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Review Article

Biodiesel as an Emerging Energy Demands and E-Production with Special Reference to Algal Species: A Review

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A B S T R A C T

The world has moved towards the energy crisis due to much more use of resources. In last century there was total reliance on fossil fuels for fulfilling the energy demand. It has been estimated that approximately, two billion gallons' diesel is consumed in Pakistan annually. And as we know Pakistan depend greatly on foreign oil so the only solution of this problem is to seek new resources for decreasing energy crisis. In Pakistan the production of crude oil is 58000 barrels per day but even entire production is advanced but it will fulfill the 14 % of national demand. The Government of Pakistan tried to replace it with 5% use with biodiesel. In these renewable fuels biodiesel has so much importance. It is ecofriendly fuel due to its expedient effect on atmosphere. Paying attention to world energy demand, decreasing petroleum reserves and mainly global warming effects, there is a requirement to produce such type of fuels that should be less pollutant and fulfill the world energy requirement. There are mainly two ways for the production of biodiesel i.e.

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agriculture crops including soybean, sunflower, rapeseed and palm as well as microbes such as methanogens and microalgae. Among microbes, microalgae produced huge amount of biofuels because of more oil contents present in it, fast growth rate and increased biomass production. The main objective of this project was production of biodiesel from Algae.

Energy demand: Energy demand has increased a lot due to increasing human population, modernization and many other factors are also involved in this dilemma such as, human deeds, industries transports, dwellings, trade, commerce and even agriculture also.It has become essential that there should be energy prosperity in country to solve the major problems like poverty, hunger, disease and illiteracy also (Amir *et al.,* 2014).Fullfiling the energy demand has become important for welfare of the human beings and for raising the standard of life (Asif, 2011). Several different types of technologies are present for the development of new energy resources to decrease the dependence on fossil fuels and imported petroleum but any solitary technology not proved itself so successful.If we use different technologies in combine form then better results can be achieved. These technologies help to overcome the energy crisis. As energy demand is increasing these days similarly world is moving towards the energy crisis and it is prediction that oil production will almost decline in between one and ten decades (Crookes, 2006).

Consumed energy sources and fossil fuels: Energy resources being consumed in the world are Fossil fuels, Nuclear power and Renewable energy. Scientists are also taking Hydrogen as option for energy production. Due to excessive use of fossil fuels the CO2 is increasing in atmosphere (Wang *et al.,* 2008). Supply of fossil fuels will be finish in about 2050. Fossil fuels consist of hydrogen bonds and carbon, these are deposits of organisms that were live present many years ago (Munshi *et al*., 2002). Here are problems with fossil fuels. Fossil fuels are cause of sulfur oxides release which are great source of environmental pollution. Sulfur oxides are main cause of acidic rain, so it's necessary to control the pollution and acidic rain by reducing sulfur oxides in fuel. In crude oils the heavy sulfur contents are so much high and these are problems for refineries. These heavy sulfur contents are also causing problems in straight run and secondary processed diesel. It has become so much obligatory to reduce the sulfur contents in oil (Gray *et al*., 2003: Monticello, 2000).

Efforts of Government and private companies for new energy sources: Government and private companies are trying to examine new energy resources. Other nonrenewable energy resources for example uranium and coal are limited and will decline soon. Government is also taking action, establishing research institutes, industries and arranging such type of programs to stimulate the people for energy security by using alternative biofuels (Altiparmak, 2007; Vincent *et al*., 2014).

EP ACT for use of biofuel: In 2005 according to energy policy act (EPACT) raise the spirit of people to use biofuels for example ethanol and biodiesel. According to this act the use of agriculture resources as a huge biomass source for biofuel production increased and in this act there were advices for secretary of Energy about petroleum fuel. The EP ACT clearly warned to decrease the use of petroleum fuel and to increase the biofuel usage. To decrease the greenhouse gas release and extra harm full components of fossil fuels there is solution in the form of bioenergy. The chief source of greenhouse gasses release is petroleum. To

contaminate the air, in these greenhouse gasses particulate matter, volatile organic compounds, CO, Sox and NOx are included (Sharif Hossain *et al.,* 2008).

