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1. Introduction

The field of research focused on biofuels from algae is still relatively new, as compared to more established biofuel feedstocks such as corn, sugar cane, oil palms or rape seed. In addition, the aquatic nature of algae make them fundamentally different in terms of large scale cultivation, harvest and processing, The scientific results of recent years have clearly demonstrated the potential of algae as a feedstock for biofuel manufacturing and much progress has been made in understanding algal molecular and physiological mechanisms as well as the engineering necessary for large scale cultivation. However much remains to be investigated further.

This document is not intended to give a complete overview of all that is unknown about algae, but to define the most important needs for further research for the coming 5-10 years. This includes molecular and biochemical understanding of the pathways involved in growth, tolerance to extreme conditions and synthesis of sources for biofuel (i.e. starch, lipids or cellulose). Understanding how algae produce these molecules and under which conditions is crucial to reach a high areal biofuel yield, either by optimizing the conditions for the algae, or by optimizing pathways through genetic modification.

Besides what is happening inside the cell, the more general physiology and behaviour on ecological level are also subject of research. To determine the optimal growth conditions, essential nutrients or optimal time for harvest (e.g. in case of macro algae), but also to assess the impact or large scale algae cultivation on the surrounding ecosystem in terms of water usage, waste disposal or in case of GMO species the prevention of proliferation in the wild. Finally the technical issues related to large scale cultivation are discussed. For macro algae these can be large floating farms in the ocean, whereas for microalgae controlled open pond or photobioreactor systems are being developed. Strategies for, for instance, optimal dimensioning, mixing, (de)gassing or cooling are subject for research.

Maybe even more challenging are the steps that follow cultivation, such as large scale harvesting, dewatering and separation to obtain the desired sources for biofuels. Especially because the overall energy balance needs to remain positive and therefore the energy input in all process steps has to be very limited, existing techniques usually are not adequate and newer, more energy efficient methods need to be developed.

Finally, the specific technologies required to produce different types of biofuels from lipids, starch, or whole biomass are discussed. Fuels such as biohydrogen, bioethanol, biodiesel, biogas or bio-oil each require very different kinds of technologies. Some are very similar to what is common in the petrochemical industry, while others are still very new and need to be optimized and developed further in order to become truly viable.

This document focuses on the most urgent and the most promising research needs for algal biofuel identified by the members of the AquaFUELs consortium. In the appendix an overview is given of what research needs are identified in scientific literature and by other major international stakeholders in the field. Through focused research on these targets we can bring this field to full maturity and offer a real and viable alternative to fossil fuels.

2. Microalgae

Despite being a potential source of renewable fuels, the production capacity for microalgae is presently limited in comparison to land based energy crops. The current worldwide microalgal manufacturing infrastructure (producing the equivalent of approximately 5000 tons of dry algal biomass) is devoted to extraction of high-value products such as carotenoids and ω-3-fatty acids used for food and feed ingredients. The total market volume is €1.25 billion, implying an average market price for microalgae of €250/kg dry biomass (Pulz and Gross 2004). As an example for comparison with land based oleaginous crops, the world production of palm oil is nearly 50 million tons with a market value of approximately 0.50 €/kg (faostat.fao.org). Production of microalgae for biofuels needs to take place on a much larger scale at much lower costs. If all transport fuels were to be replaced by biodiesel in Europe, there would be an annual need for nearly 0.4 billion m^3 (IEA). If this biodiesel were to be supplied through microalgae, 9.25 million ha (almost the surface area of Portugal) would be needed in order to supply the European market, assuming a productivity of 40,000 litres per ha per year. This productivity is based on a 3% solar energy conversion to biomass (theoretical maximum is 9%) and a biomass oil content of 50%, under the solar conditions of Portugal. A leap in the development of microalgae technology is therefore required; on a practical level, the scale of production needs to increase at least 3 orders of magnitude with a concomitant decrease in the cost of production by a factor 10 (Norsker et al 2010). In the past few years there has been a rather polarized debate between researchers in the field about technology readiness and the prospects for productivity enhancement, with some parties pressing for scale-up and commercialization now, while others cautiously stress the need for additional research leading to more careful step-by-step development (Wijffels and Barbosa 2010). Production of microalgae for co-production of biodiesel and bulk chemicals can become economically feasible. If the technology develops we expect that the cost price of production of microalgae will reduce gradually. Microalgae are now produced for high-value products in niche markets, however, if the cost price of production goes down it is expected that with every step in reduction new markets will open. Initially most probably production of edible oils for food and fish feed will become feasible, but after some time also production of bulk chemicals, biomaterials and biodiesel may become feasible. For that the technology needs to develop from a small size activity to an industrial scale technology. We expect that such a development will at least take 10 years. For that a multidisciplinary approach needs to be developed and integrated research is required at several levels.

2.1 Molecular level

Systems biology in microalgae has hardly been developed. In the first place (micro)algae originate from different families, varying from prokaryotes like cyanobacteria to eukaryotes like green algae (chlorophytes), which makes it difficult to develop one system to improve certain aspects in microalgal metabolism.

Establishing industrial production with algae requires among others, in depth knowledge of basic biological functions and tools for steering the metabolism, with the objective to improve for example the photosynthetic efficiency in photobioreactors or to enhance lipid productivity. This can be done by an optimal design of conditions inside the reactor or by metabolic engineering.

Key information in the successful application of both optimal conditions design and metabolic engineering is the availability of well annotated genomes and quantitative tools for genome-scale metabolic models that permit understanding and manipulation of the genome. There are still very few algae for which full or near-full genome sequences have been obtained and transvection systems have hardly been developed.
An additional challenge remains to integrate the genome data sets with data sets from other levels of biological organization. An integrated approach using state of the art -omics technologies, such as genome sequencing, transcriptomics, metabolomics, proteomics, metabolic modeling (fluxomics) and bioinformatics, is needed in order to gain the best possible insight into metabolic pathways leading to the product of interest. This systems biology approach is the base for the enhancement of the physiological properties of algae strains and optimization of algae production systems.

Even though this described approach is more and more used in microbial and plant sciences, it is still new in algal biology. It is expected that research in this field will develop quickly and tools will become available to improve photosynthesis in algae, to enhance productivity of lipids in microalgae and many other feature that may lead to reduction in cost prices or a higher reliability of the whole process chain. Especially in the US large research programs on genetic engineering of algae have been developed. If Europe wants to keep up with the activities in this field research has to be stimulated significantly in this area.

Annotated genome of the top 10 microalgae general

The complete genome of the top 10 microalgae genera must be sequenced in order to reveal the adaptation of these organisms, to understand algae diversity and to access to high relevant genomes; this information can be further use in various industrial processes such us to increase fatty acids yield and profiles.

DNA chip for fast identification / screening

Species-specific identification (complementary to microscopy for rapid and specific detection of microalgae).

The development of a number of transgenic algal strains

Development of high recombinant protein expression in transgenic algae.

Establish a number of indispensable genomic tools

i.e. to generate a large-scale mutant banks; use of microarrays; to develop routine and fast techniques for map-based cloning and others.

To develop algae bioinformatics

Assembly with known algal genes; Phylogenetics analysis; BLAST search for homologues.

Using molecular-assisted alpha taxonomy (MAAT)

Potential technique in reassessing biodiversity.

Genetic modification of the lipid pathways of algae

e.g. by up-regulation of fatty acid biosynthesis or by downregulation of β-oxidation.

Genetic Engineering (limitations)

By engineering or selecting your algae to perform better for a specific metabolic pathway, you will necessarily miss out on some other metabolite; It must also be kept in mind that biomass production at a large scale is likely to happen initially in open systems where dissemination is not controlled; therefore there is a risk that genetically modified cultivated populations would mix with wild type populations.

2.2 Biochemical level

For commercial production of biodiesel from microalgae it is essential to have high lipid productivity. In non-stressed growing algae, lipids are mostly present in the form of phospholipids in the cell membranes. Some microalgae when exposed to stress conditions (e.g. nutrient deprivation, high salt concentrations, high light intensities), accumulate lipids in the form of triacylglycerols in so called oil bodies. This is done at the expense of energy used for growth, leading to a decrease in growth rate and a consequent decrease in productivity. Knowledge of the biosynthesis mechanism of triacylglycerols and their accumulation in oil bodies is limited and often based on analogies with higher plants (Hu et al. 2008). If the mechanisms were known, it could open the possibility to have lipid accumulation in oil bodies without having to apply a stress factor, which could lead to higher productivities for a prolonged period of time. A detailed insight into metabolic pathways may lead to strategies to induce lipid accumulation based on process conditions, defined nutrient regimen and/or use of metabolic engineering techniques. For this well annotated genomes and quantitative tools for genome-scale metabolic models that permit understanding and manipulation of the genome need to be available.

Genome based metabolic flux models are in its infancy and are expected to be developed in the coming years. With these metabolic flux models we will be able to understand and steer metabolism in microalgae with the objective to improve for example the photosynthetic efficiency in photobioreactors or to enhance lipid productivity. Metabolic flux models can both be used to design the conditions in a reactor such that a better process is obtained but can also be used to target metabolic engineering approaches.

Complete elucidation of the metabolic pathways for pigments and lipids produced by

Each algae will have its own lipid profile thereby it is crucial to utilize species that have a suitable lipid profile for biodiesel production, understand the metabolic factors involved in fatty acids biosynthesis.

Biosynthesis mechanisms of protective compounds as glycerol or starch

Protective mechanisms by which algae cells become more halotolerant.

Metabolic engineering approaches

The identification of proteins or metabolites gives clues to rate-limiting process in the cell, which can be backed up by the determination of metabolic flux.

Identifying differentially expressed proteins

Utilization of enzymes/proteins that are either directly involved in lipid biosynthesis and degradation or that are co-ordinately regulated for improving oil production.

Utilization of "key enzymes"

These enzymes can improve both quantity and quality of lipids (chain length and saturation grade); the ester content dictates the stability and performance of the fuel.

Induce the accumulation of lipids

Under certain growth conditions, microalgae can be induced to accumulate substantial contents of lipids; some species can reach 90% w/w_{DW}.

Fatty acid profile

The fatty acid profile of the algae cells must be determined since the heating power of the resulting biodiesel hinges upon that composition.

Biochemical engineering approaches

Enhancement of lipid production by microalgae using biochemical engineering approaches relies on creating a physiological stress such as nutrient-starvation (e.g. nitrogen) or high salinity (salt concentration) to channel metabolic fluxes to lipid accumulation. Tolerance to this stress factors can be also used as control tool to avoid contaminations or strain dissemination.

Metabolic "shifts" in response to changes in environmental conditions

i.e. growth under autotrophic, heterotrophic and mixotrophic conditions in order to enhance lipids production by microalgae, although heterotrophic and mixotrophic growth compromise the sustainability benefits of algae as a source for biofuels (because additional feedstocks are required).

Scale-up of the lipid production

(i.e. nitrogen limitation; extra CO_2 supply and iron availability).

Microalgae cultivation

Species to be cultivated have to be selected according to stability of the strain; time of generation; nutritional requirements, yield in oil and fatty acids profile.

2.3 Cellular level

In the past most research developed was on algal physiology. The knowledge on cell cycles, growth and nutritional requirements is present for commercial strains used at this moment. Nowadays the focus shifted towards lipid accumulation. Production of lipids needs to be done at much larger scale, in continuous processes, with residual nutrients (N and P) and CO_2 feedstocks and for lipid accumulation. Current knowledge we have is not applicable for these bulk applications and research is required especially dedicated to these aspects. In this bulk production area we believe Europe can obtain a much stronger position than US. Technology for production of microalgae in US is largely based on pre-cultivation in bag cultures and scale-up in batch open ponds. While research on optimal process conditions, like nutrient utilization, in both open and closed systems is required as the full potential in process engineering for microalgae has not been reached yet.

