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The application of marine microalgae

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Abstract:

Food and fuel are becoming limiting. microalgae seem to be a possible solution to both problems. Algae need far less space compared to land plants.

But if they show such great potential why don't we all eat algae and use fuels derived from algae?

Open ponds are a cheap and relatively easy way to culture algae. But only extreme algae can be cultured this way and therefor open systems are limited to a few species.

Bio-reactors have far more control and a larger range of algae to be cultured. Costs are often limiting but a lot of research is being done to optimize closed systems. They show great potential for future algal culturing.

It is unlikely microalgae will become our main food source due to high costs. But in time fuel derived from algae should be able to compete with fossil-fuels.

Keywords: Open pond, Bio-reactor, Bio-fuel

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1. Introduction

Nowadays the world experiences a large number of problems. An example is global warming, due to fossil-fuel burning, along with decreasing fuel reserves and the rapid increase of the world's population. Therefore new methods for the production of our energy and food resources are needed.

In an attempt to increase sustainability, oil products derived from plants are being used as transport fuels. But these oil products, like palm oil or rapeseed oil, can never be sustainable (Chisti, 2008). The area needed to satisfy the world's need is far greater than the planet's surface. If we want to replace only ten percent of our transport fuel with biofuel, we would need to sacrifice thirty to seventy percent of our crop area (Charles et al. 2007). This in turn will put pressure on food availability, therefore only increasing another problem. Nevertheless the worldwide production of oilseed increases each year with an estimated annual production of 418 million tons in 2008-09 (Gayathiri Bragatheswaran, 2008). This still only accounts for 0,3 percent of all the transport fuel used every year (Schenk et al., 2008).

Fig 1: Chlorella and Spirulina tablets used as food supplement (www.earthrise.com).

Phototrophic algae show a promising alternative with yields 9 to 800 times higher than oilseed plants, depending on the oil content (Chisti, 2007). These higher yields not only reduce the area needed for the production, algae can be grown on non fertile grounds as well. Therefore the competition with agricultural land will not be as great as oil derived from land plants. Because of their high protein content (Becker, 2007) and yield per m^2, algae are already used for human consumption. Mainly sold as health food products (see fig 1).

Most of the cultured algae are freshwater species. But besides food and fuel, freshwater is also becoming more and more scarce. By culturing marine, instead of freshwater algae, less freshwater is needed (Horsman et al. 2008).

But if microalgae show such great potential why don't we all eat algae and drive on fuels won from algae?

In this review we will take a closer look on the feasibility of micro-algal culturing. A short introduction to the most common culturing methods will be presented. The following questions will be addressed: Which products are already for sale? What culturing methods are used? What are the restraints for such a seemingly great product? Why are algal products not a bigger part of our lives?

2. Presently applied culturing methods

2.1 Open ponds

The most common way of mass culturing algae is in open pond systems. These systems are outdoor, mainly circular or oval basins (raceway ponds, see fig 2) with a depth of 10 to 50 cm. In deeper basins mutual shading becomes a problem (Atcc et al. 2003). The depth is optimal when cultures get sufficient light and the pond is deep enough for mixing (Kunjapur & Eldridge, 2010). Rotating paddle wheels or stirring arms are used to ensure mixing and movement of the cultures.

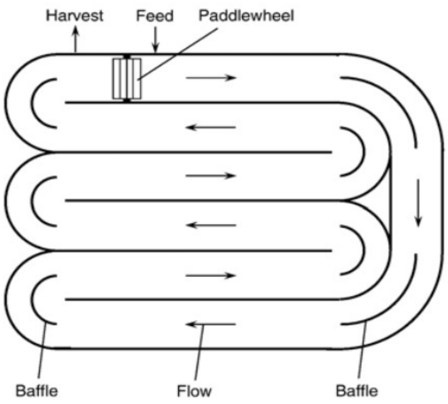

Fig 2: Raceway pond, paddlewheel to create flow, directed by baffles (Chisti, 2007).

One of the biggest advantages of open pond systems is the low costs. No energy input is required as sunlight is the main energy source. Also the manufacturing costs are low compared to bio-reactor systems, another culturing method (Tredici & Materassi, 1992). Also no fertile land has to be used, keeping land prices low.

