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Review

COULD ALGAE BE A REAL SOURCE OF FUEL?

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Summary: The idea to develop biofuels from algae is not a new one, but prior to 21st century algal proponents did not gain enough fame. However, since 2007 there has been a significant increase of interest in algal biofuels and the prospects of their application in the future has been greatly overhyped. This review deals with different kinds of biofuels – biodiesel, bioethanol, hydrocarbons, biogas and whether algae can be applied in their production. Our conclusion is that while there is some advancement in growing algae and processing of biomass, harnessing biofuels from them is still a daunting task and far from the promised green future.

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1. Introduction

On August 10th, 2013, the world celebrated 120 years since Rudolph Diesel fired the engine, now bearing his surname, for the first time. This first engine was designed to run on fuel, derived from peanut oil. Later, when Henry Ford started the production of the Model T automobile in 1908 (cited as the first affordable automobile), he planned the car to run on ethanol, derived from corn (DiPardo, 2000). Henry Ford predicted in 2025 that "the fuel of the future is going to come from fruit like that sumac out by the road, or from apples, weeds, sawdust – almost

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anything" (Rapier, 2012). However, the low cost of gasoline, combined with the Prohibition of alcoholic beverages in the United States between 1920 and 1933, made the ethanol fuel impractical for times to come.

It is also worth mentioning that at the time when the diesel engine was invented, the total Earth population was estimated to have been 1.7 billions of people. The higher population is, the bigger consumption of fuel we have.

Conventional oils are classified as non-renewable (or finite) resources. There is a growing public awareness about the future of humankind in a post-petroleum era and about the negative environmental impact of the traditional energy sources. Public surveys show that society unequivocally supports the use of so called renewable energy resources even in the case of higher energy costs. But citizens are poorly informed about the general aspects related to production and consumption of energy and about specific aspects related to the use of renewable energy sources, especially biomass and bioenergy (Segon et al., 2004) The problem of renewable sources is now part of the politics of the European Union and under 2009 EC Renewable Energy Directive (RED) 10% of all transport fuel must come from renewable sources by 2020 (Levidow, 2013). The topic is also important for USA as the country seeks to end dependence on foreign sources. Former President Bush has stated that America is addicted to oil and the best way to end this addiction is through technology (Danigole, 2009).

Renowned scientists have been proponents of plant biofuels for years and one of the most notable names in the past is that of Melvin Calvin, who is famous for being one of the researchers who discovered the Calvin-Benson-Bassham (CBB) cycle, the pathway of carbon fixation and conversion of carbon dioxide info organic compounds. Most famous are his views that plants from genus *Euphorbia* can be used, as 35% of its dry weight contains simple organic extracts. Calvin has also noted that there are algae which oil productivity is of interest, so they may be used for fuel oils (Calvin, 1987). So far, however, mass production of biofuels according to his view hasn't started.

Although, as it has been noted from above, algal biofuels exist as an idea for decades, they did not come to prominence until 21st century. As is shown in Table 1 the topic was mentioned scarcely before 2007, but after that the popularity has raised enormously. Nowadays it is often assumed that algae can be used in production of many types of biofuels. According to publicly available information (Biofuel), algal biofuels are thought to be the third generation of biofuels (the first generation is biofuel from corn, sugarcane, soybeans, vegetable oil, the second generation is biofuel from waste or plant biomass that has already fulfilled its food purpose).

This paper deals with all possible types – algal biodiesel, algal bioethanol, biogas, hydrocarbons, direct burning of algal biomass, and surveys current trends, promises and hindrances concerning these fuels.

2. Biodiesel: methyl or ethyl esters of fatty acids

Worldwide there are many manufacturers producing microalgal biomass, and none of them has ever

Table 1. Frequency of appearance of the expressions “algae biodiesel” (I), “algae biofuel” (II), “algal biodiesel” (III), “algal biofuel” (IV) in Google.Scholar.

