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Using microbes to remove microplastics from wastewater and sewage sludge

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Headlines

Issues

- Microplastics are a widespread form of plastic pollution. There is increasing evidence that they are a threat to human health and the environment.
- Microplastics in domestic and industrial wastewater become concentrated in sewage sludge during wastewater treatment processes. In 2020, water companies in England produced more than 800,000 tonnes of sewage sludge from urban wastewater.
- More than 90% of UK sewage sludge is spread on agricultural land as a fertilizer and soil conditioner. This provides a pathway for microplastics to enter the terrestrial environment.
- There is currently no UK legislation defining safe limits for microplastics in sludge and soils but future regulation is a possibility.
- There is currently no technology available to remove microplastics from wastewater treatment processes or the resulting sludge.

Solutions

- Safe limits for microplastics in treated sewage sludge, soils and water bodies should be identified. This will require a survey of the extent of microplastic pollution throughout the UK, including concentration, identity and characteristics of microplastics in each environmental reservoir, and understanding how microplastics affect different living organisms.
- Microbes or fungi that break down plastic could be added to existing wastewater treatment processes to remove microplastics and prevent their release into the environment. Alternatively, only the active enzymes (rather than the live microorganisms) could be added to the process.
- Currently, only polyester microplastics (11% of the total microplastic burden) could be treated in this way. Different biological agents would have to be discovered or developed to tackle other common microplastic polymers such as polypropylene or polyethylene.

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Objectives of the briefing paper

The Institute for Molecular Science and Engineering has brought together experts in the fields of polymer engineering, wastewater engineering, environmental toxicology, chemistry, synthetic biology and environmental policy to consider using microorganisms and/or enzymes to break down microplastics during the wastewater treatment process. In this briefing paper we will:

- Give examples of common polymers that form microplastics, and their uses.
- Provide an overview of sources and flows of microplastics.
- Explore the effects of microplastics in the environment and on human health.
- Discuss the wastewater treatment process and the impact of microplastics in sewage sludge on microbial digestion and biogas generation.
- Show how microorganisms and enzymes (biotechnological methods) could be used to remove polyester-based microplastics from sewage sludge to prevent their release into the environment.

Introduction

Plastics are versatile and inexpensive materials that can be moulded into complex shapes for a wide variety of applications. For example, flexible plastic packaging film keeps our food fresh, prevents contamination and reduces food waste. Rigid plastics are widely used in the transportation sector as they are lightweight and help reduce fuel consumption. Many clothing items are made of plastic fibres. As a result, plastics are ubiquitous materials which are important in modern everyday life. In 2021, global plastics production was estimated to be 390 million tonnes (Mt).¹ The OECD has estimated that on current trends, annual global plastic use will reach 1,231 Mt by 2060.² In tandem with production growth, the market value of plastics is also expected to grow. In 2021, the global plastics market was valued at US\$593B, and this is projected to reach a value of US\$810B by 2030.³

The main ingredient of a plastic material is a polymer. Figure 1 shows some common polymers, with their structural subunits called monomers, together with some common objects made of each. However, a plastic product does not just consist of the polymer but also additional chemicals⁴ to change how the total plastic formulation behaves, e.g. colourants, plasticisers and flame retardants. These additives may be harmful if leaked into the environment.⁵

Plastic in the environment

As plastic use is forecast to increase, so too is the amount of plastic waste generated. The OECD business-as-usual scenario predicts that annual global plastic waste will triple from 353 Mt in 2019 to 1,014 Mt in 2060, with 34–55 Mt finding its way into the environment.⁶ This is problematic as plastics are materials with long environmental persistence, with degradation time ranging from 10 to 1,000 years.⁷ The degradation time of a polymer depends on exposure to environmental conditions, particularly light, oxygen, water and microorganisms, which cause photo-degradation, oxidative degradation, hydrolytic degradation or biodegradation respectively.⁸ Polymer degradation rates also depend on the polymer's molecular structure. For example, polyethylene (PE, see Figure 1) degrades very slowly in the natural environment. The C-C bonds in PE are not biodegradable, do not undergo hydrolysis readily, and their oxidative degradation is undetectably slow at ambient temperatures. Nevertheless, this does not mean that PE does not degrade: it just takes a long time.

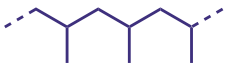
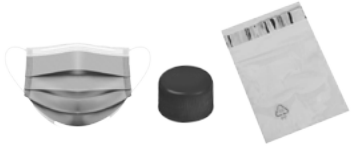
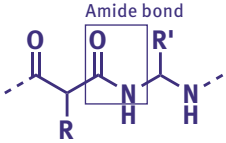
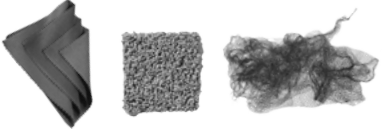
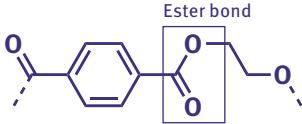
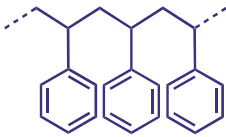
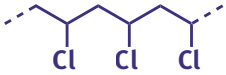
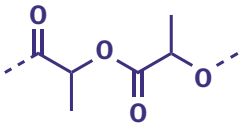
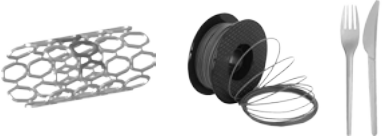
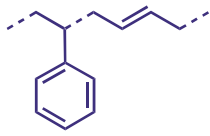
PE Polyethylene	 
PP Polypropylene	 
PA Polyamide	 
PET Polyethylene terephthalate	 
PS Polystyrene	 
PVC Polyvinyl chloride	 
PLA Polylactic acid	 
SBR Styrene butadiene rubber	 

Figure 1. Some of the most common polymers which form microplastics, with their chemical structures and examples of the kinds of plastic objects they are used in. PE, PP, PS, PVC and SBR all have either stable carbon-carbon single bonds (C-C) or carbon-hydrogen single bonds (C-H), making biodegradation difficult. Ester bonds in PET and PLA and amide bonds in PA make biodegradation easier.

