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Microalgae-based products for the food and feed sector: an outlook for Europe

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Editors: Mauro Vigani, Claudia Parisi, Emilio Rodríguez Cerezo

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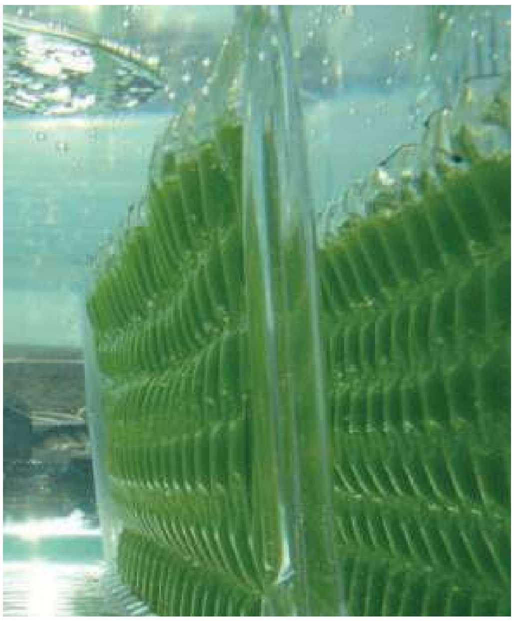

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Executive summary

The European Union has adopted recently an ambitious strategy for developing the Bioeconomy in Europe. The bioeconomy is based on the innovative use of sustainable biological resources to cover the growing demand of the food, energy and industrial sectors. In this context, algae represents an emerging biological resource of great importance for its potential applications in different fields, including food and feed.

The waters of the world host a large variety of organisms which are able to use light as source of energy to fuel their metabolism. Within these organisms, algae are a group of relatively simple, plant-like organisms, most of which capable of performing photosynthesis. Algae size ranges from micrometres of unicellular micro-algae to macro-algal seaweeds of tens of metres. Algae contain several high-value molecules, such as lipids (oil), proteins and carbohydrates (sugars), and for this reason there is a growing interest in algae as production organisms.

Algae, especially marine algae, have been already used as food, feed and fertilizers for centuries, and nowadays approximately 200 species are used worldwide in different sectors. Recently, algae have been used for the production of ethanol or biodiesel and research is on-going on genetic engineering of micro-algae, especially for the production of pharmaceuticals and cosmetics. However, no genetically modified micro-algae are currently on the market.

This report presents the results of an analysis of the technological and market developments in the field of micro-algal production systems, especially for food and feed products. Based on literature search and on interviews and survey to experts, the report provides the current state-of-the-art of microalgae as systems for producing food/feed products and discusses the future challenges for Europe to become a key player in this field.

The production systems of micro-algae may vary from open-air ponds to closed systems such as photo-bio-reactors. While open-air ponds present a great dependence on weather conditions, a high risk of contamination and an elevated consumption of water, they have low construction costs and require easy maintenance. On the other side, closed systems present higher costs but also higher production efficiency. Extraction expenses come on top of all costs for microalgae-derived products.

The global market for micro-algae-based food and feed supplements/nutraceuticals is well developed and with a great potential for growth. Micro-algae are currently used both as dried whole algae and for the extraction of high-value food/feed supplements and colorants. Although the total production volumes and market size of food and feed supplements/nutraceuticals derived from micro-algae are still relatively small with respect to alternative sources, they have increased 5-fold since the beginning of the century, and now some micro-algae applications have already a long tradition. On the other hand, the large-scale commercial production of microalgae as a source of protein/carbohydrates with impact on food-feed security can still be considered an infant industry.

Spirulina and *Chlorella* dietary supplements used as dried whole algae have by far the largest production volumes worldwide (5,000 and 2,000 tons of dry matter/year respectively), with an estimated global production values of about $40 Millions/year each. On the other hand, micro-algae-based high-value molecules (such as astaxanthin, omega-3 fatty acids and β-carotene) have smaller production volumes but larger market potential. For example, the production volumes of poly-unsaturated fatty acids (DHA/EPA) from micro-algae are only 240 tons/year, but the market value of this production (mostly extracted from ocean fish) is estimated to be higher than $300 Million/year. Therefore, microalgae are becoming a sustainable alternative for the production of these products. In the future, micro-algae based proteins and oils will also become available as food ingredients.

At present, the low volumes and high production costs of microalgae encourage exclusively the production of high-value supplements and nutraceuticals for human consumption. The microalgae-based molecules have specific advantages with respect to their synthetic and traditional alternatives that makes their use commercially viable for the food sector compared to the corresponding alternatives, despite the higher production costs. The higher quality of microalgae-based molecules compared to the corresponding alternatives is mainly due to their chemical conformation that is much more effective for food applications than the syntetic variants. However, the bulk production of carbohydrates and proteins for the food and feed sector is not yet forseen in the short run, because it would require higher production volumes and, consequently, the boosting of the cost effective scale-up with dramatic reduction of production costs. For these reasons, the actual contribution of microalgae-based food and feed products to global and European food security is rather limited.

The production of micro-algae is currently concentrated in a small number of producers, except for *Chlorella*. Most producers are located in Asia or Australia, but recently some European firms have acquired a number of leading companies in Australia and in the United States. The production of microalgae-based food and feed products from European firms is currently estimated at around 5% of the global market. Additionally, according to the analysis of pipeline products, many European producers are stepping into the micro-algae-based high-value molecules markets.

Most experts consulted estimate that Europe can become market leader in micro-algae based products for the food and feed markets in the next decade. The two most important factors that may contribute to the expected European market position are scientific and technological developments in the field of micro-algae research and in the food and feed market. However, experts highlight two major factors limiting the European potential: the insufficient European domestic demand for these products and the difficulties in achieving commercial authorization of algae-derived products in the EU markets due to the complexity of the regulation of novel foods in Europe. Hence, new microalgae-based products obtained in Europe may be intended mainly for foreign markets, and the increased production share by European companies will be mainly a result of strategic acquisitions of foreign companies.

An important source of innovation in microalgae production systems will come from biotechnology. Research on algae and genetic modification of algae is rapidly expanding due to high expectation with respect to the production of biofuel, bio-chemicals and other bio-products by algae. Large investments from governments and industries stimulate the research on GM algae. However, the technology is still immature and further research and development is needed before commercial production of products from GM algae will take place.

With respect to the potential risks of microalgae-based food products and their management, full knowledge about the possible production of toxins, allergens or other harmful compounds by the algae strain(s) that are used in industrial production is of utmost importance. For that reason, strain identity is an important parameter for determining the potential risk of mass cultivation of industrial algae.

This report illustrates the regulations applied to the commercial introduction of microalgae-based food products in two of the main global markets: the EU and the USA, which differe substantially both in the approach and in the requirements for their regulations on micro-algae products. One of the main differences between the European and USA regulation concerns the criteria for defining novel food and, consequently, the corresponding authorisation procedures. Moreover, the differences on the GMO regulation between US (based on the characteristics of the final product) and the EU (based on the technology employed to obtain the final product) may affect the future developments of algae biotechnology.

In conclusion, Europe presents important strengths in the field of micro-algae applications. Its strong position in science and technology, related to the high priority in R&D funding policies, makes Europe very active in this area. Moreover, Europe has specific structural economic and logistical assets that enhance its position in micro-algae research and applications. Europe has an outstanding tradition in high-quality agriculture production and a strong food and feed industry with multinationals operating on the global scale. Europe also benefits from high levels of human capital, workforce with adequate engineering and technical skills to work in micro-algae research, development and applications. Moreover, the spill-over effects from research on micro-algae for the biofuel sector and for sustainable production of food, are likely to contribute to the improvement of the European competitiveness in the micro-algae sector in the near future.

On the other side, some weaknesses of the European microalgae sector may hinder its competitiveness. Europe's main weaknesses are its relatively suboptimal climatic conditions with high levels of rainfall, low levels of sun hours and intensity (especially in winter) and low temperatures for most countries outside southern Europe. For these reasons, Europe lacks of optimal surface areas for the production of micro-algae. Moreover, Europe has some structural financial-economic disadvantages: relatively high labour costs, lack of venture capital and seed capital for start-up companies, low entrepreneurial activity among researchers and engineers, low R&D investments by large companies, high land costs and low internal demand for microalgae-based food and feed products.

1. Introduction

1.1 Growing importance of micro-algae as source of food and feed

The waters of the world, oceans, seas, rivers, creeks, lakes and even ice, host a large variety of organisms which are able to use light as the only energy source for their metabolic processes. Algae are a group of relatively simple, plant-like organisms, most of which are capable of performing photosynthesis: They capture light and use its energy to convert CO_2 into sugars and oxygen. In this way, they largely contribute to the global oxygen production (between 50 to 87 per cent). There are 80,000 to 100,000 different algae species with widely varying characteristics; many of which have been investigated. Algae size ranges from micrometres of unicellular micro-algae to macro-algae seaweeds of tens of metres. The organisms considered in this report are micro-algae growing in aquatic environments.

Globally, there is growing interest in algae as production organisms. Algae contain lipids (oil), proteins and carbohydrates (sugars), and, especially marine algae have been used as food, feed and fertilisers for centuries. Commercial farming of macro-algae (seaweeds) has a long history, especially in Asia. In the 1950s algae were considered a candidate for protein supply for the increasing world population. In 1950s on, a search for biologically active substances from algae began. Commercial large-scale cultures of the micro-algae species *Chlorella* were started in the early 1960s followed by Arthrospira (*Spirulina*) in the 1970s.

Since 1980, large-scale micro-algae production facilities were established in Asia, India, the USA, Israel and Australia. Nowadays approximately 200 species of micro-algae are used worldwide. There is a well-established global market for micro-algae based food and feed products, but micro-algae also have other functions. More recently algae are used for the production of ethanol (fermentation) or biodiesel (conversion) and research using GM algae for the production of pharmaceuticals is currently on-going.

The use of micro-algae for the food and feed market is increasingly relevant as the components of micro-algae (such as fatty acids, colourants, vitamins) have the potential to be competitive with the same components from other sources. To develop the competitiveness of micro-algae based products, not only the technical and the economic aspects are important, but also the national regulations ruling their use play a fundamental role.

The goal of this study is to analyse:

- The current status of micro-algae production systems for different species and products, describing the most suited for food and feed applications;

- The market applications of micro-algae for food and feed;The state-of-the-art of research and development on micro-algae biotechnology and genetic engineering;

- An overview of risks, risk management and regulatory frameworks governing micro-algae research, production and commercialisation, providing a comparison between the EU and the USA.

Finally, the study provides a critical analysis of the EU-specific strengths and weaknesses in the development of commercial micro-algae products and production systems.

1.2 Methodology

This report presents the results of a study on the scientific and economic developments in the field of microalgae-based products for food and feed. It provides the current state-of-the-art and new insights on five topics concerning the European micro-algae sector. The topics under study are the following:

1. Production systems;

2. Economics of micro-algae products and production;

3. Biotechnology and genetic modification;

4. Risks and risk management;

5. Regulation.

The methodology applied is structured into three steps: 1) literature review; 2) experts interviews; and 3) experts Delphi survey. In Annex A the full detailes of the methodology adopted in each step are illustrated.

This report presents the results of the whole study, integrating the findings from the literature review, the interviews and the Delphi survey. The results of the experts interviews have been integrated with the findings of the literature review for each of the five topics analysed and are presented in chapters. Most of the results of the Delphi survey are presented in Chapter 3, while the results on the European position in micro-algae research and production are discussed in the final chapter.

1.3 Content of the report

The remainder of the report is structured as follows: the next chapter (Chapter 2) provides an overview of the current status of micro-algae production systems for different species and products. Chapter 3 addresses the economic aspects - the current and future situation of micro-algal products, their producers and the economic aspects of the production processes - and presents the results of the survey. Chapter 4 presents the state-of-the-art of research and development on micro-algae products with a particular focus on biotechnology and genetic engineering. Chapter 5 contains an up-to-date overview of risks and risk management of micro-algae and presents the European and USA regulations concerning algae production and GM algae research, production and market introduction. The last chapter concludes this report with the findings on the future EU-specific strengths and weaknesses to develop commercial micro-algae production systems for food and feed applications.

2. Micro-algal production systems

This chapter focuses on the current status of micro-algae production systems for those species and products that are most suited for feed and/or food applications. The first section (2.1) introduces micro-algae and the most used species in research and production. Section 2.2 presents the open systems, Section 2.3 the closed systems, Section 2.4 the indoor production systems and Section 2.5 illustrates the usual growth conditions applied for algae. The chapter closes with the main conclusions (2.6).

2.1 Species used in micro-algae research and production

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*). All these micro-algae are eukaryotes distinguished by the presence of a nucleus and separate organelles for photosynthesis (chloroplasts) and respiration (mitochondria). In this report, cyanobacteria (*Cyanophyceae or blue-green algae*) are also referred to as micro-algae, e.g. the species *Spirulina* (*Arthrospira platensis and A. maxima*). The cyanobacteria are part of the eubacteria and are prokaryotes lacking a membrane-bounded nucleus. There are thousands of different species of micro-algae and cyanobacteria.

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 (a storage polysaccharide).

Green algae are especially abundant in fresh water. The main storage compound of green algae is starch, although oils can also be produced. The fresh water green algae Haematococcus pluvialis is commercially important as a source of astaxanthin, *Chlorella vulgaris* as a supplementary food product or food ingredient and the halophilic algae species *Dunaliella* as a source of β-carotene.

The golden algae also produce oils and carbohydrates and are in this respect similar to diatoms. The blue-green algae (*Cyanobacteria*) are found in a variety of habitats and several of them are known for their water polluting effect due to the production of toxins.

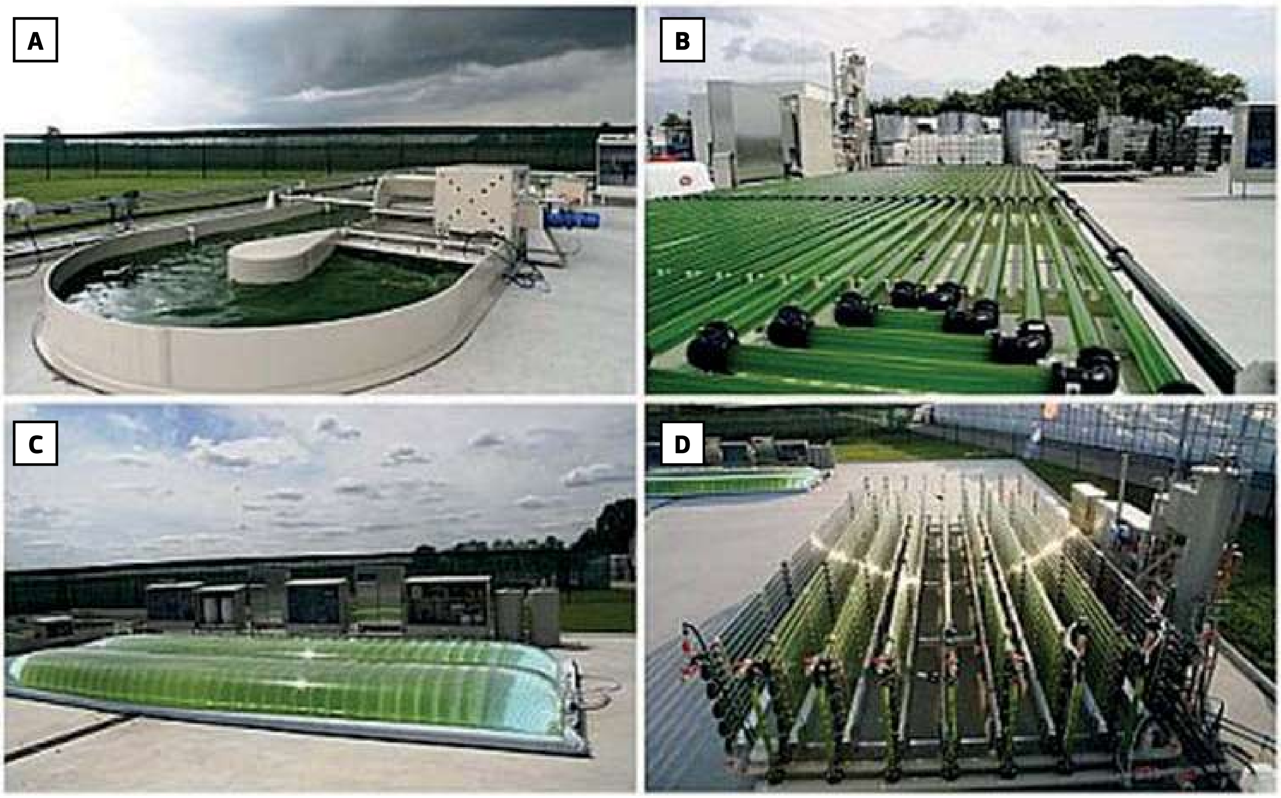
Most algae are autotrophs, while some are heterotrophic. An autotroph is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings. Autotrophs can be phototrophs or chemotrophs, depending if they use light as energy source (photosynthesis), or electron donors from organic or inorganic sources (chemosynthesis). A heterotroph is an organism that cannot fix carbon and therefore needs organic carbon for its growth. Mixotrophic algae can use both sunlight or organic carbon.

Currently, the most used phototrophic species are Arthrospira (*Spirulina,* blue green algae), *Chlorella*, *Dunaliella* and *Haematocussus* which can be grown with (sun)light as energy source. Furthermore, with emphasis on the production of omega-3 fatty acids, heterotrophic marine organisms *Crypthecodinium*, *Schizochytrium* and *Ulkenia* are cultivated, similarly to yeasts, in indoor, well controlled vessels with the addition of sugars or other carbon sources but without sunlight. For some applications *Chlorella* species are cultivated also as heterotrophic algae. For fish aquaculture (mainly to feed the larvae) the main cultivated species are: *Chlorella, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema, Thalassiosira, Haematococcus, Tetraselmis*.

Micro-algae represent potential feedstock for food and feed. However, the technology for the production of micro-algae is still immature. Research and development has been done in recent years and continues on cultivation systems. A leap in the development of micro-algae technology is required; on a practical level, the scale of production needs to increase with a concomitant decrease in the cost of production.

Micro-algae are cultivated in a wide range of different cultivation systems that can be placed outdoors or indoors. Cultivation systems range from open shallow raceway ponds to closed photobioreactors (Figure 1). The systems mostly used on a large scale and on a commercial basis are open systems (Figure 2).

Figure 1 Algae production systems at the research facility AlgaePARC, Wageningen UR NL



A. Open pond system. B. Horizontal tubular system. C. Flat Panel system (ProviAPT). D. Vertical tubular system

Source: Wageningen UR

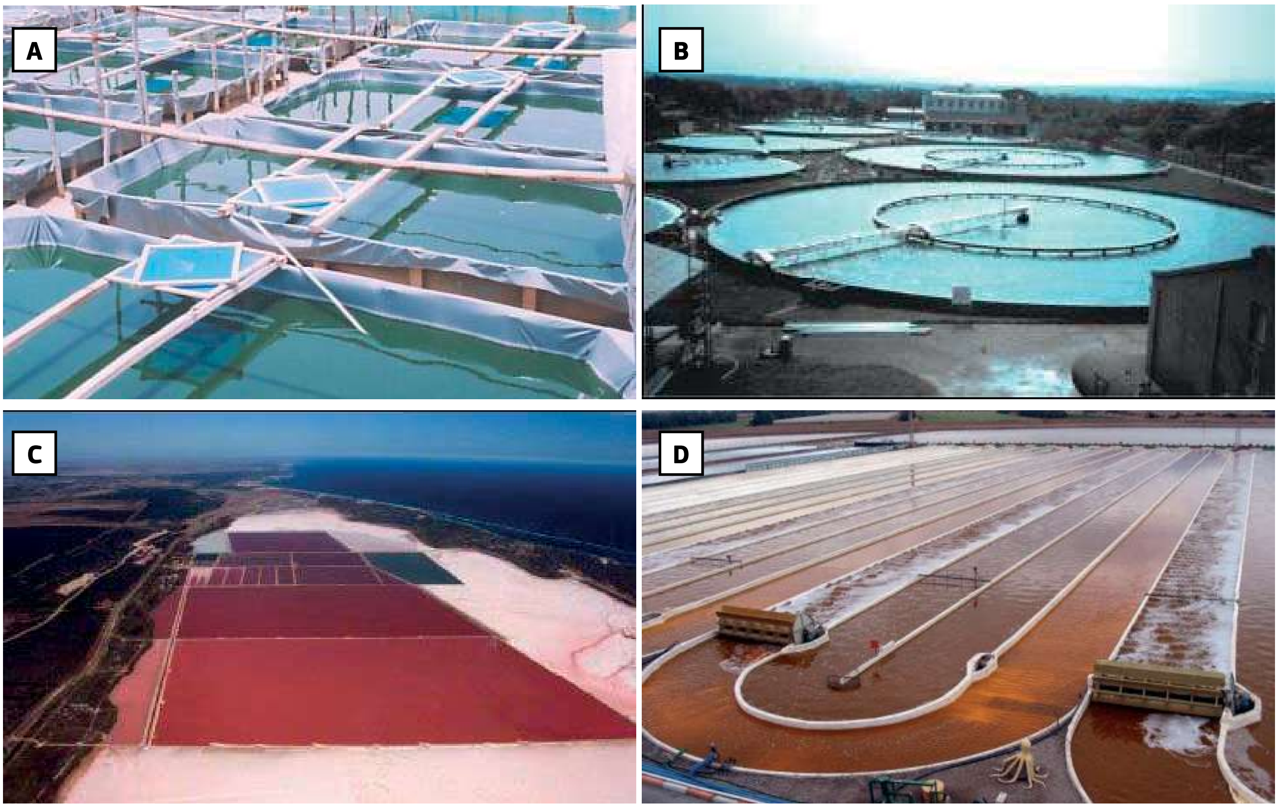
2.1.1 Open systems

The most common open systems for micro-algae production are ponds. These ponds are usually no more than 30 cm[1] deep. The circulation of the water with nutrients and micro-algae is done by a mechanical arm stirring in a circular motion in circular ponds (Centre-Pivot ponds), or more commonly by a paddle wheel in so-called raceway ponds. CO_2 or exhaust or flue gases containing CO_2 can be sprayed in the culture.

Major weaknesses of open systems are that there is almost no possibility for temperature control (unless a source of cheap surplus heat is available). Also they are very susceptible to invasion of algal predators, parasitic algae or other algal strains that grow faster at the applied conditions and therefore out-compete the desired species. Only a few species can be grown in these systems either by applying a selective environment, e.g. high salinity for *Dunaliella* salina and high alkalinity for *Spirulina* platensis, or by making use of large amounts of inoculum produced under strictly controlled conditions and taking advantage of fast-growing species such as *Chlorella*. Moreover, biomass concentration and thus volumetric productivity is very low due to the long light path, and poor mixing.

1 A certain minimal depth is needed to keep the liquid flowing through the canals using the paddle wheels.

Figure 2 Example of open pond systems



Open systems a) Small pond for Spirulina culture, Asia [2]; b) CentrePivot ponds for the culture of Chlorella in Yaiwan [3]; c) Dunaliella salina ponds of Cognis, Western Australia [3]; d) Raceway pond for production of Dunaliella bardawil at Nikken Sohansha Corporation in Eilat, Israel. Source: Wageningen UR.

On average, the biomass productivity in open systems is ca 30 tonnes of dry biomass/ha/year (value for solar conditions found in South Europe).

Open systems have already been used for commercial algae cultivation for decades. In Hawaii for instance the company Cyanotech is growing *Spirulina* and Haematococcus on 90 acres (3.6 hectare).

2.1.2 Raceway Ponds

A raceway pond is a specific form of open system built as a shallow closed loop channel that allows the water to circulate (Figure 2d). Raceway ponds are easy to build and, for this reason, they are the most common cultivation systems for the commercial production of micro-algae.

Raceway ponds are commonly built in concrete or compacted earth. Their shape may vary, but for a large area with multiple raceways, long stretched ponds with 180° curves on either end are the most compact and efficient. Raceways may be lined with plastic which allows thorough cleaning to remove contaminating organisms; it is assumed that this will increase the life-expectancy of the system and allow for easier maintenance. The materials chosen for construction should provide sufficient water retention.

