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# Oleaginous fungi as a sustainable source for biodiesel production: Current and future prospect

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**ABSTRACT**

The growing need for energy resources and the urgent needed action for climate change competing lead to conflict between all sectors on how to meet the needs without causing environmental degradation. By looking for alternatives source between the renewable resources ، the best selection meets the needs is biofuel. Out of Biofuel types, Biodiesel is a great and clean selection of energy resources. Biodiesel prepared from many feedstocks to extract fatty acid from different generations of biodiesel, each generation has its limitation thought looking for viable and cost-effective feedstock ، In this paper we discuss the use of agricultural waste as feedstock for biodiesel preparation using oleaginous fungi as mini-factories.

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## Introduction

Egypt hosted the COP 27 conference in Sharm El-Sheikh during the period from the 6-18th of November during one of the largest energy crises of the new century due to Ukraine and Russia war as a result of energy constraints, various countries back to use coal for electricity generation and different energy sectors (Bórawski et al. 2023,Harris 2023).

Due to climate change effects on energy production and the continuous mitigation efforts carried out by nations, New technology based on microorganism were applied, one of these technologies is using oleaginous fungi (Abdel-Azeem et al. 2021). All energy interested parties of the world seek to fulfill their own needs regardless environmental degradation caused by excess use of fossil fuel such as flooding, food insecurities, drought, migration and diseases increase (Johannesson & Clowes 2022).

Several of the Earth system's possible climate tipping points have already begun to activate. As a result, the 2018 International Panel on Climate Change (IPCC) Special Report on 1.5°C cautions that permitting the earth to warm beyond 1.5°C will have negative effects on human and biodiversity, including drought, floods, heat waves, and sea level rise. The likelihood of substantial damage of Arctic and coral reef ecosystems could be decreased by this half-degree differential compared to the prior internationally accepted target of 2°C. The world has about 12 years to cut global net carbon emissions in half to prevent the most serious repercussions, according to a 1.5°C maximum

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warming goal, however even if this goal is met, warming's potential effects are likely to last for decades or perhaps millennia (Malhi et al. 2020).

According to estimates, the industrial sector's energy use would rise by more than 30% between 2018 and 2050 as consumer spending rises.

Economic growth, population growth, and energy consumption were all predicted to treble by 2050. Due to resource usage and pollution, these would result in environmental degradation. It was suggested that tighter enforcement procedures be used to lower pollution emissions, and that laxer enforcement of environmental rules would worsen environmental degradation (Suproń & Łącka 2023).

Economic expansion recently has led to an increase in $CO_2$ emissions in low- and middle-income countries, particularly in the top emitting nations. China, for instance, is presently the country that contributes the most to the world's CO2 emissions, followed by the United States, Canada, Japan, Brazil, India, and Russia (Fatima et al. 2021; Grabher et al. 2023).

The use of fossil fuels must be severely reduced, and investments in fuel substitutes—particularly renewable energy—need to pick up speed. It is clear that policies supporting renewable energy have fallen short of what is required to keep fossil fuels in the ground. Therefore, a deeper analysis of fossil fuel consumption patterns is required in order to comprehend what will be required of future policies to limit the supply of fossil fuels (Johnsson et al. 2019).

Out of the seventeen SDGs, Egypt vision 2030 and focusing on these goals; ensure access to affordable, reliable, sustainable and modern energy for all, take urgent action to combat climate change and its impacts and Make cities and human settlements inclusive, safe, resilient and sustainable. We have to focus on renewable and clean energy sources to meet the need of sustainable cities, sustainable and modern energy and combat climate change (Mouneer 2021).

Various renewable and clean sources are available, but not all of them meet the need for all SDGs. Looking through the biofuel especially biodiesel, not all feedstock used to produce biodiesel are fully useful except for using of oleaginous fungi grown on local agro-waste as source of mini biodiesel factories (Manikandan et al. 2022).

This review aims to spot the light on the current status of using oleaginous microorganisms in production of sustainable source of energy.

