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Beyond the guidelines: rethinking OECD biodegradability testing for polymers in liquid formulations

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The environmental persistence of polymers in liquid formulations (PLFs) can present a significant challenge for sustainability, particularly given their widespread use and limited post-use recovery. Testing methodologies to evaluate and understand the end-of-life fate of PLFs are therefore essential. This perspective critically evaluates the existing biodegradability testing methods, with a focus on protocols from the Organisation for Economic Co-operation and Development (OECD) which are widely adopted as the gold standard. These methods were originally designed for simple, low molecular weight substances, and we explore their limitations when applied to complex PLFs, such as solubility contraints, structural complexities and alignment of test conditions with realistic environmental fates. The paper explores scientific and regulatory innovations aimed at addressing these limitations, including enhanced microbial characterisation, alternative degradation endpoints, improved simulation environments, and high-throughput screening tools. It also highlights Croda's contributions to advancing biodegradability testing through collaborative research and accelerated testing methodologies. By identifying knowledge gaps and proposing targeted improvements, this work supports the development of more robust, representative, and sustainable testing strategies for modern polymer systems.

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Sustainability spotlight

Chemical persistence is one of the key measures for understanding the environmental harm of a substance.[1] This perspective piece aims to review the current testing framework which is used to infer biodegradability, and appraise their advantages and limitations. The aim is to ensure accurate, robust and reliable test methodologies are in place, facilitating the adoption of more sustainable polymers in liquid formulations (PLFs). Sustainable PLF development is aligned with the UN's sustainability development goals, namely SDG 2 (zero hunger), SDG 6 (clean water and sanitation), SDG 9 (industry innovation and infrastructure), SDG 12 (responsible consumption and production), SDG 13 (climate action), SDG 14 (life below water) and SDG 15 (life on land).[2,3]

1. Introduction

The demand for environmentally sustainable products continues to grow as consumers become increasingly aware of the origins of ingredients, greenhouse gas emissions and also the end-of-life fate of the products they use. In response, both the private sector and regulatory bodies have established targets aimed at reducing and, where possible, eliminating the negative environmental impacts of chemical ingredients.[4]

This transition towards sustainable materials has placed renewable and biodegradable polymers at the forefront of research and industrial innovation. These materials aim to address two critical challenges: reducing dependence on finite fossil resources and minimizing the accumulation of persistent plastics. Global plastic production exceeded 430 million tonnes in 2022, with nearly 80% going to waste, underscoring the urgency for alternatives that fit within a circular economy framework.[5,6]

Renewable polymers are derived from biomass or other sustainable feedstocks such as cellulose, lignin, proteins, and plant oils. Their production leverages carbon already present in the environment, offering a lower carbon footprint compared to petroleum-based polymers. Recent advances have enabled the development of bio-based monomers and polymers with properties comparable to conventional plastics. Examples include polylactic acid, polyhydroxyalkanoates, and bio-based polyesters, which have found applications in packaging, textiles, coatings, and biomedical devices.[7–10]

Biodegradable polymers are species which break down in the presence of bacterial species under environmental conditions into smaller species, eventually mineralising into water, carbon dioxide, and biomass. Biodegradation is particularly important for single-use polymers or products that are difficult to collect

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and recycle. While many degradable polymers are also renewable, degradability can be engineered into fossil-derived polymers through chemical design such as in polycaprolactone (PCL) and oxo-degradable polyolefins.[11,12]

Despite these advances, significant challenges remain. The performance requirements of modern applications often demand durability, which can conflict with degradability. Furthermore, degradation rates vary widely depending on environmental conditions, making real-world behaviour difficult to predict. This complexity underscores the need for robust and standardized methods to evaluate polymer degradability and environmental persistence.

The Organisation for Economic Cooperation and Development (OECD) has provided guidance on assessing the biodegradability of chemicals for over 30 years, in particular with their 301 series of tests.[13] These tests were developed to assess the chemical fate of well-defined, low molecular weight substances by evaluating their biodegradability in an aerobic, aqueous environment. Additionally, the wider OECD 300 series contains tests that focus on non-aerobic and non-aqueous conditions.[14–18] Since the development of these tests, however, the landscape of commonly used chemical products has shifted to include more complex substances than those that the tests were designed to evaluate.[1]

Polymers in liquid formulations (PLFs) represent one such classification of chemical. PLFs are a diverse set of chemicals, consisting of hundreds of different polymers which can be used in agrochemicals, adhesives, coatings, cosmetics, household cleaning products, inks, lubricants, sealants, paints, personal care products and water treatment chemicals.[3,19] As of 2021, the PLF market generated $125 billion in annual sales, supporting industries that collectively produced $1.27 trillion in revenue.[3,19] Each year, more than 36.3 million metric tonnes of PLFs are produced, and they are essential for product performance.[3] Despite their significant economic value, PLFs often go unnoticed by consumers, and due to their form, they are very hard to recover post-use, making their environmental fate even more important.

Given their frequent use and large contribution to common consumer products, it is essential to have reliable, robust and accurate biodegradation tests to evaluate the environmental impact and persistence of this class of chemicals.

Recent literature has highlighted limitations of the traditional OECD tests, citing overly stringent criteria, high levels of inter-test variation, environmentally unrealistic conditions, and a lack of applicability to chemicals that differ from standard reference material (mixtures, poorly soluble materials *etc.*).[20–23]

This perspective piece aims to critically assess the advantages and limitations of OECD testing by drawing on the conclusions of recent research and technical guidance. The need for more flexible, environmentally realistic degradation tests will be explored, especially in the context of complex chemicals such as PLFs. By identifying key knowledge gaps, exploring solutions, and contributing to the ongoing discussion, this perspective piece aims to highlight opportunities to help strengthen and improve methodologies related to biodegradation testing to better reflect today's chemical landscape.

2. The OECD testing guidelines

The OECD "Guidelines for the Testing of Chemicals" are internationally recognised methods that are used for evaluating the environmental fate of chemical substances. Adopted in 1992, the 300 series focuses on biodegradability, with the 301 and 302 series specifically offering tests that are designed to assess the potential of a substance to undergo microbial degradation under aqueous, aerobic conditions. These tests have been integrated into regulatory frameworks, serving as benchmarks for determining whether a substance is "readily" or "inherently" biodegradable, terms defined by the tests.[1,13,20]

2.1. Structure and purpose of the OECD 300 series

The OECD biodegradability tests are categorised into three tiers: ready biodegradability tests, inherent biodegradability tests, and simulation tests.

