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Microalgal Biofuel

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1. Introduction

Transportation is base of economies and all other of developments of any countries, it fulfills the requirement of society. On the other hand transportation is nothing without energy/ petroleum. Petroleum, based fuel account for 97% of transportation energy. Without petroleum shipping of many important good like food and other, driving from one point to other can't be possible. This petroleum is finite and it will be finished in few years and there are serious environmental concerns fuel like hazardous emission causing global warming and air problem also. These problems can be solved if such a sensible fuel will take place on existing fuel and reduces petroleum consumption and thereby reduce emission of hazardous from fuel.

Biofuels are such a sensible fuels and it is generated from biological material. According to use of biomass as a feedstock for biofuels generation, it is classified in three generation. First-generation biofuels are produced from biomass that is edible (sugarcane and corn). It is economical viable but it is responsible for increase in food prices in poor countries. Second-generation biofuels are produced from non-food crops or it is produced from a generally less expensive biomass such as animal, forest, agriculture or municipal wastes.Third-generation biofuels are produced from extracting oil of algae. Algae have been found to have incredible production level compared to other oil seed crops like sunflower, soybean rapeseed. Table 1 below shows a comparison of oil yield for various oilseed crops.

As table 1 shows that algae is capable of producing biodiesel on a large scale compared to other oilseed crops on less area.

Plant	Gallons of Oil/Acre	lb. Oil per Acre
Algae	700	6757
Coconut	285	2070
Jatropha	201	1460
Rapeseed	126	915
Peanut	112	815
Sunflower	99	720
Soybean	62	450

Table 1. Average Production for various Oil Crops

2. World's scenario

The concept of using algae as feedstock's for biofuels was already being discussed 50 years ago but a concerted effort began with the oil crisis in the 1970s. In this series Japan and United State focused on research programs. Main focus of The United State Department of Energy's was production of biodiesel from microalgae (1978-1996), which is known as the Aquatic Species Program (ASP). Japan Government also financed some large research project, but none of them has proven economical on a large scale, due to mainly the production methods used to grow and harvest the algae.

Notwithstanding the technical challenges, the availability of suitable land, in terms of soil type, elevation and slope, in suitable climates (incident radiation, temperature, precipitation/ evaporation balances and severe weather), and the geographical nearness of this land to appropriate water and CO_2 inputs and possibly nearness to markets or transportation infra-structure may impose physical and economic limits to the contribution that algal biofuel can make to the world's future transportation fuel needs. For example, very few large CO_2 emissions sources are in close proximity to regions identified as being most suitable for year round, large scale open pond production systems. In fact, there is an absence of data that could be used in defining limits of production. Land use, land suitability and resource spatial mapping data compiled for the purpose of assessing the geographic potential of algal biofuels does not exist. Claims that algal biofuels could completely replace all petroleum derived transport fuels or even provide a significant contribution to liquid fuels on simple assessment seem improbable, but can be neither supported nor refuted. There is a need to develop this information.

There are as yet no pilot (>100 mt algal biomass/yr) photosynthetic algal biofuels production plants operating in the U.S. The few pre-pilot-scale (e.g. >10 mt) plants have operated for less than a year, with only rather smaller operations of a few hundred square meters operating for two or more years (e.g. *Seambiotic in Israel*, *Aurora Biofuels* in *Florida*, for example). As mentioned above, *Solazyme* is the front runner with the largest confirmed production of algal lipids for

energy customers to date, using a closed heterotrophic process and genetically modified algae. Three fairly advanced developers who are or will be breaking ground on the next scale demonstrations (20-200 acres) within the next year are *Phycal, Cellana, Sapphire*, and *General Atomics*. All use open pond designs and natural strains. The main interest in microalgae stems from its potential productivity on a per acre-year basis. Claims of current and future relative productivity levels range from 1000 to 5000-plus gallons per acre per year and are summarized in Table 10.

Actual productivity numbers, like other agricultural crops and industrial processes, are highly dependent on the specific site and production process used. At least one company has demonstrated actual productivity in its proprietary process of at least 1400 gal/acre/ year in 2010 for a non-optimized small experimental site in a warm-weather location and estimates productivity could be doubled in the next demonstration at the multi-acre scale. These demonstrated results and model for the next phase were validated by an independent federal agency and review team through the U.S. Pacific Command's Green initiative for Fuels Transition (GIFTPAC) interagency working group, under the leadership of the U.S. Pacific Command Energy Office, J81 Joint Innovation and Experimentation Division, Resources and Assessment Directorate. It is important to remember that these productivity numbers are only for the oil; algae organisms range from 10% of their body mass in oil and up, so for each gallon of fuel produced, a significant proportion of protein and carbohydrates are produced as well. *Cellana Co*. in *Hawaii* (a joint venture of *Shell Oil Co*. and *H.R. Biopetroleum, Inc*.) has operated a pre-pilot plant of between one and two acres to grow diatoms using the *Mera Pharmaceuticals* ponds at the *Natural Energy Laboratory of Hawaii Authority* (NELHA) near *Kona, Hawaii*. The technology was based on prior experience with production of Haematococcus pluvialis biomass by *Aquasearch Co*. in *Hawaii*. Its neighbor at *NELHA, Cyanotech*, is one of the traditional nutraceutical companies mentioned above; *Cyanotech* sold $7 million worth of algae-derived astaxanthin in 2009.

Sapphire Energy Co. of *San Diego* was awarded over $100 million in U.S. government grants and loans and is breaking ground on a 300-acre demonstration pilot plant in New Mexico. Sapphire Energy initially announced that it would produce algae oil with oil-excreting genetically modified algae (GMA), but now intends to follow the standard model of growing unmodified algae with naturally high oil content. *Phycal* of Ohio was awarded over $50 million in Department of Energy carbon recycling funds to develop a pilot plant on *Oahu, Hawaii*. *General Atomics*, in *San Diego*, received about $30 million from the U.S. *Department of Defense, Defense Advanced Research Projects Agency*, (*DARPA*) in 2008 to develop a low-cost ($3/gallon initially, $1/gallon later) process for microalgae oil production in an 18-month R&D effort to be followed by a demonstration of this technology over a further 18 months in Hawaii, *Texas*, and *California*. The economic analysis and underlying assumptions on which current projections of $3/gallon oil are based are proprietary-however they include significant animal feed co-product credits.

The contribution of algal biofuels to future liquid transportation fuel supply is assessed against the *US Energy Information Agency* growth projections. By 2030, oil consumption is expected to increase to ca. 6.2 TL yr^{-1}(106 million bbl d^{-1}) with66 % of this growth likely to occur in non-OECD countries in *Asia*. Transportation fuel use is expected to grow slightly to ca. 56% of total oil production. Over the same time period, biofuels will maintain a relatively steady share of unconventional liquid fuel production and grow to between 277 GL/yr $^{&}$ 416 GL/yr (4.8 to 7.2 million Bbl/d, or 8.0% to 12.0% of the liquid transportation fuel supply). The EIA uses a figure of ca. 340 GL/yr as a reference case for total biofuel production in 2030.

A 5% contribution of algal biofuels to total biofuels supply by 2030 would require the construction of 170 100 ML facilities. When the technical uncertainty is considered it seems unlikely that the first large scale plant would be commissioned before the middle of the coming decade, and even this would be ambitious. Approaches that rely on molecular biology to achieve breakthroughs, e.g., the partnership between *Synthetic Genomics Inc*. and *ExxonMobil Corp*., are promising but will likely take more than a decade to reach commercial viability. Assuming success in the first commercial venture and accelerated rates of adoption beyond 2015-2020, 170 100 ML facilities could conceivably be operational by 2030 as this rate of construction is lower than the recent development rate of ethanol plants in the *US* and *Brazil*. The forty-plus companies tackling the concept of algae production on a large scale for energy use have begun to differentiate into market niches, generally according to their founding technical expertise and physical location.

Companies where the founding members had deep pharmaceutical or bioengineering expertise tend to build their business models around proprietary genetically modified organisms and closed systems. Examples include *Synthetic Genomics, Solazyme, LS9, Targeted Growth, Inc., Amyris, Heliae Development*, and *Algenol*. Companies derived from other industries such as defense, wastewater treatment, and agriculture tend to prefer open pond systems and natural strains. Examples include *General Atomics, SAIC, HR Biopetroleum/Cellana, Aquaflow Bionomics,* and *Phyco Biosciences*.

Companies headquartered in colder latitudes tend to focus on closed algae production systems. Examples include *Solazyme, Amyris Biotechnologies, Algae@Work, Algaedyne,* Heliae, and *Greenfuels Technologies Inc* (now defunct). Companies headquartered in warmer latitudes tend to focus on open-pond photosynthetic systems. Examples include *Sapphire Energy, General Atomics*, HR Biopetroleum/Cellana, SAIC, and *Seambiotic* in *Israel*.

