Institution: The University of Huddersfield		
Unit of Assessment: UoA 8		
Title of case study: Novel biorefining strategies for reprocessing agricultural waste, bioethanol production from sea water and the recycling of textiles.		
Period when the underpinning research was undertaken: 2015-2020		
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:**
Chenyu Du	Professor in Chemical Engineering	Du: 2015 - current
Darren Greetham	Research Fellow	Greetham: 02.2017 - 08.2020
Period when the claimed impact occurred: 2018-2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Reducing carbon emissions to tackle climate change is a global priority, with China recently committing to lowering CO_2 emissions by 65% by 2030. Research at the University of Huddersfield has developed methods for isolating novel microorganisms from the marine environment (yeasts) and soil (fungi) that have unique properties suitable for use in biorefinery processes. Using these microorganisms, the bioconversion of agricultural crop residue into biofertiliser was developed. In field trials, the results demonstrated a reduced requirement for chemical fertiliser, improved soil properties, increased crop yield (~10%) and prevented the burning of biomass, resulting in significantly reduced CO_2 emission. Additional examples of impact, include (i) the development of a seawater based bioethanol process which has the potential of turning bioethanol production from a freshwater consuming industry into a freshwater producing industry and (ii) the development of a novel biological approach to recycle textile waste, which targets 160 million tons/annual of textile waste worldwide.

2. Underpinning research

The underpinning research reported here was conducted by Professor Chenyu Du and Dr Darren Greetham during their tenure at the University of Huddersfield. There were three distinct elements to the underpinning research, details of which are summarised below:

(i) The first element of the underpinning research involved the development of an improved method for isolating microorganisms (yeasts) from marine resources [3.1]. Yeasts that are present in the marine environment have evolved to cope with hostile environments including high exogenous salt concentrations, high concentrations of inhibitory compounds and low soluble nitrogen and carbon levels. Yeast strains with these characteristic properties could have significant industrial applications but previous methods of isolating yeasts from the marine environment suffered from bacterial and fungal contamination, combined with low numbers of yeast strains isolated. Using a three-cycle enrichment process followed by an isolation method, large numbers of yeasts (116 reported in [3.1] but now over 200) were isolated from marine samples taken in the UK, Egypt and USA. Evaluation of these strains identified 17 strains belonging to six species with greater sugar utilisation capacity compared to terrestrial *Saccharomyces cerevisiae* [3.1]. Follow up studies demonstrated their potential for use in the production of bioethanol from seawater instead of freshwater [3.2] and their increased tolerance towards inhibitory compounds such as acetic acid, formic acid, furfural, vanillin and salt compared to 78 terrestrial yeasts. In addition, significantly higher quantities of bioethanol were obtained in fermentation experiments using selected marine yeasts with media containing inhibitory compounds [3.3]. This research therefore led to the isolation of marine yeasts with distinct advantages over terrestrial yeasts and potential applications for their use in fermentation reactions using marine biomass in seawater media, leading to the production of bioethanol and other biochemical products.

(ii) Based on the methods reported in [3.1], a similar approach was adopted for the discovery and isolation of soil microorganisms. In this process, soil samples underwent enrichment processes to increase the microorganism population and then the microorganisms were screened based on their capacities of cellulase production, salt tolerance and toxic chemical removal [3.4]. The introduction of an enrichment process enables the samples to be stored longer before they arrive at capable labs. In contrast, microorganism isolation previously had to be carried out shortly after sampling close to isolate sites. The utilisation of plate reader as a cost-effective facility enables checking high numbers of microorganism at a high efficiency without using expensive robot machines. Several promising microorganisms, including *Aspergillus niger*, *Rhizomucor variabilis* and *Serratia marcescens* were obtained, and have been used for cellulase production [3.5], in-situ agriculture residue degradation [5.1] and as a heavy metal contaminated field remedy [3.4]. In terms of in-situ biomass degradation, large scale microorganism cultivation at low production cost is required. Therefore, the fermentation processes were optimised. Based on previous work at Professor Du's group on cellulase production from municipal solid waste [3.6], the impact of factors such as sugar composition, inoculation procedure, addition of minerals and the addition of nitrogen source on cellulase production were investigated. The research led to a collaboration with Professor Lu's group in Tsinghua University, China, that explored an *in-situ* biomass degradation strategy to (a) convert waste biomass to biofertiliser and (b) to avoid biomass burning in rural areas thereby reducing CO_2 emissions. The optimised microorganism cultivation protocol was used in field tests as indicated in [5.1].