For mixing and circulation, a paddle wheel (Figure 2b) is used. The paddle wheel operates continuously to prevent sedimentation, to avoid the formation of a temperature gradient, for the distribution of nutrients and carbon dioxide, for removal of produced oxygen and for transporting algae from and to the surface, which improves the total light utilisation efficiency [4]. The power required for mixing increases dramatically with flow speed.

The temperature in an open raceway fluctuates within a diurnal cycle and seasonally. Temperature is very difficult to control. Cooling can occur by means of evaporation. Evaporative water loss can be significant and causes an increase in concentration of salt and other compounds [5]. This could be counteracted by re-supplying water of sufficiently low salt content, although this may be problematic at some geographic locations. While saline groundwater is unsuitable for agriculture, it could be employed in some alga cultures. Besides sunlight, other options for heating are the use of warm wastewater or surplus heat from power generation or industry.

Due to significant losses to atmosphere, raceways - as in other open systems - use carbon dioxide much less efficiently than closed photobioreactors [5].

Nutrients are added to the water stream that is used to replace the micro-algae culture which is (semi) continuously harvested. Considering a typical depth of 30 cm , these systems are very voluminous and the micro-algae biomass concentration in these ponds is low, in the order of 0.1 to 0.5 g·L 1.

Productivity is affected by contamination with unwanted algae and micro-organisms that feed on algae [5]. Other species can outcompete the desired strain.

Raceway ponds are used for the cultivation of micro-algae or cyanobacteria with *Arthrospira (Spirulina) platensis, Dunaliella salina* and different *Chlorella* species being the most important.

In the Netherlands, raceway ponds were used by Ingrepro at Borculo (this company recently went bankrupt), by AquaPhyto at Zeewolde and Schiphol Airport (this company no longer exists), and by Kellstein Greencircle at Hallum. In addition, a raceway pond system is present at the AlgaePARC, a pilot research facility of Wageningen UR. In France there are also raceway ponds in Activ'Alg (www.activalg.fr) and Algosource Technologies (www.algosource.com). In Italy, the company Eni (www.eni.com) is using raceway ponds to fix CO_2 from a power plant. Companies in Europe mostly use closed systems.

2.2 Closed systems

There is a large variety of closed systems used for the production of algae. These closed systems prevent contact between the enclosed algae and the environment. For sampling and harvesting, precautions can be taken in order to limit the contact with the environment. Tubular systems of different sizes (placed either vertically or horizontally), polyethylene sleeves or bags and flat panels are most commonly used, but there are also new designs like biodomes and even floating bags on ocean waters. However, accidents or careless handling may breach the containment of these systems.

Closed systems are typically referred to as photobioreactors (PBRs). They can be placed outdoors but in some cases they are placed inside greenhouses to allow more controlled conditions at the expense of higher production costs.

Photobioreactors are considered to have several major advantages over open ponds, they can:

- Prevent or minimise contamination, allowing the cultivation of algal species that cannot be grown in open ponds;
- Offer better control over cultivation conditions (pH, pCO_2, pO_2, temperature, nutrient supply, etc);
- Prevent evaporation and reduce water use;
- Lower CO_2 losses due to outgassing;
- Attain higher cell concentration and therefore higher volumetric productivity.

The costs of installation and operation of photobioreactors, however, is much higher than those of open pond systems.

2.2.1 Design criteria

Design criteria for photobioreactors should aim at achieving high efficiency of light conversion and at providing the necessary reliability and stability to the cultivation process by solving the main problems encountered in photobioreactor operations such as overheating, oxygen build-up and biofouling. The fundamental design criteria for photobioreactors include reactor configuration in respect to light gradient and light/dark cycles, surface to volume ratio, mixing and degassing devices.

Surface-to-volume ratio

The surface-to-volume (S/V) ratio of the bioreactor, (i.e. the ratio between the illuminated surface of the reactor and its volume) determines the amount of light that enters the system per unit volume and the light regimen to which the cell population is exposed, and is consequently one of the most important issues in photobioreactor design. The higher the S/V ratio, the higher is the cell concentration at which the reactor is operated and the volumetric productivity of the culture. The hydrodynamic behaviour of the culture is also affected by this parameter: higher S/V ratios can lead to shorter light/dark cycles. For these reasons, in recent years a general trend towards the reduction of the diameter of tubular reactors and the thickness of flat panels can be seen.

Mixing

The type of device used to mix and circulate the culture suspension is essential in the design of a successful photobioreactor. Both the productivity of a photobioreactor and the cost of its construction and operation are influenced to a great extent by the type of device used for mixing. Mixing is necessary to:

- Prevent cells from settling;
- Avoid pH and temperature gradients along the reactor;
- Distribute nutrients;
- Remove photosynthetically generated oxygen, which after a certain value inhibits photosynthesis;
- Supply CO_2;
- Ensure that all cells experience alternating periods of light and darkness.

Yet, it must be pointed out that excessive mixing can lead to cell damage and eventually cell death. For this reason the choice of mixing intensity and mixing system must be dictated by the characteristics of the organism to be cultivated.

2.2.2 Main photobioreactors characteristics

The use of photobioreactors results in lower space requirements and lower harvesting costs per ton algae, compared to open systems. Most designs are based on the principles related to tubular photobioreactors and flat panels. Scalability of these systems is however still only possible by increasing the number of modules.

The main inputs required in addition to the algae themselves are sunlight, water, CO_2, nitrogen and phosphorus. Large-scale cultivation of micro-algae for low/medium value products must be based on sunlight as the sole source of light energy.

When working in summertime, and/or at lower latitudes, sunlight intensities are high and often oversaturate the photosynthetic cycle, limiting algae growth and leading to a drop in productivity. In recent years, much effort was put into increasing photosynthetic efficiency of micro-algae under oversaturating light (the normal condition on a sunny day) by developing new strains with smaller antenna sizes [8] and by decreasing the light path of photobioreactors while increasing mixing (turbulence) in high cell density cultures [9, 10]. Turbulence requires high-energy input and therefore is not suitable for large-scale production of biofuels from micro-algae.

One strategy to obtain high photosynthetic efficiencies under bright sunlight in systems with lower energy requirements is to reduce the light intensity at the reactor surface. This can be done by stacking the reactor units vertically. Narrow spacing in the stacks minimises loss of light to the ground surface [9]. However, if not combined with a short light path, this setup leads to voluminous reactor systems with low volumetric productivity and low biomass concentration [11].

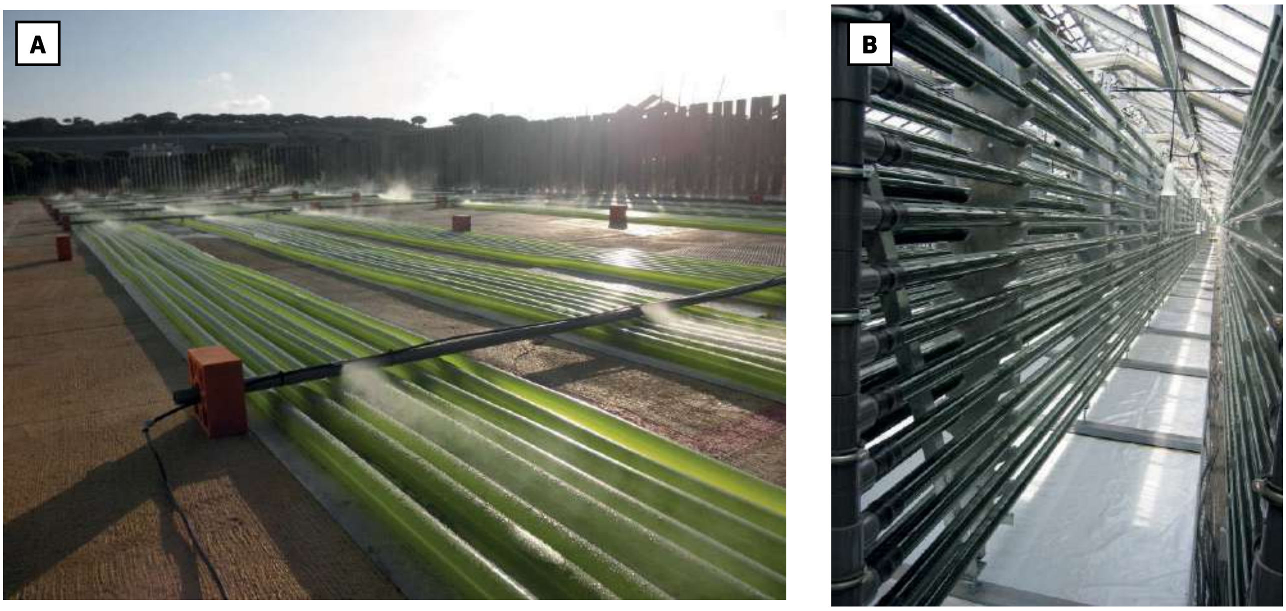
In order to reduce investment costs of these systems, vertical panels can be made from thin plastic films such as polyethylene. There are examples of thin film systems submerged in large water volumes for good temperature control and a lower associated energy requirement for cooling [12]. We expect that in the coming years many systems will be developed based on these design principles. Improvements are to be expected in material lifetime (polyethylene has a lifetime of ~1 year), ease of cleaning, and energy requirements (for example, the energy requirement for cooling can be further reduced by reflecting the near-infrared portion of the light incident on the reactor surface, which otherwise heats the system without contributing to photosynthesis) [13].

2.2.3 Tubular photobioreactors

Tubular photobioreactors are based on transparent tubes in which the algae culture is maintained and exposed to sunlight (Figure 3). Although not visible, a large number of tubes are connected to each other via manifolds and the liquid is continuously pumped through the tubes (Figure 4). This has to be done for two reasons. One reason is to prevent the micro-algae cells from settling and to have the cells continuously moving from the sunlight-exposed tube surface to the darker zones in the centre of the tubes. The other reason is related to the accumulation of oxygen in micro-algae cultures. As in any other cultivation system oxygen will quickly accumulate because of photosynthesis.

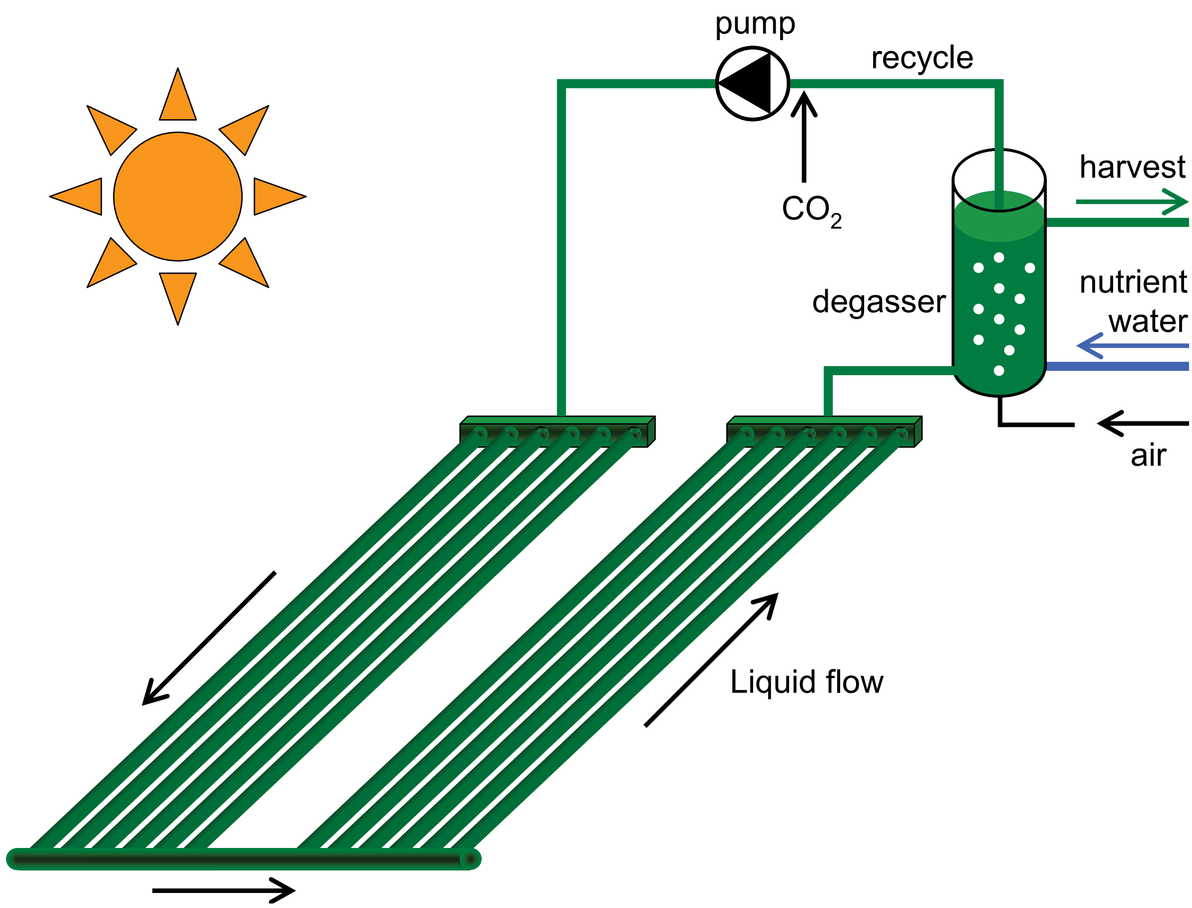
Given the low solubility of oxygen in water, the oxygen partial pressure will quickly rise to levels way above those in equilibrium with air. For this reason the micro-algae culture is continuously pumped through a so-called degassing vessel where the oxygen is allowed to escape. Within this vessel oxygen degassing is usually enhanced by aerating the degassing vessel with finely dispersed air bubbles. Obviously the length of the tubes and the liquid velocity in the tubes are important design parameters. Typical numbers are 50 to 100 m for tube length and 0.2 to 0.5 m·s-1 for the liquid flow velocity.

Figure 3 Tubular photobioreactors



On the left (a) a horizontal tubular system based on thin flexible plastic tubes in southern Spain. This system has been designed by the Dutch company Paques in collaboration with Bioprocess Engineering of WU. On the right (b) a vertical fence type structure based on rigid glass or plastic tubes placed in a greenhouse in Germany. This system was designed by the German research institute IGV. Source: Wageningen UR.

Figure 4 Simplified scheme of a horizontal tubular photobioreactor

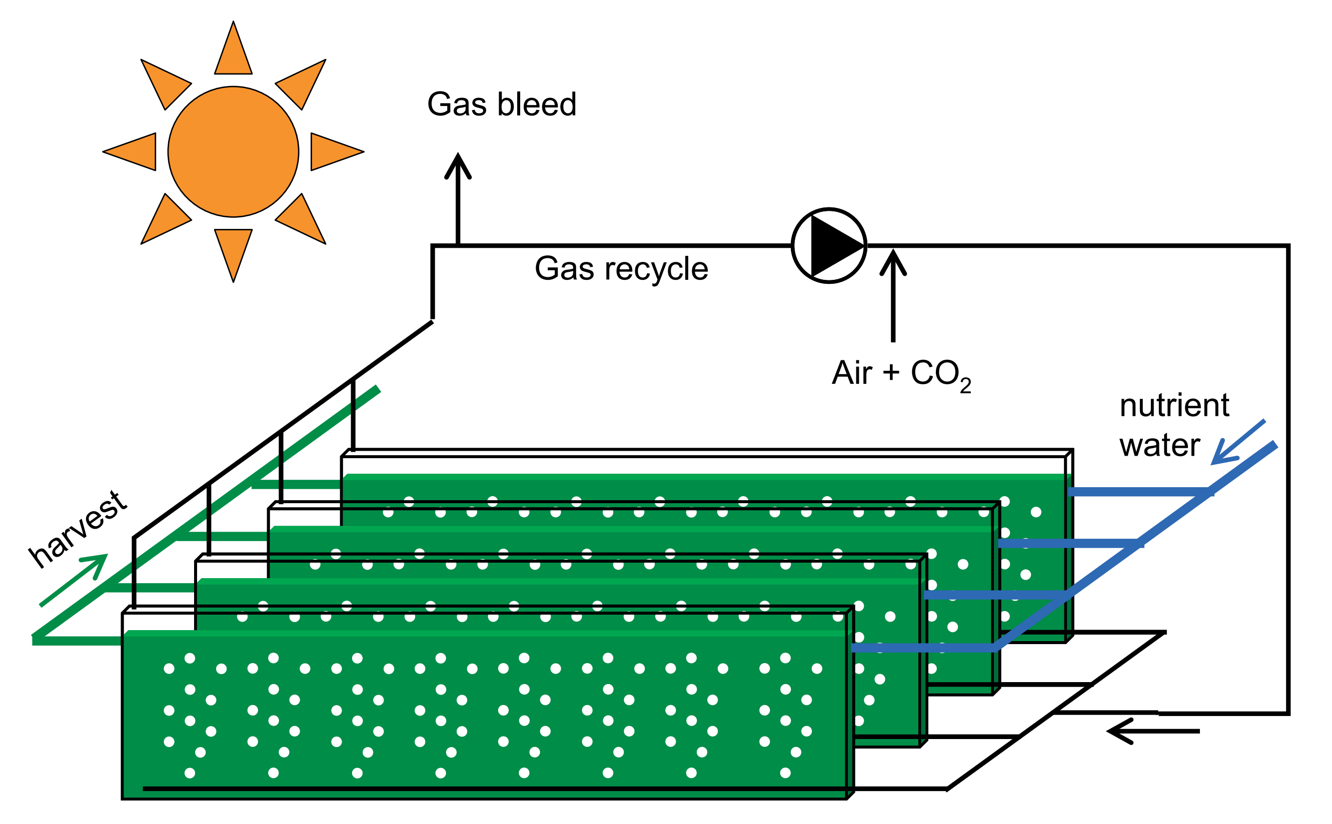


Source: Wageningen UR.

2.2.4 Vertical panel photobioreactors

Vertical panel photobioreactors are composed of thin rectangular cultivation vessels with a depth in the range of 0.01 to 0.10 m. They are mixed by gassing the panels with air which is injected over the full length of the bottom of the panels. The air is enriched with carbon dioxide (CO_2). Combustion gases rich in CO_2 can also be used. The panels are typically 0.5 to 1 m high and the length of a single panel row can be many meters. The actual length is determined by the rate of mixing of the culture across the length of the panel. The individual panels need to be well mixed and/or panel length needs to be sufficiently short in order to prevent unequal distribution of nutrients and micro-algae. Gassing the cultures requires considerable energy, therefore, mixing and panel length are important design parameters.

Figure 5 Simplified scheme of a vertical panel photobioreactor



Source: Wageningen UR

Figure 5 provides a schematic representation of multiple vertical panels making up a larger scale production plant. The gas leaving the system can be partly recycled in order to increase the residence time of the gas bubbles allowing a more efficient use of the carbon dioxide supplied.

Despite the need for substantial gassing, the micro-algae culture itself does not have to flow as fast as in a tubular system where the culture needs to be pumped through the degassing vessel and back at a high rate. For this reason, the energy requirement for mixing is generally lower in panel reactors. However, scale-up in these systems is more complicated.

The panel reactors currently running are pilot scale systems that only occupies a few tens of m2 of ground surface as illustrated by Figure 6. On the right, Figure 6 shows the so-called Proviapt system of the Belgian company Proviron. It is constituted by big plastic bags filled with water in which separate panel structures with a depth of about 1 cm are the algae growth chambers. This system is very promising since it can be produced in an automated way, based on cheap flexible plastic films. Future applied research will provide more data regarding the maximal unit size of panel photobioreactors and their scalability. Figure 6 on the left, shows the so-called green wall developed by the University of Florence, which present a design based on deeper and higher panels for use at high latitude locations. It can be composed of 2 panels per metre with a depth of 5 cm and a height of 1 m. These systems based on cheap flexible plastic films are not 100 per cent closed and are often referred to as semi-closed systems and as the best option to produce biomass for food and feed. Closed systems (e.g. tubular photobioreactors) are relatively too expensive for this type of applications.

Figure 6 Panel photobioreactors

Source: Wageningen UR

In Europe several companies are using or developing closed systems for micro-algae growth. The scale is still small (the largest plant's total surface is 1 ha) but many developments have taken place in recent years aiming at increasing the scale of production and industrialising the field. Examples of these companies are: Ecoduna in Austria with rigid flat panels (www.ecoduna.com); Phytolutions in Germany (www.phytolutions.com), Archimede Ricerche in Italy (www.archimedericerche.com) and Proviron in Belgium (www.proviron.com). All these companies use thin plastic films flat panels. Tubular photobioreactors are used by the Spanish companies AlgaEnergy (www.algaenergy.es), Algasol (www.algasol.com) and Fitoplancton Marino (www.easyalgae.com), by Roquette in Germany (www.roquette.com) and A4F in Portugal (www.a4f.pt). The last two companies represent the largest production plants in Europe, ca 1 hectare (the plant from Roquette is inside a greenhouse while the plant from A4F is outdoor).

Southern Europe and Northern Africa are the best locations for producing micro-algae biomass due to the solar conditions, but only in cases where there is sufficient fertiliser and close proximity to CO_2 sources. Wastewater from urban or industrial activities can be used as a source of nutrients (nitrogen and phosphor) for micro-algae production. Flue and fermentation gases can be used as CO_2 sources. At the moment, it can be seen that in this field technology development takes place all over Europe, however, large-scale sustainable production is likely to take place mainly in South Europe.

2.3 Indoor production systems

2.3.1 Small scale for research

For small-scale algae production - mostly for research purposes - a wide range of systems is used. Algae can be cultivated in simple Erlenmeyers, in fermenters (photo-bioreactors) and in so-called flat panel reactors, among other things. Figure 7 shows examples of these systems.

Figure 7 Three examples of contained production of algae: Erlenmeyers, fermenters and flat panel reactors

Source: WageningenUR

2.3.2 Heterotrophic production systems

Heterotrophic growth is possible for some micro-algae species and products but requires organic matter sources, thus increasing its cost and reducing its sustainability.For the cultivation of heterotrophic algae (see Section 2.1) steel fermenters are used for large-scale production (Figure 8). Depending on the size, they are placed indoor or outdoor. They are, however, completely contained. Fermenters are for instance used for the production of long chain unsaturated fatty acids by the heterotrophic algae *Crypthecodinium cohnii*, *Schyzochytrium* or *Ulkenia*. Sometimes also *Chlorella* species are cultivated in fermenters for use as a dense inoculum or for some specific applications. Based on infrastructure and knowledge from industrial biotechnology, heterotrophic algae can be cultivated in 100.000 l fermenters and at high densities (30 – 100 g/l).

2.4 Growth conditions

The growth conditions in algae production systems are diverse and depend very much on algae specific natural conditions. Eukaryotic algae are found almost everywhere on earth: in the sea, in rivers and lakes, on soils and walls, and in animals and plants as symbionts. Well-known symbionts of algae are lichens, coral, sea sponges and hydra. Algae are prominent in bodies of water, common in terrestrial environments and are also found in unusual environments, such as on snow and on ice where they can be actively growing [14]. Also cyanobacteria can be found in almost every terrestrial and aquatic habitat, from oceans to fresh water to bare rock to soil. Unlike other bacteria, cyanobacteria are able to perform oxygenic photosynthesis, i.e. they use light as an energy source for growth.

The growth conditions of algae for industrial production also shows a wide range: from dark in steel fermenters (heterotrophic) to light in glass or plastic growth systems (phototrophic or mixotrophic), from salt (seawater), brackish to fresh water. Other factors like pH, temperature, nutrients and aeration are of importance for optimal growth.

Optimisation of culture conditions is an important issue in algae research.

Figure 9 presents a number of general parameters dealing with conditions for culturing micro-algae, based on the FAO "Manual on the Production and Use of Live Food for Aquaculture" [15].

Figure 8 A 1500 litre steel fermenter at Food & Biobased Research, Wageningen UR

Source: WageningenUR

Figure 9 General parameters for algal cultivation

Parameters	Range	Optimum
Temperature (°C)	16-27	18-24
Salinity ($g.l^{-1}$)	12-40	20-24
Light intensity ($mmol/m^2/s$)	15-135 (depends on volume and density)	40-70
Photoperiod (light: dark, hours)		16:8 (minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

Manual on the Production and Use of Live Food for Aquaculture, FAO [15]

The major challenges in the cultivation of micro-algae for food and feed are:

- Reduction of production costs of feed;
- Improvement of production technologies for the safety of food products;
- Stability/reliability of large cultures and suitable strains avoiding contamination and crash of the cultures.