The downside is the lack of control giving rise to all kinds of limitations. Only a limited amount of species can be cultured in open ponds. Because the ponds are directly exposed to the atmosphere they are easily contaminated by other algae, bacteria, fungi and insects. Water can also easily evaporate changing the composition of the cultures and heavy rainfall can dilute cultures. These changes make the cultures even more susceptible to contamination by other unwanted organisms. To overcome this problem the culture environments are kept extreme (high salinity, alkalinity, nutrients) to keep out any contaminants as much as possible (Brennan & Owende, 2010). Constantly monitoring pH, salinity, nutrition and other important factors and adjusting the system to changes in these factors is crucial to maintain a somewhat constant environment. These extreme environments limit the choice of algal species, leaving only a few algae to be cultured in open ponds: *Dunaliella sp.* (high salinity), *Spirulina sp.* (high alkalinity) and *Chlorella sp.* (high nutrition). Each of these will be explained shortly..

Spirulina (Arthrospira) is a filamentous cyanobacterium that is used in health food products (it contains a lot of essential fatty acids and vitamins), animal feed (mainly aquaculture) and as food coloring (blue). It grows naturaly in alkaline lakes but is also cultured in open ponds. In 2004 the annual production for *Spirullina spp.* was estimated at 3000 tons (Shimamatsu, 2004).

Chlorella is the genus of single celled green algae, mostly cultured in open ponds in countries in the Asia-Pacific ring. *Chlorella spp.* is mainly used as a health food supplement in the form of tablets or powder (it is claiming to be a great a powerful detoxification aid and strengthening the immune system). Also live *Chlorella sp.* is cultured for the feeding of juvenile fish in aquaculture (Lee, 2000). Mostly the entire biomass of C*hlorella spp.* is used when sold as health food; this is also true for S*pirulina spp.*.

Dunaliella sp. is also a green algae, like C*hlorella sp.*. Instead of it being cultured for its entire biomass *Dunaliella* is cultured for the β-carotene it produces. The Nature Beta Technologies Ltd (NBT) in Eilat (Israel) with a pond area of 50,000 m2, produces 1.5t β-carotene or about 25t *Dunaliella bardawii* (Lee, 2000). β-carotene is a valuable pigment that is used as a coloring agent and pro-vitamin A (retinol). *Dunaliella* sp. is cultured in extremely saline pond. Therefore contaminants are rare. Also in Australia *Dunaliella sp.* is cultured. Here they use large unstirred ponds. Because of the near optimal conditions stirring is less important.

Anabaena spp. is a marine nitrogen-fixing cyanobacterium that produces the valuable phycobiliproteins, used as natural dyes in food, cosmetic industries and as fluorescent tracers (it is highly fluorescent and therefore used in microbiology to label

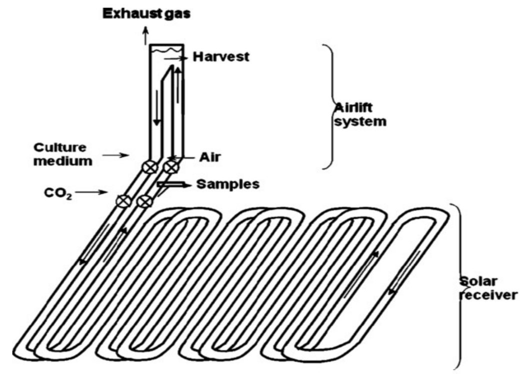

Fig 3: Tubular reactor (Kunjapur & Eldridge, 2010)

DNA and other proteins). In a small scale experiment they were also cultured in open ponds. Because of the filamentous growth and capability to fixate atmospheric nitrogen restricts the danger of contaminants. Results showed that open ponds had a higher productions compared to closed systems (José Moreno et al. 2003).

2.2 Bio-reactors

To overcome the lack of control in open pond systems, bio-reactors have been created. They are based on a closed system, instead of an open one. There are many forms of bio-reactors. The tubular reactor for instance (see fig 3), but also the flat panel reactor, column reactors and vertical reactors are a commonly used form (Xu, Weathers, Xiong, & Liu, 2009). ``Big bags´´ are perhaps the most basic bio-reactors. As the name suggest it are large bags filled with up to 1000 l of medium. Marine microalgae used as aquaculture feed are often cultured this way. Species like *Skeletonema sp., Chaetoceros sp., Thalassiosira sp., Tetraselmis sp.* and *Isochrysis sp.* (Browitzka, 1999).

