

# Status of micro-algae application in food, feed and aquaculture - recommendations to accelerate development and commercial implementation

L.M. van Dam

Master of Science Internship Report



Locations of participating institutes and companies



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MASTER OF SCIENCE INTERNSHIP REPORT

L.M. van Dam  
frauleinlvandam@gmail.com

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Supervisor:	<b>P.A.M. Besseling</b>	Ministry of Economic Affairs
	<b>J.H. Reith</b>	Wageningen University BioProcess Engineering
	<b>G. Olivieri</b>	Wageningen University BioProcess Engineering
	<b>T.P.M. Israel-Hoewelaken</b>	Centre for BioBased Economy

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# Summary

## Aim and approach of the study

In this study - commissioned by the Ministry of Economic Affairs - the current worldwide status of micro-algae production and application was investigated and compared to the status and perspectives of the micro-algae sector in the Netherlands with a focus on opportunities for the food, feed and aquaculture sector. The study was performed via desk study and interviews with 27 stakeholders in the Dutch microalgae sector incl. R&D institutes, algae producers, technology developers, end users of algae products and investment funds. Although the number of respondents is limited, several trends can be distinguished regarding the status and perspectives of the Dutch microalgae sector. Based on the results, recommendations are given for the Dutch government to accelerate the commercialisation and scale up of the micro-algae sector.

## Worldwide status and perspectives of the micro-algae sector

Worldwide production of micro-algae is growing. Current commercial applications focus on health food supplements, aquaculture, cosmetics and pharmaceuticals. Worldwide growth is seen particularly in higher value products for health, nutrition and aquaculture. In the longer term, as production costs decrease, lower value applications such as animal feeds, proteins, and other commodities (incl. bio-fuels) are expected to enter the market. The same worldwide trends apply to the microalgae sector in the Netherlands regarding best performing market segments and applications, and growth potential.

## Status and perspectives of the micro-algae sector in the Netherlands

The micro-algae sector in the Netherlands is limited in size, but well established with positive perspectives for growth. Overall there is a strong cluster of research, production and technology developers in the Netherlands.

Regarding technology there is an emphasis in the Netherlands on development and use of advanced photobioreactor systems in R&D, algae production and commercial technology development. Furthermore there is significant ongoing innovation in harvesting and processing technology as well as product development.

Commercial activities in the Dutch micro-algae sector focus on production and sales of micro-algae and derived products for food, feed, aquaculture and cosmetics. Production scale in the Netherlands is still relatively small but quality standards are high. Furthermore there is significant activity in the area of development and sales of cultivation and harvesting technology, in addition to services for processing and product development. Several end-users in the food and feed sector are involved in product development. The R&D sector focuses on optimization of cost-effective and sustainable micro-algae production methods, developing knowledge for scale-up, and development of processing technologies.

The majority of the interviewed stakeholders in this study have a positive expectation of the further development of the micro-algae sector in the Netherlands because of the availability of good infrastructure, high quality and technology standards and the top ranking of the Netherlands in agriculture and horticulture. The sector is growing, following the worldwide trend. The algae producers and developers expect a significant growth in turnover within the next 5-15 years for production and sales of

high quality algae and derived products for food, feed, aquaculture and other market sectors. Growth is also projected for the development and sales of technologies, know-how and services in the area of cultivation, harvesting and processing developed by the R&D sector and commercial technology developers.

#### Barriers for further development and scale-up of the micro-algae sector in the Netherlands

Major identified barriers for scale-up of the micro-algae sector in the Netherlands include: lack of financing possibilities - especially to bridge the gap between pilot and commercial scale - market development (consumer awareness) and obstacles caused by regulations. The most important technological R&D subjects to accelerate the development of the microalgae sector in the Netherlands are cost reduction of algal biomass production, optimisation of cultivation systems, selection of new production strains and genetic modification. In order to reduce production costs, the major challenges are: reduction of energy use, improvement of cultivation systems and growth improvement of micro-algae.

The major identified non-technological R&D subjects to promote development of the micro-algae market in the Netherlands are marketing (addressing clients, end-users, consumers and market creation) and consumer/market acceptance.

Greenhouses are identified as a suitable location for micro-algae production in the Netherlands because of the large greenhouse area, and the high level of horticultural knowledge and infrastructure availability especially for high value applications. Furthermore coastal, non-agricultural land and areas close to infrastructure offering residual streams that can be used for algae cultivation such as nutrients, waste heat and flue gasses offer possibilities.

#### Overall conclusion and recommendations

An overall conclusion of this study is that a significant growth potential exists for the micro-algae sector in the Netherlands that merits further government support. Based on the results of the study several recommendations can be given for the Dutch government to accelerate the commercialisation and scale up of the micro-algae sector

- Enhancement of funding possibilities
  - Subsidies for micro-algae projects. Subsidies are especially important for R&D and in the early phases of a company e.g. via government (co)funding of Public Private Projects. Access to existing funding programs is perceived as difficult because of the specific nature of the technology and the fact that the algae biomass can be used for multiple applications and projects are therefore difficult to place. The creation of additional subsidy possibilities is recommended.
  - Government loans & investments. In particular companies experience a funding gap between R&D and commercial scale, especially for demonstration at industrial scale. It is recommended to review existing programs and develop methods to enhance investment opportunities for private investors and governments especially to bridge the gap between R&D and commercial scale.
- Market exploration. Performance of a detailed market exploration study is recommended to analyse potential markets and the acceptance of products by commercial end-users and consumers, public awareness and information required to inform these stakeholders.
- Regulations. Several regulations have been identified as barriers for further development and scale-up due to strict contents in combination with time consuming and costly application procedures. In particular this applies to approval of the use of (new) algae strains and products in food and animal feed, regulations addressing the use of residues for algae cultivation, and the rules for obtaining an organic certification. It is recommended to review these regulations and develop appropriate adaptations in consultation with stakeholders.
- R&D. Funding of applied and pre-competitive R&D remains an important tool to further develop the technology and the market. It is recommended to further support R&D -e.g. in the form of

Public Private Partnerships (PPPs) -to address topics that accelerate the scale up of the micro-algae sector and foster creation of new business opportunities. Several themes for research are identified in this study including the optimisation of cultivation systems and productivity, reduction of production costs and the development of biorefinery technologies.

- Stronger integration with horticulture. Attention for a stronger integration between micro-algae production and horticulture (esp. in greenhouses) is recommended. Several projects are already underway. Micro-algae can be considered as an (alternative) crop for horticulture (as well as agriculture) and benefit from the availability in these sectors of good infrastructure, high level expertise and high quality and technology standards.
- Heterotrophic micro-algae cultivation. This study has focused on cultivation of phototrophic micro-algae, using light and mineral nutrients including CO<sub>2</sub>. Several commercial successes have been achieved using heterotrophic (and mixotrophic) algae cultivation in which organic compounds such as sugar are used as a substrate for algal growth instead of or in addition to light. It is recommended to further explore the potential of heterotrophic algae production for the Netherlands. This could be an interesting option in combination with the end of the EU sugar quota management system in 2017, which may enhance the availability of sugar at lower costs.
- Algae Platform. Many respondents indicate an interest for a platform or meeting place for micro-algae companies, research institutes, government bodies and investment funds to exchange information, and enhance cooperation between parties in the micro-algae value chain. It is recommended to further investigate the creation of such a platform in the Netherlands in consultation with the stakeholders.



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# Chapter 1

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## Introduction

### 1-1 Overall position of micro-algae in the bio-economy

The development of a biobased economy (BBE) aims for enhanced sustainability through innovation by using biological raw materials to replace fossil resources and covering the growing demand of food, feed, energy, materials and other industrial products [1]. Today, micro-algae still play a minor role in the biobased economy, but they form an emerging, valuable biological resource for a range of biobased products. Phototrophic micro-algae are microscopic organisms which convert solar energy to chemical energy via photosynthesis<sup>1</sup>. Phototrophic cultivation is the main focus of this study. Up to now, 40,000-60,000 species have been identified of which only a few hundred have had their chemical composition investigated and less than 15 species are used for cultivation on an industrial scale [2]. Micro-algae are a highly productive source of oils, proteins and polysaccharides and contain various valuable compounds incl. poly unsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), pigments/carotenoids such as  $\beta$ -carotene, astaxanthin and phycobiliproteins, and polysaccharides such as  $\beta$ -glucan. These compounds are found in the dominant micro-algae used for commercial production: *Chlorella*, *Dunaliella*, *Isochrysis*, *Arthrospira (Spirulina)*, *Haematococcus* and *Chaetoceros* [3]. Micro-algae and derived products can be used in the production of food, animal feed incl. aquaculture, bio-fuels, pharmaceuticals and cosmetics. This report focuses on micro-algae production for application in food and feed incl. aquaculture at pilot and commercial scale.

### 1-2 Micro-algae as a food source

Rosella (2012) stated that the food sector is the most important application area for micro-algal biomass production (around 75%), especially health food and nutritional supplements. This is followed by a strong increasing trend for aquaculture and animal farming (around 20%) [2]. Nowadays, more natural foods with health benefits are demanded by consumers. Based on this trend, the expectation is that micro-algae are going to play a more important role in the food industry in the future [4, 5]. The limited production space needed for micro-algae production in comparison to traditional crops has a potential of supplying a substantial part of the EU food and feed market on a limited surface area [6]. There are two main categories of food products from micro-algae: dried biomass, directly sold as dietary

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<sup>1</sup>Some micro-algae strains are capable of heterotrophic growth using sugars as energy source or growth using both light and sugars (mixotrophic growth). In this study the primary focus is on photoautotrophic cultivation using sun light, CO<sub>2</sub> and mineral nutrients for growth.

supplements (with the potential to be used in bulk production) or specialty products isolated and extracted from the micro-algae as e.g. food ingredients [5].

### 1-3 Suitability for animal feed and aquaculture

In this study aquaculture is separated from the category animal feed, which comprises feed for cattle, poultry, farm animals, pets and ornamental birds. The chemical composition of micro-algae can have a positive effect on the health of animals due to the enhancement of the nutritional content of conventional feed preparations. This is mostly due to the capability of the cells to synthesize all the amino acids which are essential to animal nutrition. Also the overall digestibility is high, implying no limitation in using whole dried micro-algae in feeds [7–9]. However, in order to commercialize the use of algae in feeds, the material must be examined for the presence of toxic compounds to prove their harmlessness. It is estimated that approximately 30% of micro-algae produced worldwide is used in animal nutrition, mainly due to the high protein content and quality [4, 10]. With the world population expected to reach 9 billion by 2050, the demand for critical sources of protein is expected to exceed supply [11]. In aquaculture the use of micro-algae proteins instead of fishmeal in the feed of adult fish would allow for a decoupling of aquaculture production from forage fisheries. The same applies to potential use of algal oils to replace fish oil. The lack of alternatives to micro-algae for feeding fish and shellfish larvae and juveniles assures a market for micro-algae in hatcheries and nurseries. Furthermore, micro-algae as health improving feed additives could lead to a reduced use of antibiotics [12].

### 1-4 Challenges

There are three major challenges for successful commercialization of large-scale production of micro-algae for food and feed [1]:

- Reduction of cultivation costs
- Improvement of cultivation technologies
- Stability/reliability of large-scale cultures and the availability of suitable strains avoiding contamination and population crashes of the cultures

Another challenge are regulations. The marketing of micro-algae products is affected by the European regulations on novel food and novel food ingredients on food safety, nutrition and health [13,14]. Before launching products on the market, the safety of the products must be assessed, but the pace of commercialization is delayed by the restrictive regulatory requirements. Under the Novel Food regulation currently four micro-algae species are accepted: three types of *Chlorella* and *Spirulina* [1, 15]. These regulations (the Novel Food and Food Safety Regulations) for the commercial authorization of new products together with unfavourable climatic growth conditions and insufficient domestic demand, limit the development of the European micro-algae sector [5]. However, scientific and technological capacity, R&D funding policies, competitiveness on the global agri-food markets and related infrastructures are strengths of the European Union (EU). Today, European companies produce about 5% of the global production of micro-algae based food/feed products [5]. In order to obtain a larger and stronger position in the micro-algae market for companies in the Netherlands, many steps have to be taken.

### 1-5 Aim of the study

The Ministry of Economic Affairs is the client for this study. The Ministry, has co-financed a number of research and innovation micro-algae projects, in the period 2005-2015, amounting to a total con-



tribution of ca. 13 million € (personal communication P.A.M. Besseling, Ministry of Economic affairs (2015)).

The aim of the study is to gain insights into the current status and economic potential of the micro-algae sector in the Netherlands and the options to accelerate the development. The overarching research question of the study is: **What are the policy recommendations for the Dutch government to accelerate commercialization and scale-up of the micro-algae sector in the Netherlands?**

The three sub-questions of the study are:

1. **What are the current worldwide status and trends in micro-algae technologies, production and applications with focus on food, feed and aquaculture?**
2. **What are the business opportunities for the food, feed and aquaculture sectors in the Netherlands?**
3. **What are the policy recommendations for the Dutch government?**

The first sub-question is addressed in the first part of the study, which is based on a review of literature and other sources. The second and third sub-question were addressed in the second part of the study which consisted of a number of interviews with stakeholders in the Netherlands.

## 1-6 Approach

### Literature study

A desk study was performed to gain insight into the current worldwide status of microalgae production technologies and applications in order to compare it with the current status in the Netherlands. Sources for the desk study included: Scientific literature, websites, directives, regulations, personal communications and other sources. The results are presented in Chapter 2 and a reference list is presented in the Bibliography.

### Interviews

In the second part of the study, information was gathered via 27 interviews with key stakeholders to gain insight in the current status of the market for micro-algae products and systems in the Netherlands, the market perspectives, obstacles and options to promote further development. Dutch companies and research institutes were visited and interviewed to obtain information about the market for micro-algae and derived products as well as technology development and sales in the Netherlands. In addition, interviews were conducted with several investment funds.

The 27 interviews were conducted with representatives of companies and R&D institutes active in 5 sectors in three different areas: food, feed and aquaculture (Table 1-1). The area "Food" in this study corresponds to everything that has to do with food incl. dietary supplements, infant formulas, additives, etc., the area "Feed" in this study corresponds to feed for cattle, other farm animals, birds, horses and pets and the area "Aquaculture" in this study corresponds to fish and shellfish. Other areas were not included due to the limited time frame of this study. The 5 sectors comprise research institutes (RI), producers (P), developers (D), end-users (E-U) and investment funds, that are briefly described here.

- Research institutes are organisations that conduct research on micro-algal metabolism, growth conditions, optimization of productivity, processing, cost reduction etc. as preparation for scale-up and commercialization

	Research institutes	Producers	Developers	End-users	Investment funds
Food	AlgaePARC WUR, WUR, Glastuinbouw, TNO <sup>a</sup>	AlgaeLink, LGem, Nutress, Algaspring, Photanol	AF&F, AlgaeLink, LGem, Evodos, Algaebiotech, Philips <sup>a</sup>	Unilever, DSM	Holland Food Investments, ProDock, Rabobank, Waterland
Feed	ACRRES	AlgaeLink, Nutress, Duplaco	AlgaeLink, LGem, Omega Green, Evodos, Algaebiotech	DSM, Van Benthem, diervoeders	ProDock, Rabobank, Waterland
Aquaculture	Stichting Zeeschelp	AlgaeLink, Nutress, Algaspring, Cleanalgae	AlgaeLink, LGem, Omega Green, Evodos, Algaebiotech	DSM, Zeelandsroem, Fry Marine	Aquaspark, ProDock, Rabobank, Waterland

<sup>a</sup>TNO and Philips were not interviewed via the questionnaire used for the four sectors RI, P, D and E-U but other questions were asked relevant to the subject (more information is given in Chapter 3 in Section 3-8)

**Table 1-1:** Interviewed companies in five sectors with activities in the areas food, feed and aquaculture

- Producers produce and sell micro-algae and/or are involved in the processing and sales of micro-algae and derived products
- Developers develop and manufacture cultivation systems and/or other equipment for algae cultivation and processing (e.g. harvesting)
- End-users buy produced or processed micro-algae from producers for application and sales in consumer products
- Investment funds are funds that invest in micro-algae businesses.

In Table 1-1 it can be seen that an effort was made to equally divide the interviews over the chosen sectors and areas. The distribution over the various sectors is based on the major business activities mentioned by the interviewed organisations presented in Appendix E. Overall, the respondents are considered to represent the Dutch stakeholders in a way that results are considered to be sufficiently representative. Nevertheless, the total number of respondents is limited, this must be born in mind during interpretation of the results. The treatment of waste water for nutrient recovery from agro-industrial or other effluents is excluded from this study.

The respondents in the sectors research facilities, producers, developers and end-users received 26 questions distributed over 5 different themes: company, investments, general market, location and barriers. The questionnaire for the respondents in the sector investment funds was different with 7 questions about their market perspective, willingness to invest and the conditions for investing (Appendix C). The respondents were mostly interviewed by e-mail or by phone. (All questionnaires can be found in Appendix B and Appendix C).

As remarked in the introduction, the study focuses on the status and perspectives of phototrophic micro-algae cultivation using sun light and mineral nutrients. During this study, the cultivation and processing of heterotrophic micro-algae (without light, using organic substrates) came forward as a potentially interesting alternative. Where applicable this is specifically indicated in the text. However, the strains, cultivation technology as well as products for photoautotrophic and heterotrophic algae, differ substantially.

The results of the interviews are presented and discussed in Chapter 3. The last two sub-questions and the overarching research question are addressed in Chapter 4 and Chapter 5. Chapter 4 addresses conclusions regarding the current worldwide status and trends, including a comparison with the status of the micro-algae sector in the Netherlands and business opportunities for the food, feed and aquaculture sectors in the Netherlands. Chapter 5 presents policy recommendations to accelerate commercialization and scale-up of the Dutch micro-algae sector.

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## Chapter 2

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# Current status of worldwide applications and technologies

### 2-1 Global market

The commercial farming of micro-algae knows a long history (1950s research for biologically active substances, 1960s large scale culturing *Chlorella* (Japan) and in the 1970s for *Spirulina*). In Asia, India, Israel, USA and Australia large scale production facilities have been established since 1980 [1]. In the following 40 years, the micro-algae market has become more and more diversified [9]. China, Indonesia and South Korea, during the period of 2010-2012, were the top three exporting countries of algae based products in the world of more than 125 million dollars/year each. The Netherlands, France and Ireland were the most important EU exporters in 2010-2012. However, compared to China the export value is one order of magnitude lower [5, 16]. The total global micro-algae biomass production amounts to ca. 10,000 tons of dry matter/year which is commercially applied mainly in health food supplements, aquaculture, cosmetics and pharmaceuticals (personal communication J.H. Reith, Wageningen University 2015). The micro-algal biomass market for human health food supplements generates a turnover of approximately US\$ 1.25 billion/year [9, 12, 15].