At this moment accumulation of metabolites such as triacylglycerides is most probably maximized at a certain combination of light supply and supply of certain nutrients such as nitrogen and phosphorous. We do not know where that optimum is and whether it has to take place in a 1 stage process or a 2 stage process is also not known. Rodolfi et al. (2009) recently showed lipid productivity can be enhanced at certain levels of nitrogen limitation in a continuous process. Lamers et al. (2009) showed that turbidostats are a very nice tool to search for optima in continuous cultures. Continuously operated turbidostat systems are well suited for control of both light and nutrient regimes. Specifically of importance is whether lipids can be produced under catalytic conditions. Would it be possible under continuous starvation of nitrogen and phosphorous to harvest lipids in a continuous way. Additional topics to be studied are cultivation under extreme conditions (high or low pH, high oxygen concentrations, and changing temperatures) and especially high temperatures, high salt concentration, culture stability, biofilm formation and fouling. High-throughput screening approaches probably will be of importance to test all these variables (strains and conditions).

Cell wall and cell membrane behaviour

Annotated composition of several microalgal genera, in order to understand the cell wall behaviour, regarding the optimization of the mass balance and the optimization of cell wall degradation.

Understanding cell cycles to improve growth

Acquire complete knowledge on each microalgal genera cell cycles, including circadian cycles, allowing the optimization and eventual synchronization of the production process and its inputs and outputs, maximization of added-value compounds production, etc.

Optimizing nutrition and efficiency of utilization of nutrients
Based on the molecular composition of each microalgae genera, optimizing the nutrient medium in order to minimize its wastage, and consequently microalgae production operation costs.

Optimizing of strain-specific cultivation conditions
With any interrelated factors that can each be limiting.

Efficient harvesting of biomass
Selection of the most appropriate harvesting technique depends on the density and size of microalgae.

Microalgae physiological under stress response
Information that will reveal the regulation networks that are responsible for microalgae behaviour during cultivation.

Genetic selection / strain selection
Define the "criteria for strain selection" (productivity, robustness, harvestability, biomass composition, processability/extractability, added-value of co-products); Research on selective breeding is needed to improve productivity. Research in maintenance of the culture of gametophytes (the "seed bank").

2.4 Ecosystem level

Microalgae production has impact on several different ecological factors under which water usage, CO_2 production, nitrogen and phosphorus levels, location and land use, adding to this the influences of the environment on large scale systems and their stability.

- In biofuel crop production water usage is an important sustainability parameter. For the production of 1 liter biofuel from fuel crops, approximately 5,000 litres water is needed (de Fraiture et al 2008). Microalgae need much less water quantities. For photosynthesis alone about 0.75 liter of water is needed per kg biomass produced (Kliphuis et al. 2010). Per liter of biofuel, assuming a lipid content of 50% 1.5 liter of water is required. In practice water use in production systems is much larger because water is also used for cooling of closed systems and fresh water needs to be added to open ponds to compensate for evaporation. If closed systems are cooled with large salt water buffers using heat exchangers, freshwater usage can be reduced significantly. On top of that microalgae can be grown on salt water and thus will not compete with food crops grown on arable land. If some source of water is available and supply of nutrients and CO_2 is secured, algae can be grown anywhere; even deserts would be suitable if there is access to salt aquifers. Growth could also take place in floating systems on large water surfaces, especially if this is done in areas that are protected from the wind.
- Production of large quantities of biomass requires like water a large amount of CO_2. A minimum of 1.8 tons of CO_2 is needed to produce 1 ton of algal biomass. This means that minimum 1.3 billion tons of

CO_2 would be required for the production of 0.4 billion m^3 of biodiesel to supply the European transportation market. The European Union produces 3.9 billion tons of CO_2, so production of microalgae would be a great way to use up excess of CO_2. However, the distance across which CO_2 may need to be transported to make production feasible is a matter of concern.

- The main nutrients needed for the production of microalgae are nitrogen and phosphorus. The biomass of the algae consists of 7% nitrogen and 1% phosphorus. Consequently, for the European biofuel market approximately 25 million tons of nitrogen and 4 million tons of phosphorus are needed. This is about twice the amount that is presently produced as fertilizer in Europe (van Egmond et al 2002). For sustainable production of biodiesel from microalgae it will be important to make use of residual nutrient sources (about 8 million tons of nitrogen in Europe) and recycle nutrients as much as possible.
- Important questions to be answered are the location of production and the size of a production unit. In case light is the limiting factor production should take place at locations with a lot of sunshine. However, we need also to consider the availability of water, nutrients and CO_2, availability of land, etc., and the costs of transporting feedstocks and end products.
- The size of a production unit is relevant as well. In chemical technology processes become economical in case the scale of the process increases. Reason for that is that the investment costs are relatively high. In case of microalgae this might be different as energy requirements for supply of diluted feedstock is important. It is unclear at this stage what scale of production gives the best economy.
- For biodiesel production the scale of production needs to be so large that axenic operation will be extremely difficult. The process and strains need therefore to be robust and stable under production circumstances. In addition strains should be resistant to possible infections with wild strains. Ideally it should be possible to grow microalgae at large scale under extreme conditions such as high or low pH, high temperatures or high salinity to avoid contaminations. Cultivation of microalgae at high pH (10-11) brings an additional advantage, which is a higher mass transfer of CO_2 and therefore a decrease in energy requirement. Supply of CO_2 is essential to achieve high productivities but its supply to the culture (often done by sparging) is also an important cost factor in terms of energy.
- For large scale production new control strategies must be developed that allows including the knowledge of metabolic pathways of the cells in the optimization of the yield of the process. Because to achieve the goal of producing biofuels from microalgae it is necessary to perform the overall process near to their theoretical values, the implementation of advanced control strategies similar than used in other industrial sectors as petroleum industry is recommended.

Large scale production of biodiesel and biochemical by algae is nowadays critically reviewed in life cycle analysis (LCA) studies (Campbell et al 2009, Stephenson et al 2009, Kadam 2002, Lardon et al 2009). The outcome of the LCA-studies is not unambiguous, because of the many uncertainties in the production system. LCA results are strongly influenced by assumptions regarding yearly areal productivity (Campbell et al 2009, Stephenson et al 2009) and composition of the algae mass (Lardon et al 2009).
A major problem in current design studies and LCA studies is the lack of specific information and argumentation of the information together with large uncertainties in the assumptions. For example, Norsker *et al* (2010) use an algae productivity in photobioreactors (PBR) between 40-80 ton/ha/year. Campbell *et al* (2009) use a biomass productivity of 110 ton/ha/year in open ponds as a starting point. Then to compensate for uncertainties a productivity of only 25% of the mentioned value is used in the final evaluation. Both the results of Campbell *et al* and Wijffels *et al* depend on estimates. Another limitation of

using generic data for algae cultivation is that the designs used in the studies are based on experimental work which is not necessarily performed under practical conditions. Most experimental studies concern only the effect of a few decision variables at a time. So the outcome of the studies does not reflect the practical possible performance. Finally, most laboratory and pilot plant studies have been performed under controlled light conditions. Translation of these results to large scale production units under variable light conditions is another challenge. Models will improve the insight in biomass production using large scale unit designs and LCA studies. Moreover it provides an opportunity for optimization and hence a better position to judge the feasibility of proposed algae plants for a specific location.

Microalgae – bacteria interactions
The influence of the bacteria in microalgae cultivation must be previously evaluated since they can have a positive or a negative interaction, the presence of bacteria might be beneficial to some algae species by increasing the growth rate.

Risk of contamination of the algae
In open systems axenic cultivation is extremely difficult.

Microalgae – bacteria interactions and growth phase
The ability of bacteria to augment algal growth has been shown to vary with the growth phase of the algae.

Microalgae – bacteria interactions and cultivation conditions
The relationship between bacteria and algae has been shown to vary with nutrients concentrations such as phosphate and also with the light intensity, algae generally out-competing bacteria in high phosphate and high light conditions.

Bacteria as effective competitors
Bacteria tended to be more competitive for resources due to: i) faster growth rate; ii) a greater surface area to volume ratio and; iii) faster uptake rates of phosphorus.

Environmental Impact
Environmental Impact of large-scale phycoculture (i.e. macroalgal cultivation) and mechanised harvesting.

Evaluation of the carrying capacity of a designated site for integrated aquaculture
Data collection should lead to the development of a model for seaweed cultivation, similar to those already existing for shellfish aquaculture.

Environmental impact assessment
Is needed to assess the impact of very large algal cultivation sites on the surrounding environment. Especially the intake of water from the environment and the release of used water back into the environment should be considered with great care.

2.5 Large-scale production

In order to make a significant impact on energy demand on a national or global scale, new algal biomass production systems must be developed at large scale, which will have to have the following two basic features:

• They must be environmentally sustainable systems: avoiding using fertile land; achieving a positive energy balance (with a balance of greenhouse gas emission carried out using life cycle analysis -LCA- techniques, which permit significant reductions compared to fossil fuels); which do not pollute the environment; with minimum use of fertilisers and minimum water consumption, not using water suitable for human consumption.

• They must be profitable at competitive production costs, at least in the medium and long terms. This implies having a suitable scalable production process, monitoring efficiency within adequate space and time margins (hyper-intensive systems) and ensuring the commercial exploitation of both the process and the product.

To improve the performance of microalgae production systems major research is need in the next areas:

2.6 Location

Two important limiting factors for the production of biomass for energy purposes are sunlight and, in particular the daily photosynthetically active radiation (PAR) rate, and the temperature. In other words, a suitable location would be one that allows the maximum production of the selected strain to be maintained all year round (g m-2day-1). Ideally, production farms should not stop at all due to insufficient light or temperature excess or deficit. It is important to bear in mind that to keep biomass production costs competitive, the system must operate constantly at optimum density, production and monospecificity. Otherwise, all conventional algae production plants are based on the availability of combustion gases; that is to say they presuppose the existence of centres of CO2 emission in close proximity to the algae production site. The use of industrial gases as a source of CO2 is not only necessary, but may also become imperative for the industry due to the social and economic benefits deriving from the elimination of this type of pollution.

2.7 Carbon source

Although the stimulating effect of the addition of combustion gases to the growth of algae has been demonstrated, the following drawbacks also exist:

- The absence of large CO2 emission centres in the geographic zones where it would be possible to implement microalgae production on a large scale. Industry is not generally situated in places where climatic conditions are ideal for the location of algae production facilities. The cost of transport and distribution of the industrial gases to the cultivation site would be too high, even though CO2 channelling systems are currently being developed, which could reduce these costs considerably.
- The low efficiency to capture CO2 from combustion gases: The need for the prior conditioning of the gases.
- Possible excess acidity, depending on the composition of the gases.

2.8 Fertilizers

It is important to attempt to eliminate costs deriving from the use of fertilisers, since these involve the consumption of both materials and energy. The following options exist in this respect:

- Use industrial and/or agricultural wastewater with available nutrients (ammonium, nitrates, and phosphates).
- Use of water proceeding from wastewater treatment plants -WWTP-. In this case it would be necessary to establish the means to guarantee the supply. The possible presence of various toxins or microorganisms in these waters limits the field of application of the biomass obtained to energy purposes, excluding uses for human and/or animal food, unless adequate treatment is carried out to nullify these effects.

2.9 Photobioreactors

There are two basic designs for the large-scale production of microalgae: open systems in which the crop is exposed to the atmosphere, and closed systems constructed in transparent materials such as glass and polycarbonate etc., in which exposure to the atmosphere does not take place. Open systems are essential to maximise the harnessing of solar radiation but present the following problems:

- Ultraviolet radiation and the wind cause premature shading of the photobioreactor if plastics are used as a covering.
- Contamination of raceways if these are not covered.

Open reactors are very important, but still must be improved to become reliable, stable and more productive. In a large, open industrial facility, the availability of inocula must be guaranteed through a combination of open and closed reactors.

