OCL 2021, 28, 51

https://doi.org/10.1051/ocl/2021038

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

REVIEW OPEN ACCESS

Strategies for increasing lipid accumulation and recovery from *Y. lipolytica*: A review☆

Sally El Kantar*, Anissa Khelfa, Eugène Vorobiev and Mohamed Koubaa*

Université de technologie de Compiègne, ESCOM, TIMR (Integrated Transformations of Renewable Matter), Centre de recherche Royallieu, CS 60 319, 60203 Compiègne Cedex, France

Received 22 June 2021 – Accepted 21 October 2021

Abstract – Microbial-based biodiesel is produced by transesterification of lipids extracted from microbial cells, and is considered as a potential replacement of fossil fuel due to its advantages in reducing greenhouse gas emissions. *Yarrowia lipolytica* is one of the most studied oleaginous yeasts able to produce lipids under some fermentation conditions and is considered as a potential industrial host for biodiesel production. Several approaches have been evaluated to increase the economical attraction of biodiesel production from *Y. lipolytica* lipids. In this review, we highlighted the different strategies reported in the literature, allowing this yeast to achieve high lipid accumulation. These include metabolic engineering strategies, the use of low-cost effective substrates, and the optimization of the cultivation conditions for higher lipid productivity and less operating cost. We also summarized the most effective cell disruption technologies that improve the extraction efficiencies of lipids from *Y. lipolytica*.

Keywords: *Y. lipolytica* / microbial oils / lipid accumulation / lipid extraction / biodiesel

Résumé – Les stratégies adoptées pour améliorer l'accumulation et l'extraction des lipides à partir de *Y. lipolytica*. Le biodiesel d'origine microbienne est produit par transestérification de lipides extraits de cellules microbiennes et est considéré comme un substitut potentiel des combustibles fossiles en raison de ses avantages dans la réduction des émissions de gaz à effet de serre. *Yarrowia lipolytica* est l'une des levures oléagineuses les plus étudiées capables de produire des lipides dans certaines conditions de fermentation et est considérée comme un hôte industriel potentiel pour la production de biodiesel. Plusieurs approches ont été évaluées pour augmenter l'attrait économique de la production de biodiesel à partir des lipides de *Y. lipolytica*. Dans cette revue, nous avons mis en évidence les différentes stratégies rapportées dans la littérature, permettant à cette levure d'atteindre une accumulation élevée de lipides. Ceux-ci incluent des stratégies d'ingénierie métabolique, l'utilisation de substrats carbonés et azotés à faible coût et l'optimisation des conditions de culture pour une productivité lipidique plus élevée et des coûts d'exploitation plus faibles. Nous avons également résumé les technologies de lyse cellulaire les plus efficaces qui améliorent l'efficacité d'extraction des lipides de *Y. lipolytica*.

Mots clés : *Y. lipolytica* / huiles microbiennes / accumulation des lipides / extraction des lipides / biodiesel

Highlights

- The potential use of *Y. lipolytica* as a promising host for biodiesel production is highlighted.
- Biosynthesis and degradation pathways of lipids in *Y. lipolytica* are described.
- Strategies to improve the economical attraction for microbial oil production in terms of higher lipid productivity and downstream recovery are discussed.

1 Introduction

Fossil fuels are commonly used as a primary source of non-renewable energy. Their combustion releases large amounts of greenhouse gases, which negatively contributes to global warming. Concerns about climate change and the quick exhaustion of fossil fuel in the near future have driven research on the production of renewable biofuels as

☆ Contribution to the Topical Issue "Green and white biotechnologies in the fields of lipids and oil- and proteincrops / Biotechnologies vertes et blanches dans les domaines des lipides et oléoprotéagineux".

*Correspondences: m.koubaa@escom.fr; sally.el-kantar@utc.fr

alternatives (Mat Aron *et al.*, 2020). First, second and third generation biofuels are obtained depending on the raw material and the technology used for their production (Singh *et al.*, 2011). Ethanol produced by fermenting sugar extracted from edible feed-stocks or biodiesel obtained by transesterification of vegetable oils extracted from plants are examples of first generation biofuels (Tan *et al.*, 2010; Singh *et al.*, 2011; Xie, 2017). The main limitation of first generation biofuels is the use of edible feed-stocks such as sugarcane and corn starch that can present a serious conflict with food industries (Datta *et al.*, 2019). Besides competition with food crops, the cultivation of biofuel crops requires large amounts of land area, fertilizers and water, which result in a high production cost (Paschalidou *et al.*, 2016; Enamala *et al.*, 2018). Therefore, the use of non-edible biomass such as agricultural lignocellulosic biomass is favored for the production of second-generation biofuels. However, the production of lignocellulosic biofuels requires complicated pretreatment of the biomass to obtain fermentable sugars, consequently compromising its economic feasibility (Paschalidou *et al.*, 2016; Ali *et al.*, 2020). Consequently, third generation biofuels obtained from microorganisms such as bacteria, yeasts or microalgae have received increasing attention as sustainable and renewable alternatives to plant-based oils (Abghari and Chen, 2014). Microalgae have shown a promise potential to be used as a source for biofuels production. However, their usage is still limited due to the high cost of cultivation, low biomass concentration and the lack of genetic engineering and transformation tools to create dependable models of algal strains (Li *et al.*, 2008; Gimpel *et al.*, 2015; Xue *et al.*, 2021). Compared to algae, bacteria have a higher growth rate and their cultivation is easier but they accumulate lipids in their membrane which make them difficult to extract (Meng *et al.*, 2009). Therefore, among the oleaginous microorganisms, yeasts are the most effective in terms of lipid accumulation (Darvishi *et al.*, 2017).

Yarrowia lipolytica is a non-conventional yeast which has received particular interest for single cell oil production because of its easily manipulated genome and its extensively studied metabolism which permit the engineering of high lipid accumulation species (Ledesma-Amaro *et al.*, 2015). *Y. lipolytica* belongs to the Ascomycota phylum. It was previously named as *Candida*, *Endomycopsis* or *Saccharomycopsis lipolytica*. The name "*Yarrowia*" suggested in 1980, refers to the researcher David Yarrow who has identified this genus. The species name "*lipolytica*" is related to the ability of the yeast to hydrolyze lipids (Zieniuk and Fabiszewska, 2019). *Y. lipolytica* strains are isolated from lipid or protein-rich environments such as dairy products and sewage (Carsanba *et al.*, 2018). *Y. lipolytica* as Generally Recognized As Safe (GRAS) is a non-pathogenic yeast which has the ability to assimilate hydrophilic and hydrophobic substrates to produce value-added byproducts such as long chain dicarboxylic acids mainly used in the synthesis of polyesters and polyamides (Abghari *et al.*, 2017), organic acids (Papanikolaou *et al.*, 2002b) and lipids through *de novo* and *ex novo* pathways (Harzevili, 2014; Brabender *et al.*, 2018; Amalia *et al.*, 2020).

Cellular lipid production in *Y. lipolytica* depends on the genetic background of the strain, the type of substrates used in the culture media and the cultivation conditions. For example, some wild-type strains of *Y. lipolytica* can accumulate respectively up to 40% lipids using volatile fatty acids (Fontanille *et al.*, 2012) and 58.5% lipids using sugar cane bagasse hydrolysate (Tsigie *et al.*, 2011). Moreover, lipid accumulation can exceed 80% in obese *Y. lipolytica* strains through genetic modifications (Nicaud, 2012). Concerning growth conditions, nitrogen starvation is known to favor the accumulation of lipids in this yeast. However, most of the wild-type strains cultivated on non-fatty substrates such as glucose or glycerol are not capable to accumulate high lipid levels even under nitrogen limited conditions, since those are degraded to the benefit of organic acids production (Papanikolaou *et al.*, 2002a, 2009). Using a double nitrogen and magnesium limited-media containing glucose or glycerol as substrates, allowed an increase in lipid accumulation while organic acid production was low (Bellou *et al.*, 2016). Lipids produced by *Y. lipolytica* are accumulated in subcellular compartments called lipid bodies. These lipids consist mainly of triacylglycerols (TAG) and sterol esters and comprise of up to 80% of unsaturated fatty acids with a high proportion of linoleic acid which gave them an important added value in terms of biofuel production (Gálvez-López *et al.*, 2019). Interestingly, the lipid profile of *Y. lipolytica* oil can be modified by using low-cost fatty substrates to provide tailor-made lipids (Papanikolaou and Aggelis, 2010). For example, *Y. lipolytica* cultivated on stearin produced lipids with significant amounts of stearic acid and low amounts of unsaturated fatty acids which gave them a composition similar to that of cocoa butter (Papanikolaou *et al.*, 2001, 2003).

