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MICROALGAL BIOMASS PRODUCTION AND OIL EXTRACTION FOR SUSTAINABLE BIODIESEL PRODUCTION

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***Abstract*- Knowing the limitations caused by fossil fuels various researches are being conducted in search of a substitute fuel. One such substitute fuels are biodiesel. A microalgal feedstock is considered as the ideal third generation biodiesel as they are a highly efficient and sustainable source of fuel due to their high accumulation of lipid and are cultured without agricultural land or ecological landscapes, they help withglobal warming and treatment of wastewater. Even though they provide us with all these benefits, they pose various challenges. This review paper focuses on the important aspects of the production of biodiesel and addresses the challenges posed in the process. We discuss the process in detail from the efficient way to select the microalgal strain, effects of biotechnology and genetic engineering in improving the efficiency of the microalgae, the most efficient culture system and bioreactor used, and the optimum conditions that help the accumulation of the most amount of oil, detailed harvesting methods where both the physical, chemical, and biological methods are discussed in detail. The details of oil conversion to biodiesel by transesterification method followed by the recent developments to increase the oil production by the addition of nano-particles, advantages, and prospects have been discussed.**

***Index Terms* - Biodiesel, Microalgae, Photobioreactors, Downstream processing, in-situ transesterification.**

I. INTRODUCTION

Governments, policymakers, scientists, and researchers have been forced to find alternatives to fossil fuels due to their rapid depletion, global climate change, and rising crude oil prices.

Sustainability can be achieved both environmentally and economically through alternatives to fossil fuels, such as biofuel production from renewable sources. The sustainable production of biodiesel from microalgae through oil extraction from microalgal biomass is in very high demand currently. Microalgae are microscopic organisms that usually live in colonies. With an estimated annual turnover of $5 billion, both macro-and microalgae play an important role in the current globale conomy.

Algal biomass has several benefits in the manufacture of biodiesel: (I)The production of algal oil is higher than that of conventional oilseed crops due to their capacity to thrive throughout the year; (II) Increased tolerance for high carbon dioxide levels; (III) In algae cultivation, water is consumed at a relatively low rate; (IV) Algae production does not necessitate the use of herbicides or pesticides ; (V) Algal species have a relatively higher growth potential in contrast to other organisms;

(VI) Other sources of wastewater containing nutrients such as nitrogen and phosphorus, in addition to providing other nutrients, can be used for algal cultivation; and (VII) the capacity to thrive in tough environments such as salty, brackish, and coastal seawater, which do not influence traditional agriculture (Dis mukes et al., 2008; Dragone et al., 2014; Spolaore et al., 2006). As a result, a major focus has been on algal biomass and its use in the biofuel field.

II. ALGAE AS A THIRD-GENERATION BIO FUEL

Biofuels are generally understood to be solid, liquid, or gaseous fuels derived from organic matter (Nigam & Singh, 2011). Biofuels are divided into two types: primary fuels and secondary fuels. The detailed classification of biofuels is shown in (figure 1) (Alam et al., 2012; Dragone et al.,2014).

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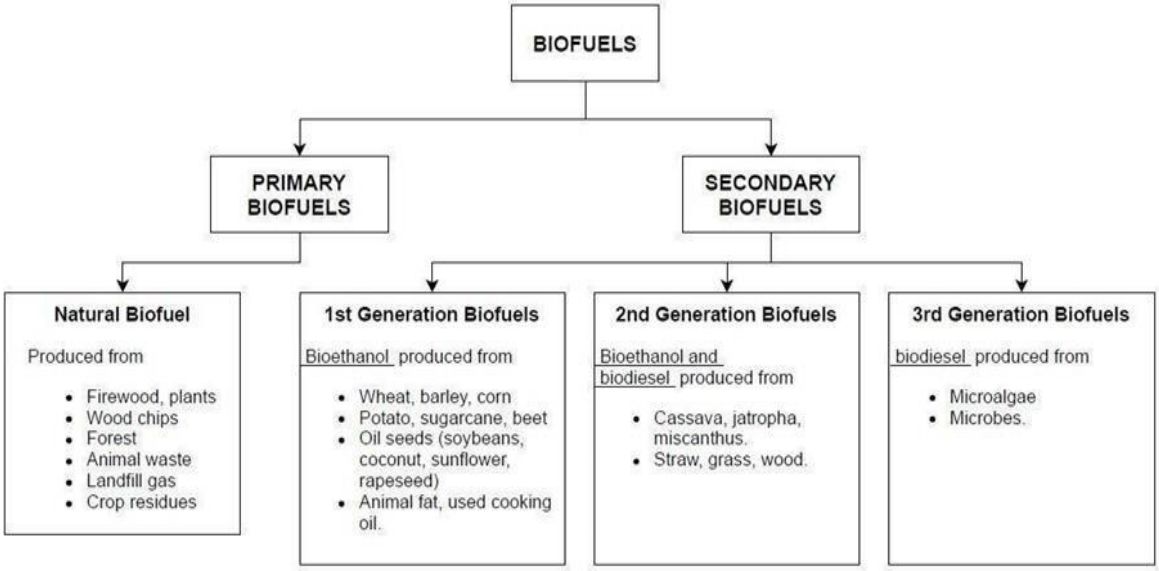

Fig.1 – Biofuel production sources

Biofuels of the first generation contain notable economic, environmental, and political concerns because of the need for more arable land to produce biofuel. This results in less land for producing food for humans and animals. Additionally, the production of first-generation biofuels also adversely affects the environment. Researchers sought second-generation biofuels since first-generation biofuels are not viable. Second-generation biofuels are not commercially profitable due to the use of expensive and sophisticated technologies in their production (Brennan & Owende, 2010; Dragone et al., 2014). Therefore, more research was focused on third-generation biofuels.

Biofuels derived from microalgae can be used to overcome the disadvantages of first- and second-generation biofuels, since third-generation biofuels are feasible alternative renewable energy resources (Chisti, 2007; Dragone et al., 2014; Li et al., 2008; Nigam &Singh, 2011). Due to their rapid growth, greenhouse gas fixation (zero net emissions), and high lipid production capacity, microalgae appear to be the ideal third- generation feedstock for biofuels, according to current research. They can also be grown on non-arable land and in saline water, and they do not compete with food or feed crops (Dragone et al., 2014; Schenk et al., 2008; Scott et al., 2010). Microalgae are typically measured in micrometers and live in ponds and other bodies ofwater.

Naturally, microalgae have higher lipid content than macroalgae and grow faster as well (Lee et al., 2014).More than 50,000 microalgal species are present, but roughly 30,000 have only been selected for scientific investigation (Rajkumar et al., 2014; Surendhiran D & Vijay M, 2012; Richmond A & Qiang H, 2013). Algae's short harvesting cycle is a crucial benefit for its relevance since it is superior to other traditional crops that are harvested once or twice a year (Chisti, 2007; Schenk et al.,2008).

As a result, there has been a lot of emphasis on algal biomass and its application in the biofuel field. As already discussed, we are aware of the advantages of using algae for biodiesel production, however, algal biomass has several drawbacks as a feedstock, including a greater growing cost than traditional crops. Similarly, harvesting algae requires a lot of energy, which accounts for around 20-30% of the entire cost of manufacturing. Centrifugation, flocculation, floatation, sedimentation, and filtering are common processes used in harvesting and concentrating algal biomass (Demirbas, 2010; Ho et al., 2011).

Algae can be converted into several renewable biofuels, including bioethanol, biogas, biodiesel, photobiological generated biohydrogen, Syn-Gas, and bio-oil via liquefaction and gasification, respectively (Kraan, 2013). The technologies for converting algal biomass to energy sources are classified into three types: biochemical, chemical, and thermochemical conversion, and an algal biorefinery can be built using any of these, as illustrated in (figure 2). (Wang et al.,2008).

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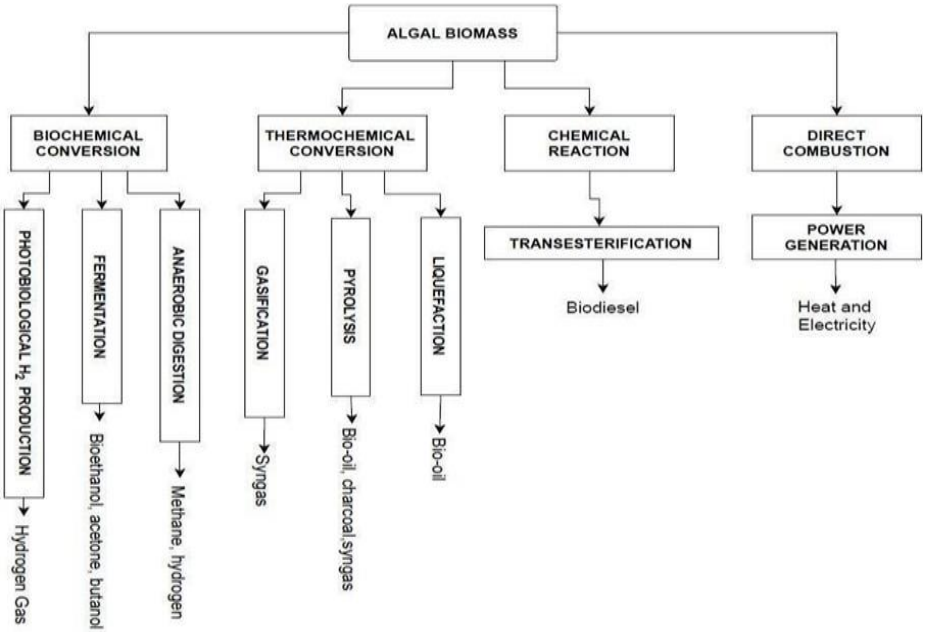

Fig.2 – Algal biomass conversion processes for biofuel production.

III. SELECTION OF OLEAGINOUSALGAE

Aside from technical considerations, environmental, economic, and social factors can all influence the selection of microalgae strains (Benyuktahtakin et al., 2014). One of the most important characteristics of microalgae in the production of biodiesel is growth rates, lipid levels, fatty acid profiles, and ease of harvest (White et al., 2010). Microalgae harvesting cycles typically last between 1 and 10 days. Their biomass can multiply in as little as 3.5 hours. Given the potential for the rapid growth of microalgae, they are an excellent source of biodiesel raw materials (Bello et al., 2012). Microalgae biomass can have a lipid content ranging from 4.5 to 80%, with lipids in oil. More biodiesel can be produced for high lipid producing strains.

