Commercial exploitation of marine microalgae: updating old methods of biomass and compound production

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Abstract
Microalgal exploitation has been going on for millennia. In recent years the interest in biotechnologically interesting compounds produced by microalgae has increased, especially since old sources of various commercial applications are becoming scarcer or are being forbidden. However some of the microalgae applications have been used for years now and the applied species or cultivation methods might be outdated. This literature research aims to see if the following applications can be updated: microalgal production of biofuel, antifouling compounds, nutrition and pharmaceutical biochemicals. To get an answer various aspects of microalgal exploitation are looked at, such as: biotechnologically relevant microalgal species, benefits of using microalgae versus other systems (e.g. yeast and bacteria), different methods for large-scale microalgae biomass production and different forms of commercial exploitation of microalgae. The commercial applications that can be updated are production of antifouling compounds, vitamin production, eicosapentaenoic acid (EPA) production and pharmaceutical biochemicals production. In addition, for commercial applications to be effectively updated more research is needed into the screening of microalgal compounds and into the design and testing of new large-scale bioreactors for the mass cultivation of microalgae.

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1. Introduction

Microalgae have been exploited by humans for millennia. *Nostoc sp.* was used in China as food since thousands of years, as well as *Arthospira sp.* in Chad and Mexico (*Spolaore et al.*, 2006). The first industrial exploitation of microalgae began after World War II in the USA, Germany and Japan. These countries were looking for a potential food source as the human population increased after the war. After the war microalgae have also been grown for the use in waste water treatment, for atmospheric regeneration in biospheres (e.g. spacecraft) and as renewable fuels for transportation. Since the 1960s microalgae are commercially exploited as a natural source of compounds for health and medical applications. Recently the interest in microalgal metabolites has increased because these metabolites can be a source of novel types of compounds, for example drugs not found in higher plants. A few commonly used species of microalgae that are exploited these days are species from the genera *Dunaliella*, *Haematococcus*, *Chlorella* and *Euglena* (*Walker et al.*, 2005), but many other microalgae possess useful properties for commercial exploitation. Studies to explore these unknown properties are being done for various commercial applications (e.g. *Hellio et al.*, 2002; *Rosenberg et al.*, 2008). These studies however are not only limited to finding new compounds, but also researched are the various possibilities of growing microalgae (e.g. *Chen*, 1996) or research for improving species that are already in use via genetic modification (e.g. *Vila et al.*, 2008).

However since most exploitations of microalgae are being used for a long time, they might be outdated. The commercial applications that will be discussed are using microalgae for the production of biofuel, antifouling agents, nutrition and pharmaceutical biochemicals. This literature research aims to check if any of these applications can be updated by using species or cultivation methods (found in other studies into microalgae compounds and cultivation methods) that have been found to give a higher production rate of the desired compounds. To get a clear picture of the commercial exploitation of microalgae, various aspects of this exploitation will be looked at by answering questions. The questions are:

- Which are the biotechnologically relevant microalgal species and why are these relevant?
- Which are the benefits of biotechnological production using microalgae versus other systems?
- Which different methods are available for large-scale microalgal biomass production?
- Which different forms of commercial exploitation of microalgae are there?

In the discussion the main question will be answered, which is:

- Can the commercial applications discussed be improved by using other species of microalgae or cultivation methods, which have been proven to increase production rate?

2. Which are the biotechnologically relevant microalgal species and why are these relevant?

Eukaryotic algae are used for commercial exploitation. Several algal groups have been found to posses useful commercial properties, such as high growth rates or thin cellulose walls. Some of these properties are characteristic for a specific algal group. The groups that are used are the Chlorophyta, the Rhodophyta, the heterokont algae, the Dinoflagellata, the Cryptophyta and the Euglenida. The first two groups of algae arose from the progenitor of the algae at the same time. The other groups appeared much later in time and are the result of a secondary endosymbioses of either a red algae or a green algae turning into a plastid within an eukaryotic host (*Pulz and Gross*, 2004). Each of the groups will now be discussed separately to explain its relevance.

a. Chlorophyta: The first group, the Chlorophyta, used for commercial exploitation belongs to the green algae lineage, of which the Conjugaphyta is also a part. The latter group has not yet been used for biotechnological applications. The Chlorophyta can be subdivided into four classes. The largest class is the Chlorophyceae, containing about 2500 species in 350 genera. Of these species most are unicellular or filamentous and live in fresh water. The reason this group of algae is used is the fact that some species, e.g. *Dunaliella salina* and *Haematococcus pluvialis*, produce and store high concentrations of carotenoids under certain cultivation conditions (*Pulz and Gross*, 2004). In fact the extraction of carotenoid pigments out of *D. salina* is already at a large-scale production, with over 80% of the world's supply of natural β-carotene coming from this species (*Walker et al.*, 2005). Efforts have also been made to produce the carotenoid astaxanthin, valuable for fish aqua-cultures, using *Haematococcus pluvialis*. The reason this species is used is because it can accumulate up to 3% of its total dry weight in astaxanthin (*Pulz and Gross*, 2004). *Chlorella spp.* are also used as live feed in marine fish aquacultures (*Wikfors and Ohno*, 2001). The Prasinophyceae are the second class of Chlorophyta. This group contains about 120 species in 13 genera and consists of flagellated unicellular algae that are covered by organic scales. Most species live in marine or brackish water. The class is mainly used as live feed for aquacultures of marine mollusks and crustaceans (*Wikfors and Ohno*, 2001). Three genera play an important role in this, because they have high growth rates under the right conditions. These genera are *Tetraselmis*, *Pyramimonas* and *Micromonas*. Some other species of the group Prasinophyceae can also reach high cell densities under mixotrophic conditions (*Pulz and Gross*, 2004). Both the Ulvophyceae and the Charophyceae classes consist mostly of macroalgal species. The few microalgal species that are in these classes are of no biotechnological relevance, except for one genus called *Spirogyra*, which produces bactericides (*Pulz and Gross*, 2004) that maybe can be used as antibiotic.

