

**International Society for**  
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**NEWSLETTER**



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Message from the President, Dr. Susan Blackburn

Dear Friends and Colleagues

I am delighted to introduce this ISAP Newsletter, the first for 2014 and the second from our current Newsletter Working Group of the ISAP Executive Committee, ably led by Amha Belay. In keeping with the approach taken in the previous Newsletter, we have two really informative articles that will stand the test of time as we move forward into the future of algal applications. Many thanks to Stephen Mayfield and Vitor Verdelho Viera for their considered thoughts on algal fuels, past, present and future.

The other exciting news is that planning and preparation for **ISAP 2014 -The 5th Congress of the International Society for Applied Phycology** is proceeding well, with less than 3 months to go. I hope to meet up with as many of you as possible in Sydney for an exciting week of symposia on a range of topics, other presentations, workshops and some extraordinary algal events. Please visit the ISAP 2014 web site [www.isap2014.com](http://www.isap2014.com) for updates.

We would appreciate your feedback on the Newsletter and would be delighted if you think you have something to contribute to our next Newsletter. Please contact Amha Belay, myself or any of the ISAP Executive Committee.

See you in Sydney!

Warm regards.

Susan Blackburn

## Message from the Editor – Amha Belay

In the last issue of the ISAP Newsletter, we invited Prof. Amos Richmond to provide an insight into the state of knowledge on microalgal biomass production and its potential and challenges in the context of algal biofuels production. We believe that the article has generated interesting discussion on the subject. Indeed, Michael Borowitzka has started a discussion forum on the same topic. In this issue we present two articles, one from Dr. Stephen Mayfield of UCSD providing his view as to when algae-based transportation fuel will be viable, and the second from Vítor Verdelho Vieira who provides an extensive and deeper look at future pathways and needs for microalgal products and technologies by drawing examples from the European experience.

As we stated in the past, the newsletter will attempt to present topics on algal biotechnology in general without endorsing any view as such. In so doing, we hope to provide a forum for a healthy and fruitful discussion that may help advance the field further on more realistic grounds.

The first two issues of the newsletter have focused on microalgae. The next issue will highlight the other side of the equation – macroalgae. We hope these articles will generate further discussion. We look forward to your suggestions to make the newsletter an educational experience.

## **Dr. Stephen Mayfield: When Will Algae-based Transportation Fuels Be Economically Viable?**

**Stephen Mayfield, Director California Center for Algae Biotechnology, University of California San Diego, La Jolla California, USA**

I am often asked, “When will algae-based transportation fuels be commercially available?” My answer tends to be that they are available now, and have been since roughly 1900. In fact, algae-based fuels are the only kind you can buy! I say this somewhat facetiously, because crude oil and the fuels that come from it are all derived from algae. Granted, these are ancient fossilized algae that have undergone some chemical transformations over millions of years, but more or less all of the crude oil processed today, including things like shale oil and tar sands, are ultimately derived from algae.

I realize that what people generally mean by these questions is “when will it be possible to grow, harvest, and process living algae into drop-in hydrocarbon fuel that can compete economically with fossil fuel”, but I answer the way I do, in order to make one essential point: that fuel from algae is not a scientific hypothesis, or some new scientific discovery that needs to be validated. It is a done deal, a proven and known commodity; fuels from algae power most of our cars, trucks, ships and planes today. This is an important distinction, because the multi-trillion dollar - yes, trillions with “T” - transportation infrastructure that enables commerce in this country, and worldwide, runs on petroleum, or algae oil. Because it is not realistic to suggest that a “new” alternative fuel infrastructure of this global scale can be deployed in any meaningful timeframe, any alternative fuels that may be developed need to be “drop in” or fungible fuels, that are immediately compatible with the existing infrastructure, such as pipelines, refineries, fueling stations, and the engines in our cars, trucks, and airplanes. Algae-based alternative transportation fuels have already demonstrated this ability, having been refined into gasoline, diesel and jet fuels, which have in turn been used to drive personal automobiles, fly commercial planes, and power Navy ships and aircraft.

So if fungible fuels derived from algae are compatible with our existing transportation network today, when will these algae-based fuels – fuels made from recently living algae – be available at a large enough commercial scale to be economically competitive with fuels made from fossil crude oil? That is actually a very complex question that has many variables associated with it. Some of those variables have to do with future cost, of both crude oil as well as renewable algae oil, and neither of those costs are easy to predict, but we do see trends. So let me try to put some of these into perspective as we project out to the time when fossil algae oil and renewable algae oil will each “cost” approximately the same.

Let us start with the direct cost of crude oil. Today that is about \$100 dollar a barrel for West Texas Intermediate (WTI) and \$110 for Brent, with the difference in price having to do with local supply and demand, but more or less, we will put that figure at \$100 a barrel. Oil has been roughly \$100 per barrel for the last three years, but going back only about ten years, historically oil has traded for under \$20 a barrel. So what are the variables driving the cost of crude oil? One of the most important is that at \$100 per barrel, it is economical to use fracking to produce shale oil. Fracking is not new technology and the fields where it is being used now are certainly not newly discovered fields, but at \$100 a barrel, it is profitable to extract oil from shale using fracking technology. So, what that really means is that \$100 a barrel is the new “floor” on oil prices; once it dips below that price, fracking becomes economical unviable, the supply will drop, and prices will climb again. Therefore, this sets the floor but not the ceiling, or even the average price. It’s hard to guess what that ceiling or average price will be, but current trends indicate that it will continue on the path of the last ten years, which is to go up on average about 10% per year, meaning that five years from today it is realistic to assume oil prices at roughly \$150 a barrel.