According to experts opinion, these challenges are expected to be addressed in about 5-7 years time.

2.5 Conclusions

This chapter has given an overview of the current status of micro-algae production systems for those micro-algae species and products that are most suited for feed and/or food applications. A distinction is made between open systems (including raceway ponds) and closed systems (including photobioreactors).

The technology for the production of micro-algae is still immature. Research and development has been done in recent years and is continuing on cultivation systems. A leap in the development of micro-algae technology is required; on a practical level, the scale of production needs to increase with a concomitant decrease in the cost of production.

Nowadays, there are many designs available and work is continuing on the improvement of existing systems and process strategies and on the development of new concepts. Presently, the main challenges are scale-up, reduction of production costs and attaining stable and reliable processes.

3. Current markets, products and future developments for micro-algae

This chapter concerns the market of microalgae-based products: its current situation and future developments. In particular, Section 3.1 presents the micro-algae products that are currently on the market and in the pipeline, and their producers. Section 4.2 illustrates the global market figures for micro-algae products, and highlights the main producers. Section 3.3 discusses the economic aspects of the production process, focusing mainly on cost structures of production technologies. Finally, Section 3.4 presents the outcomes of a survey and interviews with key experts on future developments in micro-algae research and production and their driving factors (see Annex B).

3.1 Products and producers

3.1.1 Food and feed products and their producers

Traditionally, micro-algae such as *Spirulina* and *Chlorella* are directly sold as dietary supplements, without any kind of processing except drying. The development of these products is relatively mature and they are produced by a relatively large number of producers. *Spirulina* production is concentrated in Asia and the USA, *Chlorella* mostly in Asia, although both are also produced in a small number of other countries with warm climates [83]. *Chlorella* is now also produced in Germany in the Klötze plant [69].

Besides the sales of the whole dried algae, nowadays also specific high-value components from micro-algae are being produced. In general, micro-algae based molecules are less competitive than standard synthetic and traditional alternatives. However, some micro-algae based molecules have specific advantages over their conventional alternatives which make their use commercially viable. For instance, from a chemical point of view, synthetic molecules are only available in specific isomers, which are generally much less effective than natural variants for specific applications, such as in infant formula, fish pigment enhancers or dietary supplements [87].

Astaxanthin (a carotenoid used as pigment) from dried *Haematococcus Pluvialis* is the most developed product in this domain. Astaxanthin is either available as dietary supplement (mostly USA-produced), or as food additive. Other micro-algal sources of carotenoids are presented in Figure 10.

Figure 10 List of carotenoids that are possible to obtain by selected micro-algae

Micro-alga source	Active compound
Dunaliella salina	β-carotene
Haematococcus pluvialis	*Astaxanthin, cantaxanthin, lutein*
Chlorella vulgaris	*Cantaxanthin, astaxanthin*
Coelastrella striolata var. multistriata	*Canthaxanthin, astaxanthin, β-carotene*
Scenedesmus almeriensis	*Lutein, β-carotene*

Figure 11 illustrates the most important high-value molecules currently on the market, split into pigments and fatty-acids, including a description of products applications as presented by the producers, while Figure 12 lists main global producers. A relatively large group of companies produces *β*-carotene, a food additive and ingredient, from *Dunaliella Salina*. Although most producers are located in Asia or Australia, European multinationals such as BASF and DSM have acquired a number of leading producers in Australia and the USA. More recently, a selected number of producers in Europe and the USA started producing omega-3 EPA and DHA from micro-algae, to be used for dietary supplements or food ingredients. The pigment phycocyanin is produced from *Spirulina* by a small number of companies.

Figure 11 High-value molecules produced with micro-algae that reached the food and feed market

Figure 11 High-value molecules produced with micro-algae that reached the food and feed market

High-value component	Chemical name	Micro-algae source	Application (Function/ Nutrient)	Traditional/ synthetic alternatives
		Pigments		
β-carotene (Pigment/ Carotenoid)	1,3,3-Trimethyl-2-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-1-en-1-yl) octadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]cyclohex-1-ene	*Dunaliella* *Salina*	Colourant (food colourant) Additive for feed (provitamin A) Food supplement (provitamin A, anti-inflammatory effect, chemopreventive (not proven)	Synthetic form (but only all-trans isomers, cis-isomers only come from natural sources)
Astaxanthin (Pigment/ Carotenoid)	3,3'-dihydroxy-ß-carotene-4,4'-dione	*Haematococcus Pluvialis*	Feed additive (pigment enhancer for fish) Food supplement (anti-oxidant, restores UV damage)	Synthetic form Phaffia yeast extract
Phycocyanin (Phycobili-protein)	Protein complex	*Arthrospira Porphyridium*	Food pigment Reagents (Fluorescent markers)	Synthetic pigments
		Fatty acids		
EPA	Eicosapentaenoic acid	*Nannochloropsis, Phaeodactylum, Nitzschia*	Food supplement (omega-3 fatty acid, brain development for children, cardiovascular health)	Fish oil
DHA	Docosahexaenoic acid	*Schizochrytium, Cryptocodinium*	Food supplement (omega-3 fatty acid, brain development for children, cardiovascular health)	Fish oil

Adapted from Spolaore [87]

Figure 12 Major products and producers for food and feed

	Products currently on market (Form/Application)	Producers of current products	Micro-algae source
Food	Astaxanthin (Dietary supplement)	Cyanotech (US, Hawaii) [51]	*Haematococcus Pluvialis* [51][52][54][66][67]
		EID Parry (India) [53]	
		Mera Pharma (US/Hawaii) [66]	
		BioReal (Sweden) [71]	
		US Nutra (US) / Parry Nutraceuticals (India) [74]	
	Astaxanthin (Food Ingredient/Additive)	AlgaTech (Israel) [65]	
		Blue Biotech (Germany) [54]	
		Fuji Chemicals (Japan) [52]	
		Mera Pharma (USA/Hawaii) [66]	
		BioReal (Sweden) [71]	
	Spirulina (Dietary supplement)	Cyanotech (US, Hawaii) [51]	*Spirulina* (algae as product)
		Earthrise (US, California) / Dainippon (Japan) [56]	
		EID Parry (India) [53]/ USA Nutra [74]	
		Blue Biotech (Germany) [54]	
		Inner Mongolia Biomedical Eng, (Mongolia) [55]	
		Panmol (Australia) [67]	
		Spirulina Mexicana (Mexico) [67]	
		Siam Alga Co (Thailand) [67]	
		Nippon Spirulina (Japan) [67]	
		Koor Foods Co (Israel) [67]	
		Nan Pao Resins Chemicals (China) [67]	
		Hainan Simai Pharmacy (China) [67]	
		Myanmar Spirulina (Myanmar) [67]	
		Blue Continent (NA) [67]	

Products currently on market (Form/Application)	Producers of current products	Micro-algae source
Chlorella (Dietary supplement)	Blue Biotech (Germany) [54]	*Chlorella* (algae as a product)
	Earthrise (US) / Dainippon (Japan) [56]	
	Roquette Kloetze (Germany) [69]	
	Chlorella Co. (Taiwan) [67]	
Chlorella (Food ingredient)	Phycom (Netherlands)	*Chorella*
Other Dietary supplements (Dietary Supplement)	Innova IG (France) [67]	*Porphyridum* (algae as a product
EPA/DHA (omega-3) as dietary supplement	Ocean's Alive (USA)	*Nannochloropsis*
	Flora Health (USA) [72]	*Schizochrytium* [67]
EPA/DHA (omega-3) (food ingredient)	Martek/DSM (USA/NL) [55]	*Chrypthecodinium* [55][157]
	Blue Biotech (Germany) [54]	*Nannochloropsis* [57]
	InnovaIG (France) [67]	*Odontella* [67]
	Photonz [75] (New Zealand)	(NA)
	Xiamen Huison Biotech Co. (China)	*Schizochrytum* [157]
	Lonza 2010 [157]	Ulkenia [157]
β-carotene (as additive/vitamin/colourant)	EID Parry (India) [60]	*Dunaliella Salina* [67][70]
	Cognis Australia/BASF (Australia/DE) [55]	
	Betatene/BASF (DE)	
	Natural Beta Technologies (Australia) [62]	
	Tianjin Lantai Laboratory, China [62]	
	Nature Beta Technologies (Israel) / Nikken Sohonsa (Japan) [67]	
	Aqua Carotene Ltd (Australia) [67]	
	Pro Algen (India) Biotech [70]	
	Shaanxi Sciphar Biotechnology Co. [67]	
	DSM [67]	
Phycocyanin (colourant)	Blue Biotech (Germany) [54]	*Spirulina* [75][54]
	SandaKing (Japan) [75]	
	DIC Lifetec (Japan) [interview]	

	Products currently on market (Form/Application)	Producers of current products	Micro-algae source
Feed	*Spirulina* (Dietary supplements)	Blue Biotech (Germany) [54]	*Spirulina* [54]
		Ocean Nutrition (Canada)[68]	
	Chlorella (Dietary supplements)	Blue Biotech (Germany) [54]	*Chlorella*
		Necton (Portugal)	
	Astaxanthin (Dietary supplement)	Blue Biotech (Germany) [54]	*Haematococcus pluvialis* [54][71]
		BioReal (Sweden) [71]	
	Biomass for aquaculture (Living algae as feed for fish in aquariums)	Blue Biotech (Germany) [54]	*Nannochloropsis* [54] *Isochrysis* [54]
		Necton (Portugal) [157]	*Pavlova* *Phaeodactylum* *Chaetoceros* *Skelotenma* *Thalassiosira* *Tetraselmis [Pulz]*
	Astaxanthin (Colourant for living fish)	Blue biotech (Germany)[54]	*Haematococcus pluvialis* [54]
		BioReal (Sweden) [71]	
	Algaepaste	Innovative Aqua (Canada) [72	*Nannochloropsis* [72] *Isoschrysis* [72]

NA =Not available

3.1.2 Food and feed products in the pipeline

3.1.2.1 Food and feed products in the pipeline found through desk research

Figure 13 shows the micro-algae products in the pipeline identified through desk research and their producers. The table combines new producers entering existing markets, such as sugar producer EID Parry (India) producing EPA/DHA, but also first-to-market innovations such as Algalin Flour (substitute for other lipids) developed by Solazyme Roquette Neutraceuticals [63]. The table also indicates the level of development in the pipeline.

Figure 13 Pipeline products for food and feed (based on desk research)

	Products in pipeline	Producers	Pipeline phase	Micro-algae source
Food	EPA/DHA (omega-3) as dietary supplements	Seambiotic (Israel) [61] EID Parry (India) [53]	Commercialised pipeline[2]	(NA)
Food	Algalin Flour (Lipid Additive)	Solazyme Roquette Nutraceuticals [63]	Commercialised pipeline	(NA)
Food	Phycocyanin (colorant)	EID Parry (India) [53]	Commercialised pipeline	*Spirulina* [53]
Food	Phycocyanin (colorant)	Inner Mongolia Biomedical Eng. (Mongolia) [64]	NA	*Spirulina* [64]
Feed	DHA/EPA Omega 3	Seambiotic (Israel) [61]	Advanced development[3]	*Nannochlorosis* sp. [61]

(NA) = Not available

3.1.2.2 Food and feed products in the pipeline from survey response

Survey respondents (see Section 3.1.2) were asked to provide information on pipeline products that they are aware of. This information was used to confirm the results from the literature review, and add product developments that are not yet published but are known by the field experts. The experts were asked to classify these products in four specified categories:

- Commercialised pipeline: product/innovation that have been authorised for production in at least one country, but are not yet marketed;
- Regulatory pipeline: product/innovation in the regulatory process to be marketed in at least one country;
- Advanced development: product/innovation for which there are multiple¬-location field trials and more than one proof of concept;
- Early development: product/innovation for which there is only one proof of concept.

The results are presented in Figure 14. The results roughly confirm the developments described above, but also yield additional insights. Firstly, more producers are stepping into the EPA/DHA, astaxanthin, carotenoids and phycocyanin markets. Products in the advanced development stage which were not identified in the literature review are fucoxanthin (similar to astaxanthin), proteins, β-glucan (a polysaccharide) and phycoerithrin (a pigment). Products that are in the early development stage include Lutein, but most products in this stage are non-food/feed applications. None of the products mentioned in the table below is from GM algae, since GM algae nowadays are only used at a research stage.

Figure 14 Pipeline products from the survey

Product	Development Stage	The number of respondents that have mentioned this product
EPA/DHA	Advanced development	21
Aquaculture feed	Early Development - Advanced development	10
Proteins	Advanced development	10
Astaxanthin	Advanced developments	8
Omega-6 oils for nutritional applications	Commercialised	7
Animal feed	Commercialised	7
Antioxidants	Early development	7
Carotenoids	Advanced developments	6
Phycocyanin (pigment)	Advanced development	5

2 Authorised for production, but not yet marketed

3 Multiple proofs of concept, multi-location trials

Product	Development Stage	The number of respondents that have mentioned this product
Pigments	Advanced development/Regulatory	4
Whole biomass	Advanced development	4
Fuel	Early development	3
Fucoxanthin (comparable to Astaxanthin)	Advanced development	2
Cookies holding whole dried micro-algae	Commercialised	2
Anti-fungal biomass replacing pesticides in food production	Early development	1
Phycobiliproteins from blue green algae other than Spirulina	Early development	1
Novel bioreactors (process)	Early development	1
Algae harvesting membranes system (process)	Commercialised	1
Bulk oils for food/feed development	Early development	1
Feed premix for land farming	Early Development	1
Lutein (carotenoid)	Early development	1
Enzymes	Early development	1
Cosmetics	Commercialised	1
Noodles with whole algae	Commercialised	1
Anti microbial	Early development	1
Terpene Synthase (enzymes producing carotenoids)	Early development	1
Probiotica	Early development	1
Soil amendments	Advanced development	1
Novel carbohydrates	Early development	1
Phycoerythrin (pigment)	Commercialised	1
Vaccines	Advanced development	1
Beta-glucan (polysacharide)	Advanced development	1
Extra-cellular polysacharids	Advanced development	1
Adjuvants from green chemistry (enhancing immunology)	Early development	1
Fatty-acids	Advanced development	1
Waxes	Early development	1
Resins	Early development	1

Source: survey

3.1.3 Products on the market other than food and feed

Although not the main focus of this study, the desk research also included a brief inventory of other micro-algae applications than food and feed. Figure 15 shows the main products, most of them in the cosmetics market. A small number of European (especially French) and USA companies dominate the market of micro-algae components that are used in skin care products. More niche applications are in the life sciences, in which the Dutch chemical company DSM has a strong position. There is no indication of market parties actively pursuing applications in the pharmaceutical sector.

Figure 15 Other products and producers

	Products currently on market	Producers of current products	Micro-algae / product from micro-algae
Cosmetics	Personal care skin products	Solazyme (US) + Unilever Fuji Chemicals [77]	(NA)
		Soliance (France) [78]	*Spirulina*
		LVMH (France) [87]	*Chlorella*
		Daniel Jouvance (France) [87]	(NA)
		Algenist /Solazyme (USA, California) [79]	'Alguronic acid' (trade name for a undetermined mix of polysaccharides produced by micro-algae clogging filters in algae cultures)
	Anti aging skin product (lipid)	Soliance (FR) [78]	*Skeletonema costatum*
		Exsymol S.A.M. (Monaco) [87]	Arthropira (*Spirulina*)
		Pentapharm (Switzerland) [87]	*Nannochloropsis* *Dunaliella Salina*
	Hydrating skin product	Soliance [78]	*Porphyridium cruentum*
		Codif (France) [87	*Chlorella*
	Anti – inflammation (peptide)	Soliance [78]	*Phaeodactylum tricornutum*
	Slimming products	Soliance [78]	*Dysmorphococcus globosus*
Other products	Fluorescent protein markers	Martek/DSM	(NA)
	Stable isotope biochemicals	Spectra Gases/Martek/DSM [87]	(NA)

(NA) = Not available

3.1.4 Pipeline for products other than food and feed

The most important non-food/feed application of micro-algae in the pipeline is the production of biofuels. Since there is a quite broad range of companies currently developing these applications, and given the fact that this is a relatively distinct market not related to food and feed, these firms have not been included in this analysis [94]. An exception was made for the companies Solazyme [77] and Seambiotic [61] that are developing biofuel applications but are also active in other markets.

Micro-algae based pharmaceutical products are mentioned as promising applications (see for instance Pulz and Gross, 2004). Our analysis shows that this is only addressed in academic research; no concrete indications for products in the pipeline have been found.

Figure 16 shows an overview of a small number of other micro-algae applications that are in the early development stage, notably in cosmetics and niche products serving the life science sector. There are early stage developments in the pharmaceutical sector by research organisations about vaccines and anti-microbial paste. None of these products is from GM algae.

Figure 16 Other applications in the pipeline

	Products in pipeline	Producers	Pipeline phase	Micro-algae source
Cosmetics	Suncare product	Cyanotech [51]	Early development (1 proof of concept)	(NA)
Other pro-ducts	Dielectric Insulating Fluids	Solazyme + Dow Chemical [79]	Early development	(NA)
	Bioplastics	Algix (US) [93]	Early development	(NA)

(NA) = Not Available

3.2 Markets

Although the total production volume and market size of micro-algae in general are still relatively small, they have been characterised by high growth since 1999. In 1999, global production volumes of micro-algae were estimated at only 1000 tonnes dry weight. This has increased to 5,000 tonnes dry weight representing € 1 bn by 2004 [83], which represents a 5-fold increase in five years [87]. In 2011, the total production volume has risen to 9,000 tonnes dry weight [94]. The value of the global marine biotechnology market in 2011, with micro-algae as its main component, was estimated at €2.4bn, with an expected yearly growth of 10 per cent [91]. Note that this volume is still small compared to other food commodities. Global wheat production for instance is around 700 million tonnes annually [4], 70,000 times as much. Although some micro-algae applications have a long tradition, large-scale commercial production of micro-algae can still be considered an infant industry.

Over the past decades, over 75 per cent of the production volume of micro-algae was used in the health food market as dietary supplements [67]. The algae based high value food additives and ingredients such as DHA represent a growing market. For instance, Martek's (now DSM) algae-derived DHA is found in 99 per cent of all baby food in the USA [85]. Other large companies in the food ingredients market - BASF, Unilever and Dow Chemical - have noticed the potential of these applications and their associated growth and have made major acquisitions.

Generally, micro-algae are produced for one specific application. Multiple component production (or fraction) is not yet commercialised. There is no market for the left-over biomass after the valuable component – e.g. Astaxanthin - has been extracted, given the low volumes that are produced.

Figure 17 shows the most recent information available on the different sub-markets of micro-algae(-derived) products. Key players are mentioned between brackets in cases where the total production volume is dominated by these specific producers. The potential market for derived product is measured by the market size of synthetic (e.g. Astaxanthin) or traditional (e.g. fish oil) alternatives. The results clearly show that *Spirulina* and *Chlorella* still have by far the largest production volumes, but also point to the large potential markets for high-value products such as DHA/EPA, β-carotene and Astaxanthin. In general, the production is quite concentrated on a small number of players, except for *Chlorella* production that is distributed across relatively many small players. All data are world estimates, and due to the opacity of production volumes of individual producers, shares for specific countries/regions cannot be deducted. Information on the size of the European market is therefore not available. The interviewees confirmed that there are no independent data on production figures in Europe, but one expert estimates Europe's production share at around 5 per cent globally.

4 FAOSTAT figure

Figure 17 Market figures of micro-algae based products

Current product based on micro-algae	Production volume (tons/ year dry weight)	Number of producers (key players)	Value of production volume (yearly turnover)	European share in produc-tion (%)	Potential market (synthetic / traditional forms)
Food and feed products: whole dryed micro-algae biomass					
Spirulina	5,000 tonnes[5]/ year (2012) [92]	>15 companies (Cyanotech / Earthrise) [83]	US $ 40m (2005)[83]	(NA)	No synthetic alternative
Chlorella	2,000 tonnes/ year (2003) [92]	> 70 companies (NA) [83]	US $38m (2006)[87]	(NA)	No synthetic alternative
Food and feed products: micro-algae components					
Astaxanthin (based on Hae-ma¬tococcus)	300 tonnes/year (2004)[92]	>8 companies (Fuji Chemicals, Cyanotech)	US $10m (2004) [87]	(NA)	US$200m (2004) [83]
Phycibiliprotein colourants (incl phycocyanin)	(NA)	>2 companies [83]	(NA)	(NA)	> US $ 50m (2004) [83]
EPA/DHA (Omega-3 PFA) (based on *Chrypthecodinium*)	240 tons[6]/year (Martek) (2003) [83]	>4 companies (Martek/DSM) [85]	> US $300m for Martek (2004) [83]	(NA)	±US 14.39bn (2009)[88]
β-Carotene (based on *Dunaliella Salina, Schizochrytium, Nannochloropsis*)	1,200 tons per year (2010) [87]	> 10 companies (Cognis/BASF) [83]	(NA)	(NA)	US $ 285m [83] (2012)
Other products					
Stable isotope biochemicals	(NA)	> 1 company (Martek/DSM) [87]	> $13m (2006) [87]	(NA)	(NA)
Skin products	(NA)	> 4 companies			
(Solliance, Solazyme/ Unilever)	(NA)	(NA)	(NA)		
Fluorescent protein markers	(NA)	> 1 company			
(Martek DSM)	(NA)	(NA)	(NA)		

** year for which estimation was made, past and future in brackets. NA = Not Available*

3.3 Micro-algal production costs

In order to gain better insight on the potential market opportunities in micro-algae production, cost structures are of high importance. Figure 18 provides recent estimates of the cost structure of different technologies currently used for micro-algae production. As can be seen, there is substantial discrepancy between estimates from different studies. Unfortunately, no large-scale comparative cost studies have been performed [94], and as such these estimates rely on individual examples.

Currently, three main types of production technologies for micro-algae exist: open ponds, horizontal tubular photobioreactors (PBRs) and flat panel PBRs (see 2.2 and 2.3 above). Open ponds require more land, but are generally cheaper compared to the other options, since PBRs are still relatively inefficient [91]. However, PBRs have a much better technical capacity to enhance future yields, since conditions can be controlled precisely, in contrast to open-pond systems.

5 tonnes - European tonne, 1 tonne = 1,000 kg

6 ton – US ton, 1 ton = 907.1847 kg

These figures were derived for (theoretical) installations of 100 hectares (ha) and 200 ha plant size. Note that in reality the largest plant producing *Spirulina* is only 44 ha [83], so actual costs per kg dry weight in current production sizes are usually higher. A more realistic estimate of total costs per kg dry weight in more typical plant sizes is around $8 - $11 [62]. Costs have already come down considerably since 2004 [94]. Note that these are average figures with all commonly used micro-algae types provided by Norsker et al (2010) [92], and the Scenedesmus almeriensis provided by Acien [92]. The total production costs of *Haematococcus* for Astaxanthin production is for instance higher than $30 per kg dry weight [91].

In micro-algae production, economies of scale play a large role due the large fixed capital expenditures and minimum-bound labour costs. This is reflected by a study showing that for 1 ha the average cost per kilo dry weight amounts to €17.72, for 100 ha this results in an average cost of €4.95 [92]. It is unclear whether further scale increases would result in lower costs, although it is likely to be expected.

When comparing these cost figures for algae biomass with other biomass sources, it becomes clear that micro-algae is currently far away from being a cheap source of biomass, as for instance wheat straw biomass sells for €0.03 per kg [62].

For micro-algae-derived products, extraction costs come on top of micro-algae production costs. There is hardly any information available on the costs of extraction technologies in the micro-algae production process. One estimate for oil extraction is a cost of around €1.32 per kg (2007) [5]. For making pharmagrade (i.e. 99 per cent pure) Astaxanthin, expensive extraction techniques are required, which add between €10-€15 per kg to the total costs. With conversion rates and losses, the total production costs for Astaxanthin from micro-algae approaches €465.58/kg.

The use of micro-algae for fuel applications, currently in development, is economically difficult: according to recent research, near optimal production is required to compete with current biomass prices, as for instance palm oil biomass is sold for €0.50 per kg [91] [94].

