MICROALGAE – BIODIESEL POTENTIAL PRODUCERS: A REVIEW

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Abstract:

Declining quantity of fossil fuels force scientific community to think about alternative energy sources. Thus, it is critical to focus on renewable resources and development of new technologies. Solar energy seems to be sufficient for actual energy demand. The question is what is the most efficient method for its capture, storage and distribution. As diesel stands for the third part of fossil fuels used in transport, cultivation of microalgae and extraction of lipids out of cells is one of the possibilities. Biodiesel produced from microalgae-derived lipids offers notable environmental benefits e.g. reducing the greenhouse effect by utilization of CO_2 emissions or sewage treatment.

The review includes descriptions of species selection for biodiesel production, genetic modifications leading for to higher efficiency and production process improvement, biomass and metabolites recovery from cultures, transestrification process leading to biodiesel, waste utilization and business value.

Microalgae can potentially offer substantially higher yields than other oil-producing crops and they can grow beside fresh water also in saline water or even sewage. Additionally microalgae do not compete with productive farmland thus there is no competition with food chain.

In spite of many advantages of biodiesel production from algae, there are a lot of limitations blocking its real competition with petrodiesel. Production costs seem the most substantial problem. Out of all recognized methods of microalgae production, the culture of microalgae in photobiopanels seems the most favorable for biodiesel production, however the costs are discouraging. Probably small modular systems may be an attractive solution with positive economical rationale.

Key Words: Microalgae, biodiesel, transestrification, renewable energy

Introduction

Petroleum originating fuels are currently regarded as largely environment-hostile due to their influence on carbon dioxide accumulation in the atmosphere which contributes towards greenhouse effect formation. Moreover, petroleum resources undergo progressive stock-out for their seam renew is very slow. Deficiency of fossil fuels may result in global famine as technological development, including modern agriculture advance, is predominantly petrol dependent (Hall 2009).

Renewable fuel sources are permanently searched for, especially ones suitable for transportation. One of the resources are products originating from living organisms (animals, plants and micro-organisms). Fuels from these sources are called biofuels whereas biodiesel is a combustible material used in compression-ignition engines (by Diesel).

At present, algae seem the only recognized source which, quite potentially, is able to replace totally fossil fuels (Fig. 1) in transportation (Chisti 2007), despite the fact that existing cultivation and harvesting technologies have to be improved to meet the economical requirements for fuel production. The term “algae” comprise both prokaryotic and eukaryotic, mono- and multicellular organisms of sizes from several micrometres to over of dozen metres (Sheehan, Dunahay et al. 1998). They can occur in all fresh and salt, cold and warm waters of all geographical zones. Their production reached in 2011 up to 10000 tonnes of dry mass (Griffiths, Dicks et al. 2011). The main products recovered form the algae were high value compounds such: carotenoids, polyunsaturated fatty acids, cosmetics and nutrient supplements.

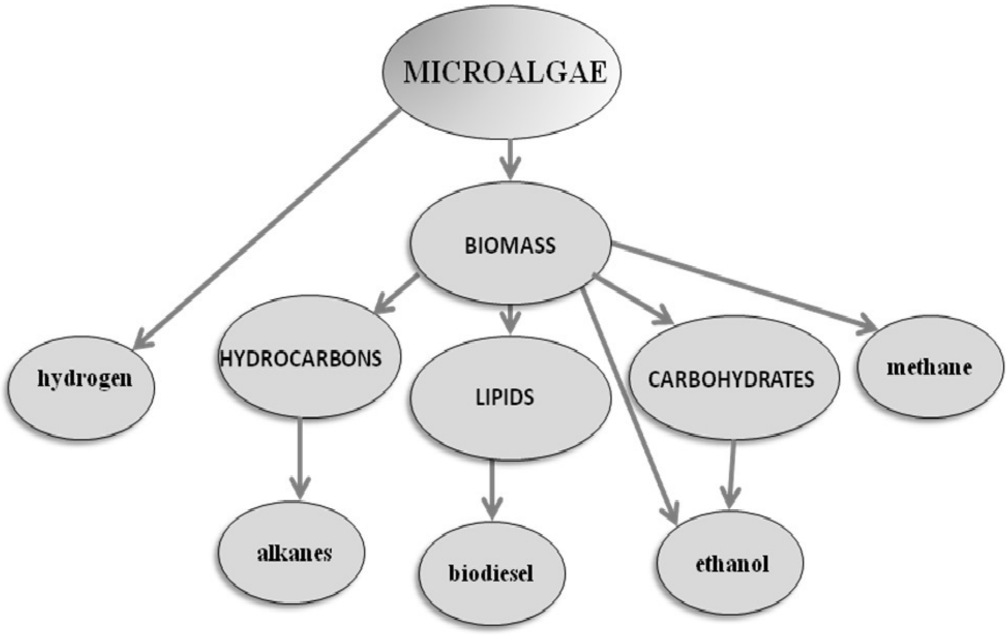

Fig. 1: Potential fuels from microalgae.

Microalgae as biodiesel sources

Microalgae is the term assigned to microscopic organisms which are several to a few hundred micrometres long. They are predominantly monocellular organisms often living in colonies. Microalgae can be the source of several types of biofuels: methane produced during anaerobic digestion of algae biomass (Spolaore, Joannis-Cassan et al. 2006), hydrogen produced photobiologically in anaerobic conditions (Ghirardi, Zhang et al. 2000) and biodiesel derived from lipids accumulated as reserve material in algae cells (Xu, Miao et al. 2006; Demirbas 2008). The most convenient fuel for transport is biodiesel, but it needs further processing of microalgae biomass for the better recovery of energy. The complete utilisation of algal biomass my involve the combination of technologies mentioned (Wiley, Campbell et al. 2011).

The algae are potentially more efficient in oil production in comparison to common oil seed crops due to the higher productiveness per area. Higher oil yield arises from high biomass production rate and high lipid content (Wiley, Campbell et al. 2011).

Many among classified species of algae have growth rate below 1 d^{-1}. While algae do no have organs characteristic to the higher plants whole cell surface can be involved in photosynthesis process. Moreover higher oil yield arises from the fact that lipids are accumulated in whole cell, while in oil crops only seeds contain significant amount of oil and are collected and processed (Griffiths, Dicks et al. 2011).

Algae species capable of accumulation large amount of lipids are found in many taxonomic groups (Fig. 2). However, chlorophytae represent the biggest group within which the species with the average content of 25.5% of lipids in dry biomass have been identified. Examinations of cyanobacteria did not reveal highly oil-bearing species. In this group lipids average contents in dry mass reached only 9.8% and did not show neutral lipids accumulation (Basova 2005; Hu, Sommerfeld et al. 2008).

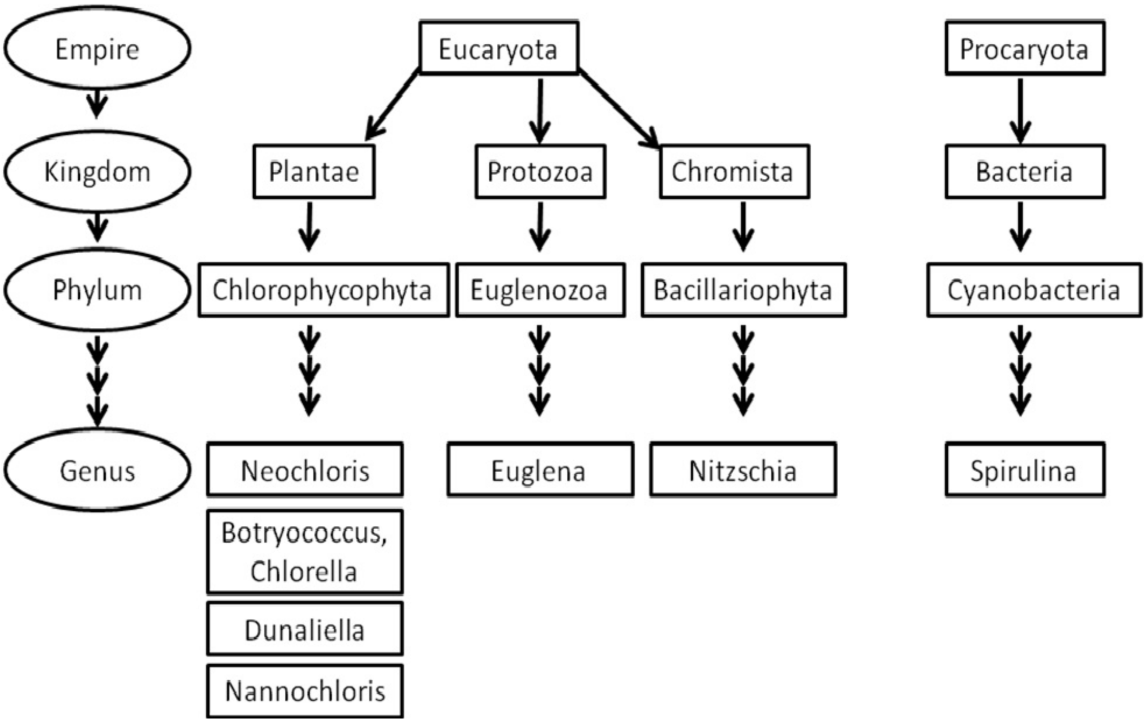

Fig. 2: Taxonomy of some algae species potentially useful for biodiesel production.

Algae is a large group of polyphyletic organisms. Most of the species potentially useful for biodiesel production belongs to green algae (*Chlorophycophyta*) and diatoms (*Bacillariophyta*). Although Cyanobacteria species accumulate lower levels of lipids (e.g. *Spirulina* 9%) but within this group there are species which are able to fix atmospheric nitrogen which could be useful for culture nutrition. Taxonomic classification based on (Guiry and Guiry 2010)

Moreover the amount of lipids in chlorophytae may be raised up to 45% of dry weight by stress or nutrient starvation (Hu, Sommerfeld et al. 2008). The increase of lipid concentration in stress exposed and ageing cells refers mainly to neutral lipids and triacylglycerols in particular. The observed phenomenon is a result of lipids metabolism shift from membrane lipids synthesis to neutral lipids storage. This can cause the increase of triacylglycerols (TCG) synthesis *de novo*, as well as the conversion of present membrane lipids to TCG. As a result, triacylglycerols may constitute up to 80% of lipids total contents in the cell (Hu, Sommerfeld et al. 2008). It is very important to notify that high lipid content does not necessarily reflects the overall lipid production (Griffiths, Dicks et al. 2011). During the starvation period the growth rate is reduced and the total lipid production may be lower in comparison to well nourished culture.

On the other hand the lipid composition has considerable influence on the technology of biodiesel production and product quality (Li, Du et al. 2013). Lipids derived from algae cultured without stress contain significant amounts of polar lipids (phospholipids and glicolipids) and limited content of TCG (up to 40 % of total lipids) (Harwood and Guschina 2009; Wang and Wang 2012). The best material for biodiesel production are TCG while polar lipids are unfavourable since they are a cause of emulsification and catalyst depletion (Mendow, Monella et al. 2011). Lipids other than TCG may also reduce the fuel quality by increasing the content of sulphur and phosphorus (Mendow, Monella et al. 2011). Despite reduced growth rate and total lipid production rate, starvation of algae may be beneficial due to the increased content of TCG.

Fatty acids composition in algal cells

Algae produce both saturated and unsaturated fatty acids with different number and position of unsaturated bonds and various length of carboxylic chain. However, both saturated and unsaturated fatty acids with even number of carbon atoms prevail there (Cobelas and Lechado 1989; Makri, Bellou et al. 2011).

Fatty acids in algae are more diverse in comparison to higher plants. Acids with three or more double bonds (up to six) are present. Another special feature of algae is high content of polyunsaturated fatty acids (PUFA) with very long chains (longer than C22) (Hu, Sommerfeld et al.

2008). Algae are the source of DHA rich oil used in vegetarian diet supplementation instead of cod liver oil (Masuda, Tanaka et al. 2003; Pyle, Garcia et al. 2008).

While algae could be excellent source of PUFA in dietary supplementation, in biodiesel production the amounts of fatty acids with four or more double bonds should be as small as possible. Such acids as well as their esters are definitely more susceptible to oxidation during fuel storage decreasing their quality (Fukuda, Kondo et al. 2001; Chisti 2007; Hu, Sommerfeld et al. 2008). It seems especially important in the case of biodiesel which is to be used in vehicles. European standards (Standard EN 14214) allow only 1% mol contents of methylic or ethylic esters of 4 or more double bonds fatty acids (Knothe 2006). Many of algae derived oils are not up to this standards. However, they can be used in biodiesel production due to partial catalytic lipids hydrogenation – the technology used during margarine production but increasing the process costs (Jang, Jung et al. 2005; Dijkstra 2006)

Algae species selection for biodiesel production

The amount of lipids in algae biomass varies largely between species. For the last decades, thousands of algae have been analysed in respect of lipids content and culture facility both in a laboratory and on a industrial scale (Hu, Sommerfeld et al. 2008). The collection of 3000 species of high lipids productivity have been made up which, after tests, isolation and thorough characteristics were limited to 300 most valuable species including mainly green algae and diatoms (Sheehan, Dunahay et al. 1998).

Rodolfi's team (Rodolfi, Chini Zittelli et al. 2009) examined 30 algae strains isolated from both fresh and salt waters. They were initially cultured in a laboratory in order to define the strains characteristic for relatively big efficacy in biomass production as well as high lipids content. Elicited results allowed to select two fresh-water species (*Chlorella* sp. F&M-M48 and *Scenedesmus* sp. DM) and two salt-water species (*Nannochloropsis* sp. F&M-M24 and *Tetraselmis suecica* F&M-M33). These microorganisms were subsequently moved to culture tubes under artificial light to examine the influence of limited access to nitrogen and phosphorus. The highest lipids accumulation simultaneously with the smallest biomass production drop was revealed for *Nannochloropsis* sp. F&M-M24. Observation of light intensity and starvation reaction in a mount rack photobioreactor was another stage followed by the culture movement to 110 litre photobioreactor GWP type (Green Wall Panel) to carry on an experiment in conditions close to large-scale culture.

Described methodology is suitable for the screening of a large number of algae strains. For strains with high growth rate and lipid accumulation the economical simulation can be prepared. In order to do this, NER (Net Energy Ratio) is calculated. NER is the ratio between the produced energy (the energy embedded in lipids used in biodiesel production and potential energy from biomass remains) and the energy introduced to the system during the cultivation and processing of biomass. If the value of NER for particulate system is higher than 1 it may be economically profitable (Jorquera, Kiperstok et al. 2010).

Genetic modifications

The genetic modifications of algae are not necessary to form achieve relatively high lipids contents and productivity. However, they can be used for the significant increase of processes business value (by e.g. elimination of photoinhibition) (Rodolfi, Chini Zittelli et al. 2009).

The use of genetically modified algae may be helpful in improving culture stability. In large scale, the contamination of selected strains often occurs. Competition between organisms reduces production efficiency. In cultures of algae resistant to herbicides the growth of undesirable microorganisms could be hindered with this chemical compounds (Gressel 2008).

Algae are very sensitive to temperature shifts. Maintaining stable temperature in cultivation vessels (heating and cooling during sunny days) is very expensive, thus obtaining strains resistant to this changes would enable the reduction of costs (Shlyk-Kerner, Samish et al. 2006; Gressel 2008).

The ability of light utilisation by algae is characterized by a light saturation constant, that is the intensity of light at which the specific biomass growth rate is half of its maximum. Light saturation constants for microalgae tend to be much lower than the maximum sunlight level that occurs at midday. Moreover intense light can lead to photoinhibition resulting from damage of the photosynthetic apparatus to the reduction of growth rate. Uneven light intensity (the highest at the surface) is also the problem of photobioreactors. Adequate genetic modifications can unsensitize photosynthetic apparatus to intense light and suppress photosaturation and photoinhibition (Chisti

2007). On the other hand, in low light intensities growth of modified algae will be reduced in comparison to wild type organisms (Polle, Kanakagiri et al. 2003; Gressel 2008). Green algae have a tendency to assemble large arrays of light-absorbing chlorophyll antenna molecules in their photosystems, which in higher light intensity absorb more photons than photosynthesis can utilize, resulting in dissipation of light energy by the first layers of cells. To advance light access to the cells located in "dark zone", antenna size could be reduced. Decreasing antennas size (e.g. by chlorophyll particles number half-restriction), light access to deeper culture layers is considerably improved. Besides, such a modification would definitely enlarge photosaturation constant value which would allow cells further growth at high light intensity (Polle, Kanakagiri et al. 2003).

Lipids accumulation can be stimulated by redirecting metabolic pathways to lipids from starch. In *Chlamydomonas reinhardtii* when starch biosynthesis was blocked lipids bodies content increased 30-fold (Wang, Ullrich et al. 2009). In addition, *C. reinhardtii* used in this experiment was cell wall-less mutant (Davies and Plaskitt 1971) enabling much easier and cheaper lipids extraction.

Lipids composition and structure can be improved by mutation within desaturases. The result of such a procedure is flax cultivar called Linola with modified fatty acids proportions (Łukaszewicz, Szopa et al. 2004). In the case of algae for biodiesel production, modification (repression) should aim at FAD2 desaturase equivalent. In higher plants species this enzyme is responsible for the synthesis of PUFA (Krasowska, Dziadkowiec et al. 2007). The elimination of this protein may result in the reduction of PUFA and increase of monounsaturated fatty acids with chain average length like oleic acid (18:1).

The modification of metabolite synthesis gives great possibilities being intensively developing domain on both basic and applied researches levels. Quite theoretically, there is possibility to obtain fatty acids ethylic esters directly in the cell which would significantly decrease biofuel production costs and solve the problem of glycerol disposal being waste product on biodiesel production.

Biodiesel synthesis out of triacylglycerols

Biodiesel, being methylic or ethylic esters of fatty acids, is synthesized in the process of transesterification of TCG with methanol or ethanol. This reaction can also be based on other alcohols like propanol, butanol or amyl alcohol, however, methanol and ethanol are predominantly used due to lower costs (Fukuda, Kondo et al. 2001). Methanol is most often produced out of natural gas or coal so, in contrast to ethyl ones, methyl esters cannot be fully derived from renewable energy sources. Transesterification needs 3 alcohol molecules for every TCG to produce 3 molecules of methyl esters. To achieve 95% efficacy of esters generation, the reaction is performed in alcohol significant excess (Fukuda, Kondo et al. 2001). Thus, there are still many possibilities of the process optimization.

Transesterification catalysis methods

TCG transesterification reaction may undergo catalysis in three ways: by the use of acids, bases or enzymes (Sharma, Chisti et al. 2001; Meher, Vidya Sagar et al. 2006). Only recently, transesterification process optimization has been found to be possible with microwaves application. Acidic catalysis is slow but it is mostly suitable for transesterification of oils with water and high content of free fatty acids (Fukuda, Kondo et al. 2001).

Basic catalysis is the highest-speed transesterification method (it arrives 4000 times faster than acidic catalysis with the use of the same amount of catalysts), however, it can be applied only in the case of water-free oils, otherwise saponification occurs and newly formed soaps decrease catalysis effectiveness and disturb glycerol separation from post reaction mixture. Recently basic transesterification method was optimised enabling single transesterification stage, lack of industrial wastes, the bleaching stage without absorbers and possibility of using raw material "first-pressing" (Kołodziej, Vogt et al. 2008).

Enzymatic catalysis with the use of lipases is also possible, however, its costs are very high (Fukuda, Kondo et al. 2001; Li, Du et al. 2013). Only biocatalysis with whole BSP (Biomass Support Particles) immobilised cells use provides lipases with high stability and long activity thus the whole process seems simple and easy to apply in industry (Ban, Kaieda et al. 2001; Ban, Hama et al. 2002).

Biomass and metabolites recovery from algae cultures

To collect TCG out of algae cultures, the following steps should be executed: 1) recovery of biomass out of the culture, 2) extraction of the compound, 3) purification of the compounds from crude extract (Molina Grima, Belarbi et al. 2003). There are three basic methods of biomass recovery:

sedimentation, filtration and centrifugation, all of them being burdened with substantial defects. Each of the methods can be preceded by flocculation (Uduman, Qi et al. 2010). Other techniques, like electrolytic methods are still being investigated and are used to a lesser extent (Show, Lee et al. 2012). Choosing the method, the features such as biomass density, volume and microalgae cells size (most often of 3-30 µm diameter) should be considered (Molina Grima, Belarbi et al. 2003). It is important to point out that biomass recovery may amount to 20-40% of the production total costs (Gudin and Therpenier 1986), so the optimization of this step might be essential for increasing the production of biodiesel from microalgae.

The process of filtration brings positive effects in the case of small amounts of biomass, especially when microorganisms used for biofuel production are bigger than bacteria. Unfortunately, the species which seem potentially promising in biodiesel production (e.g. *Chlorella* sp. or *Dunaliella* sp.) have the sizes similar to those of bacteria's, so filters regain requires relatively high energy and equipment input, and the process is relatively slow (Molina Grima, Belarbi et al. 2003). Although, as the membrane filtration is an intensively developing and more and more widely used in industry, the adaptation of this technology for the larger amounts of algal biomass seems of great promise (He, Bagley et al. 2012).

Flocculation reinforced sedimentation is a widely used method in sewage-works which potentially requires the smallest input (Shelef 1978). After such a waste treatment process, biomass contain large amount of water which increases dehydration costs (Mohn 1978). Exact and precise dehydration is especially important at base catalysis due to saponification reactions (Fukuda, Kondo et al. 2001).

Centrifugation is the most effective but also most expensive method for the recovery of biomass from algae cultures. This process is short and recovered biomass includes small amounts of water (Molina Grima, Belarbi et al. 2003). For the recovery of more than 95% of cells the centrifugal force as high as 13 000g is needed. Therefore, harvesting of microorganisms is energy-consuming. The centrifugation of 1 m^3 of algae culture consumes 1 – 1.3 kW/h (depending on the system used) whereas the filtration consumes from 2 to 3 times less energy (Sim, Goh et al. 1988). With reducing centrifuge acceleration, the amount of harvested biomass is quickly decreasing. At 6000g only 60% of cells are recovered, which is why it is of no use in the reduction of biomass recovery costs.

Collected biomass should be immediately subjected to further processes, otherwise it can get spoiled within several hours. The first stage of biomass transformation is dehydration, which is another, high energy-consuming process (Show, Lee et al. 2012). There are several methods of microalgae biomass dehydration: sun rays seasoning, spray drying, sublimation drying or drying drums application. Each of these techniques has its own merits and drawbacks. Selection of the method for biomass drying should be therefore considered carefully taking into account the scale of operation (Show, Lee et al. 2012).

Lipid containing cells should be broken in order to extract lipids which, in massive processes, is based on homogenizers application. It allows far more efficient extraction of lipids out of biomass (Chisti and Moo-Young 1986; Middelberg 1994). Oil extraction from disrupted cells can be performed with organic solvents like hexane (Chisti 2008). If the remaining biomass is considered as a feedstock source, organic solvents should be replaced with non-toxic ones e.g. supercritical CO_2 can be used as an extracting agent (Pereira and Meireles 2010; Soh and Zimmerman 2011).

Wastes recycling

Contemporary technologies should be complete and consider disposal of all substrates and their products (Fig. 3). Potentially, the biomass remaining after the extraction of lipids contains large amounts of proteins. In order to make full use of them for animal feed purpose, they cannot contain insanitary residues e.g. organic solvents used in lipids extraction. Additionally, seaweeds exploited species should be approved safe and applicable for animal feeding and alimentary purposes to procure added value from the biomass remaining after the extraction of lipids.

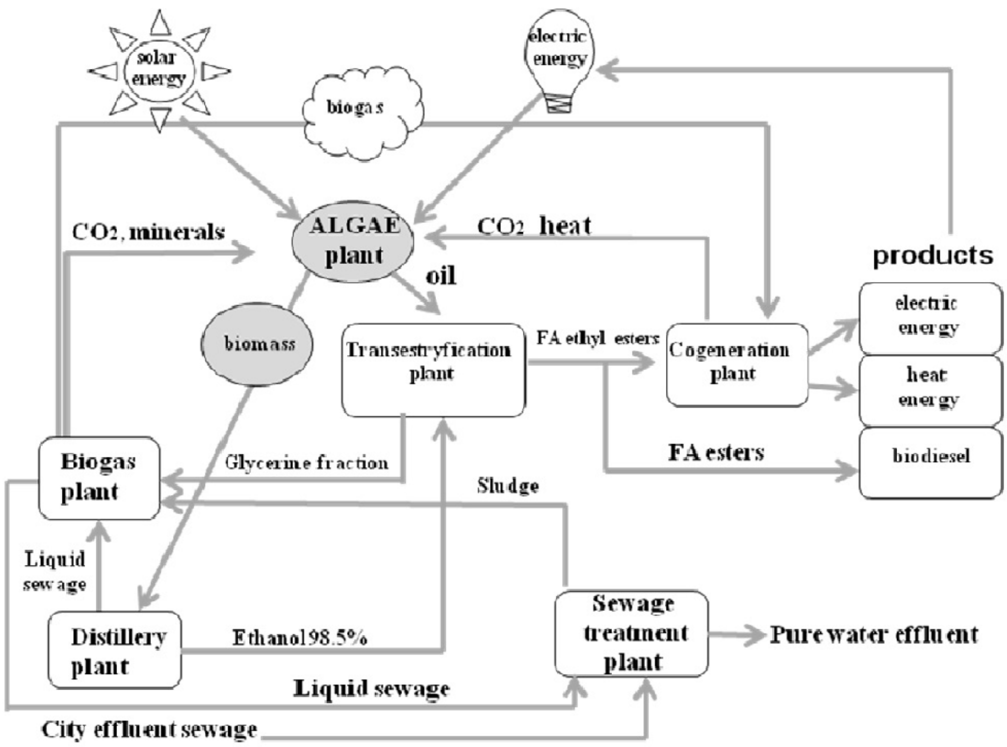

Fig. 3: Global-scale concept of the waste treatment and the biofuel-energy cogeneration centre

Another by-product in algae lipid processing is glycerol from transestrification. Recent technology of glycerol application in fodder-yeasts has been introduced by Skotan (http://www.skotansa.pl). The method has been invented and elaborated at Wrocław University of Environmental and Life Sciences with the use of *Yarrowia lipolityca* species (Rywińska and Rymowicz 2010). The surveys carried out by FDA have revealed *Y. lipolityca* as GRAS (generally recognized as safe) and confirmed applicability in e.g. animal feeding (registered by FDA 21 CFR 170.36). These types of tests should be also carried on algae which could be used in biofuels production.

Glycerol can be also used as a substrate for production of biogas. Addition of glycerol efficiently improved production of biogas from substrates such as: sewage sludge (Fountoulakis and Manios 2009) and cattle slurry (Robra, Serpa da Cruz et al. 2010). It is possible that anaerobic digestion of biomass obtained from algae could be improved with glycerol supplementation. Another interesting possibility of glycerol recycling is microbiological conversion of this by-product to more valuable compounds such as: citric acid, erythritol (Rywińska and Rymowicz 2010), ethanol and butanol (Yazdani and Gonzalez 2007).

Association of biodiesel production from algae with anaerobic digestion process could arise other benefits. The utilization of process water from the production of biogas is a serious problem. High concentration of biogenic elements such as nitrogen and phosphorus makes this water dangerous for the environment. On the other hand these elements are essential for growth of microorganisms and could be used to improve the cultivation of algae. Also, sewage from other industrial processes and household wastewater can be potentially used as a source of nutrients for microalgae.

The second method for increasing the production of algae biomass is recirculation of carbon dioxide produced in process of anaerobic digestion. Photosynthesis efficiency is often limited due to low concentration of CO_2, and photooxidation process which is the effect of low rubisco specificity. Previously, algae strains that can tolerate up to 12% CO_2 were identified (Pulz 2001). Increased availability of CO_2 should reduce this energy-consuming process and improve biomass production.

Business value of algae derived biodiesel

The production of biodiesel from algae is relatively expensive. The production costs of 1 kg of algae biomass amounts from 2.95 to 3.80$ per kg (Chisti 2007), but may be decreased in the future to 0.34$. Seambiotic LTD from Israel is one of enterprise which have work intensely to achieve comparatively low costs of dry algae production (http://www.seambiotic.com/). The costs of growing and processing of algal biomass for the providing of 1 L of oil were therefore estimated between 1.40$ and 1.81$. To get the final price of algae-derived biodiesel these costs have to be doubled (for processing, distribution, etc.), which makes from 2.80$ to 3.62$ per L (Chisti 2007). This numbers should be compared with the price of traditional biodiesel (B99-B100) as high as 1.12$ per L in January 2013 (1) or petroleum price of approximately 105$ per barrel (0.88$ per L) in March 2013 (2). In the situation of economic stagnation and problems in public finances of majority of European countries and United States, the production biofuels derived from algae seems rather doubtful due to difficulties with capital raising.

On the other hand, scale-down rather than scale-up has been observed in industry lately. In industrial output, a series of parallel operating microbioreactors can be applied which would prove extremely effective if carried from laboratory to industrial scale. It gives basis for the presumption that small-scale modular technologies adapted to particular conditions will prove very useful in future and will decrease costs of the production of biodiesel for local use. Additionally, use of different wastes as a source of nutrients and carbon for microorganisms can increase the profitability of algae biodiesel production. Also local governments show interest in the idea of surface waters treatment. The combination of these factors can make small investment easier for financing.

Conclusions

In spite of many advantages of biodiesel production from algae, there are a lot of limitations blocking its real competition with petroleum derived diesel. Production costs seem the most substantial problem. Although many companies culture algae on a large commercial scale (omega-3 and omega-6 acids production – Eau Plus or cosmetic component – Fitoplancton Marino), microalgae culture for biodiesel production still remains within small-scale laboratory interests. There are many companies working upon R&D but no project concerning biofuels derivation from algae has started so far on industrial scale (Wagner 2007).

Out of all recognized methods of microalgae production, the culture in photobioreactors seems the most favourable for biodiesel production, however the costs are discouraging. The investments cost of whole plant is one of the major limiting factors. It seems that small modular systems may be an attractive solution with positive economical rationale. Algae production plant should also be integrated with other existing installation like sewage treatment, biogas and power cogeneration plant (Fig. 3). Co-localisation these production processes results in lowered operating and investments costs. Also, such a waste treatment and the biofuel-energy centre generate a number of additional by-products that can be sold on or used for self-consumed.

Algae derived biodiesel is environmentally friendly which in the case of petroleum shortage may replace petrodiesel. However, to make this product cost-effective, petroleum price should rise or the costs of biodiesel derivation from algae should drop significantly. The presence of alternative technologies of fuels production are optimistic in the face of discussion about petroleum stock out.

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