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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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## HIGHLIGHTS

- Five activated sludge inhibition techniques were tested.
- The lowest effective concentrations of chemical inhibitor were determined.
- Chemical and thermal techniques cause alteration to the activated sludge structure.
- The gas purging technique preserves the activated sludge structure.
- There is no perfect inhibition technique for anaerobic activated sludge.

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## ABSTRACT

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 $mgHgCl_2\ g_{TSS}^{-1}$ 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.



## 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