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# Hazard identification of pharmaceutical wastewaters using biodegradability studies

**A. Žgajnar Gotvajn and J. Zagorc-Končan**

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Hajdrihova 19, SI-1000, Ljubljana (E-mail: *andreja.zgajnar@ki.si*)

**Abstract** A reliable wastewater characterization is an integral part of treatment and management strategies for industrial effluents. This is especially true for the pharmaceutical industry, which exhibits significant differences in its line of activity, generating effluents of very specific and complex natures. Any hazard or risk assessment of wastewater and/or determination of its treatability must include an evaluation of its degradability. Usually various non-standardized laboratory or pilot-scale long-term tests are run by measuring summary parameters for several days to determine the biodegradation potential of the effluent. A complex approach, based on stabilization studies, was proposed to determine the hazardous impact of wastewaters in terms of biodegradable and persistent toxicity.

The objective of our work was to carry out complex hazard evaluation of pharmaceutical wastewaters. Whole effluent toxicity was determined using two different toxicity tests. First, we measured the inhibition of oxygen consumption by activated sludge. The test indicated toxicity of the wastewater and thus we performed an additional acute toxicity test with luminescent bacteria *Vibrio fisheri*. The next step was the determination of whole effluent ready biodegradability. It was determined with simultaneous measurement of oxygen consumption (ISO 9804) and carbon dioxide production (ISO 9439) in a closed respirometer, accompanied by DOC/IC measurements. The pharmaceutical wastewater degraded readily (83%, lag phase was 2 days, biodegradation rate was 0.339 $day^{-1}$) on the basis of $O_2$ measurements. The biodegradation, calculated from the $CO_2$ measurements, was comparable. We also applied mass balances of DOC/IC at the beginning and at the end of biodegradation experiments to confirm the extent and rate of biodegradation. The determination of hazardous impact and treatability of the effluent was concluded with aerobic stabilization studies. Biodegradation of the wastewater during the study was followed by relevant biochemical analysis and DOC/IC mass balance.

**Keywords** Biodegradability; kinetics; mass balance; stabilization; toxicity; wastewater

## Introduction

A complex wastewater characterization is an integral part of treatment and management strategies for industrial effluents (Lindgaard-Joergensen, Nyholm, 1988). This is especially true for the pharmaceutical industry, generating effluents of very specific and complex natures. Although it is of fundamental importance to the global economy, the pharmaceutical industry is often associated with environmental release of a wide range of organic and inorganic pollutants. Many pharmaceutically active compounds, residues and their metabolites could resist wastewater treatment plant discharges and they could be transported into the surface waters. The widespread of antibiotics has led to the development of antibiotic resistant bacteria, capable of penetrating and surviving different treatment processes.

Because of several limitations, such as lack of analytical techniques, data on ecotoxicity, accumulation and possible bioaccumulation of representative as well as predominant constituents of the wastewaters, an assessment of effluents is not possible by using only a chemical specific approach (Ates, Orhon, Tunay, 1997). Different reactions that occur in the effluent could also change its hazardous impact regarding its physico-chemical composition. Therefore an additional approach, employing bioassays with biosensors, is needed to overcome the mentioned limitations (Nyholm, 1996).

