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Rhodotorula Mucilaginosa as a Microbial Platform for Sustainable Lipid Production: Achievements, Bottlenecks, and Future Perspectives

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Abstract

The growing demand for sustainable alternatives to fossil-derived products has intensified interest in microbial lipids, also known as single-cell oils (SCOs), as renewable feedstocks for biofuels, oleochemicals, food ingredients, cosmetics, and other biobased products. Among oleaginous microorganisms, *Rhodotorula mucilaginosa* has emerged as a particularly promising yeast due to its metabolic versatility, ability to accumulate substantial amounts of intracellular lipids, and capacity to utilize a wide range of renewable and low-cost substrates. Over the last two decades, research on *R. mucilaginosa* has evolved from laboratory-scale investigations focused on lipid accumulation under controlled conditions to more sophisticated approaches involving agro-industrial waste valorization, process intensification, integrated biorefineries, and the simultaneous production of lipids and high-value metabolites such as carotenoids. This review critically examines the historical development, current advances, and future prospects of *R. mucilaginosa* as a microbial platform for lipid production. Particular attention is given to the physiological and metabolic traits underlying lipid biosynthesis, the evolution of cultivation strategies, and the utilization of alternative carbon sources, including crude glycerol, molasses, lignocellulosic hydrolysates, and food-processing residues. Recent advances in fed-batch cultivation, bioreactor operation, process optimization, and co-production strategies are discussed, highlighting the transition from single-product processes toward integrated biorefinery concepts. Despite significant progress, several challenges continue to hinder large-scale implementation, including substrate variability, limited volumetric productivity, downstream processing costs, and the need for economically competitive lipid recovery technologies. Future perspectives are explored in the context of metabolic engineering, synthetic biology, artificial intelligence-assisted bioprocess optimization, and carbon-efficient biorefineries. The evidence reviewed indicates that *R. mucilaginosa* has considerable potential as a versatile microbial platform for sustainable lipid production and multiproduct biomanufacturing, provided that biological performance can be coupled with scalable, economically viable, and environmentally responsible process design.

Keywords: Oleaginous Yeast, Single-Cell Oil, Microbial Lipids, Biorefinery, Agro-Industrial Residues

1. Introduction

The global transition toward a low-carbon economy has intensified efforts to replace fossil-derived resources with renewable and sustainable alternatives. Among the various strategies currently under development, microbial biotechnology has attracted considerable attention due to its ability to convert renewable carbon sources into fuels, chemicals, materials, and food ingredients [1-4]. In particular, microbial lipids, commonly referred to as single-cell oils (SCOs), have emerged as promising substitutes for vegetable oils and animal fats because they can be produced independently of arable land availability, climatic conditions, and seasonal agricultural fluctuations [5-7].

The growing interest in SCOs is closely linked to increasing concerns regarding the environmental impacts associated with conventional oil production. Although vegetable oils remain the primary feedstock for biodiesel manufacturing worldwide, their large-scale utilization has raised questions related to land-use change, deforestation, biodiversity loss, freshwater consumption, and competition with food production [5, 6, 8, 9]. Consequently, alternative lipid sources capable of supporting the expanding demand for biofuels and biobased products are increasingly being investigated.

Oleaginous microorganisms constitute one of the most promising alternatives for sustainable lipid production. These organisms

are capable of accumulating lipids exceeding 20% of their dry cell weight, with some species reaching intracellular lipid contents above 60% under nutrient-limited conditions [6, 10-12]. Among them, oleaginous yeasts offer several advantages, including rapid growth rates, high substrate conversion efficiencies, tolerance to industrial cultivation conditions, and the ability to utilize diverse carbon sources [5, 6, 10, 11, 13]. Furthermore, yeast cultivation can be integrated into existing industrial infrastructures and biorefinery schemes, contributing to waste valorization and circular economy strategies [5, 6, 8, 9, 14].

Among the numerous oleaginous yeast species investigated to date, *Rhodotorula mucilaginosa* has emerged as one of the most versatile and industrially attractive microorganisms. This basidiomycetous yeast exhibits remarkable metabolic flexibility and can grow on a wide variety of substrates, including glucose [15, 16], glycerol [9, 14], sucrose [15], molasses [17, 18], lignocellulosic hydrolysates [19-21], food-processing residues [7, 20, 22], and several agro-industrial by-products [14, 21, 23-27]. In addition to its lipid-producing capacity, *R. mucilaginosa* is also known for synthesizing valuable carotenoids, such as β-carotene, torulene, and torularhodin, creating opportunities for integrated production systems capable of generating multiple revenue streams from a single fermentation process [3, 14, 25, 28].

Historically, research involving *R. mucilaginosa* was primarily focused on maximizing lipid accumulation for biodiesel applications. Early investigations explored cultivation conditions, carbon-to-nitrogen ratios, and substrate utilization patterns to improve intracellular lipid content and biomass productivity [10, 19]. As the field matured, researchers increasingly shifted their attention toward the use of low-cost renewable feedstocks, aiming to reduce production costs and improve process sustainability [5, 27, 29]. In this context, significant advances were achieved through the utilization of crude glycerol generated by biodiesel industries [9, 14], sugarcane molasses [24, 25], lignocellulosic hydrolysates [9, 21, 29], soybean-processing residues [26, 27], and other industrial by-products [7, 30].

More recently, the scientific focus has expanded beyond lipid production alone. Advances in bioprocess engineering have enabled the development of integrated biorefinery concepts in which *R. mucilaginosa* simultaneously produces microbial lipids, carotenoids, and other value-added metabolites [14, 25, 28]. This shift reflects a broader recognition that the economic viability of microbial lipid production may depend on multiproduct approaches rather than on the commercialization of lipids alone. Consequently, modern studies increasingly combine process optimization, waste valorization, and product diversification to improve overall process profitability.

Despite the substantial progress achieved over the last two decades, several technological and economic challenges continue to limit the industrial implementation of *R. mucilaginosa*-based lipid production. Key obstacles include the variability of waste-derived substrates, relatively low volumetric productivities compared with established industrial fermentations, high downstream processing costs, and the absence of large-scale commercial facilities [5, 6, 31]. At the same time, emerging technologies such as synthetic biology [32], metabolic engineering [6, 33], machine learning-assisted process optimization [31, 34], and advanced biorefinery integration [5, 8] offer new opportunities for overcoming these barriers.

Therefore, this review aims to provide a comprehensive and critical assessment of the evolution of *Rhodotorula mucilaginosa* as a microbial platform for lipid production. Particular emphasis is placed on the physiological basis of lipid accumulation, historical and recent technological developments, substrate diversification strategies, process optimization approaches, industrial bottlenecks, and future directions that may shape the role of this yeast in the emerging bioeconomy.

2. Biological basis of lipid accumulation in *Rhodotorula mucilaginosa*

Fig 1 shows *R. mucilaginosa* as a multiproduct microbial platform for sustainable biomanufacturing, which will be presented in this section.

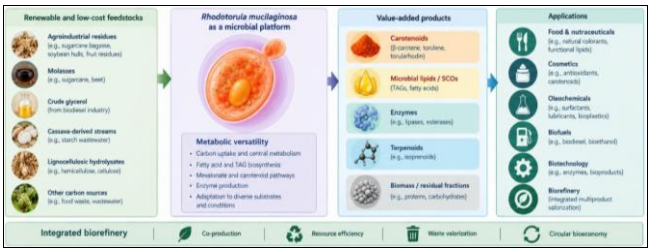

Fig 1: *Rhodotorula mucilaginosa* as a multiproduct microbial platform for sustainable biomanufacturing

2.1 Taxonomy, physiology, and industrial relevance

Rhodotorula mucilaginosa is a basidiomycetous red yeast belonging to the phylum Basidiomycota [35], class Microbotryomycetes, and family Sporidiobolaceae [28]. The species is widely distributed in terrestrial and aquatic environments and has been isolated from soils [36], freshwater ecosystems [37], marine habitats [38], plant surfaces [39], food products [28], and industrial residues [14]. Its remarkable ecological adaptability reflects an extensive metabolic versatility that enables growth under diverse environmental conditions and on a broad range of carbon substrates.

Among the members of the genus *Rhodotorula*, *R. mucilaginosa* has attracted considerable attention due to its ability to accumulate intracellular lipids while simultaneously producing carotenoid pigments, including β-carotene, torulene, and torularhodin [25, 28, 36]. This dual metabolic capability distinguishes the species from many other oleaginous yeasts and provides opportunities for integrated biorefinery approaches aimed at maximizing process profitability through multiproduct generation.

Like other oleaginous microorganisms, *R. mucilaginosa* is classified as an oleaginous yeast because it can accumulate lipids exceeding 20% of its dry cell weight under suitable cultivation conditions [12]. Several studies have reported lipid contents above 40–50%, depending on substrate composition, cultivation strategy, and nutrient availability [21, 30]. Furthermore, the species exhibits robust growth on renewable feedstocks such as crude glycerol [14], molasses [40], lignocellulosic hydrolysates [27], starch-derived sugars [21], and agro-industrial residues [3, 24], making it particularly attractive for sustainable biotechnological applications.

The industrial relevance of *R. mucilaginosa* extends beyond lipid production. Its ability to convert low-cost carbon

sources into microbial oils, carotenoids, and biomass simultaneously aligns with current trends in circular bioeconomy and waste valorization. Consequently, the species has increasingly been investigated as a microbial platform for the production of biofuels [9], food ingredients [7], feed additives [41], cosmetic compounds [42], and specialty chemicals [8, 9].

2.2 Carbon source utilization and metabolic flexibility

One of the most remarkable characteristics of *R. mucilaginosa* is its ability to metabolize a diverse array of carbon sources. This metabolic flexibility has been a key factor driving its growing industrial relevance, particularly in the context of waste valorization and biorefinery integration.

The species efficiently utilizes conventional substrates such as glucose [27], fructose [43], and sucrose [44], which support rapid biomass formation and high lipid accumulation. However, increasing attention has been directed toward the utilization of low-cost and renewable feedstocks. Numerous studies have demonstrated successful cultivation of *R. mucilaginosa* on crude glycerol generated by biodiesel industries [9, 14], sugarcane molasses [40], starch hydrolysates [21, 40], lignocellulosic hydrolysates [21, 26, 29], soybean-processing residues [19, 27], and food-processing by-products [7, 20, 22].

The ability to assimilate multiple sugars simultaneously is particularly advantageous when lignocellulosic feedstocks are employed. Although glucose generally remains the preferred substrate, several strains of *R. mucilaginosa* can also metabolize xylose and arabinose, albeit with lower efficiencies [20, 27]. This capability expands the range of potential industrial feedstocks and contributes to the feasibility of integrated biorefineries utilizing agricultural residues.

Furthermore, the species exhibits tolerance to several inhibitory compounds commonly present in industrial hydrolysates, including weak organic acids, furans, and phenolic compounds [22, 45]. Such resilience reduces pretreatment requirements and may contribute to lower production costs at industrial scale.

2.3 Relationship between lipid and carotenoid biosynthesis

A distinctive feature of *R. mucilaginosa* is the simultaneous production of lipids and carotenoids. While lipid biosynthesis serves primarily as a carbon and energy storage mechanism, carotenoids play protective roles against oxidative stress and environmental challenges [46].

Both metabolic routes are closely interconnected through central carbon metabolism. Acetyl-CoA functions as a precursor not only for fatty acid biosynthesis but also for the mevalonate pathway, which ultimately leads to carotenoid production [25, 47]. Consequently, cultivation conditions affecting carbon flux distribution can significantly influence the relative synthesis of lipids and pigments.

Carotenoids produced by *R. mucilaginosa* include β-carotene, torulene, and torularhodin [48, 49], all of which possess antioxidant properties and commercial value in food, cosmetic, pharmaceutical, and feed industries. Recent studies have demonstrated that environmental stresses such as high carbon-to-nitrogen ratios [2], osmotic stress [50], oxidative stress [46], and light exposure [50] can stimulate carotenoid biosynthesis while simultaneously promoting lipid accumulation.

This dual-production capability has contributed to a paradigm shift in the development of *R. mucilaginosa*-based bioprocesses. Rather than focusing exclusively on microbial oil production, contemporary research increasingly explores integrated strategies aimed at the simultaneous recovery of lipids and carotenoids [14, 25, 40], thereby improving process economics and enhancing the competitiveness of microbial biorefineries.

2.4 Fatty acid composition and biodiesel suitability

The industrial attractiveness of microbial oils depends not only on lipid yield but also on fatty acid composition. Numerous studies have shown that lipids produced by *R. mucilaginosa* are predominantly composed of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) [25-27, 51].

Among these fatty acids, oleic acid generally represents the major fraction, frequently accounting for more than 40% of total fatty acids [25]. This composition closely resembles that of several vegetable oils currently used in biodiesel production, including canola and sunflower oils [52].

High concentrations of monounsaturated fatty acids contribute to favorable biodiesel properties, including oxidative stability [53], cold-flow performance [54], viscosity [53], and cetane number [55]. Consequently, microbial oils produced by *R. mucilaginosa* have repeatedly been considered suitable feedstocks for biodiesel manufacturing.

