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POSSIBILITIES OF CARBON DIOXIDE FIXATION BY MICROALGAE IN REFINERY

Abstract

Capture and sequestration of carbon dioxide is one of the most critical challenges today for businesses and governments worldwide. Thousands of emitting power plants and industries worldwide face this costly challenge – reduce the CO₂ emissions or pay penalties. One possibility for carbon dioxide sequestration is its fixation in microalgae. Microalgae can sequester CO₂ from flue gases emitted from fossil fuel-fired refinery plants and units, thereby reducing emissions of a major greenhouse gas. One ton microalgae require for their growth 1,8 tons of CO₂ (theoretically). Microalgae thus are large consumer of CO₂. Combine their affinity for CO₂ with the fact that microalgae can grow practically anywhere, there is an exciting opportunity for emitting industries and power plants – to absorb the CO₂ by microalgae what can be used for the next generation of biofuels production in return. The advantages of microalgae over higher plants as a source of transportation biofuels are numerous: microalgae synthesize and accumulate large quantities of neutral lipids/oil and grow at high rates. Oil yield per area of microalgae cultures could greatly exceed the yield of best oilseed crops. It can be cultivated in saline brackish water in coastal seawater on non-arable land, and do not compete for resources with conventional agriculture. Microalgae tolerate marginal lands that are not suitable for conventional agriculture and utilize nitrogen and phosphorus from a variety of wastewater sources (e.g. industrial wastewater), providing the additional benefit of wastewater bioremediation. Microalgae produce value-added co-products or by-products (e.g. biopolymers, proteins, poly-saccharides, pigments, animal feed and fertilizer) and do not need herbicide and pesticide. Microalgae grow in suitable culture vessels (photobioreactors) throughout the year with higher annual biomass productivity on an area basis. In the current exploratory phase, VURUP Research Institute Bratislava in co-operation with Slovnaft Downstream Development learn which of the available naturally occurring microalgae strains are best suited to capture and consume CO₂. The study is concentrated on the issue – what are the main CO₂ producers within the oil refinery, what quality are flue gases with the emphasis on pollutants (SO_x, NO_x, CO, H₂S, metals, solid pollutants, etc.), how each microalgae strains can live and reproduce in real conditions in photobioreactors built

near the main CO₂ sources at refinery and how fast the microalgae grows, how resistant it is to temperature, pH and how much CO₂ it consumes. The results will help to determine further analysis in a larger second phase.

Key words: refinery, microalgae, CO₂ capture, photobioreactor

MOGUĆNOSTI FIKSACIJE UGLJIČNOG DIOKSIDA U RAFINERIJI PUTEM MIKROALGA

Sažetak

Hvatanje i vezivanje ugljičnog dioksida je jedan od najvažnijih izazova današnjice za tvrtke i vlade diljem svijeta. Tisuće elektrana i industrijskih pogona širom svijeta suočavaju se s ovim skupim izazovom - smanjiti emisije CO₂ ili platiti kazne. Jedna od mogućnosti za vezivanje ugljičnog dioksida njegova je fiksacija pomoću mikroalga. Mikroalge mogu vezati CO₂ iz dimnih plinova ispuštenih iz rafinerijskih postrojenja i jedinica na fosilna goriva, čime se smanjuje emisija glavnog stakleničkog plina. Jedna tona mikroalga za svoj rast treba 1,8 tona CO₂ (teorijski). Time postaje razvidno da su mikroalge veliki potrošač CO₂. Kombiniranjem njihove sklonosti prema CO₂ s činjenicom da mikroalge mogu rasti gotovo posvuda, javlja se uzbudljiva prilika za industrije koje imaju emisije i elektrane - apsorbirati CO₂ mikroalgama što se dalje može koristiti za proizvodnju sljedeće generacije biogoriva. Prednosti mikroalga pred višim biljkama kao izvorom biogoriva za vozila su brojne. Mikroalge sintetiziraju i akumuliraju velike količine neutralnih lipida / ulja i brže rastu. Prinos ulja po površini kulture mikroalga uvelike premašuje prinos najboljih uljarica. Mogu se uzgajati u slanoj bočatoj vodi u priobalnom pomorskom i neplodnom području, koje ne ulazi u resurse s konvencionalnom poljoprivredom. Mikroalge uspijevaju i na nekvalitetnom tlu koje nije prikladno za konvencionalnu poljoprivredu i koriste dušik i fosfor iz otpadnih voda iz različitih izvora (npr. industrijske otpadne vode), što predstavlja dodatnu korist u bioremedijaciji otpadne vode. Mikroalge proizvode nusproizvode dodane vrijednosti (npr. biopolimeri, proteini, polisaharidi, pigmenti, stočna hrana i gnojiva), a ne trebaju herbicide i pesticide. Mikroalge rastu u prikladnim posudama (fotobioreaktori) tijekom cijele godine uz veliku godišnju produktivnost po jedinici površine. U trenutačnoj fazi istraživanja, VURUP Research Institute Bratislava u suradnji sa Slovnaft Downstream Development nastoje utvrditi koji od dostupnih prirodnih sojeva mikroalga su najprikladniji za hvatanje i potrošnju CO₂. Studija je usredotočena na pitanje - koji su glavni proizvođači CO₂ u naftoj rafineriji, koja je kvaliteta dimnih plinova s obzirom na onečišćavala (SO_x, NO_x, CO, H₂S, metali, kruta onečišćavala, itd.), kako pojedini soj mikroalga može živjeti i razmnožavati se u stvarnim uvjetima u fotobioreaktorima instaliranim u blizini glavnih izvora CO₂ u rafineriji i koliko brzo rastu mikroalge, koliko su otporne na temperaturu, pH i koliko CO₂ troše. Rezultati će pomoći utvrditi daljnje analize u narednoj fazi.

