SARA COLLATON

NATURE'S INDUSTRIALISTS

By blending biotechnology and engineering, Canadian researchers are ushering in a future where commodity chemicals are not made, but grown.

By **Tyler Irving**

Radhakrishnan Mahadevan's desk is piled high with scientific papers, and the walls of his office present whiteboards covered in complex differential equations. Such an environment is entirely appropriate to the convoluted task that Mahadevan, an associate professor in the Department of Chemical Engineering and Applied Chemistry at the University of Toronto, has set for himself: to model the metabolic pathways of microorganisms, and to determine how they can be manipulated to optimize the production of valuable chemicals.

Every day, nature simply and efficiently performs chemical transformations — some on a truly immense scale — that would confound even the most talented chemical engineer. Of course, nature has a secret weapon: enzymes. Over millennia, these protein-based biochemical catalysts have evolved to facilitate reactions that would otherwise seem impossible. In the last decade, the plummeting costs of techniques like DNA/RNA sequencing and protein mass spectrometry have greatly accelerated research into understanding how enzymes work. Now, the time is ripe to see if these lessons from nature can be used to solve human problems such as breaking our addiction to crude oil or making more efficient use of forest resources. In these endeavours, chemical engineers like Mahadevan are leading the way.

"In many ways, a biological system is no different than a chemical factory: they both take raw materials and process them into products," says Mahadevan. A process control engineer by training, Mahadevan became interested in microbes about ten years ago, when he attended one of the first ever conferences in what's now called systems biology. "That term means different things to different people, but from an engineering point of view, we try to break a biological system down

Radhakrishnan Mahadevan's computer models of metabolic processes describe how to tweak the genetics of microorganisms to maximize the production of valuable chemicals.

into parts, see how the parts link up together, and then reconstruct a model of the overall system," he says.

He points to a poster on his wall which depicts the rough outline of a cell, thickly covered with a spider's web of arrows and boxes containing the names of enzymes and chemical intermediates. It's a metabolic map, describing all of the reactions by which the bacterium *E. coli* converts one molecule into another in order to extract energy or build cell components. In Mahadevan's computer models, this network of enzymes, metabolites and reactions is represented by sets of equations and variables. Changing the parameters of the model can predict the effect of over-expressing, down-regulating, or even deleting the gene that encodes for a specific enzyme.

Last year, Mahadevan and his graduate student Laurence Yang published a paper in *Metabolic Engineering* in which they describe a new computer algorithm called EMILiO: Enhancing Metabolism with Iterative Linear Optimization. EMILiO searches through existing metabolic models and identifies which modifications are most likely to increase production of a given chemical target. "Previous algorithms have been proposed for this, but ours can solve really large-scale problems in a fraction of the time," he says. "We're going from days and weeks to sixty seconds."

While such detailed models may be new, the idea of producing a valuable chemical from a microbial process is not; the yeast *Saccharomyces cerevisiae* has been used to ferment sugar into ethanol for thousands of years. But in most cases, biologically-produced chemicals currently cost more than their oil-based equivalents. That may change as oil reserves diminish or if carbon pricing becomes widespread, but for now turning valuable sugar cane or corn into a low-value biofuel makes questionable economic sense, and researchers are focusing their efforts elsewhere. "A lot of chemical engineers working in biotechnology are focused on commodity chemicals rather than fuels," says Pratish Gawand, a PhD candidate in Mahadevan's lab group whose passion for biotechnology shows through in his rapid-fire speech and encyclopedic knowledge of key papers. "Commodity chemicals still have a relatively large market, but are sold at a higher price than ethanol, so it's easier to justify the cost of production."

Gawand cites a 2004 U.S. Department of Energy report that identifies 12 classes of non-fuel chemicals that could be produced from biomass sugars using known metabolic pathways. At the top of the list was succinic acid. This four-carbon molecule can be used as a building block in everything from polymers to pharmaceuticals. It's also part of the citric acid cycle, the series of biochemical reactions that provides energy in all aerobic organisms. Since microbes like *E. coli* already produce succinic acid as part of their metabolism, getting them to produce lots of it should be a matter of tweaking only a few genes. Predicting which genes to tweak is exactly what Mahadevan's models are designed to do.