Some companies are pursuing a hybrid approach. One example is *Ohio-based Phycal Inc*., which plans to use an open-pond system at its *Hawaii* demonstration site to grow out the algae, then put them into a closed heterotrophic for "fattening" prior to harvest. *HR Biopetroleum/Cellana* also uses a hybrid system, where the seedstock are grown in closed photobioreactor systems to reduce contamination and then inoculated into open ponds for bulking up in volume prior to harvest.

Every algae company has at least one other major revenue stream in its business model beyond just lipid production for biofuels markets. That co-product tends to affect its selection of sites, strains, production processes, etc. Some examples include a valuable co-product stream from

animal feed (*General Atomics*), human food or nutraceuticals (*Solazyme, LiveFuels*), specialty chemicals (*Amyris*), carbon capture and storage (*Phycal Inc., Algae@Work*), and wastewater treatment (*Aquaflow Bionomics*). Within the closed process market niche is a group of companies that use a non-photosynthetic approach to grow their algae. This "heterotrophic" process involves feeding the microalgae sugar in the absence of light to get them to boost their proportion of oil relative to carbohydrates and proteins. An example is *Solazyme*, which is notable in being the first algae Energy Company to complete commercial sales of algae oil specifically for fuel, by delivering over 20,000 gallons of jet fuel (JP5) and marine diesel (F-76) to the *Defense Logistics Agency*.

3. Classification of biofuels

In three generation of biofuels, First-generation biofuels are the biofuels which are directly related to a biomass that is generally edible. First-generation biofuels are in trend around the world and these are also economically viable, but there are some issues related to this kind of biofuels such as utilization of arable lands which are directly affect food availability in most of poor countries so it leads food versus fuel debate. In some countries where sugar market play vital role in their economy the production of ethanol from sugarcane is facing competition with sugar market and on the other hand where ethanol from corn is also responsible for increasing value of food on the world's market. Some problems are with biodiesel market, which is limited by the price of vegetable oils. So these are some reasons which are leading interest towards second generation biofuels.

Second generation biofuels are also known as advanced biofuels. In this type of biofuels, various types of biomass can be used as a feedstock for manufacturing of biofuels. Biomass is source of organic carbon that is part of carbon cycle so it is available as renewed after completion of carbon cycle and is produced from a generally less expensive biomass such as animal, forest, agriculture or municipal wastes. Generally these biomasses are residual non food parts of crops that are not used for food purpose and food crops can be used as second generation biofuels, if they have already fulfilled their food purpose.

Two transformative technologies for production of second generation biofuels are usually done:

Biochemical: in this modification of the bio-ethanol fermentation process including a pretreatment process and

Thermochemical: in this modification of the bio oil process to produce methanol, fisher – Tropsch diesel or dimethyl ether.

Third generation biofuels are produced from extraction oil of algae. Its production has a very high growth yield and low cost. There are many advantage associated with third generation biofuels production such as fastest growing biomass, less land required compared to agriculture product used in other generation and some environment benefits like it cleans water it uses by removing nutrients & other pollutants, adds oxygen and it consumes CO_2.

4. Algal basics

Algae grow almost everywhere in the world. They live all over the world from the ocean to the desert and from hot springs to snow and ice. Algae are important for aquatic ecosystem because they provide food and shelter to other organism. Algae are also important because they have ability of an aquatic ecosystem to absorb nutrients and heavy metals. Algae use sunlight and chlorophyll to make food. Algae are organisms that are like plants and vegetables. Algae grow in almost any aquatic environment and use light and carbon dioxide (CO_2) to create biomass. Algae range in size from a few micrometers to over 30 m in length.

One most important benefit of algae over conventional crops is algae can be grown under conditions which are unsuitable for conventional crop production.

4.1. Microalgae vs. macroalgae

There are two types of algae, Macroalgae and Microalgae. The word "Macro" means big so microalgae are large in size (in size of inches and greater) and multi cellular. Seaweed is example of largest Macroalgae which can be well over 25 m in length. The largest seaweed, giant kelp, is known to grow as fast as 50 cm/day, and can reach a length up to 80 m. Microalgae cells can double every few hours during their exponential growth period Macroalgae have some advantages. Mainly because of their relatively larger sizes than micro, these can be harvested more easily.

On other side "Micro" means very small (in size of micrometers) and contain one cell so called "Unicellular" organisms. Microalgae are more preferred because of the fact that they grow very quickly and also because they have much higher lipid content than Macroalgae.

The main advantages of using microalgal organisms in a variety of industrial applications are:

- they grow rapidly and have a higher solar conversion efficiency than most terrestrial plants;
- they can be harvested batch-wise or continuously almost all year round;
- algal production facilities can be collocated on otherwise non-productive, non-arable land;
- they can utilize salt and waste water sources that cannot be used by conventional agriculture;
- they can use waste CO_2 sources thereby potentially mitigating the release of GHG into the atmosphere; and,
- they can produce a variety of feedstocks that to generate nontoxic, biodegradable biofuels and valuable co-products.
- biodegradable biofuels and valuable co-products.

4.2. Major compositions of microalgal biomass

Microalgae are one of the best alternatives to traditional forms of biomass for biofuels production, due to its ability to be cultivated on marginal lands, fastest growing biomass, high productivity, and potential to utilize carbon dioxide (CO_2) from various sources.

Microalgal biomass is unicellular organisms which mean they have only one cell. Microalgae biomass contains compounds like protein, carbohydrates, lipids and nucleic acid. The percentages of compounds vary with the type of algae. Under good condition, biomass of green algae can be double in less than 24 hours [20]. Green algae can have huge lipid contents, continuously over 50% [23]. Oil content found in green algae may be different; a comparison of the oil content in algae is shown in table 2. Microalgae are capable of fixing CO_2 in the atmosphere, thus facilitating the reduction of increasing atmospheric CO_2 levels

Species	Oil content (percentage based on dry weight)
Chlorella sp.	28-32
Cylindrotheca sp.	16-37
Nitzschia sp.	45-47
Nannochloropsis sp.	31-68
Schinzochytrium sp.	50-77

Table 2. Oil content of algal species

Many microalgae species can be induced to accumulate substantial quantities of lipids [105] thus contributing to a high oil yield. The average lipid content varies between 1 and 70% but under certain conditions some species can reach 90% of dry weight [20, 74, 75, 109].Table 3 presents both lipid content and lipid and biomass productivities of different marine and freshwater microalgae species, showing significant differences between the various species [74, 95, 96, 109]. As shown in table 2, 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, Crypthecodinium, Cylindrotheca, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Nitzschia, Phaeodactylum, Porphyridium, Schizochytrium, Tetraselmis) have oil levels between 20 and 50% but higher productivities can be reached.

Chlorella seems to be a good option for biodiesel production. Yet, as other species are so efficient and productive as this one, the selection of the most adequate species needs to take into account other factors, such as for example the ability of microalgae to develop using the nutrients available or under specific environmental conditions. All these parameters should be considered simultaneously in the selection of the most adequate species or strains for biodiesel production.

Also significant is the composition of fatty acids of the different microalgae species, as they can have a significant effect on the characteristics of biodiesel produced. These are composed of saturated and unsaturated fatty acids with 12–22 carbon atoms, some of them of v3 and v6

families. The analysis of seven fresh water microalgae species for the fatty acid compositions shows that all of them synthesized C14:0, C16:0, C18:1, C18:2, and C18:3 fatty acids.

The relative intensity of other individual fatty acids chains is species specific, e.g. C16:4 and C18:4 in *Ankistrodesmus sp.*, C18:4 and C22:6 in *Isochrysis sp.*, C16:2, C16:3 and C20:5 in *Nannochloris sp.*, C16:2, C16:3, and C20:5 in *Nietzsche sp.* Different nutritional and environmental factors, cultivation conditions and growth phases may affect the fatty acid composition. For example, nitrogen deficiency and salt stress induced the accumulation of C18:1 in all treated species and to some extent C20:5 in *B. braunii*.

5. Current usage of microalgae

Microalgae have a specialty that it useful in various ways likes humans use algae as food, for production of useful compounds, as nutrient and fertilizer, wastewaters treatment and other pollutants removal from wastewater, as indicators of environmental change, in space technology, and as laboratory research systems. Microalgae are capable of fixing CO_2 in the atmosphere because when its grow using photosynthesis, also need CO_2, which is waste of various sources and responsible for global warming worldwide.

5.1. Food

Algae are rich in iodine, potassium, iron, magnesium and calcium. Algae are a complete protein with essential amino acids that are involved in major metabolic processes such as energy and enzyme production. Algae contain high amounts of simple and complex carbohydrates which provide the body with a source of additional fuel. The sulfated complex carbohydrates are thought to enhance the immune system's regulatory response. Algae contain an extensive fatty acid profile, including Omega 3 and Omega 6, which also play a key role in the production of energy.

5.2. Nutrient removal and fertilizer

Algae is such a biomass important which take part in treatment of ponds, pollution control and useful as a fertilizer. Nitrogen, phosphorous, potassium is important fertilizers which are requisite for the growth of the plant, knows as Nutrient. Silica, iron and some other is also useful nutrient for growth of an area such as silica is a vital nutrient for growth of diatoms (phytoplanktonic organism) which is important part of various marine food series. Same as iron is responsible for restrain of phytoplankton.

