Sustainable Materials and Technologies 48 (2026) e02011

Contents lists available at ScienceDirect

Sustainable Materials and Technologies

journal homepage: www.elsevier.com/locate/susmat

Biodiesel production using wild-type oleaginous microorganisms grown on whey: A state-of-the-art review

Andrea Cantarini [a], Seraphim Papanikolaou [b], Afroditi Chatzifragkou [c], Vesna Milanović [a,*], Andrea Osimani [a], Lucia Aquilanti [a], Cristiana Garofalo [a]

[a] Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, Brecce Bianche, 60131 Ancona, Italy
[b] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece
[c] Department of Food and Nutritional Sciences, University of Reading, Whiteknights Campus, Reading RG6 6DZ, UK

ARTICLE INFO

Keywords:
Whey
Microbial lipids
Biofuel
Fermentation strategies
Downstream processing
Biorefinery
Circular economy

ABSTRACT

The global demand for sustainable biofuels necessitates the valorisation of abundant, low-cost feedstocks such as whey, a major and highly polluting dairy industry by-product. This state-of-the-art review critically examines the use of wild-type oleaginous microorganisms (microalgae, yeasts, and bacteria) for converting whey into microbial lipids suitable for biodiesel production, focusing on non-genetically modified strains. Our synthesis of current knowledge highlights that oleaginous yeasts (e.g., *Cutaneotrichosporon oleaginosus*) achieve the highest reported lipid yields, exceeding 38 g L^{-1} with optimized whey permeate supplementation. Most importantly, literature evidence suggests that the best way to overcome the intrinsic nitrogen limitation of whey is two-stage fed-batch fermentation, which effectively decouples growth and lipogenesis, representing a significant technical advancement toward high lipid production. Furthermore, this review establishes that economic feasibility and environmental impact reduction are contingent upon a full biorefinery approach, integrating lipid production with the co-generation of valuable metabolites, such as proteins and polysaccharides. Collectively, this review establishes a comprehensive framework for leveraging robust wild-type oleaginous strains to shift whey valorisation from an environmental liability to a competitive, circular bioeconomy strategy, provided that remaining scale-up and downstream processing bottlenecks are addressed.

1. Introduction

The global energy landscape is marked by escalating demand driven by population growth, industrialization, and economic development. Fossil fuels, which dominate the energy supply, are non-renewable and contribute significantly to greenhouse gas emissions, exacerbating climate change [1].

Global energy demand increased by 2% in 2024, reaching a record 592 exajoules (EJ), with fossil fuels accounting for nearly 87% of the total energy mix [2]. The International Energy Agency (IEA) reported a 1% increase in energy-related CO_2 emissions in 2024 alone [3]. These trends underscore the urgent need for alternative, sustainable energy solutions capable of reducing environmental impact while meeting global demand.

Biofuels have emerged as a promising renewable alternative to fossil fuels, showing significant growth in their contribution to the global energy sector. In 2022, biofuels accounted for over 3.5% of global transport energy demand, growing at nearly 6% annually, excluding the 2020 Covid-19 pandemic period [4]. Forecasts suggest that by 2030, biofuels could represent 9% of transport energy demand in the Net Zero Emissions (NZE) Scenario [5]. Global biofuel production reached 960 thousand barrels of oil equivalent per day in 2023, with projections to increase to 4570 thousand by 2028, indicating a 2.3% annual growth rate [6]. This expansion underscores the crucial role of biofuels in transition to a sustainable energy future.

Biofuel has evolved through four main generations, each distinguished by its feedstock type and technological maturity. First-generation biofuels rely on food crops such as corn and sugarcane, raising ethical and sustainability concerns due to competition with food production. Second-generation biofuels utilize lignocellulosic biomass and agricultural residues, offering improved sustainability but requiring complex pre-treatment processes. Third-generation biofuels, derived from microorganisms such as yeasts, bacteria, fungi, and algae, provide a flexible approach by using non-food feedstocks and exhibiting high

* Corresponding author.
E-mail address: v.milanovic@staff.univpm.it (V. Milanović).

https://doi.org/10.1016/j.susmat.2026.e02011
Received 9 December 2025; Received in revised form 2 April 2026; Accepted 16 April 2026
Available online 17 April 2026
2214-9937/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

lipid productivity. Fourth-generation biofuels, based on advanced biotechnologies such as carbon capture and genetic engineering, currently remain largely experimental [7].

Within the third generation, oleaginous microorganisms are of particular interest due to their ability to accumulate intracellular lipids under nutrient-limited conditions [8]. These microbial lipids can be converted into biodiesel through transesterification, representing a potential feedstock for sustainable fuel production [9]. A primary advantage of microbial systems is their ability to utilize low-cost, renewable substrates, including food waste and agro-industrial by-products. Utilizing such waste-derived matrices not only potentially lowers production costs, but also contributes to environmental protection by reducing the ecological footprint of organic waste disposal, thereby supporting circular bioeconomy models [10].

A particularly promising substrate in this context is whey, a by-product of cheese and dairy processing. Produced in large volumes worldwide, whey poses significant environmental challenges due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD). However, it is also rich in nutrients (primarily lactose, proteins, and minerals) making it an excellent candidate for microbial fermentation [11]. Repurposing whey as a substrate for lipid-producing microorganisms offers a dual benefit: mitigates the environmental burden of dairy waste and supports the development of cost-effective biofuel production systems.

This state-of-the-art review aims to critically evaluate recent developments on microbial lipid production for biodiesel, using whey as fermentation substrate. Natural (non-genetically modified) microbial strains are evaluated due to their ecological robustness and potential for large-scale application. The review covers a wide range of interconnected aspects, including whey characterization, microbial diversity, growth optimization, lipid biosynthesis pathways, downstream processing techniques and technoeconomic analysis. By integrating microbial biotechnology with waste valorisation, this work explores sustainable solutions for renewable energy generation, while acknowledging the engineering challenges inherent in scaling up these processes.

2. Search procedure

A systematic literature search was conducted in Scopus (http://www.scopus.com), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), and ScienceDirect (http://www.sciencedirect.com), covering the period from January 2000 to September 2025. The primary keyword “microbial biodiesel” was combined with secondary terms including “oleaginous microorganisms,” “*de novo* lipid synthesis,” “fermentation strategies,” “whey valorization,” and “biorefinery.” An initial search yielded 2669 publications, showing a significant research acceleration from 2016 to 2025 (1742 hits).

Peer-reviewed articles in English were included based on their technical relevance to lactose-to-lipid bioconversion. Studies focusing on *ex novo* synthesis (exogenous hydrophobic substrates) and grey literature (theses, conference proceedings) were excluded. Relevant data were extracted into a structured database focusing on: (i) microbial species (yeasts, microalgae, bacteria); (ii) substrate composition and C/N ratios; (iii) fermentation configurations (batch, fed-batch, continuous); (iv) lipid yields and fatty acid profiles; and (v) downstream processing and co-product generation.

3. Whey as a substrate: production, composition, environmental impact, and suitability for oleaginous microorganisms

The increasing demand for food and the growing stringency of environmental regulations have made waste management a critical issue for the agri-food industry. The dairy sector, a fundamental component of the European food system, is a significant generator of organic residues [12]. Among these, cheese whey represents the most abundant by-product, resulting from the coagulation of milk during cheese manufacturing [13]. It is estimated that for every kilogram of cheese produced, approximately 9 to 10 L of whey are generated. In Italy alone, annual whey production reaches around six million tonnes (Fig. 1) [14]. Without appropriate management, this by-product poses substantial environmental risks due to its significant organic load and sheer volume.

Cheese whey retains approximately 85 to 95% of the original milk volume and holds roughly 55% of the milk's nutrient load, including lactose, proteins, minerals, and trace quantities of fat and vitamins. On average, it contains around 65.00 g L^{-1} of total solids per litre [15,16]. Lactose is the dominant component, comprising approximately 44 to 46% of the dry matter [17]. Its high lactose content, combined with the presence of soluble organic compounds, results in elevated BOD and COD, typically ranging from 30.00 to 50.00 g L^{-1} and 60.00 to 80.00 g L^{-1}, respectively [18]. Consequently, untreated whey is a potent pollutant; its discharge into water bodies can lead to eutrophication, while land disposal (particularly in the case of acid whey) may cause soil acidification and long-term degradation [19].

Whey composition varies according to milk source, cheese variety, and processing methodology, but is generally classified into two categories: sweet whey and acid whey [15,20]. Sweet whey results from enzymatic coagulation, typically using rennet during the production of cheeses like cheddar or parmesan [21]. It has a near-neutral pH (6.0–6.5) and typically contains 6–10 g L^{-1} protein, 5–6 g L^{-1} fat, 46–52 g L^{-1} lactose, and 2.5–4 g L^{-1} minerals. Acid whey, produced by acid coagulation methods (either via bacterial fermentation or direct acid addition), has a lower pH (4.5–5.8), slightly less lactose, and a higher mineral content [21,22]. Fig. 1 illustrates the compositional profiles of these variants.

Managing whey, especially for small and medium-sized cheese producers, is often hindered by the substantial capital investment required for treatment infrastructure. Many facilities lack the technical capacity to convert whey into value-added products, leading to underutilization. Currently, approximately half of the whey produced by these dairies is transformed into powdered or condensed forms, while the remainder is frequently discarded [23]. Various physical, chemical, and biological treatments have been explored to mitigate environmental impacts and recover valuable resources.

Physical methods largely involve membrane-based technologies, such as microfiltration, ultrafiltration, nanofiltration, diafiltration, and electrodialysis, are commonly used to concentrate proteins, recover lactose, and reduce the organic load of the effluent. These techniques are especially effective when integrated into larger processing systems, but their cost and maintenance requirements often limit their adoption in small-scale operations [24].

Chemical treatments, on the other hand, are aimed at modifying the molecular structure of whey constituents. One widely studied reaction is the isomerization of lactose through the Lobry de Bruyn–Alberda van Ekenstein transformation, which can be catalyzed under acidic or basic conditions using agents such as sodium hydroxide, potassium hydroxide or calcium hydroxide [25]. In addition, coagulation and flocculation methods using compounds like aluminium sulphate, ferric chloride, or ferrous sulphate are employed to precipitate organic matter and reduce turbidity [26].

However, biological treatments are increasingly recognized for their versatility and potential sustainability. Both aerobic and anaerobic systems have demonstrated the ability to reduce the organic load of whey while enabling its conversion into useful compounds [12,27]. This biological approach provides a foundation for using cheese whey as a substrate in biotechnological processes. Its high lactose content makes it a suitable carbon source for microbial fermentation. As a result, it can be converted into a broad spectrum of bio-based compounds, ranging from traditional products like ethanol, lactic acid, and organic acids to higher-value substances such as enzymes, bacteriocins, and functional proteins [21].

In particular, the potential for transforming whey into biofuels has

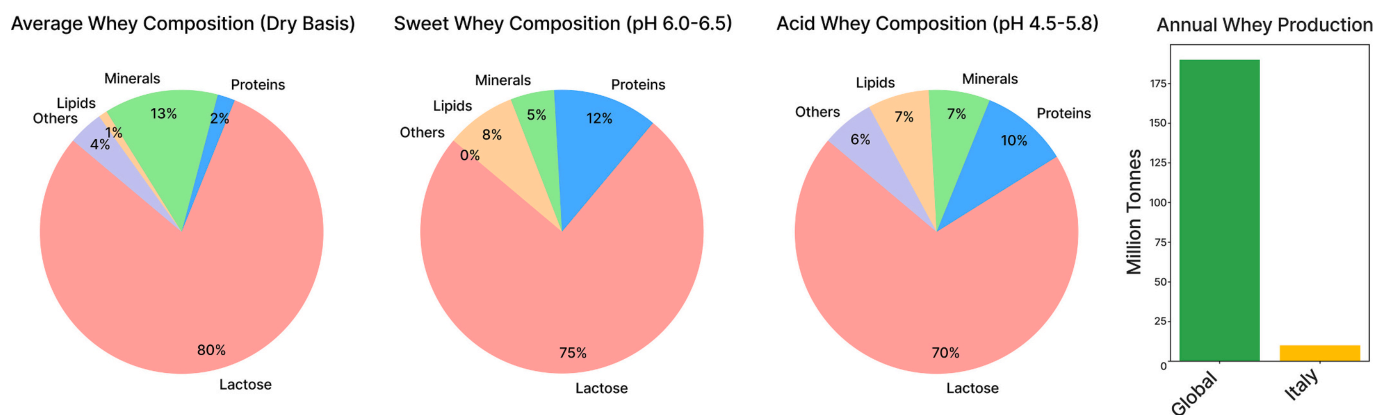

Fig. 1. Compositional Profile of Cheese Whey – Average, Sweet, and Acid Variants with Global and Italian Production Inset. The figure presents the compositional breakdown of whey (on a dry basis) across three variants: Average Whey, Sweet Whey (pH 6.0–6.5), and Acid Whey (pH 4.5–5.8). Each pie chart displays the mass percentages of the major components: Lactose, Proteins, Minerals, Lipids, and Others. The accompanying bar chart (inset) shows the comparative Annual Whey Production in Million Tonnes (Mt) for Global production versus production in Italy.

gained significant attention in recent years. Through various microbial pathways, whey can be transformed into bioethanol, biogas, methane, hydrogen, and butanol [23,28]. Furthermore, specific microorganisms, such as oleaginous yeasts, can accumulate lipids when cultivated on whey-based media. These microbial lipids represent a technically viable precursor for biodiesel production, offering a renewable alternative to fossil fuels [29–32].

The valorisation of cheese whey through these strategies addresses environmental concerns while providing prospective economic opportunities for the dairy sector. Transitioning from a linear to a circular production models may reduce waste and support sustainable bioeconomy frameworks, although the widespread industrial adoption of these systems remains contingent upon overcoming existing engineering and logistical bottlenecks.

4. Wild-type oleaginous microorganisms for biodiesel production

Oleaginous microorganisms are a diverse group of microbes capable of accumulating substantial amounts of lipids (often exceeding 20% of their dry biomass) under certain growth conditions [8]. Under optimized growth parameters, including a high carbon-to-nitrogen (C/N) ratio, certain species are capable of lipid accumulation constituting up to 70% of their biomass by weight [33,34]. Lipids typically consist of unbranched fatty acids with chain lengths from 4 to 28 carbons [35]. These fatty acids may be either saturated or unsaturated, with the degree of unsaturation classified as monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA) depending on the number of double bonds present [36].

These organisms, including various species of bacteria, yeasts, fungi, and microalgae, store lipids primarily as triacylglycerols (TAGs) in intracellular lipid bodies, which can be extracted and converted into biodiesel through transesterification [37]. Their ability to utilize low-cost substrates such as whey, positions them as potential key players in third-generation biofuel strategies, aiming to reduce reliance on food crops while simultaneously addressing waste management issues [31]. The choice between wild-type and genetically modified (GM) strains is governed by the specific requirements and economic constraints of the bioprocess. While GM strains frequently demonstrate higher specific lipid yields and faster lactose assimilation under optimized laboratory conditions, wild-type oleaginous microorganisms are often examined for their inherent metabolic plasticity and resilience when utilizing crude dairy side-streams [9,31]. Utilizing non-GM platforms avoids the metabolic burden associated with maintaining synthetic genetic circuits—which can divert energy away from primary lipogenesis—and eliminates the costs of specialized selective agents, such as antibiotics or inducers, which are often technically difficult and expensive to manage at an industrial scale [38–40]. However, a primary technical challenge in optimizing wild-type lipid yields is the inherent 'metabolic trade-off' between biomass productivity and lipid accumulation; since lipogenesis is typically triggered by nitrogen limitation, it occurs only after the growth phase has slowed [41,42]. Achieving competitive titers thus demands precise, multi-stage control of environmental parameters to maximize cell density before inducing the metabolic switch [14,43]. While engineered strains may offer higher theoretical conversion rates in refined media, the operational stability of wild-type organisms is often superior when utilizing crude, heterogeneous substrates like cheese whey, where maintaining strict selective pressures is economically unfeasible [9,44,45]. Table 1 summarizes studies investigating whey and various related dairy processing residues as substrates for lipid accumulation across different oleaginous microorganisms.

4.1. Oleaginous microalgae

Oleaginous microalgae are among the most promising candidates for sustainable biofuel production, combining high photosynthetic efficiency with minimal land requirements and rapid biomass accumulation potential [55]. Unlike conventional crops, microalgae are not restricted to a single nutritional strategy. Depending on the available resources, they can grow autotrophically, mixotrophically or heterotrophically, utilizing either inorganic carbon (CO_2) or organic carbon sources [56,57].

Lipid synthesis occurs mainly in the chloroplast and endoplasmic reticulum, where fatty acids are produced, assembled into glycerolipids, and stored as TAGs in oil bodies [58,59]. Under specific stress conditions, TAGs can reach up to 90% of the cell's dry weight, compared to a baseline range of 1–70% [60,61]. Nutrient limitation, particularly in nitrogen and phosphorus, is among the most effective triggers for lipid accumulation inducing a metabolic shift toward energy storage [62]. Furthermore, lower cultivation temperatures can promote the synthesis of unsaturated fatty acids [63]. Likewise, environmental pressures such as high light intensity, shifts in salinity or pH, and exposure to specific chemicals can prompt cells to store additional lipids as a protective strategy [64]. Controlled application of such stressors may allow for the optimization of lipid yields, although often at the expense of overall biomass productivity.

Recent research demonstrates the technical effectiveness of heterotrophic and mixotrophic cultivation methods to improve microalgal biomass and lipid production. Morowvat & Ghasemi [65] showed that *Chlorella vulgaris* grown heterotrophically on glucose yields biomass and

Table 1
Comparative data on oleaginous microorganisms grown on whey.

