MICRO- AND MACRO-ALGAE: UTILITY FOR INDUSTRIAL APPLICATIONS

Outputs from the EPOBIO project

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1 INTRODUCTION

The purpose of EPOBIO, a Science to Support Policy Consortium funded by the European Commission, is to realise the economic potential of sustainable resources – non-food bioproducts from agricultural and forestry feedstocks. To date, EPOBIO desk studies have produced eight reports addressing a range of bioproducts and feedstocks and assessing their potential for developing biorenewables with high value and utility to society. The assessment has involved a holistic analysis of the science-based projects within a wider context of environmental impact, socio economics, regulatory frameworks and attitudes of public and policy makers. This EPOBIO process has allowed costs and benefits of each application, product and process to be defined, thereby providing a robust evidence base for strategic decisions, policies and funding.

The opportunities offered by land-based agriculture, forestry and their many applications for non-food industrial products are well recognised. Most recently, the use of lignocellulose biomass for generation of transport fuels is a much debated topic in the design of future energy production systems, again illustrating the versatility of plant raw materials for both energy and non-energy products. In this context, the potential of marine biomass is increasingly discussed, given the size of the resource in that more than three quarters of the surface of planet earth is covered by water. These aquatic resources, comprising both marine and fresh water habitats have immense biodiversity and immense potential for providing sustainable benefits to all nations of the world. Some 80% of the world's living organisms are found in aquatic ecosystems.

Thus, this final report from EPOBIO will address emerging opportunities presented by phototrophic organisms of the aquatic environment. Of net primary production of biomass, it is generally accepted that 50% is terrestrial and 50% aquatic. Policies of Governments have focussed almost exclusively on the use of land plants, with little consideration so far of the non-food applications and utility of macro- and micro-algae and their products. The limitations of agricultural land and the impacts of global climate change on agricultural productivity are factors of increasing relevance in the decisions that must be taken on land use for food, feed, chemicals and energy. Clearly, this increasing competition for land is driving the current consideration of the potential of the aquatic environment for the production of biofuels and industrial feedstocks.

The technical potential of micro-algae for greenhouse gas abatement has been recognised for many years, given their ability to use carbon dioxide and the possibility of their achieving higher productivities than land-based crops. Biofuel production from these marine resources, whether use of biomass or the potential of some species to produce high levels of oil, is now an increasing discussion topic. There are multiple claims in this sector but the use of micro-algae as an energy production system is likely to have to be combined with waste water treatment and co-production of high value products for an economic process to be achieved. These current biofuel discussions illustrate two issues. First, the potential broad utility of these organisms that are capable of multiple products, ranging from energy, chemicals and materials to applications in carbon sequestration and waste water remediation. Second, the need for a robust evidence base of factual information to validate decisions for the strategic development of algae and to counter those claims made on a solely speculative basis to support commercial investment.

The current regulatory framework under development in Europe notes that an all embracing maritime policy should aim at growth and more and better jobs, helping to develop a strong, growing, competitive and sustainable maritime economy in harmony with the marine environment. An aim is to integrate existing and future EU, regional and national policies affecting marine issues. The emphasis of the proposed framework is on use of the marine environment at a level that is sustainable where marine species and habitats are protected, human induced decline of biodiversity is prevented and diverse biological components function in balance. Whilst it is recognised that innovation may help to find solutions to issues such as energy and climate change, there is little in policy proposals that addresses the utilisation of available marine biomass.

This report will explore opportunities for energy and non-energy products, encompassing both marine and fresh water macro- and micro-algae. Salt water agriculture and the use of tidal flats will not be discussed nor will the harvesting of aquatic plants other than algae. The first chapters will briefly introduce the range of organisms and their habitats, together with the production systems that are already in development and use for their large-scale cultivation. The later chapters will summarise the diverse range of products that have arisen or could be developed in this sector, including the utility of genes, made possible by the recent completion of genome sequencing programmes and the development of post-genomic technologies.

2 HABITATS AND PRODUCTION SYSTEMS

2.1 Definition of terms

The organisms considered in this report are photosynthetic macro-algae or micro-algae growing in aquatic environments. Macro-algae or “seaweeds” are multicellular plants growing in salt or fresh water. They are often fast growing and can reach sizes of up to 60 m in length (Mc Hugh 2003). They are classified into three broad groups based on their pigmentation: i) brown seaweed (Phaeophyceae); ii) red seaweed (Rhodophyceae) and iii) green seaweed (Chlorophyceae). Seaweeds are mainly utilised for the production of food and the extraction of hydrocolloids. Micro-algae are microscopic organisms that grow in salt or fresh water. The three most important classes of micro-algae in terms of abundance are the diatoms (*Bacillariophyceae*), the green algae (*Chlorophyceae*), and the golden algae (*Chrysophyceae*). In this report the cyanobacteria (blue-green algae) (*Cyanophyceae*) are also referred to as micro-algae, this applies for example to Spirulina (*Arthrospira platensis* and *A. maxima*). Diatoms are the dominant life form in phytoplankton and probably represent the largest group of biomass producers on earth. It is estimated that more than 100,000 species exist. The cell walls of diatoms contain polymerised silica, and they often accumulate oils and chrysolaminarin. Green algae are especially abundant in fresh water. The main storage compound of these algae is starch, although oils can also be produced. The fresh water green algae *Haematococcus pluvialis* is commercially important as a source for astaxanthin, *Chlorella vulgaris* as a supplementary food product, and the halophilic algae *Dunaliella* species as a source of β-carotene. The golden algae are similar to the diatoms and produce oils and carbohydrates. The blue-green algae (cyanobacteria) are found in a variety of habitats and are often known for their toxic water polluting products.

2.2 Macro-algae

2.2.1 Habitats for red, green and brown macro-algae

Seaweeds or macro-algae belong to the lower plants, meaning that they do not have roots, stems and leaves. Instead they are composed of a thallus (leaf-like) and sometimes a stem and a foot. Some species have gas-filled structures to provide buoyancy. They are subdivided in three groups, the red, green and brown macro-algae.

In their natural environment, macro-algae grow on rocky substrates and form stable, multi-layered, perennial vegetation capturing almost all available photons. Due to the fact that seaweeds are fixed to their substrate, values for maximum productivity may be 10 times higher for a seaweed stand than for a plankton population, and can be as high as 1.8 kg C m^{-2} y^{-1}. The maximum chlorophyll content is 3 g m^{-2} illuminated surface, corresponding to an algal biomass of about 10 kg m^{-2} (Lüning and Pang 2003). The productivity of plankton is much lower because most of the photons are absorbed or scattered by abiotic particles, and the algae are so thinly distributed.

Commercial farming of seaweed has a long history, especially in Asia. The kelp *Laminaria japonica* is the most important with 4.2 Mio t cultivated mainly in China (Lüning and Pang 2003). Approximately 200 species of seaweeds are used worldwide, about 10 of which are intensively cultivated, such as the brown algae *Laminaria japonica* and *Undaria pinnatifida*, the red algae *Porphyra, Eucheuma*, *Kappaphycus* and *Gracilaria*, and the green algae *Monostroma* and *Enteromorpha* (Lüning and Pang 2003).

Several species having a range of specific requirements for their living environment appear to be especially suited for large-scale cultivation (Table 1). These requirements are nutrients, salinity, temperature, light, depth, and currents. Factors that affect cultivation also include predation, growth of epiphytes, and pollution.

An example is giant brown kelp (*Macrocystis pyrifera*) (Figure 1), which has a high light absorptive capacity, and doubles its weight every six months. Tests in the open sea (off-shore) revealed a range of difficulties which included access, mooring, nutrient supply (by upwelling), and harvesting (Chynoweth 2002).

Table 1. Suitable macroalgal species for large-scale cultivation. Marine Biomass Workshop, Newport Beach, Florida, 1990 (Chynoweth 2002)

Seaweed genus	Remarks
Alaria	Possesses floating structure, occurs in arctic waters
Corallina	Calcareous, spread widely, small, can possibly be grown together with other species
Cystoseira	Moderate climate zone, floating reproduction structures
Ecklonia	Subtropical and moderate climate zone, one floating species
Egregia	Moderate climate zone, floating structure, very robust species
Eucheumia	Already cultivated in tropical areas, relatively small size
Gracillaria	Widely occurring, often cultivated, high productivity
Laminaria	Extensively grown in moderate climate zones
Macrocystis	In semi-culture, seasonal harvest, moderate climate zone
Pterygophora	Moderate climate zone, very robust species
Sargassum	Widely occurring (including Sargasso Sea), many species, floating structures, in moderate and tropical climate zones

2.2.2 Production systems

The world production of seaweeds was some 8 Mio t in 2003 (McHugh 2003). The seaweeds are used in the production of food, feed, chemicals, cosmetics and pharmaceutical products. Seaweeds are mainly produced for these end uses in Asian countries such as China, the Philippines, North and South Korea, Japan and Indonesia. The USA, Canada and European countries such as France, Germany and the Netherlands are attempting to establish large-scale seaweed cultivation (Pérez 1997; Buck and Buchholz 2004; Reith et al. 2005).

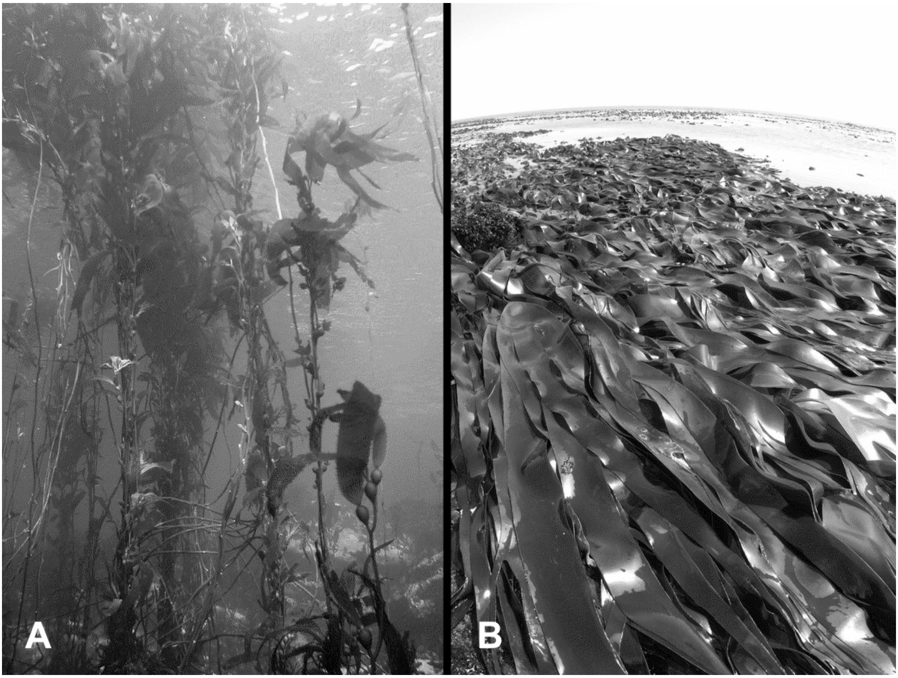

Figure 1. Some commercially exploited seaweeds. A) *Macrocystis pyrifera*; B) *Laminaria digitata* (reproduced with permission from Michael Guiry and Dirk Schories; www.algaebase.org).

In the American Biomass Program several types of large-scale cultivation systems were designed and tested for applications in the open sea (Chynoweth 2002). This concerned free-floating cultivation systems (dynamically positioned by ships), or systems anchored to the seabed or buoys. A typical problem was that anchors were lost, causing the line system to get tangled. In other tests, the structure of the line-system remained intact, but the seaweeds were flushed from the lines. This was attributed to the different dynamics of the line system and the seaweeds. Thus, it was recommended to limit movement of the lines by choosing a good geometry or putting the lines under tension. Circular ring structures (15 meter diameter) were also tested, and found to be well suited for the cultivation of *Macrocystis* (kelp).

A recent Dutch study investigated the potential of using the off-shore wind farm infrastructure (Reith et al. 2005), based on the US Marine Biomass Program (Chynoweth 2002), French (Pérez 1997), and German studies (Buck and Buchholz 2004) (Figure 2). The Dutch study discusses in detail the different available systems and studies. For example, the tests by Buck and Buchholz (2004) showed that cultivation systems were easily damaged, however, a small ring system (5 meter diameter) was best suited. The concept of using off-shore wind farms for multipurpose aquaculture seems promising, however harvesting of the algae is labour intensive and the economics of this approach need to be further investigated.

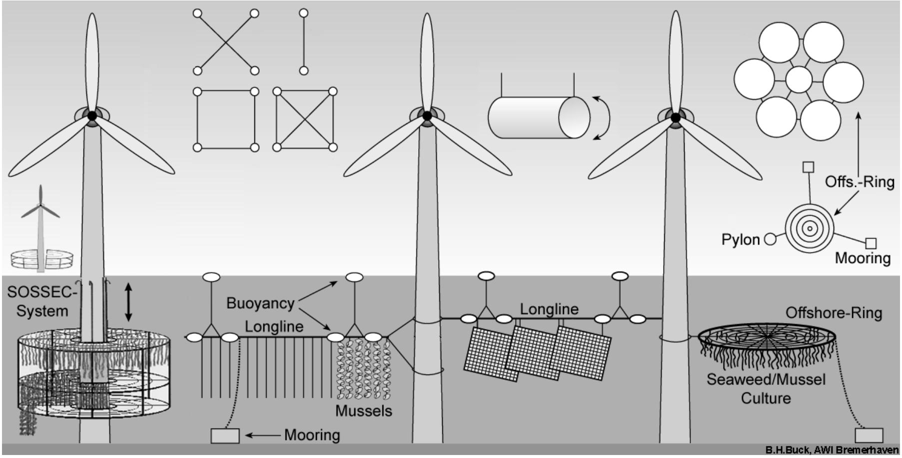

Figure 2. Multiple use of off-shore wind energy parks for the co-cultivation of seaweed and seafood (reproduced with permission from BH Buck, AWI Bremerhaven, Germany).

For floating seaweed species such as *Sargassum* it may be possible to use floating cultivation, and apply a structure that keeps the seaweed in a limited area. This would lead to significant cost-savings compared to line-based systems.

2.3 Micro-algae

Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land-based plants, but due to a simple cellular structure, and submerged in an aqueous environment where they have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass.

These organisms constitute a polyphyletic and highly diverse group of prokaryotic (two divisions) and eukaryotic (nine divisions) organisms. The classification into divisions is based on various properties such as pigmentation, chemical nature of photosynthetic storage product, the organisation of photosynthetic membranes and other morphological features. The most frequently used micro-algae are *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including the diatoms) and *Chrysophyceae* (including golden algae). Many micro-algae species are able to switch from phototrophic to heterotrophic growth. As heterotrophs, the algae rely on glucose or other utilisable carbon sources for carbon metabolism and energy. Some algae can also grow mixotrophically.

2.3.1 Applications of micro-algae

Micro-algae find uses as food and as live feed in aquaculture for production of bivalve molluscs, for juvenile stages of abalone, crustaceans and some fish species and for zooplankton used in aquaculture food chains. Therapeutic supplements from micro-algae comprise an important market in which compounds such as β-carotene, astaxanthin, polyunsaturated fatty acid (PUFA) such as DHA and EPA and polysaccharides such as β-glucan dominate (Table 2) (Pulz and Gross 2004; Spolaore et al. 2006).

Table 2. A selection of microalgal species and their products and application areas (modified from Pulz and Gross 2004).

Species/group	Product	Application areas	Prod. facilities	References
Spirulina (*Arthrospira platensis)* / Cyanobacteria)	Phycocyanin, biomass	Health food, cosmetics	Open ponds, natural lakes	Lee (2001); Costa et al. (2003)
Chlorella vulgaris / Chlorophyta	Biomass	Health food, food supplement, feed surrogates	Open ponds, basins, glass-tube PBR	Lee (2001)
Dunaliella salina / Chlorophyta	Carotenoids, *β*-carotene	Health food, food supplement, feed	Open ponds, lagoons	Jin and Melis (2003); Del Campo et al. (2007)
Haematococcus pluvialis / Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives	Open ponds, PBR	Del Campo et al. (2007)
Odontella aurita / Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food	Open ponds	Pulz and Groß (2004)
Porphyridium cruentum / Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Tubular PBR	Fuentes et al. (1999)
Isochrysis galbana / Chlorophyta	Fatty acids	Animal nutrition	Open ponds, PBR	Molina Grima et al. (1994); Pulz and Gross (2004)
Phaedactylum tricornutum / Bacillariohyta	Lipids, fatty acids	Nutrition, fuel production	Open ponds, basins, PBR	Yongmanitchai and Ward (1991); Acien-Fernandez et al. (2003)
Lyngbya majuscule / Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition		Singh et al. (2005)
Muriellopsis sp. /Chlorophyta	Carotenoids, Lutein	Health food, food supplement, feed	Open ponds, PBR	Blanco et al. (2007); Del Campo et al. (2007)

Exploitation of micro-algae for bioenergy generation (biodiesel, biomethane, biohydrogen), or combined applications for biofuels production and CO_2-mitigation, by which CO_2 is captured and sequestered, are under research (Scragg et al. 2003; Miao et al. 2004; Miao and Wu 2004; Kruse et al. 2005; Tsukahara and Sawayama 2005; Rupprecht et al. 2006, Xu et al. 2006; Chisti 2007; Hankammer et al. 2007; Huntley and Redalje 2007; Li et al. 2007; Ono and Cuello 2007).