5.3. Wastewater treatment and detoxification

Wastewater may be produced by municipal, agriculture, industrial and other activities. Algae are feasible for treatment of wastewater by removal of nutrient. Algae biomass can be used in wastewater treatment for the removal of bacteria, reduction of both chemical oxygen demand (COD) and biochemical oxygen demand (BOD), removal of N, P, and for the removal of heavy metals.

5.4. CO_2 emissions

Microalgae can generate biomass by absorb CO_2, which is produced at large-scale due to power plant gases. CO_2 utilization capacity around 1.8 tonnes of CO_2 will be utilized per tonnes of algal dry biomass produced, which is varies with algae species. CO_2 is harmful for environment as it leads some serious issue like global warming microalgae protect environment, through photosynthesis metabolism, microalgae absorb CO_2 and release oxygen.

5.5. Biofuels

Algae can be used to convert various types of fuels which depend on both technique and part of cells used. Biodiesel can be extracted from lipid and oily part of the algae biomass using similar process which is used for other vegetable oil. Alternatively or following lipid extraction, the carbohydrate content of algae can be fermented into bioethanol or biobutanol

Heat and electricity can be generated by burning of algae. Some algae can produce H_2 Gas (hydrogen gas) under some specific condition. Microalgae grow quickly and contain high oil content as compared with terrestrial crops [19].

6. Classification of microalgae

Microalgae were among the first life forms on earth [34]. As prefix "micro" mean small, so microalgae are very small in their size (in size of micrometers).Microalgae known as unicellular organisms because it has one cell. Microalgae can make their own energy and this energy is stored in the cell. They are capable of fixing large amounts of carbon dioxide (CO_2) while contributing to approximately 40 percent to 50 percent of the oxygen in the atmosphere thereby helping to support the majority of life on our planet.

Microalgae are highly productive on a global scale, with cell doublings of 1-4 per day. While microalgae make up only 0.2 percent of global biomass generated through photosynthesis, they account for approximately 50 percent of the total global fixed organic carbon [36].

Microalgae, like terrestrial plants, grow and multiply through photosynthesis, a process whereby light energy is converted into chemical energy by —fixing atmospheric CO_2.

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m²/day)
Ankistrodesmus sp.	24.0–31.0	–	–	11.5–17.4
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chaetoceros muelleri	33.6	21.80	0.07	
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	–	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	–
Crypthecodinium cohnii	20.0–51.1	–	10	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Ellipsoidion sp.	27.4	47.3	0.17	–
Euglena gracilis	14.0–20.0	–	7.70	–
Haematococcus pluvialis	25.0 – 0.05	–	0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	–	0.32–1.60	–
Isochrysis sp.	7.1–33	37.8	0.08–0.17	–
Monodus subterraneus	16.0	30.4	0.19	–
Monallanthus salina	20.0–22.0	–	0.08	12
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0	8.8	–	21.6
Oocystis pusilla	10.5	–	–	40.6–45.8

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m²/day)
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	–
Skeletonema costatum	13.5–51.3	17.4	0.08	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19

Table 3. Lipid content and productivities of different microalgae species [111]

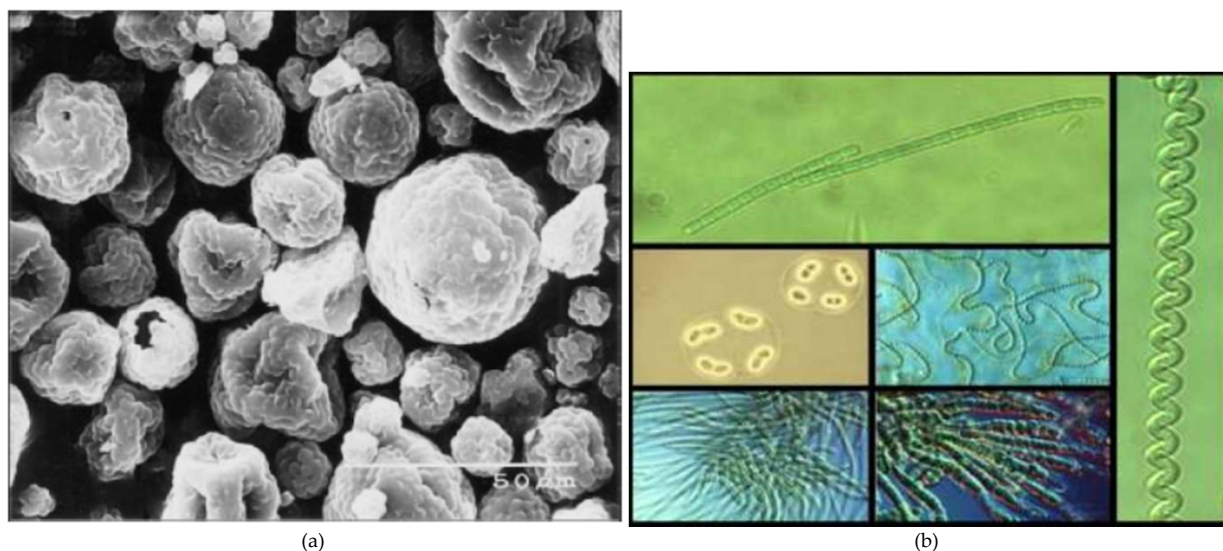**Figure 1.** (a). Scanning electron micrograph of a microalgae (Chlorella)[47]; (b).Cyanobacteria range from simple unicellular organisms to colonies [122]

Over 40,000 separate species of algae have been identified, and that number almost certainly represents a small fraction of the true population (perhaps as high as 10,000,000 different species [55]. Because of the diverse nature of algae, it has been difficult to settle on a universally accepted classification system. For example, some experts will exclude cyanobacteria because

of their simple cellular structure relative to other classes of algae. Others will focus on a separation of unicellular (microalgae) and multicellular (macroalgae).

Much of the classification of algae depends upon photosynthetic pigments, whole organism morphology, cellular anatomy and ultrastructure, and metabolism and physiology. The biological divisions that encompass the various classes of algae are;

• Cyanophyta (cyanobacteria)

• Prochlorphyta

• Glaucophyta

• Rhodophyta (red algae)

• Cryptophyta (cryptomonads)

• Chlorophyta (green algae)

• Euglenophyta

• Chloroarachniophyta

• Pyrrophyta (dinoflaggellates), and

• Chromophyta (heterokonts)

Of these classes, those that produce significant amounts of lipids are considered to be of interest for the production of Biofuels. Macroalgae typically require deep bodies of water for growth, and generally are viewed to lack the potential to make a significant contribution to the world's future liquid transportation fuel needs. Notwithstanding this view macroalgae production is increasing and there is interest in the EU and Japan in its use as a feedstock for methane production by anaerobic digestion and ethanol production by saccharification and fermentation.

Most of the algae known to produce more than 20% of their biomass as lipids fall into the divisions *Cryptophyta*, *Chlorophyta*, and *Chromophtya*. Cryptomonads are biflagellate unicellular algae carrying the photosynthetic pigments chlorophyll a and c, α-carotene and β-carotene giving them the colours green, olive, brown, yellow, red, or blue. They are found in waters ranging from fresh to hypersaline, sometimes in great abundance. *Rhodomonas salina* (also known as *Chroomonas salina)* is a cryptomonad known to produce lipids at high levels.

Chlorophyta or green algae range from unicellular forms to large seaweeds. Their photosynthetic pigments are similar to those in higher plants and include chlorophyll a and b, α-, β-, and γ-carotene, and various xanthophylls. Their cell walls contain cellulose and they often use starch as an energy reserve (attributes of potential feedstocks for ethanol production). *Chlamydomonas reinhardtii*, a chlorophyte, was selected as a model system for the study of plants, and is one of the few algae whose entire gene sequence is known. *C. reinhardtii* can grow autotrophically on a simple medium of inorganic salts and in the presence light and CO_2, but can also grow heterotrophically in total darkness using acetate as a carbon source and O_2.

Several Chlorophytes are known to produce high levels of lipids including *Botryococcus braunii*, *Chlorella vulgaris*, *Neochloris oleoabundans*, and *Nannochloris sp.*The chromophyta contain chlorophyll a and b, α-, β-, and γ-carotenes, zeaxanthin and several other xanthophylls. They comprise many different classes of algae including the *Chrysophyceae* (golden-brown algae), *Bacillariophycease* (diatoms), *Xanthophyceae* (yellow-green algae), *Eustigmatophyceae*, and *Prymnesiophyceae*. Examples of each of these classes are known to produce high levels of lipids including *Ochromonas danica*, *Phaeodactylum tricornutum*, *Nitzschia palea*, *Monallantus salina*, *Nannochloropsis sp.*, and *Isochrysis sp.*

Unlike the other divisions of algae, cyanobacteria or blue green algae is prokaryotic, that is, they lack nuclei and are members of the bacterial kingdom. They contain many different photosynthetic pigments including chlorophyll a and d, phycobilins, β-carotene, zeaxanthin, and other xanthophylls, and phycobilins. Although a *Nostoc* commune has been shown to produce triacylglycerides, cyanobacteria rarely produce more than 20% of their cell weight as lipids, but they will be included in this discussion because they have been shown to accumulate high levels of glycogen (as much as 60% of dry weight) as a storage material, and it is possible to divert the carbon flux from carbohydrate production to lipid production. In addition, cyanobacteria have long-established commercial production methods (mainly for food supplements and nutraceuticals) and genetic techniques have been developed for many different strains.

