

CHAPTER 4

VALUE CHAIN OF ALGAE

4.1.Introduction

This chapter outlines the various stages in the value chain of algae for biofuel production. It also provides a comprehensive understanding of each and every stage along with all possible methodologies and technologies developed till date for the production of green diesel. Once the value chain is in place, this chapter would build up the base for the further life cycle study for the calculation of net energy balance and net energy ratio for algae green diesel production system. Each and every stage of the value chain has influence on the Life Cycle Assessment (LCA) result.

4.2.Algae

The microorganisms, such as, bacteria, yeasts, fungi and algae, which have been shown to possess oil-producing capabilities [130, 131], and can accumulate lipids at more than 20% of their biomass, are defined as oleaginous species [131].

Microalgae represent a large group of [60] an exceptionally diverse but highly specialized [35, 58], photosynthetic [60, 132, 133] and microscopic [55, 132] organisms adapted to various ecological habitats [35, 58, 134], such as, freshwater, brackish, marine and hyper-saline, with a range of temperatures and pH, and unique nutrient availabilities [58]. They are present in all existing earth ecosystems, not just aquatic, but also terrestrial, representing a large variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed [35].

Algae are the most efficient biological producers of oil on the planet and a versatile biomass source [60]. They are sunlight driven oil factories which convert carbon dioxide into different types of renewable biofuels, including methane produced by anaerobic digestion of the algal biomass, biodiesel, and photo-biologically produced bio hydrogen [135, 60], foods and high value compounds [60] such as; vitamins (C, E, and biotin), pigments (carotenoids and ficobiliproteins) and metabolites with pharmacological activities [71].

US Department of Energy's Aquatic Species Program (ASP) has provided an excellent basis of work for the potential use of algae to produce biodiesel [63]. An important conclusion of the said previous work was that algae has the potential to produce more than two orders of magnitude higher oil production per acre than conventional crops, such as, soybeans and canola. Furthermore, algae are not a food crop like most vegetable oil sources currently used for biodiesel production [63]. According to some estimates, the yield (per acre) of oil from algae is more than 200 times than that of the best-performing plant/vegetable oils [136].

Although the relative merits of cultivated terrestrial plant biomass versus microalgae, as feedstocks for biofuel production, are still a subject of debate [66, 137], microalgae have numerous characteristics that favor their use as a biofuel source.

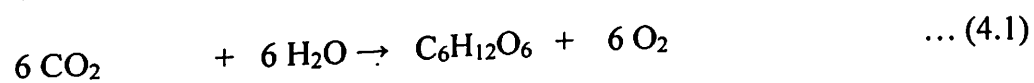
4.3. Algal Physiology for lipid synthesis

A better understanding of how plants synthesize fatty acids and triacylglycerols will ultimately allow the development of novel energy crops. For example, knowledge of the regulation of oil synthesis has suggested ways to produce triacylglycerols in abundant non-seed tissues [138]. Further the algae oil content and composition is strongly dependent on the metabolic status of the cells [69]. Therefore, only if we have an increased understanding of algal physiology, we will be able to use the

knowledge to better understand the ability of algae species to enhance lipid and oil production during cultivation.

4.3.1. Physiology of lipid synthesis

In addition to starch and protein, many plants synthesize and store large quantities of lipid in the form of fat and oil [139]. The carbon sources for lipid synthesis are the photosynthate i.e. the carbohydrates formed during the process of photosynthesis (starch, sugar etc.). In photosynthesis, the plant uses solar energy to oxidize water, thereby releasing oxygen, and to reduce carbon dioxide, via calvin cycle, thereby forming large carbohydrates, primarily sugars [139]. The process is depicted by equation (4.1).



Carbon Dioxide Water Carbohydrate Oxygen

The carbohydrates synthesized by the Calvin cycle are converted into storage forms of energy and carbon mainly sucrose and starch. These stored carbohydrates provide the carbon source, via glycolysis pathway, for lipid synthesis. Anaerobic metabolism of glucose is known as glycolysis [139].

The end product of glycolysis is pyruvate. One molecule of glucose breaks down into two molecules of pyruvate, which are then used to provide further energy, in one of many ways. Pyruvate is a key intersection in the network of metabolic pathways. Pyruvate can be converted into carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-co-enzyme A (acetyl-CoA), to the amino acid alanine, and to ethanol. Therefore, it unites several key metabolic processes [139].

Pyruvate dehydrogenase converts Pyruvate into acetyl-co-enzyme A (acetyl-CoA), which is the main input for a series of reactions, including

fatty acid synthesis. Fatty acid biosynthesis involves the cyclic condensation of two-carbon units in which acetyl-CoA is the precursor. In plants, fatty acids are synthesized exclusively in the plastids. The enzymes of the pathway are thought to be held together in a complex that is collectively referred to as fatty acid synthase [139].

The first committed step in the pathway (i.e., the first step unique to the synthesis of fatty acids) is the synthesis of malonyl-CoA from acetyl-CoA and CO₂ by the enzyme acetyl-CoA carboxylase. The tight regulation of acetyl-CoA carboxylase appears to control the overall rate of fatty acid synthesis acid synthase [139]. Figure 4.1 shows the diagrammatic representation of various steps involved in lipid synthesis.

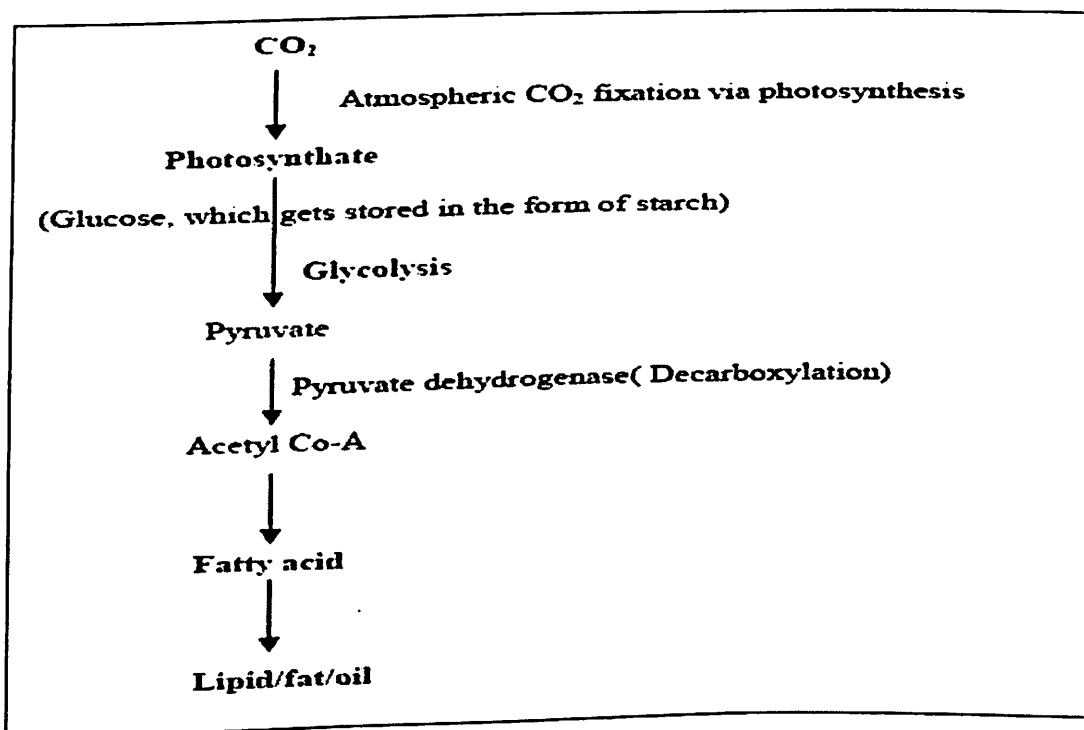


Figure4. 1: Various Steps Involved in Lipid Synthesis

Since biodiesel is produced from lipid content, biofuels ultimately derive their energy from the sun through photosynthesis [138]. Therefore, only if we can understand this phenomenon better, and its effect on lipid synthesis, we can apply this information for better culture of microalgae.

The photosynthesis process can be divided into two reaction stages, namely the light and the dark stage [52, 69]. In microalgae, as well as in higher plants, the light and dark reactions can operate independently. In the light reactions stage the light energy is absorbed by pigments of the photosystem antennae and converted into a biochemical reductant NADPH₂ (nicotinamide adenine dinucleotide phosphate diaphorase) and a high energy compound ATP (Adenosine triphosphate, coenzyme used as an energy carrier in the cells of all known organisms). The pigments, that absorb the light energy, are chlorophylls, which absorb the red light region (650–700 nm), carotenoids, which absorb the blue light region (400–500 nm), and phycobilins, which absorb the orange-red light region (600–650 nm) [52]. The latter are pigments which are present only in the cyanobacteria and the red-algae [139, 52].

Dark reaction stage is light independent [69]. In this stage the products of the light reactions are subsequently consumed [52, 69] by the reduction of CO₂ to carbohydrates [52] through calvin cycle [139].

For photosynthetic eukaryotic cells, such as microalgae, growth is the result of the biomass increase caused by photosynthesis in chloroplasts (anabolism) and its partial degradation by respiration in mitochondria (catabolism). The total growth rate can thus be expressed as the sum of two terms i.e. the photosynthetic growth and the respiration process [70].

Photosynthesis is affected by many external factors like light, temperature, carbon dioxide etc. Thus, these factors indirectly affect the rate of lipid synthesis and also lipid composition. Lipid content and lipid composition of microalgae could be adjusted through changing growth medium composition [64]. Several studies have shown that the quantity and quality of lipids within the cell can vary as a result of changes in growth conditions [70, 133, 140], which include temperature, light intensity [71, 69], cell culture density, pH and alkalinity, contamination by other

microorganisms [52] and nutrient media characteristics, like, concentration of nitrogen, phosphates, and iron [71, 69]. The possibility of oil accumulation through the manipulation of environmental culture conditions has a great potential in oil production. The culture conditions of the microalgae can be optimized in order to maximize lipid synthesis [72].

Therefore, systematic studies are needed to gain a fundamental understanding of the relationship between the combination of environmental parameters and algae production system configuration in order to produce a high cell density, a rapid turnover rate, and higher lipid content [69].

4.3.2. Effect of various external factors on physiology of lipid synthesis

1) Light

Light is the primary energy source for microalgae growth, enabling it to carry out all the necessary metabolic processes. Eight photons of photosynthetically active radiation (PAR) are required to fix one molecule of CO₂ into carbohydrate, resulting in a maximum photosynthetic efficiency (not including respiration) of about 12%. When respiration is taken into account, the maximum efficiency falls to 9% [56].

Photosynthesis is strongly dependent on the quality and the quantity of light. The first is expressed as the wavelength of light and the latter as photon-flux-density (PFD) [52]. The photosynthesis of aquatic plants is saturated in the range of 20–50% of full sunlight, or about 400–1000 $\mu\text{E}/(\text{m}^2\text{s})$ [69].

When microalgae are subjected to illumination, the light reactions are automatically activated and are deactivated if the quality and quantity of light decreases below threshold for photo pigment stimulation [52]. Algae

cultures grow differently when exposed to different colors of light i.e. different wavelengths of light [74].

Microalgae growth rates are enhanced by increasing light density up to the point of light saturation, at which point photosynthetic activity reaches its maximum [52]. This stage is critical because the excess energy is dissipated in the form of heat [74]. At high light densities, the photosynthetic capacity decreases and growth is inhibited. This phenomenon is called photo-inhibition, and it occurs at light oversaturation, a situation in which the photo-system II (PSII) is negatively affected. Photo-inhibition depends mainly on light temperature, strain and cultivation type (indoor or outdoor) [52].

Light and dark cycles also strongly influence the growth of algae [52]. The combination of factors, such as, the length of the light and dark cycles, and the light intensity result in the overall light regime in a photobioreactor [52]. In an experiment on *Porphyridium cruentum* it was seen that highest lipid accumulation level was achieved at 12:12 hours light dark cycle [141].

In another experiment the longer light period significantly increased the cell density of *D. tertiolecta* and the photoperiod of 24 hours light produced the highest biomass during the same cultivation time. Thus, light harvesting efficiency is important for bioreactor engineering encompassing those microorganisms [69].

Biomass losses might reach as high as 25% during the night, depending on the light intensity during the day, the temperature during the day, and temperature at the night [52]. During night there is an overall decrease of biomass because sunlight is absent and biomass is lost due to dark respiration [142].

