Applied Microbiology and Biotechnology (2019) 103:3651–3667
https://doi.org/10.1007/s00253-019-09742-x

MINI-REVIEW

Oleaginous yeasts for sustainable lipid production—from biodiesel to surf boards, a wide range of "green" applications

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Received: 18 December 2018 / Revised: 3 March 2019 / Accepted: 5 March 2019 / Published online: 25 March 2019

Abstract

A growing world population and a growing number of applications for vegetable oils are generating an increasing demand for these oils, causing serious environmental problems. A sustainable lipid production is then fundamental to address these problems. Oleaginous yeasts are a promising solution for sustainable lipid production, but, with the current knowledge and technology, they are still not a serious alternative in the market. In this review, the potential of these yeasts is highlighted and a discussion is made mainly focused on the economics of the oleaginous yeast oil production and identification of the key points to be improved to achieve lower production costs and higher income. Three main stages of the production process, where costs are higher, were identified. To render economically feasible the production of oils using oleaginous yeasts, a reduction in production costs must occur in all stages, lipid yields and productivities must be improved, and production must be targeted to high-value product applications.

Keywords Oleaginous yeasts · Vegetable oils · Sustainability · Biodiesel · Lipids

Introduction

The global demand for vegetable oils has been growing every year and is expected to keep growing not only because of the population growth that raises the need of vegetable oils for food applications but also because of the growing number of applications for the vegetable oils, like its application for biofuels, food additives, biopolymers, and pharmaceutical and cosmetic industries. Its use for the production of biodiesel is the application that has promoted the biggest increase on the demand for vegetable oil due to the search for cleaner fuels to reduce greenhouse gas emissions to fight global warming, but that raised many concerns related with the sustainable use of food crops (Anuar and Zuhairi 2016). Like biodiesel, also several of the other applications of the vegetable oils have environmental concerns associated, as there is a growing demand for greener bio-based alternatives to petrochemicals and others. This increasing demand creates a lot of pressure for expanding crops fields, promoting bigger destruction of forests and raising the water usage, creating serious environmental problems. In this way, what is supposed to be an environmental positive solution like biodiesel and green bio-based products can be a big problem unless we find sustainable ways to produce vegetable or vegetable-like oils. Furthermore, according to the United Nations, the world population is expected to reach 8.6 billion in 2030 and 9.8 billion in 2050, which raises big concerns about food and water resources. To overcome that problem, a raw material production that requires smaller amount of land and water to obtain high productivities must be developed. Oleaginous microorganisms (OM), which can produce oils similar to vegetable oils, have been presented as a possible solution since they have higher growth rate and oil productivity, are easier to cultivate, and use less land and water (Gerbens-Leenes et al. 2009; Yang et al. 2011). Many studies have been conducted with microalgae, yeasts, molds, and bacteria to access their potential. Oleaginous yeasts (OY) are one of the most promising OM for the production of oils similar to vegetable oils (Qin et al. 2017), since they can accumulate up to more than 70% lipids in their composition (Ratledge 1991) and exhibit several advantages for lipid production over other sources. Those advantages are related to

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their rapid growth, the requirement for smaller areas for their cultivation, and the fact of being much less affected by climatic conditions than other production systems (Ageitos et al. 2011). Oleaginous yeasts were intensively studied in Germany during World War I and II due to the need on finding ways for internal food, feed, and fuel production (Sitepu et al. 2014). Those studies were of major importance to the research in this area, showing the potential of these yeasts for lipid production. After the Second World War, the researches in this area have diminished. In the last decades, due to the growing interest in biodiesel, oleaginous yeasts got again the attention of the researchers in order to find a sustainable way of producing vegetable oil equivalents for biodiesel and other applications. However, its industrial production is still not feasible because it is not cost-competitive with the current technology (Probst et al. 2017) (Whiffin et al. 2016), although there are studies pointing to cost-competitiveness in particular cases (Park et al. 2017) (Ricardo et al. 2017).

In this paper, the main research that has been done aiming to increase productivity and decrease production costs of oils with OY is reviewed, the potential of the most promising OY is highlighted, and the main hurdles to overcome to turn OY oil production to be cost-competitive are discussed together with the potential applications of these oils. It is a more practical review in this area, more focused on the economics of lipid production and on the identification of the main points to improve towards a competitive vegetable oil equivalent production. Furthermore, since different authors present the results in different units, or just show the results in lipid percentage and lipid yield, in this review, we present the best results obtained to date in terms of lipid productivity and lipid coefficient, which are the units that give a real idea of its potential to become cost-competitive. That way, we can better relate the recent developments to the real impact in the economics of oleaginous microorganism oil production.

Potential of the main oleaginous yeasts

From the more than 600 yeast species known, fewer than 30 are known to accumulate more than 20% of their biomass as intracellular lipids (Sargeant et al. 2014). Yeasts that are able to accumulate more than 20% are designated as oleaginous yeasts. Most of the more promising yeasts are from the genera *Yarrowia*, *Candida*, *Rhodotorula*, *Rhodosporidium*, *Cryptococcus*, *Trichosporon*, and *Lipomyces* (Ageitos et al. 2011). From those genera, the most studied species for lipid production have been *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Rhodosporidium toruloides*, *Cryptococcus curvatus*, and *Lipomyces starkeyi*, although high productivities have been obtained with other species also, like with *Trichosporon fermentans* (Zhu et al. 2008; Huang et al. 2009, 2012, 2014).

Oleaginous yeast lipid profile

The oils produced by the main oleaginous yeasts have a lipid profile similar to that of some vegetable oils (Table 1). Fatty acid profiles have been shown to be quite consistent within a species if grown under consistent conditions but can change depending on the culture conditions and the time of cultivation, inducing the obtainment of different lipid profiles for the same yeast in different studies (Sitepu et al. 2013). OY lipid profile is characterized by a predominance of oleic acid but can be changed not only by manipulating culture conditions but also by using selective inhibitors or genetically manipulating the yeasts in order to obtain lipid profiles for the desired application (Sargeant et al. 2014).

Carbon sources, productivity, cultivation methods, and lipid yields

Oleaginous yeasts are able to utilize a wide range of carbon sources and were successfully cultivated using low-cost carbon sources in different media mainly composed by wastes and/or wastewaters. High productivities and lipid yields were obtained using different low-cost carbon sources and simple cultivation methods. For a better understanding of the order of magnitude of the productivities that can be achieved with oleaginous yeasts, it can be pointed out that the productivity obtained by Ricardo et al. (2017) (0.44 g/L/h) using sugarcane juice as carbon source for the cultivation of the yeast *Rhodosporidium toruloides* to produce microbial oil for biodiesel production resulted in a biodiesel yield (L/ha of land) 6.3 times bigger than the yield of standard biodiesel from soybean oil (microbial biodiesel 4172 L/ha of cultivated sugarcane; soy biodiesel 661 L/ha of cultivated soybean). Productivities as high as 1.6 g/L/h were achieved using *Lipomyces starkeyi* (Lin et al. 2011), and lipid yields reaching up to 0.29 g of lipid per gram of carbon substrate using *Rhodosporidium toruloides* (Fei et al. 2016) or *Cryptococcus curvatus* (Ykema et al. 1988) were already obtained and can further be improved considering that theoretical lipid yields can reach 0.35 gl/gs and cultivation methods can be improved. Since oleaginous yeasts can obtain high productivities using wastes, wastewaters, or lignocellulosic hydrolysates, there is no competition with food production and there is no need to destroy more forests to grow more crop fields. Also, with the ability of using such a wide range of different carbon sources, the simple cultivation methods, and the fact that there is no need for light to grow them allow for their cultivation anywhere in the world.

