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Review Article

Between FAMEs-Qualitative and Biomass-Quantitative: A review study on oleaginous fungi

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ABSTRACT

Energy scarcity remains a major global challenge, particularly in the transition toward sustainable and secure energy systems. Fossil energy plays a pivotal role in exacerbating environmental pollution. Consequently, modern bio-refineries and bioenergy have been crucial in utilizing biomass to produce a range of high-value bio-chemicals and biofuels, all in the effort to achieve a climate-neutral future. Fungal microbial lipids could represent essential alternative raw materials for biodiesel production, which may perhaps contribute to partially addressing the problem of declining global oil production. Oleaginous fungi accumulate lipids exceeding 20% of their dry biomass, predominantly as triacylglycerols comprising saturated and unsaturated fatty acids, which closely resemble fatty acids found in vegetable oils that are currently utilized in biodiesel production. Despite the significant advantages of these fungi, large-scale industrial production of fungal lipids has not yet been commercialized. One of the most important challenges that oleaginous fungi must overcome is their ability to produce very large quantities of biomass by using very cheap raw materials, in addition to the fact that these masses contain large quantities of oils of high quality to achieve economic feasibility. This review highlights the investment possibility of oleaginous fungi in biomass-based biodiesel production, their characterization as well as *in vitro* screening advantages, and indicates the need for further research to improve their industrial applications and increase their efficiency.

1. Introduction

Environmental pollution has been growing as one of the key problems in the world; as a result of population growth and developments in the industrial field. This problem has become threatening to human life with significant risks. Among the reasons for such threats is the excessive use of fossil fuels, which contributes to the increase of pollutants in the environment. Accordingly, the scientific community have been prompted to find treatments to reduce pollution and decrease its devastating effects.[1,2] Environmental problems are escalating quickly and have not been adequately addressed. Increasing industrial and human impacts on the environment have led to a rise in the prevalence and severity of these issues.[3] Ecology and the economy are at a crossroads in today's world. There is growing awareness of the need to move progressively toward green growth with sustainability because of the accelerating speed of economic liberalization and globalization, which gave rise to the modern civilization's explosive growth in consumption.[4]

Modern bio-refineries and bioenergy are essential in converting biomass into a wide range of high-value biochemicals and biofuels, contributing to the goal of a climate-neutral future.[5] Bio-refining has flourished over the past few decades, leading to the production of value-added bio-chemicals such as alcohols, along with fossil fuel alternatives.[6] The development of a sustainable bio-economy is supported using bio-based technologies. To facilitate the shift from a linear to a circular economy in the European setting, the bio-economy must include sustainability and circularity at its core.[7] By 2060, the International Energy Agency anticipated that the world's need for bioenergy will nearly triple.[8] Sustainable biomass resources, such as algae, crops, and waste materials must be utilized as effectively as possible for such a significant increase. Additionally, to satisfy future goals for bioenergy and bio-chemicals, creative bio-cascading approaches and circular economy systems must be developed to efficiently utilize biomass

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resources and minimize waste.[5]

Raw materials such as corn or vegetable oils can be used to make first-generation biofuels.[9] However, when those materials are used, they compete with food resources and incur significant material costs.[8] As stated by Azócar et al.[10] that waste lipids including waste frying oils, waste fats, and soap stock have been suggested as inexpensive alternatives to conventional biodiesel feedstocks. Economically attractive non-edible plant oils include rubber seed, pongamia (*Millettia pinnata*), and jatropha (*Jatropha curcas*). Microbial lipids, often called single cell oils (SCOs), produced by yeasts,[11] filamentous fungi, microalgae, and some bacteria, are also proposed as feedstocks can be converted into fatty acid methyl ester (FAME) synthesis; microbial strains with lipid contents of roughly ≥20% dry cell weight are of particular interest. Alternative feedstocks frequently contain elevated free fatty acids (FFAs), which increase the acid value of the oil and can cause saponification during base catalyzed transesterification; such oils therefore often require pretreatment (for example, acid esterification) or alternative catalytic systems. Microbial lipids offer a potential route to biodiesel that does not compete directly with food crops and may help mitigate declining conventional oil reserves. Many fungi are capable of accumulating substantial amounts of storage lipids, primarily triacylglycerols (TAGs).[12] All eukaryotic organisms including plants, fungi, and animals, use triacylglycerols as large-scale backup compounds, whereas bacteria rarely use them.[13,14]

Fungi are vital organisms with unique characteristics that set them apart from other forms of life. One of the key characteristics of fungi is their ability to accumulate substantial amounts of lipids. Such fungi that can accumulate lipids over 20% in their dry cell weights have been referred to as the oleaginous fungi.[15] The use of fungal lipids as raw materials for producing biodiesel has several characteristics, including the fact that they contain both saturated and unsaturated fatty acids, which closely resemble those found in vegetable oils like mystic acid 14:0, palmitic acid C 16:0, stearic acid C 18:0, palmitolic acid C 16:1, oleic acid C 18:1, linoleic acid C 18:2, and behenic 22:0,[16] in addition to, oleaginous fungi are characterized by the presence of arachidonic acid,[17] over and above, they have more interested features, such as ease of cultivation and growth, minimal land requirements, and a short life cycle,[18] and no competition for food sources.[19]

Therefore, it can be stated that, due to the numerous fungal applications and their ability to be produced from a wide range of sources, biodiesel is one of the most important and widely used types of biofuels. However, producing high-quantities dry weight biomass (DW Biomass-Quantitative) that can be ideally used to produce biodiesel from fungi remains crucial for economic viability,[20] which is related to the qualitative value produced from the FAMEs (FAMEs-Qualitative) such as saturated fatty acids (SFA), monounsaturated (MUFA), polyunsaturated FA (PUFA) of these fungi, which can be determined using various techniques[21] such as gas chromatography (GC) and GC-mass spectra (GC-MS) analysis etc.[22] Accordingly, this review paper will focus on the overall prospects of current progress in the potential of fungi to produce biomass that can be encouraged to produce clean, economical energy in the form of biodiesel, as well as the characterization of these fungi and methods for screening them in the laboratory.

2. Biofuel

Peanut oil was the first vegetable oil used as a biofuel by Rudolf Diesel. Then, with the availability of petroleum and its derivatives at low prices, the use of fossil fuels has increased significantly. With the accumulation of pollutants in recent years, environmental scientists have begun looking for alternatives to fossil fuels that are less harmful to the environment. Animal fats, vegetables, and algae oils were used as renewable sources.[23]

Biofuel is referred to as fuels that can be produced in different ways and from multiples sources. It may be produced from agricultural products as well as industrial or agricultural waste, or the byproducts

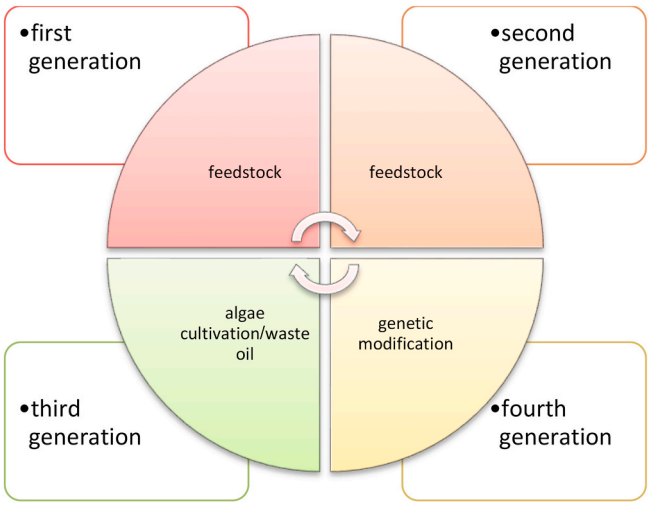

Fig. 1. Four generations in biodiesel production.

may produce it. Biofuels typically exist in three forms: liquid fuels like biodiesel, ethanol, and vegetable oils; solid fuels such as plant waste (e.g., wood) or gas like methane, which is produced from the decomposition of waste, plants, and animal manure.[24,25]

Biomass-based biofuels, whether gas, liquid, or solid, are biodegradable alternative fuels derived from renewable and biodegradable resources created from various sources, including vegetable oils and animal fats.[26]

Biofuels are characterized as clean, non-toxic, and safer than fossil fuels.[27]

Sources of biofuel production such as biodiesel have evolved through what are known as the four generations. The first generation was based on seed sources and plant grains includes vegetable oils such as rapeseed oil, soybean oil, palm oil and even animal fats,[28] while the second generation depends on sources of biomass, such as oils extracted from forest waste, agricultural residues, and food waste,[29,30] and the third generation was based on microorganisms such as microalgae oils.[31] However, genetic engineering attempts to produce the fourth generation through the genetic modification of some organisms such as oleaginous microorganisms (Fig. 1).[32]

3. Biodiesel

Conflict between all sectors over how to meet needs without creating environmental degradation is brought on by the competing demands for energy resources and the urgent need for action to combat climate change. The greatest option that satisfies needs when comparing renewable resource alternatives is biofuel. Biodiesel is an excellent and sustainable option for energy sources among the various varieties of biofuel. Each generation of biodiesel has its limitations, and several feedstocks are used to extract the fatty acids from the fuel. To overcome these limitations, practical and affordable feedstocks are sought for.[33]

Biodiesel helps reduce pollutants and enhance global climate conditions by lowering emissions of carbon dioxide (CO_2), carbon monoxide (CO), unburned hydrocarbons, and particulate matter from diesel engines.[34,35] Biodiesel can be used purely, as it is referred to as the B100,[36] but it is better to use a mixture of petroleum diesel with biodiesel.[37]