Because the system is closed and algae produce oxygen, a degassing method is needed. Too much oxygen in the systems is bad for algal growth. A degassing column that is bubbled with fresh air resolves this problem.

Some algal species have the tendency to stick to the walls of bio-reactors. Increasing flow rate of the system or cleaning it once in a while resolves this problem. Increasing flow rate is favored over cleaning, since this is less costly, but increasing flow rate may damage cells.

Open ponds seem to have reached there limits after years of research but bio-reactors are relatively new. The main advantages are better control, often better productivity, no evaporation, able to culture more species and a better temperature profile. Products from a closed system can be called more “clean” because contaminants can't get in the system (Borowitzka, 1999). One of the biggest advantages is that photobioreactor systems are not being restricted to only a few algae strains. Therefore more types of product can be grown in closed systems. Also yield per m2 is often higher in bio-reactors but economically open ponds seem to be more efficient due to lower costs. Therefore high value products are mostly being cultured in closed systems.

The biggest downside of photobioreactors is the costs. Bio-reactors are much more expensive to produce and they also cost more to maintain. As stated above the absence of evaporation is positive since there is no need for large amounts of water (also no rain can come in so there is no dilution). In open ponds though, the evaporation also cools the system. Because it lacks this natural type of cooling, expensive cooling systems are often needed in closed systems (Tredici & Materassi, 1992).

A lot of research is being done to develop new and better working bio-reactors. Take the Green Solar Collector (GSC) for example (Zijffers et al. 2008), where a light guiding system is created to ensure all incoming light is distributed equally throughout the culture and thus increasing efficiency. As more scientists come to the conclusion that open pond systems are near their maximum, bio-reactors are seen as the system of choice to optimize. See table 1 for a summary of the advantages and disadvantages of open and closed systems.

2.3 Hybrid systems

The hybrid system is also a possibility. Both open ponds and bio-reactors are combined in these setups. *Haematococcus pluvialis* a green alga is cultured for its astaxanthin (a red carotenoid). First it is cultured in bio-reactors with enough nutrients, but when transferred to nutrient poor open ponds the algae starts producing astaxanthin. Finaly the algae are harvested, the ponds are cleaned and the process start over again (Schenk et al., 2008).

But also in the production of bio-fuels a two stage culturing method is often used. The first stage is to multiply the alga, the second stage is to deprive the alga from nutrients. In this stage the alga start forming products that can be used as fuel. More about bio-fuels will be discussed later on.

Table 1: Summary of advantages and disadvantages of open systems compared to bio-reactors

System:	Advantages	Disadvantages
Bio-reactor	Better control, no water loss, better yield, more species can be cultured	High costs, cooling often required
Open pond systems	Low cost, easy to maintain, no competition for agricultural land	Limited amount of species, easily contaminated, temperature fluctuation, poor mixing

3. Algae culturing for consumption

Man started using microalgae as a source of food a long time ago, it is believed that the Aztecs consumed natural *Spirulina sp.* (Pulz & Gross, 2004). Won from freshwater lakes. The culturing of microalgae on the other hand started at the beginning of the 1960s in Japan (Borowitzka, 1999). *Chlorella (Chlorella vulgaris)* was the first mass cultured micro algae.

A decade later *Spirulina (Arthrospira platensis)* was harvested from Lake Texcoco in Mexico. Here *Spirulina sp.* grows naturally but in the 1980s Thailand started culturing *Spirulina sp.* and more countries followed. Also, due to increasing industry in Mexico City, Lake Texcoco became more contaminated, which led to the demise of the *Spirulina sp.* business there. It was forced to close and production shifted to Asia and the United States. As seen in figure 4 the production of *Spirulina sp.* increased rapidly starting in the 1970s. Production mainly concentrated in Asia but it was quickly followed by the USA. Both *Chlorella sp.* and *Spirulina sp.* are mainly sold as health food products. Due to the increasing demand and competition in the production of *Spirulina sp.* and *Chlorella sp.* the emphasis became more about quality. Closed systems are less susceptible to contamination and therefore ensuring better quality. In the year 1996-1997 heterotrophic growth of *Chlorella sp.* was even bigger then open culture systems in Japan (Lee, 2000).