Any hazard or risk assessment of wastewater and/or determination of its biological treatability must include an evaluation of its degradability in receiving water or in a wastewater treatment plant. For pure chemicals and individual components of the wastewaters many standardized methods exist (ISO 15462, 1998), which are linked to a test strategy with three straightened tiers in the European Union: ready biodegradability assessment tests, tests for determination of potential biodegradability and simulation tests for specific environmental compartments, such as surface waters, sediments or biological wastewater treatment plants. No such approach has yet been developed for biodegradability assessment of industrial effluents (Nyholm, 1996). Usually various non-standardized laboratory or pilot-scale long-term tests are run by measuring summary parameters for several days to determine the biodegradation potential of the effluent. These methods do not allow the determination of the biodegradability of minor wastewater components that could have harmful impacts. A complex approach, based on stabilization studies, was proposed to determine hazardous impact of wastewaters in terms of biodegradable and persistent toxicity. The above mentioned procedure starts with a compilation of all relevant data on the effluent, performance of simple chemical analysis (summary parameters such as COD, $BOD_5$, etc.), followed by an aerobic stabilization study with a rather high concentration of the wastewater in order to produce enough sample containing residues to be tested again. A stabilization study has usually been accomplished in batch reactors (several litres), well mixed and aerated. The type and number of physico-chemical analysis and toxicity determinations, as well as their schedule, should be selected according to the behaviour of the diluted effluent during the stabilization study. A combination of different toxicity methods assures data on effluent toxicity regarding short- and long-term toxicity (Chapmann, 2000).

The complete picture of effluent biological treatability and its risk assessment is concluded with complementary biodegradability determination as for pure chemicals in order to relate the composition of the wastewater to its environmental impact (Nyholm, 1996).

## Materials and methods

The objective of our work was to carry out complex hazard evaluation of pharmaceutical wastewaters. Wastewaters were random grab sampled twice (Sample 1 in November 1999 and Sample 2 in September 2001). Wastewaters were collected from an equalization basin, where wastewaters from batch production of antibiotics are mixed with other wastewaters from the factory. Then the effluent enters a biological wastewater treatment plant, where after mechanical pretreatment it is treated aerobically. Prior to toxicity and biodegradability tests pharmaceutical wastewater was analysed (pH, COD (IS0 6060, 1989), $BOD_5$ (ISO 5815, 1989), TOC (ISO 8245, 1987), Kjeldahl nitrogen, $NH_4^+$-N (ISO 5663, 1984) and $NO_2^-$-N as well as $NO_3^-$-N (ISO 10304-1, 1992). Whole effluent toxicity was determined using two different toxicity tests:

- We determined the inhibition of oxygen consumption by activated sludge (ISO 8192, 1986). We measured oxygen consumption of unadapted microorganisms after 30 minutes of incubation. The activated sludge was taken from the aeration basin of the laboratory wastewater treatment plant, where municipal wastewaters are treated predominantly (30 l/day). The unit was additionally fed with 0.9 mg/l of P as $K_2HPO_4$ and 0.06 g/l of peptone to maintain constant organic load and reproducible quality of microorganisms. Tests were performed with high concentration of microorganisms (0.8 to 1.0 g SS/l) to evaluate the toxic impact of the wastewater in conditions comparable to a conventional aerobic wastewater treatment plant.
- The above test indicated toxicity of the wastewater and thus we performed an additional acute toxicity test with luminiscent bacteria *Vibrio fisheri* (EN ISO 11348-2, 1998).

The next step was the determination of whole effluent ready biodegradability to assess its biodegradability in common environmental conditions. The combination of two biodegradability assessment tests from the first level of the tiered protocol for pure substances was employed (Nyholm, 1996). Biodegradability of the effluent was determined with simultaneous measurement of oxygen consumption (ISO 9804, 1991) and carbon dioxide production (ISO 9439, 1990) in a closed respirometer (Micro Oxymax, Columbus Instruments, USA). Tests were performed with 5 vol. % of the wastewaters. Their theoretically expected oxygen demands were 100 mg/l, calculated on the basis of experimentally determined COD (ISO 6060, 1989). The theoretically expected $CO_2$ productions were evaluated on the basis of DOC (ISO 8245, 1989) measurements of the raw wastewaters. As inoculum we used activated sludge generated in the same laboratory pilot plant as for toxicity studies (30 mg SS/l). All necessary nutrients were added. Abiotic elimination after addition of 10 ml/l of $HgCl_2$ stock solution (1 g/l) was determined at the same concentration of the wastewater as in the test vessel. All of the tests were run in duplicate. Gathered data were used to plot degradation curves and to calculate the rate of biodegradation. We simplify the calculations neglecting changes in biomass yield and data fit the first order kinetics (Stumm, 1990). In Eq. (1), c corresponds to the concentration of the substance, which in our case was represented with one of the measured parameters such as $O_2$ consumption, $CO_2$ production or DOC elimination. We presumed that each of them correlates to the concentration of the substance in particular time interval.

