Institution: Imperial College London

Unit of Assessment: 5 Biological Sciences

Title of case study: 3 - The development of a commercial biofuel from waste process and the success of TMO Renewables Ltd

1. Summary of the impact (indicative maximum 100 words)

Working with TMO Renewables Ltd, work in Dr Leak's laboratory at Imperial College demonstrated that thermophilic bacteria of the genus Geobacillus could use a novel (i.e. non-textbook) route to produce ethanol. TMO used this information to develop strains that produced ethanol in commercially useful quantities from lignocellulose breakdown products. This allowed them to: (i) compete for and win a major contract ($500M over 20 years) with Fiberight in the USA in 2010, to turn fermentable components of municipal solid waste into biofuel, (ii) enter into partnership agreements with the China National Offshore Oil Corporation and the China National Cereals, Oils and Foodstuffs Corporation in 2011, and (iii) enter into agreements in 2012 and 2013 with Usina Santa Maria Cerquilho to build a bioethanol production facility in Brazil. The most recent agreement with Brazil will create more than 150 new jobs in the UK. At the end of 2011, TMO Renewables reported a net worth of almost £11million.

2. Underpinning research (indicative maximum 500 words)

TMO Renewables Ltd was founded in 2002 after the demise of Agrol Ltd, a company set up by Prof Brian Hartley on his retirement from Imperial College and working on a similar theme. Dr Leak (at Imperial 1984-2012, Reader in Applied Microbiology) worked with Prof Hartley during his time at Imperial, with Agrol and TMO Renewables studying fundamental and applied aspects of Geobacillus spp. [TMO was formed by Prof Tony Atkinson (who was a consultant to Agrol) and Joachim Lukas (who had been CEO at Agrol).]

The classic route to ethanol production involves metabolism via an enzyme pyruvate decarboxylase, which is found in yeast. Routes to make ethanol in other organisms by cloning and expressing this enzyme in other organisms are covered by publications (Ingram, et al (1987) Appl. Environ. Microbiol. 53:2420-2425 and many papers thereafter) and extensive patents to the University of Florida. Although other organisms do make ethanol, they use a route which is difficult to convert into one which will only make ethanol as its end product i.e. other products are made. The discovery we made was both timely and mechanistically novel as a metabolic route i.e. we didn't simply take a pathway from another organism but came up with a completely new pathway. In early work (San Martin et al 1993-1994 [1,2]), it had been shown that mutants of Geobacillus thermoglucosidasius (formerly known as Bacillus thermoglucosidasius), which were impaired in their natural fermentation pathways (particularly lactate production), were able to produce more ethanol than theory would predict. We were interested in exploring why this happened and, suspecting that it might involve upregulation of expression of the enzyme pyruvate dehydrogenase (Pdh), ran a set of experiments under aerobic and anaerobic conditions in wild-type and mutant strains, looking at Pdh enzyme activity and transcript levels. Under the direction of Dr Leak, studies were done by Kostas Gialamas (2002-2006) and Mark Taylor (2003-2007) during their PhDs at Imperial. Mark Taylor was a CASE student working with TMO.

The work led to a successful application for a BBSRC-IPA grant (2007-2010, [G2]), backed by TMO Biotec (as they then were). The main results were incorporated into a seminal paper which appeared in 2009 [3], which described the development of the fermentation pathways which underpin the current TMO process.

What we had done in our early work is show that, when stressed, G. thermoglucosidasius could use this novel pathway to make ethanol. What we had not done was then to deliberately up-regulate this pathway to show that this could be used as a tool in a newly engineered strain. Although we may have had some claim to the IP involved, an understanding was reached, which

allowed TMO to exploit these findings (US patent 2010/0173373) and take the work forward to commercialisation. It could be argued that the up-regulation of the pathway was obvious, but this demonstration by TMO was enough to allow them to secure the patent.

3. References to the research (* References that best indicate quality of underpinning research)

Original observations:
[1] *San Martin, R., Bushell, D., Leak, D.J. & Hartley., B.S., '*Pathways of ethanol production from sucrose by a mutant thermophilic Bacillus in continuous culture'*, Journal of General Microbiology, 139**,** 1033-1040 (1993). DOI.
[2] *San Martin, R., Bushell, D., Leak, D.J. & Hartley, B.S., '*Cultivation of an L-Lactate dehydrogenase mutant of Bacillus stearothermophilus in continuous culture with cell recycle*', Biotech. Bioeng., 44, 21-28 (1994). DOI.

Key output:
[3] *Cripps, R.E., Eley, K., Leak, D.J., Rudd, B., Taylor, M., Todd, M., Boakes, S., Martin, S. & Atkinson, T., '*Metabolic engineering of Geobacillus thermoglucosidasius for high yield ethanol production*', Metabolic Engineering, 11**,** 398-408 (2009). DOI.

