Yarrowia lipolytica: an industrial workhorse

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Yarrowia lipolytica is one of the most extensively studied ‘‘non-conventional’’ yeasts, being a strictly aerobic microorganism capable of producing important metabolites and having an intense secretory activity, which justifies efforts to use it in industry (as a biocatalyst), in molecular biology and in genetics studies. Moreover, *Y. lipolytica* has been considered an adequate model for dimorphism studies in yeasts. *Yarrowia lipolytica* presents the ability to grow on Olive Mill Wastewater (OMW) as well as to degradate organic compounds, including aliphatic and aromatic hydrocarbons, often accompanied by biosurfactants production. One of the most important products secreted by this microorganism is lipase which can be exploited for several applications in the detergent, food, pharmaceutical, and environmental industries. In addition, *Y. lipolytica* is able to produce citric acid and aroma from a variety of carbon sources, including sugars, alkanes, plant oils, starch hydrolysates, ethanol, and glycerol. Thus, this chapter presents an overview of *Yarrowia lipolytica* features and its major biotechnological applications.

Keywords *Yarrowia lipolytica*; morphology; physiology; bioreactors; lipase; biosurfactant; organic acids; aroma; environment

1. General remarks

Yarrowia lipolytica is one of the most extensively studied ‘‘non-conventional’’ yeasts, being a strictly aerobic microorganism capable of producing important metabolites and having an intense secretory activity, which justifies efforts to use it in industry (as a biocatalyst), in molecular biology and in genetics studies. It is considered as nonpathogenic and several processes based on this organism were classified as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA, USA).

Moreover, *Y. lipolytica* has been considered an adequate model for dimorphism studies in yeasts, since it has an efficient system for genetic engineering transformation, and is easy to distinguish between its morphological forms, in contrast to *Saccharomyces cerevisiae*, which does not produce true filaments and exhibits pseudo-hyphae growth under nitrogen-limited conditions. The yeast-to-mycelium transition is associated with unipolar growth, asymmetric division, large polarly located vacuoles and repression of cell separation after division. It is believed that yeast dimorphism is related to a defense mechanism to adverse conditions, such as temperature and nutritional changes.

Y. lipolytica has become a reliable, a versatile, and a popular system for the expression of heterologous proteins for academic purposes as well as for possible commercial applications. The inherent ability of this yeast to secrete a variety of proteins via cotranslational translocation offers added advantages. Low overglycosylation, high secretion efficiency, good product yield, and performance reproducibility are additional features of *Y. lipolytica*.

One of the most important products secreted by this microorganism is lipase, which is an enzyme that attracts the interest of scientists and industrial researchers because it can be exploited for several applications in the detergent, food, pharmaceutical, and environmental industries. Being strictly aerobic yeast, its growth and metabolite secretion are affected by the amount of oxygen available in the culture medium. Perfluorodecalin addition as an oxygen carrier to culture media benefited *Y. lipolytica* growth rate and its extracellular enzyme production, enhancing lipase productivity. Another approach to overcome the oxygen limitation in the culture medium is the utilization of hyperbaric air. The effect of pressure on the yeast growth depends on the gas composition and on the pressurization mode, as well on the microorganism and the strain. Lipase productivity was also enhanced by oxygen transfer rate improvement at increased pressure, contrarily to what happened with cell growth, which is an indirect evidence that oxygen demand is higher for lipase production than for cell growth. In addition, it is known that oxygen and total pressure have an important role in the regulation of intracellular enzymes such as the ones of the β-oxidation pathway involved in the biotransformation of ricinoleic acid into aroma. Besides castor oil, its derivatives such as methyl ricinoleate are used as substrates for aroma production, which proved to be lipase inducers in *Y. lipolytica*.

Another ability of *Yarrowia lipolytica* strains is to grow on Olive Mill Wastewater (OMW) based medium and produce high-value compounds. In fact, this yeast has been used for bioremediation applications due to its cell wall characteristics and surfactant production. In addition, *Y. lipolytica*, when grown under nutrient-limited conditions, is able to produce citric acid from a variety of carbon sources, including sugars, alkanes, plant oils, starch hydrolysates, ethanol, and raw glycerol (the main by-product of biodiesel production units).

The ability of many species of *Y. lipolytica* in degrading a variety of organic compounds, including aliphatic and aromatic hydrocarbons, is often accompanied by biosurfactants production. These molecules are predominantly

glycolipids, but other types have also been reported using different substrates. The growth of microorganisms on a hydrophobic substrate (HS) requires the transport of the HS from the organic phase to the cell surface and the contact between the HS and the cell. This contact can occur through a direct adsorption of hydrophobic droplets to the cell surface, or it can be mediated by a surfactant. In fact, with *Y. lipolytica*, evidence of both mechanisms has been reported in literature in which the interaction between the cells and the hydrophobic surfaces or molecules is mediated by proteins or glycoproteins of the cell wall and the secreted surfactant may further enhance this interaction.

This text aims to throw light on how a single organism can be versatile with respect to its metabolic abilities, being exploited for a variety of purposes.

2. Physiology

2.1 Carbon Metabolism

Y. lipolytica is unique strictly aerobic yeast with the ability to degrade efficiently hydrophobic substrates such as *n*-alkanes, fatty acids, fats and oils for which it has specific metabolic pathways [1]. The genome sequence of the fungus has revealed that the organism is distantly related to the conventional yeast *Saccharomyces cerevisiae*. However, the underlying genetic mechanisms appear to be significantly different. Particularly, the genome displays an expansion of protein families and genes involved in hydrophobic substrate utilization [2-4].

2.1.1 Sugar

Y. lipolytica is able to degrade several hexoses, such as glucose, fructose and mannose. However, cell membranes are not freely permeable for a variety of solutes, sugars among them. Therefore, transport is the first step in carbohydrate metabolism, except in those cases in which a di- or tri-saccharide is hydrolyzed outside the cell. Transport across the membrane is carried out by specific transporters, sometimes called permeases. Transport of the common monosaccharides, glucose, fructose or mannose in *S. cerevisiae* is a facilitated diffusion process; however, the situation may be different in other yeasts. For *Y. lipolytica* there is a transport system for glucose with two components and their activity are independent of the glucose concentration in the medium [5,6].

The intracellular hexoses enter the glycolytic pathway after a phosphorylation step. Glucose, fructose and mannose are phosphorylated by hexokinases. Most of the hexokinases are inhibited by threalose-6P, an important component in the glycolysis regulation in *S. cerevisiae*. The hexokinase from *Y. lipolytica* presents the strongest trehalose 6-P inhibition yet found [5].

As a strict aerobe, high glucose concentrations do not affect the rate of respiration, the content and molecular ratio of cytochromes or mitochondrial properties in *Y. lipolytica* [7]. However, lipase production by *Y. lipolytica* IMUFRJ 50682 undergoes glucose repression and the derepression does not depend on inducer presence [8]. Sucrose cannot be utilized by wild type strains of *Y. lipolytica* because of the lack of the sucrose-cleaving enzyme invertase [8].