$$-\frac{dc}{dt} = k_1 c \tag{1}$$

c = concentration of the substance (mg/l)
$k_1$ = rate constant ($day^{-1}$)
t = time (day)

In fact we have many organics degrading according to different rates with overall first order kinetics. For calculation of rate constants, Eq. (1) was modified to enable direct calculation of degradation rates:

$$\ln\left[1-\frac{D_t}{100}\right] = -k_1 t \tag{2}$$

$D_t$ = biodegradation at time t (%)
$k_1$ = rate constant ($day^{-1}$)
t = time (day)

Material balances were also used to determine relations between different forms of carbon during biodegradation of pharmaceutical wastewaters at particular time intervals, enabling more accurate evaluation of biodegradation. Summarized Eq. (3) could be written for organic and inorganic C separately (Eq. (4) and Eq. (5)) (Stumm, 1990):

$$TC_{i,l} = TC_{t,l} + TC_{t,biomass} + CO_{2,t}(gas)\uparrow \tag{3}$$

$$\frac{dDOC}{dt} = -k_1 DOC - k_2 DOC \tag{4}$$

$$IC_{i,l} = IC_{t,l} + IC_{t,inorganic,biomass} + CO_{2,t}(gas)\uparrow \tag{5}$$

$TC_{i,l}$ = Concentration of total C at time 0 in liquid phase (mg/l)
$TC_{t,l}$ = Concentration of total C at time t in liquid phase (mg/l)
$TC_{t,biomass}$ = Concentration of total C incorporated into biomass at time t (mg/l)
$CO_{2,t}$ (gass) = Concentration of total C released from the liquid phase until time t as $CO_2$ (mg/l)
DOC = Dissolved organic C (mg/l)
$k_1$ = Biodegradation rate constant ($day^{-1}$)
$k_2$ = Biomass production rate ($day^{-1}$)
$IC_{i.l}$ = Concentration of inorganic C at time 0 in liquid phase (mg/l)
$IC_{t.l}$ = Concentration of inorganic C at time t in liquid phase (mg/l)
$IC_{t,inorganic,biomass}$ = Concentration of inorganic C incorporated into biomass until time t (mg/l)

The simplified approach to biodegradation kinetics with material balances for DOC was then used also for evaluation of gathered DOC data in stabilization studies, which was conducted with the second sample of pharmaceutical wastewater. Wastewater was diluted (1 litre to 5 litres) with unpolluted river water on the basis of toxicity studies to avoid significant toxic impact. The study was accomplished in a 5 litre reactor and some parameters like DOC (ISO 8245, 1989), pH, temperature, oxygen saturation and toxicity according to luminiscent bacteria were monitored periodically during 28 days of the aerobic aging (EN ISO 11348-2, 1998). The mixtures were inoculated with 1 ml/l of settled effluent from laboratory treatment plant. Toxicity to luminescent bacteria (EN ISO 11348-2, 1998) and physico-chemical characteristics of the wastewaters (as for the raw effluent) were determined at the beginning and the end of the stabilization to allow comparison of measured values in terms of persistent and biodegradable toxicity. A blank test was run simultaneously.

## Results and discussion

Physico–biochemical and toxicological analysis of samples of raw pharmaceutical wastewater are presented in Table 1.

