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Assessment of toxicity and biodegradability on activated sludge of priority and emerging pollutants

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Abstract

Several methods for evaluating the toxicity and biodegradability of hazardous pollutants (chlorinated compounds, chemical additives and pharmaceuticals) have been studied in this work. Different bioassays using representative bacteria of marine and terrestrial ecosystems such as *Vibrio fischeri* and *Pseudomonas putida* have been used to assess the ecotoxicity. Activated sludge was used to analyze the effect of those pollutants in a biological reactor of a sewage treatment plant (STP). The results demonstrate that none of the compounds is toxic to activated sludge and only ofloxacin to *P. putida*. The additives tested can be considered moderately toxic according to the more sensitive *V. fischeri* assays, whereas the EC_{50} values of the pharmaceuticals depend on the specific microorganism used in each test. Regarding the biodegradability, respirometric measurements were carried out for fast biodegradability assessment and the Zahn-Wellens test for inherent biodegradability. The evolution of the specific oxygen uptake rate (SOUR) showed that only diethyl phthalate was easily biodegradable and acetylsalicylic acid was partially biodegradable (98 and 65% degradation, respectively). The persistence of dichloromethane, ofloxacin and hidrochlorothiazide was confirmed upon the 28 d of the Zahn-Wellens test whereas 1,1,1-trichloroethane showed inherent biodegradability (74% removal). Most of the chlorinated compounds, pharmaceuticals, bisphenol A and EDTA were partially degraded in 28 d with TOC reduction ranging from 21 to 51%. Sulfamethoxazole showed certain biodegradation (50% removal) with TOC decrease around 31% which indicates the formation of non-biodegradable byproducts.

Keywords

Priority and emerging pollutants, Toxicity, Activated sludge, Biodegradability, Wastewater

1. Introduction

The presence of priority pollutants in treated wastewater whose quality standards are included in Directive 2008/105/EC,[1] together with a diverse group of unregulated contaminants (emerging pollutants) raises an increasing concern on the risks on humans and the environment when considering water reuse.

Biological systems based on the activity of microbial consortia are widely used for wastewater treatment. However, the microbial activity may be affected by the presence of xenobiotic compounds such as chlorinated compounds, polyaromatic hydrocarbons, dies, pesticides and pharmaceuticals. [2-4] Thus, before performing a biological treatment it is necessary to assess the toxicity and biodegradability of the wastewater on the activated sludge.[5,6] It is important to recognize that toxicity and biodegradability are not always mutually exclusive. Thus, substances which are regarded as toxic compounds can be removed efficiently in a bioreactor at concentrations below their EC_{50},[7] whereas other less toxic or inhibitory compounds poorly biodegradable would be released to the environment with the risk of accumulating and reaching concentrations over the NOAEL (No Observed Adverse Effect Level) for different environmental compartments.

Several bioassays have been developed to study the toxic effect of chemical compounds to microorganisms.[4, 8] The type of microorganism used, the biomass to substrate ratio and the length of the test are different depending on the bioassay. Therefore, the information provided by each test is different and it is unlikely that a single test can serve for a wide spectrum assessment because of the different response of microorganisms to pollutants. Toxicity is evaluated from different parameters related to bacterial activity. These bioassays include acute toxicity test (*Daphnia magna* and *Vibrio fischeri*), Adenosine TriPhosphate (ATP) luminescence,[9] growth inhibition test on *Pseudomonas*,[10] activated sludge inhibition,[11] and enzyme inhibition.[12]

Respirometric measurements are commonly used to determine the effects of toxicants on the activated sludge. [3,13-16] Although the respiration rate can be quantified by different methods, the oxygen uptake inhibition rate [14,17] is most frequently used to evaluate the toxicity.[2,6,13] A test using activated sludge for toxicity assessment will not indicate total toxicity since the bacterial density and diversity is high and only the inhibition of the most abundant organisms which are growing faster under the tests conditions is indicated.[18] Therefore, other tests for the assessment of the toxicity of the target compounds against microbial populations are also used in this work. *P. putida* is representative of the large group of Gram-negative environmental bacteria often used in bioremediation.[19] *V. fischeri* is a marine bacterium which does not represent the behaviour of microorganisms in activated sludge since it is not likely to be found in a WWTP. However, it is widely used because of some advantages derived from sensitivity, reproducibility and ease application with organic and inorganic pollutants,[20] allowing comparisons with other related studies.[21,22] The Microtox® test is the most widely used commercial assay using *V. fischeri*.

Biodegradability can be evaluated by the tests recognized by the Organization for Economic Co-operation and Development (OECD): ready biodegradability tests,[23] based on the evaluation of Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) and Biochemical Oxygen Demand (BOD), inherent biodegradability or Zahn-Wellens test,[24] and simulation test.[25] Equivalent tests are described by the ISO protocols. [26-28] Ballesteros et al. [29] conducted a comparative study of different tests to determine biodegradability. They found that *P. putida* and Zahn-Wellens tests are more reliable and reproducible than BOD_5/COD ratio and respirometry assay. Respirometry is a short time consuming method, however, the duration of the test (30 min) and the low amount of activated sludge used could be insufficient to degrade some slow biodegradable compounds and be the responsible of the poor reproducibility of this test. In this sense, the fast

biodegradability test [6] can be considered to evaluate the impact of the presence of micropollutants in raw wastewater because it uses an operation time near to hydraulic retention time and a biomass to substrate ratio approaching real conditions. When the response of the unacclimated sludge in the fast biodegradation test of the target compound is negative, the Zahn-Wellens inherent biodegradability test serves to evaluate the possibility of biodegradation in an acclimated activated sludge unit.[30]

The aim of this work is to evaluate the toxicity and biodegradability of several priority and emerging pollutans in order to learn on their potential removal upon biological treatment. Several chlorinated compounds, additives and pharmaceuticals (antibiotics, an analgesic and a diuretic agent) were evaluated in this work. Different assessment procedures have been used for toxicity (bioluminescent inhibition test with *V. fischeri*, respirometry assay with activated sludge and growth inhibition test with *P. putida*) and biodegradability (fast biodegradability and Zahn-Wellens tests).