2.1.2 Organic acid

Rodrigues and Pais [9] have shown that *Y. lipolytica* is capable to use acetic, lactic, propionic, malic, succinic, citric and oleic acids as the sole carbon and energy source, this capacity being, in most cases, independent of the pH of the culture media. Diauxic growth was observed when the yeast was grown in glucose and citric or lactic acid suggesting that the utilization of these two acids is subjected to glucose repression. Propionic, butyric and sorbic acids also had inhibitory effects on yeast growth.

Most strains of *Y. lipolytica* grow very efficiently on acetate as sole carbon source. Concentrations up to 0.4% sodium acetate are well tolerated, higher concentrations reduce the growth rate and, concentrations above 1.0% inhibit the growth [10].

2.1.3 Alcohol

As reviewed by Barth and Gaillardin [10] *Y. lipolytica* uses ethanol as carbon source at concentrations up to 3%. Higher concentrations of ethanol are toxic. Several NAD^+- and $NADP^+$-dependent alcohol dehydrogenases were observed in *Y. lipolytica*. There probably exist two NAD^+-dependent alcohol dehydrogenases which differ in substrate specificity. Synthesis of both enzymes seems not to be repressible by glucose or inducible by ethanol [11].

Glycerol may be also utilized as a carbon source under aerobic conditions by many yeasts [12], being assimilated via glycerol-3-phosphate or dihydroxyacetone pathways. Several yeasts are thought to assimilate glycerol via dihydroxyacetone. Initially, glycerol is oxidized to dihydroxyacetone by glycerol dehydrogenase and then phosphorylated to dihydroxyacetone phosphate by dihydroxyacetone kinase [13]. Papanikolaou et al. [14] have successfully used raw glycerol for *Y. lipolytica* growth and citric acid production. High initial glycerol media (40 $g.L^{-1}$)

with nitrogen limitation led to citric acid excretion of up to 35 g L^{-1} (yield of 0.42–0.44 g acid/g glycerol consumed). Lipid production was also appreciable by this yeast using the same carbon source [15].

2.1.4 Hydrophobic

The yeast *Yarrowia lipolytica* is often isolated from lipid containing habitats, such as diary products [10], polluted environments [16] and raw poultry [17] and is, particularly, adapted to hydrophobic substrates [1].

It is believed that, evolutionarily, microorganisms that live in aqueous medium where the carbon source is hydrophobic, and therefore, are disperse in the medium as drops, have developed mechanisms to facilitate the access to this substrate since there is a small probability of contact between the microorganisms and the hydrophobic droplets, in constant movement.

The contact between the oil droplets and the cells seems to be the mechanism through which most of the hydrophobic substrate is transported. Microscopic observations show the adhesion of methyl ricinoleate droplets in *Y. lipolytica*'s surface [18,19], as shown in Figure 1. The contact area between the microorganism and the substrate includes the huge droplets of substrate where the microorganisms adhere and the small ones that are adsorbed in the cell surface [20]. In this case of direct contact, several mechanisms may be involved, as hydrophobic, Lewis (acid or base), electrostatic, or van der Waals interactions. Some tests have been developed to characterize the hydrophobic properties (MATH, microbial adhesion to hydrocarbons) [21] or the electron donor or electron acceptor (Lewis acid-base) properties (MATS, microbial adhesion to solvents) of microorganisms [22]. These tests and some others such as contact angle or ζ-potential measurement [23] elucidate information on the surface properties of the cells, which can be useful to predict the microbial behavior toward a surface.

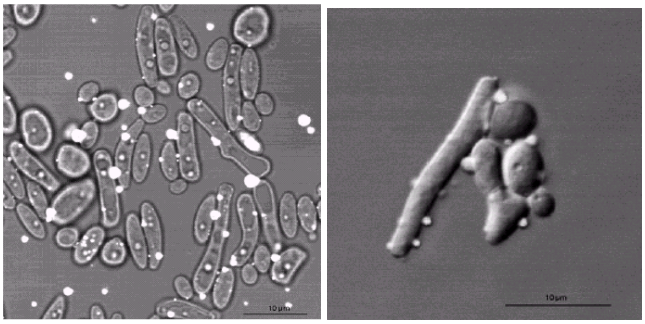

Fig. 1 Microscopic observations of *Yarrowia lipolytica* grown on methyl ricionoleate. The droplets of the oily phase are adsorbed on cell surface (Images obtained in Aguedo et al. [18,19]).

Aguedo et al. [18] have investigated the surface properties of *Y. lipolytica* W29 by the MATS test and observed a hydrophilic character of the surface of cells grown in glucose or oil, with a more electron donor/acceptor character in the presence of methyl ricinoleate. In contrast, another *Y. lipolytica* strain (*Y. lipolytica* IMUFRJ 50682), isolated from Guanabara Bay in Rio de Janeiro, Brazil, presented a very high cell adhesion to non polar solvents, an indication of higher hidrophobicity [24], showing that the mechanisms of the hydrophobic substrate uptake vary from strain to strain.

In hydrocarbon fermentations, the oily phase is dispersed as droplets in the aqueous phase and the interfacial tension that acts between the oily phase and the aqueous phase tends to maintain the droplets in spherical form against the shear stress that tends to deform it. These droplets coalesce continually and its size distribution depends on the interfacial tension, the hydrocarbon volume fraction and the agitation intensity. Gutierrez and Erickson [20] observed the reduction in the medium diameter of the oil droplets simultaneously to the superficial tension reduction in the medium during growth of *Candida lipolytica* in hexadecane and they attributed the phenomena to the tensoactive agents production by the yeast. In fact, Cirigliano and Carman [25] have detected and isolated an emulsifier capable of stabilizing water/oil emulsions, in *C. lipolytica*'s alkane growth medium. This bioemulsifier, named Liposan, was composed of 83% carbohydrate and 17% protein. Another *Y. lipolytica* strain was also capable of producing bioemulsifier, with similar composition, only in the presence of glucose as carbons source, showing that the production of those tensoactive agents is a constitutive characteristic of this yeast [26].

Therefore, hydrocarbon assimilation by this yeast involves the modification of cell adhesion properties, for the direct contact, including the creation of protrusions at the cell surface, decreasing thickness of cell wall and periplasmic space, membrane invaginations and electron-dense channels associated to the endoplasmatic reticulum (ER) [27,28] and its pseudo-solubilisation by surface-active compounds [20]. There are hypothesis that *n*-alkanes attached to the protrusions or hydrophobic outgrowths may migrate through the channels via the plasma membrane to ER [29].

