Conference Paper

COMPARISON OF DIETHYLENE GLYCOL AND PHENOL BIODEGRADABILITY BY DIFFERENT TEST METHODS*

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Received March 2003

Biodegradation is generally recognised as an important removal mechanism for pollutants in natural ecosystems. It determines the concentration of substances in the environment. According to the legislation of the European Union, there are three levels for biodegradability testing protocols for chemicals which cover different test conditions. This paper describes one such multilevel approach to biodegradation testing. Four different tests were performed on diethylene glycol and phenol. Diethylene glycol did not fulfil the requirements for ready biodegradable substances, while it degraded well in wastewater treatment plant simulation test. Phenol in turn, resulted ready biodegradable, and its biodegradability depended less on experimental conditions than the biodegradability of diethylene glycol. This corroborates the importance of combining different test methods to obtain relevant biodegradability data.

KEY WORDS: *aerobic biodegradation, aquatic environment, biodegradability testing, biodegradation measurement, multilevel protocol*

Increasing industrialization and demand for chemicals on one hand, and growing environmental awarenes on the other have led to a greater concern about the release of toxic contaminants in the environment. Their impact on natural ecosystems depends on their fate in the environment and their partitioning over environmental compartments (1). Concentrations of chemicals entering an ecosystem change due to different physicochemical (adsorption, volatilisation, accumulation, abiotic elimination such as hydrolysis and photochemical reactions, etc.) and biological processes (biodegradation, bioaccumulation) (2, 3). This is especially true for biodegradation which can be defined as a biologically-catalysed reduction in the complexity of a chemical or as a complete mineralisation. Both are generally recognised as important removal mechanisms for pollutants in natural ecosystems (4). Because the concentration of a substance is affected by the above mentioned processes, it is logical that a knowledge of their transport and degradation pathways is of great interest. This knowledge can help to determine relevant exposure concentrations and make a reliable risk assessment through quality toxicity data aplicable in a real ecosystem. Biodegradability is also an important criterion for ecological characterisation of substances, which is a part of ecological risk assessment.

Biodegradability of chemicals is affected by many factors (4, 5). The most relevant are:

1. *Structure and concentration of the substance*. The structure is important because it determines the solubility and volatility of the substance, which are essential for its bioavailability. It also determines the accessibility of active sites in molecules, which are attacked by enzymes of degrading microorganisms (6). The concentration determines the toxicity of a substance to microorganisms (2).

* Presented at the 1st SloTOX Workshop on Environmental Bioindicators and Refreshment in Basic Toxicology in Ljubljana, Slovenia, 25-26 October 2002

2. *Source and amount of microorganisms*. Various species of microorganisms live in different environmental compartments. Some are able to immediately degrade chemicals, completely or to some extent, while others have to develop appropriate enzymatic mechanisms for degradation (7). Biodegradation could be a result of action of a single species of microorganisms, but more often it occurs due to a combined activity of several microbial species. In addition, a mixed culture usually has a higher biodegradation potential and is the actual carrier of biodegradation processes in the environment (8, 9).
3. *Physicochemical conditions* are important because they determine the behaviour of substances and microorganisms. The amount and the quality of nutrients (phosphorus, nitrogen, etc.) affect the growth of microorganisms (10). The amount of oxygen present, pH, temperature and light affect the performance of microorganisms and the behaviour of the chemical (11).

As Quantitative Structure Activity Relationship (QSAR) models for degradation are far from generally applicable, experimental testing is encouraged (12). There is a number of standardized and non-standardized methods for measuring biodegradability, all of which fall in two main categories: those involving direct measurement of bioconversion of a test compound and those involving indirect measurement such as cumulative oxygen uptake (5). The main differences between the two test categories are in the concentration of the test substance, amount and source of added microorganisms and in the applied techniques to follow biodegradation (13). The common principle of all methods is to expose the chemical to microorganisms in a test medium (inoculum) in the presence of excess oxygen (1, 5). Abiotic elimination (adsorption, hydrolysis, photo-oxidation, etc.) should also be measured to evaluate the contribution of non-biological processes to final mineralization of the substance (14, 15).

According to the legislation of the European Union, the extent of biodegradability testing of chemicals depends on the type of the chemical, produced or marketed quantity, persistency, toxicity, bioaccumulation and other factors (5, 16-18). There are three levels of testing protocols.

