonic treatment. Each line employed an aeration tank (16 l) and a settler (6 l, 0.05 m<sup>2</sup>). A synthetic influent was used to simulate municipal wastewater with a chemical oxygen demand (COD) of 485 mg O<sub>2</sub> l<sup>-1</sup>, a COD/BOD<sub>5</sub> ratio of 1.2 and no inert suspended solids (Table 1). A pump provided a continuous supply of this wastewater at a flow rate of 2 l h<sup>-1</sup> (Q<sub>0</sub>). Aeration tanks were continuous stirred tank reactors placed in a large reservoir in which a coolant circulated to maintain the solution temperature at (25 ± 3)°C. They were aerated with fine-bubble diffusers at 0.5 m<sup>3</sup> h<sup>-1</sup> providing an average dissolved oxygen concentration of 1–3 mg O<sub>2</sub> l<sup>-1</sup>. The activated sludge systems were operated with a volumetric loading rate of 1.45 kg COD m<sup>-3</sup> d<sup>-1</sup>. Concentrated sludge was recycled from the settler back to the aeration tank at a flow rate of 4 l h<sup>-1</sup> (R.Q<sub>0</sub>). The excess activated sludge (Q<sub>E</sub>) was periodically removed to maintain a constant concentration in aeration tanks around 3–4 g TS l<sup>-1</sup>. Effluent (Q<sub>L</sub>) was collected for 24 h and analyzed.

A 20-kHz sonotrode (SODEVA) was used as a sonicator. It consists of a piezoelectric transducer linked to a horn (22 mm in diameter). The probe was immersed in the ultrasonic reactor (0.7 l) incorporated into the US-line. Some of the recycled sludge (a.Q<sub>0</sub> ≈ 0.5 l h<sup>-1</sup>) coming from the settler was disintegrated and returned directly to the aeration tank. High-intensity ultrasonic

treatment was placed on return sludge because it is preferable to treat the more highly concentrated sludge. The efficiency of ultrasonic treatment clearly increased with sludge concentration. This phenomenon can be explained as follows: with highly concentrated suspensions, more particles are exposed to the shear forces and particles act as nuclei, thus increasing cavitation [25, 26]. Moreover, it would not have been wise to place the treatment before the settler, because even if the settling speed was improved by this treatment, the quality of the supernatant would be severely damaged. Ultrasonic power and intensity determined using the calorimetric method were 300 W and  $42 \text{ W cm}^{-2}$ , respectively. Ultrasonic treatment was done for 1 min every 15 min.

## **Analytical methods**

### *Solids*

Total solids (TS) were quantified daily in mixed liquor in an aeration tank (X) and in excess sludge removed at the settler bottom ( $X_E$ ) according to standard methods [27]. In effluent ( $X_L$ ), turbidity was measured (spectrophotometry at 450 nm) rather than using the standard method because the effluent concentration was very low. Volatile solids (VS) were quantified in the aeration tank according to standard methods.

### *Organic matter*

Chemical oxygen demand was measured according to the Hach dichromate micro-method approved by the EPA. Total chemical oxygen demand (tCOD) was measured for the whole sample after being completely mixed. tCOD was measured in the influent (tCOD<sub>0</sub>), the effluent (tCOD<sub>L</sub>) and the excess sludge (tCOD<sub>E</sub>). The soluble chemical oxygen demand was measured for the effluent (sCOD<sub>L</sub>) after filtration through a membrane with a mesh diameter of 1.2  $\mu\text{m}$  (Whatman GF/C). COD tests were made in triplicate and average values were calculated. The particulate chemical oxygen demand (pCOD) was calculated as the difference between the total and the soluble COD.

### *Physical sludge characteristics*

Particle size distribution of the biological sludge in the aeration tank was analyzed using a Malvern Mastersizer laser beam diffraction granulometer. The instrument measures a range from 0.10 to 800  $\mu\text{m}$  but the precision below 0.50  $\mu\text{m}$  is very low. The results were expressed as the volumic percentage of the particles (liters of particles per liters of sludge) as a function of their size. The term “particles” applies equally well to isolated microorganisms and bacterial flocs, which contain mostly water.

### *Biological sludge characteristics*

Microorganism species present in the activated sludge were determined by microscopic examination.

The BI-2000® Electrolytic Respirometer (Bioscience Inc.) was used for measuring the respiration rate of activated sludge in aeration tanks. Eight closed flasks coupled to an oxygen generating system composed the respirometer. The concentration of oxygen in the flasks was kept at a constant value, so the oxygen generated was equal to the oxygen consumed by bioactivity. The 200-mL samples were placed directly in the flasks and the oxygen consumption was measured over 120 h without adding substrate.

When studying the effect of ultrasound on filamentous organisms, an epifluorescence staining method using the LIVE/DEAD® Bacterial Viability Kit (BacLight™) was applied to estimate both viable and total counts of bacteria [28]. BacLight is composed of two nucleic acid-binding stains: SYTO 9™ and propidium iodide. SYTO 9™ penetrates all bacterial membranes and stains the cells green, while propidium iodide only penetrates cells with damaged membranes, and the combination of the two stains produces red fluorescing cells.

## RESULTS

Two phases of experiments were conducted to show the utility of ultrasonic treatment. The first phase was conducted without sonication to acclimate biological culture (stabilization phase). After 25 days, the system was presumed to be at steady state. On the 26<sup>th</sup> day, ultrasonic treatment started on the US-line; a fraction of return sludge ( $a.Q_0$ ) was sonicated. Tests were performed to evaluate removal efficiencies of organic substrate and particulate matter, excess sludge production and the physical and biological characteristics of the bioflocs. Note that several experiments were undertaken, but only one is presented in this paper; the results of the others are similar.

### Total solids in the aeration tanks and organic loading

The concentration of mixed liquor in the aeration tanks was measured every day during the experiment. The value was maintained between 2.3 and 4.8 g TS  $\Gamma^{-1}$  by extracting excess sludge (Figure 2). The average value of the REF-line and the US-line was  $(3.9 \pm 0.7)$  and  $(2.9 \pm 0.5)$  g TS  $\Gamma^{-1}$ , respectively. The aeration tank of the REF-line was operated with a specific loading rate of approximately 0.37 kg COD  $\text{kg}^{-1}$  TS  $\text{d}^{-1}$  while the US-line was operated with a specific loading rate of roughly 0.50 kg COD  $\text{kg}^{-1}$  TS  $\text{d}^{-1}$  (Table 2). These experimental results show that the total solids in the aeration tanks and the hydraulic and organic loading rates were maintained at identical levels in the two lines. Note that the organic loading rate calculated in this way is a global parameter. In fact, excess biosolids disintegrated by ultrasound returned to the aeration tank and were an additional substrate that contributed to the organic loading. Therefore the actual organic loading rate was higher in the US-line than in the REF-line.