2) Cell density

Cell density in a culture is principally limited by limitations in light penetration [69]. Optimal cell density is specific to each strain and needs to be maintained in order for light intensity and light penetration to remain at optimal levels [74]. Both low and high biomass concentration result in a loss of biomass productivity. If the biomass concentration is too low, some of the light is transmitted through the culture (low absorption). Conversely, if the biomass is too high, a dark zone appears in the depth of culture (favoring the light limitation regime). Both situations result in a loss of biomass productivity. In the first case, the light is not fully absorbed, while in the second case, the dark zone, where respiration is predominant, has a negative influence [70].

At high cell densities, cell mutual shading takes place and light intensity decreases due to the increase in turbidity of the culture, causing a reduction in the photosynthetic activity [52]. Thus, high frequency of light/dark cycle is highly recommended in algal culture when the growth is light-limited. In practical cases, utilization of high-intensity light would be enhanced only by inducing turbulent streaming in culture suspension [143], which can be achieved through mixing) [143] .

In algal cultivation, the productivity of algal systems, and cost of reactor construction and operation are dependent on the mixing system employed. Mixing serves a variety of purposes, including prevention of cell settling, elimination of thermal stratification, distribution of nutrients and carbon dioxide, removal of photosynthetically produced oxygen, and improvement of light utilization efficiency [143]. Mixing distributes radiation evenly to all cells in the culture and reduces diffusion barriers around the cells, and thus, it increases the frequency of cell exposure to light and dark volumes of the reactor. To ensure adequate mixing, a minimum liquid circulation velocity should be maintained [143].

3) Temperature.

Maintaining proper temperature is very important for the growth of microalga during cultivation [144]. Although algae may be able to grow at a variety of temperatures, optimal growth of microalgae is limited to a narrow range specific to each strain [117]. Therefore, optimum temperature for biomass production is genera and strain dependent [52]. Table 4.1, shows that a sharp drop in microalgae growth rate was seen when the temperatures were further increased or decreased [71].

Table4. 1: Effect of temperature deviation on microalgae growth rate

Algae strain	Optimal Temperature	Effect of Temperature deviation
<i>C. vulgaris</i>	30 °C [71] (25 to 30 °C [144])	At 38°C, there was an abrupt interruption of microalgal growth, and later the cells died [71] .
<i>N. oculata</i>	20 °C [71]	Below optimal temperature, growth rate more than halved [71] .

Seasonal, and even daily fluctuations in temperature, can interfere with algae production. In addition, a lower temperature appears to reduce the loss of biomass due to respiration during the night [117]. Temperature strongly influences the oxygen evolving activity of the photo-system II (PSII). There is a connection between temperature, light and photo-inhibition. At low temperatures photo-inhibition occurs by high light intensities, and thus, temperature can be considered as the most important limiting factor in outdoor cultivation during the winter. However, photo-inhibition can be considerably reduced by an increase in temperature [52].

4) Oxygen

Biomass production is accompanied by oxygen production [142]. A high presence of oxygen around algae cells is undesirable. The combination of intense sunlight and high oxygen concentration results in photo-oxidative

damage to algal cells. As a general guideline, oxygen concentrations should be maintained below 400% of air saturation value [74].

5) Nutrients

Nutrient assimilation is the process by which nutrients acquired by plants are incorporated into the carbon constituents necessary for growth and development. These processes often involve chemical reactions that are highly energy intensive and thus may depend directly on reductant generated through photosynthesis [139]. Several studies have demonstrated that alteration in nutrient concentrations can modify the growth and secondary metabolism of microalgae. Furthermore, microalgae growth depends not only on an adequate supply of essential macronutrient elements (carbon, nitrogen, phosphorus, silicon) and major ions (Mg^{2+} , Ca^{2+} , Cl^- , and SO_4^{2-}) but also on a number of micronutrient metals such as iron, manganese, zinc, cobalt, copper, and molybdenum [145].

a. Carbon

Carbon is an essential nutrient for algae cultivation and can be taken up from inorganic and organic forms. Inorganic carbon is utilized through the CO_2 concentrating mechanism, an active function that enables the microalgae to acquire and concentrate inorganic carbon from the extracellular environment. Microalgae have the ability to utilize both CO_2 and bicarbonate ion (HCO_3^-), as an inorganic carbon source. As intercellular carbon is in the form of HCO_3^- , it is converted to CO_2 by the enzyme carbonic anhydrase (CA). The CO_2 dissolved in water generates one of the most important buffer systems, a weak acid/base buffer system, namely the bicarbonate–carbonate buffer system [52]. The formation of an inorganic carbon species is a function of pH and temperature. In pH values up to about 10.5, bicarbonate species dominate; while in higher pH values carbonate (CO_3^{2-}) species dominate. The bicarbonate–carbonate buffer system provides carbon for photosynthesis. Although the dissolution of

CO₂ in water results in acidification due to the forming of carbonic acid, the photosynthetic process of CO₂ fixation causes a gradual rise in pH due to accumulation of OH⁻. Moreover, the tendency of pH to rise is related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher [52]. Each strain of algae also has a narrow optimal range of pH [73]. Rising pH can be regulated by the addition of mineral acids, such as hydrochloric acid (HCl) or organic acids [146].

Therefore, CO₂ is an essential substrate for photosynthesis, and is an important factor determining algal growth [69] and biosynthesis of fatty acids [146]. CO₂ concentrations usually have to be kept within narrow margins [69]. CO₂ concentrations from 1% to 5% by volume often lead to maximum growth [74, 146]. An excess of CO₂ can also be detrimental to photosynthesis and cell growth [74]. However, highly CO₂ tolerant species that can grow under high CO₂ concentration also occur. One of such CO₂ tolerant species is the green alga *Chlorococcum littorale*, which has a great potential for aqua cultural fatty acids production [146].

Chlorophyceae can grow with up to 18% dissolved CO₂ in the cultivation medium. CO₂ fixation efficiency can amount up to 38% in *Spirulina* sp. cultures [52]. *Dunaliella tertiolecta* grew very slowly at 100% CO₂ concentrations, while it did not grow at all without CO₂ [69].

Some researchers have studied CO₂ concentration effects on biosynthesis of fatty acid using several microalgae, and have concluded that CO₂ seems to control intracellular fatty acid composition and content. Increasing CO₂ concentration is generally adjusted by supplying a desired level of CO₂ gas in air, which when used on *Chlorella vulgaris*, increased the composition of saturated fatty acids over that of unsaturated fatty acids. Therefore, bubbling gas concentration is an important factor in microalgal growth [146].

In an experiment on *Chlorocuccum littorale* it was seen that, though fatty acid content was almost constant for the CO₂ concentrations ranging from 5% to 50% under nitrate-rich conditions, but after nitrate depletion, the content drastically increased with a decrease in CO₂ concentration. HCO₃³⁻/CO₂ ratio in the culture media was found to be a controlling factor for fatty acid production after the nitrate limitation phase. For a CO₂ concentration of 5%, the fatty acid content was 34% by weight at maximum, which is comparable with other land plant seed oils [146].

b. Nitrogen

Nitrogen is also an important nutrient for the production of microalgal biomass. The nitrogen content of the biomass can range from 1% to more than 10%, and is dependent upon the amount, the availability and the type of the nitrogen source. Nitrogen can be utilized as nitrate (NO₃⁻), nitrite (NO₂⁻), or ammonium (NH₄⁺) [52, 147], and also as N₂. The preference order in which nitrogen is utilized is NH₄⁺ > NO₃⁻ > N₂. When NH₄⁺ is available, microalgae do not utilize other nitrogen sources until all the ammonia is utilized. When only nitrate is available, it is reduced intracellularly by nitrate reductase to nitrite and nitrite is reduced by nitrite reductase to ammonium. The uptake of nitrate is light energy dependent, and since the reduction of nitrite consumes energy, microalgae prefer to utilize already reduced nitrogen such as ammonium. High concentrations of ammonium will inhibit uptake of nitrate, because ammonium represses the synthesis of nitrate reductase, while high nitrate concentration inhibits ammonia uptake. The form in which nitrogen is present in a solution is pH and temperature dependent. In pH values higher than 9.25, free ammonia begins to dominate over ammonium. Also, high temperatures favour the formation of free ammonia. Free ammonia is generally toxic to photosynthetic organisms, but the toxicity appears to be reduced in alkalophilic species such as *S. platensis* [52].

Nitrogen starvation induces several physiological changes in algae, deeply altering intracellular composition and growth kinetics [70]. Experiments on various algal species [148] showed that applying stress in the form of limited nitrogen triggers lipid accumulation. However, this stress application also curtails the growth rate [74, 70, 71, 148, 149]. Nitrogen deficiency appears to inhibit the cell cycle, cell division [148], and the production of almost all cellular components [149]. Table 4.2 shows the effects of changes in nitrogen concentration on lipid yield of microalgae.

Table 4. 2: Effect of Changes in Nitrogen Concentration on Lipid Yield of Microalgae

Algae strain	Changes in Nitrate concentration from standard media	Effect on lipid yield	References
<i>N. oculata</i>	Reduced to half and quarter	Almost a duplication of lipid content.	[71]
<i>C. vulgaris</i>	Reduced to half and quarter	Threefold increase in lipid content.	[71]
<i>C. vulgaris</i>	In nitrogen deficient medium	Lipid content became more than two times.	[148]
<i>Nanochloropsis</i> sp.	In nitrogen deficient medium	Almost a duplication of lipid content.	[80]

Generally microalgae accumulate lipid under nutrient limitation when energy source (light) and carbon source (CO₂) are available and when the cellular mechanisms for the photosynthesis are active [80].

Interestingly, nitrogen deprivation also promotes the accumulation of the antioxidant pigment astaxanthin in the green alga *Haematococcus pluvialis* [149, 82]. Both of these adaptive responses help to ensure the cells' survival during times of stress, while lipids serve as energy stores. In this regard, astaxanthin seems to play a role in the protection against reactive oxygen species [149]. Lipid accumulation during nitrogen

deprivation proved to be mainly TAGs, best suited to biodiesel production [70]. In case of *Haematococcus pluvialis*, it was oleic acid in particular, known to be a desirable component of biodiesel feedstock [82].

In the range of 3–20 mM (mili moles) NaNO_3 , the highest lipid productivity was obtained at 5 mM NaNO_3 , but not at 3 mM NaNO_3 where the highest lipid content was achieved [148] which proves that the lipid productivity is the product of both biomass productivity and lipid content [148]. However, with nutrient limitation as the trigger for lipid accumulation, high lipid content and high lipid productivity appear to be in contradiction with each other. It was found that, when the lipid content was highest under nitrogen or phosphorus limitation, lipid productivity was not at its highest [80]. Therefore, the optimal culture conditions for lipid accumulation should be the balance between high biomass and high lipid content [71, 150]. The trade-off between productivity and lipid content stems from the high metabolic cost of lipid biosynthesis [74].

However, genus *Nannochloropsis* is an exception to the rule and has both enhanced lipid content and lipid productivity in an N-deficient environment [74].

c. Phosphorus

Phosphorous is also an essential macro-nutrient for microalgae growth. Phosphorus is an important growth limiting factor, especially in natural environments where phosphorus is limited. Low phosphorus concentration is related to low cell densities. The form of phosphorus, which is utilized by microalgae, is the orthophosphate (PO_4^{3-}) form [52].

The utilization of phosphate is energy dependent and its uptake rate is slower in dark than in light environments [52]. When experimented on *Syndesmus* sp. it was found that phosphorus could be almost 100% removed at any N/P ratios in the culture, but the nitrogen removal was

obviously influenced by the N/P ratios. The optimum N/P ratio, at which both nitrogen and phosphorus are removed efficiently, is strain specific. A decrease in nitrogen removal efficiency was seen due to phosphorous limitation [80].

It was found that under the condition of phosphorus limitation, the total cellular lipid content of starved cells increased, mainly due to the dramatic increase in TAG levels from 6.5% up to 39.3% of total lipids [80].

d. Other macro and micro-nutrients

Besides the essential nutrients mentioned above, microalgae also require for their growth a number of other macro-nutrients in considerable amounts, including Sulphur (S), Calcium (Ca), Magnesium (Mg) and Potassium (K). Micronutrients required include Molybdenum (Mo), Iron (Fe), Nickel (Ni), Copper (Cu), Zinc (Zn), Cobalt (Co), Boron (B), Manganese (Mn) and Chloride (Cl). The presence of ions also influences the uptake of phosphate; namely, lack of ions such as K^+ , Na^+ and Mg^{2+} decreases the phosphate uptake rate [52].