The most commonly used micro-algae in the world are *Spirulina* and *Chlorella*, with a market volume of about 40 million dollars/year each, and 5000 and 2000 tons of dry matter/year respectively [5, 9]. Overall, the study showed a lack of reliable and consistent market data. The Hainan Simai Enterprising, now named Hainan-DIC microalgae Co. Ltd, is located in the Hainan province of China and has an annual production of 350 tons of algal powder (*Spirulina*) [17]. This accounts for 25% of the total national output and more than 10% of the world output. It belongs to the Japanese DIC Corporation (Dainippon Ink and Chemicals, Japan) [2]. At the Earthrise®Nutritionals farm, located at Calipatria, USA California, the largest plant in the world can be found, which stretches over an area of 44 ha using high rate algal ponds with an annual production of 450 tons [2, 9, 18]. In Kona, Hawaii, a plant is operated by Cyanotech producing *Spirulina* Pacifica® with open ponds accounting for 33 ha. The obtained micro-algal biomass is further manufactured as pure powder filled in bottles or pressed in tablets [2, 19, 20]. Another production site where *Spirulina* is grown is in Yangon, Myanmar, where it is grown in natural volcanic lakes [2]. The largest producer of *Chlorella* is Taiwan Chlorella Manufacturing and Co. in Tapei, Taiwan with 330 tons of dried biomass produced per year and a further 300 tons in another production site in Hai-Nan, China using open ponds [2, 21]. *Chlorella* is produced by more than 70 companies and in Europe, the company Roquette Klotze GmbH & Co. KG in Klotze, Germany is the largest producer with 100 tons of dry biomass per year using glass tube PBRs, 500 km long and a

volume of 700 m<sup>3</sup> operated in a greenhouse covering an area of 1.2 ha [2, 22]. The largest producer of *Dunaliella salina* for  $\beta$ -carotene is Cognis Australia, now part of BASF located at Hutt Lagoon, Western Australia, with a production plant consisting of natural salt lakes covering 400 ha. Dietary supplements and functional foods for human nutrition and animal feed are produced from the *Dunaliella* powder and sold. The manufacturing under the product name Betatene® consists of microalgae extracts, a mix of several carotenoids [2, 23]. Lacking a rigid polysaccharide wall, the dried biomass of *Dunaliella* is easily digestible by humans and animals [2]. *Dunaliella salina* is cultivated outdoors in open ponds, which is possible due to the extreme conditions under which it grows (hypersaline, high solar radiation levels and low availability of nitrogen). Other production plants producing *Dunaliella salina* are located in Israel, US, China and other locations in Australia [9, 24, 25]. For the astaxanthin market from the algae *Haematococcus pluvialis*, Israel is the leading country. The company Algatechnologies produces astaxanthin on 4 ha of arid desert land in outdoor modular tubular PBRs with about 300 km long glass tubes [26]. Solazyme, Inc. in San Francisco, USA is producing micro-algae heterotrophically on a large scale for production of oleochemicals in foods or cosmetics and is a renewable oil and bio-products company. Heterotrophic micro-algae require sugar as substrate for growth and Solazyme obtains these sugars by enzymatic degradation of biomass residues: sugarcane bagasse, miscanthus, corn stover and switchgrass. A research and development agreement was signed in 2010 with Unilever, in which algal oils were successfully incorporated into Unilever personal care products [2, 27]. In 2017 the EU sugar quota management system will be ended (Gemeenschappelijk Landbouwbeleid (GLB) 2013). Due to less regulated production, supply of sugar in Europe may increase which may open new application areas such as biotechnology including cultivation of heterotrophic micro-algae. Although biofuels are outside the scope of this study, it is still worth mentioning because big projects and investments are going on around the world concerning biofuel production with micro-algae. In several cases co-products such as proteins for application in feed are being developed in order to improve the economics. Four companies working on algae biofuels are highlighted in this report:

- Cellana LLC in California and Hawaii, USA (2004) has the aim to grow micro-algae and to produce vegetable oils for the conversion into biofuel. A 2 ha facility in Kona, Hawaii has been developed based on the patented ALDUO™ technology and the plant has been producing since 2007 [2, 28].
- Sapphire Energy Inc. in San Diego, USA (2007) has a 40 ha plant operating in Luna Country, New Mexican desert [29]. Investment for this company in 2009 was over US\$100 million from investors like Gates's Cascade Investments and the Rockefeller family's venture-capital firm Venrock [30].
- Algenol Biofuels Inc. in Florida, USA (2006) has two big projects running for the production of ethanol by cyanobacteria with a production system licensed as Direct to Ethanol® technology. In Fort Myers Florida, USA a pilot facility of 10 ha is under construction (partially financed by governmental funding and licensed to BioFields S.A.P.I the C.V. in Mexico) to demonstrate the commercial viability of this technology [2, 31].
- Chiclana de la Frontera, South-West of Spain, is a small Spanish resort town that has begun a pilot programme in which sewage and wastewater is converted into high yield algae production and biofuel. The project is run by a consortium of European private companies and universities (Allgas, co-financed by the EU commission within the FP7 programme) and owned by the world's largest private water company, Aqualia, and majority funded by the EU. The plant (10 ha) costs 12 million € and will become operational in 2016. It aims to produce algal biomass of up to 120 ton/ha/year and to run municipal vehicles (public buses and garbage trucks) in the region of Cadiz, Spain on algal fuels [32, 33].

The biggest investor in algae is oil giant ExxonMobil. In 2009 it had announced to invest US\$600 million over the next five to six years in a partnership with Synthetic Genomics, a company in La Jolla, California [30]. The website of Synthetic Genomics only states a partnership with ExxonMobil [34]. Another interesting application for micro-algae biomass can be the production of biogas. Favourable harvesting and transport conditions are advantages of micro-algal biomass for biogas production, because no high cell concentrations are needed and biogas plants can be located next to a PBR [2]). However,

since biogas is a low value product, the use of algae biomass for exclusive production of biogas is not economically feasible and valuable co-products are required.

The biofuel market encounters the problems of high biomass production costs and the lack of a net positive-energy-balance for the process. The minimum cost price for micro-algae when produced on large scale is now between 4€ and 5€/kg, but the value of biodiesel is so low that the cost price of algal biomass production needs to be lower than 0.26€/kg dry biomass [35–37]. Biorefinery of the algae biomass into multiple valuable products next to biofuel is a potential solution to improve economics. Product processing and reactor development are two of the many factors that need improvement to diminish the challenges for the biorefinery of micro-algae (more on biorefinery in Section 2-7). In order to stimulate biorefinery, adjustment of the market demand for high-valued products and bulk biomass for the energy sector is needed. The market volume for higher value specialties is inevitably much smaller than for biofuels, causing market saturation and a drop in market value for the co-products. No balance is present in the product demand for both of these sectors. In order to achieve this balance, the market for high value products should increase [2, 38]. A major advantage of micro-algae over traditional land based crops is that use of agricultural land and valuable fresh water resources is not required thus alleviating food versus fuel conflicts. Extreme climatological conditions, limited biomass availability and land unsuitable for agriculture can become advantageous factors for micro-algae cultivation as well as the presence of industries with residual nutrients in effluents and CO<sub>2</sub> from flue gas. Possible locations for micro-algae cultivation include: oceans, flat roofs, deserts, roadside verges, degraded/marginal land and contaminated soil [39, 40].

## 2-2 Food

Micro-algae are an interesting source of food products, because high amounts of proteins, polyunsaturated fatty acids, polysaccharides and carotenoids can be found in micro-algae that could be used as valuable food and health promoting products. The term nutraceuticals is used for food or food products providing health and medical benefits, including treatment and prevention of disease with compounds such as PUFAs and carotenoids. The term functional foods is used as food affecting beneficially one or more target functions in the body in a way that reduces risk of disease and improving the state of health [26]. Looking at microalgae as a source for food commodities (proteins, starch and edible oils) eukaryotic microalgae are more interesting than the prokaryotic cyanobacteria, because natural triacylglyceride (TAG) oil is produced by eukaryotic microalgae. This lipid class is used to store fatty acids under stress conditions and this accumulation is well established in a number of algae strains [6, 41–44].

Long chain polyunsaturated fatty acids (LC-PUFAs) are recommended by the World Health Organization as an important component of the human diet because of their health benefits: preventing cardiovascular diseases, type-2 diabetes and obesity [45]. The most important PUFAs obtained from micro-algae are the omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the omega-6 fatty acids: arachidonic acid (AA) and  $\gamma$ -linolenic acid (GLA). The fastest growing application section within the omega-3 and 6 fatty acids are the new food supplements and functional foods [2], the naturally produced fatty acid market was valued at US\$ 7,2 billion in 2011 [11] with a market price for algae omega-3 oil of about US\$ 140/kg (Borowitzka 2013). The biotechnological potential for PUFAs from micro-algae as nutritional supplements in food and feed in these application areas are very promising due to the high quality, reduced risk of chemical contamination, better purification and stability and absence of unpleasant odour [2, 46]. Martek biosciences Corporation (recently acquired by DSM) in Maryland, USA and Lonza Ltd in Basel, Switzerland are the major producers of DHA, but with heterotrophically grown micro-algae. These micro-algae feed on sugars: not on sunlight and carbon dioxide [2]. Since 1999 DHA has been recommended by several health and nutrition organizations for the healthy development of brain and eyes in infants. DSM has several customers for DHA for supply of infant formula applications including Mead Johnson Nutritionals, Pfizer, Danone and Abbott Nutrition [26]. Infant food is one of the most important application sectors for micro-algal food with a global turnover of about US\$ 10 billion/year [2, 47]. The heterotrophic *Cryptothecodinium* and *Schizochytrium*

are the micro-algae to date from which DHA has been commercialized. ARA is an essential omega-6 fatty acid, critical for neonatal brain development and included in infant milk formulas [45].  $\beta$ -carotene from *Dunaliella salina*, astaxanthin from *Haematococcus pluvialis* and phycocyanin from *Spirulina* are the only commercially established micro-algal biochemicals [45, 48]. Exploited whole as nutraceuticals and health food additives are the cyanobacterium *Spirulina* and the green algae *Chlorella*.

Micro-algal species can be divided in three categories based on their exploitation potential for high-value biochemicals production: single high-value product, exploitation by biorefinery approaches and novel species. In the first category only three microalgae species are well characterized for their high product content for the production of a single product: *Dunaliella sp.* with 10-14% (w/w)  $\beta$ -carotene, *Haematococcus pluvialis* with 4% (w/w) astaxanthin and *Parietochloris incisa* with 20% (w/w) arachidonic acid (AA) [9, 45].  $\beta$ -carotene can reduce the risk of atherosclerosis and cancer [4, 49] and can be used as a pigment in butter, cheese, etc. Due to the high stacking density of cell membranes in which carotenoids are stored, microalgae have a high areal productivity. Recombinant heterotrophic bacteria cannot compete with these high levels of intracellular concentrations of carotenoids in micro-algae, but nowadays the carotenoid market is covered for 90% by synthetic  $\beta$ -carotene and 95% by synthetic astaxanthin with as main producers DSM in Heerlen, the Netherlands and BASF in Ludwigshafen, Germany [2]. Natural antioxidants have a higher bioavailability and therefore a higher protective efficacy than synthetic antioxidants [11]. The market value for  $\beta$ -carotene was around US\$ 270 million in 2013 with 20-30% of estimated natural  $\beta$ -carotene and the price for natural  $\beta$ -carotene is varying in relation to demand from US\$ 300 to 3000/kg [11, 26]. Astaxanthin is a powerful antioxidant, effective as an anti-inflammatory and has immune enhancing properties in humans and animals and is widely used in fish feeds [4]. The price of 5% astaxanthin is about US\$ 1900/kg [11]. The global carotenoid market was US\$ 1.2 billion in 2010 [11, 26].



**Figure 2-1:** Micro-algal products including pigments at ACRRES (Netherlands).

The carotenoid lutein can be applied as a natural colourant in foods, drugs and cosmetics and as a food supplement. The health benefits are preventing blindness, delaying chronic diseases, hampering the development of cataracts and the progression of early atherosclerosis, decrease in vision-loss caused by age-related macular degeneration. Furthermore it stimulates the immune response. Lutein has a recent market value of US\$ 233 million in 2010 [11]. Pilots have been set up for the production of lutein with the micro-algae *Muriellopsis sp.* and *Scenedesmus sp.*, but no established commercial systems for the production of lutein do exist yet [11, 50]. Another pigment class that is well known are phycobiliproteins, present in *Spirulina* and *Porphyridium* as phycocyanin (blue pigment) and phycoerythrin (red pigment) respectively. It is an anti-inflammatory, an antioxidant and hepatoprotective [2, 4, 51]. The pigment is used as fluorescent indicators in clinical diagnostics, and as natural colorant in food

products (chewing gum, ice creams, sweets, dairy products) and in cosmetics and nutrition [4, 15, 49]. The total market value for phycobiliprotein products was estimated to be greater than US\$ 60 million in which the annual market of phycocyanin was estimated at US\$ 5-10 million [11, 26]. *Spirulina*, also known as the cyanobacterium *Arthrospira*, has a high protein content of 60-71% of the dry matter and is therefore used as human food supplement. It is one of the richest natural plant sources of vitamin B12 and it has various health promoting benefits including attenuation of hyperlipidemia, protection against renal failure, suppression of hypertension, prebiotic stimulation of the growth of intestinal *Lactobacillus acidophilus* and suppression of elevated serum glucose level [2, 15, 52].

At present only four micro-algae species are accepted as food in the European Novel Food regulation: *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella luteoviridis* and *Arthrospira platensis* (*Spirulina*). A Spanish company (Fitoplancton Marino) has recently acquired approval for use of the marine micro-alga *Tetraselmis chuii* in condiments. A condiment is an addition in food to boost or add flavour. The company has obtained the first authorisation from the Novel Food for marine micro-algae in Europe, as well as a Generally Recognized as Safe (GRAS) rating in the US [53]. The product is called Plancton Marino Veta la Palma® and contains freeze dried *Tetraselmis chuii* to season dishes [54].

## 2-3 Animal feed

The second largest area of application is the use of micro-algal biomass as animal feed, 30% of the current algal production globally is sold as a supplement for animal feed: pets, farm animals, ornamental birds, aquarium fish and as feed for aquaculture operations. Aquaculture is described in detail in Section 2-4. The algae mainly used for this purpose is *Spirulina*, more than 50%, about 2000 tons [2, 9, 55]. *Spirulina's* positive effects are colour enhancement, increased growth rate and improved general tissue quality, but with the most promising effect immune enhancement, resulting in antiviral and anti-bacterial properties with only low addition in the feed [55].

Toxins, heavy-metal components and nucleic acids (DNA and RNA) are constituents of micro-algal biomass that may represent some constraints on its incorporation into feeds together with digestibility and high amount of salts. Nucleic acids from micro-algae should not exceed 2.0g/day in feed, but for heavy metal contents no official standards exist for micro-algal products. When speaking of digestibility, the overall digestibility of algae carbohydrates is good. However, for each algae this has to be identified when using dried whole cell micro-algae or even further, it has to be treated effectively by disrupting the cell wall, making the algal constituents accessible to digestive enzymes [7, 55].

Nowadays soy is imported mainly from Argentina and Brazil for its high protein value and protein digestibility, good amino acid profile and low cost price. However, locally produced alternatives are of interest in the Netherlands itself and the rest of Europe. Thanks to local production, transportation may be greatly reduced [56]. Micro-algae are an interesting alternative for soy because of their high protein content, but nowadays soy is not replaced by micro-algae because it is not yet economically feasible to apply micro-algae as animal feed. However, with the exclusion of extreme values, the feed quality of researched micro-algae are after appropriate treatment comparative or even better than conventional vegetable proteins [56, 57]. A food and toxicological evaluation has already been executed which proved the suitability of micro-algae biomass as valuable feed supplement [49, 56]. Micro-algae contain essential amino acids (which humans and animals cannot synthesize) and have a high protein content. Due to the amino acid profile (which is comparable with that of egg or soybean) and the protein content, micro-algae are considered to be a useful, non-conventional source of proteins [4, 10, 49]. The micro-algae that can be used as an alternative for soy proteins include: *Chlorella*, *Spirulina*, *Nannochloropsis*, *Tetraselmis*, *Chaetoceros*, *Scenedesmus*, *Nitzschia*, *Haematococcus*, *Navicula* and *Cryptocodinium* (and the macro-algae: *Ulva*, *Laminaria*, *Padina*, *Gracilaria* and *Pavonica*) [56].

As a source of food, for both meat and egg production, 50 billion chickens are annually reared [11]. In poultry feed rations, micro-algae can be added in a dose up to 5-10% without causing negative health effects. A higher percentage can result in long term decreased protein and energy efficiency and unfavourable feed conversions [56, 57]. The most important positive effects attributed to feeding algae are an

enhanced yellow colour in egg yolk and a better colour of broiler skin and shanks [11]. The "Institut für Getreideverarbeitung" (Bergholz-Rehbrücke, Germany) produces a natural feed named Algrow with the micro-algae *Chlorella* and *Arthrospira* [11, 55]. A study was conducted by Ginzberg et al. (2000) into the impact of micro-algae *Porphyridium* as feed supplement on the metabolism of chicken. Proportions of 5% and 10% were used in the standard chicken diet resulting in consumption of less food (10%), lower serum cholesterol levels (11% and 28% with the proportions of 5% and 10% respectively), reduction of cholesterol levels in egg yolk (10%), increased levels of arachidonic acid and linoleic acid (24% and 29%) and a darker colour of egg yolk indicating a higher carotenoid level produced (2.4 fold) [55, 58]. The study by Gatrell et al., (2014b) researched the feasibility of creating omega-3 fatty acid enriched eggs and chicken with various micro-algae. In the thigh muscle, breast, plasma and liver of broiler chicks EPA and DHA was increased [59, 60]. Other studies observed in turkey fed with *Spirulina* at the level of 1-10g/kg an increase in growth rate and in a lower non-specific mortality rate [55]. In pig farms, feeding with micro-algae showed enhanced health by 25% [56, 57]. A study was conducted by Abril et al. (2003) with the DHA-rich micro-algae *Schizochytrium sp.* administered in the diet of growing swine. Changes were higher weight gain and feed conversion efficiency. Doses up to five times the commercial dose shows no adverse effects [55, 61]. In a study by Lum et al., 2013, a higher concentration of omega-3 fatty acids was found in milk with a variable effect on milk fat content [56, 62]. with health promoting effects and improvement of external appearances on the pet: beautiful feathers and shiny hair, which are of importance for consumers. Studies have been conducted on rabbits and mink providing evidence of such effects [15, 55].

The minimum price for micro-algae when produced on large scale is now 5€/kg, when competing with other animal feed biomasses this cost price should be reduced to 0.30€/kg or when based on soy it should not be greater than US\$ 0.60/kg (based on a soybean price of US\$ 600/ton) [26, 56]. Major factors for this high price are the costs for energy use and installations of ponds and photobioreactors, as well as the costs for the post cultivation treatments [56]. Cost price reduction methods could involve: reduction of energy use, efficient nutrient use, growth improvements, improved cultivation, harvest and drying methods, etc. An argument for the use of micro-algae as feed additive are the positive effects on animal health and a potential reduction of antibiotic use. Many countries want to decrease the use of antibiotics in animal feed [12, 56]. This additional value of micro-algae for animal feed is a possibility to compete with e.g. soy.