### Removal efficiencies of organic substrate and particulate matter

#### *Cumulative COD removal*

In order to compare sludge production and removal efficiencies of the pilot plant's two lines during the experiment, all the results will be expressed as the cumulative quantity of substrate eliminated, noted "cumulative COD removal". To determine this, the substrate was balanced over the system. In steady state, the organic matter balance can be written as equation (i). The soluble COD is assumed to be equal in excess sludge and effluent, as shown in equation (ii). Therefore, equation (i) can be explained as equation (iv).

$$Q_0.t\text{COD}_0 = Q_L.s\text{COD}_L + Q_E.s\text{COD}_E + r_s.V \quad (\text{i})$$

$$s\text{COD}_L = s\text{COD}_E \quad (\text{ii})$$

$$\text{and } Q_0 = Q_L + Q_E \quad (\text{iii})$$

$$r_s.V = (t\text{COD}_0 - s\text{COD}_L).Q_0 \quad (\text{iv})$$

Cumulative COD removal can be calculated versus time as follows:

$$\sum \text{COD removal} = \sum_{t=0}^t r_s.V.\Delta t = \sum_{t=0}^t (s\text{COD}_0 - s\text{COD}_L).Q_0.\Delta t \quad (\text{v})$$

The beginning of the ultrasonic treatment on the 26<sup>th</sup> day corresponds to a cumulative COD removal of 420 g  $\text{O}_2$ .

#### *Organic matter removal efficiency*

The organic matter removal efficiency ( $R_{\text{COD}}$ ) was quantified by measuring COD at various points of the pilot plant: total COD in the influent and soluble COD in the effluent. It can be written as:

$$R_{\text{COD}} = \frac{t\text{COD}_0 - s\text{COD}_L}{t\text{COD}_0} \quad (\text{vi})$$

The overall organic matter removal efficiency measured in the US-line was not significantly different from that achieved in the REF-line. The average efficiency was  $86 \pm 6\%$ . These results led to the conclusion that the ultrasonic treatment did not impair the biological efficiency.

#### *Particle removal efficiency*

Ultrasounds sharply alter the characteristics of the flocs and modify their settling. In the batch reactor, ultrasonic treatment improved the settling parameters (settling rate and Sludge Volumetric Index) but also deteriorated the quality of supernatant [25]. Therefore, it was necessary to study the settling in a continuous reactor integrating ultrasonic treatment. Figure 3 shows cumulative particulate COD (pCOD<sub>L</sub>) and soluble COD (sCOD<sub>L</sub>) in effluent during the experiment; Figure 4 reports cumulative total solids (X<sub>L</sub>). The results are reported according to the quantity of cumulative COD removal so that the results of the two pilot lines (REF and US) could be compared. The results show clearly that in a continuous reactor, the quality of the settler supernatant was not impaired by sonication.

#### **Excess sludge production**

The excess activated sludge ( $\Phi_{X_{\text{excess}}}$ ) formed in the process results from the conversion of substrate into biomass. It can be evaluated as the sum of three terms (equation vii). The first one ( $Q_L \cdot X_L$ ) is the sludge measured in the effluent due to an imperfect separation in the settler. As mentioned above, this term in the US-line was roughly same as that in the REF-line (Figure 4). The second term ( $Q_E \cdot X_E$ ) is the extracted flow of sludge at the settler bottom. The third term stems from the possible accumulation of sludge in the system. This accumulation can be approximated to the product of the variation of the total solids concentration (X) by aeration tank volume (V).

$$\Phi_{X_{\text{excess}}} = Q_L \cdot X_L + Q_E \cdot X_E + \frac{\Delta X}{\Delta t} \cdot V \quad (\text{vii})$$

Cumulative excess sludge production can be explained versus time by the following equation:

$$\sum \Phi_{X_{\text{excess}}} = \sum_{t=0}^t Q_L \cdot X_L \cdot \Delta t + \sum_{t=0}^t Q_E \cdot X_E \cdot \Delta t + (X_t - X_{t=0}) \cdot V \quad (\text{viii})$$

Figure 5 shows cumulative excess sludge production expressed in total solids measured in the REF-line and the US-line as a function of cumulative COD removal. During the stabilization phase, excess sludge production was identical in the two pilot lines. During the second phase with ultrasonic treatment, the average amount of sludge production per day was  $8.3 \text{ g d}^{-1}$  and  $5.0 \text{ g d}^{-1}$  in the REF-line and the US-line, respectively. This test, carried out on a laboratory-scale pilot plant, showed that sludge production can be reduced more than 30% with an ultrasonic treatment of some of the recycled sludge.

#### *Sludge retention time*

The sludge retention time (SRT) is defined as the ratio of the total amount of biomass in the aeration tank to the rate of excess biomass production. Knowing cumulative excess sludge production, sludge retention time was calculated on the period from the 26<sup>th</sup> to the 56<sup>th</sup> day. Its value was 7.5 and 9.3 days in the REF-line and the US-line, respectively. Therefore, it seems that sonication results in a higher SRT. However, the sludge retention time calculated by applying equation (ix) is an apparent SRT that takes into account the totality of microorganisms and not only active microorganisms which can consume substrate (alive with access to the substrate).

$$\text{SRT} = \frac{X \cdot V}{X_E \cdot Q_E} \quad (\text{ix})$$

#### *Apparent yield coefficient*

During the biological treatment of wastewaters, a portion of organic pollutants is used for respiration activity during catabolism. The other portion is assimilated and produces new biomass.

To reduce the amount of excess biomass produced, the ultrasonic treatment must promote catabolism and must limit anabolism. This point is demonstrated by calculating the experimental apparent yield coefficient (Y). The apparent yield coefficient representing the conversion of organic substrates into biomass provides a useful indicator to evaluate the efficiency of the ultrasonic treatment. It can be defined as the ratio of the quantity of sludge produced to the quantity of COD removed:

$$Y = \frac{\Phi_{X_{\text{excess}}}}{(t\text{COD}_0 - s\text{COD}_L) \cdot Q_0} \quad (\text{x})$$

By monitoring biomass production and COD removal, the yield coefficient was calculated on the period from the 26th to the 56th day. Its value was  $(0.35 \pm 0.04)$  and  $(0.24 \pm 0.04)$  g TS g<sup>-1</sup> O<sub>2</sub> in the REF-line and the US-line, respectively. Consequently, the lower apparent biomass yield coefficient was achieved in the line operated with ultrasound, revealing that the conversion of the carbonaceous pollutants into carbon dioxide was optimized.

