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Microalgae- Biomass to Biodiesel: A Review

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Abstract

Microalgae are key elicitor for biodiesel production due to its high growth rate and non- competing with the arable land with oil producing crops. Rapid depletion of fossil fuel enhanced the use of microalgae for biodiesel production. Large-scale biomass production using open raceway pond and photobioreactor is a promising technology for biodiesel industry. The cost-effective production of biodiesel from microalgal biomass is of great interest in recent days. The advancement in biomass production strategy through photobioreactor engineering and enhancing cell biology through metabolic and genetic engineering are two modern eras of research. The optimization of lipid extraction techniques was also established for better lipid productivity. The nutrient starvation, light irradiance, heavy metals and temperature also enhanced lipid accumulation in microalgae in the form of triacylglycerol (TAG). The use of algal biorefinery concept increases the cost effective production of biodiesel and other bioactive compounds. In this review algal production strategy, improvement of biomass production, lipid extraction and economics were discussed.

Key words: Biodiesel, Biomass, Cultivation, Energy, Fossil Fuel, Microalgae

1. Introduction

The term ‘energy’ is applied as basic and fundamental requirement for human existence and activities. Mitigation of green house gases and production of fossil fuel substitutes now a day’s most important component of bioenergy. The increasing industrialization and population increases the need of energy continuously. Petroleum, natural gas, coal, hydro and nuclear are the basic sources of energy. Conventional diesel fuel or petroleum-based fuel can create more pollution to the environment. Continued use of petroleum-based fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of green house gases to the environment. The green house gases include CO_2, NOx, SOx, CO, particulate matter and volatile organic compounds (Klass, 1998; Goldenberg, 2000; Borowitzka and Moheimani, 2010). The accumulation of these green house gases after burning of enormous amount of fossil fuel causes global warming (Hill et al., 2006; Satpati et al., 2016). Rapid decline of fossil fuel increasing global demand for energy has led to substantial interest and activity in developing renewable biologically produced fuels, especially ethanol and biodiesel (Borowitzka and Moheimani, 2010).

Biodiesel is biodegradable, non-toxic, carbon neutral and renewable diesel fuel and can be used to neat or blends with petroleum diesel fuels. Biodiesel has many advantages compared to diesel fuels. It has higher cetane number than diesel fuel and contains no aromatics, and almost no sulfur and 10-12% oxygen by weight (Sivaramakrishnan and Rajkumar, 2012). The cetane number, iodine value, calorific value, viscosity, relative density, flashes and fire point are the most important physico-chemical properties of engine quality. Biodiesel has all these properties than the petroleum-based fuel. The high cetane number (CN) influences the combustion process and engine performances. Biodiesel also improves the lubricity, which results in no longer engine component life (Kinast, 2001; Boehman, 2005; Gerpen, 2005). Recently biodiesel is widely produced using oil from crop plants such as

soybean, sunflower, canola etc. (Ma and Hanna, 1999). The wide application of biodiesel using this vegetable oil has limited feedstock. Now a day's algae have been tested as feedstock for biodiesel production because certain microalgal species were known to accumulate large amount of lipids (30-60% of dry weight) (Satpati and Pal, 2015; Satpati et al., 2015). Algae also exhibit higher growth rates, photosynthetic efficiency and CO_2 fixation rates compared to conventional agricultural crop plants (Sheehan, 1998; Chisti, 2007; Hu et al., 2008).

2. Microalgae versus macroalgae:

As renewable energy resource, algae pay a great attention. Use of algae other than the crop plants due to high biomass yield per unit of light and area, high starch content, which is converted to oil, do not require agricultural land and minimal nutrient supplements. Two types of algae both micro- and macroalgae have been used as energy resource (Satpati and Pal, 2011; Barman et al., 2012; Satpati et al., 2015, 2016; Gorain et al., 2018). A wide variety of microalgae such as single unicellular to multicellular form can be substitute for biodiesel. The use of microalgae as energy feedstock seems to be promising because of their high growth rate (Satpati and Pal 2015). The doubling times during exponential phase are commonly as short as 3.5 h (Chisti, 2007). Their simple cellular structure and large surface area to volume ratio are able to uptake large amount of nutrients (Lee, 1980). Oil content in microalgae can exceed 80% by weight of dry biomass (Chisti, 2007). Altering culture conditions and growth media also enhanced the cellular lipid productivity (Naik et al., 2006). The major carbon source for growth of the microalgae is atmospheric carbon dioxide and they can produce 30-100 times more energy per hectare as compared to terrestrial crops (Schenk et al., 2008; Demirabs, 2010). Whereas macroalgae are less versatile, there are few species to cultivate. The anaerobic digestion is only the process of conversion of macralgal biomass to renewable energy to produce biogas other than the diesel.

3. Microalgae as bioenergy feedstock:

Microalgae are diverse group of photosynthetic microorganisms convert light energy into chemical energy in the form of carbohydrate and lipid. Recent days taxonomists have distinguished different groups of microalgae by genetic analysis and cellular morphology. The microalgae can be classified on the basis of their pigment compositions, storage products and the basic cellular structure. The most dominant and diverse group are diatoms with 100, 000 species comprising largest groups of algae (Satpati et al., 2017). They are widely distributed in fresh, brackish and marine water regions and contain chrysolaminarin and triacylglycerol (TAGs) as major storage products (Satpati and Pal, 2015; Satpati et al., 2015, 2016). The second largest group is green algae comprising approximately 8000 species. They are commonly found in fresh water regions, sometimes in brackish and marine regions. Starch and TAGs are the major storage components of this group. The blue-green algae or cyanobacteria are a group of 2000 species living in different habitats and contains starch and TAGs as major storage material (Barman et al., 2012). The last group of microalgae is golden algae or chrysophytes with 1000 species, widely distributed in fresh water regions having TAGs and carbohydrates as storage products. Some macroalgae or seaweed viz. *Gracilaria* (Red algae), *Sargassum* (Brown algae), *Rhizoclonium* (Green algae) currently studied for their suitability for biodiesel production (Rajvanshi and Sharma, 2012; Satpati et al., 2015). The lipid content of different groups of microalgae has been studied several times. The total lipid content in dry weight basis varies subsequently from 5-80% in different algal species (Table 1).

Table 1: Total lipid content in different microalgal species (modified from Chisti, 2007; Rajvanshi and Sharma, 2012; Satpati and Pal, 2015).

Microalgal species	Lipid content (% dry wt. basis)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Cryptothecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Isochrysis sp.	25-33
Monallanthus salina	20-25
Nannochloropsis sp.	20-35
Neochloris oleabundus	31-68
Nitzschia sp.	35-54
Phaeodactylum tricornutum	45-47
Schizochytrium sp.	20-30
Tetraselmis suecica	50-77
Chlorococcum infusionum	15-23
Navicula minima	30-42
Scenedesmus obliquus	28-45
Chlorella variabilis	25-35
Euglena gracilis	30-35
Chlorella ellipsoidea	20-30
Chlorococcum infusionum	15-20

4. Growth rate of Microalgae:

The unicellular algae reproduce asexually so that the cell size and biomass of individual cells increase with time, resulting in biomass growth. The genome divides equally followed by the division of cytoplasm into progenies of more or less identical size. The population number therefore increased results population growth in the culture (Richmond, 2004; Satpati et al., 2014).

4.1. Doubling time, specific growth rate and growth yield:

The time required for doubling the number of viable cells is termed generation time or doubling time. The time taken to grow and produce a generation of algal cells is generally 3-4 hrs. The exponentially growing cells in the culture could be mathematically described as follows:

$2^0N_0 \longrightarrow 2^1N_0 \longrightarrow 2^2N_0 \longrightarrow 2^3N_0 \longrightarrow 2^nN_0$

N_0= Initial number of cells; n= Number of doublings or generations.