Figure 18 Production costs of micro-algal systems data from different studies

Production technology	Capital costs per kg	Labour per kg	Other variable costs (utilities, fertilizer) per kg	Total costs per kg for a large (100 ha[92]/ 200 ha[94]) plant	Optimal theoretical total costs per kg dry weight
Open ponds	€3.02 [94]	€0.15 [94]	€1.57 [94]	$25 (2004) [94] €4.95 [92] $8 - $11 [62]	€0.68 [92] €0.21 [62]
Horizontal tubular FBR	€2.74 [92] €9.8 [94]	€0.88 [94] €0.36 [92]	€0.92 [94] €1.65 [92]	€4.15 [92] €12.6 [94]	(NA)
Flat panel FBR	€2.01 [92]	€0.35 [92]	€1.01 [92]	€5.96 [92]	€1.8 [94]

Figures for 2010/2011. PBR = photobioreactor, NA = Not available

3.4 Future developments of the microalgae-based products for food and feed in the EU

This section reports the results of the Delphi-method electronic survey that was used to explore the future perspectives for the EU, and drivers and constraints for research and production. Six of the experts selected for the interviews also answered the survey questions. The results of this part of the interview have been combined with those of the respondents of the electronic survey.

The results of the Delphi part of the questionnaire (Europe's market position and micro-algae as substitute) are presented in sections 4.4.1 and 4.4.2. The results of the questionnaire on production costs are presented in section 4.4.3 and those on Europe's competitive position in section 4.4.4.

3.4.1 Market position of EU firms

3.4.1.1 Statement

The introductory text and related statement presented to the respondents on Europe's market position (and on which the experts had to answer the questions) are the following:

Introduction: At the moment there are five micro-algae based components and two algae ¬biomass products on the food and feed market worldwide. The first category includes Astaxanthin, *β*-carotene, Phycocyanin and two omega-3 fatty acids (EPA and DHA) and the second *Spirulina and Chlorella*. In the future, micro-algae based proteins and oils will also become available as food ingredients. At the moment production mainly takes place outside Europe, but the market position of Europe-based companies is growing, mainly by acquisition.

Statement: **Europe is leader in micro-algae based products for the food and feed markets**.

The respondents were asked for the time frame in which the situation mentioned in the statement will be realised, and for the factors that have a positive influence on the achievement of the situation described in the statement.

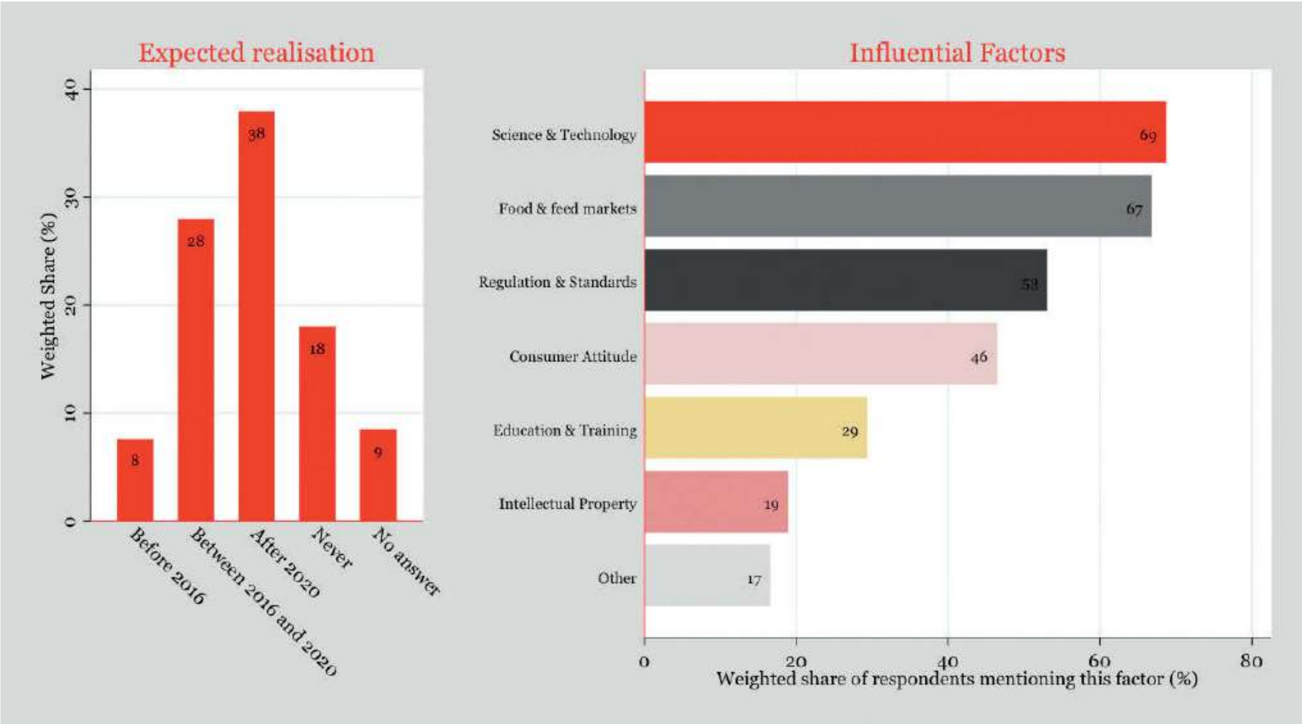
They could choose a maximum of five from the following list:

- Scientific and technological developments in the field of micro-algae research;
- Education and training in the field of micro-algae research;
- Developments in the food and feed market including the position of Europe versus other world regions and the competition of micro-algae based products with those from other sources;
- Intellectual property rights, including patent positions of companies in the field;
- Regulation and standards;
- Consumer attitude, referring both to consumer trends such as 'natural' food, 'healthy' food, but also to GM food.

3.4.1.2 First round of results

Figure 19 shows the main results for this statement in the first round. Clearly, a large proportion of respondents expects that Europe will only become a market-leader in algae based products after 2020 (38 per cent). Very few respondents believe this will happen before 2016, and a significant proportion does not see this happening at all. There is no significant difference between levels of expertise, although respondents with the highest level of expertise are more likely to indicate 'Never', and mid-level experts are more likely to say 'After 2020'.

Figure 19 Europe's market position (first round)



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Looking at positive influential factors, Science & Technology and Food and Feed markets are clearly the most important, whereas IP and Education and Training are of notably less concern, according to the experts. Other positive factors mentioned are almost all related to the 'availability of finance and risk capital'. One respondent mentioned the following:

> "*Europe has many first mover opportunities based on the advancements in algae biomass commercialisation by European algae technology companies. This can be exploited further, but there needs to be a transition from research funded support to commercialisation funding support.*"

Other respondents commented on the fact that Europe has a competitive disadvantage due to strict regulatory standards, mainly related to the Novel Foods Regulation:

> *The only factor why Europe will never be market leader is because of the regulatory environment. Novel food regulation (and to a lesser extent health claim regulation) severely hampers products going to market. So many people with good ideas, proven science and production models, but getting to market takes too long and costs too much money.*

The more sceptical respondents, who do not see market leadership happen, often refer to the European climate and domestic market for algae products:

> "*[...] Europe has neither the climate for large-scale algae production nor the markets to become a world leader.... China already produces over 85% of all micro-algae and macro-algae biomass produced*".

The interviewed experts stress that a leading market position will only be applicable to the high-value molecules market, or market control through large multinationals, but not local production. Other experts stress that such scenarios are generally optimistic (not necessarily unrealistic), but do require an increased level of investment, technical solutions and entrepreneurship for the statement to be achieved.

3.4.1.3 Second round of results

In the second round (presented in Figure 20), the respondents were asked to specify the expected date of achievement of the scenario described in the statement. The largest group (30 per cent) of the respondents that answered the Delphi-questions selected the period 2020-2022 in the second round, which confirms the results of the first round. A significant share of respondents (22 per cent) believes this will already happen in 2016-2018. We also asked what driving factor is the most important of all: this is clearly Science & Technology.

3.4.2 Micro-algae based products as substitutes

3.4.2.1 Statement

The introductory text and related statement provided for the question about the potential for using micro-algae based components as substitutes for synthetic or other natural material are the following:

Introduction: Food and feed micro-algae based components can be extracted from micro algae biomass, but also from other natural sources or they can be synthesised by the chemical industry.

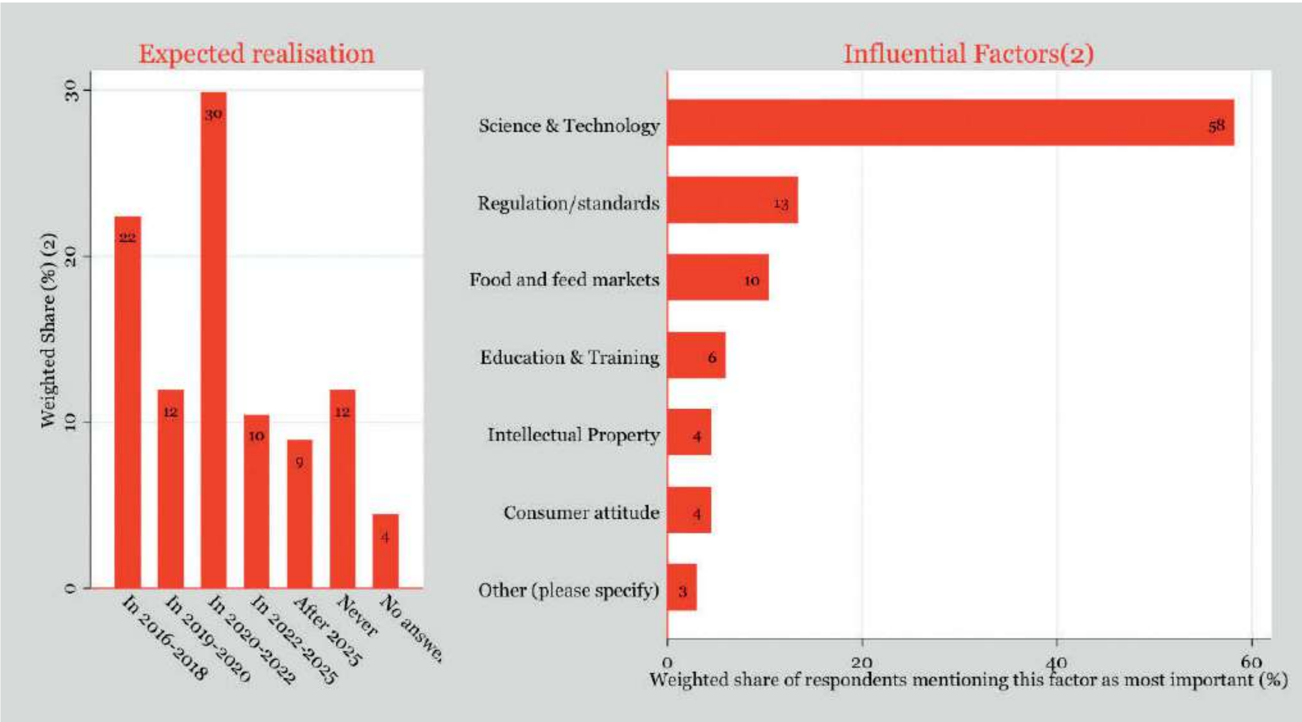
Actual situation at the moment:

- Astaxanthin: most is produced synthetically;
- β-carotene growing volumes are produced using micro-algae, but also extracts from carrots;
- Phycocyanin: mainly from cyanobacteria (blue algae);
- EPA and DHA: extracted from fish fat and walnuts.

Statement: **Astaxanthin, β-carotene, Phycocyanin, EPA and DHA for food and feed applications are mainly from algae resources**.

The respondents were asked for the time frame in which the situation mentioned in the statement will be realised, and for the factors that have a positive influence on the achievement of that situation. The respondents could choose from the list presented in Annex B.2.2.

Figure 20 Europe's market position (second round)



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3.4.2.2 First round of results

Figure 21 shows the aggregate results for the questions on products substitution in the first round. Most respondents expect that high-value components such as EPA/DHA and Astaxanthin will be mainly produced from micro-algae somewhere between 2016 and 2020, although an almost equally large group expects this to happen only after 2020. Only 11 per cent of respondents think this situation will never arise, although even fewer think that it will happen before 2016.

The most important driver is again 'Science and Technology', chosen by almost 70 per cent of respondents as one of the most important factors. Another important factor is 'Food & Feed markets', including market developments of alternative competing sources such as mentioned by one of the respondents:

> "*Extraction from fish will become more and more expensive; this favours micro-algae*"

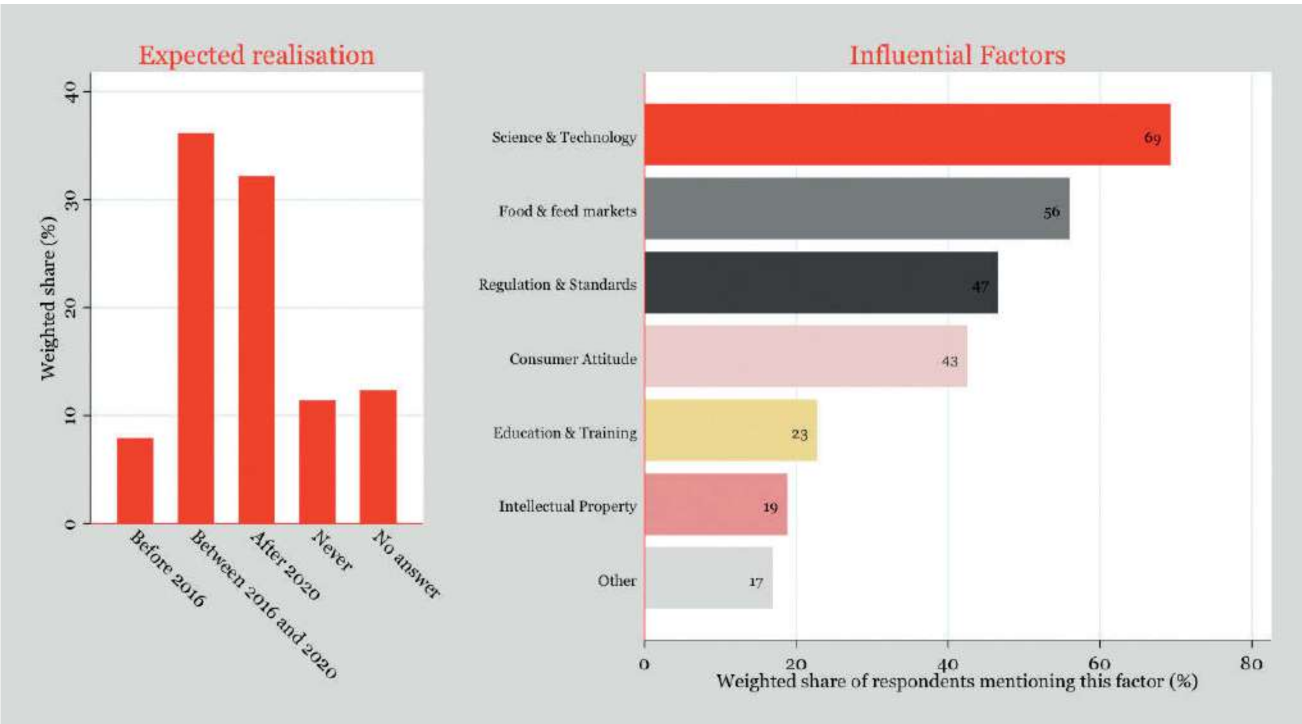
'Regulation and Standards' and 'Consumer Attitude' are also relatively important, according to experts. Again, those respondents noticing other factors mainly referred to finance issues.

One respondent noted that the trends diverge for Astaxanthin and Phycocyanin on the one hand, and EPA/DHA on the other hand:

> "*Astaxanthin and Phycocyanin will mostly come from algae in the future. This will not be the case for EPA/DHA, as the sheer volume produced world-wide is simply much larger*"

Interviewed experts who claim to be very familiar with the topic are more sceptical than those claiming to be rather familiar, although the difference is not significant. 18 per cent of those very familiar expect the situation to be unachievable, against 7 per cent of those rather familiar. In the interviews, one expert indicated that substitution by micro-alga based products will increase due to overfishing of salmon, leading to higher prices for alternative sources of fish oil. More sceptical experts indicate that other bio-sources are perhaps more competitive than micro-algae, such as yeast, fungi and bacteria.

Figure 21 Micro-algae as substitute for food/feed high-value products (first round)



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3.4.2.3 Second round of results

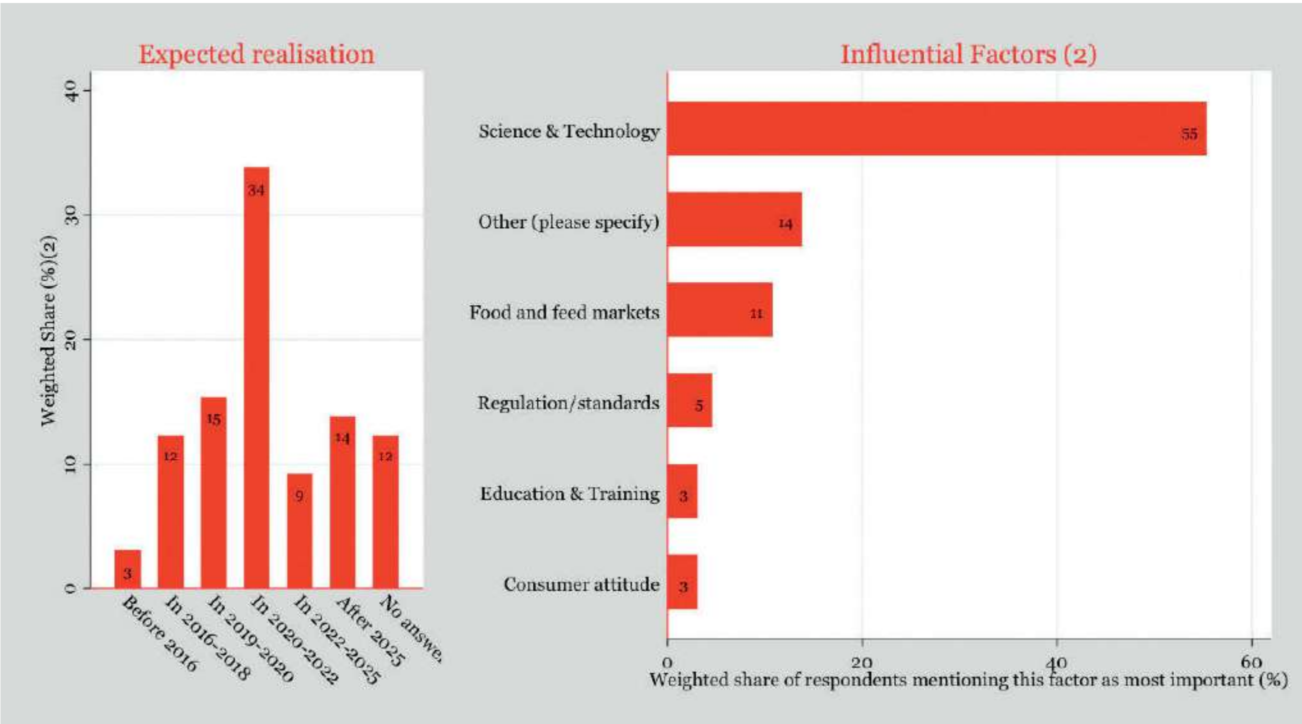
Figure 22 shows the results of the second Delphi-round for the statement on products substitution. The expected achievement data converges on the 2020-2022 period, with 34 per cent of respondents opting for this choice. For this aspect as well, 'Science and Technology' is considered by the experts as the most important driving factor. The 'other' category mainly includes demand-side factors such as market interest in sustainable products and cost of alternative biomass sources.

Respondents were asked to indicate which technical challenges contributed to the achievement of the situation in the statement.

They could choose a maximum of five from the following list:

- biomass production technologies;
- harvest technologies;
- extraction technologies;

Figure 22 Micro-algae as substitute for food/feed high-value products (second round)



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3.4.3 Production costs

3.4.3.1 Statement

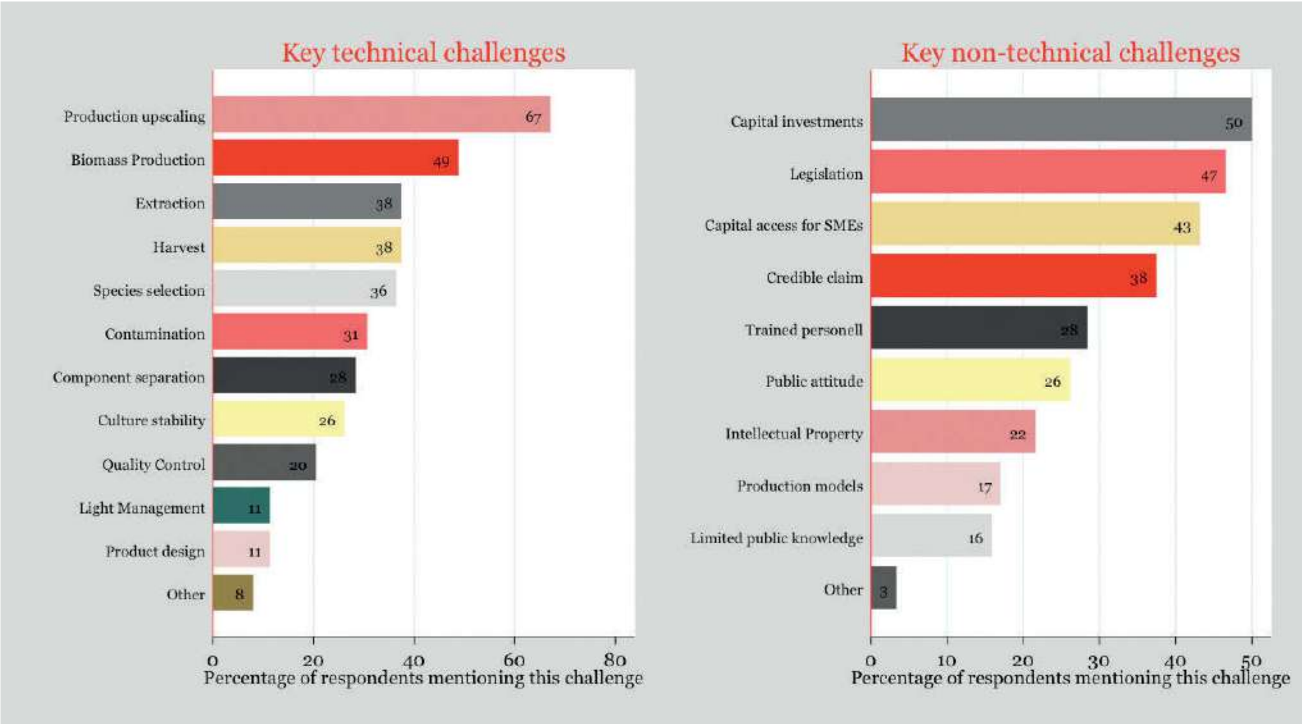
Respondents were asked to name the key challenges that need to be addressed for the following statement to be true:

Introduction: Production costs of micro-algae based components for the food and feed market are still relatively high, as compared to chemical synthesis or extraction from plants of the same components.

Statement: **In 2020, production costs of micro-algae based products for the food and feed market are so low that in Europe most of these products are now micro-algae based**.

- up-scaling of production systems;
- component separation technologies;
- product design;
- micro-algal species selection;
- culture stability;
- contamination/ predator invasion/ weed algae invasion;
- quality control monitoring;
- light management;
- other: ....

Figure 23 Challenges in production costs (respondents could choose five challenges for each category)



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In addition they were asked to indicate which non-technical challenges contributed to the achievement of the situation in the statement.

They could choose a maximum of five from the following list:

- credible product claims of food companies (that use micro-algae or their components in their end-products) in product advertisements and on the products' label;
- access to credit by small companies active in the field of micro-algae research and production;
- capital investment of large companies in micro-algae research and production;
- intellectual property rights;
- personnel trained in micro-algae research and production;
- access to production models that are suitable to micro-algae production and production of its components;
- lack of public knowledge on the value of micro-algae as food and feed product;
- public attitude towards bio-based products referring to the discussion in public debates on the use of biomass for food or for fuel and related issues such as access of third world countries to new agrifood technologies;
- legislation, regulations and standards;
- other: .....

