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Tributyltin in Wastewater: Influence on the Performance of Suspended Growth Biological Processes

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Abstract: The aim of this study was to evaluate the potential effect of tributyltin (TBT) on the performance of suspended-growth biological processes. The influence of TBT was evaluated for (i) the endogenous and exogenous respirations of heterotrophic micro-organisms in laboratory-scale batch reactors, taken from a municipal wastewater treatment plant and (ii) chemical oxygen demand (COD) removal, sludge production and oxygen consumption of a pilot-sale membrane bioreactor (MBR) system inoculated with heterotrophic micro-organisms taken from a MBR system. The batch experiments showed that the presence of TBT was likely to modify the activity of bacterial populations in endogenous conditions. The increase in endogenous oxygen needs suggested an increase in the maintenance requirements, essentially to manage the chemical stress induced by the presence of TBT. If the addition of TBT did not perturb COD removal in an MBR system, it limited sludge production and increased oxygen requirements; it is assumed that these modifications were linked with the necessity for the biomass to adapt in this stressful environment, as reflected by an increase in the maintenance requirements. These results emphasised that the respiratory activity of the bacterial cultures was modified by the presence of TBT, in the sense that an excess of oxygen was required to adapt to this chemical stress.

Keywords: tributyltin; membrane bioreactor; chemical stress; municipal wastewater treatment plant

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1. Introduction

Tributyltin (TBT), an organic compound of tin, is one of the most toxic substances to have been introduced by man into the environment [1]. The adverse effects of this molecule are numerous, indeed TBT acts on the immune, nervous, digestive and endocrine system; the effects are neurotoxic, mutagenic, carcinogenic and immunotoxic [2]. In aquatic environments, TBT can cause chronic effects at very low concentrations (in the ng/L range; [3,4]). The latter can, therefore, significantly disrupt the metabolisms of sensitive organisms such as algae, zooplankton and fish larvae. Other effects have been demonstrated in aquatic bacteria [5] and certain molluscs, such as oysters and gastropods [6]. TBT and its derivatives are classified (in a list of 33 substances) as high priority and dangerous in the field of water [7]. Since 2003, the International Maritime Organization (IMO) totally prohibits the marketing and use of tin derivatives for anti-fouling paints on boats. The problem of contamination by organotin compounds and tributyltin has long been focused on the marine environment. However, the effective use of organotin compounds in many other industrial activities (e.g., the production of plastics), and their use as biocide in a wide range of domestic products (e.g., detergents, sponges, and textiles) has led to their detection in continental systems [8–12]. This has even led to the establishment of very low environmental quality standards (EQS) for TBT by the European Framework Directive including inland surface waters that encompass rivers and lakes). The proposed EQS expressed as an annual average value (AA-EQS) 0.2 ng/L and EQS expressed as a maximum

allowable concentration (MAC-EQS) 1.5 ng/L) is extremely low and reflects the hazard and concern associated with the presence of this compound in aquatic ecosystems [13]. Released in inland waters, TBT they may contaminate drinking water and wastewater [10,14–17]. In the effluents of wastewater treatment plants, TBT concentrations of this priority substance frequently exceeded the respective EQS. In wastewater treatment plants, various studies have shown that these compounds are very strongly adsorbed on solid matrices (i.e., sludge) and poorly biodegraded [14–21]. This strong adsorption of TBT on solids, related in particular to a strong hydrophobicity of this compound (LogKow of 4,1), is likely to disturb the biological systems of treatment plants by representing a source of stress for the bacteria. The reactions, in relation to this stress, could translate into a fall in the depollution performance, modifications in the consumption of oxygen, a release of bacterial by-products, and modification of the flocculated character of the suspension.

Various studies have investigated the potential of certain micropollutants, including pharmaceutical residues, to interfere with the performance of aerobic dispersed-growth biological processes [22,23], but none have specifically investigated the case of organotin compounds, to date.

The objective of this work was to evaluate the impact that TBT presence can have on aerobic bacterial populations present in dispersed-growth biological sewage treatment processes, i.e., activated sludge processes (CAS) and the membrane bioreactor (MBR).

The first part of this work concerned batch condition experiments, at laboratory scale, in order to study the behaviour of dispersed-growth biomass in contact with the pollutant, through to the oxygen consumption, which is recognised as a relevant indicator of biomass activity [24]. The second part of the study consisted of studying, at pilot-scale, the synthetic effluent treatment performance of an MBR system; the influence of the TBT presence on the process performance was estimated through the monitoring of the chemical oxygen demand (COD) removal, the sludge production and the oxygen consumption.

2. Materials and Methods

2.1. TBT Characteristics and Analysis

Chloride forms of tributyltin (TBT) (96%) were obtained from LGC company (from Promochem, Molsheim, France). Tripropyltin chloride (TPrT, 98%) was purchased from Strem Chemicals (Bischeim, France). Stock organotin solutions containing 1000 mg(Sn) L^{-1} were prepared in methanol. Methanol, nitric acids and isooctane were purchased from Fisher Bioblock Scientific (Illkirch, France). De-ionised MilliQ water (18.2 MΩ·cm^{-1}) was used. Glassware was decontaminated overnight in a 10% (v/v) nitric acid solution and rinsed thoroughly with de-ionised water prior to use [11,25].