2. Materials and methods

2.1. Chemicals

Dichloromethane, 1,2-dichloroethane, trichloroethylene and 1,1,1-trichloroethane were purchased from Panreac; chloroform and carbon tetrachloride were supplied by Fluka. The additives (bisphenol A (BPA), ethylenediaminetetraacetic acid (EDTA), diethyl phthalate (DEP), methyl-tertbutyl ether (MTBE)) and the pharmaceuticals (ofloxacin, metronidazole, sulfamethoxazole, tetracycline hydrochloride, acetyl salicylic acid and hydrochlorothiazide) were purchased from Sigma-Aldrich. All chemical were of analytical-grade purity.

2.2. Inoculum and culture medium

Unacclimated activated sludge, collected from a municipal sewage plant was used in the respirometric tests for toxicity and biodegradability determination. The sludge was maintained with sodium acetate (150 mg COD/L) and glucose (150 mg COD/L) as carbon sources in a sequencing batch reactor (SBR) operated at 25 ºC. Ammonium sulphate and phosphoric acid were used as nitrogen and phosphorous sources. A COD:N:P:micronutrients ratio of 100:0.5:0.1:0.05 (w/w) was fixed. The mineral solution added as micronutrients supply consisted on $FeCl_3$, $CaCl_2$, KCl and $MgSO_4$. [31].

P. putida strain CECT324 was purchased from the Spanish Type Culture Collection (Colección Española de Cultivos Tipo, Valencia, Spain). Stock cultures were maintained at -40 ºC in a nutrient medium supplemented with 15 % (v/v) of glycerol. *P. putida* was transferred to a nutrient medium containing 1 g beef extract, 2 g yeast extract, 5 g peptone and 5 g NaCl per liter of deionised water. The cell suspension resulting from the late exponential growth phase was subcultured in a mineral salts medium [31] with 2 g/L glucose as carbon source to be used in the growth inhibition test performed according to the ISO 10712.[10]

2.3. Toxicity assays

In order to assess the effects of the pollutants on several environmental sceneries, different methods were used to evaluate the toxicity.

Luminescence inhibition assay. Toxicity was determined by the Microtox® Acute Toxicity Test (SCI 500 Analyzer) following the ISO 11348-3 standard.[32] The toxic effect was evaluated from the percent decrease of the light emission of *V. fischeri* after 15 min of incubation at 15 ºC. The results were expressed as the EC_{50}, namely the effective concentration of a sample that causes 50 % reduction of bioluminescence.

Respiration inhibition test for activated sludge. It was carried out in a Liquid-Static-Static (LSS) respirometer.[33] The respirometer was operated with two independent reactors

simultaneously to check the reproducibility. The reaction vessels, placed in a thermostatized orbital shaker, were stoppered so that oxygen transfer from air to the liquid can be neglected.[6] The toxicity was evaluated using the method proposed by Ricco et al. [3], based on the respiration inhibition test [14] for activated sludge. The procedure consisted in short-term (30 min) respirometric measurements using unacclimated sludge (350 mg/L) with an easily biodegrable substrate (sodium acetate) alone, as reference, and with different concentrations of the target compound. Inhibition was also estimated in terms of the EC_{50} defined as the concentration of the toxicant causing 50 % reduction of the specific exogenous oxygen uptake rate for the reference substrate ($SOUR_{exR}$). In order to check the sensitivity of the sludge, 3,5-dichlorophenol was used as control [6] according to the recommendation of the OECD 209 protocol.[14]

Growth inhibition test. The growth inhibition test was performed according to the ISO norm 10712.[10] The test uses several Erlenmeyer flasks with different concentrations of the target compound and one blank. The Erlenmeyer flasks were inoculated with 40 mg/L of *P. putida* CECT324 at a starting optical density (OD) of 0.04 (λ = 436 nm) for 16 h ± 1h at 30 ºC in a thermostatized orbital shaker. At the end of the test the OD was measured using a UV spectrophotometer (Varian, mod. Cary 50 conc., California, USA). The effect on bacterial growth was determined by comparing the bacterial density in the flask containing the target compound with that of the blank. The EC_{50} was defined as the concentration of the compound causing a 50 % reduction of cell multiplication.

2.4. Biodegradability tests

Fast biodegradability test. The biodegradability of the priority and emerging pollutants was assessed in a LSS respirometer, following the fast biodegradability test proposed by Polo et

al.[6] This method was designed on the basis of the typical working conditions of an activated sludge process, following both the activity of the microorganisms and the removal of toxicant. The synthetic water (1 L) was mixed with biomass (350 mg VSS/L) at 25 ºC and aeration was maintained for 24 h. The SOUR, TOC and pollutant concentration were continuously measured.

Inherent biodegradability test. The Zahn–Wellens test was carried out according to OECD protocol 302 B.[24] A mixture of target compound and activated sludge (using a biomass/substrate ratio of 4 mg VSS/mg TOC) in mineral salt medium [24] was stirred and aerated at room temperature in diffuse light for 28 days. Degradation was evaluated by measuring TOC and/or compound concentration. Each compound was tested in parallel in two different bottles to check the reproducibility. In order to evaluate the activity of the sludge, a run using ethylene glycol as reference compound was also carried out.

