Determination of sorption properties of micropollutants: What is the most suitable activated sludge inhibition technique to preserve the biomass structure?

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Determination of sorption properties of micropollutants: What is the most suitable activated sludge inhibition technique to preserve the biomass structure?

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Apart from providing a total activated sludge (AS) inhibition, an efficient AS inhibition technique must preserve the biomass structure in order to maintain the real sorption phenomenon. Many inhibition techniques with different modes of action were used in previous studies for AS inhibition. But, the effectiveness of AS deactivation and the adverse effects on the biomass structure were rarely related. In this paper, five common AS inhibition techniques were evaluated: thermal, three chemical and gas purging techniques. The lowest chemical effective concentrations were determined in order to limit the negative impact on the AS structure. 100 mgHg_2SO_4 g_{TSS}^{-1} and 30 mg$HgCl_2$ g_{TSS}^{-1} within 2 h of reaction were enough to provide a complete AS inhibition. However, after 20 h of reaction a full AS inhibition has never been achieved with sodium azide at 200 mgNaN_3 g_{TSS}^{-1}, even by increasing NaN_3 concentration.

The analysis of the AS apparent viscosity, the median size D_{50} of the flocs and the supernatant turbidity showed that the thermal technique destructured the AS completely. A significant AS deflocculation is induced by the three chemical reagents depending on the mode of action and the concentration used. Thermal and chemical inactivations are therefore not suitable to determine sorption properties. The only technique which kept the initial AS structure unchanged has several drawbacks since (i) a reaction might occur between the gas and the analyte of interest, and (ii) anaerobic activated sludge are not inhibited by this technique. Therefore, the establishment of anaerobic conditions without gas injection is recommended for implementing sorption experiments on aerobic AS.

*Keywords:*Activated sludge inhibition Sorption, Activated sludge structure Deflocculation, Azide, Respiration

1. Introduction

In biological wastewater treatment plants (WWTPs) pollutants can theoretically be removed through several mechanisms: biotransformation, sorption to the activated sludge (AS) flocs, air-stripping and phototransformation. Biotransformation and sorption were reported as the two most important removal mechanisms for pharmaceuticals [1]. Indeed, phototransformation is limited by the high turbidity of the mixed liquor, which blocks sunlight and removal by air-stripping depends on Henry coefficient of the pollutant.

The partitioning between biotransformation and sorption is commonly evaluated by inhibiting the AS in order to avoid biotransformation mechanism. Thereafter, the sorption properties are determined by adding the pollutant into the inactivated biomass. The removal in the liquid phase is solely attributed to the sorption mechanism. Many different AS inhibition techniques

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were used in previous studies with different modes of actions: chemical, physical, gas purging, sterilisation and freeze-drying. A correct assessment of sorption properties requires a total bacterial activity inhibition to avoid any pollutant biotransformation along with no adverse effect on the AS structure induced by the inhibition technique. An incomplete AS inhibition would involve biotransformation of the target compound and AS deflocculation could offer more possibilities for the pollutant to sorb on the AS. Thus, the mixed liquor chemistry must remain unchanged after application of the inhibition technique. It was demonstrated that a pH modification could induce a speciation of some molecules which have basic or acidic functionalities, in a more or less hydrophobic form that affects their sorption affinities [2]. The mixed liquor conductivity must remain constant as well, since the adsorption mechanism is based on electrostatic interactions.

However, the effects of the inhibition techniques were rarely evaluated in previous studies, except for sodium azide [3–6] and different chemical biocides [7]. These studies showed that sodium azide, a chemical reagent used in many works to determine sorption properties, has an impact on the AS apparent viscosity [3,5], on the sludge-water distribution of several compounds [4] as well as on the AS conductivity [7]. Sodium azide might also react with the analyte of interest and its use for sorption experiments must be carefully evaluated, especially for concentrations higher than 100 $mg_{NaN3}\ L^{-1}$ [6]. There does not seem to be any consensus about the reliability of chemical deactivation to determine sorption properties. Indeed, some authors assume that chemical reagents might have an influence on the sorption processes due to the inactivation of cells and the consecutive cell lysis or because of the possible reaction with the target compounds [6,8], whereas other works reported that sodium azide addition was not inducing cell lysis [9] or polysaccharides releases [3].

As far as chemical inhibition is concerned no clear inhibition protocols, except for sodium azide [3], and validation of the methods have been reported. The concentration and inhibition kinetic were rarely mentioned and only few information reported the AS inhibition state. In addition, the inhibitor concentrations are not always related to the same parameters (biomass concentration, weight ratio, volumetric ratio).

Regarding this situation, the aim of this study was to compare different chemical inhibitors with alternative inhibition techniques in order to suggest the most appropriate inhibition technique to determine the sorption properties. The optimal parameters of each selected inhibition technique were determined in order to reach a sufficient inhibition state and to limit the impact on the biomass structure. Among the alternative AS inhibition techniques freeze-drying (lyophilisation) and autoclaving (sterilisation) were not evaluated in the present work since they were previously reported to alter the texture of sludge flocs and thus the sorbent structure [10,11]. AS sterilisation is carried out in an autoclave at 120 °C for 30 min. Freeze-drying is applied at a vacuum pressure of 0.5 mbar and a temperature of −40 °C.

