The value of biodegradation screening test results for predicting the elimination of chemicals’ organic carbon in waste water treatment plants

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Abstract

Based on the test results of a large number of chemical substances, the comparability of different biodegradation test methods was assessed in terms of passing or failing threshold values commonly used for the biodegradability characterisation of chemicals. The comparison revealed a high consistency of the ready biodegradability evaluations in OECD 301 tests regardless of the analytical parameter used, i.e., by mineralisation (CO₂ evolution or biological oxygen demand/BOD) or by organic carbon removal measurements. Substances meeting the ready biodegradability threshold values in OECD 301 tests exhibited a high (>80%) carbon removal in model waste water treatment plants (OECD 303A). Results from the Modified Zahn–Wellens test (OECD 302B) representing a screening test with high sludge concentration, were also shown to be useful for the prediction of the organic carbon removal under WWTP conditions provided some prerequisites are met. The analysis of the compared data pairs formed a solid basis for the conclusion that positive results of a chemical in biodegradability screening tests can be used for a reliable prediction of their carbon-based removal in waste water treatment plants.

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1. Introduction

Biodegradability screening tests are a basic tool for the assessment of the environmental fate of chemicals. Standardised screening tests have been used by industry, competent authorities and scientific institutions for many years to obtain a first characterisation of organic compounds in terms of their accessibility to microbial degradation under environmental conditions. The OECD tests for ready biodegradability (OECD 301 series) (OECD, 1993) represent the most prominent group of internationally used biodegradation screening tests and play an important role in the EU environmental classification of chemicals (EEC, 1992) and in the exposure assessment within the environmental risk assessment process (EEC, 2003). Data from a further group of screening test methods represented by the OECD tests for inherent biodegradability (OECD 302 series) (OECD, 1993) may also be taken into account in the
environmental classification and exposure assessment procedures. Such screening tests have a number of common characteristics. For instance, they exhibit a simple test design, being batch tests which contain the test substance as the sole source of organic carbon that may be used for growth by the microbial inoculum added at the start of the test. It is important to note that although they do not simulate a specific environmental situation (e.g., a river or a sewage treatment plant) they do allow a general prediction of the biodegradation behaviour of organic chemicals in the aquatic and terrestrial compartments to be made. Due to the stringent test conditions prevailing in the OECD ready biodegradability tests, it is generally assumed that chemicals meeting the pertinent biodegradation criteria will be rapidly biodegraded in the environment. Conversely, a poor test result is not necessarily proof of their poor biodegradation under more realistic conditions (OECD, 1993). On the other hand, while poor results obtained in an inherent biodegradability test are considered sufficiently indicative for poor biodegradability, positive results are not necessarily predictive of biodegradability under real environmental conditions (OECD, 1993).

Compared to biodegradability screening test data, information about chemical substances based on tests simulating a specific environmental situation like in a sewage treatment plant is scarce. This is mainly due to the considerably higher efforts in time, technical equipment and cost for conducting biodegradation simulation tests like the continuous activated sludge tests (OECD 303A) (OECD, 1993). Results from such waste water treatment plant (WWTP) simulation tests provide an immediate measure of the extent to which a chemical is eliminated from the aqueous phase and, hence, will be precluded from entering the receiving water. Therefore, the prediction of a chemical’s elimination in sewage treatment conditions is an indispensable element of the exposure assessment and, hence, is essential for any risk assessment. If concrete data from a simulation test are not available, the removal of a chemical in a WWTP may be conservatively predicted from the results of biodegradation screening tests and physical-chemical data on volatility and sludge/water partitioning (EEC, 2003). Nevertheless, there is a broad evidence from environmental risk assessments of chemical substances (HERA, 2005) that the predictions of a chemical’s concentration in WWTP effluents based on simulation test data are considerably more realistic than those based on the biodegradation screening test data.

