



# Can biotechnology turn the tide on plastics?

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Accumulation of plastic pollution in aquatic ecosystems is the predictable result of high demand for plastic functionalities, optimized production with economies of scale, and recalcitrance. Strategies are needed for end-of-life conversion of recalcitrant plastics into useful feedstocks and for transition to materials that are biodegradable, non-bioaccumulative, and non-toxic. Promising alternatives are the polyhydroxyalkanoates (PHAs), a vast family of polymers amenable to decentralized production from renewable feedstocks. Establishment of a global-scale PHA-based industry will require identification of PHAs with tailored properties for use as 'drop-in' replacements for existing plastics; use of low-cost renewable/waste-derived feedstocks; high productivity cultures that may be genetically modified microorganisms or non-axenic mixed cultures maintained by selection pressures that favor high PHA-producing strains; and low-cost extraction/purification schemes.

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## Current plastics production rates are not sustainable

Plastic production has grown exponentially with a 14-year doubling time since the 1950s [1<sup>\*</sup>]. A conservative linear increase in plastic production going forward is 10.8 million metric tons per year (Figure 1). This increase far outpaces the expected increase in recycling of ~0.8 million metric tons per year for 2018 [1<sup>\*</sup>]. The inevitable result is cradle-to-grave accumulation in landfills and ecosystems (Figures 2 and 3) [1<sup>\*</sup>]. While landfilling sequesters carbon, space for landfills is limited, especially in dense urban environments [2], increasing the probability that plastic wastes will enter sensitive ecosystems. This situation will be exacerbated by China's recent decision to halt plastic waste imports as

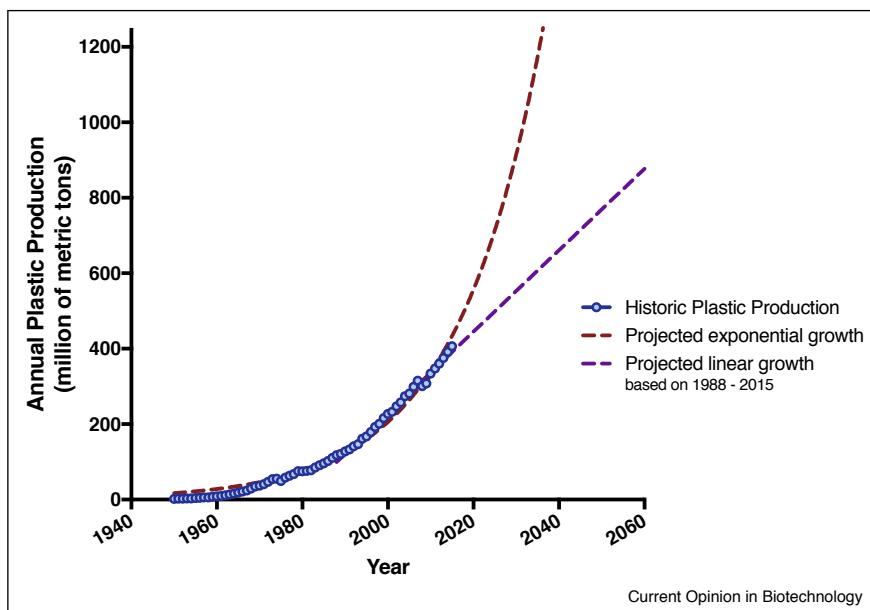
China has managed ~45% of all plastic waste generated since 1992 [3]. Accumulation of plastic in aquatic ecosystems already threatens wildlife health [4<sup>\*\*</sup>]. Human health may also be harmed by exposure to plastic additives, especially via microplastics in food, such as table salt [5,6].

Plastic-contaminated urban waters warrant special attention because these water bodies are both hubs of recreational and economic activity and delivery points for plastic and microplastics from rivers and storm water outfalls [7–9]. One of the few economic assessments of marine debris estimated that communities on the west coast of the United States spend \$13 per resident annually to clean up trash [10]. If extrapolated to the current global coastal population (40% of 7.6 billion people), such an effort would incur an annual price tag of \$40 billion. These prohibitive costs and the well-documented harm to ecosystem health [4<sup>\*\*</sup>] are compelling incentives to develop sustainable alternatives.

