

## Review Article: Biodiesel Production by Micro-Organism

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### ABSTRACT

Due to various dwindling resources as well as the significance of such resources to the building of greenhouse gases in the atmosphere, ongoing usage petroleum-based energies has also now generally regarded as irresponsible. For sustainable food production, alternative, dioxide transportation hydrocarbons were required. Gasoline made using oilseed crops has the ability to just be a sustainable or nitrogen biofuel. Consequently, gasoline fuel made using energy crops, leftover sunflower oil, including sunflower oil may provide a tiny portion of the current requirement for fuel sources. Organisms are often the primary basis of sustainable biofuels to provide the rising demands for fuel sources, as proven elsewhere. That research has carried out to determine the correct conversion, quantity of producing biofuels (ester), but also biofuel physical features. Cyanobacteria, like trees, absorb sunlight to produce lipids, but they do so extremely effectively. Many microalgae have oils efficiency that much surpasses those of the strongest seed oils. It really is explored how to make microalgae biomass biofuel financially viable against petroleum diesel.

**Keywords-** biodiesel production, physical properties, transesterification, species, microalgae.

### I. INTRODUCTION

Biodiesel is a transportation fuel that really has gained a lot of traction in the last years. As fossil fuel stocks diminishing, it's more crucial than before to find transport fuels which could replace petroleum products fuel like petrol or gasoline. Soybean, sunflowers, rapeseed, canola, or oil palm are all popular biofuel feedstocks. Recently, there's been a rising debate regarding the utilisation of alternative dietary resources for energy generation. To tackle these difficulties, researchers have shifted their emphasis away from the commonly used fuel that are now looking into the usage of non-food linked fuel sources like petroleum from microalgae. Microalgae are a wide as well as diversified collection of basic macrophytes creatures that come in a variety of morphologies, from unicellular through multicellular<sup>[1]</sup>. Such cells may transform CO<sub>2</sub> from the atmosphere into cellulose, which may then be treated farther upstream to generate biofuel, fertiliser, as well as

other valuable goods. Algae need CO<sub>2</sub>, moisture, or sunshine to develop photosynthetically. So, order to be have optimum growth circumstances, the temperatures ought to be between 20 and 30 degrees Celsius. For order to grow, algal also require artificial fertilizers such as phosphate or ammonia.

Because micro algae thrive in aqueous systems, they have easier accessibility to H<sub>2</sub>O, CO<sub>2</sub>, as well as other minerals, that illustrates why they have the potential to generate more petroleum per unit area than other plants. Algal varieties have different chemical components. Microalgae has various properties that make it a potential biofuel source worth investigating further. Biodiesel has been an excellent worldwide fuel for operating automotive motors ever since initial introduction like a replacement for petroleum diesel about a year earlier. That achievement has been linked to a number of intriguing aspects<sup>[2]</sup>. Diesel, for example, is inherently non-toxic, renewable, but easy to make by esterification reaction at moderate circumstances. It may be used immediately in traditional petroleum-diesel motors with optimum efficiency, thanks to extremely low sulphur or fragrant content with suitable flashing, clouds, or pouring characteristics, in order to make a limited contribution to Carbon dioxide as well as other air pollutant emissions. Despite the fact that worldwide consumption for biofuel is expected to quadruple or treble in most countries by 2020 or later, while related research is ramping up, a variety of crucial concerns have still to be solved. The interaction of triglycerides esters in oils with monohydric alcohol is required to convert them to mono-alkyl esters (bio - diesel). Several scholars have proposed lesser monohydric alcohols (methanol to propanol) with no clear rationale according to that meets the greatest viscous criteria in accordance with ASTM (American Society of Testing and Materials) and similar international authorities<sup>[3]</sup>. On the other side, the expected sharp increase through the cost of eatable plant oils, combined with the hazard of hunger and sediment depletion affiliated with big producing biofuel, has compelled numerous organisations, including meals and agrarian organisations or financial modellers, to dismiss the alternative as untenable. Thus far, canola, soybean, rapeseed, or sunflowers oils, as well as processing used or animal fat, have received the

most attention. Companies have a similar challenge of determining the best catalysts or response circumstances. Originally, the major esterification reaction catalysts are hydroxides or alkoxides (methoxides or ethoxides) of groups IA and IIA, such as NaOH, KOH, NaOCH<sub>3</sub>, KOCH<sub>3</sub>, Ca(OH)<sub>2</sub>, Mg(OH)<sub>2</sub>, LiOH, NaOCH<sub>2</sub>CH<sub>3</sub>, KOCH<sub>2</sub>CH<sub>3</sub>, among others. Unfortunately, these homogenous substances come with a slew of issues that make them unsuitable for long-term use<sup>[4-5]</sup>. These catalysts could be retrieved at the conclusion of transesterification, despite the fact that quicker biodiesel might be achieved in a very short response period. Like a result, it should be properly neutralised, resulting in the generation of enormous amounts of trash. Catalysts are economically costly, so fatty acid levels, even in tiny amounts, may alter them. Due to much slow response rate increases, tough thermal prerequisites, elevated reactants (petroleum to liquor) proportions, catalyst densities, as well as highly corrosive troubles, relatively homogenous lipids such as H<sub>2</sub>SO<sub>4</sub>, HCl, H<sub>3</sub>PO<sub>4</sub>, or natural sulphuric acid enzymes of the form RSO<sub>3</sub>H (R 14 alkyl or aryl) were also categorised as undependable<sup>[6-7]</sup>. These multiple issues revealed have prompted academic or commercial researchers to look into better options, including such a focus on feedstock adaptability, cleaner catalytic technologies, as well as the transformation of wasted glycerin into more usable industrial goods. Several organisations have subsequently advocated for the use on microalgae like a biodiesel feedstock but a variety of composite material as environmental catalysts. As a result, we've compiled a comprehensive review of the pertinent research on the advances achieved in just this area. This study also goes over some of the more interesting aspects regarding homogeneous improvement of glycerin generated like a consequence of the transesterification Biologically generated fuel have been widely highlighted for prospective alternative resources of energy like a result of huge current changes with oil costs and considerable worldwide worries regarding global warming<sup>[8]</sup>. Several people believe that biodiesel are one of the most potential solutions to reduce the world's reliance on fossils fuels, reduce CO<sub>2</sub> pollutants, or, in certain circumstances, help local farming as well as emerging economy. Biofuels made from sustainable biological resources, in particularly, are viewed as such a desired way of addressing aeronautical as well as other global transportation. There has been a lot of buzz about the biodiesel potential of photosynthetic organisms, that create storing fats called triacylglycerols (TAGs) which may be exploited to make biodiesel fuels with straightforward transesterification processes. Diesel fuel has become a leading company in refined petroleum, but especially in nations wherein petrol is the predominant fluid energy, diesel vehicles play an important role in a variety of sectors of the economy. This same European Union, for instance, wants to update 5.75 percent of all transport fossil energy with bioenergy by 2010 and 10% by 2020, as well as other industrialised nations

throughout the globe have frequently mentioned. Notwithstanding, even if it were recommendable to transform this essential meal asset into diesel, the worldwide yearly manufacturing of TAGs from oilseeds would not be enough to satisfy this same U.S.' current diesel requirements of 44 billion gallons annually, and it has been approximated that the merged manufacturing of biofuel production from conventional oil seeds plus waste vegetable oil oils as well as lipids would not be enough to satisfy the worlds largest requirement for transportation fuels<sup>[9]</sup>. Additional biofuels biomasses would be required to fulfil projected resource needs, as diesel from algae may be the only sustainable supply of oil capable of meeting global travel fuel needs. Microalgae - based bio - fuels are made from of the concentration of microalgae, as well as they have the possibility to be utilised as biomass resources for such a range of elevated intensity fuel sources, like biofuel, eco-friendly diesel, clean aviation fuel, as well as green fuel oil. The residual organic layer can be transformed to biofuel production via physiochemical or thermochemical converting paths. While the distinct value of farmed commercial plants material vs phytoplankton as biofuels biomasses are currently a point of contention, phytoplankton offer a number of advantages which make them a viable biofuel source<sup>[10]</sup>. Traditional land plants are poor in absorbing light, transforming less than 0.5 percent of renewable radiation collected at average mid-latitudes into biomass production; however, algal photosynthesis performance may possibly surpass 10%. Furthermore, microalgae require significantly fewer acreage, may be turned to petroleum products using easier technology than cellulosic, or give ancillary benefits that coal and oil do not. Algal biofuel may be utilized in non-modified diesels with ease, so it offers many benefits above regular diesel, including being sustainable, recyclable, or potentially producing fewer sulphur oxide or particle pollutants once burnt. Additionally, since algae are minuscule that may be cultivated continuously in well-mixed suspension environments, they may be able to provide the advantages of regulated high-output production seen in industrial fermenting. Algal farming on a big scale should be made viable as expense if it is to fulfill future world transport fuels needs. Screening or engineering of microalgae species which can be cultivated in translucent photobioreactors have been the subject of much investigation<sup>[11]</sup>. Notwithstanding, we are oblivious of a certain high-production PBR presently in procedure for biofuels, as well as we reach the conclusion that this same economically viable utilisation of PBRs form large very little area than ground bio - fuels attempts, as well as can provide extra bonus advantages by lowering contaminant release dates to the surroundings as well as needing substantially lower water tax incentives. Furthermore, we provide three crucial ecosystem concepts that may be used to guide the configuration of lake microalgal biomass facilities.