Microbial group	Microbial identity	Substrate	Biomass production (g L^{-1})	Lipid production (g L^{-1})	Organic load reduction (%)	Cultivation mode	References
Microalgae	*Scenedesmus obliquus*	BBM + 40% v v^{-1} whey permeate (WP)	4.90 ± 0.20*	0.49*	Lactose rem.: 54.40 (13 days)	Mixotrophic	[46]
		Lake water (LW) + 40% v v^{-1} WP	3.50 ± 0.10*	0.37*	–	Mixotrophic	
	Scenedesmus spp.	Pure cheese whey (1.5 g L^{-1} lactose)	1.55	0.10	TOC rem.: 67.6	Mixotrophic	[47]
		Pure cheese whey (2.5 g L^{-1} lactose)	3.51	0.08	TOC rem.: 46.58	Mixotrophic	
		Pure cheese whey (3.5 g L^{-1} lactose)	4.35	0.17	TOC rem.: 54.60	Mixotrophic	
		Pure cheese whey (5 g L^{-1} lactose)	5.50	0.15	TOC rem.: 42.20	Mixotrophic	
		Cheese whey and salts (2.5 g L^{-1} lactose)	4.27	0.15	TOC rem.: 49.04	Mixotrophic	
		Pure cheese whey permeate (2.5 g L^{-1} lactose)	2.09	0.02	TOC rem.: 45.71	Mixotrophic	
		Permeate and salts (2.5 g L^{-1} lactose)	2.33	0.07	TOC rem.: 59.90	Mixotrophic	
	Desmodesmus spp.	Cheese whey under controlled condition (CWCC)	9.03	4.00	–	Mixotrophic	[48]
		Cheese whey under environmental condition (CWEC)	10.37	2.37	–	Mixotrophic	
Yeasts	*Yarrowia lipolytica* B9	Deproteinized whey under optimized non-sterile conditions	7.40	4.29	Lactose rem.: 99.66 (120*h*)	Submerged batch	[49]
Yeasts	*Cystobasidium oligophagum* JRC1	Untreated cheese whey (UCW) at 100% conc.	20.98 ± 0.36	4.57 ± 0.13	COD rem.: 86.82 ± 0.07	Aerobic batch	[50]
	Cutaneotrichosporon oleaginosus	Whey permeate supplemented with YE and Urea + Mango Syrup (WPYESU+M)	84.0 ± 1.27	38.1 ± 1.00	–	Two-stage fed-batch	[14]
	Cryptococcus curvatus ATCC 20509	Treated second cheese whey	22.40	3.40	Lactose rem.: 98.33	Batch	[51]
	Cryptococcus curvatus NRRL-1511	Treated second cheese whey	20.60	3.20	Lactose rem.: 98.33	Batch	
	Debaryomyces hansenii ACA-DC 5079	Treated second cheese whey	8.30	1.20	Lactose rem.: 30.17	Batch	
	Papiliotrema laurentii NRRL Y-2536	Treated second cheese whey	22.00	1.20	Lactose rem.: 96.67	Batch	
	Papiliotrema laurentii NRRL YB-3594	Treated second cheese whey	14.70	1.00	Lactose rem.: 92.83	Batch	
Fungi	*Mucor circinelloides*	Hydrolyzed whey permeate (HWP)	9.03 ± 0.74**	2.20 ± 0.25**	Sugar cons.: 83.37	Submerged batch	[52]
Bacteria	*Rhodococcus opacus* PD630	Dairy wastewater +25% v v^{-1} mineral salt media	~1.50	1.10	COD rem.: ~93.00 (96 h).	Fed-batch	[53]
	Rhodococcus opacus PD630	Dairy wastewater +25% v v^{-1} mineral salt media	~1.70	1.80	COD rem.: >99.00	Continuous	
	Rhodococcus opacus PD630	Dairy wastewater +25% v v^{-1} mineral salt media	~4.00	3.40	COD rem.: ~97.00	Continuous with cell recycling	
	Rhodococcus opacus PD630	Whey permeate	6.10	2.80	–	Aerobic batch	[54]
	Rhodococcus opacus MR22	Whey permeate	6.30	3.00	–	Aerobic batch	
	Rhodococcus jostii RHA1	Whey permeate	1.80–2.90	<5% of cell dry weight	–	Aerobic batch	
Bacteria	*Rhodococcus opacus* PD630	Deproteinized CW under optimized non-sterile conditions	7.40	4.29	Lactose rem.: 99.66 (120 h)	Submerged batch	[54]

Abbreviations used: Lactose rem., Lactose removal; TOC rem., Total Organic Carbon removal; COD rem., Chemical Oxygen Demand removal; BBM, Bold's basal medium; YE, Yeast Extract; CW, Cheese Whey. **Process parameters**: The following operational conditions represent the key cultivation variables reported for the respective study and strain in the literature, which includes temperature (T°C), pH, rotational speed, aeration rate, inoculum size, and cultivation period. **Microalgae** (All Strains): Cultivation parameters include T°C: 22.5–27 °C; pH: 6.4; rotational speed: 120–150 rpm; photoperiod: 12 h light/dark; aeration: 0.7 vvm; cultivation period: 7–13 days. **Yeast** (All Strains): Cultivation parameters include T°C:15–30 °C; pH: 5.5–6.8; rotational speed: 150–180 rpm; cultivation period: 168 h. **Fungi** (*Mucor circinelloides*): Submerged batch fermentation at T°C: 33.6 °C; pH: 4.5; rotational speed: 200 rpm; cultivation period: 7 days. **Bacteria** (All Strains): Cultivation parameters include T°C: 28–30 °C; pH: 7; rotational speed: 250 rpm; aeration rate: 1 L min^{-1}; cultivation period: 100–120 h. **Notes:** * Data recorded at day 13 of cultivation; ** Data recorded at day 7 of cultivation.

lipid content significantly higher than autotrophic cultures, though the high cost of refined glucose cost remains a primary economic limitation. This has driven interest in using low-cost, waste-derived substrates. Mixotrophic systems, which combine photosynthesis with organic carbon assimilation, have a potential to enhance growth and productivity in large-scale applications [66]. Girard et al. (2014) showed that *Scenedesmus obliquus* cultivated mixotrophically on lake water enriched with (40% v v^{-1}) cheese whey permeate achieved higher growth rates and biomass than either heterotrophic or autotrophic cultures, partly through extracellular lactose hydrolysis. However, further optimization is still required to improve biomass yields and ensure complete sugar utilization.

Borges et al. (2016) found that while bio-oil production in *Scenedesmus* spp. was not enhanced using whey permeate alone, cultivation in raw cheese whey (2.5 g L^{-1} lactose) supported both bio-oil production and organic load removal, underscoring the potential dual environmental and biotechnological benefits. Similarly, Abril Bonett et al. (2020) reported lipid production of 2.37 g L^{-1} from *Desmodesmus* spp. grown on cheese whey under environmental conditions. Collectively, these studies emphasize the promising role of heterotrophic and mixotrophic cultivation of microalgae, though economic and environmental advantages remain contingent upon the scaling of these integrated systems.

4.2. Oleaginous yeasts and fungi

Oleaginous yeasts represent a small but highly relevant subset of the yeast domain. Fewer than 30 species have been documented capable of accumulating lipids more than 20% of their cell dry weight [67]. Species most frequently associated with high lipid yields include *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Rhodosporidium toruloides*, *Cryptococcus curvatus*, and *Lipomyces starkeyi* [68]. These microorganisms store lipids primarily as TAGs, which typically constitute 80–90% of the total lipid fraction, while mono- and diacylglycerols generally comprising the remainder [69]. When hydrophilic carbon sources such as sugars or glycerol are converted into microbial lipids, the process depends on a nutrient imbalance. Specifically, induction of lipid biosynthesis in oleaginous yeasts is closely linked to the ratio between carbon availability and other essential nutrients. When carbon substrates are present in excess and a key element (typically nitrogen) becomes limiting, metabolic flux is redirected away from protein synthesis toward TAGs deposition [70,71]. This metabolic shift increases overall lipid content and determines fatty acid composition. The resulting profile is typically enriched in palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids. Myristic (C14:0) and linolenic (C18:3) acids generally occur in smaller proportions [72]. However, in several cases, this nutrient imbalance favours the accumulation of storage *endo*-polysaccharides instead of lipids. Alternatively, cells may secrete low molecular weight metabolites (i.e. citric acid, polyols) into the growth medium [73,74].

From a bioprocess perspective, oleaginous yeasts combine rapid proliferation with metabolic versatility. They can utilize diverse carbon sources and suggest scalability for potential industrial fermentation. Recent studies demonstrate that cheese whey can serve as both an inexpensive feedstock and an environmental remediation strategy. Taskin et al. (2015) cultivated cold-adapted *Y. lipolytica* B9 on deproteinized whey under optimized non-sterile conditions. The study achieved biomass and lipid concentrations of 7.40 g L^{-1} and 4.29 g L^{-1}, respectively. The 58% lipid content was dominated by monounsaturated C16 and C18 fatty acids, making it ideal for biodiesel in cold climates. Similarly, Vyas et Chhabra (2019) showed that *Cystobasidium oligophagum* JRC1 grown on deproteinized whey reached lipid contents of 44%. This process achieved >84% COD removal and produced fatty acid methyl esters (FAMEs) compliant with American Society of Testing and Materials (ASTM) and European Norm (EN) biodiesel standards. These findings underline the potential of whey-based yeast bioprocesses for waste valorization and high-quality single cell oil (SCO) production. Furthermore, the “industrial” strain *C. curvatus* ATCC 20509 (also known as *Apiotrichum curvatum* ATCC 20509, *Cutaneotrichosporon oleaginosus* ATCC 20509 and *Candida curvata* D) has been reported to produce significant lipid quantities during growth on cheese whey in various fermentation configurations [51,75–77]. Other strains, such as *C. curvatus* NRRL Y-1511, have also proved capable of synthesizing yeast biomass containing variable amounts of storage lipids using this substrate [78,79].

Oleaginous fungi include various species, most of which fall within the *Zygomycota* phylum [80]. High-yielding examples include *Mucor circinelloides*, known for substantial lipid synthesis under aerobic, glucose-rich cultivation [81]. Other notable *Zygomycota* genera with oleaginous potential include but are not limited to *Mortierella* and *Cunninghamella* [29,80,82,83]. Additionally, several *Aspergillus* species within the *Ascomycota* phylum, such as *A. terreus A. niger*, and *A. flavus,* have demonstrated lipid contents exceeding 38% of biomass, with fatty acid profiles enriched in oleic and stearic acids [84,85]. A distinctive feature of these fungi is their ability to synthesize long-chain PUFAs of nutritional and pharmaceutical value alongside biodiesel-compatible lipids. These include docosahexaenoic acid (DHA), γ-linolenic acid (GLA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) [86].

Liquid cultures studies often report lower lipid yields than other systems [87]. These constraints have limited the use of oleaginous fungi on complex agro-industrial residues. Furthermore, literature regarding filamentous fungi grown on lactose or whey-based media is rare. This scarcity is due to the inability of many fungi to assimilate lactose [29,52,88–90]. Only recently has the potential for β-galactosidase production by Zygomycetes been studied [91]. The highest dry cell weight (DCW) reported for Zygomycetes on cheese-whey based media was achieved by *Morteriella isabellina* ATHUM 2935. This strain produced 32.00 g L^{-1} with approximately 25% lipid content. Further incubation increased DCW to 42.30 g L^{-1}, though lipid values were simultaneously reduced [29]. Additionally, Chan et al. (2018) demonstrated that *M. circinelloides* can grow on hydrolyzed whey permeate. This strain achieved 9.40 g L^{-1} biomass and 24% lipid content at 33.6 °C. In this case, the lipid fraction was dominated by oleic and palmitic acids. The relatively high γ-linolenic acid content indicates potential for both biodiesel production and nutraceutical applications [52].

4.3. Oleaginous bacteria

Although oleaginous bacteria produce TAGs, their application in biodiesel production remains less prevalent than that of microalgae and yeasts [31]. While most bacterial species store energy and carbon reserves as polyhydroxyalkanoic acids, a specific subset is capable of accumulating substantial lipid quantities [92]. Among Gram-positive strains, notable TAGs producers include *Rhodococcus opacus*, *Acinetobacter calcoaceticus*, and related *Acinetobacter* spp. [93], which have been reported to reach up to 87% oil content in dry biomass. Other taxa with oleaginous potential include *Streptomyces*, *Bacillus*, *Mycobacterium*, *Serratia*, *Dietzia*, *Gordonia*, and *Nocardia* [39,94]. In contrast, TAGs accumulation in Gram-negative bacteria remains insufficiently characterized for bioprocess development [95].

Bacteria can metabolize a wide range of carbon sources. Although glucose supports efficient lipid accumulation, its high price limits economic feasibility for large-scale production [96]. A more sustainable strategy involves the utilization of low-cost industrial or agricultural residues, provided they can support high-density growth. Dairy industry by-products represent potential substrates in this context. Gupta et al. (2018) demonstrated that *R. opacus* cultivated on dairy wastewater achieved its highest lipid yield (3.40 g L^{-1} with 97% COD removal) when operated in a continuous culture with cell recycling. This configuration outperformed both fed-batch (1.10 g L^{-1}) and standard continuous (1.80 g L^{-1}) modes due to enhanced biomass retention. However, it should be noted that maintaining cell-recycling systems at

an industrial scale introduces significant operational complexity. Likewise, Behera et al. [97] reported that strain DS-7, isolated from dairy effluent scum, could accumulate lipids up to 72% of its biomass when grown on dairy wastewater. Cheese whey has also been investigated as a substrate by certain *Rhodococcus* species; Herrero & Alvarez (2016) found that *R. opacus* produced 6.10–6.30 g L^{-1} biomass with 45–48% lipid content from whey. This performance far exceeding the <5% lipid yields of the other rhodococcal strains tested (*R. jostii, R. erythropolis, R. fascians, and R. equi*).

5. Lipid synthesis in wild-type oleaginous microorganisms: de novo and *ex novo* pathways

Oleaginous microorganisms synthesize TAGs through two main metabolic routes: de novo and *ex novo* synthesis [34]. In the de novo pathway, lipid accumulation is triggered by an excess of carbon alongside a deficiency of nitrogen or other key nutrients such as phosphorus, magnesium, or trace metals [98,99]. The metabolic transition from lactose uptake to TAG accumulation is illustrated in Fig. 2. Nitrogen limitation is particularly effective. It halts cell proliferation and diverts metabolic flux toward storage lipid formation [100]. Under these conditions, Adenosine monophosphate (AMP) deaminase activity releases ammonium (NH_4^+) from AMP. This lowers intracellular AMP levels, which in turn inhibits isocitrate dehydrogenase (ICDH) within the tricarboxylic acid (TCA) cycle. Consequently, isocitrate accumulates and equilibrates with citrate. This citrate is exported to the cytosol. There, ATP–citrate lyase (ACL), an enzyme present only in oleaginous species, cleaves it into oxaloacetate and acetyl-CoA. Acetyl-CoA is then carboxylated by acetyl-CoA carboxylase (ACC) to form malonyl-CoA [101]. This step is the first committed and rate-limiting stage of lipogenesis. In wild-type microorganisms, its efficiency is governed by the intracellular AMP/ATP ratio, making the process highly sensitive to C/N ratios [102]. Variations in nutrient availability impact synthesis efficiency through a 'threshold effect': if the initial nitrogen concentration is too high, the metabolic switch to lipogenesis is delayed, leading to excessive biomass at the expense of the lipid-to-substrate yield ($Y_{L/S}$). Conversely,

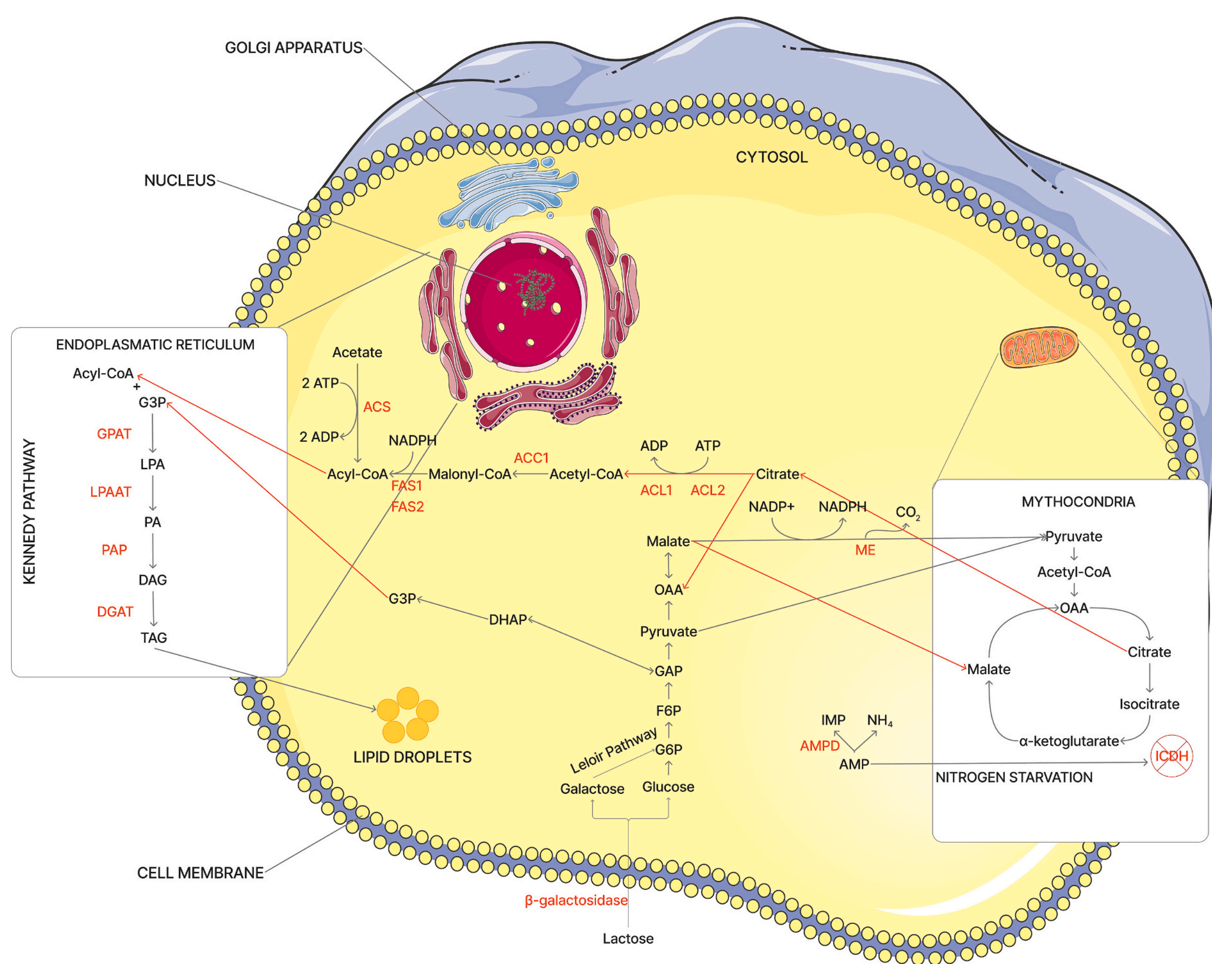

Fig. 2. Metabolic landscape of de novo lipid synthesis from lactose in oleaginous microorganisms. The diagram illustrates the biochemical integration of lactose catabolism and triacylglycerol (TAG) biosynthesis. Lactose is hydrolyzed into glucose and galactose, which enter the glycolysis pathway to generate pyruvate. Following transport into the mitochondrion, pyruvate is converted into acetyl-CoA and enters the TCA cycle. Under nitrogen-limited conditions, the activation of AMPD leads to a decrease in mitochondrial AMP, inhibiting ICDH activity. This results in the accumulation of citrate, which is exported to the cytosol. Cytosolic citrate is cleaved by ACL to provide acetyl-CoA, the primary building block for fatty acid synthesis mediated by ACC and the FAS complex. NADPH, required for these reductive steps, is supplied primarily by ME or the pentose phosphate pathway. Finally, fatty acids are incorporated into the glycerol backbone via the Kennedy pathway (GPAT, LPAAT, PAP, and DGAT) to form TAGs, which are sequestered within lipid droplets. **Abbreviations:** ACL, ATP-citrate lyase; ACC, acetyl-CoA carboxylase; ACS, acetyl-CoA synthetase; AMP, adenosine monophosphate; AMPD, adenosine monophosphate deaminase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; FAs, fatty acids; FAS, fatty acid synthase; F6P, fructose-6-phosphate; G3P, glycerol-3-phosphate; GAP, glyceraldehyde; GPAT, glycerol-3-phosphate acyltransferase; G6P, glucose-6-phosphate; ICDH/IDH, isocitrate dehydrogenase; IMP, inosine monophosphate; LPA, lysophosphatidic acid; LPAAT, lysophosphatidic acid acyltransferase; ME, malic enzyme; NADPH, nicotinamide adenine dinucleotide phosphate; OAA, oxaloacetate; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; TAG, triacylglycerol.

premature nitrogen exhaustion limits the total biocatalyst (biomass) available, reducing overall volumetric productivity even if the individual cells are lipid-rich [100,102].

Strategic strain selection is vital here, as different wild-type species exhibit varying flux capacities through the ACC and FAS complexes, directly affecting the final lipid production [103]. Malonyl-CoA serves as the substrate for fatty acid synthase (FAS)-mediated chain elongation [101]. Type I FAS operates in yeasts and fungi, while type II FAS operates in algae and bacteria, where individual enzymes repeatedly add two-carbon units [104–106]. This reductive process demands large amounts of NADPH. In most eukaryotic oleaginous species, the malic enzyme (ME) reaction (malate → pyruvate) provides this supply under non-growth conditions [107]. However, the main contributor varies by species. For example, *Y. lipolytica* and other species lacking cytoplasmic ME activity rely primarily on the pentose phosphate pathway (PPP) [108,109]. In *R. toruloides*, over 60% of NADPH comes from the PPP despite the presence of ME activity [110]. Similarly, *Rhodococcus* and related bacteria generate NADPH via the PPP [111]. In autotrophic oleaginous microalgae the primary source appears to be ferredoxin–$NADP^+$ reductase of photosystem I, although ME activity is detected, [104,112].

Critically, the industrial value of these lipids for biodiesel depends on the fatty acid profile produced, as not all TAGs are suitable for fuel. While FAS primarily creates saturated chains (C16:0 and C18:0), the final fuel quality is decided by the activity of native desaturase enzymes (Δ9, Δ12, and Δ15) [103]. According to international standards (ASTM D6751 and EN 14214), high-quality biodiesel must balance three conflicting properties: oxidative stability, cold-flow performance, and ignition quality (Cetane Number). As emphasized by Meng et al. (2009), oleic acid (C18:1)—the product of wild-type Δ9-desaturase—is the "ideal" fatty acid. It provides high oxidative stability due to its low number of double bonds while maintaining a low melting point for winter use. In contrast, an abundance of PUFAs from Δ12 and Δ15 activity creates reactive 'bis-allylic' positions. These sites facilitate radical-induced oxidation, leading to fuel polymerization and engine deposits [102,113].