The cost of oil and biodiesel production from *Y. lipolytica* could be extrapolated from the estimated numbers presented in the study of Koutinas *et al.* (2014). They found that the cost of biodiesel production from 10 000 ton of microbial oil/year is relatively high. About M$ 34.1/year are needed for lipid production using glucose ($ 400/ton) as a substrate (Koutinas *et al.*, 2014). Therefore, one of the main challenges for industrial lipid production from *Y. lipolytica* is to achieve high lipid yields and productivity especially in the engineered strains that use low-cost substrates. Moreover, the improvement of the fermentation and downstream processes represent another challenge to decrease the cost of the overall process (Abghari and Chen, 2014). In this regard, the first part of this review summarizes the strategies applied to boost lipid production in the oleaginous yeast *Y. lipolytica*. These include metabolic engineering strategies, controlling fermentation conditions (*e.g.*, agitation, pH, etc.), and the use of low-cost effective substrates. The second part provides an overview of the cell disruption technologies that have been used to increase lipid recovery from *Y. lipolytica*.

2 Biosynthesis and degradation of lipids in *Y. lipolytica*

Y. lipolytica stores its synthesized fatty acids (FAs) in lipid bodies which mainly consist of about 85% TAG and 8% sterol esters (SE) (Xie, 2017). It can synthesize TAG *via* two pathways. The first one is the *de novo* pathway, which requires hydrophilic substrates (*e.g.*, sugars, organic acids, alcohols, etc.) to produce FAs from the precursor acetyl-CoA, and assemble them into TAG. The second one is the *ex novo* pathway, which requires the hydrolysis of hydrophobic substrates (*e.g.*, alkanes, TAG, etc.) into FAs and glycerol

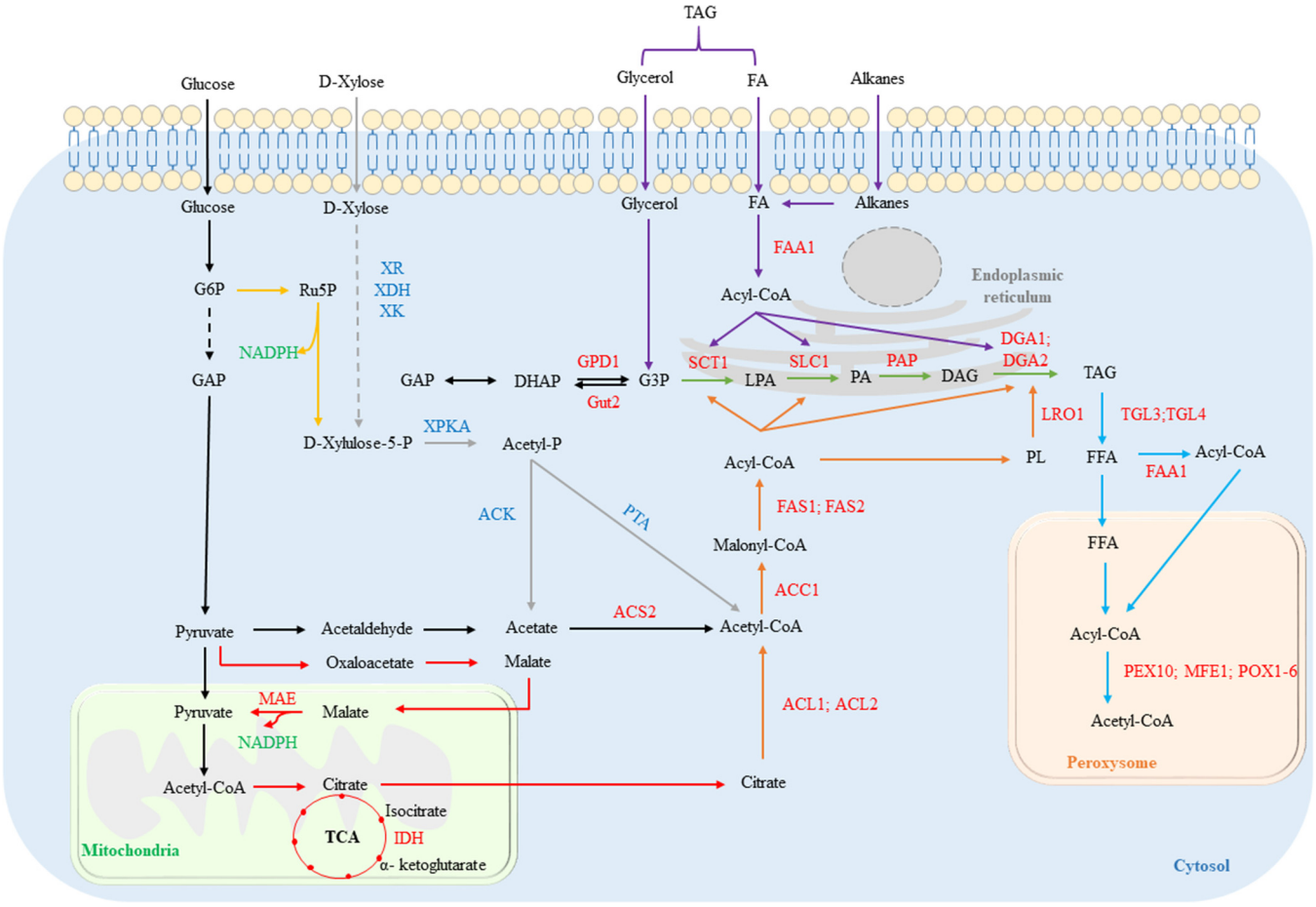

Fig. 1. Synthesis and degradation of TAG in *Y. lipolytica*. Colored arrows indicate different metabolic pathways: black: glycolysis; grey: xylose pathway; yellow: oxidative pentose phosphate pathway; red: tricarboxylic acid (TCA) cycle and related intermediate reactions; orange: de novo TAG synthesis pathway; purple: ex novo TAG synthesis pathway; green: *Kennedy* pathway; blue: β-oxidation pathway. Dashed lines represent multiple metabolic steps. Abbreviations: G6P: glucose-6-phosphate; GAP: glyceraldehyde-3-phosphate; Ru5P: ribulose-5-phosphate; G3P: glycerol-3-phosphate; DHAP: dihydroxyacetone phosphate; LPA: lysophosphatidic acid; PA: phosphatidic acid; DAG: diacylglycerol; TAG: triacylglyceride; FFA: free fatty acid.

and their transport inside the cell for TAG synthesis. Figure 1 summarizes the TAG synthesis pathways in *Y. lipolytica*.

De novo lipid synthesis is activated under nitrogen-limited conditions and requires carbon substrates such as sugars and alcohols (Mathiazhakan *et al.*, 2016). The decrease in nitrogen amount improves the activity of the enzyme adenosine monophosphate deaminase 1 (AMPD1) which changes adenosine monophosphate (AMP) into inosine monophosphate (IMP) and ammonia. The decrease in AMP concentrations inhibits the action of the enzyme isocitrate dehydrogenase (IDH) responsible for the catalysis of the oxidative decarboxylation reaction of isocitrate, giving α-ketoglutarate and carbon dioxide. As a result, the tricarboxylic acid (TCA) cycle is downregulated and the accumulated citrate is transported from the mitochondria into the cytosol. Citrate is then cleaved by ATP-citrate lyase (ACL1 and ACL2) to obtain cytosolic acetyl-CoA, the starting material for lipid synthesis (Lazar *et al.*, 2018; Wang *et al.*, 2020). Several reactions take place in thy cytosol to produce FAs from acetyl-CoA substrate. First, acetyl-CoA carboxylase (ACC1) catalyzes the conversion of acetyl-CoA into malonyl-CoA. Then the FA synthase complex (FAS1 and FAS2) allows the transformation of malonyl-CoA into acyl-CoA, and the elongation of acyl-CoA to 16 or 18 carbon atoms. In a next step, the obtained C16:0 and C18:0 molecules are transported to the endoplasmic reticulum for further elongation and desaturation (Ledesma-Amaro *et al.*, 2016).