According to size, color/ pigment, shape, lifecycle and their cellular structure, microalgae are classified in four classes as abundant in below table 4.

6.1. Diatoms (Bacillariophyceae)

Diatoms (Bacillariophyceae) are a type of algae. Mainly diatoms are unicellular but have different in shape such as stars, zigzag, ribbons, fans, spheres, elliptical and triangles when they exists as colonies. Carbon is stored in the form of oil in Diatom. This oil and water current helps them to move within the water to find their food and nutrient. Diatom cells have a unique feature is that, they are enclosed within a cell wall made of silica which is called a frustules. This silica is used to protect the cell.

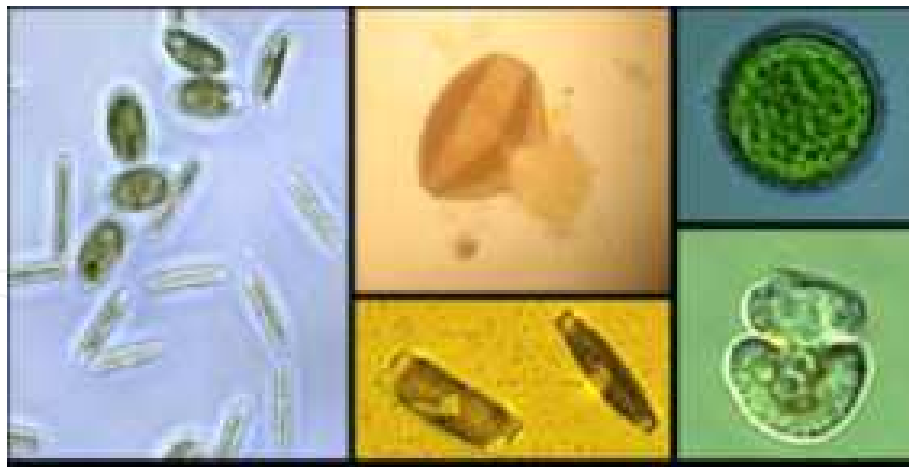| Microalgae	Known Species	Storage Material	Habitat
Diatoms (Bacillariophyceae)	100,000	Chyrsolaminarin (polymer of carbohydrates)	Freshwater and brackish water
Green Algae (Chlorophyceae)	8000	Starch	Freshwater
Blue Algae (Cyanophyceae)	2000	Starch and TAGs	Different
Golden Algae (Chrysophyceae)	1000	Natural oils & carbohydrates	Freshwater

Table 4. Microalgae Classification

Figure 2. Microalgal Biodiversity (diatoms and green algae) [105]

6.2. Green Algae (Chlorophyceae)

Green Algae (Chlorophyceae) can be unicellular or colonial, generally it found quite abundant in fresh water. They have flagella (tails) attached to each cell, they use these flagella to swim. They include some of the most common species, as well as many members that are important both ecologically and scientifically. There are approximately 350 genera and 2650 living species of chlorophyceans. They come in a wide variety of shapes and forms, including free-swimming unicellular species, colonies, non-flagellate unicells, filaments, and more. They also reproduce in a variety of ways, though all have a haploid life-cycle, in which only the zygote cell is diploid. The zygote will often serve as a resting spore, able to lie dormant though potentially damaging environmental changes such as desiccation.

The Chlorophyceae includes three major groups distinguished primarily by basic differences in the arrangement of their flagellae:

• *Volvocales, Chaetophorales, & Chlorococcales* - together make up more than half of all chlorophyceans. Members of these orders have an offset flagellar arrangement (1 o'clock-7 o'clock).

• *Chlorellales* - Members of this order have opposed flagellae (12 o'clock-6 o'clock), though some have only vestigial flagellae and so have not been definitively associated with this group. Similarities with members of the Chlorococcales make distinctions difficult.

• *Oedogoniales* - Members of this smallest group have a complex multiflagellate crown on their swimming spores. All are filamentous, oogamous, and have net-like chloroplasts.

6.3. Blue Algae (Cyanophyceae)

Blue Algae (Cyanophyceae) grow in both fresh and salt waters of dams, rivers, creeks, reservoir, lakes. Blue Algae are a type of bacteria but due to some ways it act like plant by using to manufacture carbohydrates from carbon dioxide and water and release oxygen, through a process of photosynthesis [105].
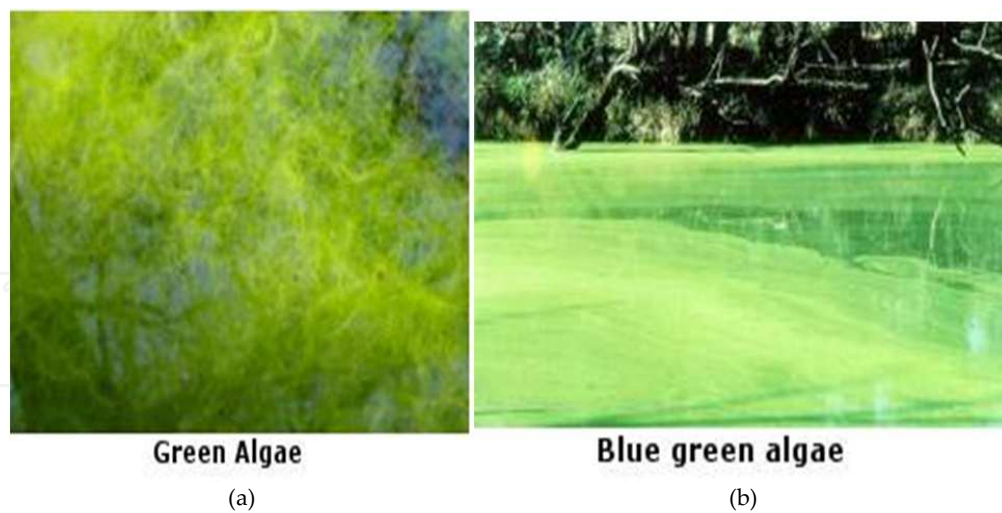
Figure 3. (a) Green Algae [112] Figure (b) Blue Green Algae [112]

6.4. Golden Algae (Chrysophyceae)

Golden Algae (Chrysophyceae), similar to diatoms in pigment and biochemical composition, are mostly found in fresh water. A single species "Prymnesium parvum" are referred as Golden The chrysophyceans (golden algae) are heterokontophyte algae with golden chloroplasts. Many chrysophycean algae are unicellular, but colonial or simple multicellular species are also known. The chrysophycean algae are basically autotrophic but there are many mixotrophic & colorless heterotrophic species. Heterotrophic chrysophyceans such as *Spumella and Paraphysomonas* play an important role as lower consumers. The chrysophycean algae mainly inhabit in freshwater, but some species (especially heterotrophs) are common in marine.
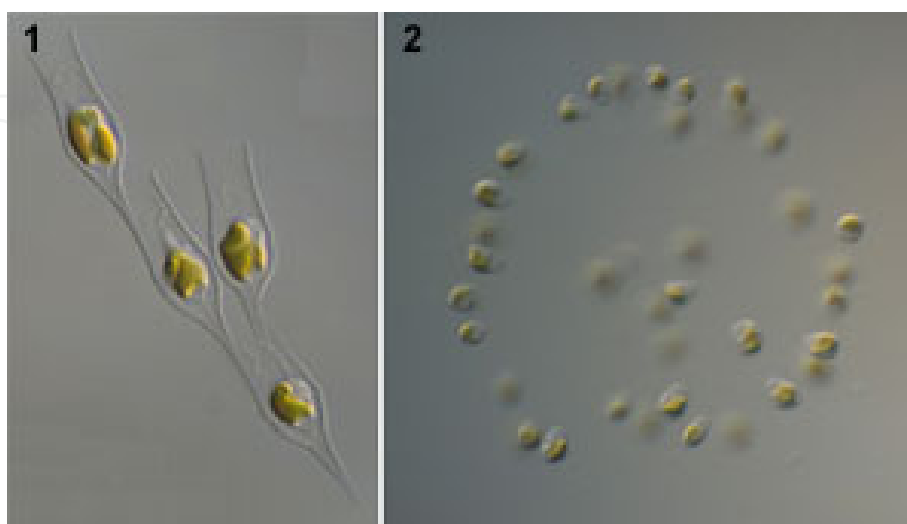

Figure 4. a: Dinobryon b: Uroglena [82]

The cells are naked or covered by scales, lorica or cell wall. The flagellate cell usually possesses two heterodynamic flagella but posterior (a) flagellum is sometimes reduced. Tubular mastigonemes on anterior (b) flagellum possess lateral filaments. Mixotrophic and heterotrophic species engulf particles (e.g. bacteria) through splitted R2 microtubules. Because major photosynthetic carotenoid is fucoxanthin, chrysophycean chloroplasts are golden-yellow in color. Asexual reproduction by means of binary fission, sporogenesis etc. Sexual reproduction has been reported in some species. The chrysophycean algae produce cysts surrounded by siliceous wall, statospore via sexual or asexual reproduction. Statospores form microfossils to be used for paleoenvironmental reconstruction.

