Designing biodegradable alternatives to commodity polymers

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Table S1 Primary Organisation for Economic Co-operation and Development (OECD) biodegradation tests.[1]

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration/ replicates	Test material and validity criteria
301 A: Dissolved organic carbon (DOC) die-away test **301 B: CO_2 emission** **301 C: Ministry of International Trade and Industry (MITI), Japan** **301 D: Closed bottle** **301 E: Modified OECD screening** **301 F: Manometric respirometry**	Activated sludge from a sewage-treatment plant (predominantly domestic sewage), sewage effluents, surface water (rivers or lakes), soils or from mixture of these	22 (±2)	Determination of primary biodegradation under aerobic conditions 301 A and E: Determination of Dissolved Organic Carbon (DOC) 301 B: Theoretical CO_2 production ($ThCO_2$) 301 C and F: Theoretical Oxygen Demand (ThOD) 301 D: O_2 consumption (Biological oxygen demand: BOD)	28 days Extended beyond 28 days if curves shown that biodegradation started but that the plateau has not been reached by day 28 2 replicates	Water-soluble organic compounds or mixture in water passing through a 0.45 µm filter A material is considered *readily biodegradable* if 60% (for OECD 301 B-D and F) or 70% (for OECD301 A and E) of the degradation reached within a 10 day window and within 28 days total. The 10 day window is defined as beginning when 10% of the degradation is reached and ends after 10 days from this point (but before 28 full days of the test). If materials fail, they may be tested using OECD 302
302 A: Modified semi-continuous activated sludge (SCAS) test **302 B: Zahn-Wellens / EMPA test** **302 C: MITI 2** **302 D: Concawe Test**	Activated sludge in aqueous medium, soil	20-25 (±1)	Determination of inherent biodegradability under aerobic conditions 302 A-C: Determination of DOC and/or O_2 consumption (Chemical oxygen demand: COD) 302 D: Theoretical maximum Inorganic carbon production (ThIC)	Min: 28 days, max: 4 months 2 replicates	Water-soluble organic compounds or mixture in water passing through a 0.45 µm filter 302 A-C: Biodegradation ≥ 20% may be regarded as evidence of inherent, primary biodegradability, biodegradation ≥ 70% may be regarded as evidence of inherent, ultimate biodegradability 302 D: Biodegradation ≥ 60 %, substance is inherently and ultimately degradable, biodegradation > 20 %, the substance is inherently, primary biodegradable. Biodegradation ≤ 20 % the substance is not inherently biodegradable in under the test condition If materials fail, they may be tested using OECD 303
303 A: Activated sludge unit simulation **303 B: Biofilm simulation**	Synthetic sewage, domestic sewage or activated sludge from plant or laboratory	20-25 (±1)	Determination of elimination and primary and/or ultimate biodegradation under aerobic conditions Determination of DOC (or COD)	Min: 9 weeks, max: 12 weeks	Water-soluble organic compounds or mixture in water, passed through a 0.45 µm filter Elimination and primary biodegradation observed if DOC or COD ≥ 80% after 2 weeks and no unusual observations have been made. If degree of biodegradation is ≥ 90 %, test chemicals are deemed to be readily biodegradable
306: Seawater **306 I: Shake flask method** **306 II: Closed bottle method**	Sea water	15-20 (±2)	Determination of the biodegradability of the test substance in the marine environment. DOC (306 I) or ThOD (306 II)	306 I: 60 days 306 II: 28 days	Highly soluble (> 50 mg DOC/L), non-volatile, and non-adsorbing organic compounds A positive result (> 70% DOC removal for the shake flask method, or > 60% of ThOD for the closed bottle method)

Table S2 Standards for the assessment of biodegradation of plastics in aerobic environments issued by American Society for Testing and Materials (ASTM).

