

Institution: The University of Edinburgh		
Unit of Assessment: UoA 5 Biological Sciences		
Title of Case Study: Cultivation and genetic manipulation of cyanobacteria boosts production of natural blue food colouring, and investment at the SME ScotBio		
Period when the underpinning research was undertaken: 2013 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Dr Andrew Free	Senior Lecturer	2011 – present
Dr Alistair McCormick	Reader in Plant Synthetic Biology	2013 – present
Dr Baojun Wang	Lecturer in Synthetic Biology	2013 - present
Dr Attila Molnar	Senior Lecturer in Applied Plant Biology	2013 – present
Period when the claimed impact occurred: Aug 2013 – Dec 2020		
Is this case study continued from a case study submitted in 2014? Y/N No		
1. Summary of the impact <p>Attribution: Research on the cultivation and genetic manipulation of cyanobacteria by University of Edinburgh scientists, Andrew Free, Alistair McCormick, Baojun Wang and Attila Molnar has allowed commercial partner ScotBio to produce more consistent and purer natural blue dye (C-PC) in demand worldwide.</p> <p>Impact on economy: ScotBio has adopted new processes, developed in partnership with researchers at the University of Edinburgh that have led to an increase in yield & quality of their product: C-PC. These improvements resulted in ScotBio winning a GBP2,500,000 investment to scale up and further grow their business. C-PC, a high value product, has been brought to market (initially in a naturally blue version of Firkin gin - Firkin Blue). 19 staff have been recruited at ScotBio and the company has entered new markets (pharmaceutical grade C-PC and synthetic biology tools for <i>Spirulina</i>).</p> <p>Beneficiaries: ScotBio is a Scottish SME focusing on food grade production of blue pigment. This pigment is now used in food, cosmetics, textiles and pharmaceuticals, providing benefits for companies operating in those markets and the consumers thereof.</p> <p>Significance and Reach: Global awareness of food additives has increased demand for natural and safe colourings significantly so that demand for naturally blue phycocyanin has outstripped supply. The international market for C-PC is currently USD44,000,000 (2020) and is expected to reach USD121,000,000 by the end of 2025 with natural food colourings and pharmaceutical industry as key markets. ScotBio has recently raised GBP2,000,000 in funding to scale up in order to access this market.</p>		
2. Underpinning research <p>Cyanobacteria are an evolutionarily ancient and diverse phylum of photosynthetic prokaryotic organisms. They are increasingly used in industrial biotechnology because of their ability to produce high value products such as the blue pigment protein C-Phycocyanin. The SME,</p>		

ScotBio (known as *Scottish Bioenergy* prior to March 2018), specialises in the development of photobioreactor systems and downstream processing methods. They initiated a series of collaborations with researchers from the School of Biological Sciences to optimise yield and increase purity and quality of C-PC from cyanobacteria for sale in the food and pharmaceutical industries.

Alistair McCormick and Baojun Wang have developed a molecular toolbox (CyanoGate) [3.1] for cyanobacteria using synthetic biology techniques. The research produced a standardised modular cloning system, based on the Golden Gate cloning system that was not previously available for cyanobacteria. This brought cyanobacteria in line with bacterial, yeast and mammalian systems. McCormick and Wang constructed and characterized a suite of known and new genetic components (level 0 parts for MoClo assembly), designed an additional level of acceptor vectors (level T) to facilitate integrative or replicative transformation in cyanobacteria and characterized assembled level T vectors in the model species *Synechocystis* sp. PCC 6803 and *Synechococcus elongates* UTEX 2973 [3.2]. In collaboration with The Edinburgh Genome Foundry (EGF), they also developed an online tool for assembly of CyanoGate and Plant MoClo vectors to assist with the adoption of the CyanoGate system [3.1]. This collaboration led to the development of a successful application to build a genome knockout library of *Synechocystis* sp. PCC 6803 for the cyanobacterial research community called CyanoSource using the EGF facilities (BBSRC grant number BB/S020128/1) [3.3].

Using CyanoGate, McCormick is creating novel synthetic genetic sensor-regulator circuits to increased yields and quality of high value products such as C-PC. They are also working towards producing thermo-tolerant versions of C-PC [3.4]. The group have developed a robust and scalable downstream processing pipeline to optimize yield and purity of C-PC in order to facilitate the commercial scale up of C-PC production. This includes the development of a scalable ion exchange chromatography protocol for purification. McCormick is developing a 3D-printed anion column to assist with purification of C-PC from crude biomass, which could save significant cost in the purification process [3.5].