Two potential countermeasures to this problem are to reduce plastic use (and therefore waste) and to substitute conventional plastics with biodegradable plastics (Box 1):

- In the UK, legislation has been introduced to reduce the use of plastics, such as introducing a charge for single-use carrier bags,⁹ banning the manufacture and sale of products containing microbeads,¹⁰ and banning some single-use plastic items, like straws and plates.^{11,12} The introduction of a plastic packaging tax¹³ and an Extended Producer Responsibility Scheme for packaging (pEPR)¹⁴ aim to promote the use of recycled plastic and the responsible management of plastic waste.
- Biodegradable plastics are made from polymers which can be degraded relatively quickly by microorganisms, where the degradation involves reducing polymer molecule length.¹⁵ However, their degradation depends on the presence of specific environmental conditions. Where these are not present, degradation of biodegradable plastics can take as long as for non-biodegradable plastics.¹⁶

Despite these measures, plastics of all kinds will undoubtedly still enter the environment. We need to identify the specific routes by which this happens and develop tailored ways to prevent it.

Box 1. Bioplastics explainer

Bioplastics is an umbrella term for plastics which are bio-based and/or biodegradable.

Bio-based plastics are made (in total or in part) of polymers obtained from renewable resources such as plants and plant materials e.g., sugarcane, lignin and starch, or from biopolymers produced by microorganisms.¹⁷ They are not necessarily biodegradable. Some conventional fossil-based plastics can also be made from renewable resources, e.g. bio-PET obtained from ethanol. However, bio-PET has the same properties as its fossil-based counterpart.

Biodegradable plastics can degrade due to the action of naturally occurring microorganisms, which sever long polymer chains into progressively shorter ones.¹⁵ An example is polylactic acid (PLA) in Figure 1. Biodegradable plastics can be made from fossil or bio-based resources. When conditions are right, they can degrade quickly. Outside these conditions, biodegradation may be partial (into microplastics), slow or non-existent.

Microplastic sources

Plastics in the environment can fragment into smaller pieces, which also degrade very slowly. These vary in size, but a common category is microplastics, which are defined as having a diameter of less than 5 mm in any dimension. They are categorised as primary or secondary according to source (Box 2). Some of the most common polymers found in microplastics are listed in Figure 1. Biodegradable plastics must also be considered as these can generate microplastics too.^{7,16,18–20}

Box 2. Sources of microplastics

Primary microplastics are purposely manufactured to be less than 5 mm in diameter. These include nurdles (tiny pellets of plastic that are used to manufacture plastic products), microbeads used in personal care products such as shower gels and toothpastes (although banned in the UK since 2018),¹⁰ and bead-blasting media. They also arise from:

- manufacture and laundry of synthetic textiles made with polyester (PET) and polyamide (PA),²¹
- fragmentation of plastic-containing wet wipes when in the aquatic environment,²²
- wear and tear of tyres,^{23,24} brake pads and road markings,
- dislodging and transport of crumb rubber (produced from old tyres and used as infill for artificial sports pitches) into the wider environment by weather, pitch maintenance and in sports clothing/shoes.

Microplastics and micro rubbers are closely related but have distinct physicochemical properties and sources.

Secondary microplastics can arise from the fragmentation of larger plastics such as plastic bags, bottles, fishing gear and agricultural plastic mulch films found within the environment or landfill. These are degraded by exposure to UV radiation, thermo-oxidation, hydrolysis, abrasion from wave action and particles of sediment, and microbial activity.

Microplastics in the environment

Microplastics can flow between environments via a variety of processes (Figure 2). They can enter the atmosphere through soil and urban dust emissions and via sea spray. They can enter aquatic environments in runoff from roads and from agricultural land, and in leachate from landfill. Furthermore, they can enter the terrestrial environment through the application of sewage sludge to agricultural land, from leachate from landfill, and from fragmentation of discarded macroplastics (plastic items with a diameter $\geq 5\text{mm}$).