Like previously mentioned, the yeasts *Rhodotorula glutinis*, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, *Cryptococcus curvatus*, and *Lipomyces starkeyi* were the most studied, and, with each one, different carbon

Table 1 Relative mass percentage of the main fatty acids present in some seed oils and single-cell oils

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	References
Rapeseed oil	3	–	1	64	22	8	Fassinou et al. (2010)
Soya oil	12	–	3	23	55	6	Fassinou et al. (2010)
Sunflower oil	6.40	0.01	2.90	17.70	72.90	–	Fassinou et al. (2010)
Jatropha oil	14.70	0.65	6.75	40.05	36.60	0.15	Fassinou et al. (2010)
Cocoa butter	23.31	0.95	24.51	28.74	3.93	–	El-Saied et al. (1981)
Palm oil	43.03	0.19	4.31	39.47	10.82	0.29	Fassinou et al. (2010)
Cryptococcus curvatus	18	–	16	50	16	–	Meesters et al. (1996)
Yarrowia lipolytica	15	2	11	47	21	3	Papanikolaou and Aggelis (2002)
Rhodosporidium toruloides	20	1	15	47	13	3	Li et al. (2007))
Rhodosporidium toruloides 68-264	12	0.4	20.9	54.2	5.6	1	Sitepu et al. (2013)
Cryptococcus victoriae 10-939	21	5.5	20.5	42.5	6.6	0.8	Sitepu et al. (2013)
Lipomyces starkeyi	37	4	6	49	1	–	Zhao et al. (2008)
Rhodotorula glutinis	23.80	5.90	2.00	54.80	10.70	1.70	Vieira et al. (2014)

sources were tested and different productivities and lipid yields were obtained.

With *Rhodotorula glutinis* (Table 2), the highest productivity obtained was 1.028 g/L/h using glucose as carbon source and a fed-batch cultivation method (Pan et al. 1986). Good productivities were also obtained using cheap carbon sources, like molasses (0.24 g/L/h) (Alvarez et al. 1992) or undetoxified corncob hydrolysate (0.168 g/L/h) (Liu et al. 2015). The highest lipid yield obtained with *Rhodotorula glutinis* was 0.182 gl/gs, still significantly lower than the theoretical maximum (Johnson et al. 1995).

With the yeast *Yarrowia lipolytica* (Table 3), the highest productivity obtained was 1.2 g/L/h using glucose as carbon source and a fed-batch cultivation method (Qiao et al. 2017). Good productivities were obtained using cheap carbon sources, like acetic acid (0.8 g/L/h) (Xu et al. 2017) or glycerol with volatile fatty acids (0.330 g/L/h) (Fontanille et al. 2012). The highest lipid yield obtained with *Yarrowia lipolytica* was 0.27 gl/gs (Qiao et al. 2017).

Using *Rhodosporidium toruloides* (Table 4), the highest productivity was obtained by a fed-batch cultivation method utilizing glucose as carbon source. Also, good productivities

Table 2 Productivities for *Rhodotorula glutinis* using different media and different cultivation methods

Yeasts	Medium	Cultivation method	Productivity (g/L/h)[a]	References
Rhodotorula glutinis	Glycerol + yeast extract	Fed-batch	0.030	Karamerou et al. (2016)
Rhodotorula glutinis	Potato wastewater + glycerol	Batch in flask	0.031	Kot et al. (2017)
Rhodotorula glutinis	*P. euramevicana* leaves hydrolysates + yeast extract + peptone	Airlift Bioreactor	0.066	Dai et al. (2007))
Rhodotorula glutinis	Crude glycerol + thin stillage	Fed-batch	0.066	Yen et al. (2012))
Rhodotorula glutinis	Monosodium glutamate wastewater + glucose	Fed-batch	0.070	Xue et al. (2008)
Rhodotorula glutinis	Crude glycerol + $(NH_4)_2SO_4$ + Tween 20	Fed-batch	0.084	Saenge et al. (2011)
Rhodotorula glutinis	Hydrolyzed pineapple pulp residue + $(NH_4)_2SO_4$	Batch in flask	0.100	Tinoi and Rakariyatham (2016)
Rhodotorula glutinis	Molasses + glucose	Fed-Batch	0.120	Johnson et al. (1995)
Rhodotorula glutinis	Undetoxified corncob hydrolysate + $(NH_4)_2SO_4$	Fed-Batch	0.168	Liu et al. (2015)
Rhodotorula glutinis	Molasses + $(NH_4)_2SO_4$ + $(NH_4)_2HPO_4$	Continuous	0.240	Alvarez et al. (1992)
Rhodotorula glutinis	Sucrose; $(NH_4)_2SO_4$; $MgCl_2 \times 6H_2O$; $CaCl_2 \times 2H_2O$; $MgSO_4 \times 7H_2O$; myo-inositol; KH_2PO_4; K_2HPO_4; trace elements solution and vitamin solution	Fed-batch	0.795	Lorenz et al. (2017)
Rhodotorula glutinis	Glucose; KH_2P04; $Na2HPO_4$; $(NH_4)_2SO_4$; $MgSO_4.7H_20$; $CaCl_2.2H_20$; yeast extract	Fed-batch	1.028	Pan et al. (1986)

[a] Calculated based on references

Table 3 Productivities obtained by *Yarrowia lipolytica* using different media and different cultivation methods

Yeasts	Medium	Cultivation method	Productivity (g/L/h)[b]	References
Yarrowia lipolytica	Lard + yeast extract + Arabic gum + potassium phosphate buffer	Batch in flask	0.055	Lopes et al. (2018)
Yarrowia lipolytica	Glycerol + YNB + $(NH_4)_2SO_4$	Batch in flask	0.066	Dobrowolski et al. (2016)
Yarrowia lipolytica	Sugarcane bagasse hydrolysate + peptone	Batch in flask	0.073	Tsigie et al. (2011)
Yarrowia lipolytica	Glycerol + minimal medium + olive oil	Batch in flask	0.102	Magdouli et al. (2017)
Yarrowia lipolytica	Crude glycerol; KH_2PO_4; Na_2HPO_4; $MgCl_2.6H_2O$; $CaCl_2$; $FeCl_3.6H_2O$; $ZnSO_4.7H_2O$; $MnSO_4.H_2O$; $MgSO_4.7H_2O$; $(NH_4)_2SO_4$; yeast extract	Continuous	0.120	Papanikolaou and Aggelis (2002)
Yarrowia lipolytica	Crude glycerol + NH_4OH	Fed-batch	0.199	Sara et al. (2016)
Yarrowia lipolytica	Glycerol + volatile fatty acids + $(NH_4)_2SO_4$	Fed-batch	0.330	Fontanille et al. (2012)
Yarrowia lipolytica	Acetic acid + ammonium sulfate + sodium acetate + yeast extract + YNB + acetate	Semi-continuous fermentation system	0.8	Xu et al. (2017)
Yarrowia lipolytica[b]	Glucose + YNB + $(NH_4)_2SO_4$	Fed-batch	1.2	Qiao et al. (2017)

[a] Engineered strain

[b] Calculated based on references

were obtained using cheap carbon sources, like sugarcane juice (0.44 g/L/h) (Ricardo et al. 2017) or corn stove hydrolysate (0.4 g/L/h) (Fei et al. 2016). The highest lipid yield obtained with *Rhodosporidium toruloides* was 0.29 gl/gs revealing high capability to use lignocellulosic hydrolysates as carbon source (Fei et al. 2016).