The dominating species of micro-algae in commercial production includes *Isochrysis*, *Chaetoceros*, *Chlorella*, *Arthrospira* (*Spirulina*) and *Dunaliella* (Lee 1997). The microalgal biomass market currently has a size of about 5,000 t y^{-1} of dry matter and generates a turnover of ca. US$ 1.25 billion per year (Pulz and Gross 2004). Another study mentions a production of 10,000 t y^{-1}, almost all of it grown in open ponds, and mainly for use as nutritional supplements (van Harmelen and Oonk 2006).

2.3.2 Production systems

2.3.2.1 Photobioreactors

Photobioreactors are different types of tanks or closed systems in which algae are cultivated (Richmond 2004). Algal cultures consist of a single or several specific strains optimised for producing the desired product. Water, necessary nutrients and CO_2 are provided in a controlled way, while oxygen has to be removed. Algae receive sunlight either directly through the transparent container walls or via light fibres or tubes that channel it from sunlight collectors. A great amount of developmental work to optimise different photobioreactor systems for algae cultivation has been carried out and is reviewed in Janssen et al. (2003), Choi et al. (2003), Carvalho et al. (2006), and Hankamer et al. (2007).

It has also been suggested to grow heterotrophic algae in conventional fermentors instead of photobioreactors for production of high-value products (Jiang and Chen 1999; Wen and Chen 2003). Instead of light and photosynthesis, heterotrophic algae are relying on utilizable carbon sources in the medium for their carbon and energy generation (Ward and Singh 2005).

2.3.2.2 Open pond systems

Open pond systems are shallow ponds in which algae are cultivated. Nutrients can be provided through runoff water from nearby land areas or by channelling the water from sewage/water treatment plants. The water is typically kept in motion by paddle wheels or rotating structures, and some mixing can be accomplished by appropriately designed guides. Algal cultures can be defined (one or more selected strains), or are made up of an undefined mixture of strains. For an overview of systems used see Borowitzka (1999) and Chaumont (1993).

2.3.2.3 Comments on the different production systems

The high capital cost associated with producing micro-algae in closed culture systems is the main challenge for commercialization of such systems (Borowitzka 1999). Open systems do not require expenses associated with sterilization of axenic algal cultures. However this leads to high risk of contamination of the culture by bacteria or other unwanted microorganisms. A common strategy therefore to achieve monocultures in an open pond system is to keep them at extreme culture conditions such as high salinity, nutrition or alkalinity (Lee 2001). Consequently, this strictly limits the species of algae that can be grown in such systems. To our knowledge, from the public literature, currently only *Dunaliella* (high salinity), *Spirulina* (high alkalinity) and *Chlorella* (high nutrition) have been successfully grown in commercial open pond systems (Lee 2001).

The necessity for a large cultivation area has been pointed out as a limitation in using open ponds to grow micro-algae for mitigating the CO_2 released from power generating plants. It has been estimated that a raceway pond requires 1.5 km^2 to fix the CO_2 emitted from a 150 MW thermal power plant (Karube et al. 1992). The large area requirements are partly due to the comparable lower productivity of open pond systems. It was pointed out that improving the control of limiting parameters in open ponds such as culture medium temperature and contamination and thereby increasing productivity could be accomplished by using a transparent cover over the ponds, such as a greenhouse (Hase et al. 2000).

Selection of a suitable production system clearly depends on the purpose of the production facility. For example, closed bioreactors will not be suitable for wastewater treatment, because the costs for treating wastewater in this system will be too high in relation to the low value added during the production process. On the other hand, high quality/value products that are produced only in small amounts might require production in bioreactors. A comparison of the different production systems is presented in Table 3.

Table 3. Adapted from (Pulz 2001).

Parameter or issue	**Open ponds and raceways**	**Photobioreactors (PBR)**
Required space	High	For PBR itself low
Water loss	Very high, may also cause salt precipitation	Low
CO_2-loss	High, depending on pond depth	Low
Oxygen concentration	Usually low enough because of continuous spontaneous outgassing	Build-up in closed system requires gas exchange devices (O_2 must be removed to prevent inhibition of photosynthesis and photooxidative damage)
Temperature	Highly variable, some control possible by pond depth	Cooling often required (by spraying water on PBR or immersing tubes in cooling baths)
Shear	Low (gentle mixing)	High (fast and turbulent flows required for good mixing, pumping through gas exchange devices)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR life-time
Contamination risk	High (limiting the number of species that can be grown)	Low
Biomass quality	Variable	Reproducible
Biomass concentration	Low, between 0.1 and 0.5 g l^{-1}	High, between 2 and 8 g l^{-1}
Production flexibility	Only few species possible, difficult to switch	High, switching possible
Process control and reproducibility	Limited (flow speed, mixing, temperature only by pond depth)	Possible within certain tolerances

Table 3 continued.

Parameter or issue	**Open ponds and raceways**	**Photobioreactors (PBR)**
Weather dependence	High (light intensity, temperature, rainfall)	Medium (light intensity, cooling required)
Startup	6 – 8 weeks	2 – 4 weeks
Capital costs	High ~ US $ 100,000 per hectare	Very high ~ US $ 1,000,000 per hectare (PBR plus supporting systems)
Operating costs	Low (paddle wheel, CO_2 addition)	Very high (CO_2 addition, pH-control, oxygen removal, cooling, cleaning, maintenance)
Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial applications	5000 t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

2.3.3 Harvesting of micro-algae

Conventional processes used to harvest micro-algae include concentration through centrifugation (Heasman et al. 2000), foam fractionation (Csordas and Wang 2004), flocculation (Poelman et al. 1997; Knuckey et al. 2006), membrane filtration (Rossignol et al. 2000) and ultrasonic separation (Bosma et al. 2003).

Harvesting costs may contribute 20 – 30% to the total cost of algal biomass (Molina Grima et al. 2003). The micro-algae are typically small with a diameter of 3 – 30 μm, and the culture broths may be quite dilute at less than 0.5 g l^{-1}. Thus, large volumes must be handled. The harvesting method depends on the species, on the cell density, and often also on the culture conditions.

2.4 Regulatory framework

Regulatory frameworks are designed to protect aquatic environments from harmful effects that might arise when resources are exploited or produced.

2.4.1 International

The term **United Nations Convention on Law of the Sea** (**UNCLOS**), also called the Law of the Sea Convention refers to the international agreement that resulted from the third United Nations Convention on the Law of the Sea that took place from 1973 to 1982 with modifications that were made by the 1994 Agreement on Implementation. The Law of the Sea Convention is a set of rules for the use of the world's oceans. The Convention was concluded in 1984 and came into force in 1994, and to date, 154 countries and the European Community have joined the Convention. The United States has not joined because it claims this treaty is unfavorable to America's economy and security. The Convention defines the rights and responsibilities of nations in their use of the seas, establishes clear guidelines for businesses, protects the environment and improves the management of marine natural resources.

UNCLOS lays down a comprehensive regime of law and order in the world's oceans and seas, establishing rules governing all uses of the seas and their resources. It embodies, in one instrument, traditional rules for the use of the oceans and at the same time new legal concepts and regimes addressing new concerns.

States have the obligation to protect and preserve the marine environment. They have the sovereign right to exploit their natural resources pursuant to their environmental policies and in accordance with their duty to protect and preserve the marine environment. States must prevent, reduce and control pollution, including that resulting from the introduction of species to a particular part of the marine environment.

The UN also records details of claims to maritime jurisdiction including claims for exclusive economic zones which, in many cases, extend 200 miles from coastlines. Information (www.un.org) also covers national legislation of coastal states and treaties dealing with the detail of maritime boundaries.

2.4.2 Regional

In Europe, the current regulatory framework under development (Proposals for a Framework for Community Action in the field of Marine Environment Policy 16976/06) notes that policy should provide a basis for a strong, growing and competitive maritime economy in harmony with the marine environment. The aim is that marine environmental policy ensures that the use of the marine environment is at a level that is sustainable with marine species and habitats protected, human induced decline of biodiversity prevented and diverse biological components allowed to function in balance.

It is likely that Member States will take forward these proposals in a way that enables them to manage their marine environments to secure maximum benefits as well as protecting biodiversity. It is recognised that innovation may help to find solutions to issues such as energy and climate change but there is little in current policy proposals that addresses issues relevant to the production and utilisation of biomass.

2.4.3 Land-based systems

Land-based systems for the production of aquatic biomass will inevitably need to respect local planning requirements. In Europe there are also controls in respect of discharges from land-based facilities and Directive 91/271/EEC concerns the collection, treatment and discharge of urban waste water and the treatment and discharge of waste water from certain industrial sectors. Its aim is to protect the environment from any adverse effects due to discharge of such waters.

Industrial waste water entering collecting systems and the disposal of waste water and sludge from urban waste water treatment plants are both subject to regulations and/or specific authorisations on the part of the Member States' competent authorities.

3 PRODUCTS

The species of marine macro-algae (seaweeds) that are industrially used currently belong to the divisions *Rhodophyta* and *Phaeophyta*, and about 7.5 – 8 Mio t of wet seaweed is harvested annually. The world market of products from macro-algae has been estimated to have a size of some US$ 5.5 - 6 billion per year (McHugh 2003; Pulz and Gross 2004). US$ 5 billion is generated by the food industry, of which US$ 1 billion is from “nori”, a high-value product worth US$ 16,000 t^{-1}; a further US$ 600 Mio was generated by hydrocolloids (55,000 t) extracted from cell walls of macro-algae.

The market size of products from micro-algae was estimated by Pulz and Gross (2004) to have a retail value of US$ 5 – 6.5 billion. US$ 1.25 - 2.5 billion were generated by the health food sector, US$ 1.5 billion from the production of docosahexanoic acid (DHA) and US$ 700 Mio from aquaculture (Pulz and Gross 2004).

Integrated marine biomass systems which could produce a combination of end products, for example, of energy, chemicals, food, cosmetics, fertiliser, wastewater treatment and/or CO_2 sequestration, would reduce production costs, since there would be a variety of outputs and sources of revenue from the main product and co-products to carry capital and operating costs. As production rates and costs are critical to the economic and commercial success of algal products, these issues are discussed first. Subsequent sections discuss the different products that have been considered in the various studies available in the public domain. The major question is whether the production of fuels or CO_2 mitigation is economically feasible as the sole income stream.

3.1 Productivity and price

3.1.1 Photosynthetic efficiency and productivity

Critical and controversial issues are the potential biomass yield that can be obtained by cultivating macro- or micro-algae, and the production costs of the biomass and derived products. The basis of the estimates is usually a discussion on three parameters: photosynthetic efficiency, assumptions on scale-up, and on long-term cultivation issues.

With respect to photosynthetic efficiency, there appears to be a general consensus that up to 9% of incident solar energy can be converted to biomass, this equates to an efficiency of 27% of PAR (photosynthetically active radiation, about 45% of total light). This value corresponds to a productivity of somewhat over 300 t ha^{-1} y^{-1} of conventional land plants (Benemann and Oswald 1996). This productivity has never been achieved in practice.

Current and reproducible yields for algae are in the 20 – 50 t ha^{-1} y^{-1} range, as demonstrated by the Aquatic Biomass Program and elsewhere (Chaumont 1993; Sheehan et al. 1998; Pulz 2001; Chynoweth 2002; Scragg et al. 2002; Huntley and Redalje 2007). Higher values have been observed under closely controlled conditions in short-duration experiments (for example Lee and Low 1991). However, these conditions (turbulent mixing, low cell density, optimal temperature and medium light intensity below levels causing saturation or inhibition) cannot be transferred to commercially viable large-scale systems.

Macro-algae are cultivated and harvested for food products, agar, carrageenan, alginate and minor products. Obtaining reliable data on biomass yields is difficult, as most macro-algae are harvested from wild populations. In a large study on the feasibility of methane production from macro-algal biomass (Chynoweth 2002), base case scenarios assumed yields of 11 dry t ha^{-1} y^{-1}, based on data from commercial growers. For optimised cultivation systems, yield of 45 dry t ha^{-1} y^{-1} were assumed.

In some highly controlled environments, such yields were actually obtained, however, at costs precluding scale-up and commercialization (Chynoweth 2002).

For micro-algae the productivity of raceway ponds and photobioreactors is limited by a range of interacting issues. While it may be possible to tackle individual issues, it has not yet been possible to effectively combine the solutions. This may be because the solutions are simply not additive, are mutually exclusive, or because of escalating associated costs. For example, several possible target areas to improve productivity in large-scale installations have been proposed (Grobbelaar 2000; Suh and Lee 2003; Torzillo et al. 2003; Carvalho et al. 2006) as follows:

1. *Culture depth or optical cross section*: thinner tubes or shallower ponds have been suggested to improve growth rates since algal cultures progressively absorb light and cause shading for algae at lower depths/inside tubes.
2. *Mixing*: greater turbulence would theoretically bring all cells into bright(er) light
3. *Nutrient content and supply*: nitrogen and CO_2 can be optimised
4. *Cultivation procedure*: batch, (semi)-continuous or multistage processes.
5. *Photosynthetic system*: reduced antenna size leading to higher quantum yields.

However, all of these solutions that have been suggested suffer from major drawbacks. For example, increased population densities do improve light utilization but require thorough mixing to avoid mutual shading: all cells should be exposed regularly to bright light. This can be accomplished by reducing the light path (thin tubes, shallow ponds), but this reduces the effective volume per surface area, and increases the cost per product unit. Thorough mixing may in principle be used to expose all cells to bright light regularly. However, to benefit from the flashing light phenomenon (which operates on the microsecond timescale), the required turbulent flow would lead to an energy input that exceeds energy output by far. Such interacting problems and bad engineering designs have caused many attempts at scale-up to fail, most notable the installations in Santa Ana, Murcia, Spain and La Rioja, Argentina (Richmond 2000). Reducing the antenna size helps to counter the photosaturation effect. Algae have evolved to effectively use dim light, but typical do not use 80% of bright light. At smaller antenna sizes, higher efficiencies have been obtained, but not in a linear way. In addition, under dim light (morning, evening, deeper parts of ponds), these modified algae would perform less well. However, a small antenna reduces photoinhibition in cells exposed to higher light intensities (Mussgnug et al. 2007).

The changes in cultivation that have been suggested to remedy low yield have mostly been ineffective. As yet the average biomass yields in commercial raceway ponds have stalled at 10 – 30 t ha^{-1} y^{-1}, and the highest reproducible productivities stand at 50 – 60 t ha^{-1} y^{-1}. This is comparable with yields in conventional tropical agriculture where typical dry biomass yields of 20 - 25 t ha^{-1} y^{-1} (sugarbeet, maize, sorghum, sugarcane) are routinely obtained, and yields of up to 50 t ha^{-1} y^{-1} for sorghum and 72 t ha^{-1} y^{-1} for sugarcane are possible on test plots (Muchow et al. 1994).

In commercial photobioreactors, higher productivities may be possible. A commercial plant in Klötze, Germany, has claimed a production capacity of 130 – 150 t ha^{-1} y^{-1} of *Chlorella* algae. However, actual production under the local climate conditions was lower with some 50 t ha^{-1} y^{-1} (Ullmann personal communication). At a selling price of some €50 kg^{-1}, the product of the Klötze plant is much more expensive than *Chlorella* from China at US$ 10 (€ 7.4) kg^{-1}. In Klötze, however, the algae are cultivated under tight control to comply with highest purity standards (Ullmann personal communication).

Table 4 lists data on yield per surface area from the scientific literature, reports, and commercial enterprises. It must be noted that the higher (more optimistic) numbers are typically from short-duration, small-scale experiments, or projections based on expected progress. There is considerable controversy around this issue, due to commercial and other vested interests (Biopact 2007).

Table 4. Photosynthetic efficiency and productivity.