### 1.1 Biodiesel

Biofuel is a sustainable, alternative energy made in the United States from soybean oil, animal proteins, or eatery greasy. Gasoline satisfies the Biofuels Format's biofuels gasoline and total invented requirements. Biofuel is not the same as sustainable gasoline, sometimes known as "green diesel."

| Biodiesel's Physical Characteristics |               |
|--------------------------------------|---------------|
| Specific gravity                     | 0.88          |
| Kinematic viscosity at 40°C          | 4.0 to 6.0    |
| Cetane number                        | 47 to 65      |
| Higher heating value, Btu/gal        | ~127,960      |
| Lower heating value, Btu/gal         | ~119,550      |
| Density, lb/gal at 15.5°C            | 7.3           |
| Carbon, wt%                          | 77            |
| Hydrogen, wt%                        | 12            |
| Oxygen, by dif. wt%                  | 11            |
| Boiling point, °C                    | 315-350       |
| Flash point, °C                      | 100-170       |
| Sulfur, wt%                          | 0.0 to 0.0015 |
| Cloud point, °C                      | -3 to 15      |
| Pour point, °C                       | -5 to 10      |

It's a natural energy that may be utilised as beef tallow, plant foods, culinary fats, and more. Ethanol is a non-toxic, chlorine, renewable, and environmentally favourable fuel. It is classified as a fuel source since it contains mono-alkylesters of lengthy fatty that fulfil a set of criteria (ASTM OR European standards). Esterification was indeed a key step in the biodiesel production. Ethanol has a low viscosity, which comes from of the infusion and detonation features in cooking oil<sup>[12-13]</sup>. Its friction coefficient from sunflower oil might well be lowered to increase the efficiency of diesels, which can be accomplished by transforming sunflower oil to biofuels. Biofuels is a cleaning solution alternative to crude oil petroleum diesel derived using sustainable resources including fresh and old unsaturated fats. Diesel was made by combining liquor with biodiesel, beef fat, or reused culinary waste. It is harmless and sustainable. Biomass, including petroleum-based diesel, is being used to power contraction (diesel) vehicles. Biomass may be mixed at either ratio of diesel oil, ranging B100

(pure biodiesel) and Biodiesel blends (the most common mixture).

Even with its eco - friendly materials and the reality that it will be derived from sustainable, biofuels has lately gained popularity. The expense of biofuels, on the other hand, is the most significant impediment to its commercialisation. For cut the price of biofuel, increasing utilisation of spent plant oils for feedstock, adaptation of a continuous transesterification, and recovering of top-grade glycerol form biofuel by-product (glycerol) among the main possibilities to explore<sup>[14]</sup>. Direct usage and mixing, nano emulsions, thermal stress (pyrolysis), or transesterification are the four main methods for biodiesel synthesis. Transesterification using biofuels is perhaps the most frequent process. The molar mass of triacylglycerols to liquor, catalysis, catalyst loading, process duration, and lipid acid and water content in cooking oils all influence the transesterification process. The esterification process method and dynamics illustrate how well the process takes place and develops. The transesterification and its upstream procedures are also discussed.

- Algal species.
- Esterification reaction Subcritical.
- Lignocellulosic.
- Petroleum byproducts.
- Polyunsaturated Fats that are unrestricted.

### 1.2 Making Biodiesel

The reaction of cooking oil or beef fat with only an alcoholic (methanol or ethanol) and an enzyme produces biodiesel. The glycerol being separated from either the oil and butter throughout this procedure. As a consequence, biodiesel becomes lighter than petroleum as well as butter, but it improves performance with gasoline engines. Biodiesel produced means the process of biodiesel synthesis via chemical methods like isomerization reactivity and epoxidation<sup>[15]</sup>. Short rope distilled liquors are used to interact with vegetables including mammal fatty acids (typically methanol or ethanol). The liquors utilised must have a comparably modest molar weights; alcohol will be the most preferred due of its affordable fee, while methyl may convert more biodiesel. The manufacturing technique is capacity due to the decomposition, which was selected since it reduces the duration and has a cheap catalytic expense. This method is less expensive that acidic esterification. Neutral catalysis, on a other hand, has a good specificity including both moisture and saturated fats inside the oil.

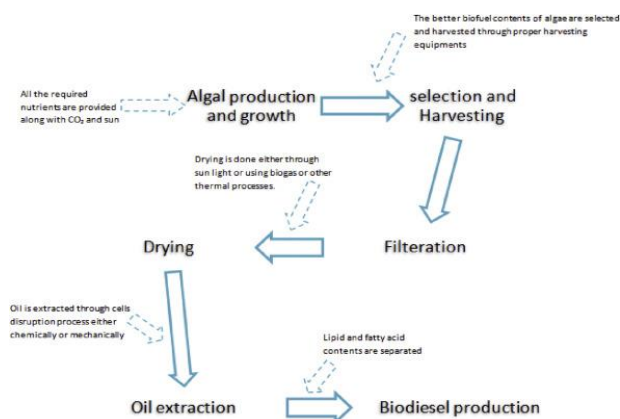
### 1.3 Biodiesel production

Biodiesel seems to be a blend with ethylene glycol along with terephthalic acidic comprising lengthy saturated fats [fatty acid methyl ester (FAME)] made up of different types of sustainable lipids biomasses and biofuels. It could be used immediately in a variety of diesels. Microalgae as a substrate for such manufacture of petroleum products has indeed been studied since the mid-1980s. Throughout World War II, several German physicists sought to harvest oils using microalgae in order to solve their energy shortage. Microalgae has a

greater oil supply than oilseeds, enabling for cost-effective conversion to biofuels applying distinctive processes. Several results shows as even a comparison with algae biofuel development with agricultural vegetation regarding such generation of biofuels. Petroleum production (the amount of petroleum generated per unit mass of microalgae - based broth each day) is determined by the algae growth rate and the organism' biomass composition<sup>[16-17]</sup>. Microalgae having high amounts of polyunsaturated FAME, including such *Kirchneriellalunaris*, *Ankistrodesmus fusiformis*, *Chlamydocapsa bacillus*, and *Ankistrodesmusfalcatus*, are often favoured for producing biofuels. Throughout explosive increase, microorganisms frequently quadruple their content with an effectively doubling period of 24 hours. The oil extracted of microalgal species is often observed to be quite considerable, exceeding up to 80% by weight of the dry substrate. Microalgae could yield 5,000–15,000 gallons of bio - diesel per hectare annually, indicating the possibilities.

Conversely, other guidelines, including the International Petroleum Specification of Automobiles and indeed the American Society for Testing and Materials (ASTM), requires microalgae biofuels to have certain physiochemical qualities in order to be accepted as a fossil energy alternative<sup>[18]</sup>. The Microalgae fluids have a larger percentage of polyunsaturated than plant oils, making them more vulnerable to degradation during preservation and limiting their use. Several researches had indicated that biomass energy has various benefits for biodiesel synthesis characterized by a high population development and oil yields when compared to the finest oil seeds.

The mixing process provides just a straightforward method to separate fats and oils and separate biofuels on a micro or laboratory basis. This procedure is broken down into multiple parts, as indicated in Figure 1.



**Figure 1.** This figure illustrates the general process of biodiesel production from algae on small scale and for experimental purpose. This method could be used to compare different algal species for the oil contents. Dotted arrow indicates the addition to specific steps which have been highlighted with bold letters and full lined arrows

#### 1.4 Different production method

In lines and batches processors, extremely or medium shearing are used. Roughly in line as well as batches Extreme and Medium Stress Reactor designs provide permanent, moderately, and bio - diesel manufacturing. That cuts down on development time while also increasing output volume. This reaction happens in the Extreme or Heavy Drag mixing console energetic region, which reduces its volume of immiscible water phase like oil or butter and methyl. As a result, the shorter the droplet volume, the bigger the contact surface, allowing for more catalytic activity. Procedure that is extreme.