Microalgae lipid is both neutral and polar. A neutral lipid or triglyceride (tag) is the most desirable component for the production of biodiesel from microalgae (Griffiths & Harrison, 2009; White et al., 2010). The amount (by dry weight) and lipid quality of the microalgae strain are critical for biodiesel production. Industrial biodiesel production necessitates the use of a strain capable of producing more than 50% of the dry weight of oil that can be extracted (Bello et al., 2012). Increased SUFA (super unsaturated fatty acid) and MUFA (monounsaturated fatty acid) percentage (with composition) yield biodiesel with higher energy outcomes, oxidative stability, and cetane numbers; however, this high percentage yields biodiesel with poor cold flow quality. Biodiesel made from high-PUFA (polyunsaturated fatty acid) strains will have a greater oxidation level but the character of the acceptable cold flow (Nascimento et al., 2013; Stansell et al., 2012; Anjorin R, 2011) Saponification of microalgae oil due to high FFA content (free fatty acids) produces low biodiesel results during transesterification (Bello et al., 2012). To achieve the best balance of cold streams and other properties, high-quality biodiesel profiles must have mass ratios of 5: 4: 1 of C16: 1, C18: 1, and C14: 0. (Stansell et al., 2012). One of the most difficult aspects of biodiesel microalgae production is harvesting microalgalbiomass.

The concentration of biomass microalgae about the amount of fluid in growth media is typically between 3 and 5 g / l; however, for industrial-scale operations, this biomass-to-liquid ratio must be concentrated at around 300-400 g / L (dry weight) (Coward et al., 2013). Harvesting is an expensive and energy-intensive procedure that accounts for approximately 20-30% of total production costs due to microalgal size (2-20lm) and the high-water content of growth media (Molina Grima et. al.,ND).

The strain determines the biodiesel produced from different microalgae on the property. The chemical composition of microalgae strains can be influenced by growth conditions and nutrients. According to Vishwanath et al., one strategy for obtaining the best choice with productive features is the screen based on natural habitat. All-important qualities include growth, oil content, fatty acid profiles, toughness, and contamination resistance (White et al., 2010). Environmental factors also influence the selection of microalgae strains. A microalgae strain grown in a tropical climate, for example, may have significantly different characteristics (lipid content, biomass amount, and growth rate) than one grown in a cooler climate.

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The lipid content of microalgae species will normally remain stable if produced under the same conditions (Singh & Olsen, 2011). MCDA (Multi-criteria decision analysis) techniques can be used to solve the problem of selecting an oleaginous microalgae strain for biodiesel production while taking into account all of the above-mentioned parameters such as growth rate, ease of harvesting, fatty acid profile, lipid content, and soon, as well as numerical analysis of the alternatives. The MCDA method computes an overall score for the option under consideration. A significant advantage of MCDA is the provision of a highly organized decision-making approach. Goals, inputs, alternatives, criteria, and weights are the main components of MCDA. (Table 1) shows how microalgae can be isolated and selected instantly for larger-scale biodiesel production.

Table 1 - Recommended 5-step procedure for accelerated microalgae selection for biodiesel production.

Step No.	**Process**	**Method**	**Reference**
1	Sampling	Microalgae with better survival strategies could be separated from water and sediment accumulated in habitats with variable and occasionally undesirable conditions (e.g., ability to accumulate lipids). Examples include rock pools, tidal coastal zones, and rivers.	Duong et al., (2012)
2	Isolation	• Dilutionseries • Plate cultivation to isolate single colonies • Single-cell isolation byamicro manipulator • Cytometric cell sorting (flowcytometry).	Duong et al., (2012)
3	Lipid Screening	Staining with Nile red for flow cytometry and/or fluorescence microscopy.	Singh & Olsen, (2011)
4	LipidProductivity	Assay for lipid productivity (Laboratory conditions).	Singh & Olsen (2011)
5.1	Lipid production optimization	Enhance factors for development, lipid induction, cultivation, and oil extraction of the most promising cultures in mid-scale (10-20 L) containers in outdoor settings.	Singh & Olsen (2011)
5.2	Algal biodiesel production	Large-scale cultivation, harvesting, oil extraction, and transesterification.	Duong et al., (2012)

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IV. CONVENTIONAL MEANS FOR IMPROVINGOIL PRODUCTION IN MICROALGAE

To increase the content of lipid produced the culture is subjected to stressors, like subjecting the cells to a different pH, temperature, light intensity, CO_2 concentration, photoperiod, salinity, and nutrient content in a controlled reactor (Shah et al., 2014). The composition of the growth medium must be optimized to raise culture in a large-scale medium, however, the optimization of nitrogen and carbon source is the most important (Puja Tandon, Qiang Jin, 2017; Irshad Ahmad et al., 2015). The optimization carbon to nitrogen ratio is important to be optimized in a growth medium in order increased lipid production and is economically viable (Helberth Júnnior Santos Lopes et al., 2020). Methods such as dual-stage cultivation at optimum conditions (CarlaDiasetal.,2015)and co-culture (S. Magdouli et al., 2016) have been noticed to increase lipid production. One of the most important methods for the increase in lipid production and optimization is strain selection. The yield of high lipid and biomass can be noticed by the use of highly productive strains (Puja Tandon, Qiang Jin, 2017). Apart from being subjected to nutrient starvation, there are various other methods such as gene manipulation that target to increase the lipid production in the microalgae. There are various types of regulation such as enhancing the fatty acid synthesis, manipulation of carbon partitioning, blocking of TAG hydrolysis, increasing the TAG content, manipulation of transcription regulators, and various other methods are employed (Prabin Kumar Sharma et al., 2018). Various genetic engineering approaches that have been done to various microalgae to increase lipid production are mentioned in (table 2).

Table 2 - Genes Manipulated for oil enhancement

Genetic Engineering Strategies applied	**Genes Modified**	**Species**	**Mode of action**	**References**
Enhancing fatty acid biosynthesis	ACCase	Cyclotella crypt	Overexpression	Dunahayi et al., (1996)
Enhancing fatty acid proportion	ACS	schizochytnum	Overexpression	Yan et al., (2013)
Modifying fatty acid profile	Acyl-ACP thioesterase	Chlamydomonas reinhardtii	Over expression	Kenneth Wei Min Tan, Yuan Kun Lee, 2017
Increase in TAG content	Acyl-ACP reductase	Cyanidioschyzon merolae	Over expression	Nobuko Sumiya et al., 2015
Shunting of carbon precursors for an increase in TAG level	ADP-glucose pyro-phosphorylase	Chlamydomonas	Suppression	Yantao Li et al., 2010
Regulating carbon for increased lipid production	Pyruvate dehydrogenase kinase	Nannochloropsis salina	Suppression	Xiaonian Ma et al., 2017
An increase in NADPH production increases polyunsaturated fatty acid content	Glucose-6-phosphate dehydrogenase	Phaeodactylum tricornutum	Over expression	Songcui Wu et al., 2019

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Enhancing availability of pyruvate for increasing synthesis of lipids	Malic enzyme isoform 2	Chlamydomonas reinhardtii	Over expression	Jongrae Kim et al., 2019
Manipulation of carbon portioning	Citrate synthase	Chlamydomonas reinhardtii	Suppression	Xiaodong Deng et al., 2014
Manipulation of carbon portioning	Glycerol kinase	Synechocystis sp.	Over expression	Ramachandran Sivaramakrishnan, Aran Incharoensakdi, 2018
Manipulation of carbon portioning	Phosphoenolpyruvate	Chlamydomonas reinhardtii	Suppression	Pei-Hsun Kao, I- Son Ng, 2017
Manipulation of substrate and product pathway	Phospho-fructokinase	Yarrowia lipolytica	Suppression	Annapurna Kamineni et al., 2021
Manipulation of carbon portioning	UDP-glucose pyro-phosphorylase	Phaeodactylum tricornutum	Suppression	Bao-Hua Zhu et al., 2016
Regulating lipid production	PNPLA3	Phaeodactylum tricornutum	Over expression	XiangWang et al., 2015
Increase in TAG content	GPAT	Chlamydomonas reinhardtii	Over expression	Umidjon Iskandarov et al., 2015
Increase in TAG content	LPAAT and GDP1	Chlamydomonas reinhardtii	Over expression	Chaogang Wang et al., 2018
Catalyze TAG pathway	Type 2 diacylglycerol acyltransferase	Scenedesmus obliquus	Expression	Chun-Yen Chen et al., 2016
Manipulation of TAG biosynthesis pathway	Type 2 diacylglycerol acyltransferase	Chlamydomonas reinhardtii	Expression	Irshad Ahmad et al., 2015
Manipulation of TAG biosynthesis pathway	Phospholipid diacylglycerol acyltransferase	Chlamydomonas reinhardtii	Expression	Zhen Zhu et al., 2018

V. GENETIC ENGINEERING STRATEGIES FOR LIPID ENHANCEMENT IN MICROALGAE

The ability to manipulate the enzymes or steps involved in the lipid synthesis pathway or other parallel pathways is at the heart of the majority of genetic engineering strategies. Over expression of enzymes such as fatty acid biosynthetic enzymes, increasing the availability of precursor molecules such as acetyl-CoA, optimizing the length of fatty acid chains using thioesters, and inhibiting fatty acid catabolism are some strategies used to improve the lipid profile and increase its content for biodiesel production (Krishnamoorthy Hegde et al., 2015).

Even though the expression of multiple components of an

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oil synthesis metabolic pathway or the control of the activity of multiple components of an oil synthesis metabolic pathway has gained interest in recent years, the most widely used technique is the manipulation of individual genes that encode for various steps of the metabolic pathway (Prabin Kumar Sharma et al., 2018).

Precise control of the mechanism that leads to increased cellular lipid production and biomass under normal conditions can be achieved through genetic modification of microalgae. There are several database resources available, including KEGG, Meta Cyc, and BRENDA, which provide us with genome sequencing databases and pathway databases useful for implementing targeted gene manipulation (Xianhao Xu, et al., 2020).