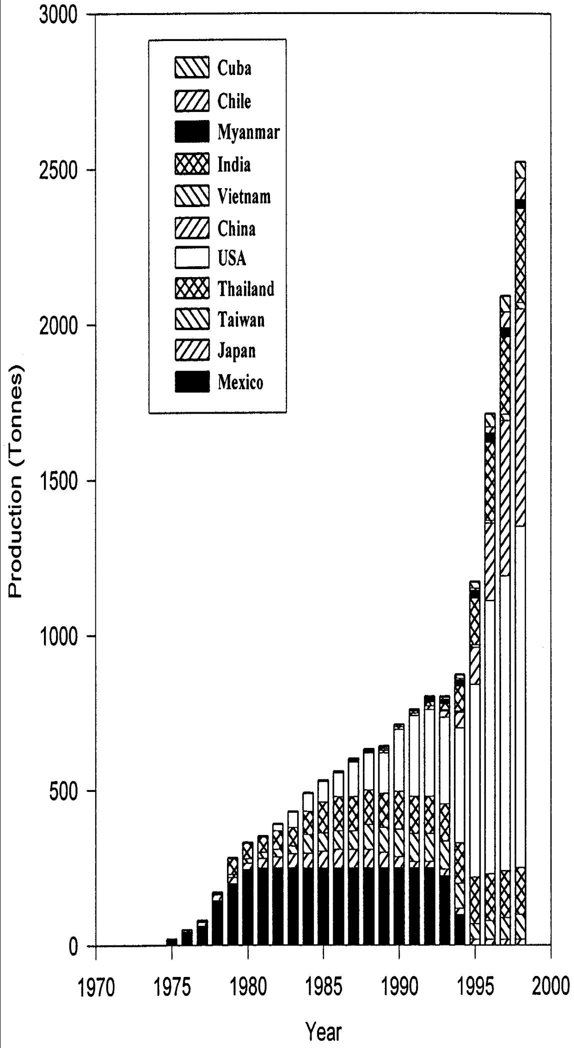

Fig 4: Spirulina production in tonnes/year (Borowitzka, 1999)

Sugars are added, instead of light as the main source of energy for growth. This system ensures great homogenous cultures but energy input is required and therefore will not be discussed.

In Klotze (Germany) a closed bio-reactor was build for the phototropic production *Chlorella vulgaris.* On their website (www.algomed.de) they promote the advantages of a closed system opposed to open pond systems.

Fig 5: Algomed facility in Klötze Germany

A new trend in the production of algae for consumerism are functional foods. This are common food products with added substances such as algae to increase health benefits (Plaza, Cifuentes, & Ibanez, 2008). This might be a step towards more algae in our daily food intake. This has already been tested in different types of food, like noodles and bread. However, due to fishy taste of the alga the product does tend to be distasteful, making it hard to corporate algae in everyday food (Becker, 2007). But also the financial aspect has to be taken in to account when looking at algae as a food source. When comparing prizes of open pond against bio-reactor cultured *Chlorella vulgaris* there are some differences. The German company (www.algomed.de see fig 5.) sells their *Chlorella vulgaris* for 300 euros per kilo. The Californian company Earthrise (www.earthrise.com) grows their *Chlorella vulgaris* in outside open ponds and sells their product for 200 euros per kilo. The differences in price can be explained by the quality. *Chlorella vulgaris* produced in closed systems is less likely to get contaminated.

Annually 3000 tonnes of *Spirulina spp.* and 2000 tonnes of *Chlorella spp.* (in dry weight) is produced at the cost of 36 euros per kilo on average (Brennan & Owende, 2010). So although the profit on algae is almost ten times the costs, kilo prices are still a lot higher than more common food sources. With these prices it seems impossible to feed the world with algae. But there is more potential for algae in the food industry. High value products such as polyunsaturated fatty acids (PUFAs) derived from microalgae have a promising future. PUFAs are particularly found in marine microalgae (Harwood & Gurschina, 2009). Since only plants and alga can create these PUFAs, harvesting them directly from the algae instead of fish for example has its benefits. Contaminants are less likely to be in the product and there will be no fishy taste to it (Pulz & Gross, 2004). Docosahexaenoic acid (DHA) derived from the marine microalga *Crypthecodinium cohnii,* a microscopic dinoflagellate, is sold for 43 euros per gram. *C. cohnii* is grown in fermenting tanks and not with the use of sunlight (Swaaf et al. 2003).

Even cremes and lotions contain algae, claiming it has essential minerals and vitamins that rejuvenate the skin. It seems that the term "algae" has become hot and a product will seem exclusive if it contains algae (Pulz & Gross, 2004).