Grant support:

[G1] EPSRC, LINK scheme, GR/K32708/0, '*Ethanol from hemicellulosic wastes*', 01/05/94-30/04/96, PI: David Leak, Co-I: D Stuckey, Project Partners: Pre Nexus Migration, £162,255.
[G2] BBSRC-IPA (with TMO renewables), BB/E002994/1, 'Developing strategies and a toolbox for metabolic engineering of thermophiles for ethanol production', 01/02/07-31/05/10, PI: David Leak, £338,088
[G3] 1 BBSRC CASE student with Agrol Ltd (Ann Thompson), 1 with TMO prior to US patent 2010/0173373, 2 current.

4. Details of the impact (indicative maximum 750 words)

As described in Section 2, we demonstrated the principle of a novel pathway to make ethanol and we continued to work with Agrol and TMO. Surprisingly, Agrol did not take the initial observation forward. With Kostas Gialamas, we provided biochemical/physiological confirmation of what had been suggested in the 1993 paper [1], but TMO saw the opportunity to capitalise on the presumed mechanism by making the deliberately engineered change to their organism [A].

In June 2008, TMO commissioned an industrial scale Process Demonstration Unit (PDU) in Surrey to demonstrate the commercial capability of the TMO Process. Covering an area of 12,000 square feet and costing in excess of £7.8 million, the PDU is used to conduct feasibility studies on a wide range of feedstocks to determine the optimal process for each material for clients at a commercially relevant scale. The PDU, which was the U.K.'s first cellulosic demonstration facility, also houses a smaller "Scale-Through" system that mirrors the details of the larger demonstration facility [B, C].

The use of an upregulated Pdh pathway is fundamental to the TMO process. It provides a mechanism to convert any fermentable substrate which the organism is capable of using (and future engineered strains) to ethanol. In Autumn 2010, TMO announced that it had been awarded an exclusive $500M contract with leading clean technology company **Fiberight LLC** in the USA. The contract is to convert the fermentable components of municipal solid waste to ethanol. The agreement involves building 15 commercial-scale cellulosic ethanol production facilities, linked to Fiberight's sorting facilities, across the USA over the next 5 years [D]. The contract was awarded against competition from US companies, such as the renewable fuel company Mascoma. In 2011, work started on the first commercial plant in the US state of Iowa. The facilities will allow the conversion of municipal solid waste to ethanol at a more efficient, cost-effective pace. According to Stuart-Paul, CEO of Fiberight, *"Integrating TMO's process with our own will give Fiberight the edge compared with other ethanol producing technologies, allowing us to be more efficient with waste than our competitors….Together, the companies are on track to become one of the largest*

producers of cellulosic ethanol in the U.S. during 2011, helping to divert millions of tons of waste away from landfills every year" [D].

More recently, TMO has been developing collaborations in China and Brazil. In China, where it has favoured company status with the Chinese Government, it is developing its lignocellulosic ethanol process to work with cassava stalks. In 2011, TMO entered into two separate partnership agreements with **China National Offshore Oil Corporation (CNOOC) New Energy Investment** and the **China National Cereals, Oils and Foodstuffs Corporation (COFCO)** – large state owned companies [E, F]. Both agreements entail TMO embarking on testing programs jointly with each partner to develop the country's first fully commercial second-generation ethanol plants using cassava residue and cassava stalk. The agreement with COFCO was for a joint testing program to manufacture ethanol from cassava residue and stalk and to finalize a design package for the first fully commercial 2g ethanol plant in China. The agreement with CNOOC was also for a joint testing program. Again focusing on the manufacture of ethanol from cassava residue and cassava stalk, the aim of the agreement with CNOOC is to develop an integrated 1g and 2g 180,000 ton plant that CNOOC has applied to build in Nanning, Guangxi province. TMO, COFCO and CNOOC continue to work closely together to build a commercial process (relying on the Pdh technology) on its preferred cassava feedstock [E, F].

In August 2012, TMO signed a Memorandum of Understanding to secure a 20-year large volume biomass feedstock supply for future biofuel production facilities from the **Heilongjang State Farm** (HSF), the largest state owned farming corporation in China [G, H]. TMO will assess the potential of the HSF-sourced feedstock at its PDU in Surrey. The MOU is a first step towards building the first of a future series of second-generation biofuel production facilities in China.