Ključne riječi: rafinerija nafte, mikroalge, CO₂ hvatanje, fotobioreaktor

Introduction

Carbon atoms in crude oil essentially end up in CO₂, when fuel products from a refinery are consumed by end users. The refinery business chain starts from crude oil production (upstream), its transportation, followed by refining operations and finally products transportation. It is known that refining operations are the major contributor to CO₂ emissions in this crude oil – products business chain.

1. Carbon dioxide emissions – a challenge for crude oil refineries

There are several possibilities for reducing, capture and utilization of CO₂ from flue gases in a refinery as follows:

- Improving energy efficiency of technological processes
- Higher utilization of nuclear energy and/or renewables
- Chemical utilization of CO₂ for ethylene carbonate (EC) and dimethylcarbonate (DMC) production
- Chemical washing system based on MEA or DEA and utilization of clear CO₂ in food industry (beverages)
- Chemical use of CO₂ (mainly in reaction with C, CO, CH₄ and H₂O) for synthesis gas production (alcohols, fuels as final products)
- Carbon capture and storage of CO₂ in underground geological formations (CCS)
- CO₂ mitigation and fixation by microalgae

Carbon dioxide reduction (possibly its capture) is a serious challenge for all companies burning fossil fuels. Thousands of CO₂ emitting industrial plants face basic question: reduce the CO₂ emissions or to pay penalties ? As we could see, there are a number of options for reducing of carbon dioxide in the refinery.

For a selection of the best carbon capture options for the refinery it is necessary:

- to set the target,
- to perform carbon dioxide balance,
- to indicate and select applicable technology
- to propose design solutions to achieve goal
- to develop reliable site specific cost estimates.

To make carbon capture economically attractive, the CO₂ needs to have a value of at least 4 - 5 times current value of EU ETS of today (10 €/t).

In a crude oil refinery there exist some separate sources (emittents) of flue gas with GHG emissions and CO₂ respectively. Middle refinery can emit yearly roughly 1 Mt CO₂ altogether.

A contribution of individual emittents can be seen on the figure 1.

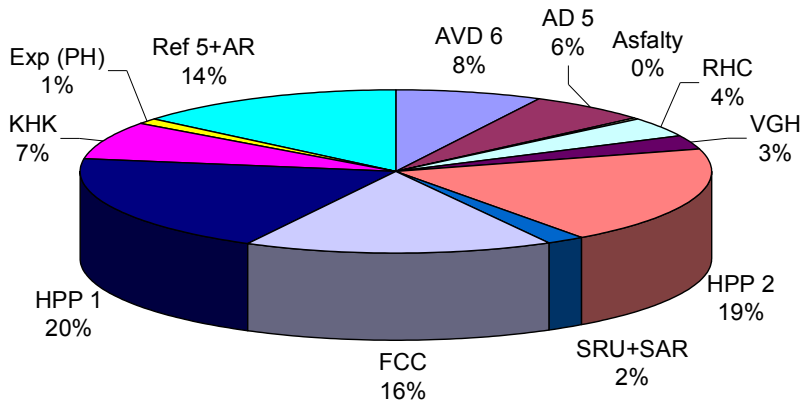
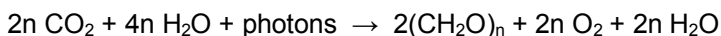


