

# Fossilising fossil fuels with green alternatives

Green Biologics are a renewable chemicals company who are not only changing the face of renewable chemicals, but are changing the world while they are at it. **Dr Liz Jenkinson** is one of the lead researchers at the company, and it is her work that is providing the answer to the question: is there an alternative to fossil fuels? Her work proves that the answer is yes, and that it only relies on three key components – bacteria, genetic engineering and sugar.

With the unprecedented threat of climate change making the planet's temperature warmer year on year, there is a growing need for greener, environmentally-friendly alternative products. It has been well documented over the years that fossil fuels are in limited supply and yet, they are the source used to power the world. They run our cars, heat our homes, and are even used to produce a variety of products such as medicines, cosmetics, plastics and lubricants. If you brushed your teeth this morning, or if you have ever played tennis, the toothpaste and balls you used were probably produced using fossil fuels.

So, the question is: if we are to reduce our dependency on fossil fuels, how are we going to continue our modern way of living?

This is where Green Biologics and the excellent work of a dedicated team of molecular microbiologists, analytical chemists and fermentation scientists comes in. The Oxford-based institution and its US-based subsidiary have recently opened the first renewable ABE manufacturing plant in the USA since the second World War, to convert the sugar from corn into the products acetone and n-Butanol along with by-products of corn oil and animal feed. These chemicals and their modified derivatives can then be used in a wide range of everyday products directly replacing the same chemicals that are currently made from fossil fuels.

**FROM MANCHESTER TO OXFORD**  
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**Green Biologics's research follows on from the original acetone–butanol–ethanol fermentation work carried out in 1912, using a bacterium called *Clostridium* as a biocatalyst to create n-Butanol and acetone**



in Manchester back in 1912. This discovered a method that could use bacterial fermentation (the conversion of sugar into products) to produce acetone, n-Butanol, and ethanol from carbohydrates such as starch and glucose. Over a hundred years later, Green Biologics have taken this concept many steps further. Their research follows the same principle of bacterial fermentation, using a bacterium called *Clostridium* as a biocatalyst to create n-Butanol and acetone but Green Biologics have improved both the bacterium and the process to produce cost-effective, higher-quality chemicals when compared to the fossil-derived versions. These chemicals can then be used directly or reacted to make derivatives, before being used in products such as paints, fragrances, cosmetics, lubricants and even as ingredients for food.

## JUST A SPOONFUL OF SUGAR...

The whole process, in effect, revolves around breaking down sugar, and converting it into n-Butanol and acetone via bacterial fermentation using *Clostridia* microbial strains. The simpler and more accessible the sugars, the more efficient this process is. For example *Clostridia* will quite happily ferment glucose which is a C6 sugar or xylose which is a C5 sugar. However, the feedstocks used for a commercial fermentation process are rarely simple and without this, the *Clostridia* microbial strain is unable to ferment correctly to produce the required products quickly enough and at high enough concentrations.

## ...HELPS THE CLOSTRIDIA FERMENT...

The team at Green Biologics have overcome this issue through a combination of advanced engineering and strain improvement methods. Using methods such as adaptive lab evolution, they have developed improved clostridial strains to use in the process of breaking down C5 and C6 sugars and for overcoming a number of other challenges associated with this type of fermentation. Not only that, but as these strains have been produced without the need for genetic modification (GM), they are natural and safe to use at the Minnesota plant.

### ...IN THE MOST DELIGHTFUL WAY

This plant – known as Central Minnesota Renewables – currently uses the ABE fermentation process with *Clostridia* to ferment sugars found in corn. However, it is hoped that future research and technological developments will move the process towards using sugars found in lignocellulosic feedstocks (i.e., corn stover, bagasse, woody biomass). Currently, the C5 and C6 sugars contained within these feedstocks are inaccessible to *Clostridia* and are unable to be broken down directly. Future research will therefore look to establish hydrolysis pre-treatments that will allow the sugars to be accessed and converted.