Ready biodegradability tests (RBTs). The ready biodegradability tests (OECD tests 301 A–F, 310) are the most stringent set of tests and determine if a material will rapidly, and comprehensively, aerobically degrade in the presence of a microbial inoculum when both are dissolved in an aqueous medium.[13,24] The degree of degradation is determined by measuring a single parameter such as oxygen uptake, carbon dioxide release or dissolved organic carbon (DOC) removal for the duration of the test.[13]

The material is considered to be readily biodegradable if either 70% of the dissolved organic carbon is removed, or if 60% of the theoretical maximum oxygen uptake or carbon dioxide production is reached within a 28 days period. Additionally, for mono-constituent chemicals, these pass values must be reached within a 10 days period (usually mentioned as 10 days window), which begins when 10% of the maximum value has been reached.[13,25] The 10 days window criterion does not apply to biodegradability tests for mixtures of structurally similar chemicals. This includes PLFs, which, even in their most purified commercial forms, consist of components with varying chain lengths, degrees, and/or positions of branching.[26]

A reference compound is tested in parallel to the test material as a positive control. This reference material is commonly aniline, sodium acetate or sodium benzoate, all of which will degrade in an RBT even if no inoculum is present.[13] If a material passes an RBT, it is assumed to be unlikely to persist in the environment.[20]

Inherent biodegradability tests. Inherent biodegradability tests (OECD 302 A–C) make up the second tier of testing and are less restricted, allowing for longer durations and more concentrated sources of microbial inoculum. They are designed to test for slower or partial degradation and can provide a higher chance of passing than an RBT.[21]

The 302 tests can all be used to test moderately to highly soluble materials and work by regularly measuring either the DOC die away or biochemical oxygen demand of the sample. If

over 20% degradation is reached within the testing period, the material is said to possess inherent primary biodegradability. If it reaches as high as 70%, the material is classified as possessing inherent ultimate biodegradability.[27–29] The duration of the test is less strict than the RBT, usually taking 28 days but can be prolonged to as long as 60 days if the adaptation phase occurs in the final days of the test period.

Chemicals that pass an inherent test (by reaching above 70% DOC die away or 60% of the theoretical maximum oxygen uptake or carbon dioxide production within the extended timeframe) are regarded to be biodegradable under many conditions (such as in wastewater treatment plants) and are therefore often classified as non-persistent.[20]

Simulation tests. The third and final tier in the OECD persistence testing hierarchy are the simulation tests. These tests are more complex and environmentally realistic, giving them better overall predictive capabilities.[14,15,20]

Simulation tests can be run in aerobic or anaerobic conditions and exist for various natural systems such as soil, water sediment, and surface water (OECD 307–9), as well as for wastewater treatment plant (WWTP) conditions (OECD 303 and 314).[14–18,30] These tests, however, are very costly and difficult to automate, often requiring more analytical work and setup time than the other tests.[20]

2.2. OECD test assumptions

OECD biodegradation tests rely on several assumptions regarding both the nature of the test substance and the conditions that the test must reflect.

One assumption made by many of the tests is that the material under investigation is sufficiently water soluble. For many materials, the test conditions require a concentration well above their solubility limit. Testing poorly soluble materials in these conditions can lead to false negatives as the lack of sufficient bioavailable material can lead to an underestimation of the true biodegradability of the substance.[1,22,31] Substances of this nature can also be limited in more robust soil and sediment tests, where their high affinity to solid particles may again hinder their bioavailability and lead to deceptively low degradation outcomes.

Furthermore the guidelines assume the material being tested is a simple, single-component substance. This might be true for some products, but this assumption is not valid for many substances within the chemical landscape, especially PLFs. Mixtures and substances of unknown or variable composition can be hard to define using the OECD guidelines, leading to final results that may not reflect their true biodegradability.[20,23,32]

Furthermore, several biodegradability tests use mineralisation (by measuring either oxygen consumption or CO_2 production) as the primary endpoint. These tests typically require a threshold of 60% mineralisation to classify a substance as biodegradable. However, work dating as far back as 1985 suggest that relying solely on this value may underestimate the extent of biodegradation.[20,33,34] In one study by Painter and King, mineralisation rates below 60% corresponded to the removal of up to 90% DOC from a sample, indicating a substantial degree of degradation.[33,34] These results suggest that, given the current OECD thresholds, biodegradable PLFs could be incorrectly labelled as persistent.[21]

Finally, the tests contain no measures to characterise the microbial inoculum used. The microbial community is assumed to be representative of environmental conditions and does not take into account seasonality, geographic location or source (*i.e.* activated sludge, sewage effluents, surface waters, soils or mixtures of these). However, this can lead to a significant variation in test results, with several studies finding direct correlation between the composition of the inoculum and the overall biodegradation value for the test.[1,35–38] Additionally, one study by Thouand *et al.* found that the use of traditional inoculum density measurements, such as total cells, suspended solids *etc.*, may not be wholly suitable for ensuring a fair assessment as these values offer no information regarding the actual make-up and diversity of the microbial community.[39] Instead the practice of measuring specific microbial degrader counts was recommended as a better practice and led to better predictions of biodegradability.[37,39] Additionally, another study found that some standardised inoculum preparation protocols (such as those used in the OECD 302 C modified MITI test) cause shifts in microbial community structure, reducing diversity and limiting biodegradation potential.[29,40] These findings highlight the need for careful consideration of inoculum handling and characterisation to ensure environmentally relevant biodegradability testing.

3. Challenges and limitations of OECD testing for PLFs

Given the complexity of PLFs related to their molecular weight, polydispersity and solubility, their biodegradability can often be difficult to assess using the traditional OECD tests.

3.1. Chemical complexity

Each OECD test compares the result obtained for a sample against its theoretical maximum. For example, when performing the OECD 301 F manometric respirometry test, the amount of oxygen consumed by the sample is compared against the maximum theoretical oxygen demand (ThOD) to obtain a biodegradation percentage value.[13]

The ThOD is calculated using the structural formula of the sample under investigation. However, some PLFs lack a well-defined structural formula (*e.g.*, acrylic copolymers, which are used in paints and adhesives, or polyethylene glycol (PEG) products like PEG-40 hydrogenated castor oil or PEG 120 methyl glucose dioleate which are used in shampoos, body washes *etc.*), making accurate ThOD calculation difficult. Whilst some guidance does exist for modifying the ThOD in these conditions, it is by no means comprehensive. In fact, the only change that can be made to the ThOD calculation exists to account for nitrification of the product. For substances with complex formulations and ambiguous structures, the chemical oxygen demand (COD) can be experimentally determined. However,

this approach is generally discouraged and is described in the test as less satisfactory. Additionally, the following is stated in the OECD 301 guidelines:

"If the ThOD cannot be calculated, the COD should be determined, but falsely high values of percentage biodegradation may be obtained if the test substance is incompletely oxidised in the COD test."