Energy Independence and Security Act (EISA): In 2007 US National Government passed an act for energy security, that act is called energy independence and security act (EISA). According to this act the renewable energy production should be annually more and more. And in 2022 the production should reached up to 36 billion gallons per year. After this act 28 more states passed their own law in 2009 about energy security because energy is the fundamental and essential aspect for people round the world. The energy production and security is important for increasing the economy and improving the standards of life. The energy demand will be increased 50% more in 2030 than todays (Shah *et al*., 2013). Due to many advantages of biofuel it has attracted the scientists (Refaat, 2010).

Renewable energy sources: The renewable resources are wind power, geothermal energy, hydropower energy, solar energy and biomass energy. These can be solution of energy's problem. Pakistan is a developing country facing all the energy problems due to poor policies and careless and wrong attitude of concerning authorities (Amir *et al*., 2014). Now a days it is necessary to find more and more alternative fuels to overcome these energy crisis.For increasing the economy of any country diesel fuels have an important role. Lot of use of fossil fuels in industries and in homes are the cause of pollution.

Alternative fuels: The alternative fuels are biofuels like biodiesel, bio hydrogen and biogasetc (Chellapandim *et al.,* 2010). Production of biofuels is from range of sources. These sources are vegetable oils, fats of animals and different crops. Rapeseed oil was the major source for biodiesel production throughout the world in 2007 (Komers *et al.,* 2001; Mohibbeazam *et al*., 2005). This was reported by International Grains Council 2008 (Hamad, 2008). Oils and fats also have the characteristic of insolubility. These oils and fats are made up of triglycerides. For increasing the production of triacylglycerides many genetic approaches have been developed (Kalpesh *et al.,*2012).

Biofuels produced from these sources are called second generation fuels. The second generation fuels are called advanced fuels. These second generation fuels create a dispute among food and fuel. Because food prices become so high due to their usage in fuel production (MohibbeAzam *et al*., 2005). In 2008 a big clash was raised against using crops for energy production. And blame of increasing food prices was totally going on farmers that they don't grow feed crops for human or animal consumption but even grow crops for energy production and starvation was also a great issue for the uses of crops (Mostafa, 2010).

Crude glycerol: Crude glycerol is chief by product of vegetable oil having low value due to impurities (Schieck *et al*., 2010). It is necessary for commercialization to refine and filtrate biodiesel (Dasari, 2007). From each gallon of biodiesel about 1.05 lb. glycerol was produced. Thisdatashows that 11,500 tonnes of 99.9% pure glycerin will be produced from 30 million gallon of biodiesel. There was estimation at that time biodiesel will produce up to 37 billion in 2016, so attention is converting towards the Crude glycerol because it can be used and applied in thousands of ways.

Biodiesel: Biodiesel is non-petroleum based fuel comprise of long chain fatty acids (Fukuda *et al*., 2001). One good point of biodiesel, it is not so involving in pollution of greenhouses by harmful

gases (Bajpai and Tyagi, 2006). Different techniques are available for production of biodiesel, these techniques are pyrolysis, and micro emulsions direct/oil blends, transesterification and many others (Turkenburg W.C., 2000; Ramadhas *et al*., 2004).

Depending upon the feedstock composition the biodiesel exhibit different chemical composition (Wyatt *et al.*, 2005). When 10% volume of palm oil was mixed with diesel for checking the performance of IDI (indirect injection) type engine consequences was not satisfactory. The performance of engine became lower. Engine released highly toxic gases and the performance of engine became so poor (Sangsawang *et al.,* 2009). Easy way of decreasing dependence on petroleum is by using biosurfactants (Mulligan, 2009).

Alcohols used in biodiesel production: Alcohols that are widely used in the production of biodiesel are, butanol, propanol, ethanol etc. But mostly methanol is used in the biodiesel production. Methanol is preferred because it is not expensive and easily available in most countries of the world. Biodiesel obtained from butanol is totally biobased (Stavarache *et al.,* 2003).Biodieselis mono alkyl esters of the oil and fats.Neat vegetable oil was not used for fuel production because neat vegetable oil was the cause of problem in diesel engine. Reason was its increased viscosity which caused deposit formation in injector choking (Alptekin and Canakci, 2008; Gerhard Knothe, 2010).