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m^{-2} day^{-1})	Typical productivity (t ha^{-1}y^{-1}) (Maximum)	Comment	Reference
Land plants					
C3 land plants	< 6.6 (theor.)	Not applicable	10 – 18 (24) 8 -10 (30)	Sugarbeet (temperate climate) Willow (max. on test plots)	Kenter et al. (2006) Keoleian and Volk (2005)
C4 land plants	< 13.4 (theor.)	Not applicable	10 – 30 (72) 10 – 20 (50) 15 – 20 (40)	Sugarcane Sorghum Miscanthus	Muchow et al. (1994), Samson et al. (2005) Habyarimana et al. (2004), Clifton-Brown et al. (2001), Heaton et al. (2004),
Macro-algae				Biomass yield difficult to determine in the absence of sustained harvests	
Laminaria offshore	Not reported	1 – 5	7 - 16	Natural populations and commercial harvesting	Mann (1973); Chynoweth (2002), page 39
Macrocystis, Gracilaria, *Laminaria* and *Chondrus* in culture chambers	Not reported	3 – 10	10 – 34 (127)	“probably not achievable on a commercial scale” (Chynoweth 2002)	Chynoweth (2002), page 11-15

Table 4 continued.

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m^{-2} day^{-1})	Typical productivity (t ha^{-1}y^{-1}) (Maximum)	Comment	Reference
Laminaria in offshore farm	Not reported	Not reported	28 – 46 (expected values, prevented by storm!)	High values can only be obtained by supplying nutrients at excessive costs	Brinkhuis and Levin (1987)
Uncultivated brown algae	Not reported	Not reported	10 - 36	Review	Gao and McKinley (1994)
Micro-algae in open ponds					
Micro-algae in commercial raceway ponds	Not reported	3 - 8	10 – 30	*Chlorella*, *Arthrospira*, and *Dunaliella* sp.	Jimenez et al. (2003)
Algae in experimental raceway ponds (Aquatic Biomass Program)	< 10	3 – 40 (winter to summer)	30 – 50	Summary of ABP-program run from 1978 – 1996	Benemann and Oswald (1996); Sheehan et al. (1998)
Heamatococcus pluvialis	3 – 4.4	10 – 15 (uncorrected)	20 – 30	Annual yield corrected for space occupied by PBRs	Huntley and Redalje (2007)
Arthrospira (Spirulina)	Not reported	2 – 15	30	450 m^2	Jimenez et al. (2003)

Table 4 continued.

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m^{-2} day^{-1})	Typical productivity (t $ha^{-1}y^{-1}$) (Maximum)	Comment	Reference
Dunaliella salina	Not reported	2	Not reported	Small outdoor photo-bioreactor, 55l, 2.2 m^2	García-González et al. (2005)
Pleurochrysis carterae	Not reported	3 – 33 (winter to summer)	60	Small system (1 m^2), 13 months 21.9 t/ha/y lipids 5.5 t/ha/y $CaCO_3$	Moheimani and Borowitzka (2006) (see Table 3 for a list of similar experiments)
Scenedesmus obliquus	Not reported	48 (3 months in summer)	Not applicable	20 m^2 raceway pond unpublished results	Grobbelaar (2000)
Tetraselmis suecica	6 – 7 average 13 – 18 max	20 60 – 70	Not applicable	Duration less than 1 month Single day result	Laws et al. (1986)
Micro-algae in photobioreactors					
Chlorella vulgaris	Not reported	Not reported	130 – 150 (claimed)	Tubular PBR (700 m^3) in 1.2 hectare greenhouse	Moore (2001); Pulz (2001)
Phaeodactylum tricornutum	15 - 20	61 – 73 (depending on tube diameter) 14 – 17 (calculated for total area)	Not applicable	PBR with optimised dilution rates, extrapolated yields	Acien Fernandez et al. (1998)

Table 4 continued.

Plant system	Photosynthetic efficiency of PAR (%)	Typical productivity range (g m^{-2} day^{-1})	Typical productivity (t ha^{-1}y^{-1}) (Maximum)	Comment	Reference
Chlorella vulgaris	5.1 – 6.4	0.57 – 0.97	Not applicable	Helical bioreactor, artificial light	Scragg et al. (2002)
Chlorella sp.	7.1	43	Not applicable	Low level artificial light Turbulent culture	Tamiya (1957)
Chlorella sp.	< 47	Not reported	Not applicable	Value obtained under extremely low light with alternative photosystems	Pirt et al. (1980); Richmond (2000)
Arthrospira (Spirulina)	5.45	5.44	Not applicable	Helical bioreactor, artificial light	Watanabe et al. (1995)
Arthrospira (Spirulina)	2 - 5	7 – 25	33	215 days outdoor cultivation period in central Italy	Torzillo et al. (1986)

3.1.2 Production costs

An early and clear conclusion of the NREL Aquatic Biomass Program was that photobioreactors are neither practical nor economical for large-scale biomass production, and were thus not considered in the main program (Sheehan et al. 1998). Tredici et al. (1998) claim a “relatively low cost” of US\$ 50 m^{-2} for a PBR system, while John Benemann, co-author of the close-out report of the Aquatic Biomass Program (Sheehan et al. 1998), and present manager of the International Network on Biofixation of Carbon Dioxide and Greenhouse Gas Abatement with Microalgae (Programme 2007) noted that photobioreactors require at least 10 times higher capital investments (i.e. more than US\$ 100 m^{-2}) than open pond systems (Hallenbeck and Benemann 2002). Based on these numbers Benemann has described the use of these “complicated and labour intensive systems” for the production of algal biomass as “absurd” (Schneider 2006). One analyst (Jonas Van Den Berg) said: "growing algae in reactors (or in plastic covered ponds) is like growing sugarcane in greenhouses, it makes no sense."

Various estimates for the production costs of algal biomass in photobioreactors range from US\$ 30 – 70 kg^{-1} (Moore 2001; Molina Grima et al. 2003; Olaizola 2003), which is almost three orders of magnitude more expensive than waste biomass from conventional agriculture (Table 5). A much lower cost estimate for biomass production is given by Yusuf Chisti, who projects a cost of US\$ 2.85 kg^{-1} for photobioreactors (Chisti 2007), based on the assumption that economies of scale will reduce costs significantly. However, many of the costs are not very sensitive to the scale of the operation (Benemann and Oswald 1996; Vonshak 1997; Molina Grima et al. 2003). Moreover, many of the assumptions on yield and costs are extremely optimistic. The large ponds and photobioreactors that should demonstrate such cost reductions have not yet been constructed, or have failed commercially and technically soon after start-up (Tredici 1998).

Table 5. Production costs of algal biomass

System	Operating costs (US$ kg^{-1})	Capital costs (US$ ha^{-1})	Total costs (US$ kg^{-1})	Remarks	Source or reference
Agriculture			0.04		Cost of wheat straw (Germany 2007)
Forestry			0.04 – 0.055		Cost of fire wood (Germany 2007)
Macro-algae					
Ocean			0.045 – 0.31 (1981)		Bird (1987); Gao and McKinley (1994); Chynoweth (2002)
Raceway ponds					
Commercial raceway ponds		100,000	2 – 15	Based on discussions with producers	Benemann and Oswald (1996); Lee (2001); Reith et al. (2006); John Benemann, personal communication
50,000 m^2 raceway pond	7 – 10	300,000	> 8 – 11 [1]	Costs at prod. sites in Thailand & USA	Vonshak (1997)
Raceway ponds	Not specified	Not specified	3.8	No calculations available	Chisti (2007)
Raceway ponds	0.14	100,000	0.24	Projected cost based on 110 t ha^{-1} y^{-1}	Benemann and Oswald (1996)
Raceway ponds	€ 0.07	100,000	€ 0.21	Projected cost based on 100 t ha^{-1} y^{-1}	van Harmelen and Oonk (2006)

Table 5 continued.

System	Operating costs (US$ kg^{-1})	Capital costs (US$ ha^{-1})	Total costs (US$ kg^{-1})	Remarks	Source or reference
Raceway ponds for phycoremediation			2 – 4	Main product is industry water	Reith (2004)
Photobioreactors					
PBR (*Chlorella*)	Not specified	Not specified	40-60 (selling price)	1.2 ha PBR in Klötze, Germany	Moore (2001)
PBR (*Haematococcus*)	Not specified	Not specified	> 30 [2]	Minimum price to compete with synthetic astaxanthin	Olaizola (2003)
PBR (cost analysis)	19.4	12.6 (capital costs 11% per y)	32	Manpower 13% Raw materials 17% Overall capital charge 34%	Molina Grima et al. (2003)
PBR (cost analysis)	Not specified	Not specified	2.95	Based on Molina Grima et al. (2003), with assumptions on scale-up benefits	Chisti (2007)

Note: numbers not corrected for inflation. [1] Total costs were not given; here a capital charge factor of 15% was used to obtain approximate total costs, which could be considered an annual charge for mature technology required to pay off the facility in 20 years (Benemann and Oswald 1996). [2] To produce astaxanthin at the price of synthetic astaxanthin (2,500 US$ kg^{-1}), the algal biomass must be available for less than US$ 30 kg^{-1} (Olaizola 2003). Natural astaxanthin now sells for US$ 7,000 kg^{-1}. www.israel21c.org/bin/en.jsp?enZone=Health&enDisplay=view&enPage=BlankPage&enDispWhat=object&enDispWho=Articles%5El986 and www.algatech.com).

Table 5 also shows the cost of producing algal biomass in raceway ponds. The cost of about US$ 10 kg^{-1} is about 2 orders of magnitude higher than that of biomass produced in conventional agriculture. These high costs clearly restrict the use of micro-algae biomass as feedstock for the manufacture of low value products.

Based on discussions with commercial producers a price-range of US$ 8 – 15 kg^{-1} has been reported (Benemann and Oswald 1996; Vonshak 1997; Lee 2001). According to John Benemann, current US delivered prices from China for 20 t container shipments are US$ 5 kg^{-1} for *Spirulina* and twice as high for *Chlorella*. Lolke Sijtsma claims that a production price range of 2 – 5 US$ kg^{-1} is possible (Reith et al. 2006). *Spirulina* and *Chlorella* are both destined for relatively higher priced health food products.

A small number of companies produce algae in photobioreactors. Algatech (Israel: www.algatech.com) and Mera Pharmaceuticals (Hawaii: www.merapharma.com, which took over Aquasearch in 2001) grow *Dunaliella pluvialis* for astaxanthin production, while Bioprodukte Prof. Steinberg GmbH (Germany: www.bioprodukte-steinberg.de) operates a large photobioreactor in Klötze, Germany, to produce *Chlorella* for the Health Food market.

Several reports present calculations on the projected cost of producing algal biomass in large-scale raceway ponds. The investments per ha of raceway ponds (at least US$ 100,000) are much higher than investments for new land plant cultivation (< US$ 10,000) (Benemann and Oswald 1996; van Harmelen and Oonk 2006). To illustrate this, the individual cost items are specified in Table 6. For the same scenario, operating costs of US$ 21,300 ha^{-1} were calculated (Table 8.3 in Benemann and Oswald 1996). The resulting cost for algal biomass is US$ 0.24 kg^{-1}. At the time, a yield of 30 g m^{-2} day^{-1} (110 t ha^{-1} y^{-1}) or even 60 g m^{-2} day^{-1} (220 t ha^{-1} y^{-1}) was assumed. However, according to the author, this is not realistic: 50 t ha^{-1} y^{-1} is achievable. Thus, the cost of algal biomass in this scenario should be raised to at least US$ 0.72 kg^{-1} (corrected for inflation, and assuming that other costs do not change).

Table 6. Capital costs of raceway ponds for a productivity of 30 $g^{-1}m^{-2}day^{-1}$ (based on Table 8.3 in Benemann and Oswald 1996).

Item	Remarks	Cost US$ ha^{-1}
Land preparation, grading, compaction	Percolation control by natural sealing	2,500
Building of pond walls & levees		3,500
Paddle wheels for mixing		5,000
CO_2 transfer sumps & carbonation		5,000
CO_2 supply (pipelines and scrubbers)	Assuming flue gas	5,000
Harvesting and processing equipment	Settling Flocculation Centrifugation and extraction	7.000 2,000 12,500
Anaerobic digestion and nutrient recycling	Lagoon	3,250
Other capital costs	Water and nutrient supply Waste treatment Building, roads, drainage Electricity supply & distribution Instrumentation & machinery	5,200 1,000 2,000 2,000 500
Subtotals of above		59,450
Engineering, contingencies	15% above	8,900
Total direct capital		68,350
Land costs		2,000
Working capital	25% operating cost	3,800
Total capital investment		74,150
Inflation corrected	2.5% inflation, 11 years	97,300

A similar low cost estimate (€ 210 t^{-1}) appeared in “Micro-algae biofixation processes: applications and potential contributions to greenhouse gas mitigation options” (van Harmelen and Oonk 2006). However, the authors specifically state that “*even with the most favourable assumptions about algae production costs*

(€ 210 t^{-1}) and revenues for biofuels (€ 120 t^{-1} algae) and GHG abatement (€50 t^{-1} algae), the process would still not be economically feasible. Thus, fuel-only algal systems are not plausible, at least not in the foreseeable future and additional revenues are required, either from wastewater treatment or higher value co-products". It must be noted that this scenario is also based on a highly optimistic yield of 100 t ha^{-1} y^{-1}, and costs should be corrected accordingly.

Thus, the present and foreseeable future cost of producing algal biomass is prohibitive for any of the bioenergy applications discussed below. Only if the algal biomass is a by-product of wastewater treatment systems, or high value compounds such as astaxanthin or β-carotene, commercially viable processes become feasible (van Harmelen and Oonk 2006). However, this limits the scale of energy production and GHG abatement from algae by default to the amount of algal biomass produced to support the profitable applications (Reith 2004).

For polyunsatured fatty acids, carotenoids, and colorants, approximate production scales can be estimated. Astaxanthin has an annual market size of US$ 250 Mio or roughly 100 t, which can be produced from 10,000 t of algae. Present worldwide annual demand for eicosapentaenoic acid is claimed to be about 300 t (Sanchez Miron et al. 1999); production from *Phaeodactylum cornutum*, which contains about 2% eicosapentaenoic acid would require production from 15,000 t of algal biomass. Docosahexaenoic acid is produced by Martek (www.martekbio.com) and has a similar market size. For colorants, a market size of 800 t and an average content of 5% of dry weight has been proposed (Reith 2004), resulting in a scale of 16,000 t. Thus the total requirement for these four high value applications is less than 100,000 t. However, it should be noted that annual fish oil sales amount to 1 - 1.5 Mio t (Jackson 2006). Most of it is used in aquaculture to obtain fish with the desired ω-3 fatty acid content. Completely replacing fish oil by algal products would require an annual production of 2.5 – 3.5 Mio t of algae (Reith et al. 2006).

The scale of wastewater treatment systems is more difficult to estimate. The main application of phycoremediation is in tertiary treatments to remove nitrogen and phosphate. The composition of the algae is not likely to be controlled and may also contain pollutants rendering it unfit for food and feed applications.

The major conclusion of the cost analysis is that the cultivation of algae solely for biofuels and CO_2-mitigation is not cost-competitive by 1 – 2 orders of magnitude. As it is difficult to identify areas where significant costs savings can be made, and yields cannot be increased easily without increased costs, these applications are not likely in the foreseeable future. Current high value products from algae would not support sufficient quantities to underpin development of alternative uses such as biofuels or CO_2-mitigation.

3.2 Energy

3.2.1 Biomass

Macro- and micro-algae are currently mainly used for food, in animal feed, in feed for aquaculture and as bio-fertiliser. Biomass from micro-algae is dried and marketed in the human health food market in form of powders or pressed in the form of tablets. Important species used for micro-algal biotechnology of biomass for human food belong to the groups of *Spirulina*, *Chlorella*, *Dunaliella*, *Nostoc* and *Aphanizomenon*. For example about 3500 t of *Spirulina* biomass was produced worldwide in 1999 (Pulz and Gross 2004).

Aquatic biomass could also be used as raw material for co-firing to produce electricity, for liquid fuel production via pyrolysis (bio-oil), or for biomethane generation through fermentation. Macro- and micro-algae for bioenergy production should fit several criteria: i) they should be highly productive; ii) they should be easily harvestable by mechanical techniques; iii) (for macro-algae) they should be able to withstand water motion in the open ocean, and iv) they should be produced at a cost equal or lower than other available biomass. Currently, the production cost of algal biomass is too high to cultivate it solely for these purposes.