Figure 1: A contribution of individual emitents of carbon dioxide in refinery

2. From CO₂ emissions to microalgae

Algae can fix CO₂ from flue gases emitted during fossil fuels burning. Combination of its affinity to CO₂ together with the fact, that algae can grow practically anywhere, it gives a big chance for industry to capture and fix emitted CO₂ and an opportunity at the same time to cultivate biomass, directly applicable for the next generation of biofuels or biogas production. Algae will do whatever it takes to survive. Algae reproduce themselves using photosynthesis to convert both CO₂ and sun energy into chemical energy, completing an entire growth cycle every few days. They can grow almost anywhere. It is estimated that more than 50 000 species exist, but only a limited number, of around 30000, have been studied and analyzed yet.

Microalgae yield up to 100 times more oil per hectare than other common biodiesel feedstock's, When grown photosynthetically, microalgae has two environmental benefits - CO₂ mitigation plus a renewable energy source. Microalgae can capture sunlight 20-40 times more efficiently than plants, and unlike corn-, sunflower- or soy-based feedstock's, they do not create a "food or fuel" dilemma. Microalgae may assume many types of metabolisms (e.g. autotrophic, heterotrophic, mixotrophic, photoheterotrophic) and are capable of a metabolic shift as a response to changes in dilemma the environmental conditions. In autotrophic metabolism CO₂ is microalgae's favorite food. Approximately half of the microalgal biomass dry weight is carbon, typically derived from CO₂ or carbonates, and is fed continually during daylight (sunlight), when photosynthesis runs:



Each 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide. CO₂ needs to be added as a gas or in bicarbonate form because cultivated microalgae grow too fast to be able to take sufficient CO₂ from the water. Compressed air blended with CO₂ up to 20%, typically provides carbon for algal photosynthesis. Microalgae can sequester CO₂ from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing GHG emissions. Algae belong to the fastest-growing plants in the world. It is critical to understand how to select the right algae species, create an optimal photobiological formula for each species, and build a cost-effective photobioreactor that can precisely deliver the formula to each individual algae cell, no matter the size of the facility, or its geographical location. The research in VURUP Bratislava is aimed on integrated decreasing of GHG production within the refinery by utilization of waste CO₂ to microalgae production and its consecutive use for biogas and /or biofuels of second generation production. The experiments were comprised of three parts: the cultivation of algae, the algae harvesting and digestion of algae to for biogas. Two algae strains were used for the tests: *Chlorella vulgaris* (CHV) (obtained commercially as a dry biomass from the Research Institute at Třeboň, Czech Republic), *Chlorella sorokiniana* (CHS), Shihira et Krauss (SAG 211-8k, CCAP 211/8k, UTEX 1230). Nutrient medium generally consists of a mixture of chemical salts and water. For the cultivation of algae a standardized culture medium BBMdn (Bold's Basal Medium with doubled nitrate) was used.

3. The research of microalgae in VURUP Research Institute Bratislava

3.1 Microalgae cultivation

A strain of algae *Chlorella sorokiniana* was cultivated in a plate photobioreactor, using the growth medium BBMdn, with the initial biomass inoculated at level 5×10^3 cells/ml. The temperature was maintained at 30 °C instantaneous water heaters. The whole culture of algae in the inner panel (working volume 22 l) was continuously aerated using an air compressor, and filtered air was mixed with extra CO₂ (3% v/v).

The panel was continuously illuminated with fluorescent lamps on both sides of photobioreactors, the average luminance of 17,000 lux. During cultivation, the algal suspension was recorded for the following parameters: cell density, growth rate, pH, temperature, dissolved oxygen, phosphate (PO₄³⁻), nitrate (NO₃⁻), inorganic carbon, dry weight biomass and elemental composition of dry biomass of biogenic elements N, C, H, and S. Cultivation took 14 days, during which the fresh medium was added in the 4th and 10th day (by volume 4 liters) due to sampling. After a cultivation, algae were settled down using a flocculants Al₂(SO₄)₃·18H₂O resp. by centrifugation (4000 rev/min, 10 min). Sedimentation was performed by raising the pH to 11 with KOH.