VI. MICROALGAE GENOME EDITING FOR STRA IN IMPROVEMENT

In recent times, genetic engineering methods have gained popularity and attention from researchers as they are considered novel and are tunable tools. Moreover, genetically modifying microalgae can help obtain better lipid production under natural conditions. Genetic engineering of microalgae for increased production of lipids seeks to suppress, inhibit or overexpress one or even more than one gene that is related to the production of lipid or biomass such as the photosynthetic process, growth rate, metabolic pathway, resistance to extreme conditions such as pH, temperature and salinity (Hossein Alishah Aratboni et al., 2019).

To overcome gene inadequacies gene-editing tools provide us with easy mechanisms that are crucial for enhancing lipid production in the microalgal strains. There are numerous genome dieting tools present. Some of the important and most used tools are ZFN (Zinc Finger Nucleases), TALEN (Transcription activator-like effector nucleases), CRISPER (Clustered regularly interspaced short palindromic repeats), and RNAi (RNA interference).

Details regarding these genome editing tools are present in (table 4).

Table 3 - Genome Editing Tools Used for Stain Improvement

Tools	**Full-Form**	**Function**	**Efficiency**	**Species**	**References**
RNAi	RNA interference	The destruction of specific mRNA leadstogene expression inhibition by the RNA	Not the most effective method to be employed.	Nannochloropsis oceanica	Wei et al., 2017
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats	The DNA binding the complex of Cas DNA endonuclease and processed CRISPR RNA utilizes guide RNA to form base pair with the targeted DNA	Most efficient technology is present due to its versatile design to implement and cost-effective	Synechococcus sp. Chlamydomonas reinhardtii	Zhang et al., 2019;Fajardo et al., 2018
ZFN	Zinc Finger Nucleases	Recognition of DNA triplets by DNA binding of synthetic proteins followed by DNA cleavage at specific binding sites	Not the most effective method but efficient in higher-orderplants	Diatom-Phaeodactylum tricornutum microalgae-Chlamydomonas reinhardtii	Zhang et al., 2019;Fajardo et al., 2018
TALEN	Transcriptional Activator like Effector Nucleases	Similar to ZFN but instead recognition of a single nucleotideoccurs	Not much effective but easier to use than ZFN	Diatom-Phaeodactylum tricornutum microalgae-Chlamydomonas reinhardtii	Zhang et al., 2019;Fajardo et al., 2018

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In recent times most commonly used genome editing tool is CRISPR/Cas. Even though CRISPR is known for its effectiveness in generating targeted mutants the predominant advantage is the ease of multiplex editing which provides a less complicated and more programmable approach for the editing of the genome (Prabin Kumar Sharma et al., 2018). Although ZFN and TALEN can be used for multiplex genome editing that could form mismatched dimers that can aggregate off-target effects (Hyongbum Kim1 and Jin-Soo Kim., 2014). The efficient expression of the selection marker can mark a successful transformation.

Sometimes the strains fail to yield the transformants due to the lack or the inefficiencies of expression of the transgene. Enthought the exact mechanism that inhibits the expression of the transgene is unknown (Seunghye Park et al., 2019). some strategies that are used to enhance the transgene expression are mentioned in (figure 2).

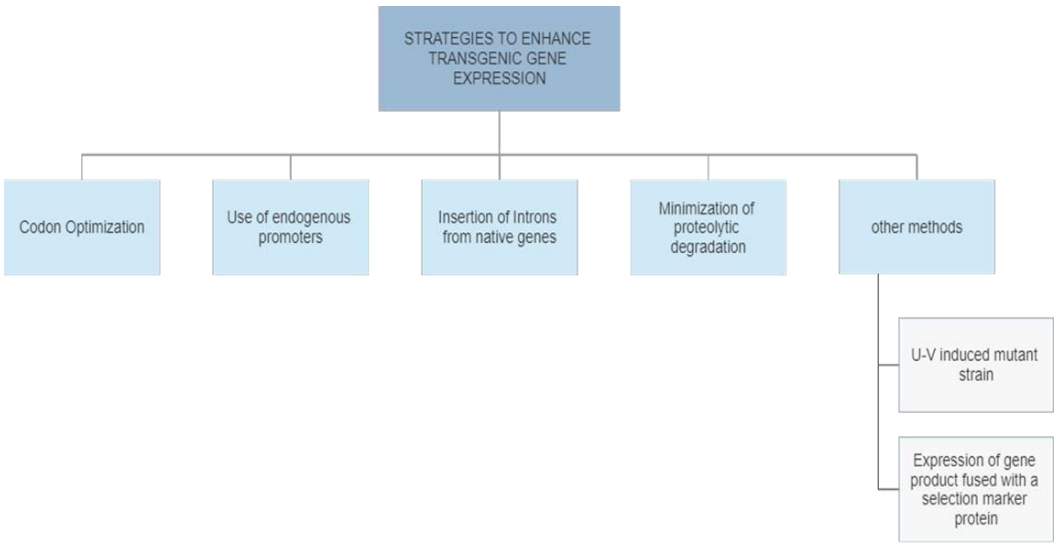

Fig. 3 – Strategies to Enhance Expression of Transgenic Genes

VII. ENHANCING THE BIOMASSYIELD

The primary factors that govern the biomass productivity in the phototrophic microalgae are abiotic stress, carbon fixation, and the efficiency of utilization of lights (Prabin Kumar Sharma et al., 2018). The CO_2 acts as the carbon source and the light energy is used for the metabolic activity of them icroalgae.

Four types of metabolic activity are adapted by the microalgae namely, photo-autotrophic, heterotrophic, photo-heterotrophic, and mixotrophic (Zhao Sheng Li et al., 2011). Carbon fixation plays a vital part in the photosynthesis of an organism. The Calvin cycle is the initial pathway for the fixation of carbon (CO_2 from the atmosphere) and to require an efficient way to modify the photosynthetic efficiency of the microalgae the breakthrough through this pathway is required. The enzymes Rubisco, SBPase, and aldolase are Important as they help in the carbon fixation in phototrophic plants. Increased biomass production and photosynthetic efficiency were increased in different microbial strains due to the overexpression of the genes that code for aldolase and SPBase as they regenerate substrate for the Rubisco enzyme (Prabin Kumar Sharma et al., 2018).

The efficiency of photosynthesis is also determined by the efficiency of capturing the photons (light absorption) by the light-harvesting antenna protein pigment complex of the chloroplast thylakoid membrane. The energy captured by the antenna is then transferred to the reaction center complexes (PSI and PSII) where the charge separation occurs (Amanda N. Barry et al., 2015). For large-scale production, the manipulation of the light enhancement strategy can improve the microalgal yield. Even though the most viable option is sunlight, the control ofthe spectrum to achieve its beneficial componentsis not possible. Hence alternate methods such as the utilization of wavelength filtering agents to shift the unusable wavelength to PAR (Photosynthetically active radiation). The conversion of UV to the visible range is made possible by the use of fluorescent paints increasing the number of photons in turn increases the intensity of the light. The application that has the largest potential for success is the use of organic dyes that can concentrate the unused and scattered light within the PAR range. The combination of the methods can also be used to eliminate the disadvantages of individual methods (Luveshan Ramanna et al., 2017). Gene modifications in the

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microalgae to increase its efficiency to absorb light would be to reduce the number of chlorophylls from the light-harvesting complexes (LHC). The downregulation of the genes coding for the LHC pigment binding protein has led to the development of mutants in the truncated antenna that showed an increased yield of biomass in the laboratory scale culture (Prabin Kumar Sharma et al.,2018).

VIII. PRODUCTION OF MICROALGAL BIOMASS:

Microalgae are currently the most unique and valuable microorganisms, not only economically but also environmentally, as they are the first biological exchanges of CO_2 and O_2 on this planet, as well as the most important primary producers of biomass.Many biotechnological production technologies have been developed to produce microalgae on a large scale in a sustainable manner for commercial exploitation. Many efforts have been made to improve the long-term production and lipid content of microalgae by implementing appropriate changes in production technologies, culture systems, and optimal growing conditions. Micro-algae Production Technologies:
Microalgae can be grown on a large scale using various methods and media. Microalgae have three main production mechanisms: heterotrophic production, photoautotrophic production, and mixotrophic production. In the heterotrophic production mechanism, microalgae require raw material and organic nutrients such as glucose to stimulate algal growth on a large scale. In the photo-autotrophic mechanism, the microalgae are autotrophic, thus they synthesize the necessary energy for growth using natural resources such as sunlight, water, CO_2, and optimal temperature. In the mixotrophic mechanism, the microalgae are both autotrophic and heterotrophic, in which they utilize the combination of heterotrophic assimilation of energy and carbon. The photo-autotrophic mechanism is regarded as the most economically viable method forproducing algae biomass on a large scale. (Brennan and Owende, 2010, Borowitzka, 1997).
Under the photo-autotrophic mechanism, there are two culture systems available for the large-scale production of microalgae, which are based on the open pond and closed photobioreactor (PBR) technologies. (Borowitzka, 1999). The industrialization of microalgae products requires large-scale culture systems to minimize the production cost. The photoautotrophic production of micro-algae is carried out in open culture systems as well as closed photo-bioreactor systems. The summary of the microalgae production technologies and the subsequent classification of the culture systems is presented in (figure 4).