TBT concentrations were measured using a gas chromatograph (Focus GC Thermo Fisher Scientific®, Waltham, MA, USA) coupled with an inductively coupled plasma mass spectrometer (ICP-MS X Series II-Thermo Fisher Scientific®, Waltham, MA, USA) [26]. The analysis methodology has been optimised beforehand, taking into account the possible matrix effects linked to the nature of the samples analyzed [27]. An aqueous solution of 2% $NaBEt_4$ (97%, Sigma Aldrich, Germany) in ultrapure water was used as a derivatising agent, and an acetic-acetate buffer (Fisher Bioblock Scientific, Illkirch, France) (2 mol·L^{-1}, pH 4.8) was used to control the derivatisation. This buffer was prepared using sodium acetate (Honeywall, Germany) and acetic acid (Carlo Ebra reagents, France). Ethylated derivatives were simultaneously extracted by isooctane (Carlo Ebra, France). After derivatisation and liquid extraction, quantification was carried out by standard additions. The accuracy of this methodology was validated by analysing the sediment's standard reference material, PACS-2 (National Research Council, Canada). The recovery percentage of the method with respect to the certified material is almost 100%. All analyses were performed in triplicate and the limits of quantification ranged between 0.1 and 0.6 ng(Sn) L^{-1}.

2.2. Sludge Origin and Biological Activity Characterisation

2.2.1. Origin of the Sludge

The studied biological suspensions (i.e., sludge) were sampled from two different suspended growth treatment processes.

The first sludge (sludge (a)) was collected in the activated sludge tank of a municipal wastewater treatment plant (WWTP), about 5000 p.e. with a mass load of 0.094 $kgBOD_5 \cdot kgMVS^{-1} \cdot d^{-1}$ and a solids retention time (SRT) of 19 days. The sludge concentrations in total suspended solids (TSS) and volatile suspended solids (VSS) were equal to 2.6 and 2.0 g/L, respectively. The floc size, measured by laser diffraction (Malvern 3000), was associated with a Sauter diameter close to 90 µm.

The second sludge (sludge (b)) was collected in a pilot-scale membrane bioreactor (MBR, situated in a municipal WWTP) about 150 p.e., with a mass load of 0.055 $kgBOD_5 \cdot kgMVS^{-1} \cdot d^{-1}$ and a SRT of 25 days. The sludge concentrations, in terms of TSS and VSS, were equal to 6.0 and 4.5 g/L, respectively. The Sauter diameter was close to 77 µm.

After sampling from the WWTP, the biomass was systematically placed under aeration and agitation for 24 h in endogenous conditions without any exogenous substrate input. The objective was to ensure the complete removal of the soluble organic carbon and ammonium initially present in the sludge.

2.2.2. Biological Activity Characterisation

The biological activity was estimated through the monitoring of a parameter classically used as a relevant indicator over time, i.e., the oxygen uptake rate (OUR). The experimental chosen procedure was inspired from the Organisation for Economic Co-Operation and Development (OECD) procedure [28].

The methodology of the OUR (in $mgO_2 \cdot s^{-1} \cdot L^{-1}$) measurement was based on the monitoring of the dissolved oxygen concentration in sludge samples taken from experimental reactors (cf. 2.3, i.e., batch reactor (control and test reactors) or membrane bioreactor MBR system). During data acquisition, the sample was maintained for a few minutes in a non-aerated cell (the oxygen exchange with the external environment was assumed to be negligible), under a slight agitation. The oximeter (Oximeter WTW Oxy 340 i, FischerScientific, Illkirch, France) was linked to a data acquisition of $mg(O_2) \cdot L^{-1}$ (Multilab pilot), allowing a rapid quantification of the OUR, corresponding to the slope of the line representing the consumption of the dissolved oxygen in the non-aerated cell over time. Measurements were initially taken with and without the addition of N-Allylthiourea (an inhibitor of autotrophic microorganisms); no significant difference (Student's *t*-test, confidence interval 0.95) was observed between these two types of measurement, certainly due to the complete loss of the nitrifiers activity during the first 24 h in endogenous conditions. In this study, we chosen to focus on the activity of heterotrophic bacteria, considered as less sensitive to autotrophic ones; it was supposed hat if the heterotrophic activity was perturbed by TBT presence, the autotrophic one would be also perturbed.

Where required (notably in exogenous conditions), this biological activity was also approximated through the follow-up of the concentrations in chemical oxygen demand (COD), TSS and VSS, according to spectrometric methods (HACH-USEPA Reactor Digestion Method- Method 8000) and the APHA method (2540 D). For soluble COD measurement, the samples were filtered through a 1.2 µm filter before analysis.

2.3. Influence of the Presence of TBT on the Biological Activity of Sludge

2.3.1. Batch-Reactors Methodology

The behaviour of sludge (a) in the presence of TBT was first studied in batch-reactor conditions. A laboratory-scale 2L-reactor in high-density polypropylene was used to minimise adsorption phenomena on the reactor wall. This reactor was continuously aerated ($[O_2] > 5\ mg \cdot L^{-1}$) and maintained at a constant temperature of 20 °C.

The experimental methodology, either with (test reactor) or without (control reactor) the addition of TBT, consisted of monitoring the bacterial community activity maintained

(i) in endogenous conditions (without an exogenous substrate) and (ii) in exogenous conditions (with an exogenous, organic, easily biodegradable substrate).