After the uptake and transport to the cells, the *n*-alkanes are then hydroxylated by a cytochrome P-450 monoxidase system localized in the endoplasmic reticulum. The fatty alcohol formed after the first step is then oxidized by a membrane-bound fatty alcohol oxidase, which results in fatty aldehydes [10]. The oxidation of the fatty aldehyde to fatty acid is catalysed by fatty aldehyde dehydrogenase [1]. When triglycerides are present in the medium, *Y. lipolytica*

can utilize it as carbon source. In order to do so, lipolytic enzymes (lipases) are produced by this organism to hydrolyze the triglyceride in glycerol and fatty acids. Ota et al. [30] discovered that this yeast can produce extracellular and cell-bond lipases and Pereira-Meirelles et al. [31] described that the lipases linked to the cells are secreted when lipidic carbon source becomes scarce in the medium, i.e., in the transition to diauxie (when more than one substrate is used) or to stationary phase.

With the fatty acid available at the medium, in most cases, this carbon source diffuse into the cell, but it can also be facilitated by a transporter, like the structures resembling channels crossing the membrane mentioned above [32]. Once inside the cytoplasm, fatty acids might interact with fatty-acid-binding proteins, which *Y. lipolytica* possesses at least one palmitate-inducible [33].

As reported by Fickers et al. [1], the main steps of alkanes and fatty acids degradation pathways in yeasts are: (i) The primary or monoterminal oxidation of alkanes in ER and peroxisomes to corresponding fatty acids of the same chain lengths, initiated in the ER by a cytochrome P450 catalysed terminal hydroxylation. Additionally diterminal or ω-oxidation leading to dicarboxylic acids can occur. (ii) The activation of free fatty acids to their corresponding CoA esters which are subsequently degraded to acetyl-CoA and propionyl- CoA (in case of odd-chain alkanes) via peroxisomal β-oxidation, or the direct incorporation of fatty-acyl moieties into cellular lipids after chain elongation and desaturation. Depending on environmental conditions, cells may accumulate free fatty acids into lipid bodies. (iii) The synthesis of tricarboxylic- acid cycle intermediates from acetyl-CoA via the glyoxylate cycle followed by gluconeogenesis, and activation of the methyl citrate cycle for propionyl-CoA utilisation when using odd-chain alkanes.

The monoterminal or primary alkane oxidation and fatty-acid diterminal or ω-oxidation in yeast involves three steps. The first step of both involves a terminal hydroxylation by a P450-dependent alkane monooxygenase system or fatty-acid ω-hydroxylase, respectively. This results in fatty-alcohol production from alkane, or ω-hydroxy fatty-acid production from fatty acid. The second step is performed either by membrane-bound or soluble NAD^+- or $NADP^+$-dependent fatty-alcohol dehydrogenases or by hydrogen peroxide-producing fatty-alcohol oxidases, which convert the terminal hydroxy groups of 1-alkanols, 1,ω-diols, or ω-hydroxy fatty acids into corresponding fatty aldehydes. The third step involves the oxidation of the fatty aldehyde to a free fatty acid catalyzed by endoplasmic reticulum or peroxisomal $NAD(P)^+$-dependent fatty-aldehyde dehydrogenase. These oxidation steps finally result in fatty-acid production from alkane, or dicarboxylic acid production from fatty acid [1].

2.2 Oxygen requirements

Y. lipolytica's growth and metabolite secretion are affected by different environmental factors. The amount of oxygen available to this microorganism seems to be an important parameter. During continuous cultivation of *Yarrowia lipolytica* N 1, oxygen requirements for growth and citric acid synthesis were found to depend on the iron concentration in the medium [34]. The addition of perfluorocarbons (PFCs) to the culture medium of *Y. lipolytica* was reported as a novel approach to enhance the oxygen uptake [35]. The oxygen permeability on the PFC is much higher than in water with the solubility being 10–20 times higher in PFC than in water. Higher specific growth rates of *Y. lipolytica* were found with increasing PFC concentration and agitation rate. Amaral *et al*. [24] also observed a curious partition of the yeast between the aqueous and organic PFC phase, with an unexpected preference of the yeast for the organic solvent. These peculiar interactions may be related to the ability of this microorganism to degrade hydrophobic substrates.

Oxygen consumption is mediated by two terminal oxidases, i.e., the cytochrome oxidase and the alternative oxidase, albeit to varying extents at different growth phases, with the greater participation of the alternative pathway upon reaching the stationary growth phase. The oxidative phosphorilation system plays an important, if not exclusive, role in the cell energy budget of this yeast [7]. The degree of involvement of cyanide-resistant alternative oxidase in the respiration of *Y lipolytica* mitochondria was evaluated by Medentsev et al. [36]. The data indicate that the alternative system is unable to compete with the cytochrome respiratory chain for electrons. The alternative oxidase only transfers the electrons that are superfluous for the cytochrome respiratory chain.

The adaptive response of the yeast *Yarrowia lipolytica* to the oxidative stress induced by the oxidants hydrogen peroxide, menadione, and juglone has been studied by Biryukova et al. [37]. The adaptation of yeast cells to such oxidant agents was associated with an increase in the activity of cellular catalase, superoxide dismutase, glucose-6-phosphate dehydrogenase, and glutathione reductase, the main enzymes involved in cell defense against oxidative stress. Lopes et al [38] used a pressurized biorrreactor to increase oxygen solubility in *Y. lipolytica* cultivation. The increase of oxygen availability caused the induction of the antioxidant enzyme superoxide dismutase, which indicates that the defensive mechanisms of the cells against oxidative stress were effective and cells could cope with increased pressure. Fivefold and 3.4-fold increases in the biomass production and in specific growth rate, respectively, were also observed under 6 bar in this work.

3. Dimorphism and cell stress

Dimorphism is the capacity displayed by different fungi to grow yeast-like or in the form of a mycelium, depending on the environmental conditions. The phenomenon of dimorphism is particularly important since in a number of fungi

pathogenic for humans or plants, the dimorphic capacity is directly related to their virulence [39,40]. This is a complex phenomenon that involves extensive modification of cellular machinery in response to environmental signals [41]. The yeast-to-mycelium transition is associated with unipolar growth, asymmetric division, large polarly located vacuoles and repression of cell separation after division [42].

The conditions that induce the dimorphic transition of yeast-to-mycelium or vice versa are extremely variable. Among these, changes in temperature, pH, the gaseous atmosphere of growth, or the presence of specific compounds in the culture media may be cited [43,44,45]. In common media, *Yarrowia lipolytica* grows as a mixture of yeast-like and short mycelial cells. It is important to note that the conditions that affect dimorphism in *Y. lipolytica* are different in solid or liquid media and depend on the strain used [46].