b. Rhodophyta: The second group, the Rhodophyta, contains high amounts of the pigment phycoerythrin, which gives the red color, in addition to chlorophyll a. Almost all species in this group are macroalgae and only a few can be considered as microalgae. The microalgae live in marine, fresh-water and terrestrial environments. Only the *Porphyridium* species are used biotechno-logically for the production of pigments (e.g. phycoerythrin), extracellular polysaccharides and arachidonic acid. This is because these species don't need the addition of vitamin B_{12} to an artificial seawater medium, which is necessary for most of the other red microalgae to grow (*Pulz and Gross*, 2004).

c. Heterokont algae: The third group, the heterokont algae, consists of a number of phyla, including the Phaeophyta (brown algae), the Xanthophyta (yellow-green algae), the Chrysophyta (golden algae) and the Bacillariophyta (diatoms). All these phyla are generally brown in color because of the high concentration of fucoxanthin within the cells. This pigment is used for photosynthesis. The Phaeophyta consists almost exclusively of macroalgae, so will not be discussed. The Xanthophyta consists mostly of unicellular or

filamentous species which preferably live in freshwater or on land. In these groups about 600 species can be identified in over 90 genera. Some species, such as *Tribonema sp.*, show high growth rates and form late winter blooms, but despite this these species are not used. These species could however be used as winter-feed for aquacultures. The Chrysophyta also consists mainly of unicellular flagellated freshwater species, which are hard to grow *in vitro* without any addition of vitamins, other organic nutrients or soil extracts to the medium. About 1.000 species in 120 genera have been identified in this class (*Pulz and Gross*, 2004). The final phylum belonging to the heterokont algae, the Bacillariophyta, are most likely the largest biomass producers on earth. At least 100.000 species in 250 genera have been identified. These diatoms can be found nearly anywhere and are unicellular and unflagellated in their vegetative stage. There are three different forms of diatoms: the centric diatoms (Coscinodiscophyceae), the pennate diatoms without a longitudinal striation (Fragilariophyceae) and the pennate diatoms with a longitudinal striation (Bacillariophyceae). Especially under nitrogen starved conditions all these forms of diatom produce oil as a food reserve. This oil can accumulate up to 60% dry weight (e.g. in the species *Phaeodactylum tricornutum*), which, together with their high productivity may represent a new alternative to fossil fuels. Unusual fatty acids, such as eicosapentaenoic acid (EPA), are of interest to biotechnology as well as food supplements (*Pulz and Gross*, 2004; *Tonon et al.*, 2002). Furthermore diatoms of the genera *Chaetoceros*, *Thalassiosira* and *Nitzschia* are also commonly used as live feed in aquacultures of mollusks and crustaceans (*Wikfors and Ohno*, 2001).

d. Dinoflagellata: The fourth group is the Dinoflagellata, which consists of about 4.000 species in 550 genera. The species are all unicellular and flagellated and can be found in both marine and fresh water. The dinoflagellates contribute significantly to the primary production in coastal waters, although approximately 50% of the species are non-photosynthetic. Some species produce an extremely harmful toxin and thus pose a threat to fisheries and aquacultures. In contrast, *Gymnodinium spp.*, such as *Gymnodinium splendens* (*Rodriguez and Hirayama*, 1997), are the preferred live feed for fish larvae and mollusks. This is because dinoflagellates produce metabolites, which are also of biotechnological interest, such as sterols and fatty acids (e.g. docosahexanoic acid (DHA)) (*Pulz and Gross*, 2004). Both sterols and DHA have been shown to be essential in the diet of mollusks (*Wikfors and Ohno*, 2001). One of the problems that is encountered when trying to use dinoflagellates to produce DHA and sterols is that most species are difficult to grow under laboratory conditions. This is due to of the requirement of complex growth media and special lighting conditions. However these high costs due to the requirements for growth justify the costs of large-scale cultivation, because of the high value of DHA (*Pulz and Gross*, 2004).

e. Cryptophyta: The Cryptophyta group consists of about 60 species in 20 genera. The species are often found in oligotrophic seawater and fresh water and are unicellular. The bloom of this group is in the winter and early spring. Carotenoids are present in the Cryptophyta. However certain species, such as *Rhodomonas sp.* and *Cryptomonas sp.*, of this group thus far are only used as live feed for mollusks and crustaceans in aquacultures (*Pulz and Gross*, 2004; *Wikfors and Ohno*, 2001).

f. Euglenida: The final group is the Euglenida. This group is commonly found in eutrophic wastewater or polluted areas. Around 800 species in 43 genera have been identified. All species are flagellated and unicellular, with a highly flexible wall. Euglenida are of interest to biotechnology because this group produces various bioactive compounds and has a high resistance against heavy metals as well as the ability to accumulate these heavy metals. However no commercial exploitation of this group is being done at the moment (*Pulz and Gross*, 2004)