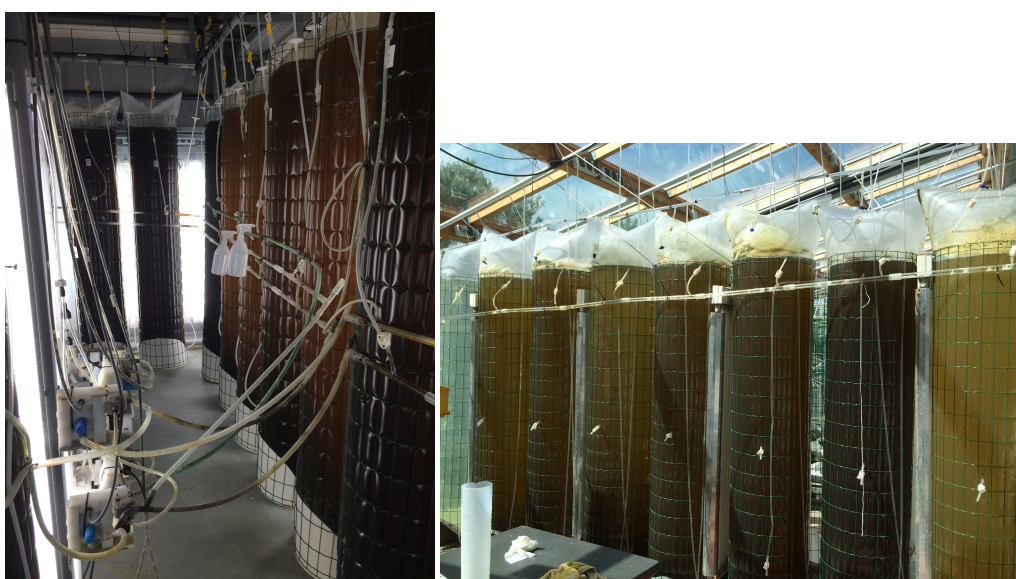
## 2-4 Aquaculture

Algae form the basis of the natural food chain in marine ecosystems and micro-algal biomass is therefore an important resource for aquaculture. Commercial and experimental fish or mollusc hatcheries generally incorporate a micro-algal production system in parallel to their hatchery, because of the whole life cycle dependence of marine invertebrates on feeding with micro-algae [63]. Two different ways of using the micro-algae are important aspects for aquaculture: 1) the biomass can be directly used as food source for zooplankton (copepods, rotifers), fish larvae and molluscs (oysters, sea ears, scallops, carpet shells) [2, 64]. 2) The biomass can be used as an additive to fish feed. Feeding with micro-algae promotes the important biological processes (enhanced growth, firmer flesh, brighter skin, better flavour and resistance against diseases) and it improves the colouring of farmed salmon. Due to the unsaturated fatty acids and the high protein content, the flesh of the fish becomes healthier and tastier [2, 65].

Not every strain is suitable for aquaculture, the micro-algae produced for aquaculture need to meet various criteria: nontoxic, correct size and shape to be ingested, easily cultured, high nutritional qualities, a digestible cell wall to make nutrients available, fast growth rates and stable to fluctuations in temperature, light and nutrient profile [2, 9, 63]. The main types which meet the above-mentioned criteria together with the suitable nutritional value by the content of unsaturated fatty acids, proteins and vitamins are: *Tetraselmis*, *Scenedesmus*, *Chlorella*, *Pavlova*, *Phaeodactylum*, *Spirulina*, *Skeletonema*, *Chaetoceros*, *Isochrysis*, *Thalassiosira*, *Nannochloropsis* and *Nitzschia* [2, 15, 66]. System crashes or



culture contaminations in hatcheries are avoided by maintaining uninterrupted supplies of microalgae [66]. The estimation of the global market for aquaculture products is more than US\$ 40-50 billion/year with an annual growth of 8% since 1970 [2, 15, 64]. Due to this growth, a continuously growing demand for specific knowledge in the area of system innovation, production systems, feed, breeding, vaccination, logistic and culture technology is expected [67]. Numerous small-scale micro-algal production facilities worldwide are located in aquaculture hatcheries that produce juvenile finfish and shellfish for food, but only a small number of micro-algal strains are used in aquaculture hatcheries [66]. The Akkeshi Town in Hokkaido for example nurtures young shells at an oyster nursery centre. *Chaetoceros calcitrans* and some other micro-algae are cultured in sealed tanks and fed to incubated oysters [68]. The main types of micro-algal cultivation systems used in aquaculture are: open ponds or tanks, closed PBRs (usually tubular, less frequently flat panel PBRs) and bubble or airlift columns (mostly vertically, less commonly horizontally placed, Figure 2-2), [66]. These types of cultivation systems are explained in the Section 2-5.



**Figure 2-2:** Column bag systems. Micro-algae cultivation for aquaculture at Zeelandsroem and Stichting Zeeschelp (Netherlands)

Most of the micro-algae suitable for aquaculture are cultured in closed systems, because in open systems only species with a highly selective environment (*Chlorella* and *Spirulina*) can be cultured remaining relatively free of contamination [63]. A specific cultivation method is the 'pseudo-green water' rearing technique. Cultured micro-algae are added to fish larval rearing tanks. In this way higher larval stocking densities are sustained, required in most commercial marine fish hatcheries in Europe and North America. *Isochrysis sp.*, *Tetraselmis sp.* and *Nannochloropsis sp.* are commonly used for this purpose [66]. Several effects have been reported when using the green water technique: improved chemical water quality, greater larval absorption of soluble organics, regulation of bacterial population, stimulation of immunity, probiotic effects, etc. [66, 69]. The microalgae used as livestock and aquaculture feeds have been credited with promotion of stress resistance, stimulation of lipid metabolism and an improved immune system [66, 70–72]. Highly unsaturated fatty acids (HUFAs) incl. polyunsaturated fatty acids (PUFAs) are eminent for human health for prevention and treatment of diabetes, hypertension, arthritis, coronary heart disease and other autoimmune and inflammatory disorders. These PUFAs should be included in the daily diet because humans and animals cannot synthesize them. Farmed fish and shellfish offer these rich sources of PUFAs, but due to the global shortage and increasing costs of fish oil and fish meal, researchers are looking for alternatives. In aquaculture the feeding fish to fish principle is used, but more than 1 kg of fish feed is needed to produce 1 kg of farmed fish, making it unsustainable. The expectation of the continuous growth of aquaculture makes researchers around the

globe concerned: increase of fishing pressure on wild stocks to supply both fish meal and fish oil, which would threaten the fish species in question [73]. Furthermore, toxins can be accumulated in the PUFAs derived from fish sources, together with unpleasant taste, smell and poor oxidative stability limiting the application of fish oil as a food additive [11]. Microalgae could be an interesting alternative, because high amounts of PUFAs are found in some microalgae [66] and the PUFAs derived from (several) microalgae do not have an unpleasant smell, have a reduced risk of chemical contamination and have better purification and stability [2, 46]. However, no protein-rich or lipid-rich algal meal is on the market for an affordable price, all categories of algal products are currently much higher in cost than the commodity feedstuffs used in animal feeds and aquaculture [66].

Biomass availability, composition and cost will be the factors depending on micro-algae biomass used as supplement or bulk feedstuff to supply protein and energy in animal feeds and aquaculture [66].

Known applications of micro-algae for food, feed and aquaculture are summarized in Table 2-1.

Species	Group	Major producers	Product	Application
<i>Arthrospira (Spirulina)</i>	Cyanobacteria	USA (California), Hawaii, Japan, India, Germany, Mongolia, Australia, Mexico, Thailand, Israel, China, Myanmar, Canada	Spirulina, Phycocyanin, biomass, <i>gamma</i> -linolenic acid, whole cell	Dietary supplement, colorant, cosmetics, feed additive, infant formulas
<i>Aphanizomenon</i>	Cyanobacteria	USA		Dietary supplement
<i>Nostoc</i>	Cyanobacteria	Asia, USA		Health food
<i>Lyngbya</i>	Cyanobacteria		Immune modulators	Pharmaceuticals, nutrition
<i>Chlorella</i>	Chlorophyta	Netherlands, Germany, USA, Japan, Taiwan, Portugal	Biomass, ascorbic acid, <i>beta</i> -glucan, whole cell	Dietary supplement, food ingredient, cosmetics, aquaculture, health food
<i>Dunaliella</i>	Chlorophyta	India, Australia, China, Israel, Japan	<i>beta</i> -carotene, biomass	Additive, vitamin, colorant, cosmetics, health food, pharmaceutical, aquaculture, animal feed
<i>Haematococcus</i>	Chlorophyta	USA, Hawaii, India, Sweden, Israel, Germany, Japan, China	astaxanthin	Dietary supplement, health food, food ingredient/additive, pharmaceutical, cosmetics, colorant
<i>Porphyridium</i>	Rhodophyta	France, Israel	AA (omega-6). Phycoerythrin, polysaccharides	Dietary supplement, cosmetics, infant formulas, pharmaceuticals, colorant
<i>Nannochloropsis</i>	Eustigmataceae	Germany, USA	EPA/DHA (omega-3), biomass for aquaculture, whole cell	Dietary supplement, food ingredient, feed for fish in aquariums, aquaculture
<i>Schizochytrium</i>	Thraustochytrid	China, USA, Germany	EPA/DHA (omega-3)	Dietary supplement, food ingredient, infant formulas, aquaculture
<i>Odontella</i>	Bacillariophyta	France, Germany	EPA/DHA (omega-3)	Food ingredient, dietary supplement, baby food, pharmaceuticals, cosmetics
<i>Chrythecodinium</i>	Rhodophyta	USA, Netherlands, Germany	EPA/DHA (omega-3)	Food ingredient/additive, dietary supplement, infant formulas, aquaculture
<i>Isochrysis</i>	Chlorophyta	Germany, Canada, USA	fatty acids, algaepaste, whole cell	Feed for fish in aquariums, animal nutrition, aquaculture
<i>Phaeodactylum</i>	Bacillariophyta	Portugal	Biomass for aquaculture, EPA (omega-3)	Feed for fish in aquariums, aquaculture, dietary supplements
<i>Ulkenia</i>	Thraustochytrid	Germany	DHA (omega-3)	Dietary supplement
<i>Nitzschia</i>	Bacillariophyta	USA, Germany	EPA (omega-3), whole cell	Dietary supplement, aquaculture
<i>Chlamydomonas</i>	Chlorophyta	USA, Germany	Whole cell, proteins	Aquaculture, pharmaceuticals
<i>Tetraselmis</i>	Chlorophyta	USA, Germany	Whole cell	Aquaculture

**Table 2-1:** Various micro-algae species with their taxonomic group, major producers, products and applications [1–3, 9, 12, 74]

Open cultivation systems	(Semi-)Closed (photo)bioreactor systems (PBR)
Unmixed open ponds	Tubular reactors (horizontal/vertical)
Race way ponds	Flat panel reactors
Race way ponds in greenhouses	Bag/flexible tube reactor
Hybrid systems	Fermentation vessel (heterotrophic cultivation)

Table 2-2: Cultivation systems

## 2-5 Cultivation systems

Phototrophic micro-algae are cultivated in systems, which can be subdivided into two types: The open cultivation systems and the (semi)closed photobioreactor systems (PBR). Each system has several sub-types (Table 2-2).

### Open systems

The most common open system types for micro-algae production are extensive unmixed open ponds (e.g. pond systems used for *Dunaliella* cultivation in Australia) and especially high rate algal ponds or race way ponds Figure 2-3.



Figure 2-3: Race way ponds operated by Earthrise farms for cultivation of *Spirulina* in California, USA

The raceway ponds circulate micro-algae and water with nutrients with the help of a paddle wheel (or mechanical arm in circular basins) stirring the contents of the pond. The depth of race way ponds is normally up to 30 cm of depth. CO<sub>2</sub> is supplied by spraying it into the culture [1]. The advantages of an open system is that they are easy to build and are relatively cheap compared to closed systems. The disadvantage of these systems is that only a few species can grow in them, due to the susceptibility to an invasion of other alga strains that grow faster and out-compete the desired species and the potential contaminations with algal pathogens and predators. The few species that can grow in these systems have specific growth requirements, such as a high alkalinity (*Spirulina*), a high salinity (*Dunaliella*) or the production of a large amount of inoculum to take advantage of fast-growing species (*Chlorella*). Furthermore temperature control is almost not possible, biomass concentrations are low and due to

the long light path and poor mixing the volumetric productivity is low. The photo-conversion efficiency (PCE) for open ponds is comparable with terrestrial energy plants (maize and sugarcane) and is around 0,5-1% and usually not exceeding 2 g/L of dry micro-algal biomass concentrations depending on the depth [2, 75]. One variation of the race way system is the covered race way system placed in a greenhouse. This allows cultivation of other species such as the system operated by Algaspring in the Netherlands for the cultivation of *Nannochloropsis* (Figure 2-4). Another approach is the hybrid cultivation system operated by Cellana, USA that combines closed PBRs for cultivation of inoculum and race way ponds for the actual production stage.



Figure 2-4: Raceway system in a greenhouse operated by Algaspring (Netherlands)

### Closed systems

The most common closed systems are the horizontally tubular PBRs (Figure 2-5), vertically stacked tubular PBRs and the flat panel PBRs. The closed systems have a higher illuminated area to volume ratio (A-V ratio) compared to the open systems, resulting in higher biomass concentrations and the control of culture conditions can be better achieved (Bosma, Dam 2014).



Figure 2-5: Horizontal PBR at LGem (Netherlands)

Algae production	Ton DW/ha/year
Open pond	20
Expected yield tubular PBR	40
Expected yield for Panel PBR	60
Theoretical max. yield	135

**Table 2-3:** Projected biomass productivity of four micro-algae cultivation systems under Dutch climate conditions [79]

A centrifugal pump is used to mix and circulate nutrients and algae and the produced oxygen is removed by the use of a degasser to minimize the negative effect of high oxygen concentrations. The PCE of a horizontal tubular PBR is up to 3% with biomass concentrations in the range of 2-4gDW/L and the PCE of a vertical stacked tubular PBR is up to 7% with biomass concentrations in the range of 1-3gDW/L [76]. The PCE of vertical stacked tubular PBR is higher, because in horizontal tubular PBR higher light intensities are experienced, in which photo inhibition can occur. This is prevented in the vertical stacked tubular PBR because light is diluted due to the vertical orientation, photo inhibition is prevented and the algae experience lower light intensities [77]. The flat panel PBR also has a vertical orientation of the panels that cause light dilution and therefore a high productivity can be achieved up to a PCE of 7% which is similar to the vertical stacked tubular PBR. In the flat panel reactor mixing is done by sparging air through the culture and the typical biomass concentration is in the range of 2-5gDW/L [37, 76, 77].



**Figure 2-6:** Flat panel PBR at AlgaePARC (Netherlands)

The PBRs are still too expensive and relatively inefficient for commercial production. However, the technical capacity to enhance future yields is much better because in contrast to open systems, the conditions can be controlled precisely [1]. Good Manufacturing Practice standards are needed for high-value product production for the pharmaceutical and cosmetic industries and can be achieved by using closed systems. Low priced product production (aquaculture, agriculture and energy sectors) is only possible in closed systems when operation and investment costs are reduced [2, 78]. The projected biomass productivity under Dutch climate conditions in various cultivation systems is shown in (Table 2-3) [79].

## 2-6 Harvesting methods

When micro-algae are cultivated and have reached the desired biomass concentration, the micro-algae need to be separated from the water as the first downstream process step, resulting in an algae paste. However, no single process can be used, because of the varying characteristics: size, shape and motility that influence, to a large extent, the settling behaviour of the micro-algal species [69]. Current harvesting methods in commercial use are centrifugation, flocculation, filtration and flotation or a combination of these methods. Harvesting is followed by processing, with as first method drying of the wet biomass (Section 2-7: Drying) [80]. Harvesting costs have been estimated to account for 20-30% of the total production costs [81]. Two harvesting methods are further explained: centrifugation and flocculation. A centrifuge is a piece of equipment, generally driven by a motor that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. The centrifuge works using the sedimentation principle, where the centripetal acceleration is used to evenly distribute substances (usually present in a solution for small scale applications) of greater and lesser density [82]. Harvesting by centrifugation is generally characterized by high capture efficiency (>90%) under low flow rates and high energy consumption. However a study by Dassey shows, that increasing the flow rates (>1 L/min) leads to lower capture efficiencies (<90%), which resulted in net lower energy consumption and up to 82% decrease in harvesting costs [81]. In the study of Milledge and Heaven (2012) the technique of the company Evodos is described: the spiral plate centrifuge. From a 0.025% suspension of *Nannochloropsis*, a micro-algal paste of 31.5% dry weight can be obtained at an energy usage of 1.9kWh/kg of dried micro-algae [83], (Evodos private communication 2011). With the flocculation method, micro-algae are separated from the medium by using chemicals to induce the micro-algae cells to form aggregates. The chemicals, or flocculating agents, promote flocculation by causing colloids and other suspended particles in liquids to aggregate, forming a floc [82]. In a study by Guedes (2012), the technique flocculation was used with the adjustment of the pH, using NaOH, followed by the addition of a non-ionic polymer, Mangafloc LT-25. This resulted in a final concentration between 200- and 800-fold after harvesting and neutralizing the ensuing flocculate. This process was successfully applied to harvest cells of several aquaculture micro-algal species with efficiencies above 80%. This technique proved to be simple, inexpensive, rapid and independent of processed volume [63]. In most of the flocculation techniques, metal salts such as aluminium chloride and iron chloride are commonly used chemicals, but cause problems in contamination of algal biomass with iron or aluminium ions. Synthetic polyacrylamide polymers require lower chemical dosages to produce similar efficiency due to their higher molecular weight. These polymers were used for flocculation-sedimentation in a study by Mikulec et al. (2015) in which a 90% efficiency of flocculation was achieved [84].

## 2-7 Processing methods

### Drying (spraying, freeze drying)

Several techniques can be used for drying: spray drying, freeze drying, oven drying, steam drying, sun drying, drum drying and fluidized bed-drying. Drying is often the most expensive step of the whole processing chain [80, 85, 86]. The most commonly used techniques for high value products are spray-drying and freeze-drying (lyophilisation). Spray-drying is a fast and uninterrupted drying application of solutions and emulsions. Freeze-drying is the most gentle drying method, preserving all the cell constituents without rupturing the cell wall, but has high energy consumption and expensive equipment [80, 86]. Sun drying is amongst the slower methods, but is energy and cost effective compared to the other techniques [80].

### Extraction

New extraction methods need to be developed because the traditional methods (mainly solid-liquid extraction) have several drawbacks including: being laborious, time consuming and having low extrac-

tion yields and/or low selectivity. A new method could be the use of compressed fluids: supercritical fluid extraction (SFE), subcritical water extraction (SWE) and pressurized liquid extraction (PLE) [39,87]. A model was used to predict the extraction yield of *Neochloris oleoabundans* via PLE by the use of the food-grade solvents: ethanol and limonene. Solvents were maintained in the liquid state by a constant extraction pressure at 10MPa, an extraction temperature of 112°C and 100% ethanol as extraction solvent. This resulted in an extraction yield of 32% [39, 88]. Studies to improve biorefinery techniques are a topic in the EU FP7 project MIRACLES, Multi-product Integrated biorefinery of Algae: from Carbon dioxide and light energy to high-value specialties [89]. The aim of this project is to develop integrated, multiple-product biorefinery technologies for production of high value specialties from algae for application in food, aquaculture and non-food applications including development of novel products for these markets [39]. Several other algal biorefinery projects are underway at the national and European level. Sonication, microwave, supercritical fluid extraction, homogenization, enzymatic extraction, ultrasonic-assisted extraction, bead-beating, osmotic shock, mechanical pressing, milling and autoclaving are usually coupled with solvent extraction for improved lipid yield. In the study by Guldhe et al. (2014) the cell disruption techniques microwave and sonication coupled with a solvent was used. The microwave method resulted in the highest lipid yields compared to the sonication technique [80]. In the study by Santos 2015 the cell disruption techniques Potter homogenizer and ultrasound treatment were used together with solvents. The most efficient extraction method for lipids was the ultrasound [90]. Another method, instant catapult steam explosion (ICSE), was used by Chen et al., (2015). The technique disrupts wet micro-algal cells for efficient lipid extraction. Steam pressure is used for flash evaporation and volume expansion to explode the cell walls and release the cytoplasm which decreases the carbohydrate content and increases the lipid content in the exploded biomass [85].