3. References to the research

[3.1] Vasudevan, R, Gale, G, Schiavon osorio, AA, Puzorjov, A, Malin, J, Gillespie, MD, Vavitsas, K, Zulkower, V, Wang, B, Howe, CJ, Lea-Smith, DJ & McCormick, A. 2019. CyanoGate: A modular cloning suite for engineering cyanobacteria based on the plant MoClo syntax. *Plant Physiology*, DOI: <https://doi.org/10.1104/pp.18.01401>

[3.2] Gale GAR, Schiavon Osoria AA, Puzorjov A, Wang B, McCormick AJ. 2019. Genetic modification of cyanobacteria by conjugation using the CyanoGate modular cloning toolkit. *Journal of Visualized Experiments* 152: e60451, <https://dx.doi.org/10.3791/60451>.

[3.3] Mills LA, McCormick AJ, Lea-Smith DJ. 2020. Current knowledge and recent advances in understanding metabolism of the model cyanobacterium *Synechocystis* sp. PCC 6803. *Bioscience Reports* 40: BSR20193325, <https://doi.org/10.1042/bsr20193325>.

[3.4] Puzorjov A, McCormick AJ. 2020. Phycobiliproteins from extreme environments and their potential applications. *Journal of Experimental Botany* 71: 3827-3842, <https://doi.org/10.1093/jxb/eraa139>.

[3.5] Simon, Ursula, et al. "Demonstration of protein capture and separation using 3D printed anion exchange monoliths fabricated in one-step." *Journal of Separation Science* 8th Sept 2020, DOI: <https://doi.org/10.1002/jssc.202000722>

4. Details of the impact

C-PC is a natural blue food colouring that is FDA-approved for use in nutritional supplements, cosmetics and pharmaceuticals, and is produced by cyanobacteria, a key area of research for McCormick and Free. Cyanobacteria are challenging to genetically modify, but McCormick's CyanoGate technology is a significant step towards enabling a standardised genetic modification approach for further process improvement.

ScotBio was set up in 2013 as *Scottish Bioenergy*, to produce high quality C-PC for the food and beverage markets. Consumers are now demanding bio-based and renewable sources for their colourants, not only in food and drink, but also in cosmetics and textiles. Many major global brands are making the switch to these from non-bio-based ingredients. Over the past seven years, the research performed by University of Edinburgh academics has made a significant impact to ScotBio's value, processes, product scale and product quality. Indeed, McCormick and ScotBio won the 2019 Scottish Life Sciences Award for Best Innovative Collaboration for pioneering work on natural blue colourants [5.1]. ScotBio is one of four biotechnology companies in Scotland to have progressed past the 'valley of death' between lab-scale production (Technology-Readiness Levels (TRL) 1-4) and commercial scale (TRL 7+) via the acquisition of venture capital funding [5.2]. ScotBio produce biochemicals used in the medical research, nutrition and pharmaceutical sector.

Impact on economy: The Chief Operating Officer (COO) at ScotBio states that:

"ScotBio would not be where it is today without having had these successful collaborations with the University of Edinburgh." [5.3]

ScotBio was set up as a micro SME in 2013 with one employee and a market value of < GBP1,000,000 and has now grown to 19 employees [5.3] with a market value of GBP24,000,000 [5.4]. This recent valuation has led to plans to expand in the future with a recent capital investment of GBP2,000,000 [5.2]. While currently remaining a pre-revenue company, the COO has confirmed that:

"Expansion of the future product pipeline via projects hosted at the university has given us a positive growth trajectory and a strong financial forecast." [5.3]

Key to ScotBio's significance is the lack of a clear path for investment, in comparison to more straightforward companies such as pharmaceutical startups. Their acquisition of venture capital funding means that they are one of the few biotechnology companies in Scotland to have achieved commercial-level scale up investment.

"Industrial biotechnology companies like ScotBio have traditionally struggled to raise venture capital, as, unlike the pharmaceutical industry, there are no clear paths to investment milestones (e.g. phase 1-3 trials) or known returns on investment" [5.5]

Exploitation of C-PC by the nutrition, pharmaceutical and cosmetics industries has given this biochemical a high market value, and as a result, demand for C-PC has outstripped supply.