2.5. Analytical methods

Ofloxacin and tetracycline hydrochloride were quantified by HPLC/UV (Prostar, Varian) at 254 nm using a Phenomenex C-18 column (Luna 150 x 3 mm) as the stationary phase and a mixture of 0.1 % formic acid and acetonitrile (5% water, 0.1% formic acid) in deionized water (90/10-0/100 v/v (40 min)) as mobile phase at 0.35 mL/min. The rest of the pharmaceuticals tested and diethyl phthalate were analyzed by HPLC/UV (Prostar, Varian) with a C18 column (Microsorb MV 100-5 250 x 4.6 mm) as stationary phase. Analyses of acetylsalicylic acid, hydrochlorothiazide, sulfamethoxazole and metronidazole were carried out using a mixture of acetonitrile/H_2O (40/60 v/v) as mobile phase. The flow rate was maintained at 1.0 mL/min and a wavelength of 280 nm was used, except for acetylsalicylic acid (228 nm). Diethyl phthalate was analyzed at 225 nm using acetonitrile/ultra-pure H_2O (70/30-95/5 (0- 15 min), 95/5 (15-30 min) and 95/5-70/30 (30-35 min)) as mobile phase at 1

mL/min. TOC was measured using a TOC-Vcsh apparatus from Shimadzu. All the samples were previously filtered through 0.45 μm fiber glass filters (Alber FV-C). Biomass concentration was measured as volatile suspended solids (VSS) following the APHA Standard Methods.[34] The results reported were the average values from duplicate runs.

3. Results and discussion

3.1. Toxicity

The EC_{50} values obtained for the chlorinated compounds tested are shown in Table 1. The Microtox® EC_{50} values (19-596 mg/L) were lower than the respirometric ones (595-1962 mg/L) for all the compounds studied, except for dichlorometane, indicating that the Microtox® test overestimates the potential negative effect on the activated sludge. The EC_{50} values decrease when the number of chlorine atoms of the molecule increase. No significant differences of EC_{50} were found varying the number of carbon atoms for the same number of chlorine atoms. Thus, the EC_{50} values obtained by respirometry were near 1800 mg/L for dichloromethane and 1,2-dichloroethane. This behaviour was also observed for aliphatic compounds with three chloride atoms. The EC_{50} value for trichloroethylene (1962 mg/L) indicates that the double bond reduces the toxicity of these chlorinated compounds. The growth inhibition test did not give reasonably results for 1,1,1-trichloroethane and carbon tetrachloride, i.e, there was not a relationship between percent inhibition and chlorinated compound concentration. The other analyzed chlorinated aliphatics showed EC_{50} values higher than those obtained by respiration inhibition and Microtox® tests.

Few data are available in the literature relative to the inhibitory effect of these compounds on activated sludge. The EC_{50} value reported by Ricco et al. [3] for dichloromethane (2590 mg/L) was somewhat higher than that obtained in this study. However, EC_{50} values extremely high (>1000 mg/L) indicate the absence of toxicity and are

generally very poorly reproducible. Hall et al. [38] reported EC_{50} values for 1,1,1-trichloroethane, carbon tetrachloride and 1,2-dichloroethane of 493, 69 and 114 mg/L respectively, all of them lower than those obtained in this work. Nevertheless, they measured the reduction in endogenous respiration rate rather than the reduction in oxygen demand for the degradation of a biodegradable reference substrate used in our work. No data for growth inhibition using *P. putida* was found. Ren and Frymier [39] reported EC_{50} values of 1409, 701 and 190 mg/L for dichloromethane, 1,2-dichloroethane and chloroform, respectively using a bioluminescent toxicity assay based on genetically modified *Pseudomonad.* The EC_{50} values obtained by the Microtox® test in this work are in reasonable agreement with those found in the literature, although these last show significant differences in some cases.

EC_{50} values of the additives and pharmaceuticals tested are also shown in Table 1. The additives showed much higher toxicity to *V. fischeri* (Microtox®) in all the cases whereas the EC_{50} values obtained by the growth inhibition test are closer to the respirometric ones. BPA is the most toxic according to the three tests. The EC_{50} values obtained from the respirometric test (16 mg/L) and Microtox® (4 mg/L) are in good agreement with those found in the literature.[40, 51,52] In the case of EDTA, the values reported by Diez and Vidal [53] from the respirometric test (204.1 mg/L) and by Sillanpaa and Oikari [41] from the Microtox® test (3.2 mg/L) were also comparable to those reported in our work. EDTA can affect the efficiency of a biological treatment since as chelating agent, it can change the toxic behavior towards the activated sludge of other species such as heavy metals. Moreover, it increases the permeability of the cell membrane favouring the transfer of toxic substances to the cell body.[54]

In the case of the growth inhibition test, the European Commission reported EC_{50} values of 81-105 mg/L for EDTA and higher than 400 mg/L for DEP, which are in good agreement with those obtained in our work (Table 1). For DEP, the 50 % reduction of

microbial growth was not observed all over the range of concentration tested. Therefore, the EC_{50} data for this compound was expressed as higher than 350 mg/L. MTBE showed a very low toxicity according to the respirometric (9419 mg/L) and the growth inhibition (17060 mg/L) tests whereas it can be considered fairly toxic by the Microtox® bioassay (16 mg/L).