## *Biofuel as sustainable and renewable energy sources*

Energy obtained from biomass is referred to as bioenergy. A raw substance of biological origin that is neither fossilized or entrenched in geological formations is called biomass. The stored energy in biomass can either be turned directly into other kinds of energy or it can be processed into solid, liquid, or gaseous fuels (Perea-Moreno et al. 2019).

The proportion of biomass energy in relation to total energy consumption varies greatly between nations due to geographic, economic, and climatic variations; it can be as low as 1% in some industrialized nations like the United Kingdom to significantly more than 50% in some developing nations in Africa and Asia. Being much greater in terms of energy than the second-largest renewable energy source, hydropower, biomass is by far the most significant renewable energy source (Achuo & Asongu. 2023).

In principle, a wide variety of resources, including biomass from agricultural and forestry primary production as well as byproducts and leftovers from downstream industries and municipal trash, can be used for energetic purposes. Biomass is a substance created during the photosynthesis of plants and is principally composed of carbon, hydrogen, and oxygen (along with additional elements). The oxygenation of carbon hydrates, an exothermic reaction, is the general basis of energy production from biomass, much as it is from fossil fuels. Our energy supply is built on the heat that is emitted during oxygenation (Gaybullaeva 2021).

If biomass usage and growth are balanced, the $CO_2$ produced during the oxygenation of biomass or biofuels does not contribute to global warming.

Most of the organic material used for energy is directly burned to produce heat and/or power, but there are a wide range of additional options for producing ecologically friendly heat and/or electricity as well as transportation fuels from organic material.

The two main types of biomass fuel—unprocessed and processed—are important to take into consideration. Direct combustion often provides heat for cooking, space heating, or electricity generating when using unprocessed biofuel, while there are also small- and large-scale industrial applications for steam raising and other processes needing low- to medium-temperature process heat (Srinivas et al. 2022).

It is technically simple and environmentally responsible to convert processed biofuels with explicitly defined fuel properties into the needed usable energy. These biofuels can then be used to accomplish a supply task comfortably and efficiently by being easily traded. The following conversion routes are available to ensure this. The term "thermo-chemical conversion" refers to all processes that use heat to transform biomass into fuels that are solid, liquid, or gaseous. As a result, these processes include the creation of charcoal, gasification, and pyrolysis, only charcoal production is currently state-of-the-art and widely employed among these options (Web & Thr 2022).

Liquid fuels are produced using physical (like pressing) and chemical (like esterification) conversion techniques. As a replacement for petroleum-based diesel fuel, the most widely utilized technology to date is the manufacture of vegetable oil from oil seed and the transesterification of this vegetable oil to Fatty Acid Methyl Ester (FAME). Conversion processes based on biological processes are summed up as biochemical conversion. The two most significant options are making alcohol from biomass that contains sugar, starch, or celluloses and making biogas from organic waste. Both methods of supplying energy are cutting-edge and widely employed (Akhtar et al. 2023).