For *Cryptococcus curvatus* (Table 5), the highest productivity was obtained using whey permeate as carbon source (0.995 g/L/h) (Ykema et al. 1988), an even better productivity than the ones obtained using glucose. The cultivation method used was batch partial recycling. Also, with glycerol, better productivities (0.59 g/L/h) (Meesters et al. 1996) than with glucose (0.47 g/L/h) were obtained (Zhang et al. 2011). This yeast is the most versatile in terms of the utilization of different carbon sources, with good productivities being obtained with different carbon sources. The highest lipid yield obtained with *Cryptococcus curvatus* was 0.29 gl/gs, revealing high capability to use lactose as carbon source (Ykema et al. 1988).

With *Lipomyces starkeyi* (Table 6), the highest productivity obtained was 1.6 g/L/h, the highest of all the five yeasts considered, using glucose as carbon source and a two-stage fermentation cultivation method (Lin et al. 2011). Good productivities were obtained using cheap carbon sources, like hydrolyzed flour-based industrial waste streams (0.4 g/L/h) (Tsakona et al. 2014) or cellobiose and xylose (0.125 g/L/h) (Gong et al. 2012). The highest lipid yield obtained with *Lipomyces starkeyi* was 0.236 gl/gs (Anschau et al. 2014).

High productivities can be achieved with all these yeasts, using different low-cost carbon sources. This ability is really important since it allows in each region of the world, to use the

Table 4 Productivities obtained by *Rhodosporidium toruloides* using different media and different cultivation methods

Yeasts	Medium	Cultivation method	Productivity (g/L/h)[a]	References
Rhodosporidium toruloides	Bioethanol wastewater + glucose	Batch in flask	0.020	Zhou et al. (2013)
Rhodosporidium toruloides	Distillery wastewater + domestic wastewater	Batch in flask	0.049	Ling et al. (2013)
Rhodosporidium toruloides	Distillery wastewater + domestic wastewater + spent seed culture	Batch in flask	0.057	Ling et al. (2017)
Rhodosporidium toruloides	Crude glycerol + 2-(N-morpholino) ethanesulfonic	Batch in flask	0.102	Yang et al. (2014a)
Rhodosporidium toruloides	Crude glycerol + solid-state fermentation autolysate	Batch	0.132	Uçkun Kiran et al. (2013)
Rhodosporidium toruloides	Glucose + yeast extract + $NaNO_3$ + $MgSO_4$	Fed-batch	0.260	Saran et al. (2017)
Rhodosporidium toruloides	Jerusalem artichoke extracts	Fed-batch	0.380	Zhao et al. (2010)
Rhodosporidium toruloides	Corn stove hydrolysate + YNB	Fed-batch	0.4	Fei et al. (2016)
Rhodosporidium toruloides	Sugarcane juice + urea	Fed-batch	0.440	Ricardo et al. (2017)
Rhodosporidium toruloides	Glucose + peptone + yeast extract	Fed-batch	0.540	Li et al. (2007)

[a] Calculated based on references

Table 5 Productivities obtained by *Cryptococcus curvatus* using different media and different cultivation methods

Yeasts	Medium	Cultivation method	Productivity (g/L/h)[a]	References
Cryptococcus curvatus	Acetic acid + $MgSO_4.7H_2O$ + KH_2PO_4 + $(NH_4)_2SO_4$ + yeast extract	Batch in flask	0.023[b]	Huang et al. (2018)
Cryptococcus curvatus	Volatile fatty acids + $MgSO_4.7H_2O$ + KH_2PO_4	Batch in flask	0.052	Liu et al. (2017)
Cryptococcus curvatus	Waste office paper hydrolysates + KH_2PO_4 + K_2HPO_4 + $(NH_4)_2SO_4$ + $MgSO_4$	Batch in flask	0.080	Annamalai et al. (2018)
Cryptococcus curvatus	Corn stover hydrolysates and biodiesel-derived glycerol	Batch in flask	0.130	Gong et al. (2016)
Cryptococcus curvatus	Prickly-pear juice (diluted)	Batch	0.145	Hassan et al. (1994)
Cryptococcus curvatus	Glycerol + spent yeast lysate from brewery industry	Batch in flask	0.152	Ryu et al. (2013)
Cryptococcus curvatus	Office paper hydrolysates	Batch in flask	0.153	Zhou et al. (2017)
Cryptococcus curvatus	Sweet sorghum syrup + minimal medium	Batch in flask	0.167	Cui and Liang (2015)
Cryptococcus curvatus	Cheese whey (pretreated)	Batch in flask	0.195	Seo et al. (2014)
Cryptococcus curvatus	Corn stover hydrolysates + Tween 80 + ampicillin + cellulase + xylanase + yeast extract + KH_2PO_4 + $Na_2HPO_4{\cdot}7H_2O$ + $MgSO_4{\cdot}7H_2O$ + ethylenediaminetetraacetic + trace element solution	Simultaneous saccharification and enhanced lipid production	0.195	Gong et al. (2014)
Cryptococcus curvatus	Crude glycerol + corn steep liquor + deoiled *Cryptococcus* lysate	Fed-batch	0.248	Thiru et al. (2011)
Cryptococcus curvatus	Glucose + KH_2PO_4 + Na_2HPO + $MgSO_4{\cdot}7H_2O$ + $CaCl_2$ + $FeCl_3.6H_2O$ + $ZnSO_4.7H_2O$ + yeast extract + peptone	Fed-batch	0.470	Zhang et al. (2011)
Cryptococcus curvatus	Glycerol + minimal medium	Fed-batch	0.590	Meesters et al. (1996)
Cryptococcus curvatus	Whey permeate + NH_4Cl	Partial recycling	0.995	Ykema et al. (1988)

[a] Calculated based on references

[b] Including 0.008 g/L/h of produced extracellular lipids

Table 6 Productivities obtained by *Lipomyces starkeyi* using different media and different cultivation methods

Yeasts	Medium	Cultivation method	Productivity (g/L/h)[a]	References
Lipomyces starkeyi	Olive oil mill wastewaters	Batch in flask	0.010	Yousuf et al. (2010)
Lipomyces starkeyi	Xylose + KH_2PO_4 + $Na_2HPO_4.12H_2O$ + $(NH_4)2SO_4$ + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ + yeast extract + trace element solution	Batch in flask	0.026	Wang et al. (2014)
Lipomyces starkeyi	Spent yeast cell mass hydrolysates	Batch in flask	0.050	Yang et al. (2014b)
Lipomyces starkeyi	Hydrolyzed flour-based industrial waste streams	Batch in flask	0.060	Tsakona et al. (2014)
Lipomyces starkeyi	Corn bran hydrolysates	Batch in flask	0.065	Probst and Vadlani (2015)
Lipomyces starkeyi	Soluble sweet potato starch + $(NH_4)_2SO_4$ + yeast extract + $Na_2HPO_4.7H_2O$ + KH_2PO_4 + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$ + $FeSO_4$ + $ZnSO_4.H_2O$ + $MnSO_4.H_2O$ + $CoCl_2.6H_2O$ + $CuSO_4$	Batch in flask	0.097	Wild et al. (2010)
Lipomyces starkeyi	Hemicellulose hydrolysate + yeast extract + $(NH_4)_2SO_4$ + Na_2HPO_4 + KH_2PO_4 + $Mg_2SO_4.7H_2O$ + $CaCl_2.2H_2O$ + $ZnSO_4.7H_2O$ + $CuSO_4.5H_2O$ + $CoCL_2.6H_2O$ + $(NH_4)2Mo_2O_7$	Continuous	0.111	Anschau et al. (2014)
Lipomyces starkeyi	$(NH_4)_2SO_4$ cellobiose + xylose + yeast extract + KH_2PO_4 + $MgSO_4.7H_2O$	Batch in flask	0.125	Gong et al. (2012)
Lipomyces starkeyi	Hydrolyzed flour-based industrial waste streams	Fed-batch	0.4	Tsakona et al. (2014)
Lipomyces starkeyi	Glucose + yeast extract + peptone	Two-stage fermentation	1.6	Lin et al. (2011)

[a] Calculated based on references

most abundant and cheap local carbon source, using for that the most suitable oleaginous yeast. To choose the most suitable yeast for a certain carbon source, it is also important to know the lipid yields that can be obtained. Different lipid yields were obtained by the oleaginous yeasts using different carbon sources (Table 7). It is important to highlight that, in some cases, lipid yields were close to the theoretical maximum, using low-cost carbon sources.