Following their synthesis and the determination of their unsaturation degree, these fatty acids are incorporated into TAGs through the Kennedy pathway [114]. In this route, glycerol-3-phosphate (G3P) is acylated by G3P acyltransferase (GAT) to form lysophosphatidic acid (LPA). LPA is then acylated by lysophosphatidic acid acyltransferase (AGAT) to produce phosphatidic acid (PA). Finally, PA is dephosphorylated by PA phosphatase (PAP) to yield diacylglycerol (DAG). DAG is finally acylated by diacylglycerol acyltransferase (DGAT) or phospholipid: diacylglycerol acyltransferase to produce TAGs stored in lipid droplets [115].

In wild-type microorganisms, the fatty acid composition exhibits significant metabolic plasticity in response to the cultivation environment. This allows for a degree of control over the resulting biodiesel quality through the strategic manipulation of growth parameters, though such adjustments often involve trade-offs between yield and fuel performance. For instance, native desaturases are temperature-regulated as part of the cell's homeoviscous adaptation. By maintaining cultures in the upper mesophilic range (28–32 °C), and limiting dissolved oxygen during the late lipogenic phase, the activity of oxygen-dependent Δ12 and Δ15 desaturases is curtailed. This promotes a high-quality profile rich in MUFAs (specifically oleic acid) which enhances oxidative stability and the Cetane Number without raising the Cloud Point to the levels associated with purely saturated fats [49,103,113]. To ensure this quality-driven shift does not compromise total yield, these thermal and aerobic adjustments must be synchronized with a fed-batch feeding profile. This 'staged' approach ensures high biomass density is secured before shifting the metabolic flux toward a specific fatty acid composition during the accumulation phase, effectively balancing fuel standards (ASTM D6751/EN 14214) with industrial productivity [102].

However, lipid accumulation is not the only possible outcome under nitrogen-limited conditions. In wild-type strains, 'efficiency' is fundamentally a competition between different metabolic sinks. When grown on glucose / glycerol / lactose, microbial cells face an enzymatic bottleneck where the rate of citrate export from the mitochondria can exceed the flux capacity of the FAS complex [116]. Instead, carbon flow is diverted toward the synthesis of intra-cellular or extra-cellular polysaccharides [116–119] or other secondary extra-cellular low-molecular weight metabolites (mostly citric acid and to lesser extend polyols like arabitol, mannitol and erythritol) [120]. In the former case, microorganisms belonging but not limited to the genera/species *Metschnikowia* sp., *Papiliotrema laurentii*, and *Cryptococcus albidus* produced significant quantities of polysaccharides. This diversion at the expense of storage lipids has been recorded in many nitrogen-limited cultures of wild-type strains of the non-conventional yeast *Y. lipolytica* [117,120].

This diversion directly reduces the net synthesis efficiency, as a significant portion of the lactose is 'wasted' on non-lipid products [116,121]. This energetic and stoichiometric burden is specific to the de novo route; unlike the *ex novo* pathway—which is more efficient but largely irrelevant for lipid-poor substrates like cheese whey—de novo synthesis requires a constant, high supply of ATP and NADPH (primarily from the PPP) to reduce sugar-derived acetyl-CoA into fatty acids [105,110]. The *ex novo* pathway, by contrast, involves direct uptake of hydrophobic substrates (such as free fatty acids, TAGs, or sterol esters) from the medium [34]. Incorporated fatty acids may be partially degraded for energy or can be re-esterified (after potential transformations, i.e. desaturations catalyzed by cellular dehydrogenases) for storage, enabling rapid lipid accumulation without de novo fatty acid synthesis. This mechanism depends on lipid-rich feedstocks (e.g., waste cooking oils, oily by-products). Since cheese whey is carbohydrate-rich and contains negligible preformed lipids, TAGs production in whey-based processes relies almost exclusively on the de novo pathway.

Consequently, the efficiency of wild-type whey valorization is ultimately a function of how effectively the fermentation environment can be controlled to minimize these alternative metabolic 'leaks' [14,31,122].

6. Cltivation strategies on whey

6.1. Optimization parameters for microbial lipid yield

The accumulation of lipids in oleaginous microorganisms is highly sensitive to the physiochemical culture environment. Key parameters such as chemical nature of carbon source, the balance between carbon and nitrogen (C/N ratio), the bioavailability of nitrogen supplied, pH, temperature, and oxygen availability play decisive roles in determining both the quantity of storage lipids and their fatty acid composition (Fig. 3). Among these, nitrogen limitation is widely recognized as the principal trigger for redirecting cellular metabolism toward lipid biosynthesis in yeasts and related organisms [123].

6.1.1. C/N ratio

Numerous studies have highlighted the pivotal role of the C/N ratio in regulating lipid accumulation. The C/N ratio determines the distribution of carbon between cellular growth and storage lipid synthesis, with an excess of carbon relative to nitrogen favouring lipid formation [41]. Consequently, culture conditions that combine a carbon-rich medium with limited nitrogen are generally considered optimal for promoting lipid overproduction in oleaginous microorganisms [124]. In dairy-based media, the variability of the C/N ratio is strictly dependent on the industrial origin and processing history of the specific stream. Raw sweet and acid whey contain significant concentrations of proteins (6.00–10.00 g L^{-1}), resulting in relatively low C/N ratios, typically ranging from 12 to 30 [11,15,21]. While these conditions support high biomass yields, the abundance of organic nitrogen often prevents the metabolic shift toward lipogenesis. In contrast, secondary streams like whey permeate or second cheese whey (produced through

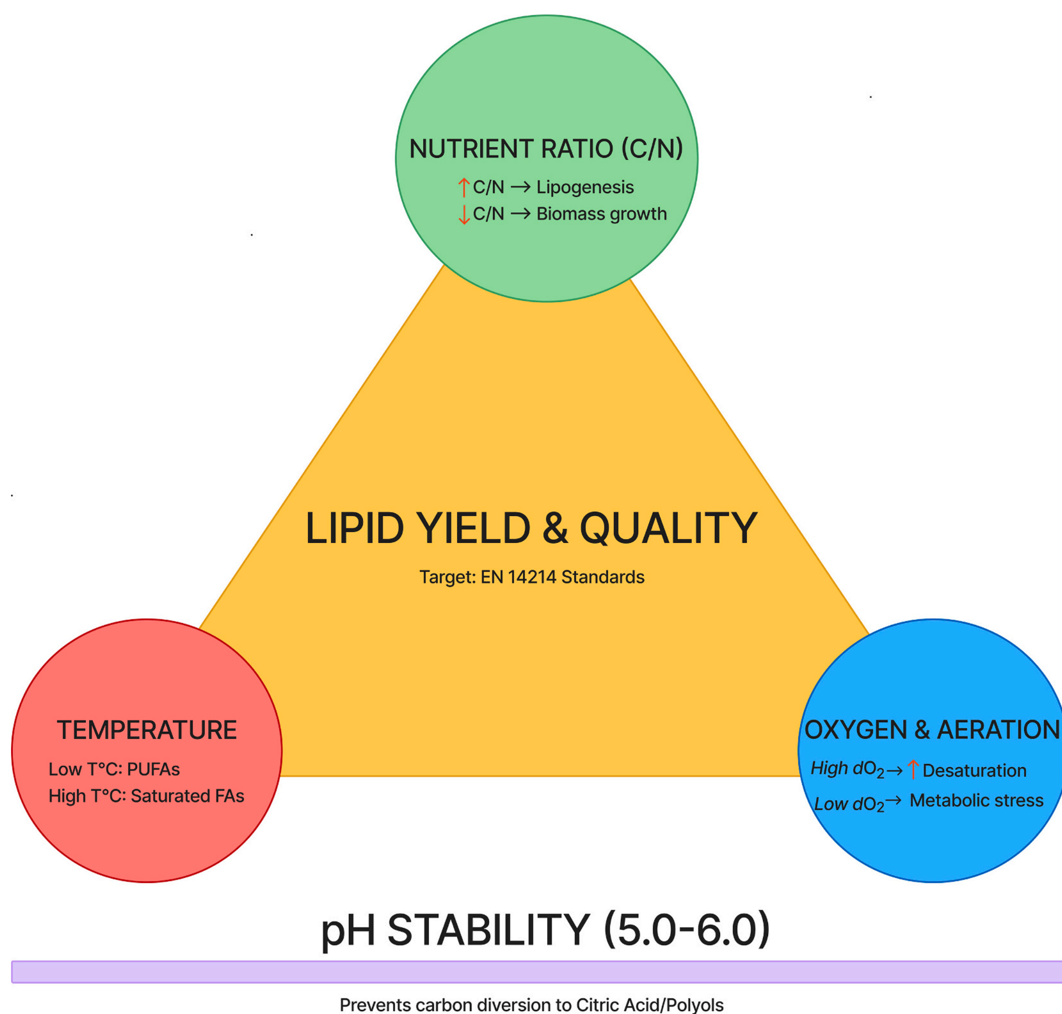

Fig. 3. Main factors affecting microbial lipid production. Conceptual map showing the relationship between nutritional and environmental parameters during yeast fermentation. The diagram highlights how the C/N ratio, temperature, and dissolved oxygen interact to control both the quantity and the quality of the accumulated lipids. Maintaining a stable pH is essential to maximize yield and prevent the formation of unwanted by-products. Abbreviations: C/N, Carbon-to-Nitrogen ratio; dO_2, Dissolved oxygen; T°C, Temperature; FAs, Fatty acids; PUFAs, Polyunsaturated fatty acids; TAG, Triacylglycerol.

the removal of high-value proteins) exhibit a natural increase in the C/N ratio compared to raw whey. However, it is important to note that the high ratios (typically exceeding 80) frequently reported in high-titer studies are rarely a natural feature of the raw substrate [11,15]. Instead, these values are typically achieved through technical interventions such as upstream concentration of the lactose or via supplemental carbon feeding during fermentation to ensure nitrogen-limiting conditions [14]. The baseline nutritional profiles of these streams, before such interventions, are summarized in Table 2.

The industrial relevance of these ratios is evidenced by the performance of specific strains. For instance, *C. oligophagum* JRC1 exhibited significantly higher lipid accumulation when cultivated on deproteinized cheese whey compared to untreated sweet whey, where the lower C/N ratio favoured biomass production over storage [50]. Similarly, Donzella et al. (2022) demonstrated the strong influence of C/N ratio adjustments on lipid accumulation in *C. oleaginosus* grown on whey permeate. In whey permeate supplemented with yeast extract (C/N ~ 60.5), the yeast reached a lipid content of ~46% of DW after 48 h. As lactose became nearly depleted, glucose feeding was introduced to restore a high C/N ratio, which further boosted lipid accumulation to 68%, among the highest values reported for this strain.

Table 2
Baseline nutritional composition and typical C/N ratios of raw whey-derived substrates.

Substrate type	Lactose (g L^{-1})	Total nitrogen (g L^{-1})	Natural baseline C/N ratio	Primary reference(s)
Sweet whey	46.00–52.00	0.80–1.50	12–25	[11,15,21]
Acid whey	40.00–45.00	0.60–1.20	15–30	[15,22]
Whey permeate	45.00–55.00	0.10–0.40	35–70	[14,15,41]
Second cheese Whey	35.00–50.00	0.20–0.40	15–45	[11,15,21]

The practical management of C/N variability in dairy-based media involves technical interventions to ensure process reproducibility. Upstream standardization is used to stabilize the nitrogen baseline; techniques such as ultrafiltration or heat-acid precipitation [11] reduce the organic nitrogen load, mitigating the ‘threshold effect’ where excess nitrogen favours biomass proliferation over lipid synthesis [50]. Furthermore, if the optimal C/N ratio (typically >80) is not naturally achieved by the substrate, the medium could be optimized through the supplementation of carbon-rich industrial by-products. For instance, the addition of condensed cheese whey-derived lactose [51] or the integration of co-substrates such as crude glycerol [14] and lignocellulosic hydrolysates [125] provides the necessary carbon excess to trigger lipogenesis. Utilizing these interventions in fed-batch ‘pseudo-two-

stage' configurations allows the process to accommodate biochemical fluctuations, facilitating high-titer lipid production from variable whey streams [126].

6.1.2. pH

pH is a critical factor in oleaginous microorganisms, modulating nutrient uptake, enzyme activity, and the partitioning of carbon toward storage lipids. Its influence is highly species- and substrate-dependent, and precise control is essential for maximizing lipid yields. Venkata Subhash & Venkata Mohan [127] identified pH as the most influential parameter in lipid production by *Aspergillus awamori*, outweighing temperature, glucose concentration, and nutrient supplementation. In microalgae, *Nannochloropsis salina* exhibited optimal growth at pH 7–8, with growth completely inhibited at pH 10 [128].

In oleaginous yeasts, slightly acidic conditions (pH 5–6.5) frequently enhance lipid synthesis [121]. For instance, *Y. lipolytica* achieved maximal lipid accumulation at pH 5–6 on glucose [129], whereas alkaline conditions (pH 8) were advantageous when cultivating on acidic substrates [130]. Interestingly, trials of the oleaginous strain *C. oleaginosus* ATCC 20509 (formerly *Apiotrichim curvatum*) on whey permeate in single-stage aerobic continuous cultures with constant dilution rate ($D = 0.04\ h^{-1}$, lactose $\approx 48.00\ g\ L^{-1}$) demonstrated a lower sensitivity to pH compared to the previous results reported in *Y. lipolytica*. In this experiments, *C. oleaginosus* grown at pH ≈ 3.5 achieved steady-state biomass and lipid concentration of ≈24.00 and = 10.90 $g\ L^{-1}$, respectively; at pH = 5.7, these values were = 20.80 and ≈9.20 $g\ L^{-1}$, demonstrating the potential of the accomplishment of the fermentations under a wide range of medium pH values imposed into the medium [131]. The ability of this strain to maintain high lipid productivity at pH ≈ 3.5 is technically significant, as it suggests the potential for operating under non-sterile conditions where low pH acts as a natural inhibitor of bacterial contamination.

6.1.3. Temperature

Temperature strongly influences both growth and lipid accumulation kinetics. In yeasts, optimal lipid production generally occurs between 20 and 30 °C [132]. However, certain oleaginous yeasts display remarkable adaptability; for example, *Rhodotorula glacialis* is capable of growth at temperatures as low as 3 °C [133], while *Blastobotrys adeninivorans* thrives under thermophilic conditions up to 45 °C [134]. Several wild-type strains of the yeast *Y. lipolytica* cultivated on glycerol have also demonstrated robust growth and storage lipid production at 20 °C [135]. In microalgae, thermal preferences are more diverse. Strains such as *Thalassiosira pseudonana*, *Odontella aurita*, *Nannochloropsis oculata*, *Isochrysis galbana*, *Chromulina ochromonoides*, and *Dunaliella tertiolecta* achieves peak lipid production near 20 °C [136]; whereas Converti et al. [137] reported that raising the cultivation temperature of *N. oculata* from 20 to 25 °C nearly doubled its lipid content (from 7.9% to 14.9%). Oleaginous bacteria typically display distinct optima ranging from 30 to 36 °C [39]. From an industrial perspective, selecting strains that thrive at ambient temperatures is crucial for reducing the energy costs associated with bioreactor cooling. This is particularly relevant during large-scale aerobic fermentations, where the metabolic activity is highly exothermic, generating significant heat that must be removed to maintain cell viability [43,138]. For example, the use of robust wild-type yeasts such as *C. oleaginosus* and *R. toruloides*, which maintain high lipid productivity at approximately 30 °C, allows for a greater temperature differential (ΔT) between the broth and the cooling medium. This minimizes the reliance on expensive refrigeration systems and reduces the required cooling water flow rates compared to cold-adapted species [76,125].

6.1.4. Aeration rate and agitation speed

In submerged fermentations, oxygen transfer and mixing strongly affect lipid production. Oxygen is vital for both cellular respiration and the enzymatic desaturation of fatty acid, while agitation ensures nutrient distribution without imposing damaging shear stress.

In oleaginous yeasts, controlled aeration and agitation significantly enhance lipid production. *Y. lipolytica* cultivated on molasses and crude glycerol reached ≈31% lipid content under 1.5 $L\ min^{-1}$ aeration and 800 rpm agitation [139]. Similarly, *C. curvatus* NRRL Y-1511 showed significantly higher biomass and lipid production in aerated bioreactor trials compared to shake-flask experiments [140]. Microalgae respond similarly, although they are more sensitive to mechanical stress; *Nannochloropsis oculata* accumulated maximal lipids (≈23%) at moderate shaking (200 rpm), whereas higher agitation caused mechanical stress and decreased yields [141].

In oleaginous fungi, oxygen supply and mixing are equally critical. In a 5-L bioreactor, *Cunninghamella bainieri* 2A1 achieved a lipid content of 38.7% under optimized aeration (0.32 vvm) and agitation (599 rpm), with regression analysis confirming these variables as the principal determinants of lipid productivity [142]. However, the scale-up of oleaginous Zygomycetes (e.g., *Mucor hiemalis*, *Cunninghamella elegans* and *M. isabellina*) remains challenging. Interestingly, studies in 3- to 20-L bioreactors did not always show significantly higher yields compared to shake-flask experiments [83,143,144]. This suggests that while shake flasks are effective for physiological studies, industrial scale-up for filamentous fungi must overcome specific rheological hurdles caused by mycelial viscosity, which can impede mass transfer in larger volumes.

6.2. Fermentation strategies

The fermentation configuration strongly determines the balance between biomass formation and lipid accumulation in oleaginous microorganisms. When whey or whey permeate is employed as feedstock, the strategy must also accommodate substrate-specific challenges, such as limited nitrogen content and the variable fermentability of lactose across species.

Batch fermentation remains the most widely used method for preliminary screenings and laboratory-scale trials. It is attractive due to its operational simplicity, low investment cost, and reduced contamination risk [138]. However, its limitations in whey valorization are evident. For example, Vasilakis et al. (2022) evaluated several wild type non-conventional yeast strains grown on second cheese whey, a by-product of Mizithra cheese production. In batch mode, *C. curvatus* ATCC 20509 achieved 22.40 $g\ L^{-1}$ biomass with 3.40 $g\ L^{-1}$ intracellular lipids, while *C. curvatus* NRRL Y-1511 reached 20.60 $g\ L^{-1}$ biomass and 3.20 $g\ L^{-1}$ lipids. Although significant amounts of biomass were obtained, lipid accumulation remained modest due to insufficient nitrogen depletion after whey deproteinization. These results highlight a primary constraint of batch setups: the difficulty in managing C/N dynamics to trigger lipogenesis effectively.

Fed-batch fermentation is frequently adopted to overcome these limitations by systematically supplying carbon sources. This allows modulation of the C/N ratio, mitigates substrate inhibition, and prolongs the productive phase of cultures. Vasilakis et al. (2022) reported that pulse supplementation with condensed cheese whey-derived lactose in fed-batch cultures of *C. curvatus* ATCC 20509 increased total dry weight to 38.10 $g\ L^{-1}$, with lipids accounting for ≈57% of cell dry weight (21.70 $g\ L^{-1}$ lipids). Similarly, Villegas-Méndez et al. (2023) demonstrated a twofold increase in lipid productivity using agro-waste hydrolysates in fed-batch mode compared to batch.

Continuous fermentation has been explored to improve process efficiency and reduce downtime. However, for oleaginous yeasts, continuous processes have consistently shown lower lipid productivities than fed-batch setups, challenging their industrial relevance. In continuous cultures of *C. curvatus* ATCC 50109 grown on whey permeate at low dilution rate ($D = 0.02\ h^{-1}$), increasing the C/N led to higher lipid content (≈45% $w\ w^{-1}$), but lower overall concentrations ≈10.00 $g\ L^{-1}$) compared to fed-batch trials, which reached ≈30.00 $g\ L^{-1}$ of lipids [77]. Koutinas et al. (2014) highlighted that the major cost drivers in microbial oil production are the capital and energy demands of large-scale

fermenters. Continuous systems, while theoretically advantageous for steady-state operation, have not yet achieved competitive performance in practice. Reported lipid titres and yields in continuous mode remain significantly below those of fed-batch cultures, where controlled nutrient feeding enables both high biomass generation and efficient lipid accumulation. Consequently, the economic feasibility of continuous systems remains a subject of debate, potentially requiring innovations in strain engineering or novel reactor configurations to offset high operational costs.