3.2.1.1 Biomethane

Research to determine the technical and economic feasibility of bio-methane production from marine biomass was conducted from 1968 until 1990 under the sponsorship of the U.S. Navy, the American Gas Association and Gas Research Institute, and the U.S. Department of Energy, and was reviewed by Chynoweth (2002). The study compared the technical potential of different biomass sources (marine algae, wood and grass species, municipal solid waste) to be used in energy farms, and concluded that marine biomass offered the highest potential. The growth rates of marine macro-algae exceed those of land plants, however, growth is often limited by the availability of nutrients. A sufficient nutrient supply in the open ocean could be achieved through upwelling of nutrients, but the study suggests that this option is too costly and that farming of macro-algae near shore with nutrient supply through recycling of wastes from conversion processes would be a better option (Chynoweth 2002). However, the production cost of biomethane from marine algae (with favourable assumptions) was a factor of 2 – 10 times higher than the cost of natural gas (Table 7).

Table 7. Production costs of methane produced from different feedstocks.

Energy crop	**Methane cost US$ GJ^{-1}**	**References**
Sorghum	6 – 8	Legrand (1993)
Poplar	3 – 7	Legrand (1993)
Kelp	3 – 14	Bird (1987)

The study by Chynoweth (2002) also concludes: "*The overall costs of producing substitute natural gas from marine macro-algae are higher than those of grass, wood, and waste systems and substantially higher than fossil fuels in the U.S. This high cost is related to the elevated cost of farming in the ocean, which may be reduced substantially by recovery of by-products and stimulation of mariculture*." High technological and economic uncertainties were associated with the large-scale production of macro-algae in the open ocean (Chynoweth 2002). Nevertheless, it was found that marine algae, such as *Gracilaria sp*. and *Macrocystis* (kelp) were excellent substrates for biomethane generation (Bird et al. 1990, Chynoweth et al. 1993). The methane yields from different biomass and waste sources are presented in Table 8.

Table 8. Yield of methane produced from different feedstocks.

Biomass	**Methane yield ($m^3\ kg^{-1}$)**	**Reference**
Laminaria sp.	0.26 – 0.28	Chynoweth et al. (1993)
Gracilaria sp.	0.28 – 0.4	Bird et al. (1990)
Sargassum sp.	0.12 – 0.19	Bird et al. (1990)
Macrocystis	0.39 – 0.41	Chynoweth et al. (1993)
Water hyacinth	0.13- 0.21	Chynoweth et al. (1993)
Sorghum	0.26 – 0.39	Chynoweth et al. (1993)
Poplar	0.23- 0.32	Chynoweth et al. (1993)
Food waste	0.54	Chynoweth et al. (1993)

Biomethane yields depend highly on the fermentation conditions, therefore the data derived in the same studies are comparable, but not necessarily the data between individual studies.

The view expressed by many authors is that the best approach to biomethane production from macro-algae is the multi purpose use of algal biomass, for example gas production arising from digestion of the residues from hydrocolloid extraction (Kerner et al. 1991, Morand et al. 1991). In this way the co-production of methane with other products would lower the production costs and could make biomethane production profitable. To our knowledge, large-scale commercial operations have not been established to date.

The cultivation of micro-algae, purely as a feedstock for biomethane generation, is highly unlikely, given the production costs as shown in Table 7.

3.2.2 Biofuel

3.2.2.1 Bio-oil

Thermochemical conversion is a process through which biomass in the absence of oxygen and at high temperature can be converted into various fuels including char, oil and gas. The resulting bio-oils present an alternative to liquid biofuels with similarities to petroleum oil (Kishimoto et al. 1994). The process can be subdivided into pyrolysis and thermochemical liquefaction (Demirbas 2000). The former is executed at high temperature (350-530°C) at which a liquid, a gaseous and a solid fraction is produced. The liquid fraction contains an aqueous and a non-aqueous phase (bio-oil or tar), which is recovered. This process requires drying of the biomass while in the latter wet biomass is treated at lower temperature and high pressure (about 300°C and 10 MPa). Since thermochemical liquefaction does not require a drying process but can use the wet material such as algal biomass (water content more than 60%) this technique has a clear advantage compared with pyrolysis. The bio-oil from the processing is composed of all organic compounds in the algae including lipids as well as proteins, fibers, and carbohydrates and therefore gives a higher yield than compared with the content of accumulated lipids in the algae cells. The feasibility of producing liquid fuel or bio-oil via pyrolysis or thermochemical liquefaction of micro-algae has been demonstrated for a range of micro-algae (Dote et al. 1994; Sawayama et al. 1999; Peng et al. 2000; Peng et al. 2001a; Peng et al. 2001b; Tsukahara and Sawayama 2005; Demirbas 2006). Ginzburg (1993) for example reported that a mixture of hydrocarbons was formed upon pyrolysis of suspensions of the micro-algae *Dunaliella* sp.

Miao et al. (2004) proposed using micro-algae harvested from lakes both to produce bio-oil via fast pyrolysis and as an environmental solution to reduce algae blooms. Up to 24% of the dry biomass was recovered as bio-oil. The pyrolysis oils had better properties than the oil from lignocellulosics, however, they still have a much higher oxygen content compared to fossil oil and their heating value is low with 29 MJ kg^{-1} compared to 42 MJ kg^{-1} of fossil oil (Miao et al. 2004). The fast pyrolysis process applied to micro-algae has been evaluated in the laboratory, and some of the challenges for commercialisation of this process are: i) to develop a low cost harvesting method; ii) to improve separation techniques for the bio-oil; iii) to design and set up large scale installations; and, iv) to reduce the cost of fuel production (Miao et al. 2004).

In a further study, Miao and Wu (2004) produced a bio-oil with improved properties via fast pyrolysis of heterotrophically grown *Chlorella protothecoides*. The heterotrophically grown algae had a higher lipid content (some 55%) compared to autotrophically grown algae (some 14%). The heating value of 41 MJ kg^{-1} was nearly as high as that of fossil oil, and the nitrogen content was reduced to about 1%. This bio-oil still had a relatively high oxygen content of some 11% compared to fossil oil with 0.05 to 1.5%. Key to improving the bio-oil quality was the increase in lipid content in the biomass. This can be achieved by growing the algae heterotrophically under laboratory conditions or in controlled closed systems, however, algae harvested from lakes do not necessarily contain a high concentration of lipids and bio-oil quality may be of lower quality.

3.2.2.2 Biodiesel

The idea of producing biodiesel from micro-algae that accumulate high amounts of oil was a main focus in the NREL project (Sheehan et al. 1998) and roughly 3000 strains of algae were collected from the northwest and the south-eastern regions of the continental U.S. and Hawaii, and screened for their oil-producing capacity.

With rising prices for petroleum and concern for global warming from the use of fossil fuels the same idea continues to attract a lot of attention (Chisti 2007; Huntley and Redalje 2007; Li et al. 2007). Many species of algae accumulate large amounts of oils that to a large extent are made up of triacylglycerols consisting of three fatty acids bound to glycerol. The fatty acids are saturated or unsaturated carbon chains of different lengths. Non- or mono-unsaturated fatty acids of 16 or 18 carbon length are preferable sources to use for the production of biodiesel. It should be noted that the vlcPUFAs in this respect are less suitable for the production of biodiesel since the polyunsaturation leads to increased oxidation problems in the fuel. However, the unsaturation of algal oil can be reduced by making use of the commonly used technology of partial catalytic hydrogenation of the oil (Dijkstra 2006; Jang et al. 2005), the same technique that is used in making margarine from vegetable oils.

The algal oil is converted into biodiesel through a trans-esterification process. Oil extracted from the algae is mixed with alcohol and an acid or a base to produce the fatty acid methylesters that makes up the biodiesel (Chisti 2007). A topic for the NREL project was to screen for algae with high accumulation of oils. A number of algae strains with good potential for making biodiesel were also identified. A selection of algae strains with potential to be used for the production of oils for biofuel is presented in Table 9.

A major current problem for the commercial viability of biodiesel production from micro-algae is the low selling price of biodiesel (less than €1 kg^{-1}). John Lewnard was Vice President of Process development with GreenFuel Technologies Corporation in 2006. With regard to biodiesel production from micro-algae he said: "*productivities of about 100 grams of algae per m^2 per day are needed to achieve commercial viability*" (Schneider 2006). This is about three times the productivity that was demonstrated during the Aquatic Species Program funded by the DOE. The challenge to produce this amount of biomass is enormous. Gerald R. Cysewski, president of Cyanotech Corporation focuses on products that sell from US$ 18 – 380 kg^{-1}. In a recent paper in Scientific American (Schneider 2006) he stated "*In the laboratory, you can create some very efficient bioreactors, but it just isn't scalable*". His response to the question whether biodiesel will ever be made this way, he responded: "*Not from micro-algae—I just can't see it*." However, Yustuf Chisti, who has recently written a review on biodiesel from microalgae states: *"It is the only renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum. Economics of producing microalgal biodiesel need to improve substantially to make it competitive with petrodiesel, but the level of improvement necessary appears to be attainable"* (Chisti 2007).

Table 9. Oil content in selected microalgal species.

Species	**Oil content (% dw)**	**Reference**
Ankistrodesmus TR-87	28-40	Ben-Amotz and Tornabene (1985)
Botryococcus braunii	29-75	Sheehan et al. (1998); Banerjee et al. (2002); Metzger and Largeau (2005)
Chlorella sp.	29	Sheehan et al. (1998)
Chlorella protothecoides (autotrophic/ heterothrophic)	15-55	Xu et al. (2006)
Cyclotella DI-35	42	Sheehan et al. (1998)
Dunaliella tertiolecta	36-42	Kishimoto et al. (1994); Tsukahara and Sawayama (2005)
Hantzschia DI-160	66	Sheehan et al. (1998)
Isochrysis sp.	7-33	Sheehan et al. (1998); Valenzuela-Espinoza et al. (2002)
Nannochloris	31 (6-63)	Ben-Amotz and Tornabene (1985); Negoro et al. (1991); Sheehan et al. (1998)
Nannochloropsis	46 (31-68)	Sheehan et al. (1998); Hu et al. (2006)
Nitzschia TR-114	28-50	Kyle DJ, Gladue RM (1991) Eicosapentaenoic acids and methods for their production. International Patent Application, Patent Cooperation Treaty Publication WO 91/14427, 3 October 1991.
Phaeodactylum tricornutum	31	Sheehan et al. (1998)
Scenedesmus TR-84	45	Sheehan et al. (1998)
Stichococcus	33 (9-59)	Sheehan et al. (1998)
Tetraselmis suecica	15-32	Sheehan et al. (1998); Zittelli et al. (2006); Chisti (2007)
Thalassiosira pseudonana	(21-31)	Brown et al. (1996)

3.2.2.3 Biohydrogen (directly produced by algae)

Hydrogen produced by photosynthetic organisms is one of a range of popular scenarios for renewable energy. It is appealing as it burns to water, is abundant in the form of water, biomass or hydrocarbons, and is non-toxic. However, it is also one of the most controversial options, as hydrogen is notoriously difficult to store and transport. Unlike biogas, which can be transported and stored using existing infrastructure, hydrogen will need a completely new, and much more expensive infrastructure. This is due to its propensity to make steel brittle, to leak out of pipelines, and the costs of compressing (Ulf Bossel).

Hydrogen can be produced by algae under specific conditions. For overviews of the current state of the art and the ongoing discussions on the prospects of hydrogen production by algae, we refer to (Levin et al. 2004; Prince and Kheshgi 2005; Rupprecht et al. 2006, Hankamer et al 2007). The following section provides a short overview of some of the technical and scientific issues.

Three different ways to produce H_2 have been proposed: direct and indirect photolysis, and ATP-driven H_2-production. Direct photolysis is possible when the resulting hydrogen and oxygen are continuously flushed away. Photosynthesis and water-splitting are coupled, resulting in the simultaneous production of hydrogen and oxygen. This results in major safety risk, and costs to separate the hydrogen and oxygen. Moreover, the hydrogenases involved in the processes are extremely oxygen-sensitive.

Therefore, indirect processes are preferred. Here, cells accumulate a carbon-storage material such as starch, the energy-content of which is partially converted to hydrogen under anaerobic and sulphur-limited conditions. This circumvents the oxygen-sensitivity of the hydrogenases, however, still remains to be demonstrated as the hydrogen produced by Melis (2000) could be attributed to acetate respiration (Prince and Kheshgi 2005).

A 2004 cost analysis seems to indicate that with very optimistic assumptions, cost effective production of hydrogen might be possible (Amos 2004). Hydrogen selling prices in different scenarios ranged from US$ 0.57 to US$ 439. The base scenario (with a minimum H_2 selling price of US$ 13.53 kg^{-1}) already makes the highly optimistic assumption that the system investments required would be only US$ 10 m^{-2} and assumes a high hydrogen production rate of 300 kg per day (which is higher than demonstrated productivities in laboratory experiments). Major factors affecting the cost of hydrogen production by micro-algae are i) the cost of the huge photobioreactor, for example an area of about 11 ha was assumed by Amos (2004), and ii) the cost of hydrogen storage facilities that guarantee continuous hydrogen supply also through the night or during cloudy periods of the day. If costs for the photobioreactor could be reduced to US$1 m^{-2}, and the hydrogen could be transferred to an existing hydrogen pipeline a minimum hydrogen selling price of US$ 2.83 was calculated, which is just above the US DOE cost goal for renewable hydrogen of US$ 2.60 (Amos 2004).

3.3 CO_2 mitigation and sequestration

3.3.1 CO_2 mitigation

To use micro-algae to fix CO_2 released from power plants via the exhaust gas and thereby mitigate the amount of carbon released into the atmosphere is an attractive idea. However, there are several major challenges before this idea becomes practical. It is known that growth of algae is negatively influenced by increasing CO_2 (Lee and Lee 2003). Strains that grow well at CO_2 concentrations of 5-10% show drastic decreases in their growth rate above 20% (Watanabe et al. 1992). An important task therefore has been to identify strains that can cope with very high CO_2 concentrations and also have high growth rates. Screening has yielded strains that grow well in CO_2 concentrations between 30% and 70% saturation (Hanagata et al. 1992; Iwasaki et al. 1996; Sung et al. 1999). Also, results by Olaizola (2003) indicate that by controlling the pH changes in the culture and releasing CO_2 to the algae on demand, growth could be sustained even at 100% CO_2. Another important property that would need to be optimised is the ability of algae strains to have high thermal stability. It has been suggested that the hot flue gases introduced in the algal cell cultures may influence the temperature (Ono and Cuello 2007).

3.3.2 Carbon sequestration

There is a worldwide awareness about global warming as a result from the rising levels of different greenhouse gases such as CO_2 released from the burning of fossil fuels. Different methods have been suggested as to how CO_2 could be sequestered or immobilised through filtering or other mechanical/chemical processes and subjected for long-term storage to avoid release into the atmosphere. In this respect the idea of biological sequestering by growing algae and take advantage of their photosynthetic machinery of capturing carbon dioxide has been suggested by many as an alternative method of reducing the amount of CO_2 released in the atmosphere (Hall and House 1993; Benemann 1997; Hughes and Benemann 1997; Sheehan et al. 1998; Chisti 2007; Huntley and Redalje 2007).

The "Aquatic Species Program" funded from 1978 through 1996 by the Office of Fuels Development (DOE) started out as a project investigating the possibilities of using algae to sequestering CO_2 emissions from coal power plants (Sheehan et al. 1998). The main direction of the program over time became focused on the specific application of developing a production of high-quality diesel from algae utilising the CO_2 in the exhaust gas from coal-fired power plants. The project screened for algae that could produce high amount of oils as well as could grow at severe conditions regarding temperature, pH and salinity. For issues that were raised in the NREL report the reader is referred to other sections in this review.

3.3.3 Carbon trading

In Europe, the Emissions Trading Scheme is one of the policies introduced across Europe to tackle emissions of carbon dioxide and other greenhouse gases and combat the serious threat of climate change. The scheme opened on 1 January

2005. The first phase runs from 2005-2007 and the second phase will run from 2008-2012 to coincide with the first Kyoto Commitment Period. Further five-year periods are expected to follow.

The scheme works on a "Cap and Trade" basis and EU Member State governments are required to set an emission cap for all installations covered by the Scheme. Each installation will then be allocated allowances for the particular commitment period in question. The number of allowances allocated to each installation for any given period, (the number of tradable allowances each installation will receive), will be set down in a document called the National Allocation Plan. Anyone who is not covered by the Scheme will be able to buy and sell allowances and this creates a tradable value for carbon saved. The aim is that the Emissions Trading Scheme is developed in such a way that there is a sound basis for the future of the carbon trading market.