The desired objectives for algae-based biomass production for energy purposes, taking into account the current situation of the sector and the market, would be:

- Continuous production of the order of 100 t of dry biomass per hectare per annum.
- Positive energy balance.
- Energy consumption of less than 40 €/m2.
- Energy consumption less than 50 W/m3.
- Biomass production costs less than 500 €/t.

Is a production technology that meets all these requirements and limitations possible? Currently, there is no unanimous answer to this question. Proof of this is that there is no consensus on algae-based biomass production, since the technology is in a stage of development, which does not allow us to possess fully substantiated data on an industrial scale. Achieving coordinated R&D&i is fundamental to reducing these costs in the near future.

The drawbacks of cultivation in raceways are that it is easily contaminated, the temperature is difficult to control and that cultivation with depths of under 15 cm (due to turbulence and flow reduction) and over 30 cm (higher agitation costs and reduction of density, with the consequent increase in harvest costs) is difficult. Another problem is that within this narrow depth margin (15-30 cm) densities are very low (0.5 g/l) and, for this reason, much larger surface areas are required (ratio of volume/total usable area between 120-150 l/m2) than those needed by photobioreactors. Sustainable production in raceways is less than 35 t ha-Iyear-I (Richmond postulates maximums of around 60 t ha-Iyear-I and Ben-Amotz maintains that potential maximums reach 75 t ha-Iyear-I).

The drawbacks of cultivation in photobioreactors is that the cost and power consumption per unit culture volume is higher than in raceways. Among different designs the flat plate photobioreactor has been reported to be the less expensive (up to 3 €/l) and power demanding (up to 50 W/m3) when comparing with tubular photobioreactors. On these the photobioreactor cost is the highest (up to 10 €/l) as well as the power consumption (up to 1000 W/m3). Otherwise, these values as far away from reported for raceways on which the photobioreactor cost (up to 0.5 €/l) and the power consumption (up to 10 W/m3) are the lowest reported. To achieve the biomass productivity of closed photobioreactors at the cost and power consumption of raceways is the goal to make the production of microalgae a real option at industrial scale.

Finally, the automation of microalgae production processes is indispensable both in order to lower production costs by reducing labour costs and, above all, to improve production. This enhancement is achieved by matching cultivation conditions to the requirements of the various strains, and by ensuring production as a consequence of maintaining the stability of the same.

2.10 Harvesting

The main problem in algae production aimed at producing biomass lies not so much in the production as in the harvesting. In the case of microalgae, harvesting of organisms measuring between 2 and 200 µm, which are usually cultivated at low harvest densities, is very costly in equipment and energy, especially if the harvest technology is based on centrifugal systems as is currently the case. Densities in raceways are lower than in photobioreactors, so costs are even higher. Thus it is essential to reduce or eliminate harvest costs. Centrifugal systems for large volumes is currently non-viable, so there is a need to find algae of a larger size that decant well, with maximum recycling of the water used, in the search for different, cheaper harvesting processes. Apart from progressing in the search for new species of microalgae, it would be interesting to study in greater depth those with the feature of not being “micro” in photobioreactors (high turbulence), but “meso”, that is to say, filamentous aggregates with sizes oscillating between 0.02 and 3 cm.

Productivity

- Enhance the microalgae growth ratio through the optimization of sunlight supply (avoiding photo-oxidation and photo-limitation phenomena, light intensity, direct vs. indirect light supply, etc)
- Increasing the outdoor productivity of microalgae culture through illumination during the night period, maintaining a positive economic and energy balance (e.g. through LED technology)
- Optimization of the culture medium formulation for each microalgae specie, in order to minimize its wastage in large-scale production, establishing a commitment between costs and productivity
- Studying the long term effects of culture medium recirculation on the microalgae productivity
- Techniques for the control of several contaminating species (bacteria, fungi, rotifer)
- Maximizing microalgae productivity through heterotrophic and mixotrophic growth

Carbonation

- Acconditioning flue gases to be used in microalgae production systems
- Carbonation and degasification processes in photobioreactors with flue gas

Temperature

- Systems for the thermoregulation of photobioreactors in large-scale production of microalgae
- Optimize the flue gas injection method in PBRs, in order to maximize the CO_2 uptake

PBRs Engineering

- Enhancing photobioreactors design (tube diameter, culture pathway, flash-light effect)
- Development of new materials that accomplish requirements of microalgae (transparency, cost, anti-fouling, etc.)
- Turbulence enhancement in tubular PBRs by the presence of static devices
- New pumping systems for energy saving
- Developing of active bio compounds for inside PBR fast cleaning
- Developing of mechanisms/automats for PBR outside constant cleaning
- Utilization of phosphorescence particles inside PBRs to dilute light inside systems

Industrial integration

- Develop the technology of microalgae production integrated in GHG emitting industries, and identify for each industry type the several points of possible cooperation
- Utilization of agricultural, industrial and domestic waste as bulk nutrients

Energy

- Evaluate synergetic interaction between microalgae and biogas production
- Reduce the uptake of mineral nutrients, specially fertilizers that are high energy demanding

Biomass harvesting

- Systems for harvesting the culture and recirculating the culture medium
- Development of models to integrate weather forecast with culture conditions for proper management of daily harvesting
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Biomass dewatering

- Systems for biomass dewatering, with reduced cost and energetic consume

Biomass compounds extraction

- Set most cultivated species as food and feed ingredient
- Biomass value increase
- Developing "clean" methods for added-value compounds extraction, as lipids, with positive economic and energetic balance

2.11 Downstream

The determination of the ideal algae-based biofuel that should be obtained is considered by some as a question to be solved prior to others related to the production of biomass. This question raises the complexity of the biotechnology-product matrix to be resolved. Possibilities include:

a) Biodiesel

The strategy of aiming to generate lipids directly from microalgae (biodiesel or jet fuel) is the prefereable for the industry. Some researchers suggest potential productions of 18,750 l oil ha-1year-1 (assuming 20% oil in the marine diatom Phaeodactylum tricornutum) and lipid rates of 5 g lipids m-2day-1). Other authors (Dr. Yusuf Christi) suggest potential productions of up to 58,760 l oil ha-1year-1, assuming 30% of oil in the biomass. According to Miguel García Guerrero reactors of 50 l/m2 with average volumetric productivity of 0.7 g of biomass l-1day-1 (or 140 l/m2 at 0.25 g l-1day-1 = 35 g biomass m-2day-1), and with fatty acid content of 30%, would produce 10 g of oil m-2day-1. The extrapolation of area would give 0.35 tonnes of biomass (0.1 tonnes of oil ha-1day-1). The area and time extrapolation considering 300 operating days per year would yield 105 tonnes of biomass, 30 t of oil ha-1year-1. The problem is not so much the production of lipids, as knowing how to extract and process them adequately. Production of biodiesel from biomass requires to separate oils from dry biomass and then to trans-esterify it, or alternatively to perform a direct transesterification process from wet biomass. Both technologies are yet under development. A more suitable approach would be through thermochemical processes of the Fischer-Tropsch type, based on an algal biomass rich in polysaccharides.

b) Bioethanol

Algal biomass contains carbohydrates, which are susceptible to be hydrolysed and fermented to ethanol by means of suitable yeasts. The monosaccharide composition of the carbohydrates present in algae is not simple, so these fermentation processes must be developed further in order to be carried out on an industrial scale. An alternative to this method is the direct production of ethanol by some cyanobacteria, the product being recovered from the culture medium.

c) Biogas

Microalgae biomass can be transformed into methane-hydrogen-CO2 mixtures by anaerobic digestion similarly to other waste are fermented into wastewater system. Alternatively the biomass can be processed hydrothermally. The efficiency of thermal process has been reported to be higher (>70%) in comparison with the methanogenic processes by anaerobic digestion (25%-35%). In both cases it is not necessary to dry the biomass, and the process begins to be profitable from 15% dry weight of biomass in the concentrate, making harvest by filtration possible (self-flocculating filamentous cyanobacteria).

d) Combustion

The application of microalgae biomass as direct fuel for production of heat and/or electricity is hardly viable since the algal paste obtained after harvest contains 80 to 90% water and very high salt content, which makes combustion difficult.

e) Hydrogen

The production of metabolic hydrogen from hydrogenase-bearing microalgae, a complex process still at the laboratory study stage, requires the production of hydrocarbon-rich algae, which, under certain conditions of oxygen limitation, constitute the energy source for cellular processes of water hydrolysis and hydrogen liberation. In addition to this option, there is another way to obtain hydrogen based on gasification, synthesis gas and reforming (enrichment of the mixture). The gas thus obtained, Syngas, may be employed as raw material for obtaining liquid biofuel or alcohol in addition to hydrogen.

Biodiesel

- Efficient recovery of lipids from wet biomass
- Development of blend oil by combining microalgae oil with other vegetable and waste oils
- Greener transesterification processes using enzymes
- Adequacy of microalgae biodiesel to standards

Bioethanol

- Development of standard procedures for saccharification of the biomass
- Optimizing yeast mixtures to efficiently transform carbohydrate mixtures into ethanol.

Biogas

- To evaluate the influence of biomass composition into the biogas methanization potential
- Determining methanization potential of waste biomass from previous processes.
- Enhancement of methanization potential of other waste by integrating microalgae biomass

Others

- Development of thermochemical processes for energy valorisation of biomass (Syngas, licuefaction, etc.)
- Designing overall processes for the valorisation of biomass (biorefinery)

3. Macroalgae

Kain (1991) described macroalgal cultivation for biomass as having no serious technological barriers, as she referred to the spectacular large-scale cultivation in China. Various cultivation trials are currently in place in Europe, however, macroalgal cultivation in a European context faces various challenges that maintain it in infancy, and cultivation seems to be afflicted by the Peter Pan syndrome (for the last 30 years). Apart from research trials, with only a few commercial applications (for low-volume/high-value production), the need for large-scale cultivation in Europe has never emerged, with demand only rising slowly although looked at with increasing interest. The FAO reported a total amount of seaweed harvested worldwide of 1,045,000 T wet weight in 2008, whereas the total cultivated reached 15,781,159 T wet weight for the same year. The macroalgal standing stock of European coastal waters will not suffice to meet biofuel demand (Horn, 2009), hence the present need for large-scale cultivation, integrated polyculture (IMTA), mechanised harvesting and processing (Biorefinery). The research required in order to meet a fully integrated large-scale production of biofuel from macroalgae will be concisely discussed at various levels (from molecular to ecosystem).

1. State-of-the-art in macroalgal research, where is the effort concentrated at the moment?

Phycology is a young branch of Botany, whilst the anthropological uses of seaweed dates as far back as 600 BC in China (Sze Teu), the scientific interest of algae in the west is concomitant with the explosion of science at the end of the XVIIIth century and the description and classification of species by Linnaeus. The current fields of study in phycology are rather widespread and involve a broad range of scientific disciplines (from chemistry to social science):

- Taxonomy: Alpha taxonomy, molecular, chemical taxonomy, bio-informatic tools.
- Ecology, Eco-physiology: Population dynamics, inter/intraspecies interactions, monitoring invasive species, elicitation/defence mechanisms, Eco-devo, Biogeography; photosynthetic studies (e.g. fluorescence).
- Cultivation: Life cycles of species, vegetative growth, effect of environmental factors on growth and production, polyculture, economics, Strain selection, etc.
- Biotechnology: Metabolites analysis, metabolites modification, Optimisation of production processes (extraction/ purification/ conservation, etc.). Activity Assays (Pharmaceuticals/ medicine/ nutrition), proteomic, environmental markers, bioremediation, etc.

2. Research needs for the development of 2nd generation biofuels.

Optimisation of each level described below is required to achieve a biologically, environmentally, economically and socially sustainable production of biofuel from macroalgae.