Results of biodegradability studies with both samples of the wastewaters are presented

**Table 1** Physico-chemical and toxicological analysis of raw pharmaceutical wastewaters

| Parameter | | Sample 1 (November 1999) | Sample 2 (September 2001) |
|---|---|---|---|
| pH | | 6.7 | 7.7 |
| COD (mg/l) | | 1620 | 1995 |
| DOC (mg/l) | | 472 | 513 |
| IC (mg/l) | | 55.2 | 57.9 |
| $BOD_5$ (mg/l) | | 1100 | 555 |
| Kjeldahl N (mg/l) | | 88.4 | 90.3 |
| $NH_4^+$-N (mg/l) | | 35.6 | 28.5 |
| $NO_2^-$-N (mg/l) | | < 0.02 | 54.4 |
| NO3$^-$-N (mg/l) | | 13.7 | 325 |
| Total P (mg/l) | | 14.1 | / |
| $PO_4^{3-}$-P (mg/l) | | 2.3 | < 0.1 |
| Bioluminiscence inhibition: | 30 minEC20 (vol. %) | 14.5 | 0.4 |
| | 30 minEC50 (vol. %) | 67.8 | 9.0 |
| | 30 minEC90 (vol. %) | / | / |
| Inhibition of $O_2$ consumption: | 180 minEC20 (vol. %) | 1.1 | |
| | 180 minEC50 (vol. %) | 3.0 | non-toxic |
| | 180 minEC90 (vol. %) | 7.9 | |

/…Not determined

in Figure 1. The first sample of pharmaceutical wastewater degraded rapidly. Its degradation considering $O_2$ measurement started after one day, when it reached 0.339 $day^{-1}$ ($r^2 = 0.950$), while its biodegradation rate according to $CO_2$ measurements was 0.108 $day^{-1}$ ($r^2 = 0.969$) through the first 6 days of biodegradation, and it had reached 0.394 $day^{-1}$ ($r^2 = 0.912$) during the last degradation period. Overall biodegradation rate on the basis of $CO_2$ measurements was 0.154 $day^{-1}$ ($r^2 = 0.888$). Final levels of degradation were comparable (83/95%) in both cases, indicating complete mineralization of wastewater.

According to DOC/IC measurements at the beginning and the end of the biodegradation study with Sample 1, biodegradation reached 86%, corresponding to biodegradation on the basis of $CO_2$ measurements (Figure 1, Table 2). Material balance for the Sample 1 in closed system based on DOC and IC measurements during biodegradation confirmed that remaining organic carbon from the wastewater (3.33 mg/l as C recalculated to 11.10 mg/l as $CO_2$) remained in the liquid phase (Table 2).

Summarizing the produced $CO_2$ (69.11 mg/l), it remained in the liquid phase and comparison to the theoretically expected production (86.79 mg/l) employing Eq. (5) enabled calculation of the portion of carbon (1.55 mg/l), incorporated into a new biomass. Produced $CO_2$ was not trapped into the liquid medium, because the enlargement of the IC concentration in the sample was only 0.391 mg/l of C or 1.419 mg/l as $CO_2$ (Table 2).

The same was noticed for the second sample of the wastewater (Table 2, Sample 2). According to DOC measurements it degraded 84%, while according to $O_2$ measurements, the final level of degradation was 92% and 87% according to $CO_2$ measurements. The remained IC confirmed effective release of $CO_2$ from liquid to the gaseous phase. The overall degradation rate in the second sample from $O_2$ consumption was 0.08 $day^{-1}$ ($r^2 = 0.833$), while it was lower based on $CO_2$ measurements (0.06 $day^{-1}$ ($r^2 = 0.861$)). In both cases biodegradation was slower in the first 16 days (0.06/0.05 $day^{-1}$) and proceeded after 5 days of plateau more intensively (0.29/0.19 $day^{-1}$). Both samples of the wastewaters were readily biodegradable. Abiotic degradation in the second case was negligible.

A stabilization study was performed with Sample 2. Initial and final values of all measured parameters are presented in Table 3, while DOC and IC changes in the reactor with the sample and in the blank are presented in Figure 2.