6) Organic substrates:

In general, microalgae are photoautotrophic organisms [52], but some microalgae species grow utilizing organic compounds as energy or/and carbon sources [74, 73, 52] to generate ATP. If organic substrates are present in adequate concentration, the light reactions are automatically deactivated [52].

4.4.Growth Techniques

Due to ability of algae to thrive both on organic as well as inorganic substrates, it can also be cultivated using photoautotrophic (or photosynthetic), heterotrophic, or mixotrophic growth techniques [74, 73]. Photoautotrophic and mixotrophic growths are influenced by light

intensity and by carbon source concentration, while heterotrophic growth is influenced only by the organic substance concentration [52]. Figure 4.2 explains the different growth techniques of algae.

In photoautotrophic, the major lipids are membrane lipids, namely glycolipids (GL) and phospholipids (PL) while in heterotrophic the major lipids are storage lipids, namely neutral lipids [75]. Since NL especially TAG has priority over PL or GL for biodiesel production due to their higher content of fatty acids, therefore, together with the much higher lipid yield, heterotrophic cells are thus more feasible as a feedstock for biodiesel production than photoautotrophic cells [72, 75]. With regard to the oil unsaturation, the iodine value of oil from photoautotrophic cells is over the European standard (120 g I₂/10 g) for biodiesel; on the other hand the iodine value of oil from the heterotrophic cells complies with the existing standard [75].

Heterotrophic cultivation of microalgae also offers many other advantages such as elimination of light requirement, good control of the cultivation process and low-cost for harvesting the biomass because of higher cell density obtained in heterotrophic culture of microalgae [77]. However, heterotrophic cultivation does not utilize solar energy and CO₂ directly, and therefore, it adds considerably to the cost of commercial scale operations. For this reason, heterotrophic cultivation should not be utilized for the large-scale outdoor cultivation of microalgae for biofuel production, but it may be useful for the rapid preparation of seed cultures [73].

Mixotrophy, on the other hand, has several advantages over phototrophy and heterotrophy. Mixotrophic algae can simultaneously drive both phototrophy and autotrophy. In mixotrophic cultures photo-inhibition is reduced, growth rates are improved, and biomass night losses due to respiration are less. Though, it is supposed that in mixotrophic cultures,

the specific growth rate is approximately the sum of the autotrophic and heterotrophic specific growth rate, but the mixotrophic specific growth rate is not the simple combination of the autotrophic and heterotrophic specific growth rate, and the two metabolic processes affect each other [52]. Under mixotrophy, an apparent synergistic effect of heterotrophy and phototrophy was observed. The biomass yield in mixotrophy was more than the sum of them in heterotrophy and phototrophy. In an experiment on *C. globosa*, mixotrophy allowed build up of 3 and 10 times more biomass than heterotrophy and phototrophy respectively. In *C. minutissima* biomass, under heterotrophy and mixotrophy, was respectively 3 and 7 times more than the biomass content in phototrophy. In case of *S. bijuga*, biomass concentration was 5 times more in mixotrophy, and heterotrophy was almost at par with phototrophic conditions [77].

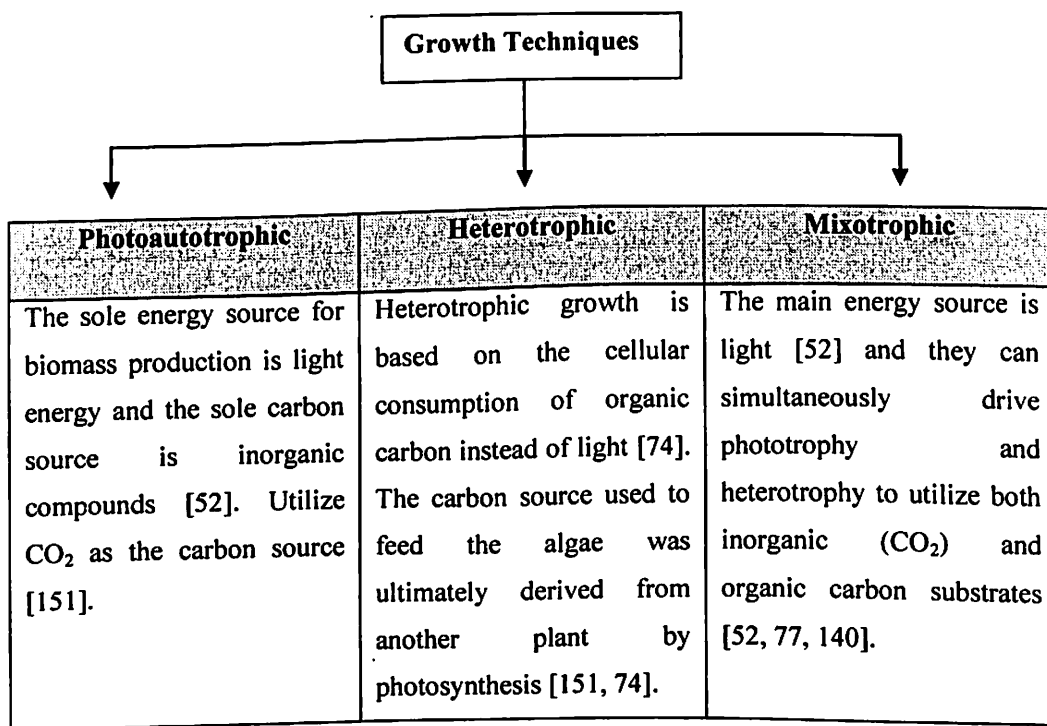


Figure4. 2: Different growth techniques of algae on the basis of energy and carbon source

Another interesting feature of mixotrophy and heterotrophy is the reduction in overall chlorophyll content. Reduction in chlorophyll could be due to reduction in the synthesis of chlorophyll, as carbon is directly incorporated from sugars and photosynthesis cannot occur. Hence the cells adopt down regulation of chlorophyll synthesis and conserve energy. This could also be due to biodegradation of chlorophyll because of metabolic regulation. In view of interference of chlorophyll with transesterification, such reduction in chlorophyll during heterotrophy and mixotrophy adds value to the process of cultivation of algae as feedstock for biodiesel [77] .

4.5.Comprehensive Value chain of Algae

The complete value chain of algae for biofuel production contains following major stages (Figure 4.3 shows the complete value chain of algae for biofuel production):

1. Algae culture & Harvesting
2. Oil Extraction
3. Oil and Biomass residue processing
4. End combustion of green diesel

4.5.1. Algae culture and Harvesting

4.5.1.1.Algae culture

A. Open and Closed systems for algae culture

Algae can be cultivated in open systems, such as large open ponds [74, 54, 152], raceway ponds [74, 75, 54], circular ponds with rotating components for mixing [74], and large bags [74], or in closed systems such as photobioreactors [74, 75, 54].

The main factors in selecting a location for algae cultivation systems are; solar radiation, temperature, land type, carbon and water supply [55]. Algal growth is directly affected by the availability of nutrients, light, the stability of pH, temperature, and the initial inoculum density [35, 132] .

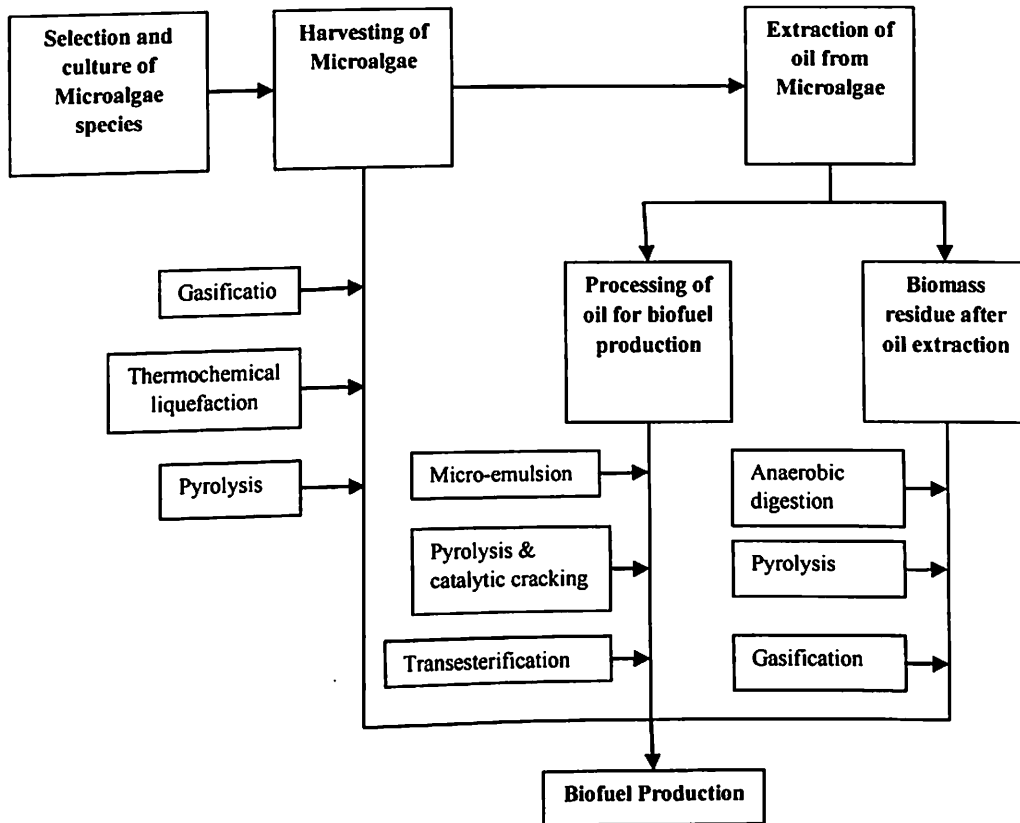


Figure4. 3: Value Chain of Biofuel Production from Algae

Among the various available open systems, raceway ponds have been the most common choice for outdoor algal production, because they cost lesser to build and operate [143] . Raceway ponds feature paddlewheels and baffles to promote mixing [74, 132]. The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with a plastic liner to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around the bends in order to minimize space. The system is often operated in a continuous mode, i.e., the fresh feed is

added in front of the paddlewheel, and algal broth is harvested behind the paddlewheel after it has circulated through the loop. The ponds are operated continuously; that is, water and nutrients are constantly fed to the pond, while algae-containing water is removed at the other end [132].

However, paddlewheel technology cannot meet certain growth requirements, such as, efficient utilization of light and extent of turbulent mixing to attain maximal productivity consistently. For efficient utilization of light, depth of algal ponds should be within certain limits, typically 12–15 cm, resulting in very dilute algal cultures [143]. Thus, optimal pond depth is a trade-off between keeping the pond shallow enough to provide sufficient light to the culture, but deep enough to enhance mixing [74, 132].

Further, in traditional raceways, mixing can be achieved only by increasing the liquid velocity, which requires additional energy input. Thus, one of the challenges in algal cultivation is to maintain adequate mixing and circulation velocity with minimal energy input. Based on conservative energetic analysis of algal biodiesel systems, nearly 28% of the energy input to the process is for algal cultivation, primarily for mixing [143].

Moreover, since the open ponds are prone to contamination, the size of the inoculums needs to be large enough for the desired species to establish in the open system before an unwanted species. To further minimize contamination issues, cleaning or flushing the ponds should be part of the aquaculture routine, and as such, open ponds can be considered as batch cultures [132].

However, when compared to closed photobioreactors, open culture systems have many drawbacks, particularly with regard to the biomass productivity obtainable [69, 143], and control of factors for optimal algae growth [74] [70]. Therefore, much attention has been paid to closed

photobioreactors. But even closed photobioreactors face many challenges which need to be addressed before profitable biodiesel can be produced from algae [75]. Therefore, a major decision to be made is whether to use closed photobioreactors or open ponds [69]. The choice of reactor system depends upon a series of factors where there is a tradeoff between capital costs and rate and reliability of biomass production [54]. Table 4.3 gives a detailed comparison of open and closed systems for algae cultivation.

A. Role of external factors and physiology in Photobioreactors design considerations

For mass scale production optimization of all the steps of microalgae cultivation is required [70], ways to which can very well be known by understanding the physiology of algae. Optimization involves controlling a range of parameters affecting productivity [70] i.e. the various environmental and operational factors, which affect the biology and habitats of the organisms, must be taken into account [52]. The growth of microalgae during the cultivation period can be affected by several factors including: biotic factors such as pathogens and competition by other algae; abiotic factors such as light, temperature, nutrient concentration, CO₂, pH, and salinity; and operational factors such as shear stress, dilution rate, and harvest frequency [73]. Figure 4.4 explains the effects of various external factors on the photobioreactor design considerations.