Use of catalysts in biodiesel production: Several catalysts were also used in production of biodiesel. These were sugars, lipases etc. Homogenous and heterogeneous acids and bases were used also in biodiesel production. A lot of other heterogeneous materials may be used as catalysts.In biodiesel production at commercial level from canned or refined oil the different catalysts are used. These catalysts are not costly and are in homogeneous form (Bryan, 2009).

Transesterification: In biodiesel production transesterification has its great role. Due to transesterification the viscosity of biodiesel is decreased and is reached to petroleum diesel (Olugbenga and Stephen, 2013). Transesterification can easily convert the Triacylglycerides into biodiesel. These triacylglycerides are stored in the form of energy in microalgae. In this process the long fatty acids are joined together with a glycerol molecule. In Transesterification process the glycerol is transferred with alcohol. Interest in biodiesel production at high level raised with increasing prices of petroleum (Thompson, 1996; Chen, 2011).

Catalytic transesterification: When the catalyst is used in the transesterification then this process is called catalytic transesterification (Dias *et al.,* 2008; Olugbenga and Stephen, 2013).

Non catalytic transesterification: If the transesterification is performed without using any catalyst this is called noncatytic transesterification (Olugbenga and Stephen, 2013).

Types of catalysts used in tranesterification: When catalysts are used these are also of two types called homogeneous and heterogeneous. Homogeneous catalysts are mostly used in industries Transesterification is of different types, Acid transesterification, alkaline transesterification and enzymatic transesterification. Acid transesterification is not as famous as the base transesterifiction. (Olugbenga and Stephen, 2013).

Alkaline transesterification: Generally alkaline Transesterification is defined as organic reaction in which esters are changed into one another by alkoxy moiety.In alkaline transesterification the pH value should be greater than seven. The base transesterification is performed at low temperature and pressure. There are little problems associated with base catalyzed transesterification because base

catalyzed transesterification is sensitive to Free Fatty Acids (FFA). (Canakci and Van, 2001). If FFA contents increased then it caused the soap formation (Haas, 2005; Olugbenga and Stephen, 2013). The product obtained with low free fatty acids is further processed in transesterification for producing biodiesel (Velasquez *et al*., 2012).

Acid transesterification: Acid transesterification is 4000 times slower than alkaline transesterification so high amount of alcohol is required for this process (Olugbenga and Stephen, 2013) Through Biofuels alcohols can be produced by two different methods which are called direct fermentation and indirect fermentation (Donaldson *et al.,* 2009). In the direct fermentation process plants important ingredients are changed into biofuel especially ethanol. The principle of this process is breaking of plants contents and converting into sugars then changing of these sugar into alcohol. This is commonly used method but indirect fermentation is not so common method so indirect fermentation depends on the pyrolysis. In the pyrolysis the plant materials are converted into gas for example Syngas. In the syngas different gasses are mixed such as carbon dioxide, hydrogen and carbon monoxide. These process are done by acetogenic bacteria (Mostafa, 2010).

Enzymatic transesterification: In enzymatic tranesterification biocatalysts are used. Enzymatic transesterification is performed at 35 to 45◦C. It is also a slow process and can be fast by using ethanol. Lipase enzyme is used in enzymatic transesterification. Lipases can be obtained from fungi and bacteria Candida Antarctica, Pseudomonas cepacia,Rhizopus oryzae and Mucor miehei. Process of Transesterification removes the glycerin and replace it with alcohol (Olugbenga and Stephen, 2013).

Advantages of biodiesel: Production of biodiesel has a lot of advantages such as biodiesel emit less harm full gasses as compared to petroleum diesel, low CO_2 release, reductions of sulphurand aromatic hydrocarbon emissions, actually improve engine life and low toxicity. Biodiesel has a more combustion than gasoline, producing unpolluted burn (Aresta *et al.,* 2005; Rakopoulos *et al*., 2006; Kocak, 2007; Hess *et al.,* 2007).