Testing begins with zero level tests for ready biodegradability (16), which provide information about the biodegradability of a substance under most common environmental conditions in order to avoid time and money consuming extensive research. Tests are based on the measurement of summary parameters [oxygen consumption, CO_2 production, dissolved organic carbon (DOC) elimination and inorganic carbon (IC) formation]. The test substance should be the only carbon source in the system. Microorganisms must not be adapted. The substance is ready biodegradable if mineralization in a 28 day period of the O_2 uptake test is at least 60 % (19). The time between the beginning of mineralization and of the final plateau (lag period) should be no longer than 10 days (ten-day window). Assuming that degradation kinetics is of the first order, the degradation rate based on the ten-day window should be higher than 0.14/day. Ready biodegradable substances are assumed to degrade rapidly in common environmental conditions, without the formation of toxic and persistent metabolites or by-products (5, 16).

Zero level tests are followed by first level tests, called inherent biodegradability assessment tests (16). They determine the biodegradability of a substance under defined conditions (high inoculum concentration, presence of other organics, possibility of use of adapted microorganisms), but the substance may not biodegrade under environmental conditions. There are two testing methods: batch Zahn-Wellens test (15), where biodegradation is measured by chemical oxygen demand (DOC or COD) elimination, and semi-continuous activated simulation test – SCAS (20), based on the same type of measurements.

The multilevel protocol is completed with simulation tests (second level testing) providing information on substance behaviour in specific, environmental conditions. Some simulation tests are available for the evaluation of substance degradation in aerobic wastewater treatment plants (21). The test simulates two biological wastewater treatment plants running in parallel with certain operating parameters. Biodegradability of substances in surface waters is assessed using a test which (22) simulates biodegradation in the surface waters.

The choice of appropriate test may depend not only on the approach, but also on the purpose of investigation. Test conditions, however, are not comparable to those in natural environment which includes seasonal and daily changes in hydraulic flows, nutrient concentrations, or in physicochemical conditions (2, 3). A systematic procedure to translate the results of a laboratory test to transformation rates under real circumstances is still lacking, and this is the reason why biodegradation of a substance in the

natural environment does not always match laboratory results (19, 23).

Biodegradability assessment of chemicals is integrated in the new EU legislation on water, such as Dangerous Substances Directive (24) or new Integrated Pollution Prevention and Control (IPPC) Directive (25), which came into force in October 1999, leading to more sustainable and environmental friendly production.

This paper describes an approach to multilevel biodegradability testing on diethylene glycol ($C_4H_{10}O_3$) and phenol (C_6H_6O).

MATERIALS AND METHODS

The prepared stock solutions contained 3.0 g/L of diethylene glycol (p. a., Merck, Germany) and 1.0 g/L of phenol (p. a., Kemika, Zagreb). Biodegradability testing was performed on all three levels. All tests also assessed abiotic elimination of test substances using uninoculated and sterilised (100 mg/L $HgCl_2$) solution of the test compound. The reference compound (sodium acetate) confirmed the activity of inoculum and adequate performance of the tests. First we tested both substances for ready biodegradability using two tests from the zero-level of testing strategy. We measured oxygen consumption in a closed respirometer Micro Oxymax (14) and in closed BOD bottles (26). In the closed bottle test, oxygen consumption due to substance biodegradation was measured using an oxygen electrode, while in the closed respirometer, oxygen consumption was measured as a change in pressure. Among ready tests, substances showed the highest biodegradability potential from zero level in closed-respirometer testing, while their potential was the lowest in the second test due to the stagnant batch system with low inoculum concentration (5). The first-level testing included Zahn-Wellens test (15), where substance biodegradation was measured by COD (27). The testing also included blank samples.

The simulation testing was performed in the laboratory wastewater treatment plant using activated sludge. Here too, we used a blank sludge sample in addition to regular ones. The treatment efficiency in the blank sample was above 90 %, indicating good performance. The concentration of activated sludge in both samples was 2-3 g SS/L. The plant had a 8.3 L aeration basin; sludge retention time was 9-11 days and hydraulic retention time was 6-7 hours. The unit was fed with synthetic municipal wastewater which consisted of 130 mg/L peptone, 0.9 mg/L phosphorous as KH_2PO_4, 70 vol. % of distilled water, and 30 vol. % of domestic sewage. The blank was also a source of inoculum for the rest of biodegradability tests. Activated sludge (inoculum) was taken from the aeration basin of the laboratory wastewater treatment plant. Before use, it was washed with tap water several times and settled to increase its concentration. This reduced the input of other organics and inpurities in the test system.