Now let us look on the other side of the ledger, at the cost of algae oil. There have been a range of recent estimates of the cost of producing a barrel of renewable algae oil, with estimates as low as \$28 a barrel, to as high as a few thousand (1). The low estimates get to their costs by assuming significant offsets from co-products or other waste stream offsets, while the high estimates get to their costs by making

(often outlandish) assumptions on the price of building an algae production facility (2). A price for algae oil was recently determined in a paper by Benneman et al (2012) and placed at \$240 a barrel, based on some conservative assumptions. So let us say that \$240 a barrel is today's price for algae oil, and then assume that as a result of logical biological and process decisions, algae fall into an agricultural model of increased productivity and reduced price over the next ten years. Viewing American agriculture for the last 50 years, it is clear that crop yields have increased dramatically over this period, with corn yields for example going up almost 400%. It seems reasonable to assume that algae yields will follow a similar path, especially over the next few years, when large gains should be easier to come by as genomic and molecular technologies begin to be applied to a crop that has yet to benefit from these proven innovations. A reasonable extrapolation would be that a 50% yield increase can be achieved in the next five years, and a doubling of yield in the next ten. So, even if no other breakthroughs emerge in process development, harvesting, extraction or any other technology (which is unlikely), based on agricultural productivity and innovation precedents, algae oil costs should reasonably decrease by 50% with the next five years and be half of today's cost within a decade. Process engineering developments will also contribute to decreased cost and perhaps increased yields, so even assuming no new inventions, these trends point to algae oil nearing the \$100 a barrel cost floor within five years, and potentially a further decrease in cost over the five following years. This increase will not have the same impact, but will reduce cost below the \$100 a barrel floor.

Some will point out that these cost estimates are based on today's dollars, and that over ten years the costs of input materials and labor for algae oil production will go up, and therefore so will the cost of algae oil. This is likely true, and so in many ways a dollar cost is only a reasonable comparison for today. What really matters in the future is the energy return on energy invested, or the EROI. If this number is not significantly above 1, meaning that more energy comes out of the process than is put in, then cost is meaningless, because the system in question is not sustainable. EROI is a reasonable measure of commercial viability, and cost will follow. The EROI on fossil oil has historically been as high as 100, meaning for every 1 barrel of oil worth of energy put in, 100 barrels of oil could be recovered. This is no longer the case, and today the EROI on fossil fuels ranges from 2.5 to about 4.5, with fracking and tar sands being on the lower end of those returns. A recent life cycle analysis of algae oil production from the Sapphire Energy pilot facility in New Mexico (3) returned a pilot scale EROI of 1, and a projected EROI of 2.5 to 3 when the full commercial scale system is completed. For sake of comparison, a present-day commercial facility for cellulosic ethanol has an EROI just below 1, while corn ethanol at full commercial scale has an EROI below 1 as well.

So what does this mean in terms of when algae oil will be cost competitive with fossil fuels? It means we could see parity within five years, but a few things have to happen in order for that to become reality. First, it is critical to sustain and enhance investments in renewable energy from algae. Many people thought that oil at \$100 a barrel would stimulate that investment, but it did not; it simply stimulated enormous investments into extracting more fossil fuel. Therefore, it is important that we reinvigorate investment in drop-in renewable fuels that are compatible with the existing transportation infrastructure. This continued investment is needed to incentivize construction of the commercial scale facilities that will allow us to achieve the economies of scale required to get the cost of algae oil lower. This investment will also enable the continued research that will allow us to improve both the algae strains and the production systems – progress that will help reduce cost further. Second, it is essential that we take a hard look at all of the potential renewable fuel options out there, and make the right decision on which options to fund, and which to back off on. An enormous amounts of money has been invested in cellulosic ethanol research here in the US, more than \$1 billion dollars in the least seven years, and probably that much again from private investment over the same period. This investment has achieved very little in terms of bringing cellulosic ethanol to commercial viability. From a purely market perspective, it is difficult to justify continuing such investments given that cellulosic ethanol has an EROI of less than 1. Perhaps part of the reason we continue down this path is that cellulosic ethanol research has now become an industry onto itself. There are just so many people funded to do this work, and so much money has been invested, that it is difficult to acknowledge that this vein is tapped out, and move on to more productive investments.

So, my prediction ... algae oil achieves economic parity with fossil crude oil at \$140 a barrel in 2019 and beats it by \$50 a barrel by 2024 when algae oil is at full commercial production scale.

1. Life Cycle Assessment for Microalgae Oil Production. Benemann, J., Woertz, J., Lundquist, T. *Disruptive Science and Technology*. 2012, 1(2): 68-78.

2. California's energy future – the potential of biofuels. Youngs, H., Somerville, R. California Council on Science and Technology. May 2013

3. Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. Liu X, Saydah B, Eranki P, Colosi LM, Greg Mitchell B, Rhodes J, Clarens AF. *Bioresour Technol*. 2013 Nov;148:163-71.

## Vítor Verdelho Vieira: Microalgae for Fuels and Beyond: the leading trends in 2013

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Future trends result from: present and emerging needs plus accumulated evolution achievements. To understand the possible future pathways it is necessary (1) to study the historical evolution, (2) the existing knowledge and experience, (3) and combine that with future needs - that can be fulfilled with microalgae products and technologies.

At present we are going through a paradigm change: “there are several emerging market needs that can be best fulfilled by a new agriculture crop: microalgae”

As a result of accumulated knowledge, at this moment, there is ‘critical mass’ that makes it possible to have this new agriculture crop in a transition from research to commercial scale. The author has been monitoring in detail the microalgae sector since 1989. More than 300 events (academic, industry related and political or regulatory) involving microalgae were evaluated along the last 310 years (since 1703, see Table 1), as well as a list of +900 companies currently in operation and +350 that went out of business have been being looked out on a regular basis. Three simultaneous inputs were spotted as the change-making factors:

**(1) Accumulated knowledge:** The first description of microalgae was reported 310 years ago. There was a slow progress in early times, then a linear growth in the 20th century – and an exponential change during the last ten years. This growth happened both in the number of persons working with microalgae at the research level and in the number of start-up companies. The first known company was Chlorella Industry in Japan in 1964 and forty years later in 2003, there were less than 100 companies worldwide involved in the continuous production of microalgae. However in 2013 there are more than 1,000 companies and only less than 20% of companies existing in 2003 still exist now. The number of research groups and research organizations with projects related with microalgae went from less than 300 worldwide to more than 5,000 in the same period. One major example following the oil crisis in the 1970s, in the United States the Carter administration through the Department of Energy under the Office of Fuels Development, funded the Aquatic Species Program where the National Renewable Energy Laboratory scientists isolated around 3,000 algae species over a period of nearly 20 years from 1978 to 1996.