Two-stage cultivation strategies provide an effective compromise by decoupling biomass accumulation from lipid induction. In the first stage, microorganisms are cultivated in a nitrogen-sufficient medium to maximize cell density; in the second stage, nitrogen limitation is imposed to stimulate lipid accumulation [145]. Donzella et al. (2022) developed a two-step fermentation using *C. oleaginosus* on whey permeate. By supplementing with urea in the first phase and a carbon-rich syrup in the second, they increased lipid concentrations from 18.00 g L^{-1} to 38.00 g L^{-1}. In studies involving oleaginous bacteria such as *R. opacus*, high lipid yields achieved in fed-batch cultivation are directly attributable to the exhaustive consumption of nitrogen, which mimics a two-stage effect by triggering the genomic pathways responsible for TAGs biosynthesis [53,54]. Similarly, for microalgae such as *S. obliquus* and *Chlorella protothecoides*, the most successful mixotrophic fed-batch protocols effectively separate growth from accumulation by providing a carbon-loading phase once a target cell density is reached [46,47]. Filamentous fungi like *M. circinelloides* and *M. isabellina* also exhibit superior lipid accumulation in submerged fermentation when growth and induction are functionally separated. This allows for high biomass formation before mycelial viscosity creates rheological hurdles that limit oxygen transfer during the induction phase [52,81,86,89].

With regards to industrial scalability, a physical two-stage process involving the transfer of biomass between separate bioreactors is often unfeasible due to high capital expenditure and contamination risks. Consequently, for large-scale whey valorization, evidence suggests that a "pseudo-two-stage" fed-batch approach (conducted in a single vessel) is the most viable strategy. This configuration allows the transition to occur through the natural exhaustion of the initial nitrogen source followed by concentrated lactose supplementation [42,125]. However, the industrial competitiveness of any strain depends not only on fermentation yields but also on the efficiency of downstream processing. Biomass recovery and lipid extraction remain the primary technological bottlenecks that determine the final economic sustainability of the process.

7. Comparative analysis of waste-to-lipid feedstocks

The industrial feasibility of microbial lipids is governed by the metabolic synergy between the microbial host and the chemical architecture of the organic feedstock. As documented by Lee et al. (2024) and Cho & Park (2018), technical competitiveness depends on the host's resilience to growth inhibitors and the operational complexity of necessary pretreatments, as each substrate presents distinct biochemical bottlenecks that influence final biomass density and lipid productivity.

Oleaginous yeasts represent a highly flexible platform due to their ability to utilize both hydrophilic and hydrophobic molecules. Saccharides and glycerol are assimilated via de novo biosynthesis, where carbon is channelled through glycolysis to produce acetyl-CoA, the precursor for triacylglycerol (TAG) synthesis. In contrast, hydrophobic substrates like waste cooking oil (WCO) utilize the *ex novo* pathway, where fatty acids are directly incorporated into cellular TAGs. For example, *Cutaneotrichosporon curvatus* utilized sonicated WCO emulsions and achieved lipid yields of 20.30 g L^{-1} concurrently with cell growth, bypassing the nitrogen-limitation requirement typical of sugar-based fermentations [146]. However, sugar-rich substrates like lignocellulosic residues (rice or wheat straw) require intensive thermochemical pretreatment, which often releases inhibitory furan aldehydes and phenols. While inhibitor-tolerant strains like *Trichosporon dermatis* can reach lipid concentrations up of 31.00 g L^{-1} [147], the energy and cost of detoxification remain significant hurdles. Similarly, crude glycerol deriving from the biofuel industry requires at least pH neutralization and often further purification to remove inhibitory methanol and salts. Studies on *Naganishia diffluens* indicate that while crude glycerol can support lipogenesis, impurities can shift the fatty acid profile—notably decreasing MUFAs by 11%—and may necessitate co-valorization with nutrient-rich sludge to achieve industrial productivities [148,149]. In this context, whey permeate offers a comparatively streamlined integration into bioprocessing; unlike the extensive chemical detoxification required for lignocellulose or the multi-step purification of glycerol, whey preparation is primarily limited to standard operational adjustments.

Microalgae offer a dual benefit of nutrient remediation and lipid synthesis, though they typically achieve lower biomass concentrations in high-strength organic wastes compared to yeasts. *C. vulgaris* cultivated on sewage sludge reached a biomass concentration of 2.10 g L^{-1}, with productivity heavily influenced by pH control and the concentration of volatile fatty acids (VFAs). While food waste hydrolysates rich in glucose and amino acids can support species like *Schizochytrium* and *Chlorella*, high ammonium levels in digested effluents often act as growth inhibitors [150]. Creative substrate coupling has been shown to improve performance; for instance, mixing nitrogen-rich brewer fermentation waste with carbon-rich crude glycerol allowed *Chlorella protothecoides* to reach a biomass concentration of 12.70 g L^{-1} and a 6.60 g L^{-1} lipid content [151]. Agricultural hydrolysates from sugarcane bagasse have also been utilized, where sugars like glucose and xylose provided the energy for *Chlorella* spp. to reach a biomass concentration of 5.80 g L^{-1}, resulting in a lipid yield of 1.97 g L^{-1} [152]. Generally, the fatty acid profiles of these microalgal strains are rich in palmitic, stearic, or oleic acids, making them compatible with biodiesel standards.

Oleaginous bacteria, particularly members of the genus *Rhodococcus*, exhibit an exceptional ability to catabolize complex and toxic substances. When grown on gasification wastewater, *R. opacus* achieved a lipid content of 54.3% of dry cell weight, which corresponds to a volumetric lipid yield of 0.38 g L^{-1} from a biomass concentration of 0.70 g L^{-1} [153]. In lignocellulosic applications, *R. opacus* can simultaneously degrade sugars and aromatics, producing a lipid profile primarily composed of C18:1, C16:0 and C18:0 [153]. Other species, such as *Bacillus subtilis*, have achieved lipid yields of 2.27 g L^{-1} (from 5.70 g L^{-1} biomass) using cotton stalk hydrolysate, though the process requires careful control of the C/N ratio [154]. While bacteria generally exhibit lower lipid accumulation capacities compared to yeasts or algae, their rapid doubling times and inherent metabolic robustness provide a distinct operational advantage for the valorization of heterogeneous industrial effluents that contain inhibitory constituents. Table 3 outlines the volumetric lipid performance and fatty acid profiles achieved using various organic waste streams.

8. Methods of quantification of biomass and lipid production

Accurate assessment of lipid production is fundamental for identifying the most promising oleaginous strains prior to scale-up. In early-stage screening, optimal methods are those that provide rapid, reliable information on lipid accumulation without requiring destructive extraction procedures, whereas conventional methods remain valuable for detailed characterization once candidate strains have been selected.

Biomass monitoring is typically the first step in evaluating culture performance. Conventional approaches such as DCW measurements and colony forming unit (CFU) counts remain standard but are time-consuming and retrospective. Optical density (OD) is widely used as a faster proxy for cell concentration; however, its correlation with biomass frequently weakens under stationary conditions or when cells undergo morphological changes [164]. To overcome these limitations, flow cytometry (FC) has emerged as a powerful at-line tool [165]. Beyond providing real-time information on cell counts and viability, FC can also

Table 3
Performance evaluation of waste-derived microbial lipids.

Feedstock category	Microbial group	Species name	Biomass (g L^{-1})	Lipid yield (g L^{-1})	Fatty acid profile highlights	References
Whey permeate	Yeasts	*Cutaneotrichosporon oleaginosus*	50.0–150.0	18.0–38.0	C16:0 (18–25%), C18:0 (10–15%), C18:1 (50–65%)	[14]
Rice straw	Yeasts	*Trichosporon dermatis*	50.6	31.0	C16:0, C18:1 (major), C18:2	[147]
Waste cooking oil (WCO)	Yeasts	*Cutaneotrichosporon curvatus*	35.6	20.3	C16:0 (15%), C18:0 (12%), C18:1 (55%)	[146]
Crude glycerol with sludge	Yeasts	*Cutaneotrichosporon oleaginosus*	42.7	17.9	C16:0, C18:0, C18:1	[148]
Crude glycerol	Yeasts	*Candida viswanathii*	26.6	13.6	C18:2 (45%), C18:1 (29%), C16:0 (10%)	[155]
Food waste leachates	Yeasts	*Yarrowia lipolytica*	21.0	10.3	C16:0, C18:1 (major), C18:2	[156]
Brewer fermentation waste with crude glycerol	Microalgae	*Chlorella protothecoides*	12.7	6.6	C16:0, C18:0, C18:1	[151]
Sugarcane bagasse hydrolysate	Microalgae	*Chlorella* spp.	5.8	1.97	C16:0, C18:0, C18:1	[152]
Hydrolysate of cotton stalk	Bacteria	*Bacillus subtilis*	5.7	2.27	C16:0 (40%), C18:1 (30%), C18:0 (15%)	[154]
Dairy waste	Bacteria	*Rhodococcus opacus*	6.6	2.2	C16:0, C18:0, C18:1	[157]
Gasification waste	Bacteria	*Rhodococcus opacus*	0.7	0.38	C16:0 (46.9%), C18:0 (42.7%)	[158]
Sewage sludge	Microalgae	*Chlorella vulgaris*	2.1	0.38	C16:0, C18:0, C18:1, C18:2, C18:3	[159]
Wheat straw	Yeasts	*Trichosporon cutaneum*	30.7	12.7	–	[160]
Soybean hull	Yeasts	*Lipomyces starkeyi*	26.5	11.3	–	[161]
Pine needle	Yeasts	*Lipomyces starkeyi*	35.9	19.6	–	[162]
Crude glycerol	Yeasts	*Naganishia diffluens*	20.3	6.8	C18:1, C16:0 (shifted profile)	[149]
Wheat bran	Yeasts	*Lipomyces starkeyi*	17.1	6.4	Oleic acid (approx. 70%)	[163]

be combined with lipid-specific fluorescent dyes such as Nile Red or BODIPY 505/515, enabling direct quantification of intracellular lipids in living cells. This non-extractive approach allows for rapid screening of strains with superior lipid accumulation potential [166,167].

In contrast, traditional lipid quantification relies on solvent extraction followed by gravimetric determination or chromatographic analysis. While gravimetric assays are simple and accurate for total lipid content, they are destructive, laborious, and unsuitable for high-throughput screening [168]. Chromatographic methods such as gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography (HPLC) provide detailed fatty acid profiles, including chain length and degree of unsaturation, which are essential for assessing biodiesel quality [169]. However, these techniques also require extraction and derivatization steps, making them more appropriate for final validation of selected strains rather than initial screening.

To bridge this gap, several non-destructive and extraction-free spectroscopic methods have been developed. Fourier transform infrared (FTIR) spectroscopy is particularly advantageous, as it enables rapid, non-invasive lipid detection using minimal biomass. When combined with multivariate analysis, FTIR can reliably quantify lipids and monitor macromolecular allocation, offering a practical high-throughput screening tool. Recent applications include the use of FTIR in microtiter plate systems for evaluating oleaginous yeasts and fungi [170–172]. Similarly, Raman spectroscopy and hyperspectral imaging provide label-free detection of intracellular lipids and are being explored as additional screening strategies [173].

Taken together, these developments suggest a complementary workflow. In the screening stage, techniques such as flow cytometry with fluorescent dyes, FTIR, and Raman spectroscopy allow for the rapid, in situ identification of high-performing strains without the need for solvent extraction. In the validation stage, conventional gravimetric and chromatographic methods remain indispensable for confirming total lipid yields and fatty acid composition prior to scale-up.

9. Downstream process

Oleaginous microorganisms produce intracellular lipids that serve as a fundamental feedstock for biodiesel production. The downstream processing of SCOs involves biomass harvesting, lipid extraction, and purification of the final product. Each stage requires methods tailored to the specific microbial strain and cultivation medium. In the context of whey-based substrates, the high water content and relatively low initial lipid concentration present substantial challenges for cell separation and concentration, directly impacting the cumulative energy demand and overall production costs. Fig. 4 illustrates a downstream process flowchart for biodiesel production from oleaginous microorganisms.

9.1. Biomass harvesting

Harvesting microbial biomass is a critical economic bottleneck in SCO production, often accounting for 20–30% of total operational costs [174]. The primary objective is the efficient separation of small, dilute cells to produce a concentrated slurry for extraction. Centrifugation is widely utilized for its high efficiency and scalability, though it remains energy-intensive and may induce mechanical stress on sensitive cells [175]. Filtration separates biomass based on cell size, utilizing conventional filters for larger aggregates or specialized membranes for smaller taxa; however, efficiency is frequently compromised by membrane fouling, and capital costs can be prohibitive for large-scale operations [176–178].

Flocculation, using chemical agents, polymers or bio-flocculants (via co-cultivation), offers a lower-energy alternative. Nevertheless, chemical flocculants may introduce toxicity or complicate downstream purification, while bio-flocculation introduces risks of microbial contamination [179,180].

In whey-based media, harvesting efficiency is often enhanced through hybrid strategies; for example, flocculation can facilitate the formation of larger aggregates that are subsequently recovered via filtration. While drying the harvested biomass can improve the efficiency of subsequent extraction, it significantly increases the total energy footprint of the process [181]. As with other systems, no single approach is universally effective; the choice depends on the microorganism, the properties of whey, and the intended application.

9.2. Lipid extraction

Efficient lipid recovery requires effective pretreatment to disrupt the robust cellular structures and enhance access to intracellular lipids. Mechanical disruption strategies utilize physical forces to compromise cell walls, including bead milling, high-pressure homogenization, and microfluidization. Energy can also be applied via ultrasonic waves, microwaves, or pulsed electric fields [182]. While effective, these

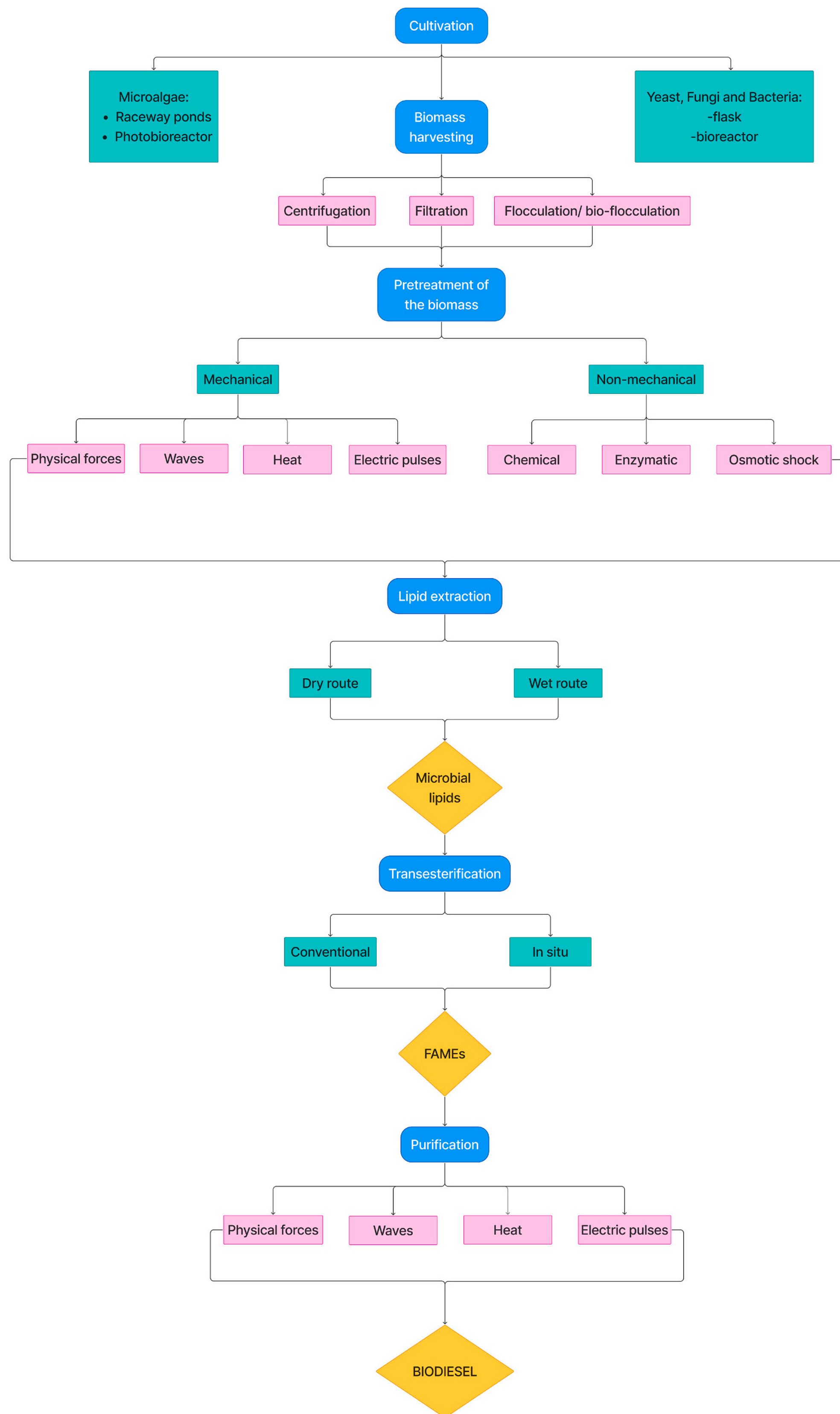

Fig. 4. Flowchart showing the downstream process for biodiesel production from oleaginous microorganisms. The diagram outlines the full sequence of steps, starting with Cultivation (using Microalgae in Photobioreactors or Yeast, Fungi, and Bacteria in Bioreactors), followed by Biomass harvesting methods (Centrifugation, Filtration, Flocculation/Bio-flocculation), and Pretreatment of the Biomass (via Mechanical or Non-mechanical methods) to disrupt the cells. This is followed by Lipid extraction (Dry or Wet route), and subsequently Transesterification of the microbial lipids (conventional or in situ) to produce FAMEs (Fatty Acid Methyl Esters). The final step is Purification (wet/dry washing or Membrane methods) to yield the final Biodiesel product.

methods often require high power inputs, long processing times and sometimes the use of hazardous solvents, making them less suitable for wet biomass or industrial-scale applications. Conversely, non-mechanical approaches, such as enzymatic or chemical cell lysis and osmotic shock, operate under milder conditions and consume less energy, though their current high costs and prolonged reaction times limit their industrial implementation [181].

Following pretreatment, lipid extraction can proceed via dry or wet routes. The dry route, while simplifying solvent penetration and improving analytical yields, is highly energy-intensive due to the requirement for oven drying or lyophilization, particularly when processing dilute substrates like cheese whey [9]. In contrast, the wet route extracts lipids directly from freshly harvested or minimally dewatered biomass, bypassing the drying step. This approach significantly reduces energy consumption and is increasingly recognized as a more industrially relevant strategy [9]. Conventional methods such as Bligh & Dyer and Folch utilize chloroform and methanol mixtures (2:1 v v^{-1}) to separate lipids from the cellular matrix [183,184]. While Bligh & Dyer method is often cited as providing higher lipid purity and greater suitability for pilot-scale operations; the Folch method is recognized for its speed, though it may exhibit lower sensitivity in certain matrices [185]. Recent modifications to these protocols, including the incorporation of acid pre-treatments or the substitution of solvents with methyl-tert-butyl ether (MTBE), have been shown to improve overall lipid recovery while specifically preserving the integrity of polyunsaturated fatty acids [186]. Furthermore, greener alternatives such as 2-ethoxyethanol (2-EE), are being explored to mitigate environmental and safety risks without compromising extraction efficiency [187]. Among emerging techniques, supercritical CO_2 extraction (SC-CO_2) has attracted significant attention due to its solvating properties, non-toxicity, and minimal environmental footprint [188]. While SC-CO_2 represents a potential alternative to high-energy mechanical cell disruption, its efficiency is typically maximized when applied to dried biomass. Although SC-CO_2 is highly effective for extracting neutral lipids and can theoretically be applied to wet biomass, the general consensus in the literature indicates that high water content reduces overall extraction yield, primarily due to diffusion limitations [93,189]. Interestingly, some studies have reported that the presence of water can act as a selective modifier, potentially promoting the extraction of specific target components, such as MUFA [189]. While this selective effect may partially mitigate the requirement for intensive drying, SC-CO_2 alone remains limited to the recovery of non-polar lipids. Consequently, to efficiently capture the complete spectrum of microbial lipids, including polar fractions, the addition of a polar co-solvent (such as ethanol) remains necessary for high yields [185,190]. This integrated approach reduces reliance on hazardous organic solvents and minimizes processing steps; however, its transition to large-scale operations is still subject to the optimization of high-pressure equipment costs and energy consumption.

9.3. Transesterification

Following lipid extraction, the subsequent phase in biodiesel production is transesterification, in which TAGs are converted into fatty acid methyl esters (FAMEs) via reaction with an alcohol (typically methanol or ethanol), yielding glycerol as a byproduct [191]. The efficiency of this conversion is governed by several parameters, including the microbial lipid profile, reaction temperature, duration, alcohol-to-oil ratio, and the catalyst employed.