The formation of TAG from three FAs and one glycerol-3-phosphate (G3P) follows the Kennedy pathway (Dulermo and Nicaud, 2011). G3P is converted to lysophosphatidic acid (LPA) under the action of G3P acyltransferase (SCT1). LPA is subsequently transformed to phosphatidic acid (PA) by LPA acyltransferase (SLC1). Diacylglycerol (DAG) is then generated through the dephosphorylation of PA by PA phosphatase (PAP). In a final step, TAG can be synthesized by 2 different reactions. The first one is catalyzed by DAG acyltransferase (DGA1 or DGA2) and uses acyl-CoA as the final acyl group donor. The second one is independent from acyl-CoA and uses glycerophospholipid as the acyl group donor under the action of phospholipid DAG acyltransferase (LRO1) (Athenstaedt *et al.*, 2006; Lazar *et al.*, 2018; Wang *et al.*, 2020).

Y. lipolytica can also synthesize lipids through the *ex novo* pathway using hydrophobic substrates such as TAG, alkanes and free fatty acids (FFAs) (Lopes *et al.*, 2018). Therefore, many studies have investigated the potential use of this yeast for the valorization of waste and by-products. Animal fats such as tallow (Papanikolaou *et al.*, 2007) and pork fat (Patrignani *et al.*, 2011), vegetable (Louhasakul *et al.*, 2020), cooking (Lopes *et al.*, 2019) and fish (Fabiszewska *et al.*, 2021) oils waste have been used as substrates by *Y. lipolytica* to produce single cell oil *via ex novo* pathway. However, some hydrophobic substrates may be toxic to the cells according to their chain length. For example, no growth was observed when alkanes and fatty acids shorter than C9 are used as carbon sources. Moreover, *Y. lipolytica* which could use a large variety of fatty acids with a chain length over C9 at concentrations above 3% is not able to grow on some type of C9 fatty acids such as nonanoic acid at a concentration higher than 0.02% (Moreno, 2018). Similarly, *Y. lipolytica* exhibited partiality to short chain FAs (C12:0, C14:0 and C16:0) which were totally consumed in a fermentation media containing glucose, glycerol and stearin (Papanikolaou *et al.*, 2003). The hydrolysis of TAG substrates into glycerol and FFAs is regulated by extracellular lipases (Donot *et al.*, 2014). The hydroxylation of alkanes entering the cells into fatty alcohols occurs in the endoplasmic reticulum *via* the cytochrome P450-dependent alkane monooxygenases. Fatty alcohols are then oxidized to fatty aldehydes and further to FAs respectively by fatty alcohol and fatty aldehyde dehydrogenases (Fukuda, 2013). The obtained FAs are converted to acyl-CoA, the substrate for TAG synthesis, through the acyl-CoA synthetase (FAA1) (Wang *et al.*, 2020).

When carbon is insufficient, TAG degradation occurs to maintain cellular metabolism. In a first step, the intracellular lipases TGL3 and TGL4 degrade TAG into FFAs (Dulermo *et al.*, 2013). Subsequently, the enzyme FAA1 generates acyl-CoA from the released FAs. Four reactions ensure the cleavage of the long acyl-CoA chains in the peroxisome through the β-oxidation pathway to obtain acetyl-CoA. The first reaction is catalyzed by six acyl-CoA oxidase (POX1-6) (Beopoulos *et al.*, 2008). The second and third reactions occur under the action of a multifunctional enzyme (MFE1) and the last reaction is catalyzed by 3-ketoacyl-CoA thiolase (POT1) (Blazeck *et al.*, 2014; Lazar *et al.*, 2018).

FAs profile of *Y. lipolytica*'s oil depends on the substrate used in the fermentation media. When tallow, rich in saturated fatty acids, was used as a carbon source, the oil produced by *Y. lipolytica* was characterized by a high content in saturated FAs (78% of C18:0 and 17% of C16:0) and only small amounts of unsaturated FA (5% of $^{\Delta 9}$C18:1) (Papanikolaou *et al.*, 2007). Likewise, when fish oil was used as carbon source, the content of lipids accumulated in the cells was comparable to the substrate and was characterized by the presence of very long chain FAs (Fabiszewska *et al.*, 2021). Similarly, when stearin and hydrolyzed oleic rapeseed oil were used as carbon sources, saturated FAs were the most prevalent and their percentage proportionally increased with that of stearin. This is due to the fact that unsaturated FAs (oleic acid) are utilized for growth needs, while saturated FAs are used for lipid storage (Papanikolaou *et al.*, 2001). However, the use of glucose as co-substrate with stearin favored the production of unsaturated FAs due to the novo FAs biosynthesis (Papanikolaou *et al.*, 2003). Some studies have also investigated the effect of cultivation conditions (pH, aeration, nitrogen depletion) on FA profile of cellular lipids (Fabiszewska *et al.*, 2021). For example, higher quantity of oleic acid was obtained when *Y. lipolytica* was cultivated in highly aerated bioreactor compared to flask trials (Papanikolaou *et al.*, 2007).

3 Enhancing lipid accumulation in *Y. lipolytica*

Various research studies have worked on the improvement of lipid accumulation in *Y. lipolytica*, by upregulating lipid synthesis or knocking out lipid degradation pathways and modifying the fermentation conditions (*e.g.*, carbon sources, pH, aeration, fermentation mode, etc.). Table 1 summarizes the different strategies and approaches applied to enhance lipid accumulation in *Y. lipolytica*.

3.1 Metabolic engineering strategies

Since wild-type strains of *Y. lipolytica* are not the most efficient for lipid production (Lazar *et al.*, 2018), researchers have used various metabolic engineering approaches to improve lipid productivity. The enzymes targeted in metabolic engineering strategies are shown in Figure 1.

3.1.1 Modulating endogenous gene expression

The potential of *Y. lipolytica* to accumulate lipids can be improved by adjusting the expression of native genes related to lipid biosynthesis and degradation. Blazeck *et al.* (2014) adopted a combinatorial strategy to simultaneously over-express and delete multiple key genes associated to lipid productivity in *Y. lipolytica*, which generated 57 different genotypes. The most advantageous engineered strain was obtained by eliminating both MFE1 and peroxisome biogenesis (PEX10) genes and overexpressing the DGA1 gene. Compared to the wild-type strain, the engineered strain represented 60-fold improvement in terms of lipid production in the optimized bioreactor conditions (Blazeck *et al.*, 2014). Silverman *et al.* (2016) studied the effect of overexpressing 44 native genes on lipid biosynthesis in *Y. lipolytica*. The highest increase in lipid yield (246%) was obtained in the strain that overexpressed the DGA2 gene when glucose was used as a substrate (Silverman *et al.*, 2016). Sagnak *et al.* (2018) evaluated the overexpression of DGA2 gene simultaneously with glycerol-3-phosphate dehydrogenase 1 (GPD1) gene. The resulting strain showed a 4-fold increase in lipid content compared to the control (Sagnak *et al.*, 2018). Similarly, Amalia *et al.* (2020) overexpressed the LRO1 gene which catalyzes the binding of an acyl group into DAG for the formation of TAG. As a result, the lipid content increased from 7.5% (wild-type stain) to 12% (Amalia *et al.*, 2020). Tai and Stephanopoulos (2013) used a push-and-pull strategy by overexpressing simultaneously ACC1 (under the hp4d promoter) and DGA1 (under the TEFin promoter) involved respectively in the first step of FAs synthesis and last step of TAG synthesis pathway. The lipid content increased from 8.7% in the control to 41% in the engineered strain that produced 0.143 g of lipids/L of culture/hour in a 2 L bioreactor (Tai and

Table 1. Summary of strategies for increasing lipid accumulation in *Y. lipolytica*. The symbol Δ represents a gene knockout and gene expression is represented by all uppercase letters.