3.1 Molecular level (Genetic engineering)

Any organism disposes of a certain amount of energy that can be orientated in one metabolic pathway or the other, one physiological characteristic or the other. One cannot excel in reproduction, metabolic production, defence, etc. at the same time. Such a Dynamic Energy Budget (DEB) defines the developmental strategy of a given specie. From this basic statement, and considering the ways to valorise a large scale production of algae (i.e Biorefinery concept), it follows that by engineering or selecting your algae to perform better for a specific metabolic pathway, you will necessarily miss out on some other metabolite. By increasing the production of mannitol you will possibly affect the anabolism of protein, hence impeding the valorisation of the latter in the Biorefinery process. On the other hand, one could argue that we can enhance or force the production of fermentable metabolites, but in the perspective of a biorefinery, biofuels (ethanol, methane) are of rather low value compared to protein or pigments for food/feed/cosmetic industries. Although you could shunt, for example, reproduction in favour of metabolite production by silencing genes that trigger sporocystes development, the thallus only becomes reproductive after the harvesting period. So again, in this case genetic engineering is not relevant. It must also be kept in mind that biomass production at a large scale is likely to happen at sea where dissemination is not controlled; therefore there is a risk that genetically modified cultivated populations would mix with wild stock populations.

3.2 Cellular level

Metabolic production pathways

- High levels of phlorotannins tend to inhibit Anaerobic Digestion (AD) (Horn, 2009), therefore the reduction of phlorotannin synthesis in brown algae could favour AD. However there is a chance that the selection of low-level phlorotannin strains of brown algae would reduce the defence of the whole crop towards grazers, fouling and infections. The solution could lie in the Biorefinery process where phlorotannins could be separated from the fermentable metabolites. The phlorotannins could then be valorised as natural antioxidants for food/feed/cosmetic industries.
- The ethanol yield from brown algae is 46% for mannitol and laminaran (Horn, 2009) therefore research should focus on enhancing metabolic pathways for both mannitol and laminaran in brown algae.

3.3 Biochemical level

- Influence of cultivation methods on biochemical composition, morphology, need to monitor biochemical composition over the period of cultivation.
- Toxicity: inhibition of methanisation can result from high concentrations of substances such as phenols, heavy metals, sulphides, salts and volatile acids (Kelly and Dworjanyn, 2008).

3.4 Organism level

- Genetic selection/ strain selection according to the criteria described in D1.4 Biology and Biotechnology, “criteria for strain selection” (productivity, robustness, harvestability, biomass composition, processability/extractability, added-value of co-products).
- Research on selective breeding is needed to improve productivity.
- Research in maintenance of the culture of gametophytes (the “seed bank”).

3.5 Population level

The following topics should be addressed:

- Optimisation and control of population densities on long lines.
- Rotation of species on the long lines to obtain a poly-crop and continuity of supply year-round.
- Assess the extent to which the fouling (e.g. byrozoans) of cultivated algae can affect fermentation. Is the strain selection of fouling free thallus necessary? Do we need macroalgae as clean as the ones produced for food purposes, or shall we aim for total biomass?
- Emission of volatile halogenated organic compounds (VHOCs) by macroalgae in aquaculture systems.
- The yield (ton/ha/year) should be obtained for large-scale phycoculture as the scaling-up calculation from current cultivation trials are misleading.

3.6 Ecosystem level

- Environmental Impact of large-scale phycoculture (i.e. macroalgal cultivation) and mechanised harvesting.

Although Fei (2004) advocates for the efficiency of large-scale cultivation in solving eutrophication issues in China, no studies have been carried out in a European context as no large scale commercial facility exists in Europe to date. Efforts should focus on:

- The evaluation of seaweed farms as Carbon dioxide sinks, nutrient load regulators (especially in eutrophied coastal area where summer macroalgal blooms are causes of nuisances). Studies should be initiated on the associated benthos (biodiversity around the farm, presence or absence of recruits, etc.), the impact of phycoculture on hydrodynamics, the pest and diseases outbreak in monoculture/polyculture systems.
- Evaluation of the carrying capacity of a designated site for integrated aquaculture. Data collection should lead to the development of a model for seaweed cultivation, similar to those already existing for shellfish aquaculture.
- Environmental impact assessment is needed in the case of beach collection of macroalgal blooms, as well as for the removal of subtidal bloom stock (e.g. *Ulva sp*. blooms).
- Removal of invasive species and uses for biofuels has to be assessed (e.g. *Sargassum muticum*).

Environmental assessment and potential risks should be compared with those of current situation for fossil fuels in order to give a realistic magnitude of benefits/risks from biofuels production.

- LCAs.

The lack of large-scale cultivation facilities impedes the development of LCAs. A couple of European projects currently include LCAs as part of the deliverables, with results expected not before 2013 (Supergen, Energetic algae, etc.).

3.7 Digestion/Fermentation

f) Methane production

- Assessment of pre-treatments, to maximise methane yields, are required such as mechanical treatment (simple chopping, crushing or ultrasonic grinding), enzymatic, heating and/or spontaneous pre-treatments such as natural hydrolysis. Pre-treatment is also the stage at which value-added compounds may be extracted before sending the processed material to energy generation.
- Mix algae with other feedstocks (such as mixed seaweed biomass, municipal sludge waste or matnur, fish and shellfish wastes, wastes form another process, e.g. glycerol a by-product of the production of biodiesel, or the residues form the alginate extraction industry, rich in mannitol and laminarian should be assessed as performance enhancers in modern digesters (Kelly an Dworjanyn, 2008).
- The first step of anaerobic digestion consists in breaking down macromolecules (protein, polysaccharides, lipids) into mono and olicomers. This microbial hydrolysis is carried out by extracellular enzymes which production is energy demanding and only happens under specific conditions. The brown algae *Desmarestia sp*. Accumulates sulphuric acid (McClintock, 1982), the addition of the latter in the first step of anaerobic digestion could provide biomass, hydrolictic conditions and energy saving. Research should be carried out to assess whether the addition of sulphuric acid from *Desmarestia sp*. In the medium could possibly inhibit further processes.

g) Ethanol production

- The current production of ethanol was performed form seaweed extracts; therefore direct fermentation of macroalgae should be investigated instead of extraction (Horn, 2009).
- Alternative to sterilization of ethanol production culture (Horn, 2009).
- Novel isolates of matine bacteria should be screened for ethanol producing capability based on their ability to mitigate the effects of polyphenol-induced inhibition and their adaptability to grow and produce biofuel under the high salt concentrations inherent to seaweed biomass and its extracts. Chemical mutagenesis and the subsequent selection of mutant strains displaying improved rates and yields should be adopted (Kelly and Dworjanyn, 2008).
- Data is required on the performance of seaweeds in modern digesters.

3.8 Others

Research in the engineering sector is crucial to develop cost effective methods for cultivation/harvesting/processing in a European context. Biofuels from macroalgae will not happen unless it is based on a mechanised production. Research in law studies regarding coastal management, and its homogenisation at a European level, for multitrophic aquaculture, or specific licence attributed to seaweed aquaculture (related to its status of primary producer) by the department of marine of the member states.

- Development of a European database for seaweed cultivation

3.9 Conclusion

Time has come to scale up cultivation trials (in the range of several hectares at sea), with associated funding programs and supportive legal framework in order to perform realistic studies on biomass production from macroalgae. Large-scale trials would have to be performed in various parts of Europe in order to reflect the diversity of habitats and species to be cultivated. The condition *sine qua non* for the development of macroalgal biofuels is the research input in the optimisation of every aspect of cultivation, coupled with the initiation of mechanised production.

4. Conclusion

Research on algae as a source for biofuels and more specifically the challenges involved in generating highly productive strains and production system is still a relatively new discipline. This offers great opportunities for the future, but also requires good strategic planning on which bottlenecks need to be addressed first to keep making progress at the highest speed. This document gives an overview of the most urgent needs in all scientific disciplines relevant for producing biofuels from algal biomass.

On the molecular, biochemical and cellular level, most challenges are involved with the fact that very few algal species have been fully sequenced and annotated and even fewer have been investigated in full detail regarding their relevant metabolic pathways, photosynthetic properties or ability to grow with a high growth rate to high biomass densities. Although many things can be learned from other plants that have been fully annotated and investigated, this still leaves much unknown for most species that are highly interesting candidates for large scale manufacture of algal biofuel.

On the ecosystem and environmental level mainly the impact of large scale algal cultivation on its immediate surrounding area should be assessed. In case of the use of GMO algae, proliferation in the environment should be prevented. But also contamination of the algal cultivation plant with native wild species of algae is a main concern. In addition, the impact of taking in and releasing back huge amounts of water necessary for algae cultivation can have a big impact on the ecosystem. Besides water, large amounts of CO2 and nutrients are required that require a logistics infrastructure to allow continuous supply. Taken together, a large scale algae cultivation facility needs to be carefully located and designed to minimize impact on the environment. Much more research is necessary in order to be able to make sound decisions.

On the technological level the biggest challenge is the sheer scale of an algal biofuel plant. Open systems are challenging enough, and closed photobioreactor systems still need much development before they can be applied at a large scale. Mixing, gassing, water management, cleaning, logistics, control of cultivation parameters, etc are all challenges when deployed at a large scale. Nonetheless, much progress has been made in the recent years and the increased costs associated with photobioreactors as compared to open systems are expected to decrease due to further refinement of the technology. This applies mainly to microalgae, because macroalgae always need an open system when cultured at a large scale. Nonetheless these “floating farms” possess their own technological challenges. Also the harvest and biorefinery of the algal biomass is still a new field for research with little or no experience at a very large scale.

The final technological step is the final conversion into the actual biofuel. This can be partly based on existing techniques and/or chemistry already used at large scale. Especially for biodiesel, biogas and bioethanol, much of the technology is already developed and in operation. A further refinement step still has to be made to accommodate the specifics of algal carbohydrate or lipid composition, however. The high water content or algae may provide additional challenges, especially for the pyrolysis-based techniques. Also hydrogen production is still a challenging technique. However, in principle this final conversion step will not be the main bottleneck for algal biofuels. Algae have clear benefits over other feedstocks for biofuels in terms of sustainability, land use and CO2 capture. This documents shows that although much still remains to be done, bigger and smaller breakthroughs can be achieved in the foreseeable future so that algae can live up to their promise. Special attention should be paid to supporting macro-algae, which currently represent the main way to produce biofuels from algae at an affordable price, but have received a minor share of the funding for research so far.