Most recently, during a business delegation to Brazil led by David Cameron in September 2012, TMO signed a letter of intent (LOI) with **Usina Santa Maria Cerquilho**. Usina Santa Maria Cerquilho is an associate of Copasucar, the largest Brazilian sugar and ethanol commercialization operation with integrated production. The agreement is to secure a 25-year feedstock supply, initially to support a 10 million litre per annum second generation ethanol refinery to be built alongside a sugar mill owned by Usina Santa Maria Cerquilho. The LOI provides for 400,000 tonnes per year of bagasse (leftover sugarcane biomass) [I]. Prime minister David Cameron endorsed TMO's achievements by commenting:

> "*this visit (…) has given TMO a foothold in the Brazilian market. It reflects TMO's expertise in this pioneering technology and highlights the opportunities for British companies in this fast growing market. TMO's success in China and now in Brazil demonstrates exactly what the government is working to achieve – getting more British companies linked up with new markets to boost growth and create jobs back at home*." [I]

Following on from the delegation to Brazil, in April 2013 it was announced that TMO and **Usina Santa Maria Ltda** has signed an MOU to build Brazil's first Second Generation bioethanol production facility in São Paulo state - the first commercial production plant in Brazil to convert bagasse to cellulosic bioethanol. The facility will be followed by the construction of full-scale industrial plant, scheduled to go into construction in 2014, with the bioethanol primarily being used to power Flex-Fuel vehicles in Brazilian market. The agreement will create jobs in Brazil and over 150 new jobs in the UK [J].

The success of TMO Renewables and the collaboration with Imperial College has been highlighted on the BBSRC web pages [K] and the company has recently been visited by David Willetts. In addition to the USA, China and Brazil, TMO is working closely with a number of other partners around the world. The latest Annual Accounts submitted to Companies House for TMO Renewables Ltd, for the year to 31/12/2011, reported 'cash at bank' of £6,620,000, 'liabilities' worth £2,735,000, 'net worth' of £10,890,000 and 'assets' worth £8,447,000 [L].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[A] TMO Group website, http://www.tmo-group.com/ (archived at https://www.imperial.ac.uk/ref/webarchive/53f on 6/11/13)
[B] Green Car Congress, 'TMO Renewables signs MOU with Province of Heilongjiang for biomass feedstock for biofuel production', 23/8/12, http://www.greencarcongress.com/2012/08/tmo-20120823.html (archived at https://www.imperial.ac.uk/ref/webarchive/47f on 6/11/13)
[C] TMO demonstration plant, http://www.tmo-group.com/demonstration-plant/ (archived at https://www.imperial.ac.uk/ref/webarchive/c4f on 6/11/13)
[D] Ethanol Producer Magazine, 'Fiberight, TMO to build 15 plants in 5 years', 23/9/10, http://www.ethanolproducer.com/articles/6999/fiberight-tmo-to-build-15-plants-in-5-years/ (archived at https://www.imperial.ac.uk/ref/webarchive/8nf on 24/7/13)
[E] Green Car Congress, 'TMO Renewables partners with COFCO and CNOOC on second-generation ethanol in China; cassava residue and stalks', 9/5/11, http://www.greencarcongress.com/2011/05/tmo-20110509.html (archived at https://www.imperial.ac.uk/ref/webarchive/73f on 6/11/13)
[F] BioFuels Digest, 'Advanced Biofuels in China: TMO, COFCO, CNOOC New Energy join fast-growing ranks', http://www.biofuelsdigest.com/bdigest/2011/05/10/advanced-biofuels-in-china-tmo-cofco-cnooc-new-energy-join-fast-growing-ranks/ (archived at https://www.imperial.ac.uk/ref/webarchive/83f on 6/11/13)
[G] 'TMO Renewables sign MOU with Province of Heilongjang', TMO press release, 23/8/12, http://tmo-group.com/tmo-renewables-signs-mou-with-province-of-heilongjiang/#more-283 (archived at https://www.imperial.ac.uk/ref/webarchive/9nf on 24/7/13)
[H] Biomass Magazine, 'TMO Renewables, Chinese officials sign MOU for biomass supply', 29/8/12, http://biomassmagazine.com/articles/8019/tmo-renewables-chinese-officials-sign-mou-for-biomass-supply (archived at https://www.imperial.ac.uk/ref/webarchive/93f on 6/11/13)
[I] 'UK Prime Minister David Cameron endorses TMO's expertise and achievements during UKTI delegation to Brazil', TMO press release, 2/10/12, http://tmo-group.com/uk-prime-minister-david-cameron-endorses-tmos-expertise-and-achievements-during-ukti-delegation-to-brazil/#more-481 (archived at https://www.imperial.ac.uk/ref/webarchive/0nf on 24/7/13)
[J] 'Anglo-Brazilian Joint Venture to launch first 2G Commercial Cellulosic Ethanol Production Plant', TMO Group, 11/4/13, http://www.tmo-group.com/anglo-brazilian-joint-venture-to-launch-first-2g-commercial-cellulosic-ethanol-production-plant/ (archived at https://www.imperial.ac.uk/ref/webarchive/f4f on 6/11/13)
[K] 'Big score for British biofuel technology', BBSRC news, 27/1/12, http://www.bbsrc.ac.uk/news/industrial-biotechnology/2012/120127-f-british-biofuel-technology.aspx (archived at https://www.imperial.ac.uk/ref/webarchive/bpf on 24/7/13)
[L] http://companycheck.co.uk/company/04405622/financial-accounts (archived at https://www.imperial.ac.uk/ref/webarchive/g4f on 6/11/13)

Individuals who can be contacted to corroborate the impact:
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