
UNIVERSITY OF CAMBRIDGE
Cambridge Algae Symposium 2013
2 September 2013
Sainsbury Laboratory
Cambridge University

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EnAlgae is a Strategic initiative of the INTERREG IVB North West Europe (NWE) programme that brings together the expertise and resources of 19 partners. It aims to reduce CO_2 emissions and dependency on unsustainable energy sources in NWE. It will do this by accelerating the uptake of **sustainable algal technologies** for bioenergy and other marketable products, processes and services.

A network of algal pilots across NWE

EnAlgae operates an integrated network of nine pilot facilities to collect and share data for the mass cultivation of micro and macroalgae. Best practice is being demonstrated to stakeholders via pilot open days, meetings and technical reports.

Sustainable algae to energy market for NWE

EnAlgae is carrying out work to identify political, economic, social and technological opportunities which promote the adoption of algal biomass within the region. The aim is to assist policy makers, industry and investors in regards to understanding which algal production systems, standards and end-use markets are applicable to the region.

Decision support tool for practitioners, policy makers and investors

EnAlgae is developing a comprehensive and user-friend ICT tool which will facilitate decision making, identify gaps in current knowledge and capability, and provide a roadmap by which stakeholders can focus future actions in the region.

For news, events and to join the mailing list, visit: www.enalgae.eu

Contact us at: info@enalgae.eu

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Welcome

09.00-09.50 Informal showing of the Algal Innovation Centre at the Botanic Garden
(Matt Davey & Steve Skill)

09.00-10.00 Registration at Sainsbury Laboratory; Coffee

10.00-10.15 Welcome & Introduction:
Brenda Parker & Beatrix Schlarb-Ridley
(representing ABC/Incrops/EnAlgae)

Session 1: PHYSIOLOGY

Chair: Matt Davey

10.15-10.45 John Day, CCAP Oban
ABC: Algal Bioresource Conservation

10.45-11.15 Jessica Adams, BEACON Aberystwyth University
Macroalgal biofuels, biorefining and BEACON

11.15-11.45 Kevin Flynn, Swansea University
Using models to optimise commercial algal production

11.45-12.00 David Russo, University of Sheffield (Young Researcher)
Metaproteomics: A Tool in Synthetic Ecology to Create Effective Mixed Communities for Open Pond Algae Cultivation

12.00-13.30 **LUNCH**
Cold buffet lunch/industry stands/posters

Session 2: GENETICS AND BIOTECHNOLOGY

Chair: Stephen Rowden

13.30-14.00 Andrew Spicer, Algenuity
Shifting eukaryotic microalgae from Molecular Biology 1.0 to SynBio 1.0: challenges, failures and successes

14.00-14.30 Johnathan Napier, Rothamsted Research
Metabolic engineering of Phaeodactylum tricornutum for enhanced accumulation of omega-3 long chain polyunsaturated fatty acids

14.30-15.00 Alla Silkina, Swansea University
Microalgal biotechnology to improve bioremediation process

15.00-15.15 Xander Anderson, University of Cambridge (Young Researcher)
Algal plasma membranes and power output in Bio-Photovoltaic devices

15.15-15.45 **TEA BREAK**

Session 3: CULTIVATION, DOWNSTREAM PROCESSING AND SCALE UP

Chair: Christopher Howe

15.45-16.15 Dorinde Kleinegris, Wageningen UR Food & Biobased Research
AlgaePARC, cultivation and scale-up

16.15-16.45 Steve Skill, Plymouth Marine Laboratory and Nottingham Boots
The Nottingham microalgae biorefinery

16.45-17.15 Jerome Vaughan, Aragreen
Algae production under glass ceilings — challenges to scaling up

17.15-17.30 Francesco Ometto, Cranfield University (Young researcher)
Algal biomass pre-treatments for energy recovery

17.30-17.45 Concluding remarks: Dr Beatrix Schlarb-Ridley

17.45-19.00 **DRINKS RECEPTION and POSTER SESSION**

ABC: Algal Bioresource Conservation

John Day, CCAP Oban

Uniquely in the context of biotechnological exploitation of micro-organisms the routine serial transfer of algal cultures (cyanobacterial and protistan) is accepted by many practitioners as the optimal method of long-term maintenance. Whilst there is limited robust evidence of genotypic change, or loss of functionality, there are examples of changes in cyanobacteria and eukaryotic algae on prolonged "serial sub-culturing" including morphological changes and loss of metabolite production. Furthermore, there are risks, not only of loss of a valuable trait, but of mislabelling, or even catastrophic failure of the master stock-cultures.

Modern algal biotechnology will almost inevitably be based on the exploitation of transgenic algae, but as yet there is little or no evidence on the long-term genotypic or functional stability of these organisms. Investors in biotechnology will insist that their "assets", whether wild-type, conventionally mutated, or transgenic strains are "secure". This will necessitate the application of long-term conservation methods. Master stock-cultures of most microbial resources exploited are conserved by either lyophilization, or cryopreservation at ultra-low temperatures. Both approaches work on the basis of removal of water rendering it unavailable for biological activity. Whilst lyophilization has potential for the conservation of some axenic cyanobacteria it is not applicable to eukaryotic algae/protists. Cryopreservation, specifically with storage below approximately –135°C, can effectively guarantee stability for 100's of years and is the method of choice, where applicable, in Biological Resource Centres (BRCs). However, there are significant challenges to the cryopreservation of algae and many taxa, including some that are currently exploited, are problematic, or as yet cannot be cryopreserved. This paper discussed the issues involved and outlines the current status of methodologies available capable of conserving wild-type and genetically manipulated algae. Furthermore, strategies needed to ensure sustainability of the sector with respect to the conservation of master stock-cultures will be discussed.

Without a guarantee of long-term stability of production strains irrespectively of whether they are: wild-type, generated through conventional strain selection/ mutagenesis, or transgenic, algal biotechnology will not be economically sustainable.

Macroalgal biofuels, biorefining and BEACON

Jessica Adams, BEACON Aberystwyth University

Jessica Adams began investigating macroalgae (seaweed) as a biomass source for biofuel production in 2008 and within her work has produced bio-ethanol, bio-methane, and bio-oil through pyrolysis. She has also measured inorganics, determined heating values for combustion and related these outputs to seasonal variation in composition. She is now looking at bioprospecting for novel enzymes capable of either degrading macroalgae or other plant material through macroalgal-associated microbe sources and investigating properties and applications for extracted macroalgae compounds. These latter areas are being investigated as part of BEACON, a £10.6m project funded by the European Regional Development Fund through the Welsh Government involving Aberystwyth (lead), Bangor and Swansea Universities. BEACON's mission is to build integrated 'green supply chains', with a focus on developing new routes to functional, cost-competitive products using biomass rather than oil. This is achieved through establishing company interactions and delivering solutions to benefit industry across a range of sectors and to support economic growth. It is capable of demonstrating scale-up processes through the biorefining pilot plant, now at the heart of Aberystwyth University's innovation campus site. Currently a short-listed project for the European-wide RegioStars award, its demonstrated ability to create impact and to have a genuine effect on SMEs is well noted.

Using models to optimise commercial algal production

Kevin Flynn, Swansea University

Efficient growth of microalgae for commercial applications requires the optimization of many parameters; light, optical path length, N & P nutrients, continuous vs discontinuous cultures, among them. To conduct a full suite of experiments to achieve such an optimisation would be prohibitive in time and cost. The alternative is to use adaptive mechanistic models of microalgae. Such models have an established pedigree in algal ecophysiology and are readily applied for commercial purposes. When merged with a cost-model it is then possible to optimise for life cycle analysis (LCA), or similar financial considerations. The approach and opportunities for such applications, including as an aid to PBR design and operation, for optimising for biomass vs biofuels or lipids, for strain selection and for development of designer (GM, etc.) microalgae will be explored.

Metaproteomics: A Tool in Synthetic Ecology to Create Effective Mixed Communities for Open Pond Algae Cultivation

David Russo, University of Sheffield

The cheapest way to cultivate algae for biofuels is using open, mixed raceway ponds. Although a major limitation is the increased risk of invaders (e.g. bacteria, other algae, grazers etc.), constructing a synthetic community resistant to these attacks has the potential to produce more robust systems. A metaproteomic platform was used here to measure metabolic changes during algal cultivation in an open pond format to further understand mixed communities for the algal biotechnology sector.

The objective was to simulate an open pond system vulnerable to invasion and was therefore mixed in terms of species richness and abundance. 30L buckets, inoculated with an environmental sample, were grown in constant light and temperature using a high nutrient concentration media. Nutrient measurements (NH_4^+, NO_3^-, PO_4^-), dissolved organic carbon (DOC) and dissolved oxygen (DO) were undertaken. Cells were concentrated using tangential flow filtration.

Protein was extracted by grinding cells, in liquid nitrogen, with a mortar and pestle. Soluble proteins were fractionated using SDS-PAGE and HILIC chromatography. Tryptic peptides were run on a Q-TOF MS/MS system and spectra interpreted using Mascot search engine. Proteins were functionally clustered using COG.

Temporal analysis of nutrient levels shows that the initial decrease in nitrate, that occurred simultaneously with an increase in DO, pH and temperature, are signs of an increase in algal metabolic activity. The late increase in DOC signals a larger bacterial contribution towards the end of the experiment. The metaproteomic profiles show an initial dominant contribution from native algae species with a rise in bacterial activity in the latter phases of growth (e.g. decomposition, recycling of biomaterials and nitrogen fixation).

Metaproteomics provides a signature of ecosystem function combined with standard ecology data. The metabolic profiles were interpreted in terms of bacterial-algae interactions, providing enhanced understanding of algae cultivation for process predictability and control.

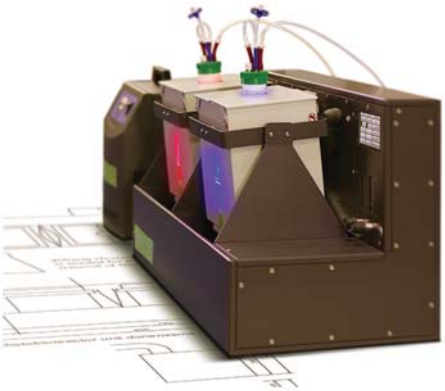
Shifting eukaryotic microalgae from Molecular Biology 1.0 to SynBio 1.0: challenges, failures and successes

Andrew Spicer, Algenuity

Eukaryotic microalgae, including diatoms, represent an as largely untapped resource of biodiversity with regard to their application as commercially relevant microorganisms for the industrial scale production of socially and economically valuable chemicals and polypeptides. Microalgae offer intriguing industrial possibilities including their ability to harness light energy for growth as well as their relatively simple culture requirements, alternate growth modes and comparatively rapid growth rates. The ability to manipulate microalgal strains in a predictable and reproducible way with regard to genetic modification strategies has been limited largely to a handful of strains that have been transformable. These approaches fall under a collective umbrella that I will refer to as Algal Molecular Biology 1.0. In order to be competitive as an industrial platform we must start to develop innovative approaches as well as translate the best that synthetic biology and high throughput technologies can offer into commercially relevant microalgal strains. Transformation efficiencies are notoriously low or non-existent for many commercially desirable strains and it is clear that no one-size fits all approach can be applied. Codon optimisation remains largely a black box, particularly for sequences targeted to the nucleus. Microalgal strains, including *Chlamydomonas reinhardtii*, have significant unknown properties that make predictability of successful expression of any given sequence at best 50:50; other strains faring marginally better. We have endeavoured to develop a suite of approaches and molecular tools in order to shift microalgae from Molecular Biology 1.0 to SynBio 1.0, the goal being predictability and precision in engineered strains. I will present an overview of our challenges, failures and successes along this path.

algem

environment modelling labscale photobioreactor



making strain-specific growth data easily accessible

identifying optimum growth parameters

improving algae growth performance

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Features

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Uses standard 1L Erlenmeyer lab flasks
Easily accessible and easy to use, clean, and autoclave.

Dynamically controlled LED lights
LED light system with intensity control, photoperiod control, flashing light effect, and independently controlled, blendable LEDs (red, white, and blue). System avoids ambient light contamination.

Temperature control with chiller included as standard

Global meteorological database with the ability to define photoperiods and temperature cycles
Input latitude, longitude, and month to simulate environments of almost anywhere in the world. Includes a global meteorological database covering 80 years.

Continuous growth monitoring with advanced software
Optical density is continuously monitored and corresponding data collected. Algal growth curves are produced.

Gimbal-based mixing system with controllable rates
Custom mixing profiles can be specified, ranging from continuous to pulsed, low shear mixing.

Flask lid with inlets for CO_2 gas for continuous sampling and operation

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Metabolic engineering of *Phaeodactylum tricornutum* for enhanced accumulation of omega-3 long chain polyunsaturated fatty acids

Johnathan Napier, Rothamsted Research

We have engineered the diatom *Phaeodactylum tricornutum* to accumulate the high value omega-3 long chain polyunsaturated fatty acid docosahexaenoic acid (DHA). This was achieved by the generation of transgenic strains in which the Δ5-elongase from the picoalga *Ostreococcus tauri* was expressed to augment the endogenous fatty acid biosynthetic pathway. Expression of the heterologous elongase resulted in an eight-fold increase in docosahexaenoic acid content, representing a marked and valuable change in the fatty acid profile of this microalga. Importantly, DHA was shown to accumulate in triacylglycerols, with several novel triacylglycerol species being detected in the transgenic strains. In a second iteration, co-expression of an acyl-CoA-dependent Δ6-desaturase with the Δ5-elongase further increased DHA levels. Together, this demonstrates for the first time the potential of using iterative metabolic engineering to optimise omega-3 content in algae.

Microalgal biotechnology to improve bioremediation process

Alla Silkina, Swansea University

Microalgae remove nutrients from their surrounding growth medium in order to grow and replicate, therefore there is the potential to use microalgae to recover nutrients (nitrogen and phosphorous) from waste streams. In this study, agricultural, fish farm and Anaerobic Digestion (AD) municipal waste was successfully tested as a nutrient source for the cultivation of both marine and freshwater microalgae species in 400L and 600L horizontal tubular photobioreactors (PBRs). Close monitoring of the cultivation process focused on the assessment of physico-chemical and biological parameters of the algal cultures. The species were grown in PBRs for 25 days in batch mode. The maximum rate of nitrogen uptake was recorded when *Chlorella minutissima* was grown using an agricultural waste source (186 μmol L^{-1} $d^{-1)}$. A similar result was achieved using the freshwater microalgae *Scenedesmus sp.* (146 μmol L^{-1} d^{-1}). The maximum rate of P-uptake was 36.4 μmol L^{-1} d^{-1} by a multi species algal assemblage isolated from an industrial steel production site using AD municipal waste as a nutrient source. The maximum nutrient removal rates achieved in this study are encouraging for the use of microalgae for bioremediation in an integrated biorefinery, particularly one using horizontal, tubular type bioreactor systems.

Algal plasma membranes and power output in Bio-Photovoltaic devices

Xander Anderson, University of Cambridge

Algae have the potential to be used as a clean and renewable power source, converting sunlight into electrical energy in devices called Bio-Photovoltaics. Such devices harness electrons from the outer surface of algal cells that have been transported from photosynthetic reactions in the chloroplast. A key barrier to electron extrusion is the cell plasma membrane and we believe that the power output of these Bio-Photovoltaic devices may be improved by better understanding the mechanisms by which the algae transport electrons across the plasma membrane. For my PhD I have been investigating a group of electron-transporting enzymes called NADPH Oxidases, which are important for growth and toxicity in several other algal species. By measuring activity of these enzymes in different strains of *Chlamydomonas reinhardtii*, I have been probing the link between enzyme activity, gene expression and power output, in a Bio-Photovoltaic device. I have found that activity varies amongst wild-type and cell wall-less strains, which seems to correlate with expression of two putative *Chlamydomonas* NADPH Oxidase genes. By cloning these genes under *Chlamydomonas* specific promoters I am attempting to overexpress the NADPH Oxidases to instigate higher activity, possibly leading to increased electron transport and power output in Bio-Photovoltaic devices.

AlgaePARC, cultivation and scale-up

Dorinde Kleinegris, Wageningen UR Food & Biobased Research

Algal production needs to develop from a craft to a major industrial process. Major challenges are to reduce production costs and energy requirements and increase production scale. Although microalgae are not yet produced at large-scale for bulk applications, recent advances – particularly in the methods of systems biology, genetic engineering, process control, and biorefinery – present opportunities to develop this process in a sustainable and economical way within the next 10 to 15 years.

AlgaePARC, an applied research centre on microalgae has been set up at Wageningen UR to compare present technology, and to develop new reactor concepts and process control strategies to achieve lower production costs and energy requirements and to gain knowledge for the design and process control of large-scale microalgae facilities.

The goal of AlgaePARC is to fill the gap between fundamental research on algae and full-scale algae production facilities. This is done in flexible pilot scale facilities in order to perform applied research and obtain direct practical experience. The set-up is fully automated and allows for fast changes between photobioreactor types, layout, and process control strategies. The initial systems chosen reflect the present development of several reactor concepts at laboratory scale, by different research groups and companies, and will enable a rigorous comparison between systems, selection and, ultimately, the development of a more efficient system and optimized operational concepts.

A five year research program started at AlgaePARC in 2011 and is supported by circa 19 companies in the food, oil, chemical and technology development sectors.

The Nottingham microalgae biorefinery

Steve Skill, Plymouth Marine Laboratory and Nottingham Boots

Boots and PML have been working together since 2002 to identify opportunities to reduce the reliance on petrochemical ingredients in Boots' cosmetics products. Boots operate a modern 15MW gas turbine power station supplying both heat and power requirements for their manufacturing base in Nottingham, and in 2007, Steve Skill proposed utilising the emissions from the power plant as a carbon source for phototrophic microalgal cultivation.

In 2008, PML embarked on a collaborative project (CCIF) with funding from the UK Technology Strategy Board, Boots, Cognis GmbH (BASF) and Carlton Power Ltd, to develop a microalgal biorefinery to produce sustainable ingredients.

Several ingredients including antioxidants, sunscreens, colorants and plastics for packaging are derived from fossil fuels, and PML targeted cyanobacterial strains that offer alternative feedstocks for these compounds.

A robust cyanobacterial strain that accumulates targeted ingredients and bioactive compounds was cultivated in a proprietary 16m^3 photobioreactor (S. Skill) coupled to the emission stack of Boots' power plant. Energy efficient harvesting and multi-stage downstream processing protocols were developed to produce cosmetic ingredients and bioactive extracts.

In order to develop a broader range of viable bio-products, the development of molecular toolkits and efficient transformation systems in cyanobacterial chassis strains were also investigated.

With EU funding support from the EnAlgae project, the biorefinery continues to operate, acquiring long term microalgal productivity data for the UK climate.

Algae production under glass ceilings — challenges to scaling up

Jerome Vaughan, Aragreen

Algae have been identified as a key area of opportunity to provide sustainable feedstock for a range of industries from high value chemicals to biofuel; however its use at industrial scale remains elusive. Indeed, the largest algae farm in the world is the 250ha facility of Hutt Lagoon in NW Australia, owned by BASF. Here algae is produced using the same techniques as employed by the Azetcs on Lake Texcoco prior to the 16th century.

Taking algae from lab to industrial scale remains a key challenge for the industry. Even at lab scale, challenges remain. A survey of some of the existing lab scale bioreactors available to algae researchers, will discuss to what extent this existing infrastructure matches demands and objectives from potential users (both corporate and academic).

Existing industrial production technologies including open raceway ponds, fermenters and bioreactors will also be discussed, along with a brief overview of various operators. Data from some of these existing technologies is discussed, along with an examination of the challenges facing these various forms of production before they can establish themselves at industrial scale.

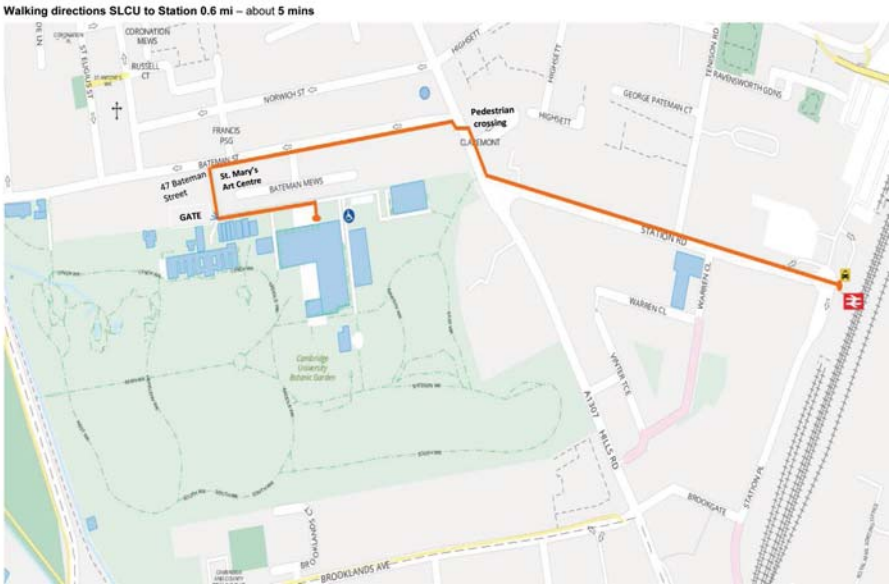
In terms of academic input into the subject of algae commercialisation, the talk will ask what is driving university research into the general topic of algae and, to what extent, research is being developed in a "cross-departmental" manner or in isolation.

Finally, the talk will examine some key characteristics of the existing approaches to algae research, development and commercialisation and pose questions as to how sustainable these approaches are, given the requirements being placed on algae. Along with some suggestions as to future pathways to successful scale-up and use of algae which may assist with breaking through current glass ceilings.

Algal biomass pre-treatments for energy recovery

Francesco Ometto, Cranfield University

Anaerobic digestion of untreated microalgae biomass yielded between 30 to 60% of the theoretical methane production. As confirmation of an incomplete digestion, residual intact cells were detected in the post digestion sludge. Different biomass pre-treatments (thermal, thermal hydrolysis, ultrasound and enzymatic) were compared in terms of cell solubilisation and digestion improvement using *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Arthrospira maxima*. Thermal treatments were carried out at five different temperatures between 105 and 165°C for 30 minutes. Compared to the thermal treatment, thermal hydrolysis was characterised by steam injection. Using ultrasound, the energy inputs were fixed at 0.35, 3.5, 10, 20 and 35 MJ kg^{-1} solids. Five different enzymes, commonly applied in the food industry, were tested at 50°C, pH 6 for 24 hours at different dosages. Overall, between all pre-treatments, despite the tested algae, only the use of enzymes and the thermal hydrolysis allowed a positive or neutral energy balance. Optimal thermal hydrolysis conditions showed an increment between 60 to 100% in biogas production depending on the algae species. The use of enzymes allowed even higher improvements (> 200%), as some enzymatic activity continued during the anaerobic digestion process. Compared to these two treatments, the ultrasound showed the least benefit. Detailed microscopic analysis of the microalgae cells underlined significant differences on the impact of the three pre-treatments on the cell wall structure, confirming thermal hydrolysis as the most direct and efficient treatment able to break the microalgae cells.



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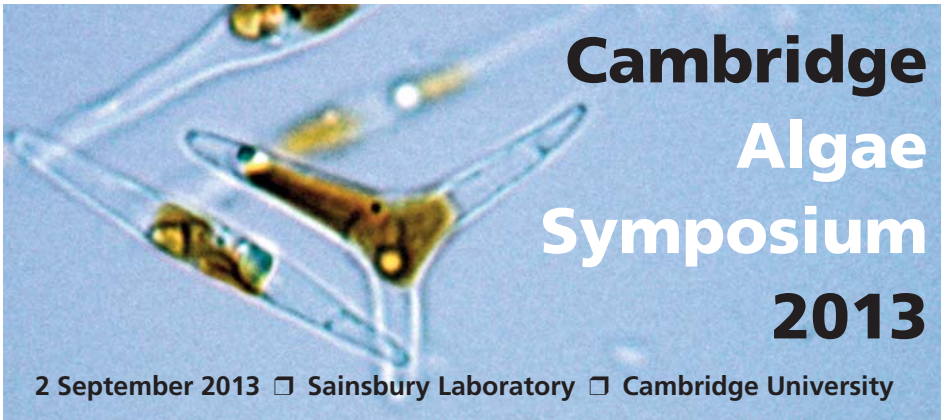

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