Three different chemical inhibitors used in previous studies were selected: sodium azide (NaN_3) [3–5,7,9,12,13], mercury sulphate (Hg_2SO_4) [14–16] and mercury chloride ($HgCl_2$) [17]. Sodium azide was also frequently used to inhibit microbial degradation in order to assess sorption properties of environmental contaminants to soil [18–21]. The alternative inhibition techniques were based on thermal and gas purging inhibition processes. The thermal technique applied basically consists in drying the sludge by heating [22,23]. The gas purging technique consists in injecting a gas into AS in order to force oxygen out and was used in few studies with argon [24] and nitrogen [25,26]. None of these studies stated on the reliability of these AS inhibition techniques to determine sorption properties.

2. Material and methods

2.1. Activated sludge

The activated sludge (AS) came from an urban wastewater treatment plant (Aix-en-Provence, France, 175,000 eq. inh., 35,000 $m^3\ d^{-1}$, organic load 0.12 $kg_{BOD5}\ kg_{MVS}^{-1}\ d^{-1}$). Samples were taken from the recirculation loop between the aeration tanks and the secondary clarifiers and were then transported to the laboratory with no aeration (30 min). The initial total suspended solids (TSSs) concentration varied from 3.5 to 5.1 $g\ L^{-1}$. The amount of volatile suspended solids varied from 76% to 80% for all the experiments. The AS was concentrated by gravimetric filtration using paper filters (average size pores around 100 μm) in order to obtain a TSS content of $10 \pm 1\ g\ L^{-1}$. Before any experiment was performed, sludge was aerated during 4 h without substrate addition and the oxygen uptake rate (OUR) of small samples was monitored to ensure endogenous respiration state.

2.2. Respiration inhibition

Activated sludge respirometry was monitored by the OUR calculation. Air injection is stopped and the decreasing dissolved oxygen concentrations were recorded every 10 s. The OUR is the value, which corresponds to the slope of the linear decrease of the oxygen concentration over time. The specific oxygen uptake rate (SOUR) relates the OUR depending on the mixed liquor volatile suspended solids concentration (MLVSS). A SOUR null value means that the AS inhibition state is reached because microorganisms cannot consume the dissolved oxygen. The inhibition state of the biomass was then monitored by calculating the SOUR drop for the five inhibition techniques in comparison to the initial SOUR of the AS. The dissolved oxygen concentration was measured with continuous oxygen probe (HQ 40d, Hach LDO, Germany).

2.3. Chemicals

Sodium azide (NaN_3, 99%, Sigma–Aldrich), mercury chloride ($HgCl_2$, 99.5%, Sigma–Aldrich) and mercury sulphate (Hg_2SO_4, 99%, Chem-Lab) were used as chemical inhibitors.

2.4. Thermal technique

The Thermal inhibition technique simply consists in drying the sludge. In this paper the protocol applied for thermal inhibition was established by Delgado [22]. AS is firstly centrifuged during 20 min at 5000 rpm. Solids are collected and rinsed with distilled water in order to reduce the amount of exopolymeric substances. The clean sludge is then centrifuged again during 20 min. The sludge is dried at 80 °C during 2 days to ensure the inactivation and the complete drying. The biomass is finally ground until obtaining a uniform size of grains.

The grains are put in a batch reactor with the AS supernatant to reach a concentration of 10 $g_{TSS}\ L^{-1}$. Solubilisation of the grains could not be reached after a long stirring time, the mixture remained completely heterogeneous. SOUR measurement indicated that the complete AS inhibition was achieved.

2.5. Gas purging technique

The inhibition protocol used in this study was based on the method developed by Seira et al. [25]. Firstly, oxygen was injected into AS to remove the residual substrates. Then, the aeration was stopped in order to remove the nitrates under anoxia conditions. Finally, nitrogen gas was injected to AS in order to force oxygen

out. The dissolved oxygen concentration quickly reached a null value, avoiding AS respiration.

2.6. COD and NH_4^+ degradation tests

800 mg L^{-1} (180 mg g_{TSS}^{-1}) of chemical oxygen demand (COD) as glucose and 50 mg L^{-1} (12 mg g_{TSS}^{-1}) of ammonium were added to the inhibited biomass during 2 h under aeration in order to assess the effectiveness of each inhibition technique on the removal of easily biodegradable compounds. COD and ammonium were chosen to distinguish the efficiency of the inhibition techniques on heterotrophic and autotrophic bacteria. Substrate degradation would show an incomplete inhibition of the related bacteria population.

2.7. Bioreactor and rheological devices

The bioreactor was equipped with a double helical ribbon impeller (HRI) and filled with a volume of 1.9 L of activated sludge. The impeller had a 9.5 cm diameter and height each, while the bioreactor was 12 cm in diameter. Sludge temperature was maintained constant at 20 ± 1 °C with water circulating in a double envelope.

To perform the in situ viscosity curves determination of AS suspensions, the HRI of the bioreactor was connected to a shear rate imposed rheometer (Rheomat 30, Contraves) with 30 available values of the imposed rotation speed in the range 0.408–257 rpm. The stirring torque was measured for each value of the rotation speed.

Before each torque measurement, the suspension was presheared at the maximum rotation speed for 30 s to ensure its homogeneity. The rotation speed was then decreased to the desired value and the torque was recorded for 15 s after 15 s of stabilisation.

