

Popular summary

What do clothes, carpets, tire cords, conveyor belts, and brushes have in common? That they are usually made of nylon. Nylon is known to be a durable fibre used in textiles and plastics, normally made of petroleum.

If we take a closer look at the nylon's composition, we can see that it is generally made of two components, one of them is adipic acid (AA). Every year, millions of kilograms of adipic acid are produced mainly for the production of nylon.

With the growing interest in getting rid of petroleum-based products, greener ways of obtaining these components have been invented. In this study, the route performed consisted in culturing bacteria that can produce adipic acid. The bacteria used, called *Gluconobacter oxydans* can be found in fruit, cider, beer, wine... and it has the ability to produce adipic acid if supplied with a certain substrate, 1,6-hexanediol (1,6-HD), which can potentially be made from sugar-based building blocks.

In order to perform the experiments, the optimal conditions to culture the bacteria are screened on a small scale (approximately 50 mL, less than a glass of water). When these conditions are settled, the process is scaled up to 22 litres, obtaining a huge number of bacteria.

To use the bacterial cells for the production of AA, the 22 L was split into batches of 4L. This 4 L is centrifuged and the cells in it are resuspended in 1 L of 1,6-HD, then the reaction starts. The 1,6-HD is converted by the cells to AA but not immediately, the cells need approximately 24 hours to convert all the substrate into product. During this time, several samples are taken to monitor the conversion. One experiment consisted in a fed-batch, which is a technique where substrate is supplied to the cells several times. The product is accumulated and the final amount is much more than if the substrate is given to the cells once. But we found out that after supplying 5 times substrate to the same cells, the cells get "tired", and every time take a longer time to produce AA. Then AA is purified through a simple process that avoids the use of organic solvents.

For a better understanding of the reaction, we wanted to know why the cells lose activity. Those responsible for carrying out the reaction are the enzymes. There are a lot of enzymes in a cell, and we wanted to figure out which ones were working for this reaction. For this reason, a number of unknown genes encoding for enzymes from *G. oxydans* were selected and expressed. It is not yet clear what enzymes they are, but work is underway to find it out.