Table 1. Biotechnologically relevant microalgal species

Phylum	**Species**	**Habitat**	**Application**	**Reference**
Chlorophyta	*Dunaliella salina*	Marine	Carotenoid (β-carotene) production	Pulz & Gross (2004)
	Haematococcus pluvialis	Freshwater	Carotenoid (Astaxanthin) production	Pulz & Gross (2004)
	Chlorella vulgaris	Marine	Live feed for marine aquacultures Health food, food supplement, feed surrogates	Wikfors & Ohno (2001) Pulz & Gross (2004)
	Isochrysis galbana	Marine	Animal nutrition	Pulz & Gross (2004)
	Tetraselmis sp. *Pyramimonas sp.* *Micromonas sp.*	Marine/ brackish water	Live feed for marine aquacultures	Pulz & Gross (2004)
	Spirogyra sp.	Freshwater	Bactericide production	Pulz & Gross (2004)
Rhodophyta	*Porphyridium spp.* *Porphyridium cruentum*	Freshwater/ terrestrial	Pigment (phycoerythrin) production Polysaccharides production	Pulz & Gross (2004)
Bacillariophyta	*Phaeodactylum tricornutum*	Marine	Biofuel production EPA production	Pulz & Gross (2004) Tonon *et al.* (2002)
	Odontella aurita	Marine	Fatty acids production	Pulz & Gross (2004)
	Chaetoceros sp. *Thalassiosira sp.* *Nitzschia sp.*	Marine	Live feed for marine aquacultures	Wikfors & Ohno (2001)
Dinophyta	*Gymnodinium splendens*	Marine	Live feed for marine aquacultures	Rodriguez & Hirayama (1997)
Cryptophyta	*Rhodomonas sp.* *Cryptomonas sp.*	Marine/ freshwater	Live feed for marine aquacultures	Wikfors & Ohno (2001); Pulz & Gross (2004)
Euglenozoa	*Euglena sp.*	Wastewater	Heavy metal removal	Pulz & Gross (2004)

3. Which are the benefits of biotechnological production using microalgae versus other systems?

Apart from microalgae there are a number of other systems that can be used to commercially produce substances of biotechnological interest. These other systems are bacteria, yeasts, mammalian cell cultures, transgenic animals and transgenic plants. The production features are important when deciding which system to use for biotechnological production, since not every system is as efficient as the other. Because commercial exploitation is of importance here the features that need to be looked at are the production time, production costs, scale up costs, cost/storage, production scale, multimeric protein assembly, protein yield, risk and ethical concerns (*Walker et al.*, 2005). Each feature will be discussed separately and comparisons will be made between microalgae and the other systems.

The production time is the time needed before the product is available. For both microalgae and bacteria this time is short, because of the high growth rates under the right conditions. The other systems take longer to grow before the product is available. The production costs are very low for the microalgal system compared to the other systems. Especially mammalian cell cultures and transgenic animals cost a lot of money for biotechnological production (*Walker et al.*, 2005).

The scale up cost is high for bacteria, yeast, mammalian cell culture and transgenic animal systems. This is mostly due to the fact that these systems will require large-scale fermenters and other associated equipment to work properly. Transgenic plants and microalgae however only need to be planted in a bigger field or put into a bigger pond to scale up the production. This is much more cost efficient (*Walker et al.*, 2005). However, when producing specific chemicals in large-scale ponds there is a risk of contamination of the culture by unwanted organisms, such as bacteria. This can be prevented by using an intensive open pond system in which conditions are maintained specifically for the microalga used. Another way to prevent contamination is by using photobioreactors, which is a closed system (*Chen*, 1996).

The cost/storage of various strains that are used in the systems varies. Transgenic plants are the easiest and cheapest, because these can be stored at room temperature. Microalgae, bacteria and yeast are also cheap, but need to be stored at -20ºC. The systems involving mammalian cell cultures and transgenic animals are expensive and these need to be stored in liquid nitrogen (*Walker et al.*, 2005).

The production scale indicates where on earth this production using these systems could take place. For all the systems this is limited, which is due to the fact that specialized equipment is needed for production that is often expensive. Exception to this is the transgenic plants. As stated earlier this system needs a field to grow, so no extra equipment is needed. That's why this systems can be applied worldwide. Microalgae can also be used worldwide. However intensive pond systems or photobioreactors are required. Extraction of high-value compounds produced by microalgae isn't needed, since commonly used microalgae of the genera *Dunaliella*, *Haematococcus* and *Chlorella* are classified as food sources falling into the GRAS (Generally Regarded As Safe) category. Consequently, many of these compounds are administered as a powder or freeze-dried algae with no extraction undertaken. Since the structure of microalgae is simple, there are no concerns about processing compounds out of complex tissues (*Walker et al.*, 2005).

The multimeric protein assembly indicates if the system is capable of binding several proteins together to form the multimeric protein that is of biotechnological interest. Bacteria, yeast and mammalian cell cultures are not capable to do this, which means additional steps are needed to form the end product. Microalgae, transgenic animals and transgenic plants are capable to do this. Furthermore the protein yield for each system is medium to high, with the exception of the microalgae system. In this system it is unknown how much protein can be harvested (*Walker et al.*, 2005).

There is also a risk that needs to be taken into account when commercially exploiting these systems. This risk is the contamination of human therapeutic proteins with viral sequences, oncogenes or endotoxins. For several systems this risk is unknown, one of these being the microalgae system. However the contamination risk is there for bacteria, mammalian cell cultures and transgenic animal systems (*Walker et al.*, 2005).

A feature that is also important when dealing with commercial exploitation of organisms is the ethical concerns. Not every system is ethically justified in the eyes of the consumer. Especially the use of transgenic animals is frowned upon. The concerns for the use of bacteria however are minimal. Microalgae are in this regard right in the middle, together with yeast, mammalian cell cultures and transgenic plants (*Walker et al.*, 2005).

Microalgal systems couple several benefits from other systems into one system. The high productivity of plants is combined with large-scale microbial production approaches. Although the microalgae are simple in structure, being unicellular, filamentous or colonial, the same basic photosynthesis mechanisms are used as in higher plants. However, since microalgae don't need to invest energy into maintaining differentiated structures like higher plants do, the energy can be invested into photosynthesis, growth and reproduction. This leads to higher protein levels in microalgae of between 30 and 50% of the dry weight biomass. Another benefit of microalgae is that they are microscopic in size and are grown in liquid media. Because of this the nutrient levels can be maintained to an optimal condition in the media which leads to a controlled, continuous productivity. This is similar to microbial fermentation. Most microalgae are also photoautotrophs, meaning only sun, water and basic nutrients are needed for maximal growth. Because microalgae require no exogenous carbon source for energy potentially makes large-scale aquaculture in open ponds cheap (*Walker et al.*, 2005). The microalgae also use up any available dissolved CO_2, which means that there is less chance of bacteria and yeasts contaminating the cultures, since conditions aren't right for them.