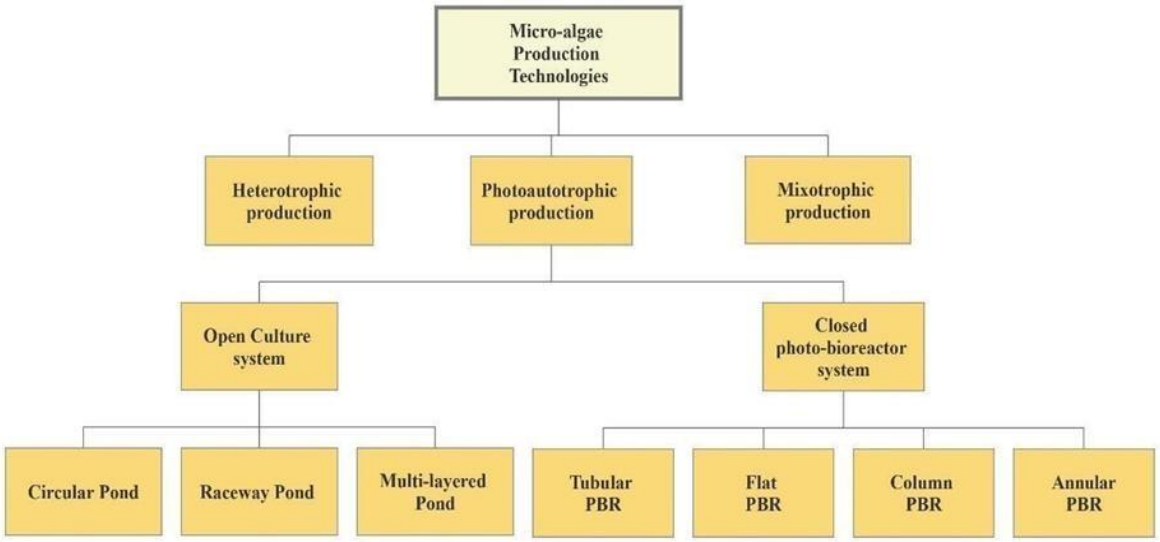

Fig. 4: Microalgae cultivation and production technologies

8.1 Open Culture System

The open culture system has been in use since the 1950s (Borowitzka, 1999). In the open culture systems, the microalgae are cultivated naturally in water resources such as lakes, and ponds. They are also cultivated in artificial ponds and containers for the optimization of the growth of microalgae (Gohain, Chutia, and Deka 2017). There are several kinds of artificial open culture systems such as raceway ponds, multi- layered ponds, and circular ponds. The most commonly used artificial system is raceway ponds, which is similar to the natural habitat of microalgae, despite a certain variability in shape (Pulz2001).
Raceway ponds, which are driven by paddle wheels, are made up of closed-loop, oval-shaped recirculation channels

having a depth of 0.2 to 0.5m (Gohain, Chutia, and Deka 2017). The main container is made up of concrete and compacted earth- lined ponds, which are supported with high-speed recirculation channels and paddle wheels, stabilizing the algae growth and maximizing productivity without sedimentation. They operate at a depth of 15cm to 20cm, with biomass productivity of more than 10–25 g/ (m2 day–1) (Pulz 2001). On the other hand, a circular pond's productivity is not as regulated and optimized as Raceway, but is more cost-effective and easier to maintain. Circular ponds are very common in Asia and Ukraine due to their cost-effectiveness and easy maintenance (Becker1994). Open culture systems are widely used for micro-algae cultivation on a large scale as it boasts multiple benefits such as low investments and capital cost as natural resources such as ponds, lakes are utilized and the sunlight is the primary energy resource, easy operation and scaling up (Pulz 2001). The advantages of open culture systems are highlighted in (table 4). Although open culture systems are widely used for various commercial applications, there are several disadvantages to be considered (table 4) such as high contamination risks, high evaporation losses, low productivity (Zhang et al. 2016). It is difficult to control the cultivation parameters such as temperature, pH, mixing, and light availability (Jerney and Spilling 2020), and it's susceptible to weather conditions (Pulz 2001). Until recently, open systems were the most important design principle for the large-scale production of microalgae. Due to the low productivity and high contamination risks, they have been proved to be unfit for the commercial production of microalgae.

8.2 Closed Culture System

The use of closed culture systems, also known as closed photobioreactor systems, for the large-scale cultivation of micro-algae has been on a rise lately, as it overcomes all the disadvantages of open culture systems. The closed PBRs are characterized by the control and regulation of all the important biotechnological parameters such as temperature, pH, mixing and light availability thus increasing productivity by multiple folds. Closed PBRs are known to have several advantages as shown in (table 4). Although the establishment of PBR demands a very high capital investment, operation, and scale-up is difficult (Pulz, 2001), it successfully overcomes all the challenges of regulating the production and controlling the biological parameters. It has been proved that the contamination risk is very low and the biomass production is very high, due to the provision of optimal growth conditions (Zhang T-Y, HuH- Y, Wu Y-H et al (2016)). In addition, they have several other advantages such as no CO_2 losses, very low evaporation loss (Pulz 2001), reproducible cultivation conditions, flexible technical design, and the ability to produce a single species with high cell mass productivity, making it ideal for sustainable and large-scale cultivation of the desired micro-algae.

Photobioreactors are designed in such a way that they can support very high productivity due to the large surface-to-volume ratio. A photobioreactor is a four-phase system consisting of a solid (cells), a liquid growth medium, a gaseous phase, and superimposed light radiation (Posten, 2009). The design of a photobiore actor is such that they have a straight tubular array, of diameter less than 0.1 m, made up of glass or plastic, capable of capturing sunlight essential for the process of microalgal photosynthesis. Recirculation of the micro-algae occurs with the help of the mechanical pump and an airlift (Gohain, Chutia, and Deka 2017). Recently, Photobioreactors have been categorized into a) Tubular b) column c) flat and d) annular based on their vary in configurations, as shown in (figure4).

Table 4 - Comparison of various microalgae culture systems

Topic	**Type of Culture System**		
	Open production system	**Closed photo bioreactor system**	**References**
Cost	Low investment and capital cost	Expensive to build and operate	Pulz (2001)
Contamination Risk	High contamination risk	Low contamination risk	Pulz (2001)
Productivity	Low productivity	High productivity	Zhang et al. (2016)
Operation	Easy to operate and scale-up	Difficult to operate and scale-up	Pulz (2001)

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Parameters	Difficult to control the cultivationparameters	Easy to control the cultivation parameters	Zhang et al. (2016)
Losses	High evaporation and gaseous losses	Low evaporation and gaseous losses	Pulz (2001)
Area	Huge amount of area isrequired	Limited amount of area is required	Pulz (2001)
Examples	Small ponds for spirulina culture	Flat photobioreactor	Zhang et al. (2016)

Optimal conditions for maximum micro-algal production in PBRS:

IX. ENVIRONMENTAL PARAMETERS:

When micro-algae are cultivated on a large scale for commercial applications, optimization of the growth conditions and environmental parameters such as temperature, light intensity, culture nutrients, CO_2 concentrations, pH and salinity would affect the PBR performance and would also make a large impact on the overall number of products produced.
The optimum conditions of the environmental parameters are summarized in (table 5).

9.1 Temperature:

The growth of micro-algae increases with an increase in temperature up to an optimal level, after which the growth rate starts decreasing. The optimal temperature ranges differ from one species to another but the average temperature range is 20°C to 35°C, for most commercial microalgae. If the temperature goes beyond 35°C, then the microalgal growth is hampered (Deb et al.2017).

9.2Intensity of light:

The amount of light that microalgae are exposed to, as well as the lighting, has an impact on biomass productivity. Microalgae growth increases with increasing light intensity and illumination until it reaches a maximum value, which is associated with the microalgae's light saturation, which varies by species. Light exposure that exceeds the maximum light saturation of the microalgae causes a phenomenon known as photoinhibition, which inhibits microalgal growth, resulting in less efficient CO_2 fixation and other nutrient intake rates. For microalgae, the average irradiance ranges between 50 and 100 E m^2 s^1 (Vejrazka et al.2011)

9.3 Culture Nutrients:

Culture nutrients are not considered as an environmental factor but as a chemical factor, which also influences biomass productivity. Nutritional factors include the composition and amount of the chemical species in the culture medium. The culture medium contains macro-nutrients such as the source of carbon, nitrogen, phosphorus, and micronutrients such as silicon, metals such as iron, copper, zinc, vitamins, etc. Carbon is the most critical nutrient for microalgae because it is their structural component and energy source. If the culture systemis autotrophic, CO_2 or bicarbonate compounds (such as sodium bicarbonate) are the only carbon supply (Daliry et al. 2017). It is thus very essential to ensure that the culture medium is rich in both the macronutrients and micronutrients because deficiencies are known to cause disturbances in metabolism and disproportionate production of intermediates of photosynthesis, in turn affecting the biomass productivity (Acién Fernández, Fernández Sevilla, and Molina Grima2013).

9.4. CO_2 concentration:

CO_2 is one of the main sources of carbon in the culture medium and is supplied to the PBRs through CO_2 enriched airflow with a certain percentage of CO_2 (such as flue gas stream) (Daliry et al. 2017). The concentration of CO_2 can be varied according to the microalgal species that is being cultivated. The ideal CO_2 concentration is 6.5%-35% but it can differ with the algal species (Acién Fernández, Fernández Sevilla, and Molina Grima2013).

9.5 Hydrogen Potential(pH):

One of the most important environmental parameters to consider is pH, which can affect the solubility and thus the availability of CO_2 for microalgal growth. A neutral pH range of 6.0–8.5 is ideal for the maximum growth of microalgae (Zhu, L.2015).

9.6 Salinity:

Salinity is another important environmental parameter that needs to be controlled in the PBRs because the deviations from the optimum osmotic conditions and salinity will cause physiological reactions thus leading to biomass productivity problems (Pulz 2001). The tolerance to salinity varies from species to species. Oligohaline microalgal species have a

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low tolerance to saline concentrations, ranging from 0.5 to 5 g.kg-1, while mesohaline and poly-haline microalgae can tolerate high salt concentrations, ranging from 5 to 18 g.kg-1 and 18 to 30 g.kg-1, respectively (Venkata Mohan and Devi2014).

Table 5 - Major Factors Affecting the PBR Performance

Environmental parameters	Optical Conditions	References
1. Temperature	20 °C to 35 °C	Deb et al. (2017)
2. Light intensity	50-100 μEm−2s−1 (The average irradiance)	Vejrazka et al. (2011)
3. Culture Nutrients	Macro-nutrients: carbon, nitrogen, phosphorus Micronutrients: silicon, metals such as iron, copper, zinc, vitamins, etc.	Acién Fernández et al. (2013)
4. CO2 concentration	6.5%-35%	Acién Fernández et al. (2013)
5. Hydrogen Potential	6.0–8.5	Zhu, L. (2015)
6. Salinity	• Oligohaline: 0.5 to 5 g.kg-1 • Mesohaline: 5 to 18 g.kg-1 • Poly-haline: 18 to 30 g.kg-1	Venkata Mohan and Devi (2014)

X. SUSTAINABLE INDUSTRIAL PRODUCTION OF BIODIESEL FROM MICRO ALGAE

The utilization of microalgae for biodiesel manufacturing has gained worldwide popularity as it is a not only cheap, eco- friendly, and renewable source of energy but the production process is also proved to be very sustainable. The main advantage of using the biodiesel obtained from the oil extraction from microalgae is that the biodiesel is eco-friendly not only when it is in use, but also while its produced. The sustainable production process of biodiesel from microalgae has been explained pictorially in (figure5).