- Any use of methyl esters at extreme degrees in a continuous method is an additional, tipping point approach for biodiesel production. The petroleum and methamphetamine are really in a separate jump just at transition situation, and the process proceeds naturally and quickly<sup>[19-20]</sup>. Because the method tolerates moisture in the substrate and converts unsaturated fats compounds to methylation ester instead of detergent, a broad range of biomasses might well be employed. In addition, the catalysts elimination phase is no longer required. Technique of using a supersonic bioreactor.

- Electromagnetic fields induce the combination was heated to develop and burst droplets equally in the ultra - sonic bioreactor technology. That agitation also offers the necessary area for mixing and scorching to complete the conversion<sup>[21]</sup>. As a result, employing an ultrasound engine for bio - diesel manufacturing decreases response time, heating, and power density significantly. As a result, so rather than employing moment multiple processors, the esterification technique would execute offline. Acoustic systems on a mass scale enable for the chemical purification of hundreds of liters each day.

#### 1.5 Biodiesel as a fuel

Biofuel satisfies the Sustainable Energy format's biofuels fuel and total invented requirement. Biomass is a fuel source that is often referred to it as Biodiesel and "neat" biofuel since it is not normalized. Biofuel, unlike petroleum - based diesel, is utilised in compaction motors. A mix with biofuel determines how everything operates in different climate conditions. The stronger biodiesel works in cold temperatures, the lower the number of biofuels in the blend. During chilly conditions, both normal No. 2 biodiesel and B5 function similarly.

#### 1.6 The simulation in the process of biodiesel production

A NRTL or RK-Soave thermodynamics characteristics have been employed in just that experiment. Although ASPEN Plus has thermodynamics statistics for some triglyceride, essential fats, and methylation ester, several critical thermodynamic parameters (such as real gases thermal capability) for such constituents in the data bases were missing. The majority of such elements are also found in the ASPEN Plus memory banks<sup>[22-23]</sup>. Thermal characteristics that aren't accessible in ASPEN Plus should be input

manually or calculated by ASPEN Plus based on the molecule of the substances. Most of the triglyceride, essential fats, and esters had their molecules drawn using ISIS sketch program, which could then be produced documents and put into ASPEN Plus. Moreover, determined by actual chemical architectures, an ASPEN Plus UNIFAC subgroup participation approach were applied.

### 1.7 Transesterification

Transesterification, often known as alcohols, a procedure of displacing alcoholic through one ester by some other, analogous to hydrolysis but with alcoholic though rather than moisture. This method has really been frequently utilised to lower triglyceride' higher solubility. Its fundamental formula for the transesterification process is shown in Fig. 1. Methanolysis refers to the use of methane inside that procedure<sup>[24]</sup>. Figure 2 depicts the triglycerides methanolysis. Transesterification is a chemical change that was carried out by combining the reaction mixture. The introduction of a catalysis (a powerful acidity or basic) does, nevertheless, speed up the production.

### 1.8 Transesterification kinetics and mechanism

Oil acids acetyl ester with glycerine are produced during triglycerides transesterification. The glycerine coating descends to the response vessel's base. The intermediaries throughout this mechanism comprise compounds showed and monoacylglycerols. Figure 3 depicts the process of transesterification<sup>[25]</sup>. These sequential processes were repeatable, and a small amount of alcohol and cigarettes is employed to change the equilibria forward into esters production. A front response is first rate in the context of excessive alcoholic, meanwhile the oxidation process becomes standard model. Transesterification considerably had been quicker whenever alkaline is used as a catalyst. Figure 4 shows the process of alkali-catalysed transesterification. The alkene ion attacks the carbonyl carbon of a triglycerides molecules during first step, resulting for the emergence of a tetragonal intermediary. Throughout the two stages, this intermediate reacts using just an alcoholic to form the alkene. This tetragonal intermediary was rearranged in the final stage, yielding the esters and a glyceride<sup>[26]</sup>. Figure 1: Transesterification solution in generalized Renewable and Clean Energy Research 248–268 251 L.C. Meher et al. Bronsted solutions, particularly sulfonate and sulphuric acids, may accelerate transesterification. Those catalyst produce extremely good yields with alkenes, though these processes remain sluggish, needing temperatures exceeding 100 8C and much more over 3 hours to accomplish. This could, nevertheless, be applied to di- and tri-glycerides. The compound is formed by complexation of a ester's carbonyl carbon, that generates a tetragonal intermediate following a nucleophilic assault of such alcoholic. To build a fresh ester and replenish the catalysis, that intermediate removes glycol<sup>[27]</sup>. In the context of 1% H<sub>2</sub>SO<sub>4</sub> and a 30:1 alcohol/oil molar ratio, the metanalysis of soy protein

were investigated. The transformation were observed to take 20 hours at even a heating rate of 65 degrees Celsius, whereas bioanalysis at 117 degrees Celsius and ethanol's at 78 degrees Celsius, who used the same amounts of catalysts and alcohol, took 3 and 18 hours, respectively.

### 1.9 Separation of biodiesel:

The biodiesel was properly recovered with sediments using a flasks divider. Sediment content (glycerine, pigments, etc.) were determined. Trying to wash: The biofuel then cleaned with 5% liquid once it became clear.

Biodiesel had been processed in a dehydrator and then stored below a moving fan over 12 hours.

Biodiesel content was evaluated with a hydrometer, pH were recorded, and the results were preserved for examination.



**Figure: 2 Lowest AV with max. esterified oil yield (43%) at 12:1, 1% H<sub>2</sub>SO<sub>4</sub>, 60°C and 400 rpm for 90 min. Max. yield of biodiesel (86.1%) at 9:1, 65°C, 0.75% KOH, and 600 rpm.**

### 1.10 Direct use and blending

In the Beginning , there was even their lot of debate over using cooking oil like a biofuel. Bartholomew discussed the idea of utilising foodstuff as an energy, stating that gasoline should become the source of energy then instead of cooking oil as well as ethanol, yet some form of renewable energy needs emerges in replace non - renewable sources. Regardless to oil shock, the far more developed research with solar fuel took place in South Africa. In Caterpillar Brazil employed pre-combustion cylinder machines with such a 10% veggio blended fuels to retain ultimate control by modifying or adjusting the engines<sup>[28-29]</sup>. Which was not practicable to replace 100 percent cooking oil for petroleum diesel at the time, but a combination of 20%cooking oil and 80% petroleum diesel worked well. In certain relatively brief studies, up to a50/50 split was employed. During August 1982, Fargo, North Dakota hosted the first World Congress on Vegetable and Plant Oils alternative Fuel sources. The expense of the gasoline, the effects of veg oil biofuels on machine quality and longevity, and fuel manufacture, specifications, and additions have been the main topics of discussion. That symposium also looked at oil and gas production, soybean refining, and extracting (ASAE) Using motor oil, a petrol engine was driven by (Anon). Utilized cooking oil was used, as well as a mixture of 95% recycled food oil and 5% petroleum products. To

adjust again for lower ambient temperatures, blending or preheating were utilised as required. There have been no issues with cooking or charcoal construct<sup>[30]</sup>. A secret was indicated to just be with lubrication grease pollution being the sole issue identified (viscosity increase due to polymerization of polyunsaturated vegetable oils). Around 4,000–4,500 miles, its lubrication oil required to just replaced. Soybean oil provide a number of benefits as a petroleum biodiesel.

**The Advantages are:**

- (1) mobility due to its liquid state,
- (2) thermal concentration (80% of petroleum diesel)
- (3) accessible accessibility,
- (4) environmental friendliness.

**The disadvantages are:**

- (1) increased stickiness,
- (2) decreased solubility
- (3) the hydrophobic alkyl store's responsiveness.

Difficulties only occur just after vehicle has also been running on plant oils for a lengthy time, particularly with straightforward motors.

**The problems include**

- (1) heating and trumpeter development on the nozzles towards the point wherein gasoline marginalisation is impaired or precluded due to clogged orifices,
- (2) charcoal formations,
- (3) lubrication block chain network, and
- (4) hardening and starting to gel of the lube oil contaminants by animal fats.

