SMALL THINGS
BIG CHANGES

THE LITTLEST FACTORIES

From genes to enzymes, how do cells make products we use?

Using microbes for a more sustainable world
Taming an ancient virus when the clock is ticking
Biomanufacturing a planet
It is now recognised that humankind stands on the brink of the Fourth Industrial Revolution, which will fundamentally alter the way people live, work and relate to one another. In its scale, scope and complexity, the transformation will be unlike anything humankind has experienced before.

Indeed, humanity’s ability to address a wide range of global problems — from climate change and resource sustainability to food security and disease — will require advances in scientific knowledge and applied technology. Central to this monumental shift are major technological advancements that are already rapidly changing how we interact with the biological world, particularly the world of microorganisms.

Humans have been harnessing the power of microorganisms for many millennia. In this magazine you’ll discover how the fermentation of fruits and grains by wild yeasts and microbes became systematic cultural knowledge that led to the earliest breweries, and most likely played a role in the dawn of agriculture. In time, this led to early concepts of germs, the discovery of antibiotics, and ultimately the rise of modern biomedicine. Incredibly, we’ve only just scratched the surface.

Today, the emergence of inexpensive, precise genome editing tools is revolutionising biology. You’ll learn how scientists are pioneering these and other technologies, including computational modelling and robotics, to enable the tailored genetic design of microbes. This is enabling us to use those microbes as tiny factories that perform complex chemistry, and in so doing allows fast, large-scale production of valuable chemicals and medicines. We call this Advanced Biomanufacturing and, as you’ll see, it’s already playing a critical role in how we care for the environment and global health.

In particular, Advanced Biomanufacturing has the potential to significantly reduce our reliance on natural gas and oil for the production of chemicals, plastics and fuels, and can even facilitate the detection and cleanup of environmental contaminants. Similarly, modified microbes and viruses are being marshalled into service for the rapid discovery and production of new medicines. These biotechnologies will not only profoundly influence the future of this planet and the people on it, they will also enable us to explore other planets. As you’ll discover, NASA scientists and their colleagues have already identified synthetic biology and biomanufacturing as critical to establishing a human settlement on Mars.

There is an exciting future ahead, and we invite you to join us.
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Humans have been taking advantage of microbes for a very long time and, in turn, these microscopic organisms have altered the course of human history many times over. Yeasts, for example, have been used in the production of alcohol and bread since the first Palaeolithic breweries and bakeries began more than 13,000 years ago. Indeed, it has been suggested that the fermentation of cereal grains by wild yeasts — a serendipitous phenomenon that led to the art of brewing — may have triggered the shift from hunter gatherer societies to those that focussed on agriculture. Beer and bread, it seems, may have encouraged humans to finally settle down. The use of microbes in medicines has a long and remarkable history, too.

Historical and archaeological records going back at least 2,500 years point to an awareness of a direct anti-infective effect of moulds found on breads, soybean paste and cheese. Indeed, with remarkable consistency, ancient healers across a wide variety of places including ancient Greece, China and Egypt, used moulds to directly treat infected wounds.

Fermentation was also a common feature in ancient medicine. Indeed, the production of medicated wines in particular has a long history in Ayurveda, a medicine system that began several thousand years ago in ancient India. Although the fermentation recipes varied, some ingredients remained constant, particularly the use of Dhataki flowers (Woodfordia fruticosa). It turns out that several species of fermenting yeast prefer to live on these flowers and, during fermentation, are able to transform chemicals in the flower into medicinally active compounds.

When it comes to microbes, ancient medicine didn’t just rely on yeasts; bacteria also played a role. Traces of the antibiotic tetracycline have been found in human bones from ancient Nubia. It seems that, by 500 AD, Nubian brewers were fermenting grains laced with the tetracycline-producing soil bacteria Streptomyces, enabling them to produce antibiotic-infused beer. This wasn’t a one-off — the levels of accumulated tetracycline in those bones suggest long-term use of the medicinal brew.
Over the course of history, contaminated liquids and mouldy foods and grains would have been identifiable by sight and smell, but even in ancient times some people harboured suspicions that there was more to the natural world than meets the eye.

In what might be considered an ancient Roman travel review, scholar Marcus Terentius Varro anticipated the fields of microbiology and epidemiology when, in 30 BC, he suggested that swamps and marshland were best avoided. In these places, he reasoned, “there are bred certain minute creatures which cannot be seen by the eyes, but which float in the air and enter the body through the mouth and nose and cause serious diseases.”

Yet, it was only within the last few centuries, aided by the invention of the microscope, that people became fully aware of the microcosm of tiny organisms that surround us.

When Dutch scientist Anton van Leeuwenhoek examined a droplet of lake water under his microscope in 1676, he saw something astounding: thousands of tiny living creatures, each moving on its own. These ‘animalcules’, as he called them at the time, were bacteria, algae, and small single-celled organisms called protozoa.

“Among all the marvels that I have discovered in nature,” he wrote, “these are the most marvellous of all.”

Two centuries later, Louis Pasteur and others went on to show that Marcus Terentius Varro had been right all along: there was indeed a link between microorganisms and disease. Pasteur’s ‘Germ Theory’, in which he proposed that infections are caused by germs, was a game changer in the field of medicine. Then along came a suite of discoveries that changed everything yet again.

By the turn of the 20th century, it became clear that many microbes were at war with one another and that some of those microbes were very good at defending themselves with molecular weaponry. This raised a tantalising possibility: if some microbes caused infections, perhaps other microbes could be used to fight those infections.

Sure enough, in 1928, Alexander Fleming discovered that bread mould fungi produced a chemical that could kill harmful bacteria. He named it penicillin. In 1941, scientists Howard Florey and Ernst Chain went further, showing that penicillin could indeed be used to treat infections. They also demonstrated that microbe cultures could be scaled up to enable mass production of the antibiotic. The age of industrial biomanufacturing had begun.
Humans have been taking advantage of microbes for a very long time and, in turn, these microscopic organisms have altered the course of human history many times over.