Also, although it is theoretically possible to characterise each individual sample to determine its ThOD, this approach is not practical or sustainable for the wide range of formulations typically encountered during product development. The time, cost, and analytical effort required would be prohibitive, especially in early-stage screening or iterative formulation processes.

Overall, the non-specific and vague nature of the guidance for complex substances with undefined structural formulas is a critical limitation, as using an estimated or alternative ThOD can significantly affect the final biodegradation result, potentially determining whether a sample meets the pass criteria or not.

3.2. Poor water solubility and bioavailability

Certain PLFs exhibit low water solubility due to their high molecular weight, extensive branching, and hydrophobic character. This significantly reduces their bioavailability in aqueous test systems, which is a critical limitation in OECD biodegradability assessments.[19,20]

The OECD protocols set out clear guidance to facilitate and improve bioavailability. For example, the respirometry tests (OECD 301 B, C, D and F) are all stated to be suitable for testing poorly soluble substances; however, it is worth noting that these are all mineralisation tests and will therefore not capture any partial degradation of the sample.[41] Additionally, testing protocols may be modified to ensure that a sample is sufficiently bioavailable in the OECD test conditions.

The modifications, taken from ISO 10634, are stated in annex III of the OECD 301 guidelines and suggest the following:

- Ensuring a sample is homogeneous before sampling.
- Agitating the sample solution for the duration of the test (though not aggressively enough to cause excessive heating, foaming for shear forces).
- Adding an emulsifier that is non-toxic and must not be biodegraded or cause foaming during the test.
- Adding a solvent with the same criteria as above.
- Adding a solid carrier for oily substances but not for solids.

If an emulsifier, solvent or solid carrier is used, an additional blank run containing solely this element must also be run.[13]

While these modifications are taken from those suggested in ISO 10634, they are applied more restrictively. For example, the original standard recommends additional measures such as:

- Direct addition of the sample to the test.
- Addition of the sample to an inert support.
- Ultrasonic dispersion.

ISO 10634 also emphasises the importance of the use of analytical methods to investigate any stock solutions prepared and generally allows a broader choice of additives to aid sample dispersion.[42]

In addition, the OECD framework also fails to fully reflect the environmental fate of PLFs. In everyday use conditions, poorly soluble substances are often introduced into wastewater systems as part of an emulsion or dispersion, with PLFs being a single component of a more complex formulation system.[19] Furthermore, if entering a sewage environment, both natural and synthetic dispersants may be present, which can re-emulsify or disperse these substances, affecting their form and availability to microorganisms.[43] As such, PLFs that appear poorly soluble under OECD test conditions may, in practice, become more bioavailable under true end-of-life conditions.[31]

Therefore, while OECD 301 provides a structured approach for testing poorly soluble substances, its conservative application of ISO 10634 methods and limited consideration of real-world dispersion dynamics may underestimate the biodegradability of PLFs in environmental systems.

3.3. Test conditions and limitations

Several of the OECD 301 test conditions present significant barriers to accurately assessing the biodegradability of PLFs. These include the use of environmentally unrealistic sample concentrations, insufficient test durations for complex substances, limitations in inoculum diversity and density, low test volumes, and the weak buffering capacity of the mineral media used for the test.[1,20,39,44]

Test concentration and duration. The recommended sample concentration varies for each of the OECD 301 tests. For example, the 301 F test requires a concentration of 100 mg L^{-1}, whereas the 301 B test recommends 10 to 20 mg DOC per L. These concentrations have often been criticised to be too high and not environmentally realistic.[20,45] A widescale review of the OECD guidelines conducted in 1995 by Painter[33] suggested that the concentration of the test chemical should be as low as possible, consistent with the detection limits of the analytical methods.[34] However, this may lead to new challenges as, below certain threshold concentrations, biodegradation can fail to take place. This may be because the microbes within the inoculum need sufficient sample to adapt and synthesise the necessary enzymes and components for degradation to occur.[46,47] In these cases, the standard 28 days test duration may be insufficient to capture the full extent of degradation, particularly for complex or slowly degrading substances. Extending the test duration allows the microbial community to adapt to more complex substances and has been shown to improve the extent of biodegradation and reduce variability, especially for polymers.[36,48]

Inoculum source and microbial diversity. The microbial inoculum used in OECD 301 tests is assumed to be representative of environmental conditions. The guidelines state that the inoculum must come from a natural source and cannot be pre-adapted to the test substance. Additionally, the suspended solid content of the inoculum in the test must be no greater than 30 mg L^{-1} and have an approximate concentration of 10^4 to 10^8 cells L^{-1}. While the measurement of suspended solids can be performed relatively easily, the method for determining the cell concentration is more prone to error. The cell

concentration is normally found using a plate colony forming method. However, only a fraction of the microbes found in naturally sourced inocula are culturable, meaning that the true cell concentration may not be accurately determined.[49] It is also important to recognise that the measurement of total cell concentration does not take into account the diversity of the microbial community, with measurements such as suspended solid content or total cells giving no information of the types of species present.[39] To ensure that biodegradation takes place, the inoculum must contain species that are capable of adapting to and sufficiently degrading the sample, these species are known as competent degraders.[21] The OECD guidelines are currently unclear on characterisation techniques to ensure that these competent degraders exist, only measuring the density of the inoculum.[13]

An alternative to extending test durations when reducing sample concentration is to modify the inoculum source to ensure proper degradation. One test by O'Malley demonstrated the ability to decrease the sample concentration by a factor of 40 when using unique microbial sources.[50] However, while some researchers advocate for standardised bacterial mixtures to improve consistency across tests, others argue that the use of standardised inocula further reduces microbial diversity, limiting the degradative capabilities and environmental realism of the test.[40,51–54] Additionally, the use of synthetic communities presents further challenges, as not all the microbes are culturable, so cell count remains ambiguous. Overall, lack of diversity within an inoculum sources can pose challenges for PLF testing as the enzymes for needed for degrading complex polymers generally belong to specific microbial species, the absence of which may lead to poor results.[55]

Additionally, some polymers which are not found to degrade under normal circumstances have shown more promise when exposed to specific microbial communities. One example is the work by Yoshida *et al.* which found that poly(eythlene terephthalate) could be effectively degraded by the bacterium *Ideonella sakaiensis* 201-F6.[56] Similarly, *Aspergillus tubingensis*, a novel fungus identified and isolated from soil in a city waste disposal site in Islamabad by Khan *et al.* was found to found to be capable of degrading samples of polyester polyurethane.[57] Finally, four bacterial strains isolated from commercial compost by Szczyrba *et al.* (classified as *Actinomycetes*) were found to measurably decrease the molecular weight and degrade the surface of samples of low-density polyethylene.[58]

In cases such as these, taxon-specific biodegradation protocols could be considered to provide insight into whether degradation is plausible in environments where these organisms can naturally occur. Such targeted assessments would help distinguish polymers that are broadly biodegradable under typical conditions from those that require highly specialized ecosystems, improving the scope of environmental persistence evaluations.