## 2-8 GMO

Genetically modified organisms (GMO), in the micro-algae domain, do not have a long history. The transformation of the cyanobacterium *Synechocystis* was reported in 1970 followed by the transformation of the green algae *Chlamydomonas reinhardtii* in 1989. Since 1989, 30 algal species underwent successful genetic transformations [1]. Genetic modification as a tool to improve algal performance is more and more considered as a necessity to achieve new and economically viable production systems [1,36,91]. Three types of targets for genetic modification of micro-algae have been distinguished: 1) improvement of photosynthetic efficiency, 2) improvement of productivity of selected products and 3) new GM algae products. Screening of algae is often based on: the use of principle component analysis and response surface modelling to identify optimal or specific conditions and the key variables controlling the production of micro-algal biomass, optimal growth and production of specific metabolites [69].

The research on genetically modified organisms is still immature in Europe. The research on improvement of production systems, improvement of genetic modified strains and commercial production based on GM micro-algae is funded by large investments from industries and governments. Through the involvement of industries and governments, GMO production of micro-algae could become more diversified and economically competitive [9]. Technological advantages will occur when regulatory factors such as transcription factors for over-expression of specific genes are altered or when engineering of metabolic pathways can be achieved to direct the flux of metabolites towards desired end products [69]. It is expected that production costs can be reduced by 50% or more and PUFA and carotenoid contents increased by 50-200% by the use of genetic modification [45]. There are however several obstacles for the development of economically viable micro-algae expression systems. These include lack of, or inconsistent recombinant protein yields, lack of understanding of micro-algal metabolism, lack of effective and consistent transformation methods for a wide variety of species and lack of production systems optimised for large-scale growth and harvesting under photoautotrophic conditions, regulations and ethical considerations [1,45].



## 2-9 Regulations

The two main European regulations for the application of micro-algae in food and feed are the Novel Food Regulation [13] and the General Food Law [14]. The Novel Food Regulation applies when a company wants to bring a new food product on the market that presents a newly developed, innovative food or food produced using new technologies and production processes as well as food traditionally consumed outside of the EU (personal communication R.J.A. Donner, Ministry of Economic Affairs 2015). A full procedure and a simplified procedure are available to obtain pre-market clearance to authorize novel foods based on the concept of „substantial equivalence” defined as „a novel food should be considered the same as a conventional food if it demonstrates the same characteristics and compositions as the conventional food” [26]. When novel foods and food ingredients are introduced on the European market, a risk assessment and a detailed scientific information report are required [13,92]. The General Food Law forms the foundation of the food and feed law. It sets out an overarching and coherent framework for the development of food and feed legislation both at union and national levels. Important related EU frameworks are the food and feed hygiene regulations, regulations on feed products, regulations on additives and regulations on genetically modified organisms (personal communication R.A.J. Donner, Ministry of Economic Affairs 2015). These European regulations are implemented in the Netherlands through several different laws and regulations. Main frameworks are the Animals Act and the Commodities Act and more specifically the Decision and Regulation on animal nutrition [93] and the decisions on novel food and food hygiene [94, 95]. The European Food Safety Authority (EFSA), established in 2002, provides guidelines for approval and scientific evaluations and assessments. The guidelines can be found in the paper by the EFSA panel on Food Additives and Nutrient Sources added to Food (ANS) [26]. The biosafety evaluation of food products is performed in the EU by the European Food Safety Authority (EFSA) and in the USA by the Food and Drug Administration (FDA) [1]. Under the Novel Food regulation the micro-algae species currently enlisted as accepted are three *Chlorella* types: *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Chlorella luteoviridis* and *Spirulina* (*Arthrospira platensis*). Products from *Dunaliella sp.* ( $\beta$ -carotene) and *Cryptocodinium cohnii* (DHA) are accepted as food ingredient by the EFSA [1, 12, 15, 96].

Below a certification scheme (GMP+) and the Dutch regulation for waste/manure use as a substrate are highlighted. This is done because the certification scheme caused misunderstanding in some articles and the ministry of Economic Affairs is working on the adaptation of the waste/manure regulation.

### Use of residues for micro-algae cultivation

When algae are used for specific product applications, various types of regulations are going to play a role with respect to allowable inputs for the cultivation process and for the quality of the final products. Every type of regulation has its own sub-types. An example is the case when micro-algae are cultivated using nutrients from waste streams such as waste water effluents or nutrients from livestock manure. In practice mostly mineral fertilizers are used as nutrient source for commercial cultivation of micro-algae. These could be substituted with nutrients from waste or even livestock manure. Every waste stream of a company is designated as waste and usually processed by waste processing companies. In general, when substances are mixed with waste or animal by-products, such as livestock manure, the resulting mixture has to be classified, in most cases, as 'waste' or 'animal by-product' This also applies in the case of micro-algae grown using waste nutrients [97]. In the present situation therefore, it is unclear what the status of the algae are if nutrient rich waste streams such as agro industrial effluents or animal manure are used as substrate for their growth. If the algae are classified as 'waste' or 'animal by-product', the micro-algae biomass or derived products cannot be fed to animals or used as food. This problem could be solved when it is possible to demonstrate that the algae can be separated from the waste stream or manure. To enable this the so called End of Waste Criterion [Einde Afval Criterium] is under development. It is known that micro-algae cultivation results in useful products and the legislation is on the way to make these procedures more flexible (personal communication R.J.A. Donner, Ministry of Economic Affairs 2015).

## Micro-algae for animal feed

In 2006 the European hygiene regulations took effect. These regulations require among others that feed and food operators guarantee the quality and safety of their products by monitoring their production processes using a quality control system based on the principles of Hazard Analysis and Critical Control Points (HACCP). The HACCP-plan addresses all risks that can lead to an unsafe product, the way in which these risks will be avoided and the required control measures to achieve this [92]. The regulation determines that businesses can submit procedures of good animal feed practices to the government for approval. Such a procedure contains an interpretation of the hygiene regulation on sectorial level (e.g. agriculture, transportation). Approval implies that the procedure contains a correct interpretation of the guidelines to the judgement of the government. The Dutch government has approved several standards of GMP+ as guide for good animal feed practices. After 2006 GMP+ was amended on different parts to anchor the starting points of the HACCP more clearly in those standards (personal communication R.J.A. Donner, Ministry of Economic Affairs 2015). Until 2004, GMP+ was a regulation, developed by the Dutch Product board for Animal Nutrition [Productschap Diervoeders]. In 2004 this regulation was replaced by the Animal nutrition law [Kaderwet diervoeders]. However the product board continued the use of GMP+ as a quality control system based on a certification scheme. It is registered in the EU council regulation no 767/2009 [98]. In 2010 the ownership of the GMP+ certification scheme was transferred to a private corporation, GMP+ international, independently from the product board. In conclusion GMP+ is not a regulation (misunderstanding in some articles), but it is a quality control system based on a certification scheme owned by GMP+ International. The content of GMP+ still consists to a large extent of the existing legislative provisions. The remaining requirements are quality standards extending further than the legally binding framework. The government does not oblige companies to participate in GMP+, but the quality control system (GMP+) contains important incentives to work with only certified companies (personal communication R.J.A. Donner, Ministry of Economic Affairs 2015). More information about the GMP+ certification scheme can be found in [99].

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## Chapter 3

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# Results of the interviews and discussion

In this chapter the outcome of the conducted interviews with companies and research institutes are presented and discussed. A short profile of the interviewed companies and research institutes can be found in Appendix A. In this study 27 interviews were conducted with companies classified into 5 sectors comprising research institutes (RI), producers (P), technology developers (D), end-users (E-U) and investment funds in the areas food, feed and aquaculture (more details in Chapter 1). The most important findings can be found in this chapter, the remaining findings are presented in Appendix D. The interviews addressed several themes as presented below.

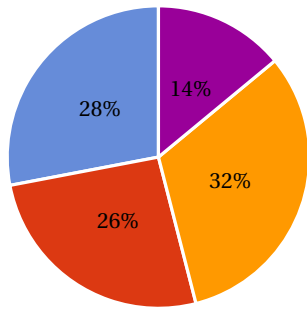
### 3-1 Theme: Company

The activity areas (food, feed and aquaculture) of the interviewed companies are presented in Figure 3-1. This figure shows that the activity areas are almost equally distributed over the sectors P, D and E-U (question 1 and 3). The applicable business case(s) for each company and research institute were identified (question 2). The business cases for all respondents are presented in Appendix E. The follow up questions in the interviews were based on these identified business case(s) (e.g. turnover).

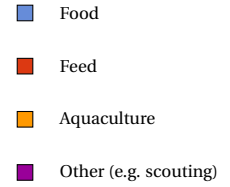
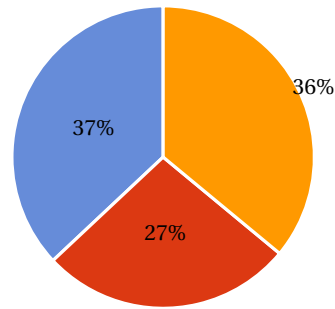
#### Development phase

Question 4 addressed the company's development phase with regard to the micro-algae related activities. For all respondents the applicable development phases were: R&D 21%, Pilot 33%, Commercial 40% and Other 6% (scouting for interesting developments and products) (Figure 3-2). Some companies or research institutes have micro-algae business cases in two development phases or in all three. The development phase for each of the four sectors research institutes, producers, developers and end-users is also shown in Figure 3-2.

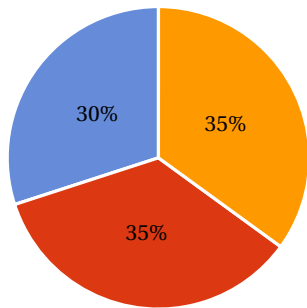
The companies' activity areas for all sectors



Producers



Developers



End-Users

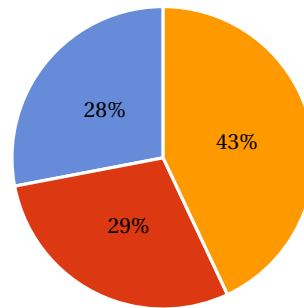


Figure 3-1: Activity areas (food, feed, aquaculture, other) of interviewed companies in all the sectors and in the sectors P, D and E-U.

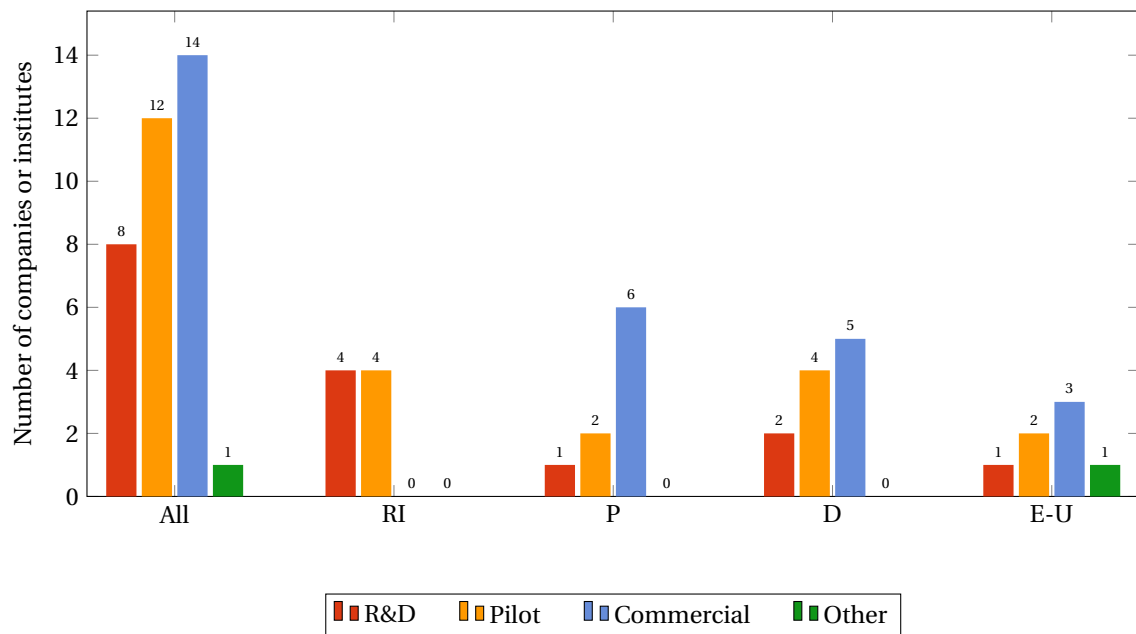
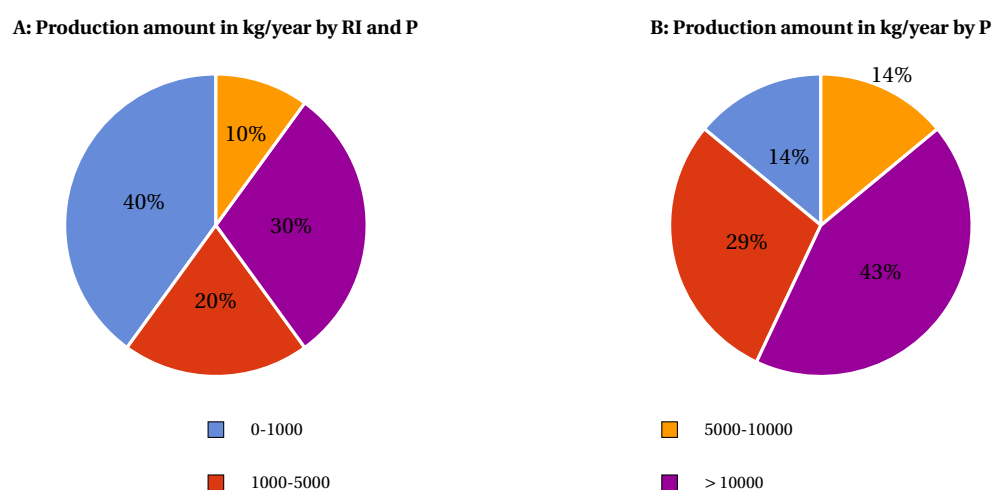


Figure 3-2: The development phase of the business cases in the various sectors. 'All' represents the results for all sectors, RI = Research Institutes, P = Producers, D = Developers and E-U = End-Users.

The research institutes (RI) are all in R&D development and pilot phase, which is logical, because the results of the research done in these institutes are intended mainly for use by other organisations. The producers (P) are mostly in a commercial development phase. Some are doing R&D internally within the company and/or have pilots running. The technology developers (D) operate in all three development phases. The end-users (E-U) differ greatly. Some focus on R&D or are waiting for the right moment to enter the market ('other'). End-users that have micro-algae already incorporated in their business case are in the pilot or commercial development phase dependent on their business area. Compared to the other sectors, the producers are in the highest development phase (commercial).

## Production capacity and turnover

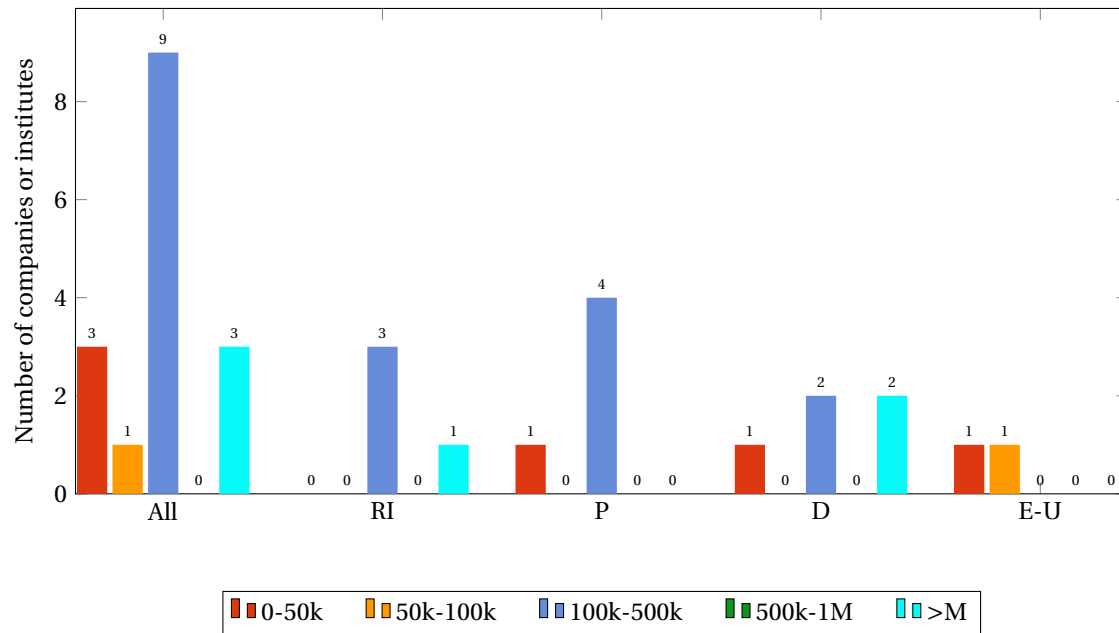
Question 5 and 6 addressed the volume of produced micro-algal biomass and derived products in kg/year and the turnover of micro-algae related business activities in €/year. Question 5 could not be answered by all companies, so this has merely been a question for producers and research institutes, because end-users and developers do not produce micro-algal biomass or products. Results are shown in Figure 3-3.



**Figure 3-3:** Volume of produced algal biomass and derived products in kg/year for producers (P) and research institutes (RI) (A) and for producers (P) alone (B)

From the four RIs, one could not give an answer due to a confidentiality agreement. The remaining RIs that could give an answer were all in the range of 0-1000 kg/year. That is why Figure 3-3, part B, is the most relevant graph because it addresses production by the micro-algae producers. This graph represents 7 producing companies of which the majority (43%) produces more than 10,000 kg/year per company. One of these companies answered for all the production facilities it operates. For some products low quantities (0-1000kg/year or 1000-5000kg/year) are already sufficient to obtain break even (i.e. high value products).

The turnover in €/year in micro-algae business activities was addressed in question 6. Most of the end-users and one developer could not answer this question. However, it was possible to obtain an overview of the turnovers based on data from 17 respondents (Figure 3-4).