According to the UK Department for Business, Enterprise and Regulatory Reform, forward trading of EU allowances has been in the range €5-15 per tonne of carbon dioxide. There is large uncertainty about forward prices and many specialists assume that they will be higher in the second phase of the EU Emissions Trading Scheme. Aquatic species could benefit from trading given their potential to mitigate and/or sequester carbon and this would contribute to the economics of production.

3.4 High-value added products from algae

High-value products can be defined as any product that when multiplied by its fraction in the algal biomass has a value of over US$ 10 kg^{-1} (the present commercial production costs for micro-algae). The number of products meeting this definition that have advanced to commercial production is low (astaxanthin, β-carotene) and most have remained in the development stage. Few new algal products cultivated in open pond systems have reached the market in recent years. There have been recent commercial advances in the heterotrophic cultivation of pure algal cultures in bioreactors. A number of recent reviews covers the state of the art and new developments (Borowitzka 1988; Borowitzka 1992; Metting 1996; Banerjee et al. 2002; Olaizola 2003; Hejazi and Wijffels 2004; Pulz and Gross 2004; Spolaore et al. 2006; Becker 2007).

3.4.1 Small molecules

A study focusing on the production of methane from algal biomass conducted in the framework of the US Marine Biomass Program concluded that of more than a dozen potential by- and co-products, only four chemicals offered the technical and economical potential for achieving significant reductions in methane cost. These are iodine, algin, mannitol, and L-fraction, the first three being commercial products. The L-fraction (apparently a lignin-related fraction) was suggested as a feedstock or a component for making specialty plastics, adhesives and timed-release substances such as pharmaceuticals or pesticides. These applications would lead to the L-fraction having a value of US\$ 1-2 lb^{-1} (US\$ 2.2-4 kg^{-1}) and up to US\$ 6-7 lb^{-1} (US\$ 13-15 kg^{-1}) depending on the particular use (Chynoweth 2002).

The microalga *Dunaliella salina* can contain up to 40% of its dry weight as glycerol (Borowitzka 1992). However, the low price of glycerol (as a co-product of biodiesel production) means that the algal product would not be competitive. Other algal species accumulate high concentrations of proline under conditions of high salinity (Benemann and Oswald 1996).

3.4.2 Polymers

Macro-algae have long been used for the production of phycocolloids such as alginates, carrageenans or agars (Lewis et al. 1988; Indergaard and Østgaard 1991; Skjåk-Bræk and Martinsen 1991; Radmer 1996; Renn 1997). These polymers are either located in cell walls or within the cells serving as storage materials. A characteristic of marine algae is the abundance of sulphated polysaccharides in their cell walls. In total these phycocolloids represent a world market of some US\$ 600 Mio y^{-1}.

For more information on the chemical composition and structure of algae cell walls the reader is referred to the classic early reviews by Siegel and Siegel (1973), Mackie and Preston (1974) and Percival (1979).

Cyanobacteria also produce the storage reserve polymer cyanophycin, multi-L-arginyl-poly-L-aspartic acid. However, current research focuses on the production of this polymer in bacteria and land plants (Neumann et al. 2005)

Table 10. Polymers from macro-algae: stable markets (from McHugh 2003).

Product	**Production ($t\ y^{-1}$)**	**Algae harvested ($t\ y^{-1}$)**	**Value (Mio US$)**	**Comments**
Carrageenan	33,000	168,400	240	mainly *Eucheuma* and *Kappaphycus*
Alginate	30,000	126,500	213	Laminaria, Macrocystis, Lessonia, Ascophyllum, and others
Agar	7,630	55,650	137	Mainly *Gelidium* and *Gracilaria*
“Extracts”			10	
Nori (food)	40,000	400,000 (wet, only Japan)	1,500	*Porphyra*

3.4.2.1 Hydrocolloids

The group of phycocolloid polymers commonly termed hydrocolloids includes the alginates, carrageenans and agars. They make up the major industrial products derived from algae (Radmer 1996, Pulz and Gross 2004). The raw materials for the production of hydrocolloids are macro-algae (red and brown seaweeds). Hydrocolloids are polysaccharides that are not found in terrestrial plants, although polymers with similar properties can be produced by certain land plants, for example gum Arabic.

Hydrocolloids possess a number of unique properties. The polymers are used in many food and industrial products to thicken, emulsify and stabilize. Hydrocolloids can be dissolved in warm water and will form a gel on cooling. The gel properties can be modified by varying the concentrations of metal ions present, the temperature and the pH, making them suitable for various applications. It is estimated that some 1 Mio t y^{-1} of wet seaweed are harvested and extracted, resulting in a total hydrocolloid production of some 55,000 t with a value of US$ 600 Mio (Mc Hugh 2003). The hydrocolloids will now be briefly described, for detailed descriptions of processing of hydrocolloids, their properties and markets the reader is referred to the comprehensive reviews of Lewis et al. (1988) and Mc Hugh (2003).

Alginates are polymers from the cell walls of a wide variety of species of the brown algae, particularly species of *Laminaria*, *Macrocystis* and *Ascophyllum*. They are polymers of composed of D-mannuronic acid and L-guluronic acid monomers. The alginates are extracted from the cell walls using hot alkali (sodium carbonate) (Radmer 1996). Alginates are commonly used in the food and pharmaceutical industries as stabilisers for emulsions and suspensions, e.g. ice cream, jam, cream, custard, creams, lotions, tooth paste, as coating for pills. They are also used in the production of paint, construction material, glue and paper, the oil, photo and textile industry (Radmer 1996). Brown seaweeds for alginate production are harvested from the wild and not cultivated for this purpose (McHugh 2003). Although these seaweeds are cultivated to produce food in China, their cultivation to provide raw material for industrial uses would be too expensive (McHugh 2003). Overall, the market value of alginate was some US$ 213 Mio in the year 2003.

Carrageenans are linear 1,3-α-1,4-β-galactans from cell walls of red algae that are substituted with one (κ-), two (ι-) or three (λ-carrageenan) sulphate groups (Mc Hugh 2003). They are extracted from the cell walls with hot water. Carrageenans are used in the food, textile and pharmaceutical industry and function as a stabiliser for emulsions and suspensions. The bulk of carrageenan is now produced from cell walls of different species of the genus *Eucheuma*, and *Kappaphycus alvarezii* (cottonii), in addition smaller amounts are isolated from *Chondrus crispus* (Irish

Moos) and *Gigartina stellata*. Most of the seaweed for carrageenan production is cultivated, because the demand for raw material cannot be satisfied from natural resources. *Eucheuma sp.* and *Kappaphycus alvarezii* are most often cultivated with the fixed, off-bottom line or with floating rafts in the Philippines, Indonesia and Tanzania. Carrageenan production was about US$ 300 Mio in the year 2003 (Mc Hugh 2003).

Agars are 1,3-α-1,4-β-galactans from cell walls of red algae that are substituted with sulphate groups (Radmer 1996). Like the carrageenans, the agars are extracted with hot water. The genera *Gelidium* and *Gracilaria* supply most of the raw material for agar production (Zoebelein 2001). *Gelidium* used for commercial agar production is harvested from the wild, whereas *Gracilaria* species have also been cultivated in Chile, China and Indonesia, in in protected bays in the ocean, on lines ropes or nets, or in ponds on land (Mc Hugh 2003). Like carrageenans, agars are used as stabilisers for emulsions and suspensions and as gelling agents. About 90% of the agar produced was for food applications and the remaining 10% were used for bacteriological and other biotechnological uses. In 2001 about 7630 t of agars with a market value of some US$ 137 Mio were produced (Mc Hugh 2003).

3.4.2.2 Ulvan

Ulvan is the name given to a group of polymers that can be extracted with water containing a cation chelator from cell walls of green seaweeds belonging to the family *Ulvales*, especially the genera *Ulva* and *Enteromorpha* (Lahaye and Robic 2007). Yields of 8% to 29% of the algal dry weight have been reported (Lahaye and Robic 2007). Ulvan samples from different species differ from each other in their composition. In general they are composed of variable proportions of different repeating sequences of rhamnose, glucuronic acid, iduronic acid, xylose and sulfate (Lahaye and Robic 2007). So far these polymers have not been commercially used, however Lahaye and Robic (2007) have recently proposed that Ulvan could be i) a source of rare sugar precursors for the synthesis of fine chemicals; ii) a source of oligosaccharides that could be used as pharmaceuticals and iii) a gelling agent for designing gels with precisely controlled textures. For a detailed review on the structure and properties of Ulvan the reader is referred to Lahaye and Robic (2007).

3.4.3 Pharmaceuticals and cosmetics

Many micro-algae produce bioactive compounds such as antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators (Metting and Pyne 1986; Borowitzka 1988). Antibiotics have been obtained from a wide range of algae and show great chemical diversity (fatty acids, bromophenols, tannins, terpenoids, polysaccharides, alcohols). The same holds for the neurotoxic and hepatotoxic compounds produced by algae. Some of these chemicals or compounds derived from them have potential applications as pharmaceuticals (Metting 1996). Algae have also been investigated as sources of vitamins and vitamin precursors, most notably ascorbic acid, riboflavin and α- β- and γ-tocopherol. Certain micro-algae, especially *Chlorella* and *Arthrospira* (*Spirulina*) are used in skin care, sun protection and hair care products (Spolaore al. 2006). So far only a few hundred of the tens of thousands of micro-algal species have been investigated for potential pharmaceuticals and nutraceuticals (Olaizola 2003). The huge biodiversity of the micro-algae makes the discovery of new metabolites very likely. There is therefore also potential for the discovery and production of high value compounds.

3.4.4 High value oils

The very long-chain poly-unsaturated fatty acids (vlcPUFAs) eicosapentaenoic (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) are well known for their nutritional importance. As they confer flexibility, fluidity and selective permeability properties to cellular membranes they have been shown to be vital for brain development, beneficial for the cardiovascular system and for other important nutraceutical and pharmaceutical targets in human and animal health (Kroes 2003; Funk 2001; de Urquiza 2000; Colquhoun 2001). For example, vlcPUFAs are found in many different product applications including formulas for infants, adult dietary supplements, animal feed, food additives and pharmaceutical precursors. These applications represent an extensive market for vlcPUFAs: the world wholesale market for infant formula alone is estimated to be about $10 billion per annum (Ward and Singh 2005).

Animals lack the capability to synthesize vlcPUFAs and therefore these essential fatty acids must be obtained from food/feed. Typically sources of PUFAs are oil-rich fish such as eel, mackerel, herring, salmon and sardines (Ward and Singh 2005). Due to concerns over declining fish stocks and the potential of the fish-oils being contaminated by a range of pollutants, possibilities of obtaining these fatty acids from other sources have been investigated (Qi et al. 2004). Interestingly the vlcPUFAs in the oil-rich fish originate from marine micro-algae that are eaten by the fish. Algal genes encoding relevant enzymes have been identified and recently several groups have reported progress on using these genes to produce DHA and ARA in transgenic plants, including crops such as soybean, linseed, tobacco and the model species *Arabidopsis* (Qi et al. 2004; Abbadi et al. 2004). By adding additional genes to the ones that are needed to produce ARA and EPA, production of DHA has been established in soybean, *Brassica juncea* and *Arabidopsis* (Robert et al. 2005, Wu et al. 2005).

An alternative approach is to use directly the algae that are the most efficient primary producers of the vlcPUFAs. Algae groups that contain vlcPUFAs include diatoms, crysophytes, cryptophytes and dinoflagellates (Cohen et al. 1995; Behrens and Kyle 1996). High amounts of DHA, for example, are produced in the algae *Crypthecodinium cohnii*, *Thraustochytrium spp*., *Schizochytrium spp*., *Isochrysis galbana* and *Crypthecodinium spp*. (Ward and Singh 2005; Patil et al. 2007). The algae, *Porphyridium cruentum* and *Parietochloris incise* accumulate AA (Zhang et al. 2002; Guil-Guerrero et al. 2000) and several species have been suggested for the production of EPA including *Nitzschia spp*., *Nannochloropsis spp*., *Navicula spp*., *Phaeodatylum spp*. and *Porphyridium spp*. (Tan 1996; Sukenik 1991; Molina Grima et al. 2003; Cohen et al. 1995). For additional information about content of vlcPUFAs in different micro-algae see (Barclay et al. 1994; Wen and Chen 2003;

Ward and Singh 2005). A slight inconvenience with using algal feedstocks directly for the production of vlcPUFAs is that in many species the accumulation of these fatty acids involves their presence in lipids other than triacylglycerides such as galactolipids. This makes their isolation more complicated.

Presently companies such as Martek, OmegaTech and Nutrinova are producing DHA from algae (Spolaore et al. 2006). For vlcPUFA production directly from micro-algae it has been estimated that the cost of producing EPA from *Phaeodactylum tricornutum* cultured in photobioreactors is about \$4602 kg^{-1} (Molina Grima et al. 2003).

3.4.5 Colourants

Micro-algae produce of wide variety of carotenoids, with over 40 carotene and xanthophylls isolated and characterized (Jin et al. 2003). Carotenoids (from all sources) have a combined market size near US\$ 1billion. The most simple is β-carotene, found in all algal species as well as other plants. In halophytic *Dunaliella* species it can accumulate to 14% of total dry weight, and several commercial facilities for β-carotene production operate in Australia, Israel, the USA and China (Del Campo et al. 2007). The largest plant (800 hectares) is run by Cognis Nutrition and Health, and produces β-carotene extracts and *Dunaliella* powder for human use and for animal feed. Prices for these products range from US\$ 300 - 3,000 (Borowitzka 1992; Spolaore et al. 2006). Lutein, canthaxanthin, zeaxanthin, lycopene and bixin are also commercially produced carotenoids, but in much smaller amounts (Spolaore et al. 2006). The most interesting carotenoid is astaxanthin, which is produced in significant amounts (1.5 – 4% of dry biomass) by green micro-algae such as *Haematococcus pluvialis*. The synthetic equivalent (95% of the market) is used in aquaculture to give a pink colour to cultured salmon, and has an estimated market size of US\$ 200 Mio with an average price of US\$ 2,500 kg^{-1} (Del Campo et al. 2007).

Although algal astaxanthin is more expensive (US $7,000 per kg is quoted on www.israel21c.org/bin/en.jsp?enZone=Health&enDisplay=view&enPage=BlankPage&enDispWhat=object&enDispWho=Articles%5El986) several commercial plants producing astaxanthin from *H. pluvialis* have been established in the USA, India and Israel (see wwwscieng.murdoch.edu.au/centres/algae/BEAM-Net/BEAM-Appl0.htm, Del Campo et al. 2007). This is possible because the natural but more expensive astaxanthin is preferred or required in specific applications, such as in koi, chicken, and red seabream diets. Nutraceutical grade astaxanthin is priced at over US$ 100,000 kg^{-1}, removing the cost for this particular application (Olaizola 2003). "*To beat the cost of synthetically produced astaxanthin,* Haematococcus *biomass would need to be produced at significantly less than $ 30 kg*$^{-1}$*. … we feel that this low cost cannot be achieved by commercial producers at this time*". Clearly, efforts by numerous companies and research groups to lower the production costs of *Haematococcus* have not met with success, despite the incentive of capturing a US$ 200 Mio market.

Another interesting xanthophyll is lutein that is used for the colouration of drugs and cosmetics. In the US, sales of lutein amount to about US$ 150 Mio annually (Jin et al. 2003). Cultures of the micro-algae *Muriellopsis* can contain up to 35 mg l^{-1} lutein.

Phycobilins or phycobiliproteins are water-soluble accessory pigments. Some are used as fluorescent markers in cell biology. However, the markets in terms of biomass are minuscule as only kg quantities are required for the world market (Benemann and Oswald 1996). Phycobilins are also of interest as colorants for food and cosmetic products. For example, a blue phycobilin from *Arthrospira*, is used to colour cosmetics and food in Japan (Metting 1996).

3.4.6 Materials

Diatoms create nanometer-patterned cell wall structures composed of silica (Figures 3 and 4). As yet, similarly structured materials cannot be made by self-assembling processes under ambient conditions. Ceramics are manufactured at very high temperatures, which prevents the production of composite materials with components sensitive to heat. Understanding how diatoms build their silica cell walls may lead to the development of new processing technologies allowing the production of nanostructured and/or compound materials with properties not available today (Mann and Ozin 1996, Morse 1999, Parkinson and Gordon 1999). For example, Kröger et al. (1999, 2002) discovered a new class of proteins, the sillafins, which are involved in the formation of silica cell walls in diatoms. Addition of the purified proteins to a solution of silicic acid under ambient conditions, resulted in the precipitation of very small silica spheres. This result showed that fundamental biological/biochemical research with diatoms can lead to a better understanding of biological processes as well as to new insights in materials technology. Other researchers proposed the utilisation and manipulation of diatoms to produce specific nanostructures (Parkinson and Gordon 1999, Drum and Gordon 2003). Currently, there is no commercial production of diatoms for biomaterials production.