### Physical sludge characteristics

An activated sludge floc is composed of biomass, exocellular polymeric substances (EPSs), inert particulates, endogenous residues and water (more than 95%). It is a highly porous and heterogeneous structure composed of nonuniformly distributed biomass concentrated in microflocs. The EPSs are made up of protein, nucleic acids, humic and uronic compounds, polysaccharides and lipids [29, 30, 31]. These compounds are mainly produced by bacteria (secretion, cell lysis, shedding of surface materials), but they may be present in the influent [32]. Many authors have proposed that these EPSs form a matrix and ensure the stability of the biofloc structure [33]. In addition, researchers have noted the major role that divalent cations such as calcium and magnesium play in the bioflocculation mechanism stemming from the negative charges in bioflocs [34]. These cations seem to be an essential attribute to link constituents in the biofloc.

All studies concerning ultrasound treatment of biosludge report significant modifications in the floc structure with a reduction in mean diameter [25, 35]. Many authors note that the disintegration of the flocs is accompanied by the solubilization of organic matter [9, 10, 11, 12, 13, 18, 19, 25]. For example, WANG *et al.* [14, 15] analyzed the polysaccharide, protein, and DNA released and they concluded that the main component is protein. They also report, like CHANG *et al.* [19], an increase in the divalent cation concentration (calcium and magnesium) in the supernatant, which is in agreement with the biofloc disintegration. CHANG *et al.* [18] assessed surface charges of the anaerobic bioflocs by measuring the zeta potential. Before anaerobic digestion, they observed that the ultrasonic pretreatment decreases the zeta potential. In addition, as digestion proceeds, the potential of the untreated sludge declines and converges to the potential of treated sludge.

All these studies were undertaken in batch processes. To understand the decrease in the excess sludge production in a continuous process, the physical effects of ultrasonic treatment were highlighted by characterizing the activated sludge properties in our experimental pilot plant. Granulometric measurement, volatile solids measurement, microscopic examination and respirometry were carried out.

#### *Disruption of biofloc*

Since biofloc size is a critical factor in the transfer of nutrients, the biofloc size distribution was evaluated. Our previous study [25] conducted in batch reactors showed a sharp reduction in floc diameter, revealing disruption of macro-flocs (>80 μm) that were more sensitive to ultrasound in smaller structures. During the first few minutes of sonication, the concentration of macro-flocs was greatly reduced with release of free and interstitial water contained in the interstices and/or inside the flocs. Smaller bioflocs (<50 μm) were more resistant to ultrasound. Their mean diameter slowly decreased, indicating erosion rather than disruption: microorganisms scattered throughout the bioflocs were progressively snatched. This hypothesis is corroborated by

the increase in small-diameter particles ( $<10\ \mu\text{m}$ ) and the microscopic observations, which clearly showed the presence of a large number of isolated bacteria. This phenomenon, inducing deterioration of settling and an additional amount of polyelectrolyte for dewatering penalizes the ultrasonic process in view of the subsequent elimination of biological sludge.

The follow-up of the median diameter carried out during our experiments conducted in the continuous flow-activated sludge reactor showed substantial reduction in biofloc size. In the REF-line, the median floc diameter was approximately  $110\ \mu\text{m}$  and it was reduced to approximately  $30\ \mu\text{m}$  for the line undergoing ultrasonic treatment. In the REF-line, the median floc diameter ranged between  $130$  and  $200\ \mu\text{m}$ . The measurement of the mean diameter is not conclusive because it does not allow demonstrating the presence of several populations. Therefore, an example of particle size distribution is reported in Figure 6, which plots the volumic percentage of the particles (liters of particles per liter of sludge) as a function of their size. The results clearly show a decrease in the concentration of larger flocs ( $> 80\ \mu\text{m}$ ) in the US-line, a rise in intermediate flocs (between  $10$  and  $50\ \mu\text{m}$ ), but no significant increase in small-diameter particles ( $< 5\ \mu\text{m}$ ). This is not in agreement with previous experimental results obtained in batch reactors. This indicates reflocculation of the biological material in the aeration tank caused by the material released during sonication. This reflocculation prevents deterioration of the settling properties of the sludge observed in the batch reactor, which reduces effluent quality. The differences observed between batch and continuous tests confirm that batch experiments should be used with caution to determine the ultimate effect of ultrasound.

### *Mineralization*

With our continuously operated process, little mineralization of activated sludge was observed in the US-line. The organic content of the sludge was expressed as a ratio of volatile solids to total solids. As shown in Figure 7, with ultrasonic treatment this ratio was reduced from  $75 \pm 5\%$  to  $62 \pm 6\%$ . The ultrasonic treatment did not directly induce mineralization phenomena, as shown by NA *et al.* [22] or BOUGRIER *et al.* [13]. These latter authors measured organic and mineral solids in a batch reactor as a function of specific energy ( $0\text{--}16\ \text{kJ kg}^{-1}\ \text{MS}$ ) during sonication of a sample with a concentration of  $18.5\ \text{g TS l}^{-1}$ . No mineralization was observed; the VS/TS ratio was constant, equal to  $81\%$ . The mineralization observed in our continuous pilot plant stemmed from a modification in biological activity.

## **Biological sludge characteristics**

### *Respiration rate*

Biomass growth and substrate consumption were assessed by monitoring the respiration rate of the mixed liquor. Samples were taken in aeration tanks during the course of the experiment and placed in the respirometer flasks for  $120\ \text{h}$ . The amount of oxygen consumed by microorganisms was measured. These values were divided by the mass of volatile solids present in the initial sample and plotted as a function of time. The oxygen utilization rate was  $6.3\ \text{mg O}_2\ \text{g}^{-1}\ \text{VS h}^{-1}$  and  $8.7\ \text{mg O}_2\ \text{g}^{-1}\ \text{VS h}^{-1}$  in the REF-line and the US-line, respectively. The respiration rate is greatly enhanced by ultrasonic treatment. This can be explained by an increase in the availability of the substrate in the US-line. For all the samples studied, we observed a higher respiration rate with sonicated sludge. However, the rate of increase varied widely from one sample to another. These results are in agreement with the ZHANG *et al.* study [24]. They measured a significant enhancement of biological activity with the ultrasonic treatment. The oxygen utilization rate increased by  $28\%$ , with a power density of  $200\ \text{W l}^{-1}$  and sonication lasting  $30\ \text{s}$ .