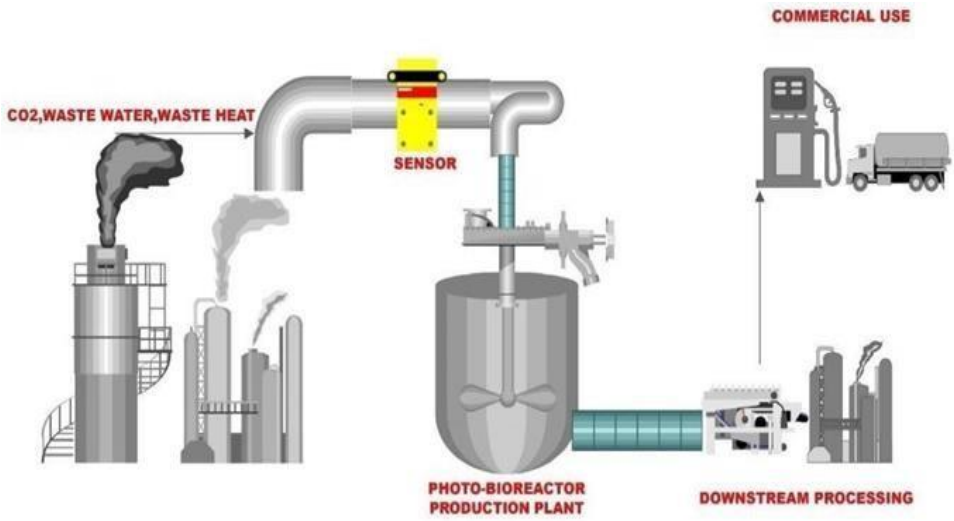

Fig. 5-Sustainable production process of biodiesel from microalgae

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The process of microalgal biodiesel production has been made ecological and economical in many ways:

- Integrating the treatment of aquaculture wastewater with algal cultivation in the Recirculation Aquaculture System (RAS): Aquaculture wastewater contains a large number of substances that can pollute the environment, such as nitrogen, phosphorus, and sulphur. Microalgae, on the other hand, can absorb these compounds and convert them into biomass. The algae produced in this process can be used in the cosmetic and pharmaceutical industries, as well as a substrate for biodiesel production. As a result, the production of biodiesel from algae and the bioremediation process is often linked (Hawrot-Paw etal,2020).
- Utilization of waste heat and CO_2 from industrial effluents: CO_2 is an essential carbon source required as a nutrient for biomass production. The required concentration of CO_2 and the heat energy required for the PBR can be obtained from industrial effluents, which if escaped into the atmosphere can cause a tremendous amount of pollution. Setting up the biodiesel production plant close to other commercial industrial areas, which emit large amounts of CO_2 and waste heat, would prove to be ecological and economical because the CO_2 and waste heat from the effluents can besupplied to the PBR through inert pipelines. The concentration and pressure of the supplies can be detected and regulated using NDIR CO_2 sensors and pressure valves. (Figure 5).

DOWNSTREAM PROCESSING

Downstream processing is an integral component of any bioprocess industry and plays a pivotal role in the isolation, purification, and formulation of our desired bio-product for different end uses. It aims to reduce the bulk of the microalgal oil by various concentration enrichment techniques. The choice of the separation methodology depends to a large extent on the nature of the product, its market volume and, the extent of puritydesired.

Bio-products can be classified into different market sectors based on market volume, market price, and purity requirements. Since microalgal oil (bio-fuel) is a bulk industrial product, it falls under market sector 3 characterized by high bulk volume, low market value, and relatively low purity requirement (Sivasankar, 2005). Hence, the bio-separation process must be tailored in a way to improve the economics of the microalgal biofuel whilst considering the purity parameters. The process should be economical, efficient, and environmental-friendly.

HARVESTING OF MICROALGAE

In addition to the lack of viable alternatives for harvesting microalgal cells, their small size (less than 10m in diameter) in diluted culture medium and density comparable to water (less than 2g/L) make microalgal harvesting one of the key bottlenecks for biodiesel production (Uduman et. al., 2010). The low sedimentation velocity of microalgae, their colloidal nature with repelling surface negative charges, and low biomass concentrations in culture broths all pose serious challenges to microalgal cell harvesting (Irena Branyikova et. al.,2018).

To achieve optimal harvesting, a synergistic technique that can improve both the economics and the efficacy of the process depending on the desired products and/or the biology of the microalgae must be developed (J. Kim et. al., 2013).Centrifugation, filtration, flocculation, and gravity sedimentation are the various physical/mechanical methods employed for the harvesting ofmicroalgae.

10.1Centrifugation

Centrifugation is a method of separating the algal biomass from the growth medium by applying centrifugal forces. After centrifugation, the algae can be separated from the culture by draining the excess medium, also known as the supernatant (Harun R. et. al., 2010). Concentrating microalgae by centrifugation significantly improves the harvest efficiency by increasing the biomass concentration within a short period. Microalgal biodiesel production is negatively affected by the intensive energy input of centrifugation (Beach et al., 2012; Sanderand Murthy, 2010). The high gravitational forces and shear forces can damage the fragile cell structure of the microalgae.

Recently, several sophisticated and newly designed centrifuges have been employed to harvest microalgae for biodiesel production. However, the high capital and operating costs of these centrifuges are discouraging. Research suggests that the application of different pre-concentration techniques before this process can reduce centrifugal energy consumption significantly making the harvesting process economical (Salim et. al., 2012).

10.2 Filtration

In the process of filtration, the nutrient broth containing the microalgal cells is passed through a micro-filter under pressure to increase the efficiency of the filtration process. Tangential filtration flow (TFF) is the preferred concentration method. It is an efficient process as there is no filter cake deposition on the filter membrane and hence, the clogging of pores and membrane fouling is prevented (Chenet al., 2012; Zhang et al., 2010). The nutrient broth is continuously run through the micro-filters.

The filtrate (liquid nutrient broth) passes through the filter plate thus depositing the retentate (microalgal cells) as thick algae paste on the membrane surface. Due to TFF, the retentate is circulated back through the recycling tank and

continues to loop through the filter facilitating further concentration (Ahmad et al., 2012). Although the process of filtration appears to be an attractive harvesting technique, it incurs extensive operating costs and hidden pre-concentration requirements (Harun R. et. al., 2010).

10.3Floatation

During the floatation process, gas bubbles form at the bottom of the floatation tank containing the algal nutrient broth. The microalgal cells become trapped in the gas bubbles and rise to the surface as a result. The microalgal biomass is then collected in the vacuole layer at the suspension's surface. Microalgal cell diameters ranging from 10–30 m to 500 m is typically preferred for effective floatation.

Microalgal pre-aggregation is an effective method of attaining the mass required for flotation because of the reduced surface charges on the microalgal cells (Hanotu et al., 2012; Henderson et al., 2010). Various parameters like the size of the gas bubble and the surface characteristics of the microalgae such as the net charge and hydrophobicity are crucial in determining the cell- bubble interaction (Cheng et al., 2010; Cheng et al., 2011). High operational costs and the need for chemical flocculants for effective concentration are the major drawbacks of this process (Okoro et al., 2019).

10.4Gravity Sedimentation

A downward-flow inclined gravity settler can be used for the concentration of microalgae by using the principle of gravity sedimentation (Zhaowei Wang et. al., 2014). It is characterized by low power consumption and low operating costs. It is a highly species-specific method and depends largely on the microalgal cell size and density. Major downfalls of this process include low sedimentation rates and low cell recovery rates. This method cannot be employed for the harvesting oflow molecular weight, less dense microalgal cells (Roselet et. al.,2019; Baros et.al.,2014).

10.5Chemical methods

Flocculation or coagulation is the most extensively used chemical method for microalgae biomass harvesting. It involves the use of chemical flocculants (organic and inorganic) for the concentration of biomass. Auto-flocculation and electrochemical-flocculation are the other flocculation techniques that are also implemented. The destabilization of colloidal suspension by an electrolyte is known as coagulation, whereas aggregation of the particles as a result of polymer addition is termed flocculation. However, these two terms are often used interchangeably (Fernandes A. et al., 2009). In flocculation, the particles aggregate through the neutralization of surface charges, electrostatic patching, or bridge building following the addition offlocculants.

10.5.1 Chemical flocculation (organic and inorganic)

The formation of flocs and subsequent sedimentation is caused by charge neutralization and electrostatic bridging between suspended algal cells and the flocculant. Multivalent inorganic compounds, biopolymers, and inorganic-organic hybrid polymers have all been widely used as algae-harvesting flocculants. For algal biomass recovery, inorganic flocculants such as Al 2(SO4)3 and FeCl3 are commonly used (Yaser, A.Z. et al.,2014).

Chitosan, cationic starches, modified tannins, and polyacrylamides are common organic polymers. The harvesting efficiency of organic and inorganic flocculants is primarily determined by physicochemical properties such as solubility and electronegativity, as well as operational parameters such as dosage and algal solution characteristics such as cell density, pH, and ionic strength (Singh G, Patidar S.K., 2018; Kim D.G et al.,2013).

10.5.2 Auto-flocculation and Electro-chemical flocculation

The spontaneous aggregation of the suspended algal cells results in the formation of large flocs that induce their simple gravitational sedimentation. Algal cells have been shown to self-aggregate in both alkaline and acidic environments by reducing the intensity of their negative surface charges (Liu J. et al., 2013). Changes in algal cell surface charge are mostly due to the considerable production of protective extracellular polymers in alkaline environments (above pH 9). Variable dissociations of carboxyl and amine groups in the algal cell wall can generate surface charge variations under acidic conditions (Shen Y. et al.,2013).