Microalgae are extremely interesting as a new crop but not as a ‘miracle making crop’. Several review papers, published in 2006 and 2007 brought together and reviewed most of the scientific and technological information. A new idea came up: if certain microalgae can have up to 70% oil; double every day and have high productivities - then, ‘microalgae’ can produce 136.900 L/ha. It was also concluded that “microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil” (this reference had +3.000 citations in peer reviewed papers). It is obvious that this syllogism, cited and repeated through the years, is wrong since no microalgae is capable of producing such high oil amounts or they would be breaking the principle of mass conservation. A similar reasoning was made about microalgae for CO<sub>2</sub> ‘sequestration’ assuming impossible levels that forgot to consider the mass conservation principle: microalgae cannot fix more carbon than their photosynthetic theoretical limit.

**(2) Regulatory factors:** ‘Microalgae for Fuels’ was pushed by political issues & regulatory factors and pulled by relevant science & technology to a level of wide awareness and commercial interest. These were the triggering factors for the recent interest on microalgae. The US Energy Policy Act of 1992 directed a demand for more studies to be done on biofuels and also gave some guidance for federal programs for the increased implementation of biofuels. In Europe a proposal to develop an EU-15

Biofuel Directive was launched during 2001. The peak in oil prices in 2008 boosted new interest in algal fuel worldwide. Research programs were initiated to investigate the different processes required to produce algal fuel.

In December 2008, the EU struck a deal to satisfy 10% of its transport fuel needs from renewable sources, including biofuels, hydrogen and green electricity, as part of negotiations on its energy and climate package. On 1 July 2011 the American Society for Testing of Materials has officially approved the use of algae and other sustainably-derived biofuels in commercial and military aircrafts. Under EU Directive 2009/28 the European Commission decided to include algae biomass in the first place of the list of substances which will count four times their energy content towards the overall 10% EU target for renewable fuels in transport. In addition, the EU Emissions Trading System (ETS), the largest multi-country, multi-sector greenhouse gas emission trading system in the world intends to support EU meeting its 20% emissions reduction target by 2020. The EU ETS covers +11,000 energy-intensive industrial installations throughout Europe, such as power stations, refineries, large manufacturing plants and was expanded to the aviation industry on 1 January 2012.

(3) **Market opportunities:** There is a current need for alternative high value omega-3 polyunsaturated fatty acids (PUFA) oils to start replacing fish oils and fishmeal. Since around 2005 aquaculture feeds has continued its strong annual growth of around 7% but the volumes of fishmeal used in aquaculture have remained steady at around 3.2 million ton and those of fish oil have even been reduced to around 600,000 ton. This has led the FAO to consider in their recently released report on the State of Fisheries & Aquaculture (2012) that “the sustainability of the aquaculture sector will be closely linked with the sustained supply of terrestrial animal and plant proteins, oils and carbohydrates for aquafeeds”. For this reason Food, Feed and Ceuticals will overcome biofuel applications relating the use of microalgae as a new crop. Some microalgae can be used as a premix to transform commodity low cost vegetable oils in added value partial replacers of fish oils and fishmeal.



## The current status and evolution pathway in microalgae technologies

### Microalgae market positioning trends

Soy: oil & meal	Fish: oil & meal	Algae: oil & meal
> 200 million ton/year of production	> 7 million ton/year of production	> 20 thousand ton/year of production
Feed applications are the most relevant	Feed applications are the most relevant	Food applications are the most relevant
Soy based feeds improved with fish meal	Fish meal feeds are improved with algae	Algae emerge as pre-mix feed ingredient
Current value < 0.5 € typically 0.35 €/Kg	Current value < 2 € typically 1.5 €/Kg	Current value < 5 € typically 10 €/kg

### Microalgae market opportunities trends

Food & Feed	(Nutra & Cosme) Ceuticals	Agriculture & Biofuels
Biomass formulations	Ingredients and Extracts	Ecological management - soil algae
Formulations: paste, frozen, dried	Extraction: super-critical, green solvents,...	Processes: inoculation for fertilizers, pyrolysis
<i>Spirulina, Chlorella, Dunaliella</i>	Astaxanthin, Beta-carotene	Future biofuel wishes dominant
Aquaculture paste in Europe since 1997	Plankton extracts	High level of social hype

### Microalgae development trends

Research	Technologies	Products
First microalgae isolated in 1703 (Tabellaria)	> First production reactor in 1957 (MIT roof)	> First product Dihe in Lake Chad
First microalgae in space in 1968 (Soyuz)	Floating devices in the ocean (Omega type)	> <i>Spirulina</i> consumption by Aztecs
> 20 species scaled-up for production	Lake based technologies (China, Australia...)	Microalgae biomass for Aquaculture
First microalgae genome sequenced 2007	Raceway based technologies (dominant)	<i>Spirulina</i> biomass in a wide range of foods
% phytoplankton formula C53.5 H7.4 O28.2 N9.4 P1.3	Photobioreactors (many configurations)	Microalgae extracts for Ceuticals
Phytoplankton as main CO <sub>2</sub> fixation source	Heterotrophic fermenters (Celsys in 1985)	Diatoms used in a wide range of industries

### Microalgae academy trends in Europe

Algae Parks	Research Groups	PhD Students
≥ 3 demo technology platforms	≥ Group => 10 researchers	≥ with 50% microalgae related topics
More than 10 small Parks in Europe (2013)	> 200 research groups (2013)	> 100 PhD under development (2013)
Reduced networking between them	Existing but reduced inter-collaboration	Networking limited to Marie Curie projects
Still in a very early stage (reduced training)	Some groups with > 30 years' experience	Increasingly applied topics

Table 1 – Key drivers for the microalgae technologies evolution pathways

## MACRO-TRENDS 1 – MICROALGAE PRODUCTS AND MARKETS

The trend is that the ‘microalgae sector’ will be increasingly **market driven** both by the actual market and also the potential or expectable market (as with biofuels). Product developments will emerge as a result of this market pressure based on existing and developing technology capacity. The current applications will become more relevant as microalgae biomass and microalgae extracts will become a possible price competitive option for formulators of food, feed and ceutical products. **Biorefinery** of microalgae biomass will make it possible to have added value products that are competitive in price, quality and performance.