### *Biological component of the activated sludge*

SEARS *et al.* [36] determined the sensitivity of heterotrophic and autotrophic organisms to ultrasonic irradiation. They observed that heterotrophic organisms were more easily released in the supernatant than autotrophic organisms. They explained this result by the difference in attachment



within the biofloc. Autotrophic organisms form dense clusters encased by a glycocalyx layer, while heterotrophs are scattered throughout the biofloc. CHANG *et al.* [19] measured the survival ratio of heterotrophic organisms and total coliform. With a volumic power of  $330 \text{ W l}^{-1}$  and a treatment time of 10 min ( $198 \text{ kJ l}^{-1}$ ), only 12% of initial heterotrophic organisms seemed to be nonviable. Coliform seem to be more sensitive to ultrasound. In the same conditions, the surviving coliforms were reduced by 20%. By increasing the duration of sonication, the survival ratio decreased: with a volumic energy of  $2380 \text{ kJ l}^{-1}$ , viable heterotrophic bacteria and total coliforms were equal to 12% and 52%, respectively, of those initially in the sludge. During our experiments, given the power input ( $470 \text{ W l}^{-1}$ ,  $140 \text{ kJ l}^{-1}$ ) and knowing that only a small fraction of the sludge was treated ( $\alpha \cdot Q_0 = 0.5 \text{ l h}^{-1}$ ), it is likely that the amount of heterotrophic bacteria disinfected by ultrasound was minor.

Concerning higher organisms, microscopic examinations have shown that these species are sensitive to ultrasonic treatment. A number of protozoa species have been identified in activated sludge in the REF-line, while their development seemed to be limited in the sludge in the US-line.

#### *Filamentous bacteria*

During activated sludge treatment, excessive development of filamentous bacteria causing floating and bulking sludge is sometimes observed. Several authors report that using high-power ultrasound treatment eliminates filamentous bacteria in batch reactors [37, 9].

First, our experiments were conducted in a batch reactor up to 4 l in volume with a bulking sludge. The ultrasonic energy input was set to 300 W and the treatment time of 20 s to 1 h was used. Samples contained a large amount of filamentous bacteria, including *Thiothrix*, *Nocardia*, and some protozoa species (*Bodo*, *Vorticella*,...). Microscopic observations have shown that only 2 min of sonication was enough to break the filaments, which were then suspended in the supernatant (Figure 8). Moreover, the longer the treatment lasted, the smaller the filaments were. The epifluorescence staining method was applied to estimate viable bacteria (Figure 9). Even with long treatment times, most of the bacteria were still viable. This result was confirmed by culturing microorganisms on agar plates. Microscopic observations have shown that filaments broken by ultrasound are able to grow again. The shear forces generated by acoustic cavitation implosion destroy the filamentous bacteria, but the cell walls of bacteria seem to be not damaged. These results obtained with the batch reactor makes it difficult to predict whether an increase or a decrease in filamentous bacteria will be observed in a continuous process.

During our continuous flow experiments, we also encountered filamentous bacteria. This enabled us to assess the impact of ultrasound treatment on their development. The microscopic observations made in the US-line clearly showed that numerous filaments of filamentous bacteria were cut. The ultrasonic treatment therefore limits the development of filamentous bacteria without removing them. This is verified by a better settling in the US-line.

## DISCUSSION

### **Effects of ultrasonic treatment on activated sludge metabolism**

During these experiments, we observed intensification of microorganism respiration and mineralization, leading to a lower sludge production. To explain these effects, a brief background on the metabolism of microorganisms is presented. In activated sludge processes, the main biological mechanisms are catabolism, anabolism, decay, cell lysis and predation [38]. Catabolism provides useful energy and metabolites by degrading substrates and reserves. The energy thus produced is consumed by maintenance functions and anabolism. The maintenance functions consist in cell mobility, turnover of cell materials, keeping concentration gradients between the interior and exterior of the cell, etc. Anabolism is biosynthesis of new biomass by using metabolites and energy. Cell death produces nonactive bacteria, which are unable to consume substrate. The dead cells that are intact cannot be directly assimilated by the active bacteria, but can be absorbed by higher organisms. These dead cells are in a transitional state preceding cell lysis. Cell lysis results in the

release into the medium of intracellular materials (proteins, lipids, nucleic acids, carbohydrates, etc.). Many of these components are biodegradable and can be used as substrate by active bacteria.

Ultrasonic treatment resulting in numerous and complex phenomena may have an action on each of these mechanisms: the improvement in mass transfer in the liquid surrounding the floc, the modification of the substrate and oxygen concentration within the bioflocs, the release of substrate through cell lysis, inducing an increase in cryptic growth and minor phenomena such as the increase in solution temperature.

#### *Improvement in mass transfer in liquid and concentration profiles within bioflocs*

In an activated sludge, microorganisms are grouped in agglomerates. The diffusion of substrate and dioxygen around and within the flocs can be a limiting factor. Therefore, the availability of substrate for biomass is an essential parameter, which can be enhanced by ultrasonic treatment. In a previous study, we demonstrated that ultrasonic treatment promotes mass transfer in the liquid by increasing the micro-mixing [39, 40]. We can therefore assume that ultrasonic treatment improves the availability of substrates and dioxygen for bioflocs.

Within the flocs, it is generally accepted that because of diffusion control, substrate and dissolved dioxygen diffuse slowly to the centre of the biofloc. This results in concentration gradients within biological aggregates. Therefore, bioflocs are stratified and different layers can be distinguished [3]: a peripheral layer supplied with both substrate and oxygen and a central starvation zone. Depending on mass transfer resistances and the kinetics of the reactions, the central zone may be deprived of oxygen and/or substrate. By modifying mass transfer within the flocs and decreasing floc diameter, ultrasonic treatment may affect these concentration profiles. The reduction in the floc diameter and the increase in the total exchange area between flocs and the liquid phase by scattering biomass are undoubtedly the main effects of ultrasonic treatment. By assuming that peripheral layer thickness is constant, the diameter reduction induces the starvation zone to become thinner and it may disappear (Figure 10). The percentage of microorganisms with access to the substrate and oxygen increases. If the floc diameter decrease can be considered an increase in active microorganisms, it is reasonable to expect that catabolism will be improved. The decrease in floc diameter may reduce the supply of substrate per unit of biomass, thus reducing the observed yield coefficient.