4. Which different methods are available for large-scale microalgal biomass production?

To make the commercial exploitation of microalgae for various applications feasible, viable and above all interesting for companies, large-scale biomass production of microalgae is needed. Currently several different production methods are being used to optimize the growth of microalgae. These methods are the open/closed pond systems, the photobioreactor and heterotrophic growth. Each method will be discussed separately.

a. Open pond systems: The open pond system is the oldest method for the cultivation of microalgae. In this system algae are grown under conditions similar to the external environment. The ponds generally used in these systems are raceway cultivators, operating at a water depth of 15-20 cm. In these ponds biomass concentrations of 0,1-0,2 g/l dry weight (DW) and productivities of 60-100 mg/l day^{-1} DW are possible (*Pulz*, 2001). The open pond system can either be operated as intensive or extensive systems. Intensive systems utilize small ponds that are modified to increase the algal biomass production. This is done by adding CO_2 for photosynthesis and by stirring the media for optimal light utilization. Contamination can also be prevented when using this intensive system. Extensive open pond systems however do have a contamination risk, which leads to the problem of maintaining a monoculture. Because of this problem only low cell densities can be achieved, which in turn leads to higher production costs. Extensive open pond systems don't have any modifications and generally make use of larger ponds. This system has several advantages compared to intensive pond systems. These are minimal cost of construction and operation and the utilization of arid/salty lands, otherwise unsuitable for agriculture. The species that are grown in these open pond systems grow in a selective and specialized environment, which is hostile

for other organisms. This is one way to prevent contamination of the culture from unwanted algae, bacteria, fungi and predators. *Dunaliella salina* and *Spirulina platensis* (cyanobacteria) are most commonly used in open pond systems, because *D. salina* can grow in media with a high salinity (>20% NaCl concentration) and *S. platensis* grows in highly alkaline water (pH >9.2). Other species that are commonly used are *Chlorella*, *Scenedesmus* and *Phaeodactylum*. These species outgrow most competitors when conditions are optimal (*Chen*, 1996). The use of extremophilic algae offers a lot of other options still. Certain red algae, e.g. *Cyanidium caldarium* and *Galdieria sulphuraria*, have been proven to be both thermo- and acidophilic, growing at temperaturs of 50 ºC and a pH of 1. Yet another group of algae, the psychrophilic algae, is potentially interesting for biotechnological applications, due to the fact that this group of slow growing ice algae contains enzymes with unique kinetic properties. Mainly extracellular enzymes may be of biotechnological interest, because the intracellular pH and salinity of the extremophilic species, such as *Cyanidium caldarium*, *Galdieria sulphuraria* and *Monoraphidium minutum*, will remain neutral (*Pulz and Gross*, 2004).

b. Photobioreactor: The photobioreactor is an enclosed system that is better than a closed pond system. Several parameters that can be closely regulated and monitored in these reactors are light irradiation, CO_2/O_2 balance, temperature, salinity, nutrients, pH and turbulence. The biomass concentrations in the bioreactor during production can be as high as 2-8 g/l DW and productivities of 100-1.000 mg/l day^{-1} DW (*Pulz*, 2001). This is much higher than can be achieved using pond systems. Aside from higher productivity enclosed photobioreactors provide many advantages over pond systems, such as better protection from contamination and better control of culture environment. There are several different types of enclosed photobioreactors. The most commonly used one is a tubular photobioreactor. Productivity in these reactors can reach up to 30-33 ton ha^{-1} y^{-1} DW, when using *Spirulina platensis* cultures. The second type is the vertical alveolar panel bioreactor. It consists of flat hollow panels in which the microalgae grow. This reactor has been developed with the purpose of maximizing the surface-to-volume ratio in order to maximize production. Cell productivity in panel reactors has already reached 24 g m^{-2} d^{-1} DW using *Spirulina platensis* cultures. Another type of reactor is the fiber optic photobioreactor, which consists of a reactor with a light source, an optical transmission system, a gas exchange unit, an ultrafiltration unit and an on-line sensor for condition monitoring. The system can effectively improve light conditions causing the algae to grow better. This leads to very high cell concentrations (30 g l^{-1} DW). However it has been proven difficult to scale-up these last two types of bioreactor. The costs also increase due to the complexity of these two systems. Another problem that is encountered is that due to the high cell concentrations in the panel and fiber optic photobioreactors, light will not penetrate. As a result the light limitation problem can be made smaller, but not entirely solved. Furthermore there is also the partly unsolved problem of the high concentrations of oxygen accumulation in the culture due to photosynthesis (*Chen*, 1996). This is a problem as high concentration of oxygen in combination with intense light produces photooxidative damage to algal cells. One way to solve this problem is to pass the culture through a degassing station once in a while, to remove excess oxygen (*Chisti*, 2007).

c. Heterotrophic growth: The methods mentioned until now rely on autotrophic growth of microalgae. This mode of growth has its problems, the most important one being light limitation. However autotrophic growth is not the only way to grow. The other mode is heterotrophic growth, in which organisms utilize organic carbon substances as their sole carbon and energy source. Light isn't required for this mode of growth. Using this mode of growth however is restricted to enclosed photobioreactors, since there may be no other carbon sources available. The optimal growth and production conditions can be easily maintained in this mode of growth. Using sterilization of the medium and aseptic operation, contamination and predators can be avoided. However these requirements cause an increase in costs. For this

method to be economically viable, heterotrophic cultures must obtain higher cell densities than other methods. Nonetheless this mode of growth is very suitable for the production of high value chemicals and pharmaceuticals. Heterotrophic growth of microalgae does has its own problems. These are the limited number of available heterotrophic algal species, potential contamination by bacteria, inhibition of growth by soluble organic substrates at low concentrations and the inability to produce some light-induced products, such as pigments. Several useful heterotrophic algal species have been found already, such as *Nitzschia alba* and *Crypthecodinium cohnii* which produce EPA and DHA, both highly valuable biotechnological compounds. The contamination by bacteria is mostly due to the fact that bacteria have a much higher growth rate than the microalgae used. This problem can be solved by rigorous sterilization and aseptic operation. The third problem is due to the fact that some organic substances inhibit growth when these are present in too high concentrations. For instance a glucose concentration of above 20 g l^{-1} has been shown to inhibit the growth of *Chlorella sorokiniana*. The fourth problem might be overcome by using mixotrophic culture. An example of this is using a mixotrophic culture to grow *Spirulina platensis* on glucose at high cell densities to produce the pigment phycocyanin. However this example has only been tested on a small scale (*Chen*, 1996).