Inoculum mass and test volume. One way to increase the likelihood that the test contains sufficient microbial diversity is to increase the mass of inoculum that is present within the setup. Overall, increasing this factor whilst maintaining a consistent dissolved organic carbon (DOC) level within the test, measurably increases the chance of detecting biodegradation (when testing a material that is biodegradable) and leads to reduced lag times (*i.e.*, The time taken to reach the 10% biodegradation threshold that starts the secondary 10 days window).[20,39,44]

Increasing the mass of inoculum must be explicitly mentioned when performing the tests and may be considered as giving the test an unfair advantage compared to environmental conditions. Another approach utilised to elevate the microbial diversity levels is increasing the overall volume of the test. This has the effect of enhancing the adaptation process by increasing the likelihood of sufficient competent degraders being present in the test solution, leading to better consistency between tests and improved reproducibility.[20,59,60]

Buffering capacity and pH control. The OECD tests involve dissolving both the sample and inoculum source in a mineral medium. The medium is designed to maintain a pH of 7.0, ensuring a favourable balance of CO_2 between the gaseous and aqueous phases. Nonetheless, the mineral media typically used in OECD protocols have a phosphate concentration of around 3.7 mM, which provides quite a poor buffering capacity.[20] Additionally, upon degradation, the pH of samples can change. For the OECD 301 B CO_2 evolution test, this pH change can impact the quantity of CO_2 measured. The guidance takes this into account by acidifying the test solution at the end of the 28 days period, ensuring any CO_2 which has not been measured is liberated. This would more accurately reflect the final CO_2 measurements, although it does not account for errors in kinetic results that would occur due to pH changes.[13] In tests where the phosphate content was considerably raised (to around 25.1 mM), it was possible to accurately assess the biodegradation kinetics of samples that cause a pH shift upon degradation (*e.g.*, organic acids or certain polymers).[44] While less environmentally realistic, this approach may be necessary to offset the amplification of sample-induced pH changes caused by the equally unrealistic high sample concentrations, closed system environments and shorter timeframes of the test.

Environmental fate and end-of-life conditions. Overall, the OECD 301 tests fail to represent the diverse and complex end-of-life pathways that PLFs undergo in real world environments. Disposal practices vary widely across the eight major market sectors in which PLFs are used influenced by factors such as consumer behaviour, product design, and local waste management. For example, PLFs in rinse-off products (such as shampoos and detergents) are typically discharged directly into wastewater systems, where they are diluted and mixed with other compounds.[19] As these treatment processes are unlikely to separate PLFs from these complex systems, these species may persist through treatment and enter agricultural soils *via* sludge application, where their mobility in soil and water can lead to widespread environmental dispersion. Additionally, PLFs used in agricultural applications (such as fertilisers and pesticides) are intentionally released into the environment. This highlights the need for tests that reflect the varied conditions to which PLFs are subjected. In certain applications, PLFs are embedded in curable formulations like paints and coatings, which adhere to substrates such as plastics, metals, and composites. These

materials are often difficult to separate and recycle, leading to increased landfill disposal.

The OECD 301 framework does not simulate these end-of-life scenarios, and consequently, it provides limited insight into the actual environmental persistence and impact of PLFs.

3.4. Incomplete consideration of degradation pathways

The environmental degradation of polymers is a complex, multi-phase process. The OECD guidelines assess biodegradability based on the final stage of degradation, mineralisation. By investigating the degradation pathways of natural and synthetic polymers, the intermediate processes that are overlooked by the OECD testing can be explored (Fig. 1).

Polymer degradation is a sequential process made up of four stages: deterioration, fragmentation, assimilation, and finally, mineralisation.[61] Each of these stages is governed by specific physicochemical/biological mechanisms.

• Deterioration involves the initial weakening of the polymer structure due to abiotic factors such as UV radiation, temperature fluctuations, and mechanical stress. This stage alters the surface and mechanical integrity of the material, facilitating further degradation by allowing better access for the microbial culture.[61,62] As PLFs are not solid structures, biodeterioration plays a smaller role in their degradation than for large solid plastics such as items made from high density polyethylene (HDPE).

• Fragmentation is the chemical breakdown of the polymer into dimer or monomer units. This step is also known as depolymerisation and only refers to the breaking of polymer linkages, not to changes in the composition of the monomer units themselves.[61] Bio-fragmentation is the breakdown of these linkages specifically caused by the excretion of enzymes by the microbial community. Tests exist that can be used to estimate how well a polymer will bio-fragment by adding specific enzymes to the material and measuring the extent of polymer link breakage.[62]

• Assimilation occurs when the compounds that are formed from bio-fragmentation are consumed by the microbial species and used as carbon/nitrogen sources, resulting in energy production and biomass formation.[61,63] This assimilation can occur *via* a combination of aerobic respiration, anaerobic respiration and fermentation and results in the overall growth of the microbes.[64]

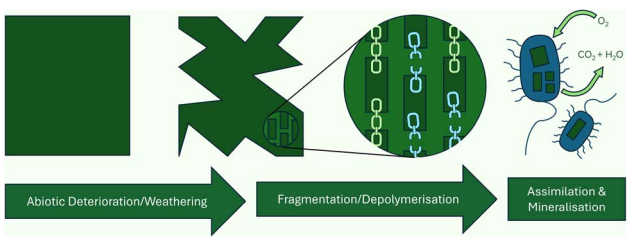

Fig. 1 A rudimentary figure depicting the steps of biodegradation.