Table 1 summarises the most important parameters of each biodegradability test. Degradation

Table 1 *Parameters of biodegradability assessment tests with diethylene glycol and phenol*

Testing level	Zero		First	Second
Test name	**Closed Bottle Test (CBRT)**	**Respirometric Test (RRT)**	**Zahn-Wellens Test (ZWT)**	**Simulation Test (ST)**
Initial DEG (mg/L)	3.0	100	331	150
Initial Phenol (mg/L)	1.0	100	210	67
Initial Inoculum (mg SS/L)	*	30	410	2500
Measured parameter	O_2 uptake	O_2 uptake	COD	COD
Number of parallels	3	3	2	1
Added nutrients	Yes	Yes	Yes	Yes
Added secondary substrate	No	No	No	Yes
Time of incubation (Days)	25	28	15	12

Legend:

* = *The test was performed with 1 mL of settled effluent from laboratory wastewater treatment plant (blank test) per 1 L of test medium*
COD = *Chemical oxygen demand (mg/L)*
DEG = *Diethylene glycol*
SS = *Suspended solids*

in percentage was calculated as a difference between the changes of the measured parameter in the test system and the blank, and divided by the theoretically expected change for each time interval. Theoretically expected oxygen consumption or COD reduction were calculated from empirical formulas of the test compounds. Abiotic elimination was calculated without considering blank test. Biodegradation curves were plotted as the percentage of biodegradation versus time, and average curves were drawn because standard deviation was below 5 % in all measurements.

RESULTS AND DISCUSSION

In this study we used diethylene glycol and phenol to illustrate multilevel biodegradability testing protocols. Diethylene glycol is a known borderline case of a ready biodegradable substance (16). Its biodegradability depends on the quantity and quality of microorganisms present its environment, as well as on the test method used. Phenol has been determined as ready biodegradable substance.

Diethylene glycol

Figure 1 shows the degradation curves of diethylene glycol obtained in all biodegradability tests. Diethylene glycol did not degrade in the closed bottle test (CBRT), but it degraded completely in the respirometric test (RRT). Its abiotic elimination in RRT reached 2 % after 28 days of exposure. It did not meet all the criteria for the classification as a readily biodegradable substance; it degraded over 60 % in 28 days, but its degradation time exceeded 10 days (the ten-day window criterion) – it was 22 days. Our experiments confirmed earlier data on diethylene glycol as the borderline case of ready biodegradable substance (1). Its biodegradation rate depends on the quality of added inoculum and experimental conditions (28) and it may not meet all the criteria required to be assessed as readily biodegradable.

The difference between the zero level test results (CBRT and RRT) clearly indicated that more than one ready biodegradability test should be performed to verify the obtained results, because zero level tests are the basis for the classification of substances (1). Had only the closed bottle test (CBRT) been performed, diethylene glycol would have been considered not readily biodegradable. In other words, it would have been assumed more hazardous than it actually is. The second zero-level test in the closed respirometer (RRT) showed its biodegradation potential. The finding was validated by the first-level, Zahn-Wellens test used in the multilevel testing strategy, which showed intensive biodegradation of the substance. Diethylene glycol degraded completely in 13 days. Its abiotic elimination was 5 %. The complete removal was also rapidly achieved in the laboratory wastewater treatment plant: 100 % degradation rate was measured after 4 days of unit operation. Diethylene glycol should cause no problem during aerobic treatment in a wastewater treatment plant, where it is to degrade completely.

Phenol

Degradation curves of phenol obtained in all biodegradability tests are shown in Figure 2. Both closed bottle test (CBRT) and respirometric test (RRT) established phenol as readily biodegradable (19). In both tests it degraded more than 60 % in 10 days. No abiotic elimination (1 %) was found in the respirometric test. It is therefore safe to assume that phenol biodegrades completely in natural environment leaving no side-effects. Its

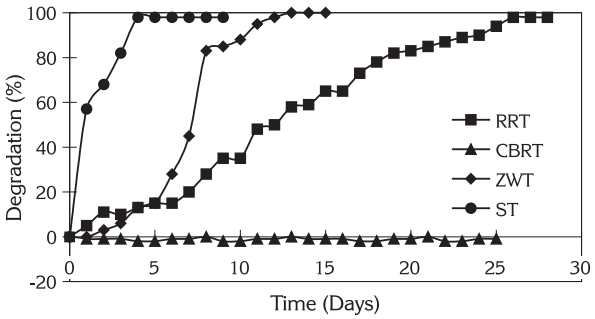

Figure 1 *Degradation curves of diethylene glycol in biodegradability assessment tests: RRT-Respirometric Ready Test; CBRT-Closed Bottle Ready Test; ZWT-Zahn-Wellens Test; and ST-Simulation Test*