The growth of the cells (N_t) at the incubation time't' in an exponentially growing culture can be determined as:

$N\,t = N_0 2^n$

$N_t = N_0 2^{t/td}$

$N_t / N_0 = 2^{t/td}$

$\ln (N_t / N_0) = (\ln 2)\, t/ t_d$

At the exponential phase, the growth rate of the cells is proportional to the biomass of the cells. The population of cells or biomass can be measured more accurately than the number of cells. In culture the cell growth equations are often expressed in terms of biomass. The cell component such as protein may be used as an alternative to direct weighing of biomass. Hence, assuming the biomass concentration at initial time '0' and final time 't' as X0 and Xt respectively can modify the above equation:

$\ln (Xt/X0)/t = 0.693/ t_d$

$d (\ln X)/ d_t = 0.693/ t_d$

$d (\ln X)/ dX.\ dX/ d_t = 0.693/ t_d$

$1/ X.\ dX/ d_t = 0.693/ t_d$

$\mu = 0.693/ t_d$

Where, 'μ' represents the specific growth rate (h^{-1}) of the microalgal culture. It states the increase of the biomass at specific time i.e. increase of certain g- biomass per hour from every g of existing biomass. Specific grow rate defines the average growth rate of all cells in the culture.

The amount of biomass produced (dX) through the consumption of a unit quantity of a substrate (d_s) can be defined by growth yield ($Y_{x/s}$, g-cell/ g-substrate). Hence the conversion efficiency of the substrate to the biomass can be expressed as:

$Y_{x/s} = dX/ d_s$

4.2. Energy requirement for growth and maintenance:

The microalgal cells require energy for growth and maintenance. The total energy consumed at a particular set of environmental condition and a constant rate can be expressed as:

$(d_s/ d_t)_m = mX$, where 'm' is the constant called the maintenance coefficient and 'X' is the biomass concentration. Thus the energy balance is defined as:

Total rate of consumption= Rate of consumption for growth + Rate of consumption for maintenance.

$\mu X/ Y_E = \mu X / Y_G + mX$

Where, Y_E = Overall growth yield; Y_G = True growth yield; m = 0.

5. Culture of Microalgae:

Microalgae are simple, microscopic, autotrophic micro organisms can grow invariably under all adverse environmental conditions (Satpati and Pal, 2015, 2016, 2017). They can live in freshwater, brackish or marine environments and can convert atmospheric carbon dioxide to biomass with the help of sunlight by photosynthesis (Satpati et al., 2011, 2012, 2013; Barman et al., 2015). The biomass mainly composed of three major bioactive components viz. carbohydrate, protein and fat. The culturing and production of biomass will basically need to convert biomass to bioenergy i.e. biodiesel. There are three modes of culture system viz. batch culture, continuous culture and immobilized culture system.

5.1. Batch culture:

This is the most common commercial method for culturing of microalgae and production of biomass. In a batch culture system, a limited amount of culture medium and inoculums are placed in a culture vessel and incubated in a sterilized, controlled environment for growth. A simple agitator or a paddle wheel is fitted for mixing the nutrients and proper gaseous exchange throughout the system. Structurally the culture vessel can be a simple conical flask or an environment controlled fermentor. The CO_2 can be supplied continuously with CO_2 enriched air and illuminated with natural or artificial light sources. In batch culture mode, a portion of the inoculums was retained for the next culture batch. Different growth phases in the culture system may reflect changes in the biomass and in its environment. In the lag phase the growth of the cells is at the sub-maximum level due to the presence of non-viable cells, physiological adjustment of cells to the newly introduced culture medium and cells exposed to higher irradiance. The cells began to grow and multiply at the end of the lag phase because the cells have adjusted to the new environment i.e. they enter into the exponential phase. At the end of the exponential phase cells began to utilize all the light energy in terms of photons and grow exponentially until the concentration of cell become high. The light become limited after absorbance of all the biologically active photons and the growth of the biomass was at constant rate i.e. in linear phase.

5.2. Continuous culture:

In continuous culture, the fresh culture medium is supplied to the homogenously mixed culture system and simultaneously the culture is removed intermittently. The rapid depletion of nutrients and accumulation of products during growth are the main approaches of continuous culture. The growth limiting substrate ceases the growth of the culture. The continuous removal of growth limiting substrate and adding of fresh culture medium sustain the algal growth.

The increase in biomass in the culture can be defined as:

$V\mathrm{d}x = V\mu X\mathrm{d}t - FX\mathrm{d}t$

Where, V= culture volume (l); dx= increase in biomass concentration ($g.l^{-1}$); μ= Specific growth rate ($l\ h^{-1}$); X= Biomass concentration ($g.l^{-1}$); dt= Infinitely small time interval (h); F= Culture flow rate ($l\ h^{-1}$).

Thus, $\mathrm{d}x/\mathrm{d}t = (\mu - F/V)\ X$.

where, F/V represents the dilution rate of the culture i.e. D with the unit of $l.\ h^{-1}$.

Hence the above equation could be written as, $\mathrm{d}x/\mathrm{d}t = (\mu - D)\ X$.

At steady state, the specific growth rate is equal to the dilution rate ($\mu - D = 0$), $\mathrm{d}x/\mathrm{d}t = 0$. That means no net increase in the biomass concentration takes place.

A constant chemical environment or chemostat is a special type of continuous culture, where the rate of addition of medium and the rate of removal of culture is the same and thus culture volume is maintained at a constant level. The all culture conditions viz. temperature, pH, nutrients were adjusted to understand the specific growth rate. This is similar to the batch culture but in batch culture it could not easily differentiate the effects of culture parameters and the specific growth rate (Lee and Soh, 1991; Molina Grima et al., 1994).

A continuous alternating light and dark period for 24 hrs is subjected to algal growth and biomass production. This is referred to as constant cyclical illumination or cyclostat (Rhee et al., 1981; Satpati et al., 2014).

The constant turbidity or turbidostat is a modified condition of chemostat where the concentration of the culture is maintained at a pre-set value by adjusting the rate of substrate (medium) flow through a feedback control loop. It is most useful culture system for slow growing algae and those with a complex cell cycle. In *Haematococcus lacustris* the growth rate is varied at various stages of cell cycle. Hence complete removal of the limiting factors in the culture medium through excessive dilution rate was applied (Lee and Ding, 1994).

In fed-batch culture mode the biomass is harvested periodically and the culture medium is added continuously or intermittently. Hence the culture volume varies with the dilution rate. It is most widely used in industrial continuous flow culture system where concentrated culture medium is fed continuously and the culture is harvested at the end of the cultivation cycle (Lee, 1997).

The biomass concentration and productivity was also increased in order to recycling the culture system by returning the cells back into the culture. The microalgae having slow growth rate are cultured in this system where cells being retained in the culture by a cell filter system (Chen and Johns, 1996).

5.3. Immobilized cultures:

There are various microalgal species that favors protective and stable microenvironment for growth. This condition allows the cells to grow at a faster specific growth rate at higher culture temperature and high shear conditions (Ding and Lee, 1994). The natural polysaccharide gels or synthetic polymers have the ability to entrap microalgal cells and improved their stability and productivity (Robinson et al., 1986). The major disadvantages of the cell entrapment system are the low stability of the alginate beads for long-term mass cultivation of microalgae. The use of phosphate, magnesium and EDTA in the culture medium progressively weakens the structural integrity of the alginate gel by removing calcium ions through precipitation and chelation, leading to leakage of algal cells. The integrity of the alginate beads were stabilized by washing it with 0.1M $CaCl_2$ solutions.