• Mineralisation is the final stage and occurs at the same time as assimilation.[61] This stage involves the conversion of organic materials into inorganic endpoints, with CO_2 and H_2O being the primary resultant species in the case of aerobic degradation, and CH_4 being the primary endpoint in the case of anaerobic respiration.[65,66]

Overall, the degradation process is complicated and involves different species of microorganisms with complex interactions. For example, some microbes may only be involved in bio-fragmentation, with other species degrading the remaining material.[61,62] While mineralisation is the easiest step to measure, looking solely at this result is not likely to represent the overall biodegradation journey of a complex material.

For example, natural polymers such as lignin often degrade slowly due to their structural complexity and functional roles in nature. These polymers would fail the OECD tests, yet they are not considered environmentally hazardous.[41] Natural polymers such as these may bio-deteriorate or depolymerise, with only small amounts of mineralisation occurring.[67,68] In fact, some natural polymers (found in materials such as coconut coir, rice hull, wood pulp) degrade so slowly that they would be classified as persistent substances.[41,69,70] Additionally, the assimilation of the resultant smaller sample fragments into organic soil matter is a competing process to mineralisation.[41] Humification, for example, where the polymer-bound carbon is not mineralised, but is instead used for natural soil formation, can even occur to polymers which normally degrade rapidly in nature.[71,72]

Although these processes are complex and harder to measure than respirometer mineralisation assessments, overlooking these elements when performing end-of-life assessments will not give an accurate account of the natural environmental biodegradation and endpoints a product will encounter.

This observation also raises a broader, critical question: should the ability of a material to degrade automatically imply environmental safety? As degradation is not a singular process, but a series of transformations, it is important to also consider whether intermediates formed during this process can themselves pose ecological or human health risks. The notion that biodegradable materials are inherently environmentally benign may oversimplify the complexity of degradation processes and the toxicology of breakdown materials.

For instance, polylactic acid (PLA), a polymer which can degrade up to 90% in optimal conditions, has been shown to degrade slower in natural conditions and to generate micro- and nano-plastic fragments during its breakdown in the human digestive system.[73,74] These breakdown products where then used as carbon sources and altered the metabolic phenotype of the gut. Additionally, the presence of these microplastics in the diet of mice was found to significantly reduce the appetite and body weight of the animals exposed.[73] Another study found a clear correlation between microplastics, and the likelihood of having inflammatory bowel disease. In this study, patients suffering from the disease were found to have significantly higher levels of faecal microplastics, indicating a strong link between microplastic ingestion and gut health issues.[75] Additionally, one study focusing on the fate of biodegradable

plastics ingested by mice found that breakdown products and plastic nanoparticles bioaccumulated in the liver, intestine and brain.[76] These findings suggest that incorporating ecotoxicological and human health evaluations may be beneficial for capturing the full spectrum of environmental risk. Whilst not discussed in detail in this article, it is important to acknowledge that current OECD biodegradability tests may also overestimate the biodegradability of PLFs (echoing the need for more robust and representative test methodologies for polymers). Although a fuller discussion on this topic is beyond the scope of this section, the potential for such overestimation should be considered when interpreting biodegradability data for PLFs, and future work will need to examine this issue in greater depth.

Transitioning to a more nuanced approach that incorporates depolymerisation rates, bio-assimilation, and stable incorporation into humic substances, along with complementary ecotoxicological and human health tests, may help support a more sustainable and environmentally safe future.

4. Bridging the gaps: scientific and regulatory innovations

Due to the limitations outlined in the previous section, there is a growing body of work aimed at modernising biodegradability testing to more accurately reflect the complexity of contemporary chemical products such as PLFs. The following section explores several pathways forward, including enhanced microbial characterisation, alternative degradation endpoints, more realistic simulation environments, improved solubilisation techniques, and the integration of computational tools (Fig. 2). These advances represent an approach toward a more flexible framework for assessing persistence that better reflects real world conditions.

4.1. Integration of microbial ecology tools

One of the largest sources of variability in the OECD biodegradability tests is the source of microbial inoculum. Recent advances in microbial ecology have made it possible to precisely characterise inoculum sources using techniques such as next-generation sequencing, metagenomics, proteomics and metabolomics.[77] These tools allow researchers to assess the diversity and functional potential of microbial communities to effectively degrade species by detecting specific genes and enzymes that are involved in these processes.[21,78] This would make it possible to ensure the presence of competent degraders within the test, decreasing variability both within single investigations and between laboratories.[79] Additionally, concepts such as biodegradation adaptation potential have been suggested where inoculum is pre-evaluated before testing to ensure that it is able to degrade substances sufficiently, ensuring a fair result for the test sample.[20] This approach uses lag time to categorise the inoculum source into different tiers to ensure that it has the ability to degrade a substance.

Another potential methodology to assess the degradation potential of a microbial community could be the use of functional gene arrays. In this method, thousands of oligonucleotide probes, each specific to genes involved in essential microbial processes, are immobilized onto a chip. DNA is then extracted from an environmental sample and labelled. When it hybridises with a matching probe on the chip, a fluorescent signal is emitted.[80] This technology could be adapted to include probes targeting enzyme sequences related to the degradation of various types of polymers, serving as a high-throughput, quantitative tool for detecting the microbial potential in complex communities. Alternatively, if these enzyme families could be isolated and produced, it would be possible to assess biodegradation potential by developing colorimetric or fluorometric assays using substrates that release a detectable signal when they are hydrolysed by the enzymes.[81]

Although the cost and complexity of these methods have traditionally been prohibitively high for regular biodegradation testing, sequencing technologies continue to become more affordable and accessible.[21,78]

To implement microbial ecology tools, inoculum sources should be pre-characterized using metagenomics or functional gene arrays before OECD testing. This involves sequencing microbial DNA to confirm the presence of competent degraders and enzymes relevant to polymer breakdown. These methods make the assumptions that detected genes correlate with enzymatic activity under test conditions and that inoculum diversity remains stable throughout the test. Validation would require correlation studies between gene presence and observed biodegradation rates.

As they become more widespread, the application of these methods will allow researchers to better understand microbial diversity, track changes in microbial communities during degradation, and effectively assess the true environmental fate of substances.

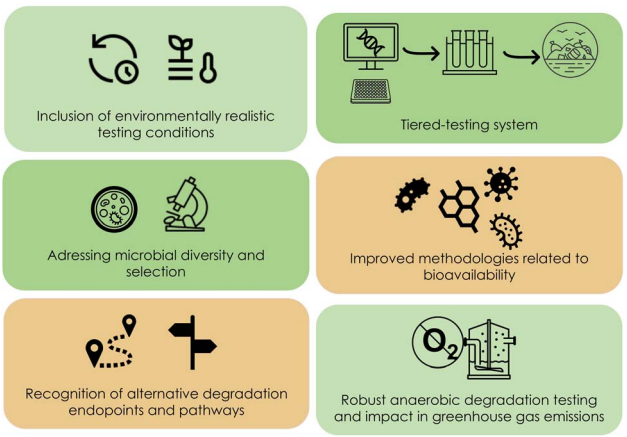

Fig. 2 A summary of the key areas for regulatory developments to focus on.