This is just one of Green Biologics' current research focuses, progressing in conjunction with Dr Jenkinson's ground-breaking work using CLEAVE™ technology. This technology is a different way of applying CRISPR gene-editing technology, designed to make highly specific changes in the clostridial DNA (for example deleting a specific region of a gene or making a single base pair change). It can also be used to integrate specific genes into *Clostridia* microbial strains. These genes can be pinpointed, edited and developed to incorporate the new functionalities required.

In other words, CLEAVE™ technology has provided Dr Jenkinson and her team with a breakthrough technology capable of expanding and diversifying their product range. Using this innovation, clostridial microbes can be effectively converted into small chemical factories which, with the application of genomic editing and synthetic biology techniques, can develop more products than the

butanol and acetone produced during ABE fermentation.

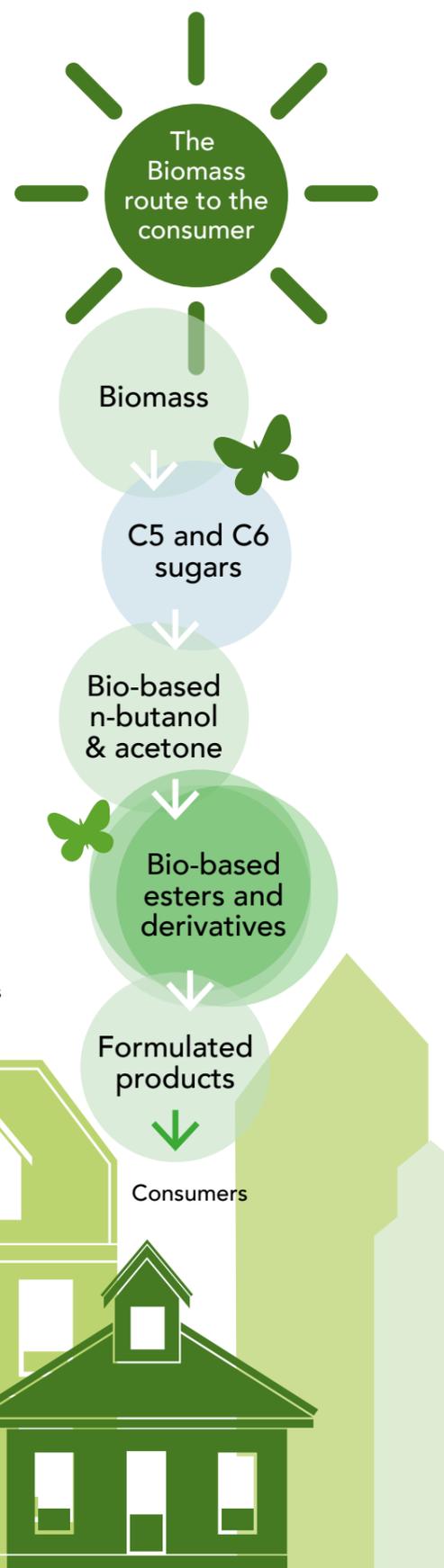
### CLEAVING CLOSTRIDIA

The ability to edit genomes and utilise synthetic biology within *Clostridia* enables new biological pathways to be added or removed. This, in turn, generates different products that can be utilised across different industries. In one example of Dr Jenkinson's work, a novel pathway was added to the fermentation process using CLEAVE™ technology to produce a chemical particularly valuable within the food industry. In another example, CLEAVE™ was used to alter the ratio of butanol and acetone produced during the ABE fermentation process, depending on the quantity required and the value of each product. Not only that, but by optimising and inserting new enzymatic genes into the *Clostridia*, the microbes can be modified to break down more complex carbohydrates, which supports and ties into Green Biologics' other area of research – accessing sugars contained within lignocellulosic feedstocks.

### A SWEET FUTURE

Although the research undertaken by Dr Jenkinson and her team is yet to be published, the emergence of CLEAVE™ technology as a potential, scientifically-proven, alternative method to fossil fuels is ground breaking work, and is likely to change the world as we know it. The ability to develop a diverse range of products using Green Biologics' methods will not only help the environment, but it will also provide a platform on which further beneficial research can take place.