Y. lipolytica has a hyphae diameter corresponding to 60–100% of its single cell stage. The true mycelium consists of septated hyphae 3–5 μm wide and several millimeters long. The apical cells frequently exceed 100 μm, while the segments measure 50–70 μm. The germination exhibits a bipolar pattern, and there is no case of overposition in germination locus [45].

pH was verified as the most important factor regulating the dimorphic transition. Mycelium formation was maximal at pH near neutrality and decreased as pH was lowered to become almost null at pH 3. It is important to stress that the initial rather than final pH of the medium was more important for dimorphism [43]. In addition, it was also reported that a transient increase in intracellular pH preceded the morphological transition of *Candida albicans* [47,48]. H^+ extrusion catalyzed by the plasma membrane H^+-ATPase also contributes to the regulation of intracellular pH and surface pH of yeast. This enzyme plays an essential role in fungal physiology since it generates a large electrochemical gradient that drives the transport of amino acids, sugars and inorganic ions [49]. Lobão et al. [44] described the relation between an increase in H^+ transport mediated by P-type plasma membrane H^+-ATPase and aluminum tolerance in *Y. lipolytica* cells. The authors concluded that although *Y. lipolytica* growth is not affected by high Al concentrations (0.5–1.0mM $AlK(SO_4)_2$), this element led to drastic changes in cell morphological development, inhibiting yeast-to-hypha transition. Furthermore, Al-treated cells showed a stronger H^+ efflux in solid medium. Thus, the results reported suggest that alterations in the plasma membrane H^+ transport might underline a pH signaling required for yeast/hyphal development.

Carbon and nitrogen sources, namely glucose and ammonium, were also important for mycelium formation. It was reported [50,51] that mycelial growth was favored by *Y. lipolyica* using animal serum. But as occurred with other morphogenetic stimuli, the effect of serum was dependent on pH, although it was the only one where a high concentration counteracted an acid pH rather successfully. Anaerobic stress also affects dimorphism in *Y. lipolytica.* Since the fungus has an aerobic metabolism and is unable to grow under strict anoxic conditions, semi-anoxic conditions provided in liquid or solid media induced the formation of extremely long hyphae [43].

Evidence exists that external signals influencing fungal dimorphic response are basically sensed through the operation of two signaling transduction mechanism: the mitogen activated protein kinase (MAPK), and the cyclic-AMP (cAMP) dependent protein kinase (PKA) pathways [52,53,54]. It means in *Y. lipolytica*, MAPK and PKA pathways are oppositely involved in dimorphism: while a MAPK signaling pathway is necessary for mycelial growth, a functional PKA pathway is required for growth in the yeast-like form. Cervantes-Chávez et al. [55] concluded that the functions regulated by TPK1, the gene encoding Pka catalytic subunit are positively regulated in *Y. lipolytica* contrary to that observed in other fungi (*S. cerevisiae* and *C. albicans*). Thus, PKA and MAPK pathways may operate differently depending on the fungal species in some systems, even acting either cooperatively or in opposition during the control of several physiological responses.

Several genetic or genomic approaches have been used to identify genes differentially expressed in yeast or hyphal forms or those involved in the regulation of the dimorphic transition in *Candida albicans.* However, its diploid nature and the lack of a sexual cycle have hampered some genetic studies and made mutant generation more difficult. *Y. lipolytica* have been proposed as better model to understand dimorphic transition mechanisms. This fungus is able to grow in both in the haploid and diploid state, in yeast, pseudomycelial or mycelial forms, which is an important advantage in making easy genetic modifications. Thus, based on two-dimensional gel electrophoresis (2-DE) studies, Morín et al. [41] compared soluble cell extracts from yeast and hyphal forms from a *Y. lipolytica* wild-type strain and the Δ*hoy*1 non-filamentous mutant. The authors detected 45 spots that displayed statistically significant changes during dimorphic transition, nine of them being already identified and, interestingly, most of them are proteins involved in carbohydrates and purine metabolism or have oxidoreductase activity, meaning that there are relevant differences in the physiology of the two morphological forms.

In addition, fungal dimorphism is generally characterized by a presence of many intermediate morphological forms, displaying a broad distribution of cell sizes and shapes. This wide morphology spectrum greatly affects fermentation performance, since it induces rheological changes and consequently leads to mass and heat transfer alterations in the bioreactor. Hence, the understanding of cell morphology became an important key to enhance and optimize productivity. *Y. lipolytica* morphology alterations under thermal and oxidative stress conditions were assayed using digital image analysis processing tools. The results demonstrate an increase in the elongation factor (F_{max}/F_{min}) of 25 % for thermal and oxidative stresses, indicating that both conditions gave similar results with respect to hyphae formation intensity. Although it is possible to attest an increase in hyphae characteristic length (F_{max}) in both stress cases, an oxidative condition enabled higher values compared with thermal ones. These results led to conclude that there is a

relationship between cell defense system and morphological changes [45]. Furthermore, cell morphology was slightly affected by pressure, particularly at 8 bar, where cells kept the predominant oval form but decreased in size, demonstrating that air pressure rise did not inflict oxidative stress to the cells [56].

4. Bioreactors and oxygen mass transfer

Due to the wide range of substrates that *Yarrowia lipolytica* can use efficiently, many industrial applications of this yeast cultures have been developed. Besides medium composition, pH and temperature optimization, other operating factors having been addressed to develop bioprocesses based in *Yarrowia lipolytica* cultures. Oxygenation is one of the crucial parameters to control, since this yeast is an aerobic microorganism, but also because many intracellular enzymatic activities are regulated by oxygen [57]. On the other hand, many cultures of this yeast consist of complex phase systems, due to the presence of hydrophobic compounds that, in some cases can be used as substrate. Thus, the system is composed of solid phase (cells), biphasic liquid phases and gas phase. In such complex systems, oxygen transfer rate from air to culture may be influenced by many factors, such as the nature of the hydrophobic phase and respective concentration, bioreactor design and operation.

Traditional stirred tank bioreactors (STR) have been extensively used for the optimization of bioprocess involving *Y. lipolytica* strains. Operating conditions optimization of parameters such as medium composition, temperature and pH may be conducted in erlenmeyers flasks [29,58,59] but conditions like oxygenation are usually carried out in STR [34,60]. STR's are most useful to study the influence of oxygen in the bioprocesses and can be analyzed trough the oxygen transfer rate (*OTR*) that is given by Eq. (1).

$$OTR = k_L a\ (O^s - O) \tag{1}$$

OTR depends on the volumetric mass transfer coefficient, $k_L a$, and on the driving force for the mass transfer which is the difference between the oxygen solubility (O^s) and the dissolved oxygen concentration (O) in the medium. For a specific bioreactor and medium, it is possible to increase $k_L a$ and, consequently, *OTR*, using high agitation and aeration rates. This approach has been applied for the production of lipase [61] and aroma [58] by *Y. lipolytica*.