Year	I	II	III	IV
2013	198	251	321	432
2012	168	281	318	378
2011	139	169	191	216
2010	101	94	108	128
2009	57	82	64	65
2008	35	27	40	20
2007	16	9	17	4
2006	4	2	3	2
2005	0	2	1	0
2004	1	1	0	0
2003	2	0	2	0

offered biodiesel as a product, with the exception out of curiosity. The idea to harvest microalgal oils and to turn them into biodiesel pops up every now and again during the last 60 years regularly, at least once per decade. But while this question was lying in the field of basic science in the past, nowadays it is no longer the case. The quest for algal biodiesel is no longer scientific; mankind already possesses the knowledge of growing algae and extracting valuable substances from them. The quantity of the algal product depends on the quantity of the biomass and usually the product is of much less quantity than the raw biomass. Many engineering efforts have been directed to speed up the growth of algae by ensuring optimal temperature, light intensity and optimal proportion of nutritional elements. However, even if the very best optimal conditions are provided, the growth of the algae (regardless of the species) has its own upper genetically determined limit including factors like enzyme activity, rate and effectiveness of photosynthesis.

There are many books devoted to algae biodiesel and the next excerpt from one of them is worth citing: “Dear Friend, Imagine...having a photobioreactor in your garage with the ability to grow algae for bio-oil for your home, health food supplements for your family, organic fertilizer for your farm or garden, working silently, automatically, 24/7, in your garage. Imagine able to do it all from one unit, at the same time...” (Sieg, 2009). The author does not make it clear how large that garage ought to be and several fundamental questions of photobioreactor scaling have not been taken into consideration. He provides some tips about how to construct a bioreactor for $215. While it is possible to build a simple bioreactor able to grow algae for a low cost, will it produce enough biomass for biodiesel? In fact, the author admits in the Forewords of the book that “no one can promise the result you'll get" and "how you implement and use them (the photobioreactors) is entirely at your risk". Knowing that ensuring good growth requires optimal conditions, good nutrition, CO_2, it is more likely that the produced biomass from the custom made photobioreactor may not cover the expenses so it would be much cheaper to buy biodiesel, rather than create your own. Such books about producing algae biodiesel at home are more suitable for enthusiasts who enjoy doing experiments, not for those who expect fast and market competitive results.

The President of USA Barack Obama, in his speech in Miami on 27 February 2012 said: "We're making new investments in the development of gasoline and diesel and jet fuel that's actually made from a plant-like substance - algae." (Bell, 2012). We ought to admit that nowadays air companies are a huge supporter of biofuels, including algal biofuels, because their business depends on petroleum. Our comprehensive opinion on the question was published 45 days before the Obama's speech (Petkov et al., 2012). What we have to add here is that the predicting mathematical models, though being created quite correctly, do not present an optimistic future for biodiesel derived from microalgae. For example, Brownbridge et al. (2014) stake on 30-35 % "algal oils", which are not oils but total lipids. The real "oil content" equals to the percentage of total fatty acids, which are the biodiesel raw material. The total fatty acids are merely 10-12 % of dry biomass. Besides, there is no place on the world where an amount of 100 t/h could be produced annually. It is equal to an average yield of 27-28 $g.m^{-2}d^{-1}$ for 365 days, which is too much even at controlled and optimal conditions. This daily yield is only achievable with a single unit of vertical photobioreactor situated on a small area. At large scale, one could stake on an optimistic yield of up to 70 t/h under controlled conditions and the produced oil will be less.

There is some progress in the area of biomass processing, namely of trans-esterification and hydrogenation at 250-350°C and 20 MPa (Bai et al., 2014; No, 2014). These conditions require an abundant amount of energy added to the expenses for biodiesel production. This problem has no direct connection to the main issue, namely growing algae and the prime cost of raw biomass, but both contribute a lot to the final cost of biodiesel. The governments invest money in Research and Development (R&D) of microalgal biodiesel and the outcome is plain: "many words and few to the point*". Investments ought to be made in the R&D of microalgal biomass production and processing, but the main purpose should be usage for food, forage additives and pharmaceuticals. Photoautotrophic production of microalgae biomass cannot compete with heterotrophic microbial industry or chemical industry where the overall process proceeds at much higher rates. Besides, the process in a chemical or in a heterotrophic reactor occurs in a large volume, but algae require big surface and less volume of cultivation vessels.