7. Microalgae for biodiesel production

Microalgae as compared to conventional crops have high photosynthetic efficiency and therefore potentially high productivity per unit area of plantation. The U.S. Department of Energy's Aquatic Species Program (1978- 1996) focused on biodiesel production from micro-algae because biodiesel is a promising fuel product in many ways because it is useful to counter "Energy Security" and "Climate Changes". Day by day the energy required in Transportation sector is increasing and petroleum will not be able to fulfill the all requirements, in that case Microalgae is one of the substitutes to petroleum. Through photosynthesis metabolism, microalgae absorb CO_2 and release oxygen; it will reduce the global warming effect.

7.1. Current biodiesel feedstock

Biodiesel can be produce from various feedstock's which is soybean oil, rapeseed, Jatropha, mustard, jojoba, sunflower, palm oil, coconut hemp, animal fats, sewage sludge and algae. A comparison of feedstock for biodiesel is as below in table 5.

Feedstock	Oil content (% dry weight biomass)	Land use (sqm/year/L of biodiesel)	Productivity of Biodiesel (Liter Biodiesel/Hectare /Year))
Maize	44	56	179
Soybean	18	15	661
Jatropha	28	13	772
Rapeseed	41	10	1014
Sunflower	40	9	1113
Palm oil	36	2	5585
Microalgae (Low Oil Content)	30	0.2	61,091
Microalgae (medium Oil Content)	50	0.1	101,782
Microalgae (high Oil Content)	70	0.1	142,475

Table 5. Feedstock for biodiesel [93]

7.2. Potential of using microalgae as biodiesel feedstock

Microalgae are emerging as a potential high-volume source of lipids for advanced biofuels. While commercial production of microalgae has been established for human nutritional products like *Spirulina*, *beta carotene*, and *omega-3* fatty acids for at least three decades, the concept of using microalgae as an aquaculture source for energy production on the mega-ton scale meaningful to the petroleum industry has enjoyed a recent resurgence. Over conventional crops, algae can be grown under conditions which are unsuitable for conventional crop production and algae can be grow on the land which is not arable land. Microalgae potential because of the fact that they grow very quickly and live in harsh conditions due to their unicellular structure even Microalgae are able to double their mass within few hours. Micro-algae are preferred over Macroalgae because Microalgae have much higher lipid content than Macroalgae.

Microalgae species	Carbohydrates (%)	Proteins (%)	Lipids (%)
Chaetoceros muelleri	11–19	44–65	22–44
Chaetoceros calcitrans	10	58	30
Isochrysis galbana	7–25	30–45	23–30
Chlorella protothecoides	10.62–15.43	10.28–52.64	14.57–55.20
Chlorella sp.	38–40	12–18	28–32
Nannochloropsis sp.	-	-	31–68
Neochloris oleoabundans	-	-	35–54
Schizochytrium sp.	-	-	50–77
Scenedesmus– obliquus	10–17	50–56	12–14
Quadricauda de Scenedesmus	-	47	1.9

Table 6. Chemical composition of biofuel source microalgae

The majority of companies trying to demonstrate commercial production of microalgae for energy and other markets were found within the past six years. The pace of innovation in systems engineering, cultivation techniques, intracellular productivity improvement techniques, and business model development has been extremely rapid. Production and productivity levels have jumped by orders of magnitude each year over the past three to four years, for example from less than 100 verifiable gallons of algae oil produced by the entire industry in 2009 to over 20,000 gallons delivered to customers in 2010.

Table 7, shows that Microalgae (low, medium and high Oil Content) have been found to have incredible production level compared to other oil seed crops.

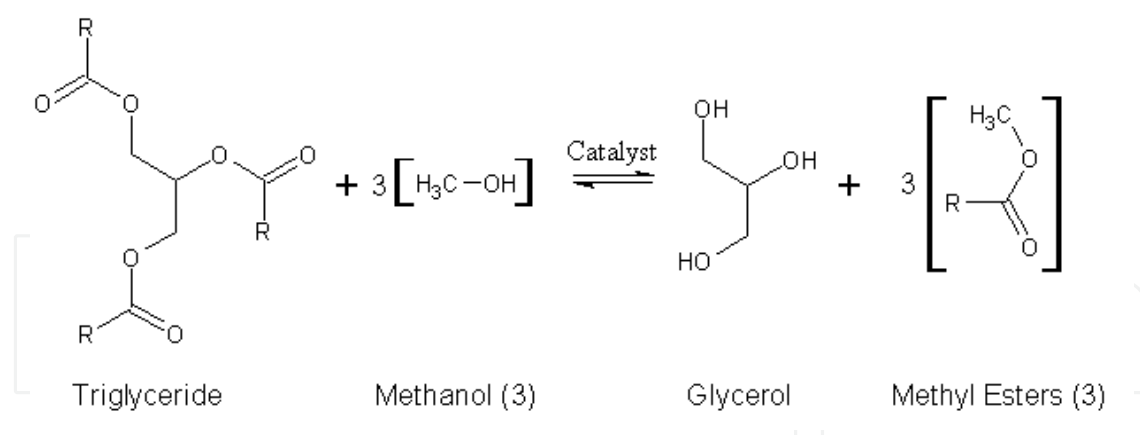| Species	Oil content	Species, %	Oil content, %
Botryococcus braunii	25–75	Isochrysis sp.	25–33
Chlorella sp.	28–32	M. Subterraneus	39.3
Chlorella emersonii	63	Monallanthus salina	420
Chlorella minutissima	57	N. laevis	69.1
Chlorella protothecoides	23	Nannochloris sp.	20–35
Chlorella sorokiniana	22	Nitzchia sp.	45–47
Chlorella vulgaris	40, 56.6	P.incisa	62
Cylindrotheca	16–37	Phaeodactylum tricornutum	20–30
Crypthecodinium cohni	20	Schizochytrium sp	. 50–77
Dunaliella primolecta	23	Tetraselmis sueica	15–23

Table 7. Oil content in some microalgae

Source	Carbohydrates (%)	Proteins (%)	Lipids (%)
Anabaena cylindrica	25–30	43–56	4–7
Chalmydomonas rheinhardii	17	48	21
Chlorella vulgaris	12–17	51–58	14–22
Dunaliella salina	32	57	6
Porphyidium Cruentum	40–57	28–39	9–14
Spirulina maxima	13–16	60–71	6–7
Baker's yeast	38	39	1
Meat	1	43	34
Milk	38	26	28
Rice	77	8	2
Soya bean	30	37	20

Table 8. Chemical composition of some food source microalgae (% of dry matter) [120]

7.3. Biodiesel production from microalgae biomass

Liquid fuel can be obtained by the process of oil extraction from algae. Hexane is an organic solvent which is used for this process. The hexane removes the oil from the algae. The mixture of hexane and oil is distilled leaving pure algae oil. This technique has significance that solvent is reused for each cycle. Algae fiber, which is remain after this process can be used as fertilizer.

The methodology mostly used for biodiesel production is based on the transesterfication reaction, as follows:

The transesterfication reaction, as above, takes place in the presence of either homogeneous or heterogeneous catalysts (traditional method). Those alternatives can be compared in search for the most efficient method of biodiesel production from microalgae lipids. The biodiesel consists of a biodegradable fuel produced from renewable sources.

Properties	Mcr algal oil Biodiesel	Biodiesel fuel	ASTM biodiesel standard
Density (kg L^{-1})	0.864	0.838	0.86–0.9
Viscosity, (mm2s^{-1}, cSt at 40^{0}C)	5.2	1.9–4.1	3.5–5.0
Flash point , (^{0}C)	115	75	Min 100
Solidifying point (^{0}C)	-12	-50 to 10	-
Cold filter plugging point, (^{0}C)	-11	-30 (Max -6.7)	Summer max 0; winter <15
Acid value, (mg KOH g^{-1})	0.374	Max 0.5	Max 0.5
Heating value , (MJ kg^{-1})	41	40–45	-
H/C ratio	1.81	1.81	-

Table 9. Properties of biodiesel from microalgal oil, biodiesel fuel & ASTM biodiesel standard [120].