Two primary process configurations are utilized. The conventional approach maintains extraction and transesterification as distinct, sequential steps. Alternatively, direct (in situ) transesterification integrates these processes into a single operation, potentially reducing processing time and solvent requirements [190].

Catalysts are essential for achieving competitive conversion yields. In homogeneous catalysis, the catalyst (acid or alkali) is in the same phase as the reactants. Alkali catalysts are fast but prone to soap formation when free fatty acids are present, whereas acid catalysts are slower but more tolerant [192]. In fact, homogeneous base-catalyzed transesterification (catalysis employed by sodium hydroxide; NaOH and potassium hydroxide; KOH catalysts) is indeed the most effective type of catalysis implicated [193]. Conversely, homogeneous acid catalysis — utilizing inorganic acids (phosphoric, hydrochloric, or sulfuric) or organic alternatives (4-dodecylbenzene sulfonic acid) — is more robust when processing feedstocks with high acidity or water content, which is frequently the case with microbial oils derived from taxa such as *Chlorella* spp. [193]. Heterogeneous acid or base catalyzed transesterification, facilitates easier recovery and reuse, representing a potential path toward lowering operational costs, though it typically requires more stringent reaction conditions [194]. Enzymatic catalysis, mainly with lipases, offers mild conditions, high selectivity, and fewer by-products. Immobilized lipases further improve stability and reusability, though high costs remain a barrier [195,196].

Alternative direct methods have also been investigated. Supercritical transesterification utilizes alcohols above their critical point, bypassing the need for biomass drying and catalyst addition. Microwave-assisted transesterification simultaneously disrupts cells and accelerates the reaction, reducing time and improving yields [197,198]. Finally other alternative state-of-the art processes include ultrasound-assisted transesterification, which utilizes acoustic waves (10–100 kHz) to increase biodiesel yields at lower temperatures. Similarly, transesterification implicating electrolysis has been successfully implemented using microbial lipids (deriving from *Spirulina* sp. and *Naganishia liquefaciens*) as feedstock for biodiesel production [193,199].

9.4. Purification

Transesterification produces crude biodiesel that requires significant refining before it can be utilized in internal combustion engines. This crude mixture typically contains residual glycerol, catalysts traces, water, soap, and unreacted lipids, all of which compromise fuel quality and stability [190]. Therefore, purification is an essential final step in the downstream process, not merely a formality.

Wet washing remains the traditional method for biodiesel refining, effectively removing water-soluble impurities. However, this approach is increasingly scrutinized due to its excessive water consumption and the subsequent need for energy-intensive drying and wastewater treatment [200,201]. To overcome these drawbacks, dry washing has gained traction. This method employs adsorbents such as silica, starch derivatives, ion-exchange resins, or even low-cost materials like chamotte, to selectively remove impurities without introducing water. While dry washing minimizes product loss and allows customization depending on the contaminants present [202], the disposal of spent adsorbents represents a secondary waste management challenge.

Emerging membrane technologies offer another pathway for biodiesel purification [203]. Membranes made from chemically and thermally stable materials, such as poly vinylidene fluoride (PVDF) and poly dimethyl siloxane or ceramics, can selectively filter out water and residual contaminants [190]. They operate under mild conditions, consuming less energy and generating a smaller environmental footprint. Nevertheless, fouling from glycerol, soap, or particulates is a practical limitation, requiring careful pretreatment or optimized solvent selection to maintain efficiency [204].

Increasingly, integrated approaches that combine methods are being explored. For example, coupling wet and dry washing sequentially can yield higher-quality biodiesel while mitigating the limitations of each method [201]. The ultimate goal is to produce a fuel that strictly meets EN and ASTM standards, while carefully balancing operational costs, environmental impact, and energy consumption. Future advances are expected to focus on the development of greener solvents and more effective adsorbents, alongside integrated processes designed to simplify purification while ensuring consistent biodiesel quality.

10. Genetic and adaptive approaches

The efficient valorisation of whey by oleaginous microorganisms is often hindered by their limited capacity to metabolize lactose and their sensitivity to complex waste compositions. Genetic engineering has provided valuable proof-of-concept solutions to these challenges. For example, *Y. lipolytica* engineered with secreted β-galactosidases from *Aspergillus niger* and optimized galactose metabolism, combined with lipid pathway overexpression (ACC1, DGA1), achieved complete sugar utilization and microbial oil production directly from acid whey [40,205]. Despite the promise of such strategies, the deployment of genetically modified strains is constrained by regulatory frameworks and consumer acceptance. Furthermore, the genetic stability of such high-yielding strains under large-scale industrial fermentation conditions represents a significant engineering challenge that has yet to be fully addressed.

By contrast, Adaptative Laboratory Evolution (ALE) provides a non-transgenic alternative to improve strain performance under whey-like conditions. ALE exposes microorganisms to defined selective pressures over extended periods, enabling the accumulation of beneficial mutations that enhance growth and metabolite synthesis [206,207]. Evolution can be conducted through serial batch transfers, which are cost-effective but prone to environmental fluctuations, or in continuous cultivation systems, which provide stable selective conditions but require more advanced infrastructure [208]. The degree of selective stress is critical, as mild pressures slow adaptation while excessive stress can cause population collapse [209].

ALE has successfully enhanced lipid productivity and robustness in several oleaginous microorganisms. For instance, *R. toruloides* adapted to hydrolysate-based media exhibited improved growth, lipid, and carotenoid accumulation [210]. Likewise, *Y. lipolytica* evolved for tolerance to lignocellulosic inhibitors showed enhanced lipid production [211].

11. Biorefinery concept for oleaginous microorganisms

The biorefinery concept applied to cheese whey focuses on the integrated production of multiple products, maximizing resource efficiency while mitigating the environmental burden of this abundant dairy by-product. Whey, rich in lactose, residual proteins, and minerals, can serve as a low-cost feedstock for oleaginous microorganisms capable of synthesizing lipids, pigments, polysaccharides, and other metabolites.

Oleaginous yeasts such as *Y. lipolytica*, *C. oleaginosus*, *C. curvatus*, and L. *starkeyi* efficiently convert whey-derived sugars into TAGs with fatty acid profiles (mainly C16 and C18 species) suitable for biodiesel production [14,72,212]. In parallel, some strains such as *Rhodotorula* spp. can produce carotenoids (e.g. β-carotene, torulene, and torularhodin) adding value for food, feed, and nutraceutical applications [213,214]. Other yeasts, including *P. laurentii*, *C. curvatrus* and *C. albidus* grown on second cheese whey, have been reported to produced significant amounts of endopolysaccharides and/or secrete extracellular polysaccharides while also accumulating intracellular lipids, thereby expanding the product spectrum toward biomaterials and functional food additives [51,116,118,119].

Practically, the transition to an integrated biorefinery depends on managing technical trade-offs between product diversity and recovery costs. For instance, recent techno-economic modelling of microbial lipids demonstrates a minimum selling price (MSP) of US$ 1.81 kg^{-1}, which can only be reduced to US$ 1.20 kg^{-1} at massive industrial capacities (~48,000 t/year) [215]. However, these models indicate that if co-products are simultaneously produced and sold in excess of US$ 1.00 kg^{-1}, the effective production price of the lipid can be reduced, heavily subsidizing the process [215]. Validating this, recent case studies using red yeasts (*Rhodotorula* spp.) on agri-food residues have demonstrated the ability to yield up to 56% w w^{-1} intracellular lipids alongside significant titers of high-value carotenoids (e.g., 140 mg L^{-1} β-carotene) [216]. Nevertheless, recovering these pigments introduces significant downstream complexity. Separating carotenoids from TAGs requires specialized extraction, which increases the total Operating Expenses (OPEX) and demands high intracellular titers to justify the separation costs.

In downstream processing, biodiesel production via trans-esterification generates glycerol as a major by-product. In a circular biorefinery model, this crude glycerol (representing approx. 10% w w^{-1} of the total biodiesel output) is often viewed as a carbon source for microbial fermentation or upgraded into platform chemicals such as 1,3-propanediol, succinic acid, or polyols [193,217–219]. However, due to the rapid global expansion of biodiesel, the raw market value of this crude glycerol has plummeted to below US$ 0.11 kg^{-1} [220]. Upgrading it to refined platform chemicals requires energy-intensive purification stages (e.g., ion exchange and vacuum distillation), which are cost-prohibitive unless managed at a large scale [221]. Microalgae have also been cultivated on cheese whey, such as *C. vulgaris,* producing both lipids and pigments such as carotenoid and chlorophyll derivatives, reinforcing the potential of whey as a versatile platform for high-value bioproducts [222].

Following lipid extraction, the microbial biomass remains rich in proteins, vitamins, and residual carbohydrates. This nutrient-dense material can be processed into animal feed ingredients, contributing to feed security while supporting circularity within the agri-food sector. Techno-economic models demonstrate that valorizing this whole yeast cell residue can drastically improve plant economics, potentially dropping the effective production cost of the primary microbial lipid from US$ 1.20 kg^{-1} down to US$ 0.81 kg^{-1} [215]. However, achieving food-grade safety standards for feed applications requires the complete removal of residual extraction solvents and moisture. This necessitates heavy thermal treatments; industrial-scale plant designs indicate that the necessary spray dryers and air heaters add over US$ 1.1 million strictly to the downstream Capital Expenditures (CAPEX) [215]. As detailed in Section 12, balancing this massive capital investment and its associated energy demands requires strictly maximizing the revenue from the resulting de-oiled biomass. Collectively, these market dynamics illustrate that the biorefinery is not merely a theoretical maximization of resources; rather, it is a delicate economic balance where the added revenue from high-value co-products must carefully offset the compounding CAPEX and OPEX required for multi-stage separation [126].

12. Techno-economic analysis (TEA) and industrial feasibility

The industrial transition of whey-based microbial lipid production hinges on a favourable balance between CAPEX and OPEX. While laboratory yields are promising, a robust economic model must account for the high cost of stainless-steel infrastructure and the energy-intensive nature of intracellular lipid recovery [102,223]. The industrial feasibility of this process is governed by the International Organization for Standardization (ISO) 14,040/14044 framework, which necessitates an exhaustive depiction of the environmental trade-offs across upstream, midstream, and downstream stages [43,122]. A primary economic driver for this process is the "negative cost" of the substrate, as untreated cheese whey represents a significant environmental liability. With COD levels reaching up to 80.00 g L^{-1}, dairies often face municipal discharge surcharges or environmental taxes ranging from US$ 0.05 to US$ 0.20 per m^3 [45,224]. In a regional facility processing approximately 539 m^3 per day, the adoption of a "Gate Fee" model creates an immediate revenue stream. This strategy achieves over 80% COD reduction while effectively decoupling the plant's profitability from the high costs of traditional sugar sources [224].

Regarding the energy demands of downstream processing, which typically accounts for 30–60% of total OPEX, the choice of recovery route serves as a critical economic variable. Mechanical cell disruption methods, such as high-pressure homogenization, require an energy input of approximately 1.0 to 2.5 kWh per kg of dry biomass [225].

While traditional dry-route extraction involves a significant energy "sink" for thermal moisture removal, consuming between 3.5 and 5.0 MJ per kg of evaporated water, the implementation of wet extraction techniques may offer potential reductions in overall energy costs [225]. However, the feasibility of wet extraction remains technically sensitive to specific process parameters, such as the efficiency of cell wall disruption in aqueous media and the complexity of subsequent solvent recovery. These methods must be optimized to ensure that energy savings are not offset by lower lipid recovery yields or increased solvent-water separation costs [102]. Furthermore, the efficiency of the final transesterification of extracted lipids into biodiesel is a key determinant of the final price. Engineering models indicate that achieving a conversion efficiency of 93–97% is necessary to maintain economic competitiveness, with the cost of the catalyst and alcohol representing significant variable inputs [226].

The initial investment for a commercial-scale plant is largely dominated by the fermentation section, where a facility with a 1000 m^3 bioreactor capacity requires an estimated CAPEX of US$ 15–25 million [44]. Beyond direct costs, environmental sustainability metrics such as Global Warming Potential (GWP) and Net Energy Ratio (NER) play an increasingly vital role in industrial feasibility. One LCA study estimated that a metric ton of lipid production can require a cumulative energy output of 29,693 kWh, resulting in emissions of roughly 1905 kg CO_2 equivalent [42]. However, these impacts can be mitigated through high-efficiency solvent recovery (>95%) and the valorization of biogenic carbon. In sugar-based models, biogenic CO_2 storage is estimated at 2.82 tons per ton of lipids; in a whey-integrated biorefinery, this carbon capture is coupled with a reduction in eutrophication potential [126,227].

To ensure a positive Net Present Value (NPV) within a 7–10 year timeframe, the facility should ideally operate as a multi-product biorefinery. By offsetting the heavy downstream CAPEX through the simultaneous sale of high-value carotenoids and de-oiled yeast biomass (approx. 0.48 tons per ton of lipid), the highly competitive target MSP for microbial biodiesel of approximately US$ 1.20 kg^{-1} becomes achievable. The economic model is further stabilized by recycling the generated crude glycerol to enhance subsequent lipid titers and the inclusion of Carbon Credits for avoided emissions, providing a fiscal buffer of US$ 50–90 per ton of CO_2 equivalent avoided [44,217,224]. Sensitivity analyses indicate that the most critical profitability threshold is lipid production; achieving >20 g L^{-1} is essential to decouple the MSP from the volatility of utility prices. While combining these revenue streams mitigates economic risks, the technical challenges of maintaining consistent product quality and process stability at industrial scales remain the primary hurdles to widespread commercial implementation [126,223].

13. Conclusions and future perspectives

Microbial lipids produced from dairy waste exhibit high concentrations of oleic, palmitic, and stearic acids, that are remarkably similar to conventional vegetable oils such as palm, canola, and soybean. This chemical composition is critical, as it ensures that the resulting SCOs meet the oxidative stability and cold-flow property requirements mandated by international biodiesel standards (e.g., EN 14214). Despite notable academic progress, the transition from laboratory-scale lipid accumulation to industrial biodiesel production remains a relatively novel challenge, with several technical hurdles hindering large-scale implementation.

The path to industrial viability is primarily constrained by significant upstream and downstream bottlenecks. Upstream, the energy-intensive nature of sterilization and the high oxygen transfer requirements necessary to maintain lipid productivity in large-volume bioreactors present major operational costs. Downstream, the recovery of lipids remains a primary economic barrier; current methods for cell harvesting and the requirement for efficient, non-toxic solvent extraction systems must be further optimized to ensure that the final biodiesel is economically competitive with fossil fuels and first-generation biofuels. The utilization of dairy by-products like whey presents a promising avenue to reduce the raw material costs that often dominate the economic profile of microbial lipids. Furthermore, regulatory frameworks such as European Union Regulation 2009/28/EC establish ambitious targets for third-generation biofuels, providing a policy environment that favours the valorisation of such industrial side-streams. Ultimately, bridging the gap between lab-scale titers and commercial deployment will require an integrated biorefinery approach that balances high volumetric productivity with cost-effective recovery technologies. By addressing these scale-up challenges, whey-derived SCOs can transition from a waste-mitigation strategy to a sustainable, industrial-scale contributor to global renewable energy targets.

CRediT authorship contribution statement

Andrea Cantarini: Writing – original draft, Methodology, Investigation, Conceptualization. **Seraphim Papanikolaou:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Afroditi Chatzifragkou:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Vesna Milanović:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Conceptualization. **Andrea Osimani:** Visualization, Validation, Methodology. **Lucia Aquilanti:** Visualization, Validation, Supervision, Conceptualization. **Cristiana Garofalo:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Funding

This work was supported by the project *"Sviluppo di un processo biotecnologico per la valorizzazione di sottoprodotti dell'industria lattiero-casearia"*, funded within the program "Dottorati Innovativi a caratterizzazione industriale", Avviso Pubblico decreto n. 86/IISP del 5/5/2025, P.R. Marche FSE+ 2021–2027, Asse 4 – OS 4a.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

References

[1] J. Love, Microbial pathways for advanced biofuel production, Biochem. Soc. Trans. 50 (2022) 987–1001, https://doi.org/10.1042/BST20210764.
[2] Energy Institute, Statistical Review of World Energy 2025, 2025 https://www.energyinst.org/statistical-review (accessed July 30, 2025).
[3] International Energy Agency, Global Energy and Climate Model 2024, 2024. https://www.iea.org/reports/global-energy-and-climate-model-2024 (accessed July 30, 2025).
[4] International energy agency, biofuels - energy System. https://www.iea.org/reports/biofuels, 2023 (accessed July 29, 2025).
[5] International Energy Agency, Transport biofuels – Renewables 2023 – Analysis. https://www.iea.org/reports/renewables-2023/transport-biofuels, 2023 (accessed July 29, 2025).
[6] Research And Markets, Biofuels Market Outlook 2024–2028, 2024.
[7] H.A. Alalwan, A.H. Alminshid, H.A.S. Aljaafari, Promising evolution of biofuel generations, Subject review, Renewable Energy Focus 28 (2019) 127–139, https://doi.org/10.1016/j.ref.2018.12.006.
[8] D.D. Nunes, V.L. Pillay, E. Van Rensburg, R.W.M. Pott, Oleaginous microorganisms as a sustainable oil source with a focus on downstream processing and cost-lowering production strategies: A review, Bioresour. Technol. Rep. 26 (2024) 101871, https://doi.org/10.1016/j.biteb.2024.101871.
[9] A. Patel, D. Karageorgou, E. Rova, P. Katapodis, U. Rova, P. Christakopoulos, L. Matsakas, An overview of potential oleaginous microorganisms and their role in biodiesel and Omega-3 fatty acid-based industries, Microorganisms 8 (2020) 434, https://doi.org/10.3390/microorganisms8030434.

[10] R.A. Dar, T.-H. Tsui, L. Zhang, Y.W. Tong, S. Sharon, O. Shoseyov, R. Liu, Fermentation of organic wastes through oleaginous microorganisms for lipid production - challenges and opportunities, Renew. Sust. Energ. Rev. 195 (2024) 114328, https://doi.org/10.1016/j.rser.2024.114328.

[11] A.F. Pires, N.G. Marnotes, O.D. Rubio, A.C. Garcia, C.D. Pereira, Dairy by-products: A review on the valorization of whey and second cheese whey, Foods 10 (2021) 1067, https://doi.org/10.3390/foods10051067.

[12] T. Ahmad, R.M. Aadil, H. Ahmed, U. Ur Rahman, B.C.V. Soares, S.L.Q. Souza, T. C. Pimentel, H. Scudino, J.T. Guimarães, E.A. Esmerino, M.Q. Freitas, R. B. Almada, S.M.R. Vendramel, M.C. Silva, A.G. Cruz, Treatment and utilization of dairy industrial waste: A review, Trends Food Sci. Technol. 88 (2019) 361–372, https://doi.org/10.1016/j.tifs.2019.04.003.

[13] S.R. Macwan, B.K. Dabhi, S.C. Parmar, K.D. Aparnathi, Whey and its utilization, Int. J. Curr. Microbiol. App. Sci. 5 (2016) 134–155, https://doi.org/10.20546/ijcmas.2016.508.016.

[14] S. Donzella, A. Fumagalli, S. Arioli, L. Pellegrino, P. D'Incecco, F. Molinari, G. Speranza, D. Ubiali, M.S. Robescu, C. Compagno, Recycling food waste and saving water: optimization of the fermentation processes from cheese whey permeate to yeast oil, Fermentation 8 (2022) 341, https://doi.org/10.3390/fermentation8070341.

[15] M.P. Ryan, G. Walsh, The biotechnological potential of whey, Rev. Environ. Sci. Biotechnol. 15 (2016) 479–498, https://doi.org/10.1007/s11157-016-9402-1.

[16] S. Kaminarides, H. Zagari, E. Zoidou, Effect of whey fat content on the properties and yields of whey cheese and serum, Journal of the Hellenic Veterinary Medical Society 71 (2020) 2149, https://doi.org/10.12681/jhvms.23640.

[17] D. Buchanan, W. Martindale, E. Romeih, E. Hebishy, Recent advances in whey processing and valorisation: technological and environmental perspectives, Int. J. Dairy Technol. 76 (2023) 291–312, https://doi.org/10.1111/1471-0307.12935.

[18] M. Valdez Castillo, V. Laxman Pachapur, S.K. Brar, M. Naghdi, S. Arriaga, A. Ávalos Ramirez, Yeast-driven whey biorefining to produce value-added aroma, flavor, and antioxidant compounds: technologies, challenges, and alternatives, Crit. Rev. Biotechnol. 40 (2020) 930–950, https://doi.org/10.1080/07388551.2020.1792407.