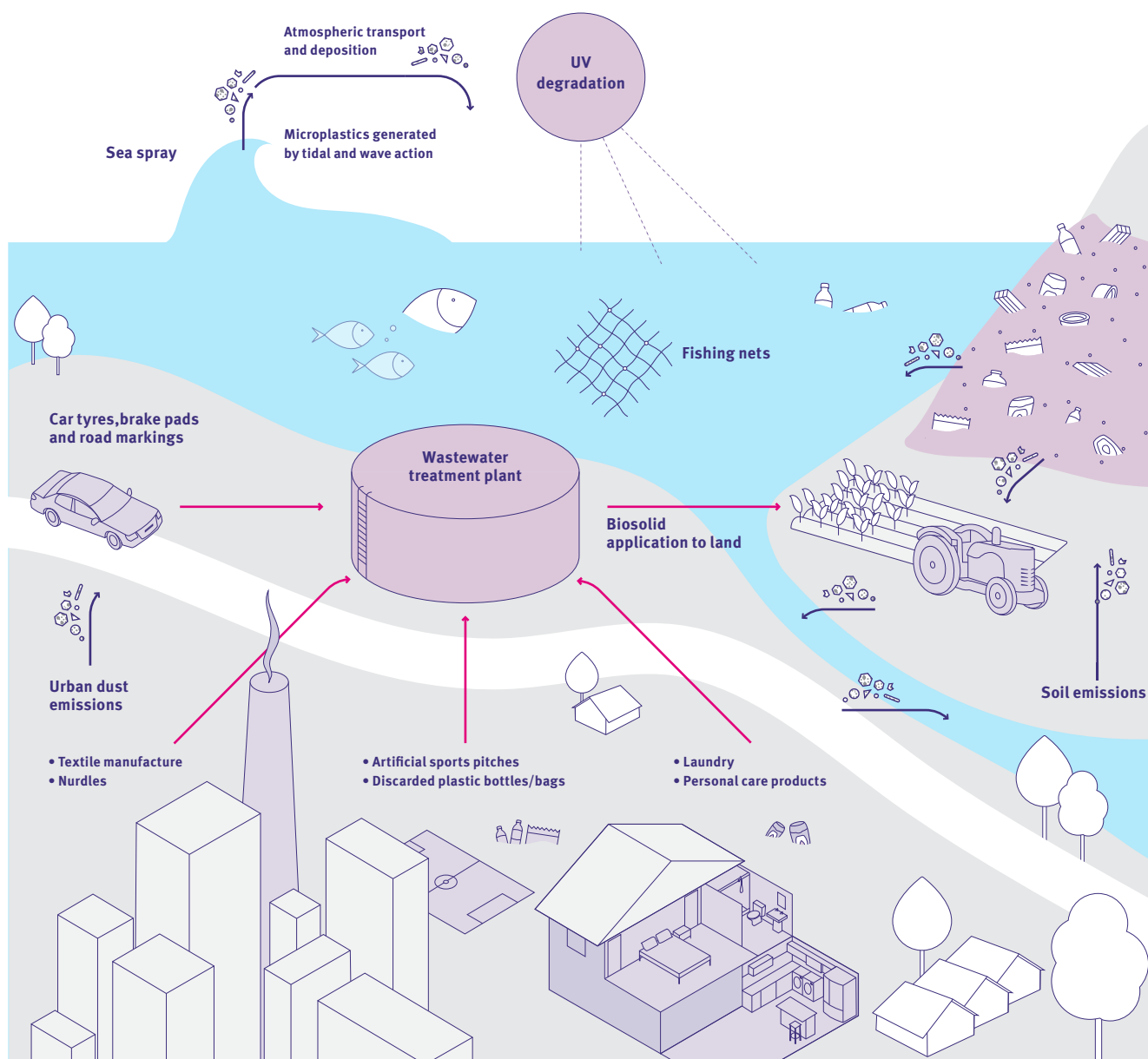


Figure 2. Sources of microplastics in sewage sludge and examples of how microplastics flow through different environments.

Microplastics in the environment could have significant consequences for different ecosystems, human health and food chains (Box 3). In the UK, the risk this presents is beginning to be recognised and preventive measures taken. In its 2023 Plan for Water,²² the Department for Environment, Food and Rural Affairs (Defra) recognised the need to prevent microplastics entering the aquatic environment,

and to better understand the levels of microplastics in this environment, and their effects on wildlife and humans. Defra's suggested solutions include developing low-cost microfibre filters for washing machines and banning the sale of plastic-containing wet wipes (subject to public consultation). The same measures are also needed for terrestrial and atmospheric environments.

Box 3. How much is known about the effects of microplastics on the environment and human health?

In both freshwater and marine environments, high levels of microplastic pollution are predicted to adversely impact plants, animals and microbial communities.²⁵ Darwinian fitness parameters, such as survival, growth, and reproduction, have experimentally been shown to be affected in a range of aquatic invertebrate and vertebrate species. Microplastics can influence the structure and function of microbial populations, which can change food web relationships and carbon cycling.²⁶ Furthermore, evidence suggests that lower trophic species in contaminated areas ingest microplastics, which thus enter the food chain from an environmental source. Seafood has been studied for its microplastic content: observations in commercial species or specimens purchased from shops or markets highlight the potential for human consumption.²⁷

Microplastics have been observed in a range of beverages including bottled water, soft drinks and beer.^{28,29} Salt, honey, milk and rice have also been studied for microplastic contamination.^{30–33} Key food groups such as cereals and grains, roots, fruits and vegetables remain understudied yet comprise a large proportion

of the global population's diet. Here, microplastics in plants are likely to derive from soil. Soil-dwelling organisms and microbial communities may be affected by microplastics either indirectly via modification of the soil properties and/or chemical leachates, and directly following ingestion. Sensitive soil-dwelling organisms, such as the earthworm *Eisenia fetida*, are predicted to experience adverse effects associated with microplastics above a threshold of 82,000 microplastic particles per kilogram.³⁴

In addition to ingesting microplastics from food, humans may also inhale them from contaminated indoor and outdoor air. Occupational epidemiology studies indicate that inhalation of high levels of respirable plastic dust can lead to chronic inflammation and interstitial lung disease.³⁵ However, it is not clear how this high exposure, single source risk compares to everyday population exposure to a mixture of microplastics, other particles and chemicals. There is also a growing body of evidence to suggest microplastic ingestion may lead to adverse reproductive and multigenerational effects. It is not clear whether this is a physical effect of the microplastic particles, and/or due to their chemical additives or any contaminants they are carrying.³⁶ In addition, these studies focus on a narrow set of microplastics, with little resemblance to the real range of microplastics that humans are exposed to.

One major source of microplastics in terrestrial environments is sewage sludge derived from wastewater treatment plants (WWTPs), a nutrient-rich waste stream that is applied to agricultural land as a fertiliser or soil conditioner.^{37,38} In WWTPs, sewage sludge is treated to remove harmful microorganisms such as *Escherichia coli* by anaerobic digestion and lime stabilisation, before it is spread on fields.³⁹ Treated sewage sludge products are sometimes called biosolids, and these include digested cake, dried granules/pellets, lime cake and digested liquid.⁴⁰ In Europe,

50% of sewage sludge is converted this way into fertiliser for agricultural use.⁴¹ This practice, along with energy recovery from anaerobic digestion, is thought to be more sustainable than the alternatives of incineration or disposal in landfill, which are the preferred disposal methods in some parts of Europe (Box 4). However, wastewater treatment and anaerobic digestion processes do not destroy microplastics. Instead, microplastics from domestic and industrial wastewater (Box 5) are concentrated in treated sludge and are then applied to agricultural soils.^{42–44}

Box 4. Sludge disposal in Europe

Methods for disposal of sewage sludge vary across Europe. The four main disposal methods are: spreading on agricultural land, composting, landfill and incineration. In 2020, water companies in England produced 807,882 tonnes of sewage sludge from urban wastewater.⁴⁵ Of this 94% was used for soil and agriculture. Eurostat data⁴⁶ show that disposal of sewage sludge from urban wastewater treatment to agriculture is also the preferred method of disposal in Spain (87%, 2018 data), Ireland (89%, 2020 data) and Bulgaria (77%, 2019 data). In contrast, sewage sludge is mainly incinerated in Germany (74%, 2019 data) and the Netherlands (96%, 2020 data). Some countries adopted both agriculture and composting as disposal methods. For example, in Finland and Hungary in 2019, 40% and 20% was used in agriculture and 58% and 74% for composting, respectively. In contrast, data for 2020 show that both Malta and Serbia landfilled 100% of sewage sludge.