Petroleum dependence: Petroleum was considered the best in 2010 in US and people thought that it is the one and only source of energy at that time. About 71% energy was used in transportation. In 2009, fuel consumption was 13 million barrels per day as transportation fuel. This data shows that there was too much dependence on petroleum based fuel and it was not feasible due to continuous finishing of oil reserves. There were also bad results by the use of fossil fuels (Chisti, 2007; Lardon *et al.,* 2009; Demirbas and Demirbas, 2011).

Problems associated with petroleum: Petroleum diesel has so many disadvantages. Petroleum diesel is great reason of pollution, also great threat of human beings and animal's health.Petroleum diesel release many toxic gasses which becomes the cause of cancer Development and progress. The results of these emissions are also respiratory problems cardiovascular effects and several other health complications. Air pollution is the common and big problem caused by the petroleum diesel. Bad effects on Water, soil and ecological effects are also due to petroleum diesel. That's why a lot of work has been done and still scientists are working to solve these problems (Lloyd and Cackete, 2001).

Release of hydrocarbons: From petroleum the hydrocarbons are released and these petroleum hydrocarbons are cause of contamination in the

atmosphere. For this purpose, the cleanup methods are present. These cleanup methods are bioremediation and phytoremediation. These methods got so much attention from last two decades to clean the environments (Liste and Felgentreu 2006; Gaskin *et al*., 2008; Li *et al*., 2008a; Chauhan and Rai, 2009).

Diesel: During gas oil fraction, petroleum separation and middle distillate the product we obtained is called diesel which is mixture of saturated hydrocarbons and aromatic hydrocarbons (Bondioli *et al.,* 2003). These are also source of pollution. These are organic pollutants so these pollute the soil when leak from underground pipelines and storage tanks. When these are released from storage tanks or pipe lines then these go into ground water and hydrocarbons mix with it. Water pollution also occur. When animals, human beings or any other living organisms consume such water it cause the diseases (Wang and Bartha, 1990).

Evolution in biofuels production: Due to modernization, environmental problems, crops and land issues evolution of biofuels increased in last decade. In 2000 to 2010 the biofuels production increased. This increase was about 12% for ethanol and 27% for biodiesel. There are many reasons which effected the development of biofuel lsuch as downfall of capital markets. But biofuel production raised again in 2010 which was 13.8%. In this production 86,870 million liters of ethanol and 19,800 million liters of biodiesel. This was the substantial increase in the liquid fuels in decade. Liquid biofuels contributed 2.7% to the transportation sector worldwide.

Algae as good sources for biofuel production: Algae have been considered good choices for biofuel production due to its simple life structure (Li *et al.,* 2008a) high growth rate of algae, huge biomass production and mainly photosynthetic property of it (Miao and Wu, 2006: Li *et al*., 2008b). Contents present in algae such as fatty acids, hydrocarbons, triacylglyceride and isoprenoids are of very much importance because they have the ability to compensate the failure of crude oil production. In crude oil and natural gas the fossil carbon is also found (Guschina and Harwood, 2006).

Selection of right specie of algae for biodiesel production: For biodiesel production the right form of algae should be selected. Botryococcus braunii and chlorella vulgaris have been considered good choices for biodiesel production (Largeau *et al*., 1980) because these species can easily grow and have high oil contents that are the main points for biodiesel production. But oil extraction is little difficult from because the cell wall of algae is too much hard and grinding of dry algae become co hard that's why the whole process from grinding to oil extraction is not easy. For using algae in biodiesel production their efficiency should be improved. The chemical composition of algae should be completely known.

Advantages of algae: Other advantages of algae are,Algae are also use as medicine. These algal strains are commercially available such as Isochrysis, *Chaetoceros, Chlorella, Arthrospira (Spirulina)* and Dunaliella and these are also use as medicine. Algae are use as therapeutic supplement and this is the great advantage of algae. The commercially available therapeutic compound of algae is astaxanthin, β-carotene, polyunsaturated fatty acid (PUFA) for example DHA and EPA and polysaccharides such as β-glucan (Indira and Biswajit, 2012). By photosynthesis algae change the CO_2 to algal biomass and reduce the global warming. Different products are obtained from algal biomass for example carotenoids, sterols,

omega-3 fatty acids, and other pigments and antioxidants, these are also use as fertilizers (Thomas, 2006).

