

Popular summary

The way plastics are currently produced, used, and discarded is not sustainable and several different ways of tackling the problem have been presented. A sustainable closed-loop solution is the circular economy, where the focus is on repair, remanufacturing, refurbishment, cascading, or upgrading used materials to put the material back into the economy again. For the circular economy, it is important to decouple plastic production from fossil resources. The present linear economy using fossil feedstock and end-of-life treatment of post-consumer plastic by incineration and landfilling contribute to large greenhouse gas emissions. This dissertation aims to find new bio-based routes of producing plastic building blocks from renewable biomass and recycled plastic as an alternative to oil-based production.

One of the most promising methods for finding sustainable substitutes for oil-based chemicals is to convert biomass or other bio-based chemicals using living cells or enzymes. These cells are usually simple microorganisms that grow quickly and naturally have different enzymes that can catalyse the conversion of biomass-based raw materials to these chemicals. The cells can be either wild-type (unmodified) or genetically engineered as it is possible to add genes from other organisms or remove existing genes to change the properties of a cell.

The production of several types of plastic building blocks has been investigated as different building blocks are needed for different types of plastics. Different production strategies have been chosen depending on how the production of these molecules fits into the metabolism of the cell. To produce the aromatic molecule (i.e. a molecule with an aromatic ring) protocatechuic acid (PCA), genetically engineered *Escherichia coli* cells were used. But, to produce the aliphatic building blocks (i.e. molecules with open carbon chain) adipic acid and 4-hydroxybutyrate, unmodified *Gluconobacter oxydans* cells were used.

E. coli is a well-developed model organism in biotechnology with many available genetic tools. These tools are needed to increase the production of aromatic molecules that are costly for a cell to produce and therefore the cell has developed several ways to control that it produces what is necessary and not more. Overcoming these control mechanisms requires tools to manipulate the cell's metabolism. The first two articles aim to manipulate some of these mechanisms to increase the production of PCA in *E. coli*. This resulted in one of the highest reported PCA amounts. The PCA yield was further improved by the use of a so-called promoter library, where a catalogue of different genetic start signals was generated that control protein concentration in a cell. The use of this library resulted in the characterization of three novel start signals which increased the PCA yield by a further 10-21%.

The other two articles used the bacterium *G. oxydans* which has the ability to oxidize a large number of chemicals and can do so selectively to produce useful compounds. *G. oxydans* has a unique respiration chain that allows rapid conversion of simple sugars and alcohols. However, this system can also be used on molecules on which the bacterium cannot grow on, which causes the products of these reactions to accumulate. This system was used to oxidize both 1,6-hexanediol and 1,4-butanediol to the adipic acid and 4-hydroxybutyrate, respectively, with very high efficiency. In the latter case, 1,4-butanediol was obtained by degradation of a commercially available biodegradable plastic PBAT (polybutylene adipate terephthalate) using the enzyme LCC.