The immobilization of algal cells by absorption is widely used in packed column bioreactors where the cells absorbed on polyurethane. The maximum number of cells absorbed on the porous surface of the system and recovered the by squeezing and washing in water. The disadvantages of this culture system is high density photoautotrophic algae cant not grow properly and in the other way low density algal cultures render the entire system cost-ineffective.

6. Large scale cultivation of microalgae:

The demand for cost effective production of biodiesel is increasing with time and therefore the production of diesel requires large amount of biomass. Microalgae obtain their energy through the absorption of light energy for the reduction of CO_2 by the oxidation of substrates, mainly water with the release of O_2. Photoautotrophic microorganisms require inorganic mineral ions as nutrient substrate and light as energy to produce biomass. Many studies were conducted to determine the optimal nutrient concentrations for various algal species (Vonshak, 1986). The minimal nutrient for cultivation of microalgae is some ionic compounds such as magnesium, sodium, calcium, sulfate and chloride. The optimal pH was maintained throughout the culture system and vitamins as supplements. Some trace elements and chelating agent such as EDTA was also used for biomass productivity (Satpati et al., 2016). The three most important nutrients for algal growth are carbon, nitrogen and phosphorous.

6.1. Carbon and CO_2:

The production of large quantities of algal biomass requires carbon as major nutrient supplements. To replenish the carbon in the culture media, atmospheric CO_2 and HCO_3^- provides externally. For high growth rate of microalgae atmospheric CO_2 cannot satisfy the C-requirements. The CO_2- H_2CO_3- HCO_3^- - CO_3^{2-} system is the most important buffer in fresh water ecosystems and it is the best means available to control and maintain specific pH levels for optimization of algal growth. It also reported that high CO_2 concentration might also inhibit algal growth (Lee and Tay,

1991). The culture of microalgae is the possible way to capture atmospheric CO_2 to prevent global warming (Doucha et al., 2005; Wang et al., 2008; Satpati et al., 2016; Gorain et al., 2018). The CO_2 removed from the power stations can be applied to the production of microalgal biomass (Benemann, 1997; Haidue et al., 2009). The algae cultivation unit was made near the power stations to minimize the distance of CO_2 supply to the cultivation system; hence preconcentration of CO_2 is required before transport (Metz et al., 2005; Borowitzka and Moheimani, 2010).

6.2. Nitrogen:

The second important macronutrient for microalgal growth is nitrogen (N). The nitrogen can be supplied as nitrate, ammonia or urea to the culture medium. The preferred N-source for microalgae is ammonia and both ammonia and nitrate can be assimilated in relation to the pH of the growth medium (Satpati and Pal, 2015). The nitrogen content of the biomass varies from species to species from 1% to 10%. The nitrogen in the culture system enhances the specific growth rate, culture stability and lipid and fatty acid compositions in the cells (Fidalgo et al., 1998).

6.3. Phosphorous:

Phosphorous is the essential mineral element necessary for the biosynthetic processes such as energy transfer, synthesis of nucleic acid, DNA etc. Phosphorous supplied to the algal cells as orthophosphate and its uptake to the cell is energy dependent. It is the most important limiting factor in algal growth because it is easily bound to other ions such as CO_3^{2-} and iron to precipitate all the essential mineral elements to make them unavailable for cell growth. The external supply of phosphate to the culture enhances the biomass productivity in terms of carbohydrate and lipids (Borowitzka, 1988; Satpati et al., 2016). The phosphorous recovered from waste water can be utilized in algal cultivation (An et al., 2003; Jiang and Mwabonje, 2009).

The uptake of nitrogen and phosphate by cell in a particular concentration was studied from earlier times. Droop developed a model for nutrient uptake of algae which is dependent on the two main factors viz. cell quota of a nutrient and the specific growth rate of algae. The nutrient uptake model by Droop can be explained as (Droop, 1968, 1983):

$\mu = \mu_{max}(1- k\text{q}/Q)$

Where, μ = specific growth rate, μ_{max} = maximum specific growth rate, kq= minimum cell quota for the limiting nutrient, Q= cell quota for the limiting resource.

The nutrient assimilation in steady state was described by the equation (Droop, 1968):

$\mu = \mu_{max}. [S]/ K_s+[S]$

Where, [S] = the steady state substrate concentration and K_s= the half saturation constant for steady state nutrient uptake.

6.4. Water:

Water is the most essential medium for large-scale cultivation of algal biomass and converting them into fuel (Dominguez-Faus et al. 2009; Mulder et al. 2010). In large-scale cultivation of algae the requirement of water varies from open raceway pond to closed photobioreactor (Grobbelaar, 2009). The availability of marine water regions than the fresh water bodies is a major issue for algal productivity. A large amount of algal biomass can be cultivated in fresh water bodies than the marine water. The focus has been done to isolate and grow the algal species in marine or brackish water (Gouveia and Oliveira, 2009; Vijayaraghavan and Hemanathan, 2009). The uses of both fresh water and saline water in the cultivation of algae using open or closed photobioreactors have been discussed.

7. Biomass production:

The large-scale production of microalgal biomass using photobioreactors has been of great interest in recent days. Photobioreactors are open or closed system where microalgal cells grown by photobiological reaction. Continuous

culture system and daylight are the two important factors for large-scale production of microalgal biomass (Molina Grima et al., 1999; Chisti, 2007). The commercial production of microalgae using raceway pond and closed photobioreactors has been discussed in detail.

7.1. Open Lakes and Natural Ponds:

These are naturally occurring ponds or lakes contain high pH, nitrate and phosphate for sufficient algal growth. There are many eutrophic lakes or natural ponds exploited for microalgae production. The people of Kanembu exploited natural pond and harvested algal biomass regularly to use as food (Abdulqader et al., 2000). The people from Central Burma cultivated *Arthrospira* as unialgal form throughout the year using alkaline water after volcanic eruption (Min Them, 1993). The productivity of the cyanobacterium is around 30 t $year^{-1}$ (Lee, 1997). The cultivation of *Dunaliella* in large natural ponds of Australia was done for the extraction of β- carotene throughout the year (Lee, 1997; Borowitzka, 1999). The disadvantage of the system is productivity does not exceed 1 gm^{-2} d^{-1} and concentration of the biomass given very low. A year-round suitable environmental condition and unmixed ponds may be cost-effective for microalgal cultivation.

7.2. Open Raceway Pond:

Open raceway pond is a type of 'U' shaped closed circulation channel where the culture system is mixed by circulation with the help of paddle wheel or simple floater system (Chisti, 2007). The culture vessel is made up of concrete unit and compacted with the earth. The biomass is harvested behind the paddle wheel after a complete circulation (Fig. 1). The use of raceway ponds for mass cultivation applied since 1950s (Dodd, 1986; Oswald, 1988; Becker, 1994; Lee, 1997; Molina Grima, 1999; Tredici, 1999; Sanchez Miron et al., 1999; Lee, 2001, Chisti, 2007). The commercial application of algal biomass after harvesting from the raceway pond is also studied. The *Arthrospira* (Lee, 1997) and *Dunaliella* (Borowitzka, 1999) were extensively cultivated using raceway ponds for commercial application. Raceway ponds are less expensive than the photobioreactors and have low biomass productivity compared to the photobioreactors. The disadvantages of the system are large area volume but less cell concentration, the water level cannot be operated much lower than 15 cm, excessive evaporative loss in hot and dry climatic conditions and increases cost of harvesting (Richmond, 1999).