Different fuels production from algae: Through microalgae different renewable fuels are produced methane, biodiesel and biohydrogen (Halim *et al.,*2012). These are better than fossil fuels. Biodiesel is produced from algal oil through transesterification (Halim *et al.,* 2011). Production of biohydrogen is by photobiological process and anaerobic digestion is the method used for methane production from algal biomass (Thomas, 2006: Widjaja, 2009). Algal biomass can also be used for the production of different supplementary products protein pigments and plastics. Further, algae can also be used for water treatment and remove the heavy metals from water (Thomas, 2006). There is another good point about algae, for oil production there is no need of any fertile area any special food source and even water also for algae. Algae also require so much less water than soybean. It may be 300 times less than soybean for oil production that is further used for biodiesel production (Phan *et al*., 2009; Belarbi *et al.,*2000).

Algal species and their uses: Chlorella vulgaris is full of protein so used as feed but it also has other benefit that it can be used in the treatment of waste water. Chlorella vulgaris is used in the oxidation process of sewage (Singh *et al.*, 2011). Cultivating algae Chlorella protothecoids in heterotrophic environment with hydrolyzed corn starch as a feed source, algae adopted this environment with high population density of 15.5 g/L. Algae adopted this environment by losingits photosynthetic organelles and doubledthe oil contents (Xu *et al*., 2006). Spirogyra spp and chlorochocum spp produce polysaccharides and starch in their hard cell wall. Then this starch is use for production of biofuel. Chlorochocum spp produce 60 % more ethanol than other spp. Cladophora sp, produce higher quantity of biodiesel than *Spirogyra* sp and there was not so change inph. of biodiesel (Khola and Ghazala, 2012).

Requirements for algae cultivation: Algae cultivation has some basic requirements Water, space, carbon from CO2 and light but these all should be provided in balance (Prez *et al*., 2004). These all should be provided in a controlled way. These are necessary for increasing the biomass and oil contents in algae. System for culturing of microalgae and macroalgae are dissimilar (Schneider, 2006; Posten and Schaub, 2009). There is need of large scale system to grow algae for energy production.These systems should be range from simple open air system to large optimized systems. Simple open air system exposes the algae to atmosphere. Technology is under process for the development of algal fuels (Chen *et al.,* 2009; Wi *et al*., 2009). There are found several species and strains of algae and these strains and species have variations in percentages of lipids and dry mass. There is need to characterize the strains of algae. For this purpose, many technologies are present. Environmental conditions have much impact on the characteristics of algae (Rosenberg *et al.,* 2008). Typical standard methods should be applied to characterize the algae (Caramujo, 2008).

Role of Fatty Acids in Algae: In characterization the fatty acids are also significant. Fatty acids are released by process of enzymatic hydrolysis (Thompson *et al.,* 2010, Schaloske *et al.,* 2006). They have unique role. The fatty acids have their metabolic role also. These are existing in all organism. They play the role as signaling molecules. Fatty acids have capacity to regulate the enzymes. Fatty acids produce much energy upon oxidation. These are stored in the form of

triacylglycerol and sterol ester. They play role as energy reservoir (Martin and Parton, 2006; Horn *et al.,* 2011).

Commercially available strains of algae: Different algal strains and species are commercially available. The commercially available dominant species of algae are Isochrysis, Chaetoceros, Chlorella, Arthrospira (Spirulina) and Dunaliella (Behera *et al.,* 2007; Ananyev *et al.,* 2008; Indira and Biswajit, 2012). Phaeodactylum tricornutum, *Nannochloropsis salina* and *Botryococcus braunii*. These algal strains have the high oil contents. *Botryococcus braunii*, the algal strain that was first chosen for generating biodiesel at big level because of more hydrocarbons contents. But there was some problem that's why Nannochloropsis salina was selected for biodiesel production (Final Report on Biodiesel Production from Microalgae - A Feasibility Study – Presented to Statoil Hydro ASA Oslo, Norway May 16, 2008).