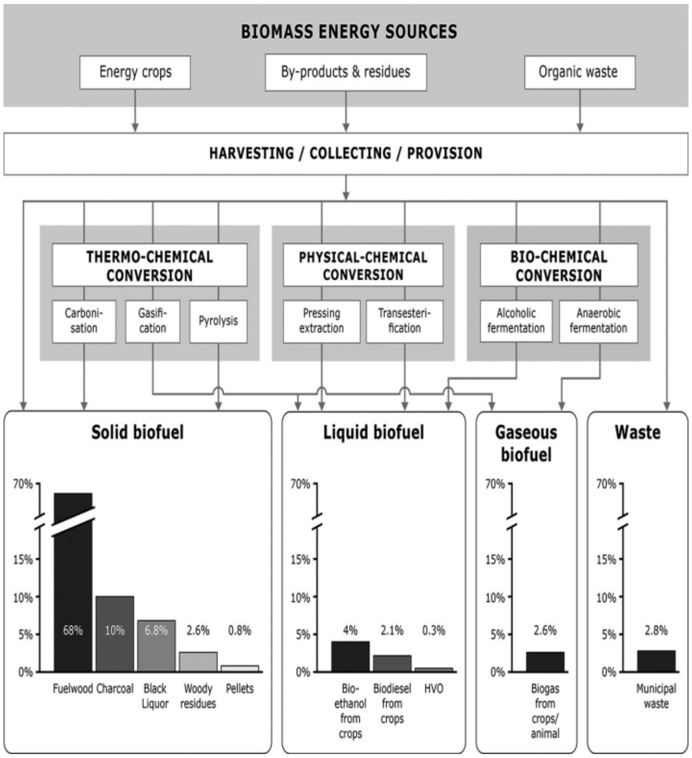

**Fig 1**. Bioenergy carriers (Web & Thr 2022).

Advanced biofuels (Fig. 1) also have the potential to require less land, reduce competition between food and fuel due to the use of residues and waste, improve GHG balances, and lessen sustainability concerns for biofuels (Web & Thr 2022).

### *Biodiesel where and to where?*

The method utilized in the 1800s to create fuel from biomass feedstock is essentially the same method employed today. The history of biodiesel is less technological and more political and economic. Early in the 20th century, gasoline-powered cars were first made available. The amount of crude oil that oil firms had to refine to produce gasoline resulted in a surplus of distillate, which is a great fuel for diesel engines and far less expensive than vegetable oils. But regarding petroleum, resource depletion has always been a problem, and farmers have consistently looked for new markets for their goods. As a result, efforts to utilize vegetable oils as fuel have persisted.

The technique of creating biodiesel from vegetable oils is not new. Transesterification is the process by which vegetable or animal fats are transformed into monoalkyl esters or biodiesel. The method of transesterifying triglycerides in oils is not new.

Transesterification was first carried out by Duffy and Patrick in 1853. When renowned German inventor Dr. Rudolph Diesel released a paper titled "The theory and building of a rational heat engine" in 1893, the diesel engine was born. The initial diesel engine was created by Dr. Diesel to operate on vegetable oil (Demirbas 2008).

Rudolf Diesel had a clear understanding of the advantages and disadvantages of renewable energy sources and foresaw the effective use of vegetable oils in his engines.

Before George Chavanne's patent in 1937, there is no known history of the use of mono alkyl esters of fatty acids from vegetable oils as fuels in the literature. Countries like France, Belgium, and the UK demonstrated strong interest in biodiesel produced from vegetable and plant oil despite not being major producers of the feedstock oil crops. The availability of the raw resources from their colonies was the main factor driving this interest. Priorities and energy policies evolved as the world's political climate did. Prior to the development of the commercial technique for producing biodiesel in the early 1970s, interest and investment in biofuel research and production fluctuated with the state of the world economy.

Brazilian researcher Expedito Parente patented the process in 1977. The first commercial biodiesel plant began operating in Austria in 1989, more than ten years later (Bandh & Malla 2022).

Petroleum-based fuels have historically required to be hard to come by in order to spur the research and production of biofuels; it has only lately been the problems facing mankind that have spurred this sector. Debate on climate change, energy policy, and food rights has been generated by issues including species extinction, ecological collapse, and resource sustainability. With its benign emission profile, biodiesel becomes a viable choice for reducing global dependency on fossil fuels and elevating energy security.

However, with the limited subsidies given to biodiesel and the frequently contested "food vs. fuel" debate, the future of biodiesel will grow more uncertain. The ability to generate renewable feedstocks, such as plants and vegetables, in a way that keeps the price of biodiesel comparable with that of fossil fuels is a prerequisite to produce biodiesel (Balasubramanian & Steward 2019).

A small number of Pacific Islands use coconut oil, especially when the outside temperature does not drop below 17 °C. In the meanwhile, the Japanese government gave tax exemptions for using pure vegetable oil in cars.

According to British Train Operating Company, the first biodiesel train was operated using a fuel mixture consisting of 80% diesel and 20% biodiesel. It was said to reduce direct emissions by 14%. On September 15th, 2007, The Royal Train Services started functioning on 100% biodiesel. The King's Highness The first passengers on the train were James Hygate, the managing director of Green Fuels, and the prince of Wales.