3.4.3.2 Results

Figure 23 shows the challenges that respondents think have priority to be addressed in order to arrive at the situation described in the statement. On the technical side, production up-scaling is the main challenge identified by two-thirds of the experts involved:

> *"Key will be to design integrated or large-scale production systems to sufficiently reduce costs"*

> *"Production volume is the issue at this moment. Producers and customers need to find each other to jointly increase market volume up to the point that capacity is not the biggest bottleneck."*

On the non-technical side, capital investments, credit access and legislation are considered as the main barriers. Other factors that were mentioned are the willingness of large companies to invest in micro-algal solutions and CO_2 pricing mechanisms. One respondent also noted that it is important to distinguish between the food and feed markets:

> *"At present, algae companies typically focus on supplements (food) rather than feed market due to low volumes and high cost of production. Cost effective scale-up needed for use of algae as a bulk feed ingredient."*

3.4.4 Europe's competitive position

3.4.4.1 Statement

Respondents were asked to identify the most important challenges for Europe's competitive position in industry-relevant R&D, based on the following statement:

Introduction: European research groups are very active in micro-algae research and the European Commission invested in this research field through several framework programmes. Also, European industry is increasingly active in the field. However, Europe does not have a top position (R&D, production) as compared to other world regions.

Statement: In 2020, Europe's micro-algae research for food and feed applications is fine¬-tuned to the needs of European industry and leads to new products and lower costs production processes, thereby strengthening the competitive position of the European industrial sector in this field.

The respondents were asked to indicate which key challenges contributed to the achievement of the situation in the statement.

They could choose a maximum of five from the following list:

- doubling of EC-budgets for micro-algae research;
- technical breakthroughs in micro-algae research;
- regulatory approval of GM algae based products;
- access to venture capital;
- academic and industrial training in micro-algae research and production;
- reduction of biomass production costs;
- better communication and cooperation between research organisations and companies;
- other:……..

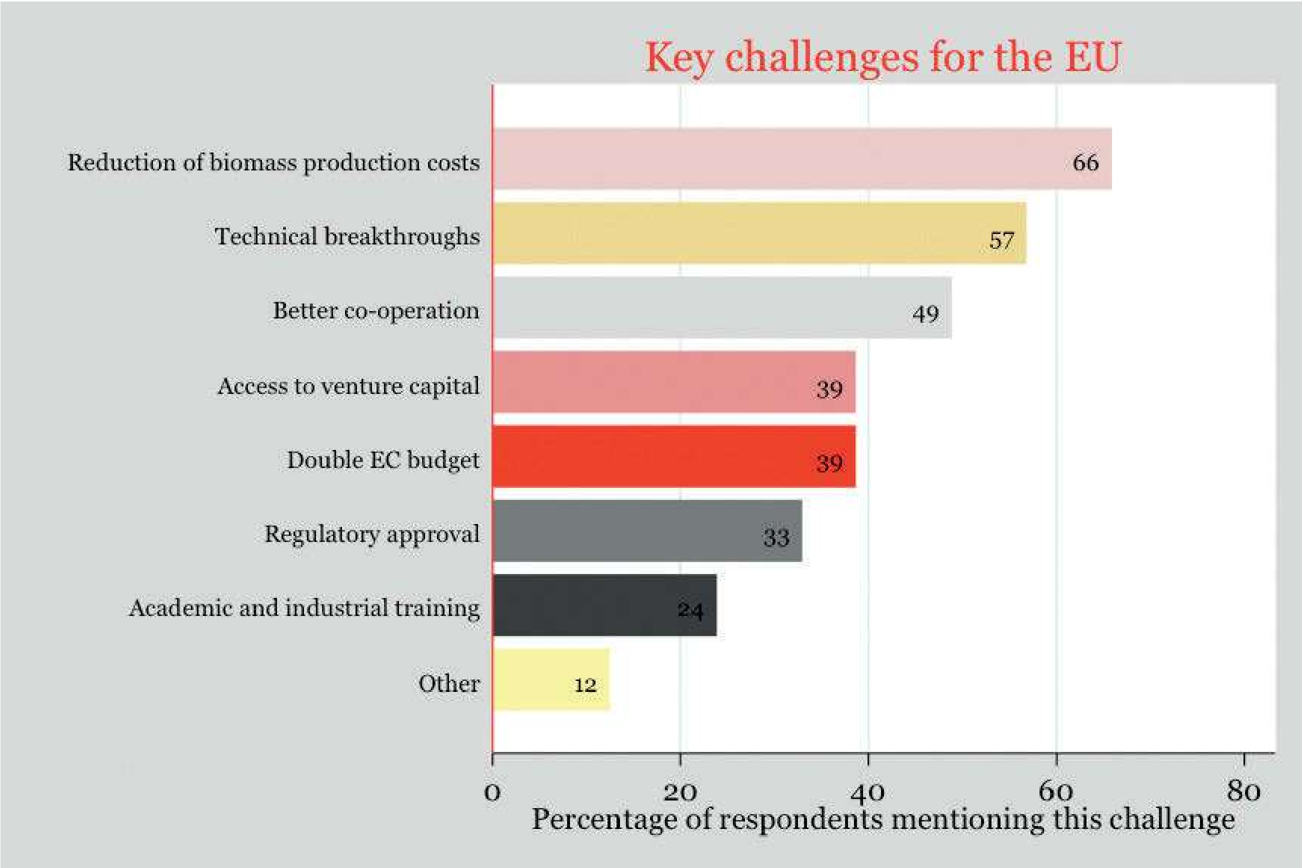
3.4.4.2 Results

The results are presented in Figure 24. In order to improve Europe's competitive position in the field of micro-algae R&D, cost-reduction and technical breakthroughs are considered as the most important challenges by experts. Better co-operation, especially between academia and industry, is considered relevant by around half of the respondents:

> "*[The Commission should (ed.)] substantially reduce investing in R&D at universities and put this into product approval teams that put products on the market, safe, cheap and fast. Consortia of industry & academia should play a key role in this.*"

> "*The European companies should take most more risks and should invest much more in R&D. The link between Research and Companies should be improved and strengthened*"

Figure 24 Competitive position of Europe



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3.5 Conclusions

This chapter presents the economic aspects of current micro-algae production and future developments, focusing on factors that can positively contribute to Europe's competitive position in this field in 2020.

Currently, the most important micro-algal products for the food and feed sector are dried micro-algae (such as *Spirulina* and *Chlorella*), mainly sold directly as dietary supplements. These products are relatively mature. Nowadays, specific high-value components of micro-algae are also being extracted; the most important high-value molecules currently on the market are pigments and fatty-acids used as dietary supplements. Although most producers of micro-algae for food and feed are located in Asia, the USA and Australia, European multinationals such as BASF and DSM have acquired a number of leading producers in Australia and the USA. Analysis of pipeline products show that more producers are stepping into the micro-algae components markets. Other applications of micro-algae and their components are mostly in the cosmetics market. There are no indications of market parties actively pursuing micro-algae applications for the pharmaceutical sector.

Although the total production volumes and market size of micro-algae in general are still relatively small, they have been characterised by high and increasing growth rates, from 1000 tonnes dry weight in 1999 to 9000 tonnes dry weight in 2011. Over 75 per cent of the production volume of micro-algae was for the dietary supplements market; however, also the algae-based high-value food additives and ingredients, such as DHA, have a growing market.

There is hardly any public information available about the cost structure of the production process (including extraction) of micro-algae components from micro-algae. Some data are available, but there is substantial discrepancy between estimates from different sources.

At the moment micro-algae production takes place mainly outside Europe, but the market position of Europe-¬based companies is growing (mainly by acquisition). Most experts consulted (in the Delphi-survey) estimate that Europe can become market leader in micro-algae based products for the food and feed markets in 2020-2022. The two most important factors that are expected to contribute positively to the European market position are scientific and technological developments in the field of micro-algae research and in the food and feed market. However, experts highlight two major factors limiting the European potential: the insufficient European domestic demand for these products and the difficulties in achieving commercial authorization due to the Novel Food regulation. Hence, new microalgae-based products obtained in Europe will be intended mainly for foreign markets.

Many high-value molecular components that can be extracted from micro-algae biomass, can also be obtained from other natural sources or can be synthesised by the chemical industry. Consulted experts expect that high-value components such as EPA/DHA and Astaxanthin will be mainly produced from micro-algae in the period 2020-2022. The main driving factor is scientific and technological development in the field of micro-algae research; others include demand-side factors such as market interest in sustainable products and high cost of alternative biomass sources.

At present, the low volumes and high production costs of microalgae permit exclusively the production of supplements and nutrients for human consumption. The microalgae-based molecules have specific advantages with respect to their synthetic and traditional alternatives that makes their use commercially viable for the food sector, despite the higher production costs. The higher quality of microalgae-based molecules compared to the corresponding alternatives is mainly due to their chemical conformation that is much more effective for food applications than the syntetic variants. However, the bulk production of carbohydrates and proteins for the feed sector is not yet forseen in the short run, because it would require higher production volumes and, consequently, the boosting of the cost effective scale-up with dramatic reduction of production costs.

Production costs of micro-algae based components for the food and feed market are still relatively high in comparison to chemical synthesis or extraction from plants. The main technical challenge to lower costs of micro-algae production is the up-scaling of the production process; while the main non-technical challenge is capital investment.

In order to improve Europe's competitive position in the field of micro-algae R&D by 2020, cost reductions, technical breakthroughs and better co-operation between academia and industry are considered by experts as the most important challenges.

4. Outlook: R&D and prospects for micro-algae biotechnology and genetic engineering

This chapter provides an overview of the developments in biotechnological research and production with emphasis on genetically modified (GM) algae. The chapter starts with a section on new developments in algae research (4.1), continues with a section on research on transgenic algae (4.2) and closes with the main conclusions (4.3).

4.1 New developments in algae research

4.1.1 Research topics

Since algae are ubiquitous and have a long history of use, there is also a long history of research on these organisms. The oil crisis in the 1970s, moreover, increased research on algae substantially: large research programs were initiated on developing micro-algal energy production systems. The boost in algal research was generated in particular by worries about oil depletion and environmental concerns such as climate change, CO_2 issues and land use. Technical developments like the introduction of new generation DNA technologies, which lead to improvements in algal genetic modification techniques, have also contributed to the expanding of research on algae. Compared to the biotechnology of bacteria, yeast and fungi, algal biotechnology is a rather young discipline.

Two main fields of biotechnological research on algae can be distinguished [see 1, 16]:

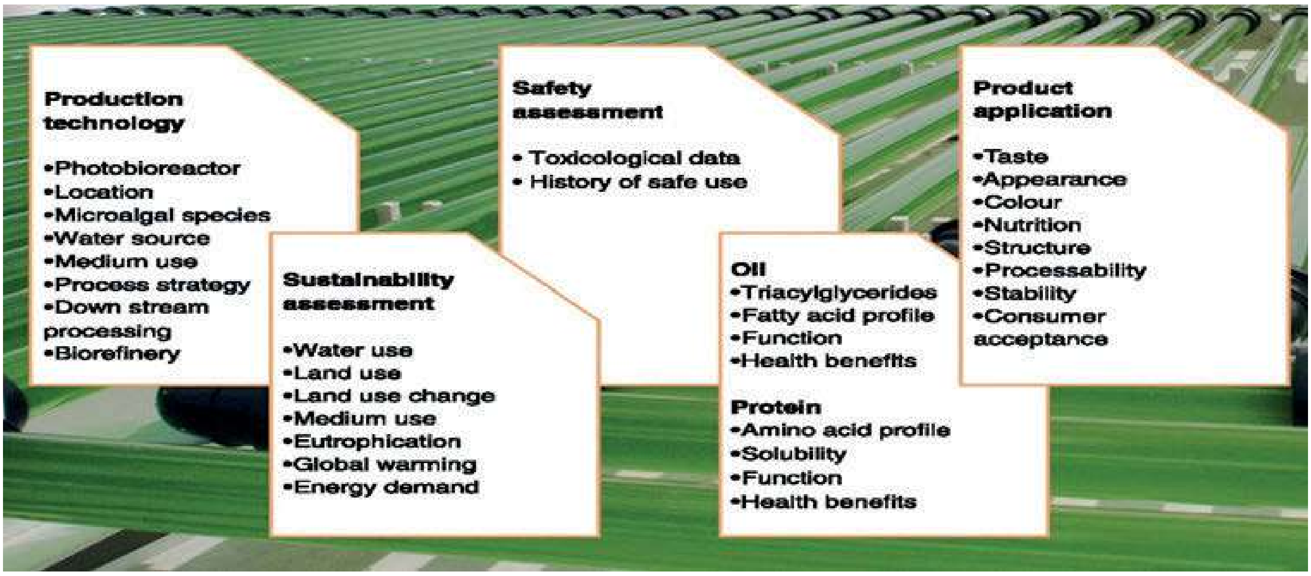
- Technological productivity improvement: this includes reactor design, process control, harvesting and extraction.
- Strain improvement: this includes strain selection, mutagenesis and genetic modification. This aspect is discussed in more detail.

Research on algal components (such as carotenes and Astaxanthin) and algal biomass for food supplements is mostly focused on improving productivity through optimisation of production systems, selection of new or better strains, optimising growth conditions and optimising extraction procedures.

More recent developments in DNA sequencing techniques, systems biology and tools to determine relevant enzymes and metabolites opened possibilities for the design and engineering of (new) metabolic pathways for the production of new algal products like biodiesel, bio-ethanol, bioplastics, recombinant proteins and new pharmaceuticals [17, 18, 19].

In 2013, the scientific literature presented perspectives regarding the benefits of using algae as natural sources of functional food ingredients [159], as well as a review on food commodities (oils, proteins) obtainable from micro-algae [160]. The authors conclude that micro-algae can be used as a sustainable source of food supply and a valuable source of bioactive compounds [158]. Furthermore, they have a great potential as feedstock for food commodities [160] (see also Figure 25). During the interviews, some experts mentioned the production of high value proteins. However, major breakthroughs are required to enable cost effective production of food ingredients from micro-algae.

Figure 25 Food commodities from micro-algae: overview of key features, which need to be considered to enable successful sustainable, cost effective and safe product applications [160].



[160]

4.1.2 Developments in micro-algae research

As mentioned in the previous paragraph, the big boost in algal biotechnological research in the past decade has been on developing micro-algal energy production systems (see [20]). In this paragraph we will briefly describe biotechnological developments in energy production systems and in food/feed applications.

Bioenergy

Worldwide, research and demonstration programmes are being carried out to develop the technology needed to expand algal lipid production, from a craft to a major industrial process, [21, 13, 22, 17, 23] with the purpose of the production of biofuels.

Research on algae is also an important topic in Europe and several algae R&D projects are on-going. In particular, the European Commission has financed many research projects through its Framework Programmes. An inventory of R&D projects related to algae and aquatic biomass was made in 2009 in the framework of the Aquafuels project [25]. Figure 26 presents the non-food algae projects, based on our knowledge of the field. These projects, additional to those mentioned in ref [25], are listed in the table from the BIOALGAESORB-project information.

Figure 26 Overview of expertise and EU-funded algae projects related to non-food applications

Project title	Acronym	Coordinator / Coordinating country	Finances
Biofuel from Algae Technologies	BIOFAT	Prof. Mario Tredici, IT and Abengoa Bioenergy, ES [121]	Project cost: €10. 4m Project Funding: €7.3 m
Sustainable Fuels from Marine Biomass Project	BIOMARA	Scottish Association for Marine Science (SAMS) at the Scottish Marine Institute UK [122]	Project costs: €6m Project funding € 4.9m from the INTERREG IVA
Biowaste and Algae Knowledge for the Production of 2nd Generation Biofuels	BIOWALK4BIOFUELS	IT [123]	
Energetic Algae	ENALGAE	UK [124]	EnAlgae has received 50 per cent of its funding through the INTERREG IVB NWE programme (€7.3m).

Project title	Acronym	Coordinator / Coordinating country	Finances
Utilisation of Micro-algae for Wastewater Treatment with Energy Purposes	ENERBIOALGAE	ES [125]	
Real Time Non Invasive Characterisation and Selection of Oil Producing Micro-algae at the Single Cell Level	FUEL MAKING ALGAE	CZ	Project cost: €45,000 Project Funding: €45,000
Marine Algae as Biomass for Biofuels	MABFUEL	Dr Julie Maguire, IE	Project cost: €1.4m Project Funding: €1.4m
Renewable Hydrogen from Sun and Water	SOLAR-H2	Margareta Uvhagen Antono, SE	
Biotechnological Exploitation of Marine Products and By Products	BIOTECMAR	Fabienne Guerard, FR [126]	
Control of Light Use Efficiency in Plants and Algae From Light to Harvest	HARVEST	Dr. Jan Dekker, NL [128]	Project cost: €4.6m Project Funding: €4.6m
Towards a Better Sunlight to Biomass Conversion Efficiency in Micro-algae	SUNBIOPATH	Prof. Claire REMACLE, BE [129]	
Enabling European SMEs to Remediate Wastes, Reduce GHG Emissions and Produce Biofuels via Micro-algae Cultivation	BIOALGAESORB	Cato Kjølstad, NoBio - Norsk Bioenergiforening NO [131]	
Development of an algal platform for production of building blocks for the chemical industry and their further conversion to products	SPLASH	Dr. Maria Barbosa, Wageningen, NL [132]	Project cost: €12.1m Project Funding: €8.9m
A sustainable chain for continuous biofuel production using micro-algae as a production platform, thereby making 2nd generation biofuels competitive alternatives to fossil fuels.	Fuel4Me	Dr. Maria Barbosa, Wageningen, NL	
This project will demonstrate on large scale the sustainable production of bio-fuels based on low-cost micro-algae cultures	All-Gas	Federico Salmon, Madrid, ES	

Project title	Acronym	Coordinator / Coordinating country	Finances
Demonstration of integrated and sustainable enclosed raceway and photobioreactor micro-algae cultivation with biodiesel production and validation.	INTESUSAL	Wilton Centre, Wilton, Redcar, UK	
Harnessing Oxygenic Photosynthesis for Sustainable Energy Production	HOPSEP	Prof. Nathan Nelson, Tel Aviv, IL	
Pilot scale algal research centre (including lipid production) Relevant to Biology & Botechnology, Economic assessment	ALGAEPARC	Dr. M. Barbosa, Wageningen, NL [133]	

Modified from: [25], [26]

Food and feed applications and other specialty products

In addition to the food and feed applications mentioned above, Ibañez and Cifuentes [159] recently indicated that algae are an interesting source of bioactive compounds to be used as functional food ingredients or as pharmaceuticals. However, several hurdles need to be overcome in order to get the information that is needed to make decisions about what are feasible applications for food and feed. Important research topics include: comprehensive screening of bioactive metabolites produced by different marine organisms, gene sequencing and characterisation, and unravelling the metabolic pathways behind secondary metabolites synthesis. Major biotechnological challenges are the development of transformation systems for a variety of micro-algae (expected to be achieved within 3-5 years for a few key species) and improved strain stability.

EU-funded research projects related to value-added food products are presented in Figure 27.

In addition to research on value-added food products from micro-algae, food safety assessment is also being investigated. For some source organisms, we can employ the concept of 'history of safe use' to assess consumer safety. For instance *Chlorella* species have already been used as food supplement in some countries and thus a history of safe use is available. Although there is not a complete package of information on toxicity of products from *Chlorella*, there is information on some relevant toxicology parameters and human consumption trials on this organism were already performed [see ref 160]. So, food safety analysis has already provided evidence for some of the products and micro-algal strains used [160] but more evidence on safety needs to be built up (see Chapter 5 for more information on safety aspects of micro-algae).

Figure 27 Overview of expertise and EU-funded algae projects related to food, feed or pharmaceutical applications

Project title	Acronym	Objective	Coordinator / Coordinating country
Novel algae-based solution for CO_2 capture and biomass production	ALGADISK	The aim of the ALGADISK project is to develop a modular, scalable, and automatic biofilm reactor for Algae biomass production, with low operational and installation costs. The reactor will be designed to capture CO_2 from industrial emissions to produce high value organic products.	SPAIN
Genetic Improvement of Algae for Value Added Products	GIAVAP	The consortium will adapt genetic engineering techniques to various algal strains of economic interest focusing on carotenoid and PUFA production and the over-expression of peptides of commercial value	Dori Schneider, ISRAEL [127]
Sustainable Production of Biologically Active Molecules of Marine Based Origin	BAMMBO	BAMMBO will screen and identify target marine organisms (e.g. bacteria, fungi, sponges, micro-algae, macro-algae and yeasts) from diverse global locations for potential as sustainable producers of high–added value molecules	Dr. D. Walsh, IRELAND [168}
AlgaeParc	Pilot scale algal research centre (including lipid production) Relevant to Biology & Biotechnology, Economic assessment	Research and production facility at pilot scale. Comparison of different production systems	Dr. M. Barbosa, THE NETHERLANDS [133]
Oil production with algae	Emerald Oils	Production of oils for food applications	Prof. Dr. R. Wijffels, THE NETHERLANDS

4.2 Genetic modification of algae

As a result of increased research on eukaryotic algae and cyanobacteria (e.g. *Spirulina*), a large amount of data, protocols and publications on the molecular biology of algae have become available [1].

This section describes the state-of-the-art of research on transgenic algae. It presents an overview of the genetically transformed algae strains (3.2.1), the DNA delivery methods employed (3.2.2) and the targets of genetic modification of algae (3.2.3).

4.2.1 Genetically modified algal strains and their stability

As presented in the COGEM report [1], transformation of the cyanobacterium *Synechocystis* was already reported in 1970 [27]. Successful transformation of the green alga *Chlamydomonas reinhardtii* was reported in 1989 [28]. *C. reinhardtii* has become the model species in molecular biology of (eukaryotic) algae and is therefore the best one described [28]. Since 1989 successful genetic transformation of approximately 30 algal species has been demonstrated [1, 19, 18 162]. Figure 28 provides an overview of algal species that have been genetically modified (as demonstrated by the expression of an antibiotic resistance gene, complementation of a mutation, or the expression of a reporter gene) and the stability of this transformed species (genetic modification of specific traits is discussed in Section 4.2).

Figure 28 Overview of genetically transformed algal species

Species	Stability of transformation*	Species	Stability of transformation*
Chlorophyta		***Heterokontophyta***	
Chlamydomonas reinhardtii	Stable	*Laminaria japonica*	Stable
Chlamydomonas reinhardtii	stable (chloroplast)	*Undaria pinnatifida*	Stable
Volvox carteri	Stable	*Phaeodactylum tricornutum*	Stable
Dunaliella salina	Stable	*Navicula saprophila (Fistulifera saprophila)*	Stable
Dunaliella viridis	Stable	*Cylindrotheca fusiformis*	Stable
Haematococcus pluvialis	Stable	*Cyclotella cryptic*	Stable
Chlorella sorokiniana;	Stable	*Thalassiosira weissflogii*	Transient
Chlorella kessleri (ParaChlorella kessleri)	Stable	*Nannochloropsis sp.*	Stable
Chlorella ellipsoidea	Stable	**Dinoflagellates**	
Chlorella vulgaris	transient	*Amphidinium sp.*	Stable
Ulva lactuca	transient	*Symbiodinium microadriaticum*	Stable
Ostreococcus tauri	stable		
Rhodophyta		**Cyanobacteria**	
Cyanidioschyzon merolae	stable	*Spirulina platensis (Arthrospira platensis)*	Stable
Porphyra yezoensis	stable / transient	*Anabaena sp*	Stable
Porphyra miniata	transient	*Synechocystis sp.*	Stable
Kappaphycus alvarezii	transient	*Synechococcus*	Stable
Gracilaria changii	transient	*Nosctoc muscorum*	Stable
Porphyridium sp	stable (chloroplast)		
Porphyridium sp	stable	**Euglenids**	
Gracilaria	stable	*Euglena gracilis*	stable (chloroplast)

**nuclear transformation unless otherwise mentioned.*

4.2.2 Methods for DNA delivery

Several methods for DNA delivery have been applied successfully to micro-algae. These methods include micro-particle bombardment (or biolistic), cell agitation with micro- or macro-particles (e.g. glass beads), protoplast transformation with polyethylene glycol, protoplast or whole cell transformation by means of electroporation and finally Agrobacterium mediated transformation [29]. Cells from the late logarithmic growth phase are commonly used for transformation. An extensive overview of transformation techniques applied to algae was recently presented by Song Qin et al. [162] and is depicted below in Figure 29.