5. Literature

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6. Appendices

Research needs in literature

Conclusion: As demonstrated here, microalgal biodiesel is technically feasible. It is the only renewable biodiesel that can potentially completely displace liquid fuels derived from petroleum. Economics of producing microalgal biodiesel need to improve substantially to make it competitive with petrodiesel, but the level of improvement necessary appears to be attainable. Producing low-cost microalgal biodiesel requires primarily improvements to algal biology through genetic and metabolic engineering. Use of the biorefinery concept and advances in photobioreactor engineering will further lower the cost of production. In view of their much greater productivity than raceways, tubular photobioreactors are likely to be used in producing much of the microalgal biomass required for making biodiesel. Photobioreactors provide a controlled environment that can be tailored to the specific demands of highly productive microalgae to attain a consistently good annual yield of oil.	Chisti, Y. Biotechnology Advances 25 (2007) 294–306	2007
The general R&D topics required for practical development and applications include: • Selection and improvement of algal strains able to be mass cultured in open ponds; • Maximization of algal productivity under sunlight conditions; • Maximization of algal biomass C-storage products; • Development of large-scale, low cost systems for algal cultivation; • Development of low cost algal-harvesting technologies; • Improvements in the processes for converting algal biomass into fuels; • Practical demonstrations in wastewater treatment, aquaculture and other near-term applications; • Ongoing engineering and economic feasibility analyses to help focus R&D priorities.	Pedroni, P. A Proposal to Establish an International Network on Biofixation of CO2 and Greenhouse Gas Abatement with Microalgae	2001
Without careful assessment of the lifecycle energy balances – including actual environmental impacts, there is the risk that many proposed schemes would be inappropriate from the point of view of sustainability; critical analysis is thus seminal to provide a clear picture of the scenarios available. Moreover, a dramatic lack of data pertaining to real-life demonstration has turned economic assessments into essentially hypothetical exercises; pilot studies are thus to be conducted under realistic setups, including typical weather conditions, so that useful estimates of biodiesel productivities likely to be achieved in practice can be generated. Major breakthroughs are still needed towards design and development of technologies that can reduce costs while increasing yields; only integrated	Malcata et al. Applied Energy (2011) Advances and perspectives in using microalgae to produce	2011

studies, following coherent and long-run, well-funded R&D program will eventually attain this goal: selection of lipid hyperproducing strains perfectly adapted to regional conditions, coupled with genetic improvement and process optimization will surely constitute contributions to bring about innovation that will eventually meet with success. A biofinery approach is by all means a must – which upgrades spent biomass after lipid extraction, at the expense of alternative bulk or fine chemical production. Time urges, as responses to climate and environmental issues cannot be systematically delayed, and food security cannot be jeopardized on a worldwide basis by an increasing market demand of biodiesel in richer countries. Novel locations for such crops are thus to be found – and marine farming employing microalgae is surely a promising avenue towards this endeavour.	biodiesel	
Microalgae offer great potential for exploitation, including the production of biodiesel, but the process is still some way from being carbon neutral or commercially viable. Part of the problem is that there is little established background knowledge in the area. We should look both to achieve incremental steps and to increase our fundamental understanding of algae to identify potential paradigm shifts. In doing this, integration of biology and engineering will be essential. In this review we present an overview of a potential algal biofuel pipeline, and focus on recent work that tackles optimization of algal biomass production and the content of fuel molecules within the algal cell.	Scott et al. Current Opinion in Biotechnology 21:277–286 Biodiesel from algae: challenges and prospects	2010
Producing algal biodiesel requires large-scale cultivation and harvesting systems, with the challenging of reducing the cost per unit area. At a large scale, the algal growth conditions need to be carefully controlled and optimum nurturing environment have to be provided. Such processes are most economical when combined with sequestration of CO2 from flue gas emissions, with wastewater remediation processes, and/or with the extraction of high value compounds for application in other process industries. Current limitations to a more widespread utilization of this feedstock for biodiesel production concern the optimization of the microalgae harvesting, oil extraction processes, and supply of CO_2 for a high efficiency of microalgae production. Also, light, nutrients, temperature, turbulence, CO_2 and O_2 levels need to be adjusted carefully to provide optimum conditions for oil content and biomass yield. It is therefore clear that a considerable investment in technological development and technical expertise is still needed before algal biodiesel is economically viable and can become a reality. This should be accomplished together with strategic planning and political and economic support. Further efforts on microalgae production should concentrate in reducing	Mata et al. Microalgae for biodiesel production and other applications: A review Renewable and Sustainable Energy Reviews,14 (2010) 217–232	2010

costs in small-scale and large-scale systems. This can be achieved for example by using cheap sources of CO_2 for culture enrichment (e.g. from a flue gas), use of nutrient-rich wastewaters, or inexpensive fertilizers, use of cheaper design culture systems with automated process control and with fewer manual labour, use of greenhouses and heated effluents to increase algal yields. Apart from saving costs of raw-materials (nutrients and fresh water use), these measures will help to reduce GHG emissions, waste amount, and the feed cost by using of nitrogen fertilizers. Also, will raise the availability of microalgae biomass for different applications (e.g. food, agriculture, medicine, and biofuels, among others) and will contribute to the sustainability and market competitiveness of the microalgae industry.		
Algae and Cyanobacteria for third generation biodiesel need transgenic manipulation to deal with "weeds", light penetration, photoinhibition, carbon assimilation, etc…	Gressel, J. Transgenics are imperative for biofuel crops	2008
More innovations are still needed for the development of technologies which reduce costs while increasing the yields. This can be realized successfully through a coherent, extensive, and well-funded R&D program. It is extremely important in the early phases of this promising, yet challenging industry, to deliberate new business models that look at the bioenergy potential of algae through the transportation fuels market, as well as production of other higher value products so as to make the economics practicable. A sustained effort from the technologists and planners can result in the successful accomplishment of this extremely potential concept towards the solution of world's future energy concerns.	Singh, J. Commercialization potential of microalgae for biofuels production Renewable and Sustainable Energy Reviews, Volume 14, Issue 9, December 2010, Pages 2596-2610	2010
Algae have great potential as a sustainable feedstock for production of diesel-types with very low-carbon dioxide emissions. Nonetheless, there are currently serious drawbacks, and it would be harmful to overestimate the greenness of this up and coming technology. Research on algae-based fuel is still in the beginning stages, and any large-scale use of algae for biofuel would require massive investments in production facilities. That would take many years and large amounts of money to develop.	Um, B. and Kim, Y. Journal of Industrial and Engineering	2009

	Chemistry	
In brief, the objective of R&D in this field is to demonstrate that it is actually possible to mass culture algae for maximal oil productivity, and harvest them cheaply, which remains to be shown, and to reduce the cost of such algal biomass production to an acceptable level. The main issue that must be addressed by the future R&D is how to bridge the gap between the current reality, exemplified by commercial production systems the goal of low-cost algae oil production, which requires much higher productivities and oil content than is currently achievable. A long litany of R&D needs and goals can be formulated, centered both on the organisms, the algae, and the engineering of the cultivation system. There is a need to isolate screen, select, test (in outdoor ponds) and genetically improve algal strains, for both higher oil content and overall productivity (e.g. photosynthetic efficiency in mass culture), as well as for resistance to grazers, invasions, temperature and other environmental factors, etc. One of the advantages of microalgae is their short generation times, as little as one day in outdoor mass cultures (e.g. at the 50% per day dilution, readily achievable under favourable conditions) and a few hours in the laboratory, and even outdoors after inoculation before the culture achieves optimal density for maximal productivity. This fast growth rate allows more rapid development of this technology than for conventional crop plants, where a single lifecycle can be months to years. Even with this advantage, the development of the algal strains and cultivation technologies to the level of productivity, efficiency, stability, and easy harvesting required for biofuels production will be very difficult and require years, assuming it proves to be actually feasible at all. Further, such developments in the "software", must be combined with the "hardware", the engineering design of the production system, including not only the large ponds, larger than anything operated thus far for intensive commercial cultivation, but also the algal processing, oil extraction, etc. Again, there is no guarantee that a sufficiently low-cost process can actually be engineered. On the other hand, there are no clear "show-stoppers" that would suggest that either the biological or engineering R&D required cannot be eventually successful.	Benemann, J.R. Opportunities and challenges in algae biofuels production Algae World 2008, Singapore	2008
Microalgae produce storage lipids only under photo-oxidative stress or other adverse environmental conditions. Nitrogen deprivation, high light intensity, low temperature, and high salt or iron concentration can stimulate lipid formation. Light is the source of energy for algal growth, but too high light intensity may result in photo-inhibition or overheating. That´s why the physics of light distribution and the efficient utilization of light inside the photobioreactor is one of the major biotechnical challenges in bioreactor design. The further	Rösch, C. Microalgae - opportunities and challenges of an innovative	2009

development of the photo-bioreactors for large-scale energy production must consider: - seasonal and diurnal changes in irradiance; - the biomass growth kinetics, photo-inhibition and flashing-light effect; - mass transfer of carbon dioxide and the fluid mechanics, and - peculiarities of the species being cultured (e.g., shear stress tolerance) Extracting lipids from microalgae is another biotechnical challenge due to the sturdy cell wall making the oil hard to get at. Although the use of the transgenic microalgae for commercial applications has not yet been reported, several examples of engineered microalgae for biotechnological applications show significant promise. The development of a number of transgenic algal strains boasting recombinant protein expression, engineered photosynthesis, and enhanced metabolism encourage the prospects of engineered microalgae. The manipulation of microalgae lipid production by genetic engineering was one of the first reported approaches for the production of bio-diesel by transgenic diatoms. Another example is the modification of photoautotrophic diatom to live in the dark with glucose as the only carbon source.	energy source 17th European Biomass Conference and Exhibition, Hamburg	
The extensive R&D of microbial oil production carried out over the past several years are still continuous and are basically aimed at improving the economic competitiveness of microbial lipids compared to plant- and animal-derived oils. Three main pathways are mentioned as follows to improve economics of microorganism biodiesel: 1. Screening for potential oleaginous microorganism - it is the first and an essential step limiting the number of strain for further study and practical use. Although several wild-type oleaginous microorganisms are able to synthesize rich oil, these strains have a limited ability to produce biomass. Making use of mutation techniques in microbial lipid production to filtrate better strain will get much more biomass than wild-type. 2. Genetic and metabolic engineering - with the development of biochemistry and molecular biology (such as DNA recombination, site-directed mutagenesis), engineer progress in microorganisms has led to speculation that certain oil components could become marketable commodities if the genomes of traditional oleaginous microorganisms were appropriately modified. It is likely to have the greatest impact on improving the economics of production of microalgal diesel. 3. Making full use of by-products - in the course of fatty acid production, there are usually some other by-products useable in industry. In addition to oils, oleaginous microorganisms contain significant quantities of proteins, carbohydrates and other nutrient contents. Therefore, how to make use of these by-products and improve their industrial value is another way to reduce the production cost in biodiesel.	Meng, X. Biodiesel production from oleaginous microorganisms	2009

Developing high lipid content microorganisms or engineered strains for biodiesel production would be becoming a potential and promising way in the future. The manipulation and regulation of microbial lipid biosyntheses open a possibility for academic research and demonstrate the potential in its industrial application in biodiesel production.		
Biodiesel production from microalgae is a goal that still needs much research. In order to set up an economically advantageous process, several aspects have to be approached. First of all, metabolic factors involved in fatty acid biosynthesis have to be deeply understood, to elucidate which are the relationships among the single factors involved. Such a study has to be performed from several points of view, such as microalgal physiology under stress response and molecular basis of the triggered mechanisms, in terms of functional genomics, proteomics, transcriptomics, and metabolomics. Such information will reveal the regulation networks that are responsible for microalgal behaviour during cultivation. Another aspect that has to be pointed out is the need of microalgal genome sequences, in order to study genetic engineering strategies to increase fatty acid yield and profiles; in such a way it would be possible, for example, to reduce the presence of poly-unsaturated fatty acids that have a negative effect on the stability of the final biodiesel mixture. In order to reduce some negative factors, such as light saturation photoinhibition and oxygen toxicity, the maximization of photosynthetic efficiency has to be reached. Other aspects are connected to the cultivation systems; therefore, though optimization of raceway ponds and photobioreactors is currently in progress, it is desirable that innovative and alternative cultivation systems, more efficient and less expensive, would be designed. Strictly linked to the cost of cultivation, the process by-products (disposal of exhausted growth medium and of exploited biomass) have to be considered as new substrate for other processes; in such a way, fatty acid production and by-products disposal, as well as using other wastes as substrate(s) for microalgae cultivation, could be coupled in an integrated system, with higher value and commercial suitability. Finally, the development of new production methods, as those based on biocatalysts, will help to get a more economic and environmentally sustainable microalgal biodiesel fuel.	Salis, A. Biodiesel from microalgae *In* Handbook of hydrocarbon and lipid microbiology, Springer-Verlag Berlin Heidelberg	2010
FURTHER IMPROVEMENTS IN PRODUCTIVITY: 1. Pure Engineering Solutions It is now generally recognized that maximum photosynthetic efficiency is a function of the average photon irradiance per cell, a factor that is influenced not only by incident light, but also by the concentration of cells, the optical path length or depth of the culture, and by the frequency	Huntley, M. CO_2 mitigation and renewable oil from photosynthetic	2006