Deval Patrick, the governor of Massachusetts, mandated that beginning in July 2010 and increasing to 5% in 2013, the fuel used for residential heating be made from biodiesel (Raj et al. 2022).

The majority of the renewable fuels utilized in the world's transportation fleet, according to the 2014 study from the Renewable Energy Policy Network for the 21th. Century (REN21) were biodiesel and ethanol. Both biofuels have an expanding market share in Brazil, just like they do in the USA and other European nations. Among all the biofuels, biodiesel saw the most increase in the previous ten years, growing by 15 times (REN21, Renewables 2014 Global Status Report).

Volkswagen intends to lower its fleet's carbon impact in Europe by 40% by 2030. As a result, the firm is increasingly focused on completely and partially electric vehicles. Volkswagen is now officially allowing cars equipped with the latest-generation 4-cylinder diesel engines for usage with paraffinic fuels. When compared to ordinary diesel, these newly created bio-component diesel fuels allow for considerable $CO_2$ reductions of 70-95 percent. Along with its rapid ramp-up efforts in electric mobility, Volkswagen is gradually expanding its existing line of combustion engines. (Volkswagen Newsroom 2023).

The Volkswagen Group contemplated using B5 and B100 gasoline blends produced of rapeseed oil in its cars as of late in 2021 since they comply with the EN 14214 standard (Raj et al. 2022).

### *Biodiesel as a vital and versatile renewable biofuel*

Biodiesel is a sustainable, oxygenated, sulfur-free, and biodegradable source of energy. Using biodiesel as fuel in a diesel engine doesn't require any modifications. In comparison to diesel fuel, biodiesel exhibits lower levels of regulated and unregulated emissions. Focus on reducing greenhouse gas (GHG) emissions, less impact on the climate, sustainable and renewable energy source, and to obtain more promising alternate fuel supply to meet the current energy demand are just a few of the reasons why biodiesel is used as an alternative fuel. (Singh et al. 2020).

All biodiesels share the same renewable source and fundamentals. They differ from early photosynthesis because they are created through the photosynthetic conversion of solar energy to chemical energy. produced using a catalyst and methanol in the transesterification of triglycerides. During the transesterification process, glycerol (glycerin) is produced as a byproduct. Methanol is typically used to make biodiesel due to its low cost and wide availability. 100% of FAME is referred to as B100, while lesser amounts, like B20, are referred to as "biodiesel blends." Solar energy is used in the production of biodiesel, which is the cornerstone of a sustainable bioeconomy (Aghbashlo et al. 2021).

The struggle for land for food versus fuel development is a major issue with renewable fuel. The first, second, and third generations of biodiesel are typically categorized according to their source, whereas the fourth generation of biodiesel is made from artificial biological tools and is only in the early stages of basic research (Singh et al. 2020).

Edible feedstocks, such as rapeseed oil, soy oil, coconut oil, corn oil, palm oil, mustard oil, olive oil, rice oil, etc., are used to make first generation biodiesels. The main drawback of using these feedstocks that raises the price of food products is the possibility of a food supply shortage. These limitations forced users to switch to additional alternative sources to produce biodiesel (Rezende et al. 2021).

The non-edible feedstocks used to make second generation biodiesels include things like neem oil, jatropha oil, nagchampa oil, karanja oil, calophyllum inophyllum oil, rubber seed oil, and mahua indica oil. various non-edible raw materials used in the production of biodiesel The main advantages of using second-generation biodiesel, as contained in these oils, are that no additional food plants or agricultural land are needed. The main non-edible plants, like Jatropha oil, Jojoba oil, and Karanja oil, see a decline in yield with second generation fuels. These raw materials can be grown on unimportant lands. For this reason, non-edible crops are required to be grown on agricultural land; this has an immediate impact on the societal economy and food production. Researchers are focusing on new, economically viable, and more easily accessible alternatives to combat the socioeconomic problems caused by non-edible oil. The disadvantages of second-generation biodiesel also include the requirement for additional alcohol amounts (Singh et al. 2020; Rezende et al. 2021).