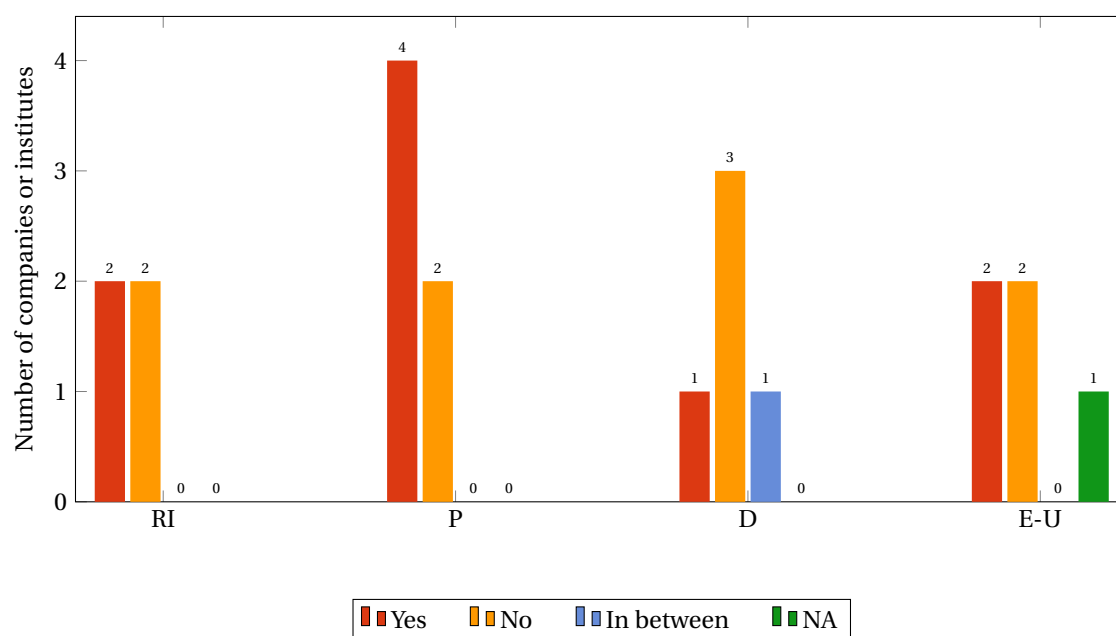


**Figure 3-4:** Turnover of micro-algae business activities in k€/year for all sectors together (ALL) and for every sector separately (research institutes (RI), producers (P), developers (D) and end-users(E-U))

Most of the research institutes' turnovers are in research, even one over 1 million€/year. The turnover of the producers depends on their business case, most have a turnover of 100-500k€/year. Typical selling prices for the companies' products ranged from <10€/kg (lower value products for food and feed) to 10-25€/kg or >25€/kg (high value products). The results show that only one of the interviewed companies (a developer) has reached a turnover of >1 million€/year, while one other developer was anticipating a turnover of >1 million€/year. The main message is that in the current situation most of the companies are relatively small businesses with a limited turnover in the range 100-500k€/year.

### Economic feasibility and expected turnover in 5, 10 and 15 years

The presented data on turnover still do not show if the companies are profitable or not. Question 7 therefore asked if the production is economically feasible/if a breakeven is reached (Figure 3-5). The results differed per sector, the majority of the producers is achieving breakeven. Overall about half of all respondents is reaching breakeven. Some respondents indicated to be close to reaching a breakeven. 'In between' indicates that breakeven depends on demands and can therefore differ for each year (Figure 3-5).



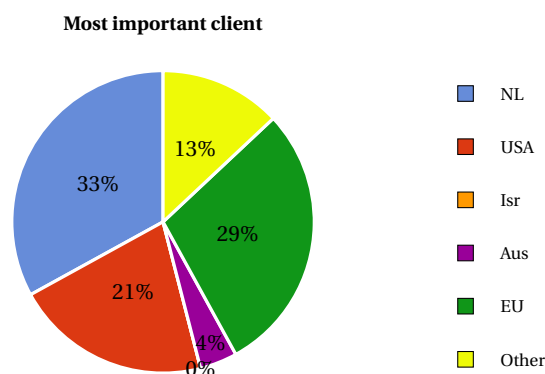
**Figure 3-5:** Economic feasibility: number of companies and research institutes achieving breakeven (RI = Research Institutes, P = Producers, D = Developers and E-U = End-Users).

The expectations about the turnover within the next 5, 10 and 15 years were addressed in question 8. Respondents that are now at breakeven, expected the turnover to be the same or to increase in the coming years.

Respondents that are not yet at breakeven and especially those that are close to breakeven, mentioned various factors for this situation. The main message is that some companies still rely on subsidies, are waiting for a contract with a partner, are waiting for other financial support (e.g. investments), experience delays from regulations or a combination of these. If these issues are resolved this will help them to push their company towards the breakeven point. Other companies that have begun doing research, cannot reach a breakeven yet. The majority of all respondents, especially the micro-algae producers and technology developers, expect a significant growth in turnover by a factor 2-5 towards a million€/year business within the next 5 years. Furthermore a positive development for the coming 5-10 and 10-15 years is anticipated by several micro-algae production and technology development companies ranging from steady growth (30-50% per year) to exponential growth to an annual turnover of several 10 millions€/year up to several 100 millions€/year.

## Collaborations and clients

Question 9 addressed cooperation with other organisations (nationally and/or internationally). The vast majority of the respondents is cooperating with other organisations both on the national and international level, in areas such as R&D and commercial partnerships. Question 10 asked in which country the most important clients are situated for all sectors. The results are presented in Figure 3-6.



**Figure 3-6:** Which country situates the most important clients of the respondents

The Netherlands and the rest of Europe together with the US stood out as major clients. More than 60% of the clients are located in the EU including the Netherlands. The main European client countries outside the Netherlands are located in the South: Greece, Spain and Italy. Especially clients of Dutch algae companies producing for aquaculture can be found in Southern Europe.

## Competitive resources and companies

Question 11 aimed to identify competitors of the companies and research institutes. Most of the answers pointed at alternative, competing resources such as yeast, bacteria, fish oil and plant based oil, plant and animal proteins (soya), heterotrophic micro-algae and large companies outside of the Netherlands e.g. companies producing synthetic product alternatives, algae producing companies and hatchery companies. Examples of some alternative resources are: *Blakeslea trispora*, a fungus, has become an important alternative resource of natural  $\beta$ -carotene competing with the algal product and for astaxanthin, small quantities are produced from *Xanthophyllomyces dendrorhous/Phaffia rhodozyma*, a yeast and from *Euphausia spp.*, krill [26]. Some companies indicated that they do not experience competition.

## Swot analysis

The last question within the theme "company" addressed Strengths, Weaknesses, Opportunities and Threats (SWOT) perceived by the respondents. A summary of the results is presented and discussed below for the four sectors: research institutes, producers, developers and end-users. For all SWOT analyses it must be said that some reflect the view of a small number of companies or in some cases of just one company. Nevertheless, the SWOT analyses present an interesting overview of relevant issues in the field.

The trend in health food is also described in Vigani et al., (2015). The consumer's preferences for natural and healthy products is constantly increasing [5]. Furthermore micro-algae have a potential yield of 40-75 tonnes per hectare compared to 3.7 tonnes per hectare for soy [36, 100].

The regulations will be discussed further in Section 3-5. The role of private equities is addressed in Section 3-9. Large companies have more opportunities in the micro-algae market because more resources are available to them. Thus, they can evolve quicker and also hold off smaller companies. Companies/start-ups that fail can cause problems, because their business case is not working, creating disinterest in their clients which has a negative impact on companies that can and do produce micro-algae. Most of the export of micro-algal companies producing for aquaculture is to Greece and these days Greece is facing a financial crisis.



Strengths	Weaknesses
<ul style="list-style-type: none"> <li>-Micro-algae are capable of high production on a small area non-competitive with agriculture</li> <li>-large network</li> <li>-knowledge of the whole value chain</li> <li>-potential for cultivation in Dutch greenhouses</li> <li>-facilities available</li> </ul>	<ul style="list-style-type: none"> <li>-insufficient proof additional value for animal feed</li> <li>-managing of the whole chain</li> <li>-competitiveness in metabolic engineering</li> <li>-yields are still low</li> </ul>
Opportunities	Threats
<ul style="list-style-type: none"> <li>-worldwide shortage of biomass for food, feed and fuel</li> <li>-growing trend in healthy food</li> <li>-cooperation</li> <li>-sharing of knowledge</li> </ul>	<ul style="list-style-type: none"> <li>-complying to regulations is time consuming</li> <li>-reduction of subsidies</li> <li>-if business case is for a niche product, uncertainty what happens if micro-algae production is growing rapidly</li> <li>-innovation and regulation arrest each other in development</li> </ul>

Table 3-1: SWOT analysis Research Institutes

Strengths	Weaknesses
<ul style="list-style-type: none"> <li>-high quality</li> <li>-the surplus of CO<sub>2</sub> can be used for production</li> <li>-knowledge</li> <li>-good price-quality relationship</li> </ul>	<ul style="list-style-type: none"> <li>-sensitivity cost-price due to niche market</li> <li>-a lot of investments needed (especially CAPEX<sup>a</sup>)</li> <li>-export mostly outside the Netherlands due to regulations and lack of domestic market</li> </ul>
Opportunities	Threats
<ul style="list-style-type: none"> <li>-growing trend in healthy foods</li> <li>-many years of knowledge</li> <li>-usage of CO<sub>2</sub> in a positive manner</li> <li>-the added value of micro-algae for food and feed</li> <li>-sustainability</li> <li>-good business models</li> <li>-worldwide growth in aquaculture</li> </ul>	<ul style="list-style-type: none"> <li>-regulations</li> <li>-if CO<sub>2</sub> is not an issue anymore</li> <li>-same product, but other competing resource(s)</li> <li>-big companies</li> <li>-companies/start-ups that fail</li> <li>-few private equities in the Netherlands</li> <li>-crisis in Greece (aquaculture)</li> </ul>

a

CAPEX stands for CAPital EXpenditure

Table 3-2: SWOT analysis Producers

Strengths	Weaknesses
<ul style="list-style-type: none"> <li>-(improvement) technology</li> <li>-strong network</li> </ul>	<ul style="list-style-type: none"> <li>-small production volume</li> <li>-cost-price</li> <li>-state of the algae industry (technologically not possible yet)</li> </ul>
Opportunities	Threats
<ul style="list-style-type: none"> <li>-algae network</li> <li>-market and product development and growing demand</li> <li>-demand for technology</li> <li>-sustainability</li> </ul>	<ul style="list-style-type: none"> <li>-time and finances (needed for development)</li> <li>-lack of market development</li> <li>-rise of alternative resources</li> </ul>

Table 3-3: SWOT analysis Developers

Strengths	Weaknesses
-good score sustainability -ingredients produced by micro-algae - large area of distribution of fry <sup>a</sup> (aquaculture)	-price -lacking proof of functionality -no supply (aquaculture), need to produce it by themselves
Opportunities	Threats
-innovation	-consumer acceptance is still lacking - Other resources

<sup>a</sup>

Fry = 'broed' (translated in Dutch)

**Table 3-4:** SWOT analysis End Users

Alternative resources such as synthetics and alternative biotechnological products can be a threat if they reach the market earlier with the same kind of products that micro-algae are able to produce. The technology standard in the Netherlands is good, but some weaknesses hold off fast growth of the micro-algae market. In Section 3-3 (question 16 and 18), important technological and non-technological obstacles and R&D topics are identified and discussed.

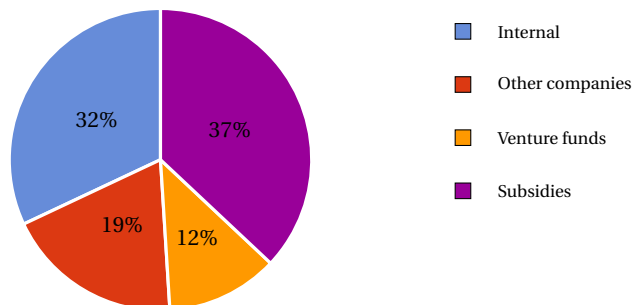
Many aquaculture companies have their own supply in micro-algae. In order to breed fish or shellfish, live micro-algae are needed in the nursery/hatchery stage. External micro-algae companies can fulfil their demand for live micro-algae needed for the nursery/hatchery, however, this has not yet been organised in most cases.

## 3-2 Theme: Investments

Question 13 addressed if future investments were needed and if yes, from whom, for what purpose and to what amount. In general most of the companies and research institutes need future investments. These include investments for improvement of technologies, research, marketing, a (new) production plant, management and business development and market introduction activities. The required investments ranged from several kEuro's to several millions Euro's. Parties mentioned by the companies as potential investors were: the government (subsidies), external businesses and investment funds.

Question 14 addressed the question which parties invested in their business case. For the results see Figure 3-7. The majority of the interviewed companies received investments via subsidies. Almost equal in size are investments from the companies themselves (internally). Investments from other companies and venture funds are much smaller. Respondents indicated that subsidies are important for R&D and in the early phases of a company.

**Who invested in the business case**



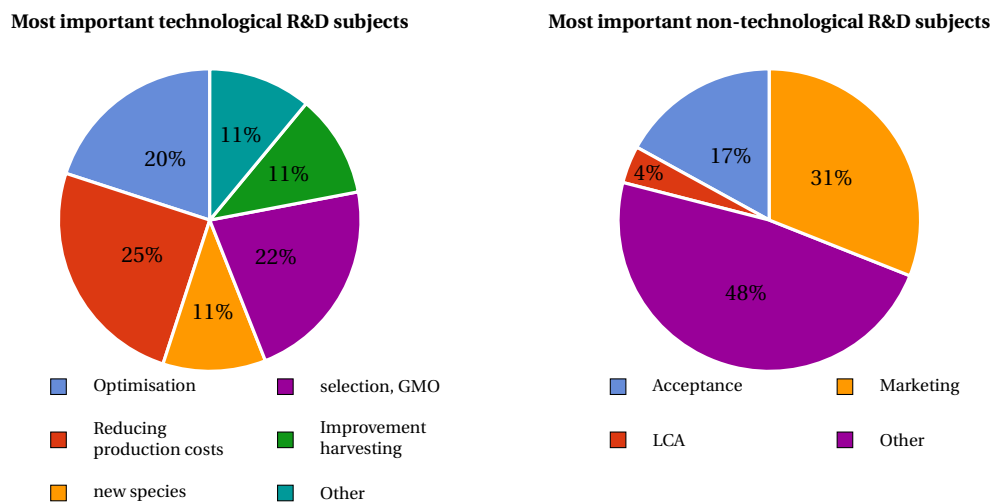
**Figure 3-7:** Internal and external investments in the current business case(s)

### 3-3 Theme: General market

Question 15, 16, 17, 18 and 19 addressed the general market for micro-algae products and technology in the Netherlands. In response to question 15, addressing the type of companies are needed to enhance the market size of the micro-algae sector. Most companies and research institutes answered that first small and medium sized enterprises (SME) were needed and after that the bigger companies are needed (Appendix D). SMEs are more flexible and it is easier for them to innovate. Bigger companies are needed subsequently to enhance the scale of operations.

#### Important technological and non-technological R&D subjects

The most important technological and non-technological R&D subjects for the coming years were addressed in question 16 and 17. Results are presented in Figure 3-8. The most important technological R&D subjects mentioned by the respondents are: Reduction of production costs, optimisation of cultivation systems and selection of new species/GMO. For most of the companies and research institutes these were the most important in order to accelerate the market development for micro-algae in the Netherlands (left side Figure 3-8). Marketing was considered the most important non technological R&D subject by most of the companies and research institutes, followed by consumer/market acceptance and LCA. A significant number of companies and research institutes answered with 'other' including logistics, licenses (aquaculture), finances, commercialization, chain connection, business development and legislation (right side Figure 3-8).



**Figure 3-8:** Most important technological and non-technological R&D subjects for further development of the micro-algae market in the Netherlands.

These results can be compared with the study by Enzing et al. (2014), in which the view of experts on the importance of technological and non-technological factors affecting R&D and competitiveness was analysed. Results are shown in the diagram depicted in Figure 3-9. This study focused on the European market, consulted experts (companies) all over the world and interviewed a large number of experts (214) [1]. In this study and by the study of Enzing et al. (2014) (Figure 3-8 and Figure 3-9) it can be seen that reduction of production costs is the most important factor. Interesting is the fourth factor mentioned by Enzing et al., (2014) i.e. access to venture capital. Some companies interviewed in this study encounter a similar problem. More information about this specific factor is presented in Section 3-9.

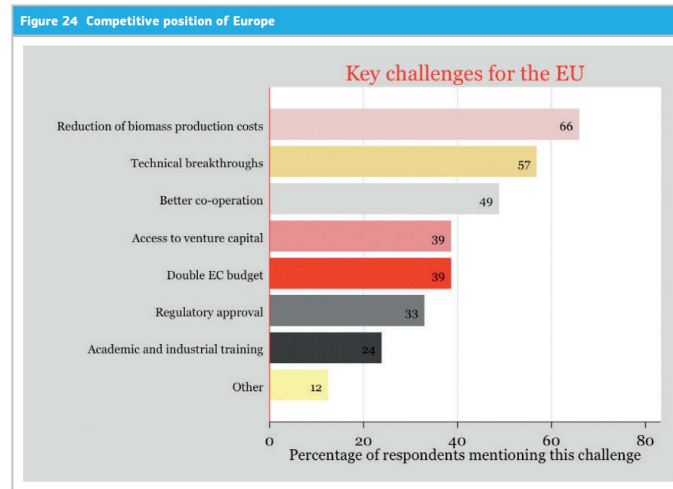


Figure 3-9: Factors affecting R&D and competitiveness in the EU [1]

## Reduction of production costs

Question 18 asked what the best approaches would be to reduce the production costs of micro-algae. The results can be found in Figure 3-10. Important to mention here is that some respondents chose multiple answers.

The most important approaches (> 15%) are: energy, systems and growth improvement (Figure 3-10) with energy, reduction of energy use, as the highest in order to reduce the production costs. This is followed by systems (including the equipment needed for the cultivation systems) and growth improvement (including the methods to achieve this during cultivation). The category 'others' included reduction of operational expenditures (OPEX), bio-refinery and scalability.

Important approaches for the reduction of production costs

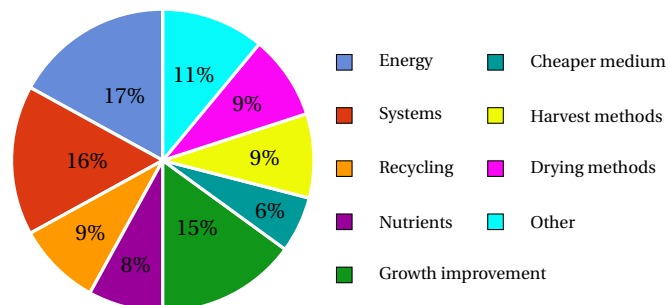
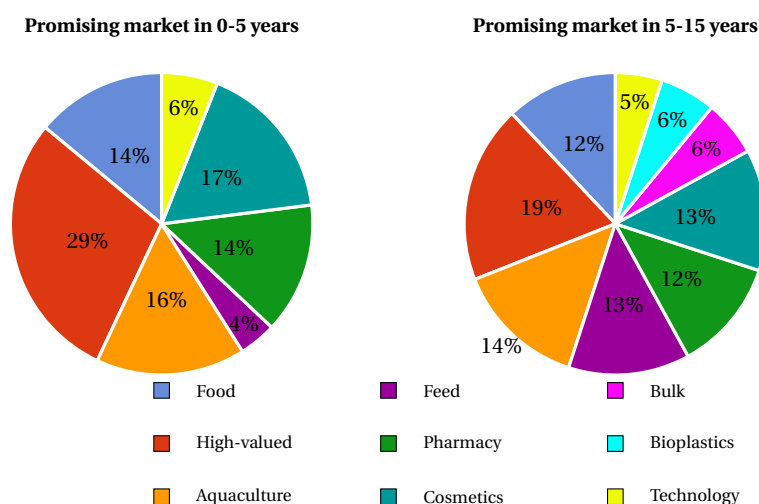


Figure 3-10: Important approaches for reduction of production costs. Energy, Systems and Growth improvement are the most important.