Since in many cases, the culture medium is an oil-in-water emulsion, such as in the case of aroma production from castor oil, the effect on $k_L a$ of the second water-immiscible phase has been taken into account trough empirical correlations Eq. (2) [62].

$$k_L a = \delta \cdot \left(\frac{P_g}{V}\right)^{\alpha} \cdot (v_s)^{\beta} \cdot (1 - X_{ORG})^{\gamma} \tag{2}$$

where *Pg* represents the power requirement of the aerated bioreactor; *V*, the bioreactor working volume; v_s, the superficial gas velocity through the bioreactor; X_{ORG}, the fraction of the total bioreactor working volume that is in the organic phase (the hydrophobic substrate or the surfactants) and δ, α, β and γ are real constants. These equations have been adapted from two-phase partitioning bioreactors modeling where the second water-immiscible phase is an inert hydrophobic compound added to the culture medium as an oxygen carrier [63]. This is the case of PFC's that have been successfully applied for the enhancement of lipase production by *Y. lipolytica* [35,64].

The enhancement of *OTR* by the presence of a hydrophobic compound is mostly attributed to the high affinity of oxygen to these compounds, thus increasing the driving force for oxygen mass transfer from gas to the bulk liquid phases. The effect on $k_L a$ may vary according with the range of concentrations of the organic phase [62]. Studies conducted in pneumatic agitated bioreactors, like bubble columns and air-lift bioreactors, have shown that the increase of the hydrophobic fraction in a oil-in-water emulsion can cause a decrease in the interfacial area (a) for mass transfer, as a consequence of the partition of the surfactant molecules between water and oil phases, leading to the increase of air bubbles coalescence [65].

Air-lift bioreactors have great potential for the development of bioprocesses based in *Y. lipolytica* cultures due to the high oxygen transfer rates capacity of these systems. The biotransformation of methyl ricinoleate into aroma by *Y. lipolytica* W29 was conducted in a lab-scale air-lift bioreactor and it was shown that the aeration conditions determined the production of aroma [66]. Moreover, dissolved oxygen concentration control was proven to be an effective way of selecting the metabolites produced in this biotransformation [67]. Thus, for many cases the intrinsic factor for oxygenation optimization is the level of oxygen in the medium instead of aeration rate.

For that reason, hyperbaric bioreactors are of great interest as an efficient meaning of *OTR* enhancement for yeast growth [68]. In these bioreactors, the increase of *OTR* is achieved by the increase of total air pressure, and consequently of oxygen partial pressure, leading to the oxygen solubility rise. *Y. lipolytica* growth was stimulated under increased air pressure [56,57]. The influence of oxygen in the β-oxidation pathway in *Y. lipolytica* was studied in a hyperbaric bioreactor (at 5 bar of total pressure) and it was observed that high oxygen levels increased the activities of enzymes involved in the γ-decalactone degradation and is less important for the pathways leading to the aroma accumulation in the medium. The applicability of hyperbaric bioreactors for *Y. lipolytica* cultures was also validated for lipase production enhancement [56] and for antioxidant enzymes induction [38].

5. Industrial Applications

5.1 Lipase

Lipases (E.C. 3.1.1.3) have the ability to catalyze several reactions of industrial interest in addition to hydrolysis, like esterification, interesterification and transesterification [69,70]. For this reason, these are the most widely used enzymes in organic synthesis and can also be exploited for several industrial applications, such as detergents, substituting chemical surfactants [71], in food, specially for flavor development [72], for the production of optically active compounds for pharmaceutical industry [73] or for the treatment of oily wastewaters [74]. One promising application for lipase is in Biodiesel production. The possibility of regeneration and reuse of the immobilized lipase, a bigger thermal stability of the enzyme due to the native state, the easier separation of product and the production of a cleaner glycerol are great advantages of using lipases in the production of this biofuel. However, intensive study is still needed to reduce the production cost of this catalyst for an economically viable process [75].

Lipase productivity is affected by different environmental factors [76]. Carbon and nitrogen sources are the most important ones. Several workers have shown that lipid substrates are the best and glucose might repress enzyme production [8,76,77]. Peptone was a much better nitrogen source than urea or ammonium sulfate for lipase production by *Y. lipolytica* UFRJ 50682 [8]. Tryptone and oleic acid were the most suitable nitrogen and carbon sources for the production of the extracellular lipase by a *Y. lipolytica* mutant [29]. Usually the lipase produced by yeasts stays inside the cell (bound to the cell wall) and it is only secreted to the culture medium when the carbon source becomes scarce in the medium, i.e., in the transition to diauxie (when more than one substrate is used) or to stationary phase [31]. For this reason, surfactants (as Tween 80) can also be used in the medium composition to increase the excretion of the cell bond catalyst [78]. The amount of oxygen available to the microorganisms is also an important parameter, since many authors have shown the dependence of lipase productivity on system aeration and agitation [60,79,80]. Moreover, the addition of an oxygen carrier has increased lipase production in 24 times [36] and total air pressure rise up to 8 bar to improve oxygen transfer rate also enhanced lipase production by *Y. lipolytica* [38].

The structures of lipases from several sources have now been determined, leading to a better understanding of their properties [81,82]. There are several sources of lipases, but microorganisms present the greater advantages, including the variety of producers. Microbial strains of the same genus or species may produce distinct lipases [83] that can be exploited for several applications. *Y. lipolytica* is a great candidate, especially because a fair amount of data both on genetics and molecular biology of this specie had been accumulated [10]. Destain et al [84] selected a *Y. lipolytica* mutant (chemical mutation) which produced a lipase with 35 times the activity of the wild type strain enzyme. Pignede et al [85] described the LIP2 gene responsible for all extracellular lipolitic activity of *Y. lipolytica* W29. Fickers et al [86] amplified the gene LIP2 in a *Y. lipolytica* mutant and obtained 26.450 U mL^{-1} of lipase activity in batch operation and 158.246 U mL^{-1} in fed-batch.

5.2 Aroma

γ-Decalactone is a peach-like aroma compound well known in several food and beverages, reason why the food industry has a high interest in its biotechnological production. γ-Decalactone can be produced biotechnologically through the biotransformation of ricinoleic acid (12-hydroxy-octadec-9-enoic acid), catalysed by some yeasts with GRAS status, conferring a natural label to the aroma, which is very important, considering the increasing health- and nutrition-conscious lifestyles. One of the yeasts able to perform this biotransformation is *Yarrowia lipolytica* [87]. The process involves the substrate biodegradation through the peroxisomal β-oxidation, leading to the formation of 4-hydroxydecanoic acid, which cyclises into γ-decalatone [88].

Ricinoleic acid is a hydroxylated C18 fatty acid that in its esterified form is the major constituent (about 86 %) of castor oil. This fatty acid is the precursor used in the production of γ-decalactone. In some cases, substrates of the process are castor oil hydrolysates, fatty acids or esters of these compounds [89], like methyl ricinoleate.

