Investigation of Alternative Test Designs for Testing the Biodegradability in Seawater

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Introduction

Within persistency assessment biodegradation in seawater is a topic of increasing interest. The established TG OECD 306 offers two alternative test designs for investigating the biodegradability in seawater, each with its own advantages and limitations. The shake flask method has the inherent disadvantage of only allowing to determine the elimination of a substance by measuring the DOC. This renders this method unsuitable for adsorbing, insoluble or volatile substances. The alternative closed bottle method allows to determine the biodegradation by measuring oxygen consumption. Nevertheless, the limited amount of dissolved oxygen within the bottle makes it prone to missing the validity criteria in case of higher background activity, especially when nitrifying bacteria are present.
Therefore, substances with certain characteristics can only be subjected to the test acc. to OECD 306 with restricted bioavailability or not at all. This might raise high costs when substances for that reason have to be tested in expensive simulations tests. Looking for alternatives to overcome some of the shortcomings of the TG OECD 306, the TG OECD 301 for ready biodegradation offers a number of test designs, of which the OECD 301 A/D and E, respectively, in principle resemble the methods from TG OECD 306. The method OECD 301F (Manometric Respirometry) allows to measure biodegradation by determination of the oxygen consumption. Due to the headspace, oxygen is not as limited as in the closed bottle method. This makes this setup less sensitive for higher background activities. Since the test procedure is less circumstantial in the respirometry method, this gives more room for pre-treatment and bioavailability improvement for difficult substances. The continuous measurements allow for a more precise description of the biodegradation curve without increasing the number of replicates. Data on biodegradation of the reference item sodium benzoate in the manometric respirometry method with seawater are shown in comparison to the original TG OECD 306 closed bottle method. Additionally, data on biodegradation of the reference item sodium benzoate in the CO_2 evolution test method (OECD 301B) with seawater are shown.

Methods

Characteristics of the Seawater

Sampling site	Biologische Anstalt Helgoland
Colony forming units	5.3×10^5 CFU/L
Weather at sampling	1.8°C, wind 5 km/h, no precipitation
Depth of sampling	Pipeline to mole, depth depending on tide
Appearance	Clear
Salinity	32.8 ‰
DOC	4.15 mg/L
pH value	7.97
NH_4^+ concentration	<0.04 mg/L
Nitrate concentration	<0.3 mg/L

Test design: Manometric Respirometry following OECD 301F

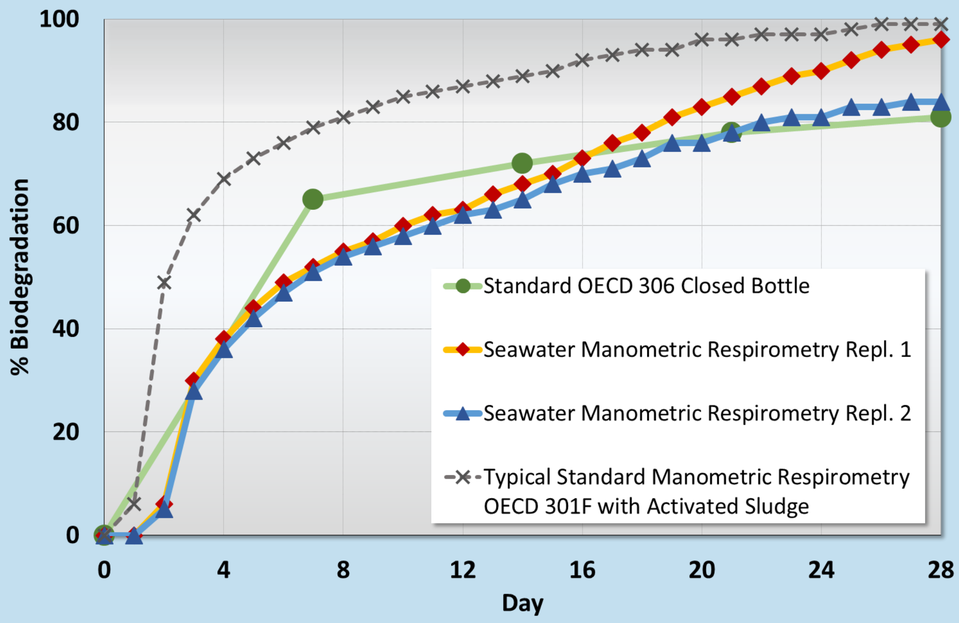
- Test concentration: Na benzoate 45 mg/L
- Volume 0.25 L
- Inoculum: Sea water and mineral salts acc. to OECD 306
- WTW OxiTop™ System
- Test duration: 28 days