[19] M. Ghasemi, A. Ahmad, T. Jafary, A.K. Azad, S. Kakooei, W.R. Wan Daud, M. Sedighi, Assessment of immobilized cell reactor and microbial fuel cell for simultaneous cheese whey treatment and lactic acid/electricity production, Int. J. Hydrog. Energy 42 (2017) 9107–9115, https://doi.org/10.1016/j.ijhydene.2016.04.136.

[20] P.M.R. Guimarães, J.A. Teixeira, L. Domingues, Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey, Biotechnol. Adv. 28 (2010) 375–384, https://doi.org/10.1016/j.biotechadv.2010.02.002.

[21] I.G. Malos, A.-I. Ghizdareanu, L. Vidu, C.B. Matei, D. Pasarin, The role of whey in functional microorganism growth and metabolite generation: A biotechnological perspective, Foods 14 (2025) 1488, https://doi.org/10.3390/foods14091488.

[22] D. Rocha-Mendoza, E. Kosmerl, A. Krentz, L. Zhang, S. Badiger, G. Miyagusuku-Cruzado, A. Mayta-Apaza, M. Giusti, R. Jiménez-Flores, I. García-Cano, Invited review: acid whey trends and health benefits, J. Dairy Sci. 104 (2021) 1262–1275, https://doi.org/10.3168/jds.2020-19038.

[23] C.S. Osorio-González, N. Gómez-Falcon, S.K. Brar, A.A. Ramírez, Cheese whey as a potential feedstock for producing renewable biofuels: A review, Energies (Basel) 15 (2022) 6828, https://doi.org/10.3390/en15186828.

[24] G.Q. Chen, Y. Qu, S.L. Gras, S.E. Kentish, Separation Technologies for Whey Protein Fractionation, Food Eng. Rev. 15 (2023) 438–465, https://doi.org/10.1007/s12393-022-09330-2.

[25] S. Cheng, M. Hummel, B. Dahal, Z. Gu, P. Kharel, S.I. Martínez-Monteagudo, A two-step process for the synthesis of sweetening syrup from aqueous lactose, LWT 117 (2020) 108659, https://doi.org/10.1016/j.lwt.2019.108659.

[26] K.O. Iwuozor, Prospects and challenges of using coagulation-flocculation method in the treatment of effluents, Advanced Journal of Chemistry-Section A 2 (2019) 105–127.

[27] M. Kasmi, Biological processes as promoting way for both treatment and valorization of dairy industry effluents, Waste Biomass Valor. 9 (2018) 195–209, https://doi.org/10.1007/s12649-016-9795-7.

[28] M. Pescuma, G.F. de Valdez, F. Mozzi, Whey-derived valuable products obtained by microbial fermentation, Appl. Microbiol. Biotechnol. 99 (2015) 6183–6196, https://doi.org/10.1007/s00253-015-6766-z.

[29] A. Vamvakaki, I. Kandarakis, S. Kaminarides, M. Komaitis, S. Papanikolaou, Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes, Eng. Life Sci. 10 (2010) 348–360, https://doi.org/10.1002/elsc.201000063.

[30] E. Carota, S. Crognale, A. D'Annibale, A.M. Gallo, S.R. Stazi, M. Petruccioli, A sustainable use of ricotta cheese whey for microbial biodiesel production, Sci. Total Environ. 584–585 (2017) 554–560, https://doi.org/10.1016/j.scitotenv.2017.01.068.

[31] H.U. Cho, J.M. Park, Biodiesel production by various oleaginous microorganisms from organic wastes, Bioresour. Technol. 256 (2018) 502–508, https://doi.org/10.1016/j.biortech.2018.02.010.

[32] F. Arous, F. Frikha, I.-E. Triantaphyllidou, G. Aggelis, M. Nasri, T. Mechichi, Potential utilization of agro-industrial wastewaters for lipid production by the oleaginous yeast Debaryomyces etchellsii, J. Clean. Prod. 133 (2016) 899–909, https://doi.org/10.1016/j.jclepro.2016.06.040.

[33] J. Cescut, L. Fillaudeau, C. Molina-Jouve, J.-L. Uribelarrea, Carbon accumulation in Rhodotorula glutinis induced by nitrogen limitation, Biotechnol. Biofuels 7 (2014) 164, https://doi.org/10.1186/s13068-014-0164-0.

[34] S. Papanikolaou, G. Aggelis, Lipids of oleaginous yeasts, Part I: Biochemistry of Single Cell Oil Production, European Journal of Lipid Science and Technology 113 (2011) 1031–1051, https://doi.org/10.1002/ejlt.201100014.

[35] P.M. Dewick, Medicinal natural products, Wiley, 2009, https://doi.org/10.1002/9780470742761.

[36] C. Ratledge, Fatty acid biosynthesis in microorganisms being used for single cell oil production, Biochimie 86 (2004) 807–815, https://doi.org/10.1016/j.biochi.2004.09.017.

[37] G.S. dos Costa, W.J. Martinez-Burgos, G.A. dos Reis, Y.P. Puche, F.R. Vega, C. Rodrigues, J.L. Serra, S.M. de Campos, C.R. Soccol, Advances in Biomass and Microbial Lipids Production: Trends and Prospects, Processes 12 (2024) 2903, https://doi.org/10.3390/pr12122903.

[38] European Commission, Directive 2001/18/EC on the Deliberate Release into the Environment of Genetically Modified Organisms. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32001L0018, 2001 (accessed August 11, 2025).

[39] D. Koreti, A. Kosre, S.K. Jadhav, N.K. Chandrawanshi, A comprehensive review on oleaginous bacteria: an alternative source for biodiesel production, Bioresour. Bioprocess. 9 (2022) 47, https://doi.org/10.1186/s40643-022-00527-1.

[40] M. Tai, G. Stephanopoulos, Engineering the push and pull of lipid biosynthesis in oleaginous yeast Yarrowia lipolytica for biofuel production, Metab. Eng. 15 (2013) 1–9, https://doi.org/10.1016/j.ymben.2012.08.007.

[41] H.J.S. Lopes, N. Bonturi, E.J. Kerkhoven, E.A. Miranda, P.-J. Lahtvee, C/N ratio and carbon source-dependent lipid production profiling in Rhodotorula toruloides, Appl. Microbiol. Biotechnol. 104 (2020) 2639–2649, https://doi.org/10.1007/s00253-020-10386-5.

[42] R. Robles-Iglesias, C. Naveira-Pazos, C. Fernández-Blanco, M.C. Veiga, C. Kennes, Factors affecting the optimisation and scale-up of lipid accumulation in oleaginous yeasts for sustainable biofuels production, Renew. Sust. Energ. Rev. 171 (2023) 113043, https://doi.org/10.1016/j.rser.2022.113043.

[43] T. Sharma, D. Dasgupta, J. Singh, T. Bhaskar, D. Ghosh, Yeast lipid-based biofuels and oleochemicals from lignocellulosic biomass: life cycle impact assessment, sustain, Energy Fuel 4 (2020) 387–398, https://doi.org/10.1039/C9SE00540D.

[44] A.B. Ozturk, Z.D. Acar, F. Yilmaz, O. Sagdic, Whey conversion scenarios for sustainable lactic acid, ethanol and hydrogen production: techno-economic aspects of biofuels and biochemical production, Biofuels Bioprod. Biorefin. 19 (2025) 1463–1482, https://doi.org/10.1002/bbb.2761.

[45] A.R. Prazeres, F. Carvalho, J. Rivas, Cheese whey management: A review, J. Environ. Manag. 110 (2012) 48–68, https://doi.org/10.1016/j.jenvman.2012.05.018.

[46] J.-M. Girard, M.-L. Roy, M. Ben Hafsa, J. Gagnon, N. Faucheux, M. Heitz, R. Tremblay, J.-S. Deschênes, Mixotrophic cultivation of green microalgae Scenedesmus obliquus on cheese whey permeate for biodiesel production, Algal Res. 5 (2014) 241–248, https://doi.org/10.1016/j.algal.2014.03.002.

[47] W.S. da Borges, B.S.A. Araújo, L.G. Moura, U. Coutinho Filho, M.M. de Resende, V.L. Cardoso, Bio-oil production and removal of organic load by microalga Scenedesmus sp. using culture medium contaminated with different sugars, cheese whey and whey permeate, J. Environ. Manag. 173 (2016) 134–140, https://doi.org/10.1016/j.jenvman.2015.11.015.

[48] J.E. Abril Bonett, P. de Sousa Geraldino, P.G. Cardoso, F. de Freitas Coelho, W. F. Duarte, Isolation of freshwater microalgae and outdoor cultivation using cheese whey as substrate, Biocatal. Agric. Biotechnol. 29 (2020) 101799, https://doi.org/10.1016/j.bcab.2020.101799.

[49] M. Taskin, A. Saghafian, M.N. Aydogan, N.P. Arslan, Microbial lipid production by cold-adapted oleaginous yeast Yarrowia lipolytica B9 in non-sterile whey medium, Biofuels Bioprod. Biorefin. 9 (2015) 595–605, https://doi.org/10.1002/bbb.1560.

[50] S. Vyas, M. Chhabra, Assessing oil accumulation in the oleaginous yeast Cystobasidium oligophagum JRC1 using dairy waste cheese whey as a substrate, 3 Biotech 9 (2019) 173, https://doi.org/10.1007/s13205-019-1701-0.

[51] G. Vasilakis, D. Karayannis, T. Massouras, I. Politis, S. Papanikolaou, Biotechnological conversions of Mizithra second cheese whey by wild-type non-conventional yeast strains: production of yeast cell biomass, Single-Cell Oil and Polysaccharides, Applied Sciences 12 (2022) 11471, https://doi.org/10.3390/app122211471.

[52] L.G. Chan, J.L. Cohen, G. Ozturk, M. Hennebelle, A.Y. Taha, J.M.L.N. de Moura Bell, Bioconversion of cheese whey permeate into fungal oil by Mucor circinelloides, J. Biol. Eng. 12 (2018) 25, https://doi.org/10.1186/s13036-018-0116-5.

[53] N. Gupta, N.A. Manikandan, K. Pakshirajan, Real-time lipid production and dairy wastewater treatment using Rhodococcus opacus in a bioreactor under fed-batch, continuous and continuous cell recycling modes for potential biodiesel application, Biofuels 9 (2018) 239–245, https://doi.org/10.1080/17597269.2017.1336347.

[54] O.M. Herrero, H.M. Alvarez, Whey as a renewable source for lipid production by Rhodococcus strains: physiology and genomics of lactose and galactose utilization, Eur. J. Lipid Sci. Technol. 118 (2016) 262–272, https://doi.org/10.1002/ejlt.201500080.

[55] G.M. Elrayies, Microalgae: prospects for greener future buildings, Renew. Sust. Energ. Rev. 81 (2018) 1175–1191, https://doi.org/10.1016/j.rser.2017.08.032.

[56] N. Nirmalakhandan, T. Selvaratnam, S.M. Henkanatte-Gedera, D. Tchinda, I.S. A. Abeysiriwardana-Arachchige, H.M.K. Delanka-Pedige, S.P. Munasinghe-Arachchige, Y. Zhang, F.O. Holguin, P.J. Lammers, Algal wastewater treatment: photoautotrophic vs. mixotrophic processes, Algal Res. 41 (2019) 101569, https://doi.org/10.1016/j.algal.2019.101569.

[57] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306, https://doi.org/10.1016/j.biotechadv.2007.02.001.
[58] O. Perez-Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic cultures of microalgae: metabolism and potential products, Water Res. 45 (2011) 11–36, https://doi.org/10.1016/j.watres.2010.08.037.
[59] J. Chen, J. Li, W. Dong, X. Zhang, R.D. Tyagi, P. Drogui, R.Y. Surampalli, The potential of microalgae in biodiesel production, Renew. Sust. Energ. Rev. 90 (2018) 336–346, https://doi.org/10.1016/j.rser.2018.03.073.
[60] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96, https://doi.org/10.1263/jbb.101.87.
[61] N. Arora, A. Patel, P.A. Pruthi, K.M. Poluri, V. Pruthi, Utilization of stagnant non-potable pond water for cultivating oleaginous microalga Chlorella minutissima for biodiesel production, Renew. Energy 126 (2018) 30–37, https://doi.org/10.1016/j.renene.2018.03.033.
[62] T.-Q. Shi, L.-R. Wang, Z.-X. Zhang, X.-M. Sun, H. Huang, Stresses as first-line tools for enhancing lipid and carotenoid production in microalgae, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00610.
[63] B. Gao, J. Hong, J. Chen, H. Zhang, R. Hu, C. Zhang, The growth, lipid accumulation and adaptation mechanism in response to variation of temperature and nitrogen supply in psychrotrophic filamentous microalga Xanthonema hormidioides (Xanthophyceae), Biotechnol. Biofuels Bioprod. 16 (2023) 12, https://doi.org/10.1186/s13068-022-02249-0.
[64] Y.A. Alkhamis, R.T. Mathew, G. Nagarajan, S.M. Rahman, Md.M. Rahman, pH induced stress enhances lipid accumulation in microalgae grown under mixotrophic and autotrophic condition, Front. Energy Res. 10 (2022), https://doi.org/10.3389/fenrg.2022.1033068.
[65] M.H. Morowvat, Y. Ghasemi, Maximizing biomass and lipid production in heterotrophic culture of Chlorella vulgaris: techno-economic assessment, Recent Pat. Food Nutr. Agric. 10 (2019) 115–123, https://doi.org/10.2174/2212798410666180911100034.
[66] J. Roostaei, Y. Zhang, K. Gopalakrishnan, A.J. Ochocki, Mixotrophic microalgae biofilm: A novel algae cultivation strategy for improved productivity and cost-efficiency of biofuel feedstock production, Sci. Rep. 8 (2018) 12528, https://doi.org/10.1038/s41598-018-31016-1.
[67] L.A. Sargeant, C.J. Chuck, J. Donnelly, C.D. Bannister, R.J. Scott, Optimizing the lipid profile, to produce either a palm oil or biodiesel substitute, by manipulation of the culture conditions for Rhodotorula glutinis, Biofuels 5 (2014) 33–43, https://doi.org/10.4155/bfs.13.64.
[68] X. Meng, J. Yang, X. Xu, L. Zhang, Q. Nie, M. Xian, Biodiesel production from oleaginous microorganisms, Renew. Energy 34 (2009) 1–5, https://doi.org/10.1016/j.renene.2008.04.014.
[69] F. Abeln, C.J. Chuck, The history, state of the art and future prospects for oleaginous yeast research, Microb. Cell Factories 20 (2021) 221, https://doi.org/10.1186/s12934-021-01712-1.
[70] A. Chattopadhyay, M.K. Maiti, Lipid production by oleaginous yeasts, 2021, pp. 1–98, https://doi.org/10.1016/bs.aambs.2021.03.003.
[71] J. Chopra, R. Sen, Process optimization involving critical evaluation of oxygen transfer, oxygen uptake and nitrogen limitation for enhanced biomass and lipid production by oleaginous yeast for biofuel application, Bioprocess Biosyst. Eng. 41 (2018) 1103–1113, https://doi.org/10.1007/s00449-018-1939-7.
[72] A. Caporusso, A. Capece, I. De Bari, Oleaginous yeasts as cell factories for the sustainable production of microbial lipids by the valorization of Agri-food wastes, Fermentation 7 (2021) 50, https://doi.org/10.3390/fermentation7020050.
[73] M. Athenaki, C. Gardeli, P. Diamantopoulou, S.S. Tchakouteu, D. Sarris, A. Philippoussis, S. Papanikolaou, Lipids from yeasts and fungi: physiology, production and analytical considerations, J. Appl. Microbiol. 124 (2018) 336–367, https://doi.org/10.1111/jam.13633.
[74] C.T. Evans, C. Ratledget, The physiological significance of citric acid in the control of metabolism in lipid-accumulating yeasts, Biotechnol. Genet. Eng. Rev. 3 (1985) 349–376, https://doi.org/10.1080/02648725.1985.10647818.
[75] M.D. Floetenmeyer, B.A. Glatz, E.G. Hammond, Continuous culture fermentation of whey permeate to produce microbial oil, J. Dairy Sci. 68 (1985) 633–637.
[76] M. Ringel, M. Paper, M. Willing, M. Schneider, F. Melcher, N.I. Stellner, T. Brück, Sustainable lipid production with Cutaneotrichosporon oleaginosus: insights into metabolism, Feedstock Valorization and Bioprocess Development, Microorganisms 13 (2025) 1988, https://doi.org/10.3390/microorganisms13091988.
[77] A. Ykema, E.C. Verbree, M.M. Kater, H. Smit, Optimization of lipid production in the oleaginous yeastApiotrichum curvatum in wheypermeate, Appl. Microbiol. Biotechnol. 29 (1988) 211–218, https://doi.org/10.1007/BF00939309.
[78] S.S. Tchakouteu, A. Chatzifragkou, O. Kalantzi, A.A. Koutinas, G. Aggelis, S. Papanikolaou, Oleaginous yeast Cryptococcus curvatus exhibits interplay between biosynthesis of intracellular sugars and lipids, Eur. J. Lipid Sci. Technol. 117 (2015) 657–672, https://doi.org/10.1002/ejlt.201400347.
[79] R.F. Castanha, A.P. Mariano, L.A.S. de Morais, S. Scramin, R.T.R. Monteiro, Optimization of lipids production by Cryptococcus laurentii 11 using cheese whey with molasses, Braz. J. Microbiol. 45 (2014) 379–387, https://doi.org/10.1590/S1517-83822014000200003.
[80] J.A. Ferreira, P.R. Lennartsson, L. Edebo, M.J. Taherzadeh, Zygomycetes-based biorefinery: present status and future prospects, Bioresour. Technol. 135 (2013) 523–532, https://doi.org/10.1016/j.biortech.2012.09.064.
[81] A.M. Shah, H. Mohamed, A.B.A. Fazili, W. Yang, Y. Song, Investigating the effect of alcohol dehydrogenase gene knockout on lipid accumulation in Mucor circinelloides WJ11, Journal of Fungi 8 (2022) 917, https://doi.org/10.3390/jof8090917.
[82] A. Chatzifragkou, A. Makri, A. Belka, S. Bellou, M. Mavrou, M. Mastoridou, P. Mystrioti, G. Onjaro, G. Aggelis, S. Papanikolaou, Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species, Energy 36 (2011) 1097–1108, https://doi.org/10.1016/j.energy.2010.11.040.
[83] A. Chatzifragkou, S. Fakas, M. Galiotou-Panayotou, M. Komaitis, G. Aggelis, S. Papanikolaou, Commercial sugars as substrates for lipid accumulation in Cunninghamella echinulata and Mortierella isabellina fungi, Eur. J. Lipid Sci. Technol. 112 (2010) 1048–1057, https://doi.org/10.1002/ejlt.201000027.
[84] G.A. Youssef, A.M. El-refaey, S. El-assar, Potential assessment of oleaginous Fungi for sustainable biodiesel production: screening, identification and lipid production optimization, Egypt. J. Bot. 0 (2021), https://doi.org/10.21608/ejbo.2021.59291.1614, 0–0.
[85] D. Li, Y. Wang, E. La Kim, J. Hong, J.H. Jung, A new fungal triterpene from the fungus aspergillus flavus stimulates glucose uptake without fat accumulation, Mar. Drugs 20 (2022) 203, https://doi.org/10.3390/md20030203.
[86] S. Papanikolaou, G. Aggelis, Sources of microbial oils with emphasis to Mortierella (Umbelopsis) isabellina fungus, World J. Microbiol. Biotechnol. 35 (2019) 63, https://doi.org/10.1007/s11274-019-2631-z.
[87] E. Carota, S. Crognale, A. D'Annibale, M. Petruccioli, Bioconversion of agro-industrial waste into microbial oils by filamentous fungi, Process. Saf. Environ. Prot. 117 (2018) 143–151, https://doi.org/10.1016/j.psep.2018.04.022.
[88] S. Papanikolaou, M. Galiotou-Panayotou, S. Fakas, M. Komaitis, G. Aggelis, Lipid production by oleaginous Mucorales cultivated on renewable carbon sources, Eur. J. Lipid Sci. Technol. 109 (2007) 1060–1070, https://doi.org/10.1002/ejlt.200700169.
[89] M. Demir, I. Turhan, A. Kucukcetin, Z. Alpkent, Oil production by Mortierella isabellina from whey treated with lactase, Bioresour. Technol. 128 (2013) 365–369, https://doi.org/10.1016/j.biortech.2012.10.078.
[90] J. Sajbidor, M. Certík, S. Dobroňová, Influence of different carbon sources on growth, lipid content and fatty acid composition in four strains belonging to mucorales, Biotechnol. Lett. 10 (1988) 347–350, https://doi.org/10.1007/BF01026163.
[91] B. Volford, M. Varga, A. Szekeres, A. Kotogán, G. Nagy, C. Vágvölgyi, T. Papp, M. Takó, Β-Galactosidase-producing isolates in Mucoromycota: screening, Enzyme Production, and Applications for Functional Oligosaccharide Synthesis, Journal of Fungi 7 (2021) 229, https://doi.org/10.3390/jof7030229.
[92] E. Carsanba, S. Papanikolaou, H. Erten, Production of oils and fats by oleaginous microorganisms with an emphasis given to the potential of the nonconventional yeast Yarrowia lipolytica, Crit. Rev. Biotechnol. 38 (2018) 1230–1243, https://doi.org/10.1080/07388551.2018.1472065.
[93] T. Dong, E.P. Knoshaug, P.T. Pienkos, L.M.L. Laurens, Lipid recovery from wet oleaginous microbial biomass for biofuel production: A critical review, Appl. Energy 177 (2016) 879–895, https://doi.org/10.1016/j.apenergy.2016.06.002.
[94] S.A. Shields-Menard, M. Amirsadeghi, W.T. French, R. Boopathy, A review on microbial lipids as a potential biofuel, Bioresour. Technol. 259 (2018) 451–460, https://doi.org/10.1016/j.biortech.2018.03.080.
[95] M. Kumar, R. Rathour, J. Gupta, A. Pandey, E. Gnansounou, I.S. Thakur, Bacterial production of fatty acid and biodiesel: opportunity and challenges, in: Refining Biomass Residues for Sustainable Energy and Bioproducts, Elsevier, 2020, pp. 21–49, https://doi.org/10.1016/B978-0-12-818996-2.00002-8.
[96] M.A.L.R.M. Cortes, C.C.C.R. de Carvalho, Effect of carbon sources on lipid accumulation in Rhodococcus cells, Biochem. Eng. J. 94 (2015) 100–105, https://doi.org/10.1016/j.bej.2014.11.017.
[97] A.R. Behera, K. Dutta, P. Verma, A. Daverey, D.K. Sahoo, High lipid accumulating bacteria isolated from dairy effluent scum grown on dairy wastewater as potential biodiesel feedstock, J. Environ. Manag. 252 (2019) 109686, https://doi.org/10.1016/j.jenvman.2019.109686.
[98] S. Wu, C. Hu, G. Jin, X. Zhao, Z.K. Zhao, Phosphate-limitation mediated lipid production by Rhodosporidium toruloides, Bioresour. Technol. 101 (2010) 6124–6129, https://doi.org/10.1016/j.biortech.2010.02.111.
[99] A. Beopoulos, J. Cescut, R. Haddouche, J.-L. Uribelarrea, C. Molina-Jouve, J.-M. Nicaud, Yarrowia lipolytica as a model for bio-oil production, Prog. Lipid Res. 48 (2009) 375–387, https://doi.org/10.1016/j.plipres.2009.08.005.
[100] J.P. Wynn, C. Ratledge, Oils from microorganisms, in: F. Shahidi (Ed.), Bailey's Industrial Oil and Fat Products, John Wiley &, Hoboken, Sons, 2005.
[101] C. Ratledge, J.P. Wynn, The Biochemistry and Molecular Biology of Lipid Accumulation in Oleaginous Microorganisms, 2002, pp. 1–52, https://doi.org/10.1016/S0065-2164(02)51000-5.
[102] K.K. Kumar, F. Deeba, A.K. Pandey, A. Islam, D. Paul, N.A. Gaur, Sustainable lipid production by oleaginous yeasts: current outlook and challenges, Bioresour. Technol. 421 (2025) 132205, https://doi.org/10.1016/j.biortech.2025.132205.
[103] S.Y. Lee, M. Weingarten, C. Ottenheim, Current upstream and downstream process strategies for sustainable yeast lipid production, Bioresour. Technol. 414 (2024) 131601, https://doi.org/10.1016/j.biortech.2024.131601.
[104] S. Bellou, I.-E. Triantaphyllidou, D. Aggeli, A.M. Elazzazy, M.N. Baeshen, G. Aggelis, Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content, Curr. Opin. Biotechnol. 37 (2016) 24–35, https://doi.org/10.1016/j.copbio.2015.09.005.
[105] J.E. Cronan, J. Thomas, Bacterial fatty acid synthesis and its relationships with polyketide synthetic, Pathways (2009) 395–433, https://doi.org/10.1016/S0076-6879(09)04617-5.
[106] J.L. Blatti, J. Michaud, M.D. Burkart, Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel, Curr. Opin. Chem. Biol. 17 (2013) 496–505, https://doi.org/10.1016/j.cbpa.2013.04.007.
[107] S. Bellou, I.-E. Triantaphyllidou, P. Mizerakis, G. Aggelis, High lipid accumulation in Yarrowia lipolytica cultivated under double limitation of nitrogen and