The accumulation of γ-decalactone in the medium depends on the rates of production and degradation by the cells. In both cases, the peroxisomal β-oxidation pathway is involved and several compounds (3-hydroxy-γ-decalactone, dec-2-en-4-olide and dec-3-en-4-olide), proceeding from the direct precursor of γ-decalactone (4-hydroxydecanoic acid), can be detected in the medium [90]. The accumulation of these compounds in the medium gives an indication about the activities of the enzymes of the pathway, namely acyl-CoA oxidase and 3-hydroxyacyl-CoA dehydrogenase. Oxygen may influence their activities since it is necessary for the regeneration of the cofactors FAD^+ and, more indirectly NAD^+ [91] and therefore, influence the production of γ-decalactone (Figure 2).

The production of γ-decalactone has been intensively investigated in order to better understand the all process and optimize it, either using selected mutant strains [92] either by controlling environmental parameters during the biotransformation [57,62,93].

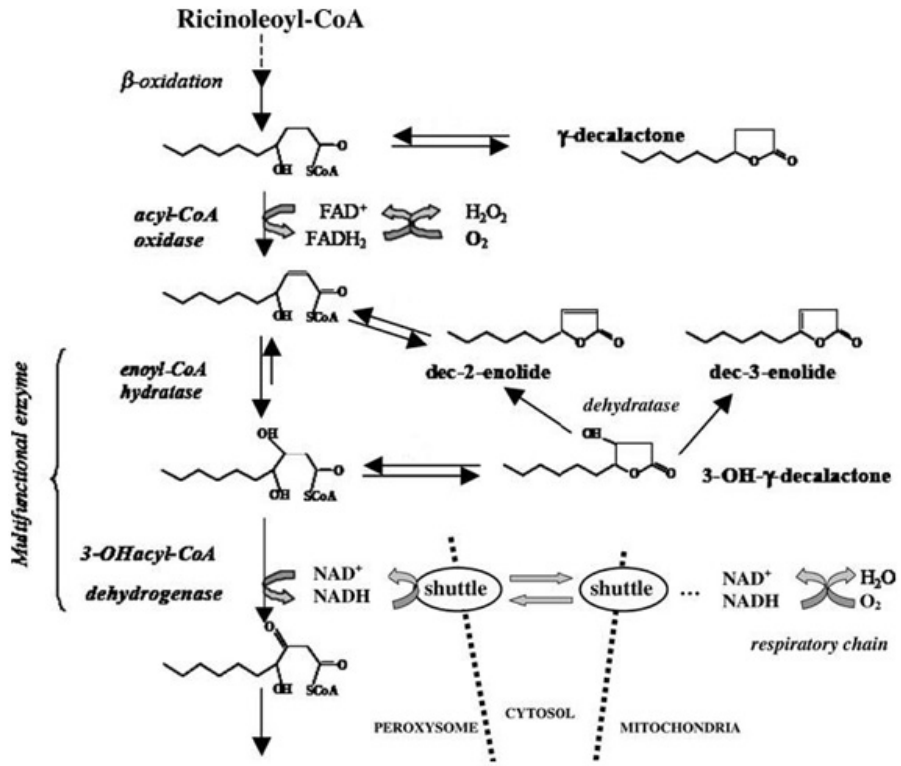

Fig. 2 β-Oxidation cycle from the direct precursor of γ-decalactone (4-hydroxydecanoic acid) during the degradation of ricinoleoyl-CoA [90].

5.3 Organic Acids

Microbial production of organic acids is a promising approach for obtaining building-block chemicals from renewable carbon sources. Most of them are natural products of microorganisms, or at least natural intermediates in major metabolic pathways. Because of their functional groups, organic acids are extremely useful as starting materials for the chemical industry. Mycelial fungi and certain groups of bacteria (e.g., acetic acid and lactic acid bacteria) were conventional producers of organic acids. In addition to the well-established filamentous fungal species, the yeast *Yarrowia lipolytica* presents the ability to produce and secrete a great variety of organic acids, including TCA cycle intermediates, like citric acid (CA), isocitric acid (ICA), α-ketoglutaric acid (KGA) and piruvic acid (PA) [34,94], as described in Table 1. The carbon sources used in these processes are n-alkanes, vegetable oils, ethanol, molasses and hydrolyzed starch in growth limited conditions caused by different nutrition factors like nitrogen source, thiamine, phosphate or mineral compounds (phosphorous, magnesium, sulphur). In conditions of nitrogen exhaustion, citric and isocitric acid secretion occurs, whereas thiamine limitation (a vitamin not synthesized by *Y. lipolytica* but necessary for α-cetoglutarate dehydrogenase activity), under lower pH, mainly leads to α-ketoglutaric (20-25%) and piruvic acids (75-80%). The beginning of the acids synthesis was provoked by the reduction of thiamine intracellular concentration. Under thiamine limitation, the cell requirement for this vitamin to piruvic acid production depends on the carbon source used. The use of glycerol leads to a production of 61.3 $g.L^{-1}$ of this acid with a yield of 0.71 $g.g^{-1}$ [95].

Table 1. Organic Acids Obtained Using the Yeast *Y. lipolytica* [95].

Acid	Substrate	Product	
		$g\ L^{-1}$	yield (% substrate)
KGA	Petrolatum	109	120
	Ethanol	50	50
PA	Glucose	50	50
	Glycerol	61	71
CA + ICA	Petrolatum	102	142
ICA	Petrolatum	60	60
	Ethanol	66	66
CA	Petrolatum	217	145
	Ethanol	120	88
	Head fraction of ethanol	116	88

This fungus has been developed as a microbial cell factory for citric acid. Currently the yearly production of citric acid is approximately 1.6 million tons (t) [96]. Its production in all developed countries follows a conventional procedure which involves the use of *Aspergillus niger* (as a producer) and molasses (as a substrate). Conventional CA production is a complex and ecologically unsafe process due to the characteristics of the raw material used. Concentrated acids and alkali are used throughout the process, and the discharge into the environment (containing cyanides and gypsum) is comparable in amount to the product [95]. Because of these ecological problems of the *Aspergillus* process, it is of interest to develop alternative processes using yeast as producing organisms. A bioprocess for CA production using *Y. lipolytica* would have several additional advantages compared to the *Aspergillus* process, including a larger substrate variety (selection of low-priced substrates would be possible), smaller sensitivity to low dissolved oxygen concentrations and heavy metals, and higher product yields. A disadvantage of using wild type strains of *Y. lipolytica* for CA production is the secretion of ICA as byproduct of the process. Contaminations of ICA above 5% of the CA produced disturb the crystallization of CA during the purification process [97]. Table 2 presents an overview of data for citric acid production.

Table 2. Current data for citric acid production (adapted by Sauer et al. [96]).