## Promising micro-algae market segments for the Netherlands

Question 19 addressed the promising segments in the micro-algae market in the Netherlands in 0-5 years and in 5-15 years respectively (Figure 3-11). The most promising market segment according to companies and research institutes in the coming 0-5 years is the market for high-value products (including additives, pigments, vitamins, fatty acids, antimicrobials and antioxidants). High value products

are processed products for all kinds of high value applications (including food, feed, aquaculture, pharmacy, cosmetics and bioplastics). They can be sold for a high price per kg to compensate for high production costs. The promising markets in the coming 5-15 years are more equally distributed. Still high value products have the most potential, followed by applications in aquaculture, feed, food, pharmacy and cosmetics. Bulk production (including biofuels) and bioplastics stay pretty low, these are seen as the least promising market for micro-algae in the Netherlands even in 15 years. Development and sales of technology was also identified as a promising market segment.



**Figure 3-11:** Promising market segments for micro-algae in the Netherlands in 0-5 years and in 5-15 years

In the National Strategic Plan Aquaculture (NSPA) (2014) the market perspective for the Dutch aquaculture sector is described. The current share of the Dutch aquaculture sector in the global production is very small and can only provide a limited contribution to the increasing global demand of seafood. The products currently cultivated in the Netherlands are bought by clients in surrounding countries. Chances for the Dutch aquaculture sector exist especially for exclusive regional products, sustainably cultivated for specific markets. Furthermore, an increasing demand will arise for specific knowledge in the area of system innovations, cultivation systems, nourishment, breeding farms, vaccines, logistics and cultivation technologies. In the Netherlands a high level of knowledge is present in companies and research institutes [67]. Micro-algae are essential in the early growth stages as feed in hatcheries and nurseries for cultivation of juvenile fish and shellfish.

### 3-4 Theme: Location

The questions in this theme addressed the feasibility of commercial production of micro-algae in the Netherlands (question 20) and at which location(s) this would be most suitable (question 21).

#### Commercial production in the Netherlands

Most of the companies and research institutes have a positive view towards the feasibility of commercial production in the Netherlands. Some arguments were: good infrastructure, surplus of CO<sub>2</sub>, high quality and technology standards and the Netherlands is one of the leading countries regarding agricultural technologies and horticulture. Most respondents have a positive view towards the feasibility of commercial production in the Netherlands. Arguments mentioned by respondents who do not have a

positive view towards the feasibility of commercial production in the Netherlands included: the Netherlands is small, has high labour costs and too low sunlight irradiation. These last two arguments were confirmed by a recent scenario study of Ruiz et al., (2015). It indicates that production of microalgae and biorefinery of the algal biomass to final products in the Netherlands has higher costs than at other locations such as Southern Spain. This is due mainly to higher labour costs and to other location specific factors incl. solar irradiance and land costs [101].

### Possible locations in the Netherlands

Suitable locations for micro-algae production in the Netherlands were addressed in question 21 (Figure 3-12). 34% of the respondents indicated greenhouses as a possible location for micro-algae production in the Netherlands. Their motivations are: 1) Due to a large greenhouse area, 2) a substantial part of the current greenhouses is not in use and 3) the high level knowledge in horticulture. The respondents argued that algae produced in greenhouses will be used especially for high value applications. Coastal areas were seen as the second best location, especially for marine micro-algae due to the access to sea water. Under the header 'other', respondents mentioned locations with residual nutrient streams, waste heat and flue gasses that can be used as a resource for micro-algae cultivation.

Suitable locations for micro-algae cultivation in the Netherlands

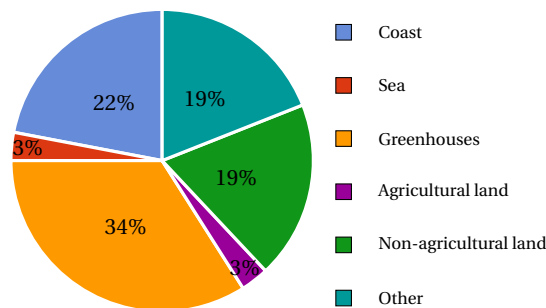


Figure 3-12: Suitable locations for micro-algae cultivation in the Netherlands

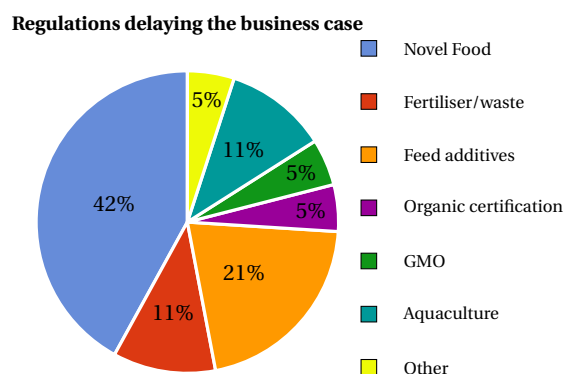
## 3-5 Theme: Regulations

This theme consisted of two questions. One question asked if regulations are delaying the development of their business case and the other question asked if and how the government can stimulate or facilitate the further development of their business case.

### Delay by regulations

Question 22 addressed the role of regulations in delaying development of the business case (Figure 3-13). The most important regulations that are delaying the business case are the Novel Food Regulation and the approval procedure for feed additives by the European Food Safety Association (EFSA).

The Novel Food Regulation is a major obstacle for micro-algae businesses, because today only four micro-algae species are accepted as food [1, 15]. The obstacles regarding approval of feed additives is further explained here: In the Netherlands since 2010 the cultivation of micro-algae as biomass for animal feed was accepted by [het Productschap DierVoeders (PDV) (now Federatie Nederlandse Diervoederketen (FND))]. A Dutch company has spent almost 10 years and ca. 30k€ to obtain approval for the use of micro-algae biomass in animal feed, resulting in a GMP+ certificate for micro-algae cultivation [102]. The firm Kelstein in Hallum, a biological dairy farm, has also acquired a GMP+ certificate



**Figure 3-13:** Regulations that delay some business cases

and more companies have followed. Kelstein cultivates micro-algae in three ponds and four photobioreactors using residual streams from a biogas installation as a nutrient source. The micro-algae are further used by Van Benthem Veevoeders en Kunstmest in algae lick-stones as a feed supplement for horses [56]. Micro-algae biomass can be added as a component in animal feed, but only when a risk analysis is executed on production methods and when an analysis is executed on the final products to ensure that products contain a minimal level of certain compounds and of pathogens [96]. These risk analyses take time and are costly.

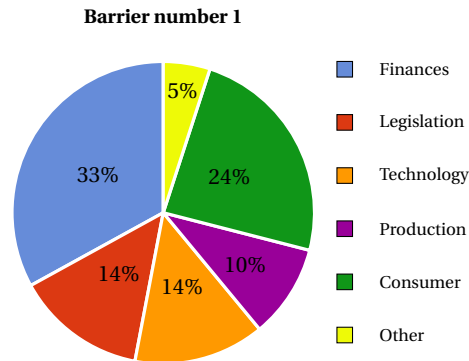
Furthermore, other relevant regulations for the micro-algae sector address organic certification, manure/waste water regulation and aquaculture regulations. The role of the organic certification and the manure/waste water regulation are strongly dependant on each other. An organic certification is only possible when micro-algae would be allowed to be cultivated on manure and sold as food/feed product, but in Section 2-9 it is explained that micro-algae cannot be cultivated on manure/waste water. Nowadays the Netherlands has a 30% surplus of manure [103] that cannot be used in the agriculture sector. Without the delaying legislations this could be an interesting substrate for algae cultivation. Aquaculture is relatively new in the Netherlands. Licenses are required to expand this market. These licenses require administrative time and have high costs [67].

### Stimulation and/or facilitation by the government

In question 23 it was asked how the government could stimulate or facilitate in the company's or research institutes' business case. The respondents gave the following answers: 'by providing subsidies', 'by adapting/changing the regulations' or 'other'. An equal amount of the respondents mentioned 'by providing subsidies' and 'by adapting/changing regulations'. Subsidies are needed in order to reduce risks and for research purposes. Regulations that need to be adapted/changed have been discussed above and in Section 2-9. In the category 'Other', respondents mentioned: integration between governmental bodies, facilitation of infrastructure, reduction of high administrative burdens and use of policies with a global perspective.

## 3-6 Theme: Barriers

In this theme the most important barriers for the further development of the business cases were ranked. Identified barriers include finances, regulations, technology, production, consumers and 'other'. Respondents were allowed to fill in more than one barrier. Figure 3-14 shows the barriers mentioned by the respondents as the most important one(s). The remaining barriers can be found in Appendix D.



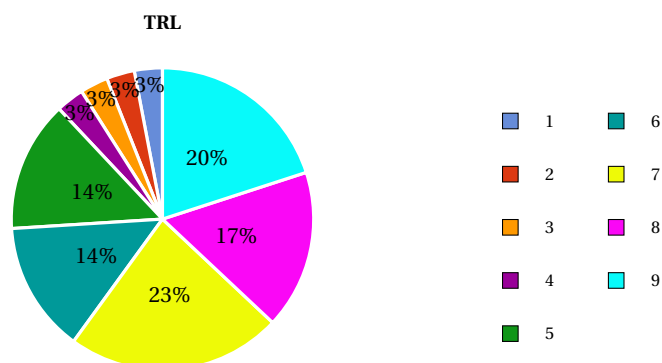
**Figure 3-14:** Number 1 barriers companies face

Examples for every chosen barrier were provided (question 25). Figure 3-14 shows that lack of finance is the most important barrier according to the majority of the respondents. The following purposes were mentioned by the respondents for financial support: To prove their business case via research, to build a demonstration plant, to expand their company, for marketing and to prove their technology/concept. The latter has to do with finances from investment funds. It is difficult to obtain finance from an investment fund without a proof of concept on adequate industrial scale. The existing funds are not investing in start-ups or some that do invest in start-ups invest in a different sector (e.g. ICT) More information is given in Section 3-9. The runner up barrier is formed by the consumers. The main reasons for this barrier are: A need for consumer acceptance, convincing of the client, lack of a demand in micro-algae products and lack of marketing showing the benefits of micro-algae.

### 3-7 Technology Readiness Level

One additional question was asked to establish the technology readiness level (TRL) of the ongoing activities.

In the TRL classification system, companies and research institutes can define their level of technology readiness up to 9 TRL levels (Figure 3-15). Various TRL classification systems exist, the TRL classification system used in this study is used by the European commission [104].



**Figure 3-15:** Technology Readiness Level (TRL) of companies and research institutes

The most common level of technology readiness for the interviewed companies and research institutes is TRL 7. TRL 7 implies: „system prototype demonstration in operational environment”. The second most common TRL for companies and research institutes is TRL 9. This is the most mature level and



implies: „actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies: or in space)”.

### 3-8 Additional information

The company Philips and the research institute TNO were not included in the survey. They were contacted separately to provide relevant information and this section gives a summary of the results.

Philips was approached because of their knowledge in the field of Light Emitting Diode (LED) technologies for horticulture which could be of interest for micro-algae production in greenhouses.

Philips' LED-horticulture business case is developing, selling and installing LEDs in greenhouses and cultivation, without daylight, of vegetables. Some research and small scale projects are ongoing with micro-algae and the interest for micro-algae lies in the high value products. Production in greenhouses with LEDs is for now too expensive in terms of production costs, but in the future for high value products economic feasibility could be achievable. A horticulturist approaches Philips LED-horticulture when LED is desired for their cultivation. Philips expects that this could also happen with cultivators of micro-algae. Philips already worked on some algae projects and they expect more projects to come. Horticulturists already have a lot of experience with optimization of cultivation conditions of their current crops, in air-conditioning, carbon dioxide dosing, water and nutrient provision and water treatment systems [105]. Another interesting observation is that production costs of micro-algae are in the same order of magnitude as the production costs of tomatoes. If the dry weight is 5%, the cost price for cherry tomatoes can be 30€/kg DW and for truss tomatoes 13€/kg DW [105, 106].

TNO was approached because of their mobile bio-refinery unit, VALORIE, that has been developed for the downstream processing of micro-algae to extract high value products from the biomass.

VALORIE stands for Versatile ALgae On-site Raw Ingredient Extractor and is a mobile bio-refinery pilot unit that can process micro-algae to produce multiple algal ingredients. The project was started by TNO, Ingrepro and Agentschap NL and was later continued by TNO, Algae Food & Fuel and RVO (Netherlands Enterprise Agency, [Rijksdienst Voor Ondernemend Nederland]). VALORIE is designed by the above mentioned partners and used by a consortium called GAIA (Getting Algae Ingredients Applied) which consists of several companies.

The scale of VALORIE is suited for the current production capacity of micro-algae in the Netherlands and is able to process 5-15 kg DW/h. The pilot facility consists of: Storage container, bead mill (for cell disruption), 3 phase centrifuge (solid phase, dissolved phase and sometimes an oil phase), solvent extractor and membrane separations such as micro- and ultrafiltration. The mobile unit has been built for use on location at micro-algae production companies to test the processing of the produced micro-algae for the long or short term.

### 3-9 Investment funds

For this study it is important to establish what the driver of investment funds is to invest, if they are interested in investing in micro-algae companies, and what are the conditions for investing. One of the conclusions of the questionnaire earlier in this study was that some companies are waiting for an investment fund to invest in their demonstration plant. This plant has to demonstrate the potential of their micro-algae business case. However, in most of the cases, investments are only granted when the technology is proven, which implies that a demonstration plant should already exist. Investment funds received questions by e-mail, by telephone or in face to face meetings. In general, questions were asked from the questionnaire (Appendix C), but in some cases questions varied. 5 investment funds were interviewed and various differences between them were established. These funds differ in when they invest (size of the company) and in what they invest (what kind of companies). The most important

question for the investment funds addressed their market perspectives/interests and their willingness to invest in micro-algae technology.

- Investment fund I sees obstacles in start-ups and is actively searching for partners, including partners with micro-algae activities, for the creation of a strategic partnership (most of the time with several branches). Investment fund I sees an opportunity for micro-algae in for example cleaning of the underwater infrastructure. They are willing to invest and their typical investment size ranges from 0,5-5 million€. Of course the companies they invest in must meet their requirements and terms/conditions.
- Investment fund II has several themes and one of them is sustainability. Micro-algae companies have appeared on their radar, but they saw that those companies were still in their 'infancy'. There is of course an interest in micro-algae, but they are only going to invest if there is a proven technology. This means that a demonstration plant should already exist with positive test results and a proven business case. Investment fund II could then invest in a second installation. This shows that the decision to invest depends on the status of the technology (infancy vs. proven technology) and the presence of a production facility on at least industrial scale. Investment fund II manages 4 billion€, therefore they only invest significant amounts, starting from 10 million€.
- Investment fund III is seeing big potential in micro-algae (also outside the Netherlands) with a concrete market perspective for fish feed, cosmetics and nutraceuticals. There is a high demand for micro-algae products, but in competition with synthetic products. There is a willingness to invest in micro-algae and some research has been done on several products from micro-algae companies, a few with attractive products. Fund III did not reveal whether they have invested or are investing in a micro-algae company.
- Investment fund IV is not a typical investment fund. It is a business incubator that focusses on early stage companies. Their view towards market perspectives for micro-algae is determined by societal trends: the growth of the world population (9.6 billion by 2050) ([107]) and the demand for more sustainable food production systems. Interesting markets for investment fund IV include high value products, proteins and fatty acids from micro-algae. Not bio-diesel or other low value applications. Investment fund IV expects that the Netherlands can have a strong market position, but this largely depends on the contribution of larger companies. A small company is often bought by larger companies when it is successful. Investment fund IV already invests in a micro-algae company, so there is no room for a second. In general, reasons for not investing in a start-up are: something is missing in the business plan or the company is not willing to contribute enough in sharing the risks (personal commitments). A problem in the Netherlands is that the investments are not available for every companies' development phase. Most investment funds do not invest in start-ups (e.g. Investment fund II). Funds are usually available for the research stage (mostly through subsidies), but for demonstration on industrially relevant scale, a venture is needed. However, venture funds only invest when the pilot/demonstration plant has proved to be successful. A catch-22 situation which creates a funding gap (the so called valley of death). A solution would be that the government can provide a loan for the realisation of the proof of concept of the starting companies in which the entrepreneur will warrant 20%. In this way an entrepreneurial climate is created which makes it possible to keep the research phase plus the early development in the Netherlands. The rest will be picked up by the market, which is international. Another remark is that although research and development continue (mostly possible due to subsidies), too few results are actually reaching the market.
- Investment fund V is not a real investor, like the ones described above, but it provides loans. There is a worldwide market of micro-algae with a good perspective for the Netherlands, due to its good position in agriculture. The opportunity lies in the high value products and there is a possibility for a strong market position in the Netherlands for micro-algae technologies and products, but cooperation between agriculture, horticulture and biotechnology is needed together with the availability of funding for demonstration plants/projects. Investment fund V is willing to

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provide loans to micro-algae companies, but it depends on the quality of the entrepreneur and the company's business plan.



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# Chapter 4

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## Conclusions

### 4-1 Current worldwide status and trends of micro-algae technologies, production and applications focusing on food, feed and aquaculture

- The total global micro-algae biomass production is ca. 10,000 tons of dry matter/year and commercially applied mainly in health food supplements, aquaculture, cosmetics and pharmaceuticals. Global production is increasing. Most production takes place in Asia, the USA and Australia while production in Europe is growing.
- The most commonly used micro-algae strains are *Spirulina* and *Chlorella* that are mainly used in health food supplements with a production volume of 5000 and 2000 tons of dry matter/year respectively. *Spirulina* is commercially cultivated particularly in Asia and the USA. *Chlorella* is produced by more than 70 companies worldwide. In addition, a limited number of other micro-algae strains are cultivated commercially for health food ingredients e.g. *Dunaliella* and *Haematococcus* and for aquaculture for which ca. 15 strains are commercially cultivated.
- Several types of open and closed cultivation systems and harvesting systems are in commercial use worldwide. Most of the current commercial production is performed in open high rate algal ponds in which only a limited number of strains can be reliably cultivated. The application of closed PBRs, in particular tubular and flat panel reactors, is growing, enabling controlled cultivation of specific micro-algae strains and higher value applications.
- With regard to the current scale of the production technology, the largest commercial cultivation system comprises 400 ha using simple unmixed open pond systems for cultivation of *Dunaliella* in Australia. This is followed by the system operated in California, USA, for cultivation of *Spirulina* which stretches over an area of 44 ha using high rate algal ponds. The current commercial tubular PBRs (e.g. for production of astaxanthin from *Haematococcus*) have a surface area up to several hectares per system. Worldwide, systems are being scaled up to enhance production and reduce costs.
- The major application of microalgae in the food sector is as health food supplements in the form of whole, spray dried or freeze dried algal biomass. In addition pigments especially carotenoids (*beta*-carotene, astaxanthin) and long chain polyunsaturated fatty acids are constituents of micro-algae that are used in health food supplements. The global turnover of micro-algal biomass for human health food supplements is approximately US\$ 1.25 billion per year.