Third generation biodiesel is the name given to the biodiesel produced using waste oils and microalgae. Less greenhouse effect, increased growth and productivity, less struggle for farming land, a higher oil percentage, and less impact on the food supply are the main advantages of third generation biodiesel. The main drawbacks are the high investment requirements, the need for sunlight, the problem of production on a larger scale, and the challenges of oil extraction. Fish oil, animal fat, microalgae, used cooking oil, and other sources are the primary raw materials for third generation biodiesel. Third generation biodiesel's feasible resources outperform earlier generations' feedstocks in terms of availability, adaptability to environmental constraints, and economic viability.(Mat Aron et al. 2020).

Fourth generation of biodiesel, Electro fuels and photo-biological solar fuels are taken into consideration. Solar energy is converted into biodiesel using raw materials to create solar biofuels; this process of conversion is a relatively new area of study. Raw materials are cheap, plentiful, and never-ending (Singh et al. 2020).

### *Oleaginous fungi as Promising Biodiesel Resource*

Oleaginous microorganisms are characterized as having a 20% microbial lipid excess. Single cell oils (SCO), or biodiesel made from microbial lipids, have received a lot of attention internationally.

There have been numerous reports of oleaginous yeasts and microalgae growing and accumulating large amounts of lipids resembling those in vegetable oil. The genetic makeup of microorganisms determines the degree of lipid accumulation because the maximum attainable lipid contents can differ greatly between species and even within a single strain (Abdel-azeem 2013; Uthandi et al. 2022).

Despite having a high lipid content, microalgae require more land to cultivate and a longer fermentation time than bacteria.

Bacteria are superior at producing biodiesel because of their quick growth (12–24 hours to reach huge biomass) and simple culture process. As an alternative, storage-lipid-accumulating bacteria, those of the actinomycetes group, may be used.

Under growth-restricted conditions, these bacteria are capable of synthesizing remarkably high amounts of fatty acids (up to 70% of the cellular dry weight), which they then accumulate intracellularly as TAGs.

Since the 1980s, moulds and yeasts have been regarded as beneficial oleaginous microorganisms. As much as 70% of the dry weight of some yeast strains' biomass can be made up of intracellular lipids, including *Rhodosporidium* sp., *Rhodotorula* sp., and *Lipomyces* sp. Triacylglycerols with a specific structure or those rich in polyunsaturated fatty acids are accumulated by oleaginous yeasts and moulds (Meng et al. 2009). These days, filamentous fungi are being used to make biodiesel because it has been shown that they can also absorb intracellular lipids (Gujjala et al. 2019).

Because oleaginous fungi contain a malic enzyme that produces Nicotinamide adenine dinucleotide phosphate (NADPH), which may reduce acetyl units and create the backbone of fatty acids, it has been claimed in many biochemical investigations that they might collect lipids (Gutiérrez et al. 2011). Malic enzyme and culture conditions both contribute significantly to lipid formation, for example, by limiting nitrogen and phosphorus in the culture medium and increasing carbon content. A high C/N ratio, which is excellent for lipogenesis, results from a high carbon to nitrogen ratio. Nitrogen is required to produce amino acids and nucleic acids during the growth phase. As a result, due to its restricted availability in the culture broth, protein and nucleic acid synthesis is often inhibited. If both carbon and the culture broth are concurrently too prevalent, then a sizable quantity of TAG will build up in the lipid bodies ( Stoytcheva &Montero 2011; Wu et al. 2011).

Other parameters, such as temperature, pH, inoculum size, incubation period, aeration, and the kind of microbe, affect the fatty acid composition and lipid contents in addition to nitrogen and carbon contents. Ex-novo lipid accumulation is a different method of lipogenesis that involves cultivating oleaginous organisms on hydrophobic carbon sources. Studies have demonstrated that hydrophobic carbon sources cause oleaginous fungus to accumulate large quantities of lipid (Subramaniam et al. 2010).