Current strategies for controlling plastic pollution in urban waters rely on wastewater treatment. In these systems, large plastic debris is removed by screening then landfilled; microplastics are mostly removed by attachment to biosolids that are then transferred to solids handling facilities [11]; a small fraction remains in the treated effluent [8,9]. When these biosolids are applied to land, the plastics can potentially re-enter aquatic ecosystems via runoff [12]. Release of plastic to such environments could be avoided if microplastics were designed to biodegrade in aquatic systems, such as wastewater treatment plants. Researchers have isolated plastic-degrading microorganisms from landfills and petroleum-production sites [13<sup>\*</sup>,14–16], but, to date, the rates achieved by isolated pure cultures have been low and almost all isolates are restricted to growth on a single type of plastic [16]. Microorganisms and enzymes are needed that can more rapidly and efficiently degrade complex plastic mixtures [16].

## From tiny bioreactors to new mitigation strategies

Recent research has identified one environment, in which structurally diverse plastics do undergo rapid degradation: the gut of arthropods, specifically insect larvae of *Tenebrio molitor* (mealworms) [17,18,19<sup>\*</sup>], *Plodia interpunctella* (Indianmeal moth) [20], and *Galleria mellonella* (wax moths) [21]. The most well studied are the mealworms, with half-lives for the conversion of ingested plastic to CO<sub>2</sub> on the order of 15–20 hours, much faster than rates observed for microbial isolates [22]. The mealworm gut microbiome can also degrade multiple types of plastics and mixtures (e.g. polyethylene plus polystyrene),

**Figure 1**

Projected increase in plastic production based on historic production data. Historic data are shown in blue circles. Dashed lines show projected growth. The red dashed line shows projected plastic production based on an exponential growth model ( $R^2 = 0.98$ ). The purple dashed line shows projected production based on a linear regression ( $R^2 = 0.99$ ) from the last three decades. Historic plastic production data from Geyer *et al.* [1\*].

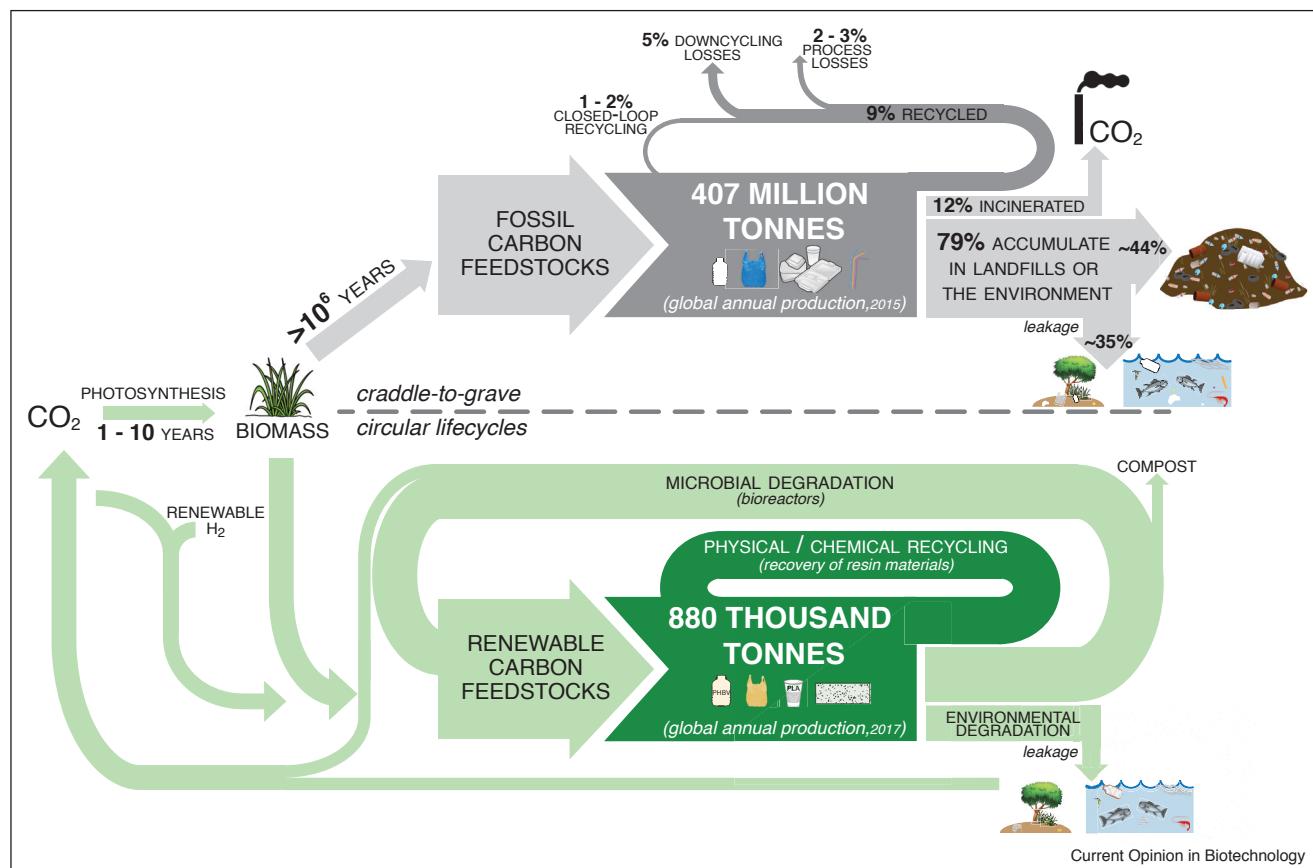
suggesting an initial non-specific oxidative attack [19\*]. A deeper understanding of the underlying molecular mechanisms, including the relative contributions of the insect itself [21] and the gut microbiome [20,22], could enable development of scalable enzyme-based strategies for plastic waste management [16]. Given that the products of mealworm degradation contain oxidized functional groups [18,19\*], it is possible that such metabolites could serve as substrates for future production of polyhydroxy-alkanoates (PHAs), converting an otherwise recalcitrant plastic into one that is biodegradable and sustainable [23]. This possibility has been demonstrated for polyethylene terephthalate (PET): enzymatic-mediated PET hydrolysis yields terephthalic acid and ethylene glycol [13\*,24\*], both of which can serve as feedstocks for PHAs [25,26].