of alternating exposure to high and low light conditions within the culture. Turbulence in the culture medium also plays an important role because it determines the frequency of exposure to alternating light conditions. In essence, the practical challenge is to expose cells to the highest possible light intensity without causing photoinhibition, which in turn depends upon the state of photoacclimation. One example of a process improvement would be the removal of auto-inhibitory growth factors by medium replacement, which can lead to a four-fold enhancement in areal productivity. This particular method could be accomplished by the two-stage cultivation process we developed, in which culture medium from the photobioreactor is significantly diluted upon transfer to the second-stage open-pond batch culture. 2. Reduction of the Light-Harvesting Pigment Complex Reduction in size of the light-harvesting pigment (LHP) complex can lead to greater photosynthetic efficiency at high photon flux density. The photosynthesis of cells with a large complement of LHP does not increase in proportion to light intensity at high PFD because excess excitation energy is dissipated to non-photochemical quenching and heat and, consequently, photosynthetic efficiency is decreased. Model simulations confirm the concept. 3. Enhancement of Oil Biosynthesis There has been much research on oil synthetic pathways in higher plants, and those pathways have simply been assumed to be similar in photosynthetic microbes. However, the quantity and quality of oils undergo major changes during nutrient deprivation in these organisms; this does not happen in higher plants. Relatively little has been done to enhance, let alone to understand, oil biosynthetic pathways and regulation in photosynthetic microbe. 4. Engineering Rubisco for Enhanced Photosynthetic Efficiency It has long been recognized that the enzyme ribulose 1,5-disphosphate carboxylase/oxygenase (Rubisco), which catalyzes the first step in photosynthetic CO_2 fixation and respiratory carbon oxidation, imposes the primary rate-limiting step on plant productivity. Rubisco's notorious inefficiency has led to the suggestion that the enzyme might be improved as a means to increase photosynthetic efficiency. Much of the research effort has been targeted at higher plants with the goal of improving agricultural yields, but one of the more promising recent developments has been accomplished with photosynthetic microbes. Using the photosynthetic bacterium Rhodobacter capsulatus as host, Smith and Tabita (2003) successfully introduced Rubisco genes from the cyanobacterium Synechococcus, obtaining a positive mutant that exhibited a significant improvement in growth rate.	microbes: a new appraisal Mitigation and Adaptation Strategies for Global Change, Springer	

Most studies reported to date have been performed on the bench-scale, and were conducted under strictly controlled conditions. As a result, little is known about the feasibility of reactor scale-up, or the effects of competition by other microbial species inadvertently introduced into the bioreactor with the feed stream. Current investigations focus mainly on closed bioreactors, whereas future research should also consider open systems, owing to the possibility of more widespread use of biological CO_2 mitigation. Among these, technologies that support the supply of adequate amounts of CO_2, nutrients and light to microalgal cells, consume minimal energy, and release less CO_2 into the atmosphere are in special demand. Enhanced CO_2 levels (typically well above its atmospheric level) are needed for efficient microalgal growth and metabolism, and currently are major contributors to the overall cost of microalgal cultivation. However, different sources of CO2 and supplementary nutrients will greatly improve the overall environmental performance of microalga-based bioenergy production. Future research should explore existing sources, such as CO2 from ammonia plants or flue gases from power stations. Furthermore, nutrients could easily be extracted from wastewater or agricultural wastes owing to their richness in nitrogen and phosphorus. Note, however, that the quality of the flue gas might hamper specific applications in the medical and food fields. Studies might also be required to discern whether microalgal biomass production using wastewater produces sufficient-quality effluent for discharge into the environment, or even eventual reuse. Waste streams are, in general, small compared with energy demands; as a result, transportation costs to concentrated production sites should be assessed in advance. Co-digestion of microalgae with wastewater sludge for biogas production should also be considered, because this strategy could be integrated into the existing wastewater infrastructure. Harvesting, dewatering and lipid extraction from microalgal biomass are still challenging issues because they consume large amounts of energy – mainly because of the small cell size and relatively low biomass levels of microalgal cultures. Research efforts are therefore warranted for cultivation under higher cell densities, which pose engineering challenges with regard to cell accessibility to light and gas. In addition, the possibility for chemically induced or auto-flocculation of microalgal cells needs to be addressed. Finally, a key challenge for microalgal biodiesel production is the use of microalgal species that can maintain a high growth rate in addition to a high metabolic rate, thus leading to significant lipid yields. This major challenge can be duly addressed via extensive bio-prospecting or target oriented genetic engineering – both of which are now starting to appear as promising approaches.	Kumar, A. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions Trends in Biotechnology 28 (2010) 371–38	2010
All of the elements for the production of lipid-based fuels from algae have been demonstrated. Algae can be grown in large outdoor cultures and	Pienkos, P.	2009

harvested. The algal lipids can be extracted and converted to biodiesel or other transportation fuels. The relevant question is not whether biofuels from algae are possible, but rather whether they can be made economically. There are, however, a number of major technical challenges that will need to be overcome to achieve this goal. Significant attention and support should be given to both basic and applied research on algae for biofuels applications and the engineering of sustainable microalgal systems. Our techno economic analysis indicates that algal productivity is the primary production cost determinant and so efforts should be focused on various aspects of algal biology that can have the greatest impact on growth rate and lipid biosynthesis. However, this work cannot be done in isolation, and it would be a mistake to equate progress in productivity made at the bench scale with success in large-scale cultivation. Hence, attention must be paid to growth under conditions that model commercial production (including climate, and input sources), with data exchanged between biologists and process engineers. In anticipation of success of this revolutionary approach to a novel twenty first-century concept of agriculture, and if using land that has never been developed for any purpose, it is essential to complete a detailed LCA and ecological impact analysis in advance of any large-scale deployment to ensure a smooth path to commercialization. An LCA should also include a very detailed assessment of the energy recoveries for algal biofuels production (i.e., net energy being recovered in the algal fuel compared to the energy input from fossil fuels in order to produce the renewable fuels). Based on recently published energy calculations, microalgal biofuels have the potential to be produced sustainably. Energy ratios, which range from 3.3 to 7.5, are dependent on a variety of parameters such as algal cell oil yields, areal biomass productivity, biogas yield resulting from an anaerobic digester, harvesting and extraction processes, waste-water treatment, and fertilizer/nutrient recycling.	The promise and challenges of microalgal-derived biofuels Biofuels, Bioprod. Bioref. 3:431–440 (2009)	

General Conclusions

It is crucial to:

- Improve algal biology through genetic and metabolic engineering;
- At a large scale, the algal growth conditions need to be carefully controlled and optimized;
- The algae behaviour varies according to the strain;
- Have a strategic planning and political and economic support (in order to improve the economic competitiveness);
- Develop further efforts in order to reduce costs in small and large scale systems;
- Study the availability of microalgae biomass for different applications;
- Develop and improve the bioreactor design
- Study the microalgal physiology under the stress response;
- Maximize the photosynthetic efficiency.

Research needs identified by other stakeholders

Research needs evaluated by the Carbon Trust

www.carbontrust.co.uk/news/news/press-centre2010/2010/Pages/uk-takes-on-world-in-global-race.aspx

19 March 2010

Carbon Trust creates 'UK dream team' of top scientists to find world beating formula for algae biofuel

The Carbon Trust today announced plans to take on the world in the global race to develop a sustainable, cost-effective biofuel from algae. The "dream team" of eleven leading UK institutions was unveiled who will work together with the Carbon Trust to find a winning formula for cultivating 70 billion litres of algae biofuel a year by 2030. This will be the equivalent to 6% of road transport diesel and a saving of over 160 million tonnes of CO2 every year. The eleven institutions were selected from over 80 initial proposals following an extensive competition and detailed assessment process.

Starting from first principles of agriculture, thousands of strains of algae will be screened to find the winning few that can produce large quantities of a substance similar to vegetable oil. Additional research will develop methods for enabling large-scale production in algae ponds and next year the Carbon Trust plans to start construction of a pilot demonstration plant in an equatorial region where algae are most productive.
Algae has the potential to deliver 5 to 10 times more oil per hectare than conventional cropland biofuels and new Carbon Trust lifecycle analysis indicates that, over time, it could provide carbon savings of up to 80% compared to fossil fuel petrol and jet fuel. With costs of algae biodiesel currently estimated to be approximately $5-$10 a litre, Carbon Trust is focussing on more cost-effective production methods to ultimately bring the cost down to less than $1 a litre.

Launching **Europe's most significant public initiative into algae biofuels**, Tom Delay chief executive of the Carbon Trust, said: "We have pulled together a dream team of over 70 UK algae scientists who have the expert knowledge to turn algae into a British biofuel success story. Applying principles this country has developed from its proud agricultural heritage and leading bioscience expertise we will be developing a truly sustainable biofuel that could provide up to 80% carbon savings compared to diesel savings in car and jet fuel.

With a market value of over £15 billion the potential rewards are high." Transport Minister, Sadiq Khan, said: "This project demonstrates our commitment to ensuring that biofuels are truly sustainable – and to show the potential from microalgae to be refined for use in renewable transport fuel development, to help reduce carbon dioxide emissions." Production of 70 billion litres will require man-made algae ponds equivalent to a landmass larger than Wales to be built in optimum locations across the world. Algae need a source of carbon dioxide and water to grow so Carbon Trust is now looking to investigate possible locations for large-scale plants which could be, for example, next to industrial facilities located near the sea.

The Carbon Trust is investing £8 million over 3 years into the projects using funding from the Department for Transport and the Department for Energy and Climate Change (DECC). Those universities and institutions selected to conduct the research are:

– University of Coventry
– London Queen Mary
– University of Manchester
– University of Newcastle (Supported on one project by Critical Processes Ltd)
– Plymouth Marine Laboratory (PML)
– Scottish Association for Marine Science
– University of Sheffield
– University of Southampton
– University of Swansea (Supported by Bangor University and PML)
– Scottish Association for Marine Science Grazer
– Coventry University

The research projects address five key challenges identified by the Carbon Trust:

1. Isolation and screening of algae strains
2. Maximising solar conversion efficiency
3. Achieving both high oil content and high productivity
4. Sustained algae cultivation in open ponds
5. Design & engineering of cost effective production systems
6. Control in algae cultivation
7. Algal economics

The Carbon Trust

The Carbon Trust is a not-for-profit company with the mission to accelerate the move to a low carbon economy, providing specialist support to business and the public sector to help cut carbon emissions, save energy and commercialise low carbon technologies. By stimulating low carbon action we contribute to key UK goals of lower carbon emissions, the development of low carbon businesses, increased energy security and associated jobs.

We help to cut carbon emissions now by

• Providing specialist advice and finance to help organisations cut carbon

• Setting standards for carbon reduction

We reduce potential future carbon emissions by

• Opening markets for low carbon technologies

• Leading industry collaborations to commercialise technologies

• Investing in early stage low carbon companies

Research needs by Large European Projects

AquaFUELS - Algae and aquatic biomass for a sustainable production of 2nd generation biofuels

The European project AquaFuels (FP 7 project) started in January 2010. AquaFUELS intends to focus on establishing the state of the art on research, technological development and demonstration activities regarding the exploitation of various algal and other suitable non-food aquatic biomasses for 2nd generation biofuels production. AquaFUELS will elaborate an overall assessment on the technology, and identify major research and industrial needs. The surveys and assessments produced by AquaFUELS will address the full life cycle analysis - from collection to fuel use - in terms of environmental, economic and social sustainability. The AquaFUELS project consortium includes European Biodiesel Board (EBB), Università degli Studi di Firenze (UNIFI), Ben-Gurioun University (BGU), Necton, and IN S.rl. Prof Mario Tredici (UNIFI) is the Scientific Coordinator of the AquaFUELS project.

