

Letters to the Editor

Comments on the article

'Optimisation of the Solid-Contact Test with *Arthrobacter globiformis*'

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Recently, a method was published, performed according to a German standard biotest (DIN 38412 L48), promising an optimised modification of the procedure (Neumann-Hensel & Melbye 2006). I criticise the proposed method because the paper takes no notice of the uncertainties of measuring fluorescent dye in soil slurries. Therefore, more knowledge of how the principle of the test system works is more than useful to avoid hidden pitfalls.

The solid-contact test with *Arthrobacter globiformis* was developed in order to characterise the effects of solid-bound pollutants (Rönnpapel et al. 1995). The basic idea is to add bacteria with a high affinity to surfaces and analyse their activity with a dye which is not stored intracellularly, either as substrate or as product. Subsequently, this assumption was confirmed in detail for the dye resazurin (O'Brien et al. 2000). Resazurin is a chemical indicator which has been used in the dairy industry for 50 years to monitor microbial contamination in milk (Thomas et al. 1963). One of the first applications for measuring chemical toxicity stressed the importance of a well-buffered medium, because low pH-conditions reduce resazurin (Thomsom et al. 1986). That is the reason why the solid-contact test is not applicable at a pH lower than 6. Thus, it is no surprise that the authors Neuman-Hensel and Melbye measured low dehydrogenase activities for soils with a low pH. This result highlighted a crucial point for measuring resazurin or resorufin in presence of soil or sediment particles and, it is worth mentioning, possible reactions with solids to be tested.

First, the simple adsorptions of resazurin or resorufin to particle surfaces are influencing factors affecting the test results. For an endpoint measurement of resazurin, the problem was overcome by an extraction of resazurin with amylalcohol, whereas the total amount of unreduced dye could be measured (Liss & Ahlf 1997). The determination of resorufin as fluorescent indicator of microbial activity is very sensitive, but generates an additional source of error. We know that particular soil or sediment components may chemically reduce resazurin to the fluorescent product. An appropriate control without bacteria should estimate the amount of that influence. However, an issue is complicating this process, because resorufin could continually be reduced to dihydroresorufin. This final product is not a fluorescent one and is obviously not detectable.

In general, the fluorimetric measurement of a substance in the presence of a totally unknown composition of components must consider a probable interference with those compounds. A fluorescent dye can lose energy without emitting light during contact with other substances. The result of such a quenching effect is a reduced fluorescent signal. Therefore, a calibration method was developed, tested and described in detail for a solid-contact test with bacteria using varied ratios of resazurin to resorufin as standards (Heise & Ahlf 2005). It is our experience that toxicity assessments of soils or sediment are not reliable without such a calibration of environmental samples if resorufin is used only as a toxicological endpoint.

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Optimisation of the Solid-Contact Test with *Arthrobacter globiformis* [JSS 6 (4) 201–207]

Helga Neumann-Hensel and Kerstin Melbye

Background, Aim and Scope. In this study, a solid contact test with added bacteria (*Arthrobacter globiformis*) was optimised (through miniaturization) for the development of a test kit with conserved bacteria. As in other tests, the results can be influenced by natural soil factors, often masking anthropogenic impacts. For this reason, a further goal of this study was the investigation of the influence of natural soil characteristics on the result of the solid contact test.

Materials and Methods. This method is based on an existing German standard (DIN 38412 L 48) using *Arthrobacter globiformis* for testing whole soils and sediments. The test principle is the measurement of the dehydrogenase activity of the test organism *A. globiformis* after an incubation time of two hours with the solid material. To attain the miniaturization in microplates, dye measurement was changed from spectrophotometrical determination of the substrate resazurine to the fluorimetric measurement of the product resorufin. A second step towards optimisation was the use of freeze-dried bacteria. Freshly spiked and polluted field soils were analysed in order to obtain information about the sensitivity of the test.

Results. It is possible to perform the contact test in microplates. The fluorimetric dye measurement can be carried out in the presence of the solid material, so the work-intensive step of centrifugation and filtration is no longer necessary. The measurement in the optimised contact test is based on the kinetics of the enzyme reaction. The investigation showed that conserved bacteria have the same activity and sensitivity as cultivated bacteria.

Discussion. The study of the uncontaminated soils demonstrated the influence of various soil characteristics on the results of the solid contact test. This information is the basis for the selection of the control and reference soils and is crucial for setting the threshold value in toxicity testing. The investigation of freshly spiked and contaminated soils showed a different sensitivity dependent on the kind of the contamination.

Conclusions. The solid contact test was successfully optimised using microplates, whereas now less than six hours are necessary for the analysis. The optimised test is rapid and sensitive, requiring small samples and no stock culture of the bacteria *A. globiformis* if using freeze-dried bacteria. In this study, the effect of natural soil factors such as pH-value was shown. This information is used to define the threshold value for toxicity. Therefore, the optimised contact test can be used for an efficient assessment of soil or soil substrates. Further studies will clear up if this optimisation is also valid for aquatic sediments and waste.

Recommendations and Perspectives. Due to its short analysis time, the test is suitable for screening different kinds of solid matter and can be used for on-site analysis. – The optimised contact test with freeze-dried bacteria as part of a battery of tests is appropriate for the assessment of contaminated soils, sediments and waste.