

Algal Biotechnology: Biofuels and Beyond - Abstracts for poster presentations

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1. Environomics for scale-up of photobiological hydrogen production

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Molecular hydrogen (H₂) has the potential to become the fuel of the future, but only if it is produced by a sustainable process. The green alga *Chlamydomonas reinhardtii* (*C. reinhardtii*) has an ability to photosynthetically produce H₂. It offers a biological route to renewable H₂ production from the abundant resources of sunlight and water. Photosynthetic hydrogen production is governed by the hydrogenase enzyme, which recombines $H^+ + e^-$, to produce H₂ and is activated under anaerobic conditions. The efficient and economical growth conditions to attain healthy and high cell densities are necessary for a commercially large-scale H₂ production. As a result, key environmental parameters e.g. light intensity, temperature and CO₂ concentration have been examined to investigate the optimum growth rate. Environomics and growth of *C. reinhardtii* have been manipulated and measured using the tubular reactor, Sartorius Biostat PBR 2s.

2. Evaluation of okadaic acid production by *Prorocentrum lima* culture using different scales of cultivation

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Prorocentrum lima produces several highly valuable compounds, such as okadaic acid, which are potentially useful in medical and pharmaceutical applications. Okadaic acid (OA) is a tumour promoter and a potent inhibitor of protein phosphatases 1 and 2A (PP1 and PP2A). OA is currently used as an important research tool in medical and physiological studies to understand cell regulation and various cellular processes. The aim of this study was to test the capability of *P. lima* in producing OA at different scales of phototrophic cultivation.

Experiments were performed in small (1 L), medium (10 L) and large (30 L) scales of batch culture, using *P. lima* PL2V strain grown in f/2 medium. Filtered natural seawater was used for the medium preparation with salinity level of 36 ppt and pH of 6.5. Cultivation was maintained at temperature of 22 °C. Average illumination was 17.16 μmol/m²/s for small and medium cultures, and 20 μmol/m²/s for large culture.

Comparison of small and medium cultures showed that optimum growth was obtained in small scale cultivation with 3.7x10⁵ cells/ml after 4 weeks of cultivation compared to 1.3x10⁵ cells/ml in medium scale culture. Analysis on the OA production also revealed that small scale culture produced much higher OA than medium and large scale cultures, with OA concentration observed at 0.475, 0.250 and 0.195 μg/ml for small, medium and large cultures respectively. This was probably caused by difficulties in maintaining homogeneity of culture and light penetration at larger scales. Other challenges identified from the cultivation experiment with large scale are: seawater requirement, benthic nature of the organism, and slow growth. This indicates that optimisation of reactor design and cultivation condition is still required for large scale cultivation of *P. lima*.

3. Glycerol production by novel strains of *Dunaliella* and *Asteromonas* isolated from Namibian marine water

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Aim: to develop a low-cost system for producing industrial quantities of glycerol in pure streams from halophytic microalgae. This study represents the first step in data acquisition for process synthesis, modelling, optimization, and process flowsheeting aimed at integrating stages that produce glycerol and assessing reliable targets for efficiency and cost. It centred on characterising halophytic microalgae isolated from marine water of Namibia.

Innovation: Glycerol has emerged as a new biofuel with important properties, and now underpins a commercial CHP technology: this is as a result of the novel Mcneil combustion cycle which allows standard production compression ignition engines to combust glycerol at high efficiencies and with very low emissions without chemical alteration or the addition of combustion enhancers. Combustion is more energy efficient than any known fossil, bio or synthetic fuel (34 – 37 % (10 – 30kW), 40 – 42% (up to 1 MW) and 42 – 48% (over 1 MW) electrical efficiency), and engine performance with glycerol has been proven. Furthermore when combusted within diesel engines glycerol produces no combustion particulate, no SO_x, reduced primary NO_x, extremely low VOC and aldehyde emissions and no catalyst poisons; these parameters have been independently verified. Glycerol also has safe handling properties: it is water-soluble and bio-degradable and could therefore be used to power ships and even tankers carrying glycerol as a distributable fuel with minimal risk to the marine environment. It holds the key to developing an entirely new environmentally-sustainable, biofuel industry with enormous commercial potential in all fields of application of engine power production. Moreover in the biorefineries of the future glycerol will serve additionally as an intermediate to replace various fossil oil-based bulk chemicals (e.g. ethylene and propylene glycols, 1,4-butanediol, epichlorohydrin and acrolein).