magnesium, J. Biotechnol. 234 (2016) 116–126, https://doi.org/10.1016/j.jbiotec.2016.08.001.
[108] C. Ratledge, The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: a reappraisal and unsolved problems, Biotechnol. Lett. 36 (2014) 1557–1568, https://doi.org/10.1007/s10529-014-1532-3.
[109] T.M. Wasylenko, W.S. Ahn, G. Stephanopoulos, The oxidative pentose phosphate pathway is the primary source of NADPH for lipid overproduction from glucose in Yarrowia lipolytica, Metab. Eng. 30 (2015) 27–39, https://doi.org/10.1016/j.ymben.2015.02.007.
[110] R.R. Bommareddy, W. Sabra, G. Maheshwari, A.-P. Zeng, Metabolic network analysis and experimental study of lipid production in Rhodosporidium toruloides grown on single and mixed substrates, Microb. Cell Factories 14 (2015) 36, https://doi.org/10.1186/s12934-015-0217-5.
[111] S.K. Spaans, R.A. Weusthuis, J. van der Oost, S.W.M. Kengen, NADPH-generating systems in bacteria and archaea, Front. Microbiol. 6 (2015), https://doi.org/10.3389/fmicb.2015.00742.
[112] S. Bellou, G. Aggelis, Biochemical activities in Chlorella sp. and Nannochloropsis salina during lipid and sugar synthesis in a lab-scale open pond simulating reactor, J. Biotechnol. 164 (2013) 318–329, https://doi.org/10.1016/j.jbiotec.2013.01.010.
[113] X. Meng, J. Yang, X. Xu, L. Zhang, Q. Nie, M. Xian, Biodiesel production from oleaginous microorganisms, Renew. Energy 34 (2009) 1–5, https://doi.org/10.1016/j.renene.2008.04.014.
[114] G.M. Carman, G.-S. Han, Phosphatidic acid phosphatase, a key enzyme in the regulation of lipid synthesis, J. Biol. Chem. 284 (2009) 2593–2597, https://doi.org/10.1074/jbc.R800059200.
[115] L. Qin, L. Liu, A.-P. Zeng, D. Wei, From low-cost substrates to single cell oils synthesized by oleaginous yeasts, Bioresour. Technol. 245 (2017) 1507–1519, https://doi.org/10.1016/j.biortech.2017.05.163.
[116] G. Vasilakis, E. Dalaka, G. Bekiaris, S. Marka, A. Kaliora, I. Politis, E. Flemetakis, G. Theodorou, S. Papanikolaou, Production and characterization of cellular and released polysaccharides by Papiliotrema laurentii strains bioreactor-cultivated on Mizithra secondary cheese whey, Carbon Resour. Convers. (2026) 100390, https://doi.org/10.1016/j.crcon.2025.100390.
[117] P. Diamantopoulou, R. Filippousi, D. Antoniou, E. Varfi, E. Xenopoulos, D. Sarris, S. Papanikolaou, Production of added-value microbial metabolites during growth of yeast strains on media composed of biodiesel-derived crude glycerol and glycerol/xylose blends, FEMS Microbiol. Lett. 367 (2020), https://doi.org/10.1093/femsle/fnaa063.
[118] G. Vasilakis, R. Tefa, A. Georgoulakis, D. Karayannis, I. Politis, S. Papanikolaou, Screening of non-conventional yeasts on low-cost carbon sources and valorization of Mizithra secondary cheese whey for metabolite production, BioTech 14 (2025) 24, https://doi.org/10.3390/biotech14020024.
[119] G. Vasilakis, A. Georgoulakis, E. Dalaka, G. Bekiaris, I. Diamantis, D. Karayannis, M.-E. Zografaki, P. Diamantopoulou, E. Flemetakis, G. Theodorou, I. Politis, S. Papanikolaou, Bioconversion of Deproteinized cheese whey to metabolites by understudied Cryptococcus-related yeasts: characterization and properties of extracted polysaccharides, Dairy 6 (2025) 69, https://doi.org/10.3390/dairy6060069.
[120] S. Papanikolaou, P. Diamantopoulou, F. Blanchard, E. Lambrinea, I. Chevalot, N. G. Stoforos, E. Rondags, Physiological characterization of a novel wild-type Yarrowia lipolytica strain grown on glycerol: effects of cultivation conditions and mode on polyols and citric acid production, Appl. Sci. 10 (2020) 7373, https://doi.org/10.3390/app10207373.
[121] M. Kuttiraja, A. Dhouha, R.D. Tyagi, Harnessing the effect of pH on lipid production in batch cultures of Yarrowia lipolytica SKY7, Appl. Biochem. Biotechnol. 184 (2018) 1332–1346, https://doi.org/10.1007/s12010-017-2617-y.
[122] Y.H. Chan, R.R. Tan, S. Yusup, H.L. Lam, A.T. Quitain, Comparative life cycle assessment (LCA) of bio-oil production from fast pyrolysis and hydrothermal liquefaction of oil palm empty fruit bunch (EFB), clean Technol. Environ, Policy 18 (2016) 1759–1768, https://doi.org/10.1007/s10098-016-1172-5.
[123] A.A. Koutinas, A. Chatzifragkou, N. Kopsahelis, S. Papanikolaou, I.K. Kookos, Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production, Fuel 116 (2014) 566–577, https://doi.org/10.1016/j.fuel.2013.08.045.
[124] S. Sarantou, N.G. Stoforos, O. Kalantzi, S. Papanikolaou, Biotechnological valorization of biodiesel-derived glycerol: trials with the non-conventional yeasts Yarrowia lipolytica and Rhodosporidium sp, Carbon Resour. Convers. 4 (2021) 61–75, https://doi.org/10.1016/j.crcon.2020.12.006.
[125] M.Á. Villegas-Méndez, J. Montañez, J.C. Contreras-Esquivel, I. Salmerón, A. A. Koutinas, L. Morales-Oyervides, Scale-up and fed-batch cultivation strategy for the enhanced co-production of microbial lipids and carotenoids using renewable waste feedstock, J. Environ. Manag. 339 (2023) 117866, https://doi.org/10.1016/j.jenvman.2023.117866.
[126] N. Bonatsos, C. Marazioti, E. Moutousidi, A. Anagnostou, A. Koutinas, I. K. Kookos, Techno-economic analysis and life cycle assessment of heterotrophic yeast-derived single cell oil production process, Fuel 264 (2020) 116839, https://doi.org/10.1016/j.fuel.2019.116839.
[127] G. Venkata Subhash, S. Venkata Mohan, Lipid accumulation for biodiesel production by oleaginous fungus aspergillus awamori: influence of critical factors, Fuel 116 (2014) 509–515, https://doi.org/10.1016/j.fuel.2013.08.035.
[128] M.L. Bartley, W.J. Boeing, B.N. Dungan, F.O. Holguin, T. Schaub, pH effects on growth and lipid accumulation of the biofuel microalgae Nannochloropsis salina and invading organisms, J. Appl. Phycol. 26 (2014) 1431–1437, https://doi.org/10.1007/s10811-013-0177-2.
[129] Y.-K. Park, C. González-Fernández, R. Robles-Iglesias, L. Vidal, P. Fontanille, C. Kennes, E. Tomás Pejó, J.-M. Nicaud, P. Fickers, Bioproducts generation from carboxylate platforms by the non-conventional yeast Yarrowia lipolytica, FEMS Yeast Res. 21 (2021), https://doi.org/10.1093/femsyr/foab047.
[130] R. Gao, Z. Li, X. Zhou, W. Bao, S. Cheng, L. Zheng, Enhanced lipid production by Yarrowia lipolytica cultured with synthetic and waste-derived high-content volatile fatty acids under alkaline conditions, Biotechnol. Biofuels 13 (2020) 3, https://doi.org/10.1186/s13068-019-1645-y.
[131] R.J. Davies, Yeast oil from cheese whey – Process development, in: R.S. Moreton (Ed.), Single Cell Oil, 1988, pp. 99–145.
[132] A. Abghari, S. Chen, Yarrowia lipolytica as an oleaginous cell factory platform for production of fatty acid-based biofuel and bioproducts, Front. Energy Res. 2 (2014), https://doi.org/10.3389/fenrg.2014.00021.
[133] A. Amaretti, S. Raimondi, M. Sala, L. Roncaglia, M. De Lucia, A. Leonardi, M. Rossi, Production of single cell oils by the cold-adapted oleaginous yeast Rhodotorula glacialis AS 4.7: effects of the growth temperature and the C: N ratio, Chemical Engineering Tansactions 20 (2010) 109–114.
[134] S. Thomas, D.R.A. Sanya, F. Fouchard, H.-V. Nguyen, G. Kunze, C. Neuvéglise, A.-M., Crutz-Le coq, Blastobotrys adeninivorans and B. Raffinosifermentans, two sibling yeast species which accumulate lipids at elevated temperatures and from diverse sugars, Biotechnol. Biofuels 12 (2019) 154, https://doi.org/10.1186/s13068-019-1492-x.
[135] E.-S. Vastaroucha, N.G. Stoforos, G. Aggelis, S. Papanikolaou, Studies of polyol production by the yeast Yarrowia lipolytica growing on crude glycerol under stressful conditions, Carbon Resour. Convers. 7 (2024) 100210, https://doi.org/10.1016/j.crcon.2023.100210.
[136] M.Y. Roleda, S.P. Slocombe, R.J.G. Leakey, J.G. Day, E.M. Bell, M.S. Stanley, Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy, Bioresour. Technol. 129 (2013) 439–449, https://doi.org/10.1016/j.biortech.2012.11.043.
[137] A. Converti, A.A. Casazza, E.Y. Ortiz, P. Perego, M. Del Borghi, Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production, Chem. Eng. Process. Process Intensif. 48 (2009) 1146–1151, https://doi.org/10.1016/j.cep.2009.03.006.
[138] E.E. Karamerou, C. Webb, Cultivation modes for microbial oil production using oleaginous yeasts – A review, Biochem. Eng. J. 151 (2019) 107322, https://doi.org/10.1016/j.bej.2019.107322.
[139] M. Rakicka, Z. Lazar, T. Dulermo, P. Fickers, J.M. Nicaud, Lipid production by the oleaginous yeast Yarrowia lipolytica using industrial by-products under different culture conditions, Biotechnol. Biofuels 8 (2015) 104, https://doi.org/10.1186/s13068-015-0286-z.
[140] P. Diamantopoulou, N.G. Stoforos, E. Xenopoulos, D. Sarris, D. Psarianos, A. Philippoussis, S. Papanikolaou, Lipid production by Cryptococcus curvatus growing on commercial xylose and subsequent valorization of fermentation waste-waters for the production of edible and medicinal mushrooms, Biochem. Eng. J. 162 (2020) 107706, https://doi.org/10.1016/j.bej.2020.107706.
[141] S.O. Ajala, M.L. Alexander, Evaluating the effects of agitation by shaking, stirring and air sparging on growth and accumulation of biochemical compounds in microalgae cells, Biofuels 13 (2022) 371–381, https://doi.org/10.1080/17597269.2020.1714161.
[142] N. Saad, P. Abdeshahian, M.S. Kalil, W.M. Wan Yusoff, A. Abdul Hamid, Optimization of aeration and agitation rate for lipid and gamma linolenic acid production by Cunninghamella bainieri 2A1 in submerged fermentation using response surface methodology, Sci. World J. (2014) (2014) 1–12, https://doi.org/10.1155/2014/280146.
[143] M.J. Kennedy, S.L. Reader, R.J. Davies, D.A. Rhoades, H.W. Silby, The scale up of mycelial shake flask fermentations: A case study of gamma linolenic acid production byMucor hiemalis IRL 51, J. Ind. Microbiol. 13 (1994) 212–216, https://doi.org/10.1007/BF01569750.
[144] G. Vasilakis, C. Roidouli, D. Karayannis, N. Giannakis, E. Rondags, I. Chevalot, S. Papanikolaou, Study of different parameters affecting production and productivity of polyunsaturated fatty acids (PUFAs) and γ-linolenic acid (GLA) by Cunninghamella elegans through glycerol conversion in shake flasks and bioreactors, Microorganisms 12 (2024) 2097, https://doi.org/10.3390/microorganisms12102097.
[145] L. Zhang, E. Lim, K.-C. Loh, Y. Dai, Y. Tong, Two-stage fermentation of Lipomyces starkeyi for production of microbial lipids and biodiesel, Microorganisms 9 (2021) 1724, https://doi.org/10.3390/microorganisms9081724.
[146] A. Patel, L. Matsakas, A comparative study on de novo and ex novo lipid fermentation by oleaginous yeast using glucose and sonicated waste cooking oil, Ultrason. Sonochem. 52 (2019) 364–374, https://doi.org/10.1016/j.ultsonch.2018.12.010.
[147] Y. Yu, Y. Sha, J. Yu, L. Zhou, X. Chen, R. Zhai, Z. Xu, M. Jin, DLC(sa) and DLCA (sa) pretreatments boost the efficiency of microbial lipid production from rice straw via Trichosporon dermatis, Fuel 309 (2022) 122117, https://doi.org/10.1016/j.fuel.2021.122117.
[148] J. Chen, X. Zhang, R.D. Tyagi, Impact of nitrogen on the industrial feasibility of biodiesel production from lipid accumulated in oleaginous yeast with wastewater sludge and crude glycerol, Energy 217 (2021) 119343, https://doi.org/10.1016/j.energy.2020.119343.
[149] M. Li, M.K.H. Alotaibi, L. Li, A.E.-F. Abomohra, Enhanced waste glycerol recycling by yeast for efficient biodiesel production: towards waste biorefinery, Biomass Bioenergy 159 (2022) 106410, https://doi.org/10.1016/j.biombioe.2022.106410.