Measurement of the biological activity in endogenous conditions: the bacterial activity was estimated through the OUR quantification (cf. 2.2.2.) 10 min after TBT addition. Different conditions of TBT concentration were studied in a test reactor, varying from 15 to 650 ngSn·L^{-1}. The evolution of the OUR was also monitored for almost three days.

Measurement of the biological activity in exogenous conditions: a pulse TBT injection was associated with a pulse injection of ethanol (easily biodegradable substrate), resulting in initial TBT and soluble COD concentrations in the test reactor of 600 ngSn·L^{-1} and 700 $mgO_2 \cdot L^{-1}$, respectively. During experiments in exogenous conditions, OUR (cf. 2.2.2), soluble COD and VSS were measured over time (sampling approximately each hour), to quantify the respiratory activity of the bacterial community, the organic substrate removal and the sludge production with and without TBT, respectively.

2.3.2. Continuous Reactor Methodology (Immersed Membrane Bioreactor System)

Continuous experiments were carried out using an experimental immersed membrane bioreactor (MBRi). The MBRi consisted of a 15-liter tank associated with an external filtration module; the hollow fibre membranes (organic membrane Polymem® with a 0.3 m^2 surface area and a 0.08 µm pore size) were immersed in the tank. Tangential flow and air scour helped limit membrane fouling. The system was continuously agitated (high-speed impeller in the tank) and aerated to maintain the dissolved oxygen concentration close to 5 $mgO_2 \cdot L^{-1}$. Temperature and pH were continuously controlled and maintained at 20 °C and 7, respectively.

The experimental MBRi was inoculated with sludge (b). The system was fed with a synthetic influent composed of ethanol (0.18 g·L^{-1}), urea (0.09 g·L^{-1}) and KH_2PO_4 (0.04 g·L^{-1}). During the first period (of approximately 30 days), the MBRi was only fed with this synthetic influent without added micropollutants. After this first period, TBT was added to the influent at a concentration of 30 ng·L^{-1}, in the same order of magnitude as that detected in French WWTPs influents [10,11,16]. The hydraulic retention time HRT, the solid retention time SRT and the average organic loading rate Cv (corresponding to the influent COD concentration by HRT) in the MBRi system were, respectively, equal to 12 h, 60 d and 0.8 $kg_{COD} \cdot m^{-3} \cdot d^{-1}$.

Over time, TSS, VSS, total COD, soluble COD (COD_s) and OUR (cf. 2.2.2) were monitored in the MBRi, in order to quantify organic removal, sludge production and oxygen consumption, respectively, before and after the addition of TBT. The influent COD concentration (COD_i) and the effluent one (COD_e) were also measured.

3. Results and Discussion

3.1. Influence of TBT Addition on the Biological Activity of Sludge in Batch Reactors

3.1.1. Endogenous Conditions

Ten minutes after the addition of TBT in the test reactor, the oxygen uptake rate (OUR_{TBT}) was measured and compared to the one measured in the control reactor ($OUR_{initial}$, close to 0.003 $mgO_2 \cdot L^{-1} \cdot s^{-1}$).

Figure 1 presents the ratio $OUR_{TBT}/OUR_{initial}$ versus the TBT concentration present in the test reactor. In the range of tested TBT concentrations (between 15 and 650 ngSn·L^{-1}) and whatever this concentration was, the presence of TBT led to an immediate increase in the rate of endogenous respiration, of the order of 20%.

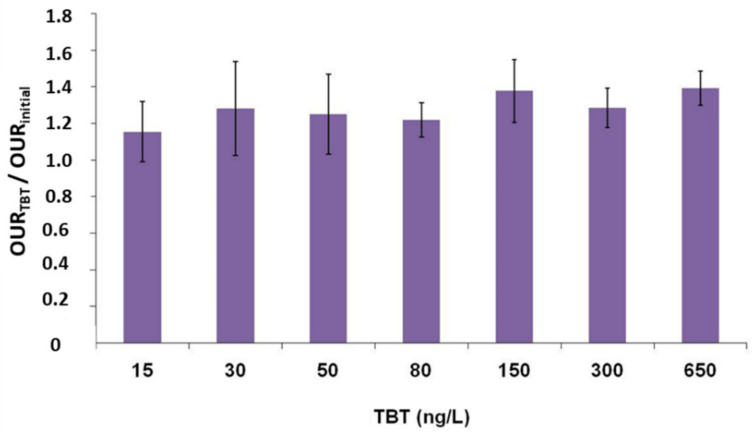

Figure 1. $OUR_{TBT}/OUR_{initial}$ in endogenous conditions for different TBT concentration in the test reactor.

According to the literature [29,30], this sudden OUR increase may be attributed to the need for bacterial populations to acclimatise to the presence of TBT, which may be considered as momentary chemical stress. This presence modified the environment and the culture medium and may change the exchanges and transfers through the biological membrane (possibly inducing higher maintenance requirements and/or bacterial diversity).

With the objective of verifying whether this sudden increase was permanent and to study the mid-term behaviour of the biomass, the OUR evolution was monitored for 3 days in a test reactor, at a concentration of 650 $ng_{Sn} \cdot L^{-1}$. The same monitoring was proposed in parallel, in a reactor without TBT (control reactor).