4.2. Supporting measurements

While mineralisation is easy to measure, relying solely on this endpoint can overlook other processes by which polymers interact with environmental systems. Mineralisation indicates complete conversion to CO_2, but real-world conditions often

involve partial degradation and incorporation of breakdown products into soil organic matter. Therefore, additional data are needed to complement mineralisation tests and provide a more holistic understanding of environmental fate.

As recognition grows that the stable incorporation of polymer breakdown products into humic substances is both valid and environmentally safe, so does the need to understand the mechanisms that drive this process.[82] Crucial to this understanding is knowledge of the degree of partial polymer degradation and the extent to which these breakdown products can successfully integrate. Several analytical techniques can be used to assess the structural changes that take place during these steps of degradation:

- SEM analysis to measure changes to polymer surfaces.[83,84]
- Gel permeation and gas chromatography to assess changes in molecular weight and chemical structure.[85–87]
- ^{1}H NMR, ^{31}P NMR, UV and ATR FT-IR analysis and spectroscopy techniques to analyse changes in chemical structure.[85,88]
- Measurements of changes to physical properties such as degree of crystallinity and melting point.[89,90]

These techniques provide valuable insight into chemical and morphological changes during degradation. However, they do not directly quantify the overall extent of degradation or define endpoints. Instead, the data obtained from these methods should be considered supporting evidence alongside mineralisation tests, helping to interpret degradation under real-world conditions. These methods could be integrated by supplementing OECD 301 tests with periodic structural analyses. For example, samples could be withdrawn at defined intervals and analysed using SEM for surface changes, GPC for molecular weight reduction, isotope labelling to trace carbon incorporation into biomass or humic substances, and NMR for chemical structure shifts. This would assume that these structural changes correlate with environmental degradation rates and that analytical techniques can detect early-stage breakdown even when mineralization is incomplete.

Additionally, some studies have used ^{13}C isotope labelling to track the incorporation of polymer degradation products into both microbial biomass and into humic substances.[87,91] This allows researchers to completely trace the path of a polymer as it degrades, vastly increasing the understanding of how it will behave when it reaches its natural endpoint.

4.3. Realistic environmental simulation testing

As the OECD guidelines do not fully reflect the complexity of real-world conditions, many researchers have explored new test designs that better simulate the natural environments and endpoints that products will encounter once they are disposed of.

One important end-of-life scenario that is under-represented in biodegradability assessments is landfill, where conditions exist so that even after 20 years, materials such as food waste and newspapers can remain sufficiently intact to be easily identified.[92–94] Adamcová *et al.*, investigated this endpoint by conducting a 12 months field trial in a municipal solid waste landfill, testing several commercial biodegradable and compostable plastic bags. Despite being marketed as degradable, each of the samples demonstrated little to no degradation after burial with only a cellulose control sample fully decomposing.[92] This highlights the discrepancy between real-world and test conditions as each of these materials was certified as completely degradable yet failed to even partially breakdown in a true end-of-life scenario.

Another field test, by Mouhoubi *et al.*,[95] compared the degradation of polyesters under both laboratory and true environmental conditions. The review concluded that lab tests often fail to capture the variability introduced by environmental factors, such as microbial diversity, compost composition, and temperature fluctuations. Additionally, longer test durations and more environmentally relevant conditions were recommended to better reflect real-world degradation pathways, particularly for polymers with slower biodegradation kinetics.[95]

Furthermore, modifying the OECD 301 tests to better reflect environmental conditions may offer a more practical alternative to immediately progressing to more complex simulation methods. One way in which the accuracy of these tests could be improved is by addressing their exclusive focus on aerobic degradation. Anaerobic conditions are common in realistic end-of-life scenarios such as wastewater treatment systems and marine sediments, both of which are relevant pathways for PLFs.[96–98] While aerobic degradation typically proceeds more rapidly, some polymers have demonstrated the ability to degrade under both aerobic and anaerobic conditions.[99] This highlights the need to adapt these tests so that they simulate the range of environmental scenarios polymers may encounter more accurately.

To address some of these limitations, a study by Kintzi *et al.*,[100] examined how small adjustments to the standard OECD 301 tests would affect the biodegradation performance of poly(aspartic acid) and ε-poly(L-lysine), which tend to have slow and variable degradation results for these tests. By using microbial inocula sourced from municipal treatment plants, the researchers assessed the impact of avoiding aeration, pre-incubating polymers with sterilized wastewater, and lowering polymer concentrations. These modifications led to reduced lag times for both polymers, improving their biodegradation profiles.[100] These findings demonstrate the importance of optimising test protocols (which were originally designed for simple molecules) to reflect realistic exposure scenario, ensuring results are relevant to the modern-day chemical landscape.

Modifications such as this could be implemented by adapting OECD protocols to include anaerobic phases, extended durations (beyond 28 days), and environmentally relevant concentrations. For example, a test could incorporate a pre-incubation phase with sterilized wastewater and avoid aeration to mimic sediment conditions. This method would assume that the inoculum remains viable under modified conditions and that extended timeframes do not introduce secondary abiotic degradation artifacts. Applicability should be validated through comparison with field trials.

4.4. Improved handling of poorly soluble and complex substances

Whilst OECD provides some guidance for the testing of poorly soluble compounds, the limitations on how extensively modifications can be made have faced some criticism.[21,31] Additionally, while the guidance suggests the use of emulsifiers that are non-toxic and do not biodegrade, no examples of any such materials are provided.[13,101] Whilst ISO 10634 does suggest the use of Tween 80 as an emulsifier, this material has been found to be readily biodegradable by the OECD tests, making it non-viable when testing poorly soluble samples.[42,102]

Recent advancements have sought to address these limitations by introducing newer methods for testing poorly soluble substances. One example is the use of trimethylated α-, β-, and γ-cyclodextrins as effective dispersants in biodegradability tests, which have been found to create 'inclusion complexes' between the test sample and inoculum source, increasing bioavailability.[101] These species were found to be non-biodegradable, making them great candidates for use in the testing of poorly soluble substances.