Final level of biodegradation of the wastewater in aerobic stabilization study was 65% from DOC measurements, 76% from COD and 100% from $BOD_5$ measurements. The biodegradation was the most intensive during the first 9 days when no lag phase was observed. The degradation rate was 7.48 $day^{-1}$ ($r^2 = 0.960$) for the first 9 days, and the maximal level of degradation (65%), calculated from DOC changes in the diluted sample, was attained on day 26.

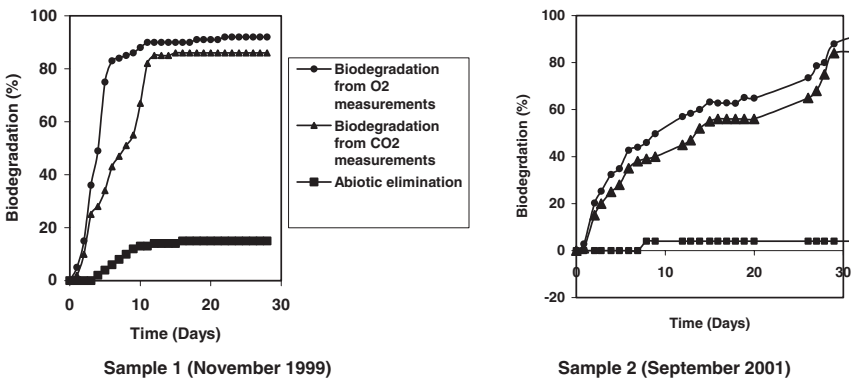


**Figure 1** Biodegradability of raw pharmaceutical wastewaters

**Table 2** Carbon balances during biodegradation of the wastewaters in closed respirometer

| Parameter | Time (days) | Sample 1 (mg/l) | Blank 1 (mg/l) | Sample 2 (mg/l) | Blank 2 (mg/l) |
|---|---|---|---|---|---|
| DOC | t = 0 | 23.67 (± 0.645) | 0.315 (± 0.006) | 27.89 (± 0.712) | 0.556 (± 0.011) |
| | t = 28 | 3.33 (± 0.03) | 0.302 (± 0.005) | 4.865 (± 0.06) | 0.491 (± 0.009) |
| Difference in 28 days | | 20.34 | 0.013 | 23.025 | 0.065 |
| IC | t = 0 | 2.76 (± 0.02) | 0.287 (± 0.004) | 3.09 (± 0.02) | 0.335 (± 0.006) |
| | t = 28 | 3.32 (± 0.04) | 0.456 (± 0.007) | 3.88 (± 0.05) | 0.698 (± 0.009) |
| Difference in 28 days | | 0.56 | 0.169 | 0.79 | 0.363 |
| $CO_2$ production | | 69.11 | 2.22 | 78.54 | 3.22 |

**Table 3** Physico-biochemical and toxicological analysis of diluted pharmaceutical wastewater (Sample 2) at the beginning and at the end of stabilization study (28 days)

| Parameter | Sample 2 (September 2001) | | Blank | |
|---|---|---|---|---|
| Time (days) | t = 0 | t = 28 | t = 0 | t = 28 |
| pH | 7.9 | 8.5 | 8.0 | 8.3 |
| COD (mg/l) | 401 | 96 | 3.5 | 2.9 |
| DOC (mg/l) | 87.75 | 31.03 | < 1 | < 1 |
| IC (mg/l) | 41.25 | 56.51 | 39.44 | 45.34 |
| $BOD_5$ (mg/l) | 111 | < 1 | < 1 | < 1 |
| $NO_2^-$-N (mg/l) | 10.75 | 5.65 | < 0.02 | 0.065 |
| $NO_3^-$-N (mg/l) | 65.25 | 10.5 | 0.111 | 0.647 |
| $PO_4^{3-}$-P (mg/l) | 0.13 | 0.18 | 0.14 | 0.169 |