Viscosity calculations were performed with Metzner–Otto's principle. It defines an apparent viscosity η_a (Pa s) based on the generalization for non-Newtonian media of the relationship in an agitated vessel (in the laminar region) between the dimensionless power Np and the Reynolds number Re (Eq. (1)):

$$N_p = \frac{P}{\rho \cdot N^3 \cdot d^5} = \frac{K_p}{Re} = \frac{K_p \cdot \eta_a}{\rho \cdot N \cdot d^2} \text{ then } \eta_a = \frac{P}{K_p \cdot N^2 \cdot d^3} \quad (1)$$

where P (W) is the mechanical stirring power related to the agitation torque C (N m) and to the mechanical rotation rate N (s^{-1}) by the following equation:

$$P = 2 \cdot \pi \cdot N \cdot C \quad (2)$$

K_p corresponds to the laminar power curve constant and d (m) to the impeller diameter.

At a rotation rate N corresponds an effective shear rate γ_{MO} (s^{-1}) related to the rotation speed of the impeller by the Metzner–Otto dimensionless constant characterising the stirrer geometry (Eq. (3))

$$\gamma_{MO} = K_{MO} \cdot N \quad (3)$$

Values of K_p and K_{MO} constants have been determined at respectively 393 and 50. A calibration of the set-up was carried out for a previous paper [27] with two model fluids at 20 °C to determine the K_p and K_{MO} constants. The fluids used were a Newtonian solution of pure glycerol and an aqueous solution of guar at 1 wt.%.

The Ostwald power law model (Eq. (1)) has been used to represent the viscosity curves of initial and inhibited AS.

$$\tau = K\gamma^n \quad (4)$$

Both parameters of this model, i.e. the consistency index (K in Pa s^n) and flow index (n) were calculated with a simple linear regression log–log scale.

Three measurements of the rheological profile were carried out to ensure the data reproducibility.

2.8. Analytical methods

TSS were measured by centrifugation of a 30 mL sludge sample for 15 min at 13,500 rpm followed by pellet drying at 105 °C until a constant weight was obtained (24 h). TVSS were measured after 2 h at 550 °C.

The degree of sludge deflocculation was estimated by the method presented by Wilén et al. [28]. Samples of AS were taken during the inhibition reaction and centrifuged at 2000 rpm for 2 min. Supernatant turbidity was then measured as the absorbance at 650 nm.

Particle size distribution of AS was measured with a laser granulometer Mastersizer S (Malvern Instruments). It measures particle size from 0.1 μm to 900 μm. The median size (D_{50}) was recorded for every AS inhibition technique. The filtrate of the mixed liquor recovered after AS concentration was used to dilute 100 times the inhibited AS samples in order to obtain an acceptable obscuration allowing the particle size distribution measurement. Three measurements of particle size distribution and the supernatant turbidity were carried out to ensure the data reproducibility.

pH and conductivity of the mixed liquor were measured for initial AS and for every inhibition techniques.

3. Results and discussion

3.1. Determination of optimum chemical inhibitor concentrations

Very little information is provided in the literature about chemical inhibitor parameters. There is no consensus about inhibitor concentration units. In this paper the expression of inhibitor concentrations was standardised into $mg_{inhibitor}\ g_{TSS}^{-1}$. Inhibitor concentrations used in previous studies were converted into $mg_{inhibitor}\ g_{TSS}^{-1}$ when enough data were available in the papers (Table 1). As it can be seen in this table, there is also no consensus on the chemical concentration used for AS inhibition.

Concerning sodium azide a wide range of concentrations, from 0.5 mg g_{TSS}^{-1} [4] to 720 mg g_{TSS}^{-1} [5], were used in previous studies for AS inhibition. However, sodium azide was previously reported to alter biomass viscosity [3,5]. It is coherent to assume that if chemical reagents induce negative effects to the AS structure, increasing the chemical concentration would emphasize the impact on AS structure. This hypothesis was confirmed by preliminary rheological measurements carried out at different sodium azide concentrations (Fig. 1). An increase in the sodium azide concentration caused a lower AS viscosity. Thereby, the lowest effective chemical concentrations were firstly determined before comparing the impact of each inhibition technique with the AS structure.

Therefore, it is required to find for each chemical inhibitor the lowest concentration at which the complete inhibition is achieved in order to limit alteration of the AS structure. Working at the lowest effective chemical inhibitor concentration makes it possible to compare the different inhibition techniques with each other equally.

3.1.1. Sodium azide

Only little information is generally mentioned by the authors about the inhibition parameters and the reliability of the method (Table 1), except for Barbot et al. [3] who tested 3 sodium azide

Table 1
Chemical inhibitor concentrations used in previous studies.

Inhibition information	Calculated concentration	Reaction time	Comments	References
NaN_3				
0.2% w/w	2 mg g_{TSS}^{-1}	–	"based on respirometry measurement, without any cell lysis or any change in the sludge hydrophobicity"	[8]
–	–	–	–	[9]
0.2% v/v	–	–	No information on the sodium azide solution concentration – TSS = 9.01 g L^{-1}	[10]
0.9 g g_{MVLSS}^{-1}	720 mg g_{TSS}^{-1}	–	Inhibition of the respirometry activity. "no polysaccharide release, viscosity was reduced of around 40% but did not modify activated sludge sorption abilities"	[4]
200 mg g_{TSS}^{-1}	–	4 h	Inhibition of around 90% after 4 h. 0.1 et 0.35 g g_{TSS}^{-1} also tested. Decrease of the apparent viscosity	[2]
190 mL sludge – TSS = 4 g L^{-1} [NaN3] = 0.2% and 1%	0.5 mg g_{TSS}^{-1} and 2.5 mg g_{TSS}^{-1}	–	Adverse effects of sodium azide on the sludge-water distribution of several compounds	[3]
Hg_2SO_4				
0.5 mL L_{sludge}^{-1} – [Hg_2SO_4] = 200 g L^{-1} – 0.1 g $_{Hg2SO4}$ L_{sludge}^{-1} – TSS = 1-3-5-7 g L^{-1}	14 – 20 – 33 – 100 mg g_{TSS}^{-1}	–	–	[11]
[Hg_2SO_4] solution = 0.2 g L^{-1} – TSS = 1 g L^{-1}	–	–	–	[12]
1 mL Hg_2SO_4 to 200 mL_{sludge} – [Hg_2SO_4] = 20 g L^{-1} – TSS = 15 g L^{-1}	6.7 mg g_{TSS}^{-1}	–	–	[13]
$HgCl_2$				
100 mg_{HgCl2} L_{sludge}^{-1} – Xbiomass = 6.2 g_{COD} L^{-1} – TSS = 5.46 g L^{-1}	18.3 mg g_{TSS}^{-1}	–	$HgCl_2$ has a smaller influence than sliver nitrate on the measurements of the investigated compounds	[14]