A progressive decrease in endogenous respiration was observed over time, both in the test reactor and in the control one (Figure 2). Thus, this evolution was essentially attributed to the absence of an exogenous substrate and to a reduction in the global respiration activity. As no significant decrease in VSS concentration was observed, this decrease was assumed to be directly linked to the reduced maintenance requirements of the bacterial communities and not to a bacterial lysis.

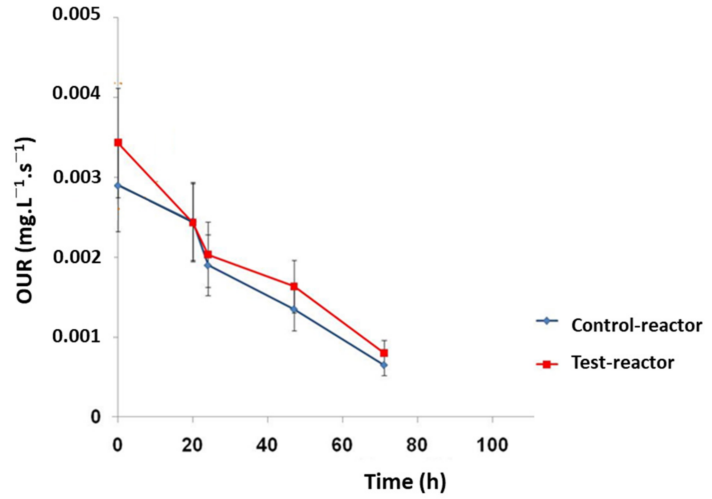

Figure 2. OUR_{TBT} and $OUR_{control}$ evolution over time in the test and control reactors.

If the presence of TBT did not modify the global behaviour of the biomass, the oxygen requirement with TBT always remained higher than the one measured without TBT (Student's *t*-test, confidence interval 0.95). This result was different from those obtained by Aubenneau et al. and Henriques et al. [22,30], who observed in a second time, after a first OUR increase due to an addition of carbamazepine and 2.4-dinitrophenol, respectively,

a return of the OUR value to the endogenous one. This observed difference in biomass behaviour could be attributed to the chemical nature of the micropollutant; the duration of the perturbation could be dependent on the chemical properties of the added substances and their ability to perturb the bacterial environment.

3.1.2. Exogenous Conditions

The collected sludge was initially maintained under aeration and agitation for 24 h, without any exogenous substrate input. Then, the pulse addition of an exogenous substrate (ethanol) was carried out in the test and control reactors, so as to obtain a concentration of 700 $mgCOD \cdot L^{-1}$ (S_0) in the two reactors. In the test reactor, TBT was added to the ethanol substrate simultaneously, in order to reach a concentration of 600 $ng_{Sn} \cdot L^{-1}$ in the reactor.

During the 8 h of monitoring, the following parameters were measured: TSS and VSS concentration, COD removal rates and the OUR. Throughout the experiments, the VSS concentration changed very little in both reactors (with and without TBT) and remained close to 2000 $mgMVS \cdot L^{-1}$ (X_0 or VSS_0). Thus, it was assumed that no significant biomass production was observed during these 8 h of monitoring; this observation was consistent with the fact that the S_0/X_0 ratio was not sufficient to allow VSS increase during the experiment [31].

A quasi-linear evolution of the COD concentration over time, which is synonymous with a zero-order kinetic (classically observed for easily biodegradable substrates [31]), was observed for the two reactors (Figure 3). After 7.5 h of monitoring, all of the added COD had been removed (COD removal efficiency of 100%), whether TBT was present or not. The COD removal rate (r_s) in the two reactors was calculated and the values were found to be close to 111 ± 4 $mgCOD \cdot L^{-1} \cdot h^{-1}$. If one of the main effects of chemical stress on the performance of a suspended growth treatment system is supposed to be the decline of the COD removal rate [30], this decline was not observed in our tested conditions. Thus, in batch conditions and in presence of an easily biodegradable exogenous substrate, the TBT addition did not disturb at all the capacity of the biomass to assimilate the substrate and thus did not represent a stress/inhibition factor.

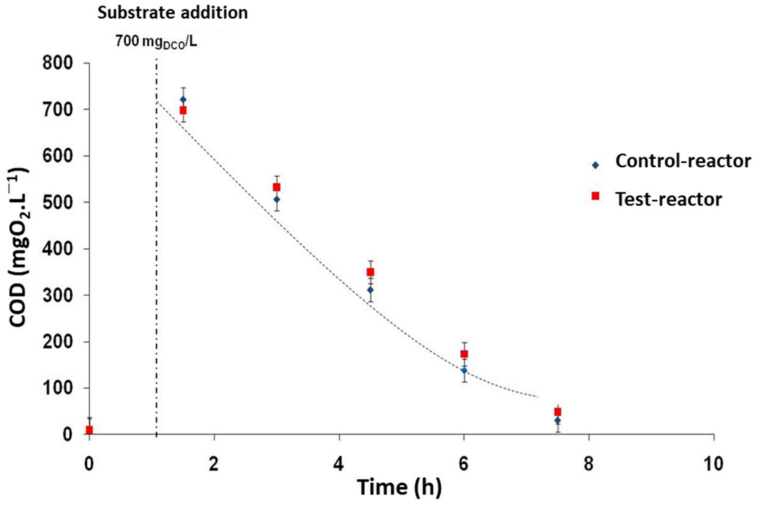

Figure 3. Evolution of the soluble COD concentration in the reactor exposed to a TBT concentration of 600 $ng_{Sn} \cdot L^{-1}$ and in the control reactor.