Systems for plastic production and recycling have yet to fully address the fate of chemical additives, such as fire retardants and plasticizers [27]. Strategies are needed to ensure their removal from recycled materials and to develop green chemistry replacements [28]. Within the proposed ‘Ecocyclable’ framework (discussed later), chemical additives within new materials would be evaluated through tests of toxicology and bioaccumulation. Such assessments could be informed by non-profit organizations that provide screening tools and services to assist in selection of safe replacement chemicals (e.g. Clean Production Action, [29]).

### From linear to fully circular economies

Figure 2 illustrates the potential transition from recalcitrant plastics in the current linear economy to a circular economy, in which renewable plastics displace conventional recalcitrant plastics. The inputs for the linear path of recalcitrant plastics are fossil carbon feedstocks that required millions of years to accumulate, and recycle loops are small; the outputs either accumulate in landfills or the environment [1\*]. By contrast, renewable plastics fit within a circular economy with large recycle loops and feedstocks that are renewable over a short time (<10 years) [30]. In order to meet current plastic demands, a roughly 500-fold increase in renewable plastics production is required [31]. In addition, production costs need to be decreased by a factor of 2–4 to achieve cost parity with recalcitrant fossil carbon-derived plastics (Table S1).

Renewable plastic will need to match the in-use functionality of current plastics while also safeguarding the environment and enabling disassembly at end-of-life (Table S1). To that end, an ‘Ecocyclable’ framework has been proposed in which new materials are compared to reference polymers in terms of degradability, susceptibility to bioaccumulation, and toxicity (<https://ecocyclable.wm.edu>, [32\*\*]). The reference polymers are cellulose, the most abundant organic polymer on Earth, and polyhydroxybutyrate (PHB), an ancient and ubiquitous biopolymer that is synthesized into biodegradable granules by numerous Bacteria and Archaea [33].

**Figure 2**

Current linear cradle-to-grave pathways for recalcitrant plastics and circular pathways of future plastic materials. Data for recalcitrant plastics from Geyer *et al.* [1\*] and Ellen MacArthur Foundation [30]. Data for biodegradable plastics from European Bioplastics [31].