Liquid algae paste was dried in an oven at 105 °C. Before extraction of the dried algae were disintegrated in a laboratory mill Polymix A 10 fi Kinematic. Extraction was carried out in 2055 Tecator Avanti apparatus at 130 °C for one hour. Total lipids were determined gravimetrically. Alga of *Chlorella vulgaris* was obtained as a dry biomass commercially from the Research institute at Trebon (Czech Republic).

Microalgae composition

The composition of fatty acid in the samples of extracted lipids was evaluated according to standards EN 5509, ISO 5509 and STN EN 14103. A sample of the extracted lipids was saponify with methanolic sodium hydroxide, mixture was then heated under reflux and the resulting soaps were converted to fatty acid methyl esters by reaction with methanolic solution of boron trifluoride. Prepared fatty acid methyl esters of C₈-C₂₄ were separated by high-resolution capillary gas chromatography in a 100 m long columns with a polar stationary phase CP-Sil 88th.

Determination of total carbohydrates was done by Anthrone methods.

Anaerobic digestion

The digester (glass column with 1 l volume) was continuously mixed with magnetic stirrer. The temperature was maintained at 40-41 °C in water bath. The reactor was fed with inoculum from biogas unit (with maize as a feedstock), Shelton medium containing nutrients and trace metals, demineralized water, algae paste or dry algae, silicone antifoam agent and CaCO₃. Biogas volume was measured by water displacement and then normalized normal condition. Biogas production is given in norm mL per gram volatile solids (273 K, 1013 mbar). The biogas composition was determined by gas chromatography and/or by GA2000 analyzer. Total solid (TS), total volatile solid (TVS), pH, chemical oxygen demand were measured in regular interval.

3.2 Results of microalgae cultivation

In terms of cultivation and biomass production value was reached at 3.30 g/l. Lipid content was 18.63 wt% of dry matter, total carbohydrate content was 7.66 wt% of dry. Lipids extracted with chloroform contain 20.66 wt% saturated TAG (C16: 0 = 17.85 wt%) and 77.78 wt% of unsaturated TAG (C18: 2 n-6 = 36.08 wt%, C18: 3 n-3-11,63 wt%). The elemental composition of dry matter after chemical flocculation using Al₂(SO₄)₃ was as follows (in wt%): N-6.00, C-38.40 H-7.48, S-2.04. Higher sulfur originated from remains from flocculant. The comparative algae *Chlorella vulgaris*, contains 7.92 %wt. total sugars, 10.41 %wt. lipids. Lipids contained 17.15 wt% saturated TAG (C16: 0 = 15.66% wt.) and 79.75 wt% unsaturated TAG (C18: 2 n-6 = 24.18 wt%, C18: 3 n-3 to 19, 91 wt%).

3.3. The growth curve

The growth curve of algae strain *Chlorella sorokiniana* is presented in figure 2.

3.4 Algae digestion

For digestion of algae three algae samples were selected. Dried and disintegrated algae CHV was used for a comparison. For other tests we chose algae CHS, which in previous cultivations had higher lipid content. Since the disintegration and drying processes are energy intensive in one test, we used wet algae flocculated with Al₂(SO₄)₃ and in the other test was dried and disintegrated algae used. The highest biogas and methane yield was achieved in case of dry and milled algae CHS. In comparison with the algae CHV the yield of biogas is higher and is correlated with a higher content of lipids. In the case of wet algae CHS the lowest biogas yield was observed. One possible explanation is the inhibition by Al³⁺ ions.