Electro-chemical flocculation is carried out by the passage of direct electric current through the electrodes into an algal culture broth wherein the algal cells act as negatively charged colloids. This induces a water-hydrolysis reaction resulting in the evolution of micro-gas bubbles. The microbubbles capture the flocculated microalgae and float them to the surface of the suspension, thus concentrating and harvestingthem.

10.6 Biological methods

The biological method of microalgae harvesting employs the use of flocculating microorganisms like bacteria, fungi, and self- flocculatingalgae.

10.6.1 Bio-flocculation

The process of harvesting non-flocculating target algae by introducing a self-flocculating microbe (or its extracellular biopolymer) to the culture broth is known as bio-flocculation. Bio-flocculants include bacteria, fungi, yeasts, and self- flocculating algae, as well as their exudate-rich

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culture supernatants. As with auto-flocculation, no chemicals are used in this process, so it can be regarded as a sustainable method of harvesting algal biomass (Prochazkova G. et al., 2015; Salim S. et al., 2014). Although the mechanism of bio-flocculation is unknown, it is thought to be a result of the reactivity of the extracellular biopolymer or the direct adsorption of self- flocculating microorganisms on the target algae (Wan C. et al., 2015; Alam M.A. et al., 2016)

(Table 6) enlists the advantages, disadvantages, and recovery rates of the various harvesting methods. These parameters could be considered for the choice of appropriate harvesting technique for the concentration of a particular microalgal cell species.

Table 6 - Harvesting methods: Advantages, disadvantages, and recovery rate

Typeof Harvesting method	**Harvesting Method**	**Biomass recoveryrate (%)**	**Advantages**	**Disadvantages**	**References**
Physical Method	Centrifugtion	>90%	High solid concentration, high harvesting efficiency, quick separation process, and simple operation	Energy-intensive, Highcapital and operating costs, cell damage, and unsuitability for bulk harvesting	Xu et al. (2011), Buck walter et al. (2011)
Physical Method	Filtration	70-90%	Wide variety of filters and membrane types available, water and nutrients can bere-used	Clogging and fouling is a major issue, slow process, only suitable for large algalcells	Christenson andSims (2011)
Physical method	Floatation	50-90%	Fasterthan sedimentation, with the option of combining with the gaseous transfer.	Algal species specificity, High capital and operational costs, and theuse of chemical flocculants, in general, necessitate the use of chemicalflocculants.	Okoro et al. (2019), Singhand Patidar (2018), Roselet et al., (2019)
Physical method	Gravity Sedimentation	10-50%	Lowpower consumption, low operating cost, low requirement forskilled labourers	Algalspecies specific, low Sedimentation rates,lowcell recovery, depends on microalgal cell size and density.	Roseletet al.(2019), Barros et al. (2015)

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Chemical method	Chemical flocculation	80-95%	Wide range of chemical flocculants available, good solid recovery	Chemical contamination, highly sensitive to Ph level, flocculants can be expensive, removal of flocculants can be difficult	Ummalyma et al. (2016), Kwonet al. (2014), Gonget al. (2011)
Biological method	Bio flocculation	80-99%	Reduced toxicity and environmental impact due to the use of biodegradable flocculants, high recovery efficiency	Microbiological contamination of the biomass, Species-specific	Alam et al. (2017), Wan et al. (2015), Van Den Hende et al. (2011)

10.7 Drying and dewatering of algalbiomass

The concentration of the dry solid content of the biomass slurry remains low even after microalgae harvesting procedures are completed (Christenson L.; Sims R., 2011). As a result, depending on the finished product's requirements, a drying procedure such as solar drying, convective drying, spray drying, or freeze-drying is frequently required (Brennan L.; Owende P, 2010). (Figure 6) denotes an interactive diagram showcasing various drying and dewatering methods that can be employed. A brief description of each process is also provided.

Fig. 6 – Drying and dewatering methods of microalgae

10.7.1 Solar Drying

The most cost-effective dewatering method is solar drying; however, it requires extended drying durations and a large drying surface area (Brennan L.; Owende P, 2010). Furthermore, traditional open solar drying methods make it difficult to preserve the end product's quality, and the sluggish drying pace caused by the low temperature can lead to biomass degradation and, as a result, an increase in the bacterial population (Prakash J. et al., 1997). Within 3–5 hours of drying, certain closed solar drying equipment can elevate the ambient temperature from 35°C to 60°C, and the moisture content in the end product is less than10%.

When compared to other drying methods like oven drying and freeze-drying, the quality of the dry biomass yielded from open solar drying appears to have no substantial impact on some important processes, such as oil extraction (Balasubramanian R.K et al., 2013; Guldhe A. et al.,2014).

10.7.2 Convective Drying

A convective hot air-drying mechanism, such as oven drying, is often used for convective drying, which is a preferred procedure for microalgae dehydration (Oliveira E.G., et al., 2009; Prakash J. et al., 1997). The ideal drying temperature range for Spirulina sp. is 40– 55°C (Desmorieux H. et al., 2005; OliveiraE.G., et al., 2010).Convective drying has better efficiency than solar

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drying. Furthermore, the dry biomass's fatty acid content is not considerably different from that of the fresh biomass (Guldhe A. et al.,2014).

10.7.3 Spray Drying

Spray drying is the preferred method for drying high-value microalgal products, and it can produce a dark green powder of dried microalgae (Milledge J.J.; Heaven S., 2013), with the aspect and color of the powder being highly dependent on the spray drying process and temperature (Oliveira E.G., et al., 2009). Spray-dried microalgal biomass is less sensitive to lipolysis than freeze-dried microalgal biomass during storage (Ryckebosch E. et al., 2011).

10.7.4 Freeze-drying

Freeze-drying is a soft drying method as all the cell constituents are preserved without rupturing the cell wall (Guldhe A. et al., 2014). It is mostly implemented when the protein content of the microalgae needs to be preserved ((Desmorieux H. et al., 2005). Since the lipid content and not the protein content of the microalgae is important in algal oil production, this method is usually not used.

A major drawback is that the algal oil recovered from microalgae dried in natural sunlight has three times the amount of free fatty acids as that extracted from microalgae that have been subjected to freeze-drying (Balasubramanian R.K et al., 2013).

(Table 7) enlists the advantages and limitations of the various drying methods. These parameters can be considered for the choice of appropriate drying technique for the concentration of a particular microalgal cellspecies.

Table 7 - Drying methods: Advantages and disadvantages

Drying/Dewatering Methods	**Advantages**	**Limitations**	**References**
Solar Drying	Low capital cost, renewable energy source, sustainable and environmentally friendly	Slow process, large land requirement, weather dependent	Ahmad et al. (2014), Salim et al.(2011)
Spray Drying	The rapid drying process, high drying efficiency	High capital and operational costs, significant deterioration of microalgal pigments	Chen et al. (2011)
Freeze Drying	High cell recovery, protects certain algal components from oxidation (such as lipids)	High capital and operational cost.	Milledge and Heaven (2013)
Convection Drying	Allows accurate temperature control, provides uniform heating	High capital and operational cost of the ovens, the process is driven by non-renewable energy source.	Sanga et al. (2000), Orsal et al. (2006)

XI. SIMULTANEOUS OIL EXTRACTION AND CONVERSION TO BIODIESEL BY DIRECT/IN-SITU TRANSESTER IFICATION

Microalgae have been identified as the potential raw material for the production of biodiesel by direct/in-situ transester ification. Microalgae can be used as the feedstock for the production of renewable energy. The properties of the microalgae such as high lipid content, ability to produce carbon-neutral biofuel, and high biomass productivity make them suitable for the production of biodiesel. The intriguing ability of the microalgae to produce 1,000 – 6,500 gallons of oil per acre annually, makes it a vital element in the production of biodiesel. (Skorupskaite, Makareviciene, and Gumbyte2016)

Various methods have been used to identify the most effective process to enhance the oil content and productivity of the microalgae. The process of extraction of oil and conversion to biodiesel by direct/in-situ transesterification involves a series of processes. Initially, the desired species of microalgae are identified. As shown in (table 8). (Mallick et al.2012)

The growth of the microalgae is affected by various factors. These factors play a vital role in enhancing the lipid content of the microalga. For example, under heterotrophic nitrogen-limited conditions in the presence of corn powder hydrolysate, the growth of Chlorella protothecoides is favored. Similarly, the brown resting state is the favored condition for the growth of Botyococcus braunii. They have the highest liquid content among the microalgae species in

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(table 8). (Taher et al. 2011).

Table 8 – Microalga growth condition and lipid content.

Microalga	**Growth condition**	**Lipid content (% dcw)**	**Reference**
Botyococcus braunii	Brown resting state	86	Shovon Mandal, (2012), Chiu et al.(2009)
C.minutissima	Nitrogen limitation	57	Illman et al. (2000)
Chlorella protothecoides	Heterotrophy with corn powder hydrolysate under nitrogen limitation.	55	Xu et al. (2006)
Nannochloris sp.UTEX LB1999	Nitrogen limitation	51	Shovon Mandal, (2012)
Chlorella Sp.	Heterotrophy with 1%sucrose. 2% CO2	33	Rattanapoltee et al. (2008)
Nannochloris oculate NCTU -3	2% CO2	50	Chiu et al. (2009)
Choricystis minor	Nitrogen and phosphorus deficiencies	60	Shovon Mandal, (2012)
Scenedesmus obliquus	Nitrogen and phosphorus limitations in presence of sodium thiosulphate.	58	Sobczuk and Chisti (2010), Shovon Mandal, (2012), Rattanapoltee et al. (2008)
Chlorella emersonii	Nitrogen limitation	63	Shovon Mandal, (2012)

The selection of the appropriate microalgae is very important for the extraction of high-quality biodiesel. As the stain isolation process is complex and expensive. The selection of microalgae plays a vital role in reducing both time and money spent on stain isolation. Hence, the selection of the microalgae species is followed by the extraction of the target stain. Various analytical and chemical techniques are used for this process. Once the desired stain is isolated, the microalga undergoes the process of biodiesel extraction.

The extraction of biodiesel is expensive. Meticulous handling of both the microalgae and the processing units is essential. A sterile environment has to be provided throughout the process. At first, the algal oil is extracted from the desired microalgae. For this, the microalgal cell is introduced to an organic solvent. The organic solvent causes the cell to rupture. Then the solvent is extracted and the lipid/solvent is separated. Once this process is completed, the next step is to subject this algal oil to the trans-esterification process (Skorupskaite, Makareviciene, and Gumbyte2016).