#### *Cryptic growth increase*

At sufficiently high acoustic power inputs, ultrasonic treatment can potentially induce cell wall breakdown, which results in the release into the medium of intracellular materials (proteins, lipids, nucleic acids, carbohydrates, etc.). This substrate released by cell lysis is available for microorganisms and contributes to the overall organic loading. The result is an increase in cryptic growth; cryptic growth is defined as the biomass growth on intracellular products. It is well known that cryptic growth is a significant mechanism for the reduction of the apparent biomass yield coefficient [3, 6]. Using ozonation, YASUI *et al.* [41, 42] induced cell lysis and suppressed excess sludge production. Indeed, a portion of substrate released by cell lysis is reused for catabolism and released as gases and is not accumulated as excess biomass.

#### *Bulk temperature increase*

Heating alone (without ultrasound) enhances solubilization of organic compounds. Moreover, ultrasonic disintegration seems to be more efficient with a high treatment temperature between 20 and 60°C [17, 19]. Therefore, we can assume that both shear stress and temperature increase contribute to the efficiency of the ultrasonic treatment. During our experiment, the ultrasonic reactor was not cooled, so the cavitation effect was not dissociated from the thermal effect. However, the increase in temperature due to sonication was only a few degrees: the bulk solution temperature measured at the outlet of the ultrasonic reactor was approximately 30°C. The effect of increasing the bulk temperature is therefore probably minor compared to the mechanical effect.

## Acoustic energy supplied

The energy consumption must be optimized in the industrial development of this process. The effectiveness of sonication is highly dependent on the energy provided. The most important parameters are volumic energy ( $\text{kJ l}^{-1}$ ) and specific energy ( $\text{kJ kg}^{-1}$ ) defined using ultrasonic power, sonication duration, sample volume and total solids concentration. As mentioned in the introduction, comparison with data in the literature is difficult because studies conducted with the continuous flow process are very rare. However, a few values of the optimal energy supplied are reported here. Note that the value of the optimal energy depends on the parameters used to evaluate the ultrasonic treatment (organic matter solubilization, biological respiration rate, biogas production, excess sludge production). Studying batch anaerobic biodegradability, BOUGRIER *et al.* [12] obtained the best results with specific energies between 6.3 and 9.5  $\text{MJ kg}^{-1}$  TS. CLIMENT *et al.* [20] measured the solubilization of volatile solids of an anaerobic sludge. They used different specific energy values between 1 and 100  $\text{MJ kg}^{-1}$  SS and they observed that beyond 40  $\text{MJ kg}^{-1}$  SS, improvements were not significant. CHU *et al.* [18] observed greatly enhanced anaerobic digestion with a volumic energy of 330  $\text{W l}^{-1}$  and a duration of 20 min (42.2  $\text{MJ kg}^{-1}$  TS). The optimal conditions were evaluated by ZHANG *et al.* [43] with a sequential batch reactor system by measuring excess sludge production. They proposed a specific supplied power of 120  $\text{kW kg}^{-1}$  TS with sonication lasting 15 min (108  $\text{MJ kg}^{-1}$  TS); 3/14 sludge was treated. The volumic energy was 11.6  $\text{kJ l}^{-1}$  in terms of wastewater. The same team [44], measuring the respiration rate of the sludge in the SBR, determined an optimal power density equal to 200  $\text{W l}^{-1}$  with sonication lasting 30 s, with a volumic energy equal to 6  $\text{kJ l}^{-1}$ . They noted that a further increase in power had no benefit.

In our study, the specific supplied energy was between 25 and 35  $\text{MJ kg}^{-1}$  TS depending on recycled sludge concentration. This applied power was not optimized and will require a more comprehensive study. The goal of treatment for our application was not necessarily cell lysis, which would require too much power and would lead to an exorbitant cost for the treatment process. The goal was to increase the exchange surface promoting the bioavailability of organic compounds. It is important to point out that some studies show very substantial effects, but they are undertaken with energies that are far too great for industrial applications.

Moreover, the portion of biomass recycled via the ultrasonic treatment loop ( $a.Q_0 \approx 0.5 \text{ l h}^{-1}$ ) will be the subject of an optimization study. The amount of sonicated biomass each day is between 42 and 60 g depending on recycled sludge concentration. It is approximately equal to the amount of biomass contained in the aeration tank. A preliminary experiment has shown that it was not beneficial to treat the entire recycling flow.

## CONCLUSIONS

Ultrasonic treatment at low frequencies seems to be a solution to reduce sludge production in a wastewater treatment plant, since the tests carried out on a laboratory-scale pilot plant resulted in reducing sludge production by approximately 30%. It was shown that there is a combination of several mechanisms which contribute to the decrease in biomass production. The physical characteristics of bioflocs are modified and mass transfer is promoted. Moreover, the ultrasonic treatment inducing biofloc breakdown and perhaps cell lysis resulted in the release of inter- and perhaps intracellular materials into the medium. A portion of biomass, recycled via the ultrasonic treatment loop and sent back to the reactor, formed substrate by cell lysis, increasing cryptic growth.

However, many points remain to be clarified. The impact of ultrasonic treatment on microorganism metabolism, its faunistic profile, and the substrate must be studied. Further studies will be essential before industrial development:

- the study of the hygienic properties of the sonicated sludge and the content of pathogenic organisms;

- the mass balance in nitrogen and phosphorous;
- the optimization of energy consumption and the portion of biomass sonicated;
- the evaluation of the increase in the total oxygen demand of the aerobic treatment. Indeed, the promotion of the respiration activity by ultrasonic treatment will increase the total oxygen demand in the aeration tank.