Production costs

Only a few studies are available about the economics of yeast oil production, and the results obtained differed significantly according to the different scenarios considered. In some studies, with the current technology, yeast oil production is still considered non cost-competitive, due to the high production costs, making it more expensive than vegetable oils (Koutinas et al. 2014) (Ratledge and Cohen 2008). Koutinas made a techno-economic evaluation of microbial oil (MO) production and subsequent production of biodiesel, using the yeast *Rhodosporidium toruloides* (Koutinas et al. 2014). They have evaluated four different flowsheets, one for the production of yeast cells, other for the oil extraction and purification, and 2 more for the biodiesel produced either by direct or indirect transesterification of MO. The experimental results obtained by Li were used for the development of the process flow diagram (Li et al. 2007). A productivity of 0.54 g/L/h and an overall glucose to microbial mass and MO conversion yields of 0.35 and 0.23 g/g, respectively, were considered in this study. The fermentation was carried out in fed-batch mode and had two stages, one for microbial growth and the other for MO accumulation. The results were obtained based on an annual production capacity of 10,000 t of microbial oil. Considering the cost of glucose as being zero, an estimated production cost of purified microbial oil of $3.4/kg was obtained. Considering a glucose price of $400/t, the price would rise to an estimated cost of $5.5/kg oil. Note also that, in this study, yeast extract was used as nitrogen source, at a cost of $800/t accounting for 16.46% of the raw material costs while glucose accounts for 79.28% if considered at a price of $400/t. Besides the evident influence of the feedstock price in the production cost, Koutinas concluded that the main costs are the capital investment and electricity consumption associated with the operation of classical fermenters (Koutinas et al. 2014). The total fixed capital investment for a 10,000 t MO production plant like this was estimated to be M$ 71.5, coming more than half of the value from the fermenters.

Other economical study was made in New Zealand using the yeast *Cryptococcus curvatum* and lactose coming from cheese and butter creameries as carbon source to obtain cocoa butter equivalent (CBE) (Ratledge and Cohen 2008). High yields and conversion ratios of lactose to oil were achieved,

Table 7 Lipid yields obtained by the oleaginous yeasts using different carbon sources

Yeasts	Carbon source	$Y_{L/S}$ (gl/gs)	References
Rhodotorula glutinis	Glycerol	0.101	Karamerou et al. (2016)
	Corncob hydrolysate	0.159	Liu et al. (2015)
	Sucrose	0.180	Lorenz et al. (2017)
	Glucose	0.182	Johnson et al. (1995)
Yarrowia lipolytica	Glycerol	0.140	Sara et al. (2016)
	Acetic acid	0.160	Xu et al. (2017)
	Glucose	0.270	Qiao et al. (2017)
Rhodosporidium toruloides	Glycerol	0.220	Yang et al. (2014a)
	Glucose	0.260	Li et al. (2007)
	Acetic acid	0.277	Huang et al. (2016)
	Corn stove hydrolysate	0.290	Fei et al. (2016)
Cryptococcus curvatus	Corn stove hydrolysates	0.159	Gong et al. (2014)
	Acetic acid	0.172	Liu et al. (2017)
	Volatile fatty acids	0.187	Liu et al. (2017)
	Glycerol	0.220	Ryu et al. (2013)
	Cardboard hydrolysates	0.224	Zhou et al. (2017)
	Glucose	0.246	Zhang et al. (2011)
	Whey permeate	0.290	Ykema et al. (1988)
Lipomyces starkeyi	Glycerol	0.150	Wang et al. (2014)
	Sweet potato starch	0.160	Wild et al. (2010)
	Glucose	0.180	Gong et al. (2012)
	Xylose	0.180	Gong et al. (2012)
	Cellobiose	0.200	Gong et al. (2012)
	Hemicellulose hydrolysate	0.236	Anschau et al. (2014)

$Y_{L/S}$, lipid yield g_L/g_S (conversion yield of lipid formed per carbon substrate consumed)

quality of the oil was entirely satisfactory as a CBE, and manufacturing cost of the yeast oil was calculated as US$ 800–1000/t based on using 200,000 m^3 of whey/year. This cost did not include plant depreciation, interest on capital investment, or manufacturing overheads. A calculated likely selling price of US$ 2000–2500/t was obtained, which would make it cost-competitive if a reduction in the price of cocoa butter had not occurred. At the time the study started, the cost of cocoa butter was about US$ 5000/t, but short time after the price dropped 40%, making the production insufficiently profitable to justify further development of the process (Ratledge and Cohen 2008). This study ends up by pointing this process not to be cost-competitive if the cost of cocoa butter keeps low. Anyway, it must be noted that the trend after 2000 was for the prices to increase due to the prevalence of harmful insects and viruses and the general failure of the cultivation techniques of the cocoa plant, being even considered the risk of their disappearing (Papanikolaou and Aggelis 2011).

Park assessed the economics of microbial lipids for biodiesel production using volatile fatty acids (VFAs) derived from organic waste as carbon source and a multistage continuous high cell density culture (MSCHCDC) process (Park et al. 2014). They made a simulation study assuming a lipid yield of 0.3 g/g VFAs, cell mass yield of 0.5 g/g glucose or wood hydrolysates, and employing process variables including lipid contents from 10 to 90% of cell mass, bioreactor productivity of 0.5–48 g/L/h, and plant capacity of 20,000–1,000,000 metric ton (MT)/year. They estimated, for a 100,000 MT/year production capacity, a production cost of US$ 1.048/kg lipid, considering carbon source costs of US$ 0.2/kg for wood hydrolysates and US$ 0.15/kg for VFAs; nitrogen source (NH_3) US$ 0.2/kg; water US$ 0.305/MT (904,166 MT per year); *n*-Hexane US$ 1.5/kg; bioreactor productivity of 9 g/L/h; 100,000 MT/year production capacity; and 75% lipid content in cell mass. For a more realistic productivity of 1.5 g/L/h, close to the maximum productivity obtained with oleaginous yeasts (1.6 g/L/h obtained by Lin et al. in a two-stage fermentation process with *Lipomyces starkeyi* (Lin et al. 2011)), the production cost was estimated to be US$ 1.422/kg lipid. In this study, the variables having the highest impact on microbial lipid production costs were the cost of VFAs and lipid yield, followed by lipid productivity, lipid content, and

fermenter cost. The carbon source (wood hydrolysates + VFAs) and the utilities were the main contributors for the final production cost representing 60.45 and 21.44% of the production costs, respectively. The total fixed capital investment for a 100,000 t microbial lipid production plant was estimated to be M$ 52.9. The main contributor for the equipment cost is the fermentation process equipment that represents 41% of the total equipment cost, followed by the medium preparation equipment (26.6%), cell mass washing and recovery (17.9%), and lipid extraction (14.5%). In this study, despite being a 10 times bigger capacity plant than the one referred in Koutinas et al. (2014), total fixed capital investment is lower, mainly because of the lower price of the fermentation process equipment. Plant capacity has also a big influence in the final production cost, since the smallest plant assessed in this study, with a capacity of 20,000 MT/year, resulted in a production cost of US$ 1.705/kg and the largest assessed plant size of 1,000,000 MT per year resulted in a production cost of only US$ 0.876/kg. The bigger the plant capacity, the more diluted will be the capital investment cost influence in the production cost.