Carbon source	Scale	*Y. lipolytica* strain	Modification	Lipid content % (grams of lipids per gram dry weight)	Lipid titer g/L (grams of lipids per liter of culture)	Lipid yield g/g (grams of lipid per gram of carbon substrate consumed)	Productivity g/L/h (grams per lipid per liter of culture per hour)	References
Glucose	Bioreactor	Po1f	Δmfe1, Δpex10, DGA1	71.0	25.30	–	0.210	(Blazeck *et al.*, 2014)
Glucose	Flask	Po1g	DGA2	–	2.4	0.55	–	(Silverman *et al.*, 2016)
Glucose	Bioreactor	JMY3501	GPD1, DGA2	55.0	–	0.140	–	(Sagnak *et al.*, 2018)
Glucose	Bioreactor	Po1g	hp4d-ACC1, TEFin-DGA1	61.7	–	0.195	0.143	(Tai and Stephanopoulos, 2013)
Glucose	Bioreactor	Po1g	TEFin-ACC1, TEFin-DGA1, TEFin-SCD	–	55.00	0.234	0.707	(Qiao *et al.*, 2015)
Glycerol	Flask	Po1d	Δgsy1	52.0	–	–	–	(Bhutada *et al.*, 2017)
Glucose	Flask	Po1g	Hp4d-LRO1	12.0	1.30	–	–	(Amalia *et al.*, 2020)
Glucose	Flask	ATCC 20362	Δsnf1	30.0	–	–	–	(Seip *et al.*, 2013)
Glycerol	Bioreactor	H222	Δsnf1, DGA1, DGA2	53.0	4.80	–	0.070	(Abghari and Chen, 2017)
Glucose	Flask	ACA-DC 50109	Δmig1	48.7	–	–	–	(Wang *et al.*, 2013)
Glucose	Bioreactor	Po1f	EMS mutagenesis	87.0	38.9	0.243	0.509	(Liu *et al.*, 2015a, b)
Glucose	Bioreactor	Po1f	Δmga2	–	25.00	0.213	0.145	(Liu *et al.*, 2015b)
Glucose	Flask	ACA-DC 50109	Δmhy1	43.1	–	–	–	(Wang *et al.*, 2018)
Glucose	Bioreactor	Po1f	ACC1, DGA1, SCD, Δpex10, Δmfe1, Δ-12D, Δ-15D (flax)	77.8	50.00	–	–	(Yan *et al.*, 2020)
Glucose	Bioreactor	NS18	DGA1 (from *Rhodosporidium toruloides*), DGA2 (from *Claviceps purpurea*), Δtgl3	77.0	85.00	0.210	0.730	(Friedlander *et al.*, 2016)
Glycerol	Flask	Po1h	ACL (from *mus musculus*)	23.1	–	–	–	(Zhang *et al.*, 2014)
Glucose	Bioreactor	Po1h	Haemoglobin (from *Vitreoscilla*)	14.5	–	–	–	(Zhang *et al.*, 2019a)
Glucose	Bioreactor	Po1g	perCAT2 (from *S. cerevisiae*), ACC1, DGA1	–	66.40	0.229	0.565	(Xua *et al.*, 2016)
Xylose	Bioreactor	Po1d	DGA2, GPD1, XDH, XR, XK, XPKA (from *Aspergillus nidulans*), ACK (from *Aspergillus nidulans*), Δpox1-6, Δtgl4	67.0	16.50	–	1.850	(Niehus *et al.*, 2018)
Glucose/xylose	Bioreactor	Po1f	XK, DGA1, Δpex10	56.7	13.50	–	–	(Yook *et al.*, 2020)
Glucose	Bioreactor	Po1g	ACC1, DGA1, MCE2 (from *Mucor circinelloide*), GAPC (from *Clostridium acetobutylicum*)	66.70	99.3	0.279	1.200	(Qiao *et al.*, 2017)
Glucose	Bioreactor	Po1g	GSR ylGPO ScZwf EcALDH	82.5	72.70	0.252	0.970	(Xu *et al.*, 2017)
Crude glycerol	Flask	TISTR 5151	–	64.0	–	–	–	(Cheirsilp and Louhasakul, 2013)
Crude glycerol	Flask	A101	–	25.0	1.69	–	–	(Dobrowolski *et al.*, 2016)
Crude glycerol	Flask	QU21	–	18.9	1.27	0.060	–	(Poli *et al.*, 2014)

Table 1. (continued).

Carbon source	Scale	*Y. lipolytica* strain	Modification	Lipid content % (grams of lipids per gram dry weight)	Lipid titer g/L (grams of lipids per liter of culture)	Lipid yield g/g (grams of lipid per gram of carbon substrate consumed)	Productivity g/L/h (grams per lipid per liter of culture per hour)	References
Crude glycerol	Bioreactor	SKY7	–		7.21			(Kumar *et al.*, 2020)
Purified glycerol			Purification of crude glycerol using phosphoric acid		19.47			
Crude glycerol	Flask	SM7	Addition of olive oil	35.80	6.13	–	–	(Magdouli *et al.*, 2017)
Palm oil mill effluent	Flask	TISTR 5151	Addition of surfactant	–	2.54	–	–	(Louhasakul *et al.*, 2020)
Acetic acid	Flask	CICC 31596	–	31.12	0.724	0.290	–	(Gao *et al.*, 2017)
Acetic acid	Flask	CICC 31596	Maintain an alkaline pH	27.22	10.11	0.144	–	(Gao *et al.*, 2020)
Glucose	Culture tubes	Po1f	Using urea as a nitrogen source	–	0.65	–	–	(Brabender *et al.*, 2018)
Glucose	Bioreactor	ACA-DC50109	Double nitrogen and magnesium limitation	47.5	5.80	–	–	(Bellou *et al.*, 2016)
Crude glycerol	Bioreactor	SKY7	pH controlled at 6.5	45.4	7.78	–	–	(Kuttiraja *et al.*, 2018)
Glycerol	Flask	W 29	pH 5.5	63.0	–	0.023	–	(Sekova *et al.*, 2019)
Glucose	Flask	ATCC 20460	Increase in OTR factor	≈50	–	–	–	(Lopes *et al.*, 2018)
Glucose	Bioreactor	H222	Drop of oxygen concentration from 50% to 1% after 20 h cultivation	–	–	0.110	–	(Kavščcek *et al.*, 2015)
Glycerol	Bioreactor	SM7	Two-stage cultivation with a dissolved oxygen shift from 60% to 30%	52.7	13.60	–	–	(Sara *et al.*, 2016)
Glucose and acetic acid	Bioreactor	MUCL 28849	Two-stage fed-batch fermentation	40.7	12.36	0.130	0.160	(Fontanille *et al.*, 2012)
Pretreated cane molasses	Bioreactor	S47	Two-stage fed-batch fermentation	57.3	12.20	–	–	(Wang *et al.*, 2019)
Glycerol	Bioreactor	LGAM S(7)1	Continuous fermentation	43.0	–	–	1.200	(Papanikolaou and Aggelis, 2002)
Glycerol	Chemostat	JMY4086	Continuous fermentation	–	24.20	0.100	0.430	(Rakicka *et al.*, 2015)

Stephanopoulos, 2013). Qiao *et al.* (2015) upregulated the simultaneous expression of ACC1, DGA1 and stearoyl-CoA desaturase (SCD) genes in *Y. lipolytica*. Total lipid yield increased by 2.93-fold in the engineered strain that showed a maximal lipid productivity close to 1 g/L/h after 78 h of fermentation in a 1.5 L bioreactor. The improvement in lipid yield in the engineered strain is due to the upregulated activity of the SCD enzyme that catalyzes the Δ9-desaturation of saturated FAs to mono-unsaturated FAs. Therefore, the possible allosteric inhibition of ACC1 by saturated FAs could be released, which improves the FAs biosynthetic flux (Qiao *et al.*, 2015). Lipid transporters have also been regulated to increase lipid accumulation in *Y. lipolytica*. Dulermo *et al.* (2014) found that Ylfat1 protein is responsible of the export of FAs from lipid bodies. Therefore, ΔYlfat1 strain accumulated 37% of lipids compared to 25% for the parent strain (Dulermo *et al.*, 2014). The knockout of pxa1 transporter of fatty acyl-CoA into peroxisome in addition to other fatty acyl-CoA oxidase genes has also resulted in 11-fold improvement of FAs titer compared to the wild-type strain (Ghogare *et al.*, 2020). Finally, redirecting the metabolic flux toward lipid synthesis has effectively enhanced lipid accumulation in *Y. lipolytica*. Bhutada *et al.* (2017) deleted the glycogen synthase gene (GSY1) involved in glycogen synthesis in *Y. lipolytica* strain engineered for high lipid accumulation. They observed a 60% increase in TAG accumulation with up to 2-fold improvement of desaturated C18 FAs compared to the control strain. They concluded that glycogen synthesis pathway competes with TAG accumulation and the elimination of GSY1 gene redirected the cellular carbon flux from glycogen to TAG synthesis (Bhutada *et al.*, 2017). In another study, Liu *et al.* (2015a) created spontaneous mutations in the *Y. lipolytica* strain Po1f Δ*pex10* Δ*mfe1* and studied the strain E26 that presented the highest lipid content. The results of genome sequencing of E26 revealed a mutation in the open reading frame UGA2 which encodes for a succinate semialdehyde dehydrogenase involved in the γ-aminobutyric acid (GABA) assimilation pathway. They concluded that a decrease in GABA assimilation pathway could reduce the flux through the TCA cycle, thus redirecting more carbon towards the lipogenic pathway (Liu *et al.*, 2015a).