- $\beta$ -carotene from *Dunaliella salina* (applied in health food supplements and food colorants), astaxanthin from *Haematococcus pluvialis* (applied as antioxidant and as food colorant for salmon) and phycocyanin from *Spirulina* (applied as fluorescent marker and food colorant) are commercially established micro-algal high value products. Furthermore, there is significant production worldwide of omega-3 and omega-6 PUFAs from heterotrophic algae cultivation
- The use of micro-algae or derived fractions as a feed component offers an interesting perspective, because of the high protein content and the suitable amino acid profile. Furthermore, research indicates that the addition of micro-algae to feed rations has positive effects on animal health and productivity for cattle, poultry, pigs and other farm animals, pets and ornamental birds.
- Micro-algae can be used as aquaculture feed in two ways: Direct use of fresh algae in hatcheries and nurseries (mostly produced on-site at the aquaculture company) or as a component in compound aqua feed as a source of oils and proteins. The application of micro-algae in aqua feeds is under active development, e.g. for salmon production, and is expected to grow significantly in the coming years. The current market volume for this application (including feed) is estimated at ca. 30% of total micro-algae produced.
- The world population is growing and there is a growing demand for nutritional and health promoting products which offers opportunities for micro-algae due to their positive effects on health. Worldwide growth is seen particularly in higher value products for health, nutrition and aquaculture. In the longer run, as production costs decrease, lower value applications such as animal feeds, proteins, and other commodities (incl. biofuels) are expected to enter the market. Worldwide there is growing attention for algae processing including the development of biorefinery concepts to manufacture multiple co-products in order to enhance revenues.
- The two main European regulations governing the application of micro-algae in food are the Novel Food Regulation and the General Food Law. Biosafety evaluation is performed in Europe by the European Food Safety Authority (EFSA) and in the USA by the Food and Drug Administration (FDA).
- The development and use of Genetically Modified (GM) algae is expected to improve algal performance significantly. However, research on GM algae is still in an early stage in Europe in part due to strict regulations. This is perceived as an obstacle which may be holding off diversification and economic competitiveness

## 4-2 Comparison of the current status of the micro-algae sector in the Netherlands with the current worldwide status

- The study shows that the micro-algae sector in the Netherlands is still limited in size but well established with positive perspectives for growth. Parties active in the Dutch micro-algae sector include: R&D institutes, commercial algae producers, technology developers and (potential) end-users of algae products. These stakeholders plus several investment funds were all represented in the interviews conducted in the framework of this study to enable a representative view towards the current status and perspectives.
- Cultivation of microalgae is technically feasible in the Dutch climate conditions, however higher productivity per hectare may in general be expected in regions with higher solar irradiance and temperature e.g. in Southern Europe. Applications in which higher productivities are needed (e.g. bulk production) are probably less suitable for production in the Netherlands.
- Production costs in the Netherlands are relatively high compared to other locations due to esp. higher labour and land/infrastructure costs. These higher costs can be compensated by a focus on high quality products and applications as is the case for several Dutch micro-algae companies.

- The current production systems in the Netherlands are significantly smaller than the systems used worldwide. The biggest (known) commercial cultivation area in the Netherlands is 1.3 ha, composed of an open system placed in a greenhouse. The limited production scale may be due to the earlier development phase of the sector in the Netherlands and the focus on high value applications.
- Regarding technology there is an emphasis in the Netherlands on development and use of advanced PhotoBioReactor systems in R&D, algae production and technology development. Furthermore there is significant innovation in harvesting and processing technology.
- The major commercial activities and business case(s) in the Dutch algae sector include sales of algae and derived products for food, feed, aquaculture and cosmetics. Furthermore there is significant activity in the area of development and sales of cultivation and harvesting technology, in addition to services for processing and product development. Several (potential) end-users in the food and feed sector are involved in development and sales of products among others in ongoing R&D projects.
- Dutch R&D institutes are active in development and optimization of cost-effective and sustainable micro-algae production methods, development of knowledge and data for scale-up, and development of harvesting and processing technologies. Furthermore R&D focuses on innovations in aquaculture and the greenhouse horticulture sector.
- The present financial turnover of the algae producers depends on their specific business case(s); most producers have a turnover of 100-500 k€/year. The turnover is highest in research institutes and technology developers, up to 1 million €/year. Overall, in the present stage, a large number of Dutch micro-algae companies are relatively small size businesses with a limited turnover (< 500 k€ per company per year).
- Regarding economic feasibility, the majority of the interviewed Dutch algae producers and about half of the technology developers achieve at least break even. Overall, about half of respondents in all sectors are achieving break even, while some respondents indicate to be close to the break even point.
- The majority of the respondents cooperate with other organisations on the national and international level, in R&D and commercial partnerships. Major clients are located in the Netherlands, the rest of Europe and the USA. More than 60% of the clients are located in the EU (including the Netherlands). The main European client countries outside the Netherlands are Greece, Spain and Italy. Especially clients of Dutch algae companies producing for aquaculture can be found in Southern Europe.
- Investments in the sector to date derive mostly from subsidies, internal funding and investments by external companies or venture funds. Government subsidies are important for R&D and the early phases of commercialization. Most companies indicated that more investments are needed (ranging from several 100's k€ to several millions) for improvement of technologies, research, marketing, a (new) production plant, management and business development and market introduction activities.
- The majority of the interviewed companies have a positive expectation of the feasibility and further development of the micro-algae sector in the Netherlands because of the availability of good infrastructure, high quality and technology standards and the top ranking of the Netherlands in agriculture and horticulture. The sector as a whole is growing, which follows the worldwide trend, which is caused by an increasing demand for food supplements, nutrition and feed (incl. aquaculture) products.
- The majority of the respondents -especially the algae producers and technology developers- expect a significant growth in turnover by a factor 2-5 towards a millions €/year business within the next 5 years. Furthermore a positive development for the coming 5-10 and 10-15 years is

anticipated by several micro-algae production and technology development companies ranging from steady growth (30-50% per year) to exponential growth to an annual turnover of several 10 millions €/year up to several 100 millions €/year.

- A number of algae producers and developers worldwide incl. the Netherlands are involved in projects combining algae production with nutrient (N,P) recovery / water purification from agro-industrial or other effluents. Water purification as business case was excluded from this study. However, the use of effluents as a source of nutrients for algae cultivation can lead to cost reduction and could thus have significant potential for specific application areas. This will require adaptation of regulations for use of residues for micro-algae cultivation.

### **4-3 Business opportunities for the food, feed and aquaculture sectors in the Netherlands, identification of R&D topics, obstacles and barriers for development**

- Significant growth is expected by producers and developers in production and sales of high quality algae and derived products for food, feed, aquaculture and other market sectors within 0-5 years and 5-15 years respectively. The main identified opportunities for the micro-algae sector in the Netherlands are:
  - High value algae products (including pigments, vitamins, fatty acids, antimicrobials and antioxidants) for applications in various categories have the largest potential within the next 5 years. Also in the longer term (5-15 years) high value products are perceived as most promising followed by production of algae for aquaculture, feed, food, pharmacy and cosmetics.
  - Algae based health food supplements and food ingredients offer an interesting potential due to a growing demand for healthy nutrition and the specific opportunities offered by algae.
  - Micro-algae have significant potential for aquaculture as fresh feed for hatcheries and nurseries and as component in compound aquafeed, due to the added value of micro-algae for fish and shellfish cultivation as a source of colourants, fatty acids and proteins. Both routes and especially the production of high quality algae for aquafeed could be an interesting opportunity for the Dutch micro-algae sector.
  - Use of micro-algae as a nutritional additive in animal feeds (other than aquaculture) has significant potential. Further R&D is required to document the added value of micro-algae for promotion of animal health and productivity
- Growth is also expected for the development and sales of technologies, know-how and services in the area of cultivation, harvesting and processing developed by the Dutch R&D sector and commercial technology developers. New concepts and technologies could be developed up to semi-commercial scale in the Netherlands followed by worldwide roll-out.
- For the further development of the algae sector the role of SME's is considered vital due to their high flexibility and innovation skills. Larger companies could 'jump in' in a later stage to enhance the scale of operations.
- The most important identified technological R&D subjects to accelerate the development of the micro-algae sector in the Netherlands are cost reduction of algal biomass production, optimisation of cultivation systems, selection of new production strains and Genetic Modification. In order to reduce production costs, the major challenges are reduction of energy use, improvement of cultivation systems and growth improvement of micro-algae.



- The major identified non-technological R&D subjects to promote development of the micro-algae market in the Netherlands are marketing (addressing clients, end-users, consumers and market introduction) followed by consumer/market acceptance.
- Greenhouses are seen by the respondents as a suitable location for micro-algae production in the Netherlands because of the large greenhouse area, the numerous empty greenhouses and the high level of horticultural knowledge and infrastructure availability especially for high value applications. Furthermore coastal, non-agricultural land and areas close to infrastructure offering residual streams that can be used for algae cultivation such as nutrients, waste heat and flue gasses offer possibilities.
- Major identified barriers for development and scale-up of the micro-algae sector and market in the Netherlands include lack of financing possibilities, market development (consumer awareness) and obstacles caused by regulations, incl. the Novel Food Regulation and regulations that hinder the use of residues for algae cultivation.



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## Chapter 5

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# Recommendations

In this chapter policy recommendations for the Dutch government to accelerate commercialisation of a micro-algae market in the Netherlands are presented.

### Algae Platform

Many respondents indicate an interest for a platform or meeting place for micro-algae companies, research institutes, government bodies and investment funds. The aim would be to exchange information, to enhance cooperation between parties in the micro-algae value chain and to discuss and improve marketing and regulations. It is recommended to further investigate the creation of such a platform in the Netherlands in consultation with the stakeholders. Membership of the European Algae Biomass Association (EABA) [108] is recommended because EABA gathers stakeholders across the EU and addresses several issues and bottlenecks identified in this study.

### Enhancement of national subsidies

The national subsidy possibilities for micro-algae projects are seen as limited. Access to funding programs (such as the Topconsortia voor Kennis en Innovatie (TKI) top sector Agri & Food) is perceived as difficult because of the specific nature of the technology and the fact that the algae biomass can be used for multiple applications and projects are therefore difficult to place. Subsidies are especially important for R&D and in the early phases of a company e.g. via government (co)funding of Public Private Projects. The creation of a targeted subsidy program is recommended.

### Government loans & investments

The lack of financing possibilities was identified as the most important barrier for commercialization and scale-up. In particular companies experience a funding gap between R&D and commercial scale, especially for demonstration at industrial scale. This phase is necessary to prove the technological concept. No subsidies are available for this phase while venture funds are less active in the biobased economy in general than in other sectors and may invest only after industrial feasibility has been proven. To bridge the gap several governmental instruments are available at the moment. They provide loans rather than subsidies. These programs are executed by RVO, e.g. GO [Garantie Ondernemingsfinanciering] and BMKB [Borgstelling MKB Kredieten] ([109]), and usually involve provision of guarantees (up to 50%) or supplementation of private investments by means of guarantees and direct financing with a repayment obligation, such as loans and equity investments in projects. Furthermore several provinces offer this type of support. It is recommended to review the existing programs and to develop methods to enhance investment opportunities for private investors and governments especially to bridge the gap between R&D and commercial scale.

### Stronger integration with horticulture

Attention for a stronger integration between micro-algae production and horticulture (esp. in greenhouses) is recommended. Several projects are already underway, some companies perform cultivation

in greenhouses. Micro-algae can be considered as an (alternative) crop for horticulture (as well as agriculture) and benefit from the availability in these sectors of good infrastructure, high level expertise and high quality and technology standards. This may help to attract investments. In addition, a relatively new application of micro-algae biomass in biofertilizers and biopesticides is performed/developed by some Dutch companies, which can enhance the integration with the horticulture and agriculture sectors.

#### R&D

Funding of applied and pre-competitive R&D remains an important tool to further develop the technology and the market. It is recommended to further support R&D -e.g. in the form of Public Private Partnerships (PPP) ([110])- to address topics that accelerate the scale up of the micro-algae sector and foster creation of new business opportunities. Several themes for research are identified in this study including the optimisation of cultivation systems and productivity, the reduction of production costs through a decrease of energy use, growth improvement, efficient nutrient use, strain selection and improvement etc. R&D is further recommended into the processing/biorefinery of micro-algae in order to reduce costs and enhance revenues through multiple products. In R&D an integral chain approach should be followed from producer to end-user and consumer. Specific attention is recommended for the translation of R&D results into innovative technology and products.

#### Market exploration

Performance of a detailed market exploration study is recommended to analyse the potential markets for micro-algae products, the acceptance of products by commercial end-users and consumers, public awareness and the information required to inform these stakeholders about added value of micro-algae for human health and the environment as documented by research. Whereas product marketing is a typical task for companies, the government could support studies addressing possible applications and public awareness.

#### Regulations

Several regulations have been identified as barriers for further development and scale-up due to strict contents in combination with time consuming and costly application procedures. In particular this applies to approval of the use of (new) algae strains and products in food and animal feed, regulations addressing the use of residues for algae cultivation, and the rules for obtaining an organic certification. It is recommended to review these regulations (of course without compromising the standards for health and environment) and develop where possible appropriate adaptations in consultation with stakeholders together with the micro-algae and aquaculture sectors. In addition there are strict regulations for GMO in Europe, that are less strict in other parts of the world. In order to compete globally, the regulations for GMO in Europe should be reviewed and possibly harmonized with the global regulations. Finally, aquaculture is relatively new in the Netherlands and in order to let this sector grow, regulations and licences should be adapted.

#### Heterotrophic micro-algae

This study has focused on cultivation of phototrophic micro-algae, using light and mineral nutrients including CO<sub>2</sub>. In the last decades commercial successes have been achieved using heterotrophic (and mixotrophic) micro-algae cultivation in which organic compounds such as sugar are used as a substrate for micro-algal growth instead of or in addition to light. It is recommended to further explore the potential of heterotrophic micro-algae production for the Netherlands. This could be an interesting option in combination with the forthcoming end of the EU sugar quota management system in 2017, which may enhance the availability of sugar at lower costs.

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# Appendix A

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## Participating Companies

Short profiles of the participating companies, research institutes and investment funds are presented below.

**ACRRES** Applied Centre for Renewable RESources (ACRRES) is the national applied research centre for sustainable energy and green raw materials. The applied research centre is a joint effort of the Lelystad research institutes Applied Plant Research and the Animal Sciences Group, both part of Wageningen UR, and ENECO together with the Province of Flevoland and Lelystad (<http://www.acrres.nl/en/home-en/about-acrres>). On the pilot site micro-algae culturing and harvesting techniques are tested for the company Algae Food & Fuel. Digestate or artificial fertilisers and CO<sub>2</sub> from the flue gases of a digester and CHP engine are used as minerals to grow the micro-algae in ponds. The produced algae biomass is at present used for high value feed applications, feed additives [96].

**Algae Food&Fuel (AF&F)** Algae Food & Fuel is a joint venture of the firms BioSoil and Tendris Solutions. AF&F designs, builds, sells and installs systems for the industrial and agricultural production of algae: algae farming. Their unique scalable systems are highly productive, improve the stability of algae based processes, are cost efficient and local resources (waste streams) are used for algae growth (<http://www.algaefoodfuel.com/home/>).

**Algaebiotech** Started in 2008, algaebiotech provides services for product development, contract processing and engineering of micro-algae (<http://www.algaebiotech.nl/>).

**Omega Green** Omega Green has developed a unique growth system in which the economy of scale is in reach. Its ambition is to play an important role on the European market for algae growth systems and is building an algae growth system (demo-unit) to demonstrate their efficient algae growth systems whilst producing sustainable and healthy ingredients for feed and food applications ([http://omegagreen.nl/?page\\_id=126](http://omegagreen.nl/?page_id=126)).

**AlgaeLink** AlgaeLink started in 2007 with a prime objective of offering a robust, highly effective photobioreactor for the cultivation of algae for high valued commodities, food, feed and fuels (<http://algaelink.nl/joomla/>).

**AlgaePARC** AlgaePARC develops cost-effective and sustainable micro-algae production methods outdoors. Furthermore it is doing assessments on economics and sustainability of the entire chain for defining the research program which integrates the biological and the engineering aspect of cultivation and bio-refinery (<http://www.wageningenur.nl/en/Expertise-Services/Facilities/AlgaePARC.htm>).

**AlgaSpring** AlgaSpring is the European number 1 producer of the marine microalgae *Nannochloropsis gaditana* and has a 1.3 hectare photobioreactor system located in Almere. For various markets AlgaSpring cooperates with specialized companies in feed, food and personal care for the development of products and sales ([http://www.feedfromwater.nl/?page\\_id=37](http://www.feedfromwater.nl/?page_id=37)).

**Aquaspark** Aquaspark is a global investment fund that provides investments in sustainable aquaculture businesses and invests in small to medium enterprises (<http://www.aqua-spark.nl/>).

**CleanAlgae** CleanAlgae focuses on the growth and harvesting of various micro-algae strains used in a number of aquaculture products offered by the company. Development and algae solutions are offered for cosmetics, health and the food markets as well as solutions for the treatment of municipal and industrial waste water using micro-algae (<http://www.feyecon.com/business-ventures/cleanalgae>).

**DSM** Royal DSM is a global science-based company active in health, nutrition and materials. By connecting its unique competences in Life Sciences and Materials Sciences DSM is driving economic prosperity, environmental progress and social advances to create sustainable value for all stakeholders simultaneously. DSM delivers innovative solutions that nourish, protect and improve performance in global markets such as food and dietary supplements, personal care, feed, medical devices, automotive, paints, electrical and electronics, life protection, alternative energy and bio-based materials (<http://www.dsm.com/corporate/about/our-company.html>).

**Duplaco** Duplaco B.V. is a privately held enterprise that was established in October 2012. By using the natural power of plankton in their technologies, Duplaco makes building blocks and products for animal nutrition & health, food & beverages and pharma & cosmetics (<http://duplaco.com/>).

**Evodos** Evodos is the only Algae harvesting solution where all valuable components (ingredients) inside the Algae cells are fully retained. The Evodos Dynamic Settlers, with the unique Spiral Plate Technology, outperform traditional separation/harvesting equipment. The separation efficiency is over 95%, and the dry weight of the output Algae paste is 1.5 - 2 times better compared to traditional centrifuges. Even more important, the Algae paste is of excellent quality, all algae cells are harvested intact and undamaged (<http://www.evodos.eu/index.php/applications/harvesting-algae.html>).

**Fry Marine** The focus of Fry Marine is to increase the quality of juvenile marine fish in hatcheries worldwide. The key for this is believed to be using more natural high quality algae and marine live feeds. Their mission is to provide high quality copepods to fish hatcheries, research institutions and other clients. Therefore a key ingredient for success in larval rearing of (marine) fish species is provided (<http://www.frymarine.nl/?q=content/about-us>).

**Holland Food Ventures** Holland food ventures (HFV) invests in entrepreneurs and companies in the food industry that want to bring new and innovative technologies/products to the markets (<http://www.hfv-bv.nl/uk/index.html>).

**LGem** LGem is the first Dutch company to use closed photobioreactors to produce microalgae on a commercial scale. Since 2007 freeze-dried algae powder has been produced and sold all over the world as a food supplement. The innovative cultivation process has been steadily improved, resulting in a stable, robust and easy-to-operate production platform. In 2009, GF Piping Systems joined the development team, introducing new components. As a result of this fruitful collaboration, the tubular PBR has evolved into a cleverly designed system, with low operating costs (<http://www.lgem.nl/>).

**Nutress** Nutress was founded in 2012 and grown into Europe's biggest and most innovative algae food company. Nutress manufactures and markets nutritional added value applications for feed, food and pharmaceuticals (<http://www.nutress.eu/>).

**Philips** Philips has been developing ways to apply lighting technology to crop farming for over 75 years. With their cutting-edge LED innovations at their command, they can custom-build a science based solution. Tailor-made light recipes mean faster growth, bigger harvests and higher quality plants and growth systems, while LEDs for sustainable crop cultivation create optimal conditions all year round (<http://www.lighting.philips.com/main/products/horticulture/>).