Inside a John Deere 6-cylinder, 6.6 L capacity, direct-injection, turbocharger cylinder, solutions using imagery soy protein as well as diesel fuel in the proportions of 1:2 and 1:1 being evaluated both engine power and crankcase lubrication consistency for something like a maximum of 600 hours Both oil pressure hardening and possible starting to gel were present in the 1:1 mix though not in the 1:2 mixture. According to the findings, a 1:2 mix might be used as a lubricant for agricultural machinery throughout times of biodiesel blends shortfalls or restrictions. Petroleum degradation with ignition delay being some serious issues related by the use of plant oils as biofuels<sup>[31]</sup>. Unsaturated fats proved particularly vulnerable to polymerase and plaque production as a result of chemical reactions while preservation or complicated oxidation and thermally polymers at temperatures greater after burning. The gum didn't entirely burn, leading in carbon dioxide and a viscosity of the lubricant film<sup>[32]</sup>. Due to the obvious high dividend and body composition (45%) of wintertime safflower, as well as the large (46.7%) erucic acid concentration in something like a petroleum, it became investigated as a petroleum diesel. The development of gingivitis in wintry sunflower oil were times quicker than in rich unsaturated (75–85%) oil. The high viscosity of 50/50 or 70/30 mixes of winter safflower petroleum with gasoline, as well as upcoming cold weather

rapeseed oil, seem to be greater above diesel (618 times). Over 850 hours, the 70/30 combination using wintertime sunflower oil or diesel were employed efficiently the run a simple singular diesel generator. There found no signs of abnormal wearing or impacts on the lubricant lubricant or energy level.

**1.11 Biodiesel production technologies**

As previously said, biofuels may be created utilising multiple procedures: physical and biochemical. Immediate usage, mixing, or nano emulsion are practical procedures that enable the oils to be utilised in their natural state. Pyrolysis and interesterification are biochemical methods that results in a physically changed version of essential oils. Both advantages and disadvantages of different biofuel manufacturing systems are shown in. Thermal decomposition has been the heat breakdown of hydrocarbons using a catalyst that does not include oxygen. Pyrolysis process substances include sunflower oil, beef tallow, and naturally fatty acids. Many researchers have looked at the decomposition of high triglycerides in order to provide viable biofuels for internal combustion engines<sup>[33]</sup>. Transesterification seems to be similar chemical technique that converting natural oils and fats produce biofuel. It involves the stoichiometric reaction of 3 mol of alcohol, such as methyl, with 1 mg / ml of triglycerides. By generally, the transesterification process happens at 60-70 degrees Celsius with such a catalytic, yielding a conventional single ester (biodiesel) as that of the major products and glycerine as a by-product. According illustrated in the transformation of triglyceride to monoglycerides happens in three irreversible steps. Methyl ester first interacts with triglycerides to form affect various aspects. The monoglyceride was formed when multiples interact with methyl<sup>[34-35]</sup>. Eventually, glycerine is synthesized when monoglyceride combines with methanol. The transesterification, which would be dependent on reactions circumstances, is influenced by a variety of factors. Whereas if circumstances really aren't optimal, the procedure will be either unsuccessful or get a considerable decline in effectiveness. As a result, each component is absolutely vital for achieving high levels of productivity in the synthesis of biofuels that performance objectives criteria. Fats, water activity, types of hard liquor and lower jaw ratios have been using, catalysed different kinds and abundances, conductivity time and heating rate, agitating rate as well as process, finished products purifying, combining motion, sustainably grown founder influence, but also dry density have been the most substantial elements that affect the fermentation.

**1.12 Advantages of biodiesel:**

When contrast to Petro diesel, biofuels generate much less carbon monoxide, particulates, hydrocarbons, and sulphates. Furthermore, particularly contrasted to petroleum diesel fuel, biofuels lower dangerous chemical pollutants by up to 85 percent.

Simple to operate: Neither car modifications nor refuelling appliances. Strength, Productivity, or Cost-Effectiveness: Energy that has been shown

- Bio - diesel is a beneficial energy because of its production, effectiveness, and efficiency. Bio - diesel has a positive impact on the planet.
- cutting Dioxide (CO<sub>2</sub>) emissions, which still decreases the impacts, helping the environment or better wellness Bio - diesel helps to cut down on the consumption of imported petroleum.
- Although biofuels become less harmful, it is easier to navigate.
- And it's a lot easier to transport than gasoline. Biomass contributes to community well-being by preserving resources.
- bucks you have at residence

### 1.13 Disadvantages of Biodiesel

- Biodiesels is now around yet another times the cost of Petro diesel.
- Generating biofuel using soybean plants takes energy, as does seeding, fertilising, and reaping.
- Additional downside of pure biofuels is it might damage leather housing in certain machines.
- Because biofuels wash the dust out of the combustion, it might gather in the fuel injector, causing blockage. As a result, filtration should indeed be updated on a frequent basis.
- Some other downside of biofuel production is that the supporting network has to be improved.

## II. UTILIZING BACTERIA TO IMPROVE BIO-FUEL

Micro - organisms are the focus of attention inside a variety of academic disciplines. Enzymes can be found in almost every ecosystem just on planet, so their utilization is propelling a tiny but substantial innovative renaissance. Organisms are being utilized to manufacture ethanol for biofuel using lingo cellulosic biomass, which would be a combination of cellulose, hemi - cellulose, plus phenol that composes the cell walls<sup>[36]</sup>. Enzymatic hydrolysis being the enzyme that breaks down viscose. Researchers have been looking for this enzyme's origins in a range of microorganisms species and environments. These bellies of termites or dirt spotted near eruptions are two of such uncommon ecosystems.

The occurs as the result Sulfurous solfatarticus was discovered in lava lakes around Mount Vesuvius. Currently, scientists have begun working using gene manipulation to boost this microbe's capacity to create the required proteins.

Trichoderma effective and efficient is a fungus that may be found in the soil all over the world. This nourishes by cells secrete a large amount of enzyme production. This fungi was first found in WWII, and that was accountable behind "jungle rot," that caused the rayon inside U.S troops' shelters or clothes to break down<sup>[37-38]</sup>. This fungus has been genetically modified by a Canadian business to create more lactase enzyme to

turn hay into sugar that might subsequently be converted into ethanol. They were successful in converting 75 percent of the straws to sugar. Microalgae may also be a viable option. Organic material is used to convert carbon dioxide into glucose that is subsequently used to make fats. Researchers are converting fats into biofuel or algae carbohydrates into bio - ethanol in comparatively tiny lab bioprocesses. Algae-based biofuels could become a big component of the mixture if they can scale it up to pre - industrial level.

There's obviously the issue of trash to consider. This is minimised since the plant matter employed is uneatable, such as straws. Like a result, a microbiological approach for biofuels is much less wasted, better moral, as well as less expensive. It also reduces greenhouse gases usage and emissions significantly.

### 2.1 Toward a Brighter Future with Bacteria with Bio - fuels

Changing climate, petroleum depletion, overcrowding, or agricultural usage are all real threats to humanity. Management of challenges like this can conceivable through new ways like the utilization or microorganisms can produce better biodiesel. Far more investigation is required to give these answers on a massive scale, although these are becoming increasingly accessible as time goes on.

### 2.2 Lipids produced by microbes

Third-generation biodiesel generated from oleaginous microbes have lately received much interest like a possible feed stock to produce electricity, decreasing directly reliance on conventional fuels. Unfortunately, owing to a number of problems relating to the life cycle evaluation or innovative viability of microorganism-based ethanol, implementation of microbiological technologies for biofuels generation stays complicated or uncertain. Several nutritive implications with increasing biomass or lipids outputs from diverse microbiological fuel sources, which serve as imperatives for the development of 3rd generation biofuels, are the subject of research paper<sup>[39]</sup>. These impacts of nutrition optimization techniques like nutrition famine, supplementing, or balanced approach on bacterial population or lipids formation are explored. Most crucially, regarding prospective technological scale-ups or implementation, the financial implications of oleaginous microbe cultivation practices are analysed or schemed employing different as well as non-conventional food resources.

Its creation of innovative goods to fulfil the demands of the global community is now centred on methods that maintain a healthy atmosphere. Single Cell Oil (SCO) is a relatively novel substance which has been recognised like a feasible option to biofuel, olefins, or culinary oils inside the previous year. SCOs made by oleaginous bacteria exhibit chemical composition comparable to crop oils. Conversely, organic chemical microorganism offers a number of benefits over vegetable crops, including the need for fewer acreage or

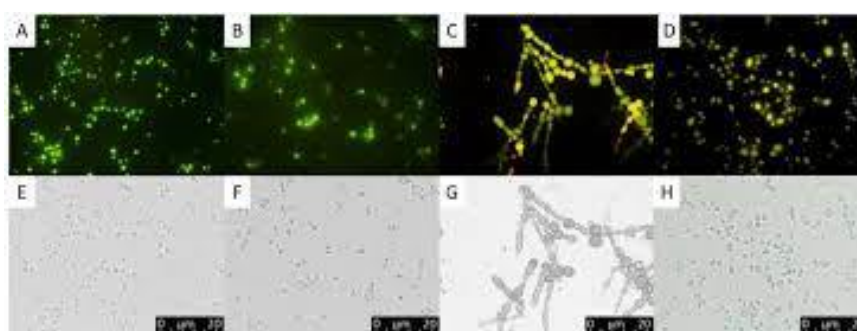
quicker growing times. Determination of optimal growing circumstances, wherein the microorganism's maximal capacity to transform the charcoal source into store lipids may be exhibited, is required for efficient SCO manufacturing. When evaluating economical uses, the substrates procurement price must be given particular consideration, since it accounts for 40–80 percent of the entire manufacturing cost<sup>[40]</sup>. As it being a renewable or plentiful platform with significant levels of culturally assimilated sugars, lignocelluloses are an excellent choice. Moreover, lignocelluloses material would be strongly intransigent due to its framework as well as macro-molecular concentration, requiring special substance, physiological, or genetic pre-treatment during which, through addition to culturally assimilated refined sugar, numerous networks which may restrict growth of microorganisms and/or lip genesis are released. Various techniques are developed to alleviate the inhibitory action of lignocelluloses biomass pre-treatment by products to boost lipids productivity levels. Pre-treatment strategies that produce lesser inhibiting chemicals, as well as a detoxifying mechanism capable of lowering their content, have been studied. Modern methods for lignocelluloses microbiological reutilization include genetic design methods evolutionary adaptability and the use of specialist

bacterial communities. Unfortunately, the current technology capability is inadequate to sustain expense SCO manufacture. The purpose of this article is to cover recent research involving lignocelluloses pre-processing as to offer additional a complete picture in organic chemical microbe proliferation or lipids synthesis on lignocelluloses materials.