Table 2 shows a short summary of some cultured algae, their applications and the country were they are mostly grown.

Table 2: Algae species and their products (Kunjapur & Eldridge, 2010)

Species	Products	Country	Culturing method
Chlorella spp	Food, cosmetics, animal feed	China, USA, Myanmar, Japan	Open ponds (also closed in Klötze but mainly open ponds)
Spirulina spp	Food, cosmetics, aquaculture	Taiwan, Germany, Japan	Open ponds
Dunaliella salina	Food, cosmetics, b-carotene	Australia, Israel, japan	Open ponds
Crypthecodinium cohnii	DHA oil	USA	Closed systems
Haematococcus pluvialis	Astaxanthin(a red carotenoid)	USA	Closed systems
Anabaena spp	Phycobiliproteins	-	Open ponds (small scale)

4. Bio-fuels

Due to the large economic importance of fuels, bio-fuels gained a lot of interest in the last decade. Some algae produce oils that can be used as fuel. With increasing fuel prices more research is being done on oil producing algae.

But also bio-fuels derived from algae seem to be the only sustainable option. The carbon the algae need to grow is removed from the atmosphere and is released again when the oil is burned. This way algal oil is completely carbon neutral and does not contribute to global warming. Other crop derived bio-fuels already exist but simply need too much space competing with food crops to be sustainable. Table 3 shows the difference in yield between seed plants and algae. If this seems to be the solution to our fuel needs, what is the bottleneck that is slowing this development down?

Table 3: Biodiesel yield comparing seed plants with algae (Scott et al., 2010)

Species	Oil content per tonne of biomass (wt% dry mass)	Biodiesel yield (L/ha/y)
Oilseed rape	40-44%	1560
Soya	20%	544
Chlorella vulgaris	Up to 46%	8,200
Nannochloropsis sp.	Up to 50%	23,000-34,000

With bio-fuels it is relatively easy to determine whether a product will be successful or not. Prices should simply be the same or lower than present oil prices. Depending on the oil contents per dry matter of algae (ranging from 15 percent to 55 percent) the costs of algal culturing has to be reduced by a at least fraction 9 times to be competitive with fossil derived oil. In this example closed systems were used to culture the algae. The alga had a oil content of 55% and oil prices were 100$ per barrel (Chisti, 2008). So when oil prices increase, the feasibility will increase as well.

After harvesting the algae they also need to be processed to create bio-fuel. But the costs for this are expected to be the same or even lower than the costs for processing crude oil. Since the cells are not a hundred percent oil, waste is created when making algal bio-fuel. This waste can be used to create food for animals or to generate electricity. Things like this can contribute to the feasibility of algal derived bio-fuels since these products can be sold for profit as well.

Due to better control bio-reactor are the system of choice. Column reactors seem to have the best photosynthetic efficiency and productivity on small scale. The problem with column reactors however is up-scaling. Multiple small units are needed which is not cost efficient ad therefore unlikely to be economically feasible (Kunjapur & Eldridge, 2010).