3. Fermentation of algal biomass and production of ethanol

Bioethanol is produced through the fermentation of sugar and starch, which are obtained from different sources, such as sugarcane, maize, or a number of other grains. The process necessary for the production of bioethanol is different depending on the type of plant biomass and typically includes pretreatment of the biomass, saccharification, fermentation, and recovery of the product. The pretreatment is an important process for the formation of the sugars used in the fermentation process. Acid hydrolysis is widely used for the conversion of carbohydrates from the cell wall into simple sugars (Miranda et al., 2012). Such pretreatment is efficient and involves low energy consumption (Harun and Danquah, 2011a). Enzymatic digestion, gamma radiation, alkaline pretreatment and hydrolysis mediated by fungi (Harun et al., 2011; Harun and Danquah, 2011b; Yoon et al., 2012) are interesting alternatives for increasing the chemical hydrolysis to render it more sustainable.

Fermentation processes from any material that contains sugar could derive ethanol. The varied raw materials used in the production of ethanol via fermentation are classified into three main types: sugars, starches, and cellulose materials. Sugars from sugarcane, sugar beets, molasses, and fruits can be converted into ethanol directly. Starches from corn, cassava, potatoes, and root crops must first be hydrolyzed to fermentable sugars by the action of enzymes from malt or molds. Cellulose (from wood, agricultural residues, waste sulfite liquor from pulp, and paper mills) must likewise be converted into sugars, generally by the action of mineral acids. Once simple sugars are formed, enzymes from microorganisms (bacteria – *Zymomonas, Clostridium thermocellum*; yeast – *Saccharomyces cerevisiae*; filamentous fungi – *Monilia* sp., *Neurospora crassa, Aspergillus* sp. *Trichoderma viride* etc.) can readily ferment them to ethanol (Lin and Tanaka, 2006).

Starch is an ultimate storage form of photosynthetically fixed carbon both in algae and higher plants, and it could easily be converted into bioethanol by fermentation. The necessity to store carbon in plants depends on the conditions. Starch content can be increased in algae by changes in temperature and light intensity. It can also be increased by starvation (nitrogen or sulfur), and also by applying specific inhibitors which affect nuclear DNA replication or protein synthesis (Zachleder and Brányiková, 2012). It is obvious that starvation cannot be applied, or the metabolic pathways cannot be blocked during the process of growing algae, because in such ways a sufficient quantity of algal biomass for ethanol production could never be obtained. That is why the scientific literature refers to a treatment of already produced commercial biomass, but not all treatment methods are suitable, for example, using inhibitors like cycloheximide is unrealistic both environmentally and economically, while the starvation by limitation of nutrition elements like nitrogen is preferred. However even this approach has drawbacks, for example, there is a short time between production of starch and cell death (Brányiková et al., 2011).

Nowadays bioethanol is mainly produced from sugarcane, a plant with C4-type photosynthesis cycle, of genus Saccharum, family Gramineae. Sugarcane is also one of the most important commercial crops, with Brazil being the leading producer with a cropland area of around 7 million hectares, which is

42% of total production (BNDES, 2008). Chisti (2008) has criticised the idea of producing bioethanol from sugarcane. He states that even under optimal conditions, sugarcane biomass yield does not exceed 100 metric tons per hectare. The author also states that bioethanol contains only 64% of energy content for biodiesel, that's why biodiesel instead of bioethanol from growing algae should be considered. We think however, that his assumptions that tubular bioreactors with natural sunlight for producing large quantities of biomass are quite optimistic. The author admits that there should be feeding of the algal culture at a constant rate, as well as maintaining a high turbulent flow with a pump. This is a process that costs money. Will the produced algal biomass pay for the expenses? The author also states that "at least once a year a photobioreactor facility must be shut down for routine maintenance and cleaning". Once per year is a highly optimistic assumption. The facility must be cleaned frequently if contamination with other algae occurs, or if the pump is still unable to prevent total sedimentation of the algae.