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration/replicates	Test material and validity criteria
ASTM D5338-15	Compost from municipal solid waste	58 (±2)	CO_2 evolution	Max 45 days 3 replicates	Plastic materials ≥ 70 % degradation of reference material and the deviation among the reference material replicates is less than 20 %
ASTM D5988- 18	Adapted or non-adapted soil, natural soil	20-28 (±2)	CO_2 evolution	Max. 6 months 3 replicates	Plastic materials ≥ 70 % degradation of reference material. The measured CO_2 or the BOD values from the blanks at the end of the test are within 20 % of the mean
ASTM D6400-23	A mixture of primary and secondary sludge that has been aerobically digested	50-60 (±2)	CO_2 evolution	Max. 6 months	Plastics and products made from plastics 90 % of the organic carbon in the whole item shall be converted to CO_2 by the end of the test period when compared to the positive control or in the absolute
ASTM D6691- 17	Preselected strains or seawater, synthetic medium	30 (±2)	CO_2 evolution	Max. 3 months	Plastic materials including formulation additives ≥ 70 % degradation of reference material
ASTM D6868-21	Compost	58 (±2)	Visual evidence for degradation; loss of dry mass and CO_2 evolution	Max 6 months	Plastics or polymers incorporated to substrate and the entire end item is designed to be composted ≥ 90 % Disintegration during composting; 90% carbon converted to CO_2
ASTM D6954-18	Soil, landfill, compost in which thermal oxidation may occur, and land cover and agricultural use in which photooxidation may also occur	Varies depending on in situ conditions	Visual evidence for degradation; loss of dry mass and CO_2 evolution	Not specified	Polymers For homopolymers or statistical copolymers, 60% carbon must be converted to CO_2 before termination of the test. For block copolymers, segmented copolymers, polymer blends, or where low molecular weight additives have been employed, 90% carbon must be converted to carbon dioxide, CO_2 before termination of the test
ASTM D7991- 22	Sediment and seawater	15-28 (±2)	CO_2 evolution; static test conditions	Max 24 months 2 replicates	Plastic buried in sand ≥ 60 % degradation of reference material

Details of standards can be found in the ASTM test and standard database.[2]

Table S2 Standards for the assessment of biodegradation of plastics in anaerobic environments issued by ASTM.

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration and replicates	Test material and Validity criteria
ASTM D5511-18	Methanogenic inoculum derived from anaerobic digesters operating only on pre-treated household waste	37 (±2) or 52 (±2)	Total carbon gas (CO_2 and CH_4) evolution	Max. 1 month 3 replicates	Plastic materials in high-solids anaerobic conditions ≥ 70 % degradation of reference material and the deviation among the reference material replicates is less than 20 % of the mean
ASTM D7475-20	Methanogenic inoculum derived from anaerobic digesters operating only on pre-treated household waste	35 (±2)	Total carbon gas (CO_2 and CH_4) evolution	Max. 300 days 3 replicates	Plastic materials in an accelerated aerobic-anaerobic bioreactor landfill test environment
ASTM D5526-18	Methanogenic inoculum derived from anaerobic digesters operating only on pre-treated household waste	35 (±2)	Total carbon gas (CO_2 and CH_4) evolution	Max. 300 days 3 replicates	Plastic in an accelerated-landfill test environment

Standards can be found on ASTM test and standard database.[2]

Table S3 Some examples of active standards for the assessment of biodegradation of plastics used in Europe.

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration/replicates	Test material and Validity criteria
BS 8472:2011[a]	Soil, landfill, compost in which thermal oxidation may occur, and land cover and agricultural use in which photooxidation may also occur		CO_2 evolution O_2 consumption (BOD)	6 months 3 replicates	Natural or synthetic polymers, copolymers or mixtures, plastic materials with additives, water-soluble polymers Requirement of ISO 17556
DIN EN 13432:2000[b]	Compost, soil or sludge Industrial compost	Varies depending on in situ conditions	CO_2 evolution Specification	6 months	Packaging ≥90 % conversion of the carbon in the test polymer to CO_2. The 90 % level set for biodegradation in the test accounts for a ± 10 % statistical variability of the experimental measurement
DIN EN 14987[b]	Microbial inocula from municipal or industrial sewage sludge	25 (±2) cold water 60 (±2) hot water	CO_2 evolution	2 months	Water soluble plastic in cold or hot water ≥ 90 % of mineralisation degree or 90 % mineralisation degree reaches in the same time by the reference material, test in parallel (relative biodegradation ≥ 90 %). The reference material is soluble starch or microcrystalline cellulose
DIN EN 14995:2006[b]	Soil, compost	20-30 (±2)	CO_2 evolution O_2 consumption (BOD)	6 months	Plastic in non-packaging application ≥ 70 % degradation of reference material. The measured CO_2 or the BOD values from the blanks at the end of the test are within 20% of the mean
DIN EN 17033:2018[b]	Soil, compost	20-30 (±2)	CO_2 evolution	24 months	Mulch film for use in agriculture and horticulture ≥90 % conversion of the carbon in the test polymer to CO_2. The 90 % level set for biodegradation in the test accounts for a ± 10 % statistical variability of the experimental measurement

a. Tests and standards issued by British Standard Institution.[3, 4]
b. Tests and standards issued by DIN[5] at the national level. Affix "EN" corresponds to standards and test approved at the regional level by the European Committee for Standardszation (CEN).[6]

Table S4 Active standards for the assessment of biodegradation of plastics in aerobic environments issued by International Standards Organisation (ISO).