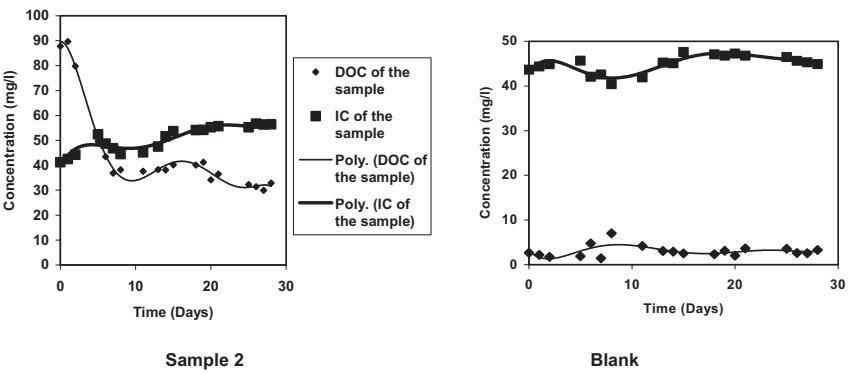


**Figure 2** DOC and IC changes in the sample and in the blank in stabilization study (Sample 2)

Changes of toxicity, based on bioluminescence determination as 30 minEC values, are presented in Figure 3. EC50 was not calculated for t = 0 days and t = 12 days, because the mixture was non-toxic. Temperature was constant during the test (19 ± 2°C), pH remained in the 8.1 ± 0.4 range, oxygen saturation was between 118 and 96%, but it dropped below 30% during the third and fourth day, when biodegradation was in the most intensive phase. Aeration had been intensified with higher mixing speed allowing oxygen to reach 90% of saturation during the next 6 hours.

IC and DOC did not change in the blank significantly. Its concentration in the diluted sample increased, as a consequence of biodegradation (9.64 mg/l). Proceeding of decomposition after the plateau phase (day 20) was also noticed as in the ready biodegradation test. At the beginning of the stabilization study, there was no toxic impact on luminescent bacteria (Figure 3). The toxicity of the mixture increased (EC50 was 37 vol. % on day 9), probably as a result of metabolites formation. Then its toxicity was decreased until day 12, when mixture became non-toxic. Then toxicity started to occur again (EC50 was 78 vol. %

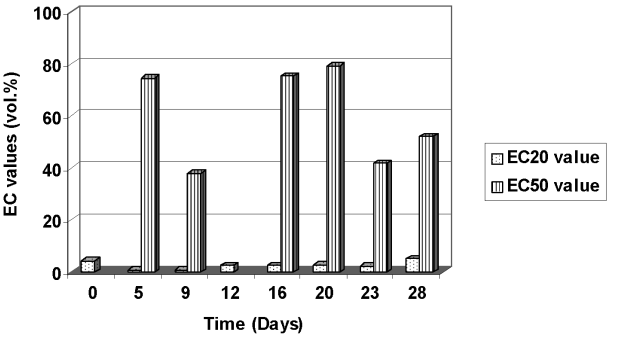


**Figure 3** Changes of toxicity during stabilization study with pharmaceutical wastewater (Sample 2)

on day 16). At the same time biodegradation started again. At the end of the stabilization study the remaining mixture was toxic to luminescent bacteria (EC50 = 52 vol.%). In spite of its biodegradability, wastewater seems to degrade to toxic metabolites, which could be harmful for aquatic organisms. Additional data from the stabilization study proved persistent toxicity of the wastewater, not detected in the common biodegradability test.

## Conclusion

In order to illustrate a method for complex characterization of pharmaceutical wastewater a study on ready biodegradability determination, toxicity studies with microorganisms and aerobic stabilization study has been reported. Good biodegradability (> 86%) has been shown by all measured non-specific summary parameters. However, stabilization study indicated the formation of toxic metabolites during aerobic degradation. Pharmaceutical wastewater consists of persistent toxic compounds or they had been formed during biodegradation, which was proven in the stabilization study with a characterization by toxicity test. It is recommended that specific analysis should be performed to identify problematic compounds to be linked with their source of origin in the pharmaceutical factory.

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