> "A lot of chemical engineers working in biotechnology are focused on commodity chemicals rather than fuels. It's easier to justify the cost of production."

But there's another problem: if you force a bug to spend all of its energy producing succinic acid, it tends not to grow very quickly. Luckily, that problem is being tackled by Nik Anesiadis, a student just finishing his PhD in Mahadevan's lab whose easygoing manner belies his knack for detail. Anesiadis' solution takes advantage of a phenomenon called quorum sensing. Many bacteria produce signalling molecules that are sensed by their neighbours; the concentration tells the cells how crowded they are. When things get too cosy for comfort, the cells turn certain processes off or on, almost like a genetic switch. Using molecular biology techniques, Anesiadis has managed to incorporate his genetic changes into this switch. The result is a line of *E. coli* cells that grow normally until their vessel is full, then start producing succinic acid like crazy. That system is still being refined, but bio-based succinic acid is already moving ahead in industry. BioAmber, an American company with a patented process for producing succinic acid using yeast, is currently building a 34,000 tonne per annum plant in Sarnia, Ont.

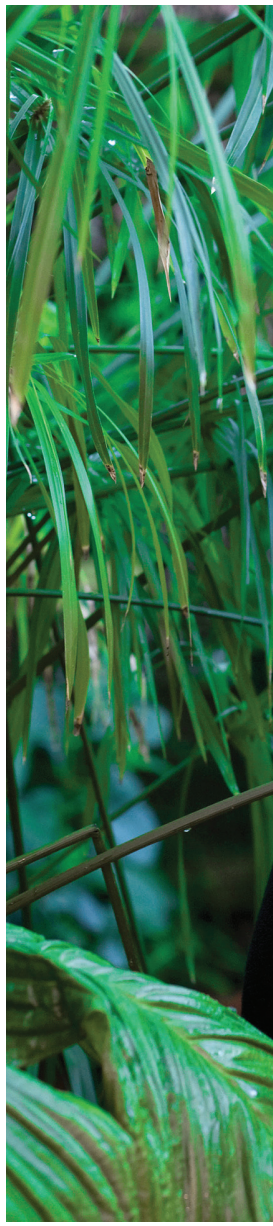
Ultimately the idea would be to create a 'forest biorefinery' where each fraction of wood — cellulose, hemicellulose and lignin — provides its own set of value-added products.

***

Where Mahadevan is a chemical engineer working with biological systems, Emma Master is the opposite: an environmental microbiologist who has found her niche in chemical engineering. Her office, one floor up in the same department as Mahadevan's, is neatly organized and the diagrams she needs to explain her work are filed away in drawers. This is ironic, as the wood-derived molecules she works with are some of the messiest around, both in terms of where they come from — think of rotting tree trunks — and in terms of the chaotic chemical structure they posses.

"Softwood remains one of the more difficult, biologically recalcitrant biomass sources to work with," says Master. That's because wood is actually a mix of three biopolymers: cellulose, hemicellulose, and lignin. Cellulose is the most straightforward: a long chain of glucose molecules joined up with a repeating beta 1,4 linkage. But hemicellulose, while sporting the same basic backbone, contains other sugar groups that branch off randomly in all directions, giving it unpredictable properties that vary wildly from one species to another. These branching groups interact with lignin (an even more complex polymer) and cellulose, binding the tree together. Although they are among the most common chemicals on the planet, making use of these three polymers requires their efficient separation.

Traditionally, the North American pulp and paper industry has focused exclusively on cellulose, using harsh chemicals to isolate it from its less-valuable cousins and refining it into newspaper, cereal boxes and hundreds of other products. But competition from developing countries and the rise of paperless communication has meant hard times, and the industry is now searching for ways to extract more value from trees. Hemicellulose and lignin offer many possibilities: they could increase the structural strength of plastic in everything from car door impact panels to lawn furniture, or act as non-toxic scaffolds in medical implants. They could even be broken down into their basic sugars and fermented into ethanol, saving corn and sugar cane for more valuable applications. Ultimately the idea would be to create a 'forest biorefinery' where each fraction of wood — cellulose, hemicellulose and lignin — provides its own set of value-added products.