## ACKNOWLEDGMENTS

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## SYMBOLS

sCOD	soluble chemical oxygen demand (kg O <sub>2</sub> m <sup>-3</sup> )
tCOD	total chemical oxygen demand (kg O <sub>2</sub> m <sup>-3</sup> )
pCOD	particulate chemical oxygen demand (kg O <sub>2</sub> m <sup>-3</sup> )
a	rate of ultrasonicated sludge (-)
Q	flow (m <sup>3</sup> s <sup>-1</sup> )
r <sub>S</sub>	rate of substrate consumption (kg O <sub>2</sub> s <sup>-1</sup> m <sup>-3</sup> )
R	recycling rate (-)
R <sub>COD</sub>	organic matter removal efficiency (-)
V	aeration tank volume (m <sup>3</sup> )
X	sludge concentration (kg TS m <sup>-3</sup> )
Y	apparent yield coefficient (-)
Φ <sub>X<sub>excess</sub></sub>	flow of excess sludge production (kg TS s <sup>-1</sup> )
0	influent
E	excess sludge
L	effluent

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- Figure 1. Schematic diagram of the activated sludge systems. The reference line (REF-line) was the same set up without ultrasonic treatment.
- Figure 2. Total solids concentration in the aeration tanks ( $\text{g TS l}^{-1}$ ) as a function of time in the REF-line ( $\blacklozenge$ ) and in the US-line ( $\blacklozenge$ ). The concentration was maintained around  $3.5 \text{ g TS l}^{-1}$  by extracting excess sludge.
- Figure 3. Cumulative organic matter measured in the effluent as a function of cumulative COD removal: particulate COD in the REF-line ( $\bullet$ ) and in the US-line ( $+$ ) and soluble COD in the REF-line ( $\blacktriangle$ ) and in the US-line ( $\triangle$ )
- Figure 4. Cumulative total solids in effluent as a function of cumulative COD removal in the REF-line ( $\bullet$ ) and in the US-line ( $\circ$ ).
- Figure 5. Cumulative excess sludge production expressed in total solids as a function of cumulative COD removal in the REF-line ( $\blacksquare$ ) and in the US-line ( $\square$ ).
- Figure 6. Particle size distribution of biomass flocs in the aeration tanks in the REF-line ( $-\blacksquare-$ ) and in the US-line ( $-\triangle-$ ).
- Figure 7. Volatile to total solids ratio as a function of COD removal in the REF-line ( $\bullet$ ) and in the US-line ( $\circ$ ).
- Figure 8. Photographs of filamentous bacteria before and after sonication
- Figure 9. Photograph of filamentous bacteria with epifluorescence staining method ( $\times 1000$ ). Viable bacteria are stained green, while damaged bacteria appear red.
- Figure 10. Effect of floc diameter decrease on concentration gradients and starvation zone
- Table 1. Diluted synthetic influent composition.
- Table 2. Characteristics of activated sludge process.

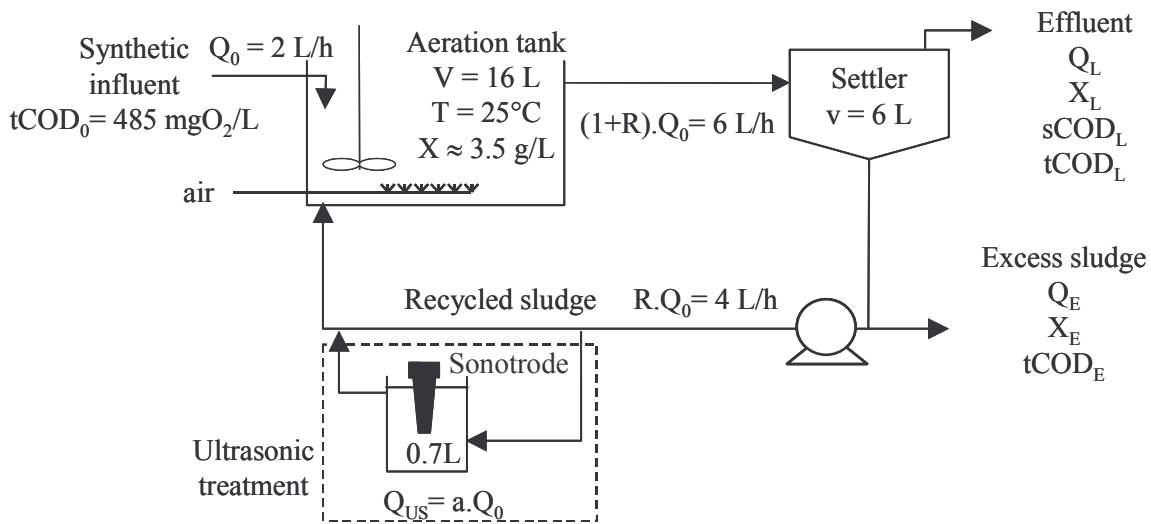


Figure 1

Reagent	Concentration (mg l <sup>-1</sup> )	Reagent	Concentration (mg l <sup>-1</sup> )
Urea	183	KH <sub>2</sub> PO <sub>4</sub>	47
Ammonium chloride	25	FeSO <sub>4</sub>	12
Sodium acetate	260	Potatoes starch	243
Peptone	35	Milk powder	232
MgHPO <sub>4</sub>	58	Yeast	105

Table 1

	Total solids in the aeration tanks (g l <sup>-1</sup> )	Volumetric loading rate (kg COD m <sup>-3</sup> d <sup>-1</sup> )	Specific loading rate (kg COD kg <sup>-1</sup> TS d <sup>-1</sup> )	Excess sludge production (g TS d <sup>-1</sup> )	Sludge retention time (d)
REF-line	3.9 ± 0.7	1.45	0.37	8.3	7.5
US-line	2.9 ± 0.5	1.45	0.50	5.0	9.3

