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ENZYME BASED RECYCLING PROCESSES

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ABSTRACT
Novel strategies allowing environmentally friendly recycling of plastics are strongly needed. Enzymes have shown high potential, especially for the recovery of building blocks from multilayer materials which will be discussed in this paper. It has been shown that enzymes can specifically hydrolyze and solubilize certain components of blended packaging materials or mixed wastes. This allows a step—wise recovery of valuable building blocks which can be used for re-synthesis or for bioproduction (e.g. recovered glucose). However, despite the high potential of biocatalysts, even more efficient enzymes are required for economic industrial implementation. In this paper, which is based on a contribution to the SUM 2022 conference, we will consequently demonstrate how enzyme discovery can lead to more powerful tools for plastics recycling and provide some examples.

1. INTRODUCTION

The application of enzymes is a powerful alternative to other recycling strategies of plastics especially when blended / multi-layer materials or mixed wastes are targeted. Enzymes are highly specific biocatalysts that lower the activation energy for the conversion of certain molecules while leaving others unchanged. For example, extracellular enzymes secreted by fungi naturally hydrolyse cellulose (wood) under mild conditions while the same process conducted in their absence in-vitro would require high temperatures and acids. Moreover, being proteins, enzymes are biodegradable. Now, such cellulases could be used to hydrolyse cellulose components in multi-layer packaging materials or blended textiles (Vecchiato et al., 2018;2019). Thereby, cellulose is converted into glucose which is water-soluble and can be easily separated and recovered. This process can be repeated for the remaining components with other enzymes specific for other components (Figure 1). Finally, the most recalcitrant component would remain in pure form ready for recycling (re-granulation, spinning etc). Needless to say, enzymes are very efficient on polymers occurring in nature such as cellulose while efforts over the past twenty years have also led to enzymes for the decomposition of synthetic materials such as polyesters (Ribitsch et al., 2013; Haernvall et al., 2022). In this paper, we will review recent work on enzyme-based recycling strategies and critically discuss current limitations and possible future developments.

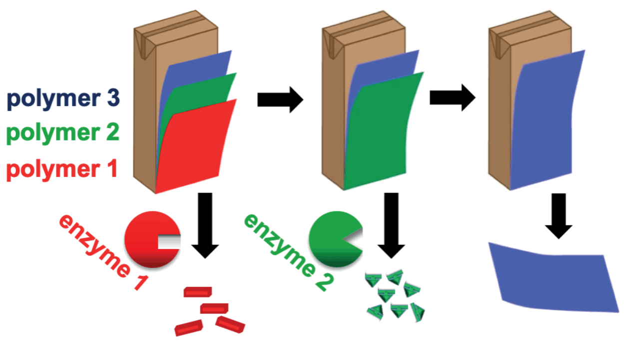

FIGURE 1: Step-wise recovery of valuable components from packaging waste. Enzymes specifically hydrolyse only certain polymers thereby solubilizing the respective building blocks allowing their recovery. Finally, remaining pure polymers layers can be recycled as such.

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2. DISCUSSION

2.1 Bioexploration of enzymes for recycling processes

Multilayer materials can consist of many components like cardboard, polyesters, polyolefins, aluminum and others. In the recycling strategy depicted in figure 1, enzymes specific to cellulose could be used in a first step allowing recovery of glucose. Such enzymes termed cellulases are known for more than hundred years and are commercially available. In contrast, nature did not have as much time for evolution of enzymes acting on synthetic polymers like poly(ethylene therphthalate) (PET). Yet, there are polyesters present in nature such as cutin occurring e.g. in apple or tomato shells. Consequently, scientists have taken these enzymes termed cutinases as starting point for further development and adaptation to synthetic PET. Nevertheless, there is still a strong demand for more efficient enzymes acting on the different (synthetic) components in multilayer materials.

Modern biotechnology offers a variety of technologies for the identification of novel enzymes (Figure 2). Proteomics can provide information on the function of unknown proteins secreted e.g., by bacteria or fungi based on information on similar proteins in databases. Hence, based on knowledge on sequence/structure/function on existing polymer decomposing enzymes, novel, potentially more efficient enzymes can be identified. Many microbes produce certain enzymes only when needed (i.e., the respective polymer is present). Therefore, comparison of the proteins secreted when the polymers of interest are present during cultivation or not can allow fast identification of enzymes involved e.g. in polyester hydrolysis (Wallace et al., 2017). Metagenomics on the other hand, does not rely on certain organisms but screens the whole genetic information of a certain eco-system / environmental sample. Putative enzymes are recombinantly expressed and identification can be either function based (activity on polymers) or sequence based (has a similar sequence like a known enzyme). The great advantage of metagemonics is the fact, that genetic information of organisms that cannot be cultivated (> 98% of all microorganisms!) can be explored. Using this approach, PET hydrolysing enzymes were identified from moss accociated organisms (Muller et al., 2017). Nevertheless, novel synthetic polymer degrading enzymes could also be identified from microorganisms using conventional cultivation-based approaches. Therefore, the potential of high-throughput robot-based cultivation and assessment of polymer degrading enzyme activities has been demonstrated recently (Weinberger et al., 2020).

Once novel and more efficient enzymes have been identified in nature, genetic engineering of enzymes is applied to further improve their performance. A variety of strategeies has been applied in the past, notworthy to mentioned that apart from engineering the active site, the potential of tuning of sorption properties to improve PET hydrolysing enzymes has been demonstrated (Ribitsch et al., 2013; Haernvall et al., 2022).