### III. MICRO - ORGANISMS THAT PRODUCE OLEAGINOUS SUBSTANCES

#### 3.1 Important Oleaginous

Genus Micro - organisms classified as oleaginous have one commonality: they can store biochemical fuel in the shape of lipids. For prokaryotic or eukaryotic, the metabolic of such microorganisms has been evolved to transform chemical substrates into storing lipids, that are retained inside the cytoplasm as lipid aggregates with maintained patterns Figure 3 depicts typical organic chemical cell with fatty particles. Membrane structures are composed by proteins or lipids, mostly afford different, in eukaryotic and only occasionally in prokaryotic cells (TAGs).



**Figure 3: Lipid droplets visualized under light microscope (400×) within yeast cells and the mycelium of a filamentous fungus as shown through fluorescent filter after Nile red staining (A–D) and without fluorescence filter (E–H). (A,E) Meyerozyma guilliermondii, (B,F) Scheffersomyces coipomensis, (C,G) Umbelopsis (Mortierella) isabellina, (D,H) Yarrowia lipolytica (Original pictures).**

Microalgae, fungus (filamentous or yeasts), bacterial, as well as parasitoid wasps all include oleaginous microorganisms. Algal isolates linked with the Chlorella, Scenedesmus, Chlamydomonas, Nannochloropsis, Chlorococcum, Isochrysis, Cylinthotheca, Tetraselmis, Auxenochlorella, and Botryococcus genera have been shown to produce high levels of lipids. Rhodococcus, Streptomyces, Nocardia, Mycobacterium, Dietzia, and Gordonia are recognised kinds of bacteria. Umbelopsis (Mortierella), Microsphaeropsis, Fusarium, Candida, Meyerozyma, Rhodotorula, Rhodosporidium, Pichia, Cryptococcus, Lipomyces, Trichosporon, or Yarrowia are characteristic oleaginous eukaryotic organisms as well as yeast strains. Increasing lipids synthesis has been observed using bacterial consortium of microalgae with bacterial or algae with fungal strains<sup>[41]</sup>. Whereas research on

consortium have revealed that synergistic effect can happen across diverse species, enabling for higher storage of 40 lipids, additional research is required to establish the unique contributions of the every partner to the lipid manufacturing processes. Because oleaginous bacteria' metabolism differs from various species, the capacity to collect lipids is influenced by a variety of species-specific variables.

### IV. LIPID ACCUMULATION-PROMOTING GROWTH CONDITIONS

The concentration of nutrients within growth media, including such nitrogen or carbon, is critical for lipid synthesis. For heterotrophic bacteria, a higher C:N



molecular ratio is generally required to induce significant lipid accumulation, and the type of the nitrogen supply is also relevant. To forecast development or fat buildup, and also resource (namely, glucose or nitrogen) intake using oleaginous bacteria, comprehensive statistical simulations has been constructed. Ammonium nitrogen promotes lipid formation in certain circumstances, whereas organic nitrogen promotes lipogenesis in another. Furthermore, chemical restrictions like mg, copper, zinc, or phosphorus, including dual mineral constraints like nitrogen plus magnesium or nitrate or phosphorus, increase their formation for lipids<sup>[42]</sup>. Additional key factors that impact lipid accumulation include warmth, pH, soluble oxygen, or cultural stimulation. Medium chain fatty acid (FA) production is reduced at temperature over 30°C, while fat buildup is enhanced at neutrality as basic pH conditions. Around 200 to 300, rpm, rates which permit for significant oxygen, cellulose or lipids formation have been observed to just be high. Conversely, when agitating rates rise, lipids synthesis declines, so carbon conversion is shifted to external compounds like alcohols ( for example, ethanol, mannitol, arabitol, or 2,3-butanediol) at low movement rates. For heterotrophic organisms like microalgae, nitrogen concentration or recognize such a various affect development or lipogenesis. Several kinds including algal were shown that develop more lipids, particularly TAGs, in nitrogen-limited circumstances contrasted to nitrogen-rich ones. Several research, on the other hand, imply that nitrogen deprivation has a negative impact on photosynthetic, as well as hence biomass or lipid content, owing to reduced NADPH biosynthesis, that is the significant source of reduction power<sup>[43]</sup>. Additionally, nitrogen deficiency may have an impact on the lipid composition or various metabolic processes of cells. Like a result, novel techniques, like genetic manipulation, might well be required to better comprehend the impact of nitrogen on algal biology or TGA production or buildup. Lipid formation is promoted in many microalgae genera under phosphate-limited environments as well as at pH 8. Furthermore, the conversion of  $\text{NH}_4^+$  to  $\text{NH}_3$  happens at  $\text{pH} > 8$ , therefore nitrogen persists as  $\text{NH}_4^+$  ions at lower pH levels, that microalgae prefer above  $\text{NH}_3$ . Additionally, illuminance,  $\text{CO}_2$  concentration, or oxidative damage produced by saltness have all been shown to enhance fatty acid profile. Temperature changes have an impact on development rates, production, or lipids content<sup>[44-45]</sup>. Because the many variables implicated in microalgal development and lipid production influence distinct organisms in various ways, their optimal levels are previously identified or perhaps stress and must be discovered systematically. This impact of reactive oxygen substances (ROS) or free - radical on lipid production or deposition in mixotrophic or auto-trophic oleaginous bacteria has subsequently been investigated. ROS, which also include superoxide ( $\text{O}_2^-$ ), hydroxyl ( $\text{OH}^\cdot$ ), per hydroxyl ( $\text{HO}_2^\cdot$ ), and the alloy radical ( $\text{RO}^\cdot$ ), as

well as non-radical species like hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) or oxygen, are produced throughout bacterial metabolism considered signals of strain ( $\text{O}_2$ ). Lipid per oxidation or proteins as well as DNA conformational changes are common side effects of their buildup. While the particular processes of ROS engagement in lip genesis have just yet become fully elucidated, it's been proven that at regulated concentrations, Radicals may enhance lip genesis. As a result, further study is needed to put stress-based techniques in place to boost bacterial lipid synthesis.

## V. SUGAR CONVERSION INTO SCO AND REGULATORY MECHANISMS IN SUGAR ASSIMILATION

Glucose molecules as well as similar hydrophobic materials are converted to SCO by de novo biosynthesis that has 3 main stages: development during regulated circumstances, malodorous in nitrogen-limited circumstances, then reserves lipids cycling when the charcoal supply inside the growing media has been depleted. Throughout the development stage, oleaginous bacteria use the glycogen synthesis or hexode phosphorus pathways to transform carbon sources into comprising multiple rich in proteins and carbohydrates, whereas only small amounts of lipids, mostly polarity fats needed for cell membrane formation, are created<sup>[46]</sup>. Acute loss of at most one key ingredient ( for example, nitrogen, sulphate, phosphorus, and magnesium) inside the early stages of development triggers the start of the oil consolidation stage, which lasts until the charcoal supply is depleted. Furthermore, TAGs were destroyed through the lipids cycle stage to create fuel for the cell preservation and, in certain cases, development Glucose metabolic, particularly xylose or dipeptides, has been widely studied, with their hypothetical molar ratio transformation to lipids predicted. Oleaginous microorganisms have a high rate of conversion of sugar as well as other carbohydrates to glycolysis, that is employed in lipid production. Another mol of glycolysis was produced from 100 g of sugar (approximately 0.56 mol), that is transformed to 1.1 mol of acetyl-coA, resulting in a maximal potential ratio of 0.32 g of lipids per g of sugar. When using xylose, an output of 1.2 mol of acetyl-coA per 100 g of xylose (0.66 mol) is predicted, corresponding to 0.34 g of lipids every g of substrates. While the theoretical values are a decent estimate, the lipids production seldom surpasses 0.22 g/g sugar or 0.23 g/g xylose in practical cultivation conditions. The effective transformation of sugar molecules like cellobiose or glucose into store lipids, on the other hand, has been documented. Estimated outputs from oleaginous bacteria grown on a particular glucose are insufficient to forecast the effective transformation of lignoc ellulose into SCO<sup>[47]</sup>. For accomplish sustained Uc manufacturing activities, concurrent absorption both xylose and dipeptides present in basic substrates from botanical origins are required. Several investigations on