Within the Ecocyclable framework, biodegradability is assessed by comparison of test materials to either cellulose or PHB in aerobic soils, anaerobic (methanogenic) conditions, and aquatic environments [32\*\*]. Materials deemed ‘Generally Ecocyclable’ biodegrade in all three environments; materials deemed ‘Conditionally Ecocyclable’ biodegrade in one or more environment, but not all three.

### Low-cost, tailored to the application, and Ecocyclable

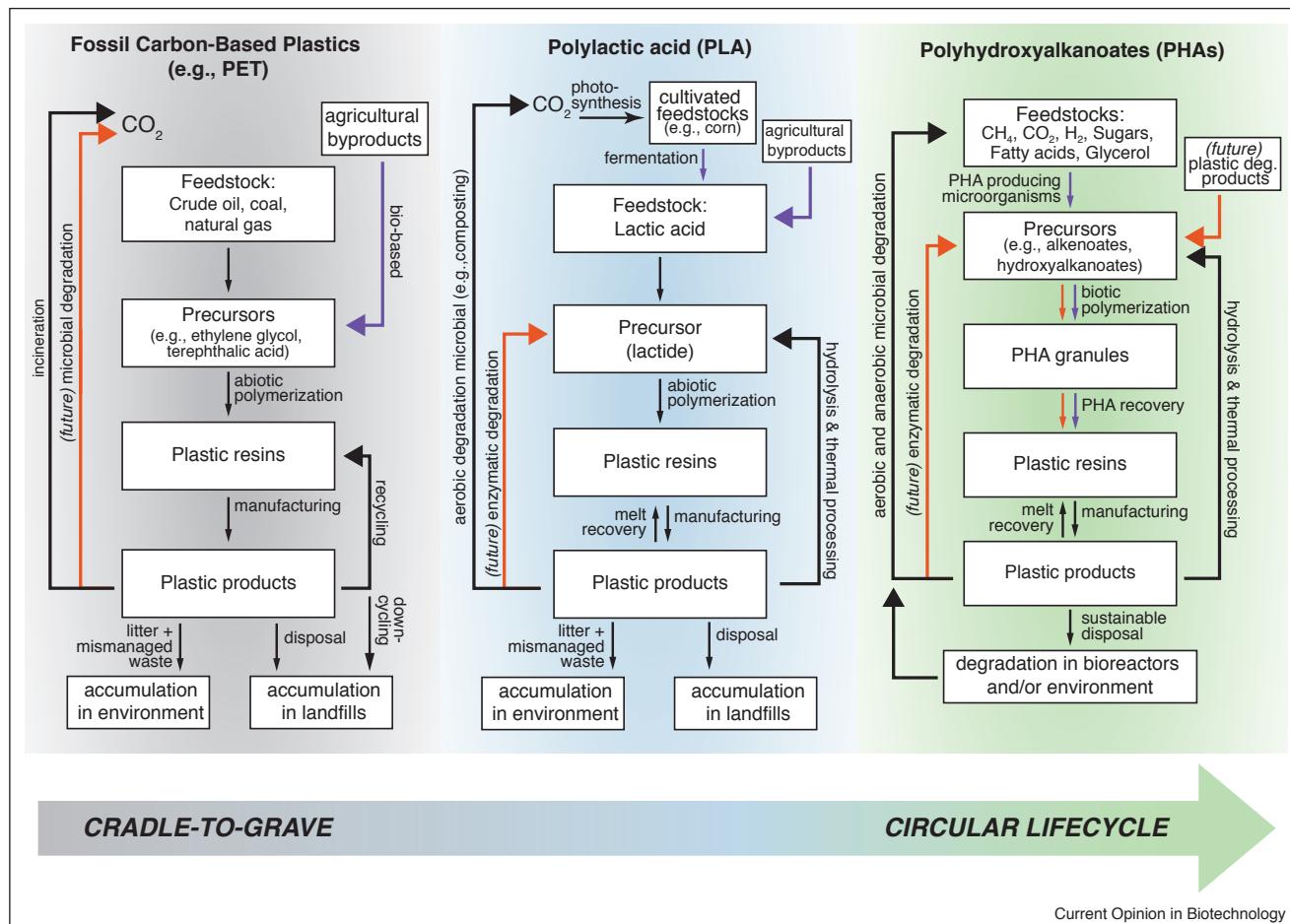
Figure 3 presents a transition sequence from polyethylene terephthalate (PET) to polylactic acid (PLA) to polyhydroxyalkanoates (PHAs). The order of this sequence reflects increasingly renewable, biodegradable, and ‘Ecocyclable’ materials. Unlike other fossil carbon-derived plastics (e.g. polystyrene), PET is recyclable without downcycling and typically does not contain toxic additives. It is recalcitrant under all environmental conditions and, therefore, not ‘Ecocyclable’. As noted previously, PET is susceptible to enzyme-mediated