The OUR evolution throughout the 8 h of monitoring is presented in Figure 4. The first point represented in the figure, at time 0, corresponds to an endogenous situation, since the addition of a substrate is not yet effective (nor the addition of TBT). The endogenous measured value (approximately 0.001 $mgO_2 \cdot L^{-1} \cdot s^{-1}$) is lower than that obtained during the previous tests ($OUR_{initial}$ was approximately 0.003 $mgO_2 \cdot L^{-1} \cdot s^{-1}$), which was certainly due to some differences in the sampled biomass.

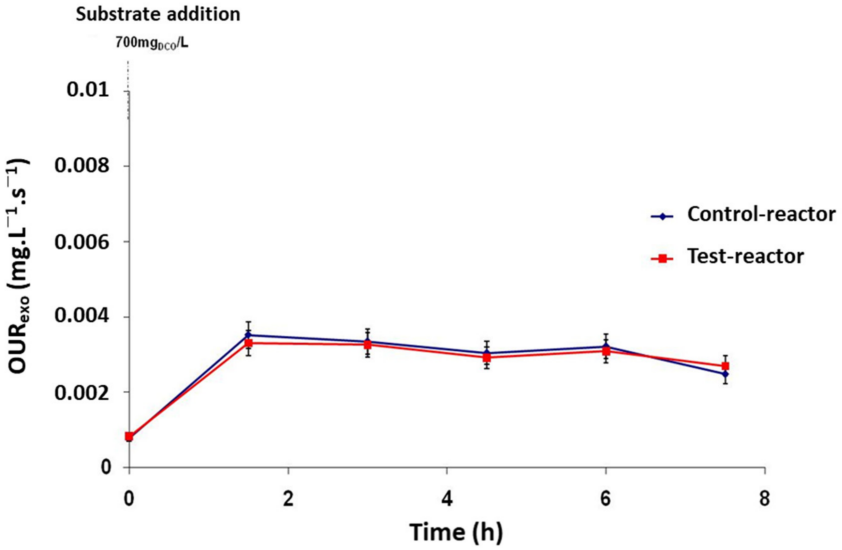

Figure 4. Evolution of the oxygen uptake rate (OUR) in exogenous conditions as a function of time in the test reactor and in the control reactor.

An increase in oxygen requirements, after contact with the exogenous substrate, was first observed. Throughout the 8-hour experiments, no significant difference between the oxygen requirements in the test reactor and the control reactor was noted (Student's *t*-test, confidence interval 0.95); the oxygen consumption was considered as constant during the exogenous phase, at approximately 0.003 ± 0.0005 $mgO_2 \cdot L^{-1} \cdot s^{-1}$, both in the presence or absence of the pollutant. According to the equation proposed by Eckenfelder (Equation (1)), it was possible to quantify a′ (the exogenous respiration coefficient) and b′ (the endogenous respiration coefficient).

$$OUR = a' \times r_s + b' \times VSS \quad (1)$$

b′, based on an OUR value approximately equal to 0.001 $mgO_2 \cdot L^{-1} \cdot s^{-1}$ (cf. above) and a VSS concentration around 2000 $mg \cdot L^{-1}$, was equal to 0.04 $mgO_2 \cdot mgVSS^{-1} \cdot j^{-1}$. a′, corresponding to the oxygen required for the exogenous substrate oxidation, was estimated equal to 0.06 $mgO_2 \cdot mgCOD_{removed}{}^{-1}$. Thus, in accordance with the results, about 70% of the oxygen consumed is for exogenous needs, and 30% for endogenous needs, whether the biomass is in the presence of TBT or not.

Tests conducted under exogenous conditions demonstrated that substrate degradation rates and oxygen requirements were not modified by the addition of TBT. We have not observed any effects of TBT on degradation activity, contrary to some studies, which showed that the presence of a pollutant can inhibit the metabolisation of the substrate [32,33]. The oxygen requirements, compared to those obtained under endogenous conditions, tend to demonstrate that the stress induced by the presence of TBT is more efficiently managed in the presence of an exogenous substrate. The presence of an easily biodegradable exogenous substrate could facilitate the adaptation of bacterial populations or reduce their sensitivity to TBT. This could be attributed to the fact that the exogenous substrate represent an external energy source, than could contribute to favour and/or supply the adaptation mechanisms of the bacterial communities.

3.2. Influence of the Addition of TBT to the Sludge Biological Activity in a Continuous Reactor (MBRi System)

The influence of TBT on the performance of MBRi feeding with a synthetic influent and inoculated with sludge (b) was estimated through the quantification of the COD removal rate, before and after the TBT addition, the sludge production, and the oxygen consumption.

3.2.1. TBT Effect on COD Removal Rate

Figure 5 presents the evolution of the soluble COD in the reactor (CODs), as well as in the outlet effluent (CODe).