microbial sources (particularly yeast strains) living on glucose combinations have revealed that different sugars are absorption is sequentially, beginning with the simplest to digest then progressing to the more complicated, exhibiting diauxic development. Hexoses, such as glucose, are often favoured carbohydrates; however certain organisms are capable of efficiently metabolising ribose, such as xylose. Two separate processes are thought to be responsible for these something behaviour, that might or might not happen at the same time<sup>[48]</sup>. The first is metabolic suppression, wherein the chosen glucose suppresses the production of enzymes involved in the metabolism of other sugars, as well as the second is antagonistic transporter rivalry, wherein the chosen sugar's carriers block the transporters of other refined sugar. These factors lead in ineffective lignocellulose utilisation or poor lipids formation rates. Another research on the development of oleaginous bacteria on glucose combinations, on either hand, has demonstrated that certain strain may eat sugars concurrently, leading in significant lipid synthesis. Whenever sugar or xylose (or mannose) are utilised as carbon - based materials concurrently, oil output for malodorous yeast strains or mixotrophic developing algae varies around 0.15 to 0.26 g / g or sugar<sup>[49]</sup>. Additionally, certain strains may take many carbohydrates at the same time. For example, *Rhodospiridium* or *Pseudozyma* isolates consume glucose, xylose, or sucrose together and glucose, xylose, and arabinose simultaneously. In varied sucrose medium, *Rhodospiridium kratochvilovae* and *Y. lipolytica* both displayed concurrent ingestion of various ribose or xylose. Furthermore, isolates of *Meyerozyma guilliermondii*, *Scheffersomyces coipomensis*, or *Sugiyamaella paludigena* obtained from rotting timber have subsequently been shown to absorb sugar, oligosaccharides, or galactose, the three most significant sugars in lignocellulose, concurrently.

## VI. METABOLIC REGULATION IN OLEAGINOUS MICROORGANISMS

Oleaginous microorganisms make up a tiny percentage of organisms that may collect lipids in excess percent 20% of its mass. Such organisms can convert recycled resources into bacterial oil with such a fatty acid content that is comparable to that of plant-based oil. Transesterification with a significant polyunsaturated fatty acid concentration might be produced by certain species. Like a result, bacterial lipids are regarded as a possible oil resource. Several characteristics of oleaginous species, as well as recent advancements in their biosynthesis mechanism or metabolism regulatory mechanisms, are summarised below.

### 6.1 Oleaginous microorganisms accumulate oil:

This same cost of raw materials that now accounts for around 75% of the biofuel cost of manufacturing is the key constraint limiting the development of biofuels industrialisation, as previously

stated. From such a raw - materials standpoint, the third stage of biofuel employs oleaginous microorganisms like a raw resources, that avoids fuel-food rivalry or lowers manufacturing costs. It offers the benefits of fast production levels, a brief manufacturing cycles, little labour needs, seasonality as well as environmental freedom, plus simplicity of scale-up for chemical processes, particularly the capacity to spontaneously accrue large triglycerides. Organic chemical microorganisms are defined by bacteria which produce or store fats through excess on 20% of their cell dry weight<sup>[50]</sup>. Bacterial lipids, also known as Single-Cell Oils (SCOs), are oils produced by microbial sources or kept inside the cell, with triacylglycerols (TAGs) being the most common kind . Yeast strains, moulds, algae, or bacteria are examples of microbial sources that are widely spread and varied. Yeasts, by instance, are better prospects for commercial lipid synthesis because they could metabolise a larger variety of energy substrates. Just 4% of 1,600 fungal isolates have been recognised as microbial sources, as per the published research.

As a result, finding new high-performance oleaginous bacteria is critical to industrialisation. Fungal organisms like *Rhodotorula* sp, *Yarrowia lipolytica*, *Meyerozyma guilliermondii*, and *Rhodospiridium fluvial* were demonstrated to have tremendous promise in bio-diesel production in current research. At the moment, microbiological fats research is mostly focused on improving fermenting technique or strains to boost lipid output.

### 6.2 Oleaginous Yeast Metabolic Engineering

Oleaginous bacteria are highly appealing as potential metabolic engineering platforms. Microbial metabolic in oleaginous bacteria produces a large flow in acetyl-CoA or NADPH, essential precursors for reduced glutathione again for synthesis of fats as well as a variety of additional bio-based compounds. This oleaginous yeast *Y. lipolytica* has been developed to produce bacterial lipid having such a concentration of 99 g L<sup>-1</sup> as well as a pace of 1.2 g/L/h like a Generally Recognized as Safe (GRAS) species. Besides reconditioning lipid as well as fatty acid metabolites, transform method of *Y. lipolytica* generated liposome chemicals like alcohols, lacquer amides, as well as unexpected essential fats like ricinoleic acid and Eicosa Penta Anolic (EPA), which are categorised as n-3 (omega-3) poly unsaturated fatty acids (PUFA)<sup>[51]</sup>. Through using biotransformation to increase the chemical spectrum, *Y. lipolytica* is able to research has looked amino substances like caustic soda or glucose alternatives like xylitol. Simultaneously, significant development has been achieved in genetic manipulation of *R. toruloides* for the production of lipid-based chemicals as well as additional molecules like indigo Idine, a blue pigment.

Several unique metabolic engineering methodologies, such as designing three - carbon metabolic or route separation, have been used to create the active cell manufacturers of malodorous bacteria. As sugar was employed like a source and charcoal inside

this malodorous fungus *Y. cyclized*, NADPH was largely produced through the oxidized Pentose Phosphate Pathway (PPP), that resulting to energy depletion as exhaled CO<sub>2</sub> again for biosynthesis of the final product. Various synthetic routes to transform glycolytic NADH → NADPH are developed in fungal cytoplasm to re-balance the oxidative state for lipid production of *Y. lipolytica*. For *Y. lipolytica*, acetyl-CoA synthesis was improved to boost TAL synthesis by altering the private dehydrogenate (PDH) unit, private PDH bypass route, or -oxidation. Pathway compartmentalization results in elevated levels of precursor's availability or enzymatic activities, as well as lower demand from other metabolic processes or an improvement in the owner's sinks capability for product buildup<sup>[52-53]</sup>. The transmembrane framework, mitochondrial, or peroxides of *Y. lipolytica* have been used to design or modify routes in addition to those generated in the cytoplasm. Additionally, by manipulating the appropriate transporter, overall transportation of compounds between various compartments might be re-wired to shift metabolism flow towards targeted production. Over expression of the genes producing a mitochondria tricarboxylate transporters of Fungus time. usually through *Y. lipolytica*, for instance, increased polycarboxylic acids biosynthesis. This production of fat droplets as a cellular environment for the retention of proteins or lipids such as triacylglycerols (TAG) and/or cholesterol esters is yet another unusual trait of oleaginous bacteria (SE). Research discovered a link between lipogenesis as well as the formation of lipid-soluble carotenoids like lycopene or recorded prior<sup>[54]</sup>. Optimizing both carotenoids production with lipid accumulation in *Y. lipolytica* resulted inside a concentration of 6.5 g/L for -carotene. Utilizing sophisticated bioprocess methodologies that capitalise on the peculiarity of oleaginous microorganisms as foundation microorganisms, it offers a viable avenue to designing effective cell manufacturers.

### **6.3 Evaluation of oil production from oil palm empty fruit bunch by oleaginous micro-organism**

The EFB (Empty fruit bunches) of oil palm trees is a widely accessible cellulosic material with the ability to be used as a co - substrate for the generation of microbiological oil. A technological research was conducted to examine the generation of microbiological petroleum from Empty fruit bunch by cultivating oleaginous microorganisms on EFB hydrolyses. EFB hydrolyzates were made by diluted acid pre-treating biomass, then detoxifying or using liquids portion of the pre-treatment like an EFB liquid hydrolyzed (EFBLH). Before being utilized as an Empty fruit bunches enzyme hydrolysis reaction, every retentive were metabolic enzymes hydrolyzed<sup>[55]</sup>. *M. plumbers* had the greatest petroleum levels (1.9 g/L of oil on EFBLH and 4.7 g/L of oil on EFBEH). A techno-economic research was conducted depending just on oil yields of *M. plumbeus* per acre of planting, following by an estimate of the

substrate price for oil and gas production, through order to assess the viability of large-scale bacterial oil output. Additional oil palm feedstocks (flower stalk or stem) were included within this investigation to see whether they may help with the economy of large-scale bacterial oil and gas production.