Website: http://www.aquafuels.eu

SHAMASH - Biofuel from microalgae autotrophs

This project started in France in December 2006. The goal of the Shamash project is to produce biofuel from autotrophic microalgae. Shamash is an integrated research project. This project includes researchers from several research organizations, INRIA, CNRS, IFREMER, CEA, CIRAD, and universities in Nantes and Aix en Provence, and two companies, Valcobio and Alphabiotech. The project associates a wide range of specialists: algae culture, physiology and metabolism, process design and optimisation, biofuels and lipids extraction and purification, engine testings. The primary objective is to assess the technical and economic feasibility of a production of methyl ester. Shamash project has a total budget of 2.8 million euros. The National Program of Research on Bioenergy (PNRB) of the ANR contributes to the tune of 0.8 million euros. The project is coordinated by O. Bernard (INRIA Sophia-Antipolis).

Website: http://www-sop.inria-fr/comode/shamash/

MAMBO - MicroAlgae, starting Material for BioOil

The Italian research project MAMBO (MicroAlgae, starting Material for BioOil) was launched in May 2009 under Novaol S.r.l. coordination together with the Italian Biodiesel Manufacturers Association – Assocostieri. The project is led by the University of Florence (Dipartimento di Biotecnoloige Agrarie and Centro di Ricerca per le Energie Alternative e Rinnovabili) in partnership with Fotosintetica & Microbiologica S.r.l, and Stazione Sperimentale Oli e Grassi. The main goal of the project is to demonstrate the economical, technical and environmental feasibility of a pilot plant for the production of algae oil to feed the biodiesel production process. The project is divided into two phases. The first part deals with strain selection, comparative analyses of existing algae cultivation systems, and testing in small and innovative pilot units of microalgae performances to analyse energetic costs and preliminary design of a demonstration plant. With positive results of the first phase, the second phase of the MAMBO project schedules the building of the demonstration plant and microalgae cultivation for the final technical, economic and environmental assessment of the process. M.A.M.B.O. (MicroAlgae: raw Material for Bio-Oil) is dedicated to evaluate the technical, economic and environmental feasibility of microalgae production systems for biodiesel generation able to produce at least 15 to 20 tons of oil per hectare per year.

SOLAR BIOFUELS CONSORTIUM

Solar Biofuels is an international consortium established by Professor Benjamin David Hankamer, from the Institute for Molecular Bioscience (IMB) at The University of Queensland (Australia). Prof. Clems Posten from The University of Karlsruhe, Germany is member of this Consortium. This consortium aims to maximise the speed of development of high-efficiency 2nd generation microalgal biofuel systems. It includes 8 international teams and conducts bio-discovery, marine biology, structural biology, molecular biology, microbiology, genomics, metabolomics, culture optimisation and bioreactor scale up within a coordinated research program of parallel research streams. The consortium also has a detailed IP management strategy and provides a single point of contact for industry partners.

Website: http://www.solarbiofuels.org/

ALGAL BIOENERGY CONSORTIUM

The Algal Bioenergy Consortium (ABC) involves a large multidisciplinary group of scientists in UK, mostly from University of Cambridge who aim to use algae for a number of different applications in the bioenergy industry. This consortium brings together molecular biologists, physiologists, engineers and chemical engineers to facilitate the development of future bioenergy solutions. We collaborate with industrial partners to test our ideas. Given the size of the challenge to provide energy security in a sustainable way, it is important to explore the entire spectrum of possible energy sources.

Website: http://www.bioenergy.cam.ac.uk/abc.html

CENIT BIOSOS - BIOrefinería SOStenible

Abengoa Bioenergy has been, lately, awarded with a Cenit Project (under Spanish Administration R&D National Program) to support its R&D activities under a 37 M€ project to be conducted by ABNT leading 14 different companies during the next 4 years The aim of the Cenit BioSos Project (2009-2012) is to follow the biomass value chain in its entirety, from the generation of the resource up until the final market product. Special importance has been given to the development of these studies to assure the sustainability of the results obtained. Five main areas are to be highlighted: Raw materials (such as microalgal biomass), Sugar platforms, Syngas platforms, Bioproducts and Environmental, economic and social sustainability.

CENIT SOST CO2 - Nuevas Utilizaciones Industriales Sostenibles del CO2

The National Strategic Consortium for Technical Research “New insdustrial and sustainable utilization of CO2”(Spanish abbreviation for CENIT SOST-CO2) are composed by 15 companies that cover a broad spectrum of market from petrochemical, energy or food industries to engineering for services to a enormous industrial sectors. The support of 28 Universities, research centers and fundations make it of the greatest scientific excellency. This project has the main objective in to tackle the life cycle of CO2 from it emission source capture, to transport, storage and it big scale valorization. It expects link it capture with subsequent valorization, following alternatives to geologic confinement. Threre topic have been integrated in this project, all of them well interrelated, from CO_2 capture, Biological transformation to subsequent multiple uses for energy to feed, waste water treatment, materials, etc… The four-year project (2008-2012) accounts for a budget of 32 M€.

Website: http://www.cenit-sostco2.com

CENIT CO2 - Consorcios Estratégicos Nacionales en Investigación Técnica del CO2

The National Strategic Consortium for CO_2 Technical Research (Spanish abbreviation for CENIT CO2) is one of the projects granted by CDTI and is integrated in the Energy and Environment Technological Area. It was presented in the CENIT 2005 Programme Call and was approved in the Resolution of May 30, 2006. Its main objective is the research, development and validation of new knowledge and integrated solutions for increasing the efficiency in CO2 reduction processes. Consequently, Spanish industrial research projects are promoted with the aim of accelerating the development of advanced technologies that might lead to promising competitive advantages for Spanish companies and industries. The consortium is leaded by ENDESA Generación, with the relevant collaboration of UNIÓN FENOSA and the participation of other 12 industrial partners and 16 research institutions. The four-year project (2006-2009) accounts for a budget of 20 M€, from which 9.5 are granted by CDTI.

Website: http://www.cenitco2.es

SYMBIOSE

The Symbiose ANR project is a three year project launched in 2009 aiming at developing an integrated system to recover methane from microalgal biomass and recycle nutrients (mainly nitrogen and phosphorus). The project (with 2.5 million Euros total budget) includes 4 French public research teams and a private company. Symbiose is aiming at combining microalgae cultivation capturing CO2 from industrial process and anaerobic digestion. Symbiose relies on recent advances in both control of microalgae cultivation and anaerobic digestion processes. It integrates ecology of high rate ponds ecosystems and ecodesign.

BIOMARA - Sustainable biofuels from marine biomass

The Sustainable Fuels from Marine Biomass project, BioMara, is a new UK and Irish joint project that aims to demonstrate the feasibility and viability of producing third generation biofuels from marine biomass. It will investigate the potential use of both macroalgae and microalgae as alternatives to terrestrial agri-fuel production. The practicalities of using algal biomass as a competitive, sustainable biofuel source will be considered in concert with wide stakeholder engagement, whilst environmental impacts of algal cultivation and extraction will be core considerations of the project. BioMara aims to facilitate commercial biodiesel production by first identifying the high-yielding microalgal strains and then determining the optimal conditions for cultivating them.

Website: http://www.biomara.org/

SOLAR-H2

SOLAR-H2 brings together 12 world-leading European laboratories to carry out integrated, basic research aimed at achieving renewable hydrogen (H2) production from environmentally safe resources. The vision is to develop novel routes for the production of a Solar-fuel, in our case H2, from the very abundant,

effectively inexhaustible resources, solar energy and water.
The project integrates two frontline research topics: artificial photosynthesis in man-made biomimetic systems, and photobiological H2 production in living organisms.
The consortium performs research and development on the genetic level to increase their understanding of critical H2 forming reactions in photosynthetic alga and cyanobacteria. These studies are directly aimed at the improvement of the H2 producing capability of the organisms using novel genetic and metabolic engineering. The project also involves research aimed at demonstrating the concept of photobiological H2 production in photobioreactors.
The project is led by the Uppsala University and includes researchers from seven countries.

Website: http://www.biomara.org/

SENS BIOSYN

The purpose of this research collaborative project is to develop sensors and biosensors for on-line monitoring growth parameters of algal biomass and their bioactive compounds produced by large scale systems, with a particular focus on a group of relevant industrial processes for the natural synthesis of antioxidant Xanthophylls.
The team, is composed of three Research Institutes - National Institute for Biological Sciences - Centre of Bioanalysis; Ben Gurion University and National Council of Research - Institute of Crystallography, and three SMEs - Biosensor S.r.l., Nanosens and AlgaTechnologies Ltd.
The main objectives of the team are as follows: **The identification** of the industrial bioprocess requirements; the definition of the biosensors specifications; and the biosensors design; **Screening, identification**, characterization and purification of wild-type and molecular engineered *Chlamydomonas reinhardtii* photosystem II complexes for biosensor development. Set the Purification protocols, calibration and validation tests setup; **The development** of a set of optical and electrochemical sensors able to assess the content and the antioxidant efficacy of accumulated xanthophylls; **Control** of the biosensors application in field analysis; sensor response validation; manuals preparation, application notes.

Website: http://www.sensbiosyn.com/

EUREKA BIOFIX

The aim of the project is to work out a procedure for algal biomass production and to test the resulting biomass quality using the thin layer culture technology, where the source of carbon dioxide for growth of algae is derived from the combustion of municipal solid waste processed by the TERMIZO company. Use of this CO2 can reduce the price of the algal biomass and extends its utilization in human and animal nutrition. Furthermore, it can serve for reducing waste carbon dioxide emitted from flue gases into the atmosphere.
The proposed project will be solved on the Czech side in cooperation with the following partners: TERMIZO A.S., LIBEREC, INSTITUTE OF MICROBIOLOGY, CZECH ACADEMIC SCIENCE, Trebon and FUEL RESEARCH INSTITUTE, Prague. The foreign project partner will be the Division of Algal Biotechnology, INSTITUTE FOR CEREAL PROCESSING, LTD., NUTHETAL, OT BERGHOLZ-REHBRUECKE, GERMANY. This institute has long-term experience with the development of equipment for laboratory and outdoor cultivation of algae in closed bioreactors and with commercial application of algal products as food/feed additives, in cosmetics and pharmacy.

EUREKA ALGANOL

During the proposed project will be modified produced micro-algal biomass with a high content of starch. Such cultivation requires modification of production conditions in order to enhance a biosynthesis of reserve compounds (starch eventually lipids) of the micro-algae. The starch-enriched biomass will be subsequently treated in order to obtain fermentable sugars for bio-ethanol production. Also the lipid fraction will be separated and utilized for bio-diesel production. This downstream process includes optimization of cell disintegration, starch and cellulose hydrolysis, ethanol fermentation, lipid extraction and esterification, etc. Not least, the advantageous utilization of protein-rich biomass residues will be investigated. The proposed project will be carried out on the Czech side in cooperation with the following partners: Termizo a.s., Liberec Institute of Microbiology, Czech Acad.Sci., Trebon Institute of Chemical Technology, Prague Fuel Research Institute, Prague. The foreign project partners will be: Division of Algal Biotechnology, Institute for Cereal Processing, Ltd., Nuthetal, GERMANY Centre of Biological Engineering, University of Minho, Braga, PORTUGAL Department of Environmental Process Engineering, International Graduate School, Zittau, GERMANY.

MABFUEL Marine algae as biomass for biofuels

The group intends to facilitate a multi-disciplinary research programme through the recruitment of experienced researchers aimed at the acquisition of new knowledge and skills in the production of biofuels from native seaweed and cultured micro-algae. The project will identify the native seaweed and cultured micro-algal processes with the most potential for fuel production, the best time and technique to harvest seaweed and the culture methodologies for micro-algae along with an economic and environmental appraisal which will identify the size of the farm required and the feasibility of a commercial size operation. This will provide the physical (biomass product) and the intellectual (methodology for production and extraction) tools to enable the bio-fuel sector to base it s business on the most suitable and profitable process.

This project involves a large group of scientists from three countries: United Kingdom, Ireland and Turkey.