The products from microalgae have currently only three possible forms: microalgae paste, dried microalgae and microalgae extracts, each of which have a wide range of formulations. Algae paste may have different concentrations, usually from 5 to 15%, which strongly dependent on the microalgae species and application. Microalgae are mostly spray-dried or freeze-dried but can also be simply sun-dried. Extracts can be obtained with solvents, super-critical, or with mechanical processes.

Only 3 possibilities:	<b>PASTE</b>	<b>DRIED</b>	<b>EXTRACTS</b>
Applications:	Aquaculture	Food & Feed Aquaculture Ceuticals	Ceuticals

There are two possible ways to evaluate the market size for microalgae products: the most usual one is to determine the approximate production quantities (ton/year) of each microalgae and consider its average value per kg. Another alternative is to consider the top 100 companies and relate their turnover with the products they have. This is possible and relatively accurate because most companies in the sector are single product base, and the top 100 represent more than 80% of the market (Table 2).

Microalgae business based on turnover of the top 100 companies – values for 2012			
Number of companies	Markets	Segments	< M US\$ / year >
	AQUACULTURE (~2%)		
	Paste	<i>Nannochloropsis</i> and other	20
	Freeze & spray Dried	<i>Nannochloropsis</i> and other	5
		ST	25
	FOOD & FEED (~41%)		
	Dried microalgae	<i>Spirulina</i>	250
	Dried microalgae	<i>Chlorella</i>	150
	Dried microalgae	Other (including AFA)	100
		ST	500
	CEUTICALS (~57%)		
	Extracts: Pigments	Beta-carotene from <i>Dunaliella</i>	100
		Astaxanthin from <i>Haematococcus</i>	80
		Other (*)	20
	Extracts: PUFA	DHA from <i>Schizochytrium</i>	400
		DHA from <i>Ulkenia</i>	80
		Other (including new <i>Cryptocodinium</i> )	20
		ST	700
		Total	1.225
	'PROJECTS'		
	Biofuel related		90
	Other		35
	<b>MICROALGAE BUSINESS</b>	<b>TOTAL VALUE</b>	<b>1.325</b>

It is a good estimation to consider that Microalgae biomass % extracts have a value of 1.5 million US\$ / year Worldwide.

- (1) Government programs to establish new species of microalgae and microalgae extracts as Novel Foods will be critical to establish them as new crops. The costs for such requests are high and will benefit the sector crosswise. This regulatory approval is mandatory for most added value applications.
- (2) Super-critical extraction and other green / environmental balanced technologies – dissemination and wide use - are essential for the valorization of microalgae extracts for food and ceutical applications.

Table 2 – Microalgae products global market value

## MICROALGAE FOR BIOFUELS: the emerging trends for sustainable solutions

From a wide range of possibilities, six technologies are emerging as main drivers for biofuels able to support the economical production of microalgae-based fuels. They comprise (i) Microalgae biorefinery for food, feed, fertilizer and energy production; (ii) Biofuel production from low-cost microalgae grown in wastewater; (iii) Biogas upgrading with microalgae production for carbon neutral low cost production of electricity (iv) Hydrocarbon milking of modified *Botryococcus* microalgae strains; (v) Hydrogen production through microalgae biophotolysis; and (vi) Direct ethanol production from cyanobacteria. They have different possible LCA frameworks and requirements for development and scale-up and there are many companies and research groups working along these possibilities.

Selected microalgae	Biofuel production from low-cost microalgae grown in wastewater	Biogas upgrading with microalgae for carbon neutral low cost production of electricity	Microalgae biorefinery for food, feed, fertilizer and energy production
GMO microalgae	Direct ethanol production from cyanobacteria	Hydrocarbon milking of modified <i>Botryococcus</i> microalgae strains	Hydrogen production through microalgae biophotolysis

**Biofuel production from low-cost microalgae grown in wastewater.** The use of microalgae for treatment of municipal wastewater has been under study for more than 50 years, although it has been used together with physical and chemical removal methods. Microalgae have been shown to be particularly tolerant to many wastewater conditions, removing large amounts of N and P. The necessary CO<sub>2</sub> may be provided from nearby industries, at low cost. By simultaneously supplying an effective and cheap growth medium for microalgal biomass production, as well as providing sewage treatment in a low-cost with the production of biomass for fuels and fertilizers.

**Biogas upgrading with microalgae production for carbon neutral low cost production of electricity.** Biogas (a mixture of methane and CO<sub>2</sub>) from landfills or other anaerobic digestion (AD) systems can be flown through microalgae cultures in PBR that consume the CO<sub>2</sub>. Biomethane can be burned to generate electricity or heat, whereas resulting CO<sub>2</sub> may be reintroduced into microalgal autotrophic cultures. The resulting biomass can be biorefined and the leftovers re-introduced in the system. The final digestate can be used for valuable fertilizers.

**Microalgae biorefinery for food, feed, fertilizer and energy production.** The majority of the mature industrial microalgae producers are focused on a single product application for the biomass, which has a high market value. For products with lower market value, as biofuels, protein or animal feed, the economic viability of the process is only achieved within the logic of biorefinery, meaning that all its secondary products or biomass residues are recovered and applied on the market. Economic viability can be achieved having biofuel application as secondary objective.

**Direct ethanol production from autotrophic cyanobacteria.** Expressive advances in the development of genetic tools to increase energy production in microalgae and cyanobacteria have recently been achieved, and are being used to manipulate central carbon metabolism in these organisms. Manipulation of metabolic pathways can redirect cellular functions towards synthesis of preferred products. It is likely that many of these advances can be extended to industrially relevant organisms. Several companies and research groups have already established the proof of concept and the scale or inclusion of co-products looks to be the requirement for a positive economic balance.