The issue of the predictive value of biodegradation screening tests for the elimination behaviour of chemicals in WWTP gains an additional importance in the context of the exposure assessment of possible recalcitrant metabolites arising from the biodegradation of a chemical. It is generally accepted that readily biodegradable chemicals according to the OECD criteria will be rapidly and ultimately biodegraded in the environment forming inorganic mineralisation products and biomass but no persistent degradation intermediates. For substances not meeting these criteria there might be a possibility that recalcitrant degradation metabolites appear and, hence, further safety assessment issues may have to be addressed. The latter conclusion has been an important driver for the revision of EU detergent legislation. The new Detergent Regulation (EEC, 2004) requires a rapid mineralisation of all surfactants used in detergent products. Surfactants not meeting these criteria will only be allowed in low-volume technical products after they have successfully passed a risk assessment of the possibly recalcitrant metabolites. Of course, such an environmental risk assessment requires an exposure analysis, i.e., an evaluation if and to what extent such recalcitrant metabolites may be formed in the degradation process. In contrast to a standard environmental exposure assessment predicting the environmental concentration of a specific chemical substance, a preliminary metabolites’ exposure assessment will have to be based on an unspecific analytical parameter, such as the organic carbon content (dissolved organic carbon/DOM or total organic carbon/TOC), because the chemical structure is normally unknown. Consequently, the predicted environmental concentration of such metabolites will be strongly dependent on the prediction of the organic carbon removal of the parent chemical under WWTP conditions. This is particularly relevant for a first conservative assessment within a tiered evaluation approach according to the detergent regulation (EEC, 2005), assuming that the parent compound-derived organic carbon passing a WWTP is due to recalcitrant metabolites. Again, suitable carbon removal data of chemical substances from WWTP simulation tests are seldom available, so the question arises whether screening test data can be used for a meaningful prediction of the substance-related carbon removal under WWTP conditions?

This paper reports on an investigation comparing the experimental results from testing a large number of chemical substances in ready biodegradability screening tests, inherent biodegradability tests and WWTP simulation tests. The study investigates the correlation

(i) between test results based on mineralisation (carbon dioxide evolution or biological oxygen consumption/BOD) and carbon-removal (DOC) measurements in ready (ultimate) biodegradability screening tests,

(ii) between carbon removal data obtained in WWTP model tests and data from ready biodegradability and inherent biodegradability tests, respectively.

The study aims to arrive at conclusions if and how reliably test data from ready biodegradability tests or from
a specific inherent biodegradability test can be used for the prediction of the carbon removal extent of specific chemical substances under WWTP simulation conditions.

2. Materials and methods

2.1. Test substances

The experimental results used in this study cover test data for 256 individual organic chemical substances. A significant percentage (ca. 40%) of the substances tested were surfactants of all types, i.e., anionic surfactants (e.g., alkylbenzene sulfonates, alkyl sulfates, alkyl ethersulfates, alkane sulfonates, methyl estersulfonates and alkylethoxy phosphates); non-ionic surfactants (e.g., alcohol ethoxylates, fatty acid ethoxylates, alkylamine ethoxylates, alcohol EO/PO adducts, alkylphenol ethoxylates, alkylamine oxides and alkyl polyglycosides); cationic surfactants (e.g., alkyl trimethylammonium compounds, alkyl benzyl dimethylammonium compounds and esterquats) and amphoteric surfactants (e.g., alkyl betaines and alkylamido betaines). The remaining test substances represent a broad range of chemical substance groups used in the chemical industry.

2.2. Test methods

The test results this study is dealing with are based on internationally used standard test methods as described in the OECD Guidelines for the Testing of Chemicals (OECD, 1993) or on methods closely related to them. The biodegradation test methods used can be subdivided into the following groups:

(i) Ultimate biodegradability screening tests. These tests encompass the OECD tests for ready biodegradability (DOC die-away test/OECD 301A, Modified Sturm test/OECD 301B, Modified MITI test/OECD 301C, Closed Bottle test/OECD 301D, Modified OECD Screening test/OECD 301E and Manometric Respirometry test/OECD 301F) as well as the BODIS test (BOD test for Insoluble Substances, ISO 10708) which is fully in line with the test conditions of the OECD 301 tests (Richterich et al., 1998). Among these tests a differentiation was made between tests which measure the progress of biodegradation via the analytical parameters of mineralisation (carbon dioxide evolution or oxygen consumption) like the tests OECD 301B, 301C, 301D, 301F, BODIS, and those which determine the extent of ultimate biodegradation by DOC removal as the OECD tests 301 A and 301 E. All these tests are batch tests containing a mineral medium, the test substance as the sole carbon source and a low concentration of a bacterial inoculum originating from a municipal WWTP. The test duration is 4 weeks.