Figure 29 Transformation techniques in algae

Methods	Characteristics
Agrobacterium tumefaciens mediatedgenetic transformation	The efficiency is highly dependent on many elements and this method is technically challenging.
Electroporation	It has simple procedure, and is used universally to different genera but constrained in brown algae.
Biolistic transformation	Exogenous DNA can be introduced into various cells and tissues. Diversified vectors can be applied to overcome the genetic background insufficiency of the substances. The manipulation is controllable and mature. But it requires specialised and high cost equipment.
Glass beads	The procedure is simple and it doesn't need high cost transgenic equipment. But it is constrained in macroalgae due to immature protoplast regeneration technology.
Silicon carbon whiskers method	It overcomes the cell wall's obstruction of exogenous DNA compared to glass beads method and is inexpensive. But it requires strict safeguards to avoid the inhalation hazard.
Microinjection	Whereas it is a highly efficient and low cost method but it has complicated and delicate procedure.
Artificial transposon method	Exogenous gene could be directionally integrated into receptor's genome.
Recombinant eukaryotic algal Viruses	It has potential application in brown algae but still needs extensive and comprehensive fundamental studies.
Trans-conjugation	It is mainly in cyanobacteria and rarely used at present.
Natural and induced transformation	It is mainly in cyanobacteria and rarely used at present.

[162]

Although cyanobacteria are generally included in micro-algae studies, they actually are bacteria (see Section 2.1) and can be transformed by established techniques for bacteria, e.g. by means of electroporation, by conjugative transfer of vectors from *E. coli* [30] and by a natural DNA uptake system which is present in *Synechocystis* sp. PCC 6803 and in *Thermosynechococcus elongatus* [31].

In the transformation experiments of algae, a number of selectable markers have been shown to be successful in obtaining genetically modified strains. Most selection systems for these algae have been tested in *Chlamydomonas reinhardtii* because of its prominent position in the eukaryotic algae molecular biology. The number of selection markers for cyanobacteria exceeds the number of markers for eukaryotic algae.

The promoters used to drive gene expression in transgenic algae are either homologous promoters, e.g. the Rubisco small subunit (RbcS2) and the ubiquitin (Ubi1) promoter, or heterologous promoters like CaMV35S and SV40. CaMV35S, the cauliflower mosaic virus promoter, is a typical promoter for strong expression in higher plants and works well in several algal strains, while the SV40, the simian virus 40 promoter, a polyomavirus promoter, has been shown to work in *H. pluvialis* and in *C. reinhardtii* [29].

Nuclear transformation of the eukaryotic algae generally results in random integration of transgenes. In *C. reinhardtii*, *C. merolae* and *Ostreococcus* homologous recombination has been achieved for a precise gene integration but the frequency is low [18]. Recently, the oil producing algae *Nannochloropsis* sp. was shown to have a high frequency of homologous recombination after transformation and selection [32]. In contrast, chloroplast transformation often results in homologous recombination [33, 34]. Contrary to the eukaryotic algae, homologous recombination is easy to achieve in cyanobacteria [35]. Moreover, also autonomously replicating vectors can be used in the cyanobacteria *Synechococcus* and *Synechocystis* [36].

RNA silencing by either antisense or RNAi technology has also been applied to algae. Several examples of RNA silencing and RNAi technology in C. reinhartii have been reviewed by Schroda [37] while RNAi has also been applied to *Euglena gracilis* and *Phaeodactylum tricornutum* and is predicted to become a valuable tool in algae genetics [38].

4.2.3 Targets of algal genetic modification

As already discussed previously [1], genetic modification as a tool to improve algal performance is more and more considered as a necessity to achieve new and economical viable production systems (Figure 30) [1, 13, 39, 22, 17, 40].

In general, we can distinguish between three types of targets for genetic modification of algae:

1. Improvement of photosynthetic efficiency;

2. Improve productivity of selected products;

3. New GM algae products under development.

Improvement of photosynthetic efficiency

This aspect has direct impact on algal productivity and consequently is relevant for biomass production for food, feed as well as biofuel production. Daylight intensity is most of the time above the maximum photosynthetic efficiency of algae and therefore growth is reduced, a phenomenon known as photoinhibition. Research in this area focuses on the light harvesting antenna complex (LHC) [41,8].

Improve productivity of selected products

The rising market demand for pigments from natural sources has promoted large-scale cultivation of micro-algae for synthesis of such compounds. Genes encoding enzymes that are directly involved in specific carotenoid syntheses have been investigated and further development of transformation techniques will permit considerable increase of carotenoid cellular contents [43]. One example of algae transformation with a gene encoding the enzyme phytoene desaturase was published in 2006 [44].

Figure 30 New products that have been made by algae through genetic modification.

Product	Algae used	Reference
Hepatitis B antigen protein (HBsAg)	*Dunaliella salina*	[47]
Human growth hormone (HGH)	*Chlorella vulgaris* *Chlorella sorokiniana*	[48]
Erythropoietin; Human fibronectin 10FN3 and 14FN3; Interferon β; Proinsulin; Human vascular endothelial growth factor (VEGF); High mobility group protein B1 (HMGB1)	*C. reinhardtii*	[49]
Bovine lactoferricin (LFB)	*C. reinhardtii*	[50]
Avian and human metallothionein type II; Antigenic peptide P57; Antigenic proteins VP19,24,26,28; Foot and mouth disease virus VP1 protein; Anti-glycoprotein D of herpes simplex virus; Anti-rabbit IgG; Human tumour necrosis factor; Bovine mammary-associated serum amyloid; Classical swine fever virus E2 viral protein; Human glutamic acid decarboxylase 65; Human erythroprotein; Anti-anthrax protective antigen 83 antibody; D2 fibronectin—binding domain	*C. reinhardtii*	[114]
Flounder growth hormone (FGH)	*Synechocystis*	[115]

Despite the above developments, a limited number of articles on applications of GM algae for food was found in reviews of the last five years, and papers of the last two years. Ogbonna [161] stated that, although a lot of work has been done to increase the contents and composition of tocopherols in higher plants through genetic and metabolic engineering, work on genetic modification of microorganisms for increased tocopherol accumulation is scarce. Tocopherols are antioxidants and have been claimed to prevent various diseases caused by oxidative stress. Similar conclusions can be drawn regarding applications in new functional foods [163]. In particular for food and feed applications, the development of transgenic algae faces problems and challenges, such as competitiveness, public acceptance, regulatory issues and biosafety [163].

As mentioned earlier in this report, research on lipid production has increased in the past decades due to interest in developing algal biofuels. Genetic modification is part of the strategy to increase lipid production with algae. Target genes are lipid biosynthetic genes, lipid storage genes and lipid degradation genes. Obviously, the first two categories of genes have to be over-expressed while the third category of genes should be inhibited [18, 17].

Another interesting aspect of algae biotechnology is the modification of lipid characteristics. This could increase the quality of the lipids with regards to suitability as diesel fuel feedstock but could also make the lipids suitable for other applications like food or feed or industrial applications [18][7]. Genes for this purpose will be identified in the group of fatty acid modifying enzymes, such as desaturases and thioesterases which have been studied in GM plants in detail already for a long time [45, 46].

7 In relation to fatty acid metabolism, within the GIAVAP project [158] scientists cloned a DGAT1-like gene (PtDGAT1) from the diatom P. tricornutum. In a yeast expression system, PtDGAT1 restored triacylglycerol (TAG), an ester derived from glycerol and three fatty acids and lipid body formation), and favoured incorporation of saturated fatty acids into TAGs. In this area the increasing interest in the production of specific fatty acids, such as the omega-3 fatty acid EPA, for health food applications [164], but also DHA can be noted.

New GM algae products under development

An emerging field in algae biotechnology is the introduction of genes or metabolic pathways in order to produce components of economic interest. Figure 30 gives an overview of new products that have been made by algae through genetic modification with a focus on recombinant proteins. The status within the product pipeline, however, is unknown.

Micro-algae integrate the advantages of microbes (such as rapid growth and ease of culture) with those of higher plants in performing post-translational modification of the obtained molecules and photosynthesis. However, several obstacles currently hinder the development of economically viable micro-algal expression systems. These include:

- lack of effective and consistent transformation methods for a wide variety of species;
- low or inconsistent recombinant protein yields;
- lack of production systems optimised for large-scale growth and harvesting under photoautotrophic conditions [116];
- lack of understanding of algal metabolism and regulation.

Systematic and concerted research efforts that are both conventional and engineering-based such as optimisation of promoters, regulatory elements and codon usage, as well as development of improved photobioreactor culture systems will be critical to the success of micro-algal production platforms (see review [117]). It is expected that within the next 10 years developments in genome mining will offer opportunities for steering the metabolism of e.g. high value products.

So far, none of the products mentioned above is commercially available. However, research on the application of algal systems for the production of these molecules is increasing [118, 119, 116, 114].

A review of recent research involving engineering of cyanobacteria for the production of valuable compounds has been published by Ducat et al. [120].

4.3 Conclusions

Although in recent decades algal biotechnology was mainly focused on food and feed (ingredient) applications, current research on algae and genetic modification of algae is rapidly expanding due to high expectations with respect to the production of biofuel, bio-chemicals and other bio-products by algae. Large investments from governments and industries stimulated the research on GM algae [1].

The technology for genetic modification of algae is still rather immature, and a lot needs to be done before commercial production of products from GM algae will take place.

Within the area of algal biotechnology and genetic engineering the main challenges are to improve:

- Technological productivity which includes reactors design, process control, harvesting and extraction in an efficient and sustainable way. Such a process may also include fractionation of several products of interest via optimised biorefinery;
- Productivity of selected targeted molecules with biotechnological means. This includes strain selection, mutagenesis and transgenesis. With regard to the latter approach, it is important to develop effective and consistent transformation methods for a wider variety of micro-algae species and obtain high expression of relevant genes and products;
- Strain stability: Due to genetic drift of haploid algae, it is important to have stable cultures in order to guarantee a secure and constant production.

5. Safety and regulatory aspects of micro-algae food and feed applications in Europe and the USA

This chapter deals with the safety of micro-algae products, including both whole dried algae products and components of micro-algae for food and feed, and the regulation concerning their market introduction in Europe and the United States of America (USA). Although GM micro-algae did not yet reach a commercial phase, the second part of this chapter is dedicated to their safety and to regulatory aspects both in Europe and the USA, to complete the overview on micro-algae regulation.

Figure 31 summarises the regulations that apply to the whole production chain of micro-algae products: research, production and commercialisation of food and feed products, including GM-algae.

Figure 31 EU and US regulation concerning research, production and market introduction of micro-algal products (including GM micro-algae) for food and feed applications

	Europe	United States
Research	- EC directives 2009/41/EC (contained use of GM algae) - EC directive 2001/18/EC (deliberate release of GM algae)	- NIH rDNA Guidelines - EPA Standards for microbiological practices, under the Toxic Substances Control Act (TSCA)
Production	- EC directives 2009/41/EC (contained use of GM algae) - EC directive 2001/18/EC (deliberate release of GM algae)	- TSCA Environmental Release Application (TERA) - Microbial Commercial Activity Notice (MCAN) under TSCA [- USDA Plant Protection Act]
Market introduction	- EC Regulation on Food Safety (EC 178/2002) - EC Regulation on Novel Foods and Novel Food Ingredients (EC 258/97) - EC Regulation on Genetically Modified Food and Feed (EC 1829/2003) - EC Regulation on traceability and labelling of GMOs and of food and feed products produced from GMOs (EC 1830/2003) - EC Regulation on Nutrition and Health claims made on foods (EC 1924/2006)	- Food, Drug and Cosmetic Act - Dietary Supplement Health and Education Act

5.1 Industrial relevant micro-algae and their safety

5.1.1 Industrial micro-algae for food and feed applications and their safety aspects

Figure 32 provides a list of micro-algae currently used in food or feed applications. Some algae have been given the GRAS (generally recognised as safe) status by the FDA (Food and Drug Administration) of the USA.

In Europe the EFSA (European Food Safety Authority) is requested to assess the safety of any new food and feed compound before they are authorized for production and commercilization. EFSA's scientific panels maintain a list of biological agents to which the concept of qualified presumption of safety (QPS) can be applied. The QPS list is reviewed and updated annually by EFSA. Although no algae are included in the QPS assessment list of 2012, β-carotene from *Dunaliella* and docosahexaenoic acid (DHA) from *Crypthecodinium cohnii* are already approved as food ingredients by EFSA[8] and *Chlorella* and *Spirulina* are commonly sold as food supplements both in the USA and in the EU.

Figure 32 Micro-algae relevant for food/feed applications and their safety aspects where information is available

Organism	Species	Safety aspect	Organism	Species	Safety aspect
Cyanobacteria	*Spirulina / Arthrospira sp.*	GRAS	**Heterokon-tophyta**	*Navicula sp.*	NT
	Synechococcus sp.	NT		*Nitzschia dissipata*	NT
Chlorophyta	*Tetraselmis sp*	NT		*Phaeodactylum tricornutum*	NT
	Chlamydomonas reinhardtii	NT		*Thalassiosira pseudonana*	NT
	Haematococcus pluvialis	NT		*Odontella aurita*	NT
	Dunaliella sp.	NT		*Skeletonema sp.*	NT
	Chlorococcum sp.	NT		*Monodus subterraneus*	NT
	Scenedesmus	NT		*Nannochloropsis sp.*	NT
	Desmodesmus sp	NT	**Haptophyta**	*Isochrysis sp.*	NT
	Chlorella sp	GRAS		*Pavlova sp3*	NT
	Parietochloris incisa	NT	**Dinophyta**	*Crypthecodinium cohnii*	GRAS
Rhodophyta	*Porphyridium cruentum*	GRAS			

Adapted from [1]. NT = no toxins known, GRAS = Generally Recognised as Safe

8 See: http://www.efsa.europa.eu/fr/efsajournal/doc/1000.pdf

Certain marine algae produce potent toxins that have an impact on human health through consumption of contaminated shellfish and finfish and through water or aerosol exposure. Toxic algae can be filtered from the water by shellfish, such as clams, mussels, oysters, or scallops, which then accumulate the algal toxins to levels which can be lethal to consumers, including humans [100, 101]. Typically, the shellfish are only marginally affected, even though a single clam can sometimes contain sufficient toxin to kill a human being. Fish and shellfish can also be subject to sub-lethal effects, including increased susceptibility to disease and reduced growth. Algal toxins can give rise to a number of different poisoning syndromes:

- NSP - neurotoxic shellfish poisoning;
- PSP - paralytic shellfish poisoning;
- ASP - amnesic shellfish poisoning;
- DSP - diarrhoeic shellfish poisoning;
- Ciguatera fish poisoning.

Algae such as *Isochrysis*, *Chaetoceros gracilis*, *Tetraselmis suecica*, *Pavlova lutheri*, *Skeletonema costatum*, *Dunaliella* tertiolecta, *Nannochloropsis sp.*, *Phaeodactylum tricornutum*, *Chlorella* sp. that are used in aquaculture or for production of food supplements do not produce toxins.

In general, over the past three decades, the frequency and global distribution of toxic algal incidents appear to have increased, and human intoxications from novel algal sources have occurred [99]. Even within the same species, large differences exist between toxic and non toxic algae. Dinoflagellates and diatoms are best known for their production of toxins that can affect humans, but for instance the Dinoflagellate strain *Crypthecodinium cohnii* has a GRAS status and is used for commercial production of the omega-3 fatty acid DHA (134, see Figure 32). Consequently, in view of application of algae for food or feed, it is very important to know their safety at strain level. The Department of Botany of the Smithsonian National Museum of Natural History has developed an Internet site with an overview of harmful Dinoflagellates and diatoms [104].

5.2 Food safety of human consumption of *Spirulina*

Another example of the importance of knowing the algae strains used for human consumption, can be found in cyanobacteria. Many of the existing cyanobacteria species are known to produce toxins (microcystins, in particular MCYST-LR, a small polypeptide that may harm liver function) [105], while *Spirulina* has a GRAS status. An overview of toxin producing cyanobacteria can be found on the Cyanosite, an Internet site of Purdue University [106]. Although there has been no conclusive evidence on the presence or absence of microcystins in *Spirulina*, only products from the blue-green algae species *Arthrospira platensis* have so far been cleared for consumption under specific conditions (USA, Australia, Canada and EU).

The case of *Spirulina* seems to be controversial. A Canadian study found that no microcystins was detected in cyanobacteria products containing only *Spirulina* [135], while a study conducted for the Oregon Department of Agriculture (ODA) published in 2000, found MCYST-LR in all the 15 *Spirulina* samples (dietary supplements) analysed [136]. Nevertheless, MCYST-LR content in *Spirulina* samples were below the regulatory level established by the ODA for microcystins in blue-green algae products (1 µg/g).

According to the European legislation, if a product was on the market as a food or food ingredient and consumed to a significant degree before 15 May 1997, like *Arthrospira platensis*, its access to the market is not subject to the Novel Food Regulation (EC) No. 258/97 [167]. However, other specific legislation may restrict the placing on the market of this product as a food or food ingredient in some EU Member States.

Spirulina has been recognised as GRAS in the USA under the "intended conditions of use" implying that it is "for use as an ingredient in foods, at levels ranging from 0.5 to 3.0 grams per serving", i.e. in relatively small amounts. Nevertheless, considering that safety level and possible hazards for consumption of *Spirulina* and *Spirulina*-related products have not been established beyond doubt, special precautionary measures would be necessary on the consumption of *Spirulina* products to some segments of the population at risk to include pregnant women, nursing mothers, people in dialysis and immune-compromised (see review [137]).

5.3 European regulation on marketing of micro-algae for food and feed

Three European regulations apply to the marketing of micro-algae or its components: on food safety, on novel food and novel food ingredients, and on nutrition and health claims for food. They will be presented in more detail in this section. The EU regulation on GM food and feed will be presented in Section 5.7.

Regulation on Food Safety

Market introduction of food products using the whole micro-algae organisms (such as *Spirulina*, or *Chlorella*) or products that include the micro-algae (like pasta with the green algae colour) are subject to food safety regulations that apply to all food products. This is the European Community Regulation on Food Safety (EC 178/2002) published in 2002 in the Official Journal of the European Communities (1.2.2002 EN L 31/1).

This Regulation provides a framework for a coherent approach in the development of any food legislation; it provides the general framework for those areas not covered by specific harmonised rules but where the functioning of the internal market is ensured by mutual recognition. It lays down definitions, principles and obligations covering all stages of food/feed production and distribution. It establishes common principles and responsibilities, the means to provide a strong science base, efficient organisational arrangements and procedures to underpin decision-making in matters of food and feed safety. Also it holds down the general principles governing food and feed in general, and food and feed safety in particular, at Community and national level. The regulation applies to all stages of production, processing and distribution of food and feed. The regulation also established the European Food Safety Authority (EFSA).

According to experts, food safety is an important issue in algae-technology and needs particular consideration when algae are produced in open-air systems, since they proved to be easily subject to contamination from other microorganisms.

Regulation on Novel Foods and Novel Food Ingredients

The Food Safety regulation mentioned above states that safety concerning food must be proven by a prolonged period of consumption. When this condition is not met - i.e. food products are new to the market without a history of safe use - these products are not authorised on the European market [140] without having performed a safety assessment first.

Only after these products have met the conditions set out in the Regulation on Novel Foods and Novel Food Ingredients (EC 258/97 [139]), they are authorised to be marketed. This Novel Food regulation applies for those foods and food ingredients that were not on the European market before May 15, 1997. For instance the EPA- and DHA-rich micro-algal oils have only recently been introduced to the market and thus fall under this regulation, despite EPA and DHA have a significant history of consumption before May 15, 1997.

A similar concept applies to colourants from *Spirulina*. *Spirulina* itself is not a novel food (as it was already on the market before 1997). However, *Spirulina*'s blue colourant, extracted from the algae and refined, is a new product and thus falls under the Novel Food Law. This implies that companies have to provide information on the safety of the food product (including results of animals testing), to the EFSA before to commercialied the colourant. This risk assessment process is usually time consuming and expensive and therefore companies argue with the regulators that these requirements are too strict [9].

The Novel Food Regulation applies to foods and food ingredients:

- which present a new or modified primary molecular structure;
- which consist of micro-organisms, fungi or algae;
- which consist of or are isolated from plants and ingredients isolated from animals;
- whose nutritional value, metabolism or level of undesirable substances has been significantly changed by the production process.

Important principles applied in this regulation are that novel foods and food ingredients must be safe for consumers (not being dangerous or nutritionally disadvantageous) and properly labelled so as not to mislead consumers.

Companies which intent to market a novel food or novel food ingredient must apply to a national authority for authorisation, presenting the scientific information and safety assessment report. Authorisation covers conditions of use, designation of novel food or novel food ingredient, and specification and labelling requirements. The national authority establishes if additional assessment is necessary or not. If the Commission and EU countries do not object the product can be finally authorised. Before the authorisation, the Commission asks the Standing Committee on Food Chain and Animal Health for an opinion. However, any decision or provision concerning a novel food or food ingredient which is likely to have an effect on public health must be referred to EFSA Scientific Committee for Food. If the assessed products are being used exclusively in food supplements, also new uses in other foods require authorisation under the Novel Food Regulation.

A novel food or ingredient can also be marketed through a simplified procedure called "notification" (see Figure 33) [174]. This is done when the applicant considers its food or ingredient to be 'substantially equivalent' to a similar product already on the EU market. The applicant applies to the European Commission directly or alternatively seeks the opinion of a Member State competent authority. The applicant must provide scientific evidence that the product is substantially equivalent with respect to composition, nutritional value, metabolism, intended use and the level of undesirable substances contained therein. The company notifies the Commission that they are planning to market a novel food or ingredient based on the opinion of a food assessment body that has established "substantial equivalence". If the novel food or ingredient is likely to affect public health, the Commission asks the opinion to the EFSA Scientific Committee for Food. See the figure below with a list of micro-algae products for which authorisations and notifications have been applied in the EU.

9 For the law a colourant is an additive. For additives there is Regulation EC 1333/2008. Each food colour authorised for use in the European Union is subject to a rigorous scientific safety assessment.

Figure 33 Applications for micro-algae products under EC 258/97: authorisations (last application December 2012) and notifications (last application April 2012)

Applications		Notifications	
Food or food ingredients and applicant	**Application date and Status**	**Food or food ingredients and applicant**	**Dates for Notification and Transmission to Member States**
Additional uses of DHA (docsahexaenoic acid)-rich oil from micro-algae Ulkenia sp. By Nutrinova, Germany	15 November 2004 Commission Decision 2009/777/EC concerning the extension of uses of algal oil from the micro-algae Ulkenia sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council OJ L278 of 23 October 2009, p. 54	Microalga Odontella aurita. Innovalg S.A.R.L. Bouin, F	9 December 2002, 19 December 2002
DHA-rich algal oil from Schizochytrium sp. For additional food uses By Martek, USA (UK-FSA)	14 January 2008 Commission Decision 2009/778/EC concerning the exten¬sion of uses of algal oil from the micro-algae Schizochytrium sp. as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council OJ L278 of 23 October 2009 p. 56	DHA (docohexanoic-acid)-rich microalgal oil (DHActive™) LONZA AG, Basel Switzerland	10 November 2003 24 December 2003
Tetrasemilis chuii (micro-alga) By Fitoplancton Marino S.L., Spain	26 July 2011 Pending	Capsules with Astaxanthin-rich Carotenoid Oleoresin extracted from Haematococcus Pluvialis (max 4 mg Astaxanthin/ capsule). Herbal Sciences International Ltd.Loughton Essex, UK for US Nutra, USA	28 June 2004 13 July 2004
Nannochloropsis gaditana (micro-alga) By Fitoplancton Marino S.L., Spain	26 July 2011. Pending	Astaxanthin in food supplements. Real AB, Gustavsberg, Sweden	17 May 2006 22 May 2006

Applications		Notifications	
Food or food ingredients and applicant	**Application date and Status**	**Food or food ingredients and applicant**	**Dates for Notification and Transmission to Member States**
DHA and EPA from Schizochytrium sp By Martek Biosciences, USA	31 January 2011. No objections may be placed on the market pursuant to Article 4.2 of Regulation (EC) No. 258/97	Food supplements with Astaxanthin-rich oleoresin extracted from Haematococcus Pluvialis. Cyanotech Corporation USA	7 March 2007 13 March 2007
		Astaxanthin rich extract from Haematococcus pluvialis. Alga Technologies Ltd. Is.	14 April 2008 13 May 2008
		DHA from Schizochytrium sp. Ocean Nutrition Canada Limited, Canada	20 April 2012 25 April 2012

[169, 170]

The Novel Food Regulation contains also specific requirements concerning the labelling of novel food and food ingredients which are additional to the general European requirements on food labelling [141]. The application must mention any characteristics (such as composition, nutritional value and intended use) and the presence of materials that may affect the health of individuals or that give rise to ethical concerns.