Box 5. Microplastics in wastewater in the UK

In some areas of the UK (including London), older sewage systems transport both domestic/industrial wastewater and surface water (rainfall runoff) to WWTPs. Domestic wastewater contains microplastics from microfibres released during laundry, and from personal care products (banned in the UK since 2018¹⁰). Surface water can contain microplastics from tyre wear particles, brake pads, erosion of road markings, and from the breakdown of larger plastics discarded in the environment.

Modern sewage systems deal with domestic wastewater and surface water separately. Domestic wastewater goes to WWTPs and surface water is discharged into existing water bodies as it is generally free of pollutants, although it is still likely to contain microplastics.

Wastewater treatment and sludge production

In WWTPs, a series of physical, chemical and biological steps filter out impurities in the liquid portion (influent) to meet specific quality standards, before discharging this effluent into waterways (Figure 3). In the **pre-screen** step, wastewater passes through a grid or vertical bars to remove large solid items, such as cans, plastic bottles, and natural debris. In the **pre-treatment** step, smaller floating solids are allowed to settle in the **primary sedimentation** tank, where they form a mass of solids referred to as sludge and are removed from the tank. The remaining wastewater is then transferred to an **aeration** tank where it is mixed with air to allow aerobic microorganisms (bacteria) to break down the organic matter (**activated sludge**). The mixture then flows into the **secondary sedimentation** tank, where the activated sludge settles through gravity. It is pumped off and combined with primary sedimentation sludge. The **clarified wastewater** may be disinfected with **chlorine, ozone or ultraviolet (UV) light** in the **tertiary stage** to destroy pathogens, before being discharged into waterways.

The combined sludge obtained from the primary and secondary sedimentations is treated through either composting or **anaerobic digestion**, where microorganisms

break down organic matter. In the presence of oxygen, compost is produced, while in its absence (anaerobic conditions) **biogas^a** is produced, which is a valuable source of renewable energy. Anaerobic digestion also destroys potentially dangerous pathogens. At some WWTPs, sludge is first sterilised by **thermal hydrolysis** before anaerobic digestion. High temperatures (around 170°C) and pressures break down organic matter and sterilise sludge by, for example, rupturing bacterial cells. Following anaerobic digestion, **dewatering** thickens the treated sludge, which can then be applied to agricultural land.

Various studies have shown how microplastics may affect water treatment processes and biogas generation (Box 6).

Current estimates of the quantities of microplastics applied to soils are variable. Microplastic concentrations of up to 14,000 items/kg of sludge have been reported.⁴⁷ A study based on wastewater-derived digestate inputs calculated that between 63,000 and 430,000 tonnes of microplastics are spread onto European agricultural soils every year,⁴¹ an amount larger than on the ocean's surface.⁴⁸ Another study based on WWTP data calculate that between 31,000 and 42,000 tonnes of microplastics are applied annually to soils throughout Europe via sewage sludge.⁴⁴ However, this is likely to be an underestimate as it did not include microplastics less than 1 mm in size.

[a] a mixture of methane, carbon dioxide and small quantities of other gases

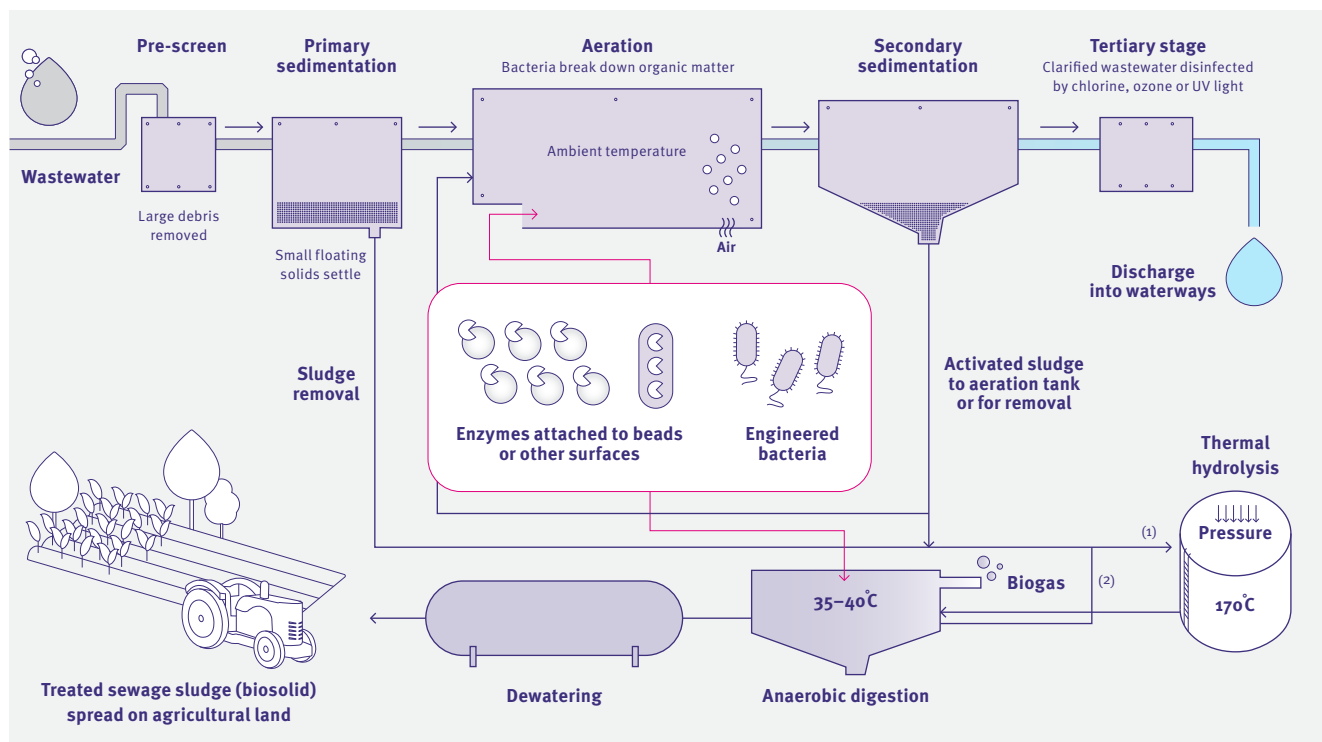


Figure 3. The wastewater treatment process, with the successive physical, chemical and biological steps which remove impurities from wastewater and concentrate sludge. The pink box and arrows indicate the stages where microbes or enzymes could be added to break down microplastics.