ALGOHUB Exploitation and use of microalgae in Nutrition & Health

Algohub is a multidisciplinary program sponsored by OSEO Innovation. Roquette and its partner firms are planning to study biodiversity, develop microalgae bioreactors, and produce micronutrients and high-added-value ingredients. That is why the Group recently acquired BPS, the German microalgae (chlorella) production specialist that owns the world’s largest freshwater photobioreactor.Approved by two national competitiveness centers: the « Nutrition Health Longevity » Centre and the « Aquatic Products » Centre, the ALGOHUB™ programme has been validated by the State body OSEO, which will enable the innovation process to be speeded up. This consortium brings together 14 partners (industries, research and training centres, SME’s, start-ups, institutes) in order to raise the level of the knowledge and effectively improve the production of microalgae. Well-known for their technological know-how and their scientific knowledge, these partners will provide state-of-the-art expertise in key sectors: pharmacology, cosmetology, marine biotechnologies, cell engineering, depollution, aquaculture, extraction of active compounds, human nutrition, animal nutrition, plastics technology, separation/knowledge and control of light.

Research needs by the SRI / DOE Report (August 1986)

Fuels from Microalgae: Technology Status, Potential, and Research Requirements

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Prepared under Task No. 4513.20 - FTP No. 513

Solar Energy Research Institute. A Division of Midwest Research Institute.

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7.0 CONCLUSIONS AND RECOMMENDATIONS

This technology assessment suggests that gasoline and diesel fuels could be produced from mass cultured microalgae at prices that will be competitive with conventional fuels. Aggressive research is needed to fulfill the performance requirements defined by the analysis, but the required improvements are within the bounds of attainability and have been closely approached under controlled conditions. Based on the achievement of these research goals, liquid fuels that are potential direct substitutes for conventional hydrocarbon fuels can be produced from microalgae for $1.60-$2.00/gal. Conclusions from the analysis and recommendations for future research fit into three categories: fuel products, resource and environmental considerations, and the biological and engineering aspects of fuels from microalgae.

7.1 **Fuel Products from Microalgal Feedstocks**

Research Recommendations. The immediate research priority in microalgal fuel product assessment is a detailed characterization of the fuel properties of microalgal lipids. Attention must be directed toward (I) the identification of techniques by which these lipids can be extracted on a large scale and (2) a detailed description of the characteristics of these lipids as they relate to their suitability as feedstocks for fuel conversion processes. Ultimately, conversion processes specifically tailored to the characteristics of microalgal lipids must be developed, either through the optimization of existing techniques or through the development of innovative conversion technologies. Such research activities will require the production of algal biomass samples on a scale suitable for extraction and fuels characterization. Samples from a number of promising species should be included, since there are strong indications that the character

ktics of lipids vary widely between taxa.

7.2 Resources and Environmental Considerations

Research Recommendations. Both the microalgal production model and the advice of experts in the field have emphasized the significance of saline groundwater as a potential limiting resource for the development of a large-scale microalgal technology in the desert Southwest. To assess the potential scale of this technology, it is critical that accurate estimates of the sustainable yields of the saline aquifers in the area be determined. Factors of interest are total aquifer volume, recharge rates, and potential changes in water composition with prolonged pumping. In addition, the effects of extensive pumping of saline aquifers on potable water aquifers in the same region will have to be assessed. Beyond an assessment of the saline groundwater resource, we also recommend an assessment of the magnitude of the possible contribution of coastal seawater and agricultural runoff to a microalgal fuels technology.

7.3 Biological and Engineering Aspects of Feedstock Production

Research Recommendations. The identification or development of microalgae strains that will meet the performance criteria of high productivity; high lipid content; and wide ranges of environmental tolerance is the single most critical research requirement for the-economic viability of a microalgal fuel technology. The ability to meet these requirements must first be established in closely controlled experimental cultures, then confirmed under conditions that more closely approximate outdoor mass culture conditions. The ultimate success of the fuels-from-microalgae concept is critically dependent on the rate and degree of species improvement. Continued investigation of harvesting techniques is recommended, as is an emphasis on a characterization of the performance of different species in various harvesting systems. The identification of species characteristics that determine their harvestability is a critical input to the selection and development of microalgal species for use in outdoor production systems: a strong interaction between species development and harvester engineering activities will be critical to the development of species that meet performance criteria and yet can be harvested economically. Finally, it is recommended that mechanisms for control of evaporation and nutrient outgassing be explored to evaluate the potential for minimizing the cost of these resources.

Research needs by the NREL / DOE Report (July 1986)

A Look Back at the U.S. Department of Energy's Aquatic Species Program - Biodiesel from Algae

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From 1978 to 1996, the U.S. Department of Energy's Office of Fuels Development funded a program to develop renewable transportation fuels from algae. The main focus of the program, known as the Aquatic Species Program (or ASP) was the production of biodiesel from high lipid-content algae grown in ponds, utilizing waste CO2 from coal fired power plants. Over the almost two decades of this program, tremendous advances were made in the science of manipulating the metabolism of algae and the engineering of microalgae algae production systems. Technical highlights of the program are summarized below:

Applied Biology

A unique collection of oil-producing microalgae.

The ASP studied a fairly specific aspect of algae—their ability to produce natural oils. Researchers not only concerned themselves with finding algae that produced a lot of oil, but also with algae that grow under severe conditions - extremes of temperature, pH and salinity. At the outset of the program, no collections existed that either emphasized or characterized algae in terms of these constraints. Early on, researchers set out to build such a collection. Algae were collected from sites in the west, the northwest and the southeastern regions of the continental U.S., as well as Hawaii. At its peak, the collection contained over 3,000 strains of organisms. After screening, isolation and characterization efforts, the collection was eventually winnowed down to around 300 species, mostly green algae and diatoms. The collection, now housed at the University of Hawaii, is still available to researchers. This collection is an untapped resource, both in terms of the unique organisms available and the mostly untapped genetic resource they represent. It is our sincere hope that future researchers will make use of the collection not only as a source of new products for energy production, but for many as yet undiscovered new products and genes for industry and medicine.

Shedding light on the physiology and biochemistry of algae.

Prior to this program, little work had been done to improve oil production in algal organisms. Much of the program's research focused attention on the elusive "lipid trigger." (Lipids are another generic name for TAGs, the primary storage form of natural oils.) This "trigger" refers to the observation that, under environmental stress, many microalgae appeared to flip a switch to turn on production of TAGs. Nutrient deficiency was the major factor studied. Our work with nitrogen-deficiency in algae and silicon deficiency in diatoms did not turn up any overwhelming evidence in support of this trigger theory. The common thread among the studies showing increased oil production under stress seems to be the observed cessation of cell division. While the rate of production of all cell components is lower under nutrient starvation, oil production seems to remain higher, leading to an accumulation of oil in the cells. The increased oil content of the algae does not lead to increased overall productivity of oil. In fact, overall rates of oil production are lower during periods of nutrient deficiency. Higher levels of oil in the cells are more than offset by lower rates of cell growth.

Breakthroughs in molecular biology and genetic engineering.

Plant biotechnology is a field that is only now coming into its own. Within the field of plant biotechnology, algae research is one of the least trodden territories. The slower rate of advance in this field makes each step forward in our research all the more remarkable. Our work on the molecular biology and genetics of algae is thus marked with significant scientific discoveries. The program was the first to isolate the enzyme Acetyl CoA Carboxylase (ACCase) from a diatom. This enzyme was found to catalyze a key metabolic step in the synthesis of oils in algae. The gene that encodes for the production of ACCase was eventually isolated and cloned. This was the *first* report of the cloning of the full sequence of the ACCase gene in *any* photosynthetic organism. With this gene in hand, researchers went on to develop the first successful transformation system for diatoms—the tools and genetic components for expressing a foreign gene. The ACCase gene and the transformation system for diatoms have both been patented. In the closing days of the program, researchers initiated the first experiments in metabolic engineering as a means of increasing oil production. Researchers demonstrated an ability to make algae over-express the ACCase gene, a major milestone for the research, with the hope that increasing the level of ACCase activity in the cells would lead to higher oil production. These early experiments did not, however, demonstrate increased oil production in the cells.

Algae Production Systems

Demonstration of Open Pond Systems for Mass Production of Microalgae.

Over the course of the program, efforts were made to establish the feasibility of large-scale algae production in open ponds. In studies conducted in California, Hawaii and New Mexico, the ASP proved the concept of long term, reliable production of algae. California and Hawaii served as early test bed sites. Based on results from six years of tests run in parallel in California and Hawaii, 1,000 m2 pond systems were built and tested in Roswell, New Mexico. The Roswell, New Mexico tests proved that outdoor ponds could be run with extremely high efficiency of CO2 utilization. Careful control of pH and other physical conditions for introducing CO2 into the ponds allowed greater than 90% utilization of injected CO2. The Roswell test site successfully completed a full year of operation with reasonable control of the algal species grown. Single day productivities reported over the course of one year were as high as 50 grams of algae per square meter per day, a long-term target for the program. Attempts to achieve consistently high productivities were hampered by low temperature conditions encountered at the site. The desert conditions of New Mexico provided ample sunlight, but temperatures regularly reached low levels (especially at night). If such locations are to be used in the future, some form of temperature control with enclosure of the ponds may well be required.

The high cost of algae production remains an obstacle.

The cost analyses for large-scale microalgae production evolved from rather superficial analyses in the 1970s to the much more detailed and sophisticated studies conducted during the 1980s. A major conclusion from these analyses is that there is little prospect for any alternatives to the open pond designs, given the low cost requirements associated with fuel production. The factors that most influence cost are biological, and not engineering-related. These analyses point to the need for highly productive organisms capable of near-theoretical levels of conversion of sunlight to biomass. Even with aggressive assumptions about biological productivity, we project costs for biodiesel which are two times higher than current petroleum diesel fuel costs.

Resource Availability

Land, water and CO2 resources can support substantial biodiesel production and CO2 savings.

The ASP regularly revisited the question of available resources for producing biodiesel from microalgae. This is not a trivial effort. Such resource assessments require a combined evaluation of appropriate climate, land and resource availability. These analyses indicate that significant potential land, water and CO2 resources exist to support this technology. Algal biodiesel could easily supply several “quads” of biodiesel—substantially more than existing oilseed crops could provide. Microalgae systems use far less water than traditional oilseed crops. Land is hardly a limitation. Two hundred thousand hectares (less than 0.1% of climatically suitable land areas in the U.S.) could produce one quad of fuel. Thus, though the technology faces many R&D hurdles before it can be practicable, it is clear that resource limitations are not an argument against the technology.

The authors identified four **major research needs** to achieve the objectives of high productivity in large-scale outdoor systems:

1. Photosynthetic efficiency for light energy and high lipid production.
2. Fundamentals of species selection and control in open pond systems.
3. Mechanisms (and control) of algal bioflocculation.
4. Effects of non-steady-state operating conditions on algal metabolism.

The appendix to this report (Benemann et al. 1982b), analyzed the ARPS system as proposed by Raymond (1979, 1981) that was in development at the time at the University of Hawaii (Section III.B.1.). First, a detailed historical review of microalgae systems designs was presented, which traced the evolution of the two concepts. The main report carried out a detailed and updated review of all prior cost analyses. The specific claims made for the ARPS systems were analyzed in detail. For example, the CuSO4-filled cover was claimed to reduce harmful IR radiation, but this was not supported by the photosynthesis literature. Also, overheating would still be a major factor even with a $CuSO_4$ cover, requiring a cooling process. In addition, the heated $CuSO_4$ could not be plausibly used as a power source. Mixing power inputs would be prohibitive for this design. Increased productivities caused by a flashing light effect were not plausible. Most important, the costs for even the cover and liner for such a system would be prohibitive by themselves, without considering any other factors.

This study clearly identified the major difficulties associated with microalgal mass culture for fuel production. Only a very low-cost system, based on open ponds without plastic liners, mixed at low velocities, and using a very simple harvesting process, could be considered in such a process. But even with these rather favorable, though plausible, assumptions, costs would still be well above those for current, or projected, oil prices.