In a more recent study, Park used volatile fatty acids, obtained via anaerobic digestion of rice straw hydrolysates, as carbon source, for microbial lipid production by *Cryptococcus curvatus*, and the estimated production cost was US$ 1.15/L lipid (1.35/kg lipid) considering a carbon source cost of US$ 0.15/kg (Park et al. 2017). The cost could be as low as US$ 0.30/L (0.35/kg) if the cost of the carbon source is considered equal to zero. In both cases, nitrogen source was considered as having cost zero and the lipid yield was 0.15 g/g. In South Korea, the overall cost of food waste was estimated to be US$ − 0.60/kg VFAs by a local government subsidy. In this study, the overall lipid costs for operational expenses other than the feedstock, including utilities, labor, waste treatment, and facility costs, were assumed to be US$ 0.354/kg, based on the previously mentioned study of Park et al. (2014).

Fei assessed the effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production and made a preliminary cost analysis of the production of lipids (Fei et al. 2011b). A lipid yield of 0.15 g/g of VFAs, a plant production capacity of 1,000,000 ton of lipid per year and a variation of VFAs cost between US$ − 20 per ton and US$ 100 per ton was considered in that analysis. In those conditions, the production cost of the lipids would range between US$ 0.16 and 1.055/kg of lipids. In this analysis, although the capital investment cost, that is one of the main contributors to the final cost according to Koutinas et al. (2014), was not considered, the obtained values are very promising.

Ryu et al. (2013) used the same method as Fei et al. (2011b) to assess the production cost of lipids obtained with *Cryptococcus curvatus* cultivated on spent yeast from brewery industries and glycerol. They calculated that the lipid production cost would be US$ 0.292/kg, coming a large part of the cost from the cost of separation of the spent yeast from fermented wastewaters. Like in the study of Fei et al. (2011b), they did not consider the capital investment cost.

Ricardo et al. developed in Brazil a successful pilot-scale process for biodiesel production from microbial oil produced by *Rhodosporidium toruloides DEBB 5533* (Ricardo et al. 2017) using sugarcane juice as carbon source and urea as nitrogen source. In their study, they were able to reach a lipid productivity of 0.44 g/L/h in a bioreactor of 1000 L working volume in fed-batch mode. They made a preliminary economic analysis that was based in the costs of the medium and energy involved in each step of the biooil and biodiesel production. They demonstrated microbial biodiesel production economically competitive (US$ 0.76/L) when compared to the vegetable biodiesel (US$ 0.81/L). The price of the carbon source was US$ 0.14/kg and the nitrogen source US$ 0.265/kg. This is a very particular case where the microbial oil obtained is used for biodiesel production in a country where the cost of sugarcane juice is very low. It should also be taken in consideration that in the economic analysis it was assumed that part of the electricity would be provided from an alcohol factory, which generates electricity from the sugarcane bagasse burn. Furthermore, in this preliminary economic analysis, only the costs of the medium and energy involved were considered and, for instance, the capital investment cost was not taken in consideration. Anyway, it remains a very promising result.

These studies allow for the identification of the production stages that have a bigger influence in the final production cost of lipids using OY. Despite that these studies were made mostly in different scenarios, the stages of production where the production costs are higher are the same. We can divide the production process in three main stages in decreasing order of magnitude (Fig. 1): medium preparation, fermentation process, and downstream processing. Medium preparation is the most costly stage, mainly because of the carbon and nitrogen source costs; the fermentation process is the second most costly stage mainly because of the investment cost and energy needed; and the downstream processing is the less costly despite of still having significant cost associated to the cell mass washing and recovery and to lipid extraction.

Main points of improvement to lower production cost and increase incomes

Many studies have been done with the goal of reducing the production costs. In this section, the main aspects to be considered for cost reduction will be discussed and solutions for process improvement will be identified in each of the stages

Fig. 1 The three main stages of the production process contributing for the final production cost, in decreasing cost order

mentioned before (medium preparation, fermentation process and downstream processing).

Medium preparation

As medium preparation is the most costly stage, most of the studies have dealt with this aspect. The main costs in this stage come from the carbon source, the nitrogen source, the sterilization of the medium, and water usage, if a traditional fermentation is going to be carried out.

The main contributor to the final cost is by far the carbon source. Glucose is considered an effective carbon source in the fermentation processes, but if glucose is used as carbon source ($500/t in 2010), it can represent as much as 80% of the total medium cost, contributing to over 60% of the total production costs in a typical fermentation process (Fei et al. 2011a). In lipid production by oleaginous yeasts, it needed a much higher amount of carbon source than nitrogen source, since high carbon-to-nitrogen (C/N) ratios are favorable to trigger high lipid accumulation and when nitrogen is limiting for the production of biomass, the carbon source can be converted to storage lipid (Ratledge 2002). That is why most of the studies are about finding low-cost carbon sources that can be used as good substitutes of glucose. To be a good substitute, it has to be cheap, abundant, and allow good productivities. Wastes and wastewaters are strong candidates to be used as carbon source since they are abundant and have no cost or represent even negative cost. Oleaginous yeasts can utilize a wide variety of different carbon sources, which allows to explore the possibility of using many different low-cost carbon sources.

Wastewaters

Oleaginous yeasts can be used with the dual purpose of treating wastewater and producing microbial lipids. Wastewaters can be at the same time carbon and water sources for the oleaginous yeasts besides providing several other nutrients. A few authors tested wastewaters of different kinds. Xue assessed the potential of using diluted monosodium glutamate wastewater as a cheap fermentation broth for *Rhodotorula glutinis* (Xue et al. 2006). Although, in the initial experiments, a low productivity was obtained, a new study (Xue et al. 2008) was made where diluted monosodium glutamate wastewater was supplemented with glucose to increase the C/N ratio. In this case, although glucose is used, the utilization of monosodium glutamate wastewater could contribute to reduce the amount of glucose and water needed for microbial lipid production. Despite a considerable increase in the productivity, the value obtained was still far from the productivities for other media. Bioethanol wastewater was also assessed for its potential as a cheap fermentation broth, with glucose being also used as supplement to increase the productivity (Zhou et al. 2013). Similar results were obtained as the ones previously reported for the use of monosodium glutamate wastewater. In both cases, further studies should be done to increase productivity and find a cheaper supplement than glucose. Better productivities, although still not very high, were obtained with a mixture of distillery wastewater and domestic wastewater without sterilization, using the yeast *Rhodosporidium toruloides* (Ling et al. 2013). In this case, besides using a low-cost carbon source, they also eliminated the sterilization step. In another study, it was tested with success the possibility to reuse the spent seed culture medium of *Rhodosporidium toruloides*, and that allowed for a reduction of around 30% in the medium cost (Ling et al. 2017). Several other wastewaters like olive oil mill wastewaters (Yousuf et al. 2010) and potato wastewaters supplemented with glycerol (Kot et al. 2017) were tested, but low productivities were obtained. Only few wastewaters were tested until now. Many other agro-industrial wastewaters can still be assessed, and the supplementation with other cheap carbon sources can possibly increase the productivities and lipid yields since with some yeasts the productivities and lipid yields were even higher when using cheap carbon sources than using glucose, like as was mentioned in the "Carbon sources, productivity, cultivation methods, and lipid yields" section.