Note: (1) sewage sludge can undergo thermal hydrolysis before anaerobic digestion or (2) go to anaerobic digestion directly.

Box 6. Microplastics and wastewater treatment

The presence of microplastics may reduce the functioning of multiple elements of the wastewater treatment process, including anaerobic digestion, biogas generation and sludge characteristics.⁵³ Wastewater treatment can also affect microplastic characteristics. For example:

- Large amounts of microplastics may lead to the generation of reactive oxygen species (ROS). These are poisonous to microorganisms in the digester, resulting in reduced cell viability.⁵³ Short exposure to PE microplastics at a concentration of 100 and 200 particles per gram of total solids significantly decreased methane production,⁵⁴ which reduced the productivity of biogas generation.
- Some chemicals leaching from microplastics can reduce digestion efficiency. This may depend on concentration, for example, a low level of Bisphenol A (BPA) leached from PVC had a positive effect on methane generation but a negative effect at higher concentrations.⁵⁵

- Microplastics may cause incomplete anaerobic digestion. PE microplastics at concentrations between 1–200 particles per gram of total solids reduced methane production by 5–11% compared to a control.⁵⁶
- Microplastics can break down into nanoplastics and can attach to cell membranes. A high dose of PS nanoplastics adhering to cell membranes of microorganisms decreased activity and methane production.⁵⁷
- Changes to the physicochemical properties of microplastics that have been through the WWTP allow them to adsorb more pollutants, e.g. the heavy metal cadmium.⁵⁸ Heavy metals may affect the activity levels of enzymes involved in anaerobic digestion, but the impact is not yet clear.⁵⁹
- Microplastics may alter sludge characteristics to change dewatering, settling or thickening properties.⁵³

UK soils may be some of the most microplastic-contaminated in Europe.⁴⁴ A study using data from one WWTP in England calculated that the volume equivalent to >20,000 plastic bank cards were spread on agricultural soils per month via sludge products.⁴⁹ Furthermore, a study in Germany found up to 56.18 microplastic fragments/kg (dry) soil from land previously treated with sewage and even after 34 years with no further sewage application, these microplastic loads were still significantly higher than in untreated soils from surrounding areas.⁵⁰ There is currently no limitation on the quantity of microplastics that can be applied to agricultural land via sewage sludge in the UK (or in most European countries⁵¹), though some legal limits have been enacted, e.g. in Germany, the limit for plastics larger than 2mm in fertiliser is up to 0.1% (w/w).⁵²

Current regulation of sewage sludge use in agriculture in the UK

Sludge mainly consists of human waste, but it can also include waste from other sources such as industry and storm or surface run-off. Therefore, it can contain potentially toxic elements (PTEs) and pathogens. The risks from these depend on, for example, current levels of PTEs in the soil and how the sludge has been treated before agricultural use. The PTEs currently monitored in sludge and soil to ensure that they do not exceed accepted safe levels include: lead, mercury, molybdenum, zinc, copper, nickel, arsenic, cadmium, selenium, chromium and fluoride.³⁹ Dry and organic matter content, pH, nitrogen and phosphorus levels are also monitored³⁹ but microplastic levels are not.

Treatment and use of sewage sludge is currently regulated by the Environment Agency through the Urban Wastewater Treatment Directive (UWWTD), the Environmental Permitting (England and Wales) Regulations (EPR) and the Sludge (Use in Agriculture) Regulations (SUiAR), supported by Sewage Sludge in Agriculture Code of Practice.³⁹ However, the Environment Agency has recently reviewed these regulations⁶⁰ to consider the evolving nature of sludge management and has decided to bring sludge (and septic tank sludge) into the EPR, so the SUiAR will no longer be used. This review included the risks emerging from microplastics (as well as those posed by organic and inorganic chemicals and anti-microbial resistance) and the increased importance of energy generation from sludge. The EA concluded that, as 96% of UK sewage sludge is recycled to land, emerging risks need to be identified and understood in conjunction with the development of effective management systems.

European legislation on the application of sludge-derived digestate on agricultural land, including the Water Framework Directive (2000/60/EC)⁶¹ and Urban Wastewater Treatment Directive (91/271/EEC),⁶² makes no reference to quantifying, limiting, or controlling microplastics. However, the European Union recently adopted a Drinking Water Directive (2020/2184/EU),⁶³ which acknowledges microplastics as a matter of emerging concern and introduces mandatory monitoring in water bodies used for the abstraction of drinking water.

Future regulation of sewage sludge use in agriculture in the UK

Future legislation setting safe limits in the UK could affect sludge use, as sludge containing levels of microplastics exceeding safe limits would require alternative disposal methods. Currently, sludge that is not applied to agricultural land is dried and either landfilled, incinerated, or sent to an energy-from-waste facility (i.e. incineration with energy recovery). Incineration (with or without energy recovery) represents the only way to effectively eliminate microplastics. However, this process is associated with significant greenhouse gas emissions (0.3–0.8 tonnes CO₂eq per tonne of sludge, depending on drying method and fuel type⁶⁴) and may release other pollutants into the atmosphere.⁶⁵ Furthermore, disposing of sludge by thermal treatments such as gasification is costly and energy-intensive compared to application on land and would require investment in new infrastructure or transport to alternative sites for processing.

