Biodegradability Which Test Is Best?

Many different test methods can be used to determine the biodegradability of organic substances such as lubricants. Typically, a biodegradation rate of 60 percent after 21 or 28 days is enough for a substance to be considered biodegradable.

However, according to research at Germany's Hermann Bantleon GmbH, this interpretation may apply to a sample containing only one kind of molecule. But a mixture of molecules, such as a lubricant, could contain components that are not biodegradable at all and still return a biodegradation rate of 60 percent.

In a presentation at the 19th Colloquium Tribology at the Technische Akademie Esslingen in January, Ben Müller-Zermini, laboratory head and head of product development, compared the precision of different biodegradation test methods and how accurate they are for lubricants.

BY RICHARD BEERCHECK

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Primary vs. Complete

Müller-Zermini explained, "The degradation process consists of a chain of many very complex biochemical reactions that eventually produce carbon dioxide and water." He said that all biodegradability tests are based on one of two principles: primary degradation and complete degradation (also known as mineralization).

There are different definitions of the term primary degradation, he said. "Usually, it is defined as the first step of a degradation process. In the detergent industry, for example, detergents are primary degraded when they have lost their surface activity."

Another definition of primary degradation is the measurement of sample concentration after some specified time period. "A primary degradation test determines whether the original sample molecules have or have not degraded," Müller-Zermini said. "Therefore, the test uses an analytical method that detects only the sample molecules."

He noted that CEC-L-103-12 and CEC-L-33-A-93 are often classified as primary degradation tests, even though they do not precisely fit the definition of primary degradation. "The CEC tests measure both the sample molecules that are not degraded and the nonvolatile, oil-soluble intermediates produced during the test," he said. They are based on analytical methods that detect all organic compounds with carbon-hydrogen bonds.

Mineralization methods are often called complete degradation tests because the molecules of the sample are broken down to carbon dioxide and water as the final byproducts. "Carbon dioxide is an inorganic reaction product; [therefore], measuring its [production] allows the percentage of mineralization of an organic substance to be determined," said Müller-Zermini.

Sometimes, methods that measure oxygen consumption are also called mineralization

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>
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methods. However, they do not measure degradation directly, he said.

Another term used in describing biodegradability tests is ultimate biodegradation, which is defined in ISO 9439 as "the breakdown of a chemical compound or organic matter in the presence of oxygen to carbon dioxide, water and mineral salts…and the production of new biomass." Müller-Zermini said, "The term complete degradation is sometimes used in place of ultimate biodegradation, but it can be misleading because complete degradation means different things when the test sample is a mixture and when it is a pure substance."

Testing Mixtures

According to Müller-Zermini, "The lubrication industry often uses ultimate biodegradation tests like OECD 301 B to test formulated hydraulic fluids. These fluids are mixtures of structurally different substances; namely, base oils and additives." If the OECD test gives a result of 60 percent or more, the product is deemed completely biodegradable. "But is this correct?" he asked.

For example, in a mixture of 70 percent paraffinic and 30 percent aromatic hydrocarbons, the biodegradability of medium- and short-chain paraffinic hydrocarbons is very good. "Potentially, 91 percent of these substances are degraded within 28 days in the OECD 301 B

test," Müller-Zermini said. On the other hand, aromatic hydrocarbons biodegrade very slowly, and only about 3 percent degrades during the 28-day test.

"However, OECD 301 B only measures carbon dioxide evolution and provides no information about the origin of the [gas]," he continued. "And this mixture can achieve 65 percent biodegradability, which would be interpreted as biodegradable, although it contains components that are not at all biodegradable."

The example shows that when measuring mixtures, OECD 301 B does not indicate complete degradation, he said. This phenomenon is called sequential degradation and is taken into consideration in the revised introduction to the OECD guidelines for testing chemicals. Section 3, Point 44, of the guidelines states, "Tests for ready biodegradability are not generally applicable for complex mixtures containing different types of chemicals."

Testing Pure Substances

Biodegradation tests measure the carbon dioxide produced by the test substance as it is degraded by microorganisms. "However, organic molecules may be very complex and can contain different kinds of carbon [bonds] that react in different ways," said Müller-Zermini. "It is possible that some of the carbon atoms of one molecule are broken down easier by microorganisms than the atoms in others." This means one segment of a molecule may biodegrade but another segment may not.

As an example, Müller-Zermini described a molecule consisting of an aromatic part containing 27 carbon atoms and a polyglycol part containing 14 carbon atoms. "Degradation happens in a series of steps," he said. "First, the polyglycol part is degraded quickly and easily by the microorganisms [to generate] carbon dioxide." On the other hand, the aromatic part of the molecule is very stable, and most microorganisms cannot degrade it.

"However, when the molecule is evaluated in the OECD 301 B test, it may be classified as biodegradable," Müller-Zermini said. "This is because it consists of 41 carbon atoms, 27 in the easily biodegradable polyglycol part and only 14 in the aromatic part. When carbon dioxide production is measured, a biodegradation rate of 66 percent – 27 divided by 41 – could be measured.