Beyond biofuels, the presence of nutritionally valuable fatty acids also creates opportunities for applications in food ingredients [1, 7], animal feed supplements [56, 57], and oleochemical industries [13, 29, 55]. Therefore, the fatty acid profile of *R. mucilaginosa* reinforces its position as a versatile microbial cell factory capable of supporting multiple industrial sectors.

3. Historical development and biotechnological emergence of *Rhodotorula mucilaginosa*

The scientific trajectory of *Rhodotorula mucilaginosa* reflects the evolution of microbial biotechnology itself, progressing from the simple characterization of an environmental yeast to the recognition of a versatile microorganism with relevant industrial potential. Initially studied due to its distinctive pigmentation and ecological occurrence, *R. mucilaginosa* has progressively gained attention because of its metabolic adaptability and capacity to synthesize valuable biomolecules. The expansion of knowledge regarding its taxonomy, ecological distribution, and metabolic capabilities has contributed to the establishment of this yeast as a promising platform for sustainable bioprocesses.

3.1 Early identification and taxonomic evolution

The genus *Rhodotorula* has historically been associated with pigmented yeasts characterized by the production of carotenoid compounds, which confer colonies with yellow, orange, pink, or reddish coloration [58, 59]. During the early stages of research involving these microorganisms, species identification was mainly based on phenotypic characteristics, including colony morphology, cellular appearance, pigment production, and physiological tests such as carbon assimilation profiles [60].

Among these species, *R. mucilaginosa* was frequently reported in environmental samples due to its characteristic pigmentation and its ability to grow under diverse conditions. However, conventional taxonomic approaches based exclusively on morphological and biochemical traits often presented limitations, particularly due to the similarity among closely related yeast species. The development of molecular phylogenetic tools substantially improved the classification of pigmented yeasts, allowing more accurate species identification and revealing the evolutionary relationships within the group [61, 62].

The incorporation of genomic and molecular approaches marked an important transition in the study of *R. mucilaginosa*, shifting the understanding of this microorganism from a phenotypically defined yeast [60] toward a genetically characterized organism [61]. These advances provided new perspectives for investigating the molecular basis of its metabolic traits, including pigment biosynthesis [28], stress tolerance [50], and substrate utilization [43].

3.2 Ecological distribution and adaptive capacity

One of the earliest recognized characteristics of *R. mucilaginosa* was its broad ecological distribution. This yeast has been isolated from a wide range of environments, including soils [36], freshwater [37] and marine ecosystems [38], plant surfaces [39], food products [28], and industrial residues [14]. Its presence in chemically and physically distinct habitats indicates a high degree of physiological flexibility and environmental adaptation.

The ability of *R. mucilaginosa* to survive under fluctuating environmental conditions has been associated with its metabolic plasticity. Like many environmental microorganisms, this yeast can adjust its metabolism according to nutrient availability [2] and external stresses [46, 50]. Such adaptability includes the utilization of different carbon sources [20, 27], resistance to oxidative conditions [46], and survival under nutrient-limited environments [2].

The ecological success of *R. mucilaginosa* is particularly relevant from a biotechnological perspective because microorganisms capable of adapting to diverse environments often exhibit metabolic pathways suitable for the conversion of heterogeneous substrates. Consequently, characteristics that initially explained the ecological distribution of this yeast later became key factors supporting its exploitation in industrial processes.

3.3 Discovery of carotenoid and lipid production potential

The perception of *R. mucilaginosa* changed considerably when its capacity to produce intracellular metabolites with commercial value was recognized. The production of carotenoids was among the first characteristics that attracted industrial interest. These pigments, responsible for the characteristic coloration of the yeast colonies, also perform important physiological functions by protecting cells against oxidative damage [63].

Among the carotenoids reported in *R. mucilaginosa*, torularhodin [48, 49], torulene [48, 49], and β-carotene [49] are particularly relevant due to their antioxidant properties and potential applications in food, pharmaceutical, cosmetic, and nutraceutical industries [25, 48, 49]. The microbial production of carotenoids has gained attention as an alternative to chemical synthesis, especially due to the increasing demand for natural bio-based compounds.

In addition to carotenoid accumulation, *R. mucilaginosa* has been progressively recognized as an oleaginous yeast capable of storing significant amounts of intracellular lipids, mainly in the form of triacylglycerols (TAGs) [6, 11, 12]. This discovery expanded its biotechnological relevance, since microbial lipids represent potential alternatives to conventional plant-derived oils. The concept of SCOs has therefore positioned oleaginous yeasts such as *R. mucilaginosa* as promising candidates for the production of renewable lipid-based compounds, including biofuels and oleochemical intermediates [9, 13, 29].

The simultaneous ability to accumulate lipids and synthesize carotenoids represents a distinctive advantage of *R. mucilaginosa*, enabling the possibility of developing integrated bioprocesses in which multiple value-added products are obtained from the same microbial platform [14, 25, 28, 40].

3.4 Transition toward industrial biotechnology and sustainable applications

The increasing demand for sustainable production systems has accelerated research involving *R. mucilaginosa*, particularly toward lower-cost cultivation and improved metabolite productivity. Recent studies have increasingly examined alternative substrates [3, 19, 43] and process strategies [5, 64-66] that can reduce dependence on refined feedstocks and improve the efficiency of microbial production.

The emergence of genomic and metabolic engineering approaches represents a more recent stage in this development [67-70]. These approaches have expanded the focus from empirical optimization toward a mechanistic understanding of biosynthetic pathways and opportunities for targeted metabolic improvement.

At the same time, the species has attracted interest in environmental biotechnology because of its tolerance to chemically complex environments and its ability to interact with inorganic contaminants and other challenging matrices [37, 38, 71]. Together, these developments marked the transition from studies of a naturally occurring pigmented yeast toward its evaluation as a versatile microbial platform.

This historical progression established *R. mucilaginosa* as a microorganism with relevance extending beyond its original characterization as a pigmented yeast, providing the basis for the metabolic, process, and application-oriented developments discussed in the following sections.

4. Metabolic features and carbon flux regulation

The ability of *Rhodotorula mucilaginosa* to accumulate substantial amounts of intracellular lipids is closely associated with its metabolic flexibility and efficient regulation of carbon fluxes. As an oleaginous yeast, *R. mucilaginosa* possesses metabolic pathways that enable the conversion of diverse carbon substrates into storage lipids while simultaneously supporting the biosynthesis of other valuable metabolites, including carotenoids [14, 28, 49]. The regulation of these pathways involves a complex interplay between carbon assimilation [26, 27], nitrogen availability [72], precursor generation [11, 12], reducing power supply [10, 11], and intracellular storage mechanisms [72], as illustrated in Fig 2.

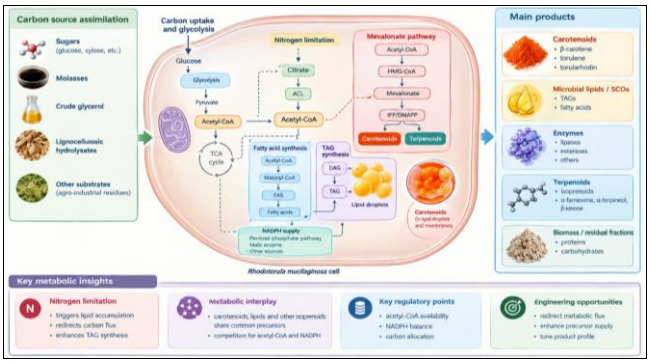

Fig 2: Metabolic bases of lipid and carotenoid biosynthesis in *Rhodotorula mucilaginosa*

Understanding the metabolic basis of lipid accumulation is essential for the development of efficient bioprocesses aimed at maximizing lipid productivity and improving substrate conversion efficiency. In recent years, advances in yeast physiology [63], metabolic engineering [6, 33], and synthetic biology [32] have provided valuable insights into the mechanisms governing carbon partitioning in oleaginous microorganisms. These studies have revealed that lipid accumulation is not merely a consequence of excess carbon availability but rather the result of coordinated metabolic responses that redirect cellular resources from biomass formation toward the synthesis of storage compounds.

4.1 Carbon assimilation and central carbon metabolism

Carbon assimilation represents the first step in the conversion of renewable substrates into microbial lipids. One of the most attractive characteristics of *R. mucilaginosa* is its ability to utilize a wide variety of carbon sources, including monosaccharides [27, 43], disaccharides [44], sugar alcohols [67], and complex hydrolysates derived from agro-industrial residues [20, 21, 26, 29]. This metabolic versatility enables the cultivation of the yeast on conventional substrates such as glucose [27], fructose [43], and sucrose [44], as well as on alternative feedstocks including crude glycerol [14], molasses [24, 25, 40], lignocellulosic hydrolysates [9, 26, 45], starch-derived sugars [21], and food-processing by-products [7, 20, 22].

Regardless of the substrate employed, assimilated carbon is ultimately directed toward central carbon metabolism. Glucose and fructose are readily incorporated into glycolytic pathways, where they are converted into phosphorylated intermediates and subsequently metabolized to pyruvate [73, 74]. Sucrose, after hydrolysis into glucose and fructose, follows the same metabolic routes [73]. Through glycolysis, these carbon sources generate adenosine triphosphate (ATP) and reducing equivalents while simultaneously providing metabolic intermediates required for cellular growth and biosynthetic activities [73, 74].

Pyruvate occupies a central position in the metabolism of *R. mucilaginosa*, acting as a key branching point between energy generation, biomass production, and lipid biosynthesis. Under growth-favorable conditions, pyruvate is primarily oxidized through the tricarboxylic acid (TCA) cycle to support cellular proliferation [73, 74]. However, under nutrient-limited conditions, particularly nitrogen depletion combined with excess carbon availability, cellular metabolism undergoes substantial reprogramming, redirecting carbon fluxes toward the accumulation of storage compounds [12, 72, 75].

The efficiency with which *R. mucilaginosa* converts different carbon substrates into biomass and lipids depends not only on substrate availability but also on the metabolic pathways required for substrate assimilation. Variations in carbon source composition can influence growth kinetics [27], carbon conversion efficiency [26, 27], lipid productivity [14], and carotenoid biosynthesis [14, 25]. Consequently, the selection of suitable feedstocks has become a critical aspect of process optimization, particularly in the context of industrial biorefineries utilizing low-cost renewable substrates.

Beyond supporting lipid accumulation, central carbon metabolism also provides the precursor molecules required for multiple biosynthetic pathways. Intermediates generated through glycolysis and associated metabolic routes contribute directly to the synthesis of amino acids, nucleotides, carotenoids, and fatty acids [11, 12, 73, 74]. Therefore, the regulation of carbon assimilation constitutes the foundation upon which the oleaginous phenotype of *R. mucilaginosa* is established, ultimately determining the distribution of carbon between cellular growth and the production of value-added metabolites.

4.2 Acetyl-CoA generation and metabolic rewiring under nitrogen limitation

A defining characteristic of oleaginous microorganisms is their ability to redirect carbon flux toward lipid biosynthesis when exposed to conditions of excess carbon and limited nitrogen availability. In *Rhodotorula mucilaginosa*, as in other oleaginous yeasts, nitrogen depletion triggers a profound metabolic reorganization that shifts cellular metabolism from biomass production to the accumulation of storage lipids [11, 72, 75].

During the growth phase, assimilated carbon is primarily utilized for the synthesis of proteins, nucleic acids, membrane components, and other cellular constituents required for proliferation [73, 74]. However, when nitrogen becomes limiting, protein synthesis and cell division are significantly reduced, despite the continued availability of carbon in the culture medium [12]. As a result, carbon uptake may continue while cellular growth is restricted, creating a metabolic imbalance that favors the accumulation of reserve compounds [11].

One of the key metabolic consequences of nitrogen limitation is the alteration of TCA cycle activity [10, 75]. Under these conditions, intracellular concentrations of adenosine monophosphate (AMP) decrease due to the activation of AMP deaminase [75]. Because mitochondrial isocitrate dehydrogenase is partially dependent on AMP for optimal activity, reduced AMP levels lead to diminished enzyme activity and a consequent slowdown of the TCA cycle [12, 75]. This metabolic bottleneck promotes the accumulation of citrate within the mitochondria [12].

The accumulated citrate is subsequently transported from the mitochondria to the cytosol through specific citrate transport systems [12, 73]. Once in the cytoplasm, citrate is cleaved by ATP-citrate lyase (ACL), generating acetyl-CoA and oxaloacetate [70]. This reaction represents one of the most important biochemical features distinguishing oleaginous from non-oleaginous yeasts, since ACL provides the cytosolic acetyl-CoA required for de novo fatty acid biosynthesis [70, 75].

The generation of cytosolic acetyl-CoA constitutes a critical metabolic checkpoint in lipid accumulation. Acetyl-CoA

serves as the primary precursor for fatty acid synthesis and ultimately determines the availability of carbon for the production of TAGs [10, 73, 75]. Consequently, the efficiency of citrate export and ACL activity strongly influences lipid productivity in oleaginous yeasts [73].