(iii) A solid-state fungal fermentation process was developed for cellulase production [3.5], which contributed to the establishment of a biological textile waste recycle strategy. A previous study in the group discovered that the cellulase enzyme produced using a specific substrate (starting material) contained a better enzyme consortium that hydrolysed the substrate better. In this study, textile waste samples with different cotton to synthetic fibre ratios were tested for cellulase production. A few different cellulase producers were tested, and a microorganism isolated from the soil microorganism projects (*Aspergillus niger*) was demonstrated be to the best one. The cellulase was then tested for the hydrolysis of the textile waste, leading to a comparable sugar hydrolysis yield (70.2%) in comparison to commercial cellulase enzyme (77.2%) [3.5]. This study, together with other investigations carried out at UoH, such as cellulase production optimisation, demonstrated the feasibility of the first step in biological textile waste recycling. The cellulase was then used to hydrolyse the cotton fibre fraction of the cloth waste at Hong Kong City University. The hydrolysed sugar was fermented to lactic acid and was then used to synthesis biopolymer lactic acid (HKCU). The leftover un-hydrolysed synthetic fibre (mainly PET) was re-spun for making new cloth at Hong Kong Polytechnic University and Industrial Technology Research Institute, Taiwan.

3. References to the research

[3.1] Zaky AS, Greetham D, Louis EJ, Tucker GA, Du C. 2016. A new isolation and evaluation method for marine derived yeast spp. with potential applications in industrial biotechnology. Journal of Microbiology Biotechnology. 05074: 1017-7825. https://doi.org/10.4014/jmb.1605.05074 (Reported a novel marine yeast isolation method, IF 3.5, citation 22 based on google scholar)

[3.2] Zaky AS, Greetham D, Tucker GA, Du C. 2018. The establishment of a marine focused biorefinery for bioethanol production using seawater and a novel marine yeast strain. Scientific Reports, volume 8, Article number: 12127. https://doi.org/10.1038/s41598-018-30660-x (Reported a new method for bioethanol production using seawater instead of fresh water, IF 4.0, citation 17, the paper was reported by 20+ journals/media)

[3.3] Greetham D, Adams J, Du C. 2020. The utilization of seawater for the hydrolysis of macroalgae and subsequent bioethanol fermentation. Scientific Reports. 10, 9728. https://doi.org/10.1038/s41598-020-66610-9 (First report to demonstrate a novel conception of biofuel production using only resource from marine environment. IF 4.0, citation 1)

[3.4] Chen Y, Zhu Q, Dong X, Huang W, Du C, Lu D., 2019. How *Serratia marcescens* HB-4 absorbs cadmium and its implication on phytoremediation, Ecotoxicology and Environmental Safety. 185, 109723 https://doi.org/10.1016/j.ecoenv.2019.109723 (A novel strain was discovered and its soil remediation property was reported. IF 4.7, citation 4)
[3.5] Hu, Y., Du, C., Leu, S.Y., Liu, H., Jing, H., Li, X., Lin, C.S.K., 2018. Valorisation of textile waste by fungal solid state fermentation: An example of circular waste-based biorefinery, Resources, Conservation and Recycling, 129:27-35, https://doi.org/10.1016/j.resconrec.2017.09.024 (This is one of early reports on the textile waste recycle using enzymatic hydrolysis method. IF 8.1, citation 38)
[3.6] Abdullah JJ, Greetham D, Pensupa N, Tucker GA, Du C. 2016. Optimizing cellulase production from municipal solid waste (MSW) using solid state fermentation (SSF). Journal of Fundamentals of Renewable Energy and Applications 6 (206) https://www.longdom.org/open-access/optimizing-cellulase-production-from-municipal-solid-waste-msw-using-solid-state-fermentation-ssf-2090-4541-1000206.pdf (Reported on-site enzymatic hydrolysis of complex, toxic cellulosic waste materials and fermentation optimisation. IF 1.4, citation 29)

4. Details of the impact

The underpinning research described in section 2 has generated and is continuing to generate impact in the following key areas (i) the conversion of agricultural waste into biofertiliser, which naturally enhances soil nutrient level, increases productivity of crops and reduces the CO_2 emissions associated with the existing method of burning of agricultural residue (ii) the production of bioethanol from marine sources, thereby conserving freshwater supplies by reducing usage and the production of fresh water from the biorefining process and (iii) the promotion of sustainability within the textile industry by the reprocessing of textile waste to regenerate synthetic fibres for the manufacture of new clothes.

(i) Improving agricultural practices in China to reduce CO_2 emissions.