Quantity of biodiesel: Biodiesel is a good alternative fuel emitting less harmful gasses that got attention of scientist due to its renewability and different other properties (Vasudevan and Briggs, 2008). Biodiesel industry developed histrionically in few recent years. Advanced and established developed countries recognized a Biodiesel Board for making policies. Biodiesel production is increasing day by day specially in European countries. The production of biodiesel increased from 1.9 million tons in 2004 to 3.2 million tons in 2005 and it was a good news at that time specially for solving the energy problems. In 2006 it reached up to 4.9 million tons. There was also massive increase in 2011 production rate was 22.117 million tones.

Algal biodiesel a good fuel: Algal biodiesel is a good selection for achieving satisfactory results. Algal biodiesel is comparably good fuel than other fuel because of increased oil production rate of algae than other plants (Ball and Morell, 2003; Albuquerque *et al.,* 2009; Bhale *et al.,* 2009). Biodiesel is used in replacement of petroleum diesel. Biodiesel is preferred to petroleum diesel because of containing some unique qualities. It is highly lubricant than the petroleum diesel (Geller and Goodrum, 2004). Biodiesel 100% replace the petroleum diesel due to its functional properties. When biodiesel is used in transport vehicles it lowers the release of smoke by 50% hydrocarbons, CO_2 80 and 60% carbon monoxide. Algae produces 30-time high energy than soybeans (Spolaore *et al*., 2006; Chisti, 2007).

Cultivation of algae in waste water: Biodiesel production from algae is expensive but here is solution for this problem. Algae should be cultivated in waste, dirty and unwanted water. The advantage of growing algae in waste water is no need for providing any special nutrients because waste water also contains all necessary nutrients that are required for algal growth. Growing algae in waste water is economical and it is not so overpriced procedure. Algae have been using for waste water treatment also and this process is from more than 50 years. *Chlorella* sp also used in the treatment of waste water. One advantage of growing algae in waste water is, addition of lipids and fatty acids becomes high and fast that is good for biodiesel production through transesterification. There is also another benefit of growing algae in waste water for example, the heterotrophic bacteria present in waste water use oxygen that is produced by algae. These bacteria then convert this oxygen into algal biomass.Algae can also be cultivated in that type of land which is inappropriate for growing food. Estimation about oil production from microalgae is almost5000 to 20,000 gallons of oil per acre per year (Chisti, 2008). Microalgae are biochemical plants. They regulate

CO2 just like other telluric plants. They produce variety of fatty acids. Hydrocarbons and other contents especially oil and it all depends on the type and specie of algae or its strains. The hydrocarbons and lipids are present in almost all of the biomass but they are found as storage products and membrane metabolites. Simply they are found as membrane products. Different algal strains, species types, such as red algae, green algae, brown algae, blue green algae, diatoms and dinoflagelates have variety of lipids and oil contents (Lebeau and Robert, 2003).

Macroalgae: There are so many varieties of algae and biomass towards Pakistani seaside area, because this type of environment is full of nutrients. A lot of macroalgae have been discovered in Pakistan (Tanaka and Shameel, 1992). Macroalgae grow very fast up to 60m in length. They grow in washbasins also and is considered a great source for solving the problem of energy crisis (Goh and Lee, 2010). Biomass of macroalgae produce high amount of sugars that can be easily used in the production of methanol. Macroalgae mostly grow over mud areas. (Siddiqui *et al.,* 2000).

Use of Macroalgae: Macroalgae are also autotrophic organisms. They change the nutrients and energy into different food products which are source of nutrition for variety of organisms. Macroalgae also provide shelter for many aquatic organisms. Macroalgae are full of protein, carbohydrates, minerals and many other food contents that's why these are used as fodder in different countries of the world (Blanchard *et al.,* 2000; Lamare and Wing, 2001; Zodape, 2001). Biomass of macroalgae can be used in several ways for example it can be used as food (Chopin and Sawhney, 2009).