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration/replicates	Test material and validity criteria
ISO 14851:2016	Activated sludge from a sewage-treatment plant predominantly domestic sewage	20-25 (±1)	O_2 consumption (BOD)	6 months 3 replicates	Plastic materials ≥ 60% degradation of reference material at the end of the test. BOD of negative control must not exceed a specified upper limit
ISO 14852:2021	Activated sludge from a sewage-treatment plant predominantly domestic sewage, Soil and/or compost	20-25 (±1)	CO_2 evolution	6 months 3 replicates	Plastic materials ≥ 60% degradation of reference material at the end of the test. CO_2 evolved from negative control must not exceed a specified upper limit
ISO 14855	Compost	58 (±2)	CO_2 evolution	6 months	Natural or synthetic polymers, copolymers or mixtures, plastic materials with additives, water-soluble polymers >70% of biodegradation after 45 days. The difference between the percentage of the reference material in the different vessels is <20% at the end of the test. The inoculum in the blank has produced more than 50 mg but less than 150 mg of CO_2 per gram of volatile solids (mean values) after 10 days of incubation
ISO 17556:2019	Soil, landfill, compost in which thermal oxidation may occur, and land cover and agricultural use in which photooxidation may also occur	25 (±2)	CO_2 evolution O_2 consumption (BOD)	24 months	Natural or synthetic polymers, copolymers or mixtures, plastic materials with additives, water-soluble polymers ≥60% degradation of reference material and the measured CO_2 or the BOD values from the blanks at the end of the test are within 20% of the mean
ISO 18830:2016	Marine sandy sediment at the interface between seawater and the seafloor from sublittoral zone	15 – 25 (±2)	O_2 consumption (BOD)	24 months 3 replicates	Non-floating plastic >60 % degradation of reference material; BOD of negative control must not exceed a specified upper limit
ISO 19679:2016	marine sandy sediment at the interface between seawater and the seafloor from sublittoral zone	15 – 25 (±2)	CO_2 evolution	24 months 3 replicates	Non floating plastic ≥ 60% degradation of reference material at the end of the test. CO_2 evolved from negative control must not exceed a specified upper limit

Standards can be found on the ISO test and standard database.[7]

Table S6 Active standards for the assessment of biodegradation of plastics in anaerobic environments issued by ISO.

Standard / Method	Inoculum	Temperature/ °C	Measurement type	Duration and replicates	Test material and Validity criteria
ISO 13975	Sludge, livestock faeces or other organic waste	35 (±3) or 55 (±5)	CO_2 and CH_4 evolution	3 months 2 replicates	Plastic materials >70% degradation of reference material after 15 days; extent of degradation (%) must differ by <20 % between replicates
ISO 14853:2016	Sludge	35 (±3)	CO_2 and CH_4 evolution	3 months 2 replicates	Plastic materials >70% degradation of reference material; pH of medium must remain between 6 and 8
ISO 15985:2014	Methanogenic inoculum derived from anaerobic digesters operating only on pre-treated household waste, preferably only the organic fraction	52 (±2)	CO_2 and CH_4 evolution	4 months 3 replicates	Plastic materials >70% degradation of reference material after 15 days; extent of degradation (%) must differ by <20% between replicates

Standards can be found on the ISO test and standard database.[7]

Table S7 Microorganisms and the enzymes involved in the biodegradation process of polymers and their constituent monomers.[8 9]

Enzyme class	Microbes	Polymers
Cutinase[10, 11]	Bacteria, fungi	Polyesters, polyurethanes, polyethylene
Dioxygenase[12, 13]	Fungi	Polystyrene, polyethylene, Polyester
Esterase[14, 15]	Bacteria, fungi	Polyesters, polyurethanes, polyolefins
Hydrolase[16]	Yeast, fungi	Polyesters, polyurethanes, polyamides, polyolefins
Hydroxylase[17]	Actinomycetal fungi, bacteria	Polyolefins
Laccase[18, 19]	Fungi	Polyolefins, polyurethanes
Lipase[20]	Bacteria, fungi, yeast	Polyesters, polyurethanes, polyolefins
Polyurethanase[21, 22]	Bacteria, fungi	Polyurethanes
Protease[23, 24]	Bacteria, fungi, yeast	Polyesters, polyurethanes