Agriculture residue from arable crops has low economic value and is seldom collected. In developing countries, it is mainly directly burnt to clear the field for next crop rotation resulting in significant air pollution. Based on the underpinning research reported in section 2 (ii) which involved the isolation and characterisation of new soil microorganisms, a field trial on 730 acres of farmland in Sandong Province, China was conducted in 2019-2020. Selected consortium microorganisms were cultured in large quantities and sprayed on field for *in-situ* degradation of agriculture residues, such as straws, hulls and leaves. This work was conducted with Shandon WoDiFeng Biofertilizer Co Ltd, who funded the trial [5.1]. The results of the trial are summarised in the testimonial letter provided by the CTO of WoDiFeng Ltd. It states "*after 30 days on-site degradation test, the results show that the soil physical structure had been improved, the organic matter in the soil increased, the waste agriculture residue had been treated and the environmental pollution (by burning the agriculture residue) was avoided. It also showed that the corn received on-site biofertiliser grew better than those did not receive the treatment*" [5.1]. The net economic benefit of utilizing the on-site degradation technology was estimated to be £50/acre. Another field test was carried out in a tobacco field in Anhui Province, China in 2018-2019, supported by Anhui Academy of Agriculture Science. 33 acres of tobacco field were treated with biofertiliser generated via *in-situ* degradation of biomass. As a result, the usage of chemical fertiliser reduced by 14%, the ratio of tobacco at top grade increased by 7%, with the net economic benefit estimated to be £120/acre. The trials demonstrated that *in-situ* degradation of agriculture waste processes not only provided economic benefits, but also reduced air pollution. It was estimated that around 2-5 tons/acre of crop residue could be generated, depending on the crop. Converting the biomass into biofertiliser instead of burning it could prevent emissions of 0.7-1.8 tons of CO_2 per acre into the atmosphere. During the two field tests, it was estimated that 900 tons and 20 tons of CO_2 emissions had been avoided in the corn/potato field and the tobacco field, respectively [5.1]. This new approach significantly reduced the utilisation of chemical fertiliser, decreased the production cost of organic fertiliser as no waste biomass harvesting process was required and prevented gas pollution by avoiding burning of the residues. After the successful field demonstration, full scale commercialisation of the technology has been under discussion with WoDiFeng Biofertilizer Co Ltd.

(ii) Developing a low water footprint bioethanol production process using marine resources

In the fight against global warming, bioethanol fuel has been widely used as a climate-friendly alternative to petrol. But current biofuel production mainly uses land-based biomass which competes with arable land for food and feed usage. It also consumes large amounts of freshwater – a precious resource in many parts of the world. It has been estimated that the water footprint for bioethanol production was 1388 to 9812 L H_2O/L bioethanol (mainly for plant irrigation), while at least 10 L H_2O/L bioethanol was directly used in the factory. The underpinning research described in section 2 (i) resulted in the isolation of 200+ marine yeasts with superior tolerance properties and then demonstrated the feasibility of utilising only marine resource: seaweed, seawater and marine yeast for bioethanol production [3.2, 3.3] (Figure 1). The idea of utilising seawater in the bioethanol production process attracted great attention in the biofuel research field, and was reported by 20+ journals and media outlets. A few examples have been listed in [5.2]. The major benefit for this process was not only in the saving of freshwater, that could be used for plant irrigation and industrial processing, but that the process generates freshwater. For example, it is estimated that at least 10 litres of fresh water could be produced for each litre of bioethanol generated. If half of the world bioethanol production could all use this process (50% of 90 billion litres), there would be enough freshwater produced (450 billion litres) to fill all bottled water sold in the world (444 billion litres, https://www.statista.com/outlook/20010000/100/bottled-water/worldwide#market-ontradeRevenueShare). The team have recently been exploring the feasibility of commercialisation of the process.