7.3. Inclined and circular pond system:

The inclined systems have received more attention for very thin culture layers. In the system turbulence is created by gravity, which mixes the biomass from top to bottom of a sloping surface (Richmond, 1999). The cell concentration increased up to 10g l^{-1} and has higher surface-to-volume ratio compared to raceway ponds. The inclined pond of 0.5-ha slop was used to produce Chlorella in Western Australia (Borowitzka, 1999). The system can produce 25$gm^{-2}d^{-1}$ throughout the year. The process was unsuccessful due to some technical reasons such as strong evaporative loss of medium, high turbulence causes sedimentation of cells, high rate of CO_2 desorption and high requirement of energy for continuous pumping of the cultures to the inclined surface. Circular ponds are type of open raceway ponds used for the cultivation of microalgae. But the system is not so used because the system requires extensive concrete structure and high energy for the circulation and mixing of the cells. This system is widely used in Taiwan, Japan and Indonesia for *Chlorella* biomass production (Lee, 2001).

7.4. Photobioreactors:

Photobioreactors are closed culture system that prevents the direct penetration of light to the culture surface. The system allows passing light through the transparent reactors wall to reach the culture system (Fig. 2). This is the culture system for phototrophs do not allow direct exchange of gases and contaminants between the culture and atmosphere (Tredici, 2004). In comparison to other open raceway ponds, photobioreactors permit single species culture of microalgae for long time. The large quantities of microalgal biomass can be produced using photobioreactors from the earlier times (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006; Chisti, 2007; Schenk, 2008; Feng, 2011, Hallmann, 2015; Satpati et al., 2016). On the basis of design and mode of operation, photobioreactors can be classified into different types.

Fig. 1. Open raceway ponds for algal cultivation, A. Raceway pond with thick dividing section (Source- SARDI, South Australia), B. Open raceway pond (Source- Arban Infrastructure Pvt. Ltd., Biotech division), C. Laboratory scale high rate algal pond (Source- New Mexico State University), D. Schematic of the horizontal pond or bioreactor (Source- Brown et al., 2015).

7.4.1. Tubular photobioreactors:

Tubular photobioreactors are 'U' shaped bends consists of an array of transparent tubes that are usually made up of glass or plastics. Gas exchange and addition of nutrients to the culture normally takes place in a separate vessel (Fig. 2). A pump or airlift is used for the circulation of the gas and nutrients throughout the culture. The tubular solar collector is applied to maximize the light penetration to the growing cultures (Molina Grima et al., 1999; Sanchez Miron et al., 1999; Chisti, 2007). The arrangement of the tube depends on the area and the mode of cultivation. Tubes are sometimes placed parallel to each other and flat above the ground known as serpentine or manifold photobioreactors and when the tubes are arranged like a fence, known as fence-like photobioreactor (Chisti, 2007). In the photostage the pH can be adjusted by supplying CO_2. The annual biomass productivity of a helical photobioreactor is 100,000 kg and aerial productivity is approximately 0.072 kg $m^{-2}d^{-1}$ (Chisti, 2007).

7.4.2. Flat photobioreactors:

Flat photobioreactors are massive applied for laboratory cultivation of microalgae. The system is made up of thin glass plates arrange horizontally or sometimes in inclined position. This system facilitates the measurement of irradiance at the culture surface (Fig. 2). Some reports are available of mass cultivation of microalgae using flat plate photobioreactors (Hu et al., 1996; Richmond et al., 2003; Feng et al., 2011) in outdoor conditions.

7.4.3. Column photobioreactors:

Column photobioreactors are simple systems in which circulation and mixing of gas and nutrients are achieved by injecting compressed air. The unit is made up of simple glass columns of 1.8m in height and 10cm in diameter. The structure is narrowed or constricted at the bottom to prevent the settling of algae (Fig. 2). *Chlorella* is a best growing algae in this system, produces 0.48g $l^{-1}d^{-1}$ in indoor condition and 0.35g$l^{-1}d^{-1}$ in outdoor condition (Tredici, 2004). Hu and Richmond (1994) successfully cultivated *Chlorella* using column photobioreactors in outdoor condition. The

system is not so popularized because its vertical structure reduces the penetration of light thus the growth of the biomass is inhibited.

7.4.4. Annular photobioreactors:

Annular photobioreactors are hollow cylinder of glass made reactors, which acts as a bubble column. The empty cylinder avoids dark phase to enhance the microalgal biomass productivity (Fig. 2). The hollow column also increases the surface area-volume ratio to maximum algal growth (Schenk, 2008).

The cost effective production of biomass, high volumetric productivity and conversion of light energy to chemical energy are the major objective to achieve successful biodiesel production. In summary, for high biomass productivity photobioreactor design criteria should include high surface area-volume ratio, orientation of the system, mixing of gas and nutrients, degassing, temperature regulation, transparency, durability of the construction material and cleaning of the system.

8. Biomass harvesting and dewatering:

The biomass harvesting involves solid-liquid separation steps. The harvesting method depends primarily on the type and size of algae. The culture medium and water must be removed to enable harvesting. The most common methods of harvesting processes are filtration, centrifugation, flotation and flocculation (Molina Grima et al., 2004). Harvesting of microalgae from open raceway ponds or photobioreactors employed these techniques to concentrate algae (Maurya et al., 2016).

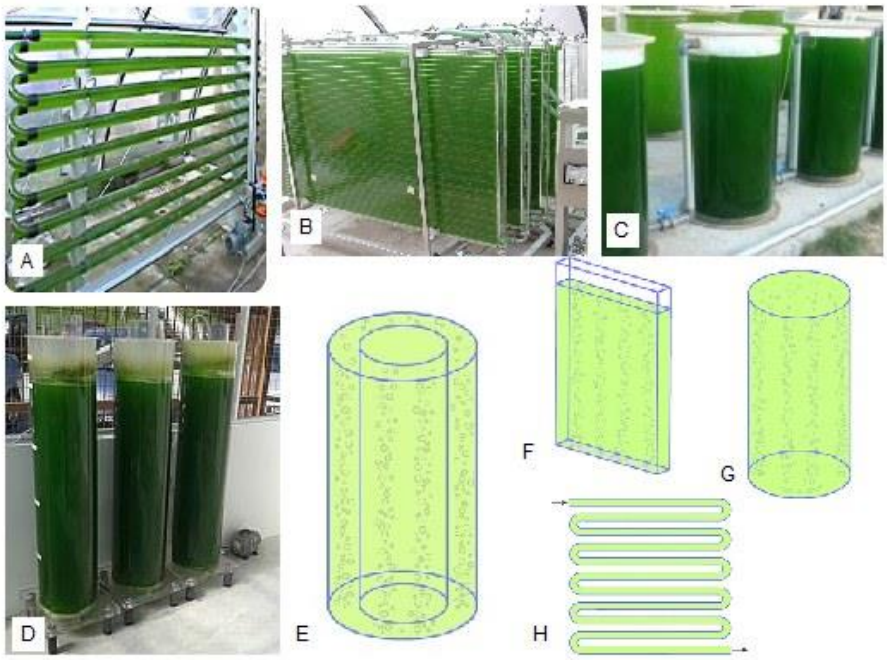

Fig 2. Showing different type of Photobioreactors for algal cultivation, A. Tubular (Source- Chempure Technologies Pvt. Ltd.), B. Plate (Source- IGV Biotech, Wikipedia), C. Column (Source-Oilgae), D. Annular (Source-Tan, Gallery), E-H. Diagrammatic representation of photobioreactors (Source- Hallmann, 2015), E. Annular, F. Plate, G. Column, H. Tubular.