Table 2

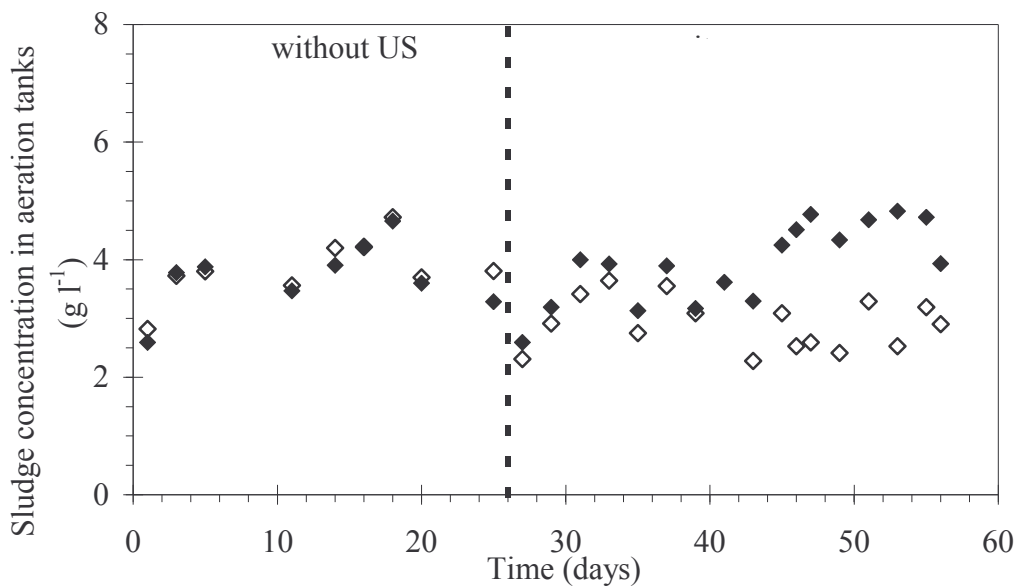


Figure 2



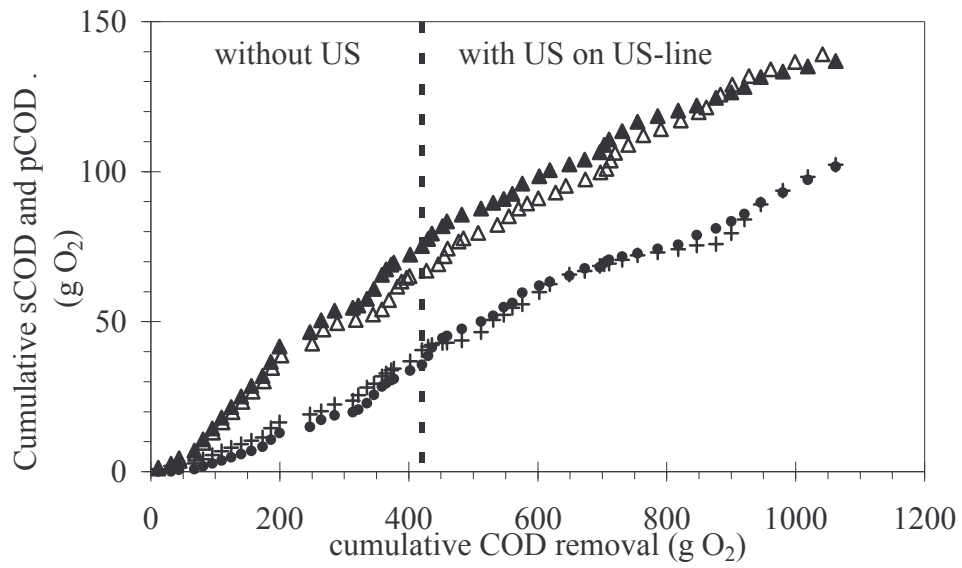


Figure 3

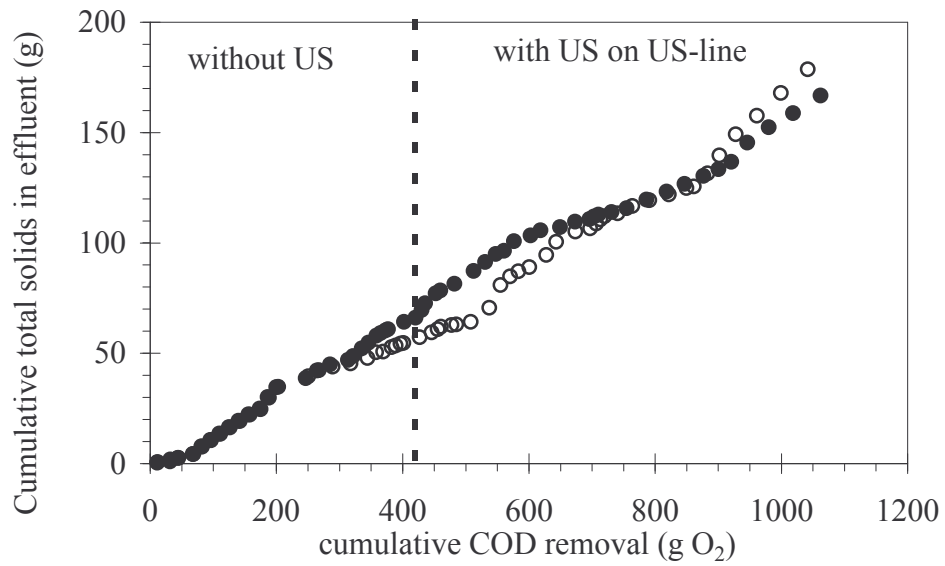


Figure 4

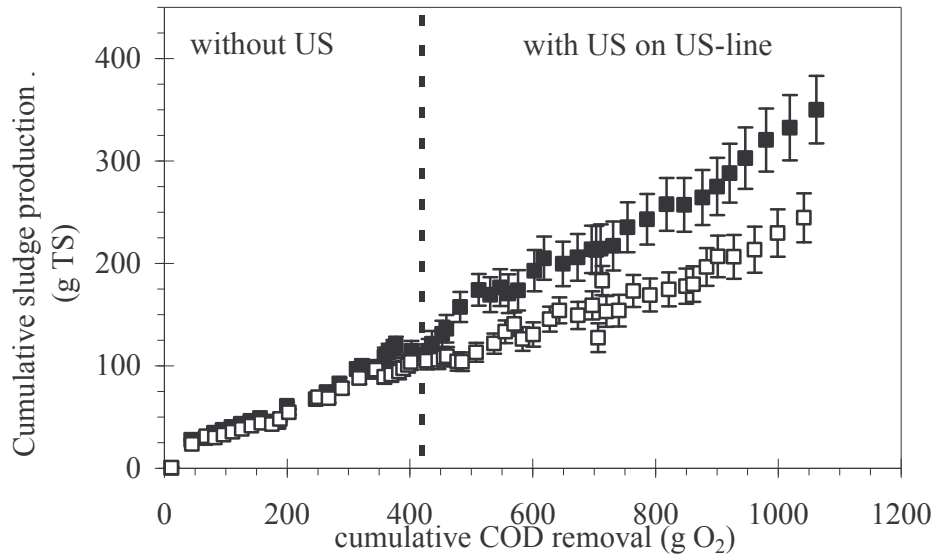


Figure 5