Finally, the question in which environment polymers would be biodegradable often arises in the course of the development of bioplastics. Decomposition in industrial composting plant does not necessarily mean biodegradation in marine environments. Today, the genome of many microorganisms from different environments has been sequenced. Hence, based on the knowledge of the sequence of a polyester degrading enzyme from typical compost organisms (e.g., Thermobifida sp.), in-silico screening can predict whether enzymes with such activities could be present e.g., in waste-water treatment plants (i.e., aquatic microbes such as Pseudomonas sp.) or e.g., biogas plants (anaerobic microbes such as Clostridium). Indeed, this allowed fast prediction of biodegradation of polyesters in various enviroments correlating to the very time-consuming CO_2 evaluation assays (Haernvall et al. 2017).

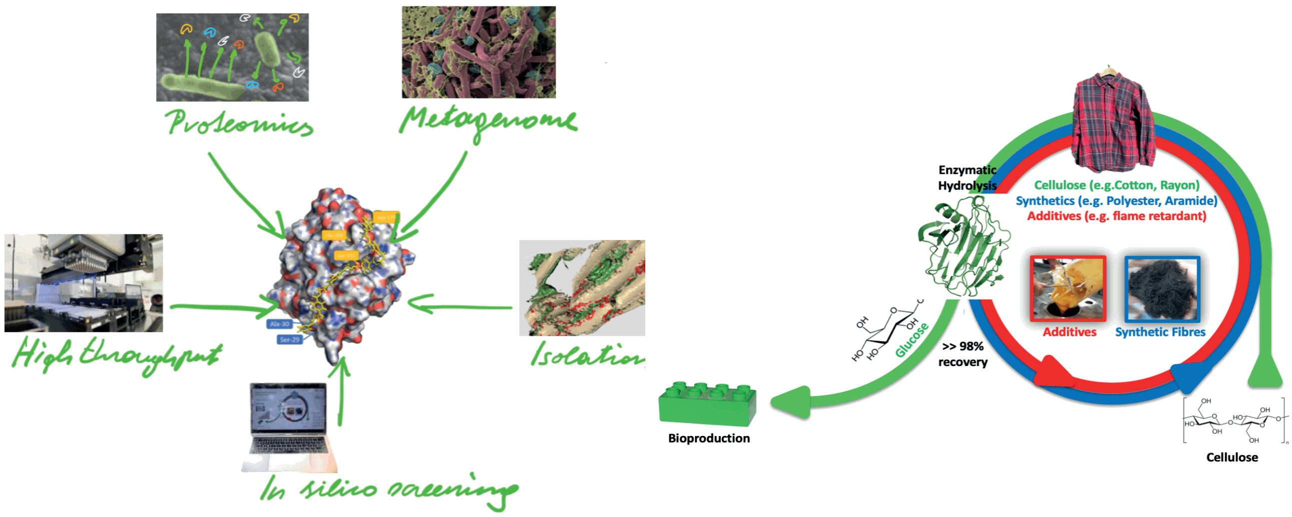

FIGURE 2: Different modern approaches for bioexploration of enzymes for recycling purposes (left) and enzymatic recycling of blended fabrics (right).

2.2 Synthetic polymers succeptible to enzymatic recycling

As elaborated above, impressive progress has been made in the last decade related to enzymatic decomposition of polyesters (i.e., PET). However, there are relatively few reports on enzymes that can decompose other synthetic polymers commonly found in plastics. For example, only recently, enzymatic hydrolysis of polyurethanes has been demonstrated (Gamerith et al., 2017). Apart from plastics/polymers currently on the market, enzymatic decomposition/recycling has recently been assessed for future bio-based/biodegradable alteratives to fossil based plastics such as poly(ethylene furanoates) (PEF) (Pellis et al., 2016; Weinberger et al., 2017) currently already being introduced e.g., for beverage bottles by major players. In addition, such recycling options are also considered for bio-based polymers more in development stage (Hou et al., 2016, Curia et al., 2018, Blackwell et al., 2018).

2.3 Examples for enzymatic recycling

We have shown that cutinases can specifically release PET building blocks from multi-layer packaging materials such as PET-polyamide sparkling water bottles or PET-polyethylene ham packing (Gamerith et al., 2017). While the released building blocks can be used for re-synthesis of polyesters, remaining pure polyamide or polyethylene, respectively, can be recycled as such. Likewise, polymer building blocks were recovered from mixed textiles wastes in a chemo-enzymatic approaches (Quartinello et al. 2017; 2018). Enzymatic recycling of dual blends of fabrics consisting of cellulose (e.g., rayon, cotton) and synthetics (polyesters, aramide) yields “purified” synthetic fibres suitable for recycling as such (Figure 2) while the resulting glucose has been successfully used for the bio-production of bioethanol or platform chemials. Interestingly, this process also allowed recovery of valuable addititive (e.g. flame retardants) which are often “forgotten” in recycling concepts (Vecchiato et al., 2018;2019). Similar processes have also been reported by Kenny et al. (2008) for enzymatically decomposed plastics (i.e. PET) where the hydrolysates have been used for the production of PHAs (polyhydroxyalkanoates).

3. CONCLUSIONS

In this paper we explained the concept and demonstrated the potential of enzyme-based recycling processes based on various examples. Since such promising concepts rely on efficient enzymes, we provided some details on current research activities and strategies towards bioexploration. Due to their high specificity, in addition to textiles and packaging, such enzyme-based processes could be implemented in many other areas such as automotive recycling where likewise multi-layer materials are used and building block could be step-wise recovered. Essentially, such processes would also be applicable for recovery of valuable molecules from mixed waste streams. However, on long terms it would be desirable to consider such processes already in the design of materials by e.g. inserting enzyme susceptible breaking points to render the recovery processes more efficient.

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