Similarly, the integration of passive dosing techniques has also been proposed, where the test substance is introduced to the microbial community over time to allow for a controlled and sustained exposure.[103] This approach aims to improve the reliability of the tests by maintaining consistent bioavailability, whilst avoiding the use of solvents or surfactants that may interfere with microbial activity.

Finally, in the context of PLFs, evaluating the polymers in blends and mixtures that are representative of the true end-of-life pathway would address challenges surrounding sample solubility. This approach would simultaneously improve the environmental realism of the test. Solvation effects and co-solute interactions have the potential to increase polymer bioavailability which may influence polymer degradation kinetics and accelerate breakdown processes.[104–106] To better explore these conditions, a more rigorous assessment paradigm should incorporate testing in both bulk polymer and solution states, thereby better reflecting real-world application scenarios and improving the ecological validity of the results. However, the OECD testing guidelines currently require that each component of a blend be tested separately. Although this approach reflects the OECD's commitment to methodological accuracy and precision, it significantly increases the complexity and duration of testing and product development.

Additionally, the testing of poorly soluble PLFs can be improved by using non-biodegradable dispersants or passive dosing techniques to maintain consistent bioavailability (assuming that the dispersants do not interfere with microbial activity and that passive dosing accurately reflects environmental exposure). The modification would need to be validated through control runs with dispersants alone and monitoring for unintended microbial inhibition.

Ultimately, by failing to recognise the integrated nature of chemical mixtures in environmental contexts and the role of formulation solvents and aqueous matrices aiding polymer dispersion and bioavailability, the OECD approach may risk undermining the ecological validity of the guidelines.

4.5. High-throughput screening tools

Although increased timescales for the ready biodegradability tests can improve environmental accuracy, the time-consuming nature of these tests has increased the demand for more rapid biodegradability assessments. High-throughput screening tools and predictive models are two such methods. These tests are generally designed to work alongside the traditional guidelines, giving an early indication of a material's potential to degrade before continuing development. This would allow the assessment of many potential candidates in a much shorter timescale than testing each using a 28 days (or longer) assessment. The final material would then be tested by a traditional test to properly assess its true biodegradability.

One recent advancement, by Peters *et al.*,[107] focused on modifying an OECD 301A protocol, to simplify and accelerate the biodegradability testing. Here, the test volume was reduced to 50 mL and the incubation time was shortened from 28 to 10 days. Additionally, the progress of degradation was measured by directly quantifying the total organic carbon (TOC) of the sample rather than measuring CO_2 production or O_2 consumption. The method demonstrated reproducibility across different inoculum sources and polymer types, yielding results close to those obtained from standard 301B and 301F tests, with results for sodium alginate, gum Arabic, and carboxymethyl cellulose deviating from the traditional tests by no more than ±52%.[107] These findings demonstrate the utility of high-throughput screening methods as a tool to assess biodegradability in the early stages of product development.

Complementing these methods, machine learning models have emerged as powerful instruments for predicting polymer biodegradability based on molecular structure. These methods rely on large amounts of accurate data and require robust external validation before they can be used.[108] More sensitive measurement techniques (*e.g.*, the use of fibre-optic CO_2 fluorescence sensors to monitor gas-phase biodegradation) can be used to increase the quality of the data being used to build the model.[109]

In 2025, Lin and Zhang[110] developed a machine learning method trained using a dataset of the OECD 301 test results of 74 polymers (1779 datapoints). The model was able to predict how well new, previously unseen polymers would biodegrade, with predictions aligning closely with experimental results. On average, the model's predictions were within 20% of the actual measured values.[110]

Machine learning models and high-throughput screening tools are particularly valuable in the early stages of product development where rapid feedback is essential to optimise innovation timescales. These tools could be implemented using miniaturized respirometry or TOC-based assay, reducing test volumes and durations to under 10 days. The tests would have to be validated to verify the assumption that the accelerated conditions correlate with OECD 301 outcomes and that inoculum diversity is sufficient at smaller scales.

Ultimately, these methods will not replace the requirement for more robust tests of final product candidates, but will complement and enhance understanding at the product design phase, ensuring biodegradability potential is optimised.

4.6. Policy and regulatory developments

The restriction of synthetic polymer microparticles (commonly referred to as microplastics) and the likely inclusion of polymers in the next revision of the European registration, evaluation, authorisation and restriction of chemicals (REACH) regulation (which aims to ensure protection of the environment), reflects the growing concerns surrounding the persistence of polymers within the environment.[111,112] The microplastic restriction requires that polymers (whether they are present as single materials or in formulations/mixtures) meet specific criteria in order to exempt them from the restriction. One of these is to demonstrate degradability through standardised testing.[111] This legislation relies on accurate degradability testing of these materials, performed to the prescribed methods and standards, in order to assess their persistence. However, the continued dependence on tests which were designed for simple materials under controlled conditions means that the regulations are somewhat limited at measuring the persistence of complex products within their realistic environmental endpoints.

To address these limitations, testing for regulatory frameworks such as REACH must evolve to reflect the modern-day chemical landscape. Key areas that must be improved include:

- The inclusion of more environmentally realistic test conditions, such as lower sample concentrations and longer test durations to more fairly assess polymers and complex materials.
- Improved methodologies related to bioavailability for poorly soluble substances, ensuring realistic representation of PLFs at end-of-life.
- Addressing microbial diversity and selection, by ensuring that sufficient component degraders exist within the test through the use of microbial ecology tools.
- The development of a tiered testing system, progressing from high-throughput screening tools for early development to more in depth and environmentally realistic tests for final product candidates.
- The recognition of alternative endpoints and degradation pathways for materials including humification, partial degradation into benign substances and, abiotic breakdown processes.
- An approach to anaerobic degradation that is as robust as the approach to aerobic testing, with a better understanding of degradation pathways and the resultant greenhouse gas emissions for this process.

By aligning regulatory expectations with these advancements, policymakers would modernise persistence testing, paving the way for developments which are hindered by the current guidelines.

We believe these developments must take place as, the longer these tests remain the benchmark, the more the use of legacy materials is entrenched; not because they are less harmful, but because the tests are not fit to recognise less persistent alternatives.

5. Our contributions: Croda's commitment to smarter, sustainable testing

Croda has taken an increasing role in addressing the limitations of current biodegradability testing frameworks, particularly for PLFs. Recognising the urgent need for more representative, rapid, and robust testing methodologies, Croda's commitments to sustainability and innovation have been at the core of the company's research strategy.