The synthesis of this fuel can be accomplished by methodologies such as cracking, esterifiction or transesterfication using animal fat or vegetable oils. Table 9 shows a comparison of characteristics of biofuels and petro diesel along with ASTM biodiesel standard [118].

7.4. Current limitations for algal biodiesel production

Algae biodiesel is still a new technology because more than 30 years, research and development program was initiated by US Department of Energy but due to lack of funding and comparative low cost of petroleum fuel than algae biodiesel, in 1996 this program was discontinued. Now further research will be required to make algae fuel more viable and efficient then petroleum [120]. Algae biodiesel have also lower stability during regular seasonal temperature because during processing, microalgae differ in polyunsaturated from which is other form of biodiesel

and polyunsaturated fats have an ability to retain their fluidity at lower temperature during winter but it will have also lower stability during regular seasonal temperature.

Microalgae species	Carbohydrates (%)	Proteins (%)	Lipids (%)
Chaetoceros muelleri	11–19	44–65	22–44
Chaetoceros calcitrans	10	58	30
Isochrysis galbana	7–25	30–45	23–30
Chlorella protothecoides	10.62–15.43	10.28–52.64	14.57–55.20
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Nannochloropsis sp.	-	-	31–68
Neochloris oleoabundans	-	-	35–54
Schizochytrium sp.	-	-	50–77
Scenedesmus– obliquus	10–17	50–56	12–14
Quadricauda de Scenedesmus	-	47	1.9

Table 10. Chemical composition of biofuel source microalgae

8. Technology for growing algae

There are two algae cultivation technologies currently in use for commercial microalgae production and proposed for algal biofuel production (viz. extensive or open ponds, intensive or raceway ponds or closed photobioreactors in many designs and closed fermenter systems).

8.1. Open pond system

Large extensive or open pond systems are currently in use for wastewater treatment and *Dunaliella salina* production. Oxidation ponds in wastewater treatment systems are not in the true sense for algae production as no algae are harvested. Cognis Australia Pty Ltd produce β-carotene from *D. salina* harvested from hypersaline extensive ponds in Hutt Lagoon and Whyalla. The halotolerant *D. salina* dominates naturally in brine at salt concentrations >100 g L^{-1} but grows relatively slowly (producing perhaps not much more than 2.2 t ha^{-1} yr^{-1}). *Hutt Lagoon* has a total pond surface area of ca. 520 ha and Whyalla is ca. 440 ha. In terms of pond surface area, *Hutt Lagoon* and *Whyalla* are among the largest algal production systems in the world. These extensive pond algae production systems have limited mixing, and rely on natural selection and the bounty of nature with minimal intervention.

Open pond system used big shallow pond which is open. This type of pond is easy to construct and operate than close pond system. Shallow pond is constructed to provide large area to algae for exposed to sunlight. Open pond system is use for cultivation of algae especially having high oil content. Both natural and artificial water pond can be used to algae biomass produc‐tion. Main advantages of open pond system are low operating cost and their simple structure.

Figure 5. Spirulina farms on Hainan Island China & Circular chlorella ponds at Yaeyama on Okinawa Island, Southern Japan [121]

Similarly their many disadvantage are also associated with open pond system are poor productivity, little control over algae production, large evaporative losses, large area required, diffusion of carbon dioxide to the atmosphere and expensive harvesting etc [93].

8.2. Closed ponds

Closed pond system mean which is not open to expose in the air. Control over environment is much better much better than open pond system and it allows more species to grown than other. It is not only more expensive system than open pond system, but also low productivity of biomass.

8.2.1. Photo bioreactor

Photobioreactors are closed systems of transparent tubes, plates, bags or hemispherical domes. Photobioreactors improve yields by protecting productive strains to some extent from contamination, pathogens, and predators, offer the benefits of some temperature control and eliminate climate related impacts of open ponds (viz. rainfall, evaporation, and diurnal and seasonal temperature fluctuations). While better mixing in photobioreactors may provide slight area productivity gains, claims of productivity, which refer to the area or footprint of the growth vessel, can be extremely high when the reactors are configured vertically and are misleading. Vertical photobioreactors must be situated far enough from each other so as to not shade, and consequently the basic limitation on productivity remains the same for both open ponds and closed photobioreactors.

Surface fouling due to bacteria, other organisms, and, in particular, algae, is a major problem with photobioreactors, and cleaning can be a major design and operational problem. Where CO_2 input and O_2 evolution must be optimized for maximum productivity, gas transfer, which is restricted to the surface area of gas liquid interfaces, can limit scalability of photobioreactor designs.

Commercial photobioreactors as shown in figure 6 are in operation at different facilities including the production of *H. pluvialis* in Israel and Hawaii and C. vulgaris in Germany.

Typical plant gate selling prices/production costs are well above \$100/kg from such systems. Consequently, biofuels production based entirely on photobioreactor systems is generally considered unlikely to be commercially viable.

Algae pumped with nutrient rich water through plastic and borosilicate tube, exposed to sunlight called photobioreactor (PBR). Algae biomass produces using carbon dioxide and light by the process of photosynthesis and nutrient from wastewater in artificial environment not in natural environment. Using photobioreactor, algae easily grow on the land which is not arable such as desert and even ocean surface also. PBR is more productive and controlled but more costly and difficult than open pond system.

Table 10 makes a comparison between PBR and ponds for several culture conditions and growth parameters. Comparison of performances achieved by PBRs and open ponds may not be easy, as the evaluation depends on several factors, among which the algal species cultivated and the method adopted to compute productivity. There are three parameters commonly used to evaluate productivity in algae production units: Volumetric productivity (VP): productivity per unit reactor volume (expressed as g/L d).Areal productivity (AP): productivity per unit of ground area occupied by the reactor (expressed as g/m^2 d). Illuminated surface productivity (ISP): productivity per unit of reactor illuminated surface area (expressed as g/m^2 d).

Figure 6. Photobioreactor for large scale algae production [122]

As stated by Richmond [96] despite closed systems offer no advantage in terms of areal productivity, they largely surpass ponds in terms of volumetric productivity (8 times higher) and cell concentration (about 16 times higher). In conclusion, PBR and open ponds should not be viewed as competing technologies, but the real competing technology will be genetic engineering [96].

9. Harvesting of algae

Algae harvesting overcome to get desired algae product that is fuel. Harvesting method use for algae harvesting depends upon type of algae. There is number of algae harvesting method but some of most common is Flocculation, Centrifugation and Microorganism. There are some issues related to algae harvesting that should carried out before harvesting Process to be done such as the water content should be within desired limit, algae must be in paste form before processing. Size of microalgae cells increase by Flocculation so that sedimentation will be easily done with large cells particle. Chemical flocculation and centrifugation is useful in high density algae because using certain chemical such as alum, lime and aluminum sulphate will coagulate and precipate the cell down or float to the surface. This method is very high costly because of the large amount of the chemical used in this process.

Algal harvesting consists of biomass recovery from the culture medium that may contribute to 20–30% of the total biomass production cost [47]. In order to remove large quantities of water and process large algal biomass volumes, a suitable harvesting method may involve one or more steps and be achieved in several physical, chemical, or biological ways, in order to perform the desired solid–liquid separation. Experience has demonstrated that albeit a universal harvesting method does not exist, this is still an active area for research, being possible to develop an appropriate and economical harvesting system for any algal species.

Most common harvesting methods include sedimentation, centrifugation, filtration, ultra-filtration, sometimes with an additional flocculation step or with a combination of flocculation–flotation. Flocculation is used to aggregate the microalgal cells to increase the effective particle size and hence ease sedimentation, centrifugal recovery, and filtration [47]. Weissman and Goebel [298] studied four primary harvesting methods for the purpose of biofuels production: microstraining, belt filtering, flotation with float collection, and sedimentation. These methods discriminate on a size and density basis in performing the biomass separation. Microstrainers are an attractive harvesting method because of their mechanical simplicity and availability in large unit sizes. The recent availability of very fine mesh polyester screens has revived interest in their use for microalgae harvesting.

Subsequent studies concluded that it would be necessary to flocculate the cells prior to microstraining. Filter presses operating under pressure or vacuum can be used to recover large quantities of biomass, but for some applications filtration can be relatively slow which may be unsatisfactory. Also filtration is better suited for large microalgae such as Coelastrum probo-scideum and S. platensis but cannot recover organisms with smaller dimensions such Scenedesmus, Dunaliella, or Chlorella [47]. Alternatively, membrane microfiltration and ultra-

Culture systems for microalgae	Closed systems (PBRs)	Open systems(Ponds)
Contamination control	Easy	Difficult
Contamination risk	Reduced	High
Sterility	Achievable	None
Process control	Easy	Difficult
Species control	Easy	Difficult
Mixing	Uniform	Very poor
Operation regime	Batch or semi-continuous	Batch or semi-continuous
Space required	A matter of productivity	PBRs $\cong$ Ponds
Area/volume ratio	High (20–200 m^{-1})	Low (5–10 m^{-1})
Population (algal cell) density	High	Low
Investment	High	Low
Operation costs	High	Low
Capital/operating costs ponds	Ponds 3–10 times lower cost	PBRs "/> Ponds
Light utilization efficiency	High	Poor
Temperature control	More uniform temperature	Difficult
Productivity	3–5 times more productive	Low
Water losses	Depends upon cooling design	PBRs $\cong$ Ponds
Evaporation of growth medium	Low	High
Hydrodynamic stress on algae	Low–high	Very low
Gas transfer control	High	Low
CO$_2$ losses	Depends on pH, alkalinity, etc.	PBRs$\cong$Ponds
O$_2$ inhibition	Greater problem in PBRs	PBRs "/> Ponds
Biomass concentration	3–5 times in PBRs	PBRs "/> Ponds
Scale-up	Difficult	Difficult

Table 11. A comparison of open and closed large-scale culture systems for microalgae [115].

filtration are other possible alternatives to conventional filtration for recovering algal biomass, which are more suitable for fragile cells and small scale production processes. Furthermore these filtration processes are more expensive especially because of the need for membrane replacement and pumping.