Therefore, a few important design aspects of photobioreactor include: lighting, mixing, water consumption, CO₂ consumption, O₂ removal, nutrient supply, temperature, and pH. It is important to note that in each category the precise conditions for optimal growth depends on the strain of algae selected for cultivation [74].

Table4. 3: Comparison of open and closed systems for algae cultivation

S.No.	Metrics/Parameters	Open Pond	Close Pond/ PBR
1	Capital investment	Requires less investment [151], and is simple and cheap to build [69, 54, 76] .	Requires high investment [151, 153, 132, 62].The cost of the construction and operation is high [73].
2	Control of growth conditions	Inadequate control of nearly all growth conditions, essential for optimal algae growth [54, 65].	Almost all growth parameters can be easily controlled in these systems [76, 49, 149] .
	Light	There is limited light availability [54] and do not offer the opportunity to optimize the light path [69].	Photobioreactors offer the opportunity to optimize the light path, the extent to which the incoming light is diluted, and also the frequency of the light-dark cycle seen by an algal cell as it travels from deep in the culture to the illuminated surface [69] .
	Temperature	Difficulty of maintaining a constant environment for the culture particularly its optimal growth temperature [69, 144, 54] .	Temperature can be easily controlled [49, 149, 76].
	CO₂ removal	Though CO ₂ requirement is same as photobioreactors [151] but are less effective in CO ₂ removal [49, 149].	More effective for removal of CO ₂ [49, 149].
3	Cell density & Biomass Productivity	Low cell density [69, 73] due to shading effects [69], thus, less growth rate [49, 149, 153].	High growth rate [49, 149, 153], due to shorter light path [151].
4	Space requirement	To avoid shading effect, it requires extensive areas of land for the raceways and substantial costs for harvesting.	Space requirement is less.
5	Electricity Requirement	Electricity required to operate is low in raceway ponds [82].	Electricity required to operate is high [82] .

S.No.	Metrics/Parameters	Open Pond	Close Pond/ PBR
6	Location consideration	The location at which the pond is situated is a critical factor in determining the type of pond selected, algal strain and amount of light for photosynthesis. Due to the lack of control involved with open systems, the pond becomes a function of the local climate, thus the location significantly contributes to the success of the cultivation [76].	Since all the parameters could be controlled, so location consideration is not an important issue.
7	Ease of scale-up	Good [151]	Variable (depends on PBR type) [151]. One of the greatest challenges of closed photobioreactor design to increase reactor size in order to benefit from economy of scale and produce meaningful quantities of biofuel [74]. The scale-up technology is not sufficiently mature for commercial deployment [73].
8	Availability of technology	Readily available [151]	Not demonstrated on large-scale [151].
9	Downstream processing cost	Very dilute cultures [151], result in high dewatering cost [153]	Low because of higher density culture [151]. In addition, photobioreactors produce a much more concentrated algal broth than do open ponds and this reduces the dewatering costs substantially. With tubular photobioreactors, it may be possible to produce dewatered algal biomass at around €4 per kilogram dry weight [153].

S.No.	Metrics/Parameters	Open Pond	Close Pond/ PBR
10	Water use	Water demand is very high [151, 77, 73, 154]. Water in great quantity is lost to the atmosphere by evaporation. Due to evaporation, the concentrations of all species present increase, and this can be a particular problem with saltwater ponds as the salinity could rise above tolerable values [74].	Water demand is low in comparison to open pond. Photobioreactors require 30% of the water demand for the open pond scenario [151].
11	Risk of contamination	Due to high risk of contamination by other microbes, such as bacteria, fungi, yeasts or other micro algal species, which compete with cultivated microalgae and inhibit its growth, it is very difficult to maintain a monoculture of one desired strain of algae [69, 149, 76, 153, 74, 52].	Closed photobioreactors maintain pure culture [75]. PBRs mitigate the risk of invasion by microbes as they are largely closed monoculture systems (though they cannot be sterilized and are not designed to be operated axenically for long periods) [151].
12	Flexibility to strain selection	Low flexibility (open to invasive species) [151]. However, contamination can be eliminated by strains from extreme habitats, with high pH and relatively high ammonia concentrations in which other strains cannot survive and thus inhibit competition [75, 52]. For example <i>Dunaliella</i> , <i>Spirulina</i> [69, 74], <i>Chlorella</i> [74] and cyanobacteria [52] strains grow in such extreme environments. But this also limits the range of microalgae species which can grow well in open pond [76].	High (closed system) [151].

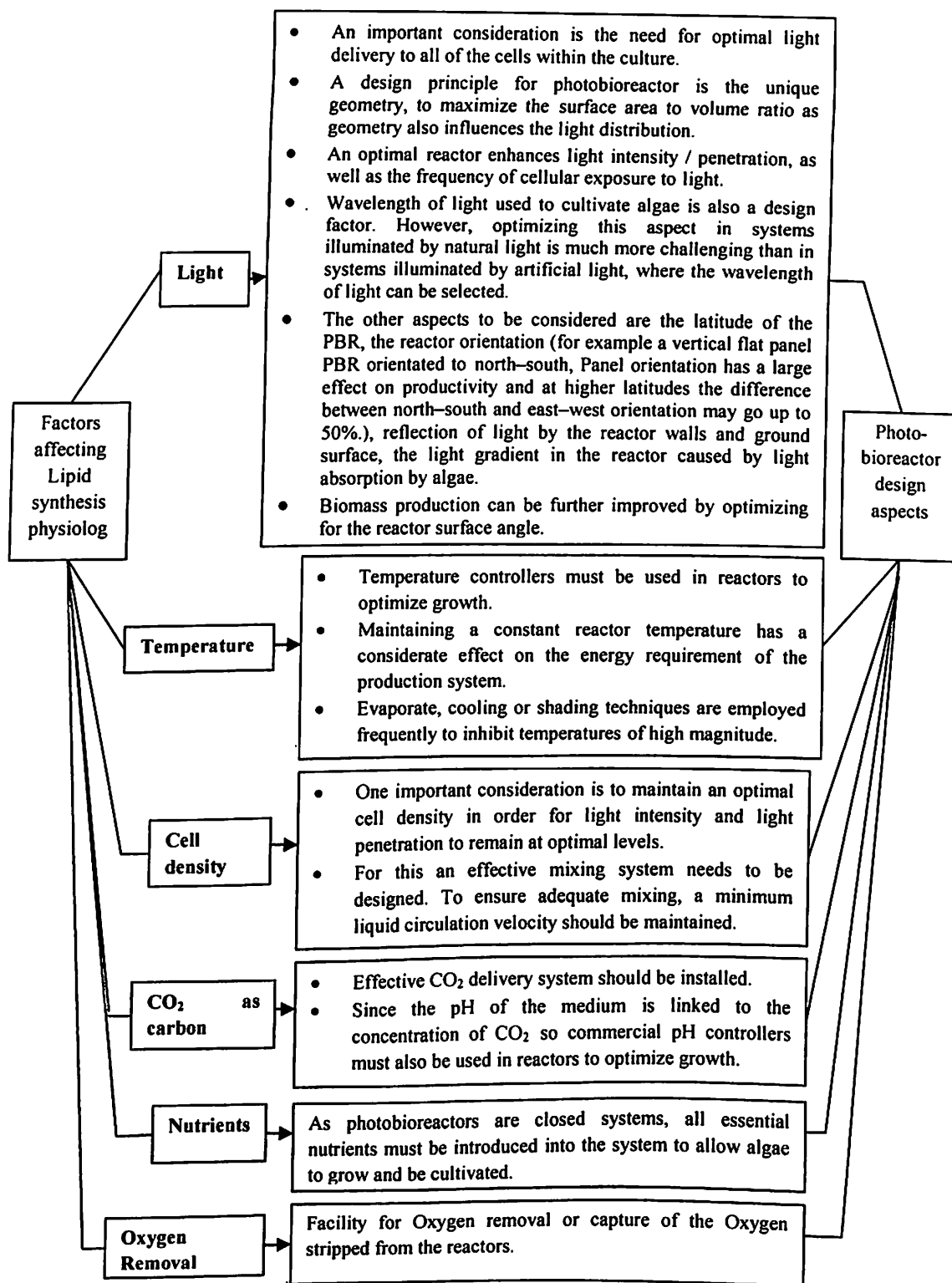


Figure4. 4: Effect of various external factors on the photobioreactor design considerations

B. Closed reactor comparison

Productivity is the most important indicator for success of technology behind a bioreactor [76]. Photosynthetic efficiency (PE) should also be used in conjunction with volumetric productivity when evaluating systems operated under similar climatic conditions [74].

Numerous types of closed photobioreactors have been designed in an attempt to best control the growth factors discussed earlier. The three main categories generally most suitable for large scale cultivation are, tubular/horizontal, column/vertical, and flat plate or flat panel (FP) reactors [74].

Tubular reactors are considered to be more appropriate for outdoor cultivation [74, 76]. They have large illumination surface [76] and over that the curved surface results in the spatial dilution of light, which significantly increases the PE [74]. A tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. The solar collector tubes are generally 0.1 m or less in diameter. Tube diameter is limited because light does not penetrate too deeply in the dense culture broth, that is necessary for ensuring a high biomass productivity of the photobioreactor [132]. Tubular reactors can be configured in a vertical, horizontal or inclined plane. The major differences in the configurations are that the vertical design allows greater mass transfer [155], under high light intensity vertical reactors experience less photo-inhibition, and under low light intensity vertical orientation captures more reflected light. A vertical orientation also requires less land area [74], while the horizontal reactor is more scalable, but requires a large area of land [76].

On the other hand, flat-plate photobioreactor is broadly in use due to narrow light path, which helps maintaining higher cell densities by more than an order of magnitude compared to other photobioreactors [76], large

illumination surface area allows high photosynthetic efficiency [132]. Additionally, these types of reactors are favorable due to low power energy consumption [74, 76, 82], high mass transfer capacity [74, 76], reduction in oxygen build up [76, 132] due to shorter oxygen path, which results in FP reactors having lower accumulation of dissolved oxygen concentration than horizontal reactors [74, 132]. However, one major drawback for FP reactors is that cell damage may occur because of high stress resulting from aeration, a problem that has never been reported in tubular reactors [74].

C. New technologies for enhanced growth, productivity and cost reduction for biofuel production from algae

1) *Lipid enhancement technologies*

a. Photosynthesis–fermentation model for enhancing lipid production

A photosynthesis–fermentation model was proposed to merge the positive aspects of autotrophs and heterotrophs. This model involved the photoautotrophic growth of *C. protothecoides* to increase biomass and subsequent heterotrophic fermentation to maximize cell density and lipid accumulation. Compared to typical heterotrophic metabolism, 69% higher lipid yield on glucose was achieved at the fermentation stage in the photosynthesis–fermentation model. CO₂ fixation takes place twice (double CO₂ fixation) in both photosynthesis and fermentation stages, which enhances carbon conversion ratio of sugar to oil, and thus, provides an efficient approach for the production of algal lipid [156].

b. Genetic engineering

The ASP recognized that the key to unlocking profitable commercialization of microalgae lies not only in species selection and optimal cultivation, but also in genetic and metabolic engineering. In regard to genetic engineering, these species are amenable to firstly,

nuclear transformation, necessary for metabolic control, secondly, chloroplastic transformation, for high levels of protein expression, and thirdly, more straightforward approaches to genetic modification compared to higher plants [149].

Knowledge of the function of a gene (or a set of genes), is the first step towards solving the puzzle of metabolic pathway [157]. Manipulation of metabolic pathways can redirect cellular function toward the synthesis of preferred products and even expand the processing capabilities of microalgae [149, 157]. As an alternative to manipulating culture conditions, metabolic engineering allows direct control over the organism's cellular machinery through mutagenesis or the introduction of transgenes [149].

Genetic engineering could enhance fuel production in a variety of ways, including improving photosynthetic efficiency, increasing biomass productivity, increasing cellular lipid content, and improving temperature tolerance of algae to reduce cooling expenses. In addition, genetic engineering could increase algal cell's tolerance to light saturation, photo-inhibition, and photo-oxidation [74].

In the longer run algal species engineered to use atmospheric nitrogen, instead of nitrogen fertilizers that are now required, will be a great step forward, as production of nitrogen fertilizers is heavily dependent of petroleum [153].