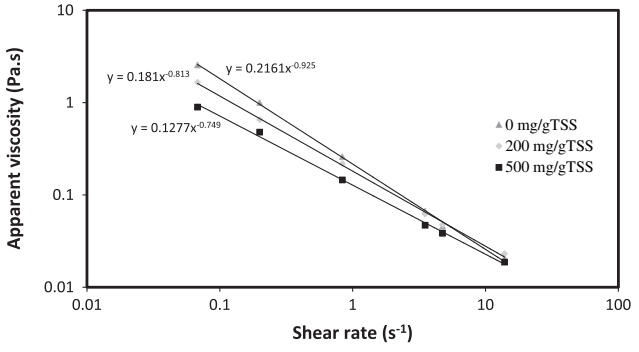

Fig. 1. Influence of azide concentration on the apparent viscosity of AS.

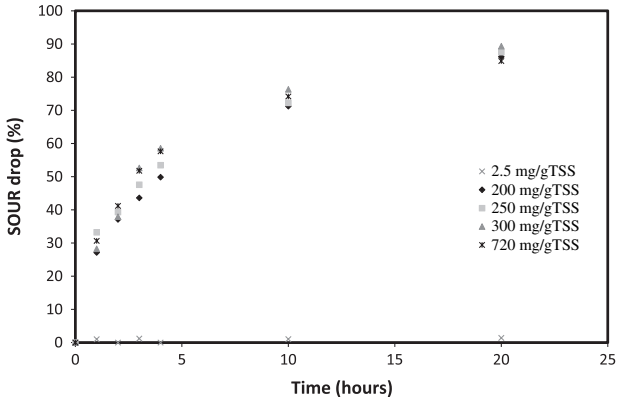

Fig. 2. SOUR drop at different sodium azide concentrations.

concentrations: 100 – 200 – 350 mg g_{TSS}^{-1}. In this study the maximum inhibition state of the microorganism respiration was obtained after addition of NaN_3 at 200 mg g_{TSS}^{-1}.

Five sodium azide concentrations were tested in this paper: the two drastic concentrations 0.5 and 720 mg g_{TSS}^{-1} and three concentrations 200 – 250 – 300 mg g_{TSS}^{-1} in order to ripen the optimum concentration found by Barbot et al. [3].

No AS inhibition was observed at 0.5 mg g_{TSS}^{-1}, this concentration being totally insufficient to impact biomass respiration. For the three concentrations 200 – 250 – 300 mg g_{TSS}^{-1} a 90% inhibition state has never been achieved after 4 h of reaction contrary to Barbot et al. [3]. 20 h were required in some experiments to reach a SOUR drop about 90% (Fig. 2). Furthermore, a poor repeatability of the results was observed, indicating that AS inhibition by sodium azide might depend on other AS parameters, such as AS initial activity or AS structure. AS inhibition with the very high sodium azide concentration (720 mg g_{TSS}^{-1}) was never above 84%, even after 20 h of reaction. It is noticeable that, in the range of the tested concentrations, an increase in the sodium azide concentration was not linked with a higher AS inhibition, meaning that beyond a threshold concentration no better AS inhibition could be reached with sodium azide.

Therefore, sodium azide seems to be inappropriate to inhibit AS because it is impossible to suggest a concentration and a reaction time allowing the achievement of a complete and systematic AS inhibition state. The concentration 200 mgNaN_3 g_{TSS}^{-1} was chosen to conduct the biomass structure comparison as no significantly better AS inhibition was achieved at higher concentrations.

3.1.2. Mercury sulphate

Hg_2SO_4 concentrations used in previous works were much lower than sodium azide concentrations (Table 1). Three concentrations of Hg_2SO_4 were tested according to the concentrations of Clara et al. [14]: 10 – 50 – 100 mg g_{TSS}^{-1} (Fig. 3). Concentrations of 10 and 50 mg g_{TSS}^{-1} provided a good AS inhibition but not complete for every test. A total AS inhibition was achieved at 100 mg g_{TSS}^{-1} in the two first hours of reaction for each test.

At half concentration and for a lower reaction length than for sodium azide the AS inhibition was complete, which indicates a different mode of action in the inhibition process. The concentration 100 mg_{Hg2SO4} g_{TSS}^{-1} was chosen for the biomass structure comparison.