**ANCIENT HISTORY**

- **~28,700 years ago**
  Earliest evidence of food fermentation (roots and tubers fermented with microorganisms), in the Solomon Islands

- **~ 13,000 years ago**
  Earliest evidence of beer fermentation in Natufian culture (ancient Israel)

- **~ 9000 years ago**
  Evidence of fermented rice, honey and fruit beverages in ancient China

- **~ 9,000 years ago**
  Evidence of milk fermentation in Mesopotamia and ancient Egypt, as well as ancient Veda scriptures and Ayurvedic texts (Indian subcontinent)

- **~ 5000 years ago**
  Beer production in ancient China

- **~ 2500 years ago**
  Evidence of soybean fermentation to produce tofu in ancient China

- **~ 2500 years ago**
  Ancient antibiotics: fungus that grows on soybean curd is used to cure boils

- **~ 2000 years ago**
  Ancient Nubians produce beer with high levels of tetracycline

- **> 2000 years ago**
  Mouldy bread used as topical treatment for infected wounds (China, Greece, Serbia, Egypt)

**EARLY MICROBIOLOGY**

- **1676**
  Anton Van Leeuwenhoek uses microscope to discover tiny ‘animalcules’

- **1881**
  Robert Koch develops technique to isolate pure bacterial cultures

- **1861**
  Louis Pasteur presents his ‘germ theory’ that microorganisms can cause disease
**20TH CENTURY**

- **1928**
  Discovery of penicillin (Alexander Fleming)

- **1940**
  Howard Florey and Ernst Chain discover penicillin can treat bacterial infections in humans

- **1942**
  Penicillin is mass produced for the first time

- **1953**
  Structure of DNA discovered

- **1966**
  The genetic code is deciphered: revealing how the order of nucleic acids in DNA and RNA determines how proteins are built

- **1970s – 80s**
  Development of DNA sequencing and DNA synthesis, as well as genetic engineering techniques

- **1973**
  First animal gene is cloned in bacteria

- **1978**
  Human insulin is produced in E. coli bacteria

- **1993**
  Frances Arnold develops ‘directed evolution’ to improve the function of enzymes and develop new catalysts

- **1996**
  Bakers’ yeast (Saccharomyces cerevisiae) genome sequence is completed

- **1997**
  E. coli bacteria genome sequenced

**21ST CENTURY**

- **2003**
  Human genome sequence completed (13 years in total)

- **2012**
  CRISPR/Cas9 genome editing tool developed

- **2015**
  Human genome can now be sequenced in approximately one day

- **2018**
  Frances Arnold shares Nobel Prize in Chemistry for her directed evolution methods, which are now used for biomanufacturing many products from pharmaceuticals to biofuels

- **2019**
  Expected completion of synthetic yeast genome, Yeast 2.0
Cells are everywhere, from the trillions of cells that make up the organs and tissues in your own body to the vast multitude of plant cells and microbes all over the world. Living cells today have evolved over billions of years to perform remarkably complex functions that enable them to develop, grow and reproduce.

In a sense, we can think of cells as tiny factories that make a variety of biologically important products such as ethanol, antibiotics and proteins.

Much like a factory, a cell acquires raw materials, such as nutrients, and then processes those raw materials through special cellular ‘departments’ called organelles, where molecular machinery carries out specialised tasks to build the final molecular product. Those products are then transported within the cell to where they are needed or are exported out of the cells.

**How we can use those little factories**

*Clostridia* is a type of gut microbe that can convert dietary fibre into short chain fatty acids, such as butyrate, which is beneficial to your immune system.

Yet butyrate is also an industrially important molecule because, with a bit of chemical modification, it can be converted into valuable chemicals and biofuels.

Importantly, butyrate-producing microbes can make this molecule much more easily and efficiently than it can be synthesised in a laboratory.

For this reason, microbes such as *Clostridium tyrobutyricum* – a relative of common gut *Clostridia* – are used to efficiently produce large volumes of butyric acid for industrial use. When provided the right conditions and nutrients, large cultures of these microbes can be coaxed into producing massive volumes of butyrate, which can then be modified as needed.

**Did You Know?**

Microbial fermentation of grains and sugarcane is the most common production method for ethanol, a simple alcohol molecule that is now used in the production of plasticisers, synthetic fibres, dyes, lubricants, detergents, pesticides and more.
Reengineering living systems

Advanced Biomanufacturing has become central to the production of industrially useful molecules including biochemicals, solvents, fuel additives, fragrances and more.

Over the last century, microbes have become increasingly central to a wide variety of industrial processes. Moreover, remarkable progress in genetics and genetic engineering technologies in recent decades — from the development of cheap, rapid genome sequencing to CRISPR gene-editing to the analysis of vast amounts of biological data with artificial intelligence (AI) — has revolutionised how microbes can be used, and ushered in a new era of Advanced Biomanufacturing.

Consequently, living cells and microbes are now vital to the production of biological molecules or ‘biologics’, such as vaccines, monoclonal antibodies, therapeutic proteins, and even components of blood. Advanced Biomanufacturing has also become central to the production of industrially useful molecules including biochemicals, solvents, fuel additives, fragrances and more.

Furthermore, modified microorganisms have the potential to replace many petrochemical-based industrial processes, and are being used to facilitate the shift away from fossil fuels. In particular, microbes are being designed to directly reduce pollution through increased absorption of greenhouse gases such as CO2 and methane, and can even be designed to produce biofuels and degrade plastics.

Professor Lars Nielsen of UQ’s AIBN explains that systems biology and synthetic biology are now playing a central role in these advancements. In other words, researchers are now taking a highly systematic approach to understanding how microorganisms and other living cells work at the molecular level. Then, with the help of cutting-edge genetic tools, they are using this information to modify or entirely redesign the molecular assembly lines within those cells to produce valuable products at high volumes.

“The science is moving from crude retrofitting of living systems with a single gene or a few genes using genetic engineering, to purposeful reengineering of living systems,” he says.

Indeed, it is now possible to transfer an entire biochemical pathway from one species to another, as well as design new biochemical pathways from scratch and encode them into an organism’s DNA.

