

Characterization of the Biodegradability of Complex Wastes

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Abstract:

A method to characterize the biodegradability of complex wastes such as industrial effluents is presented and illustrated by a case study. The course of biodegradation is followed by non-compound specific analyses and the non-degradable (persistent) fraction is tested for toxic effects and screened for potentially bioaccumulating compounds.

Introduction:

For assessing biodegradability of individual compounds, standard test procedures are available which prescribe the use of summary parameters like dissolved organic carbon (DOC), biochemical oxygen demand (BOD) or chemical oxygen demand (COD) and/or compound-specific analyses to follow the course of biodegradation of the test compound. Examples of methods are the OECD screening test (1) and the Closed Bottle test (1).

If, for instance, 70% of the DOC initially added degrades within 28 days in these tests performed with single compounds, it is concluded that the remaining 30% is also degradable (2). However, with a mixture of chemicals, it is quite conceivable that the undegraded residue contains persistent or non-readily biodegradable compounds. Methods for the determination of the biodegradability of chemical products have traditionally been based on analyses of the active components, like for instance anionic surfactants in detergents. This method, however, is not generally applicable to

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mixtures in which the identity of the compounds is unknown or for which chemical analyses are not available (2).

Combining summary parameters with bioassays (2) and analytical fingerprints (2) has proved to produce better information for judging the degradability of chemical mixtures, e.g. industrial wastes (3,4).

In the method presented here, the biodegradability of industrial wastewater samples is studied using non-compound specific analyses (DOC and group variables like adsorbable organic halides (AOX)) combined with bioassays and TLC-screenings for potentially bio-accumulating compounds. The test systems used were a (1) variant of the modified OECD screening test (1) performed with nutrient enriched natural surface water and (2) an aerobic stabilization test (2) performed with high concentrations of test material.

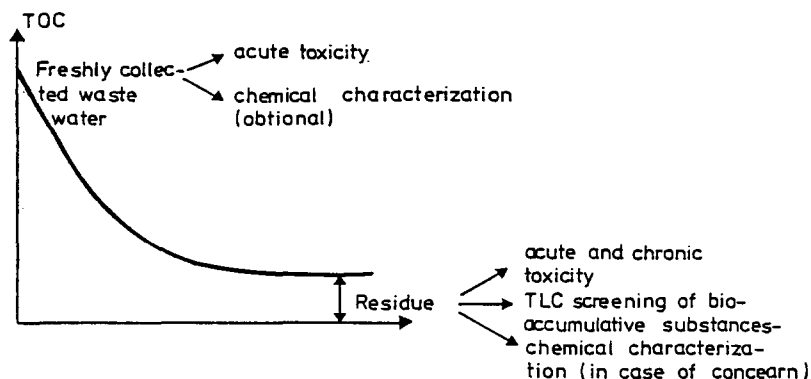


Fig. 1: Principles of the biodegradability of the organic wastes.

Fig. 1 shows the time course of biodegradation in the aerobic stabilization test. As shown, the method combines investigations of the three major parameters which make a chemical compound environmentally harmful, namely: persistency, accumulation tendency and toxicity.

MATERIALS AND METHODS

Sampling

For the case study, 24 hour time proportional samples of waste water from different petrochemical industries located at Stenungssund, Sweden were sampled in polyethylene bottles and frozen (-20°C), (10,11).

The test medium was a sample of seawater from the Kattegat collected 10 cm below the surface in 25 litre polyethylene bottles, which had been thoroughly rinsed in 0.2 N HCL. The samples were stored at the field temperature and used within 24 hours.

BIODEGRADABILITY STUDIES

The screening of the biodegradability of the effluents was performed as shake flask die-away tests (5) at 4°C and 15°C in natural seawater enriched with nutrients. The concentration of test material was 15-25 mg/l DOC (1). The flasks were incubated on a rotary shaker in a temperature-controlled room under diffuse light.

The aerobic stabilization test (3) was performed during 60 days at 15°C with mixtures of waste water and seawater (1:3 vol/vol). The DOC concentrations were 200-300 mg/l. The tests were performed in 25 litre glass bottles, stirred and aerated with GAC (granular activated carbon) rinsed air, in order to maintain an oxygen tension higher than 70% of saturation. Evaporation was minimized by passing the exit gas through a reflux condensor. The extent of biodegradation was determined by DOC and AOX analyses.

The screening tests and the aerobic stabilization tests were performed using a non-toxic concentration of wastewater as estimated in a short term respiration test with activated sludge according to the Danish standard DS 298 (5).

