Assessing the Applicability of the OECD 301B Ready Biodegradability Test Method for Volatile Compounds

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The OECD 301 Series of Ready Biodegradability Test Methods A through F plus the OECD 310 Headspace Method all have certain benefits and detractions for their selection as a test method to evaluate chemicals for 'ready biodegradability'. Each test method prescribes a certain test set-up which are applicable to certain chemical properties such as water solubility, adsorptivity and volatility. The OECD 301B 'CO_2 Evolution Test' is often recommended to determine the 'ready biodegradability' of a chemical due to its direct measurement of CO_2 and its relatively large testing volumes along with the maximum amount of inoculum allowed within the OECD 301 Series that often makes it an ideal test. The OECD 301B test is recommended for water soluble, water insoluble and adsorbing chemicals. It is not recommended though for volatile compounds.

Although this guideline is not recommended for volatile compounds since it is a flow-through system, it does have a closed bottle prior to entering the trapping solutions and given its benefits, this presentation assesses the applicability of the OECD 301B Test Method with compounds that are volatile.

The chemical compounds acetone, methanol and hexane representing the ketone, alcohol and alkane chemical groups were chosen for this experiment, conducted according to the OECD 301B guideline while aerating at a slower rate of ~20 cc/minute. The results showed that after 28 days acetone was 'readily biodegradable' and hexane was not. Methanol also appears to be 'readily biodegradable' although the 10-day window was not well defined. This experiment demonstrates that for certain volatile compounds, the OECD 301B test method may be a viable option for 'ready biodegradability' testing.

Materials and Methods

- Exposures conducted generally following the OECD 301B guideline except a lower aeration rate <30 mL/minute was used (i.e., ~20 cc/minute.
- Total inorganic carbon (i.e., CO_2) was measured using a Shimadzu TOC Instrument in the IC mode.

Results

- After an apparent lag time of approximately 2 days, CO_2 evolution occurred rapidly from the Acetone Test Solution with over 60% mineralization occurring by day 7 and over 90% mineralization occurring by the end of the study (day 28).
- The Methanol Test Solution also started showing CO_2 evolution after a few days but was slower than Acetone with an earlier plateau in the curve. Nevertheless, Methanol reached 60% mineralization by day 14 and over 85% by the end of the study.
- The Hexane Test Solution showed no significant mineralization.
- The non-volatile reference compound confirmed the suitability of the inoculum and test design while reaching over 60% mineralization by day 10.

Table 1: Time to 60% biodegradation of Acetone, Methanol, Hexane and Cellulose.

Biodegradation Summary			
Test Substance	Days to 60% CO_2 evolution	% Mineralization at Day 28	Readily Biodegradable
Acetone (249 mm Hg)	7	96.6%	Yes
Methanol (119 mm Hg)	14	87.2%	Yes
Hexane (150 mm Hg)	-	0.0%	No
Cellulose (non-volatile)	10	103%	Yes

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The OECD 301B CO_2 evolution ready biodegradation test has been shown to be successful in showing 'ready biodegradability' with certain volatile, water-soluble compounds with vapor pressures up to 250 mm Hg.

Contact Sean McLaughlin at infoERS@smithers.com for more information.

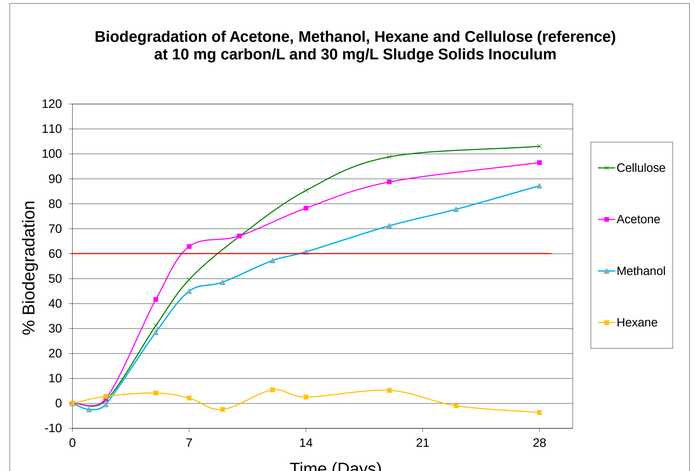


Figure 1: Biodegradation of Acetone, Methanol and Hexane over the standard 28-day time period. Acetone and Methanol were 'readily biodegradable' and hexane was not. Cellulose was used as the reference compound.

Conclusions

The OECD 301B CO_2 evolution ready biodegradation test has been shown to be successful with certain volatile compounds with vapor pressures up to 250 mm Hg (measured at 25 ºC).

- Acetone and Methanol were both shown to undergo significant mineralization to CO_2 during this experiment, concluding that they are both 'ready biodegradable' although the 10-day window for methanol (day ~3-4 to ~13-14) was not well defined. The large extent of biodegradation also indicates that Acetone and Methanol were not lost from the test system.
- Hexane was not shown to be biodegradable in this study. However, since hexane is known to biodegrade under difference circumstances, in this experiment the Hexane may have volatilized before it had a chance to start to biodegrade, especially since it's water solubility is low and density allows it to float and contact the headspace air. Therefore, this is an important consideration if considering the OECD 301B as an option to test volatile compounds.