Wastes

Glycerol is one of the most tested low-cost carbon sources. Increasing biodiesel production generates high amounts of glycerol as a byproduct, raising its availability to the point of outpacing the market demand for glycerol (Yang et al. 2012). Furthermore, the raw glycerol derived from biodiesel production to be used in the oleochemical industries needs to undergo a costly and energy-consuming purification process, making it unappealing for those industries (Yen et al. 2012). Due to this, it becomes a potentially very cheap carbon source to be used in microbial lipid production. High yields and productivities were obtained using glycerol as carbon source for the yeasts *Rhodosporidium toruloides* (Yang et al. 2014a) and

Cryptococcus curvatus (Ryu et al. 2013), respectively, making this cheap carbon source a promising feedstock. Furthermore, if the lipids produced are to be used for instance to produce biodiesel, the resulting glycerol can be recycled to produce more lipids.

Volatile fatty acids that can be obtained through syngas fermentation, lignocellulosic biomass degradation, and organic waste anaerobic digestion can also be an abundant low-cost or even negative cost carbon source. High productivities can be obtained using volatile fatty acids. Productivities of 0.28 and 0.33 g/L/h were obtained using glycerol plus volatile fatty acids and glycerol plus acetic acid, respectively (Fontanille et al. 2012). Fontanille et al. (2012) developed a two-stage fed-batch strategy where the yeast *Yarrowia lipolytica* was initially grown using glycerol, and, in a second stage, acetic acid or volatile fatty acids were added as carbon source for lipid accumulation. In this process, two low-cost carbon sources, like glycerol and volatile fatty acids, are used with success. Xu obtained a productivity of 0.8 g/L/h using diluted acetic acid as carbon source for the oleaginous yeast *Yarrowia lipolytica* in a semi-continuous system (Xu et al. 2017). The bioprocess developed by Xu managed not only to obtain high productivities but also to solve the main issue related with the dilute nature of the volatile fatty acids obtained by the processes mentioned previously, being able to sustain high-density cell culture using acetic acid at a concentration of only 3%.

Whey permeate coming as a byproduct from cheese and butter creameries can also be used as cheap and abundant carbon source, since it has about 45 g/L of lactose as the main carbon source. Ykema obtained a productivity of 0.995 g/L/h and a lipid yield of 0.29 g/g using whey permeate as growth medium for *Cryptococcus curvatus* being the best productivities and lipid yields obtained using low-cost carbon sources (Ykema et al. 1988).

Flour-rich waste streams are also a promising abundant, cheap carbon source. Tsakona successfully utilized flour-rich waste and byproduct streams generated by bakery, confectionery, and wheat milling plants as the sole raw materials for lipid production by *Lipomyces starkeyi* achieving a productivity of 0.4 g/L/h (Tsakona et al. 2014).

Lignocellulosic hydrolysates have been widely studied as cheap carbon source for fermentation processes. Some oleaginous yeasts have the ability to utilize pentoses as well as hexoses and can assimilate glucose and xylose simultaneously (Hu et al. 2011), making this carbon source really promising. High lipid yields and productivities were obtained with the main sugars present in lignocellulosic hydrolysates. Gong obtained similar lipid yields by *Lipomyces starkeyi* using glucose, xylose, and cellobiose (0.18 g/g; 0. 18 g/g; 0 .2g/g) and a lipid productivity of 0.125 g/L/h using simultaneously xylose and cellobiose (Gong et al. 2012). Also, with *Lipomyces starkeyi* and using hemicellulose hydrolysate, Anschau obtained a lipid yield of 0.236 g/g and a productivity of 0.111 g/L/h (Anschau et al. 2014). Fei, utilizing the yeast *Rhodosporidium toruloides* and using corn stove hydrolysate, was able to obtain a productivity of 0.4 g/L/h and a lipid yield of 0.29 g/g (Fei et al. 2016).

Some of the wastes and wastewaters already have nitrogen source besides the carbon source, in some cases in high quantities, making it needed to add extra carbon sources to raise the C/N ratio. One good example is the study of Ryo Byung-Gon where they use spent yeast from brewery industry as nutrient source for *Cryptococcus curvatus* (Ryu et al. 2013). In order to raise the C/N ratio, they used glycerol and were able to achieve lipid yields of 0.22 g/g and productivities of 0.152 g/L/h. In the cases the nitrogen source is not present in enough quantity, corn steep liquor and domestic animal feces or urine can be used as cheap nitrogen sources (Park et al. 2014).

One way to avoid spending energy for sterilization is to find oleaginous yeasts able to outrun the competition when growing in non-sterile media. Yeasts, able to keep high productivities at low pH or at extreme temperatures or even capable to produce antimicrobial compounds, are good candidates for that. That is exactly what Santamauro found in the yeast *Metschnikowia pulcherrima* (Santamauro et al. 2014). This yeast has the ability to grow at low temperature and pH and to produce natural antimicrobial compounds. This yeast was not classified as oleaginous, but, in their study, they were able to obtain high yields at low temperature, low pH, and using several different non-sterilized mediums with low-cost carbon sources and no yeast extract.

Further studies should be done to explore other carbon sources and to improve the productivities and lipid yields utilizing the cheap, abundant carbon sources available. A higher lipid yield would imply the use of less carbon source per unit of lipids produced, lowering in that way the production cost if there is a cost associated to the carbon source used. To improve the productivities and lipid yields, work should focus mainly in improving the fermentation process for better utilization of the low-cost carbon sources, explore the ability of some oleaginous yeasts to grow, and keep high productivities without needing to use sterile conditions and screening for other oleaginous yeast capable of achieving higher productivities and lipid yields with low-cost carbon sources or genetically engineering more robust yeasts for lipid production.

Fermentation process

As mentioned before, the fermentation process is where all the conditions must be optimized in order to obtain the highest lipid yields and productivities. To achieve that, there are different cultivation methods that can be used that require different kinds of fermenters that have different

costs. Besides that, optimum temperature, aeration, and pH must be maintained for optimum production. The main costs of the fermentation process are related with the energy spent with the aeration and keeping the ideal temperature and with the size and type of fermenter used (fermenter cost). To minimize the influence of these costs, lipid productivity and lipid yield obtained in the process should be as high as possible. Fermenter costs are strongly dependent on the lipid productivity (Ykema et al. 1988); so, higher productivities would allow to use smaller fermenters to obtain the same quantities of lipids and consequently lower fermenter cost and the energy spent. Also, the cultivation method has big influence in the lipid yields and productivities. Ykema compared four different cultivation methods (batch, fed-batch, continuous, and partial recycling culture) for lipid production with *Cryptococcus curvatus* and concluded that the highest lipid productivities will be achieved in a mode of operation that enables the cultivation at high cell densities (Ykema et al. 1988). According to Ykema, the highest productivities can be achieved using partial recycling culture of the biomass, followed by fed-batch, continuous, and batch method. Batch mode is mainly used in lab-scale studies using flasks for screening for new oleaginous yeasts, to assess the potential of the oleaginous yeasts and determine the optimum cultivation conditions. Considering that, it is possible to raise the productivities of many of the studies referenced in Tables 2, 3, 4, 5, and 6. Until now, the highest productivities by *Rhodotorula glutinis*, *Yarrowia lipolytica*, *Rhodosporidium toruloides*, and *Lipomyces starkeyi* were achieved using fed-batch cultivation methods. The highest lipid productivity obtained until now was with *Lipomyces starkeyi* as reported by Lin that used a two-stage fermentation method, where, in the first stage, cells were cultivated in a nutrient-rich medium for cell growth and, in the second stage, to promote lipid accumulation, cells were resuspended in a glucose solution, and, when the glucose was exhausted, more glucose was supplemented for more lipid accumulation (Lin et al. 2011).