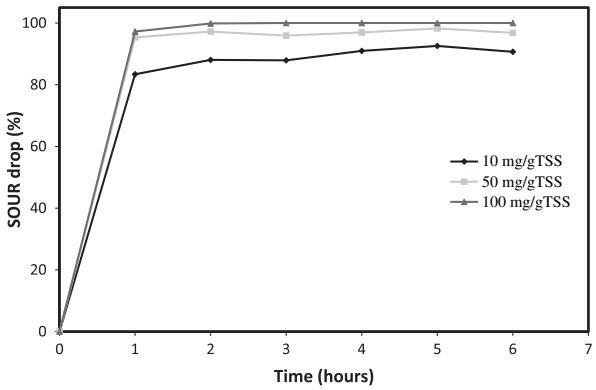

Fig. 3. SOUR drop at different mercury sulphate concentrations.

3.1.3. Mercury chloride

Only one study using $HgCl_2$ to inactivate biomass has been found [17]. Because of the lack of studies, the concentration tests of $HgCl_2$ were arbitrary set at the same concentration as for Hg_2SO_4: 10 – 50 – 100 mg g_{TSS}^{-1} (Fig. 4). The concentration of 10 mg g_{TSS}^{-1} provided a good AS inhibition but not complete for each test, whereas a complete and systematic AS inhibition was achieved at 50 and 100 mg g_{TSS}^{-1} in the two first hours of reaction. A concentration of 30 mg g_{TSS}^{-1} was tested to ripen the optimum concentration and provided a complete AS inhibition in the two first hours of reaction for each test. Mercury toxicity is known to depend on its chemical form. Therefore, the lower concentration of $HgCl_2$, in comparison with Hg_2SO_4, required inhibiting AS can be explained by the fact that mercury toxicity depends on its chemical form. The mercury (II) cation is much more toxic than the mercury (I) cation. Thus, the concentration 30 mg_{HCl2} g_{TSS}^{-1} was chosen for biomass structure comparison.

3.2. Effect of activated sludge inhibition on the biomass structure

To assess the effect of each inhibition technique on the biomass structure, the rheological profile, the supernatant absorbance and the median size D_{50} of sludge suspension were measured for the initial AS sample and for every inhibition technique when AS was considered in the inhibition state (Figs. 5 and 6 and Table 3), i.e. 2 h for Hg_2SO_4 and $HgCl_2$, 20 h for NaN_3.

The viscosity curve of the thermal inhibition technique was not represented in Fig. 5 since no rotation torque could be measured by the experimental set-up at the shear rates tested in the laminar region. Indeed, the dried and ground sludge could not solubilise at all into the supernatant and resulted in two distinctive phases, which caused an irreversible effect on the AS structure.

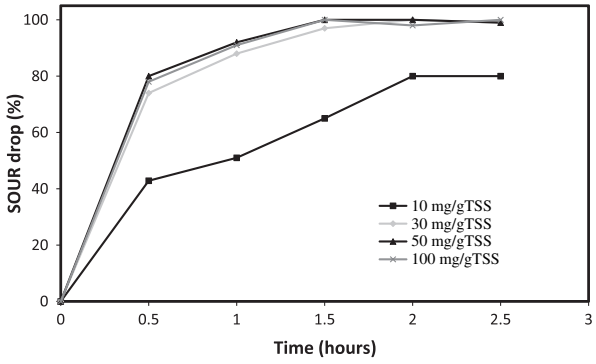

Fig. 4. SOUR drop at different mercury chloride concentrations.

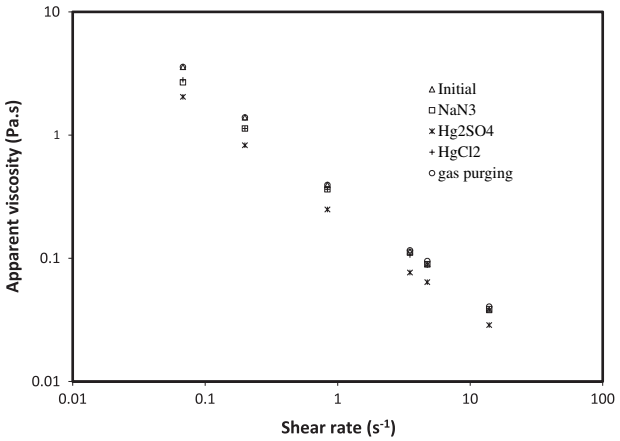

Fig. 5. Rheological behaviour of inhibited AS with the optimal chemical concentrations and the gas purging technique.

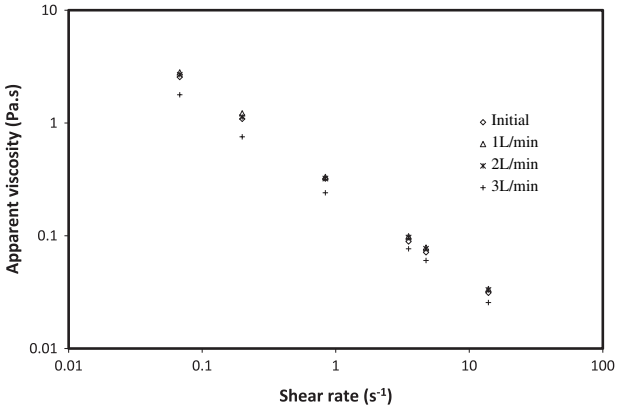

Fig. 6. Influence of N_2 flow rate on the activated sludge viscosity.