Simultaneously, additional metabolic adjustments occur to maximize carbon conservation and storage. Carbon fluxes that would otherwise support rapid biomass formation [73, 74] are progressively redirected toward anabolic pathways associated with lipid accumulation [75]. This metabolic rewiring enables cells to convert excess carbon into energy-dense storage molecules that can be mobilized later when environmental conditions become unfavorable [72, 76].

The extent of lipid accumulation depends on several factors, including the carbon-to-nitrogen ratio [72, 75], substrate type [27, 43, 44], cultivation strategy [14, 27], oxygen availability [77], and strain-specific metabolic characteristics [33]. High carbon-to-nitrogen ratios generally intensify citrate accumulation and acetyl-CoA generation, thereby enhancing lipogenic activity [10, 75]. Nevertheless, the relationship between nutrient limitation and lipid accumulation is highly dynamic, involving coordinated regulation of carbon assimilation [26, 27], precursor generation [11, 12], reducing power supply [10, 11], and intracellular storage mechanisms [72].

The formation of cytosolic acetyl-CoA marks the beginning of the dedicated lipogenic phase in *R. mucilaginosa* [10, 75]. Once generated, this precursor enters the fatty acid biosynthetic pathway, where it is converted into malonyl-CoA and subsequently incorporated into long-chain fatty acids that constitute the structural basis of microbial oils [28, 72].

4.3 Fatty acid biosynthesis and triacylglycerol formation

Following its generation in the cytosol [75], acetyl-CoA enters the de novo fatty acid biosynthetic pathway, which constitutes the central metabolic route responsible for lipid accumulation in *Rhodotorula mucilaginosa* [70, 75]. The efficiency of this pathway largely determines the capacity of the yeast to convert assimilated carbon into storage lipids and, consequently, its suitability as a microbial platform for single-cell oil production.

The first committed step of fatty acid biosynthesis is catalyzed by acetyl-CoA carboxylase (ACC), which converts acetyl-CoA into malonyl-CoA through an ATP-dependent carboxylation reaction [28, 72]. This step is considered one of the primary regulatory points of lipid metabolism because it controls the availability of malonyl-CoA, the two-carbon donor required for fatty acid chain elongation [69]. Increased ACC activity is commonly associated with enhanced lipogenic capacity in oleaginous microorganisms [28, 69, 72].

Subsequently, acetyl-CoA and malonyl-CoA serve as substrates for the fatty acid synthase (FAS) complex, a multifunctional enzymatic system responsible for the sequential elongation of fatty acid chains [72]. During each elongation cycle, two carbon atoms derived from malonyl-CoA are incorporated into the growing acyl chain through a series of condensation, reduction, dehydration, and reduction reactions [69]. These reactions continue until long-chain fatty acids, predominantly containing 16 or 18 carbon atoms, are produced [69, 72].

The biosynthesis of fatty acids requires substantial amounts of reducing power in the form of nicotinamide adenine dinucleotide phosphate (NADPH) [12, 72]. Consequently, lipid production is closely linked to cellular pathways involved in NADPH generation, which will be discussed in detail in the following section. The coordinated availability of acetyl-CoA, malonyl-CoA, ATP, and NADPH ultimately determines the overall efficiency of fatty acid synthesis [69, 72].

The major fatty acids produced by *R. mucilaginosa* are typically palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2), although their relative proportions may vary according to strain characteristics and cultivation conditions [25-27, 51]. The predominance of C16 and C18 fatty acids is particularly advantageous from an industrial perspective because these compounds closely resemble those found in conventional vegetable oils commonly used for biodiesel production and oleochemical applications [25, 52].

Once synthesized, free fatty acids are activated through esterification reactions involving glycerol-derived intermediates [78]. The assembly of storage lipids proceeds through a sequence of acylation steps that generate lysophosphatidic acid, phosphatidic acid, diacylglycerol (DAG), and ultimately TAGs [72, 78, 79]. Among these intermediates, DAG occupies a central position as the immediate precursor of TAGs formation [72, 78].

TAGs represent the principal storage lipids in oleaginous yeasts and account for the majority of accumulated intracellular oils. Their synthesis provides an efficient mechanism for storing excess carbon and energy in a chemically stable and highly reduced form [12, 72]. Under nitrogen-limited conditions, the metabolic flux toward TAGs formation can become particularly pronounced, allowing lipid contents to reach levels substantially higher than those observed in non-oleaginous microorganisms [72, 75].

The accumulation of TAGs constitutes the culmination of the lipogenic process initiated by carbon assimilation and acetyl-CoA generation [72, 78]. However, efficient lipid biosynthesis depends not only on precursor availability but also on a continuous supply of reducing equivalents. Therefore, understanding the metabolic pathways responsible for NADPH generation is essential for elucidating the mechanisms that support high lipid productivity in *R. mucilaginosa*.

4.4 NADPH supply and reducing power

The biosynthesis of fatty acids is a highly reductive process that requires a continuous supply of reducing equivalents, primarily in the form of NADPH [12, 72]. Consequently, the lipid accumulation capacity of *Rhodotorula mucilaginosa* depends not only on the availability of carbon precursors such as acetyl-CoA and malonyl-CoA but also on the efficiency of cellular pathways responsible for NADPH generation [28, 69, 72]. Insufficient reducing power can limit fatty acid synthesis even when carbon substrates are abundant, highlighting the central role of redox metabolism in oleaginous yeasts.

Among the metabolic routes involved in NADPH production, the pentose phosphate pathway (PPP) is generally considered the primary source of reducing equivalents for lipogenesis [80]. This pathway branches from glycolysis at the glucose-6-phosphate node and serves two major physiological functions: the generation of NADPH and the production of metabolic intermediates required for

nucleotide biosynthesis [80-82]. During the oxidative phase of the PPP, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase catalyze reactions that generate NADPH, providing reducing power directly linked to anabolic processes [81, 82].

The importance of the PPP in oleaginous microorganisms has been demonstrated in numerous studies reporting increased flux through this pathway during lipid accumulation [69, 72, 80]. As carbon is redirected from cellular proliferation toward storage lipid synthesis, the demand for NADPH increases substantially, promoting the activation of metabolic routes capable of sustaining reductive biosynthesis [81, 82]. Consequently, the PPP is frequently regarded as the principal contributor to the reducing power required for de novo fatty acid formation [81].

In addition to the PPP, malic enzyme (ME) may contribute to intracellular NADPH generation [83]. This enzyme catalyzes the oxidative decarboxylation of malate to pyruvate while simultaneously producing NADPH [84]. Although the quantitative contribution of ME varies among oleaginous species and cultivation conditions, its activity can complement the reducing power supplied by the PPP, particularly during periods of intense lipid accumulation [83, 84]. For this reason, reducing equivalents required for fatty acid biosynthesis are often described as being supplied primarily through the pentose PPP and, to a lesser extent, by ME activity [81, 83].

Other metabolic reactions may also contribute to maintaining intracellular redox balance, including NADP-dependent dehydrogenases associated with intermediary metabolism [81, 82]. However, their relative contributions are generally considered secondary compared with the PPP and ME [81, 83]. The integration of these pathways ensures that sufficient reducing equivalents are available to sustain the repetitive reduction reactions catalyzed by the FAS complex during chain elongation [69, 72].

The availability of NADPH influences not only the overall rate of fatty acid biosynthesis but also the distribution of carbon among competing metabolic pathways [85]. In *R. mucilaginosa*, NADPH is simultaneously required for the synthesis of other cellular components, including carotenoids [86] and antioxidant defense molecules [46]. Therefore, cellular mechanisms regulating NADPH generation and utilization play a crucial role in determining the balance between lipid accumulation [87], pigment production [46, 87], and other anabolic activities [88].

From a biotechnological perspective, enhancing NADPH availability has emerged as an important strategy for improving lipid productivity in oleaginous microorganisms. Metabolic engineering approaches targeting key enzymes of the PPP [80, 81], ME activity [81, 83], or alternative NADPH-generating reactions [80, 82, 88] have demonstrated the potential to increase carbon conversion efficiency and lipid yields. Consequently, understanding the regulation of reducing power metabolism represents a critical step toward the optimization of *R. mucilaginosa*-based bioprocesses.

The efficient generation of NADPH, together with the availability of carbon precursors, enables the synthesis and accumulation of large quantities of TAGs [80, 82, 88]. Once formed, these neutral lipids are compartmentalized into specialized intracellular structures known as lipid droplets (LDs), where they are stored and protected from degradation until required by the cell [76, 78].

4.5 Lipid droplet biogenesis and intracellular storage

The accumulation of neutral lipids in *Rhodotorula mucilaginosa* culminates in the formation of specialized intracellular organelles known as LDs, also referred to as lipid bodies [76, 78]. Rather than representing passive lipid deposits, LDs are highly dynamic organelles that play essential roles in energy storage, lipid homeostasis, membrane biogenesis, and cellular adaptation to environmental stress [89, 90]. Their formation is a defining feature of oleaginous yeasts and underlies the remarkable lipid accumulation capacity that characterizes these microorganisms.

Following de novo fatty acid synthesis, TAGs are assembled through sequential acylation reactions and accumulate within the endoplasmic reticulum (ER) membrane [78, 89]. As neutral lipids progressively increase between the phospholipid bilayer leaflets of the ER, localized lens-like structures are formed [90]. Continued TAG synthesis promotes the growth of these neutral lipid lenses until they bud from the ER into the cytoplasm, giving rise to mature lipid droplets [91]. Consequently, LD biogenesis is intimately associated with the endoplasmic reticulum and is considered an extension of its membrane dynamics [78].

Structurally, lipid droplets consist of a hydrophobic core predominantly composed of TAGs and, to a lesser extent, steryl esters [89]. This neutral lipid core is surrounded by a phospholipid monolayer rather than the conventional phospholipid bilayer found in other cellular organelles [90]. Embedded within this monolayer are numerous structural and regulatory proteins that govern lipid synthesis, droplet growth, intracellular trafficking, and lipolysis [76, 78]. These proteins also mediate interactions between LDs and other organelles, including the endoplasmic reticulum, mitochondria, peroxisomes, and vacuoles, thereby integrating lipid storage with broader cellular metabolic networks [76, 78, 89].

In oleaginous yeasts such as *R. mucilaginosa*, nitrogen limitation combined with excess carbon availability promotes extensive LD formation [27, 72, 87]. Under these conditions, carbon flux is redirected toward TAG biosynthesis, allowing lipid droplets to increase in both size and number [10, 72]. Depending on the strain and cultivation strategy, intracellular lipids may account for more than 20% of the cell dry weight, satisfying the conventional criterion for classifying a microorganism as oleaginous [6, 10-12]. In optimized cultivation systems, substantially higher lipid contents have been reported [14, 24, 27], reflecting the exceptional storage capacity of this yeast.

Beyond serving as carbon and energy reserves, LDs contribute to cellular protection under adverse environmental conditions. The sequestration of excess fatty acids into TAGs prevents the accumulation of potentially toxic free fatty acids, thereby reducing lipotoxicity and preserving membrane integrity [89, 90]. Moreover, LDs act as reservoirs of metabolic intermediates that can be rapidly mobilized when external carbon sources become scarce [92]. During carbon starvation or renewed cellular growth, stored TAGs are hydrolyzed by intracellular lipases, releasing free fatty acids that undergo β-oxidation to generate acetyl-CoA and ATP, thereby supporting cellular maintenance and survival [92, 93].

Recent studies have also demonstrated that LDs participate in broader cellular processes beyond lipid metabolism. Their

interactions with mitochondria facilitate the coordination between lipid storage and energy production, while contacts with peroxisomes support fatty acid catabolism through β-oxidation [89, 90, 92, 93]. Furthermore, LDs have been implicated in oxidative stress responses by limiting the accumulation of reactive lipid intermediates and maintaining intracellular lipid homeostasis under challenging environmental conditions [94, 95].

From an industrial perspective, the efficiency of LD biogenesis directly influences microbial oil productivity. Cultivation conditions that stimulate TAG synthesis generally promote the formation of larger and more abundant lipid droplets, increasing overall lipid yields [89, 90, 92, 94]. Consequently, understanding the molecular mechanisms governing LD formation, maturation, and turnover has become an important objective in metabolic engineering aimed at improving the performance of oleaginous yeasts in sustainable bioprocesses.

The coordinated regulation of carbon assimilation [96], precursor generation [66], reducing power availability [97], fatty acid biosynthesis [98], and lipid droplet formation [89] ultimately determines the lipid phenotype of *R. mucilaginosa*. However, these metabolic processes are not exclusively dedicated to lipid accumulation. Several biosynthetic pathways compete for common precursors, particularly acetyl-CoA and NADPH, creating an intricate metabolic network that also supports the synthesis of carotenoids and other high-value metabolites [68, 99].

4.6 Metabolic interplay between lipid and carotenoid biosynthesis

The remarkable biotechnological potential of *Rhodotorula mucilaginosa* stems not only from its ability to accumulate large amounts of intracellular lipids or synthesize valuable carotenoids individually, but also from its capacity to simultaneously produce both classes of compounds. This dual metabolic capability arises from the intricate interplay between lipid and carotenoid biosynthetic pathways, which are interconnected through shared precursors, reducing power, and regulatory mechanisms.