Although, methods like continuous cultivation have been tested with several oleaginous yeasts, methods like partial recycling culture that allowed for the highest productivity were tested with *Cryptococcus curvatus*, only. Therefore, although fed-batch is recognized as a great method for high cell density cultivation, other methods should be tested and developed to further improve the lipid productivities of the various oleaginous yeasts.

Several of the oleaginous yeasts are obligate aerobes, depending on oxygen for its energy metabolism and cellular component synthesis. For those, the aeration has a big influence in the lipid productivities obtained, since good levels of dissolved oxygen are required for higher cell growth (Yong-Hong et al. 2006) (Choi et al. 1982). At high cell density, culture viscosity is higher and mass transfer is harder, reducing oxygen availability. Pan was able to obtain much higher biomass concentrations of *Rhodotorula glutinis* and higher overall lipid productivities in a fed-batch process, using aeration with oxygen-enriched air instead of air (Pan et al. 1986). However, the effects of oxygen limitation appear to differ widely among lipogenic yeasts since for some of the yeasts the oxygen limitation seems to result in lower lipid productivities despite of the higher cell growth (Calvey et al. 2016). Higher aeration rates may improve lipid productivity of some yeasts, but it has a cost since it implies to spend a higher amount of energy. In order to reduce the aeration requirements, different aeration rates can be used promoting a lower aeration in the lipid accumulation stage, and another option is to select yeasts that require lower aeration rates. Contributions to reduce the aeration requirements were mentioned in co-culture studies of oleaginous yeasts with microalgae due to the release of oxygen to the medium by the microalgae (Xue et al. 2010; Cheirsilp et al. 2012). Xue, when assessing the lipid production of mix cultivation of *Spirulina platensis* and *Rhodotorula glutinis*, registered a rapid increase in dissolved oxygen from 7.45 to 120.5% in 5 h when *Spirulina platensis* was added to the culture (Xue et al. 2010).

Aeration not only influences the lipid productivity but also the lipid profile of some yeasts. Davies studied the effect of low oxygen uptake rate on the fatty acid profile of the oleaginous yeast *Cryptococcus curvatus* and observed that it was possible to decrease the unsaturated fatty acids percentage in the lipid profile by limiting the oxygen uptake rate of the culture (Davies et al. 1990). To decrease unsaturated fatty acids is a way to obtain cocoa butter equivalent (Hassan et al. 1994) or palm oil equivalent (Sargeant et al. 2014), but doing that by limiting the oxygen uptake rate can lower the lipid yields and productivities (Davies et al. 1990).

Temperature is another factor affecting lipid yield and productivity. In large-scale production, yeasts should be robust to withstand process disturbances, like temperature and pH variations, without affecting much the productivity, and the ability to keep good productivities at high temperatures would be advantageous to decrease the amount of cooling needed for cultivation (Lamers et al. 2016). A selection of robust yeasts and yeasts tolerant to high temperatures would be favorable for large-scale production and to reduce the energy spent in the fermentation process. Amaretti et al. (2010) and Viñarta et al. (2016) assessed the potential of several yeasts isolated from cold environments like Antarctica. The results they obtained were promising in terms of lipid yields and productivities, and the yeasts were robust being able to grow at a wide range of temperatures, but not at high temperatures. More screening should be done to find

robust yeasts able to achieve high lipid yields and productivities at high temperatures.

Downstream processing

In the downstream processing, the main costs are associated with the energy spent in the process of recovery of the lipids due to the fact that the lipids are stored inside the cells. The traditional processes involve the cell mass washing and recovery and the lipid extraction where solvents are used. High density cell cultivations are also advantageous in this stage since it contributes to reduce the energy needed in processes like centrifugation due to the less water that needs to be separated from the cells (Ling et al. 2013). The lipid extraction process is an energy-intensive process involving high amounts of toxic solvents that requires cell disruption for an effective extraction (Yu et al. 2015). The process of recovering the lipids would become much more simple and cheap if the lipids were excreted to the medium. This could possibly be achieved through genetic engineering or exploring the natural ability of secreting the lipids to the medium that some yeasts have under certain conditions (Huang et al. 2018). Many cell disruption methods are available and can be divided in mechanical and non-mechanical methods. Their efficiencies can be different depending on the microorganism used, and the lipid applications should also be taken in consideration when choosing the extraction method. For instance, when the lipids are for food industry, toxic chemicals should be avoided so that a wise choice should be done (Ochsenreither et al. 2016). Lipid recovery from wet oleaginous microbial biomass has been highly investigated since it would contribute to a significant reduction of the energy spent in dewatering the cell biomass, but the technologies developed until now are far from being ready to be commercialized (Dong et al. 2016).

To help to improve the economics of yeast lipid production, another solution could be the recovery of high-value products that some yeasts produce simultaneously with lipids, like enzymes, beta-carotene and astaxanthin. Several studies have been made about the co-production of lipids and carotenoids with promising results (Kot et al. 2017; Saenge et al. 2011). Carotenoids are high-value products, with a continuously growing global market expected to reach about US$1.4 billion in 2018 (Mata-Gómez et al. 2018); and its recovery alongside with the lipids production is appealing. The possibility of exploring other high-value products from oleaginous yeast should be further assessed to improve the economics of yeast lipid production.

In Fig. 2, the main points of improvement to lower production costs in the three main stages of the production process are presented.

Potential applications for oleaginous yeast oils

The studies on the production of lipids from oleaginous yeasts have been done mainly with the goal of using those lipids for biodiesel production. Their lipid profile similar to vegetable oils makes them suitable for biodiesel production, although different lipid profiles can be found in different oleaginous yeasts. The lipid profile of each yeast also depends on the culture conditions, making it possible to direct their lipid profile to lipid profiles more favorable to other applications. Metabolic engineering of the yeasts could also be used for tailoring the lipid profile to the desired products (Dey and Maiti 2013).

Biodiesel

In the last decades, the global biodiesel production started raising mainly due to the European Union (EU) Renewable Energy Directive (RED) that requires 10% of all transport fuels to be delivered from renewable sources by 2020 in every Member State being more than 85% of the RED transport target expected to come from biofuels (Biodiesel is the main biofuel in the EU transport sector, with a 78.2% share of total consumption, by energy, according to Eurostat 2013). This demand started raising many concerns related with the sustainability of its production, mainly the biodiesel of first generation, obtained using food crops (Anuar and Zuhairi 2016). EU specified a minimum set of sustainability criteria for biofuels and bioliquids, with a threshold of 35% savings of GHG emissions with respect to the fossil fuels they replace. The use of specific land-use categories, such as primary forest, highly biodiverse grassland, wetlands, and peatlands, is explicitly excluded due to its lack of sustainability. Oleaginous yeasts have a favorable lipid profile for the production of biodiesel, due to their high percentage of oleic acid, and they are a potential solution for all the sustainability issues related with first-generation biodiesel production. This is why so many studies have been done with oleaginous yeasts with the goal of producing lipids for biodiesel. Biodiesel production is expected to contract slightly by 2020 according to the trajectories presented by the Member States in their National Renewable Action Plans (Marelli et al. 2015), but if oleaginous yeast oils become a sustainable solution for biodiesel production, then the demand can raise again.