Furthermore, a reduction in the amount of sludge available for land application would affect biosolid markets. The value of nutrients in biosolids to the agricultural sector (mainly phosphate, nitrogen, potassium, magnesium and sulphur) is approximately £60M per annum with high demand for biosolids from farmers, as nutrients are worth around £330 per hectare.⁴⁰ Reduction in this supply may increase reliance on synthetic fertilisers, but their manufacture, transport and use have environmental impacts including greenhouse gas emissions.⁶⁶ Additionally, the manufacture of synthetic fertiliser requires phosphorus but uncertainty surrounding future supplies of this mineral have been reported.⁶⁷ Therefore, sewage sludge may become an increasingly important source of phosphorus for agriculture.

Two essential preliminary steps to legislating safe limits of microplastics in biosolids and soils are to:

- establish standardised protocols for the analysis of microplastics in sewage sludge and soils. This is essential to allow comparisons to be made between

studies, to compare microplastic output between WWTPs and across different countries.^{44,53,68,69}

- establish the typical concentration of microplastics in sewage sludge, their polymer and chemical composition, shape and size, to determine the effects and fate of microplastics in the soil environment.⁴⁴

If legislation to establish safe limits of microplastics in UK ecosystems is passed, then the quantities of microplastics reaching soils may need to be reduced to remain within safe limits. There are multiple ways to do this, including curbing plastic pollution, or using more biodegradable plastics (although these do not eliminate microplastic formation). Microplastics will continue to accumulate in wastewater from plastic fragmentation, laundry and road runoff (Figure 2) so the removal of microplastics from sludge will still be an essential part of protecting UK soils.⁷⁰

Biotechnology for the removal of microplastics from wastewater

Some of the processes already used in the treatment of wastewater can be exploited to remove microplastics from sludge. There is some evidence that anaerobic digestion and membrane filtration-based treatments do reduce the number of microplastics in sludge.^{71,72} WWTPs could also deliberately treat microplastics in sludge by adding a new biotechnological step to the wastewater treatment process. This step would involve adding plastic-degrading microorganisms (bacteria) and/or enzymes (the proteins produced by bacteria to catalyse microplastic biodegradation) into the biological stages of the water treatment process. Integrating this into existing WWTP processes would avoid major infrastructure costs. Enzymes are widely used commercially as supplements to septic tanks to enhance organic waste degradation.⁷³ They have been widely tested at

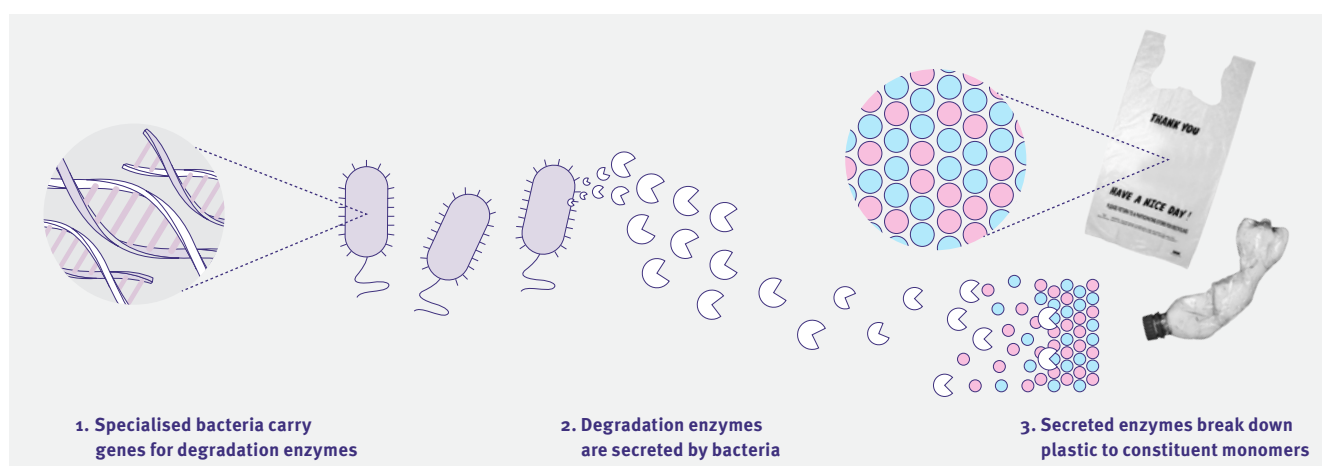


Figure 4. Plastic-degrading bacteria secrete enzymes to break down polymers into their structural subunits (monomers).

lab scale for their ability to remove major persistent pollutants (e.g. oils, pharmaceuticals, or pesticides) from wastewater. Their efficiency and effectiveness against pollutants, including plastics, have not yet been tested at WWTP scale.

How do microorganisms break down microplastics?

Some bacteria and fungi which live in environments which are highly contaminated with plastic, such as recycling facilities, can sometimes adapt to use these plastics as a source of energy. They do this by producing enzymes which break down the plastic structure into monomers (Figure 4).⁷⁴ As plastic is a relatively new-to-nature material – having only been used on a large global scale since the 1970s – most microorganisms do not possess native mechanisms to break down this class of materials. Instead, existing mechanisms within the cells adapt to enable this beneficial capability.

One group of enzymes with the potential to degrade the structure of some plastics are the esterases. These break the ester bonds which link the monomer subunits of PET (Figure 1). Esterase activity results in degradation of long polymer chains into shorter chains and eventually to the constituent monomers.⁷⁵ Other enzymes capable of degrading PET have been found in microorganisms living in habitats rich in plant material, such as compost heaps.⁷⁶ The waxy cuticle of plants is composed of the polyester polymer cutin, which is similar in structure to PET. Therefore, some enzymes capable of degrading cutin also exhibit activity against PET, e.g. leaf-branch compost cutinase (LCC), isolated from EXPO Park, Japan.⁷⁷