Richmond [96] suggested one main criterion for selecting a proper harvesting procedure, which is the desired product quality. In one hand for low value products, gravity sedimenta-

tion may be used, possibly enhanced by flocculation. Sedimentation tanks or settling ponds are also possible, e.g. to recover biomass from sewage-based processes. In other hand for high-value products, to recover high quality algae such as for food or aquaculture applications, it is often recommended to use continuously operating centrifuges that can process large volumes of biomass.

Albeit at considerable cost, centrifuges are suitable to rapidly concentrate any type of micro-organisms, which remain fully contained during recovery. Additionally, these devices can be easily cleaned or sterilized to effectively avoid bacterial contamination or fouling of raw product.

Another basic criterion for selecting the harvesting procedure is its potential to adjust the density or the acceptable level of moisture in the resulting concentrate right to the optimum subsequent process [47, 96]. Gravity sedimented sludge is generally more diluted than centrifugally recovered biomass, which substantially influence the economics of product recovery further downstream. Since costs of thermal drying are much higher than those of mechanical dewatering, in order to reduce the overall production cost, a concentrate with higher solids content is required after harvest to easy biomass dehydration (e.g. in a drum drying).

In this case a combination of methods can also be used, e.g. a pre-concentration with a mechanical dewatering step such as microstrainer, filtration, or centrifugation and then, a postconcentration by means of a screw centrifuge or a thermal drying. After separation from the culture medium algal biomass (5–15% dry weight) must be quickly processed lest it should get spoiled in only a few hours in a hot climate.

Harvesting Process	Relative Cost	Concentrated Solids	Energy Input
Centrifugation	Very High	More than 10 %	High
Chemical Flocculation	High	8-10 %	High
Direct sedimentation	Low	1-3%	Low
Bioflocculation	Low	1-3%	Low
Auto flocculation	Low	1-3%	Low
Microstrainin	Low	2-4%	Medium
filtration	High	2-6%	High

Table 12. Comparison between various Harvesting Techniques [93]

10. Algae oil extraction techniques

The general extraction techniques are mechanical extraction/ cell disruption methods and Solvent extraction coupled with mechanical cell disruption methods. Other novel methods are

Supercritical CO_2 extraction and direct conversion of algal biomass to biodiesel. Extraction methods such as ultrasound and microwave assisted are also employed for oil extraction from vegetable sources. The results indicate that compared with conventional methods these new methods can greatly improve oil extraction with higher efficiency. Extraction times are reduced and yields increased by 50–500% with low or moderate costs and minimal added toxicity. In the case of marine microalgae Crypthecodinium cohnii, ultrasound worked best as the disruption of the tough algal cell wall considerably improved the extraction yield from 4.8% (in Soxhlet) to 25.9%.

10.1. Mechanical extraction/ cell disruption methods

The first and simple extraction method is mechanical cell disruption of algal cells to extract oil without contamination of other chemicals. Mechanical pressing or French pressing of dry algal lumps involves pressurizing the algal biomass to high-pressure, where the cell walls are ruptured to releases the oil similar to oil extraction from seeds or nuts through mechanical pressing. Homogenization through bead or ball milling is a process to disintegrate the alga cells which takes place in a jacketed chamber or vessel. The shear force created by the high velocity beads which moves radially causes the disruption of cells [121]. Cell disruption in this method depends on factors like residence time, cell concentration, chamber volume, bead volume and umber of rotations. All these mechanical cell disruption are usually combined with the solvent extraction to improve the extraction efficiency. Along with the mechanical methods new pretreatment techniques ultra-sonication, microwave also getting attention. In ultra-sonication & microwave pretreatments, the biomass will be treated in a sonication [96]/ microwave [105] chamber prior to solvent extraction.

10.2. Solvent extraction

Solvent extraction is a common practice used to extract oils from the algal biomass and other biomasses. The solvent should be selected based on efficiency, selectivity towards the different classes of lipids and ability of solvent to prevent any possible degradation of lipids. In order to achieve maximum extraction, the linkages between the lipids and other organelles of the algae cells which are connected with van der Waals interactions, hydrogen bonding and covalent bonding should be broken18. The most common solvents used for extraction are n-hexane, chloroform, petroleum ether, methanol, ethanol, isopropanol, dichloromethane and mixture of any of these solvents depending upon method and desired class selection of lipids. The conventional solvent extraction methods are Bligh and dyer, folch [90], Soxhlet extraction. The steps involved in the solvent extraction at micro level were explained by halim et al. When the algal cells interacted with the organic solvents, these solvents penetrate through the cell wall and interact with the selective class of lipids depending upon its dielectric constant to form a solvent- lipid complex. This complex diffuses in to the bulk solvent due to the concentration gradient continues until this process reaches equilibrium [51]. The solvent extraction methods shows a lot of variability depending upon the organic solvent (dielectric constant) used and biological matrix being used in selection of different class of lipids [20, 51]. The cell

wall and its composition and solvents dielectric constant could be the reasons for these variable extraction properties of individual methods [51].

These solvent extraction methods have been slightly modified by many researchers to improve the kinetics of the extraction process often called as accelerated solvent extraction (ASE). Kauffmann and christen reviewed these accelerated solvent extraction techniques involving microwave heating and pressurized solvent extraction. In the microwave assisted extraction (MAE) the acceleration is achieved by faster disruption of weak hydrogen bonds the dipole rotation of the molecules caused by electromagnetic radiation. In pressurized solvent extraction (PSE) the higher temperature and pressure accelerates the extraction process as the high temperature accelerates the extraction kinetics, high pressure keeps the solvent in liquid state and forces the solvent to pass through the pores of the matrix thoroughly [68]. When coupled with the cell disruption techniques described earlier the solvent extraction will be very faster and utilizes small amounts of solvents [81]

10.2.1. Hexane solvent method

Algae oil extraction can be done thorough various techniques, hexane extraction is one of them. Hexane, Benzene and ether chemical used as a solvent extraction, in which Benzene and ether is widely used in food industry because of low cost factor. Isolation and oil press/express method are method in which hexane solvent extraction can be used for oil extraction. After oil has been extracted through expeller, remaining pulp can be mixed with cyclohexane chemical to further extraction of the remaining oil content in pulp. When the oil dissolved in the cyclohexane chemical, again pulp s filtered out from the solution and using distillation process oil and cyclohexane can be separated. Using this process more than 95 % of the total oil by the algae can be obtained.

10.3. Supercritical CO_2 extraction

Commercial applications of supercritical CO_2 extraction dates back to early 1990's. Supercritical extraction is being used in food and pharmaceutical industries due to its range of selectivity of compounds, non-toxic nature, and easy separation [87]. The principle behind this technology is, when fluids crosses both critical temperature and critical pressure they attains properties of both gases and liquids. This state of the fluid is called supercritical state of fluid, and it exhibits mass transfer properties of gas and solvent properties of liquid with greater diffusion coefficients [100]. Because of the lower critical point at 31.1ºC and 72.9 atm carbon dioxide became preferred fluid for extraction applications. The solvent properties of supercritical fluid can be modified by altering extraction pressure and the extraction temperature. As an example target compounds like pigments, proteins and neutral lipids can be extracted at their respective extraction temperature and pressure, where they interact with the solvents [51, 80]. Due to its high selectivity, lower toxicity, chemical inertness and high purity of the extracted compounds, supercritical CO2 extraction is being used in many pharmaceutical, nutraceutical and food industries worldwide [80].

11. Biodiesel production from algal oil

Biodiesel is a mixture of fatty acid alkyl esters obtained by transesterification (ester exchange reaction) of vegetable oils or animal fats. These lipid feedstocks are composed by 90–98% weight) of triglycerides and small amounts of mono and diglycerides, free fatty acids (1–5%), and residual amounts of phospholipids, phosphatides, carotenes, to copherols, sulphur compounds, and traces of water [17].