3.1.2 Modulating metabolic regulators expression

The regulation of cellular metabolic regulators has been an effective strategy to facilitate lipid accumulation in *Y. lipolytica*. Studies have shown that SNF1 is a negative metabolic regulator of FAs accumulation. Seip *et al.* (2013) concluded that the deletion of SNF1 in *Y. lipolytica* induced an upregulation of the genes ACL1 and acetyl-CoA synthetase (ACS2) leading to an improved pool of acetyl-CoA and resulting in a 2.6-fold higher lipid content in mutants compared to wild-type strains (Seip *et al.*, 2013). Other researchers combined the deletion of SNF1 regulator with the overexpression of DGA1 and DGA2 genes in *Y. lipolytica*, which resulted in a 2.47-fold increase in lipid content (Abghari and Chen, 2017). Similarly, the deletion of the glucose repression regulator MIG1 led to the upregulation of many genes associated to lipid synthesis and the downregulation of the MFE1 gene. As a result, the lipid content in *Y. lipolytica* improved from 36% to 48.7% and the biosynthesis of the FAs C18:1 increased from 45.1% to 60.2% (Wang *et al.*, 2013). Liu *et al.* (2015b) studied the role of mga2 as a regulator of desaturase gene expression in *Y. lipolytica*. They showed that mutating or deleting the mga2 gene allowed the overexpression of Δ-9-desaturase (OLE1) and resulted in the increase of the metabolism of unsaturated FAs (notably C16:1 and C18:1) (Liu *et al.*, 2015b). Wang *et al.* (2018) evaluated the regulatory function of MHY1 gene in lipid synthesis. The deletion of this gene regulated the expression of 1567 genes involved in lipid biosynthesis in *Y. lipolytica* and increased lipid content from 30.2% (wild-type strain) to 43.1% (Δmhy1 strain) (Wang *et al.*, 2018).

3.1.3 Heterologous gene expression

Several studies evaluated the expression of heterogeneous genes to improve lipid accumulation in *Y. lipolytica*. Yan *et al.* (2020) combined the overexpression of native genes (SCD, Δ-12 desaturase (Δ-12D), ACC1, DGA1) and heterologous Δ-15 desaturase (Δ-15D) gene from flax with the deletion of the endogenous PEX10 and MFE1. The resulting strain showed a maximal lipid content of 77.8% with a lipid yield of 50 g/L (Yan *et al.*, 2020). Friedlander *et al.* (2016) applied similar modifications by overexpressing DGA1 and DGA2, respectively from *Rhodosporidium toruloides* and *Claviceps purpurea*, and deleting the endogenous TGL3 involved in lipid degradation, and demonstrated that these modifications resulted in a lipid yield of 85 g/L (Friedlander *et al.*, 2016). The overexpression of ACL gene involved in the production of acety-CoA from *Mus musculus* increased the lipid content in *Y. lipolytica* from 7.3% to 23% without a significant change in the FAs profile (Zhang *et al.*, 2014). Moreover, the overexpression of *Vitreoscilla* haemoglobin in *Y. lipolytica* for improving O_2 consumption during fermentation, increased the total FAs content by 40% compared to the wild-type strain when the dissolved oxygen concentration was measured at 30% (Zhang *et al.*, 2019a).

3.1.4 Engineering alternative pathways

3.1.4.1 Alternative acetyl-CoA pathways

In *Y. lipolytica*, the ACL provides acetyl-CoA from citrate when the TCA cycle is repressed under nitrogen depletion conditions. To decouple acetyl-CoA production from nitrogen depletion and increase the amount of acetyl-CoA, Xua *et al.* (2016) overexpressed the peroxisomal carnitine acetyltransferase (perCAT2) from *S. cerevisiae* and the endogenous genes (ACC1, DGA1). They observed an increase in lipid content and titer compared to the strain that only overexpressed ACC1 and DGA1 genes (Xua *et al.*, 2016). In another study, Niehus *et al.* (2018) engineered a new strain of *Y. lipolytica* that can produce lipids using xylose as a carbon substrate. They overexpressed native xylitol dehydrogenase (XDH), xylose reductase (XR), and xylulose kinase (XK) in the Po1d strain, to allow growth on xylose. They also designed two efficient pathways for the production of acetyl-CoA from xylulose-5-P. In the first pathway, they overexpressed phosphoketolase (XPKA) from *Aspergillus nidulans* that converts D-xylulose-5-P to acetyl-P and phosphotransacetylase (PTA) from *Bacillus subtilis* that changes acetyl-P to acetyl-CoA. In the second pathway, they overexpressed the XPKA and acetate kinase (ACK) enzymes *from Aspergillus nidulans* to obtain acetate

Table 2. Main research works for increasing lipid recovery from *Y. lipolytica.*

Y. lipolytica strain	Technology	Extraction process	Lipid yield	Advantages	Disadvantages	References
ATCC 20460	Conventional solvent extraction	3 g of wet yeast biomass with 10 mL chloroform:methanol (1:2, v/v); 30 min maceration, constant stirring at room temperature	6.23 ± 0.51 g/100 g of dry weight (dw)	Easy to conduct Reproducible Easy to scale up	Limited diffusion due to strong cell walls Lower extraction yields compared to the other treatments	(Meullemiestre *et al.*, 2016)
	Ultrasound assisted-extraction	10 g of wet biomass with 50 mL chloroform:methanol (1:2, v/v) placed in a double jacket reactor; 30 min sonication at 300 W, 20 °C	8.10 ± 0.24 g/100 g of dw	Higher extraction yields compared to conventional solvent extraction	Degradation of DAG into FFAs Change in FAs profile: higher proportion of palmitic acid C16 compared to the other methods	
	Bead milling	3 g of wet biomass with 10 mL chloroform:methanol (1:2, v/v) placed in 20 mL tube with 20 g ceramic beads; 30 min treatment	13.16 ± 0.68 g/100 g of dw	Efficient for cell disruption Higher extraction yields compared to conventional solvent extraction	–	
	Microwave-assisted extraction	10 g of wet biomass with 50 mL chloroform:methanol (1:2, v/v) placed in a Teflon microwave reactor; 30 min treatment at 100 W, 110 °C	7.13 ± 0.45 g/100 g of dw	Higher extraction yields compared to solvent extraction	Degradation of DAG into FFAs	
	Cold drying under reduced pressure pretreatment	Biomass placed in a reactor; 48 h, −80 °C, −20 mbar. Then, maceration in chloroform: methanol (1:2, v/v)	13.56 ± 0.24 g/100 g of dw	–	Increase in processing time and energy High emission of carbon dioxide	
	Freezing/defrosting pretreatment	Biomass frozen for 48 h at −20 °C then placed for 12 h at 4 °C. The process is repeated 3 times followed by oil extraction in chloroform: methanol (1:2, v/v)	5.53 ± 0.43 g/100 g of dw	Decrease in the use of solvents	High energy consumption High emission of carbon dioxide	
	Bead milling pretreatment	3 g of wet biomass with 10 mL chloroform:methanol (1:2, v/v) placed in 20 mL tube with 20 g ceramic beads; 30 min treatment, 4000 rpm. Then, oil extraction in chloroform: methanol (1:2, v/v)	12.73 ± 0.41 g/100 g of dw	Decrease in the use of solvents Low energy consumption	–	
	Microwave pretreatment	10 g of biomass placed in a Teflon microwave reactor and heated; 15 min, 45 °C, 20 W.	8.18 ± 0.67 g/100 g of dw	Decrease in the use of solvents	–	

Table 2. (continued).