Naturally evolved enzymes capable of PET degradation are often inefficient: they have low activity rates, and the enzymes are only stable at low temperatures. This is not ideal as enzymatic degradation of plastic is easier at higher temperatures, close to the glass transition temperature of the polymer, when it becomes less crystalline. However, because degrading plant material produces heat, enzymes isolated from sites like compost heaps are often thermotolerant and are suitable for PET degradation at temperatures approaching 70°C.⁷⁸ Advances in protein engineering have been employed to produce enzymes with improved performance, for example, faster degradation rates, greater thermal stability, and increased binding ability between the polymer and enzyme.^{79–81} Enzyme variants are typically generated by genetic engineering, that is, modifying the DNA sequence coding for an enzyme of interest, which results in changes in the protein sequence at key sites within the enzyme structure. This produces novel enzymes with a greater suitability for use in industrial processes, e.g. for degradation of PET.⁸²

Instead of using enzymes in a WWTP, it is also possible to use the microorganisms responsible for their synthesis. These bacteria could produce the enzymes *in situ*, hence saving the costs of enzyme production and purification, by using organic

matter present in wastewater as a growth substrate. Some of these organisms can also use the monomers resulting from plastic hydrolysis as an energy source, e.g. the bacterium *Ideonella sakaiensis* 201-F6, which produces two enzymes, PETase and MHETase, which can degrade PET to intermediate products and the intermediate products into monomers respectively.⁷⁸ Unfortunately, *Ideonella* is not very efficient. Current research efforts, including ours, focus on genetically engineering other bacteria to endow them with the ability to grow robustly on PET by producing PET hydrolases, and to consume the resulting monomers for energy.

Biotechnology and wastewater treatment

So far, laboratory plastic biodegradation studies have focussed on polyester-based plastics, especially PET. French company Carbios have pioneered the application of biotechnology to break down PET on an industrial scale (Box 7), which is now used in recycling consumer plastics. This technology could be applied at a large scale via a WWTP^{83,84} to remove polyester-based microplastics in sludge, which come from polyester-based textiles. PET monomers are environmentally benign⁸⁵ and can be used as an energy source by many microorganisms, so they could be left in the resultant biosolids. An alternative option is to develop technology to recover PET monomers from WWTP tanks, for use in other industrial processes.

To avoid infrastructure costs, microbiological/enzymatic treatment for microplastics should be incorporated into existing biological treatment stages in WWTPs.^{83,84} Two potential plastic-removal stages are the **aeration** stage

Box 7. Carbios' technology for enzymatic degradation of PET from plastics and textiles

Carbios specialise in technologies which use enzymes to recycle plastic waste. They have developed an enzyme with more than 90% PET degradation efficiency over just 10 hours.⁸² This technology, known as C-ZYME®, has been implemented in a pipeline for the depolymerisation of PET on an industrial scale. This process utilises a 20 m³ reactor vessel which depolymerises 2 tonnes of PET in a single cycle. Contrary to conventional recycling technologies, which rely on heat and mechanical processes such as shredding and grinding, Carbios's system can treat mixed PET wastes including clear and coloured plastics, as well as polyester textiles. The monomers ethylene glycol (EG) and terephthalic acid (TA) are recovered from the degradation process and can be recycled into new PET or used for alternative downstream applications. New PET generated via this process is of the same quality as virgin PET produced directly from petrochemicals.⁸⁶

and the **anaerobic digestion** stage (Figure 3). In the aeration stage, light organic matter and dissolved materials are removed from settled sewage by microorganisms under aerobic conditions. In the anaerobic digestion stage, anaerobic bacteria break down organic matter in an enclosed tank without oxygen to generate biogas, water, and treated sludge and to destroy potentially dangerous pathogens. More aerobic than anaerobic plastic-degrading bacteria have so far been identified.⁸⁴ Examples of aerobic bacteria that produce plastic-degrading enzymes are *Thermobifida fusca*⁸⁷ and *Ideonella sakaiensis*.⁷⁸ Examples of anaerobic bacteria that produce plastic-degrading enzymes are two *Clostridium* strains, one naturally producing an esterase capable of hydrolysing polyester⁸⁸ and another genetically engineered to express the LCC enzyme to degrade PET.⁸⁰ In addition, *Clostridiaceae* and *Burkholderiaceae* bacteria that were part of anaerobic digestion microbial community in a WWTP demonstrated degradation activity towards the bioplastic polyhydroxyalkanoate (PHA), under relevant experimental conditions.^{84,89}

To ensure efficient biodegradation, microplastic-degrading microorganisms and enzymes will have to be active and resilient at the temperatures and pH ranges of the aeration tank and the anaerobic digester. Aeration tanks are open systems, so their temperatures vary depending on ambient temperatures. In contrast, anaerobic digesters are closed systems and most operate at mesophilic temperatures (35–40°C).⁹⁰ However, operation at thermophilic temperatures (55–60°C) or above⁹¹ would be closer to the glass transition temperature of PET (70°C),⁹² when it becomes more susceptible to biodegradation. Both digester types operate in near neutral pH conditions (pH 7.0), which is ideal for microbial growth and enzyme operation.⁹³ An additional improvement may be to immobilise the plastic-degrading enzymes on a supportive surface, e.g. beads, silica, carbon nanotubes, in order to improve their stability, recyclability and the rate of microplastic biodegradation.⁸³

The potential impact of biotechnology on microplastic concentration in sludge

At present, only microbes and enzymes capable of degrading PET have been discovered or engineered, so only this plastic type could be removed from biosolids using biotechnological methods. The levels of PET microplastics in biosolids have been increasing since the 1990s due to increased and widespread use of PET-based products, highlighting it as a material of increasing concern.⁹⁴ Microplastic abundance in UK biosolids is 14.8–17.9 microplastics per gram (wet weight),⁴⁹ and PET is reported to contribute to 11% of the microplastics found in biosolids.⁹⁴ Across the UK, 3.5 million tonnes of biosolids (wet weight) are applied annually to agricultural soil as a fertiliser and soil improvement treatment,^{40,49} over an area of 118,000 hectares of agricultural land.⁹⁵ Removing PET microplastics from biosolids, using a bio-based solution with 90% efficiency, could therefore avoid adding 5.13–6.2 trillion PET microplastic particles annually to UK fields (or 44–53 billion PET microplastic particles per hectare per year). To remove the other 89% of microplastics in biosolids, microbes and enzymes capable of biodegrading other plastic types should be identified and/or engineered, e.g. laccases and peroxidases to degrade PE.^{96,97}