At the core of this metabolic network lies acetyl-CoA, a central precursor generated from assimilated carbon sources under lipogenic conditions [70, 75]. As discussed in previous sections, excess carbon combined with nitrogen limitation promotes the export of citrate from the mitochondria and the subsequent generation of cytosolic acetyl-CoA by ACL [70, 75]. This metabolite serves as the starting point for both fatty acid synthesis and the mevalonate pathway, from which carotenoids are ultimately derived [25, 47]. Consequently, the partitioning of acetyl-CoA between these competing biosynthetic routes plays a decisive role in determining the metabolic phenotype of *R. mucilaginosa*.

In lipid biosynthesis, acetyl-CoA is converted into malonyl-CoA and incorporated into long-chain fatty acids through the activity of the FAS complex, culminating in TAGs accumulation within lipid droplets [28, 72]. In parallel, acetyl-CoA enters the mevalonate pathway, leading to the formation of isoprenoid intermediates that give rise to carotenoids such as β-carotene, torulene, and torularhodin [36, 48, 49]. Therefore, fluctuations in precursor availability, enzymatic activity, and nutrient conditions directly influence the balance between lipid storage and pigment production.

In addition to sharing carbon precursors, both biosynthetic pathways rely heavily on reducing power in the form of NADPH. Fatty acid synthesis requires repeated reduction steps during chain elongation [11], while carotenoid biosynthesis also consumes significant amounts of reducing equivalents throughout the formation and modification of isoprenoid molecules [68, 86]. As a result, intracellular NADPH availability constitutes a key regulatory factor governing the simultaneous production of microbial oils and pigments. Enhanced flux through the PPP [80] or increased ME activity [83] may therefore affect not only lipid accumulation but also carotenoid yields.

The relationship between lipid and carotenoid biosynthesis is further influenced by environmental and cultivation conditions. Nutrient limitation, particularly nitrogen depletion, generally promotes lipid accumulation by redirecting carbon away from cellular growth [72, 75, 87]. However, oxidative stress [46, 86], light exposure [50], salinity [100, 101], and other environmental stimuli may stimulate carotenoid synthesis due to the protective antioxidant functions of these pigments. Consequently, cultivation strategies designed to maximize one class of metabolites may not necessarily optimize the production of the other, highlighting the complexity of carbon allocation in oleaginous yeasts.

Carbon source composition also plays an important role in determining metabolic outcomes. Different substrates can alter carbon flux distribution, energy generation, and reducing power availability, thereby influencing the relative production of lipids and carotenoids. In recent years, the use of agro-industrial residues [3, 40] and low-cost feedstocks [29, 31] has attracted considerable attention, not only because of their economic advantages but also due to their potential to modulate metabolic pathways and favor the co-production of multiple value-added compounds.

From an industrial perspective, the simultaneous production of microbial oils and carotenoids represents an attractive strategy for improving the economic viability of *R. mucilaginosa*-based biorefineries. Integrated processes capable of generating biodiesel precursors alongside natural pigments may significantly enhance process profitability and resource efficiency [14, 40]. Consequently, metabolic engineering [6, 33, 80, 81] approaches increasingly seek to optimize carbon partitioning, enhance precursor supply [11, 12], and fine-tune the regulatory networks that govern the synthesis of these compounds [80, 83].

The metabolic interplay between lipid and carotenoid biosynthesis exemplifies the complexity and versatility of *R. mucilaginosa*. Rather than functioning as isolated pathways, these biosynthetic routes operate as components of an integrated metabolic system that responds dynamically to environmental conditions and nutrient availability [14, 28, 40, 96]. A deeper understanding of this relationship will be essential for the rational design of cultivation strategies and the development of sustainable microbial platforms for the production of fuels, chemicals, and high-value bioproducts.

5. Cultivation strategies and substrate optimization

The industrial exploitation of *Rhodotorula mucilaginosa* depends not only on its intrinsic metabolic capabilities but also on the optimization of cultivation strategies capable of maximizing biomass formation and metabolite production. Although this yeast naturally exhibits the ability to accumulate lipids and synthesize carotenoids, the efficiency of these processes is strongly influenced by culture conditions [40], substrate composition [19, 43], and operational

parameters [14], as summarized in Fig 3. Consequently, the development of economically viable bioprocesses requires a thorough understanding of the factors governing carbon conversion, nutrient utilization, and metabolite partitioning.

Fig 3: Cultivation strategies and substrate optimization for *Rhodotorula mucilaginosa*

Over the past decades, significant efforts have been devoted to improving cultivation conditions for oleaginous yeasts through the selection of suitable carbon sources [20, 27], optimization of nutrient availability [19, 40], and implementation of advanced bioreactor strategies [14]. Particular attention has been given to the use of low-cost and renewable feedstocks [29, 31], aiming to reduce production costs while promoting the sustainable generation of high-value compounds. In this context, *R. mucilaginosa* has emerged as a promising microbial platform due to its metabolic flexibility and ability to utilize a broad range of substrates.

5.1 Effect of carbon sources on lipid and carotenoid production

The choice of carbon source is one of the most critical factors affecting microbial growth, lipid accumulation, and carotenoid biosynthesis in *Rhodotorula mucilaginosa*. Carbon substrates not only provide energy and biosynthetic precursors but also influence metabolic flux distribution [11, 12], intracellular redox balance [85], and the partitioning of carbon between competing pathways [80, 81]. Consequently, variations in substrate composition can significantly alter biomass productivity and the yields of lipids and carotenoids.

Conventional sugars such as glucose [27], fructose [43], and sucrose [44] are among the most extensively studied substrates for the cultivation of *R. mucilaginosa*. Glucose is generally regarded as the preferred carbon source due to its efficient assimilation through glycolysis and its ability to support rapid cellular growth [20]. Fructose follows a similar metabolic route after phosphorylation, whereas sucrose must first be hydrolyzed into its constituent monosaccharides before entering central carbon metabolism [43]. Despite their common metabolic fate, differences in transport mechanisms and assimilation rates may influence metabolite production under specific cultivation conditions.

The metabolic response of *R. mucilaginosa* to different carbon sources extends beyond growth performance. Variations in substrate composition may affect the intracellular availability of acetyl-CoA and NADPH [28, 69, 72], thereby influencing the balance between lipid accumulation and carotenoid biosynthesis. Several studies have demonstrated that carbon source selection can modify fatty acid profiles, alter carotenoid composition, and affect the overall efficiency of carbon conversion into value-added products [14, 25, 40].

From an industrial perspective, the use of refined sugars presents economic limitations due to their relatively high cost and competition with food supply chains. As a result, increasing attention has been directed toward alternative substrates derived from agro-industrial activities. Low-cost feedstocks such as crude glycerol [9, 14], molasses [24, 25, 40], fruit-processing residues [43], lignocellulosic hydrolysates [20, 26, 27], starch-rich by-products [21, 30, 40], and food waste [7, 22] have been investigated as sustainable carbon sources for *R. mucilaginosa* cultivation.

The ability of *R. mucilaginosa* to metabolize heterogeneous substrates highlights its potential for integration into biorefinery concepts and circular bioeconomy strategies. The conversion of industrial residues into microbial oils and natural pigments not only improves process economics but also contributes to waste valorization and environmental sustainability. However, the successful implementation of alternative feedstocks depends on overcoming challenges related to substrate variability, inhibitory compounds, and process optimization. Therefore, understanding how different carbon sources influence cellular metabolism is essential for designing cultivation strategies capable of maximizing lipid productivity, enhancing carotenoid synthesis, and improving the overall performance of *R. mucilaginosa*-based bioprocesses.

5.1.1 Conventional sugars

Conventional sugars remain the most widely employed substrates for investigating the physiology and metabolic performance of *Rhodotorula mucilaginosa*. Glucose, fructose, and sucrose are particularly important because they serve not only as model substrates for understanding carbon assimilation and lipid accumulation mechanisms but also as benchmark feedstocks against which alternative substrates are evaluated. Although these sugars ultimately converge into central carbon metabolism, differences in their transport, hydrolysis, and assimilation rates may significantly affect biomass formation, lipid accumulation, and carotenoid production.

Glucose is generally regarded as the reference carbon source for oleaginous yeasts due to its efficient assimilation through glycolysis and its ability to sustain rapid growth [102-104]. Studies with *R. mucilaginosa* have demonstrated that glucose-rich media favor biomass production, indicating that this sugar is readily converted into cellular material under suitable cultivation conditions [14, 20, 21, 24]. In experiments comparing synthetic and molasses-based media, higher glucose availability resulted in greater substrate-to-biomass conversion efficiencies, suggesting that glucose preferentially supports cell proliferation rather than the synthesis of secondary metabolites [24, 25, 40].

Despite entering central metabolism through pathways closely related to those of glucose, fructose may induce distinct physiological responses. Comparative studies have shown that replacing glucose with fructose can significantly reduce biomass production in pigmented yeasts, highlighting the influence of substrate identity on carbon partitioning [16, 105]. In fact, reductions of approximately 31% in cell concentration have been reported when glucose was substituted by fructose under otherwise identical cultivation conditions [18, 106]. These observations suggest that differences in transport systems, phosphorylation reactions,

and intracellular redox balance may affect the efficiency with which carbon is converted into biomass and storage compounds.

Sucrose presents an additional metabolic challenge because it must first be hydrolyzed into glucose and fructose before entering glycolysis [18, 107]. Nevertheless, *Rhodotorula* species exhibit an efficient capacity to utilize this disaccharide through extracellular invertase activity [108]. Studies performed with sucrose as the sole carbon source demonstrated that the sugar is rapidly hydrolyzed, leading to the transient accumulation of glucose and fructose in the culture medium [107, 109]. Complete sucrose hydrolysis may occur within the first 24 h of cultivation, while monosaccharide uptake proceeds more gradually [107, 109].

Interestingly, glucose appears to be preferentially consumed over fructose following sucrose hydrolysis [15, 108]. In *Rhodotorula* cultures, extracellular glucose depletion was observed before fructose exhaustion, indicating the existence of preferential substrate utilization mechanisms [107]. Such behavior may influence both the kinetics of metabolite production and the distribution of carbon fluxes during cultivation.

Beyond its metabolic implications, sucrose is particularly attractive from an industrial perspective because it can be obtained at low cost from sugarcane-derived feedstocks such as molasses [108]. Indeed, molasses-based media have been successfully employed for the simultaneous production of microbial biomass, lipids, and carotenoids by *R. mucilaginosa*, reinforcing the economic potential of sucrose-rich substrates [18, 40]. Furthermore, sucrose-containing substrates have been shown to support substantial carotenoid production [18, 106]. In one study, molasses-derived sucrose yielded the highest carotenoid concentration (89 $mg \cdot L^{-1}$), whereas lactose-rich media resulted in the highest carotenoid content per unit biomass [110].

Taken together, these findings demonstrate that conventional sugars are not metabolically equivalent, despite converging into common glycolytic intermediates. Variations in uptake rates, hydrolysis requirements, and intracellular carbon partitioning influence the balance between biomass formation, lipid accumulation, and carotenoid biosynthesis. Consequently, understanding the physiological responses of *R. mucilaginosa* to glucose, fructose, and sucrose provides the foundation for the rational selection of alternative substrates and the development of cost-effective cultivation strategies.

5.1.2 Industrial and agro-industrial residues

The high cost associated with refined carbon sources has stimulated growing interest in the use of industrial and agro-industrial residues as alternative feedstocks for microbial lipid and carotenoid production. Due to its metabolic versatility, *Rhodotorula mucilaginosa* can assimilate a wide variety of low-cost substrates, including biodiesel-derived streams [9, 14], sugar-rich residues [18, 24, 25], and lignocellulosic hydrolysates [21, 26, 27, 40]. The valorization of these materials not only contributes to reducing production costs but also aligns with the principles of circular bioeconomy and sustainable biorefinery development.

Among the alternative substrates investigated, crude glycerol generated during biodiesel production has attracted particular attention. As a by-product rich in carbon, crude glycerol represents an inexpensive feedstock capable of supporting both biomass formation and metabolite synthesis [9, 14]. Studies employing stepwise fed-batch strategies have demonstrated that medium optimization can substantially enhance lipid accumulation in *R. mucilaginosa* [24, 25]. For example, the study conducted by Pereira *et al*. (2019) [14] reported that supplementation with $MgSO_4$ increased intracellular lipid content from 21.4 to 51.0% of cell dry weight, while lipid production rose from 3.04 to 10.72 $g \cdot L^{-1}$. Moreover, optimized cultivation conditions promoted carotenoid production of up to 2843.2 $\mu g \cdot L^{-1}$, nearly doubling the values obtained in conventional batch cultures. The lipid fraction obtained under these conditions was predominantly composed of oleic acid, accompanied by lower proportions of palmitic and linoleic acids, confirming the suitability of microbial oils derived from crude glycerol for biodiesel and oleochemical applications.

