TKP4850 Experts in Teamwork

Village: Biofuels – A Good Solution?

Project Report for Green Skulls

Title: Integrating wastewater treatment and CO_2 mitigation with microalgae to produce biofuels

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1 Contents

2 Abstract and group introduction

The purpose of this project was to write a descriptive report about using microalgae as a source of biofuels, including the abilities of microalgae to fixate CO_2 and treat wastewater. Its intention is to promote the advantages of using microalgae for biofuel production. The report is directed to a public with a technical background, but no knowledge about microalgae or biofuels. Therefore a glossary was included to clarify many of the terms in the report. First, the background and advantages of the use of microalgae is presented and the microalgae species is selected. This is followed by a description of the potential improvement of microalgae strains by genetic modification. Then, different culture reactors are presented, most importantly photobioreactors, and compared. The harvesting process is described, followed by different oil extraction methods and a comparison between them. Finally, transesterification of vegetable oils is described and an industrial process for the transesterification reaction is proposed.

While learning about the overall process was something that every person in the group did, each member had a specific part of the process to write about which had some relevance to their main field of study.

- Nam wrote about the selection of which microalgae to use, and general information about microalgae, which was due to aquaculture and microalgae being his specialty.
- Gaston's specialty is within the field of biotechnology, so he wrote about gene manipulation of microalgae.
- Jahangir wrote about the facility construction and selection of photobioreactors. It was not directly connected to his specialty, which is ship design within marine technology.
- Steffen has a specialty in chemical process technology, and he wrote about photobioreactor growth conditions and harvesting technology.
- Rufino wrote about extracting the oil from the microalgae once they had harvested, where both physical and chemical methods were be investigated. They were not directly connected to his specialty, since Rufino has a specialty in geophysics.
- Reza's specialty is within the field of chemical process technology, and he wrote about how the extracted oil could be reacted into biodiesel.

3 Glossary and abbreviations

Activated sludge	The use of microorganisms to treat wastewater sludge.
Autotrophic	Production of complex organic compounds from simple substances.
Batch cultures	Cells are grown in large constant volumes.
Biocoil	Photobioreactors which tubes are coiled forming a cylindrical shape.
Biodiesel	Diesel equivalent made from fatty acids.
Cavitation	Formation of bubbles in a liquid as the result of quick changes in pressure.
Cell membrane	Part of the cell made of a phospholipid bilayer which limits the volume of a cell.
Centrate wastewater	Fluid that is removed from sludge in the wastewater treatment process.
Diatom	A type of green algae.
DW	Abbreviation: dry weight
Enzyme	Proteins that catalyze biochemical reactions with very high specificity.
Eutrophication	The loss of oxygen in water as a result of excessive growth of photosynthetic organisms caused by too high concentrations of nitrogen or phosphate.
Fatty acid (FA)	Carboxylic acid with a long chain of carbon atoms.

Gene	A DNA sequence that contains information coding for a protein.
Gene expression	The translation of a gene into protein.
Genome	The entire DNA in an organism.
Glycerolipid	*See Triglyceride.*
Lipid	Any molecule that is soluble in fat.
Metabolism	Biochemical reactions involved in storing fuel molecules and converting them into energy.
Microalgal biomass	All the material that make up the cells of microalgae.
Monounsaturated fat	A lipid with one carbon-carbon double bond.
Nitrogen starvation	Reduced rate of growth as a result of low nitrogen concentration.
Organelle	Functional components of cells. Including, but not limited to: chloroplast, mitochondria, nucleus, ribosomes, cell wall, cell membrane.
Photobioreactor (PBR)	A translucent container in which microalgae can be grown.
Photoperiod	The amount of light per day that plants need to live.
Photosynthesis	A set of reactions that use carbon dioxide, water and light energy to produce carbohydrates and oxygen.
Phytoplankton	Microscopic photosynthetic organisms including, but not limited to microalgae, cyanobacteria.

Protein	A biomolecule made up of amino acid units, with a defined 3D structure.
Recombinant DNA	DNA made in a laboratory combining DNA from different sources.
Recombinant protein	The product of expressing recombinant DNA.
Sedimentation	The separation of dispersed particles in a suspension due to differences in their density.
Settleability	How well cells sediment. Determined by their size, specific gravity, and viscosity of the liquid.
Sludge	High concentration suspension of solid particles in water.
Terrestrial	From the Earth/ground.
Thermolysis	Chemical or physical dissociation as a result of heating.
Thylakoid membrane (chloroplasts)	A functional component in plants and photosynthetic bacteria made of a phospholipid membrane. It collects light energy and converts it to chemical energy.
Transesterification	Chemical reaction of alcohol and an oil/fat to form an ester/glycerol.
Trigger (transcription factor)	A protein responsible for causing the expression of another gene.
Triglyceride	A molecule of glycerol linked through an ester bond to three fatty acids.
Zooplankton	Microscopic animals that feed on phytoplankton.

4 Selection of microalgae species

Microalgae are microscopic photosynthetic organisms that are found in almost every environment in nature. Microalgae use **photosynthesis** to convert solar energy into chemical energy. They store this energy in the form of oils, carbohydrates, and **proteins**. These constituents are used as human food, raw material for many chemicals and livestock. Besides, algae are the primary producers for almost all natural food chains. Likewise, algae are indispensable in most types of aquaculture**.** Nowadays, microalgae can also be used as a main source of bioenergy generation (**biodiesel**) and/or combined applications for biofuel production, CO_2 mitigation, and wastewater treatment. Microalgae can be thought of as miniature biochemical factories that are much more photo-synthetically efficient than **terrestrial** plants [1]. For example, half of the dry weight of **microalgal biomass** is made up of carbon derived from CO_2. Hence, microalgae can fix 183 tons of CO_2 into 100 tons of their biomass. Furthermore, microalgae can produce oil within 3 to 5 days, while a crop cycle may take from 3 months to 3 years to yield oil. Oil from microalgae can be harvested on a daily basis (just like milk), and microalgae produce 10 to 100 times more oil than any other known plants. Yearly, microalgae can yield as much oil as 90 000 L/ha, while soybean, canola and palm can only produce about 450, 1200 and 6000 L/ha, respectively [2, 3]. It has been calculated a cost per barrel of microalgae oil ($ 20) that is 5 times lower than the price of oil in the US market ($ 100) [4]. In addition, microalgae have a large surface area to volume-body ratio, making their uptake of large amounts of nutrients [5] such as nitrogen, phosphorus, iron, and sometimes silicon highly efficient [2]. Therefore, they contribute to recycling organic and inorganic waste materials, and produce oxygen while reducing CO_2.

There are some other advantageous features of microalgae that show their great potential, such as ceaseless production which avoids long periods of establishment of conventional plants and ease of supply of optimal nutritional conditions. Moreover it is not difficult to adjust the harvest rates of algal cultures to keep optimal cell density, and it is easy to control the cell composition for high **lipid** production.

The type of lipids that are accumulated by microalgae (saturated fatty acid, poly-unsaturated fatty acids, glycolipids or triacylglycerol) and the quantity of lipids produced will depend on the microalgae species and the growth conditions. Microalgae produce storage lipids in the form of TAGs (triacylglycerols) and 20 to 50 % of their dry weight is composed of oils that are suitable for biofuel production [2, 4].