**Hydrocarbon milking of modified *Botryococcus* microalgae strains.** Among the costly downstream processing steps, it is generally accepted that harvesting / dewatering and the following extraction of fuel precursors from the biomass are the most costly and energy demanding steps. In order to decrease their cost, the possibility of integrating the steps of harvesting and extraction seems to be a potential solution. In this integrated process, algal cells can be cultured in biphasic bioreactors, consisting of an aqueous phase, where algae grow, and an organic phase, immiscible with the former, which continuously

extracts the fuel precursors. Genetically modified *Botryococcus* which naturally excretes biofuel precursors is being studied for commercial application in Japan and US.

**Hydrogen production combining direct and indirect microalgae biophotolysis.** Photobiological hydrogen production has advanced significantly in recent years. Nowadays a variety of photosynthetic and non-photosynthetic microorganisms, including unicellular green algae, cyanobacteria, anoxygenic photosynthetic bacteria, obligate anaerobic, and nitrogen-fixing bacteria are endowed with genes and proteins for H<sub>2</sub>-production. These organisms can use two natural pathways for H<sub>2</sub> production: (i) H<sub>2</sub> production as a by-product during nitrogen fixation by nitrogenases; and (ii) H<sub>2</sub> production directly.

#### MACRO-TRENDS 2 – MICROALGAE SPECIES

The trend is that (1) the microalgae that generate more publication of digital documents are the ones that are recognized for human consumption: *Spirulina* and *Chlorella*; (2) surprisingly new applications brought microalgae such as *Navicula* and *Euglena* to capture relevant interest; (3) and *Chlamydomonas*, being a model organism is also a very relevant microalgae where considerable knowledge has been accumulated.

In the Top 30 list (Table 3) one can see that the following microalgae had more than 100% growth in the last 10 years: *Thalassiosira*, *Navicula*, *Gymnodium*, *Emiliana*, *Pavlova*, *Haematococcus*, *Nannochloropsis*, *Botryococcus*, *Phaeodactylum*. Evaluating the rest of the Top70 list the following microalgas also show a growth of more than 100%: *Dysmorphococcus*, *Haslea*, *Neochloris*, *Schizochytrium*, *Botryococcus*, *Chlorococcus* (not presented here). The ‘long tail’ also has several interesting future tags. It is very relevant to see that in 2013 the Top5 species represent more than 80% of the references.

Genera + “alga”	Class	Google2003	Google2013	Fold-change in 10 Y	Scholar 2013
<i>Spirulina</i>					
( <i>Arthrospira</i> )	Cyanophyceae	303,000	8,833,000	29	57,220
<i>Chlorella</i>	Chlorophyceae	104,000	4,780,000	46	134 000
<i>Navicula</i>	Bacillariophyceae	271	1,290,000	4,760	31 400
<i>Chlamydomonas</i>	Chlorophyceae	28,000	1,170,000	42	52 900
<i>Euglena</i>	Euglenophyceae	18,800	1,020,000	54	48 600
<i>Anabaena</i>	Cyanophyceae	22,100	789,000	36	61 800
<i>Synechocystis</i>	Cyanophyceae	117,000	652,000	6	38 200
<i>Dunaliella</i>	Chlorophyceae	8,330	634,000	75	32 200
<i>Nostoc</i>	Cyanophyceae	20,400	593,000	29	36 100
<i>Scenedesmus</i>	Chlorophyceae	11,800	419,000	36	79 100
<i>Aphanizomenon</i>	Cyanophyceae	6,660	333,000	50	12 500
<i>Haematococcus</i>	Chlorophyceae	1,800	327,000	182	11 200
<i>Oscillatoria</i>	Cyanophyceae	6,520	301,000	46	30 600
<i>Nitzschia</i>	Bacillariophyceae	8,300	298,000	36	33 800
<i>Thalassiosira</i>	Bacillariophyceae	47	273,000	5,809	18 600
<i>Skeletonema</i>	Bacillariophyceae	5,140	211,000	41	21 300
<i>Chaetoceros</i>	Bacillariophyceae	5,590	209,000	37	24 500
<i>Phaeodactylum</i>	Bacillariophyceae	1,730	192,000	111	12 000
<i>Selenastrum</i>	Chlorophyceae	5,660	192,000	34	12 400
<i>Emiliana huxleyi</i>	Haptophyceae	310	186,000	600	11 900
<i>Nannochloropsis</i>	Eustigmatophyceae	1,080	180,000	167	9 930
<i>Gymnodium</i>	Cyanophyceae	166	173,000	1,042	547
<i>Cyclotella</i>	Bacillariophyceae	4,640	157,000	34	25 600
<i>Pavlova lutheri</i>	Cyanophyceae	277	154,000	556	4 830
<i>Botryococcus</i>	Chlorophyceae	1,150	143,000	124	9 790
<i>Tetraselmis</i>	Prasinophyceae	1,880	127,000	68	12 200

Table 3 – Ranking evolution along 10 years in scale Google of references for microalgae genera

If the newcomers into the sector want to obtain knowledge and experience from marine bivalve hatcheries where microalgae are produced in a m3 scale they will find the following microalgae that represent 90% of the manager choices: Isochrysis, Chaetoceros, Tetraselmis, Pavlova, Skeletonema. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained widespread use in aquaculture.

This is a Top 25 list based on an analysis from the universe of 70 genera. The google search 10 years ago was done in the month of September as well as in 2013. The objective was to evaluate the comparative relevance but now it is possible to (1) study the evolution of the interest level (growth in the number of references); (2) the current relevance level and (3) evolution of relevance level. Searches combined as simultaneous keywords the name of the genera and algae or cyanobacteria. This kind of methodology has been used in other fields with interesting results.

### MACRO-TRENDS 3 – MICROALGAE PRODUCTION OPTIONS

The **trend** is that depending on the microalgae crop and the production platform there will be different production options or carbon sources. Most microalgae production is now photoautotrophic (*Arthrospira*, *Chlorella*, *Dunaliella*...) representing 80% of production in ton/year or heterotrophic (*Schizochytrium*, *Ulkenia*, *Cryptocodinium*...) representing 20% of production in ton/year. **Mixotrophic production will become a third option** and in the near future it is expectable that each of the referred production options will have an equivalent importance in terms of production.