[150] D.Y. Shin, H.U. Cho, J.C. Utomo, Y.-N. Choi, X. Xu, J.M. Park, Biodiesel production from Scenedesmus bijuga grown in anaerobically digested food wastewater effluent, Bioresour. Technol. 184 (2015) 215–221, https://doi.org/10.1016/j.biortech.2014.10.090.
[151] X. Feng, T.H. Walker, W.C. Bridges, C. Thornton, K. Gopalakrishnan, Biomass and lipid production of Chlorella protothecoides under heterotrophic cultivation on a mixed waste substrate of brewer fermentation and crude glycerol, Bioresour. Technol. 166 (2014) 17–23, https://doi.org/10.1016/j.biortech.2014.03.120.
[152] J. Mu, S. Li, D. Chen, H. Xu, F. Han, B. Feng, Y. Li, Enhanced biomass and oil production from sugarcane bagasse hydrolysate (SBH) by heterotrophic oleaginous microalga Chlorella protothecoides, Bioresour. Technol. 185 (2015) 99–105, https://doi.org/10.1016/j.biortech.2015.02.082.
[153] T. Wells, Z. Wei, A. Ragauskas, Bioconversion of lignocellulosic pretreatment effluent via oleaginous Rhodococcus opacus DSM 1069, Biomass Bioenergy 72 (2015) 200–205, https://doi.org/10.1016/j.biombioe.2014.11.004.
[154] Q. Zhang, Y. Li, L. Xia, An oleaginous endophyte Bacillus subtilis HB1310 isolated from thin-shelled walnut and its utilization of cotton stalk hydrolysate for lipid production, Biotechnol. Biofuels 7 (2014) 152, https://doi.org/10.1186/s13068-014-0152-4.
[155] M. Guerfali, I. Ayadi, H.-E. Sassi, A. Belhassen, A. Gargouri, H. Belghith, Biodiesel-derived crude glycerol as alternative feedstock for single cell oil production by the oleaginous yeast Candida viswanathii Y-E4, Ind. Crop. Prod. 145 (2020) 112103, https://doi.org/10.1016/j.indcrop.2020.112103.
[156] D. Johnravindar, O.P. Karthikeyan, A. Selvam, K. Murugesan, J.W.C. Wong, Lipid accumulation potential of oleaginous yeasts: A comparative evaluation using food waste leachate as a substrate, Bioresour. Technol. 248 (2018) 221–228, https://doi.org/10.1016/j.biortech.2017.06.151.
[157] S. Kumar, N. Gupta, K. Pakshirajan, Simultaneous lipid production and dairy wastewater treatment using Rhodococcus opacus in a batch bioreactor for potential biodiesel application, J. Environ. Chem. Eng. 3 (2015) 1630–1636, https://doi.org/10.1016/j.jece.2015.05.030.
[158] L. Goswami, M.M. Tejas Namboodiri, R. Vinoth Kumar, K. Pakshirajan, G. Pugazhenthi, Biodiesel production potential of oleaginous Rhodococcus opacus grown on biomass gasification wastewater, renew, Energy 105 (2017) 400–406, https://doi.org/10.1016/j.renene.2016.12.044.
[159] H.U. Cho, Y.M. Kim, Y.-N. Choi, X. Xu, D.Y. Shin, J.M. Park, Effects of pH control and concentration on microbial oil production from Chlorella vulgaris cultivated in the effluent of a low-cost organic waste fermentation system producing volatile fatty acids, Bioresour. Technol. 184 (2015) 245–250, https://doi.org/10.1016/j.biortech.2014.09.069.
[160] J. Tan, D. Yu, J. Yuan, H. Wu, H. Luo, H. Zhang, X. Li, H. Li, S. Yang, Efficient delignification of wheat straw for microbial lipid production enabled by a novel ternary deep eutectic solvent containing ethylene glycol, Fuel 347 (2023) 128485, https://doi.org/10.1016/j.fuel.2023.128485.
[161] W.J. Martinez-Burgos, L. Porto de Souza Vandenberghe, S.G. Karp, A.F. Murawski de Mello, V. Thomaz Soccol, C.R. Soccol, Microbial lipid production from soybean hulls using Lipomyces starkeyi LPB53 in a circular economy, Bioresour. Technol. 372 (2023) 128650, https://doi.org/10.1016/j.biortech.2023.128650.
[162] M. Pant, T. Pant, Maximising biotransformation of pine needles to microbial lipids using Lipomyces starkeyi MTCC 1400T, Renew. Energy 206 (2023) 574–581, https://doi.org/10.1016/j.renene.2023.02.051.
[163] K.V. Probst, P.V. Vadlani, Production of single cell oil from Lipomyces starkeyi ATCC 56304 using biorefinery by-products, Bioresour. Technol. 198 (2015) 268–275, https://doi.org/10.1016/j.biortech.2015.09.018.
[164] A.P. Ferreira, L.M. Vieira, J.P. Cardoso, J.C. Menezes, Evaluation of a new annular capacitance probe for biomass monitoring in industrial pilot-scale fermentations, J. Biotechnol. 116 (2005) 403–409, https://doi.org/10.1016/j.jbiotec.2004.12.006.
[165] J.P. Robinson, R. Ostafe, S.N. Iyengar, B. Rajwa, R. Fischer, Flow cytometry: the next revolution, Cells 12 (2023) 1875, https://doi.org/10.3390/cells12141875.
[166] D. Elsey, D. Jameson, B. Raleigh, M.J. Cooney, Fluorescent measurement of microalgal neutral lipids, J. Microbiol. Methods 68 (2007) 639–642, https://doi.org/10.1016/j.mimet.2006.11.008.
[167] T.L. da Silva, J.C. Roseiro, A. Reis, Applications and perspectives of multi-parameter flow cytometry to microbial biofuels production processes, Trends Biotechnol. 30 (2012) 225–232, https://doi.org/10.1016/j.tibtech.2011.11.005.
[168] A. Patel, I. Antonopoulou, J. Enman, U. Rova, P. Christakopoulos, L. Matsakas, Lipids detection and quantification in oleaginous microorganisms: an overview of the current state of the art, BMC Chem. Eng. 1 (2019) 13, https://doi.org/10.1186/s42480-019-0013-9.
[169] R.E. Pauls, A review of chromatographic characterization techniques for biodiesel and biodiesel blends, J. Chromatogr. Sci. 49 (2011) 384–396, https://doi.org/10.1093/chromsci/49.5.384.
[170] G. Kosa, A. Kohler, V. Tafintseva, B. Zimmermann, K. Forfang, N.K. Afseth, D. Tzimorotas, K.S. Vuoristo, S.J. Horn, J. Mounier, V. Shapaval, Microtiter plate cultivation of oleaginous fungi and monitoring of lipogenesis by high-throughput FTIR spectroscopy, Microb. Cell Factories 16 (2017) 101, https://doi.org/10.1186/s12934-017-0716-7.
[171] K. Forfang, B. Zimmermann, G. Kosa, A. Kohler, V. Shapaval, FTIR spectroscopy for evaluation and monitoring of lipid extraction efficiency for oleaginous Fungi, PLoS One 12 (2017) e0170611, https://doi.org/10.1371/journal.pone.0170611.
[172] D. Ami, R. Posteri, P. Mereghetti, D. Porro, S.M. Doglia, P. Branduardi, Fourier transform infrared spectroscopy as a method to study lipid accumulation in oleaginous yeasts, Biotechnol. Biofuels 7 (2014) 12, https://doi.org/10.1186/1754-6834-7-12.
[173] B. Radwan, A. Adamczyk, S. Tott, K. Czamara, K. Kaminska, E. Matuszyk, M. Baranska, Labeled vs. Label-Free Raman Imaging of Lipids in Endothelial Cells of Various Origins, Molecules 25 (2020) 5752, https://doi.org/10.3390/molecules25235752.
[174] E. Molina Grima, E.-H. Belarbi, F.G. Acién Fernández, A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, Biotechnol. Adv. 20 (2003) 491–515, https://doi.org/10.1016/S0734-9750(02)00050-2.
[175] Z. Yin, L. Zhu, S. Li, T. Hu, R. Chu, F. Mo, D. Hu, C. Liu, B. Li, A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: environmental pollution control and future directions, Bioresour. Technol. 301 (2020) 122804, https://doi.org/10.1016/j.biortech.2020.122804.
[176] D.A. Ladner, D.R. Vardon, M.M. Clark, Effects of shear on microfiltration and ultrafiltration fouling by marine bloom-forming algae, J. Membr. Sci. 356 (2010) 33–43, https://doi.org/10.1016/j.memsci.2010.03.024.
[177] A.I. Barros, A.L. Gonçalves, M. Simões, J.C.M. Pires, Harvesting techniques applied to microalgae: A review, Renew. Sust. Energ. Rev. 41 (2015) 1489–1500, https://doi.org/10.1016/j.rser.2014.09.037.
[178] L. Brennan, P. Owende, Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sust. Energ. Rev. 14 (2010) 557–577, https://doi.org/10.1016/j.rser.2009.10.009.
[179] Y. Louhasakul, B. Cheirsilp, S. Maneerat, P. Prasertsan, Potential use of flocculating oleaginous yeasts for bioconversion of industrial wastes into biodiesel feedstocks, Renew. Energy 136 (2019) 1311–1319, https://doi.org/10.1016/j.renene.2018.10.002.
[180] L.G. Heredia-Martínez, A.M. Gutiérrez-Diánez, E. Díaz-Santos, Bio-flocculation: A green tool in biorefineries for recovering high added-value compounds from microalgae, Phycology 5 (2025) 19, https://doi.org/10.3390/phycology5020019.
[181] A. Patel, F. Mikes, L. Matsakas, An overview of current pretreatment methods used to improve lipid extraction from oleaginous microorganisms, Molecules 23 (2018) 1562, https://doi.org/10.3390/molecules23071562.
[182] Y. Ma, Z. Gao, Q. Wang, Y. Liu, Biodiesels from microbial oils: opportunity and challenges, Bioresour. Technol. 263 (2018) 631–641, https://doi.org/10.1016/j.biortech.2018.05.028.
[183] J. Folch, M. Lees, G.H. Sloane Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[184] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.
[185] R.K. Saini, P. Prasad, X. Shang, Y.-S. Keum, Advances in lipid extraction methods—A review, Int. J. Mol. Sci. 22 (2021) 13643, https://doi.org/10.3390/ijms222413643.
[186] V. Matyash, G. Liebisch, T.V. Kurzchalia, A. Shevchenko, D. Schwudke, Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics, J. Lipid Res. 49 (2008) 1137–1146, https://doi.org/10.1194/jlr.D700041-JLR200.
[187] J. Jones, S. Manning, M. Montoya, K. Keller, M. Poenie, Extraction of algal lipids and their analysis by HPLC and mass spectrometry, J. Am. Oil Chem. Soc. 89 (2012) 1371–1381, https://doi.org/10.1007/s11746-012-2044-8.
[188] B. Nozari, R. Kander, Supercritical CO2 technology for biomass extraction: review, Ind. Crop. Prod. 233 (2025) 121348, https://doi.org/10.1016/j.indcrop.2025.121348.
[189] L. Wetterwald, A. Leybros, G. Fleury, F. Delrue, A. Dimitriades-Lemaire, P. Chambonniere, A. Hertz, Supercritical CO2 extraction of neutral lipids from dry and wet Chlorella vulgaris NIES 227 microalgae for biodiesel production, J. Environ. Chem. Eng. 11 (2023) 110628, https://doi.org/10.1016/j.jece.2023.110628.
[190] S.K. Yellapu, R. Bharti, L.R. Kaur, B. Kumar, X. Tiwari, R.D. Zhang, Tyagi, recent developments of downstream processing for microbial lipids and conversion to biodiesel, Bioresour. Technol. 256 (2018) 515–528, https://doi.org/10.1016/j.biortech.2018.01.129.
[191] M. Salaheldeen, A.A. Mariod, M.K. Aroua, S.M.A. Rahman, M.E.M. Soudagar, I.M. R. Fattah, Current state and perspectives on transesterification of triglycerides for biodiesel production, Catalysts 11 (2021) 1121, https://doi.org/10.3390/catal11091121.
[192] W. Nabgan, A.A. Jalil, B. Nabgan, A.H. Jadhav, M. Ikram, A. Ul-Hamid, M.W. Ali, N.S. Hassan, Sustainable biodiesel generation through catalytic transesterification of waste sources: a literature review and bibliometric survey, RSC Adv. 12 (2022) 1604–1627, https://doi.org/10.1039/D1RA07338A.
[193] M.G. Nayak, Review and comparison of the methodology adopted for biodiesel production, Carbon Resour. Convers. 100343 (2025), https://doi.org/10.1016/j.crcon.2025.100343.
[194] R.A. Welter, H.S. Santana, L. Gaziola de la Torre, M.C. Barnes, O.P. Taranto, M. Oelgemöller, Biodiesel production by heterogeneous catalysis and eco-friendly routes, ChemBioEng Reviews 10 (2023) 86–111, https://doi.org/10.1002/cben.202200062.
[195] A.K.F. Carvalho, J.D. Rivaldi, J.C. Barbosa, H.F. de Castro, Biosynthesis, characterization and enzymatic transesterification of single cell oil of Mucor circinelloides – A sustainable pathway for biofuel production, Bioresour. Technol. 181 (2015) 47–53, https://doi.org/10.1016/j.biortech.2014.12.110.
[196] L.P. Christopher, V.P. Hemanathan Kumar, Zambare, Enzymatic biodiesel: challenges and opportunities, Appl. Energy 119 (2014) 497–520, https://doi.org/10.1016/j.apenergy.2014.01.017.
[197] P.D. Patil, V.G. Gude, A. Mannarswamy, P. Cooke, N. Nirmalakhandan, P. Lammers, S. Deng, Comparison of direct transesterification of algal biomass

under supercritical methanol and microwave irradiation conditions, Fuel 97 (2012) 822–831, https://doi.org/10.1016/j.fuel.2012.02.037.
[198] P.D. Patil, V.G. Gude, A. Mannarswamy, P. Cooke, S. Munson-McGee, N. Nirmalakhandan, P. Lammers, S. Deng, Optimization of microwave-assisted transesterification of dry algal biomass using response surface methodology, Bioresour. Technol. 102 (2011) 1399–1405, https://doi.org/10.1016/j.biortech.2010.09.046.
[199] P. Selvakumar, P. Sivashanmugam, Ultrasound assisted oleaginous yeast lipid extraction and garbage lipase catalyzed transesterification for enhanced biodiesel production, Energy Convers. Manag. 179 (2019) 141–151, https://doi.org/10.1016/j.enconman.2018.10.051.
[200] M. Berrios, R.L. Skelton, Comparison of purification methods for biodiesel, Chem. Eng. J. 144 (2008) 459–465, https://doi.org/10.1016/j.cej.2008.07.019.
[201] I.J. Stojković, O.S. Stamenković, D.S. Povrenović, V.B. Veljković, Purification technologies for crude biodiesel obtained by alkali-catalyzed transesterification, Renew. Sust. Energ. Rev. 32 (2014) 1–15, https://doi.org/10.1016/j.rser.2014.01.005.
[202] E. Arenas, S.M. Villafán-Cáceres, Y. Rodríguez-Mejía, J.A. García-Loyola, O. Masera, G. Sandoval, Biodiesel dry purification using unconventional bioadsorbents, Processes 9 (2021) 194, https://doi.org/10.3390/pr9020194.
[203] S.H. Shuit, Y.T. Ong, K.T. Lee, B. Subhash, S.H. Tan, Membrane technology as a promising alternative in biodiesel production: A review, Biotechnol. Adv. 30 (2012) 1364–1380, https://doi.org/10.1016/j.biotechadv.2012.02.009.
[204] C. Pagliero, M. Mattea, N. Ochoa, J. Marchese, Fouling of polymeric membranes during degumming of crude sunflower and soybean oil, J. Food Eng. 78 (2007) 194–197, https://doi.org/10.1016/j.jfoodeng.2005.09.015.
[205] R. Ledesma-Amaro, J.-M. Nicaud, Metabolic engineering for expanding the substrate range of Yarrowia lipolytica, Trends Biotechnol. 34 (2016) 798–809, https://doi.org/10.1016/j.tibtech.2016.04.010.
[206] V.A. Portnoy, D. Bezdan, K. Zengler, Adaptive laboratory evolution—harnessing the power of biology for metabolic engineering, Curr. Opin. Biotechnol. 22 (2011) 590–594, https://doi.org/10.1016/j.copbio.2011.03.007.
[207] N. Arora, H.-W. Yen, G.P. Philippidis, Harnessing the power of mutagenesis and adaptive laboratory evolution for high lipid production by oleaginous microalgae and yeasts, Sustainability 12 (2020) 5125, https://doi.org/10.3390/su12125125.
[208] M. Dragosits, D. Mattanovich, Adaptive laboratory evolution – principles and applications for biotechnology, Microb. Cell Factories 12 (2013) 64, https://doi.org/10.1186/1475-2859-12-64.
[209] D. Cheng, X. Li, Y. Yuan, C. Yang, T. Tang, Q. Zhao, Y. Sun, Adaptive evolution and carbon dioxide fixation of Chlorella sp. in simulated flue gas, Sci. Total Environ. 650 (2019) 2931–2938, https://doi.org/10.1016/j.scitotenv.2018.10.070.
[210] Z. Liu, M. Radi, E.T.T. Mohamed, A.M. Feist, G. Dragone, S.I. Mussatto, Adaptive laboratory evolution of Rhodosporidium toruloides to inhibitors derived from lignocellulosic biomass and genetic variations behind evolution, Bioresour. Technol. 333 (2021) 125171, https://doi.org/10.1016/j.biortech.2021.125171.
[211] C. Chen, Y.-W. Li, X.-Y. Chen, Y.-T. Wang, C. Ye, T.-Q. Shi, Application of adaptive laboratory evolution for Yarrowia lipolytica: A comprehensive review, Bioresour. Technol. 391 (2024) 129893, https://doi.org/10.1016/j.biortech.2023.129893.
[212] Y.H. Seo, I. Lee, S.H. Jeon, J.-I. Han, Efficient conversion from cheese whey to lipid using Cryptococcus curvatus, Biochem. Eng. J. 90 (2014) 149–153, https://doi.org/10.1016/j.bej.2014.05.018.
[213] P. Buzzini, A. Martini, Production of carotenoids by strains of Rhodotorula glutinis cultured in raw materials of agro-industrial origin, Bioresour. Technol. 71 (2000) 41–44, https://doi.org/10.1016/S0960-8524(99)00056-5.
[214] M. Moliné, D. Libkind, M. van Broock, Production of Torularhodin, Torulene, and β-Carotene by Rhodotorula Yeasts, 2012, pp. 275–283, https://doi.org/10.1007/978-1-61779-918-1_19.
[215] E.E. Karamerou, S. Parsons, M.C. McManus, C.J. Chuck, Using techno-economic modelling to determine the minimum cost possible for a microbial palm oil substitute, Biotechnol. Biofuels 14 (2021) 57, https://doi.org/10.1186/s13068-021-01911-3.
[216] S. Donzella, D. Romano, F. Molinari, S.B. Puga, R.P. de Souza Oliveira, C. Compagno, Production of uncommon carotenoids and lipids by red yeasts utilizing Agri-food residues and waste cooking oil, Appl. Microbiol. Biotechnol. 110 (2026) 29, https://doi.org/10.1007/s00253-025-13680-2.
[217] E.L. Almeida, J.E. Olivo, C.M.G. Andrade, Production of biofuels from glycerol from the biodiesel production process—A brief review, Fermentation 9 (2023) 869, https://doi.org/10.3390/fermentation9100869.
[218] C. Li, K. Lesnik, H. Liu, Microbial conversion of waste glycerol from biodiesel production into value-added products, Energies (Basel) 6 (2013) 4739–4768, https://doi.org/10.3390/en6094739.
[219] K.Y. Tey, J.P. Tan, S.K. Yeap, N. He, N.A. Bukhari, Y.W. Hui, A.A.I. Luthfi, S. F. Abdul Manaf, Current analysis on 1,3-propanediol production from glycerol via pure wild strain fermentation, J. Environ. Chem. Eng. 11 (2023) 110998, https://doi.org/10.1016/j.jece.2023.110998.
[220] C.A.G. Quispe, C.J.R. Coronado, J.A. Carvalho Jr., Glycerol: production, consumption, prices, characterization and new trends in combustion, Renew. Sust. Energ. Rev. 27 (2013) 475–493, https://doi.org/10.1016/j.rser.2013.06.017.
[221] C.G. Chol, R. Dhabhai, A.K. Dalai, M. Reaney, Purification of crude glycerol derived from biodiesel production process: experimental studies and techno-economic analyses, Fuel Process. Technol. 178 (2018) 78–87, https://doi.org/10.1016/j.fuproc.2018.05.023.
[222] M. Amouri, S. Belkhodja, S. Masrour, F. Kaidi, M. Aziza, Enhancement of indigenous microalgae culture using cheese whey as growth media for bioenergy and coproducts production, E3S Web of Conferences 436 (2023) 04001, https://doi.org/10.1051/e3sconf/202343604001.
[223] M. Colacicco, C. De Micco, S. Macrelli, G. Agrimi, M. Janssen, M. Bettiga, I. Pisano, Process scale-up simulation and techno-economic assessment of ethanol fermentation from cheese whey, Biotechnol. Biofuels Bioprod. 17 (2024) 124, https://doi.org/10.1186/s13068-024-02567-5.
[224] A.F. Pires, N.G. Marnotes, O.D. Rubio, A.C. Garcia, C.D. Pereira, Dairy by-products: A review on the valorization of whey and second cheese whey, Foods 10 (2021) 1067, https://doi.org/10.3390/foods10051067.
[225] M. Usman, S. Cheng, S. Boonyubol, J.S. Cross, Evaluating green solvents for bio-oil extraction: advancements, challenges, and future perspectives, Energies (Basel) 16 (2023) 5852, https://doi.org/10.3390/en16155852.
[226] J.M. Marchetti, V.U. Miguel, A.F. Errazu, Techno-economic study of different alternatives for biodiesel production, Fuel Process. Technol. 89 (2008) 740–748, https://doi.org/10.1016/j.fuproc.2008.01.007.
[227] A. Pandey, S. Srivastava, S. Kumar, Development and cost-benefit analysis of a novel process for biofuel production from microalgae using pre-treated high-strength fresh cheese whey wastewater, Environ. Sci. Pollut. Res. 27 (2020) 23963–23980, https://doi.org/10.1007/s11356-020-08535-4.