5. Which different forms of commercial exploitation of microalgae are there?

Microalgae can be used for various commercial applications, such as the production of biofuel, antifouling substances, nutrition and pharmaceutical biochemicals. These applications will be discussed separately in further detail. Several things will be looked at: different methods of the same application and how these work, which species of microalgae are commonly used for each application, what chemicals are produced by these species and the function of these chemicals and which production method (e.g. open pond system) is used for this application. Next to the applications mentioned there are some other forms of exploitation of microalgae which will be discussed shortly.

a. Biofuel: Exploiting microalgae for the production of biofuel is becoming an interesting alternative to fossil fuels, but is still in its infant shoes. This is because the use of petroleum based fuels is recognized as unsustainable due to depleting supplies and the contribution of CO_2 in the environment. Furthermore using marine microalgae for the production of biofuel has some advantages over using land plants for this production. First of all oil productivity of many microalgae is much higher than the oil productivity of the best producing oil crops, such as oil palms and soybeans (Chisti, 2007). Since algae grow in water there is no need for arable land. And by using low cost salt-water cultivation systems there will be no competition for fresh water since seawater can be used. However one problem when using microalgae for the production of biofuel is that the biomass produced is wet. As such processing of this wet biomass involves biologically mediated procedures, such as fermentation. Drying the biomass would be to energy consuming and cost too much. The remaining biomass after product separation can be used as biofertilizer (*Rupprecht*, 2009).

Two types of biofuel can be produced using microalgae. The first is called biodiesel, which can be produced in far bigger amounts in comparison to oil crop biodiesel (*Chisti*, 2008). This means using microalgae biodiesel is more economically viable as a replacement for fossil fuels than oil crop biodiesel. Microalgae produce lipids and other complex oils as food reserves. This lipid synthesis is higher at nitrogen starved conditions, which leads to the accumulation of oil in starved cells (*Rosenberg et al.*, 2008). Not all the algal oils are satisfactory for making biodiesel, but suitable oils are common. The oil levels in some species can exceed 80% by weight of dry biomass, but oil levels of 20-50% are more common. Some species with these oil levels are *Botryococcus braunii* (25-75%), *Chlorella sp.* (28-32%), *Schizochytrium sp.* (50-77%) and *Nitzschia sp.* (45-47%). How much oil is produced by the

algae is dependent on the growth rate and the oil content of the biomass. The microalgae species with the highest oil productivities are preferred for producing biodiesel. The only practical and viable methods of large-scale biomass production of microalgae for biodiesel production are raceway ponds (open pond systems) (*Chisti*, 2007). Biodiesel is theoretically already economically viable. The costs of one barrel algae oil is estimated to be around $52-$91 US/barrel, with this price being based on a productivity of 30-60 g m^{-2} day^{-1} with 50% algal lipid yield. The current price of one barrel crude oil is around $70 US. This means that biodiesel can compete commercially with crude oil. The calculation is based on 400 hectares of open ponds, with productivity assumptions of 30-60 g m^{-2} day^{-1} DW with 50% algal lipid yield (*Schenk et al.*, 2008).

The second type of biofuel that is produced using microalgae is biohydrogen (H_2). This is a unique biofuel which can't be produced by any land-based plant. Biohydrogen is a clean fuel, since when it burns only water is produced. Several species of algae, such as *Chlamydomonas sp.*, produce hydrogen, which can be greatly increased using genetic modification (e.g. 5-fold H_2-production increase in *C. reinhardtii* mutant strain (*Rupprecht*, 2009)). Due to the high temperature of combustion of hydrogen in air toxic mononitrogen oxides are produced. This can be avoided by using the special combustion engines, but results in a lower efficiency. For the use of biohydrogen as a replacement to fossil fuels to be economically viable this efficiency needs to go up to above 7% under normal conditions. Enclosed photobioreactors can be used for the production of hydrogen, since this ensures no H_2 leaks away (*Rupprecht*, 2009).

There are some drawbacks however in using microalgae for biofuel production. The first is that there is little experience with large-scale closed photobioreactor systems. There is also the high material costs for these large bioreactors. The infrastructure and production pipeline are also not existent, so need to be built too. Furthermore there is a high energy requirement for the cultivation, since for example the media needs to be mixed. The harvesting of the cells will also be expensive, since these need to be separated from the medium which is time and energy consuming. Finally some technology that is needed for this kind of production is not yet established, which will lead to costs in the development of prototypes (*Rupprecht*, 2009).