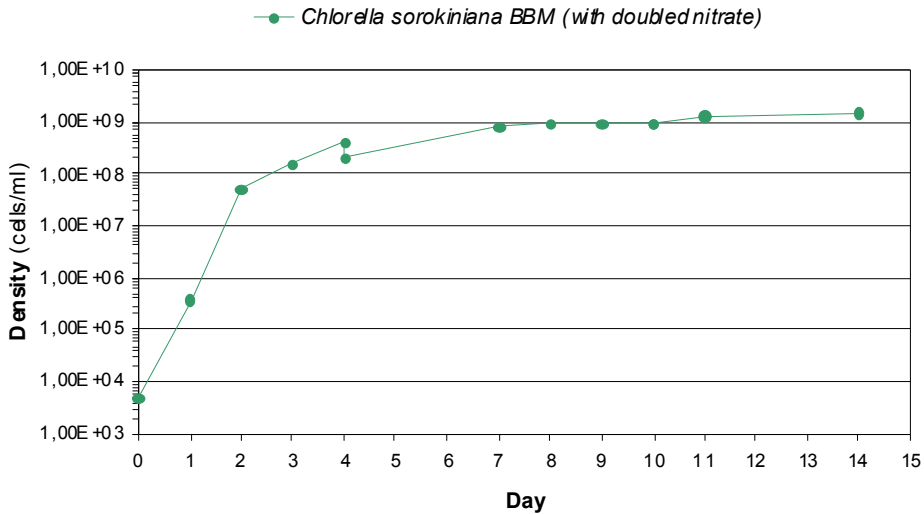


Figure 2: The growth curve (cell density) of algae strain *Chlorella sorokiniana*

3.5 Biogas yield

Biogas conversion yield fit exponential trend for all tested algae. Maximum total methane yield was in case of dry and milled CHS. Mechanical disruption of cell walls of algae has had a positive effect on the process which was reflected by a faster process of acidogenesis and higher production of biomethane. An important technological parameter is pH, which must be managed. At the beginning of digestion the pH was in range 6.5 to 7.2, gradually increased to a value of 8.1 to 8.3 which remained all the time digestion. To suppress foaming, which occurred after 6 days, to add silicone defoamer has proven. The presence of protein caused formation a substantial amount of NH_3 and H_2S . In the case of algae CHS dry & milled NH_3 concentration was 5 times lower, which had a positive impact on the course of digestion and biogas yield.

3.6 Cultivation outside – using sun light

The further step of research was performed outside using sun light and mixture of 10% CO_2 from pressure bottle with 90% of air and the same nutrients. It was tested utilization of microalgae for biogas production as well as lipids from microalgae for liquid biofuels of second generation production. There will be tested also possibilities of fixation of industrial CO_2 in a pilot system in refinery. It will be tested also a possibility of waste water treatment by microalgae by using of industrial waste nutrients (N, P) and G-phase from FAME production. All the research activities are in harmony with implementation of EU Directive 2009/28/EC on renewables.

Comparison of cumulated biogas production mL/ g TVS

Algae	Total biogas yield	Methane yield
<i>Chlorella vulgaris</i> , sp., dry & milled	221.1	189.0
<i>Chlorella sorokiniana</i>	119.1	98.2
<i>Chlorella sorokiniana</i> , dry & milled	274.1	234.4