8.1. Filtration:

Filtration is carried out commonly on cellulosic membranes with the aid of a suction pump. The microalgal cells of very low density can be harvested by this technique. Rotary vacuum filters and reverse flow vacuum are the most

modified techniques of filtration to separate the microalgal cells from the medium. The disadvantage of this technique, it is only suitable for large microalgae viz. *Coelastrum* and not for very small sized cells viz. *Scenedesmus*, *Chlorella*, *Dunaliella* etc.

8.2. Ultrafiltration:

Harvesting of microalgal cells by ultrafiltration have advantages over other harvesting techniques due to application of low shear force. High fragile microalgal species can be harvested using this technique. It can be used for both microalgal biomass harvesting and isolation of product metabolites. The application of cross flow membrane filtration for the separation of microorganisms from their culture media has been studied since 1970. But their use on microalgal biomass harvesting is often scarce. The use of membrane filtration in marine microalgal species harvesting has been applied since 1990 (Borowitzka, 1997; Rossignol et al., 1999).

8.3. Flotation:

Flotation is the method of algal harvesting in wastewater in combination with flocculation. It is the simplest method where algae can be made to float on the surface of the medium and collected as scum. There are two types of flotation methods of algal biomass harvesting, viz. dissolved air flotation and froth flotation. Dissolved air flotation is mostly applied in Kenya where the combination of both froth flotation and flocculation is made to separate the algal biomass from culture medium. Fine bubbles are supplied by an air compressor for mixing the algae/air with the flocculants such as alum (univalent and trivalent metal sulfates). Froth flotation is a method of separating algal cells from the medium by adjusting pH and bubbling air through the column to create algal froth on the surface of the medium (Satpati and Pal, 2015). This technique is very expensive for commercial use.

8.4. Centrifugation:

Centrifugation is the method of separating algae from the liquid part by a centrifugal force. A centrifuge is basically a sedimentation tank with enhanced gravitational force to increase the sedimentation rate. The biomass recovery in a centrifuge basically depends on the biomass settling rate, timing of centrifugal force and biomass settling distance. The drawback of this technique is the damage of cells due to high centrifugal force and is considered to be most expensive and energy dependent process for industrial application of harvesting microalgae (Molina Grima et al., 2004, Satpati and Pal, 2015).

8.5. Flocculation:

Flocculation is the collection of algal cells in lumps using flocculants. The flocculants are a type of polymer, which creates surface attraction on algal cells to enhance the sedimentation rate. Two major forces have been created to aggregate the algal cells. At long distances, electrostatic repulsion and at short distances, intermolecular or Vander wall forces are involved for forming algal lumps. The pH dependent flocculation or coagulation is also used for cell aggregation commonly known as autoflocculation (Uduman et al., 2010). The cationic polymers neutralize the negative surface charges of cells and cell aggregates to form lumps. Although this method is considered as most suitable for microalgal biomass harvesting, this method involves economic or technical drawbacks such as high energy cost, toxicity of flocculants and non-feasibility of scaling up (Hee-Mock et al., 2001).

9. Dehydrating or drying of microalgal cells:

After harvesting dehydration is the most common method for the application of algal biomass in different field. There are many common methods of drying are sun-drying, spray drying, drum drying and freeze based drying (Molina Grima et al., 2003). The extraction of lipid from freeze-dried biomass has been studied since earlier times (Molina Grima et al., 1994; Belarbi et al., 2000). Spray drying, freeze-drying and drum drying are widely used for drying *Dunaliella* cells for β-carotene extraction. Although these methods are mostly used for dehydrating algal cells, spray-drying method causes significant deterioration of algal pigments. Freeze-drying or lyophilization is the gentle drying method of algal biomass and widely used in research laboratories. But the method is too expensive for large-scale

commercial recovery of algal biomass. The extraction of lipid from dry algal biomass is more effective than wet algal biomass.

10. Cell disruption and lipid extraction from dried algal biomass:

Cell disruption is the most convenient method for recovering intracellular components such as lipid. It is the key method to increase the lipid extraction efficiency for biodiesel application. There are various methods for cell disruption such as microwaves, sonication, autoclaving, bead beating, osmotic pressure and ultrasound (Yon Lee et al., 2010). In microwave assisted technique a high frequency wave is applied to disrupt the algal cells whereas the cavitation effect helps to crack the cell wall and membrane in sonication based extraction of lipid. Bead beating method involves direct mechanical damage to the cells by high speed spinning with fine beads. This process is widely used both in laboratory and industrial purposes. In autoclaving based technique high temperature and pressure has been applied for cell disruption. A high osmotic pressure using 10% NaCl solution is also very effective to crack cell wall. High frequency ultrasonic sound is very useful for microalgal cell disruption.

An eluting solvent or solvent mixture is used to extract lipid from microalgal cell compartment during lipid extraction. The crude lipid after extraction was measured gravimetrically. In this section we review the two stages of lipid extraction technique, solvent extraction and supercritical fluid extraction. The application of these methods for lipid extraction is widely used in recent days (Halim et al., 2011 and 2012; Satpati et al., 2016).

10.1. Organic solvent based extraction:

Organic solvent extraction is one of the most effective for intracellular lipid extraction. When a nonpolar solvent is applied to the dried biomass, it penetrates through the cell membrane into the cytoplasm and interacts with the nonpolar or neutral lipid by Vander wall forces to form an organic solvent lipid complex. Due to concentration gradient this complex diffuses across the cell membrane and extracted out of the cell to the non-polar medium (Halim et al., 2012). The application of both non-polar and polar solvent to the cell causes penetration of both the solvent through the membrane to the cytosol. The cytosolic lipid-solvent complex then diffuses through the membrane to the outside medium. Chloroform/methanol (1/2 v/v) is universal organic solvent mixture frequently used in lipid extraction of any living tissues. The extraction of lipid using chloroform/methanol is rapid and quantitative but chloroform is highly toxic to the cell and its use is undesirable. Hexane/isopropanol (3/2 v/v) extraction is an alternative and effectively used method for its low toxicity to the cell (Halim et al., 2011). A comparative study of five different solvent mixture complexes for lipid extraction of *Cladophora* and *Botryococcus braunii* cells was done by Lee et al. (1998). The use of alcohol (butanol, ethanol or isopropanol) in combination with non-polar organic solvents such as hexane or chloroform is also effective for lipid extraction (Halim et al., 2011). The variation of temperature also increases the lipid extraction efficiency. The increasing temperature during lipid extraction of *Scenedesmus obliquus* results a significant increase of the lipid (Balasubramanian et al., 2010). It is observed that the solvent-based extraction has several disadvantages. The method requires high energy for solvent evaporation and the use of toxic organic solvents made the process slow and inconvenient. Hence the modification of the use of organic solvent extraction is necessary for improving lipid productivity. From the previous literature it is noticed that the solvent-based extraction is performed as a batch process. Though the batch extraction is limited and requires large amount of organic solvents, a continuous organic solvent extraction able to overcome this limitation. The concept of an igneous cycle for solvent evaporation and condensation was then applied with the help of Soxhlet apparatus. The apparatus continuously replenishes the cells with fresh organic solvent and subsequently minimizing solvent consumption (Wang and Weller, 2006; Halim et al., 2012). The high-energy requirement for continuous distillation is the major drawback of the system (Wang and Weller, 2006; Halim et al., 2012). In comparison to batch mode extraction, Soxhlet based continuous extraction method using hexane is more efficient for the lipid extraction of *Chlorococcum* (Halim et al., 2011).