How to convince a microbe

A chemical called 1,4-butanediol (BDO) is an industrially valuable chemical used in the production of fibres, plastics and solvents. Because of this, it is widely used in the automotive, electronics, clothing and medical equipment industries. BDO is primarily produced from petrochemicals, so a shift toward microbial production would provide a more renewable alternative.

The microbe E. coli is often used in biomanufacturing, but as Professor Nielsen puts it, “E. coli doesn’t want to make BDO, it’s much happier making the molecule acetate.”

In fact, no known organism is naturally capable of making BDO, so there was no genetic blueprint to go on.

To address this, the company Genomatica designed from scratch an assembly line of known enzymes that would be capable of converting sugar molecules into BDO. They then inserted the genes for these enzymes into an E. coli microbe and removed any genes that might interfere with that assembly line.

“They were able to convince E. coli that BDO is the product to make, and not acetate,” says Professor Nielsen.

The new microbe was indeed capable of turning sugars into BDO and at higher yields than anticipated.

Scaling Up

Natural organisms have not evolved to achieve high yields of biochemicals, they just evolved to produce what they need. When designing a new molecular assembly line in a microbe, or tweaking one that already exists, researchers endeavour to include modifications that will coax the microbe into making high volumes of the desired product.
Making models

To better understand how a microorganism works, scientists use models. Models provide a simplified representation, informed by research, of what is most likely happening inside a cell, explains Dr Esteban Marcellin at UQ’s AIBN.

“Models are a way of mentally visualising cellular systems which are poorly understood.”

“They enable scientists to link theory with experiments and guide predictions of complex behaviours.”

Many different kinds of models exist: from models of individual molecules to models of entire metabolic pathways in cells. There are also models of interactions between cells and between populations of organisms.

“Scientists can then use this information to engineer biology,” says Dr Marcellin.

For example, if you want a microbe or a cell to convert a simple sugar into a new pharmaceutical, a fragrance or a jet-fuel, then making a model of the biochemical pathways in the cell can be very useful. Such a model may reveal new ways to use the microbe’s existing genes, or could predict the effect of introducing entirely new pathways. Models can also reveal bottlenecks in the production of a product of interest, and identify genes that should be removed. In this way, models serve as a predictive tool that can guide research.

Did you know?

Yeast is one of the most commonly used organisms in synthetic biology, yielding a range of chemicals for a variety of applications.
From genetic engineering to synthetic biology

Genetic engineering refers to the direct manipulation of an organism’s DNA, and in 1973 scientists created the first genetically engineered organism when they inserted a gene for antibiotic resistance into E. coli bacteria. Since then, the ability to deliberately modify genomes has become increasingly sophisticated, yet for many years this was limited to altering, inserting or deleting one or a few genes at a time.

As any engineer will tell you, some of the most interesting work happens when you can design and re-design entire systems by making many changes all at once.

In recent years, an explosion of advanced genetic technologies have enabled scientists to do just this. The result is an entirely new field called synthetic biology, in which the core principals of engineering — Design, Build, Test, and Learn — can be fully applied to biology.

“The concept for synthetic biology comes from electrical engineering where you have defined components with known structures and behaviours, just like a capacitor or a resistor on a circuit board,” says Associate Professor Claudia Vickers, of UQ’s AIBN and Director of the CSIRO Synthetic Biology Future Science Platform.

She explains that when viewed with an engineer’s eye, you can see that cells are also made up of defined components with known structures and behaviours — such as proteins that perform chemical reactions and genetic components that carry information from one part of the cell to another — and they work together within very organised systems.

So just as electrical engineers can modify or entirely redesign a circuit to achieve a desired result, the development of a remarkable gene editing technology called CRISPR/Cas9 now enables synthetic biologists to quickly and precisely alter the DNA that encodes a wide variety of cellular componentry.

“We can control a whole lot of what happens inside the cell this way,” says Vickers.

“Almost everything we do in synthetic biology is genetic modification but synthetic biology methods and new technologies allow us to do it much faster, much more high throughput and to find solutions to problems much more quickly.”

CRISPR: The genetic scissors

When you hear about a gene editing breakthrough these days, it’s often in association with a technology called CRISPR/Cas-9. But what exactly is this new technology, and how is it used in advanced biomanufacturing?

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats — in other words, repeated sequences in DNA. A protein called Cas9 (CRISPR associated protein 9) recognises these repeated sequences and functions like a pair of scissors by cutting the DNA.

The beauty of this system is that it allows researchers to cut DNA in a very targeted and precise way. Previously, to alter DNA, researchers would have to copy the genetic sequence of interest, modify it, then insert the modified DNA into the genome in a slow, labour-intensive process.

The powerful CRISPR/Cas9 technology gives researchers the ability to quickly and efficiently edit DNA on the genome. This is achieved by altering the Cas9 protein so it recognises and cuts specific targeted sequences of interest.

The technology has opened up new possibilities for genetic modification, including in biomanufacturing, where wholesale changes to an organism’s DNA can substantially alter which chemical reactions that organism can perform and, by extension, what it can produce.
In order to reduce greenhouse gas emissions and promote healthier ecosystems, there is an urgent need to shift away from fossil fuels and toward sustainable energy sources. The development of new technologies will play an important role in that transition, including technologies based on microbes.

Indeed, microbes are now being used to develop environmentally friendly materials, to produce sustainable fuels, as well as clean up existing pollution and promote food security.

For example, with the right modifications, microbes can produce molecular chains called ‘biopolymers’, which can be used in the production of bioplastics. This, in turn, will reduce reliance on petrochemical-based plastics and materials.

“Climate change is the defining issue of our time and we are at a defining moment”
— Antonio Guterres, Secretary General of the United Nations

Meanwhile, as electricity grids begin to shift toward renewable energy sources such as solar and wind power, transportation — particularly in the airline and shipping industries — continues to rely heavily on liquid petrochemical fuels. To address this, experts in Advanced Biomanufacturing are engineering microorganisms to produce renewable biofuels, such as ethanol and biodiesel, which are easily transportable.