The microalgae nutrition mode has a high impact in the productivity, in production costs and in the possible production platforms that can be used.

only 3 possibilities:	Photoautotrophic	Heterotrophic	Mixotrophic
Production platforms:	Ocean	Fermentors	Ponds
	Lakes	Laboratory	PBRs
	Ponds		Laboratory
	PBRs		
	Laboratory		
	CO <sub>2</sub> as Carbon source	Organic Carbon as Carbon source	CO <sub>2</sub> & Organic Carbon; as Carbon sources

The term ‘micro-algae’ is most of the times used in its narrowest sense as a synonym for photoautotrophic, unicellular algae utilizing CO<sub>2</sub> and gaining energy from light through photosynthesis. Although certain species are obligate photoautotrophs, numerous microorganisms are capable of both heterotrophic and photoautotrophic metabolism either sequentially or simultaneously. Some even prefer to live heterotrophically and only survive as photoautotrophs once all other energy sources are depleted. These microorganisms may thus not be classified as pure microalgae. There is a relevant debate in the scientific community about this which may be resolved with the increase of genetic information available on these species.

**Autotrophs** can be phototrophs or chemotrophs: Autotrophic species are photosynthetic like plants. Phototrophs use light as an energy source, while chemotrophs utilize electron donors as a source of energy, whether from organic or inorganic sources; however in the case of autotrophs, these electron donors come from inorganic chemical sources. Such chemotrophs are lithotrophs. Phototrophic prokaryotes may utilize a variety of carbon sources, depending on the metabolic pathways available.

**Heterotrophic:** Heterotrophic species get their energy from organic carbon compounds in much the same way as yeast, bacteria and animals. Cultivation without light and with the controlled addition of an organic source of carbon and energy is similar to procedures established with bacteria or yeasts in multipurpose stirred closed tanks sterilized by heat. To date, only a small number of microalgal species have been cultured heterotrophically in conventional bio-reactors. Typically, heterotrophic cultures can be 5 to 10 times higher densities than corresponding autotrophic.

Mixotrophic: Organisms deriving nourishment simultaneously from both autotrophic (inorganic substances resulting from chemosynthesis and photosynthesis) and heterotrophic (organic substances) mechanisms. Chlorophyll-bearing flagellates, which are autotrophs, become mixotrophic in heavily polluted water, where they feed on organic matter in order to stimulate growth and reproduction; some of these flagellates can develop even in total darkness, that is, without photosynthesis. Mixotrophic species can use both sunlight and organic carbon, whatever they can get.

Mixotrophic cultures started to be used in Japan and Taiwan in mid 60s but during many years, the autotrophic production has been preferred because of lower production costs and easier production operation because of contaminants. Only recently, the need for higher productivities with added value microalgae re-started this approach for large-scale production of microalgae.

#### MACRO-TRENDS 4 – MICROALGAE PRODUCTION PLATFORMS

The trend is that different microalgae species will be produced in different platforms in different locations. As with other crops such a soy, sunflower or corn not all microalgae can grow in any climate. All six possible production platforms will have a different applications framework. During the next decade will be possible to see a consolidation of open ponds and PBRs and a massification in fermentation based companies. Ocean based production will emerge as a possibility for special places and Laboratory based ‘molecular farming’ will be common.

Two types of production systems will distinguish the microalgae crops from one another: open system based platforms will be agriculture related and will follow agriculture approaches, while closed system based platforms will be industry related. Different species will be produced according with the objectives and available resources.

Open:	Ocean	Lakes	Ponds
Closed:	PBRs	Fermentors	Labscale

Production platforms are of outmost importance in microalgae production, because their shape and operational conditions will dictate the final productivity rates achieved. The choice of the most suitable platform is case dependent, as well as the production mode.

**Ocean:** This possible production platform is still in a research stage. Several research groups and at least 3 companies have been working in possible approaches to grow microalgae in floating devices and built proof of concept systems. First experiments started along the last ten years and the best well known is the Offshore Membrane Enclosure for Growing Algae (OMEGA), aiming to demonstrate, in one hand, the viability and scalability of producing large amounts of algae for carbon-neutral biofuels, foods, fertilizers and other valuable products and in the other hand, treat wastewaters and sequester CO<sub>2</sub>, without competing with traditional farming for land, fresh water or fertilizers. Large amounts of algae producing units, composed of floating bags in the sea, would sequester CO<sub>2</sub> and use seawater to grow (<http://inhabitat.com/nasas-omega-project-creates-carbon-neutral-food-and-fuel>).

**Lake:** This was the first approach for the human use of natural microalgae blooms for food consumption. Harvesting of naturally occurring microalgae was reported more than 500 years ago both in Mexico and in Lake Chad. *Spirulina* blooms are still used for commercial application in China and Myanmar - and *Dunaliella* in Australia. The technology to cultivate *Spirulina* using natural lakes was first developed in the early 1970s in Lake Texcoco in Mexico (stopped operation in the 80s), and for *Dunaliella* it started in the 80s and is still in use in two locations in Australia for the production of beta-carotene and other carotenoids.

**Ponds** (circular, raceways and other): This is the most widely used production platform with raceway ponds, circular ponds or other configurations. Chlorella Industry Co., Ltd., headquartered in Tokyo, pioneered the world's first mass culture production in 1964. These facilities used circular ponds to grow *Chlorella* for human consumption. In 1966, Taiwan Chlorella Company was designed and built by Japanese experts. In the early 70s, *Spirulina* production using raceways started in in the hot desert area in Southeastern part of California (Earthrise) with a commitment to developing microalgae for food,

biochemicals and pharmaceuticals. These companies were role models for many others and knowledge spread all over the world, mostly in China for the production of *Spirulina* and *Chlorella*.

**PBRs** (many possible configurations but tubular systems are the most common): The first successful industrial tubular PBRs were established by Algatechnologies in Israel in 1999 to develop and commercialize astaxanthin for the Ceutical industry. IGV Germany also started relevant work with different types of PBR at this time and along the years a wide range of systems have been proposed.

**Fermenters:** Martek Biosciences Corporation (now acquired by DSM) developed and patented two fermentable strains of microalgae, which produce oils rich in docosahexaenoic acid (DHA) in fermenters that range in size from 80,000 to 260,000 liters. It is at the present the largest value business related with microalgae.