**Photanol** Photanol developed a breakthrough technology to convert CO<sub>2</sub> into valuable organic compounds. The company is applying this technology in a number of markets, ranging from food ingredients to chemical bio-blocks and bio-fuels ([http://photanol.com/?page\\_id=1922](http://photanol.com/?page_id=1922)). In the photanol approach synthetic biology, photosynthesis and fermentation metabolism are combined. Components are directly formed from Calvin cycle intermediates through the use of the naturally transformable cyanobacterium *Synechocystis sp.* PCC 6803 [111].

**Prodock** Prodock is an initiative of the port of Amsterdam [red. Havenbedrijf Amsterdam N.V.] and is a physical location and platform in the port of Amsterdam. The key is innovation and fast growing and innovative companies have the possibility to test and develop their new products and services commercially in a professional surrounding (translated of <http://www.prodock.nl/>).

**Stichting Zeeschelp** Stichting Zeeschelp is focused on stimulations of innovations in the (marine) aquaculture. The important objectives are execution of their own research and to provide facilities in the pilot station of the marine aquaculture (translated of <http://www.zeeschelp.nl/>).

**TNO** TNO was founded by law in 1932 to enable business and government to apply knowledge. As an organisation regulated by public law, they are independent: not part of any government, university or company. TNO connects people and knowledge to create innovations that boost the sustainable competitive strength of industry and well-being of society (<https://www.tno.nl/en/about-tno/>).

**Rabobank Westland** Rabobank Westland has been active since 1985 in Westland. It is an independent local Rabobank inside the Rabobank group. Rabobank is a bank with a cooperative foundation with connection as its core (<https://www.rabobank.nl/particulieren/lokalebanken/westland/organisatie/>).

**Unilever** Unilever is a company with more than 400 brands related to health and welfare. In November 2010 the Unilever Sustainable Living Plan was introduced to double their company together with a minimal influence on the environment. Every year €1 billion is invested in research and development to provide in human needs (translated of <http://www.unilever.nl/overons/kennismaking/>).

**Van Benthem veevoeders & Kunstmest B.V.** Van Benthem veevoeders & kunstmest B.V. is an average animal feed producer specialised in the production of cattle feed and is a real family business. Besides cattle feed they produce feed for horses, sheep, pigs, poultry and rabbits. The product 'algae lick stones' is a feed product for horses (translated of <http://www.vbvoer.nl/>).

**Waterland** Waterland is an independent private equity investment group that supports entrepreneurs in realizing their growth ambitions. It has made investments in over 300 companies and it provides entrepreneurs with a powerful position in the increasingly competitive international arena (<http://www.waterland.nu/en/>).

**WUR Greenhouse Horticulture** Wageningen UR Greenhouse Horticulture is dedicated to innovating for and with the greenhouse horticulture sector. In collaboration with the business and scientific communities and the government, we analyse issues relating to operational management and cultivation and translate them into application-oriented research and innovation procedures (<http://www.wageningenur.nl/en/Expertise-Services/Research-Institutes/Wageningen-UR-Greenhouse-Horticulture.htm>).

**Zeelandsroem** Since 2005, Roem van Yerseke owns its own shellfish hatchery. In the early phases, research to cultivate mussel seeds has been conducted. Over the last years, the focus has been on cultivating Japanese oysters, flat oysters and different species of clams. Micro-algae are cultivated in order to serve as feed for the early stages of the shellfish hatchery (<http://www.roemvanyerseke.nl/pagina/3/43/hatchery/>).





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## Appendix B

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# Questionnaire Research Institutes, Producers, Developers and End-Users

Date:

Place:

Company:

Interview with:

### **B-1 Interview**

#### **Introduction interviewer**

**Introduction of the person who is being interviewed and introduction of the company**

**Theme: company**

1. How can you characterize your company?
  - (a) Producer of algae
  - (b) Producer of (human) food/nutraceuticals
  - (c) Producer of animal feed
  - (d) Producer of fish feed
  - (e) Investor
  - (f) Reactor/material/installation developer/supplier
  - (g) R&D
  - (h) Other
2. What is your business case? (where do you or where are you going to earn your money with?)

3. What is the market in which your business case can be put? Is that the production of algae for;
  - (a) Human food/nutraceuticals
  - (b) Animal feed
  - (c) Fish feed
  - (d) Other
4. In which phase is your company?
  - (a) R&D
  - (b) Pilot
  - (c) Commercial
  - (d) Other
5. What is the capacity of your production or process?
  - (a) 0-1000 kg/year
  - (b) 1000-5000 kg/year
  - (c) 5000-10.000 kg/year
  - (d) >10.000 kg/year
  - (e) ...
6. What is your turnover rate per year in algae producing businesses/projects?
  - (a) 0-50.000€
  - (b) 50.000-100.000€
  - (c) 100.000-500.000€
  - (d) 500.000-1million€
  - (e) >1million€
  - (f) ...
7. Is your production economically feasible? (Do you break even?)Yes/no?
8. What is your expected turnover in the algae market in the coming 5, 10 and 15 years?
9. Do you cooperate with other companies? If yes, which? And internationally or nationally?
10. Which country is your biggest client/buyer?
  - (a) The Netherlands
  - (b) America
  - (c) Israel
  - (d) Australia
  - (e) Europe (if yes, which country?)
  - (f) ...
11. What is the biggest competition on your business case? Which companies? Which other biomass sources?
12. Your SWOT-analysis; can you give me your strongest and weakest point of your business case? Where is an opportunity and where a threat?

**Theme: Investments**

13. Which investments are necessary in the near future? Which and from whom? How much?
14. Who has invested in the existing business case?
  - (a) Intern
  - (b) Extern;
  - (c) Other companies
  - (d) Venture funds
  - (e) Subsidies
  - (f) ...

**Theme: general market**

15. If you want to increase the market size of the algae sector, what companies do you need therefore?
  - (a) Start-ups
  - (b) SME companies
  - (c) Big companies
  - (d) Material/reactor developers
  - (e) ...
16. What are going to be the main technological R&D subjects for the coming years?
  - (a) Optimisation production (processing, cultivation systems)
  - (b) Cost reduction (reduction energy use, re-cycling water and nutrients, bio-fouling)
  - (c) New algae species
  - (d) Selection, strain improvement/GMO
  - (e) Improvement of-watering, harvesting, multi-product bio-refinery
  - (f) ...
17. What are going to be the main non-technological R&D subjects for the coming years?
  - (a) Consumer acceptance
  - (b) Life cycle analysis
  - (c) Marketing
  - (d) ...
18. The cost price per kg for algae is high. What is the best way to reduce this high cost price? (Technically)
  - (a) Reduction of energy costs
  - (b) Improvement materials
  - (c) Improvement cultivation systems
  - (d) Re-cycling
  - (e) Efficient nutrient use
  - (f) Growth improvement
  - (g) Cheaper growth medium
  - (h) Improvement/innovation cultivation methods
  - (i) Improvement/innovation harvesting methods
  - (j) Improvement/innovation drying methods

(k) ...

19. What is a promising market opportunity for the Netherlands?

- (a) Between 0-5 year
  - i. Food
  - ii. High valued products
  - iii. Additives
  - iv. Aquaculture
  - v. Animal feed
  - vi. Pharmacy
  - vii. Cosmetics
  - viii. Oil for biofuel
  - ix. Bulk production
  - x. Bioplastics
  - xi. Other

- (b) Between 5-15 year
  - i. Food
  - ii. High valued products
  - iii. Additives
  - iv. Aquaculture
  - v. Animal feed
  - vi. Pharmacy
  - vii. Cosmetics
  - viii. Oil for biofuel
  - ix. Bulk production
  - x. Bioplastics
  - xi. Other

**Theme: Location**

20. Do you see chances for the commercial production of algae in the Netherlands (for your business case? Yes or no? Explain please.

21. Which location do you think is most suitable for algae cultivation (for the Dutch market?)

- (a) At the coast
- (b) In the sea
- (c) In greenhouses
- (d) On agricultural land
- (e) On land that cannot be used as agricultural land

**Theme: legislation and regulations**

22. Which legislation hinders your business case?

23. How can the government stimulate or facilitate in the business case? By;

- (a) Giving subsidies
- (b) Adapting/changing the legislation/regulation
- (c) ...

**Theme: Barriers**

24. What are the barriers that slow down your business case? Can you rank them?

- (a) Financial
- (b) Legislation
- (c) Technology
- (d) Production
- (e) Consumer
- (f) ...

25. Can you give an example for each barrier in question 24?

26. Have I missed something that could be of importance for you or for my research?

**Technological Readiness Level**

27. Extra question 1. For your business case, where are you concerning the TRL?

TRL-1 basic principles observed

TRL-2 technology concept formulated

TRL-3 experimental proof of concept

TRL-4 technology validated in lab

TRL-5 technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies)

TRL-6 technology demonstrated in relevant environment (industrially relevant environment in the case of key enabling technologies)

TRL-7 system prototype demonstration in operational environment

TRL-8 system complete and qualified

TRL-9 actual system proven in operational environment (competitive manufacturing in the case of key enabling technologies; or in space)



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# Appendix C

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## Questionnaire Investment Funds

Date:

Place:

Company:

Interview with:

### C-1 Interview

#### **Introduction of the interviewer**

#### **Introduction of the person who is being interviewed and an introduction about the investment fund**

1. What are the funds perspective about the market for micro-algae in the Netherlands?
2. Is there a chance for a micro-algae market in the Netherlands and which market specifically? If yes or no, please explain.
3. Do you think that the Netherlands could have a strong market position in micro-algae, or for a specific market in micro-algae? (If the second, which market).
4. Is there a will to invest in micro-algae companies? If yes, why? And if no, what has to happen to get your fund convinced to invest?
5. Did the fund ever receive a request from micro-algae companies? And what is usually done with the requests?
6. Why is there sometimes reluctance to invest in micro-algae, or why do investors not want to take the risk?
7. How is the interest of foreign investors? Do you have information on that?





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## Appendix D

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### Graphs of the interviews

Companies and Research Institutes defined

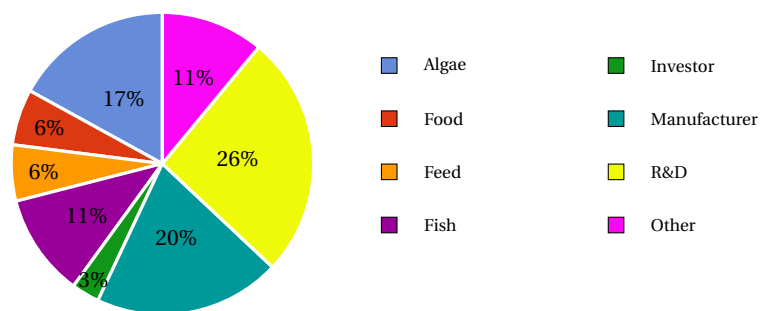


Figure D-1: Companies and Research Institutes defined

Question 1 was a general question about the definition of the company (Figure D-1) in which some companies have multiple definitions.

Question 15 addressed the companies that were needed to increase the market size of the micro-algae market in the Netherlands ( Figure D-2). Especially SMEs and bigger companies are needed.

In question 24 a ranking was asked to be made from five major barriers that companies and research institutes may encounter. The number 1 barriers are already discussed in Chapter 3. The numbers 2, 3, 4, and 5 are depicted below ( Figure D-3).

Which companies are needed to increase the market size

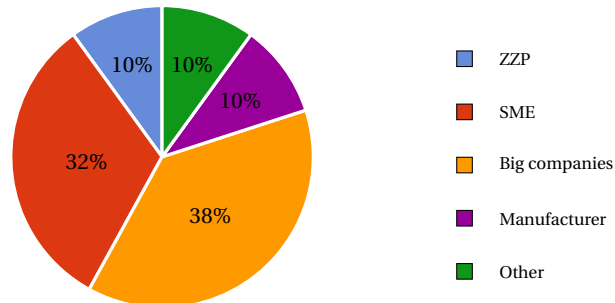


Figure D-2: Companies that are needed to increase the market size in the Netherlands

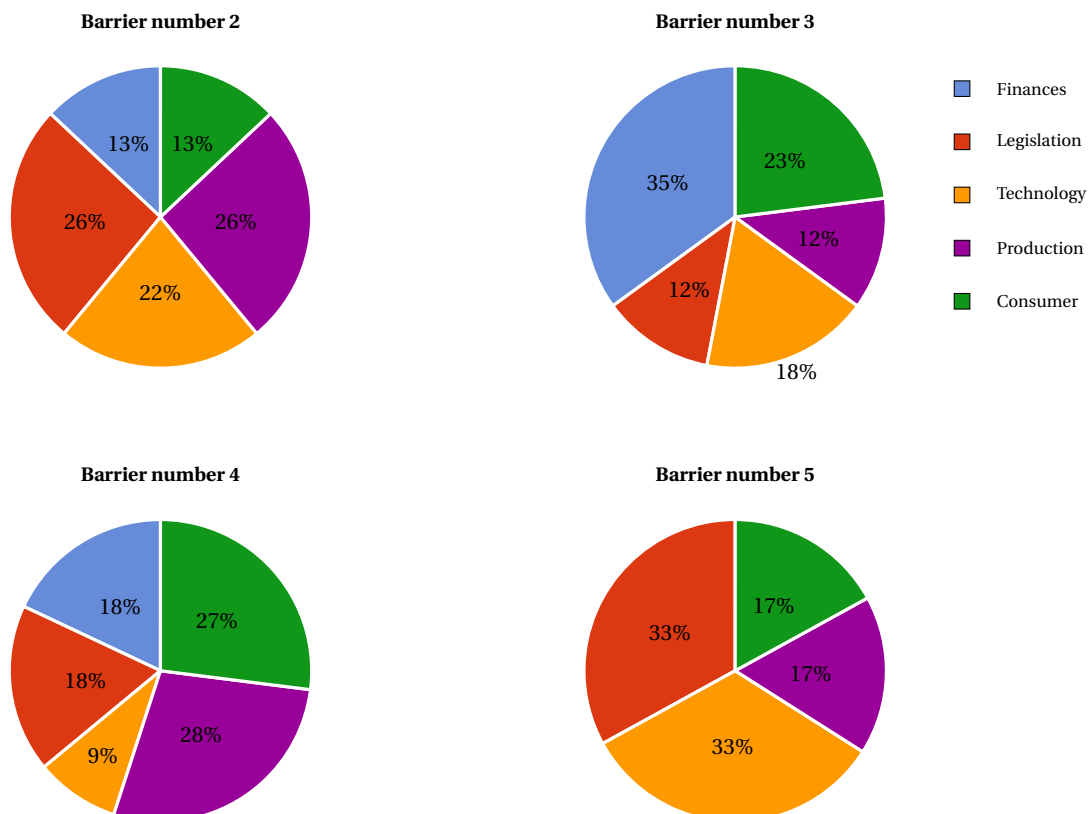


Figure D-3: Barriers ranked number 2,3,4 and 5 that research institutes and companies encounter

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## Appendix E

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# The research institutes and companies micro-algal business cases

**Evodos**

Production of materials for micro-algae harvesting

**ACRRES**

Micro-algae as feed for animals, additional value of micro-algae for animal feed

**LGem**

- Developing and supplying of photobioreactors
- Production of freeze dried micro-algae

**AlgaePARC**

Cost price reduction and optimisation cultivation systems

**WUR Greenhouse Horticulture**

Astaxanthin production for oleorasin as food supplement produced in Dutch greenhouses

**Unilever**

Eventually the use of omega-3 fatty acids and proteins from micro-algae for the consumer production (e.g. margarine) (food competing with fish and plant based oils)

**DSM**

Monitoring of developments on the area of phototrophic micro-algae for product development of high valued products for the food industry

**AF&F**

Prototype development for the production of astaxanthin

**Photanol**

Engineering cyanobacteria to make them convert CO<sub>2</sub> directly and efficiently into products, driven by sunlight. Photanol starts with low volume, high price products such as flavours, and ramps up to large volume, low price products such as fuels, as the technology matures.

**Duplaco**

Purchasing, processing and sales of micro-algae for pet food.

**Omega Green**

Supplying, construction and consultancy of cultivation systems

**AlgaeLink**

Micro-algae cultivation and sales of technology and systems; micro-algae cultivation technologies

**Zeelandsroem**

The cultivation of micro-algae in order to cultivate shellfish, (micro-algae as feed for the shellfish hatchery).

**Algaspring**

Production and supply of micro-algae for the food industry and aquaculture

**Stichting Zeeschelp**

Research in collaboration with entrepreneurs that want to innovate in aquaculture (in many projects micro-algae are needed at the start)

**Fry Marine**

Sales of small turbot to turbot nurseries and eggs of copepods to hatcheries all over the world, (micro-algae serve as feed for copepods).

**Nutress**

Production and sales of micro-algae (all kinds of applications)

**Clean Algae**

Production of micro-algae products mainly for specialty fish feed

**Algaebiotech**

Development of technology and services for industries (micro-algal process technology)

**Van Benthem Veevoerders en Kunstmest**

Production and sales of feed for cattle, sheep and horses to eventually sell more micro-algal products to these groups (algae lickstone for horses is sold, but is a really small market)





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# Abbreviations

**CO<sub>2</sub>** Carbon dioxide.

**A-V** Area to Volume.

**AA** Arachidonic Acid.

**ANS** Additives and Nutrient Sources.

**BBE** BioBased Economy.

**CAPEX** Capital Expenditure.

**cm** centimeter.

**D** Developers.

**DHA** docosahexaenoic acid.

**DNA** Deoxyribonucleic Acid.

**DW** Dry Weight.

**E-U** End Users.

**EC** European Commission.

**EFSA** European Food Safety Authority.

**EPA** eicosapentaenoic acid.

**EU** European Union.

**FDA** Food and Drug Administration.

**GAIA** Getting Algae Ingredients Applied.

**GLA** *gamma*-linolenic acid.

**GMO** Genetically Modified Organisms.

**GMP+** Good Manufacturing Practice.

**GRAS** Generally Recognized As Safe.

- ha** hectare.
- HACCP** Hazard Analysis and Critical Control Points.
- HUFAs** Highly unsaturated fatty acids.
- ICSE** Instant Catapult Steam Explosion.
- kg** kilogram.
- kWh** kilo Watt hour.
- LC-PUFAs** Long chain polyunsaturated fatty acids.
- LCA** Life Cycle Analysis.
- LED** Light Emitting Diode.
- MIRACLES** Multi-product Integrated bioRefinery of Algae: from Carbon dioxide and Light Energy to valuable Specialties.
- MPa** MegaPascal.
- NaOH** Sodium Hydroxide.
- NSPA** National Strategic Plan Aquaculture.
- NVWA** Netherlands Food and Consumer Product Safety Authority.
- OPEX** Operational Expenditures.
- P** Producers.
- PBR** PhotoBioReactor.
- PCE** Photo-Conversion Efficiency.
- PLE** Pressurized Liquid Extraction.
- PPP** Public Private Partnerships.
- PUFAs** Poly Unsaturated Fatty Acids.
- R&D** Research and Development.
- RI** Research Institutes.
- RNA** Ribonucleic Acid.
- RVO** Rijksdienst Voor Ondernemend Nederland.
- SFE** Supercritical Fluid Extraction.
- SME** Small and Medium sized Enterprises.
- SWE** Subcritical Water Extraction.
- SWOT** Strengths, Weaknesses, Opportunities and Threats.
- TAG** TriAcylGlyceride(s).
- TIS** Technological Innovation System.
- TKI** Topconsortia for Knowledge and Innovation.



**TNO** Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek.

**TRL** Technological Readiness Level.

**US** United States of America.

**VALORIE** Versatile Algae On-site Raw Ingredient Extractor.



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