Oedogonium a macroalgae specie was recognized by DNA barcoding. For assigning the names to isolates of *Oedogonium* DNA sequences were used. These DNA sequences were obtained from the Internal Transcribed Spacer (ITS) region of the ribosomal cistron. This is the marker that is used for the phylogenetic studies of green algae. Recently this marker was also used for the study of *Oedogonium*. Different variable temperature experiment and constant temperature were performed to check the growth and further identification. For controlling the growth of this macroalgae temperature was the key factor. Oedogonium Species were considered best for biomass production and its solicitations (Park and Craggs, 2011).

Microalgae: Microalgae are prokaryotic and eukaryotic photosynthetic Microorganisms. These are small organisms are of different sizes such as the microplankton, these range from 20 to 1000 μm. Another is the nanoplankton and these range from 2 to 100 μm. Next is the picoplancton and these range from 0.2 to 2 μm. Last one is the ultraplankton and these range from 0.5 to 15 μm. These all have their own role and are very significant. Their small sizes have great role in photosynthesis. Due to effective photosynthesis these changes the sun light energy reacting with CO_2 to produce the carbohydrates, lipids and protein also (Indira and Biswajit, 2012). Microalgae are present not only in aquatic environment but also in terrestrial environment. Few years back a lot of microalgae strains have been discovered in University of Coimbra (Portugal) 4000 strains and 1000 species and this was considered the largest collection at that time. Scientists thought these should also be used in different other purposes for example in pharmaceutical purpose, as a food and mainly as a source of energy (Tsukahara and Sawayama, 2005).

Microalgae used in fuel production: Microalgae can provide feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, ethanol, among others. Microalgae are

that type of cells which are sunlight-driven cell factories. Microalgae have much more oil content as compared to Macroalgae because microalgae are easy to grow and cultivate. Microalgae changes the CO2 to foods, biofuels and high-value bio actives (Walter*et al.,* 2005; Reijnders, 2008: Rodolfi *et al.,* 2009). In world almost 50000 microalgae species have been discovered that can be used as in algal biodiesel production and it mostly depends on their fatty acids composition and presence of lipid contents. There is no sulfur in algal biodiesel. Algal biodiesel decreases the release of hydrocarbons, CO, SO_X and other particular matter into environment (Teresa *et al.,* 2010). By Transesterification of algal oil mono alkyl esters is produced (Demirbas, 2010). Biodiesel is produced by Transesterification with methanol using alkali as a catalyst for example KOH and NaOH. Crude glycerol is a chief by product of biodiesel (Dasari *et al*., 2005; Dasari, 2007). Algae grown under absence of light lost its organelles of photosynthesis and doubled its oil production (Xu *et al*., 2006).

Cultivation of algae in open culture system: As microalgae is cultivated in close controlled culture systems similarly microalgae can be cultivated in open culture system such as lakes, ponds, and pools etc. example of closed culture system is photo bioreactor. Open culture system is more profitable than closed culture system, because open culture systems are not costly, long lasting, capture more energy from sun light and standardize the nutrients. Open systems have many problems such as evaporation of water, high land foot print, different ecological conditions, algae densities are low (Haag, 2007).

Race way ponds: Algae can be cultivated at largescale by a very feasible and achievable method that is called raceway ponds (Molina Grima,1999; Sánchez Mirón *et al.,* 2000). Raceway ponds are in practice from 1950 for growth of algal biomass to produce algal biodiesel. These are made up of concrete and lined with white plastic, closed loop recirculation is about 0.3m deep. During sunlight the culture is nourished constantly in front of paddlewheel where the flow begins. The largest race way ponds keep the 440,000m^2 area.

Photo-bioreactors: Algae produce 300 times more oil than other crops but it depends on the area where it is grown (Chisti, 2007). (Photo-bioreactors) PBRs are the best systems for producing algae at high level because the infectivity, pollution and contamination chances are low, biomass production high. For this purpose, there is no need of large area. Many more chances of different species adaptation and malleability also. In photo bioreactors the solar collectors are fixed to gather energy from sun. These are made up of glass tube or plastic tubes Meteorological conditions and growth environment effect the biochemical composition of algae (Lamare and Wing, 2001). Unicellular aquatic green algae by photosynthesis process can double their biomass in almost 24 hours (Christi, 2007).

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