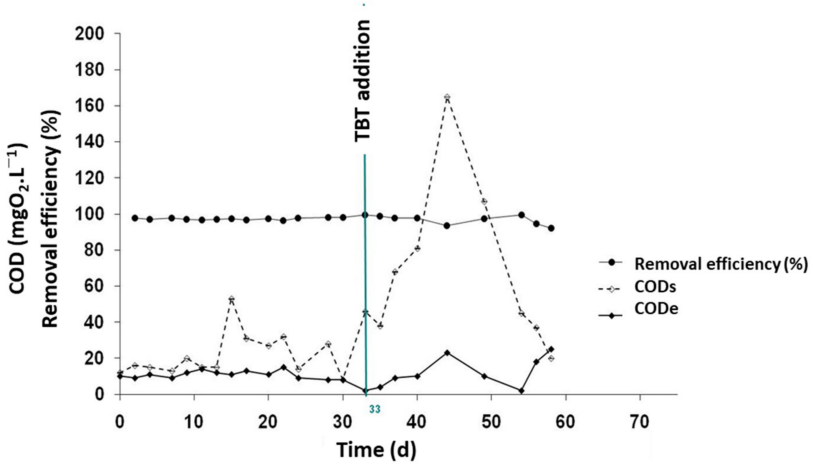

Figure 5. Evolution in soluble COD concentration (CODs), effluent CDO concentration (CODe) and COD removal efficiency in MBRi system.

The graph also shows the COD removal efficiency, calculated according to Equation (2).

$$\text{Removal efficiency } (\%) = 100 \times \frac{\text{CODi} - \text{CODe}}{\text{CODi}} \quad (2)$$

Firstly, it should be noted that the COD concentrations measured in the effluent (CODe) are always lower than 125 $mg{\cdot}L^{-1}$ (the value corresponding to the authorised discharge threshold set by the French regulations concerning wastewater treatment plant discharges). CODe concentrations were of the order 15 $mg{\cdot}L^{-1}$, which represents an average elimination rate higher than 95% (Figure 5 and Table 1). It can be seen that the addition of TBT did not affect the COD removal performances in any way, and so the average yields calculated during the periods before and during the spiking were not significantly different (Student's *t*-test, confidence interval 0.95).

Table 1. Average removal efficiency and COD removal rate values (r_s) measured during the experiments.

	Average Removal Efficiency (%)	**Average Removal Rate r_s ($mgDCO{\cdot}L^{-1}{\cdot}j^{-1}$)**
Without TBT	97 ± 1	789 ± 39
With TBT	96 ± 3	797 ± 88

A notable difference between the soluble COD concentration (CODs) in the reactor and in the effluent (CODe) was observed. This difference seemed to increase as soon as TBT was injected and was attributed to the presence of organic matter whose size ranged from 0.08 µm (pore diameter of the membrane) to 1.2 µm (cut-off point for the measurement of soluble COD).

Avella et al. (2010) distinguished two types of soluble microbial products within a biomass from an MBR, i.e., large molecules (proteins > 670 kDa and polysaccharides around 400 kDa) and smaller ones (proteins < 17 kDa and polysaccharides < 0.7 kDa) [34]. The differences measured between the CODe and CODs values could be attributed to the presence of large polymeric bacterial products; thus these products would represent an average of approximately 40% of the soluble COD before the addition of TBT, and nearly 70% after the addition of TBT. The literature indicates that the release of such compounds could be consecutive to a stress factor. Davey and O'Toole observed the secretion of polysaccharides in systems operating under stress [35]. Similarly, Aquino and Stuckey

observed the increase in the concentration of soluble substances, in cases of microbial stress caused by the presence of toxic compounds (i.e., chloroform and chromium) [36]. Bott and Love indicated that the microorganisms present in biological treatment systems would adapt physiologically to toxic shocks through the activation of a microbial response, which would involve the secretion of polysaccharides [37]. Sutherland described the role of capsular polysaccharides as a protective barrier to stress for the cells [38]. Hsieh et al. stated that these soluble substances are released into the liquid phase, either by dissolution or by the hydrolysis of certain bound bacterial polymers (floc constituents) [39]. It should be noted that these polysaccharides, or proteins, can also be used as nutrients by the cells, which could explain their quantitative evolution over time in our tests.

Consequently, if the difference between the soluble COD concentration in the reactor and in the effluent could be attributed to polysaccharide or other bacterial compounds release, we could suppose that the bacterial population reacted strongly to the chemical stress induced by the presence of TBT. The population would seem to put in place mechanisms of adaptation or protection to the stress situation.

The COD removal rate (r_s) was estimated, before and during the continuous addition of TBT, and according to mass balance on the MBRi system in steady state conditions (Equation (3)).

$$r_s = \frac{Q_e \times CODe - Q_i \times CODi + Q_p \times CODp}{V} \quad (3)$$

where Q_e, $Q_{i,}$ and Q_p are respectively the effluent, influent and withdrawal flow rate (with $Q_p << Q_i$ and so $Q_i \approx Q_e$).

CODe, CODi and CODp are, respectively, the effluent, influent and sludge COD concentration, and V is the MBR_i volume.

The removal efficiencies, with and without TBT, were almost identical, at about 800 mgDCO·L^{-1}·j^{-1} (Table 1). As for the COD removal efficiency, the presence of TBT did not affect the COD removal rate.