Glycerol is currently chemically produced from biodiesel manufacture using plant oil (approximately 10% by weight of plant oil). However we anticipate that demand for glycerol will rapidly outstrip Europe's capacity to produce glycerol from plant oil. Halophytic microalgae of the *Dunaliella* species that grow in highly saline environments such as salt pans and desalination non-potable waters across the globe will also synthesise glycerol. These have been cultivated at commercial-scale as a source of β-carotene and animal protein but not as a source of glycerol, despite the fact that the basis for the technology was established in 1978. Since the cultivation facilities depend on non-potable saline waters, by now seeking to develop and expand halophytic microalgae cultivation for glycerol to meet increased demand, cultivation for glycerol will help to meet EU SET targets and avoid food/fuel competition for land use. Moreover, a very important difference between the production of algal glycerol as opposed to algal biodiesel from lipid is that the glycerol algal product is a finished fuel and does not require chemical modification or molecular reformation. This aspect adds benefit to the algal production facility as glycerol derived from the plant can be used directly as a fuel to meet the combined heat and power requirement.

Results: In this study NaCl concentration had a strong effect on the growth rate and generation time of six strains belonging to two novel halophilic microalgae species *Dunaliella* (T35, T36 and T37) and *Asteromonas* (T33a, T33b and T33c) as confirmed by Bayesian and Neighbour-Joining analyses. Both species were shown to be capable of growth at different ionic strength of 0.5, to 4.0 M NaCl in a modified Johnson's culture medium; at 12:12 photoperiod, pH 7.5, average of 4395Wm² photosynthetically active irradiation (PAR) and 23 ± 0.2°C temperature. On exposure to hyperosmotic shock the amount of glycerol produced as a function of cell density greatly increased and was dependent on the applied NaCl concentration. After 28 days growth, for *Dunaliella* species

the highest glycerol accumulation on a cell basis was obtained from T35 (203.2 ± 3.1 pg/cell) at 4.0 M NaCl, from T36 (177.0 ± 2.6 pg/cell) at 3.0 M NaCl and T37 (234.9 ± 10.0 pg/cell) at 4.0 M NaCl concentration. Ranges of 50 to 400 pg/cell glycerol have been reported by Ben-Amotz and Grunwald (1981) for *Dunaliella* grown at 0.5 to 4.5 M NaCl and 94.26 pg/cell glycerol for *Dunaliella* species from a hypersaline river of India (Phadwal and Singh (2003)). Similar to *Dunaliella* cells, *Asteromonas* accumulated 53.8 ± 0.8 to 209.5 ± 10.1 pg/cell in modified Johnson's growth media at 1.0, 2.0, 3.0 and 4.0 M NaCl concentrations respectively. Following hyperosmotic shock glycerol accumulated both with and without illumination, indicating that the precursor for glycerol synthesis during hyperosmotic stress is also derived from sources other than photosynthesis, most probably from starch breakdown.

Conclusion: The first step towards data acquisition for process flowsheeting has been achieved using halophytic microalgae isolated from marine water of Namibia. The data provide a strategy for cultivation scale-up to achieve large-scale glycerol production in highly saline non-potable water.

4. *Chlorella emersonii* cultivation, biofuel analysis and LED photobioreactor utilisation

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Innovative and sustainable replacements are required for the rapidly depleting supplies of petroleum worldwide. Biodiesel integration during the decline in use of petroleum is successfully proven, with the infrastructure of the fuel supply network and diesel engines requiring minimal adaptation. Algae have been cultivated using various methods, both simple and more complex. Through the design, construction and utilisation of a specialised photobioreactor algae species can be screened for lipid, carbohydrate and protein composition as well as for higher value products. Gas chromatography-mass spectrometry analysis has been developed for the resultant biofuel, generally chosen to be fatty acid methyl esters. Different growth conditions of algae, particularly the species, *Chlorella emersonii*, have been investigated. To conclude, each species responds uniquely to varying growth conditions and algal growth is furthermore affected by maturity upon inoculation and harvesting of cultures.