(ii) Inherent biodegradability screening tests. Only one of the OECD tests for inherent biodegradability was used in this study, the Modified Zahn–Wellens test/OECD 302B. This is also a batch test containing a mineral medium and the test substance as the sole carbon source. However, the high sludge inoculum originating from a municipal WWTP yields a high bacterial concentration and the measured DOC removal data may also imply adsorption effects of the test substance onto sludge. While the standard test duration is 28 days, also—when available—the results after a 7-day test period were separately taken into account as data from the 7-day Zahn–Wellens test are used for a rough prediction of the elimination extent of organic waste water constituents under WWTP conditions (Germany, 1996). It is important to note that the removal data used in this study are based on DOC determinations 3 h after starting the test (by addition of the test substance) and at test conclusion (after 28-day or 7-day test duration). Hence, the % removal data should not include quick elimination processes such as spontaneous precipitation and fast adsorption, etc.

(iii) Continuous activated sludge (CAS) test. The test method used was the Coupled Units test/OECD 303A. This is a test simulating the biological stage of a WWTP and therefore contains a high bacterial concentration (“activated sludge”). It is continuously fed with a mixture of the test chemical and a high surplus of well biodegradable organic substances (“synthetic waste water”). The resulting hydraulic retention time of 3 h is considerably shorter than the typical minimum for modern WWTPs (9–12 h), so that a rather conservative prediction of a substance elimination can be expected in comparison to the actual situation in practice. The Coupled Units test determines the elimination of a test substance by DOC measurement and, at the same time, provides data of the amount of substance-derived organic carbon present in the effluent of the model WWTP.

2.3. Test data and evaluation criteria

The overwhelming part of the test data originates from biodegradation studies conducted in the laboratories of the Ecology Department of Henkel, Düsseldorf over the past 25 years. The residual data were obtained from the literature and product information sheets of raw material suppliers.
A differentiation of test substances in terms of their biodegradation/elimination results was made applying the following criteria: substances meeting the OECD ready biodegradability pass levels (≥60% CO₂ evolution, ≥60% BOD/COD, ≥70% DOC removal) in one of the ultimate biodegradability screening tests were considered “readily biodegradable” without taking the 10-day time window criterion (OECD, 1993) into account. Substances exhibiting a ≥80% DOC removal in the Zahn–Wellens test were considered highly eliminable under the test pertinent conditions. Similarly, an elimination threshold of 80% DOC removal in the CAS test was used for the differentiation between a high carbon elimination (note that this is different from substance-specific removal) and a lower elimination extent. The application of this 80% threshold for CAS studies run with an hydraulic retention time of 3 h seems justified considering that this corresponds to the average removal range of organic matter (as determined with the analytical summary parameter COD/chemical oxygen demand) measured in a municipal WWTP with a 6-h retention time (Brown et al., 1986).

3. Results and discussion

It is a general feature of screening tests that the interpretation of their results regarding biodegradability are connected to threshold values above which “ready biodegradability” or “inherent biodegradability” can be assumed, while substances not reaching this trigger value are considered to be less biodegradable. Although the definition of such thresholds is not really scientific, it represents a pragmatic differentiation criterion that also allows a comparison of the screening test results of an individual substance obtained in different tests. In addition, conclusions may be possible in terms of the comparability of tests, their stringency and—if compared with simulation test results—their predictive value for waste water treatment situation.

The threshold values employed in this comparison have already been explained (Section 2.3). In the following sections, various types of degradation/elimination screening tests are compared by determining the percentage of congruence. The extent to which results were positive or negative in both of the two test methods or lead to different evaluations was determined (i.e., surpass or fall short of the respective threshold value). This correlation assessment deliberately was not based on statistical evaluation techniques, although a high number of data pairs would have been available for such an analysis. However, it is not the intention of this study to evaluate the correlation of the individual results of all tested chemicals but to assess whether the categorisation of chemicals by fulfilling/failing a certain biodegradability or elimination limit leads to consistent conclusions.

3.1. Comparison of ready biodegradability tests: mineralisation vs. carbon removal tests

Mineralisation tests based on the measurement of CO₂ evolution or oxygen consumption determine the mineralisation of a compound, while DOC removal tests also take into account biomass formation. This difference is reflected in the higher pass level for ready biodegradability in the DOC removal tests. The analytical method employed in the latter test type does actually not allow a differentiation to be made between DOC removal due to ultimate biodegradation or that due to other elimination mechanisms, such as volatilisation, adsorption onto surfaces, etc. Therefore, carbon removal-based ready biodegradability tests are sometimes considered less reliable in terms of ultimate biodegradability assessment than mineralisation tests; nevertheless, suitable controls and the shape of the DOC removal curve often allows a clear distinction between true biodegradation and elimination. As biodegradability determinations in DOC removal tests could perhaps lead to biased conclusions, it seemed particularly interesting to analyse in a comparability study if, and to what extent the two types of ready biodegradability tests differed from each other.