A decrease of the AS apparent viscosity is induced by the three chemical methods. At the concentrations tested to ensure biomass inhibition, NaN_3 and $HgCl_2$ have the same impact on the apparent viscosity whereas Hg_2SO_4 causes a more significant drop-off. Mercury inhibitors seem to have a stronger impact on the bioflocs structure than sodium azide although NaN_3 is 6.7 times more concentrated than $HgCl_2$. AS apparent viscosity differences between Hg_2SO_4 and $HgCl_2$ could be attributed to the higher concentration of Hg_2SO_4 required for AS inhibition.

The Ostwald law parameters confirm these observations (Table 2): a 10% decrease of the consistency index K is induced by NaN_3 and $HgCl_2$ additions at inhibiting concentrations whereas a 35% decrease of K is induced by Hg_2SO_4 addition. The flow index n increased for the 3 chemical inhibition techniques in the same order of magnitude (from 0.14 to 0.19 for $HgCl_2$ and Hg_2SO_4 and 0.20 for NaN_3). The apparent viscosity remained constant for the gas purging inhibition indicating no influence on the AS rheological behaviour. However, the nitrogen flow rate has an influence on the apparent viscosity of AS (Fig. 6). A decrease of the apparent viscosity was observed from 3 L_{N2} min^{-1} indicating a slight destructuration of AS.

In order to quantify the degree of deflocculation induced by the different inhibition techniques, the supernatant turbidity was measured for initial and inhibited samples (Fig. 7). The results were consistent with the rheological measurements. The gas purging technique did not induce any deflocculation whereas the thermal technique caused the highest deflocculation of all the tested inhibition techniques. The high degree of deflocculation induced

Table 2
Ostwald law parameters of inhibited AS with five different inhibition techniques.

Inhibition technique	Concentration (g gTSS^{-1})	K (Pa s^n)	K decrease[a] (%)	n
Initial	–	0.35	–	0.14
NaN3	0.2	0.31	10.7 ± 0.6	0.20
Hg_2SO_4	0.1	0.22	36.6 ± 1.4	0.19
$HgCl_2$	0.03	0.31	10.7 ± 0.2	0.19
Gas purging	–	0.35	0 ± 2.2	0.15
Thermal	–	–	–	–

[a] Average and standard deviation based on three measurements.

by the thermal inhibition technique could result from the fact that sludge solids did not solubilise at all into the supernatant. The centrifugation (2 min at 2000 rpm) carried out to measure supernatant turbidity was not fast enough to bind a significant amount of sludge solids together.

It is noticeable that the deflocculation degree induced by the three chemical inhibitors is similar whereas the reaction duration is much longer for sodium azide (20 h) than for both mercury inhibitors (2 h). It confirms that mercury is much more toxic to AS than sodium azide. The evolution of the supernatant turbidity over 20 h showed that AS deflocculation was limited for sodium azide and mercury sulphate (0.06 absorbance unit) whereas mercury chloride was inducing a high AS deflocculation over time (0.16 absorbance unit). This confirms the more toxic nature of mercury (II) cation in comparison to mercury (I) cation. Therefore, $HgCl_2$ might have a higher negative effect to determine sorption properties than Hg_2SO_4 depending on the required sorption equilibrium.

The observation of the median size (D_{50}) of AS flocs for the five inhibition techniques showed that the three chemical inhibitors induced a reduction of the D_{50} (Table 3). Hg_2SO_4 caused the most significant D_{50} reduction of the chemical inhibitors with a D_{50} drop-off of 20%. $HgCl_2$ induced a 15% decrease of D_{50} probably due to the lower concentration used, in comparison to Hg_2SO_4, to inhibit biomass. Surprisingly, NaN_3 effect on D_{50} was very limited with only a D_{50} decrease of 6%, whereas NaN_3 impacts on the apparent viscosity and the deflocculation degree were identical to $HgCl_2$ impacts. The thermal technique had a drastic effect on the particle size distribution, mainly because of the grinding and the resolubilisation step. The very high D_{50} variability of the thermal inhibition technique shows the lack of homogeneity of the grinding step. Finally, the gas purging technique did not cause any alteration of D_{50}. The biomass structure has not been altered at all by this inhibition technique.

3.3. Effect of the activated sludge inhibition techniques on pH and conductivity

pH and conductivity of initial and inhibited AS were analysed (Table 4) for each inhibition techniques in order to check if the inhibition technique has an impact on these parameters that would disturb the determination of the sorption properties. The pH variation is not significant since pH remains approximately neutral for every inhibition technique. A slight increase in the conductivity is observed for $HgCl_2$ and Hg_2SO_4 but remains in the same order of magnitude as the initial AS.

NaN_3 induces a drastic increase in the conductivity which can be attributed to the high molecular concentration used (30.77 mol m^{-3} for NaN_3, 1.10 mol m^{-3} for $HgCl_2$ and 2.01 mol m^{-3} for Hg_2SO_4). Therefore, the addition of sodium azide would clearly modify the sorption properties because of the increase in conductivity. Once again, it is noteworthy that the gas purging technique has the lowest effect on both pH and conductivity.

3.4. Degradation of easily biodegradable compounds by inhibited activated sludge

The removal of 800 mg L^{-1} of COD as glucose and 50 mg L^{-1} of NH_4^+ were analysed for the five different inhibition techniques

Table 3
D50 values of initial and inhibited AS.