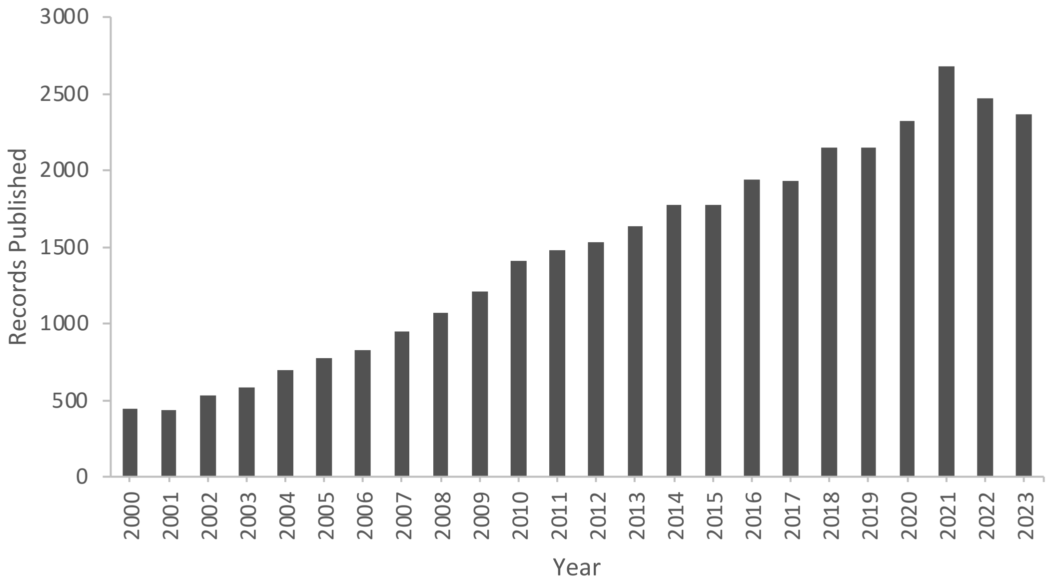

Figure S1 The number of records published each year with the keyword ‘biodegradable polymers’ in the title (Web of Science 02-06-2000 to 02-06-2023).

Table S5 Analytical methods used to evaluate the degradation processes of polymers. Adapted from Sun *et al*.,[8] Solanki *et al*.,[9] and Baidurah *et al*.[25]

Technique	**Principle**	**Advantages**	**Disadvantages**	**Reference**
FTIR	Identification of functional groups	Identify affected bonds Rapid and simple to use	Sample extraction prior to analysis required Additives present will cause discrepancies	Yu *et al.*[26]
GPC	Average molecular weights; molecular weight distributions	Direct proof of fragmentation	Suitable only as a complementary technique	Yang *et al.* `[27]
SEM	Identify surface deterioration by changes in polymer surface morphology	Minimal sample preparation Relatively quick to run and analyse data	Expensive Artifacts possible Limited for lab use as requires a vacuum environment Sample needs to be a solid Microbial support and biofilm effect may cause discrepancies	Nisa *et al.*[28]
Radio labelling	Radioactive isotope tracking e.g., ^{14}C and ^{3}H to track the formation of CO_2 and H_2O	Identify metabolites and degradative pathways Non-destructive	Restrictive for field studies Expensive to produce tracer in sufficient quantities Environmental radiative hazardous	Réjasse *et al.*[29] Zumstein *et al.*[30, 31]
Comparison to ISO, ECS and ASTM standards	Gas (CO_2) emitted from degradation	Universal Simple	Challenging for aquatic systems Difficult for use in field studies Discrepancies may be caused by other materials	Beran *et al.*[32]

FTIR: Fourier Transform Infrared Spectroscopy; GPC: Gel Permeation Chromatography; SEM: Scanning Electron Microscopy; SEM: Scanning Electron Microscopy; ISO: Internal Standard Organisation; ECS: European Committee for Standardisation; ASTM: American Society for Testing and Materials.