Modified microbes can also help treat wastewater, degrade plastics, and clean up heavy metal pollutants. They can even be used to capture greenhouse gases and transform them into chemicals or fuels. Moreover, they can be designed to act as ‘biosensors’ that rapidly identify the presence of toxins, hormones, pathogens and other contaminants in the environment.

Furthermore, tiny microbes will have a big influence on the future of agriculture. Microbial biosensors are already being designed to detect crop diseases, while modified bacteria and algae have the potential to be used as agricultural probiotics that enhance soil quality, water efficiency and nutrient availability while reducing reliance on chemical fertilisers and pesticides.
Making Tyres from Microbes and Plants

Using synthetic biology to replace petrochemicals will dramatically reduce the cost and carbon footprint of tyres.

Over 25 litres of oil is used to make one tyre and over a billion tyres are produced worldwide each year. With this in mind, major tyre producers are now using synthetic biology to produce fossil-fuel free alternatives.

When the demand for tyres outstripped the world’s supply of natural rubber, manufacturers changed the recipe to a combination of natural rubber, synthetic rubber and fillers.

Isoprene is a major building block of synthetic rubber, and is currently made almost entirely from petrochemical sources. This compound is naturally produced by some plants when under heat-stress, but like natural rubber, it is not economically viable to harvest it from plants.

To address this, researchers around the world have been turning to microbes. For example, scientists in China have recently hijacked the pathway that the marine bacterium *Synechococcus elongatus* uses for photosynthesis, altering it to produce high quantities of isoprene in the laboratory, while using carbon dioxide as fuel.

Meanwhile, researchers in the US are using carbohydrates from plants as a feedstock for specially designed yeast that ferments those carbohydrates into low cost alcohols. They’ve now developed an add-on chemical process that then converts the alcohol into isoprene.

It is not only our vehicles that these developments will benefit — building blocks for rubber are also used to produce rubber-based products used in a wide range of industries, including the medical and construction industries.

Replacing petrochemicals and providing a cheaper way to make products that are usually derived from petrochemicals will dramatically reduce both their cost and their carbon footprint.

**NATURAL VS SYNTHETIC**

*Natural rubber*

The rubber tree (*Hevea brasiliensis*) is native to the Amazon region but introduced to many parts of the tropics for rubber production. Latex is harvested from the tree (called “rubber tapping”) and used to make rubber. In the past, tyres were made entirely from natural rubber.

*Synthetic rubber*

Building blocks for rubber, such as butadine and isoprene are currently sourced from petroleum.
Atmosphere
Greenhouse Gas Layer
Radiation from the sun passes through Earth’s atmosphere. Some of the radiation is reflected or absorbed by the atmosphere. Some is radiated back into space as infra-red (IR) energy. Some of this IR energy is captured and trapped by greenhouse gases.

Acetogens to the rescue
Could the oldest living microorganisms help us fight climate change?

In 2019, atmospheric carbon dioxide (CO₂) will surpass 415 ppm (parts per million), a level not seen since the Pliocene era, around three to five million years ago.

CO₂ is not only very good at trapping heat in the atmosphere, it also lingers there for a very long time. The majority of CO₂ remains in the atmosphere for up to a century, while the remainder can take millennia to be recycled. According to future climate models, we just don’t have that kind of time.

To reduce CO₂ levels and keep global temperatures in check, many climate scientists argue that not only do we need to achieve net-zero CO₂ emissions as soon as possible, we will also need to achieve negative CO₂ emissions in the near future. In other words, it’s not enough to stop releasing CO₂, it must be actively removed from the atmosphere.

“The world has a big problem with rising greenhouse gas emissions,” says Dr Esteban Marcellin at UQ’s AIBN. “We need to make the same chemicals and fuels in a more sustainable way, and biomanufacturing can contribute to that.”

Indeed, microbes have been critical to the global carbon cycle since the emergence of life on Earth. Back then, the atmosphere was very different from what we breathe today; at that time, it was a reducing atmosphere of noxious gases which would be toxic to most life on our planet now. When single-celled life first emerged, organisms that could convert those gases into the building blocks of life had a distinct advantage.

In particular, microbes called acetogens flourished under these conditions. Over aeons, they developed molecular machinery to build larger carbon chains from CO₂ and CO (carbon monoxide) and use the carbon for growth. Luckily for us, they’re still here.

“Acetogens are one of the oldest living microorganisms on the planet and are speculated to have the same molecular pathways that resulted in the emergence of life,” says Dr Marcellin.

How does CO₂ trap heat?
The greenhouse gas carbon dioxide (CO₂) is a major contributor to the rise in global temperatures and, by extension, climate change.

- Radiation from the sun passes through Earth’s atmosphere
- Some of the radiation is reflected or absorbed by the atmosphere
- Some is radiated back into space as infra-red (IR) energy
- Some of this IR energy is captured and trapped by greenhouse gases
Ancient biology meets cutting-edge technology

How ancient biology coupled with cutting-edge technology could reduce global greenhouse gas emissions.

From deep sea vents to hot springs to the guts of mammals, acetogens turn up in the most unusual places. LanzaTech, a gas fermentation company based in the US, is particularly interested in a species of acetogen called *Clostridium autoethanogenum*, which was originally discovered in rabbit droppings. LanzaTech uses this acetogen as part of its carbon capture and reuse process, whereby industrial waste gases such as steel mill exhaust are converted into useful by-products like ethanol.

Through an ARC Linkage Project, LanzaTech teamed up with Dr Esteban Marcellin and Professor Lars Nielsen at AIBN in order to better understand the process by which the microbe is able to fix CO₂ and CO. Together they built a platform so that gasified-waste streams can be turned into industrially useful chemicals.

To achieve this, LanzaTech and AIBN researchers recently developed a computer model of *C. autoethanogenum*’s metabolic pathways.

“Because biology is so complex, the human brain can’t quite process how the many different gene interactions going on at once can lead to genetic changes resulting in superior strains,” says Dr Marcellin.

“When you look at a microorganism like *C. autoethanogenum*, there are 4,000 genes interacting there, so we use computer models to process and model combinatorial patterns.”