Sugar-rich residues from the food and sugar industries also constitute promising feedstocks for *R. mucilaginosa*. In particular, sugarcane molasses has been extensively investigated due to its low cost, wide availability, and high sucrose content [18, 24, 25, 40]. Cultivation in molasses-based media has been shown to support the simultaneous production of microbial biomass, lipids, and carotenoids, while maintaining a fatty acid profile dominated by unsaturated compounds [18, 25]. Demonstrating this, Rodrigues *et al*. (2025) [24] found that batch cultures yielded a lipid content of approximately 46.7% of the cell dry weight, compared to 34.6% in fed-batch systems. In both cases, oleic acid remained the predominant fatty acid, representing more than 75% of the total lipid fraction. These results reinforce the industrial potential of molasses and related substrates as renewable carbon sources for microbial oil production.

The use of agro-industrial residues extends beyond sugarcane-derived streams. Alternative culture media formulated with molasses [18], rice parboiling wastewater [111], corn steep liquor [24, 25], and other food-processing by-products [7, 20, 22] have been successfully employed to replace glucose-based formulations, demonstrating the adaptability of *R. mucilaginosa* to heterogeneous nutrient compositions. Such versatility is particularly advantageous for large-scale bioprocesses, where substrate cost constitutes a major economic constraint.

More recently, lignocellulosic hydrolysates have emerged as attractive feedstocks for the sustainable production of microbial oils. Since most yeasts are unable to directly assimilate lignocellulosic biomass, pretreatment and hydrolysis steps are generally required to release fermentable sugars from cellulose and hemicellulose fractions [19, 23]. For instance, Rocha *et al*. (2026) [26] performed acid hydrolysis on soybean hulls to obtain a mixture containing 11.59 $g \cdot L^{-1}$ of xylose, 4.37 $g \cdot L^{-1}$ of arabinose, 1.24 $g \cdot L^{-1}$ of glucose, and 0.60 $g \cdot L^{-1}$ of cellobiose, resulting in a total fermentable sugar concentration of 17.80 $g \cdot L^{-1}$.

Despite the successful release of carbohydrates, lignocellulosic hydrolysates often contain inhibitory compounds that negatively affect microbial metabolism. In the studies by Rocha *et al*. (2025) [27] and Rocha *et al*. (2026) [26], soybean hull hydrolysis released acetic acid and hydroxymethylfurfural at concentrations of 1.08 and 0.06 $g \cdot L^{-1}$, respectively. These inhibitors reduced lipid accumulation compared to pure glucose cultures: while glucose-supported cultivation resulted in a lipid content of 32.26%, cultures grown on the hydrolysate mixture accumulated only 5.30% lipids [26]. Nevertheless, *R.*

mucilaginosa maintained satisfactory growth under these conditions, suggesting that optimization strategies such as detoxification and process engineering may further improve the utilization of lignocellulosic substrates.

Regardless of the substrate employed, the literature shows that the fatty acid composition of *R. mucilaginosa* remains relatively conserved, with oleic, palmitic, and linoleic acids consistently representing the major lipid components [14, 24, 26, 27]. This compositional stability is advantageous from an industrial perspective, as it facilitates the standardization of downstream applications and product quality.

Overall, the ability of *R. mucilaginosa* to convert industrial and agro-industrial residues into lipids and carotenoids highlights its considerable potential as a microbial platform for integrated biorefineries. However, the successful implementation of these processes still depends on overcoming challenges associated with substrate heterogeneity, pretreatment requirements, and the presence of inhibitory compounds. Future advances in cultivation strategies and metabolic engineering are expected to further expand the range of renewable feedstocks suitable for large-scale applications.

5.1.3 Emerging feedstocks and biorefinery approaches

Recent advances in industrial biotechnology have shifted the focus of microbial lipid production from the evaluation of individual carbon sources toward the development of integrated biorefinery concepts. Rather than considering agro-industrial residues solely as inexpensive substrates, current research increasingly explores their incorporation into circular production systems capable of simultaneously generating multiple value-added products while minimizing waste generation and environmental impacts.

Within this context, *Rhodotorula mucilaginosa* has emerged as an attractive microbial platform due to its remarkable metabolic flexibility. Its ability to assimilate diverse carbohydrate-rich streams—including crude glycerol [9, 14], molasses [24, 25, 40], lignocellulosic hydrolysates [9, 26, 27], food-processing residues [7, 20, 22], and starch-based wastewaters [21, 111]—allows the integration of microbial oil production into existing industrial chains. Such versatility enables the conversion of low-value by-products into high-value metabolites, contributing to resource efficiency and process sustainability.

The biorefinery concept is particularly attractive because *R. mucilaginosa* is capable of synthesizing multiple commercially relevant products from a single cultivation process. Besides accumulating substantial amounts of intracellular lipids suitable for biodiesel and oleochemical applications [13, 29], the yeast simultaneously produces carotenoids [28, 49, 61, 111], microbial biomass [4, 104], and other metabolites with potential applications in the food, cosmetic, pharmaceutical, and feed industries [1, 7, 48, 49]. The co-production of these compounds represents an important strategy for improving process economics, as revenues can be generated from multiple product streams rather than relying on a single target metabolite.

Another emerging trend involves the sequential or integrated utilization of different waste streams according to their chemical composition. Sugar-rich residues may preferentially support rapid biomass formation [15, 16, 105], whereas glycerol-rich substrates often favor lipid accumulation [9, 14]. Likewise, lignocellulosic hydrolysates provide access to abundant renewable carbohydrates that do not compete directly with food resources [9, 26, 27]. The strategic combination of these feedstocks offers new opportunities for optimizing carbon utilization and tailoring metabolite production according to specific industrial objectives.

Despite these advances, several technological challenges still limit the large-scale implementation of integrated biorefineries based on *R. mucilaginosa*. The heterogeneous composition of waste streams, seasonal variability, nutrient imbalance, and the presence of inhibitory compounds may compromise process reproducibility and product yields. In addition, downstream processing remains a significant economic bottleneck, particularly when multiple products are simultaneously recovered from the same fermentation broth [14, 18, 40, 106].

Future developments are expected to combine optimized cultivation strategies with systems biology [12, 13, 32], metabolic engineering [68, 69, 99], and process intensification to improve substrate utilization and carbon conversion efficiency [5, 54, 66]. Advances in synthetic biology and adaptive laboratory evolution may further expand the substrate spectrum of *R. mucilaginosa*, enabling the efficient conversion of increasingly complex renewable feedstocks [29, 31]. Together, these approaches reinforce the role of this oleaginous yeast as a promising microbial chassis for next-generation biorefineries and circular bioeconomy initiatives.

5.2 Influence of culture conditions

Although substrate selection plays a fundamental role in determining the metabolic performance of *Rhodotorula mucilaginosa*, cultivation conditions are equally important in regulating biomass formation, lipid accumulation, and carotenoid biosynthesis. Environmental and nutritional factors influence carbon partitioning [93, 104], intracellular redox balance [81, 82, 85], enzymatic activity [61, 79], and stress responses [88, 94, 105], ultimately determining the efficiency of metabolite production. Consequently, process optimization requires not only the selection of suitable carbon sources but also careful control of cultivation parameters throughout fermentation.

Among the various operational factors investigated, the carbon-to-nitrogen (C/N) ratio [72, 87], temperature [28, 50], pH [36, 50], oxygen availability [25, 46, 77], and environmental stresses [86, 87] have consistently been identified as the primary determinants of lipid and carotenoid productivity.

5.2.1 Carbon-to-nitrogen ratio

The carbon-to-nitrogen (C/N) ratio is widely recognized as one of the most influential parameters governing lipid accumulation in oleaginous yeasts [2, 10, 19]. While carbon provides the precursors required for biomass formation and metabolite synthesis [10, 12], nitrogen is essential for the production of amino acids, nucleic acids, proteins, and other cellular components associated with active growth [12, 72, 73]. Consequently, the relative availability of these nutrients determines whether assimilated carbon is preferentially directed toward cell proliferation or stored as intracellular lipids.

Under balanced nutritional conditions, *R. mucilaginosa* primarily allocates assimilated carbon to biomass production, maintaining high rates of protein synthesis and cell division [10-12, 70, 72, 75, 96]. However, as nitrogen becomes limiting while carbon remains available, cellular metabolism undergoes a profound reorganization. Protein synthesis slows, cell proliferation is reduced, and excess carbon is progressively redirected toward the biosynthesis of TAGs

[11, 70], as discussed in Section 4. This metabolic transition represents the physiological basis of the oleaginous phenotype and explains why nitrogen limitation is routinely employed to stimulate lipid accumulation in industrial fermentations.

The influence of the C/N ratio extends beyond lipid production. Altering nitrogen availability also affects carotenoid biosynthesis [36, 50, 87, 96], cellular respiration [10, 46, 86], and overall carbon conversion efficiency [14, 24, 66]. Depending on the cultivation strategy, moderate nitrogen limitation may favor the simultaneous production of biomass, lipids, and pigments, whereas excessively high C/N ratios can reduce cell growth to levels that ultimately compromise volumetric productivity [14, 18, 40, 106]. Therefore, the optimal C/N ratio should be considered a balance between maximizing intracellular lipid content and maintaining sufficient biomass formation.

The optimal C/N ratio is not universal and depends on multiple factors [10, 96], including strain characteristics [24, 36, 50], carbon source composition [14, 86], cultivation mode [14, 66], and oxygen availability [46, 87]. [50, 87] As a result, C/N optimization is often performed in combination with adjustments to aeration, pH, temperature, and feeding strategies to maximize process performance [46, 75, 86].

From an industrial perspective, controlling the C/N ratio represents one of the simplest and most effective approaches for regulating carbon partitioning in *R. mucilaginosa*. Rather than requiring genetic modification, lipid accumulation can be substantially enhanced through appropriate nutrient management [10, 66], making C/N optimization a central component of process development for microbial oil and carotenoid production [14, 24, 40].

5.2.2 Physical parameters

In addition to nutrient composition, physical cultivation parameters play a fundamental role in determining the physiological and metabolic performance of *Rhodotorula mucilaginosa*. Temperature, pH, and agitation influence enzyme activity [10] and microbial growth [36, 96], membrane properties [50, 51, 72, 112], nutrient transport [46, 50, 75], oxygen transfer [75], and intracellular metabolic fluxes, thereby affecting biomass formation and the biosynthesis of lipids, carotenoids, and other metabolites [14, 24]. However, the studies compiled in this review indicate that the effects of these parameters are highly dependent on the strain, culture medium, cultivation strategy, and target metabolite. Therefore, rather than defining universal optimal conditions, the available literature provides a range of conditions associated with different biotechnological objectives.

Table 1 summarizes six experimental studies that specifically investigated or optimized temperature, pH, and/or agitation during *R. mucilaginosa* cultivation.

A comparative analysis of these studies reveals an important pattern: although the reported conditions are not identical, *R. mucilaginosa* generally exhibits satisfactory performance under moderate cultivation temperatures. Four of the six studies identified or employed approximately 25 °C as the principal cultivation temperature, whereas Naghavi *et al.* (2014) [112]. used 30 °C and Silva *et al*. (2020) [113] evaluated a broader range from 22 to 34 °C. This tendency is consistent with the physiological requirements of yeast cells, for which temperature affects enzymatic reaction rates [10], membrane fluidity [50, 51], substrate transport [46, 50], and energy metabolism [10, 73, 74]. Nevertheless, the broader temperature range investigated by Silva *et al*. (2020) [113] provides an important qualification to this apparent consensus. Their results showed that the condition favoring biomass accumulation was not the same as that favoring carotenoid production: biomass reached 12.87 $g \cdot L^{-1}$ at 34 °C and pH 5, whereas carotenoid production was favored at 22 °C and pH 7. Thus, temperature optimization should be regarded as product-dependent, rather than as a search for a single optimum applicable to all *R. mucilaginosa* bioprocesses.

The influence of pH is even less amenable to generalization. The studies investigated or employed initial pH values ranging from approximately 4.5 to 7.0, with different conditions associated with lipid, carotenoid, biomass, or enzyme production. Pereira *et al*. (2019) [14] obtained high lipid accumulation under an optimized cultivation strategy involving acidic conditions, whereas Potumarthi *et al*. (2008) [17] reported maximum lipase production at pH 7.0. Particularly relevant is the study of Maldonade *et al*. (2012) [36], in which initial pH values between 4 and 6 were evaluated and pH did not significantly affect biomass or carotenoid production within the investigated range. These findings indicate that *R. mucilaginosa* possesses considerable physiological tolerance to pH variation and that the optimal pH is likely determined by the interaction between strain characteristics, nutrient composition, metabolic objective, and cultivation strategy. Accordingly, the available evidence does not support the definition of a universal pH optimum for this species.