b. Antifouling: Since the conventional antifouling substance tributyl tin (TBT) is banned in several countries, including those within the European community, new non-toxic antifouling substances need to be developed. Methods up till now relied on toxic antifouling paints containing copper and organotin (e.g. tributyl tin oxide), which had negative effects on several marine organisms and the environment (*Hellio et al.*, 2002; *Bhadury and Wright*, 2004). Furthermore these toxic paints are not fully effective, particularly against slime-forming diatom algae (*Hellio et al.*, 2004). Marine microalgae have been proven to be a great supplier of non-toxic antifouling agents, since they produce a variety of chemical deterrents for defence purposes (*Bhadury and Wright*, 2004). Biofouling is a problem that effects maritime domains worldwide and occurs when solid surfaces, submerged in seawater, get covered by a complex layer due to the adhesion of marine organisms, such as microbial slimes, diatoms, barnacles, tunicates, bryozoans and spores of marine algae. There are four different biofouling stages that can be identified. The first stage happens as soon as a man-made object is immersed in water, causing dissolved organic matter, polysaccharides and protein fragments to accumulate on the surface of the object. The second stage is the gradual settling of bacteria (e.g. *Bacillus sp.* and *Pseudomonas sp.*) and single-cell microalgae (e.g. *Cylindrotheca closterium*, *Phaeodactylum tricornutum* and *Porphyridium cruentum* (*Hellio et al.*, 2002)) on the object, which forms a microbial film. Colonies start to form, which start to produce extracellular polymeric substances. These sticky substances in combination with the irregular microbial colonies give way to the third stage. In this stage more particles and organisms get trapped. Algae spores, marine fungi and ciliate protozoa start to appear on the

biofilm. The fourth stage involves the settlement of other marine organisms, such as mussels, tunicates, tubeworms and barnacles. From certain marine microalgal species biogenic compounds have been isolated which have antialgal, antifungal or antibacterial properties. These compounds will interfere with stage two and three, by killing or slowing the growth of the algae, bacteria and fungi. The species belong to the Dinophyceae and Chlorophyceae. Only one species showed antialgal properties, this species being a Dinophyceae, *Peridinium bipes*, of which the isolated biogenic compound is a water-soluble extract. The biogenic compound goniodomin A isolated from *Goniodoma pseudogoniaulax* has antifungal activity. Polyether compounds (gambieric acid A and B) isolated from the species *Gambierdiscus toxicus*, *Prorocentrum lima* and *Dinophysis fortii* also have antifungal activity. The Chlorophyceae *Staurastrum gracile*, *Pleurastrum terrestre*, *Dictyosphaerium pulchellum* and *Klebsormidium crenulatum* each have methanol extracts as isolated biogenic compounds that have antibacterial properties. Aqueous extracts isolated from *Chlorococcum sp.* also showed to have antibacterial properties. In addition the biogenic compound α-linolenic acid isolated from the *Chlorococcum* HS-101 strain showed antibacterial activity. Finally from the species *Chlorokybus atmophyticus* acetone extracts are isolated which also have antibacterial properties (*Bhadury and Wright*, 2004). The desired compounds can easily be harvested by putting the algae in the appropriate extraction fluid. Photobioreactors can be used for large-scale production of the antifouling biogenic compounds. However these bioreactors first need to be more efficient and effective (*Bhadury and Wright*, 2004).

c. Nutrition: Another application microalgae are commonly used for is nutrition. Microalgae as nutrition are available in different forms such as tablets, capsules and liquids. Incorporation into pasta's, snack foods, candy bars and beverages is also an option. Because microalgae have such diverse chemical properties, they can be used as nutritional supplement (e.g. vitamins, fatty acids and proteins) or as a source for natural food colorants (e.g. carotenoids and phycobiliproteins) (*Spolaore et al.*, 2006). However it can be expected that exploitation of the diversity of microalgae will be hampered by food safety regulations for human consumption (*Pulz and Gross*, 2004). Microalgae represent a valuable source of nearly all essential vitamins (*Spolaore et al.*, 2006). Vitamin production using microalgae can be very useful for food supplements, as for instance medical studies have proven that human cancer risks go down when β-carotene, the precursor to vitamin A, is taken in. *Dunaliella salina* is the preferred microalga already being exploited for its β-carotene, because the levels can reach 14% of dry weight (*Spolaore et al.*, 2006). *Spirulina sp.* is also used for the production of vitamin B_{12}, which helps the immune system (*Pulz and Gross*, 2004). The production of vitamins using microalgae can be done in photobioreactors, making sure conditions are right for maximal vitamin production, which is dependent on the environmental factors, the harvesting treatment and the method of drying the cells (*Spolaore et al.*, 2006).
Microalgae are also a valuable source of lipids. Especially the long chain poly-unsaturated fatty acids (LC-PUFAs) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are of biotechnological interest, since these lipids have been proven to have several important pharmaceutical applications. Among these are the requirement of DHA in the diet of infants in order to achieve full development potential (e.g. correct brain and eye development (*Spolaore et al.*, 2006)), the involvement of LC-PUFAs in homeostatic function in animals and the prevention and treatment of chronic diseases (e.g. coronary heart disease, hypertension, type II diabetes). Another source of these lipids is fish. However this source is a declining resource and combination with the fact that there are serious environmental consequences related to the continued exploitation of fish-stocks, microalgae provide a new source for LC-PUFAs (*Tonon et al.*, 2002). Currently, when microalgal cultivation is concerned, the only LC-PUFA that is commercially available is DHA. The species that are used for the production of this lipid are the heterotrophic microalga *Crypthecodinium cohnii*

(containing 40-50% DHA of dry weight) and the heterotrophic microalga *Schizochytrium sp.*. Even though both species have been shown to be able to produce EPA at an industrial scale, oil containing EPA is not commercially competitive yet with other resources. The species are grown in fermenters and for both species heterotrophic processes are used. Microalgae and cyanobacteria such as respectively *Chlorella vulgaris* and *Spirulina maxima* are also exploited for human nutrition, because of their high protein content. The protein content of these organisms (*C. vulgaris*: 51-58% of dry matter, *S. maxima*: 60-71%) is higher than that of meat (43%), milk (26%), rice (8%) or soybeans (37%). These species can easily be grown in large quantities using open systems such as raceway ponds, because of their high growth rate (*Spolaore et al.*, 2006). The species *Crypthecodinium cohnii*, *Schizochytrium sp.*, *Chlorella vulgaris* and *Spirulina maxima* produce no toxins that are harmful to humans and as such all fall into the GRAS category (*Walker et al.*, 2005). This means that there are no risks attached to the consumption of these microalgae and that extraction of the nutritional compounds isn't necessary.