Biodiesel can be described as mono-alkyl esters derived from long-chain fatty acids and serve as an alternative source of fuel due to its high similarity with fossil diesel. It can be described as methyl or ethyl esters derived from a wide range of renewable sources such as cooking oil, vegetable oils,[38] animal fats[39] and esters are oxidized organic compounds that can be used in internal combustion engines since they are similar to petroleum diesel in many properties.[40]

Animal fats and vegetable oils are esters for unsaturated or saturated

carboxylic acid with triacylglycerol,[41] which are in the form of triacylglycerol, vegetable oils, and animal fats viscous. However, esterification reactions act to reduce the viscosity associated with these fats.[42] These interactions are influenced by some determinants, such as temperature, type of alcohol used, type and amount of the catalyst, reaction time, water content, and fatty acid content.[43] The esterification processes make vegetable oils and animal fats that are similar to the petroleum diesel in terms of properties and viscosity reduced significantly through the conversion of triacylglycerol which is caused by the separation of fatty acids from glycerol. According to its source, transesterification is the most common method for producing biodiesel from various sources, which is divided into first, second, and third-generation biodiesels. Different factors can affect the transesterification process, which can be taken into account when trying to improve biodiesel yield. The types of the catalyst and its concentration plays a crucial role in the transesterification of the biodiesel sources.[34] Therefore, studies seek to use hybrid materials with distinctive physical and chemical properties to improve biodiesel production processes.[44] In addition to that, these interactions are a good source of glycerol.[45] By esterification processes, glycerol (Gly) can be separated from triacylglycerol (TAGs) and the viscosity can be reduced as much as possible to produce fatty acid esters (FAEs), as in the following steps included in the following equations (Equations (1)–(4))[46,47]:

$$\begin{array}{l} H_2C-O-CO-R_1 \\ | \\ HC-O-CO-R_2 + CH_3OH \\ | \\ H_2C-O-C-R_3 \end{array} \overset{\rightarrow}{\leftarrow} \begin{array}{l} H_2C-OH \\ | \\ HC-O-CO-R_2 + CH_3-CO-R_1 \\ | \\ H_2C-O-C-R_3 \end{array} \quad \text{Eq.1}$$

(TAGs) (Alcohol) (Cat) (Di-glyceride) (FAE) R1, R2, R3 = fatty acid chains.

$$\begin{array}{l} H_2C-OH \\ | \\ HC-O-CO-R_2 + CH_3OH \\ | \\ H_2C-O-C-R_3 \end{array} \overset{\rightarrow}{\leftarrow} \begin{array}{l} H_2C-OH \\ | \\ HC-OH \\ | \\ H_2C-O-C-R_3 \end{array} + CH_3-CO-R_2 \quad \text{Eq.2}$$

(Di-glyceride) (Alcohol) (Cat) (Mono-glycerride) (FAE)

$$\begin{array}{l} H_2C-OH \\ | \\ HC-O-OH \\ | \\ H_2C-O-C-R_3 \end{array} + CH_3OH \overset{\rightarrow}{\leftarrow} \begin{array}{l} H_2C-OH \\ | \\ HC-OH \\ | \\ H_2C-OH \end{array} + CH_3-CO-R_3 \quad \text{Eq.3}$$

(Mono-glyceride) (Alcohol) (Cat) (Gly) (FAE)

$$\begin{array}{l} H_2C-O-CO-R_1 \\ | \\ HC-O-CO-R_2 \\ | \\ H_2C-O-C-R_3 \end{array} + 3ROH \overset{\rightarrow}{\leftarrow} \begin{array}{l} H_2C-OH \\ | \\ HC-OH \\ | \\ H_2C-OH \end{array} + 3R-O- \begin{array}{l} O \\ \| \\ C \\ | \\ R \end{array} \quad \text{Eq.4}$$

(TAGs) (Alcohol) (Cat) (Gly) (FAEs).

Converted FAMEs types play a pivotal role in improving or decreasing the viability of oils in producing highly efficient biodiesel. These esters affect biodiesel properties such as cloud point (CP) and cold filter plugging point (CFPP).[48] Furthermore, the type of FAMEs can affect both cetane numbers and viscosity, which are vital indicators for the operation of biodiesel internal combustion engines.[49] A decrease in FAME production after esterification indicates a decline in reaction quality, which can be identified by the presence of unreacted compounds or residues. This is related to several factors, most notably the catalysts used in the reaction, the molar ratios of alcohols, temperature, and so on. These factors clearly influence the reactions, ultimately leading to various problems.[50] The type of fatty acids, whether saturated or unsaturated, is one of the major determinants in producing biodiesel that can be used in pure/blended form in internal combustion engines.[51] Ultimately, the biodiesel produced must meet or comply with the requirements set by the American Society for Testing & Materials (ASTM).[52]

4. Oleaginous fungi

Extensive research has demonstrated that yeasts and filamentous fungi are excellent candidates for lipid accumulation within their biomass, as those lipids are like the vegetable oils in terms of properties, and therefore they can be invested in the production of biodiesel in internal combustion engines instead of fossil fuels [15,53–55]. Lipid accumulation in oleaginous yeasts primarily occurs under nutrient-limited conditions in the presence of excess carbon. When essential nutrients are limited, cell proliferation is inhibited, and the surplus carbon is redirected toward the synthesis of storage triacylglycerols (TAGs). Published studies reported that limitations in nutrients such as phosphorus, magnesium, zinc, or iron can promote lipid accumulation in model oleaginous fungi.[56] Which accumulate lipids exceeding 20% of their cell dry weight.[57] Oleaginous fungi are capable of accumulating lipids within their biomass, presenting a promising alternative for biodiesel production.[58] They can accumulate lipids in their biomass, offering a promising alternative for biodiesel production. Fungi prefer plants because they have numerous advantages.[59]

Many fungal species are rich in lipids due to their ability to synthesize and accumulate large quantities of lipids, such as triacylglycerol, within their cells. These lipids typically contain high levels of long-chain fatty acids ranging from C6 to C36[60] that are very similar to the vegetable oils.[61]

Fungal lipids have the same structure and energy value as vegetable oils[62] however, their production does not displace food resources,[59] especially in the case where they depend on cheap sources of carbon.[63] Furthermore, oleaginous fungi have a short life cycle and are unaffected by weather changes in their production processes.[64] Furthermore, fungi do not require light to grow,[65] and have ability to decompose organic compounds.[63]

4.1. Lipids accumulations mechanisms in oleaginous fungi

The accumulations of lipids in the oleaginous fungi are triggered by specific nutrient limitations, such as phosphorus, nitrogen, and magnesium source. The reduction of nitrogen source (N) has been considered a sufficient condition for the accumulations of lipids (lipogenesis).[66] Nitrogen is necessary throughout the growth phase for synthesizing the nucleic acids and proteins, therefore, when the source of N is limited, the synthesis of proteins and nucleic acids is likely to stop, and the rate of growth slows down. At the same time, the carbon source distributes energy and anabolic processes that produce carbohydrates, fats, proteins, and nucleic acids.[67,68]

Lipid accumulation in oleaginous fungi is generally dependent on several factors, including the carbon source used. The type and concentration of the carbon source may also play a vital role in lipids accumulation.[69] Research suggests that by maintaining a balanced carbon concentration and minimizing nitrogen sources, fungi can achieve high lipids accumulation rates.[70] Aeration, oxygen supply,[71] and the presence of phosphorus in the nutrient medium are also important

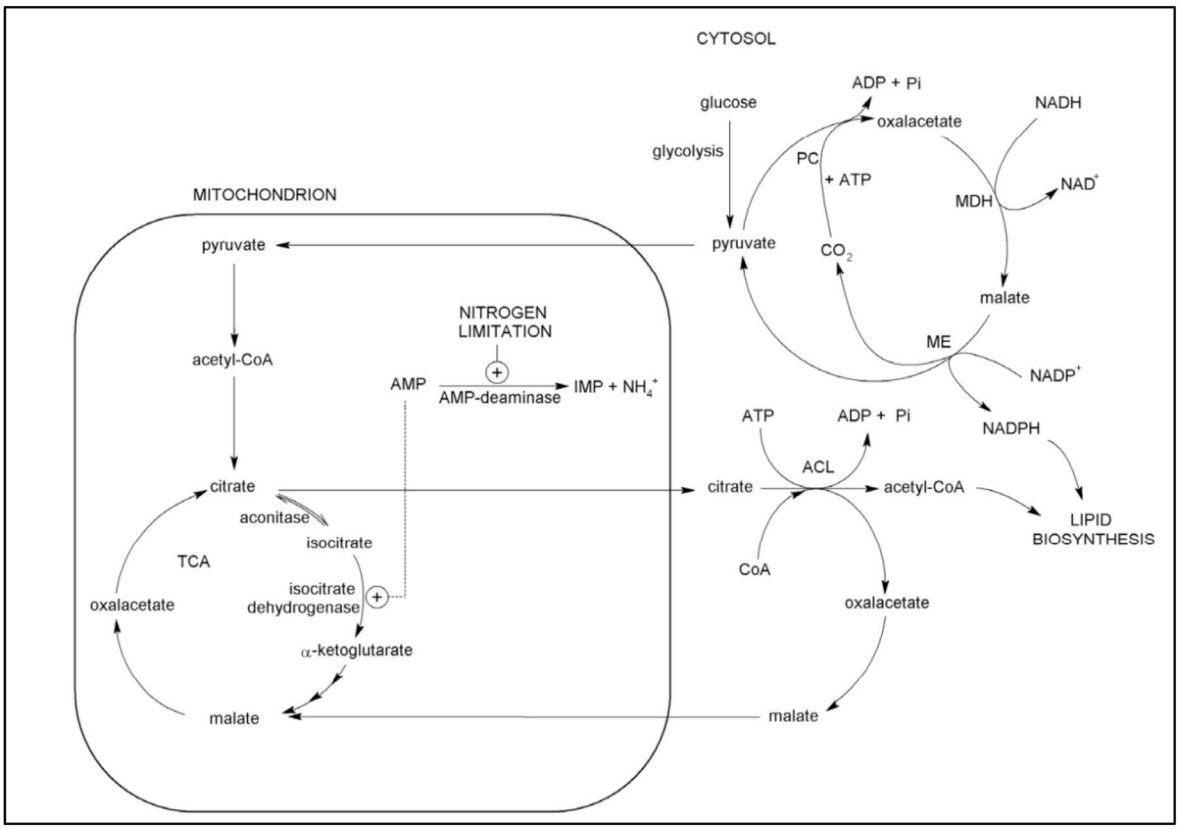

Fig. 2. Intracellular pathways of lipids biosynthesis[56] adapted from.[14]