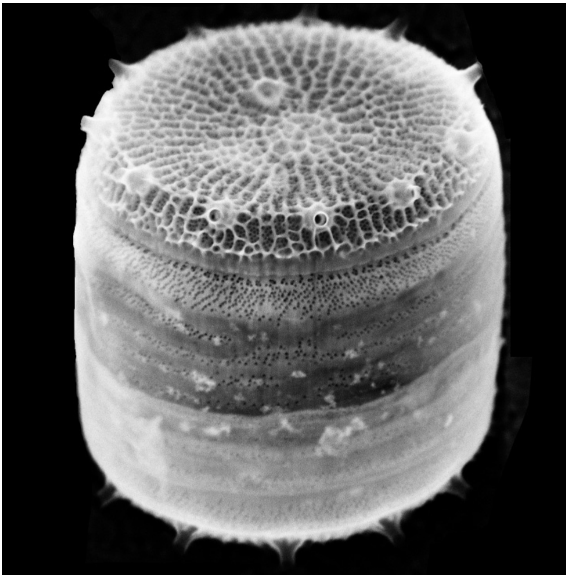

Figure 3. Scanning electron micrograph showing the intricate cell-wall pattern of the diatom *Thalassiosira pseudonana*. Photo by Nils Kröger, Georgia Institute of Technology.

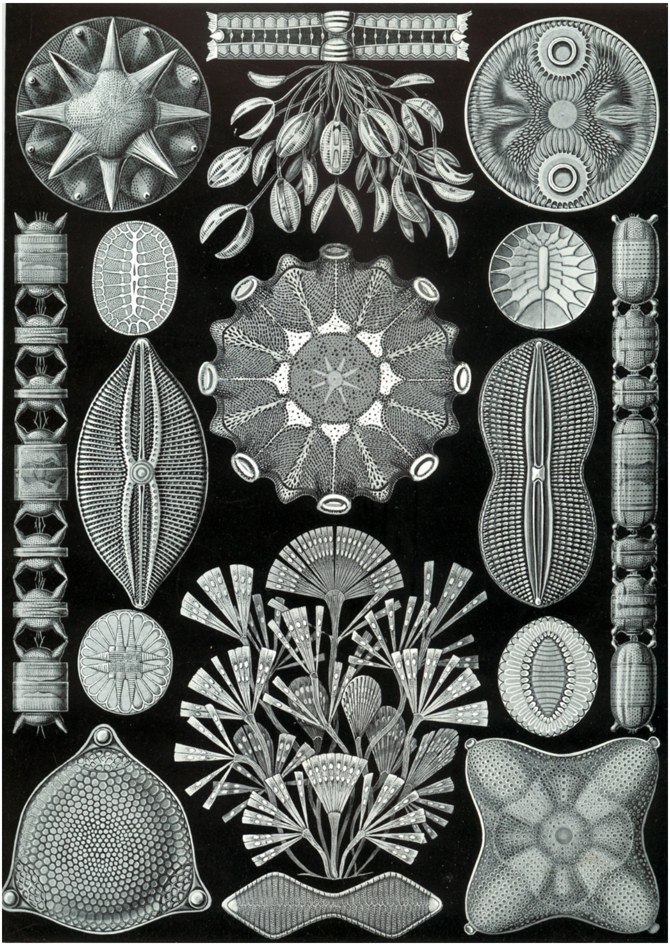

Figure 4. Illustration of diatoms. The 84th plate from Ernst Haeckel's "Kunstformen der Natur" (1904)

It should also be mentioned that diatoms are the most intensively utilised fossil algae, namely in the form of sedimentary rock (diatomite). Because of the diatom structure and their elaborate cell walls these rocks are very porous, have a high surface to volume ratio, and are used as raw material in the manufacture of industrial filters and as fillers in paint.

3.5 Waste water treatment

Macro- and micro-algae can be applied to sequester, remove or transform pollutants such as excess nutrients, xenobiotics, and heavy metals from wastewater, or CO_2 from exhausts. These applications are known as phycoremediation. The treatment processes yield an output in the form of algal biomass that can be used to produce chemicals, biofuels or biogas as by-products (Munoz and Guieysse 2006).

3.5.1 Removal of nutrients

Micro-algae are often applied in the tertiary treatment of domestic wastewater in maturation ponds, or in small-to-middle scale municipal wastewater treatment systems. Systems such as the Advanced Integrated Wastewater Pond Systems (AIWPS) Technology are commercially available (Oswald and Green, LLC) (Oswald 1991). The most common designs include facultative ponds, which are relatively deep and support surface growth of micro-algae, and high-rate algal ponds (HRAPs), which are shallow and depend on mechanical mixing to maximize algal production and removal of biological oxygen demand. HRAPs are the most cost-effective reactors for liquid waste management and capture of solar energy, and are used to treat waste from pig farms. In these systems, productivities of up to 50 t ha^{-1} y^{-1} are feasible. Algal biomass can be harvested from the HRAPs for animal feed, and can be seen as a component of integrated approaches to recycling of livestock wastes, in which algal wastewater treatment is a second step following an initial anaerobic treatment of high-strength organic wastewater (Ogbonna et al. 2000; Olguin 2003). Typically, there is no effort made to control the species composition in wastewater treatment ponds. However, having specific species that sediment, flotate or flocculate efficiently would greatly facilitate harvesting, which is an expensive step.

A Dutch economic evaluation concludes that the use of algae for the production of industrial quality water and algal biomass can be profitable with a reasonable break-even after 2-4 years, with production costs of 0.4 – 0.6 € per m^3, respectively 2 - 4 € kg^{-1} algal dry weight (Reith 2004). Biofuel production in conjunction with wastewater treatment and fertiliser recycling is seen as a near-term application (5 to 10 years), since the algae are already used in wastewater treatment (van Harmelen and Oonk 2006).

3.5.2 Removal of organic pollutants

Chlorella, *Ankistrodesmus* or *Scendesmus* species have been used to treat wastewater containing organic pollutants from pulp and paper mills and olive oil mills (Munoz and Guieysse 2006). However, heterotrophic microalgae are often out-competed by bacteria, since they have lower growth rates. Algae and bacteria, however can be combined to consortia that clean up wastewaters. The production of photosynthetic oxygen by the algae reduces the need for external aeration of the wastewater, which is especially useful when volatile pollutants must be biodegraded aerobically but should not evaporate due to mechanical aeration (Olguin 2003; Munoz and Guieysse 2006). The algae produce oxygen for the bacteria that can degrade hazardous organic pollutants. These treatments have been shown to be possible (reviewed by Munoz and Guieysse 2006). The biomass produced during wastewater treatment is unlikely to be used as a human food source or animal feed. There might be applications for production of high value chemicals. But more likely could the algal-bacterial treatment of wastewater be combined with CO_2 mitigation (see Chapter 3.3.1) and biomethane production.

3.5.3 Removal of heavy metals

Algal biomass can be used as an inexpensive biomaterial for the passive removal of toxic heavy metals. Brown algae have proven to be the most effective and promising substrates due to the biochemical composition of the cell wall, which contains alginate and fucoidan. The relative affinity of raw *Sargassum* biomass for various divalent metal cations was determined at environmentally relevant concentrations to be Cu > Ca > Cd > Zn > Fe (Davis et al. 2003). Also micro-algae have been used to remove heavy metals from waste water (Wilde and Benemann 1993; Perales-Vela et al. 2006).

3.6 Genes with high utility

The genomes of a number of algae have now been fully sequenced. These include *Chlamydomonas reinhardtii*, *Cyanidioschyzon merolae*, *Ostreococcus lucimarinus*, *Ostreococcus tauri*, and *Thalassiosira pseudonana* (Waters and Rioflorido 2007). This sequence information provides major new opportunities for increasing our fundamental understanding of the biology of macro- and micro-algae as well as establishing the molecular foundation for new industrial applications. For example, the genome of *Ostreococcus tauri* (*Prasinophyceae*), a unicellular green alga and the world's smallest free-living eukaryote, revealed a number of interesting features and previously unobserved levels of heterogeneity for any eukaryote known (Derelle et al. 2006). In terms of utility, *Thalassiosira* genes have already contributed to research and commercial efforts to produce vlcPUFAs in transgenic crop plants (Tonon et al. 2004, 2005). It will be increasingly important to develop new molecular tools to gain information about the genes in the algal genomes, particularly through the analysis and modulation of gene function *in vivo*. In this context, recent progress has been made with respect to the development of high throughput analytical methods for the diatom, *Phaeodactylum tricornutum* (Siaut et al. 2007). A number of resources for the study of micro- and macro-algae are in development, including culture collections such as the European collection (www.ccap.ac.uk), comprising strains in the public domain of which 1050 are marine algae and 1300 freshwater algae. These collections will provide immense opportunities for the application of post-genomic technologies to our understanding and application of algal species (Gachon et al. 2007).

4 PLAYERS IN THE FIELD

Research and commercial applications of micro-algae have gained more interest during the last few years. A significant number of companies proposing to use micro-algae for producing biofuels and abating climate change through CO_2 mitigation have been formed. Just recently two Canadian companies announced the formation of a new company with the purpose to convert CO_2 to algae biomass (www.insidegreentech.com/node/1446). In Table 11 some research groups, research programmes, companies and internet resources on micro-algae are listed. In addition the reader is referred to the list of attendees of the 7th European workshop (2007) on micro-algae by the European Society of Microalgae Technology (www.igv-gmbh.de).

Table 11. Research groups and companies involved in micro-algae research and application.

Name	Expertise	Contact
Biotechnology Research Group, Massey University, New Zealand	Biochemical and chemical engineering; bioreactors; downstream processing	Prof. Y. Chisti, Massey University, New Zealand. www.massey.ac.nz
Microalgal Biotechnology Laboratory, Ben Gurion University of the Negev, Israel	Development of biotechnology involved in mass production of micro-algae for various commercial purposes.	bidr.bgu.ac.il/BIDR/research/algal/index.htm
Departmento Ingeniería Química, Universidad de Almería, Spain		web.ual.es/web/pConocenos.jsp?id=6374 (in Spanish)
Instituto Bioquímica Vegetal y Fotosíntesis, Universidad Sevilla-CSIC, Spain	Development of microalgal culture systems for commercial interesting compounds production.	www.ibvf.cartuja.csic.es/main.asp
French Research Institute for Exploitation of the Sea (IFREMER), Laboratory of Physiology and Biotechnology of Algae, Nantes, France	National institute of marine research, the French public institute for marine research, Ifremer contributes, through studies and expert assessments, to knowledge about the ocean and its resources, monitoring of marine and coastal zones and the sustainable development of maritime activities.	www.ifremer.fr/anglais/
Biotechnology of Algae Group (BITAL), University of Huelva, Spain		

Table 11 continued.

Name	Expertise	Contact
Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto		
Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Moscow		
Instituto per lo studio degli ecosistemi – CNR, Florence, Italy		ise.fi.cnr.it
Dipartimento di Biotecnologie Agrarie, Universita degli studi di Firenze, Florence, Italy		
Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic	The research is aimed at optimizing the technologies of algal production and of processing the product as well as on various practical uses of algae	147.231.249.2/en/
Laboratory of Biochemical engineering, Federal Universty Foundation of Rio Grande, Brazil		
Food and Bioprocess Engineering Group, Department of Agrotechnology and Food Science, Wageningen University, The Netherlands	Photobioreactors and marine invertebrates	www.marine.wur.nl
University of Nantes, CNRS, GEPEA, Saint Nazaire Cedex, France		
Institute of biological sciences, applied ecology, University of Rostock, Germany		

Table 11 continued.

Name	Expertise	Contact
Albrecht-von-Haller Institut für Pflanzenwissenschaften, Abteilung experimentelle Phykologie und Sammlung von Algenkulturen, University Göttingen, Germany	Research and teaching on the diversity of algae	www.epsag.uni-goettingen.de
IGV Institut für Getreideverarbeitung GmbH, Nuthetal, Germany	Research and engineering of photobioreactors	www.igv-gmbh.de
Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany	Research on photosystems and hydrogen production	www.mpimp-golm.mpg.de
Institute of Cell Physiology, University of Bielefeld, Germany		www.uni-bielefeld.de/biologie/Algenbiotechnologie/kruse/
Integrative Biology, University of Queensland, Australia		
Institute of Engineering in Life Sciences, Department of Bioprocess Engineering, University of Karlsruhe, Germany		
Ruhr-Universität Bochum, Germany	The working group deals with the basic questions of the biochemistry, genetic and biotechnology of photosynthetic microorganism	www.ruhr-uni-bochum.de/pbt/

Table 11 continued.

Name	Expertise	Contact
European Research Centre for Algae (CEVA)	As a Technical Centre, the CEVA is unique in Europe, it provides services for companies interested in finalizing and developing industrial products with marine based ingredients: macro-algae, micro-algae, marine plants and sea-water, and for the local municipalities faced with the problems of increasing amounts of washed-up seaweeds and sea plants.	www.ceva.fr/en/ceva/domaines.htm
Institute for Molecular Bioscience, University of Queensland, Australia		

Research programmes

Name	Expertise	Contact
SARDI	$950,000 research program into the development of micro-algae as a biodiesel feedstock	www.sardi.sa.gov.au/pages/biofuels/biofuels_research_program.htm:sectID=877&tempID=1
International Network on Biofixation of CO_2 and greenhouse gas abatement with micro-algae		

Table 11 continued.

Name	**Expertise**	**Contact**
Alginet	Project funded by the 5th framework programme of the EU	217.114.171.142/alginet/index.html Contact: www.biozoon.com
BEAM Research Network	The network links researchers from around Australia and key overseas groups studying microalgal physiology, biochemistry, molecular biology, ecology and photobioreactor design with emphasis on applied outcomes	Prof. M.A. Borowitzka, Murdoch University, Australia. wwwscieng.murdoch.edu.au/centres/algae/BEAM-Net/BEAM.html
Companies		
Name	**Expertise**	**Contact**
BlueBiotech International GmbH	Biotechnology of micro-algae, pharmaceutical products	www.bluebiotech.de
Greenfuel Technologies Corporation	Biodiesel	www.greenfuelonline.com
Solazyme	Biotechnology company. Bioengineering of algal strains.	www.solazyme.com
Petroalgae	Aim: Oil production from algae; R&D site in Florida (4 acre) to validate lab results in field (Feb. 2007)	www.petroalgae.com

Table 11 continued.

Name	Expertise	Contact
Betatene	β-carotene production	www.betatene.com.au
Western Biotechnology	β-carotene production	www.cognis.com
AquaCarotene	β-carotene production	www.aquacarotene.com
Cyanotech	β-carotene and astaxanthin	www.cyanotech.com
Nature Beta Technologies	β-carotene production	www.chlostanin.co.jp
Inner Mongolia Biological Eng.	β-carotene production	not available
Tianjin Lantai Biotechnology	β-carotene production	not available
Algatechnologies	Astaxanthin production	www.algatech.com
Mera Pharmaceuticals	Astaxanthin production	www.merapharma.com
Fuji Health Science (BioReal Inc. (USA) and BioReal AB (Sweden)	Astaxanthin production	www.fujihealthscience.com/astaxanthin.html
Parry Neutraceuticlas	Astaxanthin, mixed carotenoids	www.parrynutraceuticals.com
Aquaflow	Produce algae in open ponds and waste water for biofuel	aquaflowgroupcom.axiion.com/Home
Solix	To produce algae in photo-bioreactors for biofuel	www.solixbiofuels.com
GS CleanTech Inc	Capture CO_2 with algae in photobioreactor	www.gs-cleantech.com/product_desc.php?mode=3
Green Star Products, Inc.		www.greenstarusa.com/news/06-11-13.html

Table 11 continued.

Name	**Expertise**	**Contact**
IGV, Potsdam, Germany	Research and manufacture of photobioreactors, and characterisation of produced substances.	www.igv-gmbh.de/index_e.htm
Global Green Solutions Inc.	Uses vertical bioreactors to mass produce algae for oil to biodiesel	www.globalgreensolutionsinc.com/s/Home.asp
Internet resources		
Name	**Expertise**	**Contact**
Oilgae	Blog for discussing oil and biodiesel production from algae	www.oilgae.com/blog/2007/02/microalgae-biotechnology-and.html
PESWiki	Resource that focuses on alternative, clean, practical, renewable energy solutions	www.peswiki.com/index.php/Directory:Biodiesel_from_Algae_Oil
Internet resources list	An annotated selection of World Wide Web sites relevant to the topics in Environmental Microbiology	Wackett (2007)

5 CONCLUSIONS

The macro and micro algal populations of the aquatic environments provide a vast genetic resource and biodiversity. This feature alone suggests that these organisms have considerable potential for offering new chemicals, materials and bioactive compounds. The completion of the genome sequencing programmes of two micro-algae also opens up major opportunities for new applications, either using the algae themselves or through using the genes in other production systems, whether fermenter-based or fields.