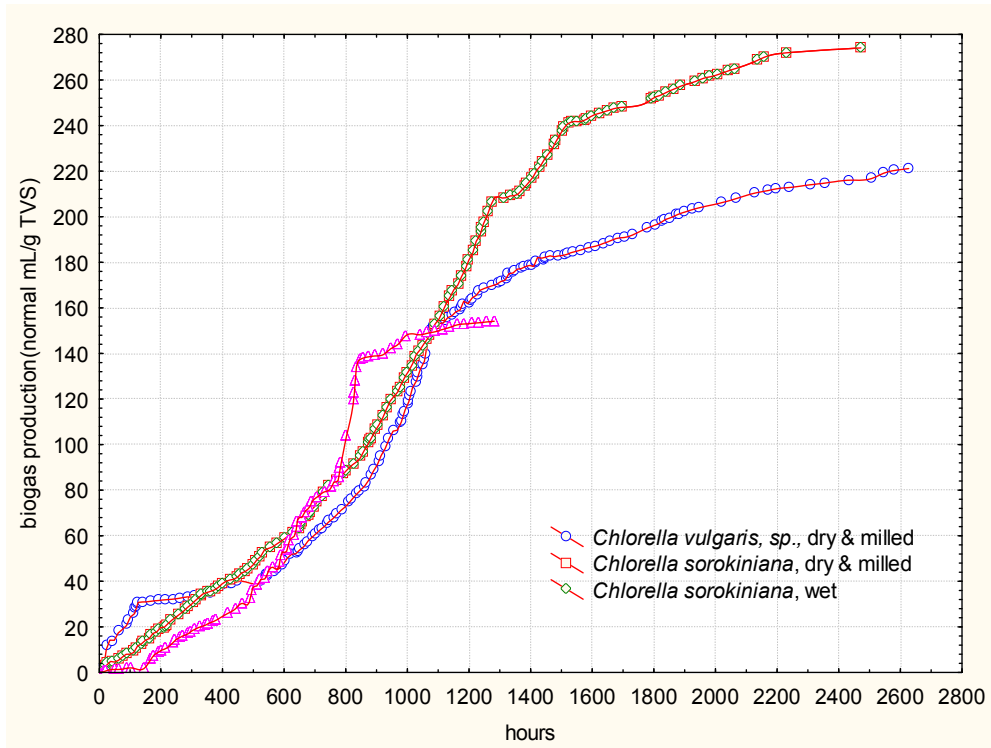


Figure 3: Comparison of cumulated biogas production

4. General optimization of cultivation conditions

Algae grow in open, closed or semi-closed systems in round, long or tubular tanks that maximize access of the entire biomass to sunlight. Growth occurs only in the top layer, about 5-6 cm, of the growing medium unless mixing occurs. New cell growth blocks the sunlight for plants below. Semi-continuous mixing is necessary to give all the algae sufficient light. Some production systems put light sources near or in the water to augment sunlight. Growth occurs based on a host of variables that not only constrain growth, but may change the algal composition. Primary variables include the following.

Light. Usually sunlight provides sufficient light but artificial light also works as well - especially for indoor growing systems. Some growing systems may be tilted to optimize orientation to the sun and reflected light. Several producers are experimenting with bent light using mirrors or glass cables and other are using LED lights that minimize energy consumption.

Mixing. Since most growth takes place in the top layer of the surface that faces the light source, mixing is imperative. Each cell needs to move in and out of the light for their light and dark growth periods as they take in CO₂ and exhale O₂. Algae are heavier than water and would sink away from their light source without mixing.

Water. Algae grow well in nearly any kind of water. They are especially good at using photosynthesis to convert dissolved nutrients and metals in wastewater to green biomass where the metals can be removed and recovered. Production systems can use wastewater, grey water and saline or ocean water, depending on the species grown. Growing systems can recycle the water so the only loss comes from evaporation.

CO₂. Approximately half of the microalgal biomass dry weight is carbon, typically derived from CO₂ or carbonates, and is fed continually during daylight. Industrial CO₂ or waste flue gasses are typical sources. Some producers such as Solazyme use an organic carbon source in the form of acetic acid or glucose.

Nutrients. Algae feed their growth with the same fertilizers used for land plants but the fertilizers can come from waste streams that are too salty for land plants. Algal growth consumes far less nitrogen and other fertilizers per pound of biomass than food grains such as corn and the nutrients are easier and less expensive to apply. Dissolved chemical fertilizer or waste stream nutrients are utilized by algae with far more efficiency than land plants because the tiny single celled algae consume the nutrients directly and do not have to transport the nutrients long distances. Unused fertilizer also may be reused with the recycled water.

pH. The acidity of water may be specific to the type of algae produced. Controlling the water's pH represents a good strategy for retarding growth of competing algae. Water pH is likely to be highest at noon due to the high photosynthetic activity, which consumes maximum CO₂.

Stability. Maintaining a stable growth environment presents difficulties with the high velocity of growth. The growing medium may retain too much of any nutrient or O₂, which may create stress and or composition changes to the plants. Some producers capture released O₂ and sell the pure gas as a value added product.

5. Chemical composition of dry algae

Chemical composition of algae on a dry matter basis (%)