Minimising risks associated with engineered organisms

There is strict legislation controlling the deliberate release of genetically modified organisms into the environment in the UK.⁹⁸ Any engineered microorganism used in a WWTP would need to be killed before treated water or sludge were released into the environment. A number of strategies can be employed here, including:

- treating wastewater with chlorine, ozone or ultraviolet light (Figure 3) will destroy engineered bacteria before discharging into waterways.^{71,83}
- thermal hydrolysis before anaerobic digestion (Figure 3), which is already used in some WWTPs. In this process, the sludge is heated to around 170°C under pressure, and when the pressure is released, microorganism cells rupture. In this way, any engineered microbes used in the aeration stage would be eradicated.
- using enzymes instead of bacteria in the aeration stage. Enzymes are proteins, not organisms, so they cannot reproduce and are safe to be released into the environment without being destroyed.
- engineering bacteria to thrive in the aeration stage but not to survive the anaerobic conditions of the digester, or the conditions outside of the water treatment process.

- adding an extra treatment stage after anaerobic digestion to destroy all microorganisms within sludge, but this would require investment in new infrastructure.
- immobilising bacteria onto support surfaces to avoid release may also be possible, in a similar way to enzymes being immobilised on metallic nanoparticles and recovered for reuse after wastewater treatment using magnets.⁸⁵

In order to choose the best option, a full assessment of the environmental risks associated with the use of engineered microorganisms or enzymes for the removal of microplastics from sewage sludge will be necessary.

Societal acceptance

There has been much controversy and debate over the use of genetic engineering (much of it related to the agricultural industry) in recent decades. This, coupled with concerns associated with microbes following the Covid-19 pandemic, shows the need to seek society's views on the application of microbial or enzymatic technology in the water treatment industry. Responsible research and innovation processes should be used at all stages of developing microbial and enzyme technologies to involve stakeholders and citizens in the research and ensure that outcomes are sustainable, ethically acceptable and socially desirable.⁹⁹ The authors have begun collecting stakeholder and public views on using biotechnology to address the plastic waste crisis through interviews, workshops and interactive presentations.

Conclusions and recommendations

Microplastics in water and soil are an emerging threat to the natural environment, wildlife and human health. Although recent legislative changes will curb some plastic pollution, plastic will continue to enter the environment. Microplastics will be generated from degradation of existing and new plastic waste, the manufacture and laundry of textiles, and the transport sector, among other sources. They will be present in wastewater entering wastewater treatment plants (WWTPs), where they may be detrimental to biosolid and energy production, and will eventually enter the terrestrial environment through the recycling of sewage sludge to agricultural land. To address this emerging threat, we recommend a number of actions for policy makers (in Defra, the Environment Agency or Ofwat, or the equivalent bodies for the devolved administrations), research funders, research performing organisations (RPOs) and water companies. Theme 3 in particular, below, is aligned with the 2023 National Vision for Engineering Biology¹⁰⁰ and would benefit from the additional investment and new infrastructure.

Theme 1: Quantify the extent of microplastic pollution (next three years)

- Develop standardised methodology^{53,68,69} for determining microplastic concentration, identity and analyses in:
 - natural waterbodies and soil environments across the UK (policy makers/research funders/RPOs).
 - wastewater influent and sewage sludge from WWTPs throughout the UK (research funders/water companies/RPOs).
- Develop an open database of concentration, identity and characteristics (colour, size, chemical composition, molecular weight, degradation by-products) of microplastics in natural waterbodies and soils across the UK which would indicate the extent of the microplastic problem within these environments (policy makers).
- Establish a surveillance programme to monitor changes in microplastic concentration, identity and analyses over time in waterbodies, soils, WWTP influent and sewage sludge, and to detect differences between regions of the UK (policy makers/research funders/RPOs).

Theme 2: Establish safe levels of microplastics (next five years)

This should encompass both biodegradable and non-biodegradable plastics in both sewage sludge and soils (policy makers/research funders/RPOs).

Theme 3: Develop biotechnological solutions for treating microplastics in WWTPs (next 10 years)

- Quantify the efficacy and efficiency of existing enzymes and microorganisms to break down polyester-based plastic, especially PET, in sewage sludge, and engineer

new microorganisms (and/or enzymes) if appropriate (research funders/RPOs).

- Establish feasibility and cost of incorporating this technology into existing wastewater treatment processes (water companies/Ofwat).
- Continue using responsible research and innovation to engage with and inform stakeholders and the public about this developing technology and gauge whether the technology is in stakeholder and public interest (policy makers/RPOs).
- Develop the technology from TRL2 (technology concept formulated) to TRL9 (actual system proven in operational environment)¹⁰¹ to ensure maximum efficacy and efficiency for polyester-based microplastic removal when integrated into existing water treatment processes (research funders/water companies/RPOs).
- Assess the environmental risks associated with the use of engineered organisms or enzymes for the removal of microplastics from sewage sludge (research funders/RPOs/policy makers).
- Test the efficacy of hydrothermal treatments such as thermal hydrolysis to destroy microorganisms and enzymes and prevent their release into the environment (research funders/RPOs/water companies).
- Establish the efficacy of the technology by monitoring polyester-based microplastic concentrations in WWTP influent and sewage sludge and by quantifying the impact on microplastic concentrations in agricultural soils and natural waterbodies (policy makers/research funders/RPOs/water companies/Ofwat),
- Develop further technologies to remove all types of microplastics from wastewater (research funders/RPOs).

It is also important to tackle microplastic pollution at source, for example, in the laundry process. The quantity of microplastics in wastewater can be reduced by fitting microfibre filters into washing machines to capture microfibres released by washing. France is the first country in the world to make microfibre filters in washing machines mandatory from 2025¹⁰² and similar legislation should be introduced in the UK. Defra already recognises the importance of encouraging the development and use of effective and cost-efficient microfibre filters for washing machines.²²

These recommendations will significantly contribute towards alleviating the burden of microplastics in terrestrial and other environments. These actions will help fulfil the United Nations Sustainable Development Goal 15 'Life on Land' which focuses on the protection, restoration and promotion of terrestrial ecosystems.¹⁰³

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