In the case of algae, only for a limited number of species, the whole genome sequence has been generated, or is currently being generated in one of the algal genome projects. Genomics of *Chlamydomonas reinhardtii*, a unicellular freshwater chlorophyte, has probably been best studied [157].

c. Two stage cultivation process

It was found that the lipid content more strongly impacts the economics than the growth rate does: for the open pond case, when lipid content is either doubled or divided in half, the net cost impact is twice as large as a similar adjustment in growth rate. This is because increasing the areal growth rate merely decreases the size of the growth system (and associated costs) relative to the amount of algal biomass being grown, while an increase in the oil content actually decreases the amount of algal biomass that must be produced to achieve a set oil production target (thereby reducing all downstream processing costs due to lower equipment throughputs). Although the photobioreactor case also shows greater cost sensitivity to oil content than to the algae growth rate, the cost savings is less pronounced; this is due to the photobioreactor growth system exhibiting a much larger fraction of the overall cost, and thus less savings opportunity to be seen with lower downstream throughputs. In either case, the key implication for research going forward is that it is economically more beneficial to target improvements in lipid content than algal growth rate. Given that, in reality there is typically a tradeoff between the two parameters [151].

However, an optimal two stage cultivation method i.e. growth and lipid accumulation is under investigation [150]. One such two-stage cultivation process for a high starch yield from *C. vulgaris* strain : a first cultivation stage using a N and Fe supplemented medium to attain a maximum growth rate and concentration of biomass, followed by a second stage, that involves cell cultivation in a N and Fe-free medium for a few days [145].

2) Cost reduction technologies

Currently the social cost of producing algal biodiesel at 52.3 € GJ⁻¹ is higher than rapeseed biodiesel (36.0 € GJ⁻¹) and fossil fuels (15.8 € GJ⁻¹)

[55]. Changes in microalgal production cost will have a large impact on the competitiveness of microalgal biodiesel [62].

A few cost effective production technologies could be biofuels production from microalgae, coupled with flue gas CO₂ mitigation, waste water treatment and production of high value chemicals [60, 158], recycling culture medium which will reduce the amount of water needed and lower the operating costs [73]. A recent study on water footprint of pond culture with and without water recycling revealed that water recycling approach can produce almost five times more energy per liter of water used [65].

a. Use of alternative and cheap nutrient source

Even though the biomass and lipid productivities during mixotrophic growth are significantly higher compared to autotrophic growth, the cost of glucose could contribute about 80% of the total cost of growth medium making mixotrophic algae cultivation economically unfeasible. Cheap carbon sources, such as, crude glycerol from biodiesel industry, sugars from industrial and agricultural waste, cellulosic materials and cane molasses offer great promise for the cultivation of mixotrophic algae [77]. A few studies have thus attempted to find less expensive organic carbon sources; corn powder hydrolysate was tested instead of sugars, with favorable results in terms of productivities of the resulting biomass and lipid content [140].

In an experiment on *Chlorella protothecoides*, to further increase lipid yield and reduce biodiesel cost, sweet sorghum juice was investigated as an alternative carbon source to glucose. The results indicated that sweet sorghum juice could effectively enhance algal lipid production, and its application may reduce the cost of algae-based biodiesel [159]. Another alternative carbon source could be glycerol but it was seen that the heterotrophic growth with glycerol as the carbon resource showed low level of cell concentration and lipid production, compared to that of

glucose [141] . However, in one experiment *C. curvatus* has been seen to produce a high biomass density, biomass yield, and cellular lipid content with glycerol [130].

CO₂ as a nutrient represents one of the most costly components in the cultivation of microalgae [74]. Supply and transfer of CO₂ accounts for nearly 1/3 the cost of algal cultivation [143]. Therefore, it may be necessary to supply it discontinuously. Using hollow – fiber membranes may improve mass transfer and reduce costs [74]. Even a system, that couples a waste CO₂ source with the cultivation of CO₂ fixing organisms, can not only reduce cultivation costs but also reduce or remove CO₂, an environmental pollutant [52]. Flue gas from power plants [52] is desirable source of CO₂ [74, 52]. Productivities and photosynthetic efficiencies were very similar under conditions of pure CO₂ versus flue gas. Moreover, the presence of NO_x and CO in flue gas did not inhibit the growth of microalgae [74].

The other source could be sodium bicarbonate (NaHCO₃) following the recovery of CO₂ by means of NaOH from combustion flue gasses. In the agro-industrial sector, CO₂ can be provided by using biogas from the anaerobic digestion of agro-industrial waste, which contains 30–45% CO₂ or by using CO₂ emissions from the aerobic composting of animal manure. In the case of biogas from anaerobic digestion, there are two aims: on the one hand to provide the algal culture with carbon and on the other hand to purify the biogas by removing CO₂ from it [52].

b. Culture in waste water

Microalgae biomass contains considerable amounts of proteins and, on the basis of biomass composition the quantity of nitrogen (N) required as fertilizer, is estimated to be 8–16 tons N/ha, which means that microalgae production involves enormous amounts of N fertilizers. The use of such large quantities of fertilizer for microalgae cultivation raises questions

about their environmental impact [52]. A variance analysis has shown that if nutrients are added as fresh chemicals to the pond, the overall energetic balance may be negative [160]. Furthermore, the use of fertilizer contributes to 10-20% of the cost of algal biomass production [77]. Algae also have a large water foot print in terms of energy returned on water invested (0.5–1.1 for enclosed and 0.05–1.0 MJ per liter for open ponds) [77]. Water consumption represents a major challenge for future biofuel production [65].

Algal cells have an ability to assimilate organic carbon (heterotrophic growth) as well as inorganic nutrients, such as; nitrogen and phosphorus from the wastewater [147] (industrial, municipal and agricultural [78, 154]. Therefore, developing algae production approaches, that effectively use wastewater resources, can minimize both water and nutrient requirements will help reduce resource constraints [50]. Phycoremediation of wastewaters has been suggested for a number of by-product applications for the biomass generated [154]. The coupling of advanced wastewater treatment and biodiesel production based on microalgae is a promising technology [80] . It provides advantages such as, cost effectiveness, low energy requirements [79] and algae receives an inexpensive medium rich in required nutrients and the wastewater is further treated in the process [80, 161].

Many experiments have already made use of wastewater from carpet mill effluents [78] and digested dairy manures from a local dairy farm [147], swine slurry [162], poultry litter, slaughter house wastes, municipal waste and wastewater, compost plant/landfill leachate and effluents from anaerobic digesters [77]. Wastewater sources are rich in the organic nutrients, but are opaque creating an issue with light penetration to support algal growth. Therefore, growth of most of the photosynthetic algae is adversely affected in these waters. There are a number of algae that are

facultative heterotrophic and prefer, if available, an organic carbon substrate over fixing carbon dioxide. Some algae are mixotrophic and can simultaneously drive phototrophy and heterotrophy to utilize both inorganic (CO₂) and organic carbon substrates, thus leading to an additive or synergistic effect of the two processes that enhances the productivity, and in turn capability of microalgae to grow in wastewaters. The mixotrophy could overcome problems associated with the growth of phototrophic algae viz. light limitation at high cell densities and dark colored (opaque) wastewaters [77].

Apart from being opaque, due to variation in the composition of wastewater, only specific algae may perform to their potential [78]. The most widely studied microalgae species for nitrogen and phosphorus removal are *Scenedesmus*, *Chlorella*, and *Spirulina* [80]. The level of salinity also influences the overall productivity as well as individual production rates of lipids and carbohydrates in each strain of algae [80, 161]. Further, in saline water culture systems periodic supplementation of freshwater is necessary, as the culture water system (pond or bioreactor) can accumulate excessive amounts of salt due to evaporative loss [65].

Marine algal strains, like *Gloeocystis* and cyanobacterial species such as *Anabaena* and *Limnothrix*, can grow very well in carpet industrial wastewaters, suggesting the presence of some unique osmotic adjustment and regulation mechanisms to tolerate hypo-osmotic stress conditions [78].

One study indicated that *Nannochloropsis* sp. cannot tolerate lower salinity or freshwater for long. This finding was consistent with other reports which showed that marine microalgae could tolerate a wide range of salinity but could not survive prolonged exposure to low salinity or freshwater due to osmotic stress [158].

According to Razon et al, elimination of the fertilizer reduces the overall energy deficit, and as result, increases the net energy ratio. However, it may be difficult to maintain the monoculture in an open pond if primary treated wastewater is used [82] . Extensive water treatment equipments need to be installed to prevent the bacterial and other microbial flora in the waste water to interfere with the algal biomass production [65].

Operations such as passing wastewater through filters and pH adjustment before inoculation may offset any savings in fertilizer [82] and can increase the cost as well energy input of the production facilities. One way to tackle this issue is to provide high government incentives to production units which use nutrient-rich waste water (e.g., municipal, carpet mills, dairy farms, treated industrial waste water) for algal biomass production [65].

Therefore, it is essential to select strains capable of growing in variety of wastewaters, improving water quality and simultaneously producing feedstock for biofuels viz. biodiesel, bio-methane and bio-ethanol [78]. Further microalgal production has been suggested as economically feasible only by combining this technology with other ones, such as; co-production of food, high-value added products or fertilizer, green house gas abatement or wastewater treatment [162].

c. Long term Outdoor cultivation through perfusion culture process

In one study, a novel outdoor cultivation process was developed that utilizes the thermal plume instead of seawater for both nutrient and heat sources for the places where the temperatures are extremely low. The thermal plume used in this study was seawater that had been used as the coolant water in a nuclear power plant. This novel design takes advantage of the thermal energy of the discharged water, and resolves the problems associated with outdoor large cultivation. Using this unique culture system, *Chlorella minutissima* was grown to produce lipids for biodiesel

under long-term outdoor cultivation conditions. Since the thermal plume was originally obtained as sea water, and was only used to cool down the nuclear tower, there was only about a 0.5% to 1% difference in $MgCl_2$ and $NaCl$ but there were no significant differences in the composition of other minerals [144].

4.5.1.2. Microalgal Harvesting

After lipid synthesis, for further processing of microalgal biomass to biofuels requires water removal from the algal culture. Harvesting alone accounts for 20–30% of the total production cost [117]. Therefore, for mass biodiesel production, efficient harvesting method is very essential [140, 163].

Selection of appropriate harvesting method is of great importance to the economics of biofuels production. The appropriate harvesting method depends strongly upon the characteristics of the microalgae chosen [117], viz. the density and size, respectively, as well as the specifications of the desired product [140]. An optimal harvesting method should be species independent, should use less chemicals and energy, and if possible, also release intracellular materials [164].

Dewatering small sized and initial dilute cultures of microalgae is one of the major challenges obstructing the emergence of algae based fuels [163]. Moreover, the cells normally carry negative charge and excess algogenic organic materials are responsible for their stability in a dispersed state [140]. All these factors make economical biomass harvesting difficult, which requires high costs [117, 140, 164] and energy [163].

Many harvesting strategies like, centrifugation, sedimentation, flocculation, flotation and micro-filtration, can be used to harvest microalgae [117], electrophoresis [140, 164] and any combination of these [117]. Microalgal harvesting can generally be divided into a two-step

process, including: The first step being bulk harvesting during which microalgal biomass is separated from the bulk culture. This step concentrates biomass to 2-7% dry weight. Then the second step, called thickening, further concentrates the algal slurry. Thickening is more energy intensive than bulk harvesting [164].

A. Harvesting Technologies

1) Centrifugation

It is a harvesting method which involves centripetal acceleration to separate algal culture into regions of greater and less densities, thereafter the algae and water is separated by draining the excess medium. Centrifugation can also be followed by centrifugation to separate the supernatant [76]. Laboratory centrifugation tests, conducted on pond effluents, have shown that about 80 to 90% microalgae {few have also shown it to be 95 to 100% efficient [76]} can be recovered within 2-5 minutes. Rapid and efficient nature of this method makes it one of the most preferred methods for harvesting of algal biomass. However, high energy intensive nature of this method makes it economically unfeasible [117]. Moreover, processing large quantities of culture consumes a lot of time, and exposure of microalgal cells to high gravitational and shear forces can also damage them [164].

2) Gravity sedimentation

It is highly energy efficient method [117] and is commonly applied for separation of microalgae from water. Microalgae like Spirulina, which settle well by virtue of their high density and large size, can successfully be separated by sedimentation method. The rate of sedimentation also depends on the induced sedimentation velocity. Microalgal harvesting can be enhanced by sedimentation through lamella separators and

sedimentation tanks [164]. However, it is a very slow process [163]. Sedimentation rate can be enhanced by addition of flocculants to the system [164]. But then flocculants additions have their own pros and cons, which have been discussed later, in Sub-section 4 of the present section.