Food industry

There are many applications for vegetable oils in the food industry. Not only the vegetable oils are sold for cooking purposes, but they are also part of many food products. Oleaginous yeast oils could get into the market as sustainable vegetable oil equivalents since their lipid profile is

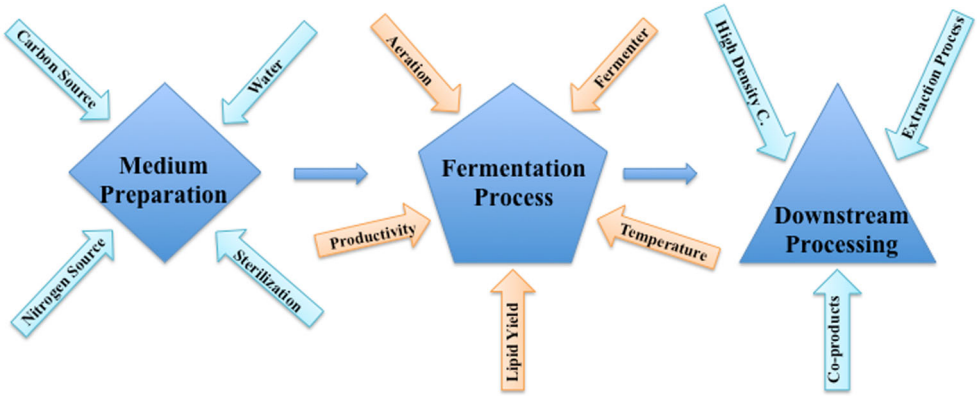

Fig. 2 Main points of improvement to lower production costs in the three main stages of the production process

similar to vegetable oils. Palm oil is one of the most used oils in the food industry, and it is produced in tropical regions where it contributes to a 1.5% annual rate of deforestation of tropical rainforests (Sargeant et al. 2014). Its lipid profile is high in saturated fatty acids, making it necessary to raise the percentage of saturated fatty acids in the oleaginous yeasts oil to obtain a lipid profile more similar to palm oil in order to be possible to be used as palm oil equivalent. The palm oil selling price is low, making it challenging to find a substitute cost competitive, but, by finding it, it would be of high environmental interest. It could be financially more interesting to develop oil yeast equivalents to high-value fats like cocoa butter. Like palm oil, cocoa butter lipid profile is also high in saturated fatty acids making it is also necessary to tailor the fatty acid profile of the oleaginous yeasts. Tailoring the lipid profile of the oleaginous yeasts to produce higher amounts of essential fatty acids for nutrition could be also advantageous.

Biopolymers

Polymers are mostly derived from petrochemicals, which raises environmental concerns. Public awareness of those environmental issues is raising the search for greener bio-based alternatives. Biopolymers obtained from vegetable oils can become a good alternative to petrochemicals if sustainable vegetable oils are used. Oleaginous yeast oils can be a sustainable alternative to vegetable oils also for biopolymer production. Furthermore, the composition of vegetable oils can vary significantly, affecting the quality of the polymer production (Zhang et al. 2017), which is not the case for the production of oil from OY.

Many polymers can be obtained using vegetable oils, mainly polyurethane, polyester, polyether, and polyolefin. Polyurethanes are synthesized by reacting polyols with petroleum-derived multi-isocyanate, both of which could be derived from triglycerides and their derivatives (Miao et al. 2014). With polyols, flexible or rigid foams can be manufactured that can be used in a wide range of products, which goes from vehicle interiors and building insulation until the core of surf boards. The production of biopolymers is also useful for biomedical applications mainly because vegetable oil is a bio-based raw material that can be metabolized in the human body, and, therefore, materials derived from them are potentially biocompatible (Ca et al. 2013). A huge market can be explored if the production of oleaginous yeasts oil becomes competitive.

Others

Other applications can be found in pharmaceutical and cosmetic industries, and several others should be researched aiming at finding high-value products to raise

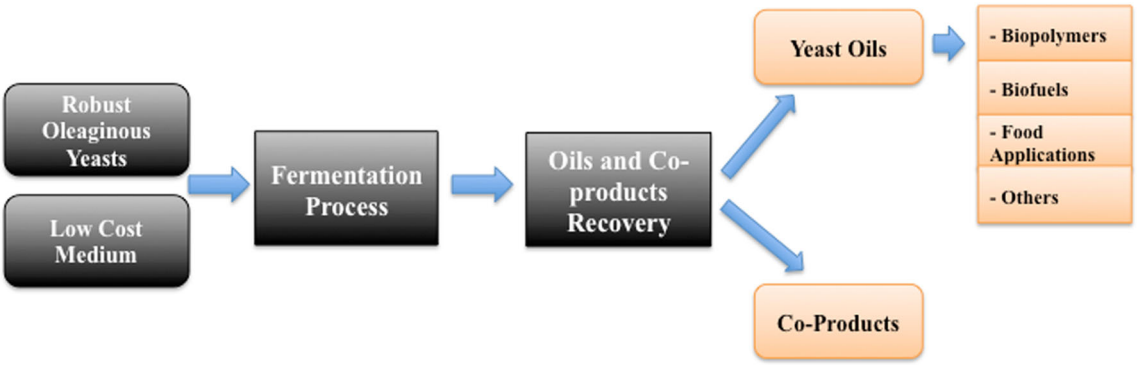

Fig. 3 Envisioned yeast lipid production and applications

the income of oleaginous yeast oils and turn its production more profitable and viable even at small scale.

Conclusions

Oleaginous yeasts have great potential for sustainable production of oils similar to vegetable oils. The high potential comes from their ability to utilize several kinds of low-cost substrates, high growth rate and lipid production, small amount of land and water requirements, and the simple cultivation methods that are not climate-affected.

Despite all the potential they have, a lot of research still needs to be done to improve the economics of yeast oil production. Screening for more robust oleaginous yeasts, or even genetically manipulating them to obtain high lipid yields and productivities, utilizing a wide variety of low-cost carbon sources in non-sterile media and the possibility of operating at a wide range of pH and temperatures, mainly high temperatures, would significantly improve the economics of the lipid production by reducing the costs in energy spent and by using carbon sources obtained locally. Considering that the carbon source can contribute to over 60% of the total production costs if glucose is used, a wider range of low-cost substrates should be evaluated, as a successful use of negative cost carbon source would have a significant impact in the reduction of the production costs. In the fermentation process, further research should be done to develop better fermentation methods to raise the lipid yield and productivities together with the use of cheaper and more energy-effective fermenters as this would also considerably improve the economics of lipid production by reducing the capital investment and energy spent and by raising the productivity. Oil recovery processes should also keep being improved to reduce the energy and solvent usage, and high-value co-products recovery should also be implemented if the amount of co-product obtained justifies the investment. The ability of the oleaginous yeasts of producing oils with different lipid profiles should be further explored, since the production of oils with different profiles could be directed for the production of different products like biodiesel, vegetable oil substitutes, food additives, biopolymers, pharmaceutical and cosmetic industries, etc., widening the market for yeast oils. To direct the lipid profile of the oils for the production of high-value products would significantly improve the economics of yeast lipid production.

In Fig. 3, we can see in a schematic way the conclusions of this work. To improve the economics of oleaginous yeast oil production, we should reduce production costs in all stages, improve lipid yields and productivities, and direct the production to high-value products.

Acknowledgements This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), FCT doctoral grant (SFRH/BD/80490/2011) attributed to Bruno Vasconcelos, and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020—Programa Operacional Regional do Norte.

Compliance with ethical standards

Conflict of interest Bruno Vasconcelos declares that he has no conflict of interest. José Carlos Teixeira declares that he has no conflict of interest. Giuliano Dragone declares that he has no conflict of interest. José António Teixeira declares that he has no conflict of interest.

Human and animal studies This article does not contain any studies with human participants or animals performed by any of the authors.

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