Natural food colorants can also be produced using microalgae. Two forms of natural food colorants are commercially produced in this way. The first are pigments called carotenoids (e.g. β-carotene and astaxanthin), which are used as photosynthesis pigments by the microalgae (*Pulz and gross*, 2004). The main nutritional value of carotenoids comes from the fact that one of these carotenoids (β-carotene) can be converted into vitamin A. Astaxanthin is mainly used as a food additive for animals and fish. Furthermore carotenoids have certain properties that are valuable for pharmaceutical reasons, such as intrinsic anti-inflammatory properties. Sometimes therapeutic chemopreventive anticancer properties are attributed to carotenoids, however this has never been proven *in vitro* or *in vivo*. An advantage of natural carotenoids over synthetic carotenoids is that isomers are produced in their natural ratio. It is accepted that the natural isomer of β-carotene is superior to the synthetic one. As mentioned previously β-carotene is produced using *Dunaliella salina* since this microalga produces up to 14% of its dry weight in β-carotene. Astaxanthin is produced commercially using *Haematococcus pluvialis* in a two-stage culture process, which can produce between 1.5% and 3% of the dry weight in astaxanthin. The first culture stage is for biomass production under perfect conditions. The second stage is for the accumulation of astaxanthin under intense light conditions and using nutrient-poor medium. β-carotene can be produced using algae that are grown in open pond systems, because of the specific conditions in which these algae can grow (*Spolaore et al.*, 2006; *Walker et al.*, 2005). The second kind of natural food colorant that can be produced using microalgae are phycobiliproteins (e.g. phycoerythrin and phycocyanin). Apart from being primarily food colorants, these phycobiliproteins can be used as a pigment for natural cosmetics such as lipstick and eyeliner. Furthermore phycoerythrin and phycocyanin are powerful and highly sensitive fluorescent reagents. This makes these proteins suitable for the use as labels for antibodies and as receptors in a fluorescence-activated cell sorter, but are also used in immunolabelling experiments and fluorescence microscopy/diagnostics. The main commercially used microalgae producing these phycobiliproteins are the unicellular *Porphyridium sp.* (Rhodophyta) (*Spolaore et al.*, 2006). Since high value molecules are being produced (up to $1500 US/mg for cross-linked pigments with antibodies (*Spolaore et al.*, 2006)), these algae are grown in large scale photobioreactors to keep conditions to an optimal level for production. When β-carotene and astaxanthin are used for nutrition, extraction of this carotenoid is not necessary, since the microalgae species *Dunaliella salina* and *Haematococcus pluvialis* being used for the commercial production of these carotenoids fall into the GRAS category (*Walker et al.*, 2005). However, when β-carotene and phycobiliproteins are used for applications other than nutrition (e.g. food colorants, pharmaceutical applications and cosmetics), the pigments need to be extracted. Several manufacturers (e.g. Cyanotech) supply these extracted pigments, but prices are high

due to extra production costs (between 300-3000 US$/kg of microalga) (*Spolaore et al.*, 2006).

d. Pharmaceutical biochemicals: Several pharmaceutical biochemicals can be produced by microalgae. Some of these have already been discussed in the nutrition section (pigments: carotenoids and phycobiliproteins). Other biochemicals that microalgae produce are antioxidants, stable isotopes, antibodies (when genetically altered) and toxins.

Antioxidants are health-related compounds of which some have shown to have anti-cancer properties (*Luiten et al.*, 2003). Carotenoids also act as biological antioxidants (Hallmann, 2007). Microalgae produce antioxidants due to their phototrophic life, in which they are exposed to high oxygen levels and radical stresses. The accumulation of free radicals and reactive oxygen species can be prevented using antioxidants to avoid the cell being damaged. The pharmaceutical application of antioxidants is the therapy of oxidation-associated diseases, such as inflammations (*Pulz and Gross*, 2004). The production of antioxidants can easily be done in photobioreactors. This is because the oxygen reaches high concentrations in these reactors, as said earlier, which promotes the endogenous detoxification process against oxidative attack by accumulation of highly effective antioxidants (*Pulz and Gross*, 2004). The microalgal species used for the production of antioxidants is *Dunaliella salina* (*Hallmann*, 2007; *Spolaore et al.*, 2006).

Microalgae are also ideal sources for stable isotopically labeled compounds. This is possible due to the ability to perform photosynthesis, which allows algae to incorporate stable isotopes (^{13}C, ^{15}N and ^{2}H) from relatively inexpensive inorganic molecules ($^{13}CO_2$, $^{15}NO_3$ and $^{2}H_2O$) to more highly valued organic compounds (e.g. lipids and amino acids) (*Spolaore et al.*, 2006). Thus, in theory any phototrophic microalgae can be used to produce stable isotopes in photobioreactors. Several purposes of stable isotopes are for use in gastrointestinal/breath tests, for use in physiological investigations (*Pulz and* Gross, 2004) and determining the structure of proteins, carbohydrates and nucleic acids at the atomic level. Several companies have begun production of stable isotope biochemicals using microalgae, but since the production efficiency is still low and the isotopes need to be harvested, the prices for stable isotope biochemicals can be very high (between 60-38.000 US$/g DW) (*Spolaore et al.*, 2006).

The commercial interest of antibody production using microalgae is also increasing, with anticancer, antimicrobial and antiviral compounds being reported. These are not yet used clinically (*Walker et al.*, 2005), although *Chlamydomonas reinhardtii*, a microalgae species capable of producing human antibodies when genetically modified, is considered as being safe for human health (*Mayfield and Franklin*, 2005). Furthermore although these antibodies have promising results *in* vitro, when used *in vivo* these microalgal antibodies are inactive or even toxic (*Walker et al.*, 2005).