Concentration (g L^{-1})	Productivity g $(L\ h)^{-1}$	Yield (g g^{-1})	Carbon source	Organism	Refs
200			Glucose	*Aspergillus niger*	[98]
113.5		0.71	Beet, cane molasse	*Aspergillus niger*	[99]
114	0.61	0.76	Cane molasse	*Aspergillus niger*	[100]
40	0.10	0.99	n-Paraffin	*Yarrowia lipolytica*	[101]
42.9		0.56	Fatty acid, glucose	*Yarrowia lipolytica*	[102]
140	0.73	0.82	Sucrose	*Yarrowia lipolytica*	[97]

Glycerol is an important renewable feedstock as it is the principle side-product of the biodiesel production process, which is nowadays applied on a large commercial scale. Furthermore, glycerol is produced by several others industries, such as fat saponification and alcoholic beverage production units. Although glycerol has been widely used as the sole carbon source in the production of 1,3-propanediol by several bacterial strains [103], only few investigations dealing with the utilization of this substrate by eukaryotic microorganisms are present in the literature [15,104]. In eukaryotes, glycerol is transformed into intermediates of the glycolytic pathway via either the phosphorylation pathway in which direct phosphorylation to glycerol-3-phosphate and subsequent dehydrogenation took place or the oxidative pathway in which dehydrogenation of glycerol and subsequent phosphorylation of the reaction product took place. The product of these reactions is dihydroxyacetone phosphate which can be transformed to citric acid, storage lipids and various other products [103,105]. Makri et al. [106] studied the growth of *Yarrowia lipolytica* on glycerol recognizing three distinct phases, namely biomass production phase, lipogenic phase and citric acid production phase along growth cycle. Different metabolic activities of NAD^+ dependent iso-citric dehydrogenase (NAD^+–ICDH) were detected, like high activity of this enzyme during biomass production phase and a significant decreased afterwards inducing lipogenesis. A drop in NAD^+–ICDH activity to minimal levels and a decrease in glycerol kinase activity were observed during the citric acid production phase. Surprisingly, citric acid production was accompanied by storage (neutral) lipid turnover, along with remarkable biosynthesis of glycolipids, sphingolipids and phospholipids. Thus, *Y. lipolytica* successfully converts glycerol via phosphorylation pathway into valuable biotechnological products, such as single cell oil and citric acid. Using the same carbon source, Levinson et al. [107] achieved with the highest yielding strain, *Y. lipolytica* NRRL YB-423, a production of 21.6 g L^{-1} citric acid from 40 g L^{-1} glycerol (54% yield). The citric acid to isocitric acid ratio produced by this strain in the initial screen was 11.3.

Different industrial wastes have been used as alternative substrates for CA production. Papanikolaou et al. [108] cultivated *Yarrowia lipolytica* ACA-DC 50109 on olive-mill (OMW) wastewater based media. In diluted OMWs enriched with high glucose amounts (initial sugar concentration, 65 g L^{-1}), a notable quantity of total citric acid was produced (28.9 g L^{-1}). OMW-based media had a noteworthy stimulating effect on the production of citric acid, since both final citric acid concentration and conversion yield of citric acid produced per unit of sugar consumed were higher when compared with the respective parameters obtained from trials without added OMW. Adaptation of the strain in OMW-based media favored the biosynthesis of cellular unsaturated fatty acids (principally of oleic and palmitoleic acids). Additionally, a non-negligible decrease of the phenolic compounds in the growth medium [up to 15% (wt/wt)], a slight decrease of the phyto-toxicity, and a remarkable decolourisation of the OMW were observed.

In the same direction, pineapple waste was used as sole carbon source by Imanid et al. [109] to produce CA using *Y. lipolytica* NCIM3589 in a solid-state fermentation. Citric acid production under optimized conditions was 202.35 g.(kg ds)$^{-1}$ (g citric acid produced/kg of dried pineapple waste as substrate).

5.4 Single Cell Oil

Depending on environmental conditions, yeast cells are able to mobilize free fatty acids or to store them as triacylglycerols and steryl esters into lipid bodies, which consist of a hydrophobic core formed from neutral lipids and

surrounded by a phospholipid monolayer with a few embedded proteins [1]. Few microorganisms are known to create significant amount of lipid bodies and when they are able to do so to a level corresponding to more than 20% of their biomass are described as oleaginous yeasts. The best known oleaginous yeasts include genus of *Candida, Cryptococcus, Rhodotorula, Rhizopus, Trichosporon* and *Yarrowia* [110]. Biodiesel production using microbial lipids, which is named as single cell oils (SCO), has attracted great attention in the whole world [111].

Lipids may accumulate via two different pathways: (i) *de novo* synthesis, involving the production, in defined conditions, of fatty acid precursors, such as acetyl and malonyl-CoA and their integration into the storage lipid biosynthetic pathway and (ii) the *ex novo* accumulation pathway, involving the uptake of fatty acids, oils and triacylglycerols from the culture medium and their accumulation in an unchanged or modified form within the cell [110]. Oleaginous microorganisms have different mechanisms to generate acetyl CoA, an essential precursor for fatty acids biosynthesis. The key to this process is the exhaustion of a nutrient from the medium (it is usually nitrogen), which alters the cell respiration process. In this case, AMP (adenosine monophosphate) deaminase, which catalyses the transformation of AMP in IMP (inosine 5'-monophosphate) and ammonia, is activated. The activation of AMP deaminase decreases mitochondrial AMP concentration and increases cellular ammonium concentration. The decrease in AMP concentration inhibits isocitrate dehydrogenase, blocking the citric acid cycle at the isocitrate level and aconitase mediates the accumulation of citrate in mitrochondria. Then, the citrate coming from the mitochondria is cleaved by ATP-citrate lyase in the cytosol providing acetyl-CoA in large amounts for fatty acid synthesis. This enzyme is absent from non-oleaginous yeasts, such as *S. cerevisiae*, but has been shown to be present in *Y. lipolytica*. Additionally, the malic enzyme, which transforms malate to pyruvate, generates the reducing power needed for the fatty acid synthesis (nicotinamide adenine dinucleotide phosphate, NADPH) [110, 111, 112].

Few lipid bodies (LB) could be observed in *Y. lipolytica* when grown in glucose medium whereas LB accumulation was observed during culture in fatty-acid or triglyceride medium [1]. Aggelis et al [113] obtained yeasts containing 43 % of lipids in dry biomass using industrial glycerol in a single-stage continuous culture of *Y. lipolytica*. Bati et al [114] evidenced a major effect of dissolved oxygen, nitrogen/carbon ratio, pH and amount of oil substrate on lipid accumulation, resulting in yeast having from 37 % up to 70 % of lipid. Papanikolaou et al [102] analyzed lipid accumulation and composition when yeasts were grown on agro-industrial residues consisting in a mixture of industrial animal fat (stearin), technical glycerol and glucose. They could modulate the level of polyunsaturated fatty acids and obtained a SCO presenting a CBE-type lipid composition. The lipids from *Y. lipolytica* present an interesting fatty acid composition: 14.7 – 23.1% of palmitic acid (16:0), 47.1 – 68.3% of stearic acid (18:0), 6.9-18.2% of oleic acid (18:1) and 2.2-8.9% of linolenic acid (18:2) [102]. Therefore, this SCO has a great potential to be used in biodiesel production.