In the absents of trans-esterification, the wet algal biomass has to be first subjected to biomass separation to produce the dry biomass. Then its dry biomass has to undergo lipid extraction in the presence of Chloroform, Methanol to produce microbial lipid. This microbial lipid is subjected to solvent recovery. Following that, a combination of lipid, methanol, and H2SO4/NaOH catalyst is used in precise concentration to yield biodiesel/ Fatty Acid Methyl Ester (FAME). Trans-esterification is a single-step process. Trans-esterification involves the direct production of algal biodiesel from temperature their solubility in biodiesel decreases and thus becomes immiscible. Hence, the algae biomass is effectively converted into FAME through trans-esterification in relatively less time. However, the properties of the fatty acid vary based onthe chosen stain, the maturity levelofthemicroalgal microalgae cells. In-situ trans-esterification combines both the extraction of the algal oil and trans-esterification into one single process. This lowers the processing cost. (Figure 7) the flow chart of the steps carried out in the biodiesel extraction (Wall, Johnson, and Kyte 2012).

After the target stain extraction from the microalgae, the cells are sent to a bioreactor or an open/close pond. Their

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nutrients and carbon sources (CO_2) are supplied in the presence or absence of light. This initiates the biodiesel production process. Then the biomass/ separation medium is produced and the spent medium is released. After which the biomass or the separation media is dewatered or dried. This stage marks the end of feedstock preparation and the beginning of the post- harvest phase (Skorupskaite, Makareviciene, and Gumbyte 2016).

The beginning of the post-harvest phase is marked by the transesterification reaction. As illustrated in (Figure 8), (Babcock et al. 2014) in transesterification, the cell is initially ruptured by the organic solvent, then the solvent is extracted and the oil/solvent is separated from the cell debris. The extracted algal oil contains two major components triglycerides and free fatty acids. The triglycerides are trans-esterified and the free fatty acid is esterified into fatty methyl esters in the presence of methanol at its critical point 239.5°C and 8.14 MPa (1,180 psi). A major advantage of this process is that there are no catalysts neither homogeneous nor heterogeneous are involved and the separation of methanol is very easy as at room biomass when harvested, and the oil extraction condition. But, when proper parameters are chosen and implemented, transesterification is a relatively better method for the extraction of the FAME.

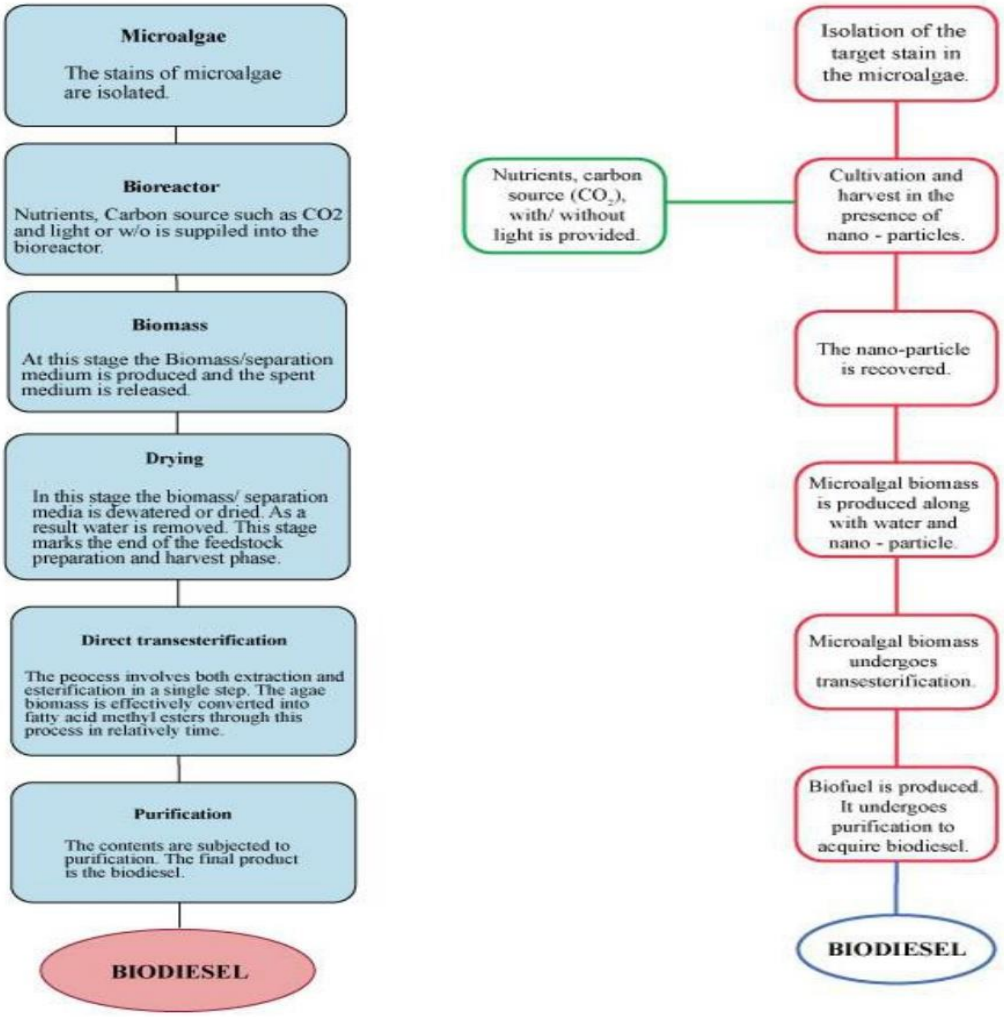

Fig. 7 – The difference between the sequence of biodiesel production in the presence and absence of nano-particles.

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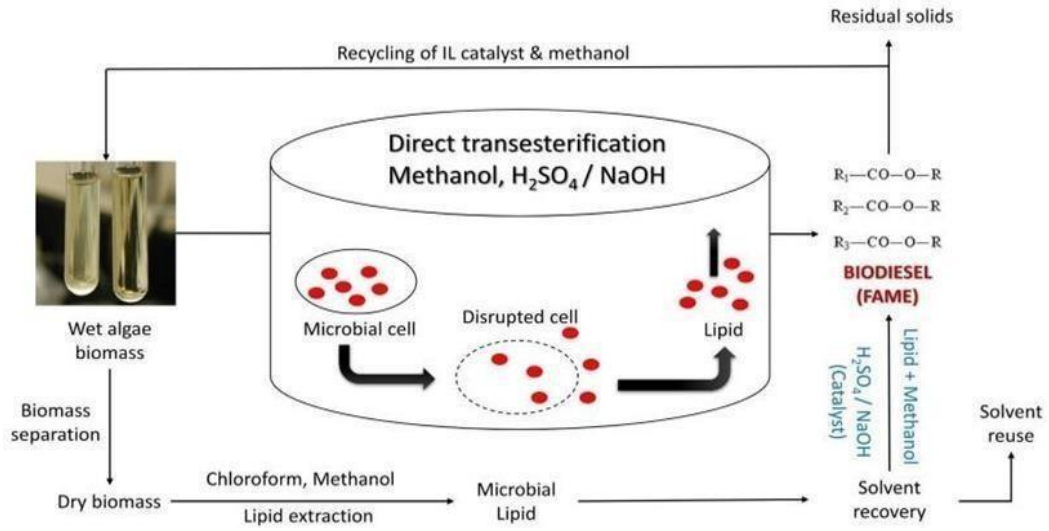

Fig.8 – Comparative illustration of direct transesterification and the direct method of biodiesel extraction.

Finally, the FAME is subjected to purification processes to extract pure biodiesel. Various purification techniques are employed based on the type of impurities. Ion exchange resin, distillation, solvent extraction, and membrane separation are some of the widely used techniques. Sometimes biodiesel is purified using dry washing techniques and water. However, the solubility of water could affect the purification process. Hence, the method is not always suitable for biodiesel purification and hence is replaced with other techniques. Recent developments have improved both the quality and quantity of the biodiesel produced (Wall, Johnson, and Kyte 2012).

XII. RECENT DEVELOPMENTS - MICROALGAE BIOFUEL PRODUCTION WITHNANO-ADDITIVES

The recent breakthrough in microalgae biofuel production is the discovery that nano-additives can help in enhancing microalgae biofuel production. Recent studies have shown that the use of nano-additives in microalgae biofuel production is playing a phenomenal role in the advancement of technology. Using nano-additives at different stages of microalgae biodiesel production have a potential mercantile outcome, almost no negative impact on the environment, and generation of valuable co-products (Karthikeyan and Prathima 2017).

Nano-additives are used to enhance a wide range of properties in the different stages of microalgae production. For instance, during the cultivation and the harvest phase, it enhances the yield of the biomass and increases the cell density. In the process of conversion of microalgae into biofuel, the nano- additives help in producing pure co-products and enhance the yield of the biofuel. They enhance the implication of microalgal biofuel in diesel and petrol. Nano-additives also help in clean and complete combustion and in improving thermal efficiency (Wu et al.2012).

As shown in (table 9) certain properties of the nano-particles can introduce or enhance a certain property of the biodiesel.