3) Filtration

In this method algae culture runs through filters, which hold back algae and allow the water to pass through them. The process takes place continually until filters contain a thick paste of algae. Microfiltration, dead end filtration, vacuum filtration, pressure filtration, ultra filtration, and Tangential Flow Filtration (TFF) are a few different filtration forms [76].

Larger algae can effectively be recovered by vacuum filtration in combination with filter aid, while micro-filtration or ultra filtrations are effective in recovering smaller algae. However, vacuum and micro-filtration are costly, that notwithstanding biomass pumping requirement makes them energy intensive. They also require frequent membrane replacements, due to fouling [117].

Another filtration method, called tangential flow filtration, is a high rate method. About 70-89% algae was recovered using this method. Another advantage of TFF is that it maintains the structure, properties and motility of the filtered microalgae [164]. Considering the output and initial feedstock concentration, according to recent studies TFF and pressure filtration are energy efficient harvesting methods [76]. However, membrane replacement and pumping limit large scale harvesting by TFF [164].

Issues like back mixing make simple filtration methods, such as dead end filtration, inadequate for dewatering microalgae culture. However, when used along with centrifugation, give better separation. Filtration methods,

in spite being an attractive dewatering option, have extensive running costs and hidden pre-concentration requirements [76].

4) Flocculation

Flocculation is a process in which solute particles in a solution join together to form aggregates called floc [163], which helps in settling [164, 165]. Conventional flocculation works by charge dispersion mechanism [117]. Microalgae carry a negative charge [76, 164], as a result of adsorption of ions originating from organic matter and dissociation or ionization of surface functional groups [164]. This common negative charge does not let them self-aggregate within suspension [166]. Microalgae can be successfully harvested only by disrupting this stable system [164]. Chemicals called flocculants help to counter this negative charge on the surface of algae [166]. Flocculants displace the negative charge and allow aggregation microalgae cells. Flocculation when combined with sedimentation or filtration increases harvesting efficiency [117].

a. Autoflocculation

Autoflocculation is the spontaneous aggregation of particles, resulting in sedimentation of the microalgae [117]. At elevated pH, CO₂ consumed during photosynthesis, precipitates in the form of carbonate salts with algal cells [167]. Carbon limitation or certain abiotic factors can induce autoflocculation [117]. Hence, cultivation of algae in sunlight, with limited CO₂, auto-flocculates algal cells, and thus, helps in harvesting. NaOH can be added to stimulate autoflocculation, as it can help to obtain the desired pH level [164].

Enhancing natural aggregation/bio-flocculation of microalgae for simple gravity settling could prove to be a promising method in terms of effluent quality (total suspended solids) as well as economics of algal biomass

recovery for biofuel production [165]. Large colonies (50–200 μm) are often formed by algal species like *Actinastrum*, *Micractinium*, *Scenedesmus*, *Coelastrum*, *Pediastrum* and *Dictyosphaerium*, which dominate high rate algal ponds used for water treatment [168]. However, more research is needed in this area and the exact mechanisms behind bio-flocculation have yet to be investigated [165].

b. Chemical flocculants

It can be applied over a wide range of microalgal species [164]. In spite of less operating cost, the chemicals added in the process can be hazardous to the environment [76]. According to the nature of the chemicals flocculants can be divided into inorganic and organic/polyelectrolyte flocculants [169]. Table 4.4 shows the comparison between inorganic and organic flocculants.

The negative surface charge on microalgal cells can be neutralized or reduced by addition of inorganic flocculants of iron or aluminium [164]. These multivalent salts vary in effectiveness due to their ionic charge [166]. Flocculants with high charge density are more effective [163]. Alums are very effective in flocculation of algal biomass during wastewater treatment but they may also later on hinder the oil extraction process of certain algal strains [117].

Organic flocculants or polyelectrolytes are cationic polymers, which physically link cells together. The aggregation strength of the polymer depends on certain specific properties. The organic flocculants to be used will depend on the charge on the algal cells, pH and biomass concentration of the algal culture [76]. High biomass concentrations help frequent cell-cell encounter thus help flocculation. Mixing at low level can also perform the same function as that by high biomass concentration, by bringing cells together. But at the same time if shear forces are high it can also disrupt the flocs. In addition to all the factors mentioned before, functional groups

on microalgal cell walls are important, because they stimulate the formation of negative charge centers on the cell surfaces [164].

Table 4. 4: Comparison of Inorganic and Organic flocculants

Parameters	Inorganic flocculants	Organic flocculants
Nature of flocculants	Multivalent salts.	Polyelectrolytes/polymers.
Key characteristics of an effective flocculant	Increasing molecular weight and charge on the polymers has been shown to increase their binding capabilities [163, 164, 76].	Flocculants that have a high charge density are therefore more effective.
Sensitivity to pH	Coagulation using inorganic coagulants is highly sensitive to pH level [164].	Coagulation using organic coagulants is less sensitive to pH [164, 76].
Dosage of flocculants required	A large concentration of inorganic flocculants is needed in order to maintain flocculation efficiency [76, 164], thereby producing a large quantity of sludge [165] and may contaminate the end product (for example addition of aluminium and iron salts).	Lower dosages of organic flocculants are required for flocculation process [76, 164], thus producing less quantity of sludge and lesser contamination probabilities.
Applicability	Although some coagulants may work for some microalgal species, they do not work for others.	Wide range of applications [164, 76], i.e. they can be used for larger number of microalgal species.

Cationic polyelectrolyte gave better flocculation, whereas no flocculation was found with the anionic polyelectrolyte. Chitosan, commonly used for water purification, can also be used as flocculent [76]. They are biodegradable and do not contaminate the microalgal biomass [164]. However, it is too expensive to be used for economic algae. Further, brackish or saline water requires an additional chemical flocculant to induce flocculation [76]. Polymeric flocculants are generally ineffective in flocculating marine microalgae (with salinity up to 36 g/L), but reducing the salinity improves the flocculation for all cationic polymers [163].

c. Combined flocculation

It is a multistep process, which involves more than one kind of flocculants. During a study on marine microalgae, it was found that combination of polyelectrolytes with inorganic flocculants or ozone oxidation followed by addition of flocculants are effective methods of flocculation [164]. Similarly, flocculation, when followed by sedimentation or filtration is a cost effective method of harvesting, as it consumes less power [117].

5) Electrolytic process

Electrolytic process or electro-coagulation takes place in three steps [164]: Firstly, the sacrificial electrode undergoes electrolytic oxidation to generate coagulants. Then particulate suspension is destabilized and breaking of emulsion takes place, after which the destabilized phase again aggregates to form flocs. In this process microalgae move towards anode, where their surface charge gets neutralized and then the microalgal cells form aggregates. This process is highly efficient and removes about 80-95% of algal cells [164].

6) Flotation

Laboratory trials have shown that flotation is suitable for harvesting small, unicellular algae [117]. Flotation is a gravity separation process in which air or gas bubbles attach to solid particles and then carry them to the liquid surface. Flotation has been found to be more effective and beneficial than sedimentation, in harvesting microalgae. In flotation the algae move upward than downward in case of sedimentation. This favors flotation, as mass cultivation of algae requires high overflow rate. Particles with a diameter even less than 500 μm can be captured by flotation. On the basis of bubble size, flotation can be divided into dissolved air flotation (DAF), dispersed flotation and electrolytic flotation [164].

a. Dissolved air flotation

In this process pressure of a water stream, pre-saturated with air at excess pressures, is reduced to bubbles of 10–100 μm in size. It uses chemical flocculants like alums and autoflocculation is achieved by photosynthetically produced oxygen, with bubbles to separate microalgae biomass. DAF removes microalgae more effectively than settling and moreover, settling requires flocculation pre-treatment [164].

b. Dispersed air flotation

In dispersed air flotation bubbles, an air injection system and a high speed mechanical agitator, form bubbles of 700–1500 μm size. The bubbles act by interacting with the negatively charged surfaces of algal cells [164]. The process can be made more effective by reducing the charge of the air bubbles, by addition of cationic surfactant or any other chemical which can give a net positive charge [117].

Ozonation-dispersed flotation is another method of creating charged bubbles. When used to harvest *C. vulgaris*, its cells showed an increase in the lipid content (from 31% to 55%) in the flotation stage. Ozone also causes lysis of the cells and releases biopolymers. These biopolymers act as coagulating agents and enhance the separation method as well as the lipid extraction process. Contamination in open ponds may prove challenging for ozonation-dispersed flotation. Moreover, it is an expensive process [117].

7) Electrophoresis techniques

It is a harvesting process, which does not require any chemical addition. An electric field makes charged algae to go out of the solution. Hydrogen, generated by electrolysis of water, sticks to the microalgal flocs and carries these to the surface. Environmental compatibility, safety, versatility, selectivity, energy efficiency, and cost effectiveness are a few

benefits of using this method. Fouling of the cathodes and systems getting damaged by high temperatures, as a result of high power requirements, are the main disadvantage of this method [164].

B. Integration of different harvesting techniques

Harvesting of microalgal biomass is one of the bottlenecks for biofuel production from microalgae [73]. It can be inferred from the above different harvesting methods that each of these have their own advantages and disadvantages, and it also shows that efficiency of one method can be increased if integrated with another method, i.e., integrating sedimentation with flocculation [164]. Another such efficient method which integrated electro-flocculation with dispersed-air flotation was used for harvesting *Botryococcus braunii* [73]. Thus, integration of different methods is an efficient technology for harvesting microalgae.

While undertaking research on harvesting, oil extraction, and refining processes for biofuel production from microalgae, nature and type of microalgal strain should be considered. Shape of algal cells, cell wall structure and oil composition vary from one algal strain to another, even two different cultures of the same strain are not similar in nature [73].

4.5.2. Oil extraction

Harvesting is followed by oil extraction. The extracted oil is then converted into biodiesel [117]. Direct transesterification of dried biomass has also been reported in some microalgal and fungal species [53].

Oil extraction is done by physical methods and chemical methods in the form of solvent extractions, or a combination of the two. Method used for extraction should be fast, easily scalable, effective and should not damage the extracted lipids [117].

Not every oil fraction is suitable for biodiesel production, and moreover, sometimes non-lipid contents also get extracted along with lipid contents. Therefore, the extraction process chosen should not only be lipid specific

but should also be selective towards desirable lipid fractions (neutral lipids containing mono, di, and trienoic fatty acid chains) [140]. Removing water, beyond 10–30 wt% dry biomasses, is energy intensive [140]. Therefore, if a lipid extraction methodology can be applied to a wet feedstock, it can save a lot of energy [170].

A. Pre-treatment: Cell disruption methods

Depending on the type of biomass, sometimes before oil extraction, pre-treatment of biomass may be required. This is generally not required for extraction from wet biomass, as solvents normally rupture the cells. Cell disruption is one such pre-treatment method. Cell disruption method will depend on the type of biomass, state of biomass and scale at which it needs to be applied at [117].

Various cell disruption methods are; microwave application, sonication, bead beating, autoclaving [117, 140], grinding, osmotic shock, homogenization, freeze drying [117] and 10% (w/v) NaCl addition [140].

Microwaves generate high frequency waves, which shatter cells via shock induction. It was recently suggested to be an efficient method for disruption of oil containing plant cells. Sonication, widely used for microbial cells, disrupts both cell wall and membrane by cavitation effect. While in bead-beating, high-speed spinning with fine beads causes mechanical disruption of the cell. Bead-beating has gained success, on both bench and industrial scales [140].

Various methods, including bead-beating, sonication, autoclaving, microwave application, and 10% (w/v) NaCl addition, were experimented for disruption of *Botryococcus* sp., *C. vulgaris* and *Scenedesmus* sp.

Bead-beating and microwave were found to be most efficient specifically in case of *Botryococcus* sp., whereas sonication had the lowest efficiency. On further experimentation on *B. braunii*, not only sonication but bead-

beating was also found to be better than other methods like french pressing or lyophilization, and homogenization. Despite its high efficiency, the only drawback of bead-beating is that, it is not easily scalable.

When experimented on *C. vulgaris*, microwave oven and autoclaving were found to be most efficient methods, while bead-beating turned out to be the worst method. Microwave can also easily be scaled-up. For *Scenedesmus* sp., the microwave oven method gave the best result, whereas the efficiencies of sonication, bead-beating, and osmotic shock methods were almost similar. In case of *C. vulgaris* and *Scenedesmus* sp, osmotic shock in spite of being simple and showing results similar to bead-beating, requires longer treatment time (48 hours). Therefore, cell disruption efficiency for lipid extraction in microalgae differs from species to species and also depends on the employed extraction method [140].