For this purpose, the test data pairs of 79 substances were compared (Table 1). The data pairs of 31 surfactants and 48 other chemical substances exhibited congruence in 74 cases (93.7%) while the mineralisation test results were less stringent in two cases (2.5%) and more stringent in three cases (3.8%). The graph of the distribution of the individual data pairs (Fig. 1) illustrates that the surfactants (including cationics often suspected to preferably provide false-positive degradability results in C-removal tests due to their strong adsorptivity) did not differ from the bulk of chemicals tested.

Altogether, 97.5% of the cases showed an equal or (to a very small extent) more stringent evaluation by the DOC removal tests compared to mineralisation tests. Considering the large data basis for this comparison the conclusion can be drawn that there is no significant difference in the stringency of the test types, i.e., DOC removal-based test results are comparably reliable in terms of ready biodegradability evaluation provided the test substances are water soluble and not volatile.

3.2. Comparison of ready biodegradability test data with C-removal data from WWTP simulation tests

A major objective of this study was to evaluate the value of ready biodegradability test results for predicting the DOC removal of chemicals in WWTPs. Based on the above consistency of mineralisation-based and DOC removal-based results from ready biodegradability tests, these test data were compared with the results from the WWTP simulation tests of 247 substances (including 110
The results of this comparison are shown in Table 1 and reveal that in 88.7% of the cases a congruent evaluation was obtained, i.e., the pass of the ready biodegradability threshold is equivalent to a carbon removal of \( \geq 80\% \). In 7.7% of the remaining cases, the screening test result was below the threshold value while the C-removal in the WWTP model was \( \geq 80\% \). Only in about 3.6% of cases was a false-positive prediction of the carbon elimination under WWTP conditions provided by using ready biodegradability test data. A specific look at the surfactants’ data pairs revealed quite comparable results: in 96.4% of the cases, the ready biodegradability test data delivered a comparable or more stringent evaluation as to the C-removal in the CAS test. The more detailed analysis of this comparison shows (Fig. 2) that the majority of the data considered false positive are

<table>
<thead>
<tr>
<th>Compared data based on</th>
<th>No. of pairs compared</th>
<th>Congruent evaluation in A and B (%)</th>
<th>A more stringent than B (%)</th>
<th>B more stringent than A (%)</th>
<th>A equal or more stringent than B (%)</th>
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<tr>
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<td></td>
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<td>C-removal RBT</td>
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<td>7.7</td>
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<td>12.9</td>
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<tr>
<td>ZW-28-day test</td>
<td>CAS test</td>
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<td>81.5</td>
<td>10.8</td>
<td>7.7</td>
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<td>CAS test</td>
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<td>8.7</td>
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</table>

RBT = ready biodegradability tests; ZW = 7/28-day Zahn–Wellens test.

![Fig. 1. Biodegradation data correlation of individual chemical substances in ready biodegradability tests (RBT) based on organic carbon removal and on mineralisation analytical parameters.](image-url)

The table shows the quantitative analysis of the data pairs of chemical substances compared in biodegradation screening tests and WWTP simulation (CAS) tests. The comparison reveals that in most cases, the results of ready biodegradability tests are congruent with those obtained in WWTP simulations.

The figure illustrates the biodegradation data correlation of individual chemical substances in ready biodegradability tests (RBT) based on organic carbon removal and on mineralisation analytical parameters.
close to the 80%-threshold of C-elimination in the CAS test and, hence, do not lead to considerably erroneous conclusions. These observations are in line with previous findings by Wierich and Gerike (1981) who showed on the basis of theoretical considerations that continuous-flow biodegradability tests like CAS test systems are, beyond a certain base value, true biodegradability tests for all soluble substances and not just tests for elimination. The conclusion that a positive ready biodegradability test result is predictive for a carbon removal of at least 80% in real sewage treatment plants is very conservative considering the findings by Painter and King (1983); they demonstrated that even when fail-safe assumptions were made, a chemical that achieved a pass within 28 days would be removed by >90% during sewage treatment.

3.3. Comparison of inherent biodegradability test data with C-removal data from WWTP simulation tests

Static inherent biodegradability tests, like the Modified Zahn–Wellens test can be considered as screening tests with a high sludge inoculum and, hence, could provide C-removal results influenced by adsorption effects. Given the relatively easy availability of such test data it was considered sensible to evaluate the suitability of such results for the prediction of the C-removal in continuous WWTP simulation test systems. Again, a large data set was available for the comparison of the carbon removal extent in the two test systems. It should be recalled that the Zahn–Wellens test results used in this study did not imply the carbon removal taking place within the first 3 h after the test substance had been added to the test system. Therefore, the C-removals determined in the period after 3 h and the end of the test may largely be attributable to biodegradation and/or adsorption of degradation intermediates.