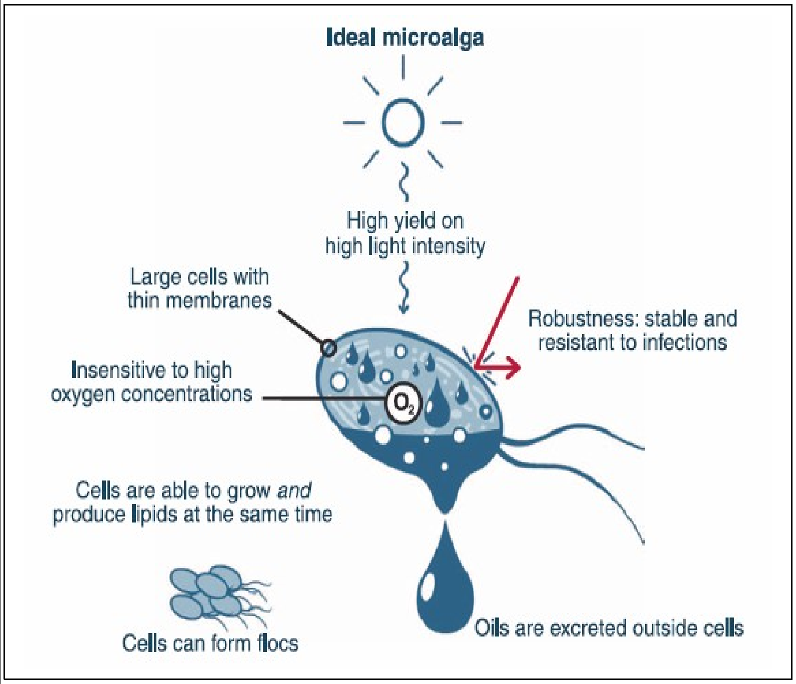

Fig 6: The perfect alga for bio-fuel (Wijffels & Barbosa, 2010)
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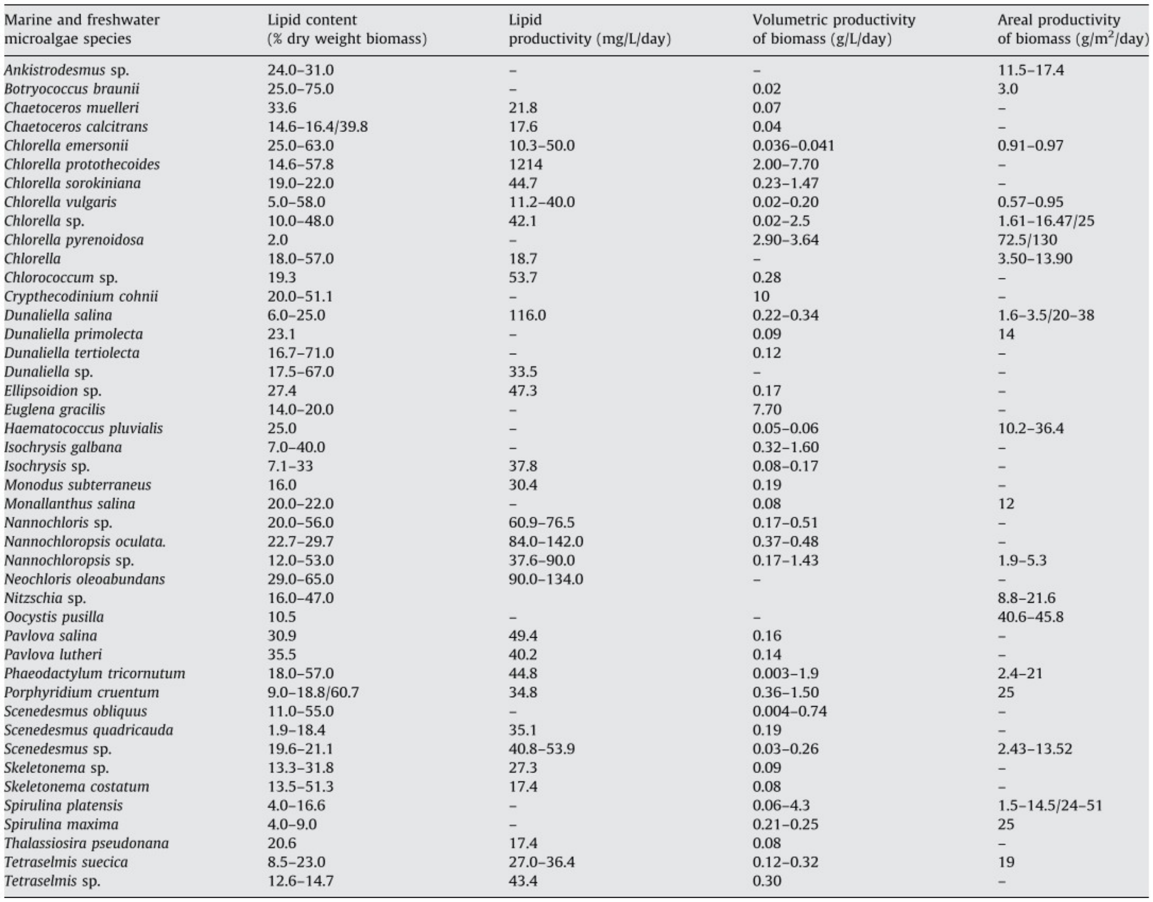
Not only the system of cultivation is important but also the strain itself. Figure 3 shows the perfect alga for the production of bio-fuel. Able to grown with high light intensity. High oxygen concentrations so degassing of the system is no longer required. Oils excreted outside the cell so further processing is cheaper and does not involve breaking up the cells. Sadly an alga like this does not exist. There are algae that have some of these characteristics but not all of them combined (Wijfells & Barbosa, 2010).