determinants of fatty acid accumulation.[72] Furthermore, pH[73] and temperature[71] may be considered determinants in achieving a balance between growth and lipid accumulation, which can promote a healthy lipid-rich biomass.

The excess carbon source (C) is used to store polysaccharides in non-oleaginous species, whereas it is often converted into carbohydrates in oleaginous species. Then, it is converted into lipids, resulting in an accumulation of triacylglycerol (TAG) in the cells' lipid bodies.[67,68] Therefore, the use of culture media with a suitable N/C ratio is vital for maximizing the production of lipids.[56] In the eukaryotes, triacylglycerol is a good source of saturated and unsaturated fatty acids, it is also a source of energy storage and carbon source, and on the other hand, there is no variation between oleaginous and non-oleaginous fungi in the bio-chemical pathway of lipid biosynthesis. The capability for accumulating high lipid amounts is dependent mainly upon the pathway of the regulation biosynthesis and precursor supply (in other words, glycerol-3-phosphate, acetyl-CoA, and malonyl-CoA) in addition to the cofactor (NADPH) (Fig. 2).[56]

The neutral lipids in the eukaryotes are always kept in specialized compartments that are referred to as lipid bodies (LB), those are formed at a specialized sub-domain of endoplasmic reticulum (ER), where majority of the structural proteins and bio-synthetic enzymes are located.[74]

According to Czabany et al.,[75] the phospholipids do not fit neutral lipids, where the phospholipids are deposited between both membrane bi-layer leaflets. On the other hand, massive neutral lipid amounts cannot be incorporated in membrane bi-layer ER. Continuous synthesis of the neutral lipid results in forming a (bud), emerging from endoplasmic reticulum as mature lipids body after reaching critical size. TAG makes up the majority of neutral lipids in the lipid bodies of most oleaginous yeast types up to 90% or more,[76] with steryl esters accounting for a small portion (SE). A substantial amount of free fatty acids (FFAs) within the lipid bodies has, however, only been reported in *Yarrowia lipolytica*.[56]

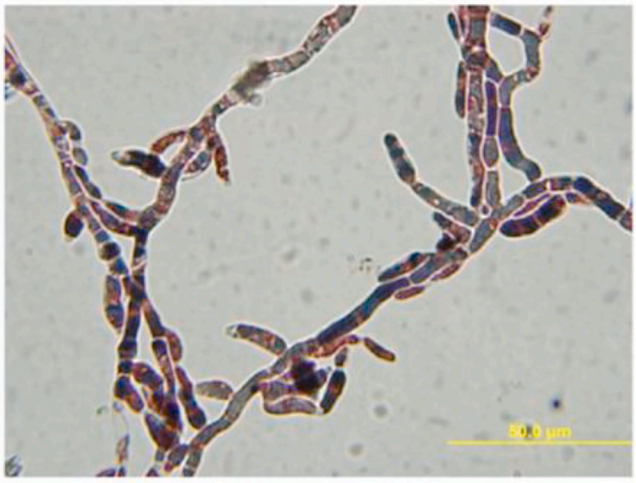

Fig. 3. Mycelium stained by Sudan Black B under an optical microscope.[78]

4.2. Screening and differentiation of oleaginous from non-oleaginous fungi

The identification and selecting of oleaginous fungi from non-oleaginous ones are an important topic for many researchers to conduct laboratory experiments. Through the experiments carried out by researchers, a part of them relied on choosing the fastest-growing species from others and accumulation of lipids in their cells, taking advantage of the ability of these species to produce higher biomass.[77]

However, this type of test may not be a reliable indicator of oleaginous potential. The use of dyes to distinguish oleaginous fungi from non-oleaginous fungi is an effective way to detect intracellular lipids and

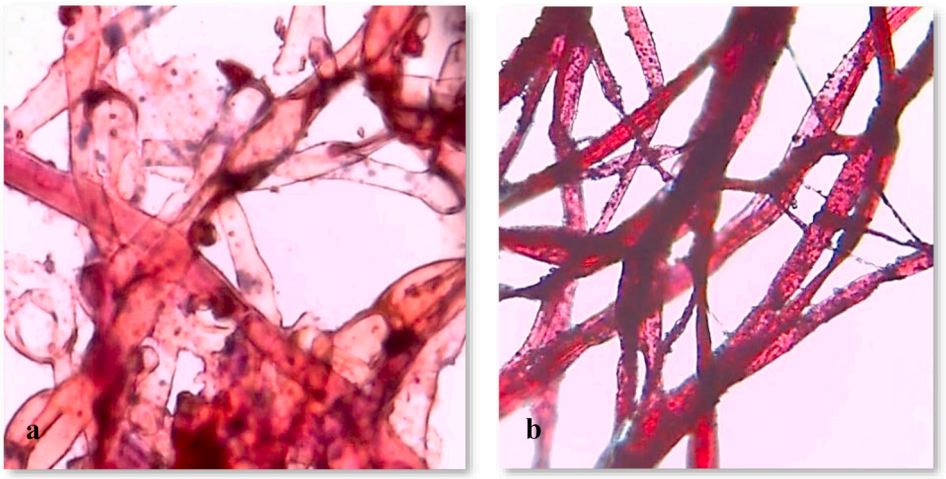

Fig. 4. Intracellular lipids stained with Sudan Black B for (a: *Aspergillus flavus*, b: *Lichtheimia corymbifera*).[88]

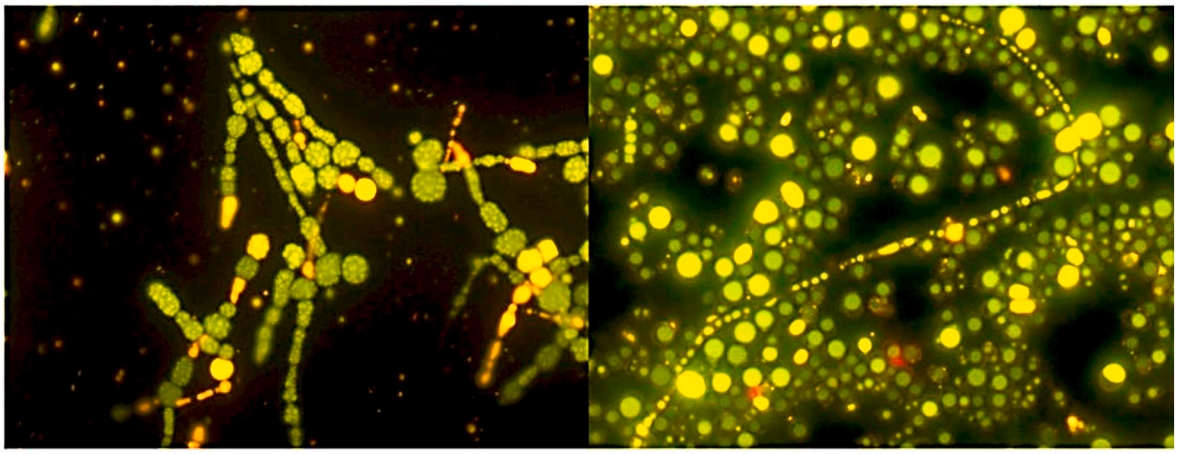

Fig. 5. Yeast and fungal cells that contain the lipids as lipid droplets that have been stained by the Nile red fluorescent stain.[79]

thus provide a good indication of these species' ability to synthesize and accumulate lipid bodies within their cells. Where Sudan black B and Nile red pigments can be used to detect the presence of neutral lipids in yeasts and fungi under a microscope (Figs. 3–5), these pigments indicate the accumulation of fungi for these types of lipids.[78,79]

Microscopic techniques have been used to detect lipid droplets deposited as intracellular lipids, including staining with fluorescent dyes,[80] which requires Nile red stain. While, Sudan Black B technique is more straightforward, rapid, and available, which is why numerous studies used Sudan Back B stain to determine lipids accumulation of different species of fungi.[78,81]