The culture of micro-algae has been studied widely through their potential for greenhouse gas abatement and this information is detailed in many reviews cited in this report. There are many conflicting statements on the potential of micro-algae for high biomass production, but there is a general agreement that the current production systems are not economically viable for biomass production alone. The difficulties include high capital infrastructure costs, problems of contamination through open pond systems and costs associated with harvesting and drying. These costs adversely affect the competitiveness of aquatic biomass production systems, compared to land-based agriculture and forestry.

Thus these negative cost considerations currently preclude the widespread use of micro-algae for biofuel production or production of other forms of bioenergy. Similarly, the macro-algal seaweeds, whilst used for some specialised applications, are also expensive to farm and harvest offshore. There are few clear drivers for using these species as biomass for bioenergy, except in specific circumstances such as maritime communities with no access to productive agricultural land or alternative energy sources.

The increasing concerns of global climate change and rising levels of atmospheric carbon dioxide have led to the recognition that carbon sequestration alone can have a tangible economic value. The value placed on a tonne of carbon within current trading schemes will determine decisions on how best to cost effectively ‘manufacture’ this product. There may be conditions in the future that would support the use of aquatic and particularly marine organisms for carbon capture and income generated through this route.

Additional value products from the micro-algae, such as chemicals, can increase the cost competitiveness. Often these are manufactured by the cells following a stress shock and under low nutrient conditions. For example, there has been a study in which the production of astaxanthin has been shown to be commercially viable using a microalgal innoculum established in photobioreactors and transferred to open ponds for three day cultivation of biomass prior to harvest of product. This system successfully avoided the problems of contamination found in open pond cultivation systems since the cycle was extremely short.

Using micro-algae for waste water treatment is not a new idea. However, combining the ability of the cells to remediate water with their use for carbon sequestration or energy production may offer an economically viable way forward for the development of multiple products.

This report highlights the need to consider carefully the economics of using organisms of the aquatic environment for industrial production.

6 INSTITUTIONS, ORGANISATIONS AND PERSONS CONTACTED

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7 REFERENCES

Abbadi A, Domergue F, Bauer J, Napier JA, Welti R, Ohringer U, Cirpus P, Heinz E (2004) Biosynthesis of very-long-chain polyunsaturated fatty acids in transgenic oilseeds: Constraints on their accumulation. Plant Cell 16: 2734-2746

Acien Fernandez FGA, Camacho FG, Perez JAS, Sevilla JMF, Grima EM (1998) Modeling of biomass productivity in tubular photobioreactors for microalgal cultures: Effects of dilution rate, tube diameter, and solar irradiance. Biotechnology and Bioengineering 58: 605-616

Acien Fernandez FGA, Hall DO, Guerrero EC, Rao KK, Grima EM (2003) Outdoor production of Phaeodactylum tricornutum biomass in a helical reactor. Journal of Biotechnology 103: 137-152

Amos WA (2004) Updated cost analysis of photobiological hydrogen production from *Chlamydomonas reinhardtii* green algae. NREL/MP-560-35593. National Renewable Energy Laboratory

Banerjee A, Sharma R, Chisti Y, Banerjee UC (2002) Botryococcus braunii: A renewable source of hydrocarbons and other chemicals. Critical Reviews in Biotechnology 22: 245-279

Barclay WR, Meager KM, Abril JR (1994) Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. Journal of Applied Phycology 6: 123-129

Becker EW (2007) Micro-algae as a source of protein. Biotechnology Advances 25: 207-210

Behrens PW, Kyle DJ (1996) Microalgae as a source of fatty acids. Journal of Food Lipids 3: 259-272

Ben-Amotz A, Tornabene TG (1985) Chemical profile of selected species of macroalgae with emphasis on lipids. Journal of Phycology 21: 72-81

Benemann J, Oswald WJ (1996) Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. US DOE, Pitburgh Energy Technology Centre.

Benemann JR (1997) CO_2 mitigation with microalgae systems. Energy Conversion and Management 38: S475-S479

Biopact (2007) biopact.com/2007/07/scientist-skeptical-of-algae-to.html

Bird KT (1987) Cost analyses of energy from marine biomass. In KT Bird, PH Benson, eds, Seaweed cultivation for renewable resources. Elsevier, Amsterdam, pp 327-350

Bird KT, Chynoweth DP, Jerger DE (1990) Effects of marine algal proximate composition on methane yields. Journal of Applied Phycology 2: 207-213

Blanco AM, Moreno J, Del Campo JA, Rivas J, Guerrero JLG (2007) Outdoor cultivation of lutein-rich cells of Muriellopsis sp in open ponds. Applied Microbiology and Biotechnology 73: 1259-1266

Borowitzka MA (1999) Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of Biotechnology 70: 313-321

Borowitzka MA (1988) Microalgae as sources of pharmaceuticals and other biologically active compounds. Journal of Applied Phycology 4: 267-279

Borowitzka MA (1992) Algal Biotechnology Products and Processes - Matching Science and Economics. Journal of Applied Phycology 4: 267-279

Bosma R, van Spronsen WA, Tramper J, Wijffels RH (2003) Ultrasound, a new separation technique to harvest microalgae. Journal of Applied Phycology 15: 143-153

Brinkhuis BH, Levine HG (1987) Laminaria cultivation in the Far East and North America. In KT Bird, PH Benson, eds, Seaweed cultivation for renewable resources. Elsevier, Amsterdam, pp 107-146

Brown MR, Dunstan GA, Norwood SJ, Miller KA (1996) Effects of harvested stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. Journal of Phycology 32: 64-73

Buck BC, Buchholz CM (2004) The offshore ring: A new system design for the open ocean aquaculture of macroalgae. Journal of Applied Phycology 16: 355-369

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: A review of enclosed system designs and performances. Biotechnology Progress 22: 1490-1506

Chaumont D (1993) Biotechnology of algal biomass production: a review of systems for outdoor mass culture. Journal of Applied Phycology 5: 593-604

Chisti Y (2007) Biodiesel from microalgae. Biotechnology Advances 25: 294-306

Choi SL, Suh IS, Lee CG (2003) Lumostatic operation of bubble column photobioreactors for Haematococcus pluvialis cultures using a specific light uptake rate as a control parameter. Enzyme and Microbial Technology 33: 403-409

Chynoweth DP (2002) Review of biomethane from marine biomass. University of Florida.

Chynoweth DP, Turick CE, Owens JM, Jerger DE, Peck MW (1993) Biochemical methane potential of biomass and waste feedstocks. Biomass & Bioenergy 5: 95-111

Clifton-Brown JC, Lewandowski I, Andersson B, Basch G, Christian DG, Bonderup Kjeldsen J, Jorgensen U, Mortensen JV, Riche AB, Schwarz K-U, Tayebi K, Teixeira F (2001) Performance of 15 *Miscanthus* genotypes at five sites in Europe. Agronomy Journal 93: 1013-1019

Cohen Z, Norman HA, Heimer YM (1995) Microalgae as a source of omega 3 fatty acids. World review of nutrition and dietetics 77: 1-31

Colquhoun DM (2001) Nutraceuticals: Vitamins and other nutrients in coronary heart disease. Current Opinion in Lipidology 12: 639-646

Costa JAV, Colla LM, Duarte P (2003) Spirulina platensis growth in open raceway ponds using fresh water supplemented with carbon, nitrogen and metal ions. Zeitschrift fur Naturforschung C-A Journal of Biosciences 58: 76-80

Csordas A, Wang JK (2004) An integrated photobioreactor and foam fractionation unit for the growth and harvest of Chaetoceros spp. in open systems. Aquacultural Engineering 30: 15-30

Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Research 37: 4311-4330

Del Campo JA, Garcia-Gonzales M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Applied Microbiology and Biotechnology 74: 1163-1174

Demirbas A (2006) Oily products from mosses and algae via pyrolysis. Energy Sources Part A-Recovery Utilization and Environmental Effects 28: 933-940

Demirbas A (2000) Mechanisms of liquefaction and pyrolysis reactions of biomass. Energy Conversion and Management 41: 633-646

Derelle E, Ferraz C, Rombauts S, Rouze P, Worden AZ, Robbens S, Partensky F, Degroeve S, Echeynie S, Cooke R, Saeys Y, Wuyts J, Jabbari K, Bowler C, Panaud O, gu B, Ball SG, Ral JP, Bouget FY, Piganeau G, De Baets B, card A, Delseny M, Demaille J, Van De Peer Y, Moreau H (2006) Genome analysis of the smallest free-living eukaryote Ostreococcus tauri unveils many unique features. Proceedings of the National Academy of Sciences of the United States of America 103: 11647-11652

De Urquiza AM, Liu S, Sjoberg M, Zetterstrom RH, Griffiths W, Sjovall J, Perlmann T (2000) Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290: 2140-2144

Dijkstra AJ (2006) Revisiting the formation of trans isomers during partial hydrogenation of triacylglycerol oils. European Journal of Lipid Science and Technology 108: 249-264

Dote Y, Sawayama S, Inoue S, Minowa T, Yokoyama S (1994) Recovery of Liquid Fuel from Hydrocarbon-Rich Microalgae by Thermochemical Liquefaction. Fuel 73: 1855-1857

Drum RW, Gordon R (2003) Star Trek replicators and diatom nanotechnology. Trends in Biotechnology 21: 325-328

Fuentes MMR, Sanchez JLG, Sevilla JMF, Fernandez FGA, Perez JAS, Grima EM (1999) Outdoor continuous culture of Porphyridium cruentum in a tubular photobioreactor: quantitative analysis of the daily cyclic variation of culture parameters. Journal of Biotechnology 70: 271-288

Funk CD (2001) Prostaglandins and leukotrienes: Advances in eicosanoid biology. Science 294: 1871-1875

Gachon CMM, Day JG, Campbell CM, Proschold T, Saxon RJ, Kupper FC (2007) The Culture Collection of Algae and Protozoa (CCAP): A biological resource for protistan genomics. Gene doi:10.1016/j.gene.2007.05.018

Gao K, McKinley KR (1994) Use of macroalgae for marine biomass production - a review. Journal of Applied Phycology 6: 45-60

Garcia-Gonzalez M, Moreno J, Manzano JC, Florencio FJ, Guerrero MG (2005) Production of Dunaliella salina biomass rich in 9-cis-beta-carotene and lutein in a closed tubular photobioreactor. Journal of Biotechnology 115: 81-90

Ginzburg B (1993) Liquid fuel (oil) from halophilic algae: a renewable source of non-polluting energy. Renewable Energy 3: 249-252

Grobbelaar JU (2000) Physiological and technological considerations for optimising mass algal cultures. Journal of Applied Phycology 12: 201-206

Guil-Guerrero JL, Belarbi EH, Rebolloso-Fuentes MM (2000) Eicosapentaenoic and arachidonic acids purification from the red microalga Porphyridium cruentum. Bioseparation 9: 299-306

Habyarimana E, Laureti D, De Ninno M, Lorenzoni C (2004) Performances of biomass sorghum [Sorghum bicolor (L.) Moench] under different water regimes in Mediterrean region. Industrial Crops and Products 20: 23-28

Haesman M, Diemar J, O'Connor W, Soushames T, Foulkes L (2000) Development of extended shelf-life microalgae concentrate diets harvested by centrifugation for bivalve molluscs - a summary. Aquaculture Research 31: 637-659

Hall DO, House JI (1993) Reducing Atmospheric CO_2 Using Biomass Energy and Photobiology. Energy Conversion and Management 34: 889-896

Hallenbeck PC, Benemann JR (2002) Biological hydrogen production; fundamentals and limiting processes. International Journal of Hydrogen Energy 27: 1185-1193

Hanagata N, Takeuchi T, Fukuju Y, Barnes DJ, Karube I (1992) Tolerance of Microalgae to High Co-2 and High-Temperature. Phytochemistry 31: 3345-3348

Hankammer B, Lehr F, Rupprecht J, Mussgnug JH, Posten C, Kruse O (2007) Photosynthetic biomass and H production by green algae: from bioengineering to bioreactor scale up. Physiologia Plantarum in press

Hase R, Oikawa H, Sasao C, Morita M, Watanabe Y (2000) Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. Journal of Bioscience and Bioengineering 89: 157-163

Heaton E, Voigt T, Long SP (2004) A quantitative review comparing the yields of two candidate C_4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass and Bioenergy 27: 21-30

Hejazi MA, Wijffels RH (2004) Milking of microalgae. Trends in Biotechnology 22: 189-194

Hu Q, Zhang C, Sommerfeld M (2006) Biodiesel from algae: Lessons learned over the past 60 years and future perspectives. Journal of Phycology 42: 12

Hughes E, Benemann JR (1997) Biological fossil CO_2 mitigation. Energy Conversion and Management 38: S467-S473

Huntley M, Redalje DG (2007) CO_2 Mitigation and Renewable Oil from Photosynthetic Microbes: A New Appraisal. Mitigation and Adaptation Strategies for Global Change 12: 573-608

Indergaard M, Østgaard K (1991) Polysaccharides for food and pharmaceutical uses. In MD Guiry, G Blunden, eds, Seaweed resources in Europe. John Wiley & Sons, Chichester, pp 169-183

Iwasaki I, Kurano N, Miyachi S (1996) Effects of high-CO_2 stress on photosystem II in a green alga, Chlorococcum littorale, which has a tolerance to high CO_2. Journal of Photochemistry and Photobiology B-Biology 36: 327-332

Jackson A (2006) The importance of fish meal and fish oil in aquaculture diets. International Aquafeed November-December: 16-19

Jang ES, Jung MY, Min DB (2005) Hydrogenation for low trans and high conjugated fatty acids. Comprehensive Reviews in Food Science and Food Safety 4: 22-30

Janssen M, Tramper J, Mur LR, Wijffels RH (2003) Enclosed outdoor photobioreactors: light regime, photosynthetic efficiency, scale-up and future prospects. Biotechnology and Bioengineering 81: 193-210

Jiang Y, Chen F (1999) Effects of salinity on cell growth and docosahexaenoic acid content of the heterotrophic marine microalga Crypthecodinium cohnii. Journal of Industrial Microbiology and Biotechnology 23: 508-513

Jimenez C, Cossio BR, Labella D, Niell FX (2003) The Feasibility of industrial production of Spirulina (Arthrospira) in Southern Spain. Aquaculture 217: 179-190

Jin E, Polle JEW, Lee HK, Hyun SM, Chang M (2003) Xanthophylls in microalgae: From biosynthesis to biotechnological mass production and application. Journal of Microbiology and Biotechnology 13: 165-174

Jin ES, Melis A (2003) Microalgal biotechnology: Carotenoid production by the green algae Dunaliella salina. Biotechnology and Bioprocess Engineering 8: 331-337

Karube I, Takeuchi T, Barnes D (1992) Biotechnological reduction of carbon dioxide emissions. Scheper, T (Ed) Advances in Biochemical Engineering-Biotechnology, Vol 46 Modern biochemical engineering; Springer-Verlag: Berlin, New York

Kenter C, Hoffmann CM, Marlander B (2006) Effects of weather variables on sugar beet yield development (*Beta vulgaris* L.). European Journal of Agronomy 24: 62-69

Keoleian GA, Volk TA (2005) Renewable energy from willow biomass crops: life cycle energy, environmental and economic performance. Critical Reviews in Plant Sciences 24: 385-406

Kerner KN, Hanssen JF, Pedersen TA (1991) Anaerobic digestion of waste sludges from the alginate extraction process. Bioresource Technology 37: 17-24

Kishimoto M, Okakura T, Nagashima H, Minowa T, Yokoyama S, Yamaberi K (1994) CO_2 Fixation and Oil Production Using Microalgae. Journal of Fermentation and Bioengineering 78: 479-482

Knuckey RM, Brown MR, Robert R, Frampton DMF (2006) Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering 35: 300-313

Kroger N, Deutzmann R, Sumper M (1999) Polycationic peptides from diatom biosilica that direct silica nanosphere formation. Science 286: 1129-1132

Kroger N, Lorenz S, Brunner E, Sumper M (2002) Self-assembly of highly phosphorylated silaffins and their function in biosilica morphogenesis. Science 298: 584-586