“Our computer model is able to predict cellular metabolism of the microbe,” said Dr Marcellin. “This can help identify the best way to modify the organism so that it can capture greenhouse gases better and convert that carbon into desired products.”

Dr Michael Koepke, Director of Synthetic Biology at LanzaTech said, “The model lets us predict what would happen if we knockout certain genes or overexpress other genes, or even introduce an entirely new pathway.”

Dr Marcellin explains that the next step is to use the computer model to identify and modify gene targets in *C. autoethanogenum*.

“We can now manipulate cells in a way we couldn’t before,” he says. “So we’re excited to see what we can achieve.”

“Today, acetogens fix around 20% of the carbon in the atmosphere, making them a major player in the global carbon cycle.”

There is now increasing interest in using acetogen cultures to help mop up greenhouse gas emissions from large industrial sources such as factories, municipal waste or steel mills.

For example, enormous tanks filled with acetogenic bacteria can be retrofitted into industrial sites so that waste gases flow directly into these cultures instead of being released into the atmosphere. Acetogens then capture the carbon and convert it into useful chemicals and fuels.

“Acetogens are the preferred organisms for gas fermentation because they can use CO₂ and CO gas as their sole carbon and energy source,” says Dr Marcellin.

Moreover, acetogens use those gases to produce useful biofuels such as ethanol, a molecule that is now used to power cars.

In other words, bacteria like acetogens can turn waste gases into valuable resources.

He is confident that with the right genetic redesign, researchers can not only improve acetogens’ ability to take in CO₂ and CO₂, but could also coax these bacteria into making an even wider variety of useful products.
Growing medicines

Here is an interesting bit of chemistry trivia: a single tablet of aspirin contains trillions of aspirin molecules, and each one consists of only 21 atoms. Meanwhile, the diabetes drug metformin has only 20 atoms and the multiple sclerosis drug dalfampridine has 13.

Lithium carbonate? Just 6 atoms.

Due to their small size and simple structures, these drugs are relatively straightforward to synthesise in a laboratory and at industrial scales. In fact, most pharmaceuticals are small molecules, but much larger molecules called biologics are on the rise and could soon account for half of all therapeutic and preventative medicines.

What are biologics?

Biological medical products, or ‘biologics’ for short, are isolated from natural sources such as microorganisms, plants, animals and humans. They include vaccines, proteins, antibodies, hormones, allergens, as well as genes and other genetic material. Cells, tissues, blood and plasma are also considered biologics.

Because biologics are complex, they can do things small molecules can’t.

They can interact in a highly specific way with targets in the body that are also complex, such as immune cells, large proteins, genomic DNA, and even entire tissues. A small molecule cannot mimic a large molecule like a protein or a stretch of genetic material, and it certainly can’t replace a blood cell.

In other words, biologics are highly specific.

They can be tailored to the task at hand, and that is incredibly valuable because it provides a much better chance of targeting the problem — such as a cancer cell, a pathogen or a poorly functioning gene — while ideally leaving healthy cells alone.
How medicines are made in cells

Microbes are a critical part of the biomanufacturing process for a wide range of medicines.

Living cells have been in the business of making complex molecules and systems ever since evolution began. When it comes to building complicated small molecules, all the way to large and elaborate proteins and genomes, living cells have a repertoire of molecular machinery capable of reading genetic instructions and then marshalling a variety of cellular systems to carry out those instructions.

Sometimes those instructions are fairly straightforward. Human insulin, for example, is a relatively small protein with a simple structure. Thus, a microbe such as yeast can be very useful for producing insulin, because it has all the equipment needed for making a relatively uncomplicated protein. If you provide yeast with the gene for human insulin, it will read those instructions and build the protein one amino acid at a time, just like it would for any of its own proteins. You can even tweak the genetic instructions to increase production. Indeed, industrial quantities of human insulin are now grown in large cultures of yeast.

Professor Lars Nielsen at UQ’s AIBN explains that microbes are a critical part of biomanufacturing a wide range of medicines, but explains that there are also many cases where microbes just aren’t up to the task.

“Sometimes the chemistry required is well beyond what any simple microbe ever evolved to do,” he says.

Many proteins are too complex for microbes to handle and often need extra tweaks that microbes don’t know how to do.

“Recombinant proteins such as hormones, growth factors, cytokines and monoclonal antibodies play an important role in modern medicine, being used to treat a variety of diseases, such as diabetes, anaemia, hepatitis and cancer.”

“But many of these proteins require a range of modifications — such as the addition of special sugar groups or phosphate groups — to ensure correct folding, activity, safety and stability. “Microbes aren’t good at this, but mammalian cells are,” he says. Consequently, monoclonal antibodies and a variety of other therapeutic proteins are routinely manufactured in large cultures of mammalian cells.
Going through a phage:
How viruses help us make medicines

We now know that phages are incredibly useful as a scientific tool. In 2018, George P Smith and Sir Gregory P Winter were awarded half the Nobel Prize in Chemistry for showing that phages can be coaxed into making many different proteins on their viral surfaces. This has become an extremely useful way to make millions of new antibodies quickly and easily.

“Through the phage’s coat, but we’ve actually tricked it into making variations of the antibodies you want,” explains Dr Martina Jones, Operations Manager of the National Biologics Facility based at the AIBN.

“We can make whole libraries of different antibody sequences this way,” she says.

In fact, it’s possible to build a library of 10 billion different antibodies — each one on the surface of a phage — in a volume of less than one millilitre.

This is an incredibly powerful way to discover new antibody therapeutics. Let’s say scientists have discovered a new protein that sits on the surface of lung cancer cells. An antibody that precisely attaches to the unique bumps and folds of this protein could help the immune system seek and destroy those tumour cells. It may even directly interfere with the tumour’s ability to grow and spread.

“All you have to do now is go fishing,” says Jones.

“That tiny volume of antibodies is like a pond and the protein of interest is like the bait,” she says. “You put the bait into the pond and pull out the antibody that binds to it.”