The specific rate of COD removal was measured as 0.2 gCOD·$gVSS^{-1}$·j^{-1}; about six times lower than that measured in batch condition (cf. 3.1.2, 1.3 gCOD·$gVSS^{-1}$·j^{-1}). The mass loading conditions (or the S/X ratio), which are different between continuous and batch systems, could explain the measured difference.

3.2.2. TBT Effect on Sludge Production

TBT addition could decrease the growth of certain bacteria, since TBT is a chemical substance that is able to interact with the lipid membrane [5,40]. Figure 6 shows the evolution of suspended matter, as well as volatile suspended matter, during the experimental campaign.

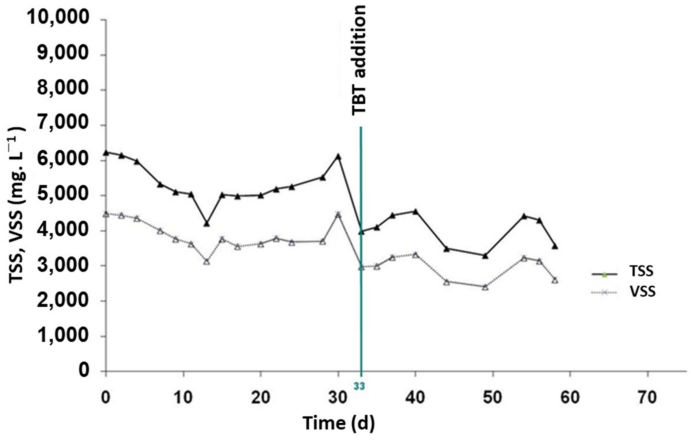

Figure 6. Evolution in TSS et VSS concentration in MBRi system.

Firstly, a decrease of approximately 2 g·L^{-1} in the TSS and VSS content can be seen at the beginning of the experiment; this can be explained by the fact that the imposed organic

volumetric loading rate was not sufficient enough to maintain the VSS concentration at such a high level. The decrease in TSS and VSS, observed shortly after the 30th day, is due to a technical incident that caused a loss of sludge. TSS and VSS concentration fluctuations were distributed around an average value close to 4000 mg·L^{-1} for the TSS and 3000 mg·L^{-1} for the VSS.

The VSS/TSS and the CODpart/VSS ratios were calculated (where CODpart = total COD–CODs), since these ratios could reflect the sensitivity of the biomass to the addition of TBT. Indeed, under chemical stress, the modification of bacterial activity could lead to changes in the composition of the biomass, namely possible mineralisation and/or modification of the nature of the organic matter present. Figure 7 presents the evolution of the VSS/TSS and CODpart/VVS ratios over time.

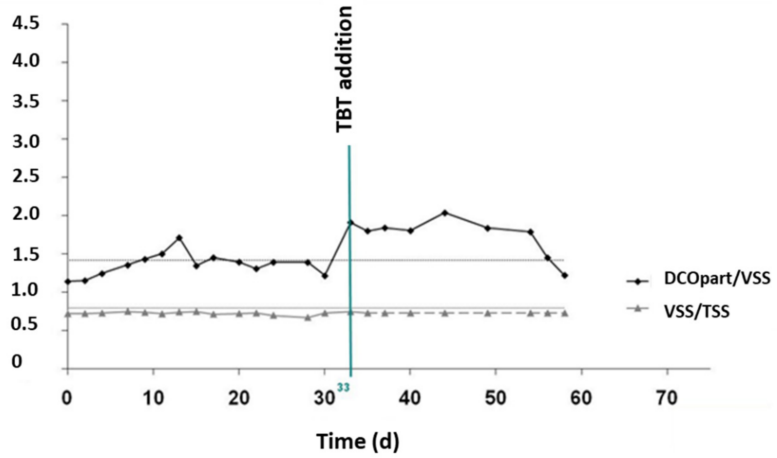

Figure 7. Evolution of VSS/TSS and CODpart/VSS ratios in MBRi system.

The VSS/TSS ratio was constant and close to 0.8; the organic content of the sludge did not seem to be modified by the addition of TBT.

The CODpart/VSS ratio was close to the reference value (1.42 for dispersed-growth biological systems (Peter and Chudoba, 1986)) during the first period, but increased suddenly after the addition of TBT and remained above 1.75. This change could be attributed to a change in the nature of the biomass that needed to adapt itself to the new, potentially stressful, environment.

The overall sludge production rate (r_x) and the observed conversion yield (Y_{obs}) were estimated according to mass balance on the MBR system in steady state conditions (Equation (4)) and are presented in Table 2.

$$r_x = \frac{Q_e \times VSSe - Q_i \times VSSi + Q_p \times VSSp}{V} \quad (4)$$

where $Q_p << Q_i$ and so $Q_i \# Q_e$, then

$$r_x = \frac{Q_e \times VSSe}{V} \quad (5)$$

Table 2. Sludge production rate and conversion rate values measured during the experiments.

	r_x (mgVSS·L^{-1}·j^{-1})	Y_{obs}
Without TBT	69 ± 8	0.09
With TBT	62 ± 14	0.08

Sludge Yield (Y_{obs}) is calculated using the following equation:

$$Y_{obs} = \frac{r_X}{r_S} \quad (6)$$

Sludge production rate, as well as the observed conversion yield, seemed to be reduced by the addition of TBT (Table 2). The alteration of bacterial growth associated with the presence of chemical stress was observed by other authors [1,5] and can be attributed to the presence of TBT.