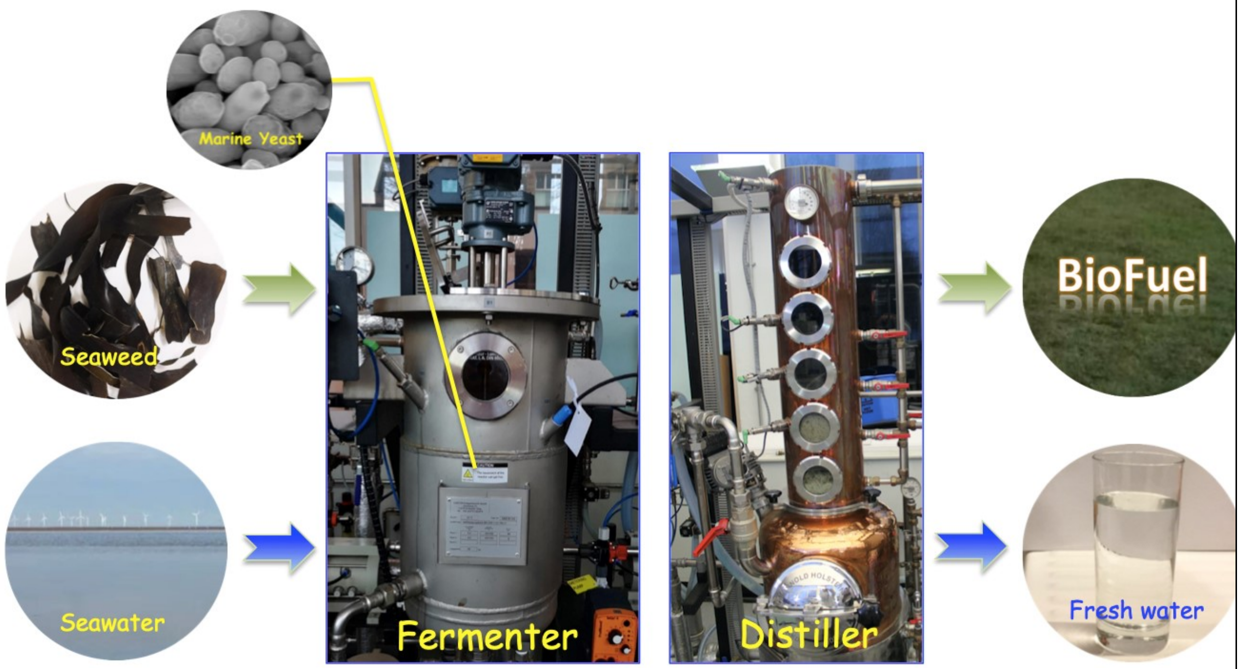

Figure 1. Schematic diagram of a marine source based biorefining process. Seaweed is used as the feedback for the biofuel fermentation by marine yeast, while the seawater is converted to freshwater via fermentation and distillation. Over 10 L H_2O/L bioethanol can be obtained.

(iii) Reducing landfill waste and increasing recycling in the global textile industry

In the UK, the textile industry produces 2,700 tons of landfill waste daily, whilst in China, this figure exceeds 70,000 tons. Textile production is both energy and water intensive and therefore methods to utilise textile waste are required. The underpinning research described in section 2 (iii) developed novel methods to directly reduce the amount of textile waste by converting it into a fermentation medium for biopolymers synthesis and indirectly decreased the environmental impact by reusing synthetic fibres for making new clothes [3.5]. This idea, which was supported by HKRITA (The Hong Kong Research Institute of Textiles and Apparel) and H&M, was presented in the 46th International Exhibition of Inventions of Geneva and was awarded a Gold Medal for innovation, with regards to cloth recycling [5.3]. The project promoted the sustainability concept in the textile/fashion design industry, in the UK and worldwide. As a consequence, several companies have since expressed their willingness to support continuous investigation on this topic. An enquiry letter from the Director of Maxwellstyle Ltd for a grant application in 2019 stated "On behalf of Maxwellstyle Ltd company, I would like to express our interest on your research on textile waste recycle project…we are concerned at the potential pollution caused by the unwanted clothes sold by us and we are willing to support research and commercialisation activities related to textile waste, aiming to reduce the plastic pollution caused by textile waste and produce value added recycled products" [5.4].

5. Sources to corroborate the impact

[5.1] Statement from the Chief Technology Officer, WoDiFeng Biofertiliser Ltd. Shandong Province, China

[5.2] Sample of media reports on marine based bioethanol process (access on 07/01/2021)

http://www.wateractive.co.uk/news/seawater_breakthrough_in_production_of_climatefriendly_fuel_bioethanol_cuts_demands_on_freshwater

https://biofuelsdigest.com/nuudigest/2018/11/20/seawater-fuel-discovery-in-production-of-bioethanol-helps-curb-freshwater-use/

https://www.alphagalileo.org/en-gb/Item-Display/ItemId/169126

https://phys.org/news/2018-10-seawater-breakthrough-production-climate-friendly-fuel.html

https://insights.globalspec.com/article/10091/seawater-can-replace-freshwater-in-bioethanol-production

http://biofuelsdigest.com/nuudigest/2018/08/22/seawater-and-yeast-strain-lowers-ethanol-water-footprint/

[5.3] Gold Medal Certificate in Geneva Invention Exhibition, April 2018.

[5.4] An enquiry letter from the Director of Maxwellstyle Ltd. for potential investment on textile recycle commercialisation.