Once they’ve identified the best antibody for the job, they need to make more of it, so researchers then make a copy of the gene for that antibody. The gene is built in bacteria, because it’s relatively straight forward to do. Unfortunately, bacteria don’t have all the molecular machinery for making antibodies, so the gene is transferred into mammalian cells. Researchers can grow large cultures of these mammalian cells and make high volumes of antibodies this way.

This approach to discovering and developing new therapeutic antibodies has been very effective.

In 2002, the antibody adalimumab (trade name: Humira®) which is used as a treatment for rheumatoid arthritis, was discovered by phage display. Currently, it is the world’s top-selling drug.
In the early 20th century, before the era of antibiotics, physicians used phages to treat life-threatening infections. At the time though, this approach was a bit hit-and-miss. A phage might eliminate a bacterial infection in one patient, but not another. There was also a problem with supply — phage cultures required constant maintenance, and hospitals often didn’t have the resources for this. Then along came antibiotics which were broadly effective and fairly easy to produce and store. Phages were largely abandoned as a therapeutic.

Now as antibiotic resistance in bacteria is on the rise, enabling the emergence of multi-drug resistant ‘superbugs’, scientists and clinicians are looking for other options. Phages are now emerging from the history books as a potential treatment for such superbugs.

We now know that the hit and miss characteristic of phage treatments is due to the fact that phages are highly specific to certain bacteria.

But what was once a disadvantage could now be very useful: it is anticipated that specific phages could soon be used to wipe out very specific bacterial infections, while leaving healthy bacteria alone.

Phage therapy is still experimental, but there are already a small number of cases where phages have been successfully used as an emergency treatment for drug-resistant infections. For example, researchers in the US and UK recently used genetically-engineered bacteriophages to treat a potentially fatal drug-resistant *mycobacterium abscessus* infection in a young cystic fibrosis patient. It’s the first time engineered phages have been used in a human and, importantly, it appears to have worked.

As phages receive increasing attention from the scientific and medical communities, biomanufacturers are exploring ways to produce phages at an industrial scale.

Ultimately, this involves letting phages do what phages do best: infecting bacteria.

The aim is to grow extremely large cultures of specific strains of bacteria in highly specialised bioreactors, then add phages of choice and let the infection spread. The phages take over the cellular machinery of the bacteria, turning each one into a phage-making factory.
In September 1994, in the Brisbane suburb of Hendra, something very dangerous happened — an ancient type of virus called a Henipavirus managed to jump from its animal host to infect a human. Within a matter of days thirteen infected horses had died. Of the two humans that had been infected, one quickly succumbed to a severe influenza-like illness.

The virus in question came to be known as the Hendra virus.

Hendra virus is a zoonotic disease, meaning it can jump from animals to humans. Most commonly, fruit bats act as a reservoir for the virus, but it can also be transmitted from bats to horses and — since 1994 — humans. By 2009, four people along with numerous horses had died after contracting Hendra, and more outbreaks were on the way. Fortunately, around the same time, researchers in the US were developing an antibody that could block a Henipavirus infection. When administered soon after exposure to a Henipavirus, this antibody binds to a protein on the surface of the virus, blocking entry to healthy human cells. The immune system can then fight off the virus.

The antibody, called m102.4, was developed by Professor Christopher Broder from the National Uniformed Services University (USU) in Washington DC. Following an outbreak of Hendra virus in 2010, Queensland Health obtained cells from USU that could produce a small amount of the antibody. The experimental treatment was administered to a mother and her 12 year old daughter who had been exposed to Hendra virus and,

Scaling up the production of antibodies has allowed researchers to fast track the development of a cure for the Hendra virus.

What is an antibody?

Antibodies are proteins that the body produces in response to the presence of an antigen, which is a molecule that is foreign to the body.

In the case of an invading pathogen, such as a bacteria or virus, proteins on the surface of those pathogens act as antigens. In response, immune cells produce very specific antibodies that can precisely recognise specific antigens and stick to them. These antibodies can serve as red-flags that will attract pathogen-destroying immune cells. Meanwhile, by binding to important proteins that the pathogen needs, antibodies can prevent pathogens from working properly.
What in the world is a zoonose?

Infectious diseases tend to prefer certain hosts, but over time those preferences can change or broaden. As a result, we have zoonotic diseases or ‘zoonoses’, which can be transmitted from animals to humans. This can happen through direct contact or through contaminated food or water. Seasonal influenza is an example of a zoonotic disease that jumped from animals to human hosts a long time ago. But sometimes that jump happens on a regular basis, such as with bird flu and Ebola virus. Consequently, global health officials are always monitoring emerging zoonotic diseases that could pose a threat to public health.

thankfully, they survived. Nevertheless, it was clear a larger supply of the antibody would be needed in the event of future outbreaks.

To address this, scientists at the National Biologics Facility at UQ’s AIBN developed a bioprocess to produce larger amounts of the antibody with high levels of purity required for clinical use in humans, and without needing to reproduce any part of the Hendra virus.

They were just in time. By July 2011, another outbreak of Hendra was underway, but there was enough antibody available. Production of the antibody for emergency compassionate use has since continued and, to date, the antibody has been administered to more than 10 people, including 3 children, who had high-risk exposure to Hendra virus. None developed the illness.

The National Biologics Facility also manufactured enough antibody to enable a recently completed phase I clinical trial.

Interestingly, the antibody can also be used against another Henipavirus, the highly lethal Nipah virus. Recently, the World Health Organisation listed Nipah virus as a priority pathogen with epidemic potential. The ability to produce the antibody at increasingly larger scales will no doubt play an important role in mitigating the impact and spread of this disease.
Welcome to the Biofoundry

How robots are playing a pivotal role in synthetic biology

The word ‘foundry’ may evoke images of a dark, noisy factory lit only by the glow of molten iron pouring into one casting after another, but in a biofoundry, something very different is going on.

Here, scientists stand in brightly lit laboratories keeping watch over gently humming machinery while robotic arms dispense droplets of liquid into multitudes of tiny wells, and transfer thousands of samples from one reaction plate to another.

In essence, a biofoundry is like a one-stop-shop for synthetic biology R&D, explains Associate Professor Claudia Vickers.