We agree that large-scale ethanol production from sugarcane has drawbacks, like damage of high-biodiversity areas, competition between food and fuel production, bad labor conditions on the field. But sugarcane is not a particularly a demanding crop in terms of soil, and it is said to adapt reasonably to soils of average fertility and high porosity/permeability sandier soil (Goldemberg et al., 2008). The question still remains about whether algal biofuels, particularly bioethanol, could be a good substitute for sugarcane. Sugarcane obtains its nutrients from the soil, and most types of soil have a good amount of nutrition elements. Even if all other problems with growing algae and processing biomass are solved, they still need to feed on a specially prepared nutrition medium which costs money. Our conclusion is that despite the obvious drawbacks of sugarcane bioethanol, algal bioethanol still raises many other problems that need to be addressed before it's implemented in practice.

4. Biogas

Biogas is typically produced from lignocellulosic biomass which exists as waste, manure, fallen leaves in cities, residues from viticulture and orcharding, sawdust. From economic point of view, it is recommendable only moist materials to be used for methanolysis. Dry biomass should be better burned, instead of turned into methane. The produced heat from direct burning is more than the heat from burning methane, produced from the same biomass.

The only meaningful production of biogas from algae is when sea macroalgae stranded by waves are used, or if there is a high amount of biomass as a result of eutrophication. Being of no other avail, these algae might be composted in a methane tank together with all other cellulose containing waste (Cuomo et al., 1995). But it has to be mentioned that the remains of some macroalgae are important part of nutrition chains at the seaside (Adin and Riera, 2003).

There are two ways to produce biogas from macroalgae – either via anaerobic digestion, or via thermal treatment. The produced biogas contains about 60% methane. In contrast to land plants, macroalgal biomass contains too much cellulose and lacks lignin. That's why it cannot be fully hydrolyzed. The amount of the produced biogas depends on ash composition and the level of stock sugars in biomass. The change of the biochemical composition affects the yield. The C/N ratio is also important for optimization of the process. Macroalgae could be used as a co-substrate in production of biogas along with food waste and agricultural slurries. The yield also depends on other factors like inoculum, stock composition and the system which is used for conversion to biogas (Hughes et al., 2012).

Several macroalgae have been used for anaerobic digestion - *Macrocystis, Laminaria, Sargassum, Gracilaria, Enteromorpha* and *Ulva*. The green alga *Ulva sp.* has a very low hemicelullose content which is about 9% of dry weight. This is a good factor for enzymatic treatment. The main problems with using *Ulva* for methane production refer to the seasonal growth of the alga, the low density in the suspension used to fill the methane tank, the high concentration of sulfur which leads to production of biogas with a high content of H_2S and presence of material that is hard to be digested (Briand and Morand, 1997). Other macrophyta have similar drawbacks.

As a conclusion, concerning macroalgae, we have at disposal so much cellulose material which rots freely in the environment, releasing green house gases, it would be pointless to deal with algae for biogas production. Biomass of cultivated macroalgae could be used as a biogas source, for example, when they are the stage of devices for biological treatment of water, which is well known. Let the wild marine algae remain a part of natural nutrition chain in the sea.

The only scientifically feasible and practical way how to include microalgae in the great quest for biofuels is not by using their high value biomass, but by harnessing the photosynthetic function. A possible scenario could be to use algae to purify biogas from carbon dioxide. A method for purification of biogas from carbon dioxide to pure methane has been devised by Alexandrov et al. (2013). Crbon dioxide is absorbed in the supernatant after centrifugation of biomass, so the erous mixture of methane and oxygen is not produced. The quest doesn't need acquiring large quantities of biomass, because only photosynthesis will be used and while biomass will be produced, it should preferably be offered as a commercial product, as an edible food. It should be stressed that this process does not lead to synthesis of methane but only to its purification. In this case the statement: "microalgae are for eating, not for burning" doesn't contradict the strategic purpose of experimental algology. Algal biomass is a valuable supplement for animals and humans.

Mann et al. (2009) survey the abilities of *Chlorella vulgaris* to grow while using biogas as a carbon source at light intensities of 53, 60 and 100 µmol m^{-2} s^{-1}. The authors estimate that the content of CO_2 decreases from 41% to 1.2 – 2.5%. Nevertheless, there is a dangerously high concentration of O_2 in the produced gas mixture.

Carbon dioxide is one of the most expensive components required to cultivate microalgae if it is supplied as a pure substrate not taken from air or waste gases. Biogas from agriculture waste contains 30-45% of CO_2. A system which combines "waste" CO_2 with cultivation of organisms that sequestrate it, like algae or cyanobacteria, may not only greatly decrease expenses, but also remove CO_2 as a pollutant. Methane, on the other hand, doesn't have a toxic effect on algae (Travieso et al., 1993; Mandeno et al., 2005; Heubeck et al., 2007). Both cyanobacteria, as well as green algae (Chlorophyceae), are able to grow at high concentrations of CO_2 (Ho et al., 2010; Markou and Georgakakis, 2011). Due to the algal photosynthesis the concentration of CO_2 decreases to 5-11.5% (Travieso et al., 1993; Converti et al., 2009). There is a linear correlation between the algal growth of *Arthrospira platensis* and the purification of methane. Carbon dioxide assimilation could reach 95% (Converti et al., 2009).

González-Fernández et al. (2012) survey how thermal treatment (at 70 and 90°C) affects the anaerobic digestion of biomass from *Scenedesmus sp*. At 90°C it has been noted 48% biodegradation and 2.2-fold higher methane production. Untreated biomass and biomass treated at70°C corresponds up to 22% and 24% of biodegradation.

The presence of cellulose in the cell wall of *Chlorella vulgaris* and other green algae is a hindrance to production of biogas from algal biomass. Addition of the bacterial strain *Clostridium thermocellum* at different concentrations to algal suspension increases the production of methane (17 – 24 %) and there is also a production of hydrogen as a result of the higher percentage of disrupted cells (Lü et al., 2013).

Mussgnug et al. (2010) survey the ability to produce biogas from seawater and freshwater algae and cyanobacteria and they show that the process depends on the species, as well as on the preliminary treatment of biomass. Indeed, the presence and the composition of the cellular wall is the main hindrance to cell disintegration and anaerobic digestion. The most suitable algae are those without cell wall. However, easily digestible *Dunaliella salina* and *Arthrospira platensis* produce less biogas than *Chlamydomonas reinhardti* because they also produce substances that inhibit methanogenic microorganisms. The combined digestion of cyanobacteria from Lake Tai Hu and corn straw leads to increased methane production and less accumulation of ammonia and fatty acids (Zhong et al., 2013). An increase of methane production has been noted during digestion of residual algal biomass from *Nannochloropsis salina* (Park and Li, 2012).

5. Algae hydrocarbons

Some algae produce hydrocarbons which are similar to those from conventional diesel. Their chain is built of 11 – 35 carbon atoms, and they are located in the membranes. The algae hydrocarbons are saturated or with 1-3 double bonds, iso- and anteiso- are present, too. The hydrocarbons C_{17}, ΔC_{17}, C_{27}, ΔC_{27} predominate significantly in the total mixture. Sometimes one of them is up to 90%. Hydrocarbons with uneven C-number predominate significantly. Normally, the content of hydrocarbons is not more than 2-3 % of the total lipids, or about 0.5 % of biomass. The hydrocarbons originate from fatty acids as a result of their peroxidation. When algae are grown at higher light intensities they usually respond with a higher content of fatty hydrocarbons. Algae like *Scenedesmus*, *Chlorella*, *Coelastrum* have a higher content of hydrocarbons in contrast to algae like *Trachydiscus* that prefer lower light intensity for optimal growth, but even these algae excrete a negligibly small amount of hydrocarbons and their concentration in the medium of *Scenedesmus* is about 10 $mg.dm^{-3}$ at 5-6 $g.dm^{-3}$ algal density (Kambourova et al., 2006).

The percentage of extracellular hydrocarbons of the green alga *Botryococcus* is significantly higher. This alga excretes terpenoid hydrocarbons which emerge on the surface (Metzger and Largeau, 2005; Eroglu and Melis, 2010). Nevertheless, the energy required for cultivation of *Botryococcus* is significantly higher than the energy income of its hydrocarbons.

A method called OMEGA (Offshore Membrane Enclosures for Growing Algae) has been actively supported by NASA as a means to grow algae in order to produce hydrocarbons, as well as oils (Trent et al., 2013). This method calls for growing algae in flexible bags with CO_2/O_2 exchange membranes, while the bags are in the ocean. It is thought to be inexpensive and affordable, because the ocean supports a constant temperature, while the algae feed on waste, take CO_2 from air and release O_2. Not only algae are being grown for biofuels, but also to purify the air from greenhouse gasses. However, OMEGA still has its limitations and unresolved problems regarding species control, harvesting of the biomass, dewatering (Ziolkowska and Simon, 2014). Moreover, algae take CO_2 from air which is not enough for fast growth required for the production of biomass.

To summarize, the low percentage of hydrocarbons makes the idea unfeasible.

6. Bio-hydrogen

The first report about hydrogen gas released from algae dates from 40s of the last century (Gaffron and Rubin 1942). Nowadays there are two manners for photobiological production of molecular hydrogen, one from algae, and another from cyanobacteria (Tamagnini et al. 2002). Studying *Anabaena variabilis*, in different nutrition media Berberoglu et al. (2008) conclude that the vanadium containing medium increases the hydrogen evolution over 5-times, because vanadium is related to the nitrogenase activity. This author has shown that the cyanobacterium release 5.6 L H_2 per 1 kg dry weight of algal biomass (80 $mg.h^{-1}$ H_2) for one hour at 30 °C and light intensity 65 - 150 $\mu mol.\ m^{-2}.s^{-1}$.

Most of the studied organisms are green algae, isolated from salt and fresh water, as well as urban environments (Skjånes et al. 2008). The most studied hydrogen producing green alga is *Chlamydomonas* (Melis et al. 2000).

Hoshino et al. (2013) reported that yield of chlorophyll content based H_2 from two *Chlamydomonas reinhardtii* strains was 220 and 176 dm^3/kg, respectively. The same authors pointed that the yield was relatively lower and the only energy conversion was increased from 30 to 53 %.

In the eukaryotic microalgal cells at the some conditions, such as sulphur deficiency, photosystem II (PSII) became inactivated and the algae switched into the H_2-production mode in the light due to “a reversible hydrogenase pathway” associated with reduction of protons to molecular hydrogen in the chloroplast stroma (Melis et al., 2000). According to Winkler et al. (2002) the H_2O oxidation in *S. obliquus* has been reduced from 13 to about 2 μmol O_2/g Chl a/h in the first 50 h of the growing of the alga under sulfate-deficiency. This is a two-step process resulting in hydrogen evolution under sulfate-deficiency (Melis and Happe 2001; Melis and Happe 2004). The hydrogen reduction was also a process which must take place in an anaerobic inner space because of the O_2 sensibility of chloroplast hydrogenase (Stripp et al. 2009).

Studying several green algae Winkler et al. (2002) established the levels of hydrogen produced by *C. reinhardtii*, *S. obliquus* and *S. vacuolatus* as follows: 200 nmol H_2/μg Chl a/h, 150 nmol H_2/μg Chl a/h, and 155 nmol H_2/μg Chl a/h. The same author found that the average of hydrogen gas from other green algae was in the interval between 50 - 460 nmol H_2/μg Chl a/h.

A lot of improvements of the conditions and the whole process have been made during the last decade. Some authors suggested genetic improvements to modify the chloroplast hydrogenase to an O_2-resistable enzyme (Greenbaum and Lee 1998; Ghirardi et al. 2000) and overexpress it (Chien et al. 2012). Studying *Scenedesmus obliqus*, Wunschiers et al. 2001 showed that the presence of ferredoxin quinone-reductase or NAD(P)-dehydrogenase in the chloroplasts also contributed to the reduction._

Other authors showed that addition of some chemical substances in the medium can improve the efficiency of the overall process. Using carbonyl cyanide m-chlorophenyl hydrazone in the medium Yang et al. (2014) inhibited the activity of PSII of the green alga *Chlamydomonas reinhardtii* and achieved hydrogen evolution 13-fold higher than the control.

Maintaining the careful titration of the sulphur nutrients in the medium contributed to carrying out a continuous hydrogen production process (Zhang and Melis 2002). Maneeruttanarungroj et al. (2010) achieved 17.3 - 61.7 μmol/mg Chl a/h hydrogen from the green alga *Tetraspora* sp., which was relatively higher than other studied microalgae. It has to be noted that the hydrogen production from *Tetraspora* sp. was carried out at low light intensity (less than 5 $\mu mol.m^{-2}.s^{-1}$). By adding 0.5 mM *β*-mercaptoethanol in the nutrition medium without S and N, the same authors doubled the hydrogen evolution from the new isolated green alga *Tetraspora* sp, which was grown at 36°C and light intensity of 48-92 $\mu mol.m^{-2}.s^{-1}$. It was also found that the hydrogen production rapidly increased when pH of the medium was enhanced.

Hahn et al. 2007 reported a different approach for the improvement of the hydrogen evolution of algae by using silica particles as a solid support of the algal cells.

Maintaining the iterating of light and dark cycles at every 1.5 h, Hoshino et al (2013) extended to 27 h the H_2 production from a chlorophyll b deficient mutant of *Chlamydomonas reinhardtii* strain and achieved 336 dm^3/kg chlorophyll content H_2 based gas.

The second approach for producing biohydrogen involved the use of nitrogenases in nitrogen fixing cyanobacteria and the estimated yield was between 0.17 and 4.2 nmol H_2/g Chl a/h (Tamagnini et al., 2002).

Alternative methods for increasing the productivity of hydrogen producing systems with algae have been developed during the last years. Some of the methods represent innovative approaches based on integration of photosynthesis of the green algae into the so called microbial fuel cells (Rosenbaum et al. 2010), as well as bioelectrochemical systems for both decreased water salinity and hydrogen production (Lou et al 2011). Such systems could evolve 1.6 ml/h hydrogen utilizing up to 10 g/l NaCl (Lou et al 2011) using salt water algae (Zhang and Chen 2007).

As a whole the achieved yield of hydrogen, though theoretically promising, is still very far from the real practical use, because of the very slow rate of photosynthesis. In fact, obtaining of hydrogen is strictly tied to the breach of the photosynthetic function, which is not leading to an effective process.

7. Direct usage of algal biomass

Extracting valuables from the algal biomass, including fatty acids and hydrocarbons, is a daunting task. But could the algal biomass be used directly, without any treatment? Does it have any useful fuel qualities?

An experiment was done which involved biomass of *Chlorella vulgaris* directly in a diesel engine. The authors conclude that a dried algal power could be used directly in a diesel engine, however, the control of the supply of dried algae to the engine was difficult and dried algae will not resuspend in diesel or biodiesel. The authors also admitted that drying algae was too expensive to be considered in the development of a fuel. Rather than dry biomass, they have offered a usage of algal slurries (suspension) and have tested a combination of standard rapeseed biodiesel (80%) and algal slurry (20%). Results showed that the emission of nitrogen oxides was lower, but there was an increase of carbon monoxide emission (Scragg et al., 2003).

Our review shows that the answer of the questions above is yes, the algal biomass could be used directly and it has fuel qualities. But knowing the hardships to produce biomass, should it be done? Our recommendation is still consistent with what was suggested before (Petkov et al., 2012) that the prime cost of algae is much more higher to be a source of fuel.

8. Genetically engineered algae

Our opinion is that it is not worth producing biofuels from algae now, due to the high costs required to grow them and obtain valuable substances from their biomass. However, could the drawbacks described in the previous chapters be overcome? If conventional methods are not enough to optimize algal growth and to enhance drastically the valuable substances, will genetic engineering work?

This was the assumption of John Craig Venter, a scientist known for his contribution to sequencing the human genome and later for creating the first living cell running on manmade DNA. He has now turned his attention to genetic manipulation of algae (Biello, 2011). His words at the New America Foundation in Washington, D.C. in 2011 are: “Nothing new has to be invented. We just have to combine genes in a way that nature has not done before. We're speeding up evolution by billions of years". However, modifying algae genetically is a daunting and a difficult task. Other than *Chlamidamonas reinhardtii* and *Volvox carteri,* few green algae have demonstrated stable, long-term expression of transgenic proteins, despite exhibiting integration of the foreign DNA. It is thought that algae possess a mechanism to suppress transposons and viral invasion, and molecular approaches toward evading transgene silencing will be an important step in the metabolic engineering of algae (Rosenberg et al., 2008). Stable transformation has been demonstrated in only a few algal species. Nowadays the interest in genetically modified algae is not universal, and further evaluation of the risks when working with these algae is necessary (Henley et al., 2013).

Recent research has shown some advancement in engineering cyanobacteria *Synechocystis* sp. PCC6803 wild type (SD100). It is claimed that cyanobacteria are modified to continuously secrete free fatty acids, which can be directly collected from the culture medium, thus avoiding the expensive biomass processing (Liu et al., 2011). While this is an impressive achievement, some drawbacks have been found according to authors, namely genetically modified cells are fragile and easily damaged at low cell density during carbon dioxide aeration. Authors admit that future work is needed, because industrial production needs robustness and cell rigidity. We would add that even biomass processing is avoided in this specific case, the costs of CO_2 supply and nutrition elements still remain a factor for production of biodiesel and it would still be expensive.

Genetic engineering of algae is considered necessary to overcome many of the drawbacks of algal biofuels. However, genomes of only a few eukaryotic microalgae have been fully sequenced and molecular biology tools required for genetic transformation are barely been developed, although some accelerating effort is being made to overcome lack of knowledge (Chisti, 2013). To sum up, genetic engineering of algae is still in its infancy.

9. Conclusions

The use of oils has changed the world economies, social and political structures, and lifestyle of people in a short time. Moreover, world agriculture is highly dependent on oil and oil supplies are limited yet (Youngquist, 1999). There is a genuine worry about what is going to happen next. Most of the world's petroleum was formed over a period of 100-300 million years and we have been consuming it about million times more rapidly than the rate at which it was produced. It is believed that the majority of the world's petroleum resources will be consumed in less than 200 years (Brown, 2004). We have to accept that the prospect of economic, cultural and technological decline is the most probable future for us (Greer, 2013).

There is a firm belief that the only solution to the imminent petroleum decline is to produce our own fuel. Algae have been called the holy grail of biofuels and their characteristics (fast growth under optimal conditions, production of lipids, consumption of CO_2 and purification of air) have been cited numerous times.

Our review, however, confirms that the prospect of growing algae for biofuel production is not optimistic. We are convinced that scientific research with algae should continue, but our belief is that they are more suitable for food rather than being used as a fuel source. People could hope for miracles, but while we share the concerns cited above, there is little use of algae in the field of biofuels so far. Their only practical application appears to be the purification of biogas from CO_2, but this is all about increasing the quality of already produced biogas, and not about production of biogas *de novo*.

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