The three distinct sources of lipid accumulation (yeasts, fungi, and microalgae) each have advantages and disadvantages that must be carefully weighed before being adopted for commercialization. Because of their higher productivity (i.e. yield of lipids per dry cell of up to 65% of DCW, and biomass per liter of production media of up to 100 g/L within 10 days), and because they can grow on a wide range of substrates, including pure sugars, lignocellulosic biomass, and waste waters, yeasts are promising candidates.

Additionally, as prospective yeasts for lipid production may be grown without light, development is guaranteed around-the-clock with low input costs. However, one drawback that frequently occurs when dealing with oleaginous yeasts is the requirement to uphold aseptic conditions within the fermenters in order to prevent any contaminating species from outcompeting the growth of the yeast and so impeding the output of lipids. Industrial use of these systems is not economically effective due to the high expense of maintaining aseptic conditions inside the production fermenters. Thorough study is being done to find ways to use oleaginous yeast to speed up the process (Spier et al. 2015).

Researchers have also looked at filamentous fungi for lipid synthesis due to its benefits, including their capacity to collect 80% lipids by dry weight and suitable fatty acids, including g-linolenic acids, for the synthesis of biodiesel. Over 98% of the lipids recovered from filamentous fungus have been shown to be saponifiable, and the biodiesel made from these lipids reportedly meets the required criteria. Additionally, solid state fermentation may be used to produce lipids from filamentous fungus with cheap startup costs, little energy requirements, and substantial lipid yields. The ability to produce filamentous fungi in the form of pellets, improving mass transfer rate and streamlining the harvesting procedure, is a vital addition to these advantages (Zheng et al. 2012).

Oleaginous microorganisms have advantages for making biodiesel, but industrial scale production makes it difficult for them to be commercially viable. According to a life-cycle assessment analysis, the main obstacles are the cost of the substrate and the method of lipid extraction. Sustainable solutions must be investigated to address these issues. Oleaginous-based oil may become more competitive by utilizing technological advancements for efficient lipid extraction, finding affordable substrates that are plentiful and easily accessible, or integrating the process for biorefinery development. Process integration between biodiesel and biorefineries is crucial for commercial viability (Lardon et al. 2009).

Lipid extraction must be carried out with the utmost care to prevent disruption of other chemicals in order to be a sustainable process and to integrate. Enzymatic pretreatment is one such workable tactic that aids in the precise cleavage of target molecules without causing any harm to other compounds, leading to the development of the biorefinery idea.

The hunt for renewable transportation biofuels has been sparked by the environmental effects of using fossil fuels and the depletion of petroleum resources. Despite being renewable and environmentally benign, manufacturing biodiesel is expensive. Oleaginous bacteria can contribute to lowering this cost as a result of their efficient usage of cheap feedstocks. Additionally, the use of environmentally friendly methods (such as green solvents and other methods) might increase the value of products made from biomass.

It has been reported that different genus of filamentous fungi has different abilities to store lipids in their bodies as *Aspergillus niger*, *Aspergillus terreus* store 9.6% and 37.4% of lipids in sequence in dry weight, while *Mucor circinelloides, Mucor plumbeus* store 23, 80, 20, and 60 % of lipids and *cunninghamella elegans* store 33 and 60% of lipids. The gratest result in *Moretierella* genus that *Moretierella isabellina and Moretierella vinacea* store 67 and 51.9 % of lipids (Gujjala et al. 2011).

Another study for fungal isolates SPSRJ27 *Lasiodiplodia exigua*, SPSRJ28 *Phomopsis sp*, SPSRJL35 *Pestalotiopsis microspore* and SPSRJL36 *Phomopsis sp.* from different plants parts for lipid storing ability was 20.2, 21.1, 26.07, and 20.42 % sequence in the total dry weight (Paul et al. 2020).

Furthermore, oleaginous yeasts could collect lipids inside their cellular compartments in a short period of time, ranging from 5 to 9 days depending on the type of yeast. Oleaginous yeasts have the unusual capacity to utilize a wide range of renewable substrates and low-cost resources, including agricultural and industrial wastes (Govender et al. 2012; Yan et al. 2014).