The EC_{50} values of the pharmaceutical compounds tested are also collected in Table 1. Given the specific activity of these compounds, their respective toxicities according to the different bioassays depend critically on the microorganisms used in each test. Ofloxacin offers a clear example, showing a high toxicity to *P. putida* (1 mg/L) whereas relatively low inhibitory effects were found to *V. fischeri* (223 mg/L) and activated sludge (165 mg/L). This fact can be explained since ofloxacin is effective against aerobic gram-negative bacteria.[18] Metronidazole showed a low toxicity by all the tests because it is effective against anaerobic bacteria.[18] Acetylsalicylic acid and tetracycline hydrochloride can be considered not toxic according to the respiration and growth inhibition tests. The information found in the literature on the toxicity of the pharmaceuticals studied by these tests is scarce. Kümmerer et al. [55] reported EC_{50} obtained by respirometry after 30 min. These EC_{50} values are higher than 100 mg/L for ofloxacin, sulfamethoxazole, tetracycline and metronidazole, which are in good agreement with those obtained in the current work. From the growth inhibition test, these authors reported also much lower EC_{50} values than ours (0.01 mg/L for ofloxacin and >64 mg/L for metronidazole). It is known that the exposure time also affects the EC_{50} values for some pharmaceuticals, particularly antibiotics. The toxicity of substances that affect biosynthetic pathways supporting growth and reproduction can only be determined if the bioassay covers the adequate period of the cell cycle.[56] In this sense, Kummerer et al. [55] observed that EC_{50} values for ofloxacin and tetracycline decrease to 1-10 mg/L whereas the toxicity of metronidazole and sulfamethoxazole did not change after a prolonged exposure of

20 h. This fact must be taken into account before establishing the concentration limit to be treated in order to preserve the performance of an activated sludge unit.

3.2. Biodegradability

The testing strategy for assessing degradability foresees that substances not passing the fast biodegradation test should be tested by inherent biodegradation tests. Compounds which reach more than 70% biodegradation within 28 d are assumed as being biodegradable, whereas substances with biodegradation extents in the Zahn-Wellens test between 20 and 70% should be regarded as partially biodegradable.[57] Considering these percentages also for the fast biodegradability test, the studied priority and emerging compounds were assayed for assessing aerobic biodegradability.

The SOUR profiles from the fast biodegradability test showed only acetyl salicylic acid and DEP yielded a positive response upon that test. Figure 1 depicts the evolution of SOUR, TOC and concentration for those two compounds. Acetyl salicylic acid showed to be partially biodegradable with 65 % conversion after 24 h. At that time, 66 % TOC reduction was achieved. DEP proved to be almost completely biodegradable, showing 98 % conversion with more than 90 % mineralization. The SOUR profile showed around one-day lag time, hence the test was prolonged until 72 h. A peak value of 22.5 mg O_2/g SSV.h was achieved at 50 h. For both compounds the degradation is simultaneous to TOC reduction. According to this test, the rest of studied compounds were not biodegradable. In this sense, these compounds are not likely to be degraded in real WWTP, which can explain why they are found in WWTP discharges. [58-60]

The Zahn-Wellens test was used to evaluate the inherent biodegradability only with compounds not biodegradable according to the fast biodegradability test. Biodegradation and TOC reduction of chlorinated compounds and additives is shown in Figure 2. Most of the

chlorinated compounds tested were partially degraded in 28 d with TOC reduction ranging from 21 to 39 % (Figure 2(a)). 1,1,1-trichloroethane showed a rapid mineralization in the first 3 days, where 50 % TOC reduction was achieved. Then a slower degradation was observed up to 74 % mineralization after 28 days. Dichloromethane showed almost negligible biodegradability. With regard to the additives, BPA and EDTA were partially biodegradable with final percentages of TOC reduction around 35 % (Figure 2(b)). MTBE biodegradation reached 60% of TOC reduction in 28 d. Bradley et al. [61] found that MTBE degradation was ranged from 15 to 66 % in several lake-bed sediments without a history of MTBE contamination. DEP was not submitted to the Zahn-Wellens test given its positive response in the fast biodegradability test.

The inherent biodegradability of the pharmaceuticals tested is shown in Figure 3. Sulfamethoxazole yielded 50 % conversion in 28 d whereas TOC reduction was about 31 % which indicates the formation of non-biodegradable intermediates by the activated sludge. Hydrochlorothiazide and ofloxacin showed very low biodegradability with compound removal below 15 %. Sousa et al. [62] reported that hydrochlorothiazide seems refractory to biological treatment. Also Gartiser et al. [63] and Kümmerer et al. [18] found that ofloxacin showed only minor TOC reduction extents in the inherent test. The lower percentage of TOC reduction (54 %) with respect to the compound degradation was also observed for tetracycline hydrochlorothiazide. The removal of tetracycline hydrochlorothiazide showed a typical degradation curve reaching the plateau phase at 76 % only after 12 d. This can be explained by the tendency of tetracycline antibiotics to form hardly soluble compounds with calcium or similar ions.[63,64]

Table 2 collects the inherent biodegradability of the compounds tested expressed as percent TOC reduction. 1,1,1-trichloroethane can be considered biodegradable whereas chloroform and carbon tetrachloride were hardly biodegradable and ofloxacin,

hydrochlorothiazide and dichloromethane were highly resistant to biodegradation. The antibiotics (sulfamethoxazole, metronidazole and tetracycline), 1,2-dichloroethane, trichloroethylene and the additives can be considered partially biodegradable.

4. Conclusions

The combination of toxicity and biodegradability tests is a suitable tool for assessing the behavior of chemicals in sewage treatment plants. Although the EC_{50} values obtained from the Microtox® test were generally lower than those from the respirometric one with activated sludge, this response is not predictable. This fact was also found using the growth inhibition test. Therefore, the respirometry inhibition test appears more suitable to evaluate the potential effects on a STP, as it directly assesses the effect on the microbial population of interest. Regarding biodegradability, the fast test used in this work allows evaluating biodegradation in a short period of time (24-48 h), thus providing a realistic approach to the biological treatment of a STP, whereas the inherent biodegradability test can be used to evaluate the convenience of treatment in adapted bioreactors. According with this strategy, diethyl phthalate and acetylsalicylic acid could be treated in a STP within concentrations lower that their EC_{50} values and the biodegradation of 1,1,1,-trichloroethane could be attempted in an adapted bioreactor. For the rest of chlorinated compounds, pharmaceuticals, bisphenol A and EDTA a chemical treatment or a coupled chemical/biological process is recommendable.