There are four important classes of microalgae: the **diatoms** (Bacillariophyceae), the green algae (Chlorophyceae), the golden algae (Chrysophyceae), and the cyanobacteria (blue-green algae) (Cyanophyceae).

It is difficult to say which one is the best species in terms of lipid yield for biodiesel production, but diatoms are the class with the most potential and the second best are the green algae [6]. Some promising algae strains that can be used for the production of oils for biofuels are summarized in Table 1 [6].

Table 1: Varying oil content (% DW) in different algal species [6]

Algal species	Oil content (% DW)
Scenedesmus TR-84	45
Botryococcus braunii	29 – 75
Chlorella spp.	29
Chlorella protothecoides	15 – 55
Cyclotella DI-35	42
Nitzschia TR-114	28 – 50
Hantzschia DI-160	66
Stichococcus	33 (9 - 59)
Nannochloris	31 (6 - 63)
Nanochloropsis	46 (31 - 68)
Tetraselmis suecica	15 – 32
Phaeodaxtylum tricornutum	31
Dunaliella tertiolecta	36 – 42
Isochrysis spp	7 – 33
Thalassiosira pseudonana	21 – 31
Ankistrodesmus TR-87	28 - 40

The world is being faced with energy challenges such as energy depletion and increasing prices of petrochemical fuels. In addition, environmental challenges include dealing with pollution from domestic municipal wastewater and CO_2 emissions**.** Most wastewaters cannot be reused or released into sea because they contain very high concentrations of nitrogen (N), phosphorus (P) and toxic metals, and expensive chemical based treatments are necessary to remove them. The total concentration of N and P found in municipal wastewater and agricultural effluents are 10 - 100 mg/L, and over 1000 mg/L, respectively [7]. If these untreated wastewaters run out to rivers and lakes, they can accumulate and create **eutrophication**. There are some common treatment methods to remove P from commercial wastewater: use chemicals to form a solid insoluble fraction or convert the wastewater into **activated sludge** by microbial activity [8]. However, these methods do not fully recover and recycle phosphorus.

It has been demonstrated that algae-based treatment of wastewater was more efficient than chemical treatment [8]. The lower cost and technology of algal treatment compared to chemical-based treatment are significant advantages. Moreover, the microalgal method does not only reduce CO_2 concentration from the atmosphere and environmental pollution, but also recycles nutrients (N, P) from wastewater to produce biomass. Algal biomass can be processed to produce biofuels. For this reason, microalgae are greatly attractive as a solution for economic and environmental problems.

There are many factors that affect growth and development of each microalgae species such as abiotic factors (physical and chemical factors), biotic factors (another organism effects),

and different algal production system (open pond/raceway pond, closed bioreactors/tubular photo-bioreactor, hybrid systems combining both open pond and bioreactors).

The efficiency of algal growth in wastewater depends on variety abiotic factors such as availability of light, temperature and pH of the growth medium, and the concentration of N, P, organic carbon, oxygen and CO_2. For example, growth of microalgae in primary settled sewage water was shown to increase significantly under long **photoperiod** conditions and following the addition of CO_2, while increasing the temperature decreases algal biomass [9]. The major difference between wastewater media from other media is the high concentration of nutrients (N, P) [9]. Wastewater may affect algal growth because it contains ammonia, toxic cadmium; mercury and organic chemicals which are often present in wastewaters from industry. Biotic factors include pathogenic bacteria and predator **zooplankton**, which may impact negatively on the growth of microalgae. They can compete with microalgae for essential nutrients, and excrete chemicals that inhibit or kill microalgae.

Each microalgae species has different tolerance to wastewater conditions, but all of them have shown to be particularly tolerant and very efficient at accumulating nutrients from wastewater [10]. For example, *Chlorella vulgaris* is more effective than *Chlorella kessleri* at accumulating N, P from wastewater, while *Scenedesmus obliquus* grew better in municipal wastewater than *Chlorella vulgaris* [10]. Microalgae that are cultured in different systems have different lipid production. The highest concentrations of lipids are obtained from **photo-bioreactor** systems or from **batch cultures** grown in a laboratory, whereas the lowest lipid production has been observed in microalgae grown in open pond systems.

There are also differences in biomass and lipid productivities by microalgae as a result of the composition of wastewaters, as shown in Table 2. There are four types of wastewater: municipal sewage wastewater, agricultural manure-base wastewater, industrial wastewater, and artificial wastewater.

Table 2: Biomass and lipid production of different microalgae species in various wastewater conditions [7]

Wastewater type	Microalgae species	Biomass (DW) productivity (mg $L^{-1}day^{-1}$)	Lipid content (% DW)	Lipid productivity (mg $L^{-1}day^{-1}$)
Agricultural (fermented swine urine)	*Scenedesmus* sp.	6	0,9	0,54
Agricultural (digested daily manure, 20 x dilution)	*Chlorella* sp.	81,4	13,6	11
Municipal (centrate)	*Chlamydomonas reinhardtii* (biocoil-grown)	2000	25,25	505
Municipal (secondary treated)	*Scenedesmus abliquus*	26	31.4	8
Municipal (secondary treated)	*Botryococcus braunii*	345,6	17,85	62
Municipal (primary treated + CO_2)	Max *Chlorella sp., Micractinium, Actinastrum sp.*	270,7	9	24,4
Artificial wastewater	*Scenedesmus* sp.	126,54	12,8	16,2
Industrial (carpet mill, untreated)	*Botryococcus braunii*	34	13,20	4,5
Industrial (carpet mill, untreated)	*Chlorella saccharophila*	23	18,10	4,2
Industrial (carpet mill, untreated)	*Dunaliella tertiolecta*	28	15,2	4,3
Industrial (carpet mill, untreated)	*Pleurochrysis carterae*	33	12	4

Recently, there have been reports about some species that can efficiently combine biofuel production and domestic wastewater treatment. For example, six microalgal species (*Ourococcus multisporus, Nitzschia cf.pusilla, Chlamydomonas Mexicana, Scenedesmus obliquus, Chlorella vulgaris,* and *Micractinium reisseri*) were examined to determine their effectiveness in both biodiesel production and piggery wastewater treatment; the study suggested that the most promising candidate was *Chlamydomonas Mexicana* [11]. Besides, Chinnasamy et al. also experimented on a consortium of 15 native algae that were cultured in untreated carpet industry wastewater where mixed algal species removed over 97 % of the nutrients from a medium enriched with 6 % CO_2 in 72 hours [12]. Cyanobacteria *Phormidium sp* has a high tolerance to extreme temperature and it is effective at treating tertiary wastewater, but has lower biomass productivity than green microalgae species (*Chlamydomonas reinhardtii, Chlorella vulgaris, and Scenedesmus rubescens*). The microalgae also showed better **settleability** and nutrient removal rate (based on the N, P balance and their assimilation into algal biomass [13]. In a recent study of municipal wastewater, a batch culture of *Chlamydomonas reinhardtii* was assessed and it showed a strong wastewater treatment ability, especially **centrate wastewater**, and yielded a total lipid content of 16,6 % **DW**. When transferred to a **biocoil**, the microalgae were able to grow consistently in wastewater for 1 month. Furthermore, lipid content from the biocoil-grown microalgae reached 25,25 % DW, provided biomass productivity of 2000 mg $L^{-1}day^{-1}$, and an estimated lipid productivity of 505 mg $L^{-1}day^{-1}$ [7]. In addition, this lipid productivity could be coupled with efficient N and P removal [14]. Similar levels of total lipid content have been

observed in *Brotryococcus braunii* grown in secondary treated municipal wastewater (17,85% DW), and interestingly these values were higher than when the microalgae were grown in synthetic growth medium (10,96%) suggesting that the stress conditions in the wastewater may induce an increase in lipid synthesis [15]. The filamentous green algae *Rhizoclonium hieroglyphicu* grown in industrial wastewater had higher biomass productivity and three-fold higher lipid productivity with added CO_2 than without (17,9 mg $L^{-1}day^{-1}$, 10,7 mg $L^{-1}day^{-1}$ and 210,72 mg $L^{-1}day^{-1}$, respectively) [16].