Table 1: Summary of experimental studies investigating physical cultivation parameters in *Rhodotorula mucilaginosa*

Study	Strain	Target metabolite/process	Temperature (°C)	Initial pH	Agitation (rpm)	Main outcome
Machado *et al.* (2019) [114]	*R. mucilaginosa* URM 7409	Carotenoids	25	6.5	130	Carotenoid production of approximately 4.16–4.32 mg·L^{-1} under optimized conditions
Pereira *et al.* (2019) [14]	*R. mucilaginosa* CCT 7688	Lipids and carotenoids	25	4.5*	180	Up to 51.0% lipid content, 10.72 g·L^{-1} lipids, and 2843.2 µg·L^{-1} carotenoids
Potumarthi *et al.* (2008) [17]	*R. mucilaginosa* MTCC 8737	Lipase production	25 ± 2	7.0	200	Maximum lipase production of 72 U·mL^{-1} at 2 vvm
Maldonade *et al.* (2012) [36]	*R. mucilaginosa*-137	Biomass and carotenoids	25	4–6	200	pH had no significant effect within the tested range; maximum values reached approximately 8.0 g·L^{-1} biomass and 745 µg·L^{-1} carotenoids
Naghavi *et al.* (2014) [112]	*R. mucilaginosa*	Carotenoids	30	5.0	150	Maximum carotenoid concentration of approximately 3.40 mg·g^{-1} biomass, with 3.93 g·L^{-1} biomass
Silva *et al.* (2020) [113]	*R. mucilaginosa* CCT 3892	Biomass, carotenoids and lipids	22–34	5–7	200	Maximum biomass at 34 °C/pH 5, whereas carotenoid production was favored at 22 °C/pH 7

* In Pereira *et al*. (2019) [14], pH 4.5 was associated with the optimized medium strategy rather than representing an independently determined universal pH optimum.

Agitation also showed a relatively consistent, although not universal, pattern. The experimental studies employed agitation rates between 130 and 200 rpm, with intermediate values predominating. Machado *et al*. (2019) [114] identified 130 rpm as part of the optimized condition for carotenoid production, while Naghavi *et al*. (2014) [112] employed 150 rpm and Pereira *et al*. (2019) [14] used 180 rpm. Other studies used 200 rpm as the principal agitation condition. Rather than indicating that a specific agitation rate is intrinsically optimal, these results suggest that intermediate agitation is generally sufficient to maintain adequate mixing and mass transfer in shake-flask systems. More importantly, agitation should not be interpreted independently from oxygen availability. Potumarthi *et al*. (2008) [17], for example, explicitly evaluated agitation together with aeration and demonstrated that changes in agitation affected oxygen-transfer parameters, including the volumetric oxygen-transfer coefficient and oxygen-transfer rate. Maximum lipase production was obtained at 200 rpm and 2 vvm, illustrating the close relationship between agitation, aeration, and microbial performance.

The interaction among physical parameters becomes particularly evident when different metabolic objectives are considered simultaneously. Machado *et al*. (2019) [114] demonstrated that the combination of 25 °C, pH 6.5, and 130 rpm substantially enhanced carotenoid production, reaching approximately 4.3 mg·L^{-1} after optimization. Conversely, Silva *et al*. (2020) [113] showed that conditions favoring biomass formation differed from those maximizing carotenoid production, demonstrating that maximizing cell growth does not necessarily maximize the accumulation of a desired metabolite. This distinction is particularly relevant for industrial process development, because the optimal operating condition should be defined according to the desired productivity and product quality rather than biomass concentration alone.

Taken together, the available evidence suggests that *R. mucilaginosa* generally performs well at moderate temperatures and under intermediate agitation rates, but that pH and temperature responses can vary substantially according to the strain and target metabolite. Therefore, the literature does not support a single universal combination of temperature, pH, and agitation. Instead, these variables should be considered interconnected process parameters whose effects emerge from their interaction with nutrient composition, oxygen availability, and the physiological state of the culture. This observation provides a strong rationale for the increasing use of multivariate experimental designs, such as response surface methodology, rather than conventional one-factor-at-a-time optimization approaches.

5.2.3 Oxygen availability and oxidative metabolism

Oxygen availability is a critical determinant of the physiological and metabolic performance of *Rhodotorula mucilaginosa*. As a strictly aerobic yeast, this microorganism depends on oxygen to sustain respiratory metabolism, energy generation, and biomass formation [10, 46, 60]. However, the role of oxygen extends beyond its function as a terminal electron acceptor in cellular respiration. Oxygen availability also influences intracellular redox balance [80, 85], lipid metabolism [70, 77], fatty acid desaturation [11, 51], carotenoid biosynthesis [50, 106], and the activation of cellular responses to oxidative stress [46, 86]. Consequently, variations in oxygen transfer may alter not only microbial growth but also the distribution of carbon toward different metabolic products [17, 38].

In cultivation systems, the amount of oxygen available to the cells is determined by the balance between oxygen transfer and oxygen consumption. This balance depends on several operational variables, including agitation, aeration, culture volume, reactor geometry, and medium properties [17, 66, 77]. In shake-flask experiments, agitation is frequently used as an indirect strategy to increase oxygen transfer by reducing the boundary layer surrounding the cells and increasing gas–liquid interfacial renewal [36, 50, 114]. However, identical agitation rates do not necessarily provide equivalent oxygen availability under different cultivation conditions. Consequently, agitation alone should not be interpreted as a direct measure of oxygen supply, particularly when comparing experiments performed in different vessels or at different scales [17, 66, 75].

The relationship between oxygen transfer and *R. mucilaginosa* performance was directly demonstrated by Potumarthi *et al.* (2008) [17], who investigated the combined effects of agitation and aeration on lipase production. Their results showed that changes in these operational parameters affected oxygen-transfer characteristics, including the volumetric oxygen-transfer coefficient (k_La) and oxygen-transfer rate (OTR). Maximum lipase production was

obtained under intermediate operating conditions, at 200 rpm and 2 vvm, rather than under the highest agitation or aeration levels investigated. These findings demonstrate that increasing oxygen-transfer intensity does not necessarily result in a proportional improvement in metabolite production and that an optimal balance between oxygen supply and cellular metabolic demand is required.

Oxygen availability is also closely associated with lipid metabolism. Oleaginous yeasts require oxygen for several enzymatic reactions involved in fatty acid synthesis and modification, particularly during the desaturation of fatty acids [10, 11, 51]. Therefore, insufficient oxygen availability may alter the fatty acid profile and limit the formation of unsaturated fatty acids [24, 25, 50, 51]. At the same time, excessive oxygen availability may favor respiratory metabolism and biomass production rather than carbon accumulation in storage compounds, depending on the nutritional and physiological state of the cells [12, 70, 77]. This dual role highlights the importance of carefully controlling oxygen transfer when the objective is to maximize lipid accumulation rather than biomass alone [66, 75, 77].

A similar relationship exists between oxygen metabolism and carotenoid biosynthesis. Carotenoids are isoprenoid compounds whose biosynthesis is connected to central carbon metabolism and cellular redox status [47-49, 86]. In addition to their commercial value as natural pigments, carotenoids perform important physiological functions by protecting cells against oxidative damage [28, 46, 48, 63]. Changes in oxygen availability can therefore influence carotenoid accumulation both through metabolic regulation and through the generation of oxidative stress [46, 50, 106]. Under conditions that increase the formation of reactive oxygen species (ROS), the accumulation of antioxidant compounds may represent an adaptive mechanism that contributes to cellular protection [46, 86, 94, 95].

This relationship between oxidative stress and carotenoid production is particularly relevant for *R. mucilaginosa*, whose characteristic pigmentation is associated with the synthesis of antioxidant carotenoids [28, 46, 48, 49]. However, oxidative stress should not be interpreted as an exclusively beneficial stimulus. Moderate oxidative conditions may activate protective metabolic responses and stimulate the accumulation of antioxidant metabolites [50, 86, 87], whereas excessive ROS formation can damage proteins, lipids, and nucleic acids, ultimately reducing cell viability and productivity [82, 85, 105]. Therefore, the relationship between oxygen availability and carotenoid production is likely to follow a balance between beneficial metabolic stimulation and oxidative damage [46, 50, 106].

The interaction between oxygen availability and oxidative metabolism also provides an important explanation for the variable effects of agitation observed across different studies. Agitation can improve oxygen transfer and support aerobic metabolism, but excessive agitation may simultaneously increase mechanical stress and alter the cellular redox environment [17, 50, 86]. Consequently, the optimal agitation rate identified in a particular study cannot be directly generalized to other strains, culture media, or reactor configurations [17, 36, 66, 114]. Instead, oxygen availability should ideally be evaluated using quantitative parameters such as dissolved oxygen concentration, k_La, OTR, and oxygen uptake rate (OUR), particularly when the objective is to transfer cultivation strategies from shake flasks to bioreactors [66, 75, 77].

From a bioprocess engineering perspective, oxygen transfer becomes increasingly important during scale-up [66, 75]. Parameters such as agitation rate or aeration flow cannot simply be maintained at the same numerical values when the cultivation volume increases because hydrodynamic conditions, mixing patterns, gas dispersion, and oxygen-transfer efficiency change substantially with reactor scale [17, 50, 66]. Consequently, the successful scale-up of *R. mucilaginosa* cultivation requires the identification of oxygen-transfer conditions capable of maintaining an appropriate balance between cellular oxygen demand and oxygen availability [66, 75, 77].

Overall, oxygen should be regarded not merely as an environmental variable but as a central metabolic regulator during *R. mucilaginosa* cultivation. Its availability influences respiratory activity, intracellular redox balance, lipid metabolism, carotenoid biosynthesis, and oxidative-stress responses [11, 46, 51, 86]. The available evidence therefore suggests that the optimization of agitation and aeration should ultimately be guided by oxygen-transfer parameters rather than by operational variables alone [50, 66, 77]. This integrated perspective is particularly important for the development of scalable processes, in which the control of oxygen availability may determine whether metabolic performance observed at laboratory scale can be successfully reproduced in larger bioreactors [17, 66, 75].

5.3 Bioreactor cultivation and scale-up

5.3.1 Bioreactor cultivation and process control

The optimization of *Rhodotorula mucilaginosa* cultivation at laboratory scale provides essential information regarding substrate utilization, nutrient requirements, and the physical conditions that favor biomass formation and metabolite production [14, 26, 27]. However, results obtained in shake-flask experiments cannot be directly extrapolated to larger cultivation systems [26, 27]. Although shake flasks remain valuable tools for preliminary screening and optimization, they provide limited control over key process variables, including dissolved oxygen concentration, pH, mixing, aeration, and substrate availability [115, 116]. As culture volume increases, these limitations become increasingly relevant [117, 118] and may substantially alter cellular physiology and metabolic performance [64, 119].

Bioreactors provide a more controlled environment in which the principal cultivation variables can be continuously monitored and adjusted. Temperature, pH, dissolved oxygen, agitation, and aeration can be regulated according to predefined operating conditions, allowing greater process reproducibility and facilitating the identification of relationships between environmental conditions and cellular responses [120, 121]. This level of control is particularly important for *R. mucilaginosa*, whose production of biomass, lipids, carotenoids, and other metabolites may respond differently to changes in cultivation conditions [14, 40, 63]. Consequently, bioreactor cultivation enables process optimization to move beyond the identification of favorable empirical conditions toward the establishment of controlled operating windows [65, 122]. Importantly, the value of this control is not restricted to maintaining constant conditions; it also enables process variables to be deliberately modified according to the physiological state and metabolic objective of the culture.

A representative example was provided by Potumarthi *et al*. (2008) [17], who investigated lipase production by *R.*

mucilaginosa MTCC 8737 in a 1.5 L stirred-tank reactor. The authors evaluated agitation between 100 and 300 rpm and aeration between 1 and 3 vvm, demonstrating that the highest lipase production, 72 $U \cdot mL^{-1}$, was achieved at 200 rpm and 2 vvm. More importantly, the study quantified the effects of these operating conditions on oxygen-transfer parameters, with k_La values ranging from approximately 19 to 32 h^{-1} and oxygen-transfer rates ranging from 17.29 to 56 $mg \cdot L^{-1} \cdot h^{-1}$. The maximum production was therefore not obtained under the highest agitation or aeration tested, highlighting that increasing oxygen-transfer capacity does not necessarily result in a proportional increase in product formation.

The same study also demonstrated that oxygen transfer cannot be considered independently of the physicochemical properties of the culture medium. Increasing the concentration of molasses increased broth viscosity and consequently affected oxygen-transfer behavior and lipase production. This observation is particularly relevant for processes based on agro-industrial residues, whose composition and rheological properties may differ substantially from those of defined laboratory media [3, 40, 50]. Consequently, process control must account not only for nominal operating variables such as agitation and aeration, but also for changes in broth properties that occur as biomass and metabolites accumulate [120, 121].

The direct relationship between aeration and metabolic performance was also demonstrated by Aksu and Eren [110], who investigated carotenoid production by *R. mucilaginosa* in batch cultivation. Increasing the aeration rate enhanced carotenoid concentration and yield up to 2.4 vvm, while the optimum cultivation conditions included pH 7 and 30 °C. Under the optimized conditions, carotenoid production reached 89.0 $mg \cdot L^{-1}$ using sucrose molasses as the carbon source. This result provides experimental evidence that oxygen supply can directly influence the formation of a value-added metabolite and reinforces the importance of considering aeration as a process variable rather than merely an auxiliary operating parameter.