But before any of that can happen, Master and other researchers like her have to conquer the inherent messiness of hemicellulose and lignin. Unsurprisingly, she's relying on enzymes to do so, and she's looking for them in some of the most unusual places. For the last two years, one of her students has been carefully raising cultures of bacteria originally isolated from beaver droppings and the stomach contents of moose. "The moose can consume pine needles, which are quite difficult to digest, and the beaver can eat bark," explains Master. "We thought that if we looked at their gut flora, we might find some organisms with interesting catalytic activity." Some of the bacterial cultures are fed with cellulose, while others are grown in the presence of lignin or other inhibitory compounds. The bugs are happily producing biogas (methane), so something in there must have the right enzymes to break down these recalcitrant chemicals. Eventually, Master hopes to use RNA sequencing to identify these enzymes in the most successful cultures.

Another place to look for hemicelluloses and lignin-degrading enzymes is in fungi, which are known for their ability to grow on rotting wood. Two months ago, Master's group published the genome of *Phanerochaete carnosa*, a fungus that is particularly good at degrading softwoods. "Of course the genome just gives you the blueprint; it doesn't tell you which genes are expressed in different conditions, or which enzymes are secreted into the culture medium," says Master. For that she needs techniques like proteomics. This involves extracting fungal proteins/enzymes from

JOHN HRYNIUK

Emma Master studies the enzymes that fungus and other organisms use to break wood down into its constitutent biopolymers — cellulose, hemicellulose and lignin — which can then be used in plastics, adhesives, medical products and more. Recently, her group introduced genes from fungus into this *Arabidopsis* plant, a common model organism for genetic studies.

her cultures and using other enzymes to digest them into smaller chunks. These chunks are then run through a mass spectrometer, the readout of which is a biochemical fingerprint for the protein of interest.

Using techniques like these, Master's group has already achieved some success. They've identified enzymes that act as glycoside hydrolases, meaning they can break off the branching sugars that make hemicellulose so tough to deal with. Using these enzymes in industrial processes could make it easier to achieve clean separation of wood's three biopolymers (current chemical methods tend to contaminate the hemicellulose and lignin streams). Enzymes may also provide a way of standardizing the quality and properties of hemicelluloses between species. The group has even managed to introduce the gene for one of these enzymes into the model plant *Arabidopsis thaliana*, causing it to partially separate its own hemicellulose and lignin as it grows.

Of course, a plant that digests its own structural materials doesn't grow very robustly, but Master already has a solution in mind. "One way to control any negative consequences is by using a genetic promoter that is only induced during later stages of plant development." The approach is similar to Anesdias's method of late expression in *E. coli*, although it uses a completely different genetic switch. Master hopes that experiments like these will help researchers identify genetic markers in trees that lead to fibre characteristics which are optimized for separation in the forest biorefinery.

It's clear that both biotechnology and chemical engineering need each other if either is to fulfill its promise of solving society's problems.

***

It's clear that both biotechnology and chemical engineering need each other if either is to fulfill its promise of solving society's problems: Mahadevan's models apply chemical engineering techniques to biological systems, while Master's work on biorefineries applies biotechnology techniques to industrial chemistry. As Pratish Gawand wryly observes, blending the two is not without its challenges. "With engineering processes, you can be sure of what's going on," he says. "Biological processes are not like that; the bugs are finicky and things don't always work the way you think they will. It's really an art. But when your strategy works and you're able to make something that could actually be useful to industry, that is very exciting." Exciting indeed, for the ultimate goal is nothing short of transformational change to some of the world's most staid industries, from petrochemicals to pulp and paper. "It's about being able to do more with what nature has given us," says Emma Master. "That's very motivating." CCN