Table 9 – The properties of nanoparticles used in biodiesel production

Nano Particles	**Properties concerning Biodieselproduction**	**Reference**
Waste cooking oil additives	At engine load, the fuel consumptions were BDF100 > BDF50 > BDF20 >JIS#2 in that order.	HakanCaliskanKazutoshi Mori, (2017)
Nano-sized water droplet	The engine's BTE was increased by 14.2 Percent when compared to pure diesel, and NOx emissions were reduced by 30.6 percent.	W.M. Yang, H. An, S.K. Chou, S. Vedharaji, R. Vallinagam, M. Balaji, F.E.A.
Nano-organic additives	The presence of water reduces NOx emissions,	W.M. Yang, H.An,

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	lowering the peak flame temperature. BTE has improved.	S.K.Chou, K.J. Chua, B. Mohan, V. Sivasankaralingam, V. Raman, A. Maghbouli, J. Li, (2013)
Titanium (IV) dioxide (TiO_2), copper (II) nitrate ($Cu(NO_3)_2$) and cerium (III) acetate hydrate ($Ce(CH_3CO_2)_3 \cdot H_2O$)	Titanium dioxides and Cerium acetate Hydrate nano-particles reduce pollutant emission.	Abdulkadir Yaşar, AliKeskin,Şafak Yıldızhan, Erinç Uludamar, (2019)
Al_2O_3-ZnO	The densities, viscosities, and thermal conductivities all increased. The amount of specific fuel consumed decreased.	Hayder A. Dhahad, MiqdamT.Chaichan, (2020)
Cu	The reduction in soot was 7–14 per cent. The probability of ignition was increased.	M.Hatami, M. Hasanpour and D. Jing, (2020)
CuO	CuO nano fuel combined with magnetic fuel conditioning has a noticeable effect on improving BTE and lowering pollutants in compression ignition engines.	M.Hatami, M.Hasanpourand D. Jing,(2020)

Nano-additives have various advantages over the available technology. They are durable, highly stable, have good adsorption efficiency, are recyclable, can be used as catalysts, have high storage capacity, an excellent yield of the biofuel, are economical, crystallinity, and are environment friendly. A wide variety of nanomaterials are used as nanocatalysts for both the direct and the indirect production of biodiesel. Some examples include nano-tubes, nano-particles, nano-sheets, nano-fibers, etc. The use of these nano-materials helps in the enhancement of the yield. They also work as carriers in the enzyme immobilization of biodiesel. For this magnetic nano-particles are used. The magnetic nanoparticles are also preferred for methanogenesis as they have powerful paramagnetic character and high coercivity hence can be used in the production of biomethane. (Hossain, Mahlia, and Saidur2019)

The employment of nano-particles at various stages of biodiesel production has helped in amplification the overall yield in the production of biodiesel through microalgae. For instance, when nano-particles of calcium oxide have been used the yield of biodiesel during large-scale biodiesel conversion via a catalytic transesterification is 91%. Different microalgae react differently to the introduction of different nano- particles. For example, Aphanizomenon and Anabaena two species of microalgae can be separated efficiently from the mixed culture by incorporating magnetic particles with aluminium sulphate. Hydrous iron (III) oxide particles even in a very small quantity can boost the microalgal cell suspension by almost 100%. Microalgae such as Chlamydomonas reinhardtii and Cynothece51142 display a 30% increase in the production of biomass during harvest when silver nano-particles are employed (Wu et al.2012).

Various nano-particles are added throughout the process of extraction of biodiesel from microalgae at different stages. For example, the addition of Poly dimethylammonium chloride along with surface functionalized iron oxide nano-particle orthe addition of Chitosan/magnetic nano-particles to Chlorella sp. increases the harvest efficiency by 97-99%. Coating Fe_3O_4 nano-particles with polyethyleneimine and the addition of both Iron oxide and cationic polyacrylamide (CPAM), doses 120 mg/l to the Chlorella ellipsoidea microalgae increases the harvest efficiency by greater than 95 % to approximately 97% (Karthikeyan and Prathima2017).

As illustrated in (Figure 7). When nano-particles are employed, there is a slit variation in the process. That is, after the isolation of the target stain. The microalgae now undergo cultivation and harvesting in the presence of a suitable nano-particle along with the presence of nutrients, CO_2, and in some cases light. Then the water, nutrients, and nano-particles are recovered. This leads to the production of clear water with nano-particle, microalgal biomass, and nano-particle (NPS) recovery. The microalgal biomass is then isolated. This isolated microalgal biomass goes through transesterification and yields biofuel. Biofuel now contains various components like bioethanol, biodiesel, biomethane, biobutanol, etc. Hence, has to undergopurification.
Once the purification process is completed then biodiesel/ FAME is available for use (Hossain, Mahlia, and Saidur 2019). The process of using nano-particles for the enhancement in the production of biodiesel from microalgae

is an energy-efficient and eco-friendly process. Using nano-particles and nano-bubbles to enhance the cultivation and harvest, efficiently increasing the yield of the microalgal-biofuel through nano-additives, and nano-droplets and finally producing clear combustion fuel engines by nano-stabilizers and nano-emulsion are some of the many applications of nano-particles in the extraction of biofuel especially biodiesel from microalgae.

XIII. ADVANTAGES OF MICROALGAE IN SUSTAINABLE BIODIESEL PRODUCTION

Microalgae have numerous advantages in the production of biodiesel. With the increases in global warming and energy, the need for an alternative source of energy that is sustainable has escalated. The growing population, rapid decline in the level of fossil fuel, and increasing industrialization demands renewable resources. Under such circumstances, the extraction of biodiesel from microalgae has been identified as a potential green renewable energy (Khan et al. 2009).

A manufacturer can choose the microalgae and extract the product by manipulating the various growth conditions as the microalga doesn't require complex growth conditions. The microalgal biomass can be improved by using certain techniques such as enhancing biomass production by genetic engineering, or by using genetic engineering for the production of biofuel. This can be achieved by manipulating the metabolic pathway of the microalgae. Although genetic engineering is a relatively expensive and crucial process, it can aid in the overall yield of biomass. The availability of the microalgae's genome sequence has helped in the genetic engineering of biomass production and biofuel production. Apart from this, the interaction with the bacterial biofilm has also helped in improving biofuel and biomass productivity (Medipally et al. 2015).

The microalgae have higher productivity than the oil crops. It is photosynthetic and produces lipids and triacylglycerols (TAGs). The TAGs can be used as sustainable feedstock in biodiesel production. Carbon sequestration when done with microalgal bases is less expensive than the general carbon capturing and sequestration process. Though their full potential has not been harvested in the Indian market. The Southern Online Biotechnologies and Naturol Bioenergy Limited (NBL) are two firms working on the extraction and production of biodiesel in India (Mata, Martins, and Caetano 2010).

Microalgae has a lot of advantages over plants in biofuel production. They can be cultivated in a photobioreactor and still produce more biomass. They don't herbicides and pesticides. The by-products and co-products also have many commercial uses. It sequesters CO_2, which, in turn, contributes to the reduction in the emission of greenhouse gas. The microalgae can bioremediate wastewater by using the nitrogen and phosphorus from the wastewater. They can also grow on marginal lands. The oil yield through microalgae is far higher than that of the oilseed crops. Their rate of growth is higher and faster than plants. They are capable of accumulating and synthesizing a larger quantity of neutral lipid (Khan et al. 2009).

Using trans-esterification for the production of biodiesel from microalgae adds to the list of advantages. Unlike the direct method which has a lot of different stages, trans-esterification is a single-step process. Trans-esterification requires lesser chemicals and almost no catalysts. Whereas the direct method requires various chemicals and catalysts at each stage of the process. Transesterification is also cost-efficient and is faster than the other methods. While applying trans-esterification, a particular property of the microalgae can be extracted. Trans- esterification also produces a relatively higher yield (Zhu 2014).

Using microalgae can help in reducing global warming. It is a sustainable feedstock for the production of biodiesel. It can work as an alternative to fossil fuels (Medipally et al. 2015). Microalgae contribute to renewable energy production. Hence, the production of biodiesel from microalgae through trans-esterification is an advantageousprocess.

XIV. CHALLENGES AND FUTURE PROSPECTS

Though there are many advantages to the production of biodiesel from microalgae, there are also a few disadvantages. At the time the biomass produced is of lower concentration and the oil content is also low. The cell size of microalgae is small, this contributes to the cost of the harvesting process. The energy required for the harvesting and drying of the microalgae biomass increases with the increase in the volume of water. Farming of microalgae is more expensive than conventional agricultural practice (Ra et al.2015).

Currently, research is being carried out to improve various aspects of the process. Improving the lipid content of the microalgae is very crucial for the production of high-grade and high-yielding microalgae. This can contribute to increasing the overall yield of biodiesel. The lipid content of the microalgae is generally started from 30% and can go up to 80% depending on the microalgal species. Research is being done to identify the high-yielding species and genetically modify them to maximum yield by controlling their nutrients, metabolic pathway, and other factors (Huo et al.2020).

Improving the harvest technology can contribute to reducing these difficulties. Developing a coproduct strategy and biorefinery can help in reducing the cost spent on microalgae biodiesel production.Developing the genetic engineering techniques to manipulate the metabolic pathway and better understanding the symbioticrelationship between the bacteria and microalgae and its effectson the production

of lipid and biomass. Making the photobioreactors highly photosynthetic. Developing a cost-efficient technique for dying and harvesting the biomass. These techniques when implemented can help in improving microalgae biodiesel production and reduce the cost (Khan et al.2009).

XV. CONCLUSION

The world's energy demand is growing at an exponential rate. As a primary source of energy, only fossil fuels are used. Because of the scarcity of fossil fuels, rising petroleum-based fuel prices, energy conservation, and increased global warming, renewable energy sources such as solar, wind, hydro, tidal, and biomass have grown in popularity around the world. Properties of microalgae that are considered during the process of strain selection are discussed. Genetic engineering processes employed on microalgae for increased lipid production aims to suppress, inhibit, or over express one or more genes involved in lipid or biomass production, such as photosynthetic process, growth rate, metabolic pathway, and resistance to extreme conditions such as pH, temperature, and salinity. Some genetic engineering strategies to improve lipid content in microalgae are discussed. The working, advantages, and disadvantages of different culture methods are discussed and conditions optimal to maximize the microalgal production in these photobioreactors are highlighted. The basic benefit of utilizing biodiesel made from microalgae oil extraction is that it is ecologically sound not only when it is used, but also when it is generated.

Since the downstream processing of the microalgae is hindered by various bottlenecks and accounts for 20- 30% of the total production cost, it is indispensable to develop a synergistic technique that can improve both the economics and the efficacy of the biodiesel production process. Microalgae oil extraction and its conversion to biodiesel is an important step in the overall biodiesel productionprocess.

The revelation that nano-additives can help to increase biofuel production is a recent and groundbreaking advancement in microalgae biofuel production. The role of various nano- additives in enhancing the production process has been highlighted. In addition to a multitude of benefits, the high lipid content of microalgae makes it an excellent candidate for the production of potential green renewable energy. Though there are many advantages to the production of biodiesel from microalgae, there are also a few challenges that need to be overcome. Extensive research is being carried out to improve the various aspects of the production process for its vast scale commercialization in the futuredecade.

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