As mentioned above, authorisation for marketing of novel foods goes through national authorities. However, most national food safety authorities do not publicise progress in the field of evaluation of applications for novel foods or novel food ingredients on their website, with the exception of the UK food safety authority (FSA) [142]. The Dutch authority only publishes dossiers that have been finalised. At the moment, EFSA leads the process of developing a new regulation under which EFSA will act as the bundler for all these national requests for authorisation.

The Novel Food Regulation was originally set up in order to deal with market introduction of GM food. However, in 2003, specific regulations for GM food and GM feed (1829/2003 and 1830/2003) were introduced (see Section 5.7), leaving a rather empty Novel Food Regulation, now only dealing with the foods that are novel on the EU market since 1997. In the regulation on Genetically Modified Organisms (GMOs), the technology employed is a main issue being the most influential factor making most of the novel foods 'novel'. There is a discussion at the moment in Europe about which technologies should and which technologies should not be included under the Novel Food Regulation, such as on the use of nanotechnology in the processing and 'formulation' of algae.

Regulation on Nutrition and Health Claims made on Foods

In 2006, the European Regulation on Nutrition and Health Claims made on Foods was introduced (Regulation (EC) 1924/2006 [144]). This regulation states that health claims on food/feed products shall be based on and substantiated by generally accepted scientific evidence (Article 6). Health claims should only be authorised in the EU after a scientific assessment of the highest possible standards. In order to ensure harmonised scientific assessment of these claims, EFSA is carrying out such assessment.

EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) provides scientific opinions on all health claims made in food/feed products. The regulation states that scientific substantiation should be the main aspect to be taken into account for the use of nutrition and health claims and the food business operators using claims should justify them. A claim should be scientifically substantiated by taking into account the totality of the available scientific data and by weighing the evidence.

5.4 USA regulation on market introduction of micro-algae

Two USA laws are applicable on micro-algae based food and feed products once they are sold on the consumer market:

- the Federal Food, Drug and Cosmetic Act (FD&C) introduced in 1938 [152], which regulates all foods and food additives;

- the Dietary Supplement Health and Education Act (DSHEA) introduced in 1994, which amended the FD&C Act to cover dietary ingredients and supplements.

The FDA regulates both acts. The FDA Center for Food Safety and Applied Nutrition (CFSAN) is responsible for regulating food ingredients and ensuring that those ingredients are safe and lawful. The authorisation of feed products falls under the FDA Center for Veterinary Medicine (CVM).

Food, Drug and Cosmetic Act

The legal status of a food substance depends on whether it is used in a conventional food, a dietary supplement or as ingredient in a dietary supplement product. For the FDA, any substance that is intentionally added to food is a food additive and is subject to premarket review and approval by FDA, unless the substance is generally recognised, among qualified experts, as safe (GRAS) under the conditions of its intended use. The assessment of each ingredient and each method of producing that ingredient stands on its own merits. In case dried *Spirulina* biomass is approved, it does not mean that an extract from *Spirulina* biomass is automatically approved. In case *Chlorella* is approved as a dietary supplement, it does not mean the oil from the same *Chlorella* species is approved [148].

The seven algae based GRAS food ingredients that have been reviewed by FDA (in the period 1998 - March 2012) are [148]:

- Micro-algal oil derived from *Ulkenia sp.* SAM2179;
- *Haematococcus pluvialis* extract containing Astaxanthin esters;
- Algal oil (*Schizochytrium sp.*);
- *Spirulina*: the dried biomass of *Arthrospira platensis*;
- *Laminaria japonica* broth and extract powder;
- DHASCO is derived from the micro-algal species *Crypthecodinium cohnii*;
- "Calcified seaweed" derived from *Phymatolithon calcareum* or *Lithothamnium corrallioides*.

Dietary Supplement Health and Education Act

The Dietary Supplement Health and Education Act (DSHEA), introduced in 1994, provides a regulatory framework for dietary supplements. It includes provisions establishing current good manufacturing procedures, mechanisms for pre-market safety notifications of new ingredients, and a mechanism for establishing claims used in product labelling [154].

According to this act, "the dietary supplement or dietary ingredient manufacturer is responsible for ensuring that a dietary supplement or ingredient is safe before it is marketed". The FDA is responsible for taking action against any unsafe dietary supplement product after it reaches the market. Generally, manufacturers do not need to register their products with the FDA nor to get FDA approval before producing or selling dietary supplements. They simply must make sure that product label information is truthful and not misleading.

Domestic and foreign companies that manufacture, package, label or hold dietary supplements, including those involved with testing, quality control, and dietary supplement distribution in the USA, must comply with the Dietary Supplement Current Good Manufacturing Practices (cGMPS) for quality control. In addition, the manufacturer, packer, or distributor whose name appears on the label of a dietary supplement marketed in the USA is required to submit to the FDA a report describing all serious adverse events associated with the use of the dietary supplement in the USA. The FDA's other responsibilities include the oversight of product information, such as labelling, claims, package inserts, website information and accompanying literature [154].

The FDA has imposed - under the authority of the FD&C Act (Title 21 Sections 301–399 of the United States Code) and the Public Health Service Act (42 U.S.C § 201 et seq.) - a number of regulatory requirements that address quality and safety of dietary supplements and claims. Dietary supplements must conform with labelling requirements imposed by DSHEA as well as other broader labelling amendments such as the Nutrition Labelling and Education Act of 1990 (Publ. L. 101- 535) and the Food Allergen Labelling and Consumer Protection Act of 2004 (Pub. L. 108-282) [155].

When companies want to market new dietary ingredients, the Federal Food, Drug and Cosmetic Act requires that the manufacturers and distributors notify the FDA about these ingredients. The notification must include information that is the basis on which manufacturers/distributors have concluded that a dietary supplement containing a new dietary ingredient will reasonably be expected to be safe under the conditions of use recommended or suggested in the labelling. The government have different option: determine that the product is not GRAS; requiring further information for the evaluation; or sending an acknowledgement letter confirming that the information provided is adequate.

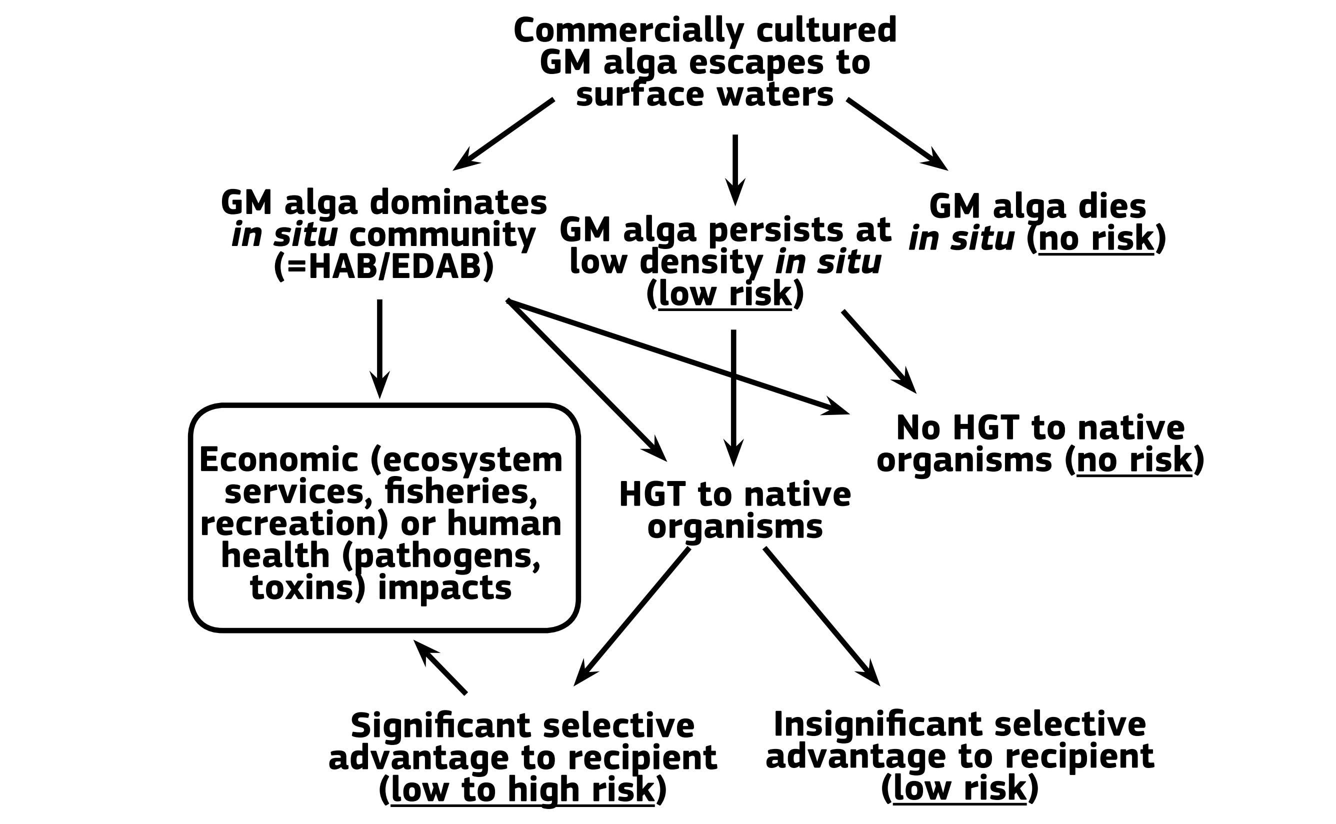
For other types of food products, it is optional for companies to ask for GRAS status from the FDA. However, since the government can ask any time for such a safety assessment, companies usually preventively perform it to pre-empt the potential request. Dietary ingredients with health claims are considered to be a drug, and fall under the drug regulations.

5.5 Overview of potential risks of GM micro-algae for human health and the environment

The issue of the biosafety of GM algae has two aspects: potential adverse environmental consequences and potential harm to human or animal health in case of food/feed or pharmaceutical applications. For human health risks, the biosafety evaluation could refer to the methods applied in higher plants to guarantee that they are safe and they do not produce toxic substances or allergens [162]. In the USA the FDA, and in Europe the EFSA, are responsible for this biosafety evaluation. For the environmental risks, Henly et al. [166] have published a comprehensive study on risk assessment of GM micro-algae for commodity-scale biofuel cultivation which is also relevant for other applications. They presented a conceptual design of the problem formulating stage of risk assessment for escaping GM algae (see Figure 34).

Inevitable escape of GM algae from any type of production facility is central to each hypothesis. In general, Henly et al. [166] predicted that most target GM algal traits are unlikely to confer a selective advantage in nature. Therefore, in a natural environment, they would rapidly diminish, resulting in low but nonzero ecological risk. Genetic and mechanical containment, plus conditional matching of GM algal traits to unnatural cultivation conditions, would further reduce this risk. These hypothetical predictions, however, must be verified through rigorous monitoring and mesocosm experiments [10] to minimise risk and foster public and regulatory acceptance. The most important aspects of the risk analysis of GM micro-algae, including safety aspects, vertical or horizontal gene transfer, strain identity, competition with wild types and strain fitness, are analysed in the following sections. For a detailed overview we refer to reference [1].

Figure 34 Conceptual diagram of the problem formulation stage of risk assessment for escaping GM algae



GM, genetically modified; HAB, harmful algal bloom; EDAB, ecosystem disruptive algal bloom; HGT, horizontal gene transfer [166]

10 A mesocosm is an experimental tool that brings a small part of the natural environment under controlled conditions. In this way mesocosms provide a link between observational field studies that take place in natural environments, but without replication, and controlled laboratory experiments that may take place under somewhat unnatural conditions.

5.5.1 Potential harmful properties of GM micro-algae

With respect to contained use, the risk assessment is aimed at identification of harmful properties of micro-algae due to characteristics of the recipient organism, the insert, the vector and the resulting properties of GM micro-algae and their products with respect to human health and the environment. In general, DNA inserted in the recipient algae has been characterised. In this respect, the choice of the selection marker (e.g. antibiotic resistance) should be taken into account.

5.5.2 Transfer of genetic material to other organisms

An important aspect to be addressed in the Environmental Risk Assessment (ERA) is the transfer of inserted genetic material to other organisms. Therefore, the point of concern is called horizontal gene transfer (HGT) and refers to the transfer of genetic material from one organism to another, which is a natural mechanism.

In cyanobacteria, HGT has played an important role in evolution [107, 108]. Indeed, ~50 per cent of extended cyanobacteria gene families putatively have a history of HGT, either between cyanobacteria and other phyla, or within cyanobacteria, or both. In these bacteria, HGT is a mechanism for real time adaptation and for that reason it is considered in the risk assessment of GM bacteria.

In eukaryotic algae, HGT has been part of the evolutionary development as well. However, in these organisms this is not a real time event and poses no additional risk in GMOs. HGT from GM plants to prokaryotes has been studied and was shown to pose negligible risks [109]. HGT from bacteria has also been studied in relation to mechanisms and barriers [110] and to risk assessment of GMOs [111].

5.5.3 Identity and taxonomy of GM micro-algae in relation to risk management

Regarding industrial applications of algae, knowledge of the specific identity of the algae strain is essential for two reasons: i) for communication and relevant exchange of information among researchers and ii) for use and application of adequate results by e.g. industry in order to build on already established safety data [1]. Establishing the strain identity is especially crucial for gathering information for risk assessment research. Taxonomists can be considered as important service providers in risk assessment also because the 'history of safe use' of algae (which is an important aspect in risk assessment) is only valid where the identity of the algae strain is known.

In particular for food applications, a history of safe use for a certain algae implies that production has proven to be safe over a longer period of time (this also implies some forms of environmental exposure). As mentioned above, when collecting knowledge on the safe use, it has to be certain that the historical data refer to the same species as the one that is intended to be used. Identity and taxonomy are relevant because in case the identity is not known, no history of safe use can be built. It is recommended to develop the concept of GILSP (Good Industrial Large Scale Practice) to be applied to algae strains.

5.5.4 Fitness and mitigation

Fitness is defined here as the ability to exist/survive in the surrounding environment. It is expected that the more domesticated the strain is and the more adapted to its production environment, the harder it is for the strain to exist in the wild environment. Technical solutions for making algae (and other micro-organisms) unfit to survive outside the defined environment include the growing of salt water algae in the country or algae that need vitamin B12 for survival. If the production system falls out and the algae wash away on the land and in the (sweet water) rivers, they will not survive. However, this presumption is not very well documented. Molecular approaches - such as metagenomics - can be used to genetically characterise the (changes in the) environment after release of the GM algae [165, 166]

5.6 European regulation on GM-algae

5.6.1 Research[11]

In general, all research - including micro-algae research - is governed by regulations in the field of good laboratory practice. Research on GM algae is governed by the EC directives 2009/41/EC and 2001/18/EC; the first deals with contained use of GMOs and the second with deliberate release into the environment of GMOs.

- contained use is defined as 'any activity in which organisms are genetically modified or in which such organisms are cultured, stored, transported, destroyed, disposed of or used in any other way and for which specific containment and other protective measures are used to limit their contact with the general public and the environment';

- deliberate release is defined as 'any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment'.

The European directives are implemented in national legislation in one or more regulations, in each member state.

11 Based on [1].

5.6.2[12] Production

Regulation concerning production of GM algae depends on the type of production system: contained or deliberate release. Cultivation of a GMO in a closed system (such as photobioreactors) falls under the regulation of contained use (Directive 2009/41/EC, Article 2c).

Natural locations (such as open ponds) are considered as deliberate release into the environment since there are no effective protective measurements to prevent the algae from entering the surrounding environment. Since the open ponds are not covered, there is contact with the environment through the air, which could also be considered as intentional introduction into the environment.

In industrial settings, a safety level of MI-I may be applied to the use of micro-organisms. The safety level MI-I is based on the concept of Good Industrial Large Scale Practice (GILSP) . This concept, already developed in the OECD 'Blue Book' [138], implies that, if a host organism has a long history of safe use in an industrial setting, the same industrial setting offers adequate containment for the use of a GMO derived from this host organism.

The rules of GILSP can be applied to the use of a GMO if:

- the host organism is non-pathogenic and has a long history of safe use under industrial conditions;
- the GMO is derived from this host organism using a 'safe' vector (if applicable) and a 'safe' insert, and the resulting GMO has a reduced fitness in the environment compared to the host organism.

The concept of GILSP implies, inter alia, that living organisms of a culture grown under GILSP may be released in the environment in as much as that is usual also for the host organism, since there is still limited practice in algae production systems. In the Netherlands, local municipalities have granted environmental approval for growth facilities for non-modified algae but have done so according to different regulations. For example the algae production systems of AlgaePARC in Wageningen needed to be contained, while for the production systems of Ingepro in Borculo risk assessment was required.

With respect to the European regulation of GM algae applied in production system, the COGEM study [1] concluded that:

- Directives 90/219/EEC and 2001/18/EC cover all issues related to a risk assessment of GM algae and GM cyanobacteria.
- Closed algae production systems could be considered contained when placed inside a building. In this case, an ERA according to the directive 2009/41/EC is applicable.
- Cultivation of GM algae and GM cyanobacteria in a closed system, which is placed outside may be considered under the regulation of contained use when it meets the following criteria:
  - the system has a long history of safe use under GILSP conditions for cultivation of the particular host organism;
  - the particular GMO is composed of a non-pathogenic host organism, a 'safe' vector and insert, and the resulting GMO has a lower fitness in the environment than the host organism, in agreement with the criteria for organisms acceptable for use under GILSP (MI-I, in Netherlands regulation).
- Cultivation of GM algae and GM cyanobacteria not meeting the criteria of GILSP in outdoor closed systems and open pond systems will be subject to an environmental risk assessment (ERA) in accordance with EC directive 2001/18/EC.

5.6.3 Consumption

Regulation on GM Food and Feed

The European food regulations presented above (2002, 1997) do not cover GMOs for food and feed (and also not food additives, flavourings, or extraction solvents). GMOs are subject to the Regulation (EC) 1829/2003 [143] on GM Food and Feed. This regulation provides the basis for ensuring a high level of protection of human life and health, animal health and welfare, environment and consumer interests in relation to GM food and feed (whilst ensuring the effective functioning of the internal market) and holds procedures for the authorisation and supervision of GM food and feed and provisions for the its labelling (Article 1).

Regulation on traceability and labelling of GMOs and of food and feed products derived from GMOs

The European Regulation (EC) No 1830/2003 [171] concerning the traceability and labelling of GMOs and the traceability of food and feed products derived from GMOs covers all products which consist of GMOs or which contain them, and foodstuffs and animal feed products made from GMOs. This regulation was made in order to inform consumers through compulsory labelling, giving them the freedom to choose and to create a "safety net" based on the traceability of GMOs at all stages of production and placing on the market. This "safety net" will facilitate the monitoring of labelling, the surveillance of the potential effects on human health or the environment, and the withdrawal of products in case of risk to human health or the environment.

12 Based on [1].

5.7 USA regulation on GM-algae

5.7.1 Research

NIH guidelines

The National Institute of Health (NIH) Guidelines for Research involving Recombinant DNA Molecules [149] apply to biotechnology research that is funded by the USA government, including GM algae. R&D conducted inside a structure (which might apply to a greenhouse or photobioreactor) is expected to follow the NIH guidelines. In general, also private companies doing biotech research follow these guidelines.

Environmental Protection Agency's standards for microbiological practices

Private companies' research is regulated by the Environmental Protection Agency (EPA) standards for microbiological practices. The EPA is in charge of the regulation concerning R&D activities; everything before commercialisation is assessed by EPA. The Toxic Substances Control Act (TSCA) introduced in 1976 provides the EPA with authority to request reporting, record-keeping and testing requirements, and restrictions relating to chemical substances and/or mixtures. The regulation addresses obligations regarding management, storage, containment use and disposal of these products. Reporting is also required for 'new' microorganisms being released into the environment and that may be subject to a new rule.

Under TSCA, microorganisms fall under the definition of chemical substances, but foods (and pesticides) are excluded from this definition. All food products and ingredients are treated as food and are exempted from EPA regulation. However, GMOs used to produce the food materials are not exempted. The EPA requirements concerning microbial products subject to TSCA (15 U.S.C. Section 2601, et seq.) are set forth in "Microbial Products of Biotechnology; Final Regulation under the Toxic Substances Control Act" (62 FR 17910 (April 11, 1997)) and codified at 40 C.F.R. Part 725. This act says, "Microorganisms resulting from the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera (intergeneric microorganisms) constitute "new" microorganisms subject to TSCA Section 5 notification requirements".

Discussion on interpretation of EPA rules

There is some discussion on the interpretation of the EPA rules. Regulation of GM algae in research, pilot plant or in production systems depends very much on the nature of the reactor in which the organisms would be used. Glass [151] concludes on the basis of his experience with authorisation for the use of GM algae in biofuel projects that: "If an open-air algal reactor is (…) judged by EPA not to be sufficiently "contained" as defined in the regulations, EPA would consider any use of such reactor with live algae to be an outdoor use, triggering the need for regulatory oversight (e.g. requiring submission of a TSCA Environmental Release Application) at the research level and possibly a greater level of scrutiny at commercial scale". He concludes that the regulatory situation is not clear yet, but: "With proper planning, advance consultation with the Agency (i.e. EPA), and given sufficient time to develop the needed data package, algae projects that might fall subject to TSCA should not encounter too much difficulty in being cleared for commercialization".

5.7.2 Production

TSCA Environmental Release Application – TERA

A TSCA Environmental Release Application (TERA) is needed for people conducting commercial research and development activities, before the initiation of the testing. EPA (following the rules set out by TSCA, as introduced above) conducts a review of these submissions to determine whether the intergeneric microorganisms present an unreasonable risk to health or the environment. The Agency can impose regulatory controls under Section 5 of TSCA.

The production process with GM algae in open systems has had no regulatory history until now. The law is there (a TERA-approval is needed), but according to one of the interviewees: "no one has been bold enough and made an application for the cultivation of GM algae in open air". As soon as such an application is being made, FDA will be in charge of it, and will consult other agencies (including EPA) about it. But such a first application will certainly be accompanied by scrutiny, as a new case has to be built about the safety aspects of GM algae for the environment.

For every open pond, a TERA approval is needed and a case-by-case approval procedure is applied. In the field of GM algae the science base has to be built from scratch and will take time to be at a level that all necessary information is available. A TERA demands extensive information. According to the regulation, a company must ask for an approval 60 days before it aims to start its production but as it takes about 6 to 9 months to review, companies apply earlier. Especially in the case of a new organism that will be used in production (GM algae), this process will take much more time. In the period 1998-2007, a total of 17 TERAs submissions have been reviewed, of which 14 have been approved (there is no information available about the number of TERAs on algae). After 2007 there have been no further submissions.

EPA conducts a review of these submissions to determine whether the intergeneric microorganisms present an unreasonable risk to health or the environment. The agency can impose regulatory controls under Section 5 of TSCA. This may be applied to outdoor micro-algae raceway pond concepts at small- and medium-scale testing facilities. There is an exemption concerning R&D for contained use.

The Animal and Plant Health Inspection Service of the US Department of Agriculture (USDA) issues authorisations for import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and GMOs [172].

Microbial Commercial Activity Notice - MCAN

People who manufacture, import, or process intergeneric microorganisms for commercial purposes subject to EPA jurisdiction under the TSCA are required to submit a Microbial Commercial Activity Notice (MCAN). MCAN is submitted under the biotechnology regulations promulgated under the TSCA (62 FR 17910, April 11, 1997; codified at 40 C.F.R. Part 725).