Inhibition technique	D50[a] (μm)	D50 decrease (%)
Initial	87.3 ± 2.1	–
Gas purging	86.6 ± 1.6	0.8
NaN_3	81.8 ± 2.4	6.3
$HgCl_2$	74.5 ± 1.1	14.7
Hg_2SO_4	69.8 ± 2.5	20.1
Thermal	118.7 ± 31.4	−36.0

[a] Average and standard deviation based on three measurements.

Table 4
pH and conductivity values of initial and inhibited AS.

Inhibition technique	pH	Conductivity (μS cm^{-1})
Initial	7.12	854
Gas purging	7.17	852
Hg_2SO_4	6.99	930
$HgCl_2$	7.37	903
NaN_3	6.97	3240
Thermal	7.03	874

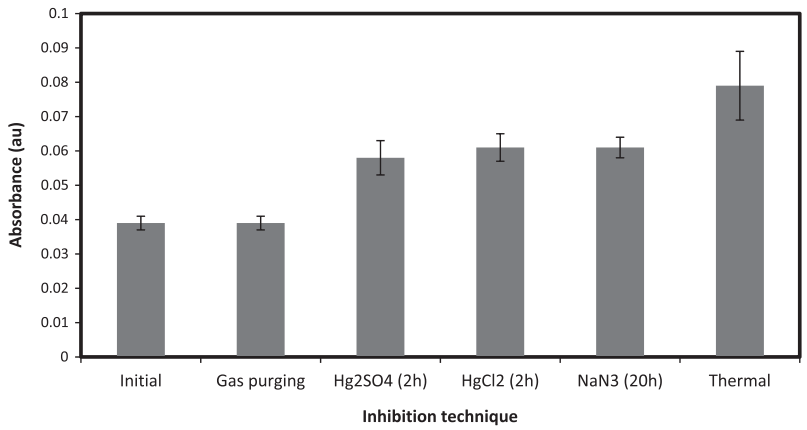

Fig. 7. Supernatant turbidity of initial and inhibited biomass.

(Table 5). An increase in COD can be observed after 2 h for every chemical inhibitor. It shows that chemical inhibition leads to a release of soluble microbial products and cell lysis. No COD removal was observed for the thermal and gas purging techniques. Ammonium concentration remained constant for both mercury reagents as well as for the thermal technique whereas a slight degradation was observed for the gas purging technique and sodium azide. The NH_4^+ degradation by sodium azide can be explained by the incomplete achieved inhibition state. One can assume that the residual SOUR after sodium azide application is partially due to autotrophic bacteria, which could perform the nitritation process. Ammonium degradation during the application of the gas purging technique was more surprising because no oxygen was present in the system. Two additional tests with the gas purging technique were performed: a control reactor with only water and NH_4^+ under continuous nitrogen injection; a reactor with AS and NH_4^+ under anaerobic conditions (without the presence of dissolved oxygen in the system) in which nitrogen was only injected at the beginning to force oxygen out. NH_4^+ degradation in water with continuous nitrogen injection was similar to NH_4^+ degradation in AS with continuous nitrogen injection. It pointed out that a reaction of NH_4^+ with N_2 occurred. NH_4^+ concentration remained constant in AS under anaerobic conditions and confirmed that N_2 reacted with NH_4^+.

3.5. Reversibility of the inhibition techniques

The reversibility of the 5 inhibition techniques was assessed 24 h after reaching the AS inhibition state by monitoring AS respirometry. Only the gas purging technique allows the recovery of a full AS activity immediately after reinjecting oxygen. No AS respiration could be recovered after inhibition by the thermal technique and both mercury reagents indicate the irreversibility of these inhibition processes causing bacteria decease. A slight AS respiration was observed with NaN_3 that can be linked to the incomplete AS state inhibition achieved with this chemical reagent. Therefore, chemical and thermal inhibition techniques cause irreversible damage on AS activity whereas the gas purging technique is the only technique investigated in this study, which allows a totally reversible inhibition process.

Table 5
COD and NH_4^+ removal efficiency for inhibited AS.

Inhibition technique	COD removal efficiency (%)	NH_4^+ removal efficiency (%)
Gas purging	0.0	14.3
Hg_2SO_4	−11.4	−0.7
$HgCl_2$	−9.9	0.9
NaN_3	−8.2	23.4
Thermal	0.0	0.0

3.6. Ranking of the tested activated sludge inhibition techniques

A recap chart of the adverse effects of the five inhibition techniques on AS structure and mixed liquor chemistry parameters investigated is presented in Table 6. Measurements of the apparent viscosity, the supernatant turbidity and the particle size distribution make it possible to rank the five AS inhibition techniques investigated in this study. The thermal inhibition technique alters drastically the AS structure resulting in a two distinct phases solution with a high floc sizes variability. Indeed, the grinding and the resolubilisation steps of the dried sludge seem crucial to recover the sludge matrix. These two steps are difficult to perform, even being extremely cautious. It explains the impossibility to measure the viscosity at low shear rates (in the laminar region) as well as the large variability of the floc sizes. The thermal inhibition technique is definitely not suitable to assess sorption properties as it damages the floc structure irreversibly. Therefore, sorption of thermally inhibited sludge would absolutely not represent the actual sorption phenomenon occurring in WWTP.