Considering the aforementioned statements and the fact that wastewater production by human activities is almost only fresh wastewater, potential microalgae candidates for biofuel production are *Brotryococcus braunii* and *Chlamydomonas mexicana*, and *Chlamydomonas reinhardtii*, all of them satisfy important criteria such as high biomass production with efficient CO_2 uptake, best nutrient removal, and high lipid accumulation (Table 3).

Table 3: Potential microalgae candidates for biofuel production

Microalgal species	Biomass productivity	Lipid content (% DW)	Nitrogen removal	Phosphorus removal	mg CO_2 removal/g biomass/day	References
Brotryococus braunii	1.88 g/DW/L	36.14	79. 63 %	100 %	144.91	[17]
Chlamydomonas mexicana	0.56 ± 0.35 g/DW/L	33 ± 3	53 mg/L	71 mg/L	Nd	[11]
Chlamydomonas reinhardtii	over 6.06 ± 1.2 $g/m^2/d$	25.25	99 %	99 %	Nd	[13]

All **autotrophic** microalgae efficiently use CO_2 in photosynthesis to produce biomass. However, the microalgae need to have CO_2 in large quantities in order to have an optimal yield of biomass. A report from the firm CK Environmental showed that the microalgae cultivated in bioreactors could reduce CO_2 concentrations in the system by 82,3 % on sunny days, and by 50,1 % on overcast or rainy days [18]. Moreover, microalgae cultured in open raceway ponds system (with dimensions:100 m x 10 m) were 50 % efficient at assimilating CO_2 [19]. Another report about the role of microalgae in reducing CO_2 showed about 60 – 80 % efficiency in using CO_2 from power plants; net greenhouse gas avoidance potential would range from 22,3 – 29,7 % if using microalgae to capture CO_2 emissions [20].

In summary, microalgae can produce biofuels, remove nutrients from wastewater, and reduce CO_2 emissions. This results in advantages such as production of sustainable biofuel, prevention of global warming, and reduction of the operational cost of microalgae production (by utilizing nutrients of wastewater). Microalgae of the genus *Chlamydomonas* would be most suitable at these three processes. Moreover, this genus has a **glycerolipid metabolism** that is less complex, so it would be feasible to manipulate its **genome** to increase lipid production.

5 Genetic modification of microalgae for enhanced oil production

As already stated, microalgae have plenty of advantages when it comes to biomass production to be converted into biofuels. Some high efficiency conversion methods have been described; however the highest efficiency from microalgae biomass conversion is expected to be the result of the genetic modification of microalgae [21]. Microalgae are naturally efficient producers of oils from fatty acids; however, microalgae produce the highest amounts of fatty acids under conditions of low nitrogen concentration [22]. They accomplish **fatty acid** synthesis by many biochemical reactions that are catalyzed by **enzymes**. The information about which enzymes to produce is stored in **genes** in the cell's DNA (FA and **Trigger** in Figure 1). The cell has a system for choosing which enzymes to produce according to its needs. Under normal conditions, the genes that contain information about the enzymes that synthesize fatty acids (FA) are "switched off" (see Figure 1A). Microalgae also have an enzyme that senses nitrogen concentration (Trigger), and when nitrogen levels are low this enzyme switches on the production of enzymes that synthesize fatty acids (see Figure 1B). Using this knowledge one would think that microalgae grown in a medium with low nitrogen would produce high amounts of fatty acids. However, nitrogen is also crucial for many other vital functions in the cell and therefore the cells wouldn't be able to grow optimally in low nitrogen conditions. Luckily genetic engineering can harbor the solution to this obstacle. By inserting **recombinant DNA** in the cell, it is possible to modify Trigger in order to force it to continuously switch on FA independently of the nitrogen levels. Then enzymes for the synthesis of fatty acids would be constantly produced from FA and the yield of fatty acids would increase – without the need to grow the cells in a medium with low nitrogen [23].

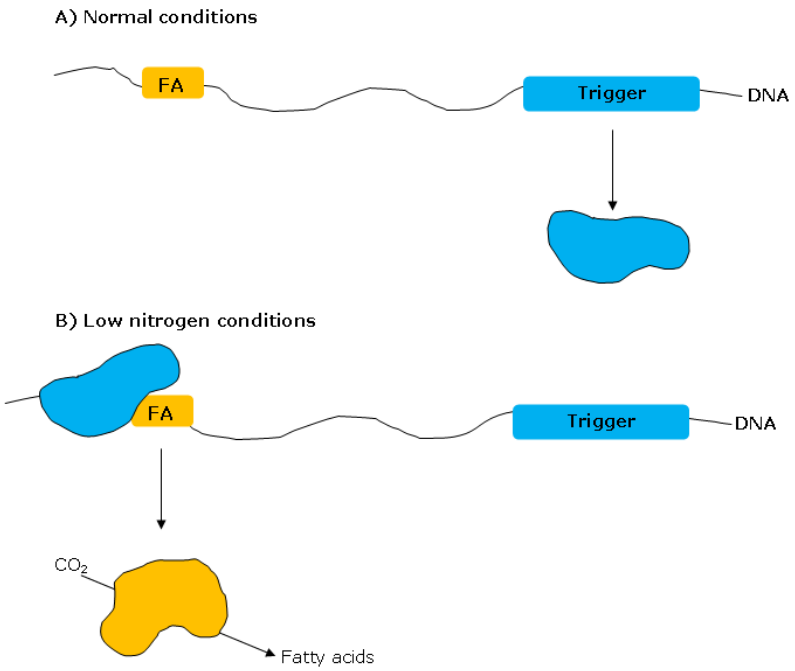

Figure 1: At normal conditions A) there are high nitrogen concentrations, the Trigger enzyme is being produced from the Trigger gene continuously, but Trigger doesn't bind to FA and thus the enzymes required for the synthesis of fatty acids are not produced. At low nitrogen conditions B), Trigger binds to FA and enzymes required for the synthesis of fatty acids are thus produced.