In addition to the use of dyes, a group of researchers used enzymatic methods to separate oleaginous fungi from non-oleaginous fungi. Some of them sought to study this method on a wide range of filamentous fungi and yeasts by restoring the lipolysis process using the lipase enzyme (Fig. 6), where the culture medium is enriched with oils, and cultured for a wide group of fungi. Finally, the species with significant lipid-degrading capacity can be an indicator of the ability of these groups to produce the enzyme lipase. Therefore, selecting these species as oleaginous involves performing lipase production tests, which are inexpensive methods used to assess the fungi's ability to accumulate lipids (Fig. 7).[15,82]

Researchers are always seeking to find methods that make the process of screening oleaginous fungi from non-oleaginous ones simpler, faster, and more economical. Along with the techniques described above, some scientists have used fluorescent dyes such as Rhodamine B. This dye binds directly to lipids, so it does not require additional steps. However, it does require the need to grow fungi in dyed media, through which oleaginous fungi can be detected from non-oleaginous ones.[82]

Oppositely, some academics have developed innovative methods for detecting triacylglycerols in microorganisms. The developed methods are characterized by being fast, direct, and highly reliable. These methods based on live cell imaging technique. Despite its advantages, these methods require special dyes such as Lipid TOX™ Green dye. They also require culture on plates and a fluorescent microscope.[83]

Despite the diversity of methods for screening oleaginous and non-oleaginous fungi, conventional methods remain the most common. Each method has its advantages and disadvantages, and the best methods are the fastest, most reliable, and best in quality. Therefore, we note that there is an accelerating pace of finding a more acceptable method through the development of numerous qualitative and quantitative screening methods. Among the most common quantitative methods are Kimura et al.[80] and Sitepu et al.[84] methods. However, they require liquid cultures to grow the fungi, in addition to fluorescent dyes and microscopic techniques. This can be a challenge for many laboratories with limited resources. In general, colorimetric detection methods are less expensive than fluorescence methods. They are also more common, time-consuming, and less safe because they use substances that can be more toxic.[82] The most common examples of these methods are the use of Sudan Black B stain 85 and Oil Red O.[86] The use of a

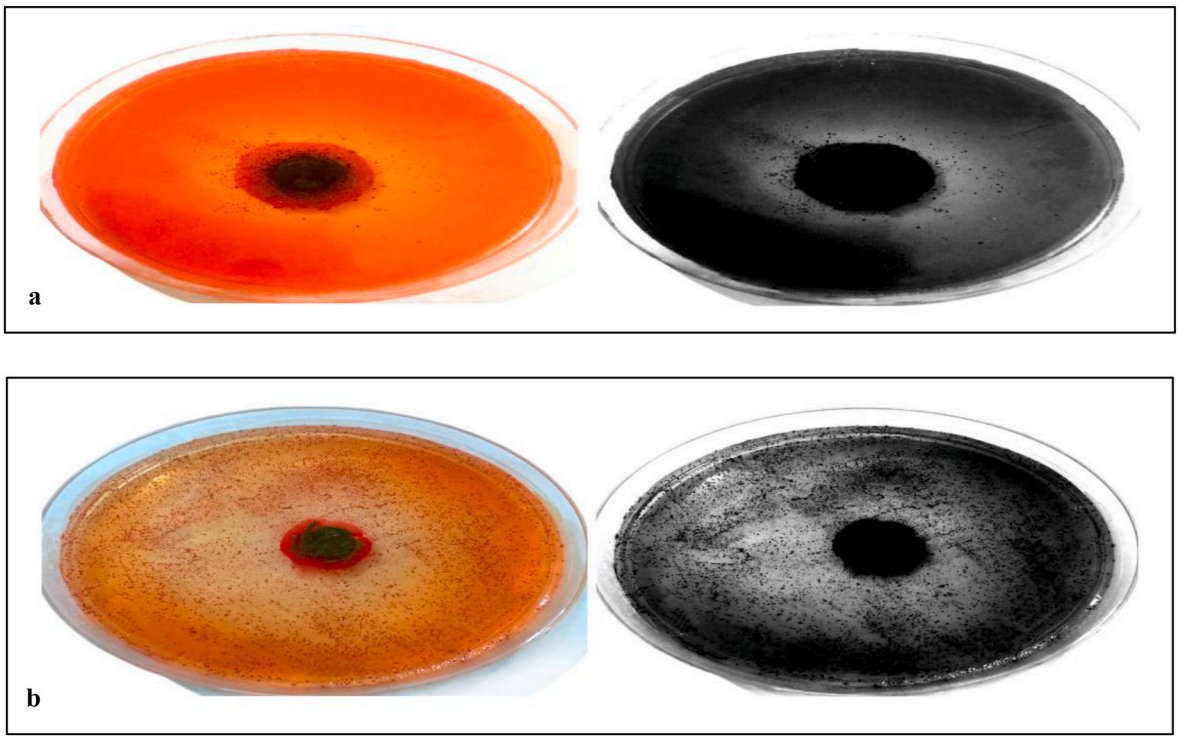

Fig. 6. Lipolytic activity is indicated by a clear zone of precipitation surrounding the colonies for (a: *Aspergillus niger*, b: *Aspergillus flavus*).[89]

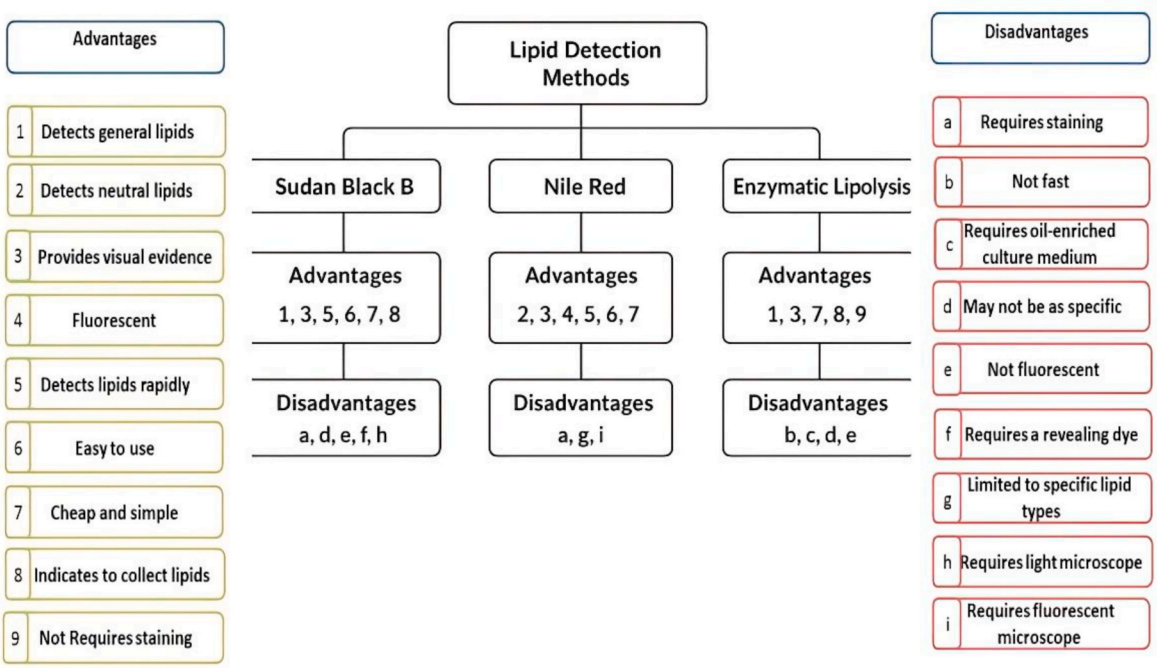

Fig. 7. Comparison of methods for screening oleaginous fungi.

BODIPY probe has also been reported to detect oleaginous yeasts.[87]

4.3. Biomass production

As reported by Gong et al.,[90] submerged fermentation (SMF) has been widely used for fungi because it is easy to control and because the concentration, temperature, pH, and rates of mass and heat transport occur at a faster rate. To produce lipids and grow, the majority of fungi require moderate amounts of oxygen; as a result, oxygen is typically transferred to a liquid medium by agitation or orbital shaking.[91] The submerged fermentation offers several advantages over conventional solid-state fermentation, such as easier control of processing parameters, the ability to handle larger volumes, shorter fermentation durations, lower labor requirements, and greater ease of scale-up.[92] Consequently, this method of cultivation is quickly taking over as the main industrial biotechnology for fungal production.