This section outlines Croda's contributions to advancing the science and policy of polymer biodegradability.

5.1. Mission Biodegradability

Foundations for the sustainable future of formulated polymers is a five-year, collaborative partnership funded by the UK Research and Innovation's EPSRC Prosperity Partnerships scheme.

Spearheaded by the University of Birmingham, the initiative brings together collaborators including Croda, BASF, Lubrizol, Unilever, the Centre for Process Innovation, United Utilities, Yorkshire Water, and the Royal Society of Chemistry (RSC) to develop new approaches for testing and evaluating PLFs.[113]

To address these challenges, the project will first focus on the design of accurate, accelerated, high-throughput tests that enable prediction of OECD biodegradation tests for PLFs.

To achieve this, the project has 4 key objectives:

(1) To create accelerated tests that correlate to OECD biodegradation behaviour for PLFs.

(2) To undertake simulated environmental tests for more reliable and robust prediction of PLF degradation in the environment.

(3) To understand how formulation components influence the biodegradation of PLFs.

(4) To create new standards to determine the environmental persistence of formulated polymers.

The initiative is one of two headline missions identified in the RSC's Sustainable PLFs 2040 Roadmap, which outlines a vision for cross-sector innovation to address the sustainability challenges associated with PLFs.[113] The roadmap calls for the development and scaling of biodegradable PLFs by 2030, alongside the creation of circular economy infrastructure and the reform of test standards to better reflect real-world environmental conditions.

Croda's participation in the partnership reflects its broader commitment to sustainable innovation, and Croda will contribute technical insight and formulation expertise to support the development of future test methodologies, to accelerate sustainable innovation and to better reflect real-world, end-of-life fate of PLFs.[114] Additionally, Croda's role as a founding member of the PLF task force highlights the company's existing efforts to reduce environmental harm and build towards a sustainable chemical landscape.

The RSC will play a central role in the policy development aims of the project, convening UK, EU and international stakeholders, supporting the development of future test standards, and representing the sustainable PLFs initiative within the project.

Mission biodegradability exemplifies the value of collaborative research in addressing these sustainability challenges. By combining scientific innovation with regulatory engagement, the partnership aims to accelerate the transition to non-persistent, PLFs that are not only high-performing but also environmentally responsible.

5.2. Industry partnerships to achieve a sustainable future

Beyond the company's involvement in mission biodegradability, Croda has undertaken a range of collaborative initiatives aimed at developing PLFs with a lower environmental burden. These efforts demonstrate the company's commitment to developing less persistent polymer products, particularly where environmental release is likely. It is also important to recognise that Croda's work forms part of a broader industry shift, with other organisations across the chemicals sector investing in more sustainable PLF technologies. This shift highlights a collective understanding that reducing polymer persistence is essential for future product stewardship.

Croda's research spans multiple polymer platforms, with both internal development and external partnerships forming key pillars of our ongoing sustainability strategy. Improving product carbon footprint, increasing renewable carbon content and optimising biodegradability are all important focus areas.

Collaborative research programmes, such as Croda's ongoing Prosperity Partnership (*Biobased and Biodegradable Polymers for a Sustainable Future*) with the Universities of York and Nottingham, which is focused on the development of biobased and biodegradable polymers, are invaluable in accelerating sustainable innovation.[115] The aim is for new polymers to be designed with biodegradability in mind from the start, and offer solutions within Croda's consumer care and life sciences product portfolio. Not only will these collaborations help Croda achieve net zero goals, but it will also give consumers greater access to safer, more sustainable everyday products.

These activities are aligned with Croda's long-term sustainability goals, including its commitment to becoming climate, land, and people positive by 2030 and net zero by 2050.[114] A growing number of countries, businesses, and other institutions are also aiming for net zero emissions. Transitioning to biodegradable, renewable materials is essential to meet these goals. By integrating biodegradability considerations into early-stage product development, Croda is supporting the transition to more environmentally responsible PLF products.

Croda continues to seek out new collaborations and partnerships, recognising that the transition to sustainable PLFs is a complex challenge – one that demands shared expertise, innovation, and collective action.

5.3. Accelerated biodegradability testing

To address challenges associated with the long timeframe of traditional OECD tests, Croda have collaborated with Impact Solutions to develop an accelerated test method designed to provide indicative biodegradability results within a significantly shorter timeframe.[116]

The method is high-throughput, utilising a 96-well plate assay to assess microbial activity *via* spectroscopic techniques, in less than 24 hours. The goal is to offer a qualitative indication of a material's likelihood to degrade under standard test conditions, enabling faster iteration during early-stage product development, significantly accelerating the innovation cycle. While not intended to replace regulatory tests, the method provides a valuable tool for screening large numbers of candidates and prioritising those with the greatest potential for environmental compatibility.

By integrating accelerated testing into its development workflow, Croda aims to reduce the time and resource burden associated with biodegradability assessment, while maintaining a strong focus on developing less environmentally harmful products. This approach supports Croda's wider initiative to reform biodegradability and persistence testing, enabling safer alternatives to thrive and replace traditional persistent materials, paving the way for a more sustainable future.

6. Conclusion

This perspective has outlined the scientific, technical, and regulatory shortcomings of the OECD biodegradability testing framework and presented a range of innovations that could bridge these gaps. These include the integration of microbial ecology tools, recognition of alternative degradation pathways, development of more realistic simulation environments, and the adoption of high-throughput and predictive screening methods.

The nature of the current framework fails to acknowledge incremental improvements in chemical design. In these tests promising, less persistent candidates may be indistinguishably grouped with legacy materials, despite offering environmental advantages. Rather than driving progress, the lack of nuance within the framework risks stalling innovation, penalising these transitional improvements that could collectively lead to a more sustainable chemical landscape.

Furthermore, Croda's active role in collaborative research initiatives demonstrates the potential for industry led innovation to drive meaningful change.

To enable the transition to more sustainable polymer systems, regulatory frameworks must evolve in parallel with scientific advancements, ensuring that persistence assessments are accurate and environmentally relevant. Only through such alignment can the development and adoption of biodegradable PLFs be effectively supported.

Conflicts of interest

The authors are employees of Croda Europe Ltd, a company that develops and supplies polymers in liquid formulations (PLFs). Croda may benefit from advancements in sustainable PLF technologies discussed in this article. However, the content of this perspective is intended to provide an objective scientific

and regulatory analysis of current biodegradability testing frameworks and does not serve as promotional material. The authors declare that the views expressed are based on scientific evidence and collaborative research initiatives.

Data availability

No data is associated with this article.

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