While the addition of TBT did not alter COD degradation rates, it seemed to impact biomass production and, therefore, conversion rates. It was envisaged that part of the degraded substrate was used for purposes other than the synthesis of new cells to ensure increased maintenance requirements, in particular.

3.2.3. TBT Effect on the Oxygen Uptake Rate

Disturbance and a slight increase in oxygen consumption were observed after the addition of TBT (Figure 8).

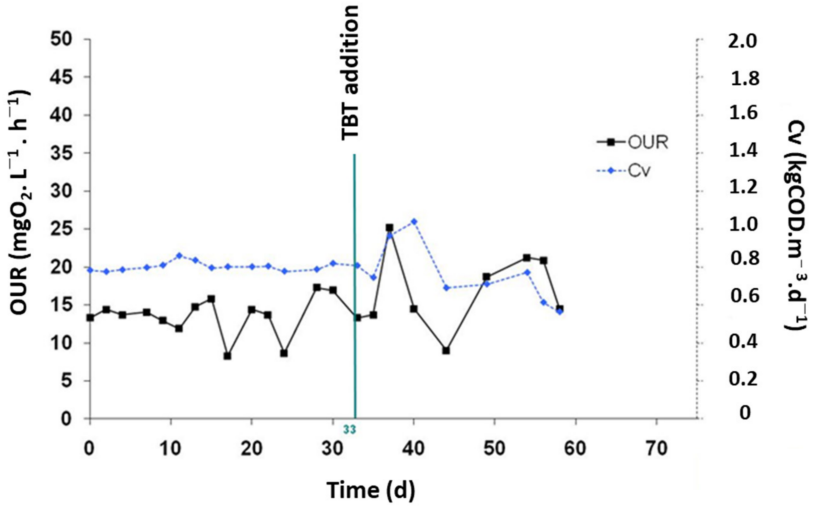

Figure 8. Evolution of the OUR and the organic volume load in MBRi system.

Table 3 summarises the average OUR values obtained during each period, as well as the oxygen consumption values related to the COD removed (note that a′* is calculated according to (Equation (7))).

$$a'^{*} = \frac{\overline{OUR}}{r_S} \quad (7)$$

Table 3. Values for average OUR and oxygen consumption rate measured during the experiments.

	OUR ($mg \cdot L^{-1} \cdot d^{-1}$)	**a′*** ($mgO_2/mgCOD_{removed}$)
Without TBT	326 ± 10	0.40 ± 0.08
With TBT	377 ± 17	0.5 ± 0.2

a′* quantification is different from a′ quantification in the sense that this parameter includes both endogenous and exogenous requirements. Thus, it was not surprising that a′* values were higher (around ten times higher) than a′ measured in batch conditions.

The OUR monitoring in the continuous system did not allow us to estimate whether the measured increase (around 25%) after TBT addition was due to more oxygen-consuming maintenance processes or to more consuming-substrate metabolisation pathways. With

respect to the tests conducted in a batch reactor, it would probably be more a question of an increase in endogenous requirements (associated with maintenance processes in particular) than an increase in exogenous requirements. Thus, the processes of protection, adaptation or acclimatisation associated with the presence of TBT would seem to generate greater energy requirements and, therefore, a higher rate of oxygen consumption.

4. Conclusions

The aim of this study was to evaluate the potential effects of tributyltin (TBT) on the performance of suspended-growth biological processes. With these objectives, the experiments were based on evaluating the influence of TBT (i) on the endogenous and exogenous respirations of heterotrophic micro-organisms taken from a municipal wastewater treatment plant, in laboratory-scale batch reactors, and (ii) on COD removal, sludge production and oxygen consumption of a pilot-scale membrane bioreactor system, inoculated with heterotrophic micro-organisms taken from an MBR system. The batch experiments showed that the presence of TBT was likely to modify the activity of bacterial populations in endogenous conditions. Effects were observed on the respiratory activity of bacterial populations. The increase in endogenous oxygen needs suggested an increase in the maintenance requirements, essentially to manage the chemical stress induced by the presence of TBT. The impact of TBT on the behaviour of the bacterial community was studied in an MBR system. If TBT addition did not perturb COD removal, it limited sludge production and increased oxygen requirements. It is assumed that these modifications were linked to the necessity of the biomass to adapt themselves in this stressful environment, as reflected by an increase in the maintenance requirement.

These results emphasised that the respiratory activity of the bacterial cultures was modified by the presence of TBT, in the sense that an excess of oxygen was required to adapt to this chemical stress. This statement has to be considered with respect to WWTPs, as aeration is the most energy-intensive operation in wastewater treatment (45–75% of plant energy costs).

In terms of perspective and with an objective to enhance these considerations, experiments taking into account the TBT degraded products, monobutyltin (MBT) and dibutyltin (DBT), would be relevant, in the sense as these products could modify also the biological behaviour. Longer MBRi monitoring could also provide interesting information about the long-term behaviour of the biological community.

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