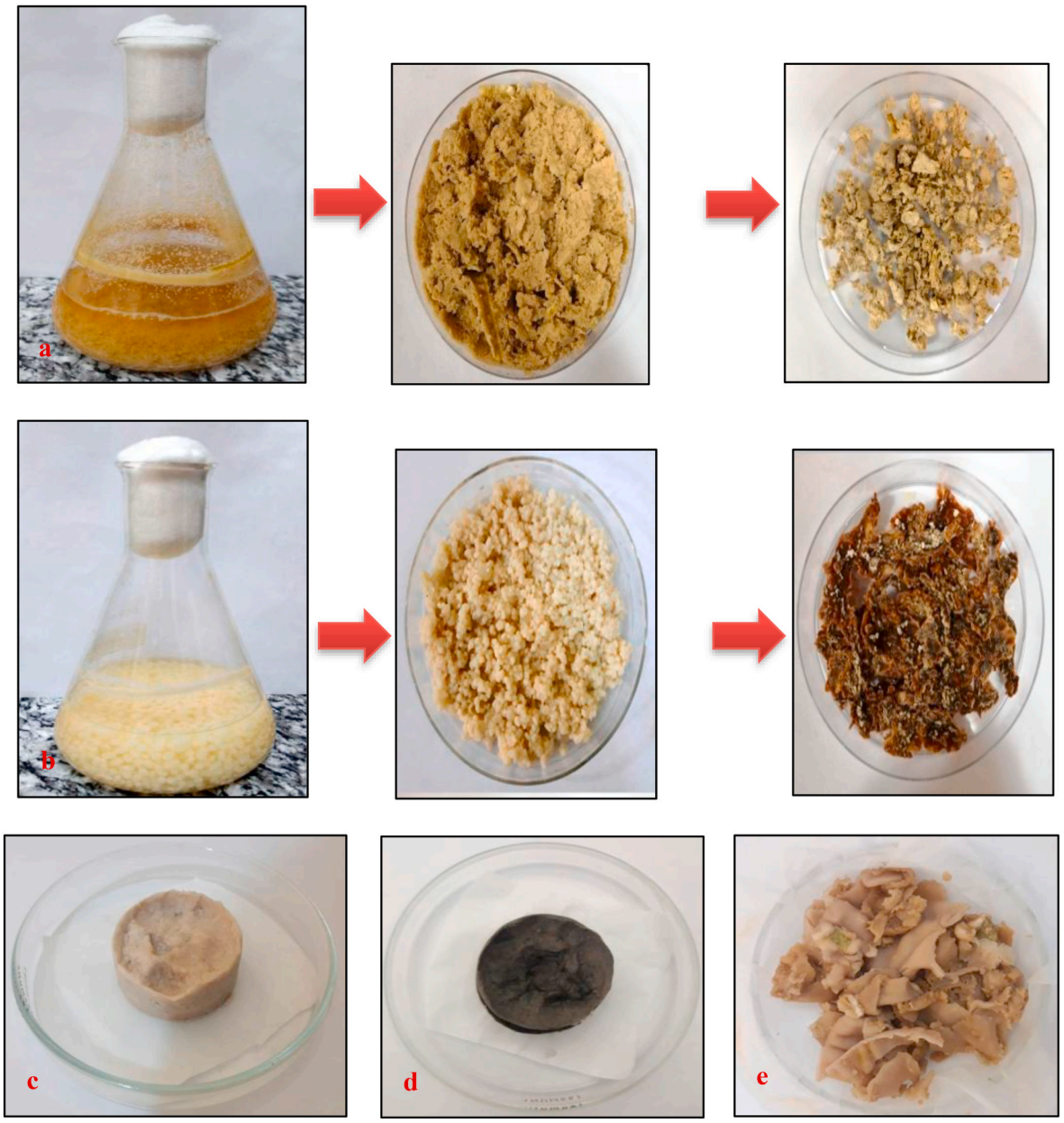

Fig. 8. Biomass production in natural media (Cheese whey) (a: *Aspergillus terreus*, b-c: *Lichtheimia corymbifera*, d: *Aspergillus niger*, e: *Aspergillus fumigatus*).[88]

However, the submerged fermentation of filamentous fungi presents specific challenges, such as the high viscosity of the fermentation medium caused by fungal morphology, which can negatively affect the yield and productivity of target products in bioreactors. During submerged cultivation in bioreactors, mycelial filamentous fungi can adopt one of three morphological forms: dispersed mycelia, clumped aggregates, or mycelial pellets, which may be either rough or smooth in structure.[93,94] Additionally, an irregular, block-like mycelial morphology is often observed during the submerged fermentation of filamentous fungi in conical flasks, a characteristic commonly seen among various species. Environmental factors and innate molecular or genetic biology can significantly and intricately influence fungus morphology.[95]

Biomass production is governed by several factors, including optimal temperature, pH levels,[96] aeration,[97] and type of C-source.[98] The C-source of glucose plays a significant impact on the productions of this biomass.[99] Glucose exemplifies the source of carbon that is most widely utilized for fungi growth. On the other hand, utilizing inexpensive and readily available natural media is essential for producing large fungal biomass (Fig. 8), which is crucial for generating substantial amounts of lipids.[100] A study in Basrah University revealed that cheese whey medium, and industrial date molasses waste were good alternative media for fungal growth. The produced biomass reached 40 g/l for *Lichtheimia corymbifera* and 16 g/l for *Aspergillus terreus*, also the same isolates produced varying amounts of biomass when wheat straw was used as a substrate and industrial date molasses wastes as an alternative source that represents C-source. In addition to that, the isolates that have been cultivated in a N limiting fermentation medium by using glucose as a source of C all oleaginous isolates gave good values of biomass *Cladosporium ramotenellum* yielded 16.08 g/l, while *Lichtheimia corymbifera* yielded 12.80 g/l,[16] and *Aspergillus ochraceus* was yielded about 20.80 g/l, whereas *Aspergillus niger* yielded 14.70 g/l.[101]

Cheese whey is considered a very cheap and available medium, as it is produced from cheese manufacture waste. The amount of biomass produced may be attributed to the rich content of proteins, sugars, and minerals, as cheese whey typically contains 10–20% protein and

Table 1
Dry weight (DW) of biomass produced based on different source of carbon.

No.	Species/strains	Biomass (DW)	Unit	Time	C-source	Production conditions: Concentration of C-source (Co), Optimal temperature (Ot), (pH), Agitation (Ag), Supplements (Su), Limitations (Li)	Ref.
1	*Aspergillus terreus*	16	g/l	8 d	Industrial date molasses wastes	Co = 150 g/l,Ot = 30 °C, pH = 6.5, Ag = 120 rpm Su = Not Applicable (NA), Li= NA	16
2	*Lichtheimia corymbifera*	40	g/l	8 d	Industrial date molasses wastes	Co = 150 g/l,Ot = 30 °C, pH = 6.5, Ag = 120 rpm, Su = NA, Li= NA	16
3	*Cladosporium ramotenellum*	16.08	g/l	8 d	Glucose	Co = 40 g/l, Ot = 30 °C, pH = 6.5, Ag = 120 rpm, Su = yeast extract, Li=N-source	16
4	*Aspergillus ochraceus*	20.80	g/l	8 d	Glucose	Co = 40 g/l, Ot = 30 °C, pH = 6.5, Ag = 120 rpm Su = yeast extract, Li=N-source	101
5	*Aspergillus niger*	14.70	g/l	8 d	Glucose	Co = 40 g/l, Ot = 30 °C, pH = 6.5, Ag = 120 rpm Su = yeast extract, Li=N-source	101
6	*Aspergillus fumigatus*	4.54	g/l	14 d	Almonds husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA, Su = NA, Li= NA	104
7	*Aspergillus terreus*	4.64	g/l	14 d	Almonds husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA Su = NA, Li= NA	104
8	*Aspergillus fumigatus*	3.82	g/l	14 d	Peanut husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA, Su = NA, Li= NA	104
9	*Aspergillus terreus*	2.24	g/l	14 d	Peanut husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA, Su = NA, Li= NA	104
10	*Aspergillus fumigatus*	2.22	g/l	14 d	Sunflower husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA, Su = NA, Li= NA	104
11	*Aspergillus terreus*	1.74	g/l	14 d	Sunflower husks	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag= NA, Su = NA, Li= NA	104
12	*Penicillium chrysosporium*	17.40	g/l	7 d	Banana peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
13	*Penicillium chrysosporium*	15.00	g/l	7 d	Pineapple peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
14	*Penicillium chrysosporium*	15.00	g/l	7 d	Papaya peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
15	*Panus tigrinus (M609RQY)*	23.60	g/l	7 d	Banana peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
16	*Panus tigrinus (M609RQY)*	15.40	g/l	7 d	Pineapple peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
17	*Panus tigrinus (M609RQY)*	13.20	g/l	7 d	Papaya peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
18	*Panus tigrinus (RO209RQY)*	24.40	g/l	7 d	Banana peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
19	*Panus tigrinus (RO209RQY)*	19.60	g/l	7 d	Pineapple peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
20	*Panus tigrinus (RO209RQY)*	16.80	g/l	7 d	Papaya peel	Co = 4 g/l, Ot = 30 °C, pH= Not Determined (ND) Ag = 150 rpm, Su = Mineral salts, Li= NA	109
21	*Rhizomucor* sp. *CCUG 61146*	65 – 140 per g glucose	mg/g	5 d	Glucose	Co = 50 g/l, Ot = 30 °C, pH= ND, Ag = 125 rpm Su = NA, Li= NA	110
22	*Rhizomucor* sp. *CCUG 61147*	65 – 140 per g glucose	mg/g	5 d	Glucose	Co = 50 g/l, Ot = 30 °C, pH= ND, Ag = 125 rpm, Su = NA, Li= NA	110
23	*Trichoderma reesei*	6.18	g/l	7 d	Lactose	Co = 10 g/l, Ot = 30 °C, pH = 6, Ag = 150 rpm, Su = NA, Li= NA	111
24	*Trichoderma reesei*	4.18	g/l	7 d	Lactose	Co = 10 g/l, Ot = 30 °C, pH = 6, Ag = 150 rpm, Su = NA, Li= NA	111
25	*Trichoderma harzianum T7*	72.75	mg/ 30 ml	10 d	Sorbitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
26	*Trichoderma harzianum T7*	145	mg/ 30 ml	10 d	Mannitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
27	*Trichoderma harzianum T7*	151.5	mg/ 30 ml	10 d	Fructose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
28	*Trichoderma harzianum T7*	184	mg/ 30 ml	10 d	Arabinose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
29	*Trichoderma harzianum T8*	47	mg/ 30 ml	10 d	Lactose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
30	*Trichoderma harzianum T8*	147.25	mg/ 30 ml	10 d	Sorbitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
31	*Trichoderma harzianum T8*	123	mg/ 30 ml	10 d	Mannitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
32	*Trichoderma harzianum T8*	112.75	mg/ 30 ml	10 d	Fructose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
33	*Trichoderma harzianum T14*	100	mg/ 30 ml	10 d	Sorbitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
34	*Trichoderma harzianum T14*	160.75	mg/ 30 ml	10 d	Mannitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
35	*Trichoderma harzianum T14*	174.5	mg/ 30 ml	10 d	Fructose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
36	*Trichoderma harzianum T14*	157.5	mg/ 30 ml	10 d	Arabinose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
37	*Arthrobotrys oligospora*	215.5	mg/ 30 ml	10 d	Sorbitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
38	*Arthrobotrys oligospora*	51.5	mg/ 30 ml	10 d	Mannitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
39	*Arthrobotrys oligospora*	122.5	mg/ 30 ml	10 d	Fructose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
40	*Arthrobotrys oligospora*	223.25	mg/ 30 ml	10 d	Arabinose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112