Until the perfect alga is found or created production and lipid content are the most important criteria when selecting an strain to produce bio-fuel. Table 4 shows a list of algae with there productivity and lipid content.



Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m^2/day)
Ankistrodesmus sp.	24.0–31.0	–	–	11.5–17.4
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	–
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	–	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	–
Crypthecodinium cohnii	20.0–51.1	–	10	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Ellipsoidion sp.	27.4	47.3	0.17	–
Euglena gracilis	14.0–20.0	–	7.70	–
Haematococcus pluvialis	25.0	–	0.05–0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	–	0.32–1.60	–
Isochrysis sp.	7.1–33	37.8	0.08–0.17	–
Monodus subterraneus	16.0	30.4	0.19	–
Monallanthus salina	20.0–22.0	–	0.08	12
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0			8.8–21.6
Oocystis pusilla	10.5	–	–	40.6–45.8
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	–
Skeletonema costatum	13.5–51.3	17.4	0.08	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	–

Table 4: Lipid content and productivity of different microalgae species (Mata et al. 2010)

5. Future prospects

Possible future solutions can be found in genetic modification. By engineering cells to cope with extreme environments, more species would be able to be cultured in open pond systems. Also modifying cells to have more efficient photosynthetic systems or metabolic systems could increase yield (Chisti, 2008).

Optimizing algal culturing is needed to increase the possibilities of algal usage. This can be done by finding new algae, optimizing the culture systems or optimizing the algae them selves. Only a small percentage of all the different algae have been described and even fewer have been thoroughly researched. When more algae are being studied it is likely that new useful substances and compounds will be found. But even before new strains and species are discovered there is a lot that can be done to change the algal market.

Open ponds seem to have reached their potential, therefore more research is done to increase the efficiency of bio-reactors. For example the work on the GSC project mentioned in before. But also a new project in Wageningen, the Netherlands, where four setups are going to be tested and researched thoroughly (see www.algae.wur.nl for more information).

Mainly in modifying strains are studied to increase productivity and growth environment. Strains of algae are being researched too, for example the increase of photosynthetic and metabolic efficiency (Melis et al. 1999). Modifying strains in such a way that they can grow in extreme environments and therefore can be cultured in open ponds is a way to overcome one of the biggest limitations of open ponds. Reducing antenna size of algae to reduce self shading and increase solar efficiency is another example (Melis et al.1999). With the help of new culturing techniques, mainly in closed systems, it should become cheaper and easier to grow algae in the future.

6. Discussion

It is clear when looking at the current application of microalgae names as *Chlorella spp., Spirulina spp.* and *Dunaliella spp.* stand out the most. This is because in biomass, they contribute the most to annual production. Also they have been cultured and consumed for a long time now.

So too answer the question which products are already for sale, mainly health food products derived from *Chlorella spp.* and *Spilulina spp.*. But also the more valuable β-carotene derived from *Dunaliella spp.*. Marine species only account for a small fraction of the total production. They are mainly produced in closed systems for high value products such as PUFAs. As bio-reactors will become more efficient and more algal species will be discovered, marine microalgae culturing will increase.

For consumption it is not likely micro-algae will ever become our main source of food. Not only because of the taste, but mainly due to high production costs.

The restraints for the culturing of algae are often costs. When looking at bio-fuel from microalgae, the costs have to decrease significantly. This can be done as described in the previous part and offers great potential.

It is often estimated algae derived fuel will be able to compete with fossil-fuels within a decade. Countries like Portugal and Spain have the right climate and enough space to accommodate large production facilities. The question remains if we have enough land space to completely shift our global fuel needs to algal derived fuels. But in due time with decreasing fuel reserves we are forced to look at other options and algae look promising.

When more progress will be made, which is only a matter of time and effort, microalgae will become a bigger part of our lives.

7. Conclusions

Although algae have been cultured for almost half a century, they still do not form a big part in most of our lives.

Open systems are limited to only a few species and bio-reactors are often to expensive and hard to scale up.

Bio-fuels offer great potential but are still too expensive to compete with fossil derived fuels.

More research is required but the prospects are looking good. Algae will probably be a bigger part of our lives in the future. Maybe one day we will eat algae and drive on fuel derived from algae.

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