degradation [13\*,24\*]; thus, future innovation could conceivably lead to a form that is ‘Ecocyclable’.

PLA is currently the most common renewable plastic [31,34]. It is made from lactic acid typically obtained from cultivated crops and would be classified as ‘Conditionally Ecocyclable’ because it degrades in some environments (e.g. thermophilic composts), but not others (e.g. marine environment) [32\*\*,35]. Copolymerizing PLA or creating PLA composites with other sustainable materials can result in better material properties and increased degradability [34]. A recent study demonstrated PLA depolymerization by microbial carboxylesterases [36], paving the way for future enzymatic control strategies like those suggested above for PET or other fossil carbon-derived plastics, potentially leading to a ‘Generally Ecocyclable’ form of PLA.

PHAs are a vast family of moldable biopolymers that could serve as ‘drop-in’ replacements for persistent plastics [37]. Most PHAs are likely to be ‘Generally Ecocyclable’ as they are close relatives of the reference

Figure 3



A transition sequence for plastic materials. Black arrows show conventional pathways. Purple arrows show existing biotechnology-based pathways. Orange arrows show future biotechnology pathways. Timeline at the bottom shows the transition in the dominant type of plastic used toward an increasingly renewable and degradable materials.

polymer PHB. PHB was first described in *Bacillus megaterium* as a homopolyester of 3-hydroxybutyric acid [38]. Interest in PHB surged following a 1958 report showing that *B. megaterium* and *B. cereus* use PHB as a carbon and energy storage polymer [39]. Studies thereafter focused on PHA synthesis in *Cupriavidus necator* (formerly known as *Hydrogenomonas eutropha*, *Alcaligenes eutropha*, and *Ralstonia eutropha*) [40••] and on the development of a genetic toolkit for recombinant *Escherichia coli* [41,42]. More recently, whole genome sequencing and phylogenetic tools have greatly expanded understanding the biodiversity of Bacteria and Archaea that produce PHA granules and on biosynthesis pathways. These data can be mined to identify PHA-producing organisms with special capabilities of interest. Examples include extremophiles, such as *Haloferax mediterranei*, a model strain for PHA metabolism in Archaea [43], and *Halomonas*, a

halophilic gammaproteobacterium, both of which can be grown at high salinities, a feature that could decrease costs for media and PHA recovery [44].

Critical bioprocess challenges for PHA production are feedstock and production costs, the need to tailor polymer structure for specific applications, maximizing productivity (g PHA/L-h), and optimizing downstream polymer extraction and purification. Costs can be decreased through economies of scale and use of waste-derived feedstocks [45•]. Economies of scale could be achieved in large biorefineries where gas pipelines deliver renewable feedstocks, such as compressed biomethane, CO<sub>2</sub>, H<sub>2</sub>, and syngas [45•,46]. Alternatively, economies of scale might be achieved through a decentralized approach in which production facilities are co-located near wastewater treatment plants, landfills, or food waste management facilities [37,45•].

Waste-derived feedstocks (e.g. H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>, glycerol, fatty acids, lignocellulose, and potentially future degradation products of recalcitrant plastic waste) avoid competition with cultivated crops for land and the associated water, energy, and fertilizer requirements [45\*].

Tailored PHAs are needed because PHB is brittle and has a narrow thermal processing window, limiting its utility as a replacement material for fossil carbon-derived plastics [46]. Fortunately, blends and copolymers, such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and more than 150 PHA variants, have material properties that can rival recalcitrant plastics (Table S1) [33,47]. The structure and in-use properties of PHA co-polymers can be adjusted by the choice of co-substrates [45\*,46], application of selection pressures on mixed cultures [46], and genetic manipulation of biochemical pathways and enzyme optimization [48]. Four classes of PHA synthase are known [42], and crystal structures are now available for two Class I synthases [49,50]. Knowledge of PHA synthase structure can provide deep insights into its mechanisms [49–51] and facilitate enzyme re-engineering for production of a diverse suite of PHA co-polymers with differing monomer lengths and sidechains and even PLA [52].

