Improvement of Bioavailability of Substances with Low Water Solubility in Ready Biodegradability Tests

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Introduction

The criterion for ready biodegradability is often failed or an unequivocal assessment is not possible because the substance is not bioavailable for the degrading bacteria due to its low water solubility. As the biodegradation of a substance strongly depends on its mass transfer and the bioavailability for the degrading bacteria, there are several technical issues which need to be addressed while testing the biodegradability of poorly water soluble substances. Due to the historic background of the guidelines for testing ready biodegradability the test concentration is relatively high (2 – 100 mg/L) and not reflecting environmental concentrations. To improve the bioavailability of poorly water soluble substances in tests for ready biodegradability the application technique and the agitation during testing need therefore special consideration. Careful investigation of the application method prior to the test start can enhance the degradation distinctly. Four techniques for the application of poorly water-soluble substances are described in the ISO standard 10634 (1995) and recommended by the REACH guidance. These methods are direct addition, ultrasonic dispersion, adsorption onto an inert support material and the use of non-biodegradable emulsifying agents. So far no single method is recommended and routinely a combination of approaches is used for application. Based on the substance properties the application method has to be chosen carefully. These investigations involve a relatively small extra effort and often more expensive simulation studies can be avoided. The influence of different application techniques on the bioavailability and biodegradation of poorly water-soluble substances was investigated.

Methods

Chemicals

Tri-alkylester, branched, chain length > C14
Solid (flakes)
Solubility ≈ 1 mg/L

Tri-alkylester, branched, chain length < C14
Liquid
Solubility < 10 mg/L

Branched carboxylic acid (> C20)
Solid (powder)
Insoluble

Testdesign

OECD 301 B

Test concentration: 10 mg C/L

Inoculum: 30 mg activated sludge suspended solids/ L

Volume 3 L

Test duration: 28 days

Up to 3 independent studies for each chemical

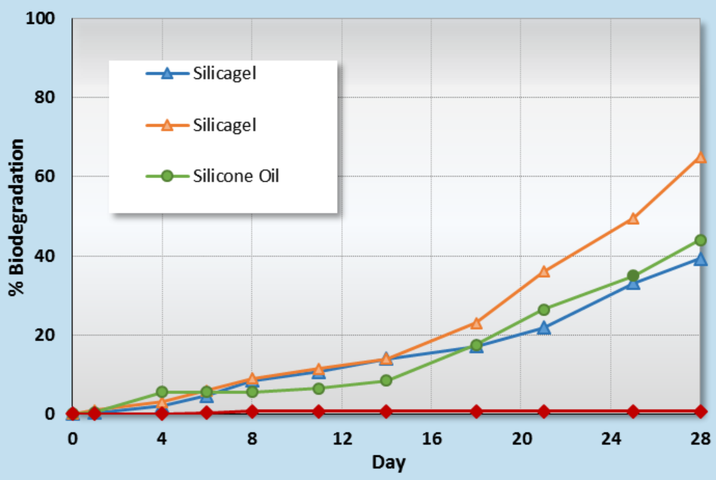
n = 2 – 6 replicates

Pretreatment

1. No pre-treatment, direct addition
2. Inert support: Silicagel
   - Coating of 500 mg Silicagel / L test solution with Chemical
3. Slow release carrier: Biologically inert silicone oil
   - Chemical mixed with 1 mL Silicone Oil / L test solution

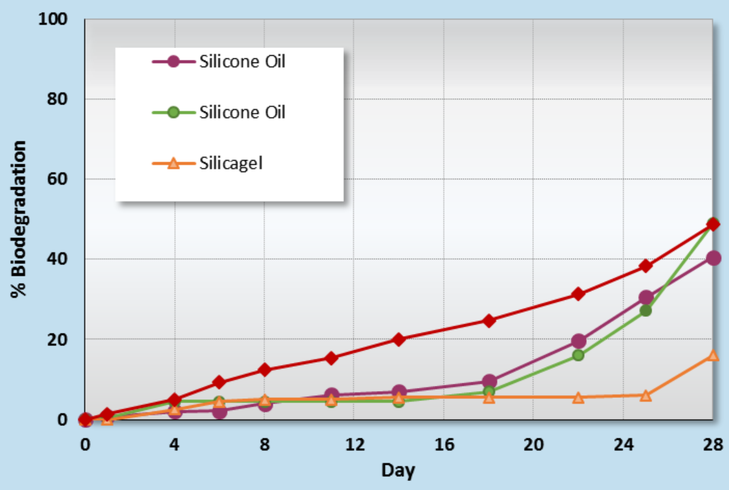
Results

Solid
Tri-alkylester



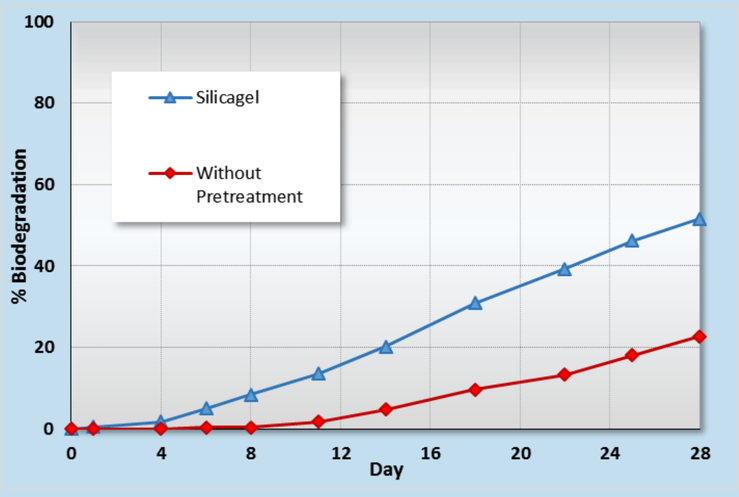
- Without pre-treatment no biodegradation was determined
- Mixture with silicone oil as well as application on silicagel improved biodegradation significantly
- 2nd study with Silicagel indicates that a higher bioavailability can be reached with silicagel as inert carrier, but possible variations due fluctuating inoculum activity cannot be excluded

Liquid
Tri-alkylester



- Biodegradation after 28 days without pretreatment and with silicone oil treatment were in the same range (41 – 49 %), but the shape of the degradation curves differs
- Without pre-treatment biodegradation started within 4 days after application and progressed steadily
- Silicone oil treatment: biodegradation started only after an adaptation phase of 18 days, but afterwards the biodegradation increased fast

Solid
Branched Carboxylic Acid



- Without pre-treatment biodegradation started after an adaptation phase of 18 days and reached 23 % after 28 days
- Coating on Silicagel improved biodegradation significantly:
  The adaptation phase decreased, biodegradation started already after 10 days and within 28 days 52 % biodegradation were reached

All single curves represent mean values of 2- 6 replicates

Conclusions

Solid Substances

Coating on silica gel and mixture silicone oil can significantly improve biodegradation compared to direct addition

→ Best application method must be chosen based on substance properties

Liquid Substances

Mixture with silicone oil can influence shape of biodegradation degradation curve compared to direct addition

→ Addition of silicone oil might improve compliance to the 10-day-window

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