### **6.4 Micro - organisms that are Heterotrophic Oleaginous**

Sustainable inside the transportation industry is unquestionably a worldwide goal; unlike direct consequence, diesel – like biofuels – research has become a hot topic. Natural oils, animal fats, or microalgae animal fats are being used to make biodiesel, and single cell oil (SCO) generation by heterotrophic oleaginous bacteria has lately piqued attention. The fact that many of those organisms may thrive on waste, like and billion tons of lignocellulose that may be dedicated to biofuels per annum inside the United States, is seen as a benefit. Through use of SCOs as biofuel resources might usher in a fresh era of mixotrophic oleaginous bacteria if the structural capacity criteria are met:

- (i) The long-term use of low-cost bio-based fuel sources;
- (ii) SCO outputs that are significant; and
- (iii) Manufacturing inside a repeatable, high-quality, or long-term manner Recent experimental effort are focused on establishing the basic parameters that influence adipogenesis in oleaginous organisms and relating findings with modeling organisms as well as modifying and optimizing culture conditions<sup>[56-57]</sup>.

For assist expose exciting options in genomic or metabolism design, Researchers discuss all afore mentioned topics, with such a focus upon current findings on transcription or protein–protein interactions regulation. This complete assessment that covers all of these domains could give the researcher a wide image or assist them assess the alternatives for future improvements. The diverse routes employed by various strains, as well as the control or comparison of lipogenesis, are discussed in this section.

Mixotrophic oleaginous microbes continue to pique attention due to their ability to collect huge amounts of lipids that makes them a prospective substrate for biofuel or oleochemicals synthesis. For such microbes, nutritional constraint, particularly nitrogen restriction, is recognized to successfully drive lipid synthesis<sup>[58]</sup>. These processes underlying lipid synthesis have indeed been explored for a lengthy period in order to generate better variants. Current omics methods can also provide system-level knowledge of their metabolism including accompanying physiological controls. This paper examines omics research is focused on

- (i) large-scale 'omic' capabilities from the top down, and
- (ii) bottom-up, computational mathematics methodologies on mixotrophic oleaginous bacteria, with a focus on metabolism design applications.

### 6.5 Enhanced biomass and oil production from sugarcane bagasse hydrolysate (SBH) by heterotrophic oleaginous microalga *Chlorella protothecoides*

Natural oils like rapeseed oil, soybean oil, or palm oil are often used to produce biodiesel (fatty acid esters). Biodiesel produced, on the other hand, is expanding, putting it in conflict with feed additives for farmland. The development of novel crop-based fuel for lipids manufacturing seems to be critical. Owing to comparable fatty acid profile to conventional sunflower oil, shorter development periods, lower labor requirements, and ease of ramping up, microbiological oils generated through malodorous bacteria had been investigated as possibilities in biodiesel synthesis<sup>[59-60]</sup>. Microalgae have attracted a lot of interest amongst microbial sources (for example bacterium, fungus, yeast strains, or algae) because to their satisfaction with social converting rate as well as greater concentrations of lipid accumulation. Microalgal lipid Productivity improvement component in rendering algal biofuel commercially feasible. This suggested mixotrophic microalgae culture regimen might easily transform organic material into algal lipids as well as produce high lipids production. This high price of mixotrophic carbon substrate (example sugar) remained the fundamental hurdle for algae lipids, though. Several studies are focusing on obtaining wastes or low-grade material as a chemical or fuel resource for mixotrophic algae rather than sugar. As example, *E. coli* has employed cassava starch hydrolysate (CSH), maize powder hydrolysate (CPH), sorghum juice (SJ), or *Cyperus esculentus* waste hydrolysate (CEWH) to make high lipids. Furthermore, mixotrophic microalgae's lipids output is eventually influenced by the effectiveness of their carbon consumption rate as well as the subsequent cell mechanisms that convert carbon into usable lipids precursor. To boost lipid formation in mixotrophic algae, an expense or effective energy supply was needed.

Bagasse is a valuable biological material that is gaining popularity owing to its high biomass production as well as diverse carbohydrates compositions. Among principal elements from hydrolysis bagasse are contributors, oligosaccharides, or glucose, which are merely potential carbon supplies inside the mixotrophic fermented manufacture of important microbes. For example, sugarcane bagasse hydrolysate (SBH) has been used as a carbon source for the manufacture of carboxylate by modified *Escherichia coli* and xylanase by *Bacillus circulans*<sup>[61]</sup>. Through fact, in microbes, distinct sugar compositions from SBH have distinct transportation or metabolic processes. According to several studies, effectors contributed significantly to the buildup of internal bioactive metabolites since it was uptake more effectively than xylose and glucose. Although sugarcane bagasse crude extract contains more sugar constituents than only glucose, it garnered particular attention. SBH, on the other hand, has made only modest efforts in the field of microalgae - based lipids. The goal of this research is to see whether

malodorous Cyan bacteria *protothecoides* can produce autotrophic microalgae - based petroleum from SBH. Bacterial impact of initial reducing sugar concentrations (RSCs) upon cellulose or lipids production of SB was studied. By using SBH like a feeding additive, a growth or traditions strategic objective regimen has also been used to boost energy as well as lipids output. As furthermore, research search for important stages in lipid production as well as the process of lipogenesis in SBH-induced *C. protothecoides* cells is continuing. It really is anticipated to make progress with the development of a mixotrophic environment for the manufacture of algal biofuels.

## VII. MICROORGANISMS THAT ARE OLEAGINOUS MIXOTROPHS

Micro-emulsification, decomposition, or transesterification may all be used to produce biofuels, as illustrated in Fig. 3. By its moderate reaction conditions, easy plant operations, cheap cost, stable bio fuel characteristics, with additional benefits, esterification has now been the primary technology for bio fuel manufacturing. According to the catalysts utilised, transesterification could be classified like homogenous acid treatment, heterogeneity hydrochloric catalyst supports, enzymes photocatalyst, as well as various innovative enzymatic approaches<sup>[62]</sup>. Like previously stated, the primary drawbacks of the base/acid method include the responsiveness of alkaline catalyst to Free fatty acid as well as moisture concentration, highly energetic or equity usage, poor reaction, or acidity catalyst' erosive character. .

Bioprocesses, in addition to basic or acidic catalyst, are an important option in biofuel generation for the following reasons:

- (1) Moderate response circumstances (60°C, atmospheric pressure);
- (2) Minimising secondary reactions like soap formation, boosting final product quality or streamlining the subsequent purifying procedure;
- (3) There will be no trash created as a result of a method that is ecologically friendly; and
- (4) Immobilization of enzymes has the potential to lower enzyme costs or improve durability.

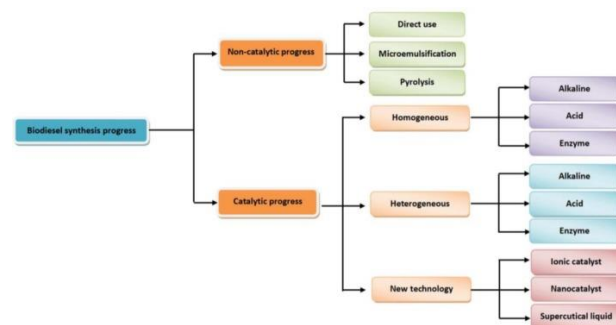


Figure 4: Ethanol manufacturing synthesis processes in general.

## VIII. ALGAE

Microalgae are multifunctional creatures that vary in size between separate microorganisms through multi cellular organisms having sophisticated or specialized forms. Algae thrive in wet locations or water bodies, making them widespread in both land and marine habitats. Algae, unlike flowers, need on three components to thrive: sunshine, carbon dioxide, and moisture. Respiration is a crucial metabolic pathway wherein trees, microalgae, and microorganisms transform sunlight into chemical energy. Peat lands, swamps, and wetlands - marshlands and high salinity - were the current significant organic producers producing algae. Lipid metabolism and acids are present in microalgal species as cellular macromolecules, storage systems, metabolism, and power providers. Lipid metabolism may make up anywhere from 2% to 40% of the volume of microalgae.