Test design: CO_2 Evolution Test following OECD 301B

- Test concentration: Na benzoate 45 mg/L
- Volume 3 L
- Inoculum: Sea water and mineral salts acc. to OECD 306
- Trapping of evolved CO_2 by $Ba(OH)_2$ and titration with HCl
- Test duration: 14 days (aborted)

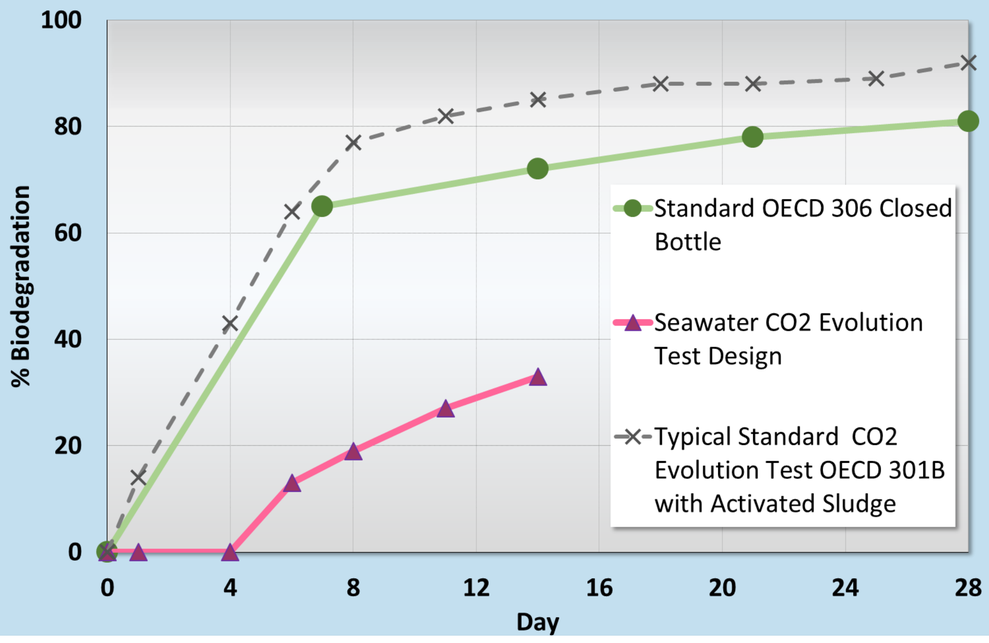
Results

Manometric Respirometry following OECD 301F



- The seawater Manometric Respirometry test design resembles the curve of the standard OECD 306 closed bottle test very well.
- The lag phase of the seawater Manometric Respirometry test design is a little longer than the lag phase of the Manometric Respirometry test with activated sludge and showed in this case a generally slower biodegradation, but reaching identical end points.
- The validity criteria defined in the TG OECD 301F for Manometric Respirometry were met.

CO_2 Evolution Test following OECD 301B



- The seawater CO_2 evolution test showed a long lag phase of 4 days that is usually not observed neither in the standard OECD 306 closed bottle test nor in the standard OECD 301B CO_2 evolution test with activated sludge. It can be assumed that the buffer capacity of the seawater prevents formed CO_2 from being released into the gas phase.
- The observed biodegradation of the seawater CO_2 evolution test did not meet the validity criteria defined in the TG OECD 301B (only 33% biodegradation were observed after 14 days).

Conclusions

The **Seawater Manometric Respirometry Test Design** is well suited for conducting biodegradation studies in seawater.

The pass level for the reference item may be needed to be adapted to this test design according to the criteria defined in the TG OECD 301F.

The **Seawater CO_2 Evolution Test Design** following the standard OECD 301B test design with trapping evolved CO_2 in $Ba(OH)_2$ solution is **NOT** suited for conducting biodegradation studies in seawater.

The buffer capacity of the sea water probably leads to an under-estimation of the actual biodegradation. The evolved CO_2 must be measured directly to solve this problem.

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