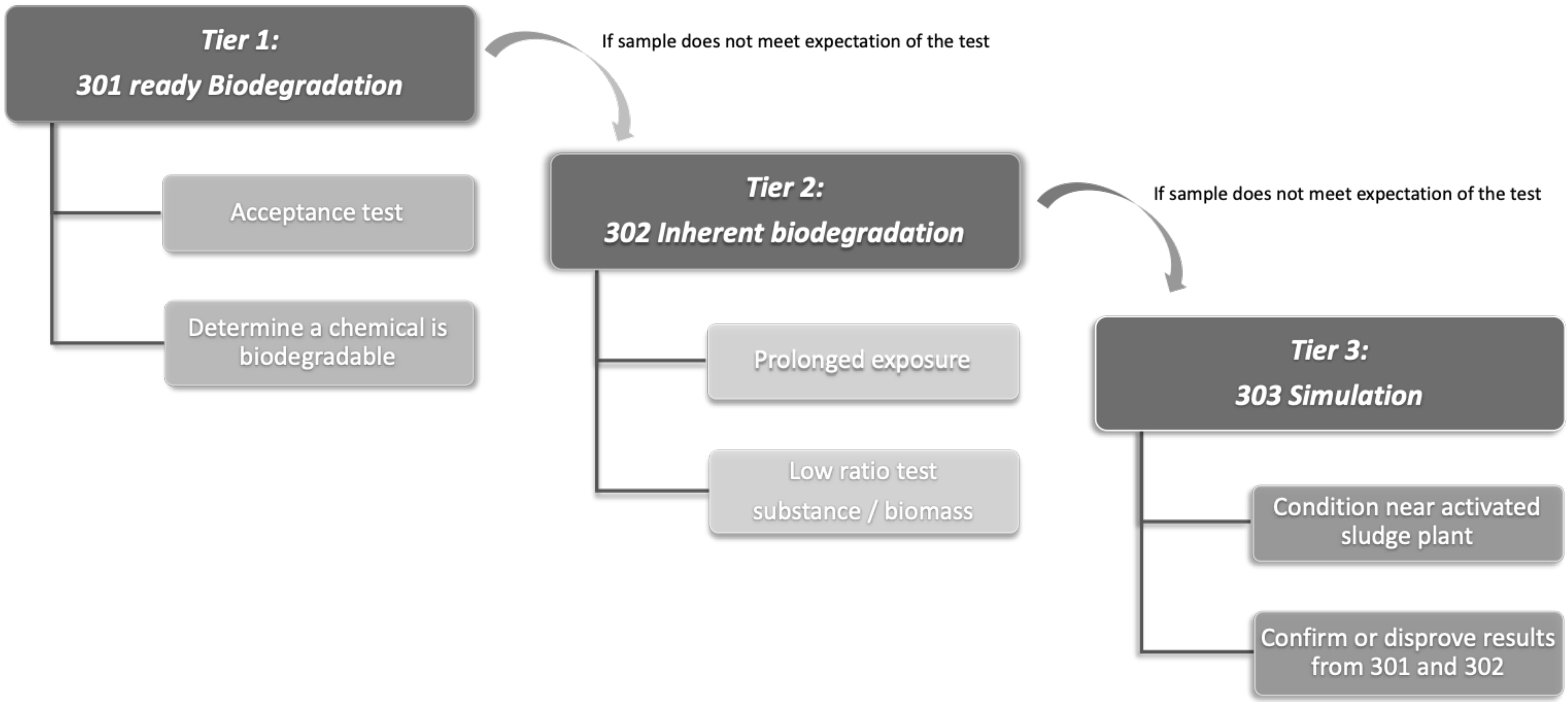

Figure S2 Principles of the tiered OECD biodegradation testing system.[33]
Tests 301 and 302 examine ready and inherent biodegradability, usually using a high substance to inoculum ratio, whilst test 303 examine simulated biodegradation, aiming to reproduce activated sludge treatment process. Failure to pass tests 301 and 302 does not lead to rejection – samples failing these tests could be demonstrated to be biodegradable using other inoculum, and/or longer exposure. Test 303 should be used for any samples that do not pass test 301 and 302. If a material is not shown to be biodegradable via test 303, an environment risk assessment can be performed.

In addition to OECD tests above, simulated biodegradation tests have been developed by OECD using radiolabelled test samples to study aerobic and anaerobic transformations in soil (OECD 307)[34] or in aquatic sediment systems (OECD 308)[35] and the aerobic mineralisation in surface water (OECD 309)[36]. The guideline methods for determining the extent and kinetics of primary and ultimate biodegradation of organic chemicals whose entry into the environment begins by discharge to wastewater is described on OECD 314.[37]

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