References

[1] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the fiend of gwater policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council.

[2] Ren S. Assessing wastewater toxicity to activated sludge: recent research and developments. Environ Int. 2004; 30:1151-1164.

[3] Ricco G, Tomei MC, Ramadori R, Laera G. Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox®. Water Res. 2004; 38(8):2103-2110.

[4] Rizzo L. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water Res. 2011; 45(15):4311-4340.

[5] Gutiérrez M, Etxebarria J, de las Fuentes L. Evaluation of wastewater toxicity: comparative study between Microtox® and activated sludge oxygen uptake inhibition. Water Res. 2002; 36(4):919-924.

[6] Polo AM, Tobajas M, Sanchis S, Mohedano AF, Rodriguez JJ. Comparison of experimental methods for determination of toxicity and biodegradability of xenobiotic compounds. Biodegradation. 2011; 22(4):751-761.

[7] Sanchis S, Polo AM, Tobajas M, Rodriguez JJ, Mohedano AF. Strategies to evaluate biodegradability: application to chlorinated herbicides. Environ Sci Pollut Res. 2014; 21(18):9445–9452.

[8] Kalcikova G, Vavrova M, Zagorc-Koncan J, Gotvajn AZ. Evaluation of the hazardous impact of landfill leachates by toxicity and biodegradability tests. Environ Technol. 2011; 32(12): 1345-1353.

[9] Dalzell DJB, Christofi N. An ATP luminescence method for direct toxicity assessment of pollutants impacting on the activated sewage sludge process. Water Res. 2002; 36:1493-1502.

[10] International Standardization Organization. ISO 10712: 1995, Water Quality: Pseudomonas putida growth inhibition test (Pseudomonas cell multiplication inhibition test).

[11] International Standardization Organization. ISO 15522: 1999, Water Quality: Determination on the inhibitory effect of the water constituents on the growth of activated sludge micro-organism.

[12] McNicholl BP, McGrath JW, Quinn JP. Development and application of a resazurin-based biomass activity test for activated sludge plant management. Water Res. 2007; 41:127-133.

[13] Guisasola A, Baeza JA, Carrera J, Casas C, Lafuente J. An off-line respirometric procedure to determine inhibition and toxicity of biodegradable compounds in biomass from an industrial WWTP. Water Sci Technol. 2003; 48: 267-275.

[14] Organisation for Economic Co-operation and development OECD. 209 Activated Sludge, Respiration Inhibition Test, OECD guidelines for testing of chemicals. Paris; 1993a.

[15] Elnabarawy MT, Robideau RR, Beach SA. Comparison of three rapid toxicity test procedures: Microtox,® polytox,® and activated sludge respiration inhibition. Toxicity Assessment. 1988; 3(4):361-370.

[16] Coello Oviedo MD, Barragan Sanchez J, Aragon Cruz C, Quiroga Alonso J M. A new approach to toxicity determination by respirometry. Environ Technol. 2009; 30(14): 1601-1605.

[17] International Standardization Organization. ISO 8192: 1986, Water quality: test for inhibition of oxygen consumption by activated sludge.

[18] Kümmerer K, Al-Ahmad A, Mersch-Sundermann V. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. Chemosphere. 2000; 40(7):701-710.

[19] Chen XC, Shi JY, Chen YX, Xu XH, Xu SY, Wang YP. Tolerance and biosorption of copper and zinc by Pseudomonas putida CZ1 isolated from metal-polluted soil. Can J Microbiol. 2006; 52(4):308-316.

[20] Onorati F, Mecozzi M. Effects of two diluents in the Microtox® toxicity bioassay with marine sediments. Chemosphere. 2004; 54(5):679-687.

[21] Altenburger R, Backhaus T, Boedeker W, Faust M, Scholze M, Grimme LH. Predictabilty of the toxicity of multiple chemical mixtures to Vibrio Fischeri: mixtures composed of similarly acting chemicals . Environ Toxicol Chem. 2000; 19(9):2341-2347.

[22] Bizani E, Fytianos K, Poulis I, Tsiridis V. Photocatalytic decolorization and degradation of dye solutions and wastewaters in the presence of titanium dioxide. J Hazard Mater. 2006; 136(1):85-94.

[23] Organisation for Economic Co-operation and development OECD. 301 Ready Biodegradability, OECD guidelines for testing of chemicals. Paris; 1993b.

[24] Organisation for Economic Co-operation and development OECD. 302 B Zahn-Wellens/EMPA Test, OECD guidelines for testing of chemicals. Paris; 1993c.

[25] Organisation for Economic Co-operation and development OECD. 303 A Simulation Test – Aerobic Sewage Treatment, OECD guidelines for testing of chemicals. Paris; 1993d.

[26] International Standardization Organization. ISO 14593: 1999, Water Quality—Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium—Method by analysis of inorganic carbon in sealed vessels (CO2 headspace test).

[27] International Standardization Organization. ISO 9888: 1999, Water Quality— Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium—Static test (Zahn-Wellens method).

[28] International Standardization Organization. ISO 11733: 2004, Water Quality: Determination of the elimination and biodegradability of organic compounds in an aqueous medium -- Activated sludge simulation test.

[29] Ballesteros Martín MM, Casas López JL, Oller I, Malato S, Sánchez Pérez JA. A comparative study of different tests for biodegradability enhancement determination during AOP treatment of recalcitrant toxic aqueous solutions. Ecotox Environ Safe. 2010; 73 (6): 1189-1195.