(*continued on next page*)

Table 1 (*continued*)

No.	Species/strains	Biomass (DW)	Unit	Time	C-source	Production conditions: Concentration of C-source (Co), Optimal temperature (Ot), (pH), Agitation (Ag), Supplements (Su), Limitations (Li)	Ref.
41	*Pochonia chlamydosporia*	235	mg/ 30 ml	10 d	Sorbitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
42	*Pochonia chlamydosporia*	188.75	mg/ 30 ml	10 d	Mannitol	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
43	*Pochonia chlamydosporia*	278	mg/ 30 ml	10 d	Fructose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
44	*Pochonia chlamydosporia*	213.25	mg/ 30 ml	10 d	Arabinose	Co = 21.053 g/l, Ot = 10-30 °C, pH = 4-8, Ag= NA, Su = Potassium nitrate as N-source, Dextrose, Li= NA	112
45	*Aspergillus carneus*	0.160	g/50 ml	7 d	Glucose	Co = 15 g/l, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Yeast 2.5 g/l, Li= NA	113
46	*Umbelopsis vinacea*	22	g/l	7 d	Glucose	Co = 80 g/l, Ot = 25 °C, pH = 6, Ag = 400 rpm, Su = Mineral salts, Li=N-source	72
47	*Rhizopus arrhizus 36017*	~2-2.5	g/l	~3 d	Waste water of potato	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
48	*Rhizopus arrhizus 36017*	~2-2.5	g/l	~3 d	Waste water of corn	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
49	*Rhizopus arrhizus 36017*	~2-2.5	g/l	~3 d	Waste water of wheat	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
50	*Rhizopus arrhizus 36017*	~2-2.5	g/l	~3 d	Waste water of pineapple	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
51	*Rhizopus oryzae* 2062	~4.5-5	g/l	~3 d	Waste water of potato	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
52	*Rhizopus oryzae 2062*	~4.5-5	g/l	~3 d	Waste water of corn	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
53	*Rhizopus oryzae 2062*	~4.5-5	g/l	~3 d	Waste water of wheat	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
54	*Rhizopus oryzae 2062*	~4.5-5	g/l	~3 d	Waste water of pineapple	Co = 100 ml, Ot = 30 °C, pH= ND, Ag = 150 rpm, Su = Peptone and yeast extract, Li= NA	114
55	*Mucor rouxii 386*	~6	g/l	8 d	Glucose	Co = 30 g/l, Ot = 28 °C, pH = 5.5, Ag = 250 rpm, Su = yeast extract, Li= NA	115
56	*Mucor* sp.*1b*	~5	g/l	8 d	Glucose	Co = 30 g/l, Ot = 28 °C, pH = 5.5, Ag = 250 rpm, Su = yeast extract, Li= NA	115

60–70% lactose.[102,103] In the study of biomass production using natural media that included medium almond, sunflower, and peanut husks, biomass (dry weight) results have shown that a higher value of the bio-mass has been obtained with the use of almonds husks (4.64, 4.54, 3.04, 3.30, 1.02, 1.00 g/l) for 6 oleaginous fungal isolates such as *Aspergillus terreus*, *Aspergillus fumigatus*, *Fusarium graminearum*, *Trichoderma harzianum*, *Penicillium* spp., and *Aspergillus flavus* respectively, followed by the peanut husks and the sunflower husk[104] (Table 1). The process of determining the biomass of fungi can be done by the conventional method of drying, which is considered the most common method. The biomass can also be determined by other methods (Table 3).

In general, variations in biomass production among isolates selected under the same conditions could be attributed the unique physiological and metabolic traits of each species. The reduction of production costs related to the fermentation process remains of fundamental significance due to its more significant role in producing inexpensive products and increasing economic viability, which is the aim of the scientists,[105] in addition to improving biomass products to make them more sustainable using strategies that help convert biomass into high-value materials[106] or energy-dense fuels.[107] One of the most important strategies used to achieve these goals is the use of catalysis processes, whether photodynamic, thermal, electrical, or enzymatic, and the associated synergistic processes.[108]

4.4. Lipids production and FAMEs profile

Several researchers have been studying various fungal groups in recent years with the intention of identifying the best fungal species capable of accumulating lipids within their cells, and to determine the best medium for producing high-lipid biomass that can be used to produce biodiesel through a series of processes. In a study conducted in Iraq to determine the possibility of producing biodiesel from fungal lipids by the use of isolates that were isolated from different environments in Baghdad city (soil and polluted water), it was found that the lipids percentage were (33.94, 46.55, 12.91, 52.44, 20.13, 50, and 36.17 %) for *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium graminearum*, *Trichoderma harizanum*, and *Penicllum* spp. respectively.[15]

Vicente et al.[116] and Khot et al.[117] noted that *Mucor circinelloides* was able to accumulate 19.9 % lipids in its biomass, while another study found that the same species accumulated 23.3 % lipids of its biomass.[117] Magdum et al.[118] also studied the ability of the same species to accumulate lipids, which were estimated at 20.69 %. Furthermore, they found that the accumulation of lipids for *Trichosporon cutaneum*, *Lipomyces starkeyi*, *Cunninghamella echinula*, *Mucar ranmanianna*, *Mortierella isabellina*, *Cunninghamella ehinulata*, and *Lipomyces starkeyi* were (46.3, 52.6, 37.6, 50, 50, 49, and 50.8 %) respectively.[119,120] Karatay and Dönmez[121] studied accumulated lipids for *Trametes versicolor*, *Aspergillus versicolor*, *Rhizopus oryzae*, and *Rhizopus arrhizus*, which were (7.8, 9.1, 8.1, and 7.3 %) respectively. At the same time, Neema and Kumari[100] studied the accumulation of lipids for *Zygowilliopsis californica* and *Galactomyces geotrichum,* which were (20 and 17 %) respectively.

Li et al.[122] studied the ability of isolates from hot springs, wetlands, sandy, saline, and agricultural lands of the Qinghai Plateau to accumulate lipids within their cells and the potential for biodiesel production. *Gibberella fujikuroi* and *Penicillium decumbens* were accumulate (14% and 8 %) of lipids respectively, when growing on carboxymethyl cellulose as fermentation medium. While it reached 30 % for *Aspergillus fumigatus* when growing on xylose medium as carbon source.

Jape et al.[123] have determined the ability of some marine fungi for accumulating lipid within their cells and estimating lipids ratios. The percentage was 24.6% for *Candida* spp., while for *Rhodotorula mucilaginosa* and *Candida tropicalis* were 18.2 % and 16.8 % respectively.

Marchand et al.[124] found that *Mucor* spp. can accumulate lipids within its biomass by up to 23 %, when growing on glucose, while, it is not exceeding 13 %, when using glycerol as a carbon source. On top of that, the ability of *Fusarium verticillioides* to accumulate lipids in their cells' using sucrose was 39 %, while the proportion in maltose was 25 % and in xylose 30 %.[78]

Furthermore, some of the oleaginous isolates, when cultured at

Table 2
FAMEs profile for some fungal oleaginous isolates.