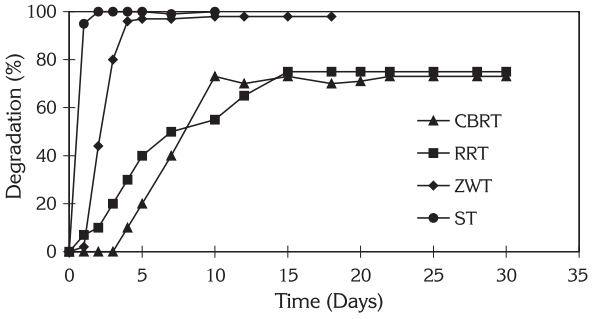

Figure 2 *Degradation curves of phenol in biodegradability assessment tests: RRT-Respirometric Ready Test; CBRT-Closed Bottle Ready Test; ZWT-Zahn-Wellens Test; and ST-Simulation Test*

biodegradability was confirmed by the Zahn-Wellens test, where the lag phase was only 1.2 days and the complete degradation time was two days. Complete degradation (98 %) was achieved on day four. Because we observed a significant abiotic elimination which contributed 60 % to the final COD elimination after 5 days, biodegradation was actually only 38 %. Phenol was completely removed in the simulation test (ST), and the constant removal rate (99 %) was reached in 2 days. Although it was completely removed in a biological wastewater treatment plant, we were unable to distinguish between its biodegradation and abiotic degradation in the test sample. This is one of the disadvantages of activated sludge simulation test (21).

It should be emphasised, that biodegradability depends on the quantity and quality of microorganisms present (1). It should also be emphasised that biodegradation rates depend on the concentration of the tested substances (5). The concentrations of diethylene glycol and phenol in this study were low enough to avoid the toxic impact on degrading microorganisms (28). At higher concentrations, the toxicity of tested substances would have reduced the biodegradation efficiency of microorganisms.

CONCLUSION

The evaluation of toxicity, biodegradability, persistency and physicochemical properties of chemical substances is essential for the assessment of their hazardous impact and risk. A number of standardised tests is available for the evaluation of biodegradability of pure substances. In the European Union, the mandatory testing protocols depend on the produced quantity of chemicals and their special properties. This paper describes the methodology used in biodegradation assessment of diethylene glycol and phenol and the differences between different testing levels.

A systematic procedure to translate the results of a laboratory test to transformation rates under real circumstances is still lacking, and this is the reason why biodegradation of a substance in the natural environment does not always match laboratory results. More work is needed to reliably estimate degradation rate constants of individual substances. The knowledge of transportation pathways in different ecosystems is essential for the application of these constants in real environment. Appropriate information about discharge can help to predict the environmental impact of a particular chemical substance and to propose means of its reduction or elimination.

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Sažetak

USPOREDBA BIODEGRADACIJE DIETILENGLIKOLA I FENOLA RAZLIČITIM METODAMA TESTIRANJA

Biološka se razgradnja općenito prihvaća kao važan mehanizam uklanjanja onečišćivača u prirodnim ekosistemima. Poznavanjem tipa i brzine takve razgradnje može se prosuđivati i koncentracija nekih onečišćivača u prirodi. Prema zakonodavstvu Europske unije procjena testova za biološku razgradnju kemikalija sadržana je u trostupanjskom protokolu uključujući različite uvjete testiranja. U ovom su radu autori prikazali višestupanjski pristup utvrđivanju biološke razgradnje na primjeru dietilenglikola i fenola. Primijenili su respirometrijski test, test u zatvorenoj boci analizom biokemijske potrebe za kisikom, statički test mjerenjem kemijske potrebe za kisikom (Zahn-Wallensov test) i simulirani test metodom polutrajnog aktiviranog taloga proveden prema propisima Međunarodne organizacije za standardizaciju. Dietilenglikol se nije uopće razgradio u testu sa zatvorenom bocom, ali se potpuno razgradio u respirometrijskom testu, i to tek nakon 28 dana. Razgradio se brzo u simuliranom testu s aktiviranim talogom. Stoga se smatra da je dietilenglikol granična kemikalija što se tiče biološke razgradnje i da njegova razgradnja ovisi uvelike o eksperimentalnim uvjetima. Za fenol je, međutim, potvrđeno da je to lako biološki razgradljiva kemikalija i da se može pretpostaviti da će se u prirodnom okolišu potpuno razgraditi, bez štetnih učinaka. Posebno je istaknuta važnost kombiniranja različitih test-metoda da bi se utvrdili stupanj i mogućnost biološke razgradljivosti testiranih onečišćivača.

KLJUČNE RIJEČI: *aerobna biološka razgradnja, mjerenje stupnja razgradnje, testiranje biološke razgradnje, višestupanjski protokol, vodeni okoliš*

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