“In the design phase, researchers try to figure out the best way that the DNA componentry of a cell can be modified to achieve a desired result,” she says.

“We might use various different software tools, bespoke knowledge, modelling tools and more to design elements of the project.”

Next comes the building phase.

“For this, we use robots that assemble the DNA components,” she explains.

These state-of-the-art instruments can assemble thousands of complex fragments of DNA very quickly, each requiring only a few drops of liquid.

They allow vast combinations of genetic designs to be engineered almost simultaneously, enabling researchers to explore a huge range of variations.

“The robotic system can then transform those genetic components into a living cell,” she says. “It might be a mammalian cell, or a yeast cell or a bacterial cell, or you might use a cell-free system.”

In the latter case, only the basic biochemical machinery for reading and carrying out DNA instructions are used to make the product.

Now it’s time to test what you’ve built.

“If you’ve modified a microbe, you’ll want to grow cultures to see how they perform, and you’ll probably use some kind of analytical chemistry,” says Associate Professor Vickers.

Here again, robotics are very useful.

“You can grow extremely tiny cultures — maybe only a fraction of a millilitre, at first — and robotised or semi-robotised systems enable you to do this in a high-throughput way, so you can test thousands of cultures at once.”

Once you’ve identified the useful strains from high-throughput screening, you can then scale up to larger volumes and do more detailed analyses. The outcomes of the testing phase informs the next round of design.

Associate Professor Vickers explains that this is the beauty of a biofoundry: the capability to quickly go through iterative cycles and, in so doing, examine a huge ‘solution space’ and rapidly zero in on the best genetic design to solve the original problem.

The UQ-CSIRO BioFoundry is a high-throughput robotic synthetic biology construction facility housed at UQ’s AIBN and is part of the Australian Foundry for Advanced Biomanufacturing (AusFAB). Joining a global emergence of biofoundries in recent years, AusFAB is an AIBN-led collaboration focused on establishing and integrating Queensland capabilities in synthetic biology for advanced biomanufacturing including biofuels, chemicals, biologics and novel bio-inspired devices. Drop by drop, they are changing the world.

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Influenza in birds represents a significant biosecurity risk for a human pandemic, as well as a major risk for poultry industries. Forms of avian influenza, such as H5N1 and H7N9, can spread rapidly in poultry flocks and some strains already have the capacity to infect humans. For example, hundreds of cases of H5N1 in humans have occurred from close contact with infected birds, with a fatality rate of 60%.

“Vaccination in poultry can reduce the spread of influenza within poultry populations,” says Professor Linda Lua, Director of the UQ Protein Expression Facility. “This can protect the poultry industry in Australia during an outbreak.”

She explains that one problem is that current influenza vaccines are not easy to make quickly. To address this, Lua and her colleagues designed a ‘capsomere’ platform.

A protein called haemagglutinin usually sits on the surface of influenza viruses, and this can vary quite a lot between strains. Lua and her colleagues took part of this protein from an avian influenza virus and attached it to a specially designed protein called VP1. When five of these VP1 proteins link together in a ring, they form a ‘capsomere’.

The resulting donut-shaped capsomere is non-infectious, but because it displays parts of the virus protein, it trains the bird’s immune system to fight a real influenza infection. Recent tests of the avian influenza capsomere vaccine show that it prevents the illness in poultry.

The way the capsomere vaccines are produced provide another advantage. Currently influenza vaccines take six to nine months to be manufactured in large enough volumes. Such lag times make it precariously difficult to respond to an outbreak.

Moreover, vaccines are normally grown in embryonated chicken eggs. While this growth process is slow, it works well for a number of other diseases. However, avian influenza doesn’t just harm birds, it harms the embryos inside the eggs as well, making this form of vaccine production problematic.

By contrast, the capsomere production method uses bacteria to make the protein components, enabling high volumes to be made.

“We use an E. coli bacteria cell factory to produce the proteins that self-assemble into capsomeres inside the bacteria, and extract the proteins.”

It’s an efficient and powerful approach for mass-manufacture, and the resulting vaccine is potentially less than 10 cents per dose.

But the major advantage of the capsomere platform is its speed. The capsomeres can be quickly tailored to match circulating viruses, then large volumes of the new vaccine can be produced within weeks, enabling swift vaccination responses to prevent an outbreak. By preventing avian influenza from circulating within bird populations, this would also substantially lower the risk of the emergence of pandemic avian influenza in humans.
NASA intends to send humans to Mars by the 2030s. Aside from the substantial challenge of safely transporting humans all that way, there is also the matter of ensuring they’ll have the supplies for long term survival once they arrive.

Dr Lynn Rothschild, the Research and Technology Lead for the NASA Headquarters Space Technology Mission Directorate, has spent a lot of time thinking about how to achieve this.

As she explains in the journal *Biochemical Society Transactions*, the supply list for sustainable settlement is huge.

"As with any other settlers, they will need habitats, clothing, food, water, medicines, waste removal and recycling," she says.

"Although the solar output will be a resource, they will need supplemental sources of power, heat and light.

"Further, the technologies used must be able to be stored until needed, flexible in their applications and reliable as resupply will be infrequent."

We simply cannot send all those necessities from Earth. That size payload would be physically impossible to launch all at once and prohibitively expensive to send in increments. Currently, it costs around US$10,000 to launch just 454 g (1 pound) of payload into Earth’s orbit — a princely sum for something roughly the volume and weight of a can of soft drink.

There’s no way around it, says Rothschild. “The requirements of a human settlement will need to be met on location.” Impossible? Not at all.

“Biology, and synthetic biology in particular, can overcome many of these challenges.”

“Biologically-created structural materials have been critical throughout human history, from wood for construction, furniture and heat, to bone for knives and needles, fibres and leather for clothes and so on. There is no reason not to continue to use them beyond our home planet.”

Specifically, she’s talking about microbes which, if selected carefully, are adaptable, reliable, and highly reproducible. They’re also very lightweight.