Y. lipolytica strain	Technology	Extraction process	Lipid yield	Advantages	Disadvantages	References
		Then, oil extraction in chloroform:methanol (1:2, v/v)				
	High pressure homogenization (HPH) pretreatment	1 kg of 15% DM cell suspension preteated with HPH (5 passes, 1500 bar). Then, oil extraction from dry biomass (after lyophilization) in *n*-hexane, at room temperature, 1 h, liquid/solid ratio of 10:1 (v:w), agitation 700 rpm	100%	High extraction yields	High energy consumption during the drying process	(Drévillon *et al.*, 2018)
JMY 5289						
	1 kg of 15% DM cell suspension preteated with HPH (5 passes, 1500 bar). Then, oil extraction from wet biomass in *n*-hexane (ratio 1:2 (w:w)) using high-speed disperser (40 min, 1000 rpm)	79.9 ± 11.5%	Low extraction yields	Lower energy consumption compared to the dry route		
	Mechanical expression pretreatment	85 g yeast suspension placed in the pressing chamber; 45 min at 5.10^5 Pa	≈ 25 ± 0.5%	–	Lowest extraction yields compared to the tested pretreatment techniques	(Drévillon *et al.*, 2019)
	High pulsed electric field pretreatment	200 g of yeast cell suspension (15% DM) placed in treatment chamber (500 pulses, 20 Kv/cm); lyophilization; oil extraction in *n*-hexane (ratio 1:10, w/v), 1 h, at room temperature with agitation at 700 rpm	29.4 ± 3%	Additional release of oil compared to the untreated cells	–	
JMY5578						
	High voltage electrical discharges pretreatment	200 g of yeast cell suspension (15% DM) placed in treatment chamber (500 pulses, 2 = 40 Kv/cm); lyophilization; oil extraction in *n*-hexane (ratio 1:10, w/v), 1 h, room temperature with agitation at 700 rpm	31.7 ± 6.5%	Additional release of oil compared to the untreated cells	Degradation of the oil Significant changes in FAs composition compared to the other treatments except for C14:0, C18:0 and C18:2	
	Ultrasound pretreatment	300 g yeast cells (15% DM) placed in US reactor (1 h, 400 W, 293 k); lyophilization; oil extraction in *n*-hexane (ratio 1:10, w/v), 1 h, room	35.5 ± 6.1%	Increase in extraction yields compared to the untreated cells (control) No significant change in FAs profile	–	

Table 2. (continued).

Y. lipolytica strain	Technology	Extraction process	Lipid yield	Advantages	Disadvantages	References
		temperature with agitation at 700 rpm				
	HPH pretreatment	Cell suspension (600 g, 15% DM) pretreated with HPH (298 K, 20 passes, 1500×10^5 Pa)	83.9 ± 4.8%	Highest extraction yields compared to the tested pretreatment techniques	Change in FAs profile	
JMY5289	HPH and bead milling pretreatment	Cell suspension (600 g, 15% DM) pretreated with HPH (25 °C, 5 passes, 1500 bar) followed by bead milling in chloroform:methanol (2:1, v:v) (stainless steel beads of 4.9 mm, and during 3 × 30 s)	99.6%	Available for large scale processing Short processing time No change in FAs profile	–	(Imatoukene *et al.*, 2020)
QU21	Liquid nitrogen with sonication	Liquid nitrogen added to biomass, then sonication 10 times for 30 s each in distilled water followed by maceration in 20 mL chloroform and methanol (2:1, v:v) for oil extraction	26.5%	Increase in the extraction yields compared to conventional maceration (14.3%)	–	(Poli *et al.*, 2013)
Po1g	Sub-critical water treatment	1 g biomass dissolved in 20 mL water and treated at 175 °C for 20 min	42.69%	Environmentally friendly technique Very similar FAs profile from untreated and sub-critical water treated samples	–	(Tsigie *et al.*, 2012)

which is then converted to acetyl-CoA by the acetyl-CoA synthetase (ACS2) (Niehus *et al.*, 2018). Similarly, Yook *et al.* (2020) introduced a xylose isomerase pathway in *Y. lipolytica*. They combined the overexpression of xylose isomerase gene XylA from *S. cerevisiae* and the endogenous XK and DGA1 genes with the deletion of PEX10 gene to prevent lipid degradation and improve lipid production from xylose substrate (Yook *et al.*, 2020).

3.1.4.2 Cytosolic NADPH pathways

TAG synthesis involves large amounts of NADPH in both elongation step and desaturation reaction (Ratledge, 2014). Previous studies showed that malic enzyme (MAE) has a key role in providing NADPH in oleaginous yeasts and its overexpression resulted in a 2.5-fold increase in lipid content in both *Mucor circinelloides* and *Mortierella alpine* (Zhang *et al.*, 2007). To understand the source of NADPH in *Y. lipolytica* during lipid accumulation, Wasylenko *et al.* (2015) used ^{13}C-Metabolic Flux Analysis of a *Y. lipolytica* engineered strain that overexpresses ACC1 and DGA1 for a high lipid production from glucose substrate. The comparison of the flux between the wild-type and the engineered strains showed that the oxidative pentose phosphate pathway (oxPPP) is the main source of lipogenic NADPH in *Y. lipolytica* (Wasylenko *et al.*, 2015). Qiao *et al.* (2017) adopted several strategies to increase NADPH supply in *Y. lipolytica*. They activated the pyruvate/oxaloacetate/malate cycle which can convert one molecule of NADH to NADPH (using 1 ATP) through the expression of the malic enzyme MCE2 from *Mucor circinelloides*. The highest lipid yield of 0.231 g/g was obtained by overexpressing simultaneously the $NADP^+$ glyceraldhyde-3-phosphate dehydrogenase GapC from *Clostridium acetobutylicum* and the heterogeneous MCE2 (Qiao *et al.*, 2017).

3.1.4.3 Oxidative stress defense pathway

Lipogenesis is generally initiated by nitrogen starvation which leads to a variety of cellular stress responses (Morin *et al.*, 2011). The primary stress response is associated with the increased concentrations of reactive oxygen species (ROS) which results in lipids oxidation and peroxidation and the generation of reactive aldehydes (Li *et al.*, 2011; Xu *et al.*, 2017). Consequently, the elevated oxidative stress may result in the inactivation of the enzymes involved in lipid biosynthesis and the consumption of the stored lipids as an energy source to maintain cellular homeostasis (Grimsrud *et al.*, 2008). Therefore, Xu *et al.* (2017) engineered an oxidative defense pathway to improve lipid production in *Y. lipolytica*. They overexpressed glutathione disulfide reductase (GSR), glutathione peroxidase (ylGPO), glucose-6-phosphate dehydrogenase (ScZwf) and aldehyde dehydrogenase (EcALDH) to resist reactive oxygen and aldehyde stress in *Y. lipolytica*. The engineered strain accumulated 2 times more lipids (82.5%) compared to the wild-type strain (40.6%) (Xu *et al.*, 2017).