The next step is inserting recombinant DNA into microscopic cells. There are currently many techniques available for doing this, but the one with the highest efficiency is called particle bombardment [24]. It consists on coating metallic microparticles with recombinant DNA molecules that in this case contain a modified version of Trigger [25]. The particles are shot at high speeds towards the cells. Most of the microalgae are destroyed when hit by microparticles, but a few of them survive the process, internalize the recombinant DNA molecules, incorporate them as part of their own DNA, and start to produce the modified version of Trigger [25]. At this point the cells are said to be genetically modified and, if everything has gone well, microalgae should now be producing 50% more fatty acids [23].

When choosing an algal species, *Chlamydomonas* is particularly well suited for genetic modification. Its genome sequence and physiology are known, which means that the result of a modification can be predicted, and it grows quickly, meaning that the results can be observed in a matter of hours [23].

Currently the **expression** of **recombinant proteins** in microalgae is low and inconsistent. Reasons for this can be that the recombinant DNA is inserted randomly in the cell's DNA, therefore it could be inserted in a region of the cell's DNA that has low expression (silencing) or that the cell down-regulates the expression of the gene (regulatory elements). In order to correct this, special DNA sequences can be added to the recombinant DNA so that the expression of the inserted genes is increased. Also, recombinant DNA can be made so that the genes code for fusion proteins – artificial, recombinant proteins that contain parts from different proteins – that are more efficient at synthesizing oils than the native ones [23].

Although optimizing the parameters required for high expression of recombinant proteins is difficult and time consuming, advances in new techniques and methods for genetic engineering will result in more efficient transformations that can be used to increase the yield of microalgal oils for industrial production of biofuels [24].

6 Reactor construction and conditions

Since micro-algae are a single cell oriented microscopic photosynthetic organism the growth of them depend on many factors, with sunlight being their main energy source and most important factor. The rest are: simple inorganic nutrients, predominantly carbon dioxide, soluble nitrogen and phosphates.

To obtain significant, cost-effective growth of microalgae, an extensive effort needs to be dedicated for the optimization of medium and controlled gardening process for algae. Currently, most industrial micro algal gardening systems are open ponds. These systems are preferred for their low capital and operational costs [26]. However, one of their major drawbacks is the lack of control over operational conditions, and therefore they can only sustain low biomass production efficiency. Beside this, algal growth is also limited to the surface of ponds, resulting in low volumetric productivity and low overall biomass concentration.

In comparison to open pond, **photobioreactor** (PBR) can support much higher photosynthetic efficiency, biomass productivity and biomass concentration [26].

6.1 Photobioreactors

A photobioreactor (PBR) is a transparent medium which provides an artificial environment for the growth of phototropic microorganisms, such as microalgae. PBR also can be defined as a bioreactor which incorporates some type of light source. Virtually any translucent container could be called a photobioreactor; however the term is most commonly used to define a closed system, as opposed to an open tank or pond. Because these systems are closed, all essential nutrients must be introduced into the system to allow algae to grow and be cultivated. A pond covered with a greenhouse could also be considered as a photobioreactor. A PBR is designed to provide optimal illumination, mixing, CO_2 mass transfer and nutrients to the phototrophic liquid suspension [27]. In a PBR, the CO_2 efficiency is much higher; up to 75 % has been reported. It is also in a much more controlled environment, so there's a smaller chance of contamination, which allows for more specialized and genetically modified microalgae. However, the cost of pumping the culture around and the capital cost are very high. The pump energy and depreciation cost are the major cost factors (20 % and 60 % respectively) [28]. Some of the pumping cost can be offset by having the installation at sea, where waves can provide the motion needed for mixing [29]. Biomass concentrations of 2 to 5 g/L have been obtained. It is difficult to scale a single PBR unit beyond approximately 100 m² due to gas exchange limitations [6]. Figure 2 shows examples of some photobioreactor designs.

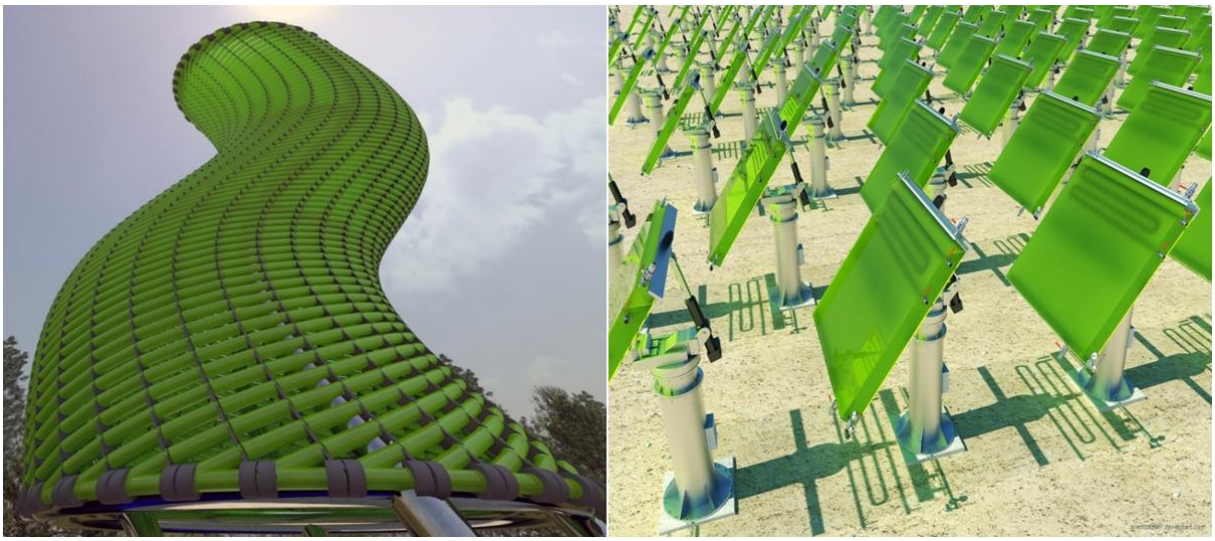

Figure 2: Example of photobioreactor designs [30] [31]

6.2 Light capturing, distribution, and utilization

Light intensity is an important role in microalgae photosynthesis. When light intensity goes below a desired critical level, light saturation and photo-inhibition may occur. Light inhibition should be avoided as much as possible. The light spectrum is also an important factor when considering the design of a PBR. Sunlight covers a wide range of spectra but only the light

within a certain range of spectra is photosynthetically active. Therefore there is a natural barrier for enhancing the photosynthetic efficiency, and the actual photosynthetic efficiency is lower because of loss as a result of light reflection and cellular respiration [32]. Another important aspect is the rhythm of light/dark cycle, which has a remarkable effect on the efficiency of solar energy capturing. In the absence of light energy, microalgae need to conduct respiration during the night time, which should be minimized as much as possible [33]. Design considerations that also affect light capture are the transparency of materials and surface/volume ratio.

6.3 CO_2/O_2 balance and gas exchange

During photosynthesis, microalgae utilize light energy to fix CO_2 and to release O_2. At high O_2, they can also utilize O_2 for photo respiration, which consumes O_2 to produce CO_2. CO_2 is the carbon source for microalgae in **auto-phototrophic** cultures and could be the limiting factor if the CO_2 concentration is low in the feed gas. To maintain an optimal balance between CO_2 and O_2, a considerable space for gas exchange is usually included in PBR.