The combining method of cell disruption and solvent-based extraction is now a day more efficient for lipid extraction. Microwave based solvent extraction is one of such method uses high electromagnetic wave for lipid extraction (Balasubramanian, 2010). Chen et al. (2011) used subcritical ethanol to extract lipid form the wet biomass of *Nannochloropsis* and found that it is more efficient for lipid extraction.

10.2. Supercritical fluid extraction:

Supercritical fluid extraction (SFE) is a modified tool for separating one component to the other using supercritical fluid as the extracting solvent. It is an emerging process of lipid extraction to replace the solvent-based method. The rising of temperature and pressure over the critical values and entering into the supercritical region is the basic principle of this green technology (Pourmortazavi and Hajimirsadeghi, 2007; Halim et al., 2012). For the production of solvent free crude lipid, SFE is more efficient because no energy is needed for extraction solvent removal. Carbon dioxide (CO_2) is the most used supercritical fluid in lipid extraction method. Co-solvents such as methanol or ethanol sometimes modify it. Use of supercritical CO_2 ($SCCO_2$) facilitates successful extraction of lipid with high temperature and pressure without any degradation of cells. $SCCO_2$ based lipid extraction is safe due to low toxicity, low flammability and lack of reactivity (Macias-Sanchez et al., 2007; Halim et al., 2012).

The sovent extraction and SFE are both applied for large-scale lipid extraction for biodiesel production. Organic solvent-based process is slow and use of high toxic or expensive solvents limited towards the desirable lipids of biodiesel production. Whereas the $SCCO_2$ based extraction is rapid, non-toxic and produces solvent free crude lipids. It is effective in both dry and wet algal cells to extract biodiesel-derived lipids (Halim et al., 2012).

11. Transesterification and formation of methyl ester:

Transesterification is the chemical conversion of glycerides to glycerol and methyl esters with the help of an alcohol and a catalyst. The catalyst increases the rate of chemical reaction and may be basic viz. sodium hydroxide (NaOH)/ potassium hydroxide (KOH) or acidic viz. hydrochloric acid/ sulfuric acid. They react as homogeneous catalysts or sometimes as heterogeneous catalysts such as metal oxides or carbonates (Basha et al., 2009). Sodium hydroxide is widely used due to its low cost and high product yield (Amin, 2009). The lipase enzyme is widely used as catalyst for transesterification and it is not frequently used now a day due to its high cost (Chisti, 2007). The optimum temperature and boiling point of the organic solvents are main criteria for transesterification. The alkali-catalyzed transesterification requires high atmospheric pressure and temperature (60-65° C) and reaction takes about 90 minutes to complete. The most common alcohols frequently used are methanol and ethanol. The methanol is found to be more effective and frequently used in commercial uses because of its low cost (Enweremadu et al., 2009; Sakthivel et al., 2011). Vegetable oils are promising feedstock's for biodiesel application since they are sustainable and renewable in nature and can be produced on a large scale and environmentally friendly (Basha et al., 2009). The rapid use of vegetable oil for biodiesel causes competition with the edible oil, which increases both the cost of biodiesel and vegetable oil (Amin, 2009; Mata et al., 2010; Carvalho et al., 2011).

The nucleophilic reaction of the transesterification happens when the carbonyl carbon of the starting ester ($RCOOR^1$) undergoes nucleophilic attack by the incoming alkoxide (R_2O^-). The reaction results a tetrahedral intermediate, which either reverts to the starting material or proceeds to the transesterified product ($RCOOR^2$) or methyl ester (Romanski et al., 2012) (Fig. 3). The transesterification reaction occurs in three major steps: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol (Chisti, 2007).

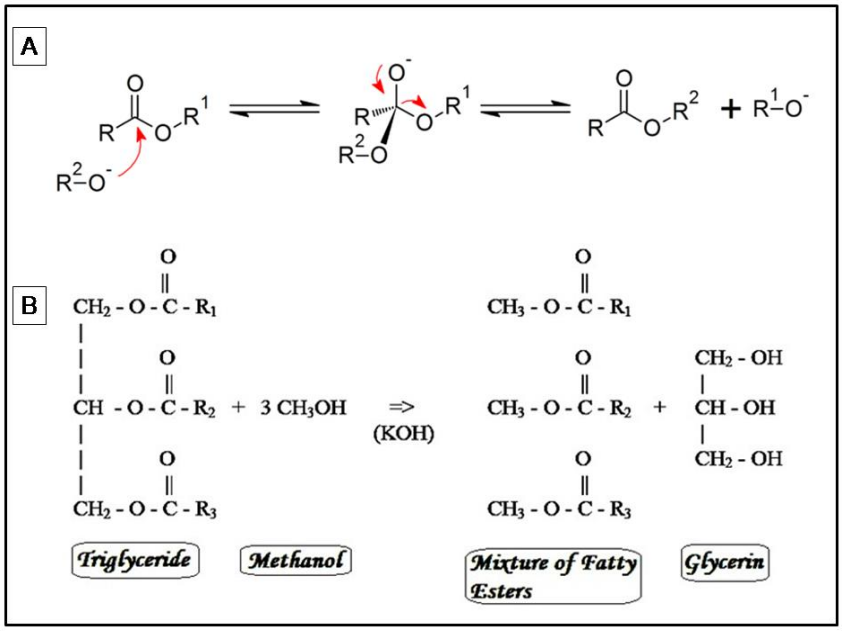

Fig. 3. Transesterfication reaction, A. Showing functional group transfer, B. Showing conversion of triglyceride to biodiesel and glycerol using catalyst.

The commercial production of methyl ester or biodiesel depends on the product quality and involved several steps of purification techniques (Fig. 4). A successful transesterification reaction involved complete separation of esters and glycerol after the reaction time. The major steps involved in this process are mixing of alcohol and catalyst, separation of different phases, alcohol removal and reuse, glycerin neutralization with acid, washing of methyl ester and absence of free fatty acids.

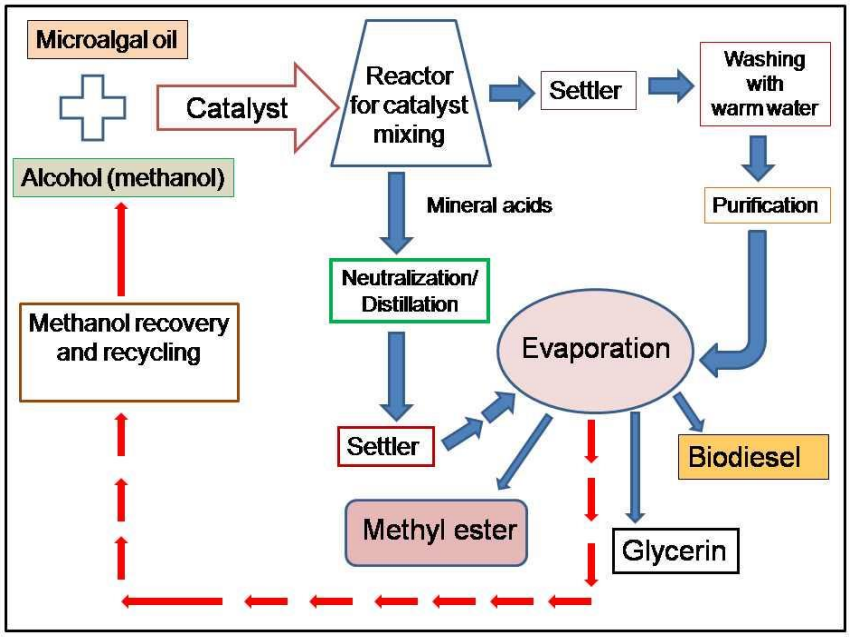

Fig. 4. Commercial production of cost effective biodiesel through transesterification.