B. Oil extraction methods

1) Solvent extraction method

In this method, extraction of algal oil is done with the use of solvents. The choice of solvent will depend on the species of microalgae chosen. Further, the solvent should be inexpensive, non-toxic, volatile, non-polar and poor extractor of other non-lipid components of the cell.

Lipids have different kinds of interactions which also need to be broken for effective extraction. Non-polar organic solvents disrupt hydrophobic interactions between non-polar/neutral lipids; polar organic solvents like alcohols disrupt hydrogen bonding between polar lipids. Strong ionic forces, if present, can be disrupted by shifting pH towards more alkaline [117].

Solvents like chloroform [170], benzene and ether have already been tried, but hexane has gained more popularity as a chemical for solvent extraction and it is also relatively inexpensive.

Hexane solvent extraction can either be used alone, or it can also be used in combination with the oil press/expeller method. After extracting oil with expeller, the oil from the remaining pulp can be extracted by mixing it with cyclo-hexane. Cyclo-hexane dissolves oil into it and the pulp is filtered out. Then, with the help of distillation oil and cyclo-hexane are separated. The two methods (cold press & hexane solvent), when used in combination, can extract more than 95% of the total oil contained in the algae. The disadvantage of using solvent extraction is related to dangers involved in use of the chemicals. Benzene is a carcinogen, while chemical solvents can also lead to explosion hazard [171]. Hexane though has been found to be less efficient than chloroform, is less toxic, has low affinity towards non-lipid contaminants, and has higher selectivity for neutral lipid fractions [170].

2) Supercritical carbon dioxide (SCCO₂) extraction

It is one of the promising green technology methods, which has the potential to displace the traditional organic solvent lipid extraction methods. A typical extraction unit consists of a feed pump for compression and transportation of liquid CO₂ to the extraction vessel, which is installed inside an oven module, and a heated micro-metering valve to depressurize incoming SCCO₂. Once the oven is heated, the compressed CO₂ enters the heated oven, in a supercritical state and extracts lipid from the microalgae.

Once completely decompressed, CO₂ evaporates as gas to the ambient, and forces the extracted lipid to precipitate out and collect in the adjoining glass vial [170]. Supercritical carbon dioxide has high solvating power and low toxicity. Intermediate diffusion/viscosity properties of the fluid

lead to favorable mass transfer equilibrium and this process produces solvent-free extract. High infrastructure and operational cost associated with this process are its main disadvantages [170].

C. Algae oil properties and composition

On average oil content of algal cells varies between 1% and 70%, but can reach 90% of dry weight under certain conditions [135]. The accumulated oil in almost all microalgae is mainly triglyceride (>80%) [135].

Algal oil contains saturated and monounsaturated fatty acids [133]. The average fatty acid contents of the algal oils are 36% oleic (18:1), 15% palmitic (16:0), 11% stearic (18:0), 8.4% iso-17:0, and 7.4% linoleic (18:2) [172, 133], where the number before the colon represents the total number of carbons; the number after the colon is the number of double bonds [139]. But most microalgal oils differ from plant oils because they are rich in polyunsaturated fatty acids [173, 135, 134, 133]. This feature limits the algal species that can be used [173], as it makes algal oil susceptible to oxidation during storage and reduce their acceptability for use in biodiesel production and performs poorly compared to its mainstream alternative [135, 133].

Highly saturated fatty acids give an excellent cetane number and oxidative stability to biodiesel [152]. Therefore, not all algal oils are adequate for making biodiesel, but suitable oils commonly occur [154]. *Chlorella* species are found to contain only saturated fatty acids and thus are suitable for biodiesel production [152]. *B. braunii* is also suitable for biodiesel production due to its high proportion of oleic acid, a mono unsaturated fatty acid [140].

4.5.3. Oil & Biomass processing

4.5.3.1. Oil processing

Presently, the common procedures of biodiesel preparation from microalgae involve the following steps: the extraction of lipids from microalgae, the removal of excess solvent, and the production of biodiesel catalyzed by homogeneous or heterogeneous catalyst [174]. Here in the case of algae oil again similar kind of engine problems occur with the direct use of neat algae oil, and thus can be overcome with the three possible processes as suggested in case of neat *Jatropha* oil in Chapter 3.

A. Oil processing methods

1) Conventional methods of oil processing

Similar to *Jatropha* oil processing, microalgae oil can also be processed via methods like micro-emulsion, pyrolysis and catalytic cracking, transesterification and hydrogenation of algal oil. Details for all the methods are given in Chapter 3, while a few points specific to processing of algal oil are explained below.

As mentioned earlier in case of *Jatropha*, in order to deal with high free fatty acids, acid catalysts could be used in conjunction with base catalysts (two stage process). Since algae oil has high free fatty acid content, acid catalysts should be the method when using oils extracted from microalgal biomass [110]. In case of algal oil, here again the two stage process i.e. acid catalysts are used in the primary stage to convert free fatty acid to methyl esters, followed by a base catalyst process to convert the remaining triglycerides to methyl esters [110].

Though the base catalyzed transesterification method is also used but the two stage process has been found to give maximum yield. For example, a maximum yield of 10 mg of FAME was obtained from 250 mg of lipids in

the following conditions: 0.6 N hydrochloric acid–methanol catalyst for 0.1 hour at 70 °C, using the lipids extracted from *Chaetoceros mulleri*. In comparison, only 3.3 mg of FAME was produced when sodium hydroxide was used as the catalyst, at the same conditions that gave maximum FAME yield [110]. In case of enzymatic transesterification of algal oil, here again if the lipase is immobilized, it can be easily separated from the reaction mixture [110, 117] by filtration, or when the lipase is in a packed bed photobioreactor (PBR) [110], no separation is necessary after transesterification [110, 117].

In an experiment on a yellow green algae, *Nannochloropsis oculata* (preferred due to its high fatty acid content), effect of CaO and MgO has been studied to avail the benefits offered by heterogeneous catalyst as discussed for *Jatropha*. Since CaO and MgO are basic catalyst, they were not found to be active for transesterification of microalgal lipid which otherwise are the most active catalysts for transesterification of oils with a low amount of free fatty acids. However, when they are mixed with Al₂O₃ (also not active for microalgal lipid transesterification), the transesterification activity increases as a function of the amount of CaO and MgO on Al₂O₃. Among the mixed oxide catalysts, 80 wt. % CaO/Al₂O₃ was the most active and could be reused at least twice [64].

2) In situ or direct Transesterification

It is one step method in which both extraction and transesterification of the algae oil take place simultaneously in the reactor [53]. It not only reduces the procedure units but also lowers the final biodiesel cost by reducing the overall process cost [174]. It also consumes lesser time than the conventional two step process [175]. After the microalgae are dried, to prevent unwanted soap formation during transesterification, it is crushed into small solid particles [53]. Methanol acts as the extractant as well as reactant. The two simultaneous processes extraction and

transesterification, demand solvents with different polarities. Therefore, methanol is mixed with a non-polar solvent in a suitable ratio. Experiments have shown that methanol and methylene dichloride in ratio of 3:1 enhance the efficiency of the extraction. Here methylene dichloride acts as a co-extractor [174].

Results showed that one-step process gave higher methyl ester yield than the conventional two step method. The biodiesel also had higher HHV. One step process could also shift the extraction equilibrium and promote the extraction efficiency. It also helped to reduce the overall heat requirement and cost of biodiesel production [174]. Table 4.5 shows comparison of two step and direct transesterification.

Table 4. 5: Comparison of Two steps/ Conventional and One step/Direct Transesterification [174]

Parameters	Two steps Transesterification	One step or Direct Transesterification
Conversion process	The conventional two-step method involves the extraction of lipid and the removal of excess solvent followed by transesterification of extracted lipid.	Both extraction and reaction of the algae oil are performed simultaneously in the reactor, thus, simplifying the conversion process by reducing the procedure units.
Overall process cost	The overall process cost is high.	Reduces the overall process cost.
Biodiesel yield	The yield reached only 22.2% through two-step method.	The highest methyl ester yield of 28.0% was obtained through one-step method operated at 65 °C for 4 hours with 45 mL mixed solvent (methanol/methylene dichloride = 3:1, V/V) and 10% catalyst .

While conventional conversion route exhibited many inherent disadvantages, like: operational complexities, high energy consumption and comparatively high cost, which limited its application on commercial

scale for biodiesel production from microalgae. Moreover, a lot of waste liquid is formed during purification of the product, disposal of which is another environmental problem [174]. However, the reported in situ transesterification reaction usually used homogeneous acid or alkali as catalyst, which resulted in complexity of products purification and environmental problem unavoidably. To overcome the above problems of transesterification, one-step transesterification was performed to produce biodiesel from (*Nannochloropsis* sp.) microalgae on heterogeneous solid base catalyst (Mg–Zr solid base catalyst), which reduced the process of the product purification and the emission of waste liquid. The catalyst was separated easily from microalgae residue [174].

B. Microalgae biodiesel

The physical and fuel properties of biodiesel from microalgae oil, in general, were comparable to those of diesel fuel. Microalgal oil is not as stable as fossil fuels.

Table 4. 6: Comparison of properties of biodiesel, diesel fuel and ASTM standard [124]

Properties	Biodiesel from microalgal oil	Diesel fuel	ASTM biodiesel standard
Density(kg l ⁻¹)	0.864	0.838	0.86-0.90
Viscosity (cSt at 40 °C)	5.2	1.9-4.1	3.5-5.0
Flash point (°C)	115	75	Min 100
Solidifying point (°C)	-12	-50 to 10	
Cold filter plugging point (°C)	-11	-3.0(max - 6.7)	Summer max 0
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 4.6 shows that algal biodiesel properties, like: density, viscosity, flash point, acid value are all in line with the ASTM biodiesel standards.

While microalgal biodiesel showed a much lesser value of cold filter plugging point of -11 °C in comparison with that of diesel fuel [124].

4.5.3.2. Biomass Processing

Both, algal biomass (with intact lipid) as well as lipid extracted algal biomass residues can be processed to produce energy via different conversion technologies. Lipid extracted algal biomass residue are rich in carbohydrates (polysaccharides), proteins [176], and pigments [73]. They can further be processed to produce a wide range of biofuels like bio-methane, bio-ethanol, bio-hydrogen and bio-butanol etc [117]. They can produce a great amount of biogas, which can improve the overall energetic and economic of the energy production system [177]. Lipid extracted algal biomass can also be utilized to produce hydrogen gas, which is a clean and efficient energy carrier and forms only water as a byproduct [177].

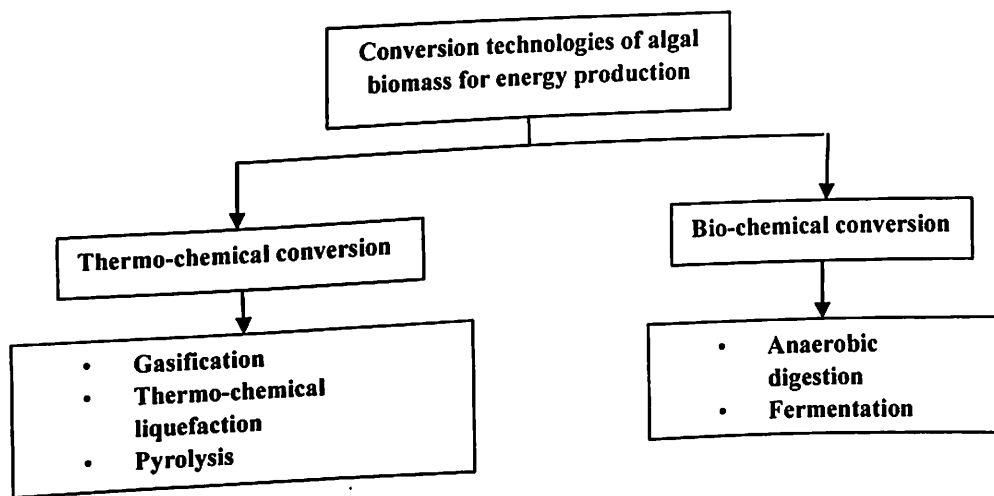


Figure4. 5: Different conversion technologies of algal biomass for energy production

Figure 4.5 shows different energy production processes from algae. There are basically two types of conversion technologies for converting microalgae biomass into biofuels, thermo-chemical and biochemical conversion. Thermo-chemical conversion techniques include direct combustion, pyrolysis, thermo-chemical liquefaction and gasification [178]. The biochemical conversion techniques include alcoholic

fermentation, anaerobic digestion, and photo-biological hydrogen production [179].