**Labscale:** This platform and scale will become relevant for specialty, high value products in the concept of ‘molecular farming’. Labeled isotope extracts are already a small business at this level. GMO microalgae will be used in most of the cases. Some of these molecules such as the diterpenoid targets in this project have market values of well over €10 million /kg.

**MACRO-TRENDS 5 – MICROALGAE PRODUCTION SCALE**

The trend is that depending on (1) the microalgae crop / market need driven - and (2) the corresponding production platforms there will be a different (3) choice of production options – and production scale. The evolution is happening in all different scales but there is strong emerging MicroFarming movement, starting in France - and a Large-Scale Farming in US and China. High potential in the 3As - Australia, Africa and Arab countries may lead to new ventures in these countries in the next 10 years. Production scale, novel food approval and marketing about microalgae species will shape the trend at this level.

Culturing microalgae in the laboratory is 150 years old and commercial farming just 60 years old. Archaeological data indicates that the domestication of various types of plants and animals evolved in separate locations worldwide, starting around 12,000 years ago. Neolithic Revolution, sometimes called the Agricultural Revolution, was the world's first historically verifiable revolution in agriculture. Microalgae will be the 21st century new agriculture crop. Micro-crops with hydroponics and related technologies will become standard technologies all over the world.

Advanced agriculture	Large-scale farming	Regular farming	Microfarming
Open systems:	> 100 ha	> 10 ha	> 1 ha
Closed systems (PBRs)	> 1.000 m3	> 100 m3	> 10 m3
Industrial biotechnology	Large-scale fermentation	Regular fermentation	Lab & Pilot fermentation
Closed systems (Fermentors)	> 1.000 m3	> 100 m3	> 10 m3

There is no optimal unique solution for microalgal production. However, several approaches have been developed by researchers, either by using alternative approaches, improving technologies in order to bypass the costly harvesting and extraction steps and operating in a general overview concept of biorefinery, as happens for example with soy where soy meal and soy oil are combined for global price sustainability. Integrated systems, with several technological options combined in the same operation unit, may provide increases in productivity and reduction in costs; examples are the utilization of photobioreactors for inoculation (5-10% of total production volume) and 90 to 95% in open systems as raceways, ponds or cascade systems. These system configurations must always be adapted to the microalgae species cultivated, final market features and production unit location.

The scale of production is still related with price performance and consideration as crop:

Protein / Lipid	Soy oil & Soy meal	Fish oil & Fish meal	Algal oil & Algal meal
Production Scale (ton/year) 2011	200,000,000s	7,000,000s	15,000s

The marketing is also a relevant bottleneck for commercialization as would be for other crops if the scientific names were used in the trade:

Cattle food	<i>Medicago sativa, Trifolium, Poaceae ...</i>	alfafa, red clover, graminae...
Fruits	<i>Malus domestica, Pyrus, Prunus persica, Prunus, Citrus sinensis ...</i>	apple, pear, peach, orange,...
Grains	<i>Triticum, Zea mays, Secale cereale, Avena sativa ...</i>	wheat, corn, rye, oat,...
Microalgae	<i>Arthrospira, Chlorella, Haematococcus, Dunaliella, ...</i>	Spirulina,
Nuts	<i>Cocos nucifera, Arachis hypogaea, Carya illinoensis ...</i>	coco, peanut, pecan
Other crops	<i>Gossypium, Rosa rubiginosa ...</i>	cotton, sweet briar,
Vegetables	<i>Solanum lycopersicum, Lactuca sativa, Spinacia oleracea ...</i>	tomato, lettuce, spinach

#### MICROALGAE RESEARCH NEEDS

The trend is that evaluating the ‘gaps’ and problems in the scale-up of microalgae technologies, the need is increasingly flowing for ‘industrial ecology’ related issues in what concerns biology related trends – this is mostly because contamination outside the laboratory is a key limitation. Technology related needs will be mostly in the discovery that it is critical to adapt and use processes that are already routine in other industries. At the same time analytical processes for fast and accurate information are essential to manage the production of microscopic organisms in a large scale which is in a level that is 1.000 x smaller than humans were used to work and where there are important need when comparing with other agriculture crops.

Research needs are a ‘best practice’ item in review papers or other publications where results were almost able to show the expected hypothesis. Under the Project AQUAFUELS, Vítor Verdelho coordinated a team with Rene Wijfels that evaluated several hundreds of ‘research needs’ statements through publications in the last 20 years. [AquaFuel FP7 Coordination Action. FP7 ENERGY – 2009 - 1. Algae and Aquatic biomass for a sustainable production of 2nd generation biofuels. Task 3.1. Research Needs. January 2011. See [www.aquafuels.eu](http://www.aquafuels.eu) for details]. This meta-analysis work with information from direct experience in microalgae biotechnologies, both academic research and industrial scale-up led to the following synthesis about applied research needs:

Bio related			Tech related		
Molecular	Cell	Ecosystem	Cultivation	Processing	Analytical

It is possible to consider that Applied Research Needs should be connected with Market Research needs and trends – but, scientific research is not connected with specific needs, but rather with (1) financing opportunities and (2) specific academic interest related with career development.