Many microalgae species (e.g. *Alexandrium lusitanicum* and *Nitzschia pungens*) also produce toxins that are harmful when ingested through eating shellfish. However these toxins also have some interesting properties that are still under investigation, such as the cytotoxic activity for use in anticancer drugs (*Pulz and gross*, 2004).

e. Rest: A few other applications for which microalgae can be used are the cleaning of heavy metal polluted soil and for environmental applications. *Euglenida sp.* have not only shown the capability to live in soil polluted with heavy metals, but also the capability to accumulate these heavy metals (*Pulz and Gross*, 2004). The environmental applications of microalgae consist mainly in fixing CO_2. Algae that are very suitable for this task are *Chlorella sp.*, because of their high growth rate and many other applications the alga can be used for.

6. Discussion

According to *Spolaore et al.* (2006) only a few hundred species of microalgae are investigated for biotechnologically interesting compounds and only a handful are used commercially for these compounds (also see table 1.). However there is potential for lots of other microalgae to be used for commercial production processes, since most microalgae produce secondary metabolites. However, only a small part is currently being screened for useful compounds. In my opinion more research needs to go into the screening of biotechnologically interesting compounds in species of algae that until now have been overlooked. This kind of research needs to be done first, before genetic manipulation is considered. There might be microalgae species with the same production properties as some mutant algae already made in laboratories. Since the production using microalgae began, various studies (e.g. *Fabregas and Herrero*, 1990; *Tonon et al.*, 2002) have proven that there might be better species (e.g. species with a broader range of compounds) already available for the various applications. As a consequence a few microalgae being used in some of the commercial applications that have been discussed can be updated in my opinion. This is also true for the way these microalgae are grown, since new techniques have been developed to better grow microalgae.

The current method of biofuel production can hardly be made better, because as mentioned the algae species with the highest oil content and growth rate (e.g. *Chlorella sp.* and *Schizochytrium sp.*) and the highest hydrogen production rate (*C. reinhardtii*) currently known are already in use. However one way to make production of biofuel higher is to use large-scale photobioreactors in which higher cell densities can be achieved (*Pulz*, 2001). This would lead to higher biomass production and thus to more biofuel being produced. Current methods still use open pond systems, such as raceway ponds, which have a lower production rate of biomass. Sadly the technology for large-scale photobioreactors is not yet available (*Rupprecht*, 2009), as biofuel would make a very interesting alternative to fossil fuel.

For antifouling, the best approach would be to continue the search for active compounds with antifouling properties and to combine the known antifouling compounds from marine microalgae, macroalgae and cyanobacteria together in a new type of antifouling paint. Studies by *Bhadury and Wright* (2004) and *Volk* (2005) showed that macroalgae and cyanobacteria also produce compounds that are toxic to specific organisms, such as bacteria, fungi and algae. The most notable macroalga is the Rhodophyceae *Laurencia obtuse*, from which four extracts were isolated with antibacterial and antifouling properties. Most notable cyanobacteria are *Anabaena lutea*, *Arthrospira* (*Spirulina*) *laxissima* and *Nostoc carneum*, from which extracts were isolated that proved to have a high to very high toxicity towards other cyanobacteria (*Volk*, 2005). The combination of antibacterial, antifungal, and antialgal properties would mean in theory that this mix of compounds is able to stop or slow down stage 2,3 and 4 of biofouling by bringing colonization of marine organisms to a halt.

For the production of vitamins, the focus shouldn't be mainly on the production of one vitamin at a time using one species (e.g. vitamin A using *D. salina*). Instead production should also be started using species that produce multiple vitamins at the same time, which would also be more cost efficient. For instance a study by *Fabregas and Herrero* (1990) has shown that certain algae species (*Tetraselmis suecica*, *Isochrysis galbana*, *Dunaliella tertiolecta* and *Chlorella matophora*) exhibit the capability to produce the precursor of vitamin A in high quantities. Next to β-carotene, these species can also produce other vitamins such as ascorbic acid (C), tocopherol (E), biotin (H), folic acid, nicotinic acid and pantothenic acid. The vitamin concentrations of four vitamins (β-carotene, tocopherol, thiamin (B_1) and folic acid) are higher in these algal species than in conventional foods considered as rich sources of vitamins (e.g. orange, carrot and soy flour). Not all the vitamins are present in the same concentration in each species. As such a mix can be made to accommodate for varying diet requirements.

Production of the lipid DHA using the microalga *Crypthecodinium cohnii* is already commercially competitive with other synthesized resources, which means that improvement isn't really necessary in my opinion. The production of EPA however isn't commercially competitive yet. Nonetheless there might be potential to make it commercially viable by using other microalgae species. For instance EPA is also produced by *Nannochloropsis sp.*, *Phaeodactylum sp.* and *Nitzschia sp.* (*Spolaore et al.*, 2006). Research should be done to see if EPA production could be made commercially viable by using these micro-algae grown in photobioreactors.

The current production of proteins and food colorants also doesn't need to be improved by using other algae species. This is because the species used have the highest production of carotenoids or lipids that is currently known and are easily produced in open pond systems due to their ability to grow in specialized environments and their high growth rates. That production of proteins and food colorants already is viable is also due to the fact that production of these compounds, especially phycobiliproteins, isn't restricted to using microalgae alone. Cyanobacteria such as *Arthospira sp.* are also used for the production of protein-rich extracts and phycobiliproteins (*Spolaore et al.*, 2006).

The production of pharmaceutically interesting compounds such as antioxidants, stable isotopes, antibodies and toxins hasn't started long ago, which means that improvement may still be needed. Studies could be done to see which microalgae could best be used for the production of antioxidants and stable isotopes. Especially since in theory every kind of phototrophic microalgae seems capable of producing these compounds, it would be interesting to see which one is commercially most viable.

In conclusion, the commercial exploitation of marine microalgae can be improved in several areas. But for these improvements to be really helpful more research has to be done. First more screening for biotechnologically interesting compounds in marine microalgae has to be done to identify new and better species for the various commercial applications. Secondly more research has to be done in designing, testing and improving industrial large-scale bioreactors, such as tubular photobioreactors, to improve the microalgae biomass production even further.

7. References

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