Analyses

Dissolved organic carbon (DOC). Two subsamples of each fifty ml were filtered through 0.2 μm cellulose nitrate filters (Satorius SM), and analysed for TOC on a Dohrmann DC 52 A carbon analyzer as described in (6). Adsorbable organic halides (AOX) were analysed in duplicate on 2 x 50 ml subsamples (preserved with 0.1 ml conc. HNO_3 and 0.1 ml 1M aqueous Na_2SO_3) on a Dohrman DX 20 TOX analyzer according to the proposed ASTM method (7). These parameters were used to follow the course of degradation.

Analyses/tests on the non-biodegradable fraction (the residue) obtained from the aerobic stabilization test included the following:

1) Short-term toxicity test with the marine diatom *Skeletonema costatum*, based on measurement of inhibition of photosynthesis (^{14}C -uptake) (8). The test duration was 6 hours. 2) TLC-screening for potentially bioaccumulating organics performed on hexane extracts of the residue on C18 reverse-phase thin layer chromatography plates (9).

Results and discussion

The results of the screening test on the two waste water samples in seawater at 4°C and 15°C are presented in Fig. 2.

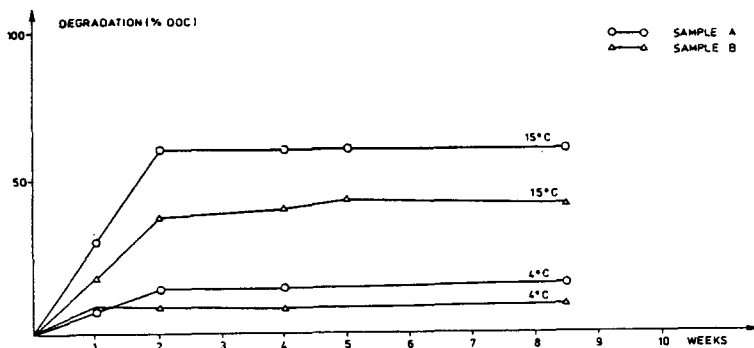


Fig. 2. Time course of biodegradation in the screening test at 4°C and 15°C as followed by DOC.

The studies revealed that approximately 10% of DOC in both petrochemical waste waters were degraded at 4°C, while 30-40% and 50%, respectively, were degraded at 15°C. For characterization of the non-degradable fraction, more concentrated samples were obtained from the aerobic stabilization test as illustrated in Fig. 3..

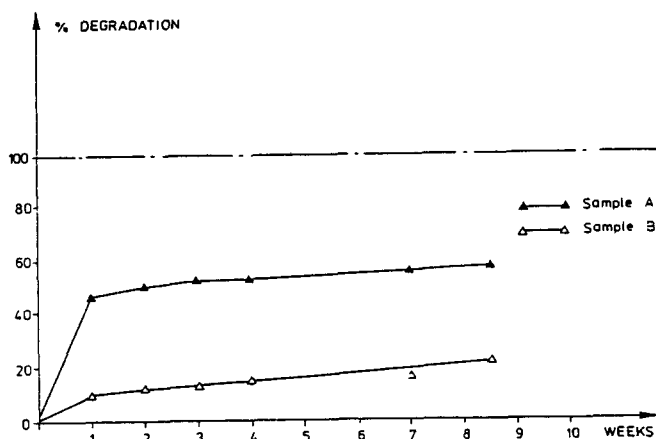


Fig. 3. Biodegradability trend in the aerobic stabilization test at 15°C followed by DOC.

As shown, 40% and 70%, respectively, were not degraded after 60 days, at which time the degradation rate was very slow.

The screening test indicates how large a fraction of the DOC or AOX is readily degradable. In the aerobic stabilization study, the inherently degradable or non-degradable fraction is isolated for further testing and analyses. The highest percentage of degradation obtained in either tests is used for the illustration given in Figure 4 of the overall degradability of the two waste water samples.

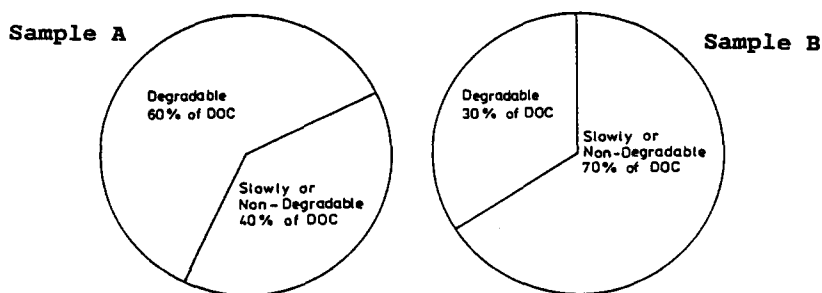


Fig. 4. Evaluation of biodegradability of waste water samples.