Kruse O, Rupprecht J, Mussgnug JR, Dismukes GC, Hankamer B (2005) Photosynthesis: a blueprint for solar energy capture and biohydrogen production technologies. Photochemical & Photobiological Sciences 4: 957-970

Lahaye M, Robic A (2007) Structure and functional properties of Ulvan, a polysaccharide from green seaweeds. Biomacromolecules DOI 10.1021/bm061185q:

Laws EA, Taguchi S, Hirata J, Pang L (1986) high algal production-rates achieved in a shallow outdoor flume. Biotechnology and Bioengineering 28: 191-197

Lee JS, Lee JP (2003) Review of advances in biological CO_2 mitigation technology. Biotechnology and Bioprocess Engineering 8: 354-359

Lee YK (1997) Commercial production of microalgae in the Asia-Pacific rim. Journal of Applied Phycology 9: 403-411

Lee YK (2001) Microalgal mass culture systems and methods: Their limitation and potential. Journal of Applied Phycology 13: 307-315

Lee YK, Low CS (1991) Effect of photobioreactor inclination on the biomass productivity of an outdoor algal culture. Biotechnology and Bioengineering 38: 995-1000

Legrand R (1993) Methane From Biomass Systems Analysis and CO_2 Abatement Potential. Biomass & Bioenergy 5: 301-316

Levin DB, Pitt L, Love M (2004) Biohydrogen production: prospects and limitations to practical application. International Journal of Hydrogen Energy 29: 173-185

Lewis JG, Stanley NF, Guist GG (1988) Commercial production and applications of algal hydrocolloids. In CA LEMBI, JR Waaland, eds, Algae and human affairs. Cambridge University Press, Cambridge, pp 205-236

Li X, Xu H, Wu Q (2007) Large-scale biodiesel production from microalga Chlorella protothecoids through heterotrophic cultivation in bioreactors. Biotechnology and Bioengineering. DOI 10.1002/bit. 21489

Luning K, Pang SJ (2003) Mass cultivation of seaweeds: current aspects and approaches. Journal of Applied Phycology 15: 115-119

Mackie W, Preston RD (1974) Cell wall and intercellular region polysaccharides. In WDP Stewart, ed, Algal Physiology and Biochemistry. Blackwell Scientific Publications, Oxford, pp 40-85

Mann KH (1973) Seaweeds: their productivity and strategy for growth. Science 182: 975-981

Mann S, Ozin GA (1996) Synthesis of inorganic materials with complex form. Nature 382: 313-318

McHugh DJ (2003) A guide to the seaweed industry. Rome, FAO. FAO Fisheries Technical Paper No. 441

Metting B, Pyne JW (1986) Biologically-Active Compounds from Microalgae. Enzyme and Microbial Technology 8: 386-394

Metting FB (1996) Biodiversity and application of microalgae. Journal of Industrial Microbiology & Biotechnology 17: 477-489

Metzger P, Largeau C (2005) Botryococcus braunii: a rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology 66: 486-496

Miao X, Wu Q (2004) High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. Journal of Biotechnology 110: 85-93

Miao X, Wu Q, Yang CY (2004) Fast pyrolysis of microalgae to produce renewable fuels. Journal of Analytical and Applied Pyrolysis 71: 855-863

Moheimani NR, Borowitzka MA (2006) The long-term culture of the coccolithophore *Pleurochrysis carterae* (Haptophyta) in outdoor raceway ponds. Journal of Applied Phycology 18: 703-712

Molina Grima EM, Perez JAS, Camacho FG, Sevilla JMF, Fernandez FGA (1994) Effect of Growth-Rate on the Eicosapentaenoic Acid and Docosahexaenoic Acid Content of Isochrysis-Galbana in Chemostat Culture. Applied Microbiology and Biotechnology 41: 23-27

Molina Grima EM, Belarbi EH, Fernandez FGA, Medina AR, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnology Advances 20: 491-515

Moore A (2001) Blooming prospects? EMBO reports 2: 462-464

Morand P, Carpentier B, Charlier RH, Maze J, Orlandini M, Plunkett BA, de Waart J (1991) Bioconversion of seaweeds. In MD Guiry, G Blunden, eds, Seaweed resources in Europe. John Wiley & Sons, Chichester, pp 95-148

Morse DE (1999) Silicon biotechnology: harnessing biological silica production to construct new materials. TIBTech 17: 230-232

Muchow RC, Spillman MF, Wood AW, Thomas MR (1994) Radiation interception and biomass accumulation in a sugarcane crop grown under irrigated tropical conditions. Australian Journal of Agricultural Research 45: 37-49

Munoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water Research 40: 2799-2815

Mussgnug JH, Thomas-Hall S, Rupprecht J, Foo A, Klassen V, McDowall A, Schenk PM, Kruse O, Hankamer B (2007) Engineering photosynthetic light capture: impacts on improved solar energy to biomass conversion. Plant Biotechnology Journal. doi: 10.1111/j.1467-7652.2007.00285.x

Negoro M, Shioji N, Miyamoto K, Miura Y (1991) Growth of Microalgae in High CO_2 Gas and Effects of Sox and Nox. Applied Biochemistry and Biotechnology 28-9: 877-886

Neumann K, Stephan DP, Ziegler K, Huhns M, Broer I, Lockau W, Pistorius EK (2005) Production of cyanophycin, a suitable source for the biodegradable polymer polyaspartate, in transgenic plants. Plant Biotechnology Journal 3: 249-258

Ogbonna JC, Yoshizawa H, Tanaka H (2000) Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. Journal of Applied Phycology 12: 277-284

Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomolecular Engineering 20: 459-466

Olguin EJ (2003) Phycoremediation: Key issues for cost-effective nutrient removal processes. Biotechnology Advances 22: 81-91

Ono E, Cuello JL (2007) Carbon dioxide mitigation using thermophilic cyanobacteria. Biosystems Engineering 96: 129-134

Oswald WJ (1991) Introduction to advances integrated waste-water ponding systems. Water Science and Technology 24: 1-7

Parkinson J, Gordon R (1999) Beyond micromachining: the potential of diatoms. Trends in Biotechnology 17: 190-196

Patil V, Kallqvist T, Olsen E, Vogt G, Gislerod HR (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture International 15: 1-9

Peng WM, Wu QY, Tu PG (2000) Effects of temperature and holding time on production of renewable fuels from pyrolysis of Chlorella protothecoides. Journal of Applied Phycology 12: 147-152

Peng WM, Wu QY, Tu PG (2001a) Pyrolytic characteristics of heterotrophic Chlorella protothecoides for renewable bio-fuel production. Journal of Applied Phycology 13: 5-12

Peng WM, Wu QY, Tu PG, Zhao NM (2001b) Pyrolytic characteristics of microalgae as renewable energy source determined by thermogravimetric analysis. Bioresource Technology 80: 1-7

Perales-Vela HV, Pena-Castro JM, Canizares-Villanueva RO (2006) Heavy metal detoxification in eukaryotic microalgae. Chemosphere 64: 1-10

Percival E (1979) The polysaccharides of green, red and brown seaweeds: their basic structure, biosynthesis and function. British Phycological Journal 14: 103-117

Pérez R (1997) Ces algues qui nous entourent. Conception actuelle, role dans la biosphere, utilizations, culture. Editions Efrimer

Pirt SJ, Lee YK, Richmond A, Pirt MW (1980) Photosynthetic Efficiency of Chlorella Biomass Growth with Reference to Solar-Energy Utilization. Journal of Chemical Technology and Biotechnology 30: 25-34

Poelman E, DePauw N, Jeurissen B (1997) Potential of electrolytic flocculation for recovery of micro-algae. Resources Conservation and Recycling 19: 1-10

Prince RC, Kheshgi HS (2005) The photobiological production of hydrogen: Potential efficiency and effectiveness as a renewable fuel. Critical Reviews in Microbiology 31: 19-31

Programme (2007) IGGRD. International Network on Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae. Retrieved June 13, 2007 from www.co2captureandstorage.info/ networks/Biofixation.htm.

Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Applied Microbiology and Biotechnology 57: 287-293

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 65: 635-648

Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart AK, Lazarus CM (2004) Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nature Biotechnology 22: 739-745

Radmer RJ (1996) Algal diversity and commercial algal products. Bioscience 46: 263-270

Reith JH (2004) Duurzame co-productie van fijnchemicalien en energie uit microalgen. ECN.

Reith JH, BDeurwaarder BP, Hemmes K, Curvers APWM, Brandeburg W, Zeeman G (2005) Bio-offshore. Grootschalige teelt van zeewieren in combinatie met offshore windparken in de Noordzee. ECN.

Reith JH, Steketee J, Brandeburg W, Sijtsma L (2006) Platform Groene Grondstoffen. Werkgroep 1: duurzame productie en ontwikkeling van biomassa. Deelpad: aquatische biomassa.

Renn D (1997) Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects. Trends in Biotechnology 15 (1): 9-14.

Richmond A (2000) Microalgal biotechnology at the turn of the millennium: A personal view. Journal of Applied Phycology 12: 441-451

Richmond A (2004) Principles for attaining maximal microalgal productivity in photobioreactors: an overview. Hydrobiologia 512: 33-37

Robert SS, Singh SP, Zhou XR, Petrie JR, Blackburn SI, Mansour MP, Nichols PD, Liu Q, Green AG (2005) Metabolic engineering of Arabidopsis to produce nutritionally important DHA in seed oil. Functional Plant Biology 32: 473-479

Rossignol N, Lebeau T, Jaouen P, Robert JM (2000) Comparison of two membrane - photobioreactors, with free or immobilized cells, for the production of pigments by a marine diatom. Bioprocess Engineering 23: 495-501

Rupprecht J, Hankamer B, Mussgnug JH, Ananyev G, Dismukes GC, Kruse O (2006) Perspectives and advances of biological H2 production in microorganisms. Applied Microbiology and Biotechnology 72: 442-449

Samson R, Mani S, Boddey R, Sokhansanj S, Quesada D, Urquiaga S, Reis V, Lem CH (2005) The potential of C4 perennial grasses for developing a global BIOHEAT Industry. Critical Reviews in Plant Sciences 24: 461-495

Sanchez Miron A, Contreras Gomez A, Garcia Camacho F, Molina Grima E, Chisti Y (1999) Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. Journal of Biotechnology 70: 249-270

Sawayama S, Minowa T, Yokoyama SY (1999) Possibility of renewable energy production and CO_2 mitigation by thermochemical liquefaction of microalgae. Biomass & Bioenergy 17: 33-39

Schneider D (2006) Grow your own? American Scientist 94: 408-409

Scragg AH, Illman AM, Carden A, Shales SW (2002) Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass & Bioenergy 23: 67-73

Scragg AH, Morrison J, Shales SW (2003) The use of a fuel containing Chlorella vulgaris in a diesel engine. Enzyme and Microbial Technology 33: 884-889

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's aquatic species program—biodiesel from algae. NREL/TP-580-24190. U.S. Department of Energy's Office of Fuels Development.

Siaut M, Heijde M, Mangogna M, Montsant A, Coesel S, Allen A, Manfredonia A, Falciatore A, Bowler C (2007) Molecular toolbox for studying diatom biology in *Phaeodactylum tricornutum*. Gene doi:10.1016/j.gene.2007.05.022

Siegel BZ, Siegel SM (1973) The chemical composition of algal cell walls. CRC Critical Reviews in Microbiology 3: 1-26

Singh S, Kate BN, Banerjee UC (2005) Bioactive compounds from cyanobacteria and microalgae: An overview. Critical Reviews in Biotechnology 25: 73-95

Skjåk-Bræk G, Martinsen A (1991) Applications of some algal polysaccharides in biotechnology. In MD Guiry, G Blunden, eds, Seaweed resources in Europe. John Wiley & Sons, Chichsester, pp 219-257

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. Journal of Bioscience and Bioengineering 101: 87-96

Suh IS, Lee CG (2003) Photobioreactor engineering: design and performance. Biotechnology and Bioprocess Engineering 8: 313-321

Sukenik A (1991) Ecophysiological considerations in the optimization of eicosapentaenoic acid production by Nannochloropsis sp. (Eustigmatophyceae). Bioresource Technology 35: 263-269

Sung KD, Lee JS, Shin CS, Park SC, Choi MJ (1999) CO_2 fixation by Chlorella sp. KR-1 and its cultural characteristics. Bioresource Technology 68: 269-273

Tamiya H (1957) Mass Culture of Algae. Annual Review of Plant Physiology and Plant Molecular Biology 8: 309-334

Tan CK, Johns MR (1996) Screening of diatoms for heterotrophic eicosapentaenoic acid production. Journal of Applied Phycology 8: 59-64

Tonon T, Harvey D, Qing R, Li Y, Larson TR, Graham IA (2004) Identification of a fatty acid ?11-desaturase from the microalga Thalassiosira pseudonana. FEBS Letters 563: 28-34

Tonon T, Qing R, Harvey D, Li Y, Larson TR, Graham IA (2005) Identification of A long-chain polyunsaturated fatty acid acyl-coenzyme a synthetase from the diatom Thalassiosira pseudonana. Plant Physiology 138: 402-408

Torzillo G, Pushparaj B, Bocci F, Balloni W, Materassi R, Florenzano G (1986) Production of Spirulina Biomass in Closed Photobioreactors. Biomass 11: 61-74

Torzillo G, Pushparaj B, Masojidek J, Vonshak A (2003) Biological constraints in algal biotechnology. Biotechnology and Bioprocess Engineering 8: 338-348

Tsukahara K, Sawayama S (2005) Liquid fuel production using microalgae. Journal of the Japan Petroleum Institute 48: 251-259

Valenzuela-Espinoza E, Millan-Nunez R, Nunez-Cebrero F (2002) Protein, carbohydrate, lipid and chlorophyll alpha content in Isochrysis aff. galbana (clone T-Iso) cultured with a low cost alternative to the f/2 medium. Aquacultural Engineering 25: 207-216

van Harmelen T, Oonk H (2006) Microalgae biofixation processes: applications and potential contributions to greenhouse gas mitigation options. Apeldoorn, TNO Built Environment Geosciences

Vonshak A (1997) Outdoor mass production od *Spirulina*: the basic concept. In A Vonshak, ed, *Spirulina platensis* (Arthrospira): physiology, cell-biology and biotechnology. Taylor & Francis, London, pp 79-99

Ward OP, Singh A (2005) Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry 40: 3627-3652

Watanabe Y, Delanoue J, Hall DO (1995) Photosynthetic Performance of A Helical Tubular Photobioreactor Incorporating the Cyanobacterium Spirulina-Platensis. Biotechnology and Bioengineering 47: 261-269

Watanabe Y, Ohmura N, Saiki H (1992) Isolation and Determination of Cultural-Characteristics of Microalgae Which Functions Under CO_2 Enriched Atmosphere. Energy Conversion and Management 33: 545-552

Waters ER, Rioflorido I (2007) Evolutionary Analysis of the Small Heat Shock Proteins in Five Complete Algal Genomes. Journal of Molecular Evolution DOI 10.1007/s00239-006-0223-7

Wen ZY, Chen F (2003) Heterotrophic production of eicosapentaenoic acid by microalgae. Biotechnology Advances 21: 273-294

Wilde EW, Benemann JR (1993) Bioremoval of Heavy-Metals by the Use of Microalgae. Biotechnology Advances 11: 781-812

Wu GH, Truksa M, Datla N, Vrinten P, Bauer J, Zank T, Cirpus P, Heinz E, Qiu X (2005) Stepwise engineering to produce high yields of very long-chain polyunsaturated fatty acids in plants. Nature Biotechnology 23: 1013-1017

Xu H, Miao XL, Wu Q (2006) High quality biodiesel production from a microalga Chlorella protothecoides by heterotrophic growth in fermenters. Journal of Biotechnology 126: 499-507

Yongmanitchai W, Ward OP (1991) Growth of and Omega-3-Fatty-Acid Production by Phaeodactylum-Tricornutum under Different Culture Conditions. Applied and Environmental Microbiology 57: 419-425

Zhang CW, Cohen Z, Khozin-Goldberg I, Richmond A (2002) Characterization of growth and arachidonic acid production of Parietochloris incisa comb. nov (Trebouxiophyceae, Chlorophyta). Journal of Applied Phycology 14: 453-460

Zittelli GC, Rodolfi L, Biondi N, Tredici MR (2006) Productivity and photosynthetic efficiency of outdoor cultures of Tetraselmis suecica in annular columns. Aquaculture 261: 932-943

Zoebelein H (2001) Dictionary of renewable resources. Wiley-VCH, Weinheim,