The international market for C-PC is currently (2020) USD44,000,000 and is expected to reach USD121,000,000 by the end of 2026 with natural food colourings and the pharmaceutical industry as key markets.

Beneficiaries: The primary benefits are to ScotBio's capabilities to produce their primary product: C-PC. Other suppliers use open pond systems that are poorly reproducible due to environmental exposure, however, research collaborations with UoE Biological Sciences have led to ScotBio's use of bioreactors to produce increased volume of C-PC in higher yields and purity and can offer traceability and security of supply via GMP manufacturing processes. As a result of research on process improvements and synthetic biology processes from McCormick and Andrew Free at UoE, ScotBio have overcome critical technical and scaling barriers for the production of analytical grade C-PC.

"The collaboration with University of Edinburgh has supported our fund-raising abilities by first enabling the scale-up of our core technology to commercial pilot scale. Secondly, many of the projects have focused on future technologies that give us a product pipeline and demonstrate continued growth for the business, which is attractive to investors." [5.3]

Current beneficiaries consist of the Scottish biotechnology landscape, evidenced by ScotBio's preeminence as a flagship company [5.5]; food and beverage companies such as Firkin Gin [5.6] and the textiles industry, such as Nostalgae [5.7].

Significance and reach: ScotBio is a leading light of the Scottish Biotechnology industry. Originally set up with an investment of GBP500,000 via an AccelerateHER Award from Angel Investors 'Investing Women'.

"A Scottish based company with such great international potential. Scottish Bioenergy has developed a unique product with a strong IP; focused on a global market where there are few competitors." [5.8]

ScotBio's partnership with Edinburgh University academics has led to the development of a consumer product in the food and beverage market and a higher grade product to be sold to the pharmaceutical industry. ScotBio has recently (2019) opened a scale-up plant in Lockerbie with a capacity of 50,000 L with additional room for growth based around the improved yields developed via [3.1] [5.9].

A partnership with Leith (Edinburgh) based drinks producer, Firkin Gin, led to the August 2017 launch of a natural blue version of their gin – Firkin Blue [5.4]. This is ScotBio's first commercial product to market and was a limited edition run, sold globally via high-end spirits suppliers [5.10], currently retailing at USD438 per bottle.

McCormick's research has led to an increase in the quality and yield of product, which allows the production of pharmaceutical and research grade C-PC which is now available for those markets. Analytical grade C-PC (purity >3.5) has a market value of GBP123 per mg [5.11], with worldwide production at 11,000,000 t.

The research described in [3.2] at the University of Edinburgh has underpinned ScotBio's commercial aims to develop synthetic biology processes for cyanobacteria. ScotBio has recently set up a subsidiary to take these to market as they reach the commercial stage [5.2].

5. Sources to corroborate the impact

- [5.1] Award for industry/academic collaboration: <https://edinburgh-innovations.ed.ac.uk/2019/03/04/natural-colouring-pioneers-win-collaboration-award/>
- [5.2] Press regarding venture capital funding for ScotBio: https://www.heraldsotland.com/business_hq/17242300.glasgow-biotech-firm-wins-2m-to-ramp-up-natural-food-colourant-production/?ref=twtrc
- [5.3] ScotBio personnel website: <https://scotbio.com/our-team>
- [5.4] Testimonial: Chief Operating Officer of ScotBio.
- [5.5] Testimonial: Research Director of IBIOIC
- [5.6] Press regarding Firkin Blue Gin, coloured with C-PC from ScotBio: <https://foodanddrink.scotsman.com/producers/leith-based-craft-drinks-producer-launches-blue-gin/>
- [5.7] Letter of support from Nostalgaie
- [5.8] Press regarding AccelerateHer Funding: <https://www.investingwomen.co.uk/six-figure-funding-bioenergy-business/>
- [5.9] ScotBio website showcasing new scale-up facility: <https://scotbio.com/press-release-201911-lockerbie-grand-opening>
- [5.10] International gin retailer stocking Firkin Blue Gin: <https://mingfathouse.com/product/firkin-blue-gin/>
- [5.11] Final Report - ScotBio / McCormick IBIOIC Grant