However, these studies also illustrate why oxygen availability should not be represented solely by agitation or aeration rates. In a bioreactor, oxygen availability results from the balance between oxygen transfer from the gas phase and cellular oxygen consumption [17]. Consequently, parameters such as k_La, OTR, and OUR, together with dissolved oxygen concentration, provide a more informative description of the physiological environment than agitation or aeration considered independently [66, 75, 77]. The relevance of this distinction becomes particularly evident when comparing different reactor configurations, working volumes, culture densities, or media with different rheological properties.

Bioreactor control also enables cultivation conditions to be modified dynamically rather than maintained at a single fixed value throughout the entire process. Pereira *et al.* (2019) [14], for example, developed a stepwise fed-batch strategy for *R. mucilaginosa* CCT 7688 based on the growth behavior observed during batch cultivation. Rather than treating the culture as a static system, the authors used the temporal progression of growth to establish feeding strategies intended to improve the simultaneous production of lipids and carotenoids. This approach illustrates an important conceptual transition in process development: once the cultivation environment can be monitored and controlled, operating conditions can be adapted to the physiological stage of the culture rather than optimized exclusively as a single set of constant parameters.

This dynamic perspective is particularly important because the conditions that maximize biomass formation are not necessarily those that maximize metabolite accumulation [14, 24, 25, 27]. As observed in previous studies discussed in Section 5.2, temperature, pH, nutrient availability, and oxygen supply may affect growth and product biosynthesis differently [10, 14, 24, 36, 96]. Accordingly, an effective bioreactor strategy may involve deliberately changing one or more process variables during cultivation to first promote biomass formation and subsequently favor the accumulation of the desired metabolite [14, 24, 25, 27]. Such strategies can potentially improve volumetric productivity while reducing the need to compromise between growth and product formation.

Taken together, these experimental studies demonstrate that bioreactor cultivation should not be regarded simply as the scale-up of shake-flask cultivation. Rather, it represents a transition toward active control of the cellular environment. The examples of Potumarthi *et al.* (2008) [17], Aksu and Eren (2005) [110], and Pereira *et al.* (2019) [14] collectively show that oxygen transfer, aeration, broth properties, and the temporal control of nutrient availability can substantially affect *R. mucilaginosa* performance. They also demonstrate that optimal process conditions emerge from the interaction among operational variables and cellular metabolic demand rather than from isolated parameter values.

Therefore, the development of *R. mucilaginosa*-based bioprocesses requires the integration of online monitoring and process control with an understanding of cellular physiology. Dissolved oxygen, pH, temperature, agitation, aeration, and substrate availability should be considered as interconnected variables whose optimal trajectories may change throughout cultivation. Once these variables are adequately controlled, the next challenge is to determine which cultivation strategy can best exploit this control while maintaining productivity as the process moves toward larger scales. This issue is addressed in the following section through the comparison of batch, fed-batch, and other cultivation strategies and their associated scale-up challenges.

5.3.2 Cultivation strategies and scale-up challenges

The choice of cultivation strategy represents a further level of process optimization beyond the control of individual environmental variables. In *Rhodotorula mucilaginosa*, the temporal availability of nutrients and the physiological state of the culture can strongly influence the balance between biomass formation and the accumulation of value-added metabolites [14, 40, 63]. Consequently, batch cultivation remains useful for strain screening and kinetic characterization [115, 116], whereas fed-batch and other dynamic strategies offer greater flexibility for controlling substrate availability and extending the productive phase of the culture [14, 24, 25].

Batch cultivation is the most frequently adopted strategy for laboratory-scale investigations because of its operational simplicity and relatively low risk of contamination [66, 115]. In this configuration, the culture medium is prepared before inoculation and the process proceeds without continuous nutrient addition [22]. Batch cultivation is particularly useful for determining growth kinetics [24, 36], substrate consumption [113], and the temporal relationship between

biomass formation and metabolite accumulation [10, 25, 27]. For example, Rodrigues *et al*. (2022; 2025) [24, 25] investigated carotenoid production by *R. mucilaginosa* using agroindustrial byproducts under both batch and fed-batch conditions, demonstrating the feasibility of modifying the cultivation mode to improve process performance. The study is particularly relevant because it directly compares static and dynamically fed cultivation strategies using low-cost substrates, thereby connecting process configuration with the broader objective of agroindustrial residue valorization.

Fed-batch cultivation provides a greater degree of control because nutrients can be supplied progressively according to the physiological requirements of the culture [66, 119]. This strategy can prevent excessive substrate concentrations, reduce substrate inhibition or undesirable overflow metabolism [45, 119], and extend the period during which cells remain metabolically active [10, 14, 70, 75, 97]. For oleaginous yeasts, controlled feeding can also be used to manipulate the balance between growth and lipid accumulation by regulating carbon availability relative to nitrogen and other nutrients. In this context, Pereira *et al*. (2019) [14] developed a stepwise fed-batch strategy for simultaneous lipid and carotenoid production by *R. mucilaginosa* CCT 7688 using crude glycerol. The feeding strategy was established based on the growth behavior observed during batch cultivation, illustrating how kinetic information obtained at an initial stage can be translated into a dynamic cultivation process.

The relevance of dynamic cultivation becomes particularly evident when biomass and product formation follow different temporal patterns. In many oleaginous yeasts, rapid biomass formation occurs during the early phase of cultivation, whereas lipid accumulation becomes more pronounced after specific nutrients become limiting [10, 70]. A similar temporal decoupling may occur during carotenoid biosynthesis [46, 50], meaning that a single set of cultivation conditions may not be equally favorable throughout the entire fermentation. Dynamic strategies therefore offer the possibility of establishing sequential process phases, initially favoring biomass formation and subsequently redirecting cellular metabolism toward the desired product [66, 87, 106]. This concept is supported by the increasing application of stepwise and two-stage strategies in oleaginous yeasts [14, 66], although their implementation specifically for *R. mucilaginosa* remains comparatively limited [1, 28, 33].

The transition from laboratory cultivation to larger bioreactors, however, introduces challenges that cannot be solved simply by increasing reactor volume. Scale-up modifies mixing time, gas dispersion, heat transfer, oxygen-transfer efficiency, and nutrient concentration gradients [17, 50, 66]. As discussed in Section 5.3.1, parameters such as agitation and aeration cannot be transferred directly from one reactor scale to another [64, 116] because the same nominal operating conditions may generate substantially different physiological environments. Consequently, successful scale-up requires the identification of appropriate engineering criteria capable of maintaining the relevant biological conditions rather than merely reproducing numerical operating parameters [17, 66, 77, 117, 118].

This challenge is particularly important when agroindustrial residues are used as substrates. Unlike defined media, these materials may exhibit considerable variability in sugar concentration, nitrogen content, mineral composition, viscosity, and the presence of inhibitory compounds [3, 26, 27, 40, 45, 103]. Such variability can affect both microbial physiology [25, 45, 70, 113] and the hydrodynamic behavior of the culture [66, 115-117]. The use of sugarcane molasses, for example, has demonstrated the potential of *R. mucilaginosa* to produce microbial oil, carotenoids, and fatty acids, but also highlights the importance of medium composition and carbon-to-nitrogen balance in determining process performance. Costa *et al*. (2020) [18] reported a maximum cell concentration of 16.42 ± 0.32 $g \cdot L^{-1}$ for *R. mucilaginosa* CCT 3892 cultivated in a synthetic medium and showed that the composition of the production medium substantially affected biomass formation.

Another important scale-up consideration is the preservation of product quality and metabolic profile. Rodrigues *et al*. (2025) [24] reported that *R. mucilaginosa* CCT 7688 maintained a fatty-acid profile dominated by oleic acid during both batch and fed-batch cultivation, with oleic acid representing approximately 60–78% of the total fatty acids. The lipid content, however, differed between cultivation modes, reaching 46.7% of dry cell weight in batch cultivation and 34.6% under the evaluated fed-batch condition. These results illustrate an important distinction for process development: changing the cultivation strategy may alter not only the amount of biomass or product obtained but also the balance between productivity and product composition. Therefore, scale-up criteria should consider the desired product characteristics in addition to volumetric productivity.

Taken together, the available evidence suggests that cultivation strategy should be selected according to the physiological objective of each process. Batch cultivation remains indispensable for initial characterization and kinetic assessment [24, 36, 113, 115], whereas fed-batch and stepwise strategies provide greater control over nutrient availability and may facilitate the separation of growth and product-formation phases [10, 14, 75, 119]. At larger scales, however, the success of these strategies depends on maintaining adequate mixing, oxygen transfer, heat removal, and substrate distribution [64, 66, 116, 117]. The development of industrially relevant *R. mucilaginosa* processes will therefore require the integration of microbial physiology with bioreactor engineering, rather than the simple enlargement of laboratory protocols.

A further limitation is the relatively small number of studies in which *R. mucilaginosa* has been evaluated under genuinely process-oriented scale-up conditions [17, 22]. The literature contains evidence that the yeast can be cultivated in conventional bioreactors and that substantial biomass can be obtained at laboratory and pilot scales [14, 17], but systematic studies linking reactor hydrodynamics, oxygen-transfer parameters, feeding profiles, and product productivity remain comparatively scarce [64, 66, 117, 118]. This gap represents an important research opportunity, particularly for processes based on inexpensive agroindustrial substrates, in which medium variability and mass-transfer limitations may become increasingly relevant as the cultivation volume increases [26, 27, 98, 115].

Overall, the transition toward industrially relevant *R. mucilaginosa* bioprocesses requires a shift from static optimization toward dynamic and integrated process design. Rather than defining a single optimum condition, future strategies should consider how substrate availability, oxygen supply, nutrient limitation, and cellular metabolism evolve throughout cultivation. Such an approach could enable the

simultaneous improvement of productivity, resource efficiency, and process robustness, providing a stronger foundation for the industrial valorization of *R. mucilaginosa* and its integration into circular biorefinery systems.

6. Industrial applications and biorefinery opportunities

The biotechnological relevance of *Rhodotorula mucilaginosa* extends beyond its ability to synthesize individual metabolites. Its capacity to simultaneously accumulate carotenoids and lipids [14, 18], produce extracellular and intracellular enzymes [28], and grow on a broad range of low-cost substrates [3, 21] makes this yeast particularly attractive as a multiproduct microbial platform. Rather than relying exclusively on refined substrates and targeting a single compound, *R. mucilaginosa* can be integrated into biorefinery schemes in which agroindustrial residues are converted into biomass and several value-added products within the same process [6, 8, 31]. This concept is consistent with the transition toward circular bioeconomy systems, in which waste streams are regarded as feedstocks for the generation of renewable chemicals, ingredients, and bio-based materials.

6.1 Carotenoids as high-value products

Carotenoid production represents one of the most established biotechnological applications of *R. mucilaginosa*. The yeast synthesizes several carotenoids, including torularhodin [48], torulene [49], and β-carotene [49, 106], which contribute to its characteristic pigmentation and provide protection against oxidative stress. Their natural origin and antioxidant properties have generated interest in food [7], nutraceutical [41], pharmaceutical [28, 48], and cosmetic [42] applications. Beyond their role as pigments, these compounds have also been investigated for additional biological activities [123, 124], although application-specific evidence and product standardization remain important considerations [25, 36].

The industrial attractiveness of microbial carotenoids depends not only on biological activity but also on product composition, stability, recovery, purification, and production economics [22, 66, 125]. Because carotenoid accumulation varies substantially with strain and process conditions, the comparison of reported titers and yields remains difficult. Nevertheless, their high value relative to bulk lipids makes carotenoids particularly relevant as a complementary product in multiproduct processes, where recovery of a high-value fraction can contribute to overall process economics.

6.2 Microbial lipids and single-cell oils

The oleaginous phenotype of *R. mucilaginosa* provides a second major route for industrial valorization. Intracellular lipids, predominantly stored as TAGs [6, 11, 12], can be recovered as SCOs and considered as renewable alternatives to conventional plant-derived oils [10, 11, 21]. Depending on their fatty-acid composition, these microbial oils may be directed toward different applications, including biodiesel production [13, 29], oleochemical synthesis [6], and potentially nutritional [25] or specialty-lipid markets [25]. The fatty-acid profile of *R. mucilaginosa* is particularly relevant in this context because several studies have reported substantial proportions of oleic acid, an unsaturated fatty acid with nutritional and industrial value [25-27].

The industrial relevance of microbial lipids depends strongly on fatty-acid composition and downstream recovery. In *R. mucilaginosa*, oleic acid is frequently a major component [25-27], supporting potential applications in biodiesel [13, 29] and oleochemical production [6, 9] and, depending on purity and composition, in specialty-lipid markets [2, 7, 28]. For example, Da Costa *et al*. (2020) [18] reported 16.50% total lipids and 0.053 $mg \cdot g^{-1}$ carotenoids during cultivation on sugarcane molasses, with 74.05% oleic acid in the fatty-acid fraction. Such results illustrate the possibility of obtaining a lipid product while retaining a higher-value pigment fraction from the same cultivation system.