A. Thermo-chemical conversion

In addition of the production of biodiesel, using oils extracted from microalgal cells, various thermo-chemical conversions have been applied for production of algae [117]. Main thermo-chemical processes include liquefaction, pyrolysis and gasification [36].

1) Gasification

It is also known as hydrothermal process [180], during which partial oxidation of biomass at high temperature of around 800 to 1000 °C, produces mixture of combustible gases. Biomass reacts with oxygen and steam water to produce mixture of gases known as syngas. Syngas consists of gases like methane, hydrogen, carbon dioxide and nitrogen. Syngas can be either directly burned to produce energy, or can be used as a fuel to run diesel or gas turbine engines [117].

Gasification is an environmental friendly process of biomass conversion into biofuels. Water is heated above its critical temperature and pressure during which physical properties of water like, dielectric constant, viscosity, density and thermal conductivity of water decrease drastically. The ionic product is almost three times higher than that of normal water. High temperature water behaves as a very good solvent and completely dissolves and breaks the organic compounds. The advantage of this process is that it does not require drying of the high water containing algal biomass and saves a lot of energy, which otherwise would have been used for drying purpose [180].

2) Thermo-chemical liquefaction

In liquefaction process, biomass is converted to liquid fuel [181]. Liquefaction takes place at temperatures between 200-350 °C in the presence of a catalyst [182]. At sub-critical condition of water, biomass breaks into small, reactive and unstable molecules [117] and then re-polymerizes to form wide range of molecular products. Alkali salts, like potassium and sodium carbonate can act to hydrolyze cellulose and hemicelluloses into smaller fragments [183]. In a liquefaction experiment on *Botryococcus braunii* at 575 K catalyzed by sodium carbonate, a maximum yield 64% dry wt. basis of oil was produced [36]. Depending on the species, liquefaction of microalgae produces between 30%-65% dry weight of oil [117].

Conversion of wet biomass into bio-crude oil is the major advantage of liquefaction process [117], which saves the energy required in drying the high water containing algae culture [36, 132]. Moreover, from energy balance point of view, liquefaction process was found to be net energy producer. When compared to supercritical carbon dioxide method of oil extraction from microalgae, hydrothermal liquefaction was found to be more effective in producing oil from microalgae [36]. However, the complex nature of the reactors makes them very expensive [117].

From the above it is clear that hydrothermal liquefaction is an effective method of biofuel production from microalgae, but in spite this, due to lack of much information about this process, more research in this area is required [36].

3) Pyrolysis

Pyrolysis is conversion of biomass into bio-oil in the absence of oxygen, in the absence or presence of a catalyst. It is a waste free and pollution free process during which biomass decomposes into charcoal, condensable

organic liquids, acetic acid, acetone, methanol and non-condensable gaseous products [117].

With increase in the temperature the amount of liquid product increases and that of charcoal decreases [132, 36]. Slow pyrolysis produces more charcoal, while fast pyrolysis produces 75%wt. of liquid bio-oil, 15–25 % wt. solid charcoal and 10–20 % wt. non-condensable gases. Flash pyrolysis, which takes place at around 500 °C with short vapor residence time, produces 95.5% of liquid biofuel. Pyrolysis of microalgae has been found to produce higher quality bio-oil than that obtained from pyrolysis of lignocelluloses [117].

The pyrolysis oil or bio-oil produced by fast pyrolysis is two to three times cheaper than gasification and fermentation processes. However, due to low quality, their use in conventional gasoline and diesel fuel engines is not possible. They have high oxygen content, are highly acidic, and also have high water content of about 25 to 50 % wt. To make them compatible with current fuels they should be deoxygenated. Several upgradation methods include hydro-treating, aqueous-phase processing and zeolite conversion [184].

It has been found that lipid containing biomass produce more bio-oil, and thus, have higher heat balances [117]. And moreover, the extra oil produced from pyrolysis of oil extracted biomass can also reduce the overall production cost.

4) Direct combustion

The biomass can also be directly combusted in the presence of air, to liberate energy for heating furnace, boilers and steam turbines. The conversion efficiency of biomass to energy is more favorable than that of direct combustion of coal. The major disadvantage of this process is the huge amount of energy required for drying of microalgae culture, which

may affect the energy balance. Therefore, in spite of more efficient than coal, the pre-treatment cost makes it less viable than coal. The overall efficiency of the process may be improved, if combusted along with coal. Limited data on viability study of combustion of biomass requires further research and development into it [117].

B. Biochemical conversion

1) Anaerobic digestion of microalgae

Anaerobic digestion of biomass takes place in the absence of air. It produces biogas which is a mixture of methane and carbon dioxide [36]. The anaerobic digestion not only converts the residual biomass left after lipid extraction but also recycles the nitrogen and phosphorous, which are added as a source of fertilizer during the algae culture. It has been found that the methane produced from lipid extracted algal biomass via anaerobic digestion, produces more energy than that obtained from the lipid [185].

Biodegradability of microalgae, due to its biochemical composition and nature of cell wall, formation of toxic ammonia [185, 36] due to high protein content (nitrogen content [36]) and presence of sodium in the marine species which affects the digester performance [185, 83, 36] are the three main bottlenecks which have been identified for this process.

However, the biodegradability can be improved by pretreatment of the biomass by acting on its physicochemical properties [185]. The pretreatment processes may include substrate concentration [83, 185], chemical treatments (acids, bases, ozonation), thermal treatment and ultrasonic lysis, which improve the disintegration of the most refractory organic fractions. These pretreatment processes increase methane yield [186].

Among the various pretreatment options, thermal treatment i.e. temperature was found to be most effective. When heated at 100 °C for about 8 hours, it was observed that methane production increased by 33%. Further, when cultured in nitrogen limited conditions, it not only increased the lipid content but also reduced the protein content, and thus, reducing ammonia release during anaerobic digestion process [185] .

Inhibiting effect of sodium inhibiting could be avoided by the use of adapted marine inoculums. Additionally, a study underlines the fact that the sodium is less inhibitory in mesophilic conditions than in thermophilic conditions, which limit the energetic consumptions of this step [83].

Theoretical methane yield depends on the composition of the microalgae. Lipid has higher methane production potential in comparison to carbohydrate and protein. Methane yield increases with the increase in the lipid content of the microalgal biomass. However, lipid hydrolyses slowly in comparison to protein and carbohydrates [185]

The lipid extracted from biomass can be processed to produce biodiesel, while the biomass residue can further be processed via anaerobic digestion process to produce methane, thus, increasing the overall energy yield and production economics. However, the potential methane yield is less from the lipid extracted algal biomass, while ammonia production increases, which may strongly limit and jeopardize the process stability [185].

It has been seen that C/N between 20 and 35 enhances the methane yield. Thus, co-digestion of high nitrogen containing substrate with poor nitrogen containing substrate, or in other words, substrate, with high carbon fractions can significantly enhance the methane yield. Moreover, co-digestion also helps to dilute certain toxic compounds and maintain their concentration under their toxic threshold [185].

Production of methane via anaerobic digestion of the raw algae does not require drying of the biomass, and thus can greatly reduce the overall production cost by removing the harvesting and drying cost, which alone is about 20% to 30% of the production cost. Further when harvesting and drying cost combine with the extraction cost, this alone is about 50% of the total production cost. And thus, use of anaerobic digestion process, could avoid a significant cost and reduce the total energy debt [83].

For algal lipid content lower than 40%, the energetic added value when recovering lipids is lower than 21% of the recovered energy [78]. Thus, when lipid content of the cell is less than 40%, anaerobic digestion is a better option with respect to energetic recovery and energy balance of the biomass [185].

2) Fermentation

Fermentation is the process which produces ethanol from sugar and starch containing crops. It has been used commercially on a large scale in many countries. As of now, corn, which contains about 60-70% starch, is the dominant feedstock of the bio-ethanol industry worldwide [132]. Algae can also be used as a feedstock for bio-ethanol production. The algal starch is converted to sugar with the help of enzymes and then by yeast, this sugar can further be converted to bio-ethanol. Initially, starch is released by using mechanical equipments or an enzyme and then the cell are allowed to degrade, after which *Saccharomycess cerevisiae* yeast is added to it to begin the fermentation process. This produces ethanol, which is taken out of the tank and fed to distillation units [36].

3) Biophotolysis

Green algae and cyanobacteria can be used to produce biological hydrogen by bio-photolysis of water [187]. Three different ways to produce hydrogen include, direct photolysis, indirect photolysis and ATP-driven

hydrogen-production. During direct photolysis the resulting hydrogen and oxygen are continuously flushed out. Both photosynthesis and water splitting take place simultaneously and produce hydrogen and oxygen. This can be a major safety risk, and also result in extra cost in separating hydrogen and oxygen. Apart from the separation cost, the other major costs include the cost of photobioreactor and hydrogen storage facility [36].

4.6. Concluding Remarks

Increasing demand for clean energy, diminishing petroleum fuel supply and their increasing cost, will further increase tremendously the need for production of more biofuels. Though there are many alternate sources for biofuel production, but among them algae has come up as one of the most potential source.

Algae are the most efficient biological producers of oil and fats, and oil synthesized by microalgal cells is similar to those present in conventional terrestrial oil crops and waste oil sources. For an adequate algae species for producing biodiesel, it needs to have both, high biomass productivity and high lipid content.

Techniques rooted in biology, can have a dramatic impact on the economics of algae production. A better understanding of physiology of fatty acids and triacylglycerols synthesis can increase the ability of algae species to enhance lipid and oil production during cultivation. It is also clear from the above review that effects of various environmental/external factors (light, temperature, cell density and mixing, contamination, oxygen, nutrients) play a very vital role in deciding an appropriate way of bioreactors design and culture of algae. Thus, the knowledge of physiology of fatty acids and triacylglycerols synthesis and effects of various environmental/external factors can further be used for system

optimization, which is required to achieve high lipid yields for large scale cultivation.

Various studies have shown that on applying stress conditions (limited nitrogen & phosphorous), the lipid content increased but at the same time it also reduced the microalgae growth, thereby affecting the overall lipid productivity. Therefore, the optimal culture conditions for lipid accumulation should be the balance between high biomass and high lipid content.

Algae can be cultured in both open and closed pond systems. Open ponds are more economical and easy to operate, but they are limited by biological factors such as organism survival due to contamination and offer very little control over factors such as carbon dioxide uptake, light, temperature etc. which affect the productivity and growth of microalgae. Further, the flexibility to strain selection is also very low. Therefore, in order to be able to utilize more number of algae species for successful cultivation and biofuel production we need to focus on closed system cultivation especially on photobioreactors.

But even photobioreactors face many challenges and need to be designed for system optimization. Photobioreactor design and configuration should be such that it best controls all the environmental and external factors.

The high cost of biodiesel is another major obstacle for commercialization. Sustainable and cost-effective systems must be put into place for large scale cultivation. A few cost effective production technologies could be biofuel production from microalgae coupled with flue gas CO₂ mitigation, waste water treatment and production of high value chemicals.

Apart from system optimization, knowledge of physiology can also be used to improve yield through the application of biotechnology and

genetics. Genetically modifying the oil-storing capabilities of algal cells make them more efficient than they are in nature. Since the whole genome sequence has been generated only for a limited number of species, there still exists an enormous potential in this field. Moreover, they can be used to generate energy in several ways. By using thermo-chemical processes, oil and gas can be produced, and by using biochemical processes, ethanol, biodiesel and bio-hydrogen can be produced.

Microalgae cultures have high water content, which must be separated in order to produce biofuels. It can be inferred from the above review that there is no exclusively best method of harvesting microalgae. The choice of preferable harvesting technology depends on algae species, size, density and desired end product. Moreover, harvesting and drying of microalgal biomass highly increases the overall operational cost of biofuel production from microalgae. Therefore, in order to produce biofuels from microalgae economically, more research and development is required to find out an efficient and commercially viable harvesting technology.

There are many processes of getting energy from algae, but each of them along with advantages also carries a few disadvantages. Research for few of them is still in very early stages, and moreover currently, biofuel production from algae is still very expensive to be commercially viable. Considering the early stage of research and high cost, it can be said that there is still a long way to go to perfect the process of optimizing the algae biofuel manufacturing process.

Therefore, based on the current research inputs, it appears that apart from identifying the most optimal methods to cultivate algae, one also needs to identify the most optimal method for efficient biofuel manufacturing from them. A lot of work is already being done in each of these two aspects, and it is hoped that there will be many more to come soon.