EPA regulates the use of intergeneric microorganisms in commerce or commercial research under the TSCA. EPA states that intergeneric microorganisms have a sufficiently high likelihood of expressing new traits or new combinations of traits to be termed "new" and warrant EPA review. The Office of Pollution Prevention and Toxics (OPPT) Biotechnology Program conducts a screening program for new micro¬organisms under section 5 of TSCA.

The average time spent by a company to prepare a MCAN is 8 to 12 months. The review takes EPA about 90 days, sometimes more. EPA can either accept the MCAN or inform the applicant that he is not yet allowed to start with the production and that more information should be provided (specified according the questions asked by EPA).

Plant Protection Act

There is uncertainty about the applicability of the Plant and Protection Act (PPA) for micro-algae. At the moment it is not applicable, as micro-algae are not a plant or animal pest. However, the regulator might at a certain moment decide otherwise in case of GM algae. The Animal and Plant Health Inspection Services (APHIS) of the USDA regulates certain GMOs that may pose a risk to plant or animal health and it also participates in programs that use biotechnology to identify and control plant and animal pests. It could be possible that at a certain moment they also consider GM algae (and their new genetic material that could exchange with other living creatures) as a pest.

5.8 Conclusions

This chapter provided an overview on the safety of micro-algae products in general and GM micro-algae in particular and of the most relevant regulations on market introduction of micro-algae products and derived products. We discussed the regulations in two of the main global markets: the EU and the USA, highlighting the directives and acts that rule these products.

Regarding the commercial introduction of (non GM) micro-algae, regulations differe substantially in the EU and USA, both in the approach and in the requirements for the authorization of micro-algae products. While in the USA the regulation applies to the product and assesses if the final product is safe or not, the European regulation focuses on the technology that is used to obtain the final product.

The most important EU regulations on production and marketing of microalgae-based products for food and feed are two: the Food Safety Regulation (EC 178/2002) and the Novel Food Regulation (EC 258/97). The latter is particularly relevant because it provideds the authorization procedures for all new food and feed products. Authorization is the first and key step for the commercialization of any new food and feed product, including the ones from micro-algae, and producers must provide all the scientific evidences that new products are substantially equivalent to the traditional ones in order to be authorized.

The USDA in its regulation does not make a distinction between GM food and non-GM food; any food must be safe. In Europe there is additional regulation on food safety and labelling of GM food and GM feed (EC 1829/2003 and 1830/2003).

6. Europe's position in micro-algae research and production for food and feed: conclusions and recommendations

We conclude this report with our findings on Europe's position in the field of micro-algae research and production for food and feed applications. First, conclusions are drawn on Europe's position in the field of micro-algae research and production (6.1). Second, a number of recommendations is being formulated (6.2).

6.1 Conclusions

6.1.1 European strengths in the field of micro-algae research and production

Europe's strengths are its science base, its active R&D funding policy and its position in agricultural and industrial production and related logistics.

Science and technology base: Europe's main strength in the area of micro-algae applications for food and fuel is its strong position in micro-algae science and technology. Europe is very active in this field and has good engineering and training skills. In addition, Europe has a number of small but very R&D-intensive companies that work on technological breakthroughs in the field of micro-algae.

Public R&D funding: This strong position in micro-algae science and technology is related to the high priority in R&D funding policies in this field of a number of member states and of the European Commission. The EC has a focused a thematic area in this field in its Framework Programmes and active sustainability policies (such as those in Horizon 2020) that support scientific and technological developments in this field.

Industrial and logistical position: Europe has specific structural economic and logistical 'assets' that enhance its position in micro-algae research and application. Europe has an outstanding tradition in high-quality agricultural production and a strong food and feed industry with multinationals operating on a global scale. An important consumer trend is the demand for natural products. Micro-algae based applications for food fit into this trend of increased focus on healthy eating (omega fatty acids) and sustainably produced food. This can further support the position of Europe's food industry. Europe has good physical infrastructure, such as large seaports capable of handling large volumes of commodities. Europe also benefits from a high level of human capital, a workforce with adequate engineering and technical skills to work in micro-algae research, development and application.

6.1.2 European weaknesses in the field of micro-algae research and production

Overall, Europe's weakness in the field of micro-algae research and production for food and feed relates to its geographical position, to a number of financial-economic aspects, and to its regulation.

Geographical position: Europe's main weakness with respect to the opportunities for micro-algae production is its relatively suboptimal climate with high levels of rainfall, low levels of sun hours and sun intensity (especially in winter) and low temperatures for most countries outside southern Europe. For this reason Europe has a lack of surface area for the production of micro-algae.

Structural financial-economic disadvantages for Europe are its relatively high labour costs, its lack of venture capital and seed capital available for start-up companies and low entrepreneurial activity among researchers and engineers in this field. Due to the focus on public research, there is relatively less focus on up-scaling and optimising production. Moreover, large companies hardly invest in R&D in this field.

Also Europe's relatively high land costs are a weakness when it comes to micro-algae production.

Regulation: Further development is also hampered by relatively restrictive regulations on GMOs (as compared to the USA), and a negative attitude of the public at large towards such technologies. Also, Europe lacks a consumer history with micro-algae, in contrast with South-East-Asia or the USA, which makes effective marketing of micro-algae based products more difficult in Europe.

6.2 Recommendations

There are numerous opportunities and challenges for Europe to increase the chance of successful large-scale applications of micro-algae in food and feed. These relate to the outputs of the high level of R&D in this field (including spill-over effects of fuel-related micro-algae research), to the use of wastewater streams and to policies.

These opportunities and challenges lead to the following recommendations:

- Due to the increasing R&D levels a considerable number of new and improved products and production/extraction processes are in the pipeline. Competition outside Europe is moving fast (China, USA) and in some regions (China, SE Asia) there is lack of IP protection.

- For that reason now is the moment for Europe, given its strong position in research in this field and the currently still very limited volumes of global production, to explore the opportunities for a first-mover advantage in high-volume high-value processing of micro-algae for food and feed.

- The continuing interest in micro-algae research for fuel applications may lead to 'research spill-over' in the area of food and feed. As the genetic make-up of many algae species is not known yet, further research (including into genetic modification and that is mostly focussing on energy applications) could yield new or more cost-effective technologies for food and feed in the future.

- In order to profit from the 'spill-overs' from micro-algae based research on bio-fuels, it is recommended to stimulate food and feed researchers (public and private) to link-up with/get involved in the bio-fuel research programmes.

- In Europe wastewater streams are increasingly well-organised; these streams are kept separate from other water streams, mainly due to environmental regulations. The waste water streams hold many components that can be used to 'feed' the micro-algae.

We recommend the exploration of opportunities for using these waste streams as input source in micro-algae production. This could become a priority in the EC FP-programmes.

- We recommend the exploration of the potential of the use of a number of European policies for increased sustainable development by using opportunities in micro-algae research and production. These include:

– A focus in R&D-programmes on co-operation between companies (including SMEs) and public research institutes could speed up technological breakthroughs and the implementation of new technologies .

– The European Emission Trading Scheme (ETS) could provide additional income for micro-algae producers which use carbon dioxide as an input.

– Europe's efforts on climate change adaptation could lead to the use of micro-algae as a diversification strategy for food and feed inputs .

– Europe's Neighbourhood Policy and our proximity to Northern Africa provide opportunities for co-operation in efficient micro-algae production.

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Annex A Methodology

Literature search

The main source used for the literature search is the database Scopus accessed through the website primo.library.wur, that is open to students and staff of Wageningen University. In addition, further on-line free databases has been searched (see below).

The targets of the literature search were five. For each of them a set of keywords and time boundaries has been identified and used for the search. Targets and corresponding keywords are the following:

1. Production systems: the keywords used for the literature search included: “reviews + microalgae”, “reviews + microalgae + cultivation”, “reviews + microalgae + photobioreactors”. For this topic, material from the master course on Marine biotechnology of Wageningen University was also used.

2. Economics of micro-algae products and production: the literature search employed the following keywords in mixed combinations: “microalgae”, “economics”, “production”, “price”, “market”, “food”, “feed”, “Astaxanthin”, “EPA”, “DHA”, “Beta-carotene”, “Europe”, “*Spirulina*”, “*Chlorella*” and “company”. Sources from the last seven years were included in the analysis. In addition to the Wageningen University Library, we made also use of Google Scholar and performed an open Internet search. Subsequently, a ‘snowball strategy’ was used for searching for relevant publications and documents that were cited in the identified publications.

3. Biotechnology and genetic modification: the keywords used for the literature search included “microalgae”, “biotechnology”, “food” and the years 2012 and 2013 in mixed combinations (previous years were covered in the COGEM-report on risks of GM algae [1]). Information on traditional food applications of *Spirulina* was based on direct personal knowledge that the authors gathered in the last ten years’ experience. Information about European research projects was found on the EC Cordis website with ‘algae’ as search term. Hits for recent projects (from 2011) were manually selected for application in food, feed and energy.

4. Risks and risk management: the main source of information was the recent COGEM-report on risks of GM algae (2012) [1]. Moreover, a further literature search on the Internet and the Wageningen library system was done using the following search terms: “microalgae” + “risk” + “food” + “2012” or “2013”.

5. Regulation: the literature search used the following search terms: “microalgae” + “regulation” + “food”, for the years 2010-2013. Additionally, Internet pages of the respective governments dealing with the relevant regulations and a number of presentations used in conferences were used.

The literature search identified a list of information gaps for each of the five topics. These information gaps were filled by interviews to experts.

Interviews with key experts

The interviews to experts were aimed at filling possible knowledge gaps identified in the literature search. The experts were also asked to revise the chapters of this report concerning their field of expertise.

The first step in selecting the experts was to create a long-list of stakeholders potentially participating to the interviews and survey. Stakeholders’ selection was based on a combination of the following characteristics:

- Having expertees in (at least) one of the following fields: micro-algae production systems; economics of the production of microalgae-based for food and feed products; biotechnology of micro-algae; risks of (GM) micro-algae; the European and/or USA regulation on the production and use of micro-algae for the food and feed sector.

- Working position, in particular in private or public companies or research centers, public institutions or regulatory agencies active in the micro-algae field.

- Having produced scientific publications and having participated in international conferences in the field of microalgae-based food and feed products.

Figure A1 – List of affiliations, fields of expertees of interviewees, including indication of participation to the Delphi survey.

Affiliation	Interview questions		Survey questions
	Field 1	Field 2	
European Algae Biomass Association / University Florence, It	Production systems		
University of Almeria, Sp	Production systems	Economics	x
University of Southampton, UK	Economics		x
Flemish Institute for Technology (VITO), Flemish Algae Platform, B	Economics		x
University of Groningen, NL	Risks		x
University of Cambridge, UK	Biotech/GM	Risks	x
Synthetic Genomics, USA	Regulation		x
DSM, NL	Regulation		
DSM, USA	Regulation		
Medicines Evaluation Board, NL	Regulation		

All the stakeholders identified fulfilling the above characteristics constituted a long-list of 219 stakeholders. From this list, 18 experts were invited to the interview by email. Criteria for selecting the experts for interviews were the working position and number of scientific publications. Some of them reacted positively, while others declined or did not answer at all. All in all 10 experts were interviewed (Figure A1). Most of them answered to questions regarding only one field, while others' expertees permitted to answer to questions related to two different fields. The experts participating to the interviews were also asked to answer the survey questions (see next section).

Survey among stakeholders

An electronic survey was used to consult stakeholders and experts on the future developments of micro-algae based products for the food and feed sectors.

The methodology employed was a combination of a standard electronic survey and elements of an expert Delphi method. The Delphi approach is a method developed in the fifties in the USA for forecasting specific developments. The Delphi method is a data collection method based on the assessment of possible future developments by selected experts who exchange their opinions with the help of a written questionnaire. The questionnaire contains a number of statements concerning future developments, which have to be assessed by a scheme of answering categories. The specific feature of the method is that the experts are asked to answer the same questions several times. In second (and possible subsequent) rounds, the experts are informed about the aggregated results of the previous round and are asked to review their answers - to the same questions - if necessary. The second round thereby serves as a confirmation to improve robustness, but also to obtain more specific answers compared to the results of first-round.

In addition to the Delphi-related questions, the electronic survey also was used for data collection on a number of other topics relevant for this study.

The Delphi-part of the questionnaire included statements and related assessment questions on:

- Europe's market position in the field of micro-algae: one statement, four questions;
- Micro-algae based products as substitutes of traditional products: one statement, four questions.
- The other topics addressed in the questionnaire were:
- Production costs: two questions;
- Europe's competitive position: one question;
- Microalgae products in the pipeline: one question;
- Strengths, weaknesses, opportunities and threats in the field of micro-algae: one question.

The list of questions for the survey can be found in Annex B.

The interviewed experts were also asked to answer the questions of the survey. Six of them did answer the questions.

Data collection was carried out via an electronic on-line survey tool (SurveyMonkey). The first round was sent on March 26th 2013 to a total of 219 stakeholders, 67 of them answered the survey. The second round (only for the Delphi-part of the questionnaire) was sent on April 22nd to the 67 stakeholders that participated in the first round, while on April 26th a reminder was sent to the 139 (206 minus 67) stakeholders who did not yet answered to the first round: this led to further 21 responses, adding up to a total of 88 responses.

A reminder for the second round was sent on April 29th. Finally 28 people also answered the Delphi questions for the second round.

An overview of the response rates is given in Figure A2. Expertise was determined both for market position (first figure) and substitution opportunities (second figure).

Annex B presents the list of questions directed to the interviewees and the text of the survey.

Figure A2 - Responses to survey results.

	First round	Second round
Total target group	219	88
Successful delivery	206	67
Number of respondents	88	28
Response rate	42.7% (88 out of 206)	41.8% (28 out of 67)
Distribution of respondents among levels of expertise for the question on market position and the questions on substitution	Very familiar 48.1% (out of 88)/38.6% (out of 88) Rather familiar 48.1% (out of 88)/ 61.3% (out of 88) Non-familiar 3.8% (out of 88)/ 4% (out of 88)	Very familiar 42.3% (out of 28)/ 34.6% (out of 28) Rather familiar 57.7% (out of 28)/ 65.4% (out of 28) Non-familiar 0%/ 0%
Distribution of respondents between industry, academic/ research institutes and other experts	Industry 59% (out of 88) Academia/Research Inst. 32% (out of 88) Other: 9% (out of 88)	Industry 57.1% (out of 28) Academia/Research Inst. 32.1% (out of 28) Other: 10.7% (out of 28)

Annex B Questions for interviews and survey

B.1 Specific part: interview questions for filling knowledge gaps

Most experts have been asked only the set of questions under one of the five headings. Some experts for micro-algae production systems have also been asked questions about economic aspects.

B.1.1 Micro-algae production systems

1. What are the three greatest achievements in the cultivation of micro-algae required for implementation of micro-algae as a source of food and feed ingredients in Europe?
2. When will this be achieved?
3. Which system you believe to be most suitable for algae cultivation for food and feed ingredients? Can you motivate your choice?
4. In which location do you think the biomass should be produced? How can we guarantee enough nutrients on site?
5. Do you think it will be possible to use residual streams as a source of nutrients for the cultivation of micro-algae? If yes, which residual streams?
6. What do you think about heterotrophic algae production for food and feed ingredients? Will this reach an important market share? What are the main advantages/ disadvantages in comparison to autotrophic growth?

B.1.2 Biotechnology/GM of micro-algae

1. What would you consider as the three major biotechnological challenges in further development of algal products for food applications?
2. What are future challenges in the field of GM-algae research and production in relation to food / feed applications. Can you name two challenges?
3. Which breakthroughs do you expect in algal research for application in food / feed within 10 years?
4. In your opinion, which GM micro-algae products and non-GM micro-algae products are most advanced in the pipeline and therefore closer to commercialisation?
5. Do you miss any opportunities or threat?

B.1.3 Economic aspects

1. Could you validate the information currently presented in the report? How robust are these figures, especially relating to total market size, given that a lot of authors cite each other?
2. Could you provide specific figures for Europe?
3. What is the current and future strategy of the multinationals that have recently taken over micro-algae producers in this domain?
4. Are there any new pipeline products not yet identified in this chapter? If yes, at which stage they are?
5. What is the current and prospective use of micro-algae based ingredients in pharmaceutical products?
6. Do you have any estimates of the cost of harvesting Astaxanthin from Haemotococcus?
7. What is the market size of food products derived from micro-algae with respect to feed products from micro-algae? Considering the market size for food and feed derived from micro-algae, what is the share for food and what for feed? Are they distinct or similar products for both markets?
8. What is the market for co-products of micro-algae derived products (i.e. dry biomass)?

9. What is the current geographical distribution of micro-algae production?

10. Do you miss any opportunities and threats (Economic, Technical, regulatory, IPR)? Which are most constraining? Why?

B.1.4 Risks

1. Are there any potential risks related to the use of micro-algae isolated from nature in food / feed applications?

2. Are there any potential risks related to the use of GM micro-algae in food / feed applications?

3. What would you suggest industry as well as policymakers to reduce the risks?

4. Should contained GM-algae in your view be treated differently from other GMOs. And Why?

B.1.5 EU vs USA regulation

1. Is the overview of regulations for micro-algae research, production and marketing of micro-algae based products food and feed products complete for Europe/USA, What is missing? What must be corrected?

2. What is your opinion on the differences between European and US regulation?

3. Are you aware of any - other - legislative uncertainty (and related discussing between companies and regulators) in the field of algae or micro-algae on legislation, especially for food and feed in Europe/the USA?

4. For the USA: Have health claims of micro-algae based products been investigated and approved?

5. Question for national food safety authority: Is *Spirulina* blue colourant an additive or a colourant (or both)? Under which regulation does it fall, for each alternative?

6. How may the current regulation affect the development of the micro-algae sector for food and feed, in the EU and in the US respectively? In particular, which stage may be affected by the regulation: research, production or commercialisation?

B.2 General part: Interview and survey questions

In the interviews additional questions are asked on the background reasons for giving the specific answer (the 'Why' and "Can you elaborate on your choice / give reasons for your choice' questions).

B.2.1 Europe's market position (Delphi)

Introduction: At the moment there are five micro-algae based components and two algae-biomass products on the food and feed market worldwide. The first category includes Astaxanthin, β-carotene, Phycocyanin and two omega-3 fatty acids (EPA and DHA) and the second *Spirulina* and *Chlorella*. In the future micro-algae based proteins and oils will also become available as food ingredients. At the moment production mainly takes place outside Europe, but the market position of Europe-based companies is growing (mainly by acquisition).

Statement: Europe is market leader in micro-algae based products for the food and feed markets.

Question A1: When the situation described in the statement will be achieved (tick the one that applies best)? Why?

- Before 2016,
- Between 2016 and 2020,
- After 2020,
- Never.

Question A2: Which of the following factor(s) have a positive influential effect on the achievement of the situation described in the statement? Can you motivate your choice?

- Science & technology,
- Education & training,
- Food and feed markets,
- Intellectual Property,
- Regulation/standards,
- Consumer attitude,
- Other: .....

Question A3: How knowledgeable /familiar are you with the subject/domain of the statement?

- Very familiar (in fact I am an expert in the field)
- Rather familiar (I know about it, I am a generalist in many fields)
- Non-familiar with specific domain

Question A4: Do you have any comments in addition to your answers to the previous questions?

B.2.2 Micro-algae based products vs other resources (Delphi)

Introduction: The five food and feed micro-algae based components can be extracted from algae biomass, but also from other natural sources or they can be synthesised by the chemical industry.

Actual situation:

- Astaxanthin: most is produced synthetically;
- β-carotene growing volumes are produced using micro-algae, but also extracts from carrots;
- Phycocyanin: mainly from cyanobacteria (blue-algae);
- EPA and DHA: extracted from fish fat and walnuts.

Statement: Astaxanthin, β-carotene, phycocyanin, EPA and DHA for food and feed applications are mainly from algae-resources.

Question B1: When the situation described in the statement will be achieved (tick the one that applies best)? Why?

- Before 2016,
- Between 2016 and 2020,
- After 2020,
- Never.

Question B2: Which of the following factor(s) have a positive influential effect on the achievement of the situation described in the statement? Can you motivate your choice?

- Science & technology,
- Education & training,
- Food and feed markets,
- Intellectual Property,
- Regulation/standards,
- Consumer attitude,
- Other: .....

Question B3: How knowledgeable /familiar are you with the subject/domain of the statement?

- Very familiar (in fact I am an expert in the field)
- Rather familiar (I know about it, I am a generalist in many fields)
- Non-familiar with specific domain

Question B4: Do you have any comments in addition to your answers to the previous questions?

B.2.3 Production costs

Introduction: production costs of micro-algae based components for the food and feed market are still relatively high, as compared to chemical synthesis or extraction from plants of these components.

Statement: In 2020, production costs of micro-algae based products for the food and feed market are that low that in Europe most of these products are now micro-algae based.

Question C1: Which of the following technical challenges have been addressed and contributed to the achievement of the situation in the statement (please pick max 5 challenges)? Why these five? Can you elaborate on your choice?

- biomass production;
- harvest;
- extraction;
- scale-up of production systems;
- component separation;
- product design;
- micro-algal species selection;
- culture stability;
- contamination/ predator invasion/ weed algae invasion;
- quality control monitoring;
- light management;
- other: ....

Questions C2: and which of the following non-technical challenges? Why these five? Can you elaborate on your choice?

- credible product claims ;
- access to capital by small companies;
- capital investment of large companies;
- intellectual property rights;
- trained personnel;

- access to production models;
- lack of public knowledge;
- public attitude towards biobased products;
- legislation;
- other: .....

B.2.4 Europe's competitive position

Introduction: European research groups are very active in micro-algae research and the European Commission invested in this research field through several framework programmes. Also the European industry is increasingly active in the field. However, Europe has not a top position (R&D, production) as compared to other world regions.

Statement: In 2020, Europe's micro-algae research for food and feed applications is fine-tuned to the needs of European industry and leads to new products and lower costs production processes thereby strengthening the competitive position of the European industrial sector in this field.

Question D: Which of the following key challenges have been addressed and contributed to achievement of the situation in the statement (please pick max 5 challenges)? Why these five? Can you elaborate on your choice?

- Doubling of EC-budgets for this type of research;
- Technical breakthroughs;
- Regulatory approval of GM-algae based products;
- Access to venture capital;
- Academic and industrial training;
- Reduction of biomass production costs;
- Better communication and cooperation between research organisations and companies;
- Other:........

B.2.5 SWOT analysis of Europe's position in micro-algae research and production

Question E: In your opinion, what are the strengths, weaknesses, opportunities and threats of Europe in the field of micro-algae research and production, for food and feed applications?

Strengths: ....

Weaknesses: ......

Opportunities: ....

Threats:....

B.2.6 Pipeline products

Introduction: So far, the questionnaire focuses on micro-algae based food and feed products that are already on the market. The last question deals with micro-algae based products for the food and feed market that are now in the pipeline and will be on the market in 2020.

Question F: Which micro-algae based products for the food and feed market are now in the pipeline in your university/research centre/company and are likely to be on the market in 2020? Could you for each product indicate in which development phase the product is at the moment, choosing from the four phases mentioned in the box?

Name Product 1: .... Now in

- Commercialised pipeline: product/innovation that have been authorised for production in at least one country, but are not yet marketed;
- Regulatory pipeline: product/innovation in the regulatory process to be marketed in at least one country;
- Advanced development: product/innovation for which there are multiple-location field trials and more than one proof of concept;
- Early development: product/innovation for which there is only one proof of concept.

development stage: ..

Name Product 2: .... Now in development stage: ..

Name Product 3: .... Now in development stage: ..

Name Product 4: .... Now in development stage: ..

Name Product 5: .... Now in development stage: ..

European Commission
EUR 26255 - Joint Research Centre - Institute for Prospective Technological Studies

Title: Microalgae-based products for the food and feed sector: an outlook for Europe

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Abstract
The European Union has recently adopted an ambitious strategy for developing the Bioeconomy in Europe, in this context algae represents an emerging biological resource of great importance for its potential applications in different fields. In particular, micro-algae are currently promoted as a new source of valuable nutrients for human and animal consumption.
This report analyses the production, markets and regulation of microalgae-based food and feed products, especially focusing on the European sector. The report is structured in the following chapters:
- Micro-algal production systems
- Current markets, products and future developments for micro-algae
- Outlook: R&D and prospects for micro-algae biotechnology and genetic modification
- Safety and regulatory aspects of micro-algae food and feed applications in the EU and the US.

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