Phenol from the Bioreactor

A team at Bayer Technology Services has managed to get bacteria to produce phenol from glucose. A technology making use of agricultural wastes could thus pave the way for the sustainable synthesis of important phenol-based polymers, such as polycarbonates.
The raw material glucose could be obtained from waste wood (above). But the cells, from which phenol can be synthesized, must first be prepared and then reproduced (below).
Cells are very versatile reactors. They can produce thousands of different molecules. No chemist can achieve this. I see unlimited possibilities for biotechnology.”

Dr. Jørgen Magnus, Process Design & Optimization, Bayer Technology Services

i.e. a species of bacteria of which there are also examples found in the human intestines.

When these coli bacteria metabolize simple glucose molecules, the conversion chain eventually leads to chorismate. And this is the very substance that Magnus tipped as a good starting point. “Phenol can be synthesized from chorismate in just two steps.” The first step leads to a substance called 4-hydroxybenzoic acid, and the second to phenol. For these two steps, the bacteria are only missing two enzymes that would induce the respective reactions. Luckily, these two enzymes can be found in other strains of coli, and the genes for these enzymes were therefore known. The only thing Magnus and his team had to do was to cut out these genes from the other bacteria and smuggle them into the genome of the bacteria with the chorismate. And so, going back to our metaphor, this would be the new highway to phenol production.

To increase the yield, Magnus had to ensure that the bacteria cells produce a larger than usual amount of chorismate from the glucose—a lot more. “We therefore copied the genes for three enzymes that are involved in producing chorismate.” That was equivalent to creating new lanes for the highway.

Unfortunately, there was one interfering factor. The bacteria continued to process the chorismate to two amino acids that are particularly important for them. As this would have impaired the phenol yield, Magnus wanted to prevent the amino acid production. “To accomplish this, we had to cut out two genes for the corresponding enzymes,” explains Magnus. It was as if they had blocked a highway exit or turnoff so as to prevent too many vehicles from leaving the highway.

And then there was one final hurdle to overcome. Cells have a feedback mechanism, which, under certain circumstances, inhibits the production of a chorismate precursor and thus the chorismate synthesis itself. In this case it was sufficient to modify the gene for the corresponding enzyme at one particular spot, thus suspending the feedback. Magnus compares this to

Utilizing nature

Nature can do an amazing number of things, and white biotechnology is increasingly making use of this talent. Unlike biotechnological applications in pharmacology and medicine (red biotechnology) or in agriculture (green biotechnology), white biotechnology involves utilizing the toolbox of nature for industrial purposes. Some examples are obtaining ethanol from biomass (bioethanol) or using biotechnological methods to produce enzymes for detergents. White biotechnology is also becoming increasingly interesting for the synthesis of important basic chemicals from renewable raw materials or even from their waste products—thus offering an alternative source to oil.

Metabolic engineering is a special field in white biotechnology or, better said, one of the methods used. In this process, existing organisms, usually single cell organisms such as bacteria, are modified so that they perform industrial tasks. One goal can be, for instance, to deliver higher yields of a substance generally produced as part of metabolism. But it can also involve reprogramming the metabolism in such a way as to induce the organisms to synthesize something completely new. This is exemplified by the case described in the main text, i.e. phenol synthesis with the help of coli bacteria.

Those who are optimistic about white biotechnology can already envision a largely biobased industry, in which renewable raw materials replace the classic fossil fuels as completely as possible. Technology is not there yet, but companies and scientists are researching towards this goal. And Bayer is one of them.

It has since been clearly proved that phenol is being diligently synthesized in this murky reactor solution.
the removal of a construction site where the traffic had previously jammed.

In the end the day came when Magnus had managed to smuggle all the necessary genetic modifications into the coli cells, and they then reproduced. A simple shaking flask had been enough to prove whether the principle functioned in practice. The flask held a mixture containing reproduced coli bacteria, some nutrient solution and, of course, sugar. After a while, Magnus checked to see if phenol had formed in the mixture. But the chromatogram of the extract he examined showed a flat line. No peak, which meant no phenol. It was a shock.

What could be the reason? Was something wrong with the culture medium? Suddenly, Magnus had a different suspicion. Had he perhaps made a mistake during the proliferation of the modified cells? This is where a trick comes into play: To prevent the coli bacteria with an unmodified genome from multiplying with the others, the modified genome is equipped with a resistance against a certain antibiotic. This particular antibiotic is then applied to the cells, with the consequence that only the bacteria cells with the modified genome (and the resistance) multiple and all the others die. Had Magnus used too little of this antibiotic? So, he increased the amount in a second try.

Wednesday, June 13, 2012: it is about 6 pm, and Magnus is the last person in the laboratory, when he injected a new sample from the shaking flask into the liquid chromatograph. After about 20 minutes, the ultraviolet detector actually registered a substance; a peak appeared. Magnus knew exactly what substance showed up at this spot in a chromatogram: phenol. In his home country of Norway, it was the time of the year when it is barely dark at night and the mid-summer night parties were in full swing. Magnus may have been far, far away in his Leverkusen lab, but he too was in a mood for partying!

Later he repeated the experiment in a bioreactor with a capacity of one liter. Here the phenol peak was even greater, and the yield higher. Despite these sensational successes, Magnus remains cautious as he is all too aware of the further hurdles to overcome. “Until now we have only confirmed the operating principle. There is still a lot to do before this principle can be transferred to an industrial scale production of meaningful dimensions.” Presumably another bacterial strain will even be necessary. “From a certain concentration phenol is toxic for the coli strain used until now,” Magnus admits. Another question is how you can extract the finished product, if possible, continuously from the bioreactor so that it cannot concentrate in the first place.

On the other hand, it is very clear where one could obtain the necessary starting material, glucose. The various plants for sugar production would be one possible source. However, cellulose, which can be obtained from plant waste, would be another possibility, Magnus stresses.

Magnus has no doubts as to the question whether small bacteria will be able to produce the industrial product on a scale of several million tons. “There are already facilities in which yeasts produce up to one million tons of bioethanol every year.” He is therefore confident that biophenol production plants of a similar size as those in the chemical industry are achievable.

But one thing is for sure: the Norwegian expert is absolutely certain about the future of biotechnology in the chemical indus-