No.	Species/strains	FAMEs profile															C-source	Production conditions: Concentration of C-source (Co), Optimal temperature (Ot), (pH), Agitation (Ag), Supplements (Su), Limitations (Li)	Ref.
		Unit	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0			
1	*Mucor circinelloides*	%	ND	0.8	NA	20	2.3	2	37	14.3	18.5	0.2	0.59	0.5	ND	1.2	ND	Co= Not Determined (ND), Ot = 26 °C, pH = 4.5, Ag= Not Applicable (NA), Su = Mineral salts, white, light (4.8W m^{-2}), Li=NA.	116
2	*Geomyces pannorum*	%	ND	1	ND	17	0.3	8.3	27	38	9	ND	ND	ND	ND	ND	Dextrose	Co = 50 ml, Ot = 22 °C, pH= ND, Ag=NA, rpm = NA, Su = NA, Li=NA.	135
3	*Rhizopus* spp.	%	29.35	ND	ND	35.30	ND	12.79	14.62	7.38	ND	ND	ND	ND	ND	ND	Glucose + Xylose	Co = 60 g/l, Ot = 28 °C, pH= ND, Ag = 200 rpm, Su = Mineral salts, Yeast extract, Li=NA.	136
4	*Aspergillus fumigatus O1*	%	ND	ND	ND	ND	1.005	ND	ND	32.745	ND	ND	ND	ND	ND	ND	Glucose	Co = 50 g/l, Ot = 27 °C, pH= ND, Ag = 120 rpm, Su = Mineral salts, Yeast extract, Li=NA.	137
5	*Mucor hiemalis URM 4144*	%	8.9	5.1	ND	44.1	ND	3.8	24.1	7.8	5.5	ND	ND	ND	ND	ND	Glucose	Co = 20 g/l, Ot = 26 °C, pH = 4.5, Ag = 250 rpm, Su = Mineral salts, Yeast extract, Li=NA.	21
6	*Penicillium citrinum URM 4216*	%	5.7	3.6	ND	37.1	ND	5.8	31.1	9.3	6.1	ND	ND	ND	ND	ND	Glucose	Co = 20 g/l, Ot = 26 °C, pH = 4.5, Ag = 250 rpm, Su = Mineral salts, Yeast extract, Li=NA.	21
7	*Fusarium solani RAS18*	%	ND	ND	ND	ND	17.89	11.12	11.81	56.81	ND	ND	ND	ND	ND	ND	Glucose	Co = 30 g/l, Ot = 28 °C, pH = 6.5, Ag = 250 rpm, Su = Mineral salts, Yeast extract, Li=N-course.	138
8	*Aspergillus candidus IBBG4*	%	ND	ND	ND	22.9	ND	23.7	26.9	17.8	ND	ND	ND	0.5	ND	ND	Agro-industrial residues	Co = 10 g/l, Ot = 30 °C, pH= ND, Ag = 120 rpm, Su = Mineral salts, Li= NA	139
9	*Aspergillus terreus*	%	2.84	14.64	ND	10.92	ND	15.91	18.51	13.20	ND	ND	ND	ND	ND	ND	Glucose	Co = 50 g/l, Ot = 30 °C, pH = 6, Ag = 150 NA, Su = Mineral salts, Yeast extract, Li= ND	140
10	*Serpula lacrymans*	%	ND	ND	ND	19-29	ND	ND	10-13	16-50	ND	ND	ND	ND	ND	ND	wheat straw	Co = 10 g, Ot = 20 °C, pH= ND, Ag= NA, Su = NA, Li= NA	141
11	*Aspergillus terreus IBB M1*	%	ND	0.3	ND	20.1	0.4	23.6	30.1	22.3	0.4	0.8	0.1	0.4	ND	0.7	Glucose	Co = 30 g/l, Ot = 30 °C, pH = 5.5, Ag = 120 rpm, Su = Mineral salts, Yeast extract, Li=N-course	117
12	*Rhodotorula mucilaginosa IIPL32*	%	0.36	0.78	ND	22.56	13.85	1.03	40.41	17.95	0.32	ND	ND	ND	ND	ND	Xylose	Co = 40 g/l, Ot = 32 °C, pH= ND, Ag = NA, Su = Mineral salts, Yeast extract, Li=N-course	142
13	*Aspergillus terreus*	%	ND	ND	ND	34	ND	ND	25	ND	ND	ND	ND	ND	ND	ND	Fruit of date	Co = 20 g/l, Ot = 30 °C, pH = 6, Ag rpm = ND, Su = NA, Li= NA	15
14	*Trametes versicolor*	%	ND	≈4	ND	≈25	ND	≈5	≈46	≈7	≈5	ND	≈1	ND	≈1	≈3	Malt extract	Co = 20 g/l, Ot = 25 °C, pH = 4.5, Ag = 150 rpm, Su = NA, Li= NA	143
15	*Ganoderma lucidum*	%	ND	≈8	ND	≈27	ND	≈7	≈38	≈6	≈3	≈0.5	≈0.5	ND	≈0.5	ND	Malt extract	Co = 20 g/l, Ot = 25 °C, pH = 4.5, Ag = 150 rpm, Su = NA, Li= NA	143
16	*Sarocladium kiliense ADH17*	%	ND	ND	ND	35.134	2.358	5.936	51.089	ND	4.611	ND	ND	ND	ND	ND	Glucose	Co = 4 g/l, Ot = 28 °C, pH = 7, Ag = 120 rpm, Su = Peptone, Yeast extract, Li= NA	144
17	*Aspergillus carneus OQ275240*	%	ND	ND	ND	6.16	ND	43.81	9.53	0.95	ND	0.53	ND	ND	ND	ND	Glucose	Co = 30 g/l, Ot = 30 °C, pH = 6, Ag = 200 rpm, Su = Mineral salts, Yeast extract, Li= NA	113
18	*Aspergillus niger SF2*	%	ND	ND	ND	22.41	1.46	ND	34.82	ND	ND	ND	ND	ND	ND	ND	Dextrose	Co= ND, Ot = 28 °C, pH = 5.5, Ag = 100 rpm, Su = NA, Li= NA	145
19	*Mucor fragilis AFT7-4*	%	0.56	ND	ND	19.41	2.28	6.69	66.77	ND	ND	0.89	0.57	0.53	ND	ND	Glucose	Co = 100 g/l, Ot = 28 °C, pH = 7-7.5, Ag = 180 rpm, Su = Mineral salts, Yeast extract, Li= NA	13, 146

(*continued on next page*)

Table 2 (*continued*)

No.	Species/strains	FAMEs profile												C-source	Production conditions: Concentration of C-source (Co), Optimal temperature (Ot), (pH), Agitation (Ag), Supplements (Su), Limitations (Li)	Ref.
		Unit	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0			
20	*Mucor circinelloides URM 4182*	%	11.8	7.2	ND	28.9	ND	2.7	21.7	10.8	13.9	ND	ND	ND	ND	ND	Glycerol	Co = 40 g/l, Ot = 26 °C, pH = 4.5, Ag = 250 rpm, Su = ND, Li=N-course.	132
21	*Mucor circinelloides URM 4182*	%	1.9	3.5	ND	35.2	ND	4.8	28.7	9.7	14.5	ND	ND	ND	ND	ND	Xylose	Co = 40 g/l, Ot = 26 °C, pH = 4.5, Ag = 250 rpm, Su = ND, Li=N-course.	132
22	*Aspergillus* sp. *EM2018*	%	0.4	0.5	ND	33.3	1.4	0.7	14.1	24.4	1.1	ND	ND	1.5	ND	15.2	Potato dextrose	Co = 50 g/l, Ot = 30 °C, pH = 5, Ag= NA, Su = ND, Li= ND.	147
23	*Fusarium oxysporum NRC2017*	%	ND	ND	ND	2.03	ND	0.33	11.13	5.57	3.04	ND	ND	ND	16.12	ND	Glucose	Co = 5 g/l mg/g, Ot = 30 °C, pH = 5, Ag = 80 rpm, Su = Mineral salts, Yeast extract, Li=N-course.	148

Lauric acid = 12:0, myristic acid = 14:0, myristoleic acid = 14:1, pentadecanoic acid = 15:0, palmitic acid = 16:0, palmitoleic acid = 16:1, stearic acid = 18:0, oleic acid = 18:1, linoleic acid = 18:2, linolenic acid = 18:3, arachidic acid = 20:0, gadoleic acid = 20:1, behenic acid = 22:0, erucic acid = 22:1, lignoceric acid = 24:0, nervonic acid = 24:1.

cheese whey medium as an alternative natural medium, showed a variation in lipids accumulation, e.g., the lipids content of *Lichtheimia corymbifera* being was about 13.2 g/l (33 %) of the total biomass product, followed by *Aspergillus terreus* at about 38 %, *Penicillium chrysogenum* at about 4.71 g/l (38 %), and *Aspergillus ochraceus* at about 19 %. Contrariwise, the ratio of lipids accumulation varies when the same fungal isolates cultivated in industrial date molasses waste and wheat straw media.[88] Utilizing organic waste to produce large quantities of lipids (single-cell oil) is highly economically feasible to produce biodiesel from extremely low-cost sources, environment friendly, and available substrate. The use of cheese whey, industrial date molasses waste, and wheat straw as fermentation media played a role in accumulating good amounts of TAG, which is the economic goal for producing cheap biodiesel.[88] The use of wastes as substrates to yield lipids for biofuels and the use of cheap carbon sources have recently been investigated for biodiesel or myco-diesel production using natural media.[125] The production of biodiesel based on fungi is expensive and does not meet industrial objectives, even if it is clean and safe compared to fossil fuels.[126] So, the decrease of production costs related to the fermentation process remains essentially crucial for increasing economic viability.[127]

By using natural media many, studies have shown that fungi accumulated both saturated and unsaturated fatty acids which are key components of biodiesel[104] (Table 2). These fatty acids include hexadecanoic acid, octadecanoic acid, tetradecanoic acid, octadecenoic acid, hexadecenoic acid, octadecadienoic acid, and octadecatrienoic acid.[104,128] In plants, the type of fatty acids is important and essential for determining fuel efficiency and the unsaturated fatty acids, which are among the best fats to improve biodiesel efficiency. The majority of fatty acids extracted from oleaginous fungi (hexadecenoic acid, hexadecanoic acid, octadecenoic acid, octadecanoic acid, and octadecadienoic) are similar to the fatty acids extracted from vegetable oil[129,130] (Fig. 9). These fatty acids are considered the best, most suitable, and available feedstocks for biodiesel production.[131] The FAMEs profile which are converted from microbial lipid determined by using several methods. Gas chromatography-mass spectrometry is widely used for FAME profile determination due to its high accuracy, while the conventional drying method is more widely used for biomass determination[132] (Table 3).