Levels	Research needs
Molecular	Annotated genome of the top 10 microalgae genera; 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 and regulation mechanisms involved in pigment and fatty acids biosynthesis; metabolic pathways elucidation.
Cell	Cell wall and cell membrane behavior; understanding cell cycles to improve growth; optimizing of nutrition and efficiency of utilization of nutrients; microalgae physiological changes under stress response; information that will reveal the regulation networks that are responsible for microalgae behavior during stress periods.
Ecosystem	Cell-cell interactions: symbiosis with bacteria (vitamins, hormones...); quorum sensing in microalgae ecosystems; cell death phenomena; biofilm formation and extracellular metabolite production; allelopathy control & contamination management; 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 due to the establishment of symbiotic relationships; viral infection management will be as important for microalgae cultures as for any other plant crop.
Cultivation	Microalgae cultivation using systems with lower contamination levels, easier to operate and to scale-up; species to be cultivated have to be selected according to stability of the strain; time of generation and culture synchronization; nutritional requirements, yield in oil and fatty acids profile; optimized and specific culture media using stoichiometric / mass balance approaches; nutrients: chemical species form interactions and change bioavailability; use of zeolites and other culture media enhancers; water recirculation and dilution strategies; energy efficiency optimization; computer simulation for technology optimization (PBR configuration, best location, cultivation strategy) based on the specific microalgae strain structural and growth reaction model.
Processing	Harvesting technologies with membranes for improved recirculation of culture media; Clean processing with specific technologies for algae - including mechanical (pressure...), chemical, biochemical, electrical (EPS...) and thermal will be extremely relevant; supercritical extraction technologies for pigments and oils; low cost preparative chromatography for purification of specific compounds.
Analytical	Remote sensing with spectroscopy based camera devices will be critical for scale-up; new analytical methods for physical and chemical parameters that are species-specific identification for rapid and specific detection of microalgae; DNA chip for fast identification / screening.

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# 5<sup>th</sup> Congress of the International Society for Applied Phycology 2014

Australian Technology Park, Sydney, Sunday 22 – Friday 27  
June 2014

## **Strengthening algal industries for the future: **key** knowledge and skills gaps**

As President of the International Society for Applied Phycology and Convenor of the conference, we are delighted to invite you to the 5th Congress of the International Society for Applied Phycology - ISAP 2014 - in Sydney, Australia.

Historically, Australia has been a leader in algal applications and currently there are many exciting research and industrial developments in algal applications happening in Australia. International interest in algal applications in Australia is strong due to our unique algal biodiversity, landscape and biogeography that lend itself to algal industry development. ISAP 2014 in Sydney will give researchers and industry representatives a wonderful opportunity to learn more of Australia's algal potential as well as to share the global achievements and future directions in this vibrant frontier at this inspiring global venue.

See [www.isap2014.com](http://www.isap2014.com) for Program and other details.

We look forward to seeing as many of you as possible in Sydney.

**Susan Blackburn & Pia Winberg**

**President & Convenor of ISAP 2014**

## European Algae Biomass Conference

Maximising Commercial Successes in Algae Biomass by Combining Focused R&D With Business Strategy

5-7 May 2014, Seville, Spain



ACI's 4th annual European Algae Biomass Conference will once again bring together senior executives from industry and academia to discuss the latest commercial and technical developments, challenges and research breakthroughs throughout the entire algae value chain.

The conference will have a heavy focus on case study examples of latest technologies in operation in the global algae industry and also includes **an exclusive site visit on Monday 5<sup>th</sup> of May 2014**. Discuss the technical challenges faced when optimising the cultivation of algae. Study the current and future commercial markets for algae products and the challenges faced during the commercialisation process including the views from different end markets.

## The Inaugural Joint Aquatic Sciences Meeting (JASM)

18-23 May 2014, Portland, Oregon, USA



“Bridging Genes to Ecosystems: Aquatic Science at a Time of Rapid Change” is the theme for this historic joint meeting of four of the leading aquatic scientific societies: Society for Freshwater Science (SFS), Association for the Sciences of Limnology and Oceanography (ASLO), Phycological Society of America (PSA), and Society of Wetland Scientists (SWS). For one week in May of 2014, these four societies will build a bridge across the disciplines within the field of aquatic science and will explore many exciting opportunities for collaboration among scientists. An attendance of over 3500 attendees is anticipated.

## Algal biomass, Biofuel & Bioproducts



15-18 June 2014, Santa Fe, USA

### Themes

- Algal Biology - Metabolic Regulation of Microalgae for Biofuels
- Algal Biology - Molecular Traits of Microalgae for Biofuels
- Algal Biology - Phylogeny of Microalgae for Biofuels
- Algal Cultivation - Heterotrophic Systems, including utilization of waste waters for algal production
- Algal Cultivation - Phototrophic Systems in Open Ponds
- Algal Cultivation - Phototrophic Systems in Photobioreactors
- Algal Harvesting and Extraction Systems
- Bioproducts from Algal Biofuels Refining, including agricultural, bioplastics, and chemical products
- Life Cycle Analysis[LCA]
- New Conversion Technologies for Algal Biofuels, including catalytic, thermal, and enzymatic systems
- Techno-economic Modeling of Algal Biofuels Systems

## **Algae Courses – Scottish Association of Marine Science (SAMS)**



The following courses are offered:

- Algal Culture for Biotechnology – June 2 & 3, 2014
- Introduction to Molecular Methods for Algae Research – June 4 & 5, 2014
- New Algal Biotechnology Masters Program – September 2014

## **2nd International Conference on Algal Biorefinery**



**A potential source of food, feed, biochemical, biofuels and fertilizer**

**27-29 August 2014, Lyngby, Denmark**

### **Themes**

- Algal taxonomy and biodiversity
- Algal genomics, transcriptomics, metabolomics and proteomics
- Algal physiology and ecology
- Algal conversions/applications (fuels, chemicals, fertilizers, foods, etc.)
- Algae for bioremediation

## **ICHA 2014 NEW ZEALAND**

**27-31 OCTOBER 2014, Wellington, New Zealand**

The international Harmful Algae science community are invited to the 16th International Conference on Harmful Algae (ICHA) to be held in the Convention Centre, Wellington City, New Zealand on 27–31 October 2014. The theme of the conference is “Advancement Through Shared Science” in recognition of the multidisciplinary nature of the field and the important role that international collaboration has played in the understanding of HAB phenomena and the mitigation of their effects.

## **2014 Algae Biomass Summit**

**<http://www.algaebiomasssummit.org>**



The Algae Biomass Organization (ABO), the trade association for the algae industry, announced that the eighth annual Algae Biomass Summit will take place September 29-October 2 in San Diego, California, a global hub of algae research and commercial activity. The ABO is now accepting abstracts and proposals for keynote speakers, panel presentations and poster sessions at the event, the world's largest algae industry conference. Speaking opportunities for the Summit are highly competitive, making the submission of high-quality abstracts before the April 1st deadline essential. Information about the event and call for abstracts can be found at <http://www.algaebiomasssummit.org>.

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