[30] Lepik R, Tenno T. Determination of biodegradability of phenolic compounds, characteristic to wastewater of the oil-shale chemical industry, on activated sludge by oxygen uptake measurement. Environ Technol. 2012; 33(3):329-339.

[31] Monsalvo VM, Tobajas M, Mohedano AF, Rodriguez JJ. Intensification of sequencing batch reactors by cometabolism and bioaugmentation with *Pseudomonas putida* for the biodegradation of 4-chlorophenol. J Chem Technol Biotechnol. 2012; 87:1270–1275.

[32] International Standardization Organization. ISO 11348-3. Water Quality: Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) - Part 3: Method using freeze-dried bacteria. Geneva; 1998.

[33] Chica A, Martin A, Vazquez FJ, Carmona FJ, Mohedo JJ. Respirometer to analyze measure dissolved oxygen and oxygen demand of microbes in leachate from municipal waste. Patent Number: ES 2283171. 2007.

[34] American Public Health Asociation. Standard Methods for the Examination of Water and Wastewater. 20th ed., Water Environment Federation. Washington DC, USA; 1998.

[35] Kaiser KLE, Palabrica VS. Photobacterium phosphoreum Toxicity Data index. Water Pollut Res J Can. 1991; 26(3):361-431.

[36] Jennings VLK, Rayner-Brandes MH, Bird DJ. Assessing chemical toxicity with the bioluminescent photobacterium (vibrio fischeri): a comparison of three commercial systems. Water Res. 2001; 35(14):3448-3456.

[37] Comission, E. European Union Risk Assessment Report: Trichloroethylene; 2004. p. 67.

[38] Hall E, Sun B, Prakash J, Nirmalakhandan N. Toxicity of organic chemicals and their mixtures to activated sludge microorganisms. J Environ Eng. 1996; 122(5):424-429.

[39] Ren S, Frymier PD. Use of multidimensional scaling in the selection of wastewater toxicity test battery components. Water Res. 2003; 37(7):1655-1661.

[40] Debenest T, Gagné F, Petit AN, André C, Kohli M, Blaise C. Ecotoxicity of a brominated flame retardant (tetrabromobisphenol A) and its derivatives to aquatic organisms. Comp Biochem Physiol C-Toxicol Pharmacol. 2010; 152(4):407-412.

[41] Sillanpaa M, Oikari A. Assessing the impact of complexation by EDTA and DTPA on heavy metal toxicity using microtox bioassay. Chemosphere. 1996; 32(8):1485-1497.

[42] Kapanen A, Stephen JR, Brüggemann J, Kiviranta A, White DC, Itävaara M. Diethyl phthalate in compost: Ecotoxicological effects and response of the microbial community. Chemosphere. 2007; 67(11):2201-2209.

[43] Werner I, Koger CS, Deanovic LA, Hinton DE. Toxicity of methyl-tert-butyl ether to freshwater organisms. Environ Pollut. 2001; 111(1):83-88.

[44] Hernando MD, Ejerhoon M, Fernández-Alba AR, Chisti Y. Combined toxicity effects of MTBE and pesticides measured with Vibrio fischeri and Daphnia magna bioassays. Water Res. 2003; 37(17):4091-4098.

[45] Ferrari B, Mons R, Vollat B, Fraysse B, Paxēaus N, Giudice RL, Pollio A, Garric J. Environmental risk assessment of six human pharmaceuticals: Are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?. Environ Toxicol Chem. 2004; 23(5):1344-1354.

[46] Calleja MC, Persoone G, Geladi P. Comparative acute toxicity of the first 50 Multicentre Evaluation of In Vitro Cytotoxicity chemicals to aquatic non-vertebrates. Arch Environ Cont Tox. 1994; 26(1):69-78.

[47] Backhaus T, Froehner K, Altenburger R, Grimme LH. Toxicity testing with Vibrio Fischeri: A comparison between the long term (24 h) and the short term (30 min) bioassay. Chemosphere. 1997; 35(12):2925-2938.

[48] Isidori M, Lavorgna M, Nardelli A, Pascarella L, Parrella A. Toxic and genotoxic evaluation of six antibiotics on non-target organisms. SciTotal Environ. 2005; 346(1–3):87-98.

[49] Kim Y, Choi K, Jung J, Park S, Kim P-G, Park J. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environ Int. 2007; 33(3):370-375.

[50] Kołodziejska M, Maszkowska J, Białk-Bielińska A, Steudte S, Kumirska J, Stepnowski P, Stolte S. Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. Chemosphere. 2013; 92(9):1253-1259.

[51] Murk AJ, Legler J, van Lipzig MMH, Meerman JHN, Belfroid AC, Spenkelink A, van der Burg B, Rijs GBJ, Vethaak D. Detection of estrogenic potency in wastewater and surface water with three in vitro bioassays. Environ Toxicol Chem. 2002; 21(1):16-23.

[52] Rehmann K, Schramm KW, Kettrup AA. Applicability of a yeast oestrogen screen for the detection of oestrogen-like activities in environmental samples. Chemosphere. 1999; 38(14):3303-3312.

[53] Diez M, Vidal G. Evaluación de la toxicidad del EDTA y metal-EDTA en microorganismos en un sistema de lodos activados que trata efluente de la industria celulosa [Toxicity evaluation of EDTA and metal-EDTA in microorganisms in an activated sludge system for treatment of cellulose industry effluent]. In: AIDIS, Forjando el Ambiente que Compartimos, San Juan, AIDIS; 2004. p 1-5.