3.2 Modifying the medium composition

Multiple nutrients are essential for microbial growth and lipid production. Monosaccharides such as glucose, fructose, galactose, sucrose, pure glycerol and organic acids are usually used as carbon sources for yeast lipid production (Hapeta *et al.*, 2017; Qin *et al.*, 2017). Several studies investigated the efficiency of low-cost substrates such as industrial by-products or lignocellulosic biomass for effective lipid production in *Y. lipolytica*. Crude glycerol has many advantages including low cost, high availability and less CO_2 emission during fermentation (Qin *et al.*, 2017). Previous studies have shown the ability of *Y. lipolytica* to convert crude glycerol coming from industrial by-products into single cell oils (Cheirsilp and Louhasakul, 2013; Dobrowolski *et al.*, 2016). Poli *et al.* (2014) observed no significant difference in lipid content when they replaced pure glycerol (1.48 g/L) with crude glycerol (1.27 g/L) in a medium containing ammonium sulfate as a nitrogen source (Poli *et al.*, 2014). However, the presence of impurities in crude glycerol could negatively affect lipid production. Kumar *et al.* (2020) purified crude glycerol to use it as a carbon source for lipid production in *Y. lipolytica*. The cultures with purified glycerol presented higher lipid yields (19.47 g/L) compared to the cultures with crude glycerol (7.21 g/L). This difference in lipid content could be explained by the presence of high potassium concentration in crude glycerol that may inhibit the ACL enzyme responsible for the conversion of citrate into acetyl-CoA. The accumulated citrate is therefore secreted out of the cell rather than converted into lipids (Kumar *et al.*, 2020). The addition of fat such as olive oil to crude glycerol improved lipid accumulation in *Y. lipolytica* due to lipase activation (Magdouli *et al.*, 2017; Ayadi *et al.*, 2018). Krzyczkowska and Kozłowska (2017) found that the addition of almond oil as a carbon source stimulated the activity of lipase enzyme, and resulted in the highest lipolytic activity after 48 h of culture (Krzyczkowska and Kozłowska, 2017). Louhasakul *et al.* (2020) highlighted the effect of adding biosurfactants to hydrophobic substrates on lipid productivity. *Y. lipolytica* cultivated in palm oil mill effluent with 2% crude glycerol produced 1.25-fold more lipids in the presence of biosurfactants (Louhasakul *et al.*, 2020). Therefore, the addition of surfactants to the fermentation media containing hydrophobic substrates, may increase the permeability of the cell membranes, leading to more nutrients uptake (Taoka *et al.*, 2011) and lipase secretion (Deive *et al.*, 2009). Gao *et al.* (2017) evaluated the growth and lipid accumulation of *Y. lipolytica* cultured on volatile fatty acids (VFAs) such as butyric, propionic and acetic acids. Among the tested VFAs, the yeast showed a fastest consumption rate of acetate that could be directly cleaved into acetyl-CoA, unlike propionate and butyrate. The highest lipid yield coefficient (0.290 g lipid/g dry cell weight) was obtained with an initial concentration of 2.5 g/L of acetic acid at pH 6 (Gao *et al.*, 2017). In a further study, Gao *et al.* (2020) improved lipid accumulation in *Y. lipolytica* cultivated on VFAs. They obtained the highest lipid concentration of 10.11 g/L from 70 g/L of acetic acid under alkaline conditions (optimal pH 8) (Gao *et al.*, 2020).

In addition to carbon, nitrogen is an essential component of fermentation media. Ammonium sulfate is generally used as a nitrogen source in the media for yeast fermentation. Brabender *et al.* (2018) conducted a study to evaluate the effect of synthetic urine (18 g/L urea) as an alternative low-cost nitrogen source for the accumulation of lipids in *Y. lipolytica* Δpex10 strain. The cultures containing synthetic urine and glucose as a carbon source (C:N = 240:1) accumulated

approximately 2 times more lipids compared to cultures containing ammonium sulfate as a nitrogen source (Brabender *et al.*, 2018). This study showed the possibility of using natural waste product such as human urine as a nitrogen source to improve lipid production in yeast.

Bellou *et al.* (2016) studied the effect of nutrients limitation on lipogenesis. When glycerol was used as a carbon source, lipid content in *Y. lipolytica* increased from 12.8% (w/w) in the media with single nitrogen limitation to 23.5% w/w in the media with double nitrogen and magnesium limitation. A further improvement in lipid accumulation (47.5%, w/w) was observed when glycerol was replaced with glucose with double nitrogen and magnesium limitation (Bellou *et al.*, 2016).

3.3 Regulating the physicochemical parameters

Changes in the cultivation parameters (*e.g.*, pH, aeration, temperature, etc.) are needed to determine the optimal conditions for lipid accumulation. Kuttiraja *et al.* (2018) showed the effect of pH controlling on lipid production in *Y. lipolytica*. They observed a 15% increase in lipid production when the pH was maintained at 6.5 in glycerol-based fermentation media compared to pH-uncontrolled fermentation (Kuttiraja *et al.*, 2018). In a separate study, Sekova *et al.* (2019) showed that a pH increase from 5.5 to 9, caused a decrease in TAG level in *Y. lipolytica* respectively from 63% to 37% (Sekova *et al.*, 2019). Studies have shown that the expression of genes involved in lipid synthesis was not pH dependent. However, some mitochondrial transporters are strongly expressed at pH 6 which results in an increase in citric acid transport into the cytoplasm and its subsequent conversion to FAs (Zhang *et al.*, 2019b). Lopes *et al.* (2018) highlighted the influence of oxygen transfer rate (OTR) factor on lipid production in *Y. lipolytica*, cultivated on glucose media. They observed a 1.6-fold improvement in lipid content when the OTR increased from 96 mg/L/h to 192 mg/L/h (Lopes *et al.*, 2018). Previous studies showed that the increase in dissolved oxygen concentration in the fermentation media upregulates the activities of ACL and malic enzymes involved in lipid synthesis (Bellou *et al.*, 2014). By contrast, other authors observed a decrease in lipid accumulation in *Y. lipolytica* cultivated in glycerol media, when the dissolved oxygen concentration increased from 30% to 40% (Magdouli *et al.*, 2018). Similarly, Papanikolaou *et al.* (2002a) observed a remarkable growth of *Y. lipolytica* at an oxygen saturation of 60% but non-significant changes in lipid synthesis when the oxygen saturation varied between 5% and 15% (Papanikolaou *et al.*, 2002a). The decrease in lipid accumulation in aerated cultures could be attributed to the increase in oil biodegradation (Martins *et al.*, 2012; Magdouli *et al.*, 2018). Therefore, it is important to find a suitable balance between oxygen needed for growth and lipid accumulation by the application of a two-stage cultivation strategy (Sara *et al.*, 2016). Kavšcek *et al.* (2015) decreased the dissolved oxygen concentration from 50% to 1% after 20 h of fermentation, which resulted in a 25-fold increase in lipid content in *Y. lipolytica* within 36 h (Kavšcek *et al.*, 2015).

3.4 Changing of the cultivation modes

Different operational modes can be applied for the cultivation of *Y. lipolytica* for lipid production. The batch mode is the most studied method for lipid production in oleaginous yeasts and it can be performed either in flasks or in bioreactors (Carsanba *et al.*, 2020). It consists of a closed system where the cells and substrates are provided at the beginning and the fermentation occurs without any external intervention (Karamerou and Webb, 2019). However, in batch fermentation, a high C/N ratio is required which could lead to stressful conditions for yeast and result in the decrease of cell growth and lipid productivity (Christophe *et al.*, 2012) . Therefore, fed-batch cultivation process has been applied to obtain high cell density and cellular lipid content in oleaginous yeasts. In the fed-batch cultivation, initial substrate inhibition can be avoided since nutrients are added intermittently or continuously to the culture vessel during fermentation (Poontawee and Limtong, 2020). The fed-batch fermentation using glucose and olive oil as substrates led to a 3-fold improvement in lipase production by *Y. lipolytica* compared to batch fermentation (Fickers *et al.*, 2009). A two-stage fed-batch cultivation has also been applied for lipid production by *Y. lipolytica*. In a first stage, the cultivation of cells in a nutrient-rich medium resulted in a high cell concentration. Then, in a second stage, the addition of carbon source without auxiliary nutrients increased lipid productivity. A two-stage fed-batch cultivation of *Y. lipolytica* using glycerol and VFAs as carbon sources, resulted in a significant increase in lipid yields compared to batch fermentation with one carbon source (Fontanille *et al.*, 2012). Moreover, Wang *et al.* (2019) applied a two-stage fed-batch fermentation using pretreated cane molasses as a carbon source for lipid production in *Y. lipolytica*. The fermentation process resulted in a lipid level of 12.2 g/L which is higher than that obtained at batch flask level (7.2 g/L) (Wang *et al.*, 2019). Continuous fermentation has also been effective to increase lipid productivity in *Y. lipolytica*. In continuous operation, cultures are continuously supplied with substrates and the fermentation broth is withdrawn at the same rate to keep the volume constant in the fermentation system. Therefore, the C/N ratio could be maintained constant in the steady state and lipid accumulation would only depend on the dilution rate (Rakicka *et al.*, 2015). The effect of dilution rates on lipid synthesis by *Y. lipolytica* in continuous fermentation process was evaluated and the highest lipid content was obtained at a low dilution rate of 0.03 h^{-1} (Papanikolaou *et al.*, 2002a; Papanikolaou and Aggelis, 2002). Rakicka *et al.* (2015) found that the continuous fermentation process was more effective than the batch-fermentation for lipid synthesis in *Y. lipolytica*. The yeast produced 24.2 g/L of lipids in continuous cultures when the dilution rate was maintained at 0.01 h^{-1} compared to 22.6 g/L in the fed-batch cultivation process (Rakicka *et al.*, 2015).