Both synthetic biology and application of feast-famine selection pressures to non-axenic mixed cultures can be used to achieve high PHA productivity [46,48,53]. High rates of PHB production have been achieved with recombinant *E. coli* strain grown on agricultural wastes (productivity >2 g PHB/L-hr [54] and by *Cupriavidus necator* DSM 545 grown on waste glycerol (>1 g PHB/L-hr) [55]. Typically, PHA is produced in two steps: in the first, growth rates are maximized during a period of balanced growth, and cells grow under replete conditions, that is, with sufficient carbon source/electron donor, electron acceptor (typically oxygen), and nutrients; in the second, PHA granule accumulation occurs under conditions of unbalanced growth, that is, with carbon/electron donor present in excess but cell division limited by lack of an essential nutrient (e.g. N, P, K) or an electron acceptor (e.g. oxygen, nitrate) [33,46]. Ultimately, cells are harvested at high density, which can exceed 100 g/L [56]. Further increases in productivity are possible through both pure culture synthetic biology and mixed culture selection strategies: mutants that generate PHA constitutively could eliminate the need for two-stage processing [57] and imposition of feast-famine regimes in non-axenic sequencing batch reactors can select for competitive dominance by high PHA-producing strains, such as *Plasticicumulans acidovorans*, a gammaproteobacterium that can accumulate >85% PHB in a second-stage fed-batch bioreactor [58,59].

Efficient processing is also needed for recovery of PHA from the cells and its purification. Current techniques

involve heat treatment or freeze-thaw to disrupt the cell membrane followed by one of several treatments: (1) solvents plus centrifugation, (2) chemical disruption plus centrifugation, or (3) use of enzymes, surfactants, and other agents to break the cells plus dissolved-air flotation [45\*,56]. These methods are time, energy, and chemical-intensive [45\*,56]. Biotechnology has the potential to improve this step through genetic modifications that enable secretion of PHA molecules into the extracellular media [60]; feeding PHA-enriched microbial biomass to animals such as mealworms that digest the bacterial cell-wall, releasing relatively pure PHA granules in the excreted fecal pellets [61]; and use of halophilic Archaea to produce PHA followed by transfer of PHA-rich cells to deionized water where osmotic pressure causes cell lysis and the release of the polymer [62,63].

### Design for disassembly

Insights from phylogenetics and metagenome screening can potentially be harnessed to expand end-of-life options. The principle of ‘design for disassembly’ [64] has been championed for years at the macro scale; advances in bioengineering now offer similar potential at nanoscale. The natural architecture of PHA granules offers inspiration with PHB depolymerases in the granule surface allowing access to amorphous regions susceptible to depolymerization [65]. It remains to be seen whether such features (e.g. plastic degrading enzymes) could be added to improve the ‘Ecocyclability’ of new materials [66]. Further knowledge of underlying biochemical degradation mechanisms might enable the design of plastics that are susceptible to disassembly upon exposure to specific environmental conditions, such as high salinity, yielding harmless, or even useful products.

### Outlook

Biotechnology opens the door for improved plastic design in terms of its production, in-use properties, and end-of-life fate. Efficient strains and mixed cultures, low cost feedstocks, optimized fermentations, and improved extraction schemes can usher in a new generation of materials that can function as drop-in replacements for existing plastics. Deeper understanding of the mechanisms of recalcitrant plastic degradation can inform design of new materials, perhaps enabling development of new materials that are readily converted into useful feedstocks.

Widespread adoption of Ecocyclable standards or their equivalent can enable nuanced regulation of plastics use, reuse, and disposal while encouraging innovation. With increased public awareness of the capabilities of biotechnology and enabling legislation, biotechnology is poised to play an important role in the remediation of plastic pollution; development of biodegradable, low-cost, and safe alternatives; and integration of ecocyclable plastics into circular economies.

## Conflict of interest statement

Nothing declared.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.copbio.2019.03.020>.

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