5. Integrated production of algal biomass

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The burden placed on natural resources by increasing human activity has fostered a concerted drive to redefine industrial problems within a sustainable context. This transition to a greener economy has been recognised as vitally important to UK industry and is highlighted as key government policy. A considerable part of this effort has been focused on the development of renewable technologies to assist in replacing the array of chemicals derived from conventional petrochemical refineries. In this respect, the expanding field of 'green biotechnology' offers great promise in providing cleaner routes to compounds made by traditional chemical synthesis. This wide-ranging potential for biomass and its associated products has been described as the 'bio-refinery' concept. Within this expanding field, recent developments in algal cultivation have been identified as having the potential to play an important role in sustainable biomass generation.

One of the major problems facing global algal production is the necessity of lowering biomass production costs in order to make the process more economically feasible. This can be achieved in a number of ways, but one of the most promising approaches is to integrate algal production within existing industry and infrastructure. This approach could prove particularly effective if coupling biomass generation to environmental remediation. Such a process would allow for the utilisation of low cost or even completely free feedstocks, which are normally considered as waste by other industries. Using sustainable biotechnology in this way can find parallels in the approaches employed by systems thinkers when describing 'industrial symbiosis', a concept in which waste products from one industry become the feedstock for another. With these considerations in mind, this research project will investigate the potential benefits that can be achieved by developing more sustainable methods of algal production, including the simultaneous design of both photobioreactor and bioprocessing loop.

6. Promising catalytic processes for the chemical conversion of microalgae into biofuels

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Durham University

The need to develop sustainable fuel sources that do not compete for land with food crops is of growing interest. Microalgae represent possible feedstocks for achieving this aim. The methods for converting algae into biofuels are numerous and integrating catalysts into these processes will help to optimise their efficiency and commercial appeal. This poster aims to give an overview of some promising processes and show how catalysts can be used to improve them.

(1) *Trans*-esterification is a reaction occurring between an alcohol and an ester of a second alcohol to interconvert the functionality. For biofuel production, this typically occurs between triglycerides (TGs) or complex fatty acids and either methanol or ethanol, to produce fatty acid methyl esters (FAMEs). These reactions are catalysed by layered double hydroxide (LDH) materials, which are optimised initially using simple esters, in preparation for use with microalgae.

(2) Decarboxylation is a method of chemically upgrading crude bio-oils *via* deoxygenation through the release of carbon dioxide. For example, *trans*-esterification produces bio-diesel (composed of FAMEs), which is oxygen rich compared to fossil fuels, therefore of limited utility. Decarboxylation can be used to upgrade the bio-diesel to obtain a more valuable product; green diesel.

(3) Thermochemical degradation incorporates techniques such as pyrolysis and hydrothermal liquefaction and differs substantially from conventional chemical methods. It relies on high temperatures and heating rates to decompose long biomolecules into shorter, less oxygenated hydrocarbons which can be burned as fuels or used to produce commodity chemicals.

Recent results exploring the efficiency of catalysts in these processes are reported, in addition to speculation on the work that still needs to be undertaken to further enhance the efficiency of microalgal processing.

7. Biofilm of oxygenic phototrophs enhance cathode efficiency

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Microbial fuel cells (MFC) are electroactive systems composed of an anode and a cathode. In such systems, anaerobic electroactive microorganisms use the anode as an electron acceptor when mineralising organic matter. These electrons pass through a circuit before arriving in the cathode where they reduce a compound of a higher redox potential, thus, producing current. Typically, in MFCs oxygen or ferricyanide accept the incoming electrons at the cathode. Power outputs are, thus, limited by the high overpotential of oxygen. To overcome such potential, mediators or catalysts are usually required. However, such abiotic cathodes increase the cost and lower their sustainability through time. For this reason biocathodes have received great interest in recent years as they can increase the power output at a lower cost and with a higher sustainability: biocathodes utilise microorganisms as biocatalyst to mediate the reduction of an oxidant either directly or indirectly. One of the numerous possibilities is the use of phototrophs.

The objective of the present study was to grow onto the cathode a biofilm composed of a mixture of oxygenic phototrophs. The aim was to increase cathode efficacy by directly producing the oxygen where it was consumed, thus, avoiding the use of any mediators. Indeed, an oxygen-supersaturation is observed in stratified ecosystems such as in lake summer stratification or as in microbial mats. Therefore, reproducing it in direct vicinity of the cathode would enhance the MFC output. The stability of this current output was also investigated in order to see if such a system could be useful for MFC applications where oxygen is a limiting factor. Figure 1 shows the relationship between power output and light/dark conditions.

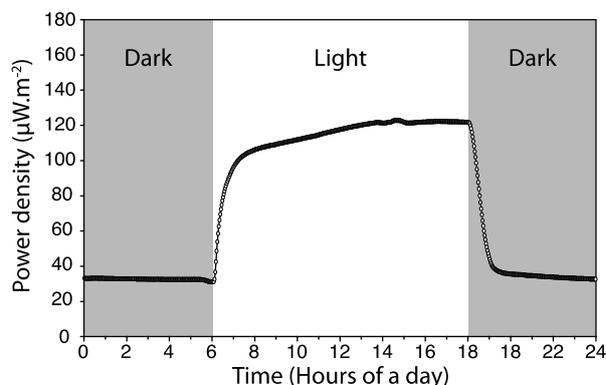


Figure 1: Mean power (n=8) output response from a phototrophic MFC under light/dark conditions. Error bars are present but are smaller than the points.

The presented results confirmed that 1) the supersaturation effects of an oxygenic biofilm growing on the cathode enhanced the current produced (Fig. 1), and 2) that this effect was stable over time.

8. Algae for cathodic oxygen reduction in MFCs

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Introduction: Microbial Fuel Cells (MFCs) are systems that use microorganisms to extract energy from organic waste. One of the MFC's major limitations is the cathodic reduction rate of oxygen which requires an efficient catalyst. Excluding non sustainable catalysts such as ferricyanide or expensive platinum and adapting cost effective and environmentally friendly materials, a biocathode

is a promising alternative (1). In this study a phototrophic biocathode was developed to provide oxygen in situ for the reduction reaction.

Materials and Methods: Five types of two chamber-MFCs were used in triplicates: 3 control abiotic cathode MFCs and 12 biocathode MFCs separated in 4 experimental groups. The difference between them was the cathode electrode used: a) carbon veil, b) carbon veil wrapped in cotton string, c) carbon veil wrapped in cellulose, d) carbon veil wrapped in stainless steel wire. These electrode configurations were used to promote algal colonisation/current collection. Cathode and anode chamber were of equal volume and electrode surface area: 25 ml and 270cm² respectively. Anode was operated in batch mode with periodic feeding with sludge and 0.1M acetate. Cathodes were operated in continuous flow mode connected to peristaltic pump and 0.5L photoreactors.

Results: Figure 1 shows the power curves of 4 tested biotic (a,b,c,d) and one abiotic MFC (veil water). The maximum power density generated by the best performing biotic MFC (veil algae) was 2.78 mW/m² which was 79% higher than the maximum performance of abiotic MFC that generated 1.52 mW/m².

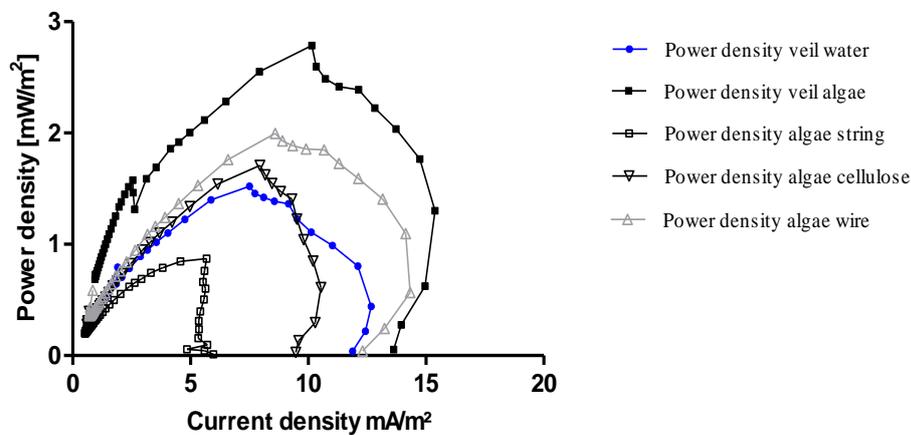


Figure 1. Power output comparison from the different cathodic systems.

Conclusions: In the present study, algal based biocathode MFCs outperformed the Microbial fuel cells with the abiotic cathode both in terms of power output and open circuit voltage.

References: 1. He, Z. and Angenent, L. (2006), Application of Bacterial Biocathodes in Microbial Fuel Cells. *Electroanalysis*, 18: 2009–2015. doi: 10.1002/elan.20060362

9. Micro-algal process flow-sheet energy balance optimisation: initial software evaluation

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The FP7 All-Gas project aims to produce biogas from the anaerobic digestion of micro-algal biomass grown in open high rate ponds using nutrients from wastewater. Carbon dioxide is supplied from the combustion of local agricultural biomass with a proportion provided from the combustion of recycled algal biogas. The dilute nature of the algal suspension poses considerable challenges in achieving favourable energy balances for the unit operations of algal biofuel production and optimising the overall process.

The ability of Life Cycle Assessment (LCA) software to produce dynamic energy and mass balance models of algal biofuel production is reviewed. An initial assessment of flow-sheet software (Superpro, UNISIM and Aspen Plus) was carried out to assess its ability to produce a dynamic model using parts of the possible FP7 All-Gas algal biofuel production process. It is concluded that although UNISIM and Aspen Plus may be capable of producing dynamic mass and energy balances for an entire algal biofuel production process it will be innovative and challenging. A very considerable amount of effort and time will be needed to produce algal biofuel dynamic models as many unit operations and components are not among the defaults available in either UNISIM or Aspen Plus.

Work is now examining the use of pinch analysis for not only optimisation of water usage but also on the possibility of the use of a novel carbon and nutrient pinch.

10. 'Oilgae' - a new generation of biofuel

Holly D. Smith-Baedorf, Prof. R.J. Scott, Prof. M.G. Davidson

University of Bath

The finite nature of petrochemical energy and global climate change are driving the need for sustainable fuels that can be readily implemented into our existing infrastructure. One such solution may be microalgal biodiesel. With high levels of productivity, fast growth rates and few nutrient requirements, these versatile organisms are an attractive option with the potential for many additional applications. The project focuses on the following obstacles to realisation of microalgal biodiesel:

1 - IDENTIFICATION OF CANDIDATE ALGAE SPECIES. As a diverse and relatively understudied group of organisms, the isolation of temperature tolerant, filamentous or flocculating strains may reduce cooling and harvesting costs significantly. As the only naturally 'hot' spring in the UK we are biospecting the roman baths for interesting species.

2 – EXTRACTABILITY. Harsh and unpredictable environments have led to the majority of microalgae possessing a very tough cell wall. This coupled with their small size makes the extraction of valuable products technically difficult, usually inefficient and expensive. The project is currently developing a screening method for the selection of cell wall mutants using staining and FACS.

11. Evaluation of froth flotation for recovery of algal biomass

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University of Sheffield

In recent years, economic, environmental and energy security issues justified an investigation for new sources of energy to replace fossil fuels. Biofuels are essentially considered as promising substitutes for the current 80% share of fossil fuels (mainly petroleum) in the energy market. Fundamental limitations of biofuels such as lack of arable land and fresh water encourage researchers to look for new generations of biofuels. Algae fuel as one of these new generations has been studied in the past decades, but high energy and production costs have been a great obstacle against economic and sustainable production of algae fuel. In order to tackle these problems, efforts have been made to decrease the overall costs of algae fuel production, especially the separation of algae biomass from its growing medium, which is called 'harvesting of algae'. In this poster a novel

harvesting method has been proposed and studied, based on a combination of the existing air flotation method, and froth separation, a method used in mineral processing for separation of ores.

12. Flocculation to harvest *Chlorella sorokiniana*

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Owing to the difficulties of algae biomass harvesting, flocculation is so far the most feasible harvesting method for large scale algal biodiesel production with relatively low costs. However, most effective chemical flocculants such as aluminum sulphate ($Al_2(SO_4)_3$) will cause a problem that recovered biomass would contain large amounts of chemical precipitation formed by the flocculants. Chitosan is a kind of cationic polymer which can induce algal flocculation mainly due to partial charge neutralization. It was proved highly effective in harvesting the green alga *Chlorella sorokiniana* at a working pH under 7. The flocculation process was monitored with the light scattering method and the floc size was found to grow to as large as several millimeters in a very short time. Flocculation can significantly improve the performance of sequential dewatering processes like centrifugation and filtration and meanwhile save much of the energy input.

13. Microalgae and cyanobacteria in waste stabilization ponds - A molecular investigation

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Waste Stabilization Ponds (WSPs) are a low cost, low energy wastewater treatment method predominantly used in developing countries. Systems usually comprise of an anaerobic pond, a facultative pond and a series of maturation ponds. The facultative and maturation ponds are populated by eukaryotic microalgae and cyanobacteria that photosynthesise providing oxygen for aerobic bacteria to treat the wastewater. The algae have also been shown to take up nutrients, removing them from the water column.

The photosynthetic component of the biomass in WSPs has not been investigated extensively. Molecular techniques have been used for bacterial communities and marine algal communities. This research focuses on adapting and testing molecular techniques to assess diversity of photosynthetic organisms in WSPs.

DNA extraction methods were tested on WSP samples, to find one that is effective and as bias free as possible. Commercially available kits were tested for DNA quality, DNA quantity, use for downstream amplification and diversity. Qiagen's Blood and Tissue Kit outperformed the other kits tested and does not require any specialised laboratory equipment, making it ideal for use in developing countries.

WSP samples have been collected in two systems in Brazil for molecular analysis, one treating domestic wastewater from homes local to the plant, the other treating a 50:50 mix of industrial and domestic wastewater. Community analysis targeting RuBisCO genes and 18S rRNA genes aims to investigate the effects of the waste stream on the organisms present. The study also looks at the changes in community composition and succession seen throughout the ponds in the system.

The effects of engineering solutions on WSPs are being investigated in a pilot scale WSP in Colombia. The effect of baffles that increase water flow and oxygen content on the community composition and the expression of photosynthetic genes will also be assessed.

14. Microalgae for sustainable wastewater treatment and feed production

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Pivotal to the basic needs of modern society are access to clean water, food and fuel. Given the added pressure of reducing our carbon intensive lifestyles and processes in order to limit potential effects on climate change, we are exploring the viability of developing a commercial wastewater treatment system also capable of producing biologically-derived added value products.

The removal or 'polishing' of nitrates and phosphates from wastewater in sewage works is an energy intensive process, which relies heavily on chemical inputs, and produces CO₂, sludge and other waste. Microalgae grown in photobioreactors offer significant potential to reduce the cost and impact of wastewater treatment whilst contributing to the security of liquid fuel supply. In addition, algae require large amounts of CO₂ to produce biomass thereby participating in the process of reducing global carbon emissions. Nitrates, phosphates, together with CO₂ and light are the main feedstocks for efficient algal biomass production.

Candidate species of freshwater microalgae are being assessed for their water polishing capabilities as well as for valuable bio-products (oils, proteins (feed, food) or pigments).

A photobioreactor pilot plant has been built, which is linked to a local treatment works -operated by Welsh Water- to provide wastewater. The pilot is now testing state-of-the-art low-energy harvesting technologies to recover algal biomass and produce water that meets strict EU discharge standards, while continued efforts in the lab are also looking at ways of reducing the overall energy input (lighting, processing etc.)

15. Integration of biological wastewater treatment and algal growth for biofuels

Philippa J. Uttley

University of Sheffield

Biological wastewater treatment is an established process that harnesses microorganisms to reduce the contaminants in domestic and industrial effluents to acceptable levels for discharge or further processing. Photosynthetic microalgae are actively under consideration for large scale biodiesel production since, under the right conditions, some species produce high levels of lipid that can be separated and converted to biodiesel. This research seeks to investigate the benefits of combining the two complementary processes via the construction of a mathematical model. By coupling our computational model for integrated wastewater treatment and algal biodiesel production to a mathematical optimisation algorithm, we can investigate the economics of large scale production of microalgae using wastewater-derived nutrients, as well as enhanced production of biogas from anaerobic digestion of algae and activated sludge. A proof-of-concept model of an integrated WWTP has been built using simplified models of activated sludge and algal growth, utilising Monod kinetics. Initial results illustrate the benefits of mass transfer of gases between the separate units,

showing an increase in COD removal and an increased rate of algal biomass production. Future versions will include anaerobic digestion and combined heat and power as producers of carbon dioxide. The final model will employ a mathematical optimisation algorithm to find cost-optimal designs for industrial-scale WWTPs.

16. Accomplishing industrial carbon capture-an Academia for Business Project (A4B)

Graham Nelson, Alla Silkina, Robin Shields

Swansea University

Wales' carbon emissions per person are highest in the UK due to the Wales' heavy industry. The 30 month project, known as **ACCOMPLISH (AlgalCarbon Capture and BiOMass Production - Linked Supply cHain)** is being supported with more than £425,000 by EU structural funds, the Welsh Government and resources from industrial partners, and aims to harness the properties of microalgae to mitigate carbon emissions and provide sustainable energy.

The three main tasks include:

- Development, installation and commissioning of an efficient, microalgae production system at Tata Steel Strip Products Ltd (TATA). This system will be set up to be applied to a range of different gas sources, including industrial steel works flue gases as well as other waste nutrient sources.
- Development of efficient processes for harvesting and drying algal biomass with the industrial partner Axium Process Ltd through the deployment of filtration equipment.
- Assessing the suitability of wet and dried micro algal biomass for bioenergy production with a third industrial partner, using Anaerobic Digestion technology. This task is to include analyses of moisture content, calorific and nutritional values to evaluate algal biomass as a candidate sustainable feedstock to be integrated into existing industrial processes.

The microalgae production system developed in the ACCOMPLISH project will supply a unique approach to provide a feasibility of steel flue gas mitigation by microalgae to reduce the carbon footprint and assure sustainable bioenergy production.

17. Carbonation of an algal cultivation system

William Nock

University of Southampton

Cultivating microalgae for the production of biofuels is currently uneconomical in comparison to fossil fuels. There is an on-going effort to improve the biological processes and engineering designs to make algal biofuels economically competitive. This poster illustrates one of these concepts, which is looking to understand and improve the supply of waste carbon dioxide into an algal cultivation system. As well as improving the algal yield by supplying carbon to the algal cells, these designs can also improve the de-oxygenation and mixing of the algal medium, which are both vital in improving the effectiveness of algal cultivation systems.

Through experimental work and numerical modeling this project has started looking into the mass transfer from carbon dioxide bubbles into water and the effects the different chemistry of algal media will have on this. The mass transfer of dissolved oxygen out of an algal medium has been proven to be very important for effective algal growth and this will also be studied to better understand the dynamics of this process. From this work it is hoped alternative designs could be developed to maximise mass transfer in an algal media, while also enhancing the mixing that the bubbling process generates.

18. Exploring the potential for therapeutic protein production in algae

Stephanie Braun Galleani, Frank Baganz & Saul Purton

University College London

Even though algae have been used for centuries especially as a food source, algal biotechnology is quite novel and still remains as a non-transgenic field in commercial processes, offering much space for development and research. *Chlamydomonas reinhardtii* is a well-studied organism that has been extensively used in the past two decades for developing genetic manipulations on algae. This current situation and the urgency to generate commercially-feasible platforms for massive recombinant protein production have put algae in a privileged and promising position.

This research aims at analysing different factors limiting recombinant protein production in *Chlamydomonas reinhardtii* by using both genetic manipulations in order to improve cell features and protein expression, and bioprocess optimisation, studying different culture parameters that may affect growth and protein productivity. By merging both approaches it is expected to develop an improved platform with recombinant protein productivity comparable to current bacterial systems.

The first approach considers the incorporation an additional light-capture system (proteorhodopsin) into *Chlamydomonas reinhardtii* aiming at increasing the ATP production in the cell, ongoing work expects to conclude if this genetic modification gives some benefit on extending the culture lifespan under nutrient starvation. A second approach considers the incorporation of a green fluorescent protein as a marker in order to have a tool for monitoring the optimisation of culture conditions, and it is expected to use this strain in lab scale cultivation in the coming months.

19. Synthesis of antibacterial proteins in the chloroplast of the green microalga *Chlamydomonas reinhardtii*

Laura Stoffels, Bambos Charalambous & Saul Purton

University College London

Endolysins are antibacterial proteins that are produced by bacteriophages to digest the bacterial cell wall for phage progeny release at the end of the lytic cycle. These efficient enzymes are highly specific for the cell wall of the target bacteria without affecting other species. Development of resistance against endolysins is very rare, because they evolved to target molecules in the cell wall that are essential for bacterial viability. Taken together, this makes them promising novel antibacterial agents. *C. reinhardtii* offers already established techniques for the expression of foreign genes in the chloroplast and is an attractive expression platform for therapeutic proteins, due to the lack of endotoxins and potentially infectious agents. Furthermore it can be inexpensively cultivated in full containment and under sterile conditions in simple photobioreactors. Two bacteriophage

endolysins targeting two major human pathogens were successfully expressed in the chloroplast of *C. reinhardtii*, and the activity in killing the bacteria was assayed *in vitro*.

20. Chloroplast engineering of green algae

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Our group has created an efficient method for chloroplast transformation of the green alga *Chlamydomonas reinhardtii* that involves a simple procedure of agitating a DNA/cell suspension with glass beads, and also avoids the use of any bacterial antibiotic-resistance marker. This method is currently being applied in our research aimed at producing novel biofuel compounds or pharmaceutical proteins in the chloroplast. For example, we have introduced a synthetic, codon-optimised gene encoding a vaccine candidate for Human Papilloma Virus, and two genes from a cyanobacterium that encode the aldehyde decarbonylase and acyl reductase enzymes necessary for alkane production. We are currently testing the transformed cell lines for synthesis of the recombinant products and exploring the influence of different 5'UTR and promoter elements on the yield of the recombinant products.

Although chloroplast transformation of green algae is currently limited exclusively to *Chlamydomonas reinhardtii*, our research has shown that a strain of *Chlorella* might make a superior host for such recombinant production since it has significantly higher growth rate and biomass productivity than *Chlamydomonas*. In order to exploit this alga's potential as a platform for the synthesis of valuable recombinant products we are developing chloroplast (and also nuclear) transformation vectors, and testing different DNA delivery and selection methods. Our progress on the chloroplast engineering of both *Chlamydomonas* and *Chlorella* will be presented.

21. Analytical methods for research on photosynthetic organisms

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Whether it is biofuel production, bio-electrochemical systems or fundamental biological research, analytical methods are needed to measure quantities, which enable us to compare versatile subjects of study and evaluate the impact of the directed change of certain factors. A couple of analytical techniques have proven to be very useful for the selective determination of these factors in biological investigations. The techniques presented on this poster are electro-chemical methods, involving rotating disk electrochemistry and the use of ultramicroelectrodes as well as continuous flow measurements. Photographs and schematics of the experimental setup are shown on the poster, the respective advantages of the method are given and examples of the obtained information are presented. Since most biological studies involve liquid phase studies, electrochemistry opens up the opportunity to conduct experiments in the natural environment of the organism. Especially the presented use of ultramicroelectrodes enables non-invasive observations on a micrometer scale. The possibility to tune electrochemistry into high selectivity results in a paramount sensitivity, which allows one to detect the slightest concentration changes of the targeted species. Additionally it holds the possibility to be directly coupled with other extremely sensitive detection methods, such as mass spectroscopy (MS) and high pressure liquid chromatography (HPLC) for metabolic profiling and flow cytometry for growth rate monitoring.