3.5 *In silico* modeling

New strains of *Y. lipolytica* with high lipid accumulation have been engineered using *in silico* modeling. Wei *et al.* (2017) have developed a genome scale metabolic model (GEM) of *Y. lipolytica* to predict metabolic engineering strategies that improve lipid accumulation. The simulation of overexpressing 6 genes enhanced the availability of acetyl-CoA and malonyl-CoA and resulted in a 34.1% improvement of TAG production. Further simulation predicted that the

addition of L-threonine or L-aspartate amino acids could lead to a 55.5% improvement in TAG synthesis rate (Wei *et al.*, 2017). Kim *et al.* (2019) also used a computational method to predict the overexpression or knockout targets on lipid accumulation in *Y. lipolytica*. One of the knockout targets implicated in one-carbon/methionine metabolism has been reconstructed and showed a 45% increase in lipid accumulation compared to the wild-type strain (Kim *et al.*, 2019). GEM was also used to predict the effect of nitrogen and oxygen depletion on lipid accumulation in *Y. lipolytica*. The model was validated experimentally using fed-batch fermentation (Kavščcek *et al.*, 2015). Therefore, GEMs are powerful tools to understand and predict the effects of genetic modifications or cultivation conditions on lipid accumulation in *Y. lipolytica*.

4 Enhancing lipid extraction from *Y. lipolytica*

Chloroform and methanol mixtures are commonly used to extract lipids from oleaginous yeasts according to the conventional Bligh and Dyer and the Folch methods (Breil *et al.*, 2017). The efficiency of Folch method for lipid extraction depends on the yeast strain. For example, Folch method was efficient for lipid extraction from *Y. lipolytica* (Sarantou *et al.*, 2021) while an acid digestion method (2.5 M, 100 °C, 30 min) was required prior to Folch method for lipid recovery from *Lipomyces starkeyi* (Tchakouteu *et al.*, 2015). However, *Y. lipolytica* cell wall is characterized by a high chitin content (7% of wall mass) which is responsible to the strength and resistance of the wall and could limit the extraction of intracellular lipids (Khot *et al.*, 2020). Therefore, after fermentation, the alteration of cell-wall permeability is required to improve and facilitate lipid extraction. For this purpose, several cell disruption technologies were investigated such as bead milling, high-pressure homogenization, ultrasound and microwave to intensify lipid extraction from *Y. lipolytica* (Tab. 2).

4.1 Bead milling

Bead milling is an efficient mechanical cell disruption method characterized by its high potential for industrial implementation. Generally, a bead miller involves a motor-driven central shaft that may carry different designs of agitators (*e.g.*, concentric or eccentric disks) inside a grinding chamber filled with glass or ceramic beads. During the operation, the movement of the beads causes compaction, shear forces and energy transfer to the cells, which results in cell disruption such as grinding (Günerken *et al.*, 2015). Meullemiestre *et al.* (2016) compared bead milling (4000 rpm, 25 °C) to other technologies such as microwave (20 bar, 110 °C) and ultrasound (300 W, 25 °C) for the extraction of lipids from *Y. lipolytica*. Results have shown that bead milling was the most efficient cell disruption technique and increased the extracted lipid yields by two times compared to the conventional maceration in methanol-chloroform (2:1, v/v) (constant stirring, 25 °C) (Meullemiestre *et al.*, 2016).

4.2 High-pressure homogenization

High pressure homogenization (HPH) is a second mechanical process that aims to push a suspension to pass through a narrow gap at high pressures. During its passage, the suspension is subjected to multiple phenomena such as friction, cavitation and turbulence which results in obtaining a fine homogenate (Tribst *et al.*, 2008). This technique was efficient for the recovery of lipids from algae (Yap *et al.*, 2015) and from the oleaginous yeast *Cryptococcus curvatus* (Thiru *et al.*, 2011). Drévillon *et al.* (2018) investigated the efficiency of HPH pre-treatment prior to high-speed maceration in *n*-hexane for the recovery of lipids from *Y. lipolytica*. They found the optimal HPH parameters of 5 passes and 1500 bar yielded a 100% recovery of lipids in dry route and 80% in wet route (Drévillon *et al.*, 2018). In another study, the authors evaluated different cell disruption techniques prior to maceration in *n*-hexane for oil recovery from *Y. lipolytica*. Among mechanical expression (ME), moderate pulsed electric field assisted mechanical expression (MPEF-ME), high pulsed electric fields (HPEF), high voltage electrical discharges (HVED), ultrasound (US), and HPH pre-treatment techniques, HPH was the most efficient technique and allowed the extraction of 83.8 ± 4.8% of oil compared to 19.8 ± 0.5% with maceration in *n*-hexane without any pre-treatment (Drévillon *et al.*, 2019). Imatoukene *et al.* (2020) combined HPH with bead milling pretreatment and evaluated the efficiency of several solvents for lipid recovery from *Y. lipolytica*. The highest lipid yield (99.6%) was obtained when bead milling was preceded by HPH and the oil extraction was performed in chloroform:methanol. Among the tested green solvents, isoamyl acetate allowed the extraction of 96.7% of lipids from pretreated biomass (Imatoukene *et al.*, 2020).

4.3 Ultrasound

Ultrasound technology is one of the most widely used method to disrupt the cell wall of oleaginous microorganisms (Patel *et al.*, 2018). It is a physical treatment based on the cavitation phenomenon. During the propagation of ultrasound waves in a liquid media, expansion (low pressure) and compression (high pressure) cycles are induced, involving the formation of cavitation bubbles, their growth and their implosion when their size reaches a critical value. The collapse of cavitation bubbles induces a local increase in temperature and pressure, respectively, up to around 5000 K and 50 to 1000 atm, which causes cell membrane disruption (Chemat *et al.*, 2017). Previous studies evaluated the effect of ultrasound on the intensification of lipid extraction from oleaginous yeasts (Kumar *et al.*, 2020) and microalgae (Araujo *et al.*, 2013). Few reports are available on the extraction of lipids from *Y. lipolytica* using ultrasound treatment. Poli *et al.* (2013) studied the effect of sonication on the disruption of *Y. lipolytica* cell walls. The experiment consisted on adding liquid nitrogen to biomass followed by sonication 10 times for 30 s each, in a mixture of chloroform-methanol. Compared to dry maceration in chloroform-methanol which yielded 14.3% of oil, ultrasound extraction technique allowed an increase in oil recovery up to 26.5% (Poli *et al.*, 2013).

4.4 Microwave

Microwave treatment was also used to enhance lipid extraction from oleaginous yeasts. Microwaves are electromagnetic radiations with a frequency ranging from 0.3 to 300 GHz. The energy produced during microwave treatment provokes a rapid and homogeneous heating of the solvent and the suspension, by dipole rotation and ionic conduction (Bouras *et al.*, 2015). The cell disruption induced by microwave treatment increased the extraction rates of lipids from *S. cerevisiae* compared to the conventional maceration in methanol-chloroform (Khoomrung *et al.*, 2013). Microwave treatment combined to acid-catalyzed transesterification was also efficient to extract and modify lipids from *Rhodotorula glutinis* in 30 s at 120 °C compared to 4 h by Soxhlet solvent extraction (Chuck *et al.*, 2014). However, in the case of *Y. lipolytica*, the treatment of the biomass with microwaves (1000 W, 110 °C) slightly increased lipid yield to 7.13 g/100 g of DW compared to 6.53 g/100 g of DW with the conventional maceration in methanol-chloroform (2:1, v/v). The obtained result was attributed to the degradation of lipids during microwave-assisted extraction. To improve cell disruption by microwaves and avoid lipid degradation, the authors tested microwave pretreatment of biomass during 15 min at a power of 20 W and a constant temperature of 45 °C followed by two washing steps in chloroform:methanol (1:2