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# Behind the Bench

## Dr Liz Jenkinson

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### Bio

Dr Jenkinson received a Bachelor's degree in Molecular Genetics in Biotechnology at Sussex University before undertaking a PhD in Biology at York University. She is currently the

head of the strain development team at Green Biologics, utilising her expertise in molecular biology to develop key tools capable of manipulating *Clostridia* microbial strains. She is also in charge of several Innovate UK funded grant projects, primarily focused on synthetic biology.

### Research Objectives

Dr Jenkinson's research focuses on using microbial engineering and synthetic biology techniques to utilise *Clostridia* microbial strains as biocatalysts. She and her team at Green Biologics aim to provide

customers with more sustainable, green alternatives for everyday products such as paints, cosmetics and food ingredients.

### Funding

Green Biologics has benefitted from private and public funding including investors Swire Pacific, Sofinnova, Capricorn Ventures, Morningside and Oxford Capital partners, and UK, European and American agencies including Innovate UK, Horizon2020, ERANET, and US Department of Energy

## Q&A

### What made you decide to get involved with Green Biologics?

When I joined, the company was still working in many different areas, including research contracts, before focusing on *Clostridia* and chemical production. For me the interest was in the science – we were working with bacteria that are not standard lab strains therefore we had lots of challenges to overcome and that was what drew me in.

### Why is finding green alternatives to products typically derived from fossil fuels so important to you?

There is a need to find alternative ways of making these chemicals. Using microbial fermentation is one way to do this whereby we can take waste and convert it into something useful. Ultimately these processes can be both renewable and sustainable, they can reduce greenhouse gas emissions, and they can provide some security regarding supply chains.

### How likely is it that your technology could replace fossil fuels as a power source (rather than as a source of chemicals to use in products) in the future?

Right now the challenge with using the bio-butanol from our process as a

biofuel is related to cost. Butanol can be used instead of petrol and the founder of Butylfuel, Dave Ramey, has shown this by driving a Buick across the US powered by 100% n-butanol (<http://www.butylfude.com/the-2005-trip.html>). At the moment, our production costs cannot compete with petrol, primarily due to the cost of feedstocks. In an ideal process our feedstock costs would be minimal, using waste that would otherwise be burnt or left to breakdown naturally. However right now, the sugars contained in these lignocellulosic feedstocks are generally inaccessible to our strains and the pre-treatment processes are either not efficient or economic to use at scale. As these technologies mature, the costs of production will come down and in the future it is feasible that bio-butanol could be used as a fuel to power our cars.

### Why did you choose to use *Clostridium* as the bacterium in the fermentation process?

Solventogenic *Clostridia* have previously been used as industrial microbes for the production of ABE; therefore, we know they are robust enough to be scaled up. The process itself, however, fell out of favour when it could no longer compete economically with petrochemical-derived products. In the last 15–20 years our understanding of these microbes, the advances made in genomics and in genetic manipulation technologies and

the advances made with fermentation technology development mean we can be competitive again, especially in the high-value market sectors. Synthetic biology has great potential for engineering organisms to make products that we need, but if we can work with nature to give us a head start, as we have with *Clostridia*, then we are more likely to be successful.

### With the recent purchase of a manufacturing plant in America, will Green Biologics continue to expand in the future?

That is the idea. Our first commercial plant at Little Falls, Minnesota began production in 2016. We are currently producing butanol and acetone and selling them to chemical companies and directly blended into consumer products. Our first product, Greenflame™ charcoal lighter fluid is now available in stores in the US ([www.greenflame.com](http://www.greenflame.com)). Over the next 12–18 months, production of acetone and butanol will be ramped up and in the meantime we are looking for potential new plants in the US or Europe. Ultimately, we will introduce new products developed through CLEAVE™ to develop biorefineries making a range of renewable chemicals.