The notion of using non-edible lignocellulosic biomass as a feedstock for oleaginous yeasts might significantly lower the cost of biodiesel production. Furthermore, the lipids collected by oleaginous yeasts have a chemical similarity to vegetable oil and animal fats. The relative lipid content of oleaginous yeasts was discovered to be C18:1 (oleic acid)> C16:0 (palmitic acid)> C18:2 (linoleic acid) =C18:0 (stearic acid). Any change in the fatty acid profile affects the characteristics of biodiesel during the transesterification process. Each microbe has a distinct fatty acid composition based on the growth circumstances (Fig. 2) and feedstocks available (Patel et al. 2016).

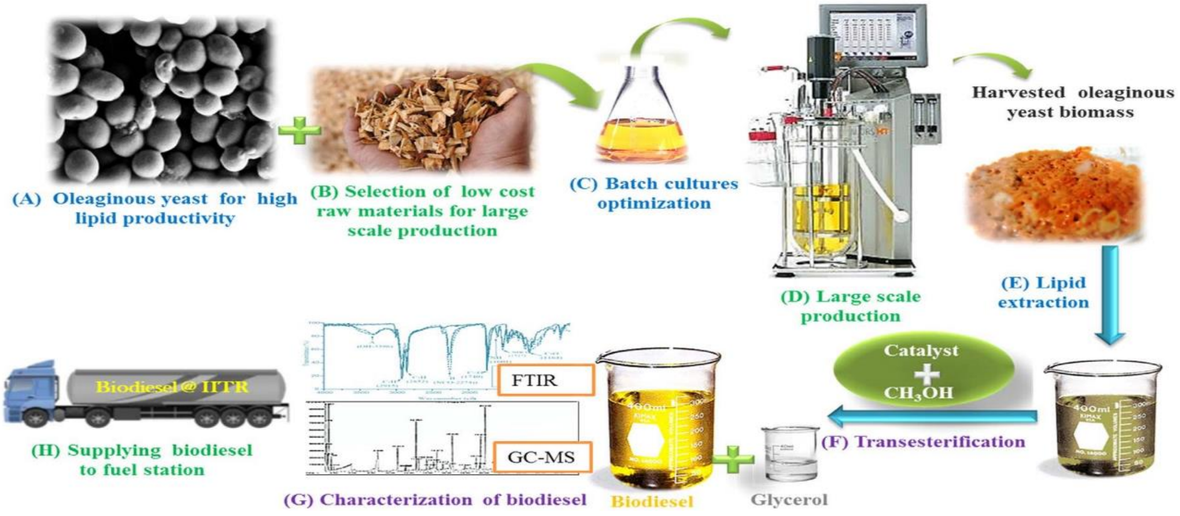

**Fig 2.** Schematic diagram of biodiesel production from the oleaginous yeast (Patel et al. 2016).

More than 20% of the total lipid content of four endophytic fungal isolates were discovered to be present in their dry biomass, demonstrating their oleaginous character. The total lipid content of several species of the same genus as well as isolates of the same species, when cultivated under identical circumstances, showed significant variance due to the diversity in metabolite synthesis in different fungal isolates. After five days of incubation, the growth media optimization determined that potato dextrose liquid medium was the best medium for lipid accumulation. Mannose and dextrose were shown to be the best sugars for fungal isolates to thrive and accumulate lipids. The amount of lipid generated was larger in the static condition than in the shaking condition, and a temperature of 28 C was ideal (Paul et al. 2020).

## Conclusion

The results of this review showed that the importance of oleaginous microorganisms as valuable and sustainable source for biodiesel production due to their ability of storing lipids above 20% of their dry weight. Also, as an emergent solution for biodiesel different generation as it depends up on agricultural waste and doesn't conflict with food production and it to meet the industrial, agricultural and transportation sectors need from energy resource to produce electricity instated of using non-renewable energy resource that result in global environmental issues such as climate change.

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