Of course, Earth’s microbes did not evolve for Martian conditions. The Martian atmosphere is almost 96% CO₂, around 2% N₂ and 2% argon. At only 0.15%, atmospheric oxygen is nearly non-existent.
Meanwhile, Martian ‘soil’ is actually regolith — a fine powder of eroded iron-rich volcanic rock mixed with hydrogen peroxide and toxic chlorine-based compounds called perchlorates. It’s not a life-friendly environment.

This is precisely why synthetic biology will be essential, says Rothschild, because it will allow researchers to genetically tailor a variety of Earth microrganisms to use Martian raw materials to produce food, fuel, clothes, medicines, and even shelter.

Microbes such as the bacterium *Sporosarcina pasteurii* and certain species of *Bacillus* can be added to a solution of sand, calcium chloride, and urea (from urine) to induce formation of calcium carbonate, which fuses the sand grains together. This process is already being used to make bricks here on Earth and researchers are developing a similar approach to form bricks and bio-cement out of Martian regolith.

Microbes can also help convert CO₂ into bioplastics or graphene-like materials. These microbes can even be 3D printed in pre-determined patterns to make complex macroscale structures. This would enable production of tools, food, smart fabrics, bio-batteries and even replacement organs.

The potential applications are myriad, says Rothschild.

“For millennia we have used biology to do chemistry on Earth. In the future, we will use biology to do chemistry beyond Earth.”

“By the end of the decade, we will have taken the first steps towards realising the vision of a synthetic biology-enabled future off planet.”

Microbes could be engineered to produce ethanol and hydrogen for use as biofuels, as well as rubber hydrocarbons, silica, latex, cellulose, and spider silk, which has a tensile strength greater than steel and can be enriched with carbon nanotubes to make fibres stronger than Kevlar. Microbes could perhaps even detoxify Martian soil.
Looking to the Future...

The UQ-CSIRO Synthetic Biology Alliance will be particularly focussed on synthetic biology applications in biomanufacturing and industrial biotechnology.

As the Fourth Industrial Revolution takes off, global industries are already changing rapidly. With this change comes economic opportunity along with significant potential to generate societal and environmental benefits in Australia and around the world.

“It’s critically important that we have a knowledge based economy going into the future, and advanced biomanufacturing is a major part of that,” says Associate Professor Claudia Vickers, a Group Leader at UQ’s AIBN and Director of the CSIRO Synthetic Biology Future Science Platform (SynBio FSP).

Synthetic biology is a critical tool that allows engineering of biological systems for efficient advanced biomanufacturing. That’s why the University of Queensland and CSIRO are building a $4.5 million initiative to boost Australia’s synthetic biology capabilities and, in so doing, will drive advances in areas such as advanced biomanufacturing, environmental remediation, biosecurity, agriculture and healthcare research.

The UQ-CSIRO Synthetic Biology Alliance involves a close collaboration between the CSIRO SynBioFSP and UQ’s Australian Institute for Bioengineering and Nanotechnology (AIBN).

“There’s incredible power in bringing together the capabilities of two really first class research organisations to capitalise on the skills and the strengths of both,” says Associate Professor Vickers.

The Alliance will be particularly focussed on synthetic biology applications in biomanufacturing and industrial biotechnology. In particular they are interested in converting cheap low cost biomass into value added products, she says.

Microbes play a central role in this conversion process, and by joining forces in terms of expertise and resources, the researchers at UQ’s AIBN and at CSIRO will be able to strategically engineer the metabolic pathways in those microbes to make them as efficient and productive as possible.

It’s an endeavour that will set us on a good path toward future industries, says AIBN Director Professor Alan Rowan.

“Our joint vision is to help create a sustainable, export-oriented biotechnology and bioproducts sector, attracting significant national and international investment, and creating regional, high-value and knowledge-intensive jobs,” he says.

“We’re delighted to be part of this,” says Rowan.

Training the next generation of biomanufacturing experts

Cells are indeed remarkable little factories, and harnessing their capacity to perform complex chemistry is driving progress in a wide variety of areas, including global health.

As we’ve seen, the ability to easily and affordably manufacture biopharmaceuticals and other complex biologics is already transforming the way many medical conditions are treated. This is exciting, but the continued development of innovative new technologies and techniques will require more than just tiny cell factories, it will require a highly skilled workforce, too.

For advanced biomanufacturing of biopharmaceuticals and other complex biologics to truly thrive as an industry and revolutionise medicine, we need to train the next generation of experts. Fortunately, this is already underway.

“Governments are putting more focus on training and professional development in the biologics sector,” says Professor Stephen Mahler, Director of the ARC Training Centre for Biopharmaceutical Innovation (CBI), which is based at UQ’s AIBN.

The CBI brings together a critical mass of expertise, world-class facilities and industrial partnerships, and has established links with other organisations including the National Biologics Facility (NBF), which is funded by the National Capability Research Infrastructure Strategy.

Together, CBI and NBF are driving a National Biologics Training Program: a continuing professional development program equipping those involved in the biologics industry with cutting edge skills and knowledge.

CBI currently supports 14 PhD students and five early career post-doctoral scientists, while its industry partners provide mentoring and placements for these students and scientists to gain valuable insight into the needs of the biopharmaceutical industry.

“With industry-driven projects directly linked to outcomes, these scientists are finding solutions to current problems, and are gaining the skills to support the growth of the industry into the future,” says Professor Mahler.

“Australia has a culture of innovation,” he adds. “Our curiosity and problem solving skills have driven the invention of some amazing discoveries and creations, from the ute right through to Wi-Fi.”

“With a focus on training combined with access to state-of-the-art facilities, Australia is well positioned to capitalise on the growing biologics industry, too, and to continue delivering high quality products and outcomes through research, development and advanced manufacturing of biopharmaceuticals both now and into the future.”
AIBN is changing the future

Learn more - talk to our Advanced Biomanufacturing Leaders

Prof Lars Nielsen
AIBN Senior Group Leader
Scientific Director of the Novo Nordisk Foundation Centre for Biosustainability

Prof Stephen Mahler
AIBN Senior Group Leader
Director of the Centre for Biopharmaceutical Innovation

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