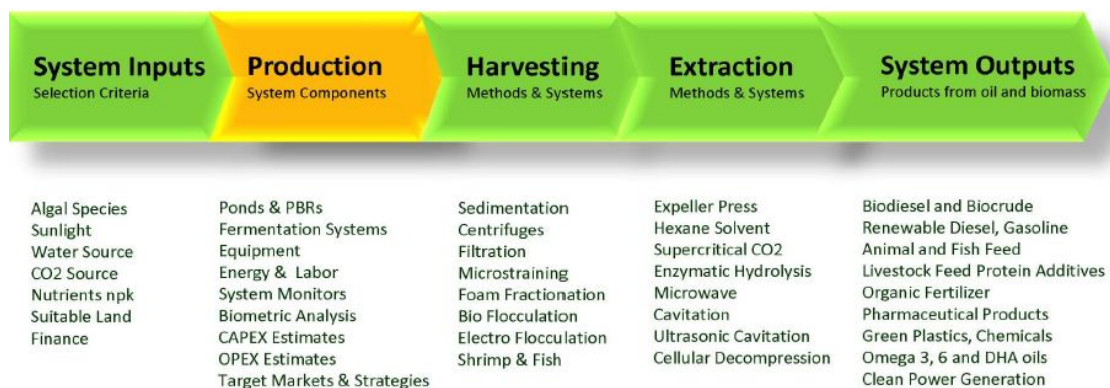
Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
<i>Scenedesmus obliquus</i>	50–56	10–17	12–14	3–6
<i>Scenedesmus quadricauda</i>	47	—	1.9	—
<i>Scenedesmus dimorphus</i>	8–18	21–52	16–40	—
<i>Chlamydomonas reinhardtii</i>	48	17	21	—
<i>Chlorella vulgaris</i>	51–58	12–17	14–22	4–5
<i>Chlorella pyrenoidosa</i>	57	26	2	—
<i>Spirogyra</i> sp.	6–20	33–64	11–21	—
<i>Dunaliella bioculata</i>	49	4	8	—
<i>Dunaliella salina</i>	57	32	6	—
<i>Euglena gracilis</i>	39–61	14–18	14–20	—
<i>Prymnesium parvum</i>	28–45	25–33	22–38	1–2
<i>Tetraselmis maculata</i>	52	15	3	—
<i>Porphyridium cruentum</i>	28–39	40–57	9–14	—
<i>Spirulina platensis</i>	46–63	8–14	4–9	2–5
<i>Spirulina maxima</i>	60–71	13–16	6–7	3–4.5
<i>Synechococcus</i> sp.	63	15	11	5
<i>Anabaena cylindrica</i>	43–56	25–30	4–7	—

Source: Becker, 1994.

6. The potential of microalgal oils

Numerous algal strains have been shown to produce more than 50% of their biomass (on a dry cell weight basis) as lipid with much of this present in the form of triacylglycerols (TAGs) (Hu et al., 2008). (It should be noted however, that like many aspects of algal biofuels research, the methodology generally used for algal lipid analysis - largely based on solvent extraction and gravimetric analysis - has yet to be standardized and thus the values published in the literature should be regarded, at best, as only an estimation of the lipid content.) Further, some algae accumulate high levels of lipids when cultivated under stress (e.g. limitations of certain nutrients) or in response to changes in culture conditions. For this reason, algal cellular lipid content can vary both in quantity and quality. Importantly, from a production point of view, accumulation of lipid produced under stress conditions is generally at the expense of significantly reduced biomass yields. Algae-derived oils contain fatty acid and triglyceride compounds, which like their terrestrial seed oil counterparts, can be converted into biodiesel (via transesterification to yield fatty acid methyl esters) (Fukuda et al., 2001), and green diesel, green jet fuel, and green gasoline.

7. From microalgae biomass to biofuels



Source: Algae 2020, Emerging Markets Online Consulting Services

Figure 4: Algal biomass production systems

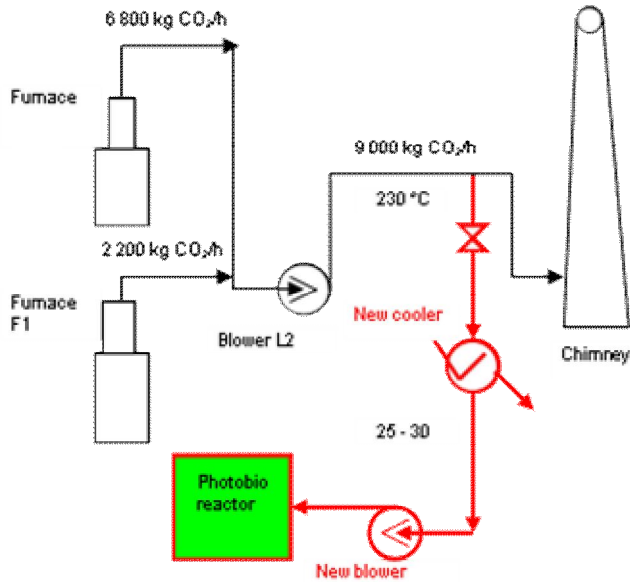
8. Chemical content of dry microalgae

Oil content in microalgae can reach 75% by weight of dry biomass but associated with low productivities (e.g. for *Botryococcus braunii*). Most common algae (*Chlorella*, *Cryptocodinium*, *Cylindrotheca*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Tetraselmis*, *Schizochytrium*) have oil levels between 20 and 50% but higher productivities can be reached. Thomas et al. Analyzed the fatty acid compositions of seven freshwater microalgae species showing that all of them synthesized C14:0, C16:0, C18:1, C18:2, and C18:3 fatty acids.

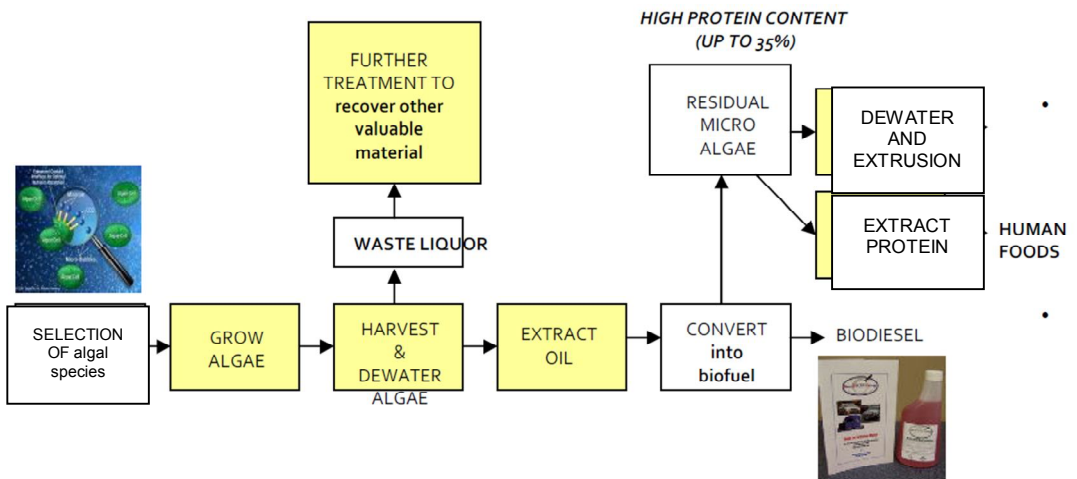
9. Harvesting, dewatering and oils extraction

Once the microalgae are cultivated, biofuel manufacturers are faced with two major technical hurdles: harvesting and dewatering. Microalgae cultures can be more than 90% water, so cells must be collected by settling, which is time-consuming, although this can be hastened with flocculating agents that cause cells to clump and precipitate. More high-tech methods like centrifugation and filtering are faster, but are more costly in both dollars and energy. Once harvested, cells may be air- or sun-dried, requiring a large surface area and significant time, or they can be dried using heat or a vacuum, again increasing the cost and reducing energy efficiency. Finally, extracting the oils is another challenge. Options include extraction with solvents like hexane, enzymatic digestion of cell walls, or physical disruption with ultrasonic sound waves or microwaves.

10. Proposal for CO₂ utilization



11. Algae to biodiesel pathway (outlook for the future)



12. Conclusions

Biodiesel production from microalgae is a goal that still needs much research. There is need for strenuous research on the biosynthesis of algal lipids, especially TAGs, if we want to understand and manipulate algae for the production of biodiesel. The concept of coupling an oil refinery plant with an algae farm provides is an elegant approach to recycle of the CO₂ from fossil fuel combustion into a useable liquid fuel. Combination of wastewater treatment and microalgal CO₂ fixation provides additional economic incentives due to the savings from chemicals (the nutrients) and the environment benefits. It provides a pathway for removing N, P and metal from wastewater, and producing algal biomass, which can further be exploited for biofuel production, without using freshwater. Use of the biorefinery concept and advances in raceway ponds engineering will further lower the cost of production.

Combining algal cultivation and biogas (methane) generation is considered to be one of the prospective environmentally feasible options of creating perpetually renewable source of pure energy for industrial and human consumption. Methane and energy generated in anaerobic fermentation facilities can be utilized as replacement for fossil fuel energy, thus reducing greenhouse gas emissions. This is caused by the fact that carbon is a biologically degradable material and algae form an integral part of the carbon cycle.

The integration technology cultivation of algae using waste CO₂ and algae digestion process has a great environmental and economic dimension. For the production of biomethane are more suitable algae which produce higher share of total lipids. A high concentration of nitrogen during anaerobic digestion of protein-containing algae have potential toxicity to methanogenic bacteria. The fatty acids in the lipids are highly influenced by the composition of growth medium.

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