From an industrial perspective, the main advantage of residue-based SCO production is therefore not simply the replacement of refined carbon sources, but the possibility of coupling low-cost feedstocks with product diversification and waste valorization. The evidence reviewed in Section 5 indicates that substrate composition can influence both productivity and product profile; consequently, feedstock selection should be considered together with downstream requirements and the intended market for the resulting oil.

6.3 Enzymes and other functional products

Although carotenoids and lipids have received the greatest attention, the biotechnological potential of *R. mucilaginosa* is not restricted to these metabolites. Enzyme production represents another relevant application, with lipases being among the best-studied examples. Lipases are industrially important biocatalysts with applications in food processing, detergents, pharmaceuticals, oleochemical synthesis, and biotransformations [126-128]. The production of lipase by *R. mucilaginosa* has been demonstrated in stirred-tank reactors, including processes using molasses as the production medium. Potumarthi *et al*. (2008) [17] reported a maximum lipase activity of 72 $U \cdot mL^{-1}$ under optimized agitation and aeration conditions, demonstrating that this yeast can produce industrially relevant enzymes under controlled submerged fermentation.

The broader enzymatic repertoire of *R. mucilaginosa* [17], together with its ability to tolerate different environmental conditions [45] and utilize diverse substrates [3, 43], suggests that additional functional products may be incorporated into future bioprocesses. However, compared with carotenoids and lipids, the industrial evidence for these products remains considerably less developed. Therefore, enzyme production should currently be regarded as a promising complementary route rather than an equally established commercial application.

6.4 From single-product fermentation to integrated biorefineries

The most compelling industrial opportunity offered by *R. mucilaginosa* may therefore lie in integrating several production routes rather than maximizing a single metabolite. Its simultaneous synthesis of carotenoids and lipids provides a direct example of this strategy, while stepwise cultivation can be used to accommodate the different physiological requirements associated with biomass formation and secondary metabolite accumulation.

A conceptual *R. mucilaginosa* biorefinery can be organized around three interconnected streams: (i) conversion of low-cost or residual feedstocks into microbial biomass; (ii) recovery of intracellular high-value compounds such as carotenoids and lipids; and (iii) valorization of the remaining biomass and process residues. The rationale is to

distribute processing costs and value generation across several products rather than relying on a single commodity.

This configuration is attractive because upstream cultivation, product formation, and residue valorization can be considered as parts of the same process rather than independent operations. However, the feasibility of such integration depends on the compatibility of extraction and purification steps, the market value of each fraction, and the additional energy, solvent, and equipment requirements associated with recovering multiple products.

Beyond the production of marketable metabolites, the physiological robustness of *R. mucilaginosa* has also encouraged investigations in environmental biotechnology. Heavy-metal tolerance [37, 71], pollutant interaction [129, 130], and the ability to transform specific inorganic compounds [131, 132] indicate that the species may have applications in bioremediation and environmental management. For example, *R. mucilaginosa* has been studied by Ollivier *et al*. (2011) [38] for tellurium reduction and methylation under different aeration conditions, demonstrating that oxygen availability can influence its interaction with inorganic contaminants. Another example comes from Vaksmaa *et al*. (2023) [130] that demonstrated that the marine yeast *R. mucilaginosa* was able to effectively mineralize and assimilate carbon from UV-treated polyethylene at a rate of 3.8% per year, highlighting fungal degradation as a potential key sink for marine plastic litter. These applications are conceptually different from metabolite production but reinforce the versatility of the species as a microbial platform.

Overall, the industrial potential of *R. mucilaginosa* is best understood as a platform rather than a single-product microorganism. Carotenoids provide opportunities for high-value applications, microbial lipids offer routes toward renewable oils and oleochemicals, and enzymes and environmental applications broaden the potential portfolio. The key industrial question is therefore not whether multiple products can be produced, but whether they can be recovered through an economically and environmentally coherent process. This perspective links the application opportunities discussed here with the scale-up, downstream, and sustainability challenges examined in Section 7.

7. Challenges and future perspectives

Despite the considerable biotechnological versatility of *R. mucilaginosa*, its development as an established industrial cell factory remains constrained by interconnected biological, technological, and economic challenges. The literature demonstrates strong potential for lipid [18, 24, 28], carotenoid [28, 36, 43], terpenoid [49, 133], and enzyme production [28, 83], but laboratory-scale titers alone do not establish industrial feasibility. The next stage of research should therefore integrate strain development, feedstock selection, bioprocess control, downstream processing, and economic and environmental assessment.

One of the most promising directions is the transition from empirical strain selection toward rational metabolic engineering. Historically, most studies involving *R. mucilaginosa* have focused on optimizing culture conditions to enhance naturally occurring products, particularly carotenoids and lipids [24-27]. Recent advances, however, demonstrate that the metabolic potential of this yeast can be actively redirected. Chen *et al*. (2024) [133], for example, engineered *R. mucilaginosa* to produce heterologous terpenoids by modifying the endogenous mevalonate pathway. Overexpression of a rate-limiting gene increased α-farnesene production to 822 $mg \cdot L^{-1}$, while the engineered strains also produced α-terpineol and β-ionone. These results demonstrate that the species can function not only as a natural producer of carotenoids and lipids, but also as a platform for the biosynthesis of non-native terpenoids.

Nevertheless, *R. mucilaginosa* remains less genetically tractable than established microbial cell factories. Zheng *et al*. (2025) [1] identified limited genetic toolboxes, insufficient exploitation of multiomics data, and incomplete understanding of species-specific metabolism as major obstacles. Future work should combine genome-scale characterization, transcriptomics, metabolomics, metabolic flux analysis, and genome engineering to identify regulatory nodes controlling carbon allocation among biomass, lipid, carotenoid, and terpenoid formation. Such approaches could shift strain development from maximizing total metabolite accumulation toward designing product-specific phenotypes.

Agroindustrial residues represent a major opportunity, but their variability is also a process constraint. Molasses [17, 18], crude glycerol [9, 14], lignocellulosic hydrolysates [27, 29, 113], and food-processing residues [7, 20, 22] can reduce raw-material costs and support waste valorization, yet their composition, nutrient balance, viscosity, and inhibitory compounds vary with origin and processing history. Accordingly, a low-cost feedstock should be evaluated by its effect on overall process performance rather than by acquisition price alone.

Recent work illustrates this transition toward a more integrated assessment. Šovljanski *et al*. (2025) [3] systematically evaluated 13 agroindustrial biowastes for carotenoid production by *R. mucilaginosa*. Although pea protein isolate provided high productivity, its relatively high market value reduced its industrial attractiveness. In contrast, untreated grape pomace and crude glycerol combined competitive carotenoid production with greater availability and lower cost. Importantly, fed-batch validation using untreated grape pomace increased carotenoid productivity by 43% compared with shake-flask cultivation. These results demonstrate that substrate selection, economic considerations, cultivation strategy, and process intensification cannot be treated as independent variables. Future studies should therefore prioritize feedstocks according to a combination of biological performance, availability, compositional consistency, pretreatment requirements, and environmental and economic indicators.

Scale-up constitutes another decisive step in industrialization. As discussed in Section 5, increasing reactor volume changes mixing [116], gas dispersion [117], heat transfer [115, 116], oxygen transfer [118], rheology [134], and the spatial distribution of nutrients and metabolites [28]; laboratory agitation and aeration rates therefore cannot simply be transferred numerically. Juyal *et al*. (2025) [135] demonstrated β-carotene production by *R. mucilaginosa* IIPL32 in a 500 L fermenter, scaling from 500 mL using medium rheology and fermenter hydrodynamics as key criteria. The 500 L system produced 3.3 kg of biomass and 5.1 g of β-carotene from a glucose-based medium. This is an important proof of scale-up feasibility, but it should be interpreted as evidence for a viable engineering strategy rather than as resolution of all industrial scale-up constraints.

Further scale-up studies should establish quantitative

relationships among dissolved oxygen, k_La, OTR, OUR, mixing time, power input, rheology, and metabolic productivity. Dynamic control will be particularly relevant when biomass formation and product accumulation occur at different physiological stages. Fed-batch feeding, oxygen modulation, and real-time monitoring may therefore provide more effective control of carbon allocation than fixed cultivation conditions.

Downstream processing is another critical bottleneck. Because carotenoids and lipids are predominantly intracellular [10, 90], their recovery requires biomass harvesting [14, 27], cell disruption [10, 11, 96], extraction [4], and, depending on the application, purification and stabilization [136, 137]. These operations can represent a substantial fraction of process cost and environmental burden. De Jesus *et al*. (2026) [4] provided a species-specific example of progress in this area by optimizing a greener extraction process for carotenoid-rich microbial oil from *R. mucilaginosa* CCT 7688, recovering 26.0% lipids with a Greenness score of 0.62. The recovered oil contained approximately 75% monounsaturated and polyunsaturated fatty acids and retained carotenoids, while the residual biomass contained substantial carbohydrate and protein fractions.

These results support the concept of sequential biomass valorization, in which carotenoids and microbial oil are followed by recovery or use of remaining protein- and carbohydrate-rich fractions. However, multiproduct processing should not be assumed to be advantageous by definition: additional extraction, purification, solvent recovery, and stabilization steps may increase capital and operating requirements. The preferred configuration must therefore be determined by product value, recovery efficiency, energy demand, and process compatibility.

This trade-off reinforces the need for techno-economic analysis (TEA) and life-cycle assessment (LCA) to be incorporated earlier in process development. Rather than evaluating biorefinery integration only after high titers have been achieved, future studies should identify which products and recovery steps actually improve the overall process. In this context, simplified economic and sustainability analyses such as those reported by Šovljanski *et al.* (2025) [3] are useful starting points because they show that the biologically best-performing substrate is not necessarily the most attractive industrial option.

A further priority is application-oriented process design. Food, feed, nutraceutical, cosmetic, pharmaceutical, fuel, and oleochemical markets impose different requirements for purity, composition, safety, stability, regulatory acceptance, and cost. Future studies should therefore report not only final biomass and metabolite concentrations, but also volumetric productivity, substrate-to-product yields, downstream recovery, energy consumption, and product specifications relevant to the intended application.

Taken together, these bottlenecks indicate that the principal gap is no longer proof of biological capability, but integration. Robust and genetically characterized strains must be matched with reproducible feedstocks, oxygen- and nutrient-controlled bioreactors, efficient downstream technologies, and product-specific quality requirements. This systems perspective is essential if the versatility of *R. mucilaginosa* is to translate into industrial performance.

Recent advances already point in this direction: engineered terpenoid production by Chen *et al*. (2024) [133], pilot-scale β-carotene production by Juyal *et al*. (2025) [135], and greener lipid extraction by De Jesus *et al*. (2026) [4] address different links of the same production chain. The next generation of studies should connect these advances within integrated process designs rather than treating strain engineering, cultivation, extraction, and sustainability as separate optimization problems.

Overall, the major challenge is not to demonstrate whether *R. mucilaginosa* can produce valuable compounds, but to determine how these capabilities can be integrated into predictable, scalable, economically viable, and environmentally responsible processes. The most promising pathway combines metabolic engineering, dynamic bioprocess control, sustainable feedstocks, green downstream technologies, TEA/LCA, and multiproduct biorefinery design. Such integration could move *R. mucilaginosa* from a versatile laboratory microorganism toward a robust platform for sustainable biomanufacturing.

8. Conclusions

Rhodotorula mucilaginosa has emerged as a versatile microbial platform with a distinctive combination of oleaginous metabolism, carotenoid biosynthesis, substrate flexibility, and physiological adaptability. These traits provide a strong basis for the production of microbial lipids and other value-added compounds, while the ability to co-produce metabolites creates opportunities that extend beyond conventional single-product fermentation.

The use of agroindustrial residues strengthens this opportunity by coupling microbial production with waste valorization and circular bioeconomy strategies. More importantly, the evidence reviewed here suggests that the long-term value of *R. mucilaginosa* may depend on integrating lipid and carotenoid production with the recovery of additional biomass fractions, rather than optimizing each product independently.

At the same time, biological potential has not yet translated into broad industrial deployment. Strain variability, limited genetic tools, heterogeneous feedstocks, scale-up and oxygen-transfer constraints, intracellular product recovery, and the scarcity of comprehensive TEA/LCA remain important barriers. Recent pilot-scale and downstream-processing advances show that these challenges are tractable, but they must be addressed as parts of the same process-development strategy.

Ultimately, the future of *R. mucilaginosa* lies in the rational integration of strain engineering, dynamic cultivation, sustainable feedstocks, efficient downstream processing, and multiproduct biorefinery design. Its main competitive advantage is therefore not simply its capacity to accumulate lipids, but the combination of metabolic flexibility and co-production potential that can support resource-efficient biomanufacturing. If these biological capabilities can be coupled with predictable process performance and sound economic and environmental metrics, *R. mucilaginosa* could become a competitive platform for circular and sustainable production systems.

9. References

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