Transesterification is a multiple step reaction, including three reversible steps in series, where triglycerides are converted to diglycerides, then diglycerides are converted to monoglycerides, and monoglycerides are then converted to esters (biodiesel) and glycerol (by-product). The overall transesterification reaction is described in Fig. 3 where the radicals R1, R2, R3 represent long chain hydrocarbons, known as fatty acids.

For the transesterification reaction oil or fat and a short chain alcohol (usually methanol) are used as reagents in the presence of a catalyst (usually NaOH). Although the alcohol: oil theoretical molar ratio is 3:1, the molar ratio of 6:1 is generally used to complete the reaction accurately. The relationship between the feedstock mass input and biodiesel mass output is about 1:1, which means that theoretically, 1 kg of oil results in about 1 kg of biodiesel.

A homogeneous or heterogeneous, acid or basic catalyst can be used to enhance the transesterification reaction rate; although for some processes using supercritical fluids (methanol or ethanol) it may not be necessary to use a catalyst [295]. Most common industrial processes use homogeneous alkali catalysts (e.g. NaOH or KOH) in a stirred reactor operating in batch mode.

Recently some improvements were proposed for this process, in particular to be able to operate in continuous mode with reduced reaction time, such as reactors with improved mixing, microwave assisted reaction [44,65], cavitations reactors [43, 44] and ultrasonic reactors [130, 68].

Transesterification is process algae oil must go through to become desired product biodiesel which is required two chemicals (Methanol and Sodium hydroxide) and following steps to be done as mix Methanol and Sodium hydroxide which make sodium methoxide now this sodium methoxide mix with algae oil and allow it to settle for about 8 hours. Now filter biodiesel to 5 microns and drain glycerin. This glycerin is used to make products such as soap and others.

12. Sustainability

Environmental protection will be one of the prominent reasons foe utilization of biomass resources. Microalgal biofuels are more important because it is useful to counter Energy Security and Climate Change problem which is main issue through worldwide. The microalgal Biomass absorbs carbon dioxide during growth, and emits it during combustion. Hence it does not contribute to green house effect. There can be a substantial reduction in the overall carbon dioxide emission as the microalgal biomass is a carbon dioxide neutral fuel. Microalgal biofuels

is also sustainable because away from conventional crops algae biomass can be grow on the land which is not arable land so it not affect food security to anywhere in the world. As discussed above, algae is fastest growing biomass on less land required to agriculture product so there is no any problem associated with row material required for biofuels generation.

Sustainability is the subject of much discussion at international scientific and governmental forums on biofuels. Emerging from this discussion is a consensus that sustainability is of foremost importance as an overarching principle for the development of biomass-to-energy agro-industrial enterprises. While sustainability criteria that are agreeable to all nations are still being expounded, the generally accepted principles of sustainability include that;

- the greenhouse gas balance of the production chain is positive;

- the biomass production is not at the expense of carbon sinks in existing vegetation and soil;

- the biomass production does not endanger the food supply and existing local business activity (i.e. local supply of energy, medicines and building materials);

- the biomass production has no impact on biodiversity (protected or vulnerable biodiversity is not affected or if possible strengthened);

- soil and soil quality are retained or improved;

- ground water and surface water are not depleted and water quality is maintained or improved;

- air quality is maintained or improved; and

- the production and processing of biomass contributes to local prosperity and to the social well being of employees and the local population.

It is self evident that where there is a natural abundance of freshwater, it is likely on arable land (that may be under agriculture and may have multiple competing uses for the water resource), or on land in its natural state with considerable biodiversity value. With few exceptions where the abundance of freshwater is the consequence of human intervention, the water has multiple competing uses.

Consequently, from the perspective of sustainability it seems obvious that algal production systems should target water resources other than freshwater. In fact, the proponents of algal biofuel claim that the production system is superior to biofuels based on terrestrial biomass because it can utilize non-arable land and waste water resources.

While the literature on the sustainability of algal biofuels is sparse, recent analyses appear to dispute the claims of superiority of algal production systems when compared to terrestrial crops.

Clarens et al. (2010) compared the environmental life cycle impacts of algal biomass production to corn, switch grass and canola production. The functional unit was 317 GJ of biomass derived energy or the amount of energy consumed by one American citizen in one year (i.e. the study sort to inform on the life cycle impacts associated with the production of 317 GJ of biomass

based on the higher heating value of the material on a dry basis). Biomass production was modeled for three locations in the USA, and for algae was based on fresh water and municipal sewerage effluents from conventional activated sludge and biological nitrogen removal treatment plants. Algae production in raceway ponds varied from 0 g m^{-2} d^{-1}(seasonal shut down) to 20 g m^{-2} d^{-1} depending on site location and climate. All four biomass production systems had net positive energy (i.e. more energy produced than consumed in the biomass production). Algae cultivation had better land use and eutrophication LCA outputs than terrestrial crops, but the terrestrial crops were found to have lower energy use, greenhouse gas emissions and water use than algae production based on fresh water or municipal sewerage effluents. When industrial grade CO_2 was used in algal biomass production the system emitted more greenhouse gases (GHG) than it sequestered. Even when flue gas was used, the algal production system consumed more energy and emitted more GHG than the terrestrial plant production systems (mostly as a consequence of high mineral fertilizer use).

Lardon *et al.* (2009) compared the environmental life cycle impacts of microalgae biodiesel production to the impacts of palm, rape and soybean oil biodiesel and petroleum diesel production. The LCA was based on a cradle to combustion' boundary (i.e. all products and processes upstream of fuel combustion in a diesel engine). The functional unit was 1 MJ of fuel in a diesel engine. The study considered four algae biofuel production scenarios, viz. production under nitrogen fertilizer rich and starved conditions and with oil extraction from wet and dry raceway ponds varied from 19.25 g m^{-2} d^{-1} (in the nitrogen starved case) to 24.75 g m^{-2} d^{-1} (in the nitrogen rich case). Of the four algae biofuel production scenarios, only growth under starved nitrogen conditions with oil extraction from wet biomass had a positive net energy. In the three other algal biofuel scenarios, the energy consumed in the production was greater than the energy in the delivered biofuel. These balances assumed 100% recovery of energy from the algae cake residue after oil extraction. Fertilizer (nitrogen) consumption had a far greater impact on cumulative energy demand than drying biomass for extraction. Algae biofuel had better land use and eutrophication LCA outputs than biofuels from the terrestrial crops, but petroleum diesel had better land use and eutrophication impacts than all biofuels. In all other assessed metrics, one or all of the terrestrial crop biofuels had lower LCA impacts than all algal biofuel scenarios (again mostly as a consequence of high mineral fertilizer use).

It should be stressed that these LCA studies are based on hypothetical operating scenarios, not real production systems. The purpose of the studies is to highlight inefficiencies in the production systems that need to be addressed to create sustainable microalgae-to-biofuel enterprises. Nevertheless, these studies created debate in the scientific community and the exchange of comments published in subsequent editions of the journal. Principal among the criticisms form algae biofuel proponents are that the authors of LCA studies that report negative outcomes use too low growth rates and too high mineral fertilizer consumption figures.

In contrast, Christi (2008), a proponent of algal biofuels, provides an opinion in *Trends in Biotechnology* titled *Biodiesel from microalgae beats bioethanol.* The claimed superiority of algal biofuel over sugarcane ethanol is based solely on land use efficiencies. In this article, Christi claims algal biofuel can sustainably and completely replace all petroleum derived transport

fuels, and quotes average annual algal biomass production in tropical regions as high as 1.535 kg m^{-3} d^{-1} in photobioreactors (a productivity/reactor volume measurement). This report has already noted that claims of extremely high growth in vertically configured photobioreactors are misleading. Vertical photobioreactors must be situated far enough from each other so as to not shade, and consequently the basic limitations on land use and productivity remains the same for both open ponds and closed photobioreactors. Christi (2007) had previously claimed very high land use efficiencies in raceway ponds (viz. 136,000 L/ha of oil for algal biomass with an oil mass fraction of 70% and 58,700 L/ha of oil for algal biomass with an oil mass fraction of 30%). Such yields are only achievable with production of greater than 340 days in a year and at a pond productivity of ca. 50 g m^{-2} d^{-1} (unrealistically high at the current state of technology). *Christi* also assumes that CO_2 is available at little or no cost (presumably in these same tropical regions); this is a challengeable assumption. Despite the liberal use of the word 'sustainable', *Christi* provides no other LCA metric than land use efficiency.

Reijnders (2008) in a rejoinder notes that Christi did not consider fossil fuel inputs during the biofuel life cycle, that previous LCA studies on *Dunaliella* and Spirulina production showed little or no net energy benefit, and that by comparison terrestrial plant production systems are characterized by much lower fossil fuel inputs. The studies of *Clarens et al.* and *Lardon et al.* support Reijnders views. It would seem probable that while the assumptions imbedded in hypothetical production scenarios do have significant impacts on LCA outcomes algal biofuel production faces significant challenges to meet sustainability criteria. Limited LCA studies indicate that significant advances need to be made in reducing fossil fuel inputs associated with nutrient use, harvesting and extraction.

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