12. Physicochemical properties of biodiesel:

The crude methyl ester or biodiesel properties were determined by standard methods. The potentiality of biodiesel to the engine was assessed by its physical and chemical properties. The properties of diesel fuel such as relative density, flash point, fire point, calorific value, viscosity and cetane number are discussed in this section.

12.1. Relative density:

Relative density or specific gravity is an important property of biodiesel. It is a ratio of mass per unit volume of any liquid at given temperature and pressure. The measurement of relative density was carried out by hydrometer or pycnometer at a temperature of 312K. Relative density can be expressed as:

RD= $P_{substance}/P_{liquid}$

Where, P= gravity or density, $P_{substance}$= density of the substance being measured; P_{liquid}= density of the liquid at given temperature and pressure.

12.2. Flash and fire point:

The flash point is the minimum temperature at which the fuel can vaporize to form an ignitable mixture in air. The fuel will ignite or vaporize on application of an ignition source. Flash point is inversely proportional to the fuel's volatility. Flash point used to help characterize the fire hazards of liquid fuel. Flash point also refers to both flammable and combustible liquids. The fire point is the lowest temperature at which the vapor continues to burn after being ignited. It does not require any ignition source. Both flash and fire point are important parameters for determining the fire hazards of diesel. Pensky-Martens closed cup apparatus and results the temperature ranges from 60- 190º C can measure the flash point.

12.3. Calorific value:

Calorific value (CV) is the amount of energy is produced after complete combustion of fuel. In this chemical method a hydrocarbon reacting with oxygen to form carbon dioxide, water and heat. It is also known as heat of combustion and can be expressed as energy/mass of fuel. CV is an important property of diesel fuel that measures the suitability of the biodiesel as alternative to diesel fuel. The CV of methyl esters can be measured in a bomb calorimeter using ASTMD240 diesel standard method.

12.4. Measurement of viscosity:

The resistance mechanism of a fluid to gradual deformation by shear stress or tensile stress is commonly known as the viscosity of that fluid. A dynamic change in the fluid motion takes place due to the friction between neighboring particles in the fluid that are moving at different velocities. A fluid's viscosity depends on the particle size and the attraction between the particles. Viscosity is measured using Redwood viscometer.

12.5. Cetane number:

The combustion quality of diesel fuel during compression ignition determined its Cetane number (CN). CN is an important property of diesel fuel, which determines the quality of the diesel fuel and responsible for the delay period. Lower delay period and smooth engine operation depends on the higher CN value of the fuel. Biodiesel has higher cetane value than the petro diesel fuel because of its high oxygen content. CN are used only for the light distillate diesel oils and diesel engine operates well with a CN 40 to 55.

Iodine values also an important parameter for determining the amount of unsaturation present in the fatty acids. The unsaturation in the form of double bonds reacts with the iodine compounds. The higher the iodine number, the more double bonds (C=C) are present in the fatty acids.

The acceptability of microalgal biodiesel depends its quality and properties after comply with existing standards. Some diesel standards are ASTM biodiesel standard D6751 of United States and Standard EN 14214 of European Union (Knothe, 2006).

13. Economics of Biodiesel Production:

Cost effective biodiesel production and enhancing lipid productivity are two important criteria for microalgal biodiesel industry. The conversion of biomass to biodiesel is not depends on the photobioreactor or raceway pond productivity. The cost of the production of biomass is most important factor in both open and closed system for producing biodiesel from microalgae (Chisti, 2007). The microalgal biomass from open raceway pond and photobioreactor costs $3.80 and $2.95 respectively. The increasing biomass productivity reduces cost of production. For example biomass productivity of 10,000 t, reduces approximately $0.60 for raceways and $0.47 for photobioreactors respectively. Oil recovery is the important step in microalgal biodiesel production contributes 50% to the cost of the final recovered oil. The estimated cost of oil recovery is $2.80/L in lower-cost biomass produced in photobioreactors. It is suggested that the microalgal oil price of $0.48/L competes with the cost of petrodiesel. Reducing the cost of microalgal diesel from about $2.80/L TO $0.48/L can eliminate the use of petro diesel. This could make the microalgal biodiesel economically sustainable.

14. Improvement of micaralgal biodiesel economy:

To reduce the cost of microalgal biodiesel production, researchers are trying to improve the production strategy. The improving capabilities of microalgae through stress physiology and genetic engineering are the most effective way towards sustainable biodiesel production. Biorefinery based production strategy is also an effective method for improving economics of microalgal biodiesel. This section of the review deals with the three major strategic improvement method for microalgal biodiesel production.

14.1. Biorefinery concept:

Biorefinery is the conversion of biomass to renewable fuel and other valuable products or chemicals. The integrated crop biorefinery is already being operated in Canada, Germany and United States. This concept can be applied to reduce the cost of microalgal biodiesel (Chisti, 2007). In addition to lipid, microalgal biomass contains significant amount of protein, carbohydrates which can be utilized as animal feed after lipid extraction. The residual biomass also can be utilized for producing biomethane gas by anaerobic digestion (Chisti, 2007). The conversion of biomass to biomethane generates electricity, which can be reutilized for microalgal cultivation. The microalgal biorefinery simultaneously produces biodiesel, biogas, animal feed and electricity (Fig. 5).

14.2. Impact of nutrients on growth and lipid productivity:

Nutrient starvation on growth and lipid productivity has been studied extensively from the earlier times. Environmental stressors such as nutrient limitation subsequently reduce cell division and frequently activate the biosynthetic pathway of fatty acid (FA). The retarded cell growth declines the membrane lipid synthesis and consequently deposits FAs into triacylglycerol (TAG) within the cytosol. The lack of nutrients retard the production of major electron acceptor NADP+ and thus growth of the algae slows down. In the other way NADPH is consumed in FA biosynthesis, therefore increased FAs production replenishes the pool of NADP+ under growth-limiting conditions.

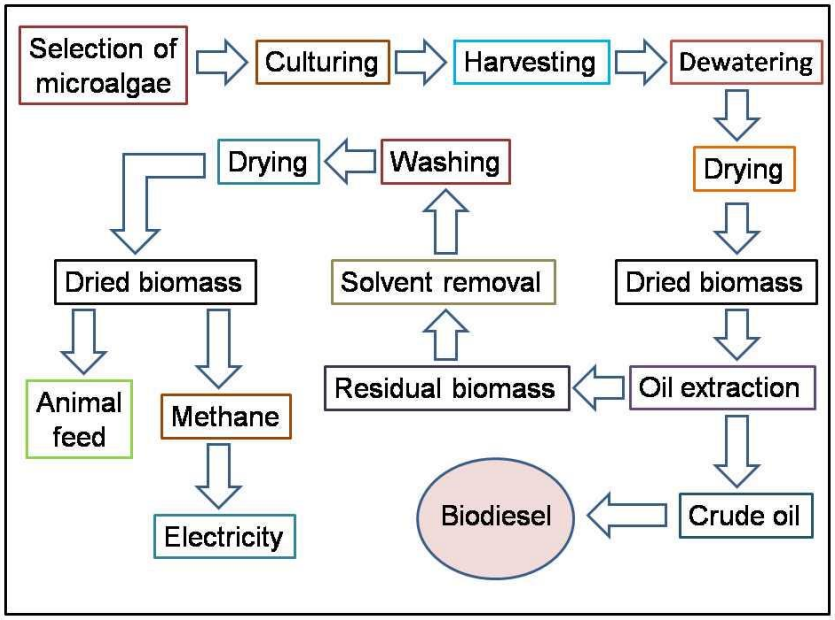

Fig. 5. Biorefinery of microalgal biomass after oil extraction.