Although the study focused on the production of fungal microbial lipids using diverse sources, achieving economic feasibility can only be achieved using very inexpensive media, which is crucial to achieving economic leadership and potential industrial applications. Achieving these goals depends on several factors, including the type of strain and its ability to accumulate lipids, the type of those lipids, the type and cost of the substrate, and subsequent treatments that can increase or decrease production costs. Achieving a high capacity to accumulate fatty acids suitable for biodiesel production is not definitive for achieving economic feasibility unless it is accompanied by low-cost fermentation processes that ensure high and optimal biomass production. Therefore, studies are ambitiously seeking to exploit waste and reduce costs in a way that ensures or significantly helps reduce production costs.[133] So, the production goal remains linked to the specific improvement method for producing microbial lipids at the extracellular or intracellular level.[134]

5. Future perspective and industrial challenges

Biotechnology researchers are increasingly seeking to use microorganisms, including fungi, to achieve effective biodiesel production as an alternative to fossil fuels. This is done by inducing fungi to accumulate lipids and exploit their unique converted FAMEs, which are very similar to converted FAMEs from vegetable lipids, which have already been produced commercially.[155] High-efficiency biodiesel production depends on the types of converted FAMEs, which can make it more acceptable and competitive with other sources,[156] despite the need to enhance the quantities of unique FAMEs, which would significantly improve biodiesel.[157]

Table 3
Description of the methods used Quantitative/Qualitative for Biomass and lipid detection.

Feature	Method	Explanation	Benefit	Limitations	Reference
Biomass-Quantitative	Dry Weight	It involves collecting the biomass, washing it, and then thermally drying it.	It is considered a more standard and accurate method.	Requires a larger biomass and a longer time	138,149
		It involves collecting the biomass, washing it, and then freeze-drying it.	It is considered a more standard and accurate method.	Requires a larger biomass and a longer time	130,150
	Optical Density	It involves measuring absorbance based on biomass concentration.	It is invested in monitoring growth and it is a fast method.	This method must include calibration and is inaccurate when the density increases.	151,152
	Weight after filtration	It includes filtration of the fungal culture and retention of the biomass.	Less expensive and easier way	It is not considered a typical method for accurate determination of biomass.	153
Quantitative FAME profiling	Gas Chromatography with Flame Ionization Detector	Gives a quantitative estimate of FAMEs.	Accurate in quantitative detection	Expensive	130,150
	Gas chromatography mass spectrometry	Using this technique, FAMEs can be identified based on their retention time and mass spectra.	Widely used and high precision	Requires expensive tools	138,152
Qualitative lipid characterization	Fourier Transform Infrared Spectroscopy	Lipids functional groups can be detected quickly.	The sample can be prepared quickly and with minimal effort.	Less accurate	153
	High Performance Liquid Chromatography	Detection of fatty acid moieties with lack of extensive derivatization.	The technology enables the separation of complex lipid mixtures well.	Semi-quantitative method	154
	Nuclear Magnetic Resonance	Lipid functional groups can be detected	Provides details of the FAME structure.	Expensive	153

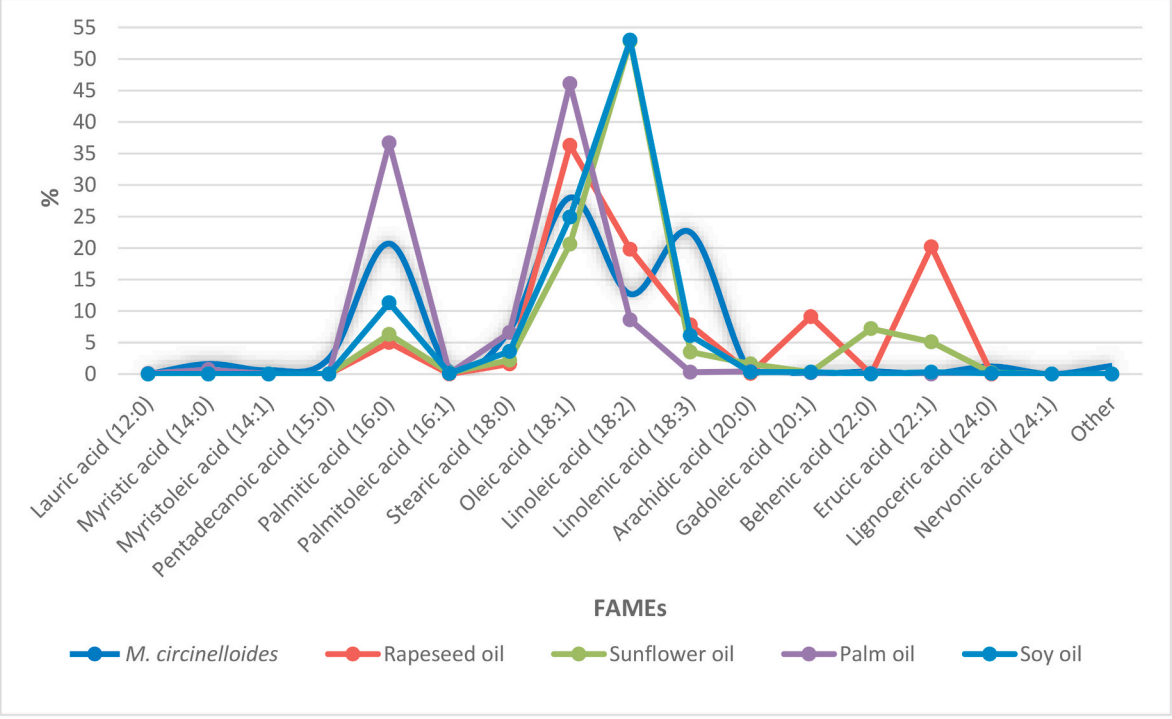

Fig. 9. Comparison of FAMEs profile in *Mucor circinelloides* and some vegetable oils.[130]

Scientists have used several techniques that can improve the quantity and quality of obtained FAMEs, including the use of gene modification or deletion methods, such as acetyl-CoA genes.[158] Some academics have used chemical agents such as sodium azide and ethidium bromide, and physical agents such as gamma rays, to quantitatively and qualitatively improve FAMEs.[159] These agents have effectively achieved their goal of increasing FAMEs, represented by the C16 to C18 types, which are considered the most efficient in producing high-efficiency biodiesel.[160] In addition to balancing the production of these quantities using very inexpensive media,[159] it can be said that the use of media derived from industrial and agricultural waste achieves the biological-environmental goals of improving their treatment processes and achieving optimal efficiency in producing high-efficiency FAMEs.

FAMEs types play a key role in improving biodiesel properties. For instance, the good chemical and physical properties of biodiesel determine its suitability for use in internal combustion engines without any modifications, as these properties achieve an ideal cetane number for biodiesel.[161] The American Society for Testing and Materials (ASTM)

prefers a cetane number of at least 47, which has been achieved in study of microorganisms' fungi.[162] In addition to the cetane number, improving injection systems is greatly affected by the viscosity of biodiesel.[163] FAMEs types are among the most important determinants in improving or reducing viscosity, which should not exceed 6 at 40 °C according to ASTM D445.[164]

When blending biodiesel with fossil fuels in internal combustion engines, density plays an important role in aspects related to transportation and combustion improvement. Depending on the acceptability of the saturated/unsaturated fatty acid esters of biodiesel, biodiesel can produce ideal density values when blended with fossil diesel, with a value not exceeding 860-900 kg m^{-3} [165–167]. Achieving an ideal density for diesel contributes significantly to the ability of this fuel to pass through filters, which is considered extremely important, especially in winter and cold regions, that is reflected in the ideal ability to start engines.[168] Over and above, achieving a balance between fatty acids in biodiesel achieves very acceptable values, for the saponification value and iodine values.[169] In addition to the higher heating value[170] and cold flow properties.[171]

Although fungi produce good FAMEs and have advantages over other sources, they are not ideal due to the limitations that may prevent their optimal industrial application. Slow growth rate is among the most significant constraints preventing the economic benefits of fungi.[172] High biomass production is subject to several constraints, the most important of which are time and the type of fermentation medium, as time is a crucial factor in any production process. Furthermore, high medium costs prevent products from achieving their economic goals when compared to competition from other sources. Processing alternative carbon sources can increase the cost of production.[173] Not all fungal species can utilize these sources and exploiting them as carbon sources and accumulating optimal amounts of lipids. This increases the economic cost, requiring enzymatic or genetic modifications.[172] On the other hand, it is not easy to control the production of secondary compounds of fungi such as organic acids and other compounds, which can negatively affect production processes to ensure that fungi.[172] In addition to the above factors, genetic variations among fungal species also play a pivotal role in producing FAMEs compatible with the requirements for high-efficiency biodiesel production.[174] It can be said that the limited application studies in the industrial production of biodiesel from fungi are due to the combination of these factors, as currently available standards are laboratory rather than applied. This reflects the need for further studies to produce bioreactors fully compatible with strategies for achieving the economic feasibility of oleaginous fungi.[57]

6. Conclusion

Despite the ability of oleaginous fungi to produce FAMEs like those produced by plants, which are currently used industrially in the production of biodiesel, as well as the advantages that fungi possess, the challenge of producing very high biomass in unsupported media remains, which may prevent the production of economical biodiesel that competes with other resources. In the hope of entering the production line of fungi, our study recommends further experiments on the possibility of achieving very high biomass using biotechnology techniques associated with cheap and economical media investment.

CRediT authorship contribution statement

Kadhim Fadhil Kadhim: Writing – original draft, Investigation. **Inaam Mahmood Najem Alrubayae:** Supervision. **Mohammed Hussein Minati:** Writing – review & editing.

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Data availability

Data will be made available on request.

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