According to the International Energy Agency (2007), alternative sources provided 14 percent of global consumption during 2005, whereas alternatives provided just 4% of overall growth in the United States. Throughout United States, oil has been the most common type of power (International Energy Agency 2007). Biodiesels were already being investigated to see whether they can make power as sustainable generating electricity<sup>[63]</sup>. The Underwater Creatures Programme began in 1970 as a nationwide attempt to create biofuels from microalgae with high oil content. Because maize as well as corn and soy oil gasoline became deemed an established technique, the renewable initiative moved their concentration to soy or corn innovations. Nevertheless, 100% of the maize and soybeans crop in the United States would only cover 12% of petroleum and 6% of biodiesel demand. To satisfy the demands of transport fuels inside the United States, a biofuels sector had to generate 530 billion barrels per year. When evaluating the various biofuels crops and their corresponding oil outputs, it becomes clear that every source consumes a substantial amount of land, resulting in major environmental effect. Organisms seem to be the greatest practical biofuels resource for such United States. Throughout quick advancement, microalgae may increase its density up to eight times every single day. Microalgae were excellent manufacturers of essential oils, they trap dioxide, lowering greenhouse gases, and they do not degrade plant resources or endanger foodstuffs. Care of the quality genotypes and choosing the right culture and processed parameters were essential for fulfilling the promise of enhanced algae biodiesel and achieving sizable commercialization<sup>[64]</sup>. Microalgae may convert energy allocations from reproducing to petroleum products when placed in demanding settings (– for example, nutritional deprivation). Using with the mechanical method named as transesterification, algal oil may be collected and converted into biofuels. For instance, in an ammonia climate, one variety of algae

went from 22 to 58 percent moisture production per weight of dry. Furthermore, found that depriving Tasteless and odourless subterranean from phosphorus changed overall lipids metabolism dramatically<sup>[65]</sup>. When compost becomes available, certain microalgae varieties could develop simultaneously biogenic as well as heterotrophically. Whenever mixotrophic algae are put in poor lighting with an artificial source of carbon, they produced a lot of protein and lipids. Nevertheless, photosynthesis growing becomes far quicker in just about all strains that mixotrophic production, and the need for organic material may make the culture method more costly and hence less commercially feasible. Because of shifting weather or interference with introduced species, reported that maintaining experimental algae growth into outdoor locations proved problematic. Further issue is that under tense events, such as nitrogen deprivation, microalgal output might drop<sup>[66-67]</sup>. Like such result, although having oil production concentration, the petroleum imports extraction under highly severe state might actually reduce owing to reduced growth. 'With a better knowledge of such dynamics, it may well be feasible to achieve a significant gain in total oil output by properly managing the time of nutrient depletion and cell extraction,' the researchers said. They presented a three-biofuel farming technique on this approach<sup>[68]</sup>. During a generator, development might well be controlled, whereas petroleum extracted could be enhanced in a regenerator. The very first step would be a continuously bioreactor with optimal marine growth nutrient availability. Several batch processors at settings that optimum the oil extracted of the microalgae species is being used in the key process. The batches then gather with the steady frequency with additional first generator, maintaining the initial phase of rapid evolution. These simulated and its results may well be regulated through employing bioreactors rather than an independent platform. The additional running expenses of an isolated circuit might well be mitigated if predicted algal yields or fatty oils are preserved in such processors<sup>[69]</sup>. Regarding microalgal, temperature is a crucial determinant of growth. This emerald seaweed Cyanobacteria was studied at degrees ranging from 15 to 30 degrees Celsius to optimum development, when it was shown that 25 degrees Celsius promotes the fastest growth. The Freshwater Biodiversity Programme discovered two varieties of Accented with green that grew temperatures ranging between 30 and 35 degrees Celsius. Furthermore, environmental stabilization must be preserved at 2C, especially for maritime strains that would be less temperature resistant. Both the duration of the flowering time and the solar irradiation may influence algal development. If indeed the cultured also isn't sustained at the proper light: dark cycling, undesirable development consequences might develop, including such hydronephrosis with something like a shorter light / dark cycle. While some species' ideal sunshine: night phases were shown to range around 12:

12 to 16: 08, constant lamp may destroy certain algae Under may produce picture in microalgae due to peroxidative damage<sup>[70]</sup>. Low light concentrations (100m) were preferred by certain algal. The present study aimed to maximise the growth of two algae strains and Antique, by manipulating ecological parameters. Both to algal strains, the goal of such tests has been to find the best amount of light, warmth, and illumination cycle. Variables of 15–40°C have been tested, as well as illumination phases of 14: 10, 16: 08, and 18: 06, for optimum development of both breeds. Light intensity of (80–200 and 40–100 ) have been chosen species and Antique respectively, data or published suggestions .

### 8.1 Algae-based biodiesel

Algal blooms biofuels has gaining popularity, particularly since many jurisdictions in United States allow biofuel to be blended with oil. The advantages or disadvantages using algal blooms biofuel are analyzed in this chapter. It is indeed vital to remember that every biodiesel is, at its core, a method of gathering energy from the sun and conserving it in a compound with a high efficiency. It is necessary to know the entire process from begin to finish in addition to making a really technology as effective as feasible. A photon is seized by a subshell atomic nucleus in a circle of conjugating verbs double securities inside a colourant chemical compound (with both the 2p exciton becoming part of a coupling bond), having caused excitation (in which the intensity level of this activation helps determine the wave length that can be 'gathered,' with colourants in photosynthetic bacteria allowing particles with wave lengths from 400 to 700 nm). Recently released research purports to properly clarify how this collected power was delivered to the active site of a rosette with approaching 100 percent accuracy<sup>[71-72]</sup>. Their own discovery of cohesive automated fluctuations among donor – acceptor colourant particles (conventionally regarded as swapping electricity through digital photoelectric absorption and emission) illustrates the wave particle duality behaviour of vibrational transfer of energy through all the fluorophore, accounting for nearly loss-free electricity<sup>[73-74]</sup>. The energy along with such photoelectron being utilized at the active site for divide CO<sub>2</sub> and H<sub>2</sub>O molecules, eventually creating carbohydrate (via the Calvin cycle's numerous phases), to just an overall system that may be described as 6CO<sub>2</sub> 12H<sub>2</sub>O photon! 6H<sub>2</sub> C<sub>6</sub>H<sub>12</sub>O Before going into the specifics of the Calvin cycle, a rough estimate of photosynthetic' quantum yield may be based on looking at the photons required to carry out the whole reaction and the energy of the by-products. The Z-scheme, that's well in photosynthetic study, states that it takes 8 photons to divide one CO<sub>2</sub> and two H<sub>2</sub>O molecule, releasing one basic carbohydrates (CH<sub>2</sub>O), one O<sub>2</sub> molecules, and one H<sub>2</sub>O peptide (which, interestingly, is not made of the same atoms as either of the two input H<sub>2</sub>O molecules). Along with total power of "photosynthetically available radiation (PAR)" photons

being around 217 kJ as well as the power density of just a solitary carbs (CH<sub>2</sub>O) being each those carbohydrates [(CH<sub>2</sub>O)<sup>6</sup>], or 467 kJ mixture, humans could really quantify a harsh highest productivity of 26.9% for transforming seized solar energy into convert the chemical energy. With PAR contributing for 43 percent of incident sunlight on the earth's crust, the theoretical limit for photosynthesis rate (based on seven photons collected per CH<sub>2</sub>O generated) is about 11.6 percent<sup>[75]</sup>. In actuality, most plants are much below this upper bound, with worldwide averages often ranging around 1% and 2%. The causes for this discrepancy typically revolve all over percentage constraints caused by factors apart from brightness (for example, H<sub>2</sub>O and nutritional accessibility), photo saturation (a few seedlings, or portion sizes of seedlings, obtain more sunlight because they can handle while others obtain less), and Unconjugated (the nutrient that finally provides a starting point for photosynthetic activity) having to accept earth's atmosphere O<sub>2</sub> (rather than CO<sub>2</sub>), going to result in photosynthetic activity.

## IX. CONCLUSION

Biodiesel made from oil seeds has the possibility to be a sustainable or carbon-neutral substitute for petroleum. Sustainable fuels, on the other hand, are not a viable alternative to petroleum fuels due to high costs or limited availability. Ethanol would become increasingly appealing to both customers as entrepreneurs like the price of petroleum fuels rises as supply decline. Microalgae seem to be the sole source of sustainable biofuel suitable for satisfying the worldwide need for petroleum products, while biofuel produced oil seeds, residual canola oil, or animals fat could sole supply a tiny part of the current demand. Microalgal biodiesel is theoretically achievable, as shown here. This is the only sustainable biofuel that has the ability to totally replace petroleum-based petroleum products. For compete with petroleum diesel, overall costs of generating microalgae biomass biofuel must significantly increase, although the amount of reduction required looks to be achievable. For produce low-cost microalgae biomass biofuels, genetic innovation must be used to enhance algae physiology.

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