Nutrient starvation is one of the key factors most widely used and applied in lipid induction (TAG accumulation) in microalgal cells has been reported in many species (Table 2). A significant increase in lipid production was noticed when algal cells of different groups were exposed to nitrogen (N) and phosphate (P) deficiency (Rodolfi et al., 2009; Sharma et al., 2012). Most recent study involves the effect of calcium, magnesium and sodium chloride on lipid accumulation of *Chlorella vulgaris* and *Scenedesmus obliquus* (Gorain et al., 2013). The effect of iron, silicon, sulphur, urea, nitrogen, phosphorus, magnesium, sodium and potassium on growth and lipid productivity was listed in table 2.

Temperature has been found to be more effective on FA biosynthesis of microalgae. The decreasing temperature increases unsaturation whereas increasing temperature causes saturation of FAs (Sharma et al., 2012). The effect of salinity ranges on growth and lipid productivity was studied extensively in the major microalgal species such as *Dunaliella*. A significant amount of C_{16} and C_{18} fatty acids were synthesized with the salinity gradients (Sharma et al., 2012). The different pH in the medium from acid to basic also enhanced the lipid productivity and accumulation of TAG in the cytosol. The effect of heavy metals such as cadmium, iron, zinc and copper has also been found to increase the lipid content in some microalgal species (Einicker-Lamas et al., 2002; Sharma et al., 2012; Satpati and Pal, 2015). Light is the most essential element for autotrophic microalgae. It has been reported that different light intensities exhibit remarkable changes in lipid and gross chemical composition. Low light intensity induces the formation of TAG and high light intensities decreases total polar lipid content (Sharma et al., 2012). An alternating light/dark cycle also has a significant effect on algal lipid composition. Effect of UV irradiance for lipid induction has also been studied in many microalgal species especially on their growth, morphology, physiology and oxidative stress. The induction of different environmental stresses on growth and lipid productivity in large commercial cultivation system differs from every microalga due to mode of nutrient supply, environmental and climatic conditions.

Table 2: Showing effect of environmental stressors on lipid accumulation in microalgae.

Microalgal Species	Stressors	Major Cellular Changes	Reference
	A. Nutrients		
Chlorella vulgaris	Mg, Ca starved	Lipid- 15.1% & 40.3%	Gorain et al., 2013
Scenedesmus obliquus	Mg, Ca starved	Lipid- 14.9% and 37%	Gorain et al., 2013
Chlorella vulgaris	N limitation	Lipid- 78 mg/L/d	Yeh and Chang, 2011
Chlorella sp.	N limitation	Lipid- 53.96±0.63 mg/L/d	Praveenkumar et al., 2012
Chlorella ellipsoidea	Absence of N	Lipid- 51.3%	Satpati and Pal, 2015
Chlorococcum infusionum	Absence of N	Lipid- 40.43%	Satpati and Pal, 2015
Chlorella sp.	Urea limitation	Lipid- 0.124 g/L/d	Hsieh and Wu, 2009
Scenedesmus sp.	N and P limitation	Lipid- 30% and 53%	Xin et al., 2010
Monodus subterraneus	P limitation	TAG accumulation	Khozin-Goldberg and Cohen, 2006
Chlamydomonas reinhardtii	S limitation	TAG accumulation	Matthew et al., 2009
Chlorella sp.	Si starvation	Changes of total lipid	Griffiths and Harrison, 2009
Chlorella ellipsoidea	Fe supplementation	Lipid- 57.36±0.41%	Satpati et al., 2015
Chlorococcum infusionum	Fe supplementation	Lipid- 48.20±0.43%	Satpati et al., 2015
	B. Temperature		
Nannochloropsis oculata	20°C-25°C	Lipid- 14.92%	Converti et al., 2009
Chaetoceros sp.	25°C	Lipid- 16.8%	Renaud et al., 2002
Isochrysis sp.	27°C-30°C	Lipid- 21.7%	Renaud et al., 2002
	C. Salinity		
Chlorella ellipsoidea	5 g/L NaCl	Lipid- 45.8±0.4%	Satpati et al., 2016
Chlorococcum infusionum	1.5 g/L NaCl	Lipid- 36.33±0.56%	Satpati et al., 2016
Nitzschia laevis	10-20 g/L NaCl	Unsaturated fatty acid increased	Chen et al., 2008
Schizochytrium limacinum	9-36 g/L NaCl	Increased level of Saturated fatty acids, C15:0, C17:0	Zhu et al., 2007

Dunaliella tertiolecta	29-58 g/L NaCl	Increase of total lipid and TAG	Takagi and Yoshida, 2006
	D. pH		
Chlorella sp.	Alkaline pH	Increase in total lipid	Guckert and Cooksey, 1990
	E. Heavy metals		
Euglena gracilis	Cd, Cu, Zn	Increase in total lipid	Einicker-Lamas et al., 2002
Chlorella vulgaris	Fe^{3+}	Lipid- 56.6%	Liu et al., 2008
	F. Light irradiation		
Tichocarpus crinitus	Low light	Increase TAG	Khotimchenko and Yakovleva, 2005
Pavolva lutheri	High light	Increase total lipid	Carvalho and Malcata, 2005
Phaeodactylum tricornutum	UV- radiation	Increase EPA and PUFA	Liang et al., 2006
Chaetoceros muelleri	UV-A	Increase MUFA	Liang et al., 2006
Nannochloropsis sp.	UV-A	Increase SFA and PUFA	Forjan et al., 2011

14.3. Genetic engineering of algae for induction of biomass and lipid:

Genetic engineering of microalgae now a day has received great attention for enhancing cell biology and lipid productivity. Genetically modified (GM) algae can be used predominantly because it increases photosynthetic efficiency, enhance biomass growth rate, increase oil content in the biomass, improve temperature tolerance to reduce the expense of cooling, enhance growth in response to increase light and reduces photoinhibition or photooxidation (Chisti, 2007). Fatty acid biosynthesis revealed several genetic modifications through biosynthetic pathways. The plastidial multisubunit acetyl coenzyme A (acetyl-CoA) carboxylase (ACCase) catalyzes the major step of fatty acid biosynthesis, acetyl-CoA to malonyl-CoA. A group of researchers and companies considered that the potentiality of microalgae as green cell factories for producing value added product such as lipid and proteins. Different genetic transformation system is developed in microalgal biotechnology for industrial application. Complete genome sequences and genetic transformation of different microalgal species was studied since 1990 (Sakthivel et al., 2011).

15. Conclusion:

The microalgae are considered as promising biodiesel feedstock for future uses to replace fossil fuel completely. The advantages of using microalgae are their high growth rate and widespread availability. There is no competition of cultivating land, minimal nutrient requirement and high oil content makes microalgae sustainable towards biodiesel production. The downstream processing of large-scale cultivation of microalgae is still in the early stages of development. The cost effective production of microalgal biomass in open raceways and photobioreactors are still in controversy. The advances of photobioreactors through engineering will further lower the cost of biomass production. The closed photobioreactors provide controlled environment, which likely to be required for better biomass productivity and subsequently making biodiesel. The improvement of algal cell biology through genetic modification and metabolic engineering also will have great interest for low cost biomass and lipid productivity. The effective lipid

extraction method also triggers the microalgae for biodiesel production. The use of biorefinery concept is tailored to the global demand of biodiesel as well as biogas, electricity and other valuable products.

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