Table 1 shows the results of the toxicity test with Skeletonema as performed on freshly collected waste water samples and on the slowly degradable fractions (corrected for dilution), respectively.

TABLE 1

Toxicity of waste water and residues from aerobic stabilization as measured in a photosynthesis inhibition test with Skeletonema costatum. Results given as ml/l undiluted water. EC 10 = Effective Concentration giving 10% inhibition.

SAMPLE	EFFECT LEVELS		
	EC 10 ml/l	EC 50 ml/l	EC 90 ml/l
Petrochemical wastewater A	0,18	0,8	3,6
Residue from Aerobic Stabilization of wastewater A	0,47	4,3	39
Petrochemical wastewater B	28	182	>500
Residue from aerobic stabilization of waste wastewater B	35	>500	>500

Sample A was found to be far more toxic to the diatom than sample B. The toxicities of the residues after stabilization were a factor of 3-5 times less than the toxicities of the freshly collected samples.

The decreased toxicity showed that some of the compounds contributing to the toxicity of the wastewaters were degradable.

The result of the screening of the samples for potentially bioaccumulable compounds is shown in Table 2.

TABLE 2

Log P_{OW} of compounds in the residue obtained from the TLC-screening.

SAMPLE	Log P_{OW}
Petrochemical wastewater sample A	6,65
	4,95
Residue from aerobic stabilization of wastewater A	4,65
	2,59
Petrochemical wastewater sample B	6,65
Residue from aerobic stabilization of wastewater B	3,05

Sample A and B contained substances with a high potential for bioaccumulation as judged from (log P_{OW}). These substances were shown to be degradable or to be transformed into less lipophilic compounds in the aerobic stabilization test. On the other hand, slowly or nondegradable compounds with a high potential of bioaccumulation (log P_{OW} = 4.65) were still present in the residue of sample A, while the compounds in the residue of sample B were less lipophilic (log P_{OW} = 3.05).

In a hazard evaluation of the environmental effects of organic wastes, characterization of the biodegradability may be considered to be sufficient in many cases if the waste consists of a degradable fraction and a slowly or non-degradable fraction which is non-toxic and at the same time free from potentially bioaccumulating compounds in significant amounts.

In this situation, the methods described are cost-effective and at the same time independent of the availability of a specific chemical

analysis for the compounds in the waste - provided, of course, that methods exist for visualization of potentially bioaccumulative compounds on TLC plates. (This can be judged from knowledge of the general classes of compounds likely to be present in the waste water.)

If, as in the case of wastewater sample A, slowly or nondegradable fractions were toxic and contained potentially bioaccumulating compounds, a more thorough chemical analysis would be advisable.

The results of GC/MS screening of the wastewater samples A and B have been reported elsewhere (10).

CONCLUSION

In order to illustrate a method for investigating the biodegradability of complex wastes, a study of the degradability of two petrochemical waste waters has been reported. The method is based upon non-specific summary parameters to follow the course of degradation in combination with a characterization by toxicity tests and TLC screening for bioaccumulative substances.

In mixture A, 45% of the DOC was degraded in screening test for ready biodegradability (shake flask die away test). This fraction of carbon might be considered as readily degradable. Another 15% of DOC was degraded within 60 days in a so-called stabilization study performed with a higher concentration of waste water. The residual 40% of DOC was slowly or non-degradable, and showed a significant toxic effect on the photosynthetic activity of algae at a concentration of 0.47 ml/l. In addition, the residue contained potentially accumulating compounds with $\log P_{ow} = 4.65$.

In sample B, waste water from another petrochemical industry, only 20-30% of the DOC was degraded in the shake flask screening test, and another 10-20% in 60 days in the stabilization test. The residual 60% DOC was slowly or non-degradable, and showed a significant toxic effect on the photosynthetic activity of algae at a concentration of 35 ml/l. The residue contained potentially accumulating compounds with $\log P_{ow} = 3.05$. In a hazard evaluation special emphasis must be put on the slowly or non-degradable compounds, having the propensity to cause long term effects as bioaccumulation or chronic toxic effects. Evaluating only the biodegradability, one might expect sample B to be most hazardous, as only 30% of DOC was degraded. The toxicity tests with algae and the TLC screening for

potentially accumulating compounds show, on the other hand, that sample A should be considered more hazardous.

In conclusion, the non-compound specific analyses in combination with toxicity tests and TLC-screening can be used to screen a mixture of chemicals for environmentally harmful non-degradable compounds. If such compounds are found, it is recommended that specific analyses be performed to identify these compounds.

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