5.5 Environmental systems

The species name *Y. lipolytica* refers to the ability of this fungus to extensively degrade n-paraffins and oils. Thus, strains of *Y. lipolytica* have been isolated and employed in the bioremediation of oil-contaminated environments. Most of the research on hydrocarbon degradation by *Y. lipolytica* is at the laboratory level. However, in situ soil bioremediation experiments have been conducted by Zogola et al. [115] with a strain of *Y. lipolytica* (A-101) that was introduced into bores drilled in petroleum-contaminated soils at a fuel base. A significant increase in the electrical conductivity of soils in the vicinity of boreholes indicated the potential of *Y. lipolytica* in the bioremediation of petrol-contaminated soils. Decontamination was suggested to be due to a joint effort of the environmental microsystem. *Y. lipolytica* possibly influenced better plant rhizoid development and increased the hydrocarbon-degrading bacterial populations thereby reducing the pollutant load [116].

Salt tolerant strains of *Y. lipolytica* have been isolated from hypersaline and marine locations implicating that this fungus may be playing a significant role in such environments [117,118]. The NCIM 3589 strain degraded the aliphatic fraction of the Bombay high crude oil most efficiently (78%) within 5 days at 30°C with shaking at 200 rpm. In addition to the degradation of the aliphatic fraction, also degraded the pure alkanes, n-hexadecane (60%), n-tetradecane (50%), n-octadecane (45%), n-decane (40%), and n-dodecane (40%) within 24 h [119].

Y. lipolytica IMUFRJ 50682 showed to be a microorganism with potential application at bioremediation process, being capable to consume n-alkanes, isoprenoids and aromatic hydrocarbons as the group of naphthalenes and the group of phenanthrene. Carbon source (glucose, glycerol or petroleum) modifies the cell wall composition, and consequently, influences the components responsible for its hidrofobicity. Thus, proteins appear to be directly related with high hydrophobic character presented by *Y. lipolytica* IMUFRJ 50682 [120].

Romero et al. [121] have suggested that aromatic hydroxylation in *Y. lipolytica* is cytochrome P450-mediated. It must be noted that cytochrome P450-dependent hydroxylation is predominant in the hydrophobic substrate degrading yeast, *Y. lipolytica* , according to Fickers et al. [1].

The simulation of natural conditions in sandy oil microcosms was performed by Schmitz et al. [122]. Such study on relative competitiveness of different microorganisms during alkane degradation revealed that two yeasts namely, *Candida maltosa* and *Y. lipolytica* were dominant and superior as compared to other yeasts such as *Pichia stipitis*, *Candida shehatae*, and *Candida tropicalis*. Competition experiments between *C. maltosa* and *Y. lipolytica* resulted in the degradation of 96% of n-tetradecane. The yeasts were able to coexist under the study conditions, and their numbers remained nearly constant in an equal proportion. An obvious advantage that yeasts display, in general, is their

preference for acidic environments. This allows their colonization in habitats such as sandy shores, deserts, or sand fillings surrounding oil tanks [116].

Cell surface hydrophobicity of *Y. lipolytica* varied with the kind of substrate that was provided. In addition to the enhanced cell surface hydrophobicity when cells were grown in a hydrocarbon-containing medium, surface protrusions have also been reported during the growth of *Y. lipolytica* on hydrophobic substrates such as oleic acid [123]. These studies indicate that *Y. lipolytica* has the ability to alter its cell surface and that such modifications play a crucial role in the utilization of sparingly water-soluble hydrophobic compounds such as oleic acid and n-alkanes, as determined by Amaral et al. [24].

There are a few reports on the significance of the morphological form of this fungus during hydrocarbon degradation. Although the fungus is dimorphic and exists in the yeast or the mycelial form, it has been established that the yeast form plays a role in hydrocarbon degradation [118,119]. When mycelia (developed under various conditions) were inoculated into an alkane containing medium, a transition to the yeast form occurred and the latter form was predominant thereafter. A rapid morphological change indicated that the yeast form was more suitable for alkane utilization (possibly for surface modifications associated with the degradation).

Different strains of *Y. lipolytica* have been used for the treatment or upgradation of a variety of wastes. In particular, they have been effective in the treatment of olive mill wastewater (OMW) and palm oil mill effluents (POME). Wastewaters from olive oil processing units cause severe pollution particularly in the Mediterranean areas and are characterized by high values of chemical oxygen demand [124]. Several physicochemical treatment methods having been proposed for this waste, as well as biological one, that have been recently reviewed [125]. The biological processes are based in the consideration of the waste as a renewable resource to be recovered. A variety of wastes have been used as alternative economical substrates for the production of enzymes, organic acids, and emulsifiers. This approach offers a twofold advantage: (a) a means of waste disposal and (b) the synthesis of a value-added product.

Y. lipolytica W29, CBS 2073, and IMUFRJ 50682 are other strains that have been tested for growth on undiluted OMW [126]. W29 reduced COD in the range of 29% to 37% (for different samples of OMW). Recently, the above mentioned wild-type strains of *Y. lipolytica* (W29 and IMUFRJ 50682) have been applied to treat OMW with a COD load of 19 g L^{-1} [56]. The effect of the treatment procedure with strain W29 was a reduction in COD by 80%, total phenol by 70%, and the production of lipases as a high-value product.

Biosurfactants and bioemulsifiers are amphiphilic molecules that display surface activity and emulsifying properties. They are used in textile manufacture, leather processing, in bioremediation, agriculture, and in the food and beverage industry [127]. Conventionally, *Y. lipolytica* produces emulsifiers in the presence of n-alkanes, oils, or glucose as substrates. "Yansan" was obtained from *Y. lipolytica* (IMUFRJ 50682) in the presence of glucose. The lipid–carbohydrate–protein complex was found to be stable in the pH range of 3.0 to 9.0. This formed water-in-oil emulsions with aliphatic, aromatic hydrocarbons, and with perfluorocarbons implicating its potential in bioremediation processes [26].

Recent trends in the production of emulsifiers have been on the use of different industrial wastes as alternative substrates. Fontes [128] investigated the use of clarified cashew apple juice (CCAJ) and crude glycerol, by-product of biodiesel production. *Y. lipolytica* IMUFRJ 50682 was able to grow and produce biosurfactant on CCAJ, achieving a maximum emulsification index (EI) of 68 % and maximum variation on surface tension (ΔTS) of 18 mN/m, when the CCAJ was diluted ten times with distilled water and sulfate ammonium (10 g L^{-1}) was added as nitrogen source. *Y. lipolytica* growth and biosurfactant production were also observed when the crude glycerol from biodiesel production (3% v/v) was used as carbon source and supplemented with sulfate ammonium (10 g L^{-1}), reaching 70.22% of EI and 22 mN/m of ΔTS. The results therein obtained indicate that the clarified cashew apple juice and the by-product of biodiesel synthesis are appropriate raw materials for biosurfactant production by *Y.lipolytica*.

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