"Even a pure substance could form persistent degradation products although the biodegradation rate according to OECD 301 B could

exceed 60 percent," Müller-Zermini said. As a result, biodegradation tests that measure ultimate biodegradability may not apply to compounds that produce intermediates that cannot be broken down within the specified test period.

Biodegradation Rate

Degradation rate is determined by measuring the change in concentration of the original substances and end products during the test. "In lubricant testing, the concentration of the remaining sample [molecule] and its nonvolatile degradation products are commonly measured in the CEC-L-103-12 or CEC-L-33-A-93 test," Müller-Zermini said.

"Every test requires special equipment," Müller-Zermini noted. "CEC-L-103-12 uses open flasks on a shaker, and the laboratory analyzes the concentration of organic substance before and after a 21-day incubation period."

Other test methods such as OECD 301 C or DIN EN ISO 9408 measure the amount of oxygen consumed. Finally, some methods measure the amount of carbon dioxide produced (OECD 301 B or DIN EN ISO 9439).

Carbon dioxide is measured by collecting it in a bottle filled with barium hydroxide solution, which acts as a trap for the gas. The amount of trapped carbon dioxide is then measured by titration.

If gases are analyzed, the test flask must be airtight. Oxygen consumption can be measured by using a closed bottle or a respirometer, a device that measures the respiration of organisms as they exchange carbon dioxide and oxygen as part of their metabolism.

"With the closed bottle, oxygen concentration in the water is measured," said Müller-Zermini. "With a respirometer, a sensor measures the pressure drop caused by the [microorganism's] consumption of oxygen."

Müller-Zermini said, "The process of biodegradation is very complex, involving many reaction steps and, therefore, many intermediates. The biodegradation of a hydrocarbon involves more than one starting product, as well as many intermediates, making the measurement of degradation rate very [difficult]." Therefore, the reliability of a particular test depends on how well it measures the degradation of each component in a sample.

Reproducibility an Issue

A key factor in rating biodegradability tests is how precise they are. Unfortunately, compared to measurements usually made in engineering, the precision of biological test methods is very poor.

"The precision of test methods is determined in round robin tests, where several laboratories measure the same sample and compare results," Müller-Zermini said. The results of a round robin test measure repeatability and reproducibility, where repeatability applies to a single laboratory while reproducibility compares the results from several laboratories.

Table 1 lists the relative reproducibility of a selection of lab tests typically performed on lubricants. It shows that some methods are very precise, like the measurement of density, refractive index or viscosity. Others are not very precise such as the four ball test or pour point.

Müller-Zermini said, "Most descriptions of biodegradation test methods do not have any data about reproducibility. The only data available can be found in the descriptions of CEC-L-103-12, DIN EN ISO 14593 and ASTM D 5864. And reproducibility data for OECD 301 was published after a round robin test in 1988." Table 2 shows the relative reproducibility of some biodegradation test methods.

Compared to typical lubrication tests, the reproducibility of biodegradation tests is very poor with the exception of OECD 301 A and CEC-L103-12. "It is notable that

Table 1 - Reproducibility of Lubrication Lab Tests

Test Method	Standard	Reproducibility (%)
Density	ASTM D7042	0.2
Refractive Index	DIN 51423-1	0.4
Viscosity	ASTM D7042	0.6
pH	DIN 51369	9
Saponification Number	DIN 51559-1	10
Flash Point (open cup)	DIN EN ISO 2719	11
Simulated Distillation	DIN EN 15199-1	12
Acid Number	DIN 51558-1	15
X-Ray Fluorescence	DIN 51396-2	30
Four Ball Test	DIN 51350-2	30
Pour Point	DIN ISO 3016	33

Source: Hermann Bantleon GmbH

Table 2 - Reproducibility of Biodegradation Tests

Standard	Test Substance	Reproducibility (%)	Measurement Method
OECD 301 A	Water Soluble	23	Sample decrease
CEC-L-103 -12	Poorly Water Soluble	24	Sample decrease
DIN EN ISO 14593	Water Soluble	48-67	Respirometer
OECD 301 B	Water Soluble	67	Respirometer
ASTM D 5864	Poorly Water Soluble	70	Respirometer
OECD 301 F	Water Soluble	117	Respirometer
OECD 301 D	Water Soluble	147	Respirometer

Source: Hermann Bantleon GmbH

these two methods are not respirometric methods," said Müller-Zermini. "Rather, both methods measure the decrease of the test sample and its nonvolatile intermediates."

He continued, "The precision of respirometric test methods is much poorer. One explanation could be the complexity of the methods, which introduces more variability into the measurements."

In conclusion, Müller-Zermini said, "Lubricants are mixtures of poorly water soluble substances. Unfortunately, it is not possible to use OECD 301 A to test [such] substances. CEC-L-103-12 is the only method for testing the biodegradability of lubricants that has reproducibility similar to that of the usual methods used in a lubricants laboratory." □