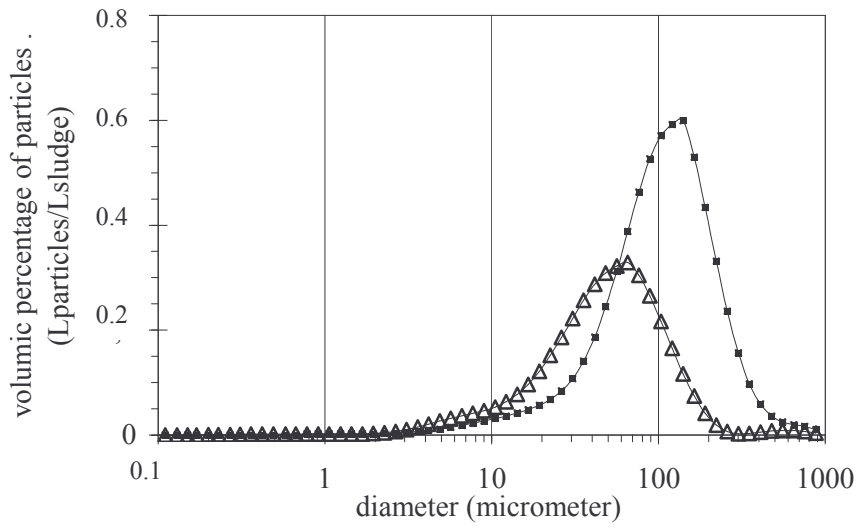


Figure 6

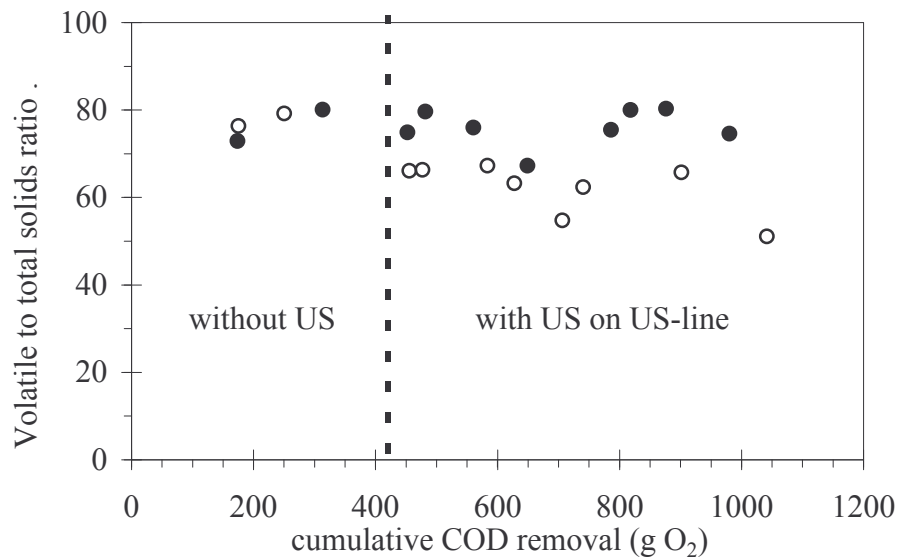


Figure 7

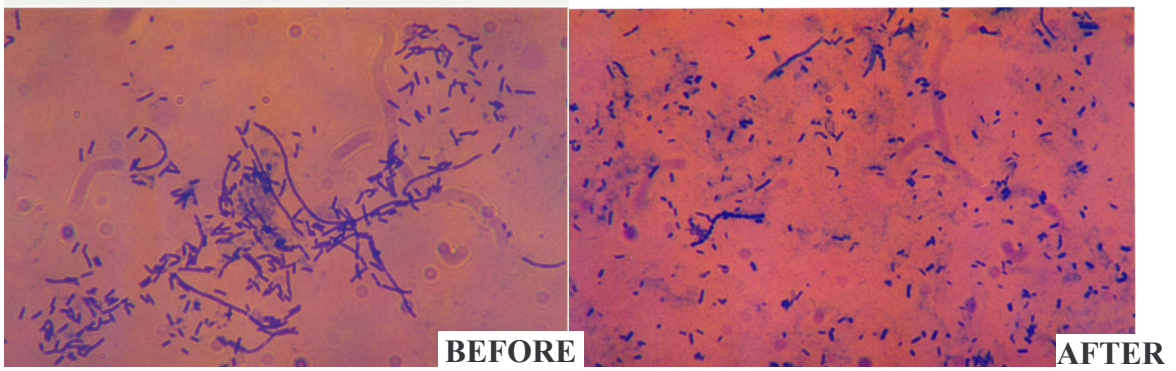


Figure 8

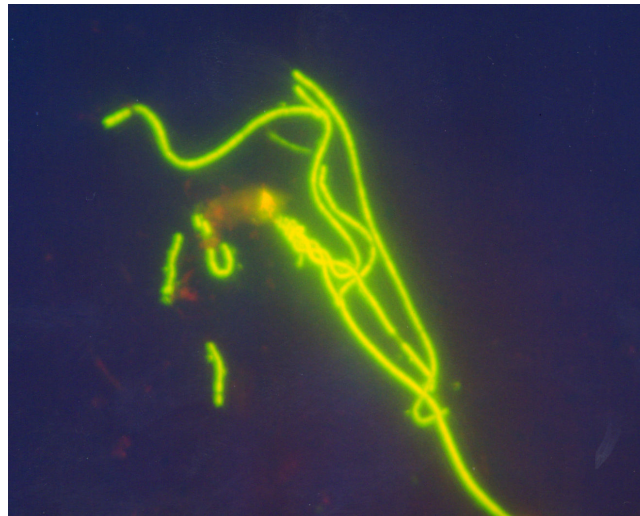


Figure 9

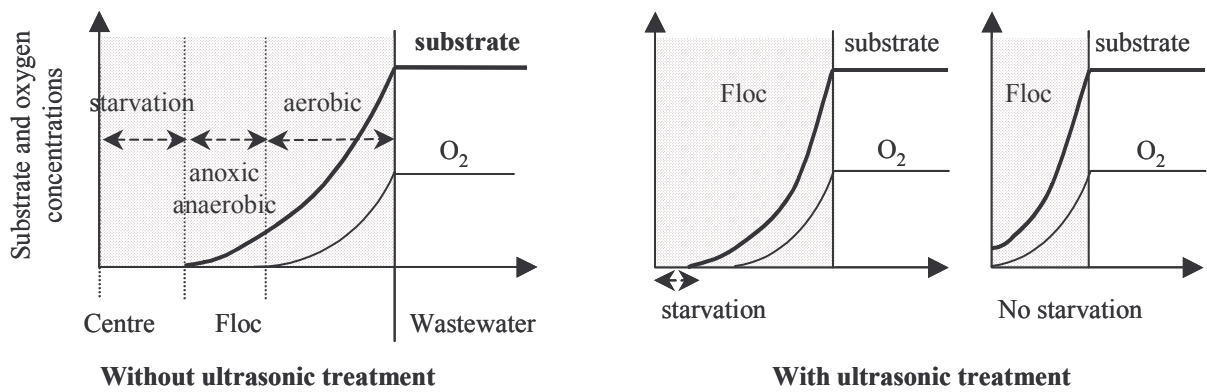


Figure 10