The comparison of 65 pairs of results from these static and continuous activated sludge test systems revealed (Table 1) that more than 80% of the 28-day Zahn–Wellens test data agree well with the CAS test data. In almost 11%, however, the results from the CAS model were higher than the predictions by the Zahn–Wellens test. Therefore, only in 8% of the cases the Zahn–Wellens test data did not deliver a sufficiently conservative C-removal prognosis for WWTPs. If this comparison is confined to surfactants (23 data pairs) no single false-positive prediction could be identified while 91% showed consistency and 9% were considered false negative. Although the comparison based on surfactants data proved satisfactory, a closer examination of the false positives found among the non-surfactant compounds (individual data not shown here) revealed significant discrepancies between the C-removals measured in the

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Fig. 2. Correlation of biodegradation/elimination data of individual chemical substances determined in screening tests and in WWTP simulation (CAS) tests.
Zahn–Wellens test (>80%) and the CAS test (<40%), respectively.

To find out if the predictive value of the inherent biodegradability test data can still be improved, a corresponding comparison of the Zahn–Wellens test data determined after a 7-day test duration was made. The 7-day Zahn–Wellens test has been broadly used for a rough and easy estimation of the carbon-based substance removal in WWTPs (Germany, 1996). Unfortunately, the data basis for this comparison was too small for obtaining reliable conclusions. Instead, the comparability was tested indirectly by assessing the correspondence of the results from the 7-day Zahn–Wellens test and the ready biodegradability tests. The available 70 data pairs (including 37 surfactants) did not show any false-positive results (Table 1) leading to the conclusion that high C-removals in the 7-day Zahn–Wellens test (excluding the C-removals in the first 3 h of the test) are also indicative for a pass of the ready biodegradability threshold. Taking this observation into account it is not surprising that the comparison of the WWTP model test results with the combined data set from ready biodegradability tests and 7-day Zahn–Wellens tests shows a high degree of conformity: 88% of the 262 data pairs revealed agreement, just 8% the screening test data were more conservative and only 4% could be considered false-positive. As shown in Fig. 2, the few Zahn–Wellens test-based false-positive results were close to the 80% removal threshold. This confirms once again the high predictive value of these data for the WWTP situation and demonstrates a corresponding superiority of the 7-day vis-à-vis the 28-day test results.

4. Conclusions

The comparison of ready biodegradability test results based on mineralisation analytical parameters (BOD or CO₂) or on DOC removal confirmed the equivalence of results from these test types if the different pass levels are taken into account. Results in ready biodegradability tests based on carbon removal and mineralisation parameters are comparably reliable provided the test substances are water soluble and not volatile.

This study also showed clearly that a positive result of a chemical in a ready biodegradability test (i.e., meeting the pass levels) has a very high predictive value for a high (≥80%) carbon removal of this chemical under WWTP simulation conditions. Considering the conservative test conditions prevailing in such CAS studies compared to WWTPs in practice, it can be concluded that such positive ready biodegradability test results can be safely used to predict a carbon removal of at least 80% in real sewage treatment plants.

The large data basis for a comparison of DOC removal data from the Modified Zahn–Wellens test and WWTP simulation test shows that in more than 90% of the cases the prediction by the screening test comes to a comparable or more conservative result. However, the results of the small group of false positives obtained in the Zahn–Wellens test revealed strong deviations from the CAS test results. Therefore, positive results (i.e., ≥80% DOC removal) in the Zahn–Wellens test are highly indicative for a similar removal under CAS test conditions. Nevertheless, a certain possibility for a significant overestimation of the removal extent still remains.

Finally, the application of stricter criteria for the use of Zahn–Wellens test results allowed further improvement of their predictive value. The excellent consistency of the results of the 7-day Zahn–Wellens test and the ready biodegradability tests formed a well-founded basis to conclude that positive results obtained in this Zahn–Wellens test version are comparably reliable as ready biodegradability data for the prediction of C-removal under WWTP conditions.

In summary, many of the available biodegradation/elimination screening test data of chemicals can be immediately used for a sufficiently reliable prediction of their carbon-based removal in sewage treatment plants.

References