The three chemical inhibitors altered the biomass structure in a more reasonable way than the thermal inhibition. AS was very sensitive to mercury as it is shown by the respiration inhibition kinetics and by the AS structure parameters. Only 2 h were required to ensure biomass inhibition at low mercury concentration whereas AS inhibition was much more difficult to control with sodium azide: complete AS inhibition has never been achieved and an increase in the NaN_3 concentration did not induce a more complete and faster AS inhibition. NaN_3 addition involved a very limited decrease of the D_{50} of the AS flocs. Besides, NaN_3 is the only chemical reagent investigated that did not inhibit the nitritation process. One can assume that part of autotrophic bacteria was not inhibited by NaN_3 addition which could explain the residual SOUR, being in the same order of magnitude independently of the initial NaN_3 concentration. Conversely, the complete and systematic AS inhibition obtained at low mercury concentrations could be the result of the high toxicity effect of mercury on cells. Therefore, mercury induces an important reduction of D_{50} which increases the contact for the reaction between mercury and the bacteria, leading to a full AS inhibition. The significant increase in the supernatant turbidity of AS inhibited with $HgCl_2$ after 20 h of reaction suggests that $HgCl_2$ alters less AS structure than Hg_2SO_4 only within a short time. Therefore, $HgCl_2$ is more suitable than Hg_2SO_4 to determine sorption properties immediately after the 2 h of reaction required for AS inhibition. Depending on the sorption equilibrium time, AS inhibition by Hg_2SO_4 would be a better option to limit AS destructuration. After 2 h of reaction the limited effect of $HgCl_2$, in comparison to Hg_2SO_4, on AS apparent viscosity and on the D_{50} can only be attributed to the lower concentration used. The increase in COD observed for the three chemical reagents after 2 h of contact with glucose confirmed the achieved inhibition state and the cell lysis induced to the biomass.

Table 6
Recap chart of adverse effects to the biomass structure and the mixed liquor chemistry induced by AS inhibition techniques.

Inhibition technique	AS respirometry	Viscosity	Supernatant turbidity	D50	pH	Reversibility	COD	NH_4^+	Ranking
Inhibition technique	AS respirometry	Viscosity	Supernatant turbidity	D50	pH	Reversibility	COD	NH_4^+	Ranking
Gas purging	/	/	/	/	/	/	/	-	1
NaN_3	-	-	-	-	/	-	-	-	3
$HgCl_2$	/	-	-	--	/	-	-	/	2
Hg_2SO_4	/	--	-	--	/	-	-	/	3
Thermal	/	---	--	---	/	-	/	/	5

/ No effect.
-, --, --- Effect degree (from low to high effect).

$HgCl_2$ seems to be the most appropriate chemical reagent for biomass inactivation as its low required concentration allows limits the adverse effects on the AS structure but only within a short time. In comparison, NaN_3 alters AS structure in the same order of magnitude as $HgCl_2$. But the difficulty to achieve a sufficient AS inhibition, which may lead to a degradation of metabolisable compounds by autotrophic bacteria, as well as the longer reaction time required makes NaN_3 less appropriate for the determination of sorption properties. However, AS inhibition by $HgCl_2$ will not allow the determination of the exact sorption properties occurring in WWTP.

The gas purging inhibition technique does not induce any effect on the biomass structure. The AS initial properties are kept unchanged after injecting nitrogen in the bioreactor. In addition, this inhibition technique is really easy to implement and, after the aerated and non-aerated phases, the inhibition state is immediately reached as the dissolved oxygen concentration is quasi instantaneously null. Only the nitrogen flow rate must be controlled in order to avoid AS deflocculation. However, it does not consist in a limiting factor since a low nitrogen flow rate is sufficient to quickly force oxygen out. Nevertheless, a gas reaction with the analyte of interest, as observed with nitrogen and ammonium, is possible and would alter the determination of sorption properties. Therefore a reaction of the gas and the analyte of interest must be totally excluded before starting the sorption experiments. The easiest way to avoid this type of reaction would simply consist in determining the sorption properties under anaerobic conditions with an inert gas or without gas injection. Indeed, the gas purging technique only consists in removing the dissolved oxygen from the system, i.e. working under anaerobic conditions. The null dissolved oxygen concentration must be controlled to make sure that there are no biotransformation possibilities since oxygen transfer to biomass might occur because of the required stirring.

However, anaerobic bacteria are not inhibited by the gas purging technique. The anaerobic bacteria fraction in activated sludge of aerobic processes can be considered as negligible because bacteria are always in contact with free and/or bound oxygen in the wastewater treatment plant. Working under anaerobic conditions is therefore appropriated for aerobic AS inhibition. There is no perfect inhibition technique for anaerobic AS. Experiments on anaerobic activated sludge inhibition should focus on chemical inhibition techniques.

4. Conclusion

The impact of five different AS inhibition techniques on AS structure was investigated in this study. The results allow to conclude the reliability of these inhibition techniques to determine the sorption properties.

The thermal technique by drying the sludge damages irreversibly the AS structure and is not suitable to assess sorption properties. Chemical inhibition causes adverse effects, which can be limited by determining the lowest effective concentration at which biomass inhibition is ensured. However, AS structure is altered even at low chemical reagents concentrations. Therefore, chemical inactivation, used in many studies, is not appropriate to determine sorption properties because sorption on altered biomass would not represent the real sorption mechanisms. The gas purging technique was the only AS inhibition technique investigated in this study which kept the biomass properties unchanged. However, a reaction between the gas and the analyte of interest might occur which would alter the sorption assessment. The establishment of anaerobic conditions without the use of gas purging avoided the possible reaction of the gas with the investigated pollutant. However, the gas purging technique is not appropriated for anaerobic AS since this technique only consists in forcing the oxygen out of the system. Chemical inhibition by HgCl2 could be the best compromise for anaerobic AS inhibition.

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