[54] Rodríguez JB, Mutis A, Yeber MC, Freer J, Baeza J, Mansilla HD. Chemical degradation of EDTA and DTPA in a totally chlorine free (TCF) effluent. Water Sci Technol. 1999; 40(11–12):267-272.

[55] Kümmerer K, Alexy R, Hüttig J, Schöll A. Standardized tests fail to assess the effects of antibiotics on environmental bacteria. Water Res. 2004; 8: 2111-2116.

[56] Froehner K, Backhaus T, Grimme LH. Bioassays with Vibrio fischeri for the assessment of delayed toxicity. Chemosphere. 2000; 40(8):821-828.

[57] Beek B, Böhling S, Franke C, Jöhncke U, Studinger G, Thumm E. The assessment of biodegradation and persistence. In Beek B, editor. Biodegradation and Persistence, The Handbook of Environmental Chemistry, vol. 2, Reactions and Processes, Part K (O. Hutzinger, Editor-in-Chief): Springer-Verlag, Berlin; 2001. p 291–320.

[58] Loos R, Carvalho R, Antonio DC, Comero S, Locoro G, Tavazzi S, Paracchini B, Ghiani M, Lettieri T, Blaha L, Jarosova B, Voorspoels S, Servaes K, Haglund P, Fick J, Lindberg RH, Schwesig D, Gawlik BM. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 2013; 47: 6475-6487.

[59] Mailler R, Gasperi J, Coquet Y, Deshayes S, Zedek S, Cren-Olive C, Cartiser N, Eudes V, Bressy A, Caupos E, Moilleron R, Chebbo G, Rocher V. Study of a large scale powdered activated carbon pilot: Removals of a wide range of emerging and priority micropollutants from wastewater treatment plant effluents. Water Res. 2015; 72: 315-330.

[60] Margot J, Kienle C, Magnet A, Weil M, Rossi L, Alencastro LF, Abegglen C, Thonney D, Chèvre N, Schärer M, Barry DA. Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon? Sci Total Environ. 2013; 461-462: 480-498.

[61] Bradley PM, Landmeyer JE, Chapelle FH. Widespread potential for microbial MTBE degradation in surface-water sediments. Environ Sci Technol. 2001; 35:658-662.

[62] Sousa MA, Goncalves C, Vilar VJP, Boaventura RAR, Alpendurada MF. Suspended TiO2-asssisted photocatalytic degradation of emerging contaminants in a municipal WWTP effluent using a solar pilot plant with CPCs. Chem Eng J. 2012; 198-199:301-309.

[63] Gartiser S, Urich E, Alexy R, Kümmerer K. Ultimate biodegradation and elimination of antibiotics in inherent tests. Chemosphere. 2007; 67:604-613.

[64] Lunestad BT, Goksoyr J. Reduction in the antibacterial effect of oxytetracycline in sea water by complex formation with magnesium and calcium. Dis Aquat Organ. 1990; 9: 67-72.

Table 1. EC_{50} values of chlorinated compounds, additives and pharmaceuticals.

Compound	Number of runs [conc. range] (mg/L)	Solubility (mg/L) x 10^{-3}	$EC_{50\ 15\ min}$ Respirometry (mg/L) (S.D.)[a]	R^2	$EC_{50\ 15\ min}$ Microtox® (mg/L) (S.D.)[a]	R^2	EC_{50} Microtox® (mg/L)		Number of runs [conc. range] (mg/L)	$EC_{50\ 16h}$ (mg/L) Growth inh. Test. *P. putida*
							Other authors	References		
Dichloromethane	5 [500-3000]	13000	1755 (150.1)	0.99	3079 (558.8)	0.92	998-3500	[35,36]	4 [406-1625]	1590
1,2-dichloroethane	11 [100-7500]	8700	1810 (351.7)	0.98	187 (29.1)	0.97	61-2170	[35,36]	4 [45-2500]	2442
Chloroform	8 [50-5000]	8000	638 (86.0)	0.96	596 (86.9)	0.99	632-2494	[15,35,36]	4 [750-2500]	1696
1,1,1-trichloroethane	5 [50-800]	1300	595 (59.2)	0.95	19 (1.5)	0.97	10-106	[5,35]	6 [20-650]	-
Carbon tetrachloride	6 [10-600]	800	608 (37.4)	0.99	28 (3.3)	0.95	6-34	[35,36]	6 [12-400]	-
Trichloroethylene	5 [50-800]	1000	1962 (106.2)	0.99	301 (42.3)	0.86	115-610	[35,37]	4 [16-63]	224
BPA	6 [5-30]	0.12	16 (2.5)	0.98	4 (1.2)	0.97	5.7-7.7	[40]	6 [1-50]	115
EDTA	7 [25-240]	1.0	262 (37.4)	0.91	9 (2.1)	0.97	3.2	[41]	6 [5-150]	114
DEP	8 [5-350]	1.1	201 (12.5)	0.98	74 (4.8)	0.99	114	[42]	6 [8-250]	>350
MTBE	7 [1 10^3-35 10^3]	26	9419 (1028.8)	0.99	16 (1.9)	0.95	41.8	[43,44]	8 [625-20000]	17060
Ofloxacin	9 [10-600]	3.4	165 (17.6)	0.93	223 (21.6)	0.99	90	[45]	6 [18-400]	1
Acetylsalicylic acid	8 [100-600]	4.6	307 (5.1)	0.97	572 (35.1)	0.99	26.1	[46]	6 [16-500]	140
Tetracycline hydrochloride	6 [50-1500]	17.8	610 (10.2)	0.94	29 (2.3)	0.98	19.9	[47]		-
Hydrochlorothiazide	6 [10-400]	0.72	36 (7.6)	0.99	68 (6.6)	0.90			12 [8-295]	Not toxic
Sulfamethoxazole	6 [25-450]	0.61	377 (25.3)	0.99	55 (3.6)	0.97	16.9-32.2 78.1	[48] [49]	6 [16-400]	477
Metronidazole	6 [300-800]	95	537 (5.1)	0.95	878 (37.9)	0.92	243	[50]	6 [16-500]	2557

[a] S.D. Standard Deviation

Table 2. Results from the Zahn-Wellens test.

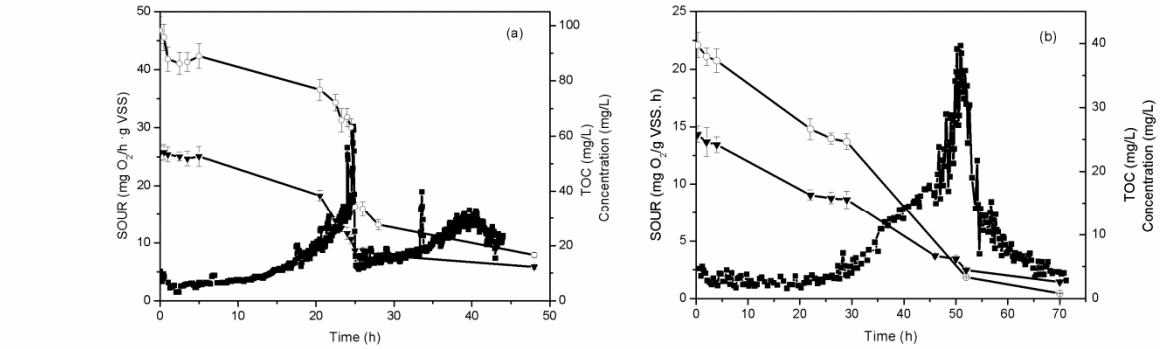
Compound	Concentration (mg/L)	% TOC reduction after 28 d
Dichloromethane	100	2.4
Chloroform	100	23.8
Carbon tetrachloride	100	21.6
1,2-dichloroethane	100	28.4
1,1,1-trichloroethane	50	73.9
Trichloroetylene	100	38.9
Bisphenol A	5	34.3
EDTA	50	33.4
MTBE	100	60.3
Ofloxacin	100	14.6
Tetracycline hydrochloride	100	54.4
Sulfamethoxazole	25	31.2
Metronidazole	20	28.1
Hydrochlorothiazide	10	14.2

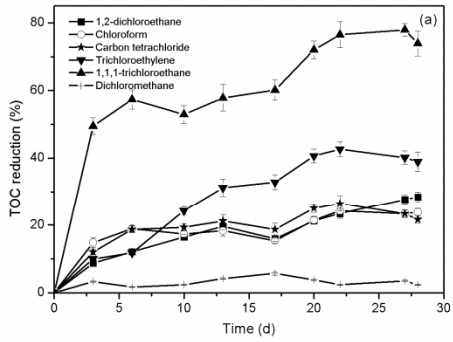
Figure captions

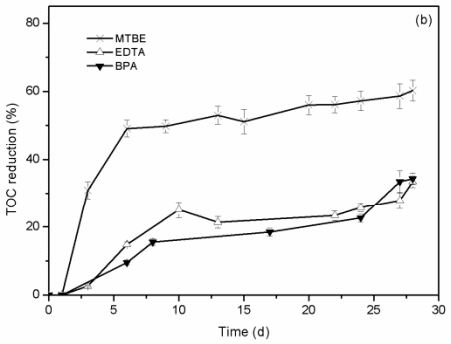
Figure 1. SOUR (square), TOC (triangle) and target compound concentration (circle) along the fast biodegradability test for a) Acetyl salicylic acid and b) DEP.

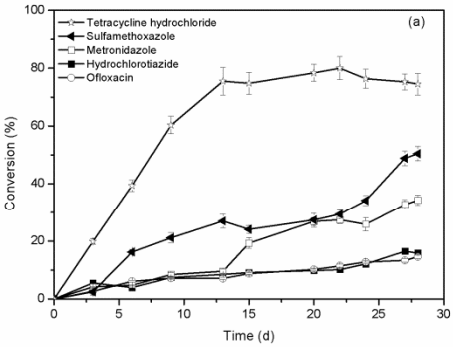
Figure 2. TOC reduction upon the Zahn Wellens biodegradability test: a) chlorinated compounds and b) additives.

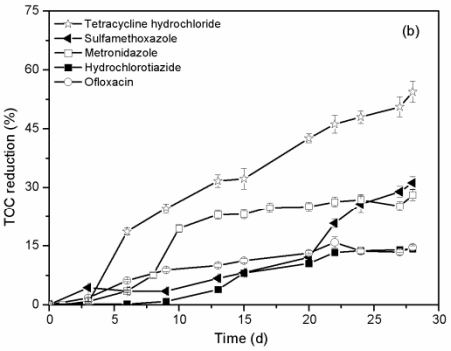
Figure 3. Zahn Wellens biodegradability test of pharmaceuticals: a) target compound degradation and b) TOC reduction.




(a)
1,2-dichloroethane
Chloroform
Carbon tetrachloride
Trichloroethylene
1,1,1-trichloroethane
Dichloromethane
TOC reduction (%)
Time (d)


(b)
MTBE
EDTA
BPA
TOC reduction (%)
Time (d)


(a)
Tetracycline hydrochloride
Sulfamethoxazole
Metronidazole
Hydrochlorotiazide
Ofloxacin
Conversion (%)
Time (d)


(b)
Tetracycline hydrochloride
Sulfamethoxazole
Metronidazole
Hydrochlorotiazide
Ofloxacin
TOC reduction (%)
Time (d)