6.4 Temperature effect on yield and fatty acid concentration

Cost-efficiency and a reliable temperature control mechanism is a significant challenge in PBR design. Without temperature control, the inside temperature of a PBR can reach higher than ambient temperature. Therefore additional cooling mechanisms are added, for example: submerging the entire culture in a water pool, spraying with water, shading or employing a heat exchanger [34].

The growth temperature is an important parameter to optimize the lipid composition in microalgae. The lipid content is the highest at growth temperatures of approximately 35ºC [35].

6.5 Mixing

Mixing in microalgae cultivation is an important feature during design consideration. A culture with high cell-density may significantly reduce the transmission of light, increase the rate of CO_2 consumption and O_2 accumulation, as well as quickly increase the inside temperature in the PBR. Mixing in microalgae cultivation is required for preventing **sedimentation** of algae, facilitating heat transfer to avoid temperature gradients, ensuring uniform exposure for all cells in PBR, and improving gas exchange between the gardening medium and the air phase [36].

6.6 Sterility (species control) and cleanability

A certain level of impurity is well accepted in microalgae cultivation. Nevertheless, care should be taken to avoid excessive contamination. For autotrophic microalgal gardening facilities, contamination of heterotrophic microalgae is usually not very important due to the lack of organic carbon sources in the system.

Cleanability is very important in PBR design consideration to prevent the formation of biofilm on inside wall of the PBR and to minimize the chances of contamination. To increase cleanability, the inside surface of a PBR should be smooth and the internal dimensions of the PBR should be large enough to reduce the number of internal bends [36].

6.7 Open pond growth systems

Raceway track systems are a 25 - 40 cm deep closed loop oval channel which is open to the air. A paddle wheel is used for water circulation and to prevent sedimentation, as shown in Figure 3. For raceway tracks there are several challenges when it comes to microalgae cultivation. Because it is open to air, there will be some contamination, and the microalgae need to be able to survive it. This lowers the energy dedicated to producing oil. Microalgae need CO_2 to grow, however raceway tracks aren't very effective at utilizing it. Only 10 – 35 % of the CO_2 used is fixated by the algae, and for a raceway track the CO_2 cost is the biggest part of the budget, at around 50 % of the total cost. Thus, acquiring cheap or free CO_2, possibly flue gas from industry, might be necessary to make it cost effective [28].

With shallower raceway tracks at 15 – 20 cm depth, it is possible to get biomass concentrations of up to 1 gram per liter and productivities of 10 – 25 g per square meter per day [37], however 0,3 g dry weight per liter is more common [38]. However, the relatively low biomass concentration increases the cost of harvesting and concentrating the biomass before drying it [38].

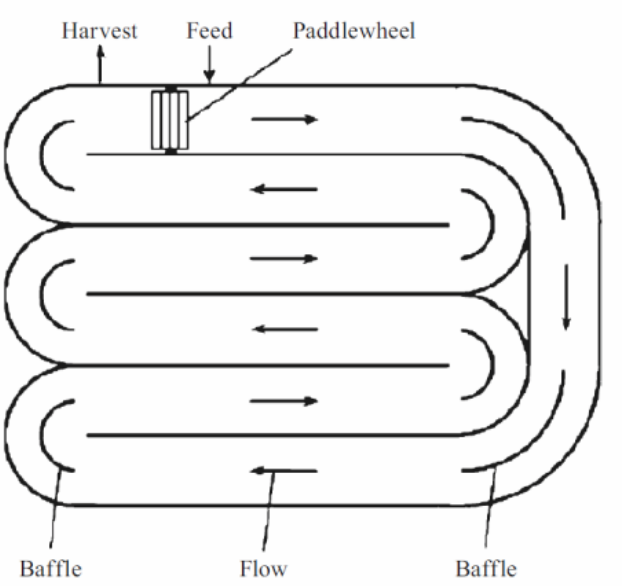

Figure 3: Top down view of raceway track [6]

6.8 Vertical column photobioreactors

Vertical column photobioreactors are usually transparent rigid cylinders with radii up to 0,2 m and heights up to 0,4 m. Vertical column PBR are distinguished by their volumetric gas transfer coefficients. The bubbling of gas from the bottom of the reactor makes for efficient CO_2 utilization and optimum O_2 removal, and the constant agitation of medium caused by this bubbling mixes the culture uniformly. Thus the amount of cell damage inside the vertical column PBR is low [39].

There are different types of vertical column PBRs as shown in Figure 4. Figure 4A is a bubble column PBR which consists of a column and a sparger situated at the bottom. Gas/liquid separation happens in the freeboard region at the top of the column. Mixing is gained by the turbulence of air bubbles. Figure 4B is an internal loop airlift PBR, which is comprised of an internal column inside the column and an air sparger. Gas separation occurs at freeboard regime at the top of the internal column and mixing is caused inside the internal column [40]. Figure 4C shows a typical split column airlift PBR in which a flat plate splits the diameter of the column and separate it into two parts, riser and downcomer region. To take the liquid upwards, air is generated at the bottom of riser. Gas separation occurs at the top of column and heavy separated gas falls downwards. The remaining Figure 4D shows a possible external loop airlift PBR in which degassing happens in the gas separation region at the top of the column and mixing is gained through an external circulation column.

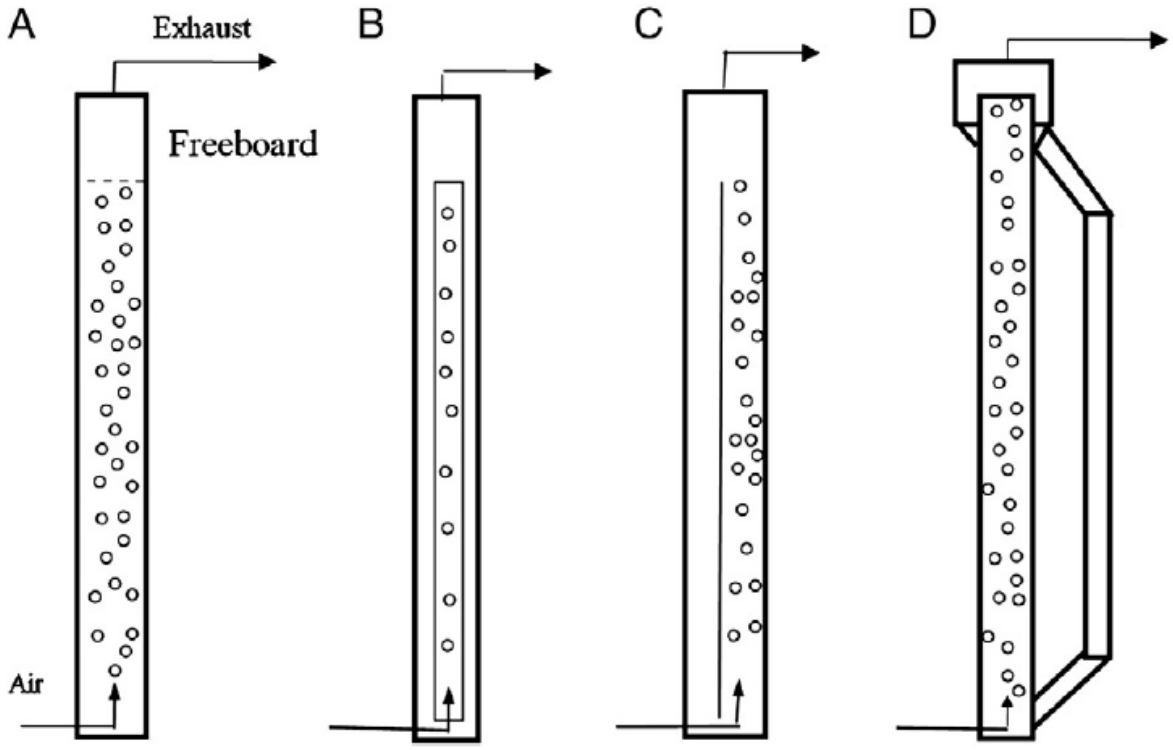

Figure 4: Schematic of A. Bubble column PBR B. Internal loop airlift PBR C. Split column PBR D. External loop airlift PBR [36]

6.9 Flat panel photobioreactors FP-PBR

A flat panel photobioreactor (seen in Figure 2) is a closed PBR with a narrow light path, and is characterized by a large illuminated surface to volume ratio. To obtain maximum opening to sunlight, it can be oriented into the direct path of light. These PBRs are categorized into two different types according to the way mixing is done: pump-driven and airlift flat panel PBR. Typical FP-PBRs suffer from deficiencies in flow control in culture, but temperature control and light inhibition are also significant challenges when designing FB-PB. During design, the plate thickness is very important since it determines surface area/volume ratio and also light path length. Smaller thickness allows for better distribution of light and diffusion. In general, the smaller light path and smaller thickness, the larger optimal cell density and biomass productivity [41]. Figure 5 below shows the schematic of a FP-PBR.

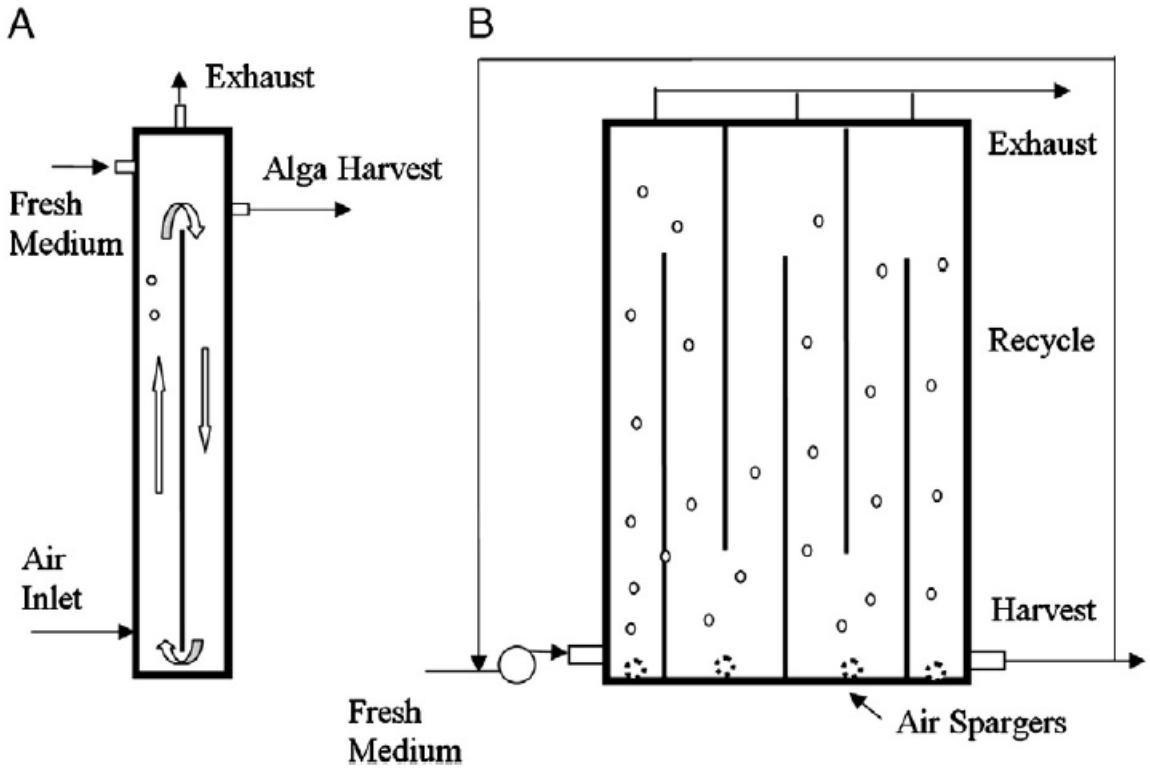

Figure 5: Schematic of A. air-driven FP-PBR B. pump-driven FB-PBR [36]

6.10 Harvesting the microalgae

Due to the low concentrations of biomass in growth cultures, harvesting the biomass is difficult. Some methods used are flocculation, filtration and ultrafiltration, centrifugation, air-flotation and auto-flotation [37]. The cost of harvesting microalgae may contribute 20 to 30 % of the total cost of the production. This is due to the small size, 3 to 30 µm, and the dilution of the culture broth which can be below 0,1 wt% [6].

Flocculation is a method to increase the particle size of microalgae. It reduces the surface charge of the molecules, often with aluminium or iron cations, which allows them to clump together. This allows for flotation or sedimentation to happen quicker. However, the use of

chemicals to induce flocculation can be a problem for downstream processing of the microalgae [42]. When the pH in a microalgal culture increases above 9, flocculation can occur spontaneously. This is called auto-flocculation [43].

Centrifugation uses a centrifuge to separate microalgae from the growth medium based on density differences. It is suitable for all types of microalgae, but the high investment cost and operation cost is prohibitive for large scale production.

Sedimentation of algae uses gravity only to separate microalgae due to density differences. It is slow compared to other methods (0.1 - 2.6 cm h^{-1}), which can cause deterioration of the biomass.

Dissolved air flotation (DAF) is using air bubbles to float particles to the surface of the liquid, where they can be skimmed off. It is a proven large scale method, and is often used for sludge removal in wastewater treatment [42].

7 Physical methods to extract oil from microalgae

There are advantages in producing oil from algae, it gives higher growth rates than what we can get from conventional crops, it can produce approximately 20 times oil per unit area more than palm oil, and there is no need to use land for food crop [44]. It is important to choose a good algal species to determine the lipid production rate, and an efficient method of lipid extraction, both which are essential in commercial fuel production. In the process of disrupting the algal cell wall, effective methods are crucial to obtain higher lipid extraction which results in greater net energy output from the process.

Direct counting and colony diameter method are used to define the effectiveness of cell disruption [45]. Bead beating is a method that produces a high yield compared to others treatment [46].

7.1 Microwave

A suspension of algal cells in water is heated by using microwaves. During this process, the cells are heated more easily than the surrounding water. This leads to the disruption of the cell walls [47]. The method can be thought of as an inverse **thermolysis**; where the breakage occurs from within the cells. This method is quicker than hot-water bath.

7.2 Hot water bath

This treatment is a relatively efficient method that causes cell disruption by thermolysis [48]. The water surrounding the algae is heated, a process that needs time to stabilize. Hot water causes the cell walls to break. The products from this method are often large debris, a characteristic that simplifies the separation of debris from lipid products [47].

7.3 Ultrasonic

Ultrasonic waves disrupt cell walls by **cavitation** bubbles – a type of mechanical disruption. Water inside the bubbles boils due to their low pressure, causing them to explode violently, and the shockwaves from the explosion is what causes cell disruption [47] [49]. Ultrasonication is a commonly used cell disruption technique to extract oil from microalgae [50].

7.4 Laser treatment

There are two proposed ways by which laser treatment disrupts the cell walls of microalgae: thermolysis and cavitation. However, studies have shown that thermolysis plays a larger role in cell wall disruption than cavitation. [47] [51].

7.5 Blender

This method has both high disruption efficiency and low energy consumption. Efficient disruption is achieved by maintaining a constant number of intact cells in the blender. Cells are disrupted by mechanical shear caused by the blades of the blender [47].

7.6 Comparison of methods

In addition to Table 4, a graph comparing the efficiencies of all presented methods as a function of time is shown in Figure 6.

The ultrasonic method is the lowest energy consuming method, but also the slowest and the least efficient one. Both the microwave and the hot water bath methods use thermolysis as a disruption mechanism. Although microwave results in a 7 % higher disruption and is faster, it is also consumes more than three times more energy than the hot water bath method. The blender method is a simple mechanical technique that results in high efficiency with low energy consumption. The laser treatment is slightly more efficient than the microwave method, and it is the fastest of all techniques. However, laser is highly energy consuming and limited to small sample volumes.

In conclusion, using the blender method seems to be the best method at giving a high percentage of cell disruption in a short amount of time and at low energy consumption. Indeed mechanical methods are the most commonly used ones in large scale processes. [47].

Table 4: Cell disruption effectiveness and energy consumption used for different treatment methods [47]

Treatment method	Disruption (%)	Energy (J/1000 mL)
Laser	96.5	16000
Microwave	94.9	74565
Blender	93.0	540
Water bath	87.7	20160
Ultrasonic	67.7	132

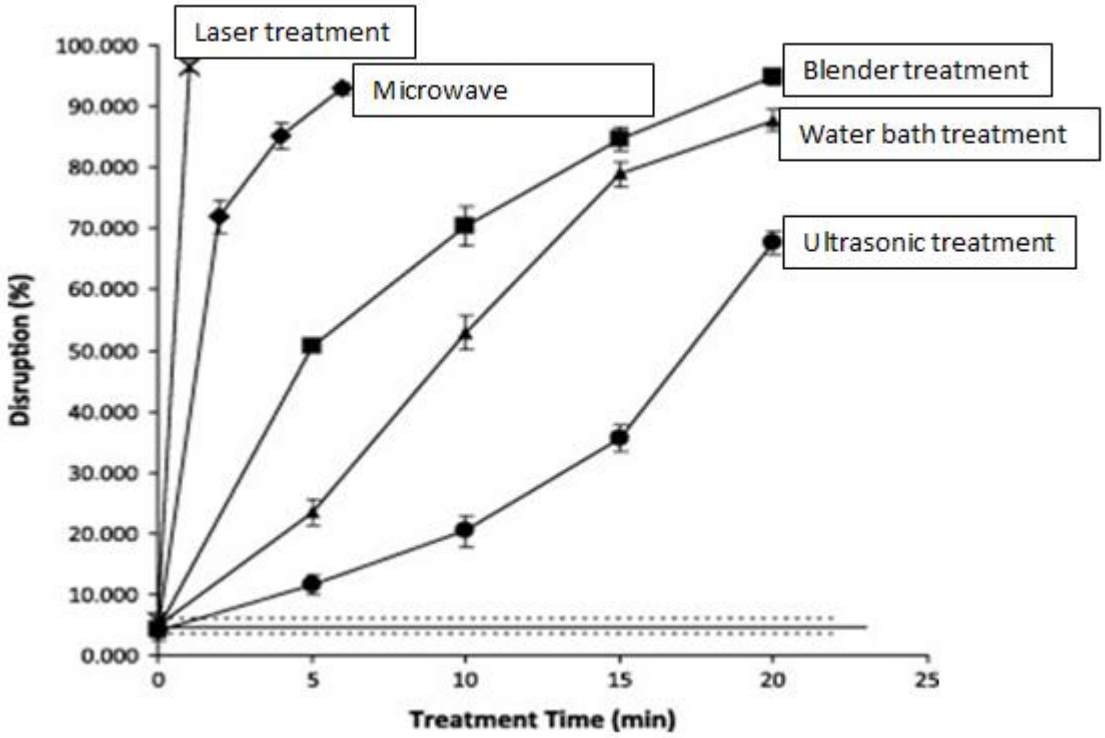

Figure 6: Effectiveness of extraction methods [47]

This graph shows the effectiveness of each method in percentage versus time in terms of cell disruption treatment. Laser treatment gives high yield (96,53%, 1 min) while ultrasonic spends more time and gets lower yield (67,66%, 20 min). The other methods such as microwave (94,92%, 20 min), blender (92,92%, 20 min) and water bath (87,72%, 20 min) are in between.

8 Biodiesel from algae oil

Biodiesel is defined as monoalkyl esters of vegetable oils or animal fats. Vegetable oils and fats are too viscous to be considered as alternative engine fuels, with a viscosity of 10 to 17 times greater than petroleum diesel fuel. The chemical conversion of high viscose oil to the corresponding fatty ester (biodiesel) is called **transesterification**. Biodiesel is the product of transesterifying the parent oil or fat; it has a viscosity close to that of petroleum diesel. Lowering the viscosity of the oil may be done with or without help of a catalyst and by using primary or secondary monohydric aliphatic alcohols, which contain one to four carbons.

8.1 Transesterification

Alcohols that can be used to produce biodiesel are methanol, ethanol, propanol and butanol. If using methanol as a reactant, the product will be a fatty acid methyl ester mixture (FAME), whereas a fatty acid ethyl esters mixture (FAEE) is obtained with ethanol. However, methanol is more favorable due to its low cost and also industrial availability [52]. Transesterification is limited by low temperature and the outcome is highly dependent on the amount of

triglyceride. Therefore, in order to have a good economic performance, the triglyceride content in microalgae must be very high [6].

In the process of transesterification each mole of triglyceride needs 3 moles of alcohol to produce 3 moles of methyl esters and 1 mole of glycerol, as shown in Figure 7.

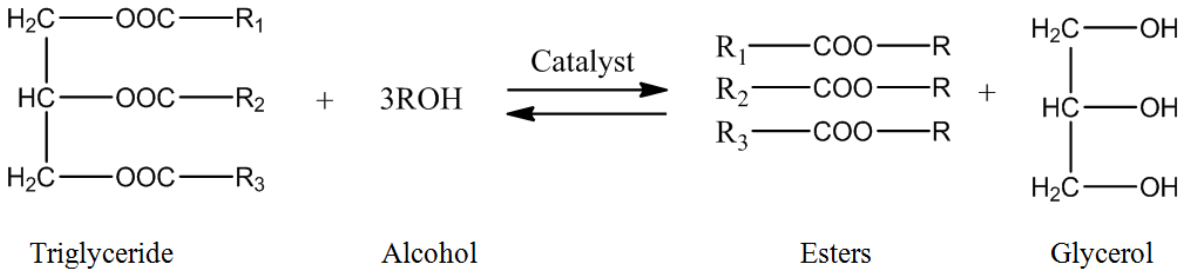

Figure 7: Transesterification reaction

This reaction is an equilibrium reaction. At the industrial level, 6 or more moles of methanol are used in order to make sure that the reaction is driven in the direction of methyl esters, i.e. towards a higher yield of biodiesel. The yield of methyl esters is about 98 % on a weight basis.

Transesterification is catalyzed by alkalis [53], acids or lipase enzymes [54]. Alkali-catalyzed transesterification is approximately 4000 times faster than an acid catalyzed reaction. Because of this, alkalis such as sodium and potassium hydroxide are better to use as a commercial catalyst. The catalyst concentration is about 1 % of the oil weight. Another catalyst which works even better than sodium hydroxide are alkoxides such as sodium methoxide. Alkali and acid catalysis involve the removal of water from the input reagents, purification of fatty acids, high energy consumption, and the production of waste. An alternative is to carry out biochemical transesterification. This process uses enzymes called lipases, which produce the same esters without the complications involved in the chemical catalysis processes. The biochemical reaction with lipases functions optimally in a temperature range of 20 - 60 ºC, drastically reducing the energy input required for the reaction [21]. However, it is not feasible to use lipases at the industrial level due to their high cost [2]. Alkali-catalyzed transesterification, which takes around 90 minutes, takes place at about 60 ºC under atmospheric pressure, due to the boiling temperature of methanol which is about 65 ºC. It is also possible to run the reaction at a higher temperature, but since higher than atmospheric pressure is also needed; it is too expensive for large scale processes. Oil and methanol are immiscible; hence the reaction mixture is made up of two liquid phases. To avoid yield loss due to saponification reactions (i.e. soap formation), the oil and the alcohol should be dry. The amount of unreacted free fatty acids left in the oil mixture should also be kept to a minimum. Recovering the biodiesel is done by repeatedly washing it with water to get rid of glycerol and methanol [2].

8.2 Process analysis

A process flow sheet is shown in Figure 8. A mixture of algal-extracted triglycerides is mixed with excess methanol and catalyst pellets (7:1 methanol:triglycerides). Then the mixture is heated and sent to a **CSTR**, in order to convert them to the FAME product and glycerol byproduct. The catalyst existing in effluent is removed by filtration and the remains are sent to a decanter. Separation of the FAME (light phase) from glycerol (heavy phase) takes place in decanter. Gravity plays the main role on the separation. Methanol is distributed between the two phases.

In order to further conversion, the light phase is sent to the second CSTR. The effluent is subjected to the same separation techniques, and afterward is sent to a distillation column where the FAME (biodiesel) is separated from methanol. The glycerol phases obtained from the decanters are combined and sent to a distillation column, in order to recover nearly-pure glycerol and methanol. The latter is reused in the next cycle of the process [53].

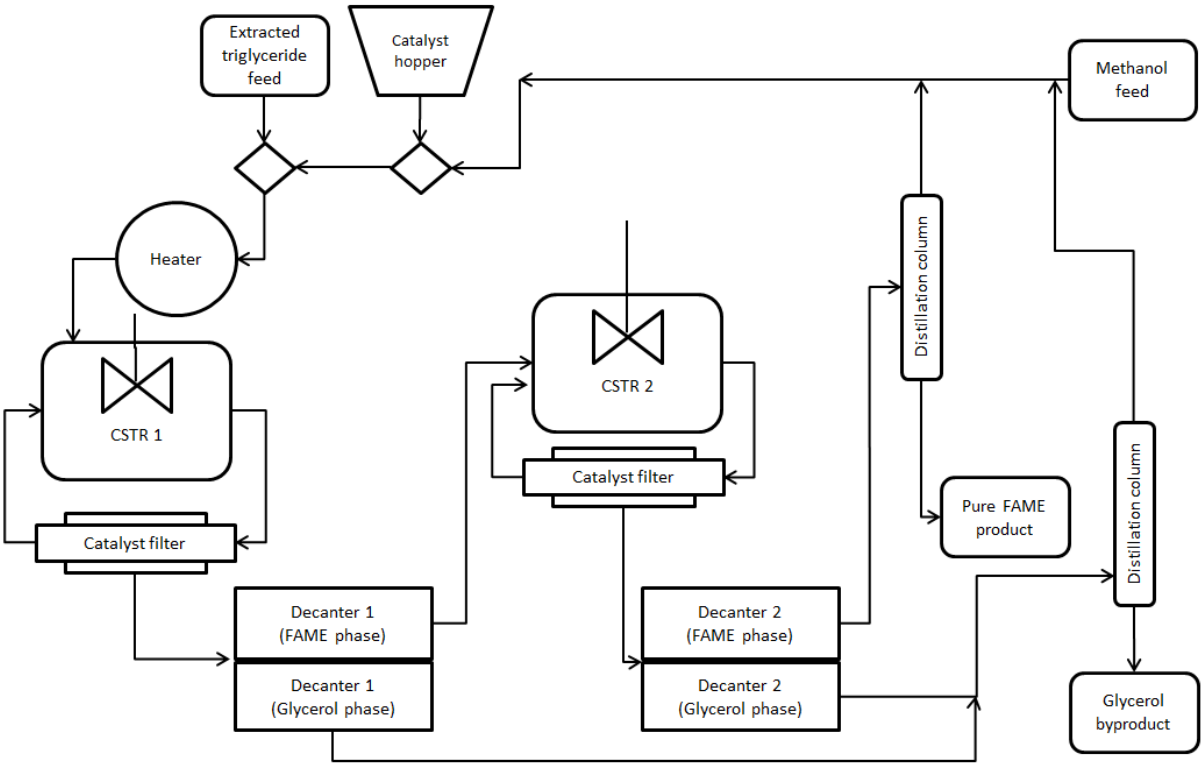

Figure 8: Transesterification process overview

9 Conclusion

The goal of this project was to write a report that described the biofuel production process from microalgae in a way that any individual with a technical (science or engineering) background would be able to understand. This has been achieved by writing in an interdisciplinary way (all of the authors have a different academic background), adding a glossary with technical and specific concepts, and writing in a descriptive way. We never strove to design a business model or convince investors to use the technology we describe. This is because the technology necessary for building the infrastructure for a biofuel producing microalgae-based plant is still under development. For example, the design of PBRs can vary greatly and while there are several companies suggesting that they have the best design, none have been actually implemented in large scale. In addition the research on genetic modification of microalgae is still incipient; the scientific community does not hold enough knowledge about the behavior of the whole genome of microalgae and therefore the overall impact of modifications in the cell's DNA cannot be determined with high accuracy. However, the ability of microalgae cultures to fixate CO_2 through flue gas utilization, and treat wastewaters by using them as nutrients for cultures, gives them the possibility to offer sustainable solutions to environmental challenges. Even though the cost-effectiveness of producing biofuel from microalgae is yet to be optimized, we believe that the technology has the potential to solve some of the energy challenges that the world is currently being faced with.

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