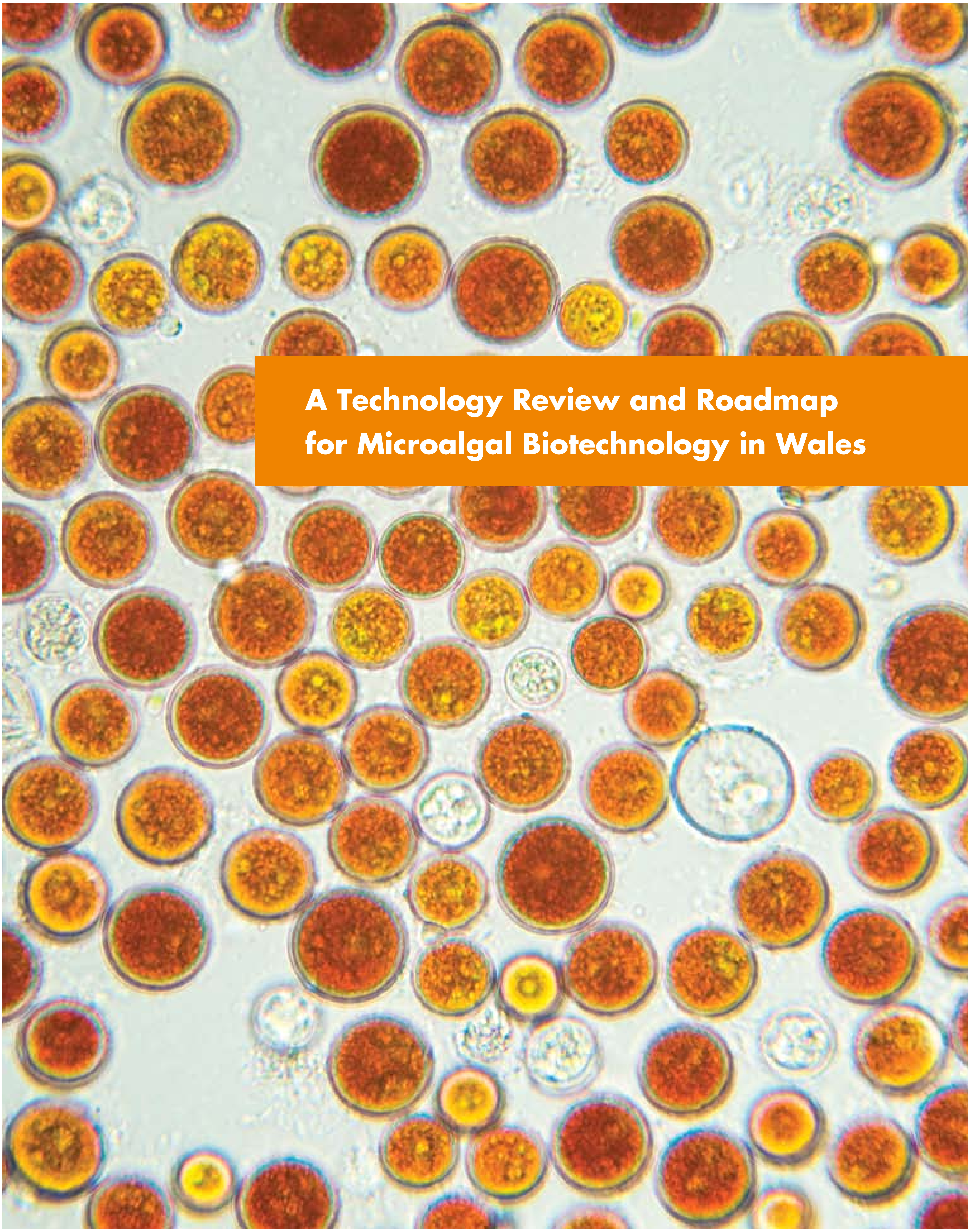

A Technology Review and Roadmap for Microalgal Biotechnology in Wales

Centre for Sustainable Aquaculture Research
CSAR

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Authors

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Centre for Sustainable Aquaculture Research (CSAR) CSAR is a recently established (2005) knowledge transfer centre located at Swansea University, focused on developing and transferring integrated aquaculture technologies for a diverse range of commercially important aquatic plant and animal species, for both food and non-food applications. A team of research and technical support staff operates from a modern facility housing a series of state of the art controlled-environment laboratories.

Dr Robin Shields is Director of the Centre for Sustainable Aquaculture Research. He obtained his honours degree in Zoology/Marine Biology and PhD (1990) in Marine Biology from University of Wales, Bangor. He has been engaged in applied aquaculture research throughout his career, within the UK Sea Fish Industry Authority and subsequently as Finfish Program Manager at the Oceanic Institute Hawaii, before joining Swansea University in 2003. His current research interests encompass dietary alternatives to industrial fishmeal and fish oil in formulated aquaculture feeds; water quality control and effluent management in recirculating aquaculture systems; and optimisation of microalgae biomass production in enclosed photobioreactors.

Prof. Kevin Flynn is a research professor in the School of the Environment and Society, Swansea University (honours degree in Marine Biology and PhD in Algal Physiology, University of Wales). Prof, Flynn's research is centred on the development of mechanistic models describing multi-nutrient interactions between plankton functional groups, with applications to oceanic impacts of climate change and optimal configuration and operation of microalgae production systems.

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Dr Ian Ratcliffe is currently a Post Doctoral Research Assistant at the Centre for Water Soluble Polymers, Glyndwr University, Wrexham. Possessing a background in biopolymers and a keen interest in biofuels and "green chemistry", he was previously employed at the School of Ocean Sciences, Bangor University. As a chemist his input included the development of extraction techniques within the algal biofuels sector.

Dr Paul Facey obtained his honours degree in Environmental Biology and a Ph.D. in plant population biology from the University of Wales, Swansea. During his postgraduate studies his interests were in the effects of natural and anthropogenic habitat fragmentation on the apportionment of genetic diversity in plant populations, as well as the interactions between native and non-native species. Furthermore, his interests also involved the study of evolving reproductive strategy in isolated plants in the British flora. More recently, his postdoctoral studies have involved the study of DNA protection proteins in Streptomyces coelicolor.

Ms Rebecca Jarvis obtained her MSc. Conservation and Land Management (2007-2008) and BSc. Marine Biology (Hons) (2005-2007) at Bangor University. She has contributed to various academic and industrial research and consultancy projects in a range of specialist fields, including the environmental impacts of drilling fluids, marine natural products, and marine biofuel technologies. Ms Jarvis has worked as a research technician within the Blue Biofuels Group at the School of Ocean Sciences, Bangor University.

A technology review and roadmap for microalgal biotechnology in Wales

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** Available on request*

Executive summary

Microalgae are a highly diverse group of microscopic single-celled organisms that occur naturally in most aquatic environments on earth. Microalgal Biotechnology involves the commercial exploitation of these organisms via mass cultivation and conversion of harvested biomass into value added products; this is a rapidly growing business sector worldwide, particularly for biofuels markets. The diverse end user applications for microalgal biotechnology includee:

- Carbon neutral Biofuel production and Bioenergy generation;
- Capture and bioconversion of carbon dioxide (CO_2) from industrial processes;
- Ingredients for human health and wellbeing products, including essential omega 3 oils, antioxidants and pigments;
- Ingredients for aquaculture and agriculture feeds;
- Bioremediation and valorisation of agricultural and industrial effluents.

The principles of microalgae exploitation using an integrated biorefinery approach are shown schematically in Figure 1, below.

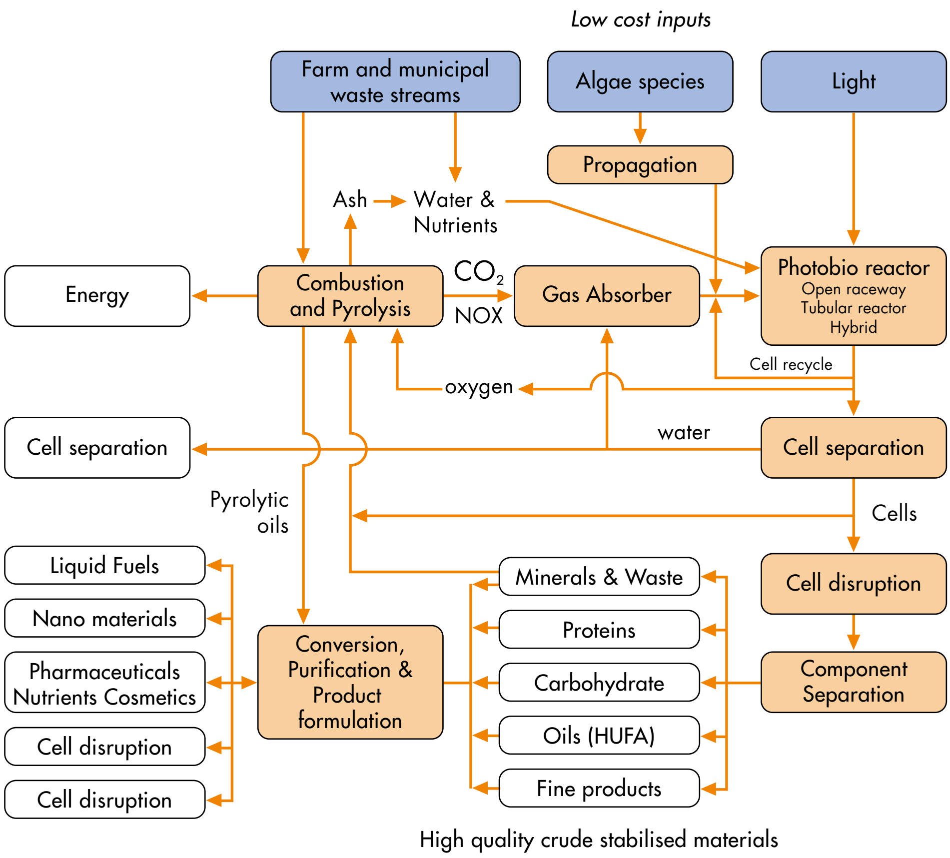


Figure 1. Schematic illustration of biorefinery approach for remediating wastes and producing value added natural products and biofuels from microalgae.

In response to a growing number of business enquiries to the Centre for Sustainable Aquaculture Research, a feasibility study was undertaken to assess the potential for Welsh businesses to adopt current microalgal biotechnologies, to examine the strategic potential among businesses, government and academia for regional development of a technology base in this sector and to map a route for the successful commercial exploitation of microalgae within Wales.

The prospects for the microalgal biotechnology sector within Wales were assessed by consulting directly with Welsh businesses, research providers and government departments (including an open invitation workshop, where 21 businesses and organisations were represented), by analysing published resources on microalgae-based biotechnologies in the UK and abroad and by critically interrogating existing technologies using computer modelling.

The information gained via these sources was incorporated into this Technology Roadmap for microalgal biotechnology in Wales (the first such roadmap in the UK), which was fine tuned by inputs from stakeholders representing industry, academia, government and NGOs.

A range of applications was identified for microalgal biotechnologies within existing Welsh businesses and utilities, encompassing:

- Nutraceuticals manufacturers;
- Cosmeceuticals manufacturers;
- Specialist feed manufacturers (agriculture, aquaculture);
- Fine chemicals manufacturers;
- Natural products biotechnologists;
- Equipment and materials suppliers (including plastics, corrosion-resistant metals, lighting, pumping, filtration, process control, analytical instruments);
- Biodiesel manufacturers;
- Energy producers and heavy industry generating CO_2 as a by-product (including both fossil fuel and biomass power plants);
- Municipal waste water treatment companies;
- Intensive livestock producers releasing "point source" effluents (both agriculture and aquaculture sectors);
- Food processors releasing nutrient-enriched waste waters.

On a longer timescale, potential was also identified for converting electricity generated from renewable energy methods that are well suited to Wales (including wind, wave, tidal, hydroelectric), to power lighting for microalgae reactors, thereby capturing and storing the variable delivery of such renewable power as a carbon neutral vegetable oil for further conversion and refining to fuels and additional products.

During the course of the road mapping exercise, strong interest was expressed by Welsh businesses in taking advantage of this expanding technology sector and in developing a coordinated pan-Wales approach to exploit the potential. Specific assistance with technology transfer and technology development was requested by businesses in the areas of:

- Developing regionally-optimised technologies for capturing industrial carbon dioxide and reusing waste heat for microalgae mass cultivation;
- Remediating and valorising agricultural, aquacultural, municipal and industrial effluent waters using microalgae;
- Identifying suitable microalgae strains for local cultivation and optimal processing;
- Diversifying biomass sources for sustainable biodiesel production in Wales;
- Developing new sources of "vegetarian" omega 3 oils;

- Producing microalgae concentrates and dried preparations for use as feed ingredients;
- Optimising artificial lighting systems for microalgal photobioreactors;
- Reusing water and nutrients in microalgae harvesting and extraction operations;
- Using sterile water techniques for bulk aseptic culture and harvesting of microalgae;
- Developing methods for super critical fluid extraction of valuable compounds from microalgae;
- Characterising and identifying new valuable products from cultivated microalgae;
- Developing techniques to counteract degradation of microalgal end products;
- Providing training in microalgae cultivation and processing techniques;
- Undertaking life cycle analyses to optimise the technical and economic feasibility of microalgae biotechnologies in Wales.

The following key components were identified to maximise industry-academic interactions for effective commercial exploitation of microalgae in Wales:

- Coordinated Industry Development Strategy;
- Improved Industry Awareness and Understanding;
- Coordinated Strategy for Research and Policy Development;
- Skills, Training and Technology Demonstrations.

These will provide established Welsh businesses in the sector, recent entrants to the field and those considering entry with:

- Up-to-date information and advice on technologies, regulatory frameworks and markets;
- Access to industry-focused research and technological development to assist with the development of new products, processes and services;
- Access to demonstration and training facilities.

Accurate information will also be provided to government and sponsors to guide investment decisions in this technology area and to establish suitable strategic and regulatory frameworks for the sustainable development of the sector.

1 Introduction

Under the Welsh Assembly Government Academic Expertise for Business (A4B) programme, the Centre for Sustainable Aquaculture Research (CSAR), Swansea University, undertook a feasibility study to review the state-of-the-art in microalgal biotechnology and to determine mechanisms by which industry within Wales can benefit by engagement with this rapidly emerging sector via transfer of technologies from Welsh HEIs/FEIs. To this end, the current report contains a non-technical overview of microalgal biotechnology, against which areas of business opportunity and existing academic and commercial strengths within Wales have been assessed. Potential barriers to engagement have been identified and a plan developed to enable successful commercial exploitation of microalgae by businesses in Wales.

Within Wales, marine algae have been used as a natural resource for hundreds of years, laverbread being a foodstuff produced from a native seaweed, Porphyra. Microalgae, single celled microscopic relatives of seaweeds, have attracted much global attention in recent years for the valuable natural products they produce and for their ability to remediate effluents, such as industrially generated CO_2 and inorganic nutrients contained in industrial / agricultural / municipal waste waters.

The main initial route for commercial exploitation of microalgae was within the aquaculture industry, where microalgae are used as a direct feed source for bivalve molluscs, or as an intermediate feed for hatchery stages of marine crustaceans and finfish. Microalgae are currently used commercially in Wales for this purpose during the production of marine finfish (sea bass) and invertebrates (polychaete worms; prawns; lobsters).

It quickly became apparent that microalgae biomass also contain accessible pigments and several species of microalgae are commercially grown internationally at very large scale to produce D-carotene and astaxanthin for use in health foods and as food colourants. Other natural products that can be commercially extracted from microalgae include omega 3 fatty acids (HUFAs, important in nutrition) and phycobiliproteins (fluorescent compounds used in biotechnology). A company has recently been established in Wales to produce microalgae for these high value, food and feed additive markets.

In addition to the above established applications, the last 3-5 years have seen a huge resurgence in interest in using microalgae as a source of high-grade vegetable oil for refining into renewable fuel, and for capturing CO_2 from industrial processes such as fossil fuel power plants. This in turn has resulted in an overall renaissance in microalgal biotechnology, since the cost effective production of biofuels from microalgae relies on:

a) Efficient and reliable production of microalgae biomass

b) Optimised biomass harvesting and conversion processes and

c) Implementation of an effective "biorefinery" approach, where all of the valuable natural products contained in microalgal biomass are extracted and exploited.

CSAR received a series of enquiries beginning in 2007 from Welsh biodiesel producers and CO_2 emitters as to the feasibility of incorporating microalgal biotechnologies for the above purposes. Enquiries were also received from aquaculture operators regarding the use of microalgae to capture excess nutrients in their effluent waters.

Against this background of established and developing worldwide applications for microalgae, together with local business interest, a feasibility study (including technology road map) was undertaken to examine the strategic industrial, governmental and academic potential within Wales for regional development of a technology base in this sector, to build a working consortium of interested business, government and academic partners, and to develop an action plan to capitalise sustainably on the business opportunities.

The current report provides a description of the roadmapping methodology used, a technology review of microalgal biotechnology internationally and presentation of TRM findings to guide engagement with this technology area within Wales.

Technology road mapping methodology 2

Technology road mapping is a powerful tool for deriving and expressing business visions, supporting technology management and overall planning. It has been widely adopted in industry in providing a framework to support national and sector foresight initiatives(a). The key elements of a TRM can be summarised as follows;

- **Time** – A TRM must have a predictive value over a given time line, in this case to 2013.
- **Deliverables** – A TRM will give desired and expected performance characteristics with benefits of the product or process. A key feature is to highlight current, intermediate and some future performance indicators.
- **Technologies** – A TRM shows groupings and interactions of technologies needed to achieve deliverables.
- **Skills** – A TRM will identify the science/ knowledge base required to deliver the technologies.
- **Legislative & Environmental perspectives** – A TRM will consider external drivers on a technology area.

Based on these key elements, a time-based Gantt chart is then drawn up (e.g., Fig. 2), allowing the user to easily visualise the evolution of markets, products and technologies and the all important linkages between them.

TRMs can be seen as outputs from empowered people within a sector of industry, which help to define a strategic direction for their sector and will be used to lobby Government Organisations, Legislators, Funding bodies, etc for the benefit of that sector.

In the current study, a technology review was initially produced by academic researchers in microalgal biotechnology and bioprocessing, along with industry liaison personnel at the Centre for Sustainable Aquaculture Research, Swansea University. On the basis of this technology review and the experience of the personnel, a draft technology road map was produced. This draft TRM provided a framework for collating the brainstorming activity (in the presence of a facilitator) of relevant component functions of the sector, i.e. manufacturing, policy, technical, business. The outcomes of the brainstorming sessions have been organised and a coherent, refined TRM produced. This technology review and roadmap are accompanied by a web-site and forum to facilitate the continuation of the roadmap and to allow all interested parties to exchange information and continue to collaborate.

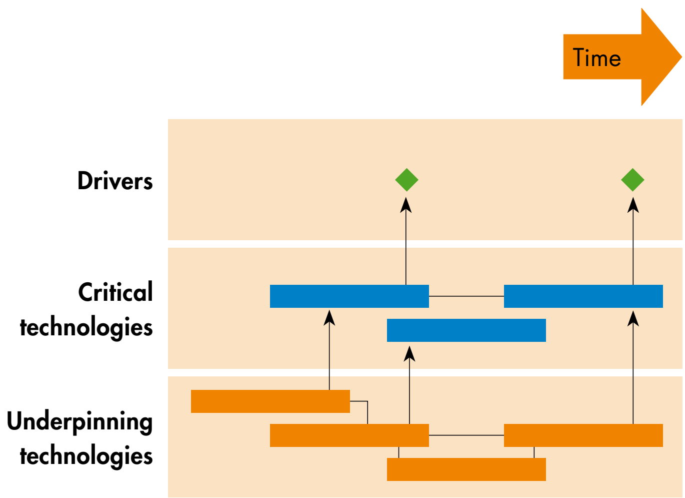


Figure 2. Example of a technology roadmap

3 Technology review – microalgal biotechnology

To date there are numerous applications for microalgae and microalgae-derived value-added products, including; pharmaceuticals, biomedical and diagnostics, cosmetics, aquaculture, food, and animal feeds. With increasing interest in environmental policy, global oil price increase and climate change, the potential for microalgal biofuel production is also of commercial and environmental interest. However, despite the range of applications already identified from microalgae, and molecules derived from them, microalgae are not yet well studied in biotechnology. In fact, despite there being over 10,000 species in existence, the chemical composition and mercantile potential has been investigated in only several hundred species, a small number of which are cultivated in quantities large enough for feasible commercial application(b). Microalgal biotechnology can be broken down into several key application areas: biofuels, nutraceuticals, pharmaceuticals and effluent remediation.

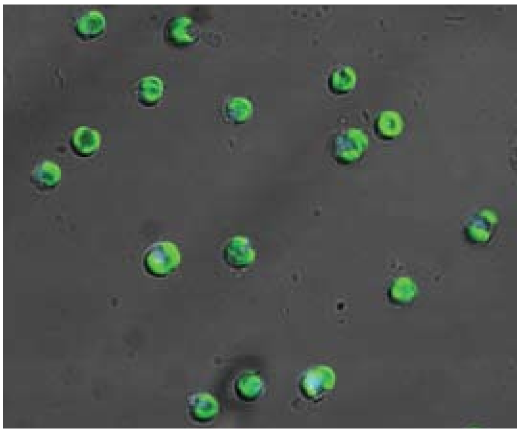


Figure 3. Photomicrograph of microalgae (*Isochrysis sp.*) courtesy of Dr Emily Roberts, Swansea University.

This section should be read in conjunction with the following appendices, whach are available on request:

Appendix 4. Summary of academic literature on microalgal biotechnology
Appendix 5. Summary of links to industry web sites on microalgal biotechnology and biofuels
Appendix 6. Summary of patent literature on microalgal biotechnology.

3.1 What are microalgae?

Microalgae are microscopic freshwater or marine organisms (e.g. see figure 3) that play a key role in nature as a food source for higher animals (eg, zooplankton, fish), for transferring nutrients in aquatic food webs and for balancing the exchange of CO_2 between the ocean and the atmosphere. Microalgae are microscopic freshwater or marine organisms. They are a highly diverse group, ranging in size from several hundredths of a mm to several tenths of a mm, taking many different shapes and existing singly or in chains or groups (see Figure 4).

Microalgae occupy a very wide range of habitats, including forms that live in open water (phytoplankton) or on surfaces (benthic), and are adapted to extreme physical and chemical conditions (eg, extremes of temperature, salinity, pH). Well known natural phenomena involving microalgae include blooms of green algae in freshwater ponds or lakes during summer and “red tides” in the sea.

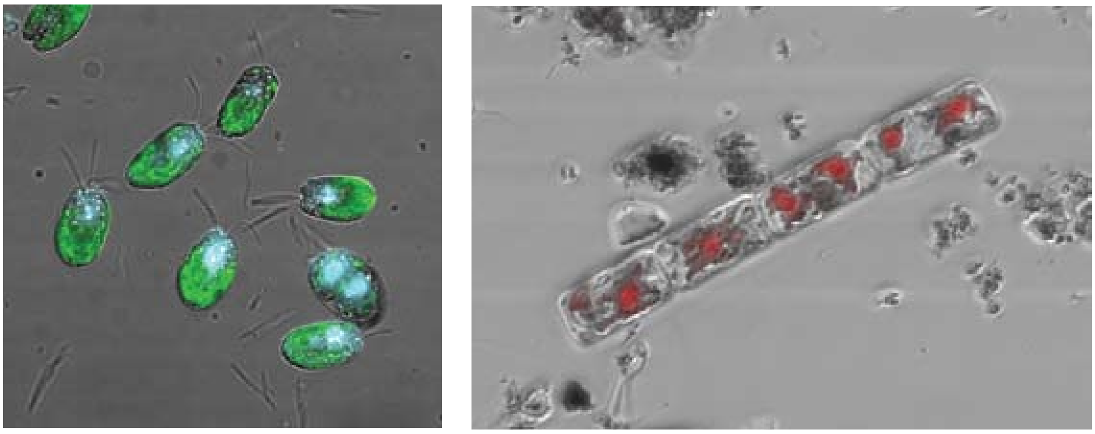


Figure 4 Photomicrographs of microalgae illustrating different sizes and shapes. Left hand, free swimming flagellate, *Tetraselmis sp*; right hand, chain-forming diatom, *Leptocylindrus sp*. Photomicrographs courtesy of Dr Emily Roberts, Swansea University

3.2 Established markets for microalgae

Table 1 summarises the production and major applications of microalgae in 2006, totalling approximately 5,000 T dry biomass pa (note, this excludes live microalgae produced and used in marine aquaculture hatcheries). Data from The Algal Industry Survey (2008) illustrates that biomass production for established markets has approximately doubled recently to 10,000 tons pa. Around half of this production takes place in mainland China, with substantial commercial production also in Japan, Taiwan, U.S.A., Australia and India, and smaller volumes produced elsewhere.

Aquaculture feeds. Microalgae are used ubiquitously as a feed source in the commercial hatchery production of juvenile marine fish and shellfish. There are thousands of marine hatcheries globally, producing billions of juvenile fish and shellfish annually. A relatively small number (~6-10) of easy-to-rear microalgae species have been adopted for this purpose.

In most cases, the microalgae are cultured on site by hatchery personnel and presented live to the fish / shellfish larvae. Under this scenario, sales opportunities to hatcheries mainly consist of the equipment and consumables required for microalgae production: photobioreactors, pumps, lights, nutrient mixes, etc. However, there is a growing trend for hatcheries to buy proprietary microalgae concentrates in order to simplify on-site operations. These concentrates are supplied by companies specialising in the large scale production and processing of microalgae. This market segment had an estimated value of $700 million globally in 2004 (see Table 1) and has grown steadily since. There is further scope to develop the sector by introducing better quality products, since it is widely acknowledged that existing concentrated microalgae products still do not match live microalgae for hatchery applications (in terms of nutritional composition; physical attributes; product stability).

Product Group	Product	Retail Value (U.S $×106)	Development
Biomass	Health Food	1,250-2,500	Growing
	Functional Food	800	Growing
	Feed Additive	300	Fast-growing
	Aquaculture	700	Fast-growing
	Soil Conditioner		Promising
Colouring substances	Astaxanthin	<150	Starting
	Phycocyanin	>10	Stagnant
	Phycoerythrin	>2	Stagnant
Antioxidants	ß-Carotene	>280	Promising
	Tocopherol		Stagnant
	Antioxidant Extract (CO2)	100-150	
	ARA	20	Growing
	DHA	1,500	Fast-growing
	PUFA Extracts	10	
Special Products	Toxins	1-3	
	Isotopes	>5	

Table 1. Global market estimates for microalgal products, 2004. Data from Pulz & Gross (Applied Microbiology and Biotechnology vol 65, pp 635-648).

Dried microalgae biomass (esp Arthrospira) is also widely used as an ingredient in formulated feeds for aquaculture species and terrestrial animals (farmed livestock, poultry, pets), where it has been demonstrated to have health promoting effects.

Pigments, antioxidants. Microalgae produce a range of valuable compounds including carbohydrates, proteins, essential amino acids, pigments and vitamins, as well as bioactive molecules. The major pigments include chlorophyll a, b and c, ß-carotene, phycocyanin, xanthophylls (astaxanthin, canthaxanthin, lutein) and phycoerythrin. These pigments have existing applications in food, feeds, pharmaceuticals and cosmetics, and there is an increasing demand for their use as natural colours in textiles and as printing dyes. The value of these pigments lies not only in their colorant properties, but also as antioxidants with demonstrated health benefits.

The worldwide market value for all commercially-used carotenoids was estimated at $887 million in 2004 and is expected to rise at an average annual growth rate (AAGR) of 2.9% to just over $1 billion by the end of the decade. Although the synthetic forms of carotenoid are less expensive than their natural counterparts, microalgal carotenoids have the advantage of supplying natural isomers in their natural ratio and are generally accepted as being superior to synthetic all-trans forms.

The largest commercial outlet of carotenoids (synthetic and natural) is in feeds, mainly because of the outstanding importance of astaxanthin and canthaxanthin, eg for colouring the flesh of farmed salmon. Increasing demand for organically farmed fish has expanded the market for microalgae-derived astazanthin. The big carotenoid marketing success in recent years has been lutein, when it was demonstrated that it can help reduce age-related macular degeneration. This pushed lutein's market value up to $139 million in 2004.

Functional foods/Nutraceuticals. The documented bioactive properties of microalgae have led to a well developed market for dried biomass as a human nutritional supplement, sold in different forms such as capsules, tablets and liquids. The most important microalgae species for this purpose are Dunaliella salina, Arthrospira sp, Chlorella sp and Aphanizomenon flos-aquae. These are mainly produced in outdoor ponds or shallow raceways, but also in closed photobioreactors at more northerly latitudes including Europe. Certain cyanobacteria, for example Arthrospira platensis and A. maxina (formerly Spirulina) are also marketed as whole food, being particularly protein-rich (up to 77% dry mass) and containing all essential amino acids, a number of important essential fatty acids (EFAs) and vitamins of the B, C, D and E groups.

This microalgae market segment is expected to grow in line with that of the wider nutraceuticals sector, which had a total global value of approximately US$ 80 billion in 2008, US$8 billion of this being European. Helping to protect the sector during the economic downturn is the strong preventive health care angle of nutritional supplements and the market's sizeable component of better-off demographics, including an aging population. The sector is currently maturing beyond basic and sometimes unproven supplements to one that delivers more subtle benefits that aid absorption of nutrients, and prevent a range of conditions relating to energy metabolism, such as diabetes. Welsh HEIs and SMEs are well placed to deliver the appropriate applied science and to develop verified microalgae-based functional foods in response to this evolving marketplace.

Cosmetics and cosmeceuticals. A number of microalgae species (esp Chlorella and Arthrospira) have become established in the cosmetics market. Some cosmetics companies (eg, Louis Vitton) have even invested in their own microalgae production capacity. Microalgae extracts can mainly be found in face and skincare products, eg anti-ageing cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers. Microalgae are also represented in sun protection and hair care products.

Omega 3 oils. Omega 3s are natural oils of marine origin containing n-3 series long chain fatty acids such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). These fatty acids are referred to as being essential in nutritional terms, since they cannot be synthesised by humans and have crucial physiological functions.

Microalgae are the main source of omega 3 oils in the marine food chain, where they become accumulated especially in the tissues of oily fish such as anchovies and sardines. The major source of commercially available omega 3 oils is currently from captured marine fish (eg, cod liver oil), contributing about 85% of the market by volume. However, the supply of marine-sourced omega 3 is being threatened by adverse environmental conditions that have contributed to lower DHA levels in fish oil especially from fish species from South American waters which are the major suppliers of fish oil and also depleting global fish stocks. These adverse environmental factors coupled with depleting fish stocks is aiding the global market growth of algal based omega 3, which is currently contributing about 3% of the total omega 3 market. Microalgae-based omega 3 oils furthermore appeal to vegetarian consumers and this sub-sector of the algal oils market is growing.

It is estimated that the EU market for algal-sourced omega 3 is currently at $56m, 90% of this volume being used for infant health products. Market analysts have predicted that the overall omega 3 ingredients market is set to grow at 24.3% annually, with a projected 2014 value of $1.6 billion for marine fish and algal sources combined. In recent years, new entrants to the microalgae omega 3 oils sector have been hampered by a network of patents attributed to just a few companies, eg Martek Biosciences (US) and Lonza (EU). However, the forthcoming expiry of key patents is expected to encourage more players into this market; furthermore new methods of microalgae production have been developed more recently that are not subject to existing patents and that Welsh businesses can benefit from.

3.3 Emerging markets for microalgae

Biofuels. Biofuels are currently attractive for a variety of reasons. In order to understand the key reasons, one must understand the history, present and future prospects of the current best available technology – fossil hydrocarbon based fuels.

Fossil fuels have powered the world's industry, heated the world's homes, driven the transport networks and provided power generation for well over a century. All fossil fuels are derived from the remains of biological material (microalgae, plants, animals) buried under sediments and subjected to the pressures and temperatures within the Earth's crust over many hundreds of thousands of years. Mankind first used coal, then oil and more recently gas derived from these fossil reserves. The last twenty years has seen the emergence of two driving forces that have rung the death knell for the future of fossil fuels. The first of these forces has led to unprecedented increases in the price of fossil oil in the last 5 years, and is not, as many might think, the threat of dwindling supply (although new sources are increasingly expensive and difficult to extract).

The real driving force for oil price hikes (as eloquently described by Nick Butler, VP of Strategy at BP in 2006) is the perceived threat to security of supply from geopolitical events (c). The reality of oil reservoir reserve levels has been put on the 50 to 70 year timeline. The second driving force is the growing awareness amongst the general population and governments that increased carbon dioxide levels are responsible for increased global temperatures – global warming – with associated potential climatic and habitat change, erratic weather patterns, and increased sea levels. Despite this general awareness of environmental impacts, the level of response by the general population is rather low – the unprecedented growth in the budget airline sector shows that many still will not trade cheap and low-cost airfares for more environmentally friendly trips.

Increased exploration and production technologies, such as deep-ocean drilling, through-tube drilling, and increased environmental protection will ensure that oil reserves are unlikely to disappear. The potential for new reserve discovery is also good. Libya in particular has massive potential that has hardly been realised thus far, with proven oil reserves of some 39 Bn barrels (bbl), but with large swathes of the country unexplored and the potential for further reserves. Brazil also has large future potential with new reserves announced in 2007. The state of global oil reserves is put into perspective when it is considered that in Brazil, there are reserves of some 14Bn bbl – over half of which has been discovered in the last five years. To put this in perspective, proven North Sea oil reserves at the start of 2006 were some 14 Bn bbl. To balance this, other reserves face problems, especially along geopolitical lines. Nationalisation of the industry in Venezuela has cost many petro-chemical companies based in the US. Iraq offers further reserves, but is again beset by problems.

However, no matter what the geopolitical situation is, or the size of potential reserves, the additional pressure of global warming and the resultant changes in public opinion and legislation will inexorably drive oil companies to search for alternative fuel sources. The International Panel on Climate Change (IPCC) reports that global greenhouse gas (GHG) emissions have grown since pre-industrial times, with an increase of 70% between 1970 and 2004. The IPCC states "CO_2 is the most important anthropogenic GHG. Its annual emissions grew by about 80% between 1970 and 2004". As a result of climate change, legislation is being introduced globally to drive the production of sustainable and alternative fuels. In the United Kingdom, for example, the Renewable Transport Fuel Obligation (RTFO) mandates that by 2010, 5% of road fuels must be from sustainable and renewable sources. The European Council has agreed a further binding target of 10% biofuels by energy for 2020. The EU emissions trading scheme provides a direct incentive for conversion to more sustainable biofuels.

It should be noted that the present day state of crude oil reserves and other pressures has not adversely affected income in the oil and gas sector; in all quarters to date in 2008 both Shell and Exxon have reported record profits, Shell setting a new all time high for a UK based company of some £14Bn.

The portfolio of alternative energy is currently rather diverse, no doubt many approaches will be filtered out as the cheapest and fastest to market approach gain acceptance. Currently photovoltaics (PV), hydrogen fuels, offshore wind power, wave and tidal power, geothermal energy and biofuels all have their strengths and key exponents.

The renowned energy scientist Dan Nocera, at MIT (Massachusetts Institute of Technology, USA), pointed out at a discussion meeting that, in general, the global climate and energy problem was unusual in that there are plenty of potential long-term solutions, but few short-to-mid term solutions (d). One major disadvantage of many renewable energy sources is that they are not compatible with the current transport, power generation and heating systems, i.e. it's not just a question of changing the fuel, but also your car, your boiler, etc. This is where biofuels score highly.

Biodiesel can be (and often is) used blended with conventional diesel at low levels, up to 15%. With some vehicles up to 85% biodiesel blend can be used. Biofuel is a catch all term used to describe fuel stocks derived from biomass. There are numerous possible sources, and numerous products, which fall in this bracket. In essence biomass comes in three varieties; protein, carbohydrate (sugar) and lipids (fat), of which carbohydrates and lipids offer routes to bioethanol and biodiesel, respectively. Some nations, e.g. Brazil and Mexico rely heavily on biofuels based on corn feed crops which are broken down to complex carbohydrates (starches), and then to the simpler carbohydrates (sugars), which is then fermented to the fuel, ethanol. Lipids are made up of two components, glycerol and fatty acids. These are the closest compounds to the hydrocarbon fossil fuels we use today. The general route is to take the fatty acid–glycerol complex and esterify it to give a fatty acid methyl ester (FAME) and glycerol. The FAME can then be used as a biodiesel.

As well as these problems with the biofuel product, there are also several issues with the production of biomass for biofuel production that have been making headlines recently. The potential fuel crops are mainly terrestrial plants: palm oil, rapeseed, corn, and high yield grasses. Owing to the high value of fuels, coupled with the vast quantities needed to drive the world's transport, energy generation and heating, pressure has increased for nations to grow these fuel crops in preference to domestic food crops. Owing to the large land expanses and increased sunlight equatorial locations are preferential for growing fuel crops, especially palm oil. This has created ethical problems as it is developing nations, already struggling to feed their populace, or stewards of areas of high biodiversity, that are growing large volumes of fuel crop.

The use of microalgae offers several notable advantages over terrestrial (land-based) biofuel crop systems. Some species of microalgae contain a much higher percentage of extractable oil than other oil crops – in excess of 50% compared to 25% extracted from rapeseed, currently the primary source of biodiesel in the UK. Crop yields are high because the microalgae grow and replicate very rapidly. The system can be contained using closed photobioreactor systems, enabling clean if not sterile conditions to be kept and gases, such as flue gases, to be directly fed into the system. The initial crop can then be released into raceways (shallow canals which are continuously stirred) with large surface areas; such systems are currently used commercially for astaxanthin production (See Section 3.4. for further information on culture systems). The footprint of such systems is still small compared to other fuel crops and doesn't require the use of fertile and prime arable land, often being carried out in coastal margins.

Use of marine (seawater) species keeps the cost of freshwater replenishment low and negates the ethical arguments against removing freshwater, a scarce commodity in many areas. Owing to the closed system approach and the use of an aqueous culture, nutrients can be readily recycled, i.e. they are not 'lost' to the soil and water courses as with conventional biofuel crops. In order for the lowest possible energy demand, the microalgae can be grown with natural sunlight, where it is intense enough, e.g. in the tropics. This does, however, create a potential problem of security of supply; shifting algal biofuel production to a small number of equatorial states with suitable coastlines. It should be noted though, that there are other nations with routes to sustainable electricity generation, but not to oil reserves. In such cases it might well be possible to use the electricity generated from renewable sources, such as geothermal, hydroelectric, wave or wind power, to run energy efficient artificial lighting for algal growth to produce a chemical energy source, i.e. oil and petrochemicals for the transport and heating infrastructure.

One of the challenges for any new technology is to compete in financial terms with existing technologies. Much of the background work on microalgal biofuel production was carried out at the US National Renewable Energy Laboratory (NREL) under the auspices of the Aquatic Species Program in the 1980's and 1990's. The main conclusion of this work was that at the time of writing, 1996, it was not economically feasible to produce biodiesel from microalgae because even using the best-case scenarios of photosynthetic productivity, the price would still be twice as high as the price of a similar quantity of petroleum diesel. However, considering that the petroleum diesel price has more than doubled over the last 11 years, microalgae-based biodiesel production should now be viable.

It is against this background that in 2008 more than $300 million dollars was realized in combined investments and commitments to microalgae fuels-based public private partnerships, private companies and first stage commercial projects. Regarding commercialization activities, positive trends are emerging via public-private partnerships involving governments, universities, research labs and private companies including

DARPA, NREL, and the UK's Carbon Trust, with continued growth in investments expected beyond 2008.

CO_2 capture. Increased awareness of the adverse consequences of global warming has resulted in the imposition of a number of policies with the objective of reducing emission greenhouse gases. Consequently, a number of CO_2 mitigation strategies have been investigated, broadly classified under chemical reaction based and biological CO_2 mitigation categories. Chemical reaction based strategies are known to be expensive because they involve a 3 stage process of separation, transportation and sequestration with cost of separation and compression coming to $30 to 50 per tonne of carbon dioxide. Therefore, because of the costly nature of the strategy, the mitigation benefits may be marginal. Biological CO_2 mitigation by terrestrial agricultural plants has attracted much attention, however it has been estimated that agriculture plants contribute only 3-6 % capture of fossil fuel emissions, largely due to their slow growth rates.

The slow growth rate limitation of agricultural plants for CO_2 mitigation has led to increased interest in microalgae as CO_2 capturing agents. It is estimated that microalgae have the ability to fix CO_2 at an efficiency of between 10 to 50 times greater than that of agricultural plants, forestry and aquatic plants. Furthermore, microalgae-based CO_2 mitigation can be made more economically cost-effective via the production of value added bioproducts.

A review of fossil fuel flue gas tolerance by microalgae has shown that high levels of CO_2 are tolerated by many algal species at moderate levels of SOx and NOx (up to 150 ppm). The marine microalga Chlorococcum littorale is known to tolerate CO_2 concentration of up to 40% per unit gas volume. Other species such as Scenedesmus obliquus and Chlorella kessleri are also known to exhibit good tolerance to high CO_2 concentrations. Pilot scale projects have demonstrated that microalgae scrubbing systems can reduce CO_2 concentrations by circa 40% and NOx by circa 86% of smokestack emissions. RTD efforts have therefore been stepped up globally for this application of microalgal biotechnology to CO_2 fixation.

Effluent remediation. It is now well known that microalgae have high potential to reduce nutrient, and organic loads from wastewaters. Removal percentages of 75%, 84% and 89% for ammonia, nitrite and phosphorous respectively have been reported. A combination of wastewater treatment and algal carbon dioxide fixation provides incentives in the form of saving in water treatment chemicals and the subsequent environmental benefits. Furthermore, a pathway for removal of nitrogen, phosphorous and metal ions from wastewater is provided and the pathway provides algal biomass which can further be exploited for biofuel production or for other innovative products.

A key point is that biofuels generally all contain far more oxygen than fossil fuels. This gives them certain advantages in terms of combustion characteristics, but does also mean that they have different flow and stability properties and it is not possible to use them as a direct fuel substitute, especially in existing petrol-driven cars. Deoxygenation of ethanol or butanol-based fuels results in a gaseous product, which is not easy to distribute. Higher molecular weight fatty acids are very difficult to deoxygenate, requiring expensive catalysts, high temperatures and high pressures. The process occurs naturally, but over very long – geological – time scales during the formation of fossil fuels.

3.4 Culture technologies for microalgae

Microalgae require water, light and nutrients for growth. The importance of water as a growth medium includes the temperature and acidity (pH). Algal growth changes rapidly with both of these factors; a change in temperature by 10ºC typically halves or doubles production, while pH not only affects growth rate but changes as the organisms remove nutrients from the water (pH regulation is required in some systems, especially freshwater systems). Light in the visible spectrum (400-700nm) is required to support carbon-fixation through photosynthesis. Nutrients are typically in the form of nitrate, ammonium, urea and phosphate and may be obtained from waste waters and (as most of the commercial products from microalgae contain little if any nitrogen and phosphorous) a high level of nutrient recycling should be achievable, and is certainly desirable.

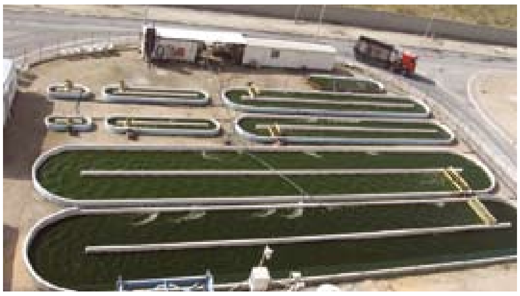


Figure 5 Example of an open raceway pond culture system (Seambiotic, Israel)

Open ponds/raceways versus closed photobioreactors. Whilst globally the most common method for commercially produced microalgae centres on the use of open, shallow ponds with paddle-like mixing devices (Figure 5), productivity in such a set up is frequently influenced by fluctuations in environmental conditions that often lead to suboptimal conditions for microalgae growth. Thus, there is an incentive to move away from open systems and develop closed reactors that not only allow for controlled monoseptic (axenic) cultures but cultures that can be easily modified to include a greater variety of microalgae species and produce higher densities of microalgae compared with open system equivalents. However, closed system photobioreactors are not without their drawbacks. Problems frequently cited with closed reactors include overheating, providing adequate controlled lighting, high oxygen tension within the culture and difficulties in harvesting the microalgae from the culture vessel. Moreover, the increased complexity of closed reactors compared to open systems presents problems with cleaning, wear and tear on mechanical parts and, perhaps more importantly, materials and parts, such as pumps, used in the construction of the closed photobiorector have to be robust enough to operate in a saline environment. Thus all of these problems have to be addressed when designing the photobioreactor.

The photobioreactor system. There are already a few purpose built photobioreactors on the market (e.g Figure 6). In general, these complete systems consist of four integrated parts: (i) the culture vessel – a light permeable, thin walled vessel that contains the microalgae culture (ii) the light delivery system consisting of, in the case of artificially illuminated reactors, banks of fluorescent tubes that provide photosynthetically usable light to the culture, or in naturally lit reactors, complex solar collection devices (iii) the gas exchange system which delivers carbon dioxide, and more importantly, removes photosynthetically generated oxygen that may inhibit metabolism or otherwise damage the culture if allowed to accumulate and (iv) the microalgae harvesting system that is involved with processing the microalgae for downstream applications. In many cases, these systems are continually monitored by a further sensory subsystem that imparts a negative feedback mechanism to keep the internal conditions stable within the culture.

Culture vessel design and problems with light delivery mechanisms There are numerous photobioreactor configurations currently described

Figure 6 Tubular photobioreactor, image courtesy of Varicon Aqua Solutions Ltd UK.

in the literature and many of these have been used successfully to culture microalgae – an indication that no a priori ideal geometry exists. Yet while a great diversity of photobioreactors have been designed, many of these can be grouped into just two categories based on the shape of the culture vessel – tubular or flat panel. The external shape is therefore fairly conserved. However, there are many aspects that have been modified, generally in an attempt to increase yield.

Perhaps the most obvious of these developments are centred on the optimisation of light penetration into the culture. Providing sufficient light to a liquid culture is difficult and this is reflected in the plethora of studies and patents trying to optimise this. For example, it is well known that light attenuation through dense cultures is sharp because of absorption and mutual shading. Thus, perhaps one of the most important aspects of a photobioreactor design should concern the optimisation of light supply to the reaction mix; since the efficiency of the reactor is often determined by this.

To overcome these problems, recent designs for photobioreactors have looked into the ways of increasing light penetration in to the culture often by reducing the optical path length. Developments have introduced small diameter culture tubes, various light types (such as fluorescent tubes and light emitting diodes; LEDs) and configurations such as laminations on the surface of the culture vessel and invaginations to reduce the surface area to volume ratio. However, despite these advances, a light gradient is frequently evident through most microalgal cultures, producing a central core of darkness surrounded by a lighter, photic zone. The problem therefore exists of how to illuminate this central zone of the culture so that a greater percentage of microalgal cells are capable of photosynthesising.

Early studies have shown that optimal lighting conditions in laboratory scale photobioreactors are easily achieved, yet scale-up projects have uncovered many problems with industrial sized reactors. Indeed, despite large theoretical and empirical advances being made in photobioreactor production, scale-up methodologies are poorly developed. Photobioreactors work very well when light is adequate, however, light decreases exponentially with the distance from the irradiated side of the reactor. This is exacerbated in large systems because many materials used in the construction of photobioreactors are not light permeable. Almost all photobioreactors are externally illuminated using either a plurality of fluorescent light tubes passing very close to the culture vessel or a single fluorescent tube spiralling around the outside of the reactor vessel. Photobioreactors can also be internally illuminated.
In these cases, designs have included in-culture fluorescent tubes, the use of light transmission rods and fibre optic technology and even the use of small reflective particles in the culture. However, there is a significant risk which needs to be addressed when running electrical components close to large volumes of water and hence there is great scope for the development of light delivering systems and light conducting materials within the reactor that deliver adequate, usable light to gain maximal yield whilst also avoiding the problems of photoinhibition, shading as well as overheating.

Mixing. While it is possible to overcome the problem of shading by using very dilute microalgal cultures, such methods, by their very nature, have significantly lower yields compared with more dense cultures. Thus one method often employed in large photobioreactors to overcome the problems of light distribution is to mix the culture using mechanical spargers. These paddle-like devices are situated in the culture vessel and agitate the,reaction mixture by rotating. Advances on this design have seen the introduction of gas spargers which implement bubble columns and air lift mechanisms – tangential inlets that create a swirling motion. Advances in mixing methods have been brought about primarily to prevent damage to fragile microalgal cells. Vigorous mixing of the culture has been shown to disrupt cell integrity through mechanical shear. Thus, there are many designs of photobioreactors that implement different types of mixing in order to overcome these problems. Efficient mixing of the culture not only keeps microalgal cells in suspension, disperses heat and creates nutrient homogeneity, but perhaps more importantly has a role in moving cells in and out of the photic zone. In dense cultures with continual mixing, cells receive intermittent light as they are moved into and out of the photic zone.

Problems with cleaning. As with all culture mechanisms, hygiene is of utmost importance, not only to maintain a monoculture but also to restrict the build up of contaminants, which can cause culture death. In order to maintain good hygiene within the reactor, there is a need for periodic cleaning of all internal parts. Therefore a significant problem exists of how to reach internal areas of the reactor, especially with larger systems. Several patents describe the use of steam cleaning for pipe work, however this requires all plastics to be steam resistant. One alternative is to autoclave smaller sections of the reactor but again, materials must be able to withstand temperatures in excess of 120ºC. Another frequently cited method of cleaning is rotary wipers inside the culture tubes. This method has many benefits, perhaps most importantly, the action of continually wiping the internal surface of the culture vessel prevents the build of dead microalgal cells and cells growing in mucus, which can further attenuate the light supply. Additionally, mixing inside the culture vessel, as described above, will also provide some degree of cleaning and has been shown to minimise the build up of biofilm on the reactor walls.

Maintaining optimal culture density. For a specific application, there is usually an optimum population density needed. While checking the population density can be performed manually, i.e. using a haemocytometer, patents exist that describe methods of continuously electronically scanning the medium by means of a photoelectric cell. The density of the culture is monitored using measurements of colour density in turbidostats. As the population density increases, pumps are operated to bring in fresh media and dilute the culture back to the optimum density and the culture flushed off during this process is harvested. From the above, it is obvious that there is significant scope for the development of an optimised photobioreactor for the production of microalgae biomass. By considering each area in turn, it will be possible to minimise the problems highlighted in the current literature.

3.5 Processing of microalgae biomass

Processing of microalgae at large-scales, with high efficiency, still presents a significant challenge to the economics of the microalgal biofuel process. Dewatering of the biomass, disruption of the microalgae cells and extraction of the oil fraction all require significant inputs of energy. Dewatering mechanisms can be described as physical (e.g. centrifugation, spray drying and filtration), biological (e.g. autoflocculation) or chemical (e.g. alum flocculant). All have their disadvantages –

centrifugation is energy expensive and requires a large capital outlay, filters are prone to clog and foul owing to the small size of microalgae cells, their physiological state and the physical properties of their exudates; autoflocculation is not a rapid process and requires the inclusion of settlement ponds for photobioreactor-based production; chemical flocculants are a large additional cost and contaminate the product, requiring separation from the biomass. Mechanisms of cell disruption and extraction include grinding, direct solvent extraction, French press, explosive decompression, freeze-drying, and supercritical fluids amongst others.

Dewatering and downstream processing of microalgae biomass represents a large component of the cost of production of microalgae biodiesel. For higher value products, the cost is not such an issue, however and increases in efficiency arising through research into microalgae biodiesel production will increase the profitability of other areas of microalgal biotechnology.

3.6 Modelling microalgae processes

Mathematical approaches (computer models, simulations) have much to offer the development and exploitation of microalgae for commercial gain. The most obvious features of interest are the immense saving in time and resources that may be achieved by using such an approach. The catch, as ever, is the need for the model to simulate reality with sufficient fidelity.

Modelling techniques are of potential importance in the following areas:

1. Optimisation of microalgal growth and production of specific end products
2. Optimisation of bioreactor design and operation
3. Production
4. Coupled operation and financial modelling and risk analysis

Optimisation of microalgal growth and production of specific end products. At the heart of any attempt to commercially exploit microalgae is the need to identify the optimal combination of microalgal strain and growth conditions. The permutations are enormous and the potential for models to help in at least identifying likely contenders is clear.

Traditional models of microalgal growth have been developed for very small-scale, 1 litre flask type, systems. There is a long and rich history of such models, and they can readily provide a simple basis for bioreactor models. That aside, traditional modelling methods may not be able to fully simulate growth in bioreactors; it is a sad fact that decades of microalgal research have not provided the types of data required to fully develop or parameterise models for commercial exploitation of microalgae.

A recognised approach for enhancement of fatty acids, and potentially for the enhanced production of other products, is the manipulation of growth conditions. The control of nitrogen sources and other conditions is important and in some instances, microalgal heterotrophic potential has been shown to be of value; microalgae are useful within some fermentation systems because, being photosynthetic, they have biochemical pathways not present in other organisms. Our understanding, and hence our ability to model the commercial viability of such approaches is weak.

There are other areas of microalgal growth and production that have attracted some modelling. These include algal-algal (allelopathic) interactions, which may be important in open-air ponds. Allelopathic interactions are only of real consequence at high cell densities and their role in nature is unclear. In commercial systems, however, both these interactions and grazing can generate important changes in biomass structure, in some instances even being counter intuitive.

The use of genetically modified (GM) microalgae was suggested and explored over a decade ago. Indeed, it is considered that the use of GM microalgae, like the deployment of GM higher plants, may be essential in order to boost production levels. Modification of the photosystems to enhance photosynthesis is an obvious target, as is enhancement of lipid (fatty acid) production. Hydrogen (rather than biomass) production is another approach that requires some level of genetic modification and/or careful manipulation of the growth medium to redirect biochemical processes to perform a production that does not naturally occur to any significant extent. It is not clear what the economic viability is for this approach.

This is an arena ripe for theoretical investigations using modelling approaches, not only to consider benefits, but also risks, but there do not appear to be any published. Some of these interactions would no doubt be of value to biologists as well; workers have expressed concern about the potential susceptibility of GM microalgae to photodamage, while a typical bioreactor simulation would suggest that at the biomass levels required for commercial production, light limitation (rather than saturation at damaging levels) is more likely.

Optimisation of bioreactor design and operation. The choice of bioreactor is as critical as that of the organism, for it governs the conditions under which the organisms grow. The two options are either for an enclosed system, rather akin to an experimental biologist's culture flask, or an open pond. The latter has far more in common with the natural growth of these organisms, potentially with inclusion of uncontrolled and/or uncontrollable interferences (light, temperature, contamination, grazers).

As mentioned above, traditional microalgal models were developed using data from small-scale cultures often using chemostats, which are akin to through-flow bioreactors. The modelling of production in pond systems may be likened to that in environmental management and there is a large literature on the modelling of microalgal growth in lakes and reservoirs. However, this is invariably directed towards minimising growth of natural microalgal populations in low nutrient systems, and understanding processes that are important to model in simulations of that growth rather than maximising growth of specific species in (very) high nutrient systems. The challenges are rather different. In theory the latter should be much easier, but the drivers (both modelling, and logistic/financial) are very different. At the interface is growth of microalgae in sewage ponds, for which modelling has been shown to have value. Modelling applications should be possible towards similar waste effluent treatment systems involving microalgae.

Models for bioreactor-type applications that are in the literature are typically deterministic ordinary differential equation structures, although an alternative approach involves fuzzy logic type modelling. In a reactor, deterministic approaches should be quite sufficient, provided that enough is known of the system. Fuzzy logic methods are more appropriate when using poorly understood systems, perhaps for microalgae with a complex life history.

At the other end of the computational scale, coupled fluid dynamics and biological modelling offers additional potential for optimisation of bioreactor design. The main parameters of importance here are dilution rates, optical path length, nutrient and light supply. At a higher level, modelling allows a consideration of the detailed physical design of the reactor. Other concepts, such a vertical sheet reactors also provide an opportunity for modelling. However, modelling studies of such systems typically employ sophisticated physics descriptions with arguably over-simplified descriptions of the biology. Such explorations have as long a history as do models of microalgae. Biological systems acclimate to changing conditions and models of microalgae growing bioreactors, especially in reactors with changing light and/or nutrient regimes, should be able to simulate physiological changes.

Modelling in the arena of bioreactor design offers many opportunities of value for commercial optimisation. For example, models have been calibrated against data for the growth of microalgae in bioreactors under different light-dark regimes, different levels of mixing and reactor design. Other workers have used models to explore the placement of lights within bioreactors. Investigations are required into the growth of different strains of microalgae under contrasting conditions to assist in model parameterisation. Likewise, growth optimisation work can be undertaken in the space of a few hours using models rather than experimental method. Scale-up of bioreactor performance is a critical issue for commercial viability and modelling offers the only realistic way of exploring very expensive alternatives short of actually building and testing them. Model formulation also acts to assist in the proper design of experiments; it is very much a two way street but traditional academic research projects have failed to take a holistic approach.

Plant operation. The other application of models to microalgal growth is in the area of systems control. It is important, in this context, to appreciate that bioreactors for photosynthetic organisms (photobioreactors) are not so simple to control as are traditional fermentors; the self-induced light limitation that is generated in a photobioreactor, coupled with the importance of a regulated gas flow, requires a complex series of control measures. So-called intelligent modelling systems have been deployed to control production in bioreactors. Models have also been applied to post-harvesting subjects, such as drying of the biomass.

Coupled operation and financial modelling and risk analysis. This is the ideal operations route for modelling, to not only aid as a guide to testing viability of the commercial exploitation of microalgae, but to aid in the operation of the enterprise. Into such a model daily changes in irradiance (for pond systems) and even commodity prices could be entered, facilitating the optimal regime for algal growth, harvesting, and down-stream processing. While the goal is clear, there is no evidence that we are close to achieving it without a significant input of resources. That said, there is more than sufficient generic knowledge to construct such a model, and to test it. For sure, the risk analysis will reveal large margins for error, but as more information is added, and the model refined, these margins of error will be decreased.

4 The opportunity for microalgal biotechnology in Wales

Figure 7 Map of Wales indicating coastal strip and marginal space relative to size of country

4.1 The location

Wales has a total area of 20,779km^2 (8,022 square miles) of which over 1,200km (750 miles) constitutes coastline, and is abundant in a variety of freshwater and brackish water sources including lakes, rivers, streams and estuaries (Figure 7).

Wales has a high proportion of coastal strip and marginal space compared to land area, and the abundance of fresh and brackish water, coupled with an advanced transportation network, demonstrates good potential for Wales in the development of the microalgal biotechnology sector.

4.2 The business environment

Wales is one of the fastest-growing regions for both internationally established and emerging bioscience businesses, with an 18% growth per year (pre-recession). The advantages of the business environment in Wales have persuaded nearly 500 international companies to locate here and include: financial incentives for research and development, capital investment, and job creation; internationally low utility costs; tariff-free access to UK and EU markets; advanced transport and telecommunications infrastructure; competent and productive workforce; supportive education and training network; a business-friendly taxation system; and a strong-tack record of profitable collaboration of academic and industrial sectors. The labour market in Wales is abundant, with over 12,000 bioscience graduates each year, and 17% of the active workforce involved in manufacturing, compared to the UK average of 14%. Wales has one of the highest staff retention rates observed in the EU, with only 3% annual turnover in some sectors, and the highest staff retention rates in the UK by a considerable amount (e).

4.3 Microalgal biotechnology expertise in HEIs and FEIs in Wales

Despite the bioscience opportunities available in Wales, the FMP-MBG Report 2005 recognised an imbalance in the UK regions with regards to marine biotechnology, with 50% of Higher Education Institutes (HEI) based in Scotland, 50% based in England, and no HEIs identified in Wales.

The Inter-Agency Committee on Marine Science and Technology recognized three sites which were noted to be involved in marine biotechnology research; Heriot-Watt (Edinburgh), Newcastle and Bangor. There is already some activity in Wales for microalgae research for mariculture, biofuels and carbon dioxide mitigation, particularly in academia. For example, CSAR (Swansea) have interests in microalgae aquaculture from the perspective of mariculture. Swansea University also has a long track record in microalgal physiology and adds to that a well developed modelling capacity; the application of mathematical modelling to microalgal biotechnology being required to optimise design and operation of bioreactors. Also, the Centre for Applied Marine Sciences, Bangor University, have managed and attracted funding for industry and EPSRC-CASE projects in this area. The production and extraction technologies used for such applications are also transferable to marine natural products in a wider sense (for which Greenwell commissioned a consultants report in 2007). At Aberystwyth University, there is existing expertise in identifying and exploiting biotechnology from bryozoans, another marine organism. In terms of microalgal biotechnology, there currently appears to be little commercial activity in Wales, despite the potential and opportunity.

There are several academic centres in Wales that can contribute to the modelling of microalgal growth and exploitation. Swansea University has an international reputation for fluid dynamics modelling (computational engineering), and also for design and use of mechanistic models of microalgal physiology (Flynn 2001) and indeed of other planktonic interactions (as may occur in pond-type systems). Bangor University, with its oceanography base, has expertise in modelling primary (microalgal) production in large-scale turbulent systems affecting by climatic variation. The universities also have a long track record in process engineering for the costing of engineering plant. Collectively there is a good core of academic knowledge from which to construct a simulation platform to progress the subject in Wales

Not directly associated with microalgal biotechnology, but very relevant to the sector, expertise exists in several related areas. Glyndwr University in Wrexham is the home of the Centre for Water Soluble Polymers. This centre has been investigating the use of agar and caragennan gels, from macroalgae, as gelling agents in food stuffs. At Bangor University, the School of Ocean Sciences has interests in microalgae technologies and the Biocomposites Centre are world leading in the field of natural product based materials. Swansea University has considerable expertise in microalgae ecosystem and physiology modelling (Department of Pure and Applied Ecology), controlled and intensive culture of marine organisms (CSAR), analysis and structural characterisation of bioactive compounds (Biological Analysis Mass Spectrometry), biological processing and modelling of complex fluids (Multidisciplinary Nanotechnology Centre).

4.4 Specific opportunities for business engagement in Wales

Microalgal biotechnology is a rapidly growing market sector, for which there are significant immediate opportunities for business engagement in Wales, aided by current industry profiles and good access to water and infrastructure along the coastal strip.

CO_2 capture. Fossil fuel (coal, oil, gas) power generators and heavy industries (eg, steel production) are large producers of CO_2 in Wales that could benefit from adopting microalgae biotechnologies for carbon capture purposes. There is furthermore interest and investment in using biomass (eg, wood) as a renewable energy source for power generation in Wales. A net carbon negative process could be generated through using microalgae to capture CO_2 from biomass-fired power stations.

Regardlesss of CO_2 source, the microalgal biomass produced could either be dried and combusted, through co-firing with other feeds, or sold. As an added advantage, power stations and heavy industry have access to sea water for cooling; waste heat to dispose of that could be used to keep microalgae cultures at maximum growth rates; around the clock lighting to allow for 24 hour growth; excellent transport infrastructure to allow for removal of the biomass if desired; large land areas and building surface areas (roofs, walls) to install photobioreactors on.

Biofuel production. There are a number of small-scale refiners of biofuels in Wales. In general, these operate on the basis of using waste vegetable oils, whether from the fast-food industry, domestic waste or oil crops. The vegetable oils are hydrolysed and converted to fatty acid methyl ester fuels (biodiesel), which can then be blended with fossil fuels. Such companies have to ensure continuous supplies of high quality oils for conversion, which is frequently a problem, owing to the wide variety of sources that the waste oil comes from. Additionally, there is an environmental cost associated with the logistics of collecting waste oil from widely dispersed small volume producers (e.g. restaurants, take-away meal outlets, etc). By teaming up with large-scale producers of microalgae biomass (such as power generation companies, etc. outlined above) a continuous source of high-grade vegetable-type oil could be ensured.

At first analysis it seems unlikely that Wales will be a suitable location for very large scale biofuel production as a wholesale alternative to fossil fuels (as is being developed in the USA and elsewhere), mainly because of suboptimal daylight levels and ambient temperatures, and restricted land area. However, mass microalgae production could potentially be integrated into wind and water-based renewable energy production, where electricity is generated in a variable and hard to store form, frequently at long distances from the large population centres where it will be used. A potential future solution may be to efficiently convert this transient electrical energy to chemical energy through supplemental lighting of large-scale microalgae bioreactors from which oils may then be harvested.

Waste water treatment works. Both commercial and domestic wastewater works are adaptable for microalgae cultivation owing to the availability of nutrient rich water, as an alternative or supplement to activated sludge bio-processing. Digestion of the microalgae biomass, or the residual biomass following initial processing for oil and high value products, might be used in certain cases to generate methane for further energy release.

Farming. Nutrient rich waste from agriculture, e.g. pig farming, is particularly useful as a feedstock of nutrients for large-scale microalgae culture. By supplying the waste as a nitrogen-rich feedstock for microalgae, the wastewater is remediated whilst yielding useful biomass and capturing carbon dioxide. Such waste often costs money to remove at present, although some level of treatment may be required prior to its use in support of microalgal growth.

Mariculture. Wales has a strong mariculture industry, with land-based intensive fish, shellfish and worm farming a particular asset. At the base of the food chain of all these technologies is a need for microalgae, either as a primary or secondary food source. Additionally, intensive aquaculture results in highly waste-rich waters that need remediation before disposal. Microalgae may result in a cleaner wastewater, and aid recycling of nutrients. Similar schemes using higher marine plants are already under development in Wales.

Natural products Wales has a well established SME base exploiting natural products from plants, invertebrates and microorganisms for health, wellbeing and medical applications. The Boots Centre for Innovation represents a significant recent addition to this sector in Wales involving a large company. The technologies involved in preparing natural products vary among companies in Wales, ranging from simple drying of biomass through extraction of specific bioactive compounds to sophisticated biotechnological approaches. This entire business sector stands to benefit from diversification into natural products from microalgae.

Anaerobic digesters/Bioagas production. Anaerobic digestion of municipal organic wastes for biogas production has been adopted as a strategic priority in Wales (£26 million WAG investment announced February 2009). The digestate slurry produced in this process is rich in nutrients that can be used for microalgae production and work is already underway in Wales to recycle AD wastes for this purpose.

Chemical feedstock/enzymes. Microalgal biotechnology for novel enzyme production and chemical feedstock has, thus far, been little explored. This is mainly because of the efforts required to secure a large quantity of biomass of consistent quality to yield enough quantities of trace amounts of bioactive compounds. Through generating large quantities of microalgae biomass for carbon capture and/or biodiesel production, the exploration for high value compounds, or novel enzymes, becomes feasible. Microalgae are prevalent in a diverse range of habitats, from the warmth of the tropics through to the extreme cold of Antarctica. As such, enzymes may be discovered that operate at lower temperatures, enabling low energy chemical processes.

Manufacturing. The construction of photobioreactors, raceway ponds and other technologies associated with microalgal biotechnology will require input from manufacturers of plastic/glass tubing, aluminium frames, geotechnical soil liners, pumping equipment, lighting and filtration technologies, amongst others. Processing of the biomass will also create opportunities for manufacturers of large volume filters, centrifuges, pumps, fluid handling equipment, and many other associated industries. Manufacturers of microalgal biotechnology production equipment have seen phenomenal growth of sales in this sector, some reporting an increase of 90% above their normal revenue from the mariculture sector.

5 Technology roadmap for microalgal biotechnology in Wales

Rationale. The main objectives of the microalgal biotechnology TRM are:

- To set an overall research and development strategy.
- To inform Welsh Assembly Government of research and policy priorities.
- To help reduce entry barriers to Welsh SMEs in terms of technical and commercial knowledge.
- To understand the link between fundamental science and microalgae biomass production/processing.
- To develop production and processing techniques.
- To identify the anticipated range of feasible application.

Outline. This technology roadmap is the first of its kind produced within the area of microalgal biotechnology in the UK. The structure of this TRM is based upon one developed for the Engineering and Physical Sciences Research Council-funded Hybrid-Net network. Technology Road Mapping is a powerful technique, allowing a strategy to be developed for the microalgal biotechnology sector in Wales so that it can achieve its overall objectives over the medium to long term, in terms of understanding and developing applications for microalgal products and technologies.

The technology roadmap document details the technology sector, the business opportunity, the stakeholders and customers in Wales, the UK and globally, with a strategy for Welsh business development in this sector incorporating a forum of interested parties to work synergistically. The market for microalgal biotechnology is burgeoning in the UK, through incentives including those of the Carbon Trust, the Technology Strategy Board and Bioscience for Business. In the petrochemical sector, Shell, BP, and Chevron all have active microalgal biotechnology programmes within their future fuels remit.

The UK's strengths in biotechnology research provide strong foundations on which to develop microalgal products and processing techniques for the benefit of companies in the UK. However, for the UK to develop a breadth and volume of industrial activity comparable and competitive with other leading nations, a number of key obstacles and deficiencies need to be addressed. It is for this purpose that the microalgal biotechnology TRM is available for dissemination to the relevant industrial and academic bodies. The study examines the strategic industrial, governmental and academic potential within Wales to allow it to develop a technology base in this sector and obtain outside funding. The study brings together representatives with capability in aquaculture technologies, reactor design, biomass separation and extraction, health, and biofuel natural product manufacture. Peripheral, but essential, sectors include mariculture, lubricants, waste-water clean up, and aviation (fuels). Areas of opportunity for engagement between parties are identified and working groups initiated to work together to develop the sector through coordinated and competitive academia-business interactions. The TRM helps to define a strategic direction and to identify specific research areas where further work needs to be carried out in the development of innovative products and processes, and thereby ultimately the requirements of this potentially lucrative market. It is important to add that the overall activity is seen as a dynamic, ongoing process that will continually update the road map, identify the areas of need and define strategies to address these needs.

Format of the Roadmap The TRM is made-up of a greater number of horizontal layers relating to different technology directions. The time scale of the Road Map is 1-5 years, through to 2013, although it must be appreciated that many of the timescales are approximations. In the US, the National Renewable Energy Laboratory is funding a programme of microalgae biofuel research designed to deliver within a 4 year period. If Wales does not engage in the microalgal biotechnology sector within a similar timeframe the opportunity will be missed.

Appendix 2 summarises the results from the facilitated Technology Road Mapping stakeholders meeting.

Where possible a colour-coding scheme has been used to differentiate between the different critical technologies. Linkages between critical technologies have been shown where appropriate.

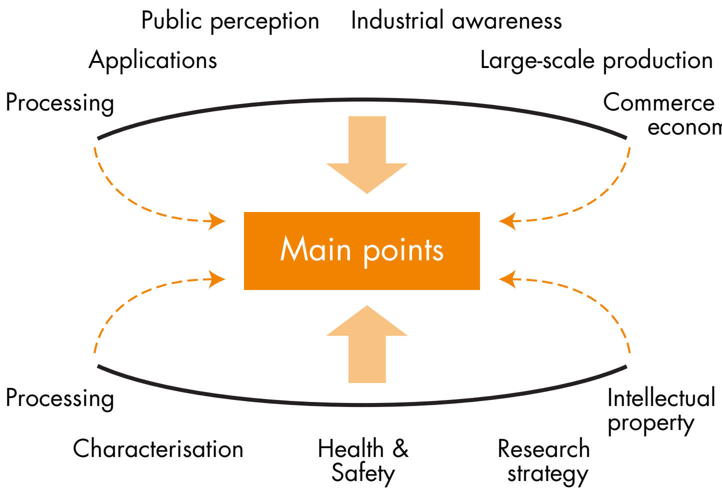
The main development tasks and milestones identified have also been included on the Road Map, where possible. In addition the TRM team scored the areas of relevance that were identified during the discussions. This was achieved by using the following scoring system:

Top priority = 3 'Points'
Intermediate priority = 2 'Points'
Low priority = 1 'Point'

Using this scoring system the TRM findings were ranked in order of relative importance. The results of this exercise are shown in Appendix 3. These were further split (nominally) into either 'Research' or 'Strategic' areas.

5.1. Technology Roadmap – summary of main points

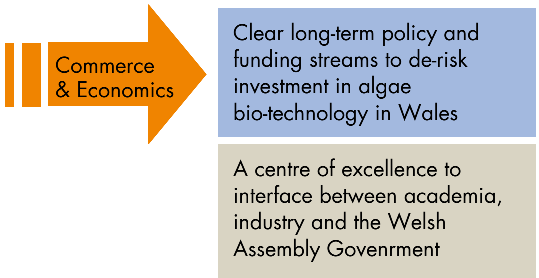
Previous research, both industrial and published, has highlighted a number of points for consideration in the TRM These have been combined with feedback from the Technology Road Mapping meeting, held in Swansea in January 2009, and are summarized in the figure above right:



5.1.1. Commerce and economics of microalgal biotechnology in Wales

For industry in Wales, particularly SMEs, there is a need to see a clear policy and funding stream from the Welsh Assembly Government (WAG) to de-risk start up and investment in microalgal biotechnology. Along with UK and EU legislation, clear drivers are needed from WAG to indicate and promote long-term markets for fuels and natural products from renewable sources. These drivers need to then be communicated to the industry, and interested parties, effectively.

An effective means to do this is through the formation of a centre of expertise within Wales to collate and disseminate all funding opportunities and policy guidelines on microalgae biotechnologies. This centre would act as a hub to communicate between academia, business and WAG and be able to provide advice across a range of disciplines from policy and financing through to core science and basic research.



5.1.2. Production

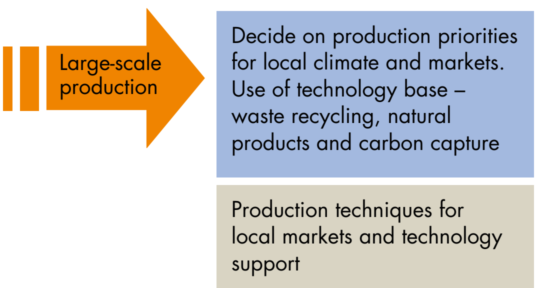
Historically, microalgae culture has been carried out in a variety of ways for mariculture and natural product production. The cost of production has been relatively high, with either the volume of end product being low (mariculture) or the value of the end products being very high (natural products). The traditional routes to microalgae production are photobioreactors for mariculture, or large-scale ponds for natural product production. There have been many attempts made to design large-scale photobioreactors for bulk microalgae production, however at the last analysis there were no truly scalable and cost efficient photobioreactors available for large-scale microalgae culture for biofuels. There has been significant interest in tailoring the biochemical composition of microalgae for mariculture and this has continued with the emergence of new microalgal biotechnology applications.

Large-scale microalgal culture. Surprisingly, there is as yet no general consensus on the optimal way to grow microalgae for biofuel applications. The relative trade off of high capital outlay, but highly productive photobioreactors versus the lower capital cost, open pond system has been discussed vigorously. For example, the UK Carbon Trust has initially ruled out using photobioreactors as the sole means of production for its Algal Biofuel Challenge funding competition. It may be that a combination of the two approaches may be the best compromise; that is photobioreactors providing cultures for growing on in ponds. Each individual case would need to be considered on its merits. Photobioreactors, for example, provide a way of growing microalgae in the unused spaces in a power station, such as the sides and roofs of buildings and can utilise some of the surplus heat generated to promote microalgae growth.

Algal culture-to-oil ratio. A key issue is actually visualising the potential challenges and the physical diminishing levels of return inherent in microalgae growth. For example, given typical figures, an ideal microalgae species may contain 20% dry weight lipids. However, an microalgae cell is some 90% water and microalgae grow in open seawater systems at 0.1-1% density. So, for every kilogram of microalgae culture, a harvest might yield 10g of wet microalgae, giving 1g dry weight microalgae. This gives 0.2g of lipids. However, only a fraction of that 0.2g will be useful fatty acid in the correct molecular weight range for direct conversion. If 20% is useful, that gives 0.04g of useful hydrocarbon per kilogram of sea water, i.e. one tonne of seawater yields just 40g of oil. Clearly, cell density and oil content need to maximised, as does the energy efficiency of any dewatering process.

Quality of water supply and control of nutrients/waste. Microalgae are available in either salt or freshwater varieties, however for algal biofuels to be a large-scale process, seawater is preferable to fresh water as a cost effective growth medium owing to the challenge of finding a large enough supply of fresh water. Conversely, cultivation of freshwater microalgae species may be preferable for remediating waste nutrients from livestock farming, etc. It is immensely difficult to carry out a research and development programme in algal biofuels, and associated technologies, based on large-scale culture in unmonitored natural (as opposed to synthetic) seawater owing to difficulties in controlling/measuring the composition and presence of any marine microbiological entities. Subtle differences in water quality can have large effects on the viability, cell density and biochemical composition of marine microalgae. Extremely rigorous filtration and water quality control and monitoring is necessary to obtain consistent data on microalgae composition. A further challenge is that microalgae require a growth medium – a blend of nutrients and minerals. Any large-scale culture of microalgae needs to optimise the cost of obtaining these nutrients. One route would be to use the waste stream from, for example, mariculture. For optimal cost-effectiveness, recovery and re-use of water containing nutrients will be essential, as will the recovery and re-use of nutrients from the cell debris after cell rupture and extraction of any lipid fraction. An understanding of the effect of filtration and chemical treatments upon water nutrient levels will also be required – any microalgae culture, even those grown in large ponds, will start off as an aseptic culture.

Summary, production. It is clear that there is some way to go in arriving at cost effective large-scale microalgae growing techniques. A favoured approach at present is to employ a combination of photobioreactors feeding into large-scale raceways. The cost-effectiveness of large-scale algal growth is inextricably linked to the processing of the biomass – realisation of maximum value from the biomass; purpose of microalgae growth programme, e.g. carbon dioxide capture, fuels or high value compounds. Within Wales, the climate (temperature and light) restricts open air growth of many microalgae species, whether in photobioreactors or in ponds. Harnessing waste heat and lighting energy from power generation or heavy industry is one suitable approach or utilising renewable electricity (hydroelectric, wind, wave or tide) for conversion to high value, and stored, natural products/fuels is another.



5.1.3. Processing

At the present time there is a misunderstanding and under-estimation of the key challenges involved. In the past the work has been, and to a large extent still is being, carried out by microalgae biologists (phycologists). The challenges do not, in fact, come from the biology. The key challenges, with one notable exception, are engineering challenges. These key challenges have been identified and include: algal culture-to-oil ratio, quality of water supply, control of nutrient/waste, dewatering of the biomass, cell-rupture at large-scale, oil extraction, identification and separation of value added compounds and the upgrading or re-use of residual cell biomass. There is a lack of understanding of how these different challenges affect the processing methods of different microalgal species.

Dewatering of the biomass As mentioned above, microalgae in open systems are typically grown at between 0.1% and 1.0% cell density in seawater. This means that for every 1g of wet microalgae cell mass, 99g – 999g of water has to be removed. The water will contain excess nutrients and other dissolved organic carbon produced by the microalgae and recycling will be key to any cost effective microalgae growth project. There are three primary ways in which microalgae may be dewatered – biological, chemical and physical. Biological methods are based on the tendency of some microalgae to auto-flocculate (clump together and sink), particularly at high pH. Such an approach is used in raceway culture of microalgae for astaxanthin production in Hawaii. Chemical methods work well and are often used in paper mills, leisure lakes and sewage works where biological molecules need to be flocculated and removed from a large volume of water. The principle method uses alum (aluminium sulphate), which is a waste product from aluminium plants and also found naturally. The alum is mixed into the water column so that the microalgae flocculates to the bottom for removal through siphoning. This method is efficient but it involves extra cost through the use of alum and also presents a problem for subsequent processing of the cells as there is a higher than normal aluminium content. Physical methods include centrifugation (used by microalgae suppliers to the mariculture industry), bubble flotation and filtration. It is likely that some method of high volume filtration would be optimal, if the energy requirements can be minimised.

Cell rupture at large-scale. In most cases, algal cells must be broken, or lysed, to extract the oil. Methods for cell rupture include osmotic shock, explosive decompression, French press, freeze drying, mechanical press, mechanical shear and biological. Currently, certain manufacturers of biofuel reactors suggest biomass drying followed by mechanical pressing as the most efficient. Other manufacturers, notably Shell, have invested heavily in biotech companies suggesting biological (enzyme) methods may offer significant advantages in the longer term. Interestingly, some microalgae degrade through the shearing action of the pumps used in bioreactors, so mechanical shear may also be an option. Small-scale biological analysis of microalgae is usually carried out using the French press method to rupture the cells.

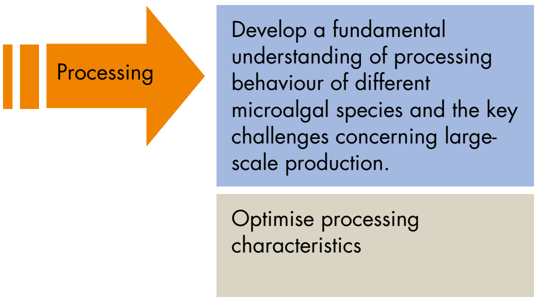
Oil extraction. Once the cell is ruptured, the lipid fraction, consisting of fatty acids and glycerol, needs to be separated from the remaining cell contents. There are a number of ways by which this might be achieved, the most common being direct extraction into a two-phase solvent system. This method is limited in that extraction of free fatty acids or highly polar acids is not always achieved and furthermore, a lot of non-polar compounds such as chlorophyll may also be extracted. Another method is reverse phase extraction, where the fatty acid and glycerol are hydrolysed and the fatty acid then extracted back. Finally, a third option is to convert the hydrolysed and free fatty acids into fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE). An advantage of this method is that FAMEs may be used as a direct oxygenated biofuel. A challenge with the solvent extraction of lipids from the biomass stream is that it is not a clean process. The interface between the aqueous and the solvent is often not clear-cut and an emulsion tends to form because of the variety of biological molecules present. The use of supercritical fluids, such as carbon dioxide, combines the cell rupture and extraction processes. This is a very environmentally acceptable approach, as no organic solvent is used. Scale up of the process may present challenges, however, despite precedents set by the coffee industry who have scaled up the process for extracting caffeine during preparation of decaffeinated coffee. A further one step cell breakage and extraction process is to dry the microalgae out and then press it, much like olive oil is produced. Drying of microalgae is, however, energy intensive.

Identification of value adding compounds and separation. Microalgae have been grown previously for a number of high value extracts. A paradox exists when considering the large-scale culture of microalgae. The high value pigments and polyunsaturated fatty acids have their value based within their rarity and cost of production. Microalgae culture on the scale needed for biofuel production is likely to devalue these products owing to the cost of production having to be decreased to a level whereby the cost of oil is competitive with fossil reserve oil. Pigments, such as astaxanthin, might sell at $3000 per kg. Clearly oil must sell at $1.25 per kg, several orders of magnitude cheaper, even to be on par with the recent $100+ per barrel record prices. That is not to demote the economic feasibility of producing high value compounds (eg, n-3 PUFAs) from microalgae, but rather illustrates the challenge of mass producing algal biofuels at a price competitive with existing fossil fuel sources.

Storage of biomass and products. There is little understanding at present about the stability of products derived from microalgae products.

Stability and storage options need to be investigated alongside the processing methods for microalgal biotechnology.

Summary, processing. There is, therefore, a very real need to establish the processing methodology for a range of microalgal species, technology and their associated end-products. The ultimate aim of any research work would be to develop understanding of processing behaviour and to optimise processing technologies to allow the 'reproducible' production of microalgal products and value added compounds (at comparable throughputs). Processing capabilities for microalgal products should run parallel to product development and form part of a wholly integrated approach for microalgal biotechnology.



5.1.4. Development of Applications

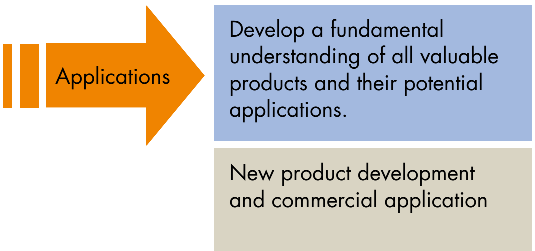
There is a need to develop a greater understanding of the potential uses and application of microalgal products, to determine their use as biofuels and value added products. Currently the most important classes of microalgal compounds determined by the literature are:

- Amino acids
- Pigments
- Polysaccharides
- PUFAs
- Lipids & Glycolipids
- Polyols
- Vitamins
- Minerals
- Metabolites

These compounds, and their product derivatives have been developed for a number of specific applications, the most notable of which are:

- Biofuels
- Food additives and nutritional supplements
- Cosmetics
- Pharmaceuticals
- Aquaculture & Animal Feeds

Summary, development of applications There is a need to understand the multi-functionality of all valuable products of interest to a variety of end users and to develop and expand upon current knowledge and application. The development of the microalgal product market is crucial in providing extra revenue in microalgal biotechnology, which in turn is of substantial commercial and technical benefit to the Welsh and wider UK biofuel industry. Another key application, which also ties into biomass production is carbon dioxide capture from power generation and industry.



5.1.5. Public perception

There has been a great deal of negative 'hype' surrounding biofuel technologies. However, 2nd and 3rd generation biofuels, derived from micro-algae, are a sustainable technology. Furthermore, algal biofuels are ethical, with no long term harmful waste (unlike the nuclear energy industry), no competition for arable land or associated ethical and social considerations (terrestrial crops have been responsible for deforestation, biodiversity loss, increased cost of food crop-based commodities as well as being linked to loss of indigenous peoples land rights, human rights abuse and the destruction of the natural resources of local communities). Algal biofuels utilise high-yield, high-cost feedstocks (more than 30 times the energy per acre than terrestrial crops) with a much higher percentage of extractable oil than other oil crops (in excess of 50% in some species, compared to 25% in rapeseed). The algal growth cycle can actually be used as a carbon sequestration mechanism because carbon dioxide is the primary input required for growth and photosynthesis. Growing microalgae is also very water efficient because of culture densities and could require only 0.4% of the water required to grow corn in the USA. Microalgae can be grown in brackish, saline and wastewater, further reducing the amount of freshwater required for growth, and the arable land required, if required at all. Within the scientific community itself the term 'biofuels' is seen very positively and substantial research monies are available for further developments. Indeed there is some frustration at the slow uptake of these technologies and the fact that they have not, as yet, achieved their promise.

In addition, there is a lot of negative publicity surrounding genetic modification (GM) of food and crops, including disputes over safety and environmental and ecological impacts within the scientific community, and negative public perceptions.

In 2004, the global area of GM crops increased to more than 47 times the area covered in 1996; the increasing use of GM 'biotech' crops in both industrial and developing countries reflects considerable progress in this area in terms of productivity, economics, the environment, and health and social benefits (f). Though the UK has approved the use of a small number of foods and animal feeds (including GM soya and some forms of maize), no GM crop has all the approvals required for commercial cultivation in the UK (g). An official report on the results of 600 meetings held in 2003 found only 2% of Britons were found to be happy to eat GM products and over half were against GM foods being available to the public (h). Environmental impacts are likely to be limited with most microalgal strains grown in controlled lab or reactor conditions, coupled with strictly controlled directives and policy. However, the use of GM algal end-products would still be subject to criticism. Whilst there is no scientific evidence for approval of all GM applications, GM has the potential to be of significant contribution to the microalgal biotechnology sector (both in terms of production and sustainability of resource) should a positive image and assured safety of GM be developed amongst the scientific and non-scientific community.

5.1.6. Skills

Within the Welsh academic and industrial communities there is a distinct skills shortage in a number of areas including microalgal biotechnology, bioengineering and bioprocessing, amongst others. To be competitive with UK and international industry and academia, Wales needs investment in industry led training programmes/academic courses in these areas.

Perception	Negative general perception Biofuels and GM
Skills	Skills shortage in microalgal biotechnology academic/ industrial realms.

5.1.7. Industry awareness and understanding

Although businesses are becoming more aware of the concept of microalgal biotechnology, there is still a lack of understanding and awareness of the potential uses of these materials. This is due to a number of reasons, most notably, the fact that many of the recent developments by industrial companies are subject to confidentiality and knowledge is not available to the industrial sector on a wider level. Also there is limited information on the cultivation, production and extraction methods of microalgae in terms of increased product yields and the upscaling of technologies for commercial application. A further problem for exploiting microalgal biotechnology is identifying the full range of products available from microalgae biomass and then linking them to existing users and markets. Technology demonstrations will be a valuable way to engage industry with microalgal biotechnology.

Industrial awareness

Lack of industrial awareness and openness of technologies/ markets will hinder microalgal product exploitation

5.1.8. Modelling

The primary challenge for the mass culture of microalgae is to balance the rate of algal production and the yield per unit volume of bioreactor. Computational modelling applications have been developed to incorporate a range of variables including; light penetration and irradiance conditions, negative feedback interactions, bioreactor size, surface area and attachment of organisms, nutritional regimes, dissolved phase nutrient and metabolite concentrations, salt load and solute concentrations, shear stress and the limitations of biological systems. Because of the multitude of interacting conditions, logic demands that initial studies are made using mechanistic models of microalgae. These models (unlike crude traditional models) have a biological basis for their construction and are thus more robust for 'what-if' testing scenarios. Models of microalgae are required for operation under different scenarios of biology, light, nutrition and physical (bioreactor design set) constraints in order to generate idealised optimum production envelopes. This would aid in the interpretation of different strategies for the deployment of microalgae for primary utilisation (e.g. algal products) and secondary uses (e.g. use of microalgae in CO_2 sequestration). Development and application of predictive modelling in this area would benefit development of microalgal products greatly.

Modelling

Developent and refinement of modelling software to balance the rate of algal production with bioreactor yield per unit volume.

5.1.9. Intellectual Property Rights & Technology applications:

The issue of IPR and its protection is very likely to inhibit knowledge transfer and the take-up of microalgal product technologies. A number of potential areas of growth in the use of microalgal products have been identified and include: methodologies for the consistent optimal growth of algal species with optimal lipid yield, high through-put cell rupture and biomass fractionation, identification and separation of algal lipid fractions and coupled models and related software for monitoring logging and optimising algal growth, harvesting and extraction.

IPR & Applications

IPR issues are thought to be restrictive to rapid expansion of microalgal product technologies and their applications

5.1.10. Research Strategy and Policy

The current research activities in the UK in this field are seen as being disparate and unconnected and there is a need for these activities to be pulled together and better communications between research groups in both the academic and industrial communities to be achieved. This is similarly true of research capacity in Wales and Convergence funds (especially ERDF priority 1) will be crucial in integrating RTD services for the benefit of businesses. There is also a very real need to ensure easier access to the existing processing and characterisation facilities, possibly through the foundation of a 'virtual' centre, or consortium. It is thought, however, that to have the necessary 'market reach' the formation of a dynamic hub within the supply chain, encompassing the excellent knowledge bases/facilities, manufacturers, suppliers centrally managed by a research organisation will bring increased profitability and increased knowledge and wealth to the emergent microalgal biotechnology industry.

Research strategy

Research activities are disparate. No coherent microalgal biotechnology strategy

5.1.11. Health and Safety

At present the risk in utilising microalgae in the biotechnology sector has not been thoroughly addressed, particularly concerning GM and the potential toxicity of certain species of microalgae if released into the environment. It is envisaged that legislation on toxicity testing will become increasingly important and a better understanding of the toxicological effects (and additional cost) of microalgal biomass-derived materials is required, so that practices for handling, processing, and disposal can be developed.

Health & Safety

At present there is limited toxicological and GM-related data with regard to the effect/ use of microalgae

5.1.12. Characterisation and identification of useful products

Robust techniques need to be developed that will allow the separation and full characterisation of algal biomass components. A number of potential techniques for the use of microalgal product characterisation have been identified and include: biological analyses via mass spectrometry and related bio-techniques, mass spectrometric and chromatographic methods, on-line analysis of complex biological extracts and detailed structural analysis via tandem mass spectrometric fragmentation analysis and accurate mass analysis. Analyses include post-genomic techniques and more focused analyses, can be implemented in the femtomole range (with lower detection limits possible via more focused analyses). Such methods can incorporate data dependent analyses in which vast data sets can be acquired with no prior knowledge of the biological components.

Character-isation

Validation and investigations into potential improvements of characterisation techniques

Strategic direction 6

In terms of the generation of specific strategy concerning the potential of microalgal products, the Road Mapping activities have highlighted that there are a number of medium- to long-term technological challenges that need to be met. In addition, a great deal of work needs to be carried out to assist industry to meet the challenge in the short to medium-term.

It is essential that the present barriers (technical, commercial and perception) are reduced to allow Welsh industry (especially SMEs) to benefit from the recent technological advances in the area of algal biofuels and biotechnology products. There is also a need for strong academic-industry and government-industry interactions in terms of both research and development to maximise commercial developments and uptake of microalgal products.

The specific steps recommended to achieve this general strategic objective are outlined below:

6.1. Commerce & economics in microalgal biotechnology

The recent demise of much of the biofuel industry across the UK owing to cheaper products from the US, rising feedstock costs and collapse of local markets has led to increased caution amongst investors and industry in engaging with the biofuel sector. In order to facilitate engagement, the industry needs to have clear long-term markets for the products. These can be driven and underpinned by WAG policy and strategy.

In order to allow for fully functional engagement between industry, academia and government in Wales a hub that will function as a "one-stop shop" is needed. This should be a recognised centre of excellence in academia, with a proven track record of industry liaison and government engagement. The centre should be able to offer advice and capacity in grant programmes, networking and training and act as a hub for consortia building.

Financing for start-ups and current industry is generally poorly understood and workshops and training courses in this key area would be welcomed amongst the community.

6.2. Defining applications for microalgal biotechnology

Though there is burgeoning interest globally about biofuels from microalgae, this has been surrounded by much hype. It is becoming clearer as the sector develops that the biofuel component of microalgae will only become commercially viable if the biomass is fully exploited to utilise all of the value added components. However, at present the field of natural product processing from microalgae is underdeveloped and aside from a handful of components, there is little in the way of full cost analysis on the best products to isolate, upgrade and commercialise. This is an area which needs to receive concerted support in the short term.

6.3. Public perception

A recent report suggests that a majority of the public know at least a little about biofuels thanks to exposure of the topic in the popular press. However what is 'known' is often incorrect and may not be applicable to microalgae. Less is known in the general public about biotechnology. Any negative attitudes are often a result of lack of information and also misinformation. Scientists, whether in industry, academia or government, have a responsibility to publicise their activities (e.g. in the popular press) in a way that not only emphasizes the social benefits, but is also sensitive to public perceptions.

6.4. Skills

It is important to foster the provision of short courses, for example in universities, which address the true needs of industry in terms of training engineers, scientists, technicians, policy makers etc. These must provide not only the state of the art in the technology, but also real practical training and technology demonstrations in, for example, processing methods.

6.5. Industrial awareness and understanding

Although a large amount of scientific information concerning microalgal biotechnology (and its potential applications) is available in the literature, within the wider industry there are still several knowledge gaps, and associated concerns, which have prevented (and are preventing) greater uptake of these technologies. Specifically there is a lack of hard evidence concerning:

- Processing techniques
- Algal culture-to-oil ratio
- Quality of water supply
- Control of nutrient/waste
- Dewatering of the biomass
- Cell rupture at large-scale
- Oil extraction
- Identification of value adding compounds and separation
- GM
- Recycling and sustainability
- Cost-benefits of microalgal biotechnology applications

To address these issues, it would be of great benefit to the industry to generate and disseminate reliable and independent short- and long-term natural product data reviews for a range of microalgae species. In addition, the production and dissemination of case-studies highlighting product developments (including cost-benefit analyses) would also be of benefit.

6.6. Integrated research strategy

With the exception of the recently launched Carbon Trust Algal Biofuel Challenge, present research into microalgal biotechnology within Wales, and the wider UK, is seen as disparate and unconnected. This is to the detriment of the research effort as there is no overarching strategy and coordination that prevents replication of work, allows integration of disciplines and components of the production process and encourages effective dissemination. This has lead to restricted interactions both within the research community and with the industrial sector. There is therefore a need to identify what research is being undertaken, where and by whom and to identify where specialist equipment is located. This will lead to strengthened academic-industry interactions and can be achieved through:

- A process to identify and collate areas of research within the research community (Universities, Research Associations and Industry).
- Development of a working consortium, including a unique website and data repository to allow greater academic-industry interaction and lead to greater industry-led research and applications development. This is currently underway at Swansea University.

One of the initial tasks of any project would be to identify what expertise and facilities are available within Wales, how to utilise these most effectively in a coordinated manner, and to target funding where there are gaps in the provision.

6.7. Sustainability

Microalgal biotechnology products have the potential to increase sustainability for a number of applications, such as: greater recycling potential, reducing fuel emission in the transport sector though biofuel technologies, utilisation of waste/nutrient sources and the use of microalgae in CO2 sequestration. As the effects of legislation become more and more significant in the near future, the industry must be made aware of how microalgal products and technologies can be used to address these issues. Therefore the industry should be made aware of forthcoming legislation and the research work that has been undertaken that may help to address these issues. In addition, the economic benefits of algal products (in relation to meeting legislative targets) must be independently evaluated and disseminated to industry.

Research and technology development programme 7

In addition to the strategic directions outlined above, a number of critical technologies that are considered to be of importance should be developed to allow businesses to process cost effective microalgal products and technologies for a range of applications. These critical technologies identified are outlined below:

7.1. Processing

If the manufacture of microalgal products is to be commercially viable, the processing methodology and the associated technologies MUST be optimised and reproducible. There is a very real need for development concerning large-scale production and the continuous identification of value-added products to provide a secondary income stream of benefit to biofuel development. Therefore there is a need to carry out research and development into both processing technology and identification of value adding compounds. The areas of processing research identified from the TRM were:

- Development of processing technologies that will give reproducible product distributions and yields.
- Development of in-line monitoring and control technologies.
- Improvement of the microalgae culture-to-oil ratio; cell density and oil content need to be maximised.
- Quality control of water supply and nutrient and waste sources.
- Development of filtration and chemical treatments.
- Assessment of the potential for water recovery and re-use.
- Development of dewatering processes that will maximise energy efficiency.
- Determination of most suitable method for dewatering the biomass at high volumes; biological, chemical, or physical.
- Determination of most suitable cell-rupture and oil extraction methods at large scale.
- Identification of value adding compounds and refinement of separation techniques
- Processing technologies must be developed that are cost effective.

7.2. Modelling

The complications associated with industrial-scale microalgal growth are the main inhibitory factor in the commercial application of microalgal biotechnology. By modelling a range of varied growth situations, and their interactions, the biological models can assist the interpretation of different growth strategies dependant on microalgae species, products and scale-up. Therefore methods such as those based on continuum mechanics breakdown and other modelling methods need to be developed and further refined to encompass more than one single theoretical aspect. The TRM highlighted the following areas of need:

- There is a lack of predictive modelling in microalgal production for biofuels.
- Better understanding of the growth mechanisms and requirements of different microalgal species under local conditions is needed to improve predictive modelling.
- Modelling for predictive design (short- and long-term) is very important to future microalgal biotechnology development, particularly when integrated with financial modelling and life cycle analysis of microalgae processes.

7.3. Technology in applications for microalgal biotechnology

There are a number of technology areas where microalgal products may be developed for specific applications. The most important applications identified within the TRM were:

- Biofuels
- Food Additives & nutritional supplements
- Cosmetics
- Pharmaceuticals
- Biomaterials
- Diagnostics
- Aquaculture & animal feeds
- Wastewater remediation

The development of applications depends strongly on effective interactions between industry and the research base and it is important that mechanisms are developed that allows this to occur effectively. The applications developed must also take account of cost as this will be the major factor in determining the use of microalgal products and technologies for specific applications.

7.4. Genetic modification

It seems inevitable that for maximising microalgae productivity, genetic modification (GM) will be considered. Industry will only start to implement GM microalgal biotechnology on a large-scale if they can be sure that the organisms and products are safe and that they can be handled and processed safely. There seems to be a very real need to develop a research programme that will look at aspects of microalgal products, in terms of GM and that address the following issues:

- Which microalgal species, products, and processing techniques should be evaluated.
- Risks associated with each species, and each product, and how these can be minimised.
- Appropriate uses and applications for GM strains.
- How microalgal products and released by-products may interact with the environment and people.
- Development of genetic monitoring techniques.

7.5. Sustainability

With the EU moving towards sustainability in manufacture there is an ever growing need for biofuels and other biotechnological products to be produced using relatively low energy and to enhance the potential for carbon sequestration. Microalgal biotechnology can potentially go some way to achieving these objectives. Life Cycle Analyses and Cost Benefit Analyses should be undertaken on microalgal technologies, applications and products to ascertain the true environmental costs of these materials and to establish how these can help manufacturers to achieve their future legislative obligations.

8 Recommendations

This Technology Road Map has identified definite areas of need to capitalise on microalgal biotechnology opportunities in Wales. It is now the responsibility of the Welsh Assembly Government, Welsh based industry and academia to utilise the research activity within Wales to directly address these issues.

This technology road mapping exercise has been carried out to achieve a positive purpose, most notably:

- To define the research strategy and priorities.
- To define a strategic direction that will allow entry barriers to SMEs in terms of technical, commercial and perception to be reduced.
- To inform government organisations, legislators and funding bodies for the benefit of the 'sector'.

To this end the TRM identifies the following recommendations:

The TRM has shown that within the Welsh academic community, research into microalgal biotechnology, processing and characterisation is of high quality. However, this research is not yet coordinated effectively with Welsh industry, particularly SMEs, and the technologies being developed are not being taken up by industry more widely. The reasons for the lack of industrial growth in this area are down to a number of factors including:

- Lack of knowledge of microalgal biotechnology, the products from microalgae and the technical and commercial benefits.
- Lack of information on funding opportunities and long-term policy/strategy for biofuels and biotechnology within Wales from the Welsh Assembly Government.
- Lack of access to equipment, know-how and technology that would enable industry to better understand and engage with microalgal biotechnology and develop applications.
- General skills shortage.

As a result of these findings the following generic priorities have been identified and should be acted on to maximise industry-academic interactions for the benefit of the sector:

- **Coordinated Research and Policy Strategy:** A coordinated research approach should be adopted that will bring together the disparate research activities and develop a strategic world-class, generic, industry-led research programme. The programme should lead to a better understanding, and bring about further development, of microalgal products, technologies, characterisation techniques and processing. The present research activities should be identified and collated (to establish the research being undertaken and equipment provision) and the industry needs established. A generic research programme should be developed that builds on the established strengths and addresses the established needs. This generic programme should be based on a national level and integrate well with developing policy frameworks within Wales to ensure the long-term development of the technology area.

- **Coordinated Development Strategy:** For industry to derive benefit from the present and future research activities there is a need for a working consortium, served through a dedicated centre of excellence with research and industry/government liaison specialists and with a website to communicate through. This consortium should allow for access to appropriate processing, testing and characterisation facilities as well as to the knowledge base. Any such consortium should allow the development of competitive applications developed from the research work.

- **Industry Awareness and Understanding.** The consortium should work together to raise awareness within the industry of the technical and commercial benefits of microalgal biotechnologies. This can be achieved through workshops and seminars and also through the generation of Case Studies relating to microalgal biotechnology that show both the technical and commercial benefits.

- **Skills, Training and Technology Demonstrations:**
Workshops, short training courses and technology demonstrations would enable industry and policy makers to learn more about the technology area, and carry out preliminary tests of technology and research ideas. Provision of training in working with industry and understanding financing would be useful additions to graduate level courses to provide suitable staff for Welsh based SMEs.

Generic Research Programme.

The TRM has highlighted that there is a need to develop a greater understanding of the link between the fundamental science and processing technologies. To achieve within a generic research programme, the following areas of priority have been identified:

- Large-scale microalgae growth (including utilisation of effluent nutrient sources)
- Rapid processing.
- Modelling.
- Applications identification and development.
- Environmental profile and Life Cycle Analysis.
- Long-term policy effects and markets.

In summary, it is believed that to effectively implement these recommendations then the TRM group and the appropriate persons must agree a common position that:

- Determines a plan of action from these recommendations.
- Determines who should be responsible for implementing these plans.
- Determines how the appropriate resources can be made available.
- Communicates these decisions and maintains the network through an internet based forum.

If supported by the whole group, this plan of action will be an effective tool with which to lobby the appropriate government organisations, legislators and funding bodies. This should hopefully go some way to securing funding to implement this plan of action for the sector.

9

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Name	Organisation
John Fordham	Penstar Process and Technical Services Ltd
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Jan Cliff	Sundance Renewables
Carolyn Crieg	Sundance Renewables
Robert Serwata	Dragon Feeds Ltd
Andrew Shearer	Neem Biotech Ltd
Steve Rist	Merlin Biofuels
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Harvey West	Vogen Energy
Fiona Dudden	Corus Strip Products UK
Robert Eden	Rawwater Ltd
James Killian	Recovery Solutions
Andrew Stephens	Environmental Energy Recovery Solutions
Ralph Bettany	Ralph Bettany Associates Ltd
David Williams	Welsh Assembly Government
Marcia Jones	Welsh Assembly Government
Nick Ward	Welsh Assembly Government
Richard Griffiths	Welsh Assembly Government
Lindsey Dodds	World Wildlife Fund
Brian Minty	Coleg Sirgar
Alex Marshall	Ramblers Association
Derek Tolley	AquaSol Enterprises
Chris Williams	International Business Wales
Margaret Houldsworth	Axium Process
Karin Jonsell	Swansea University
Adam Powell	Swansea University
Craig Pooley	Swansea University
Ilseon Jung	Swansea University
Geertje van Keulen	Swansea University
Yvonne Jones	Swansea University
Ed Pope	Swansea University
Heine Fabian	Swansea University
Ali Parker	Swansea University
Rachel Williams	Swansea University

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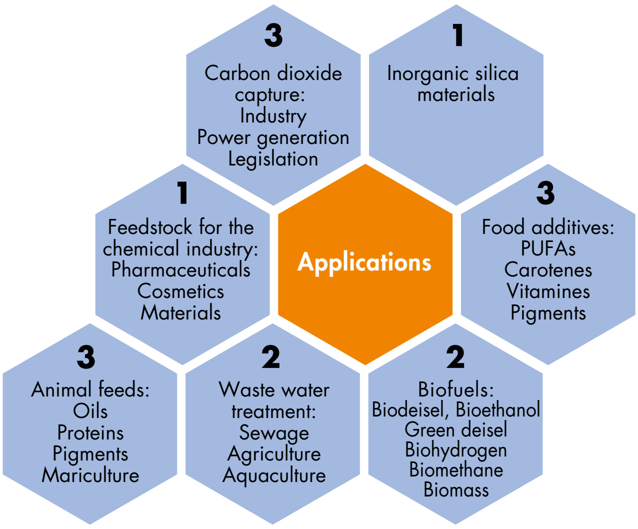
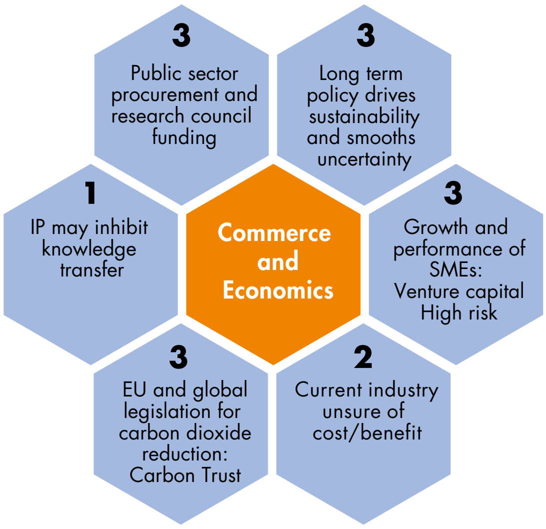
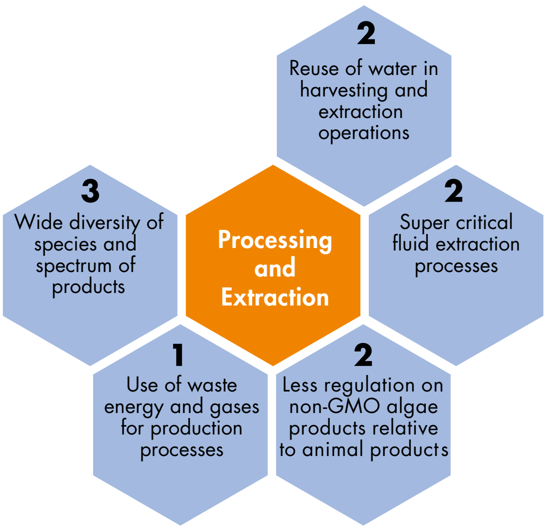
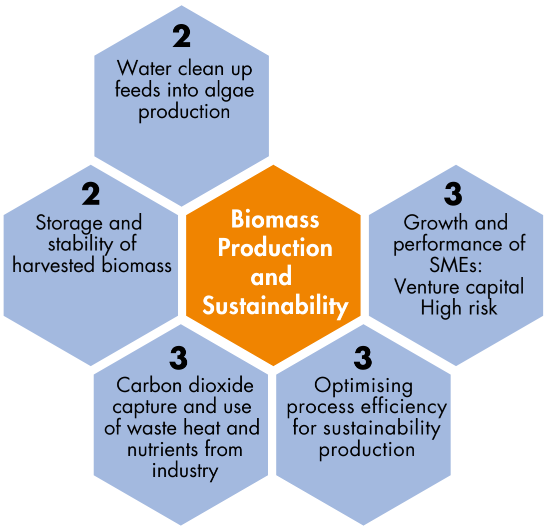
Appendix 2: TRM hexagons

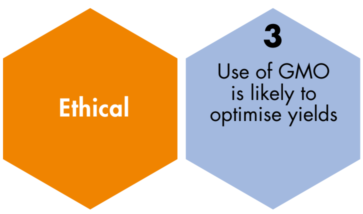
1 Of 3 : Market / Industry trends and drivers:











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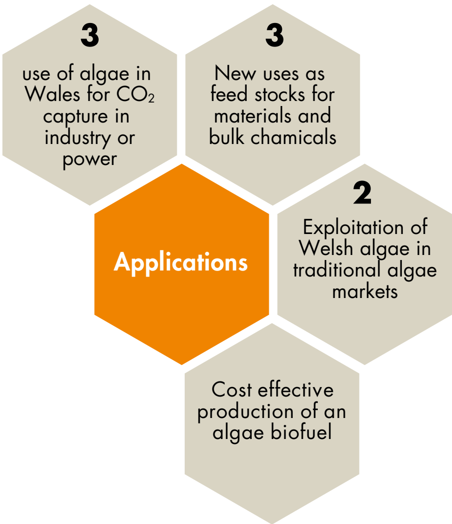
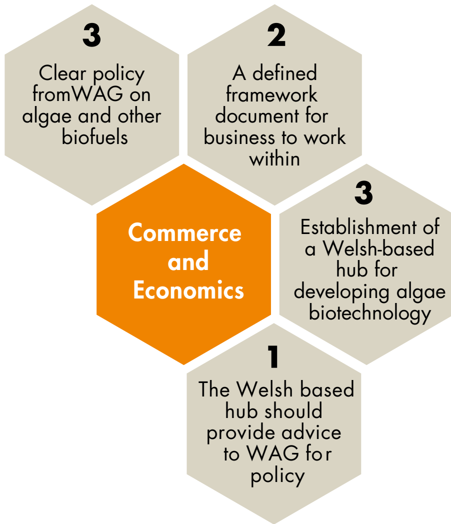
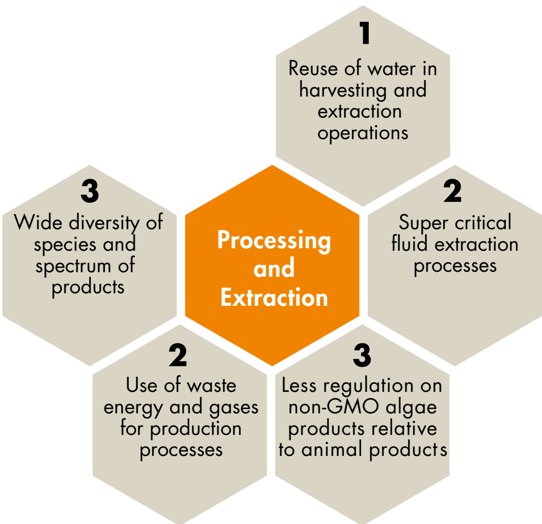
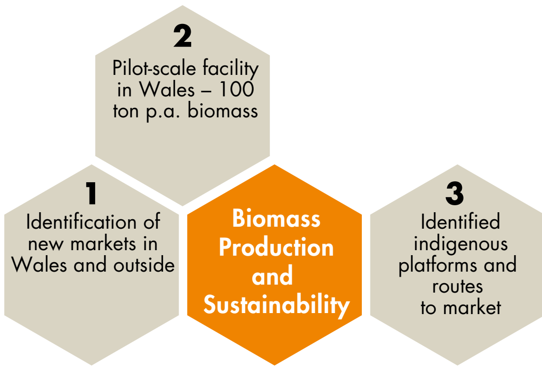
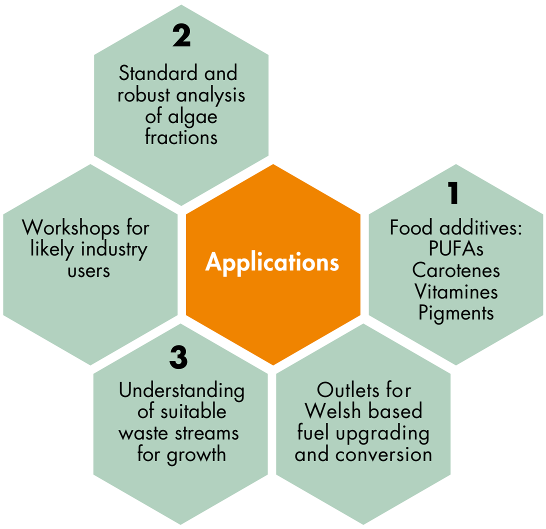


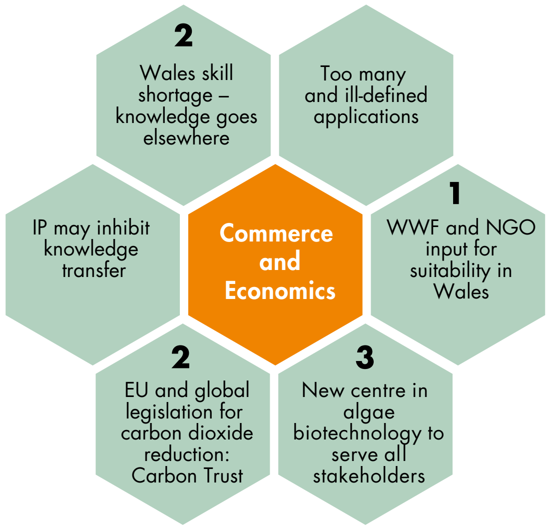


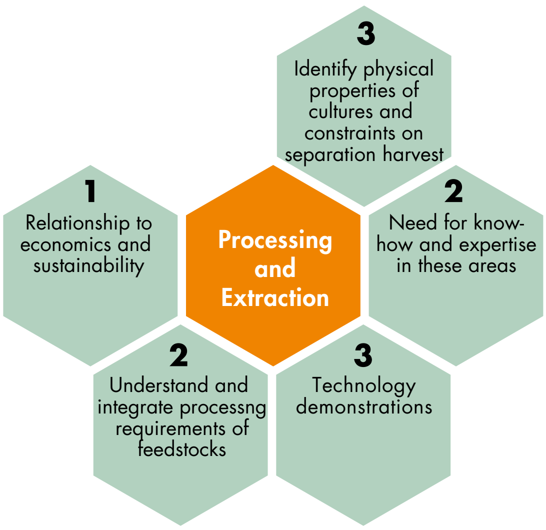


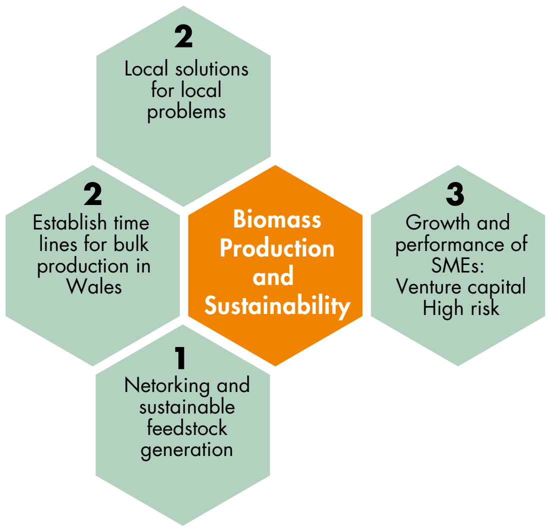


3 of 3 : Technology directions:









Appendix 3: Scoring and ranking of areas of importance

Issue	Score	Total Score	Research or Strategy
Commerce & Economics for Microalgal biotechnology:			
De-risking algae biotechnology through clear WAG policy & funding	11		
Development of microalgal biotechnology hub to bring funding routes to fore and coordinate applications	7		
UK and EU legislation on carbon capture as a driver for research	3		
Sustaining growth and performance in SMEs through funding	3	32	Strategy
Industry awareness of cost / benefit	2		
Providing relevant training to avoid future skills shortage	2		
Access to advice on financing and capital raising for start up	2		
Sustainable microalgal biotechnology for Wales	1		
Processing & Extraction for Microalgal biotechnology:			
Understand processing requirements of wide range of products and species	9		
Use of waste water and energy for processing	4		
Establish feasibility of supercritical extraction methods	4		
Integration of techniques and methods	4		
Understand degradation of products to prevent wastage	3	32	Research
The need to demonstrate technologies to end users	3		
Regulation reviewed on GMO microalgae and their products	2		
Maximise processing to maximise biomass utilisation	2		
Understanding sustainability and economics of processing	1		

Issue	Score	Total Score	Research or Strategy
Applications:			
Carbon dioxide capture	6		
Alternative feedstocks	5		
Waste water clean up	5		
Animal & mariculture feeds	3	29	Strategy
Food additives	3		
Local solutions and markets	3		
Robust analysis methods	2		
Biofuel production	2		
Biomass Production & Sustainability:			
Producing biomass at sufficient scale in Wales	7		
Establish markets in Wales (and outside)	6		
Carbon dioxide capture and use of waste streams	5	27	Research
Optimising process efficiency and sustainability	4		
Storage and stability of harvested biomass	2		
Tailoring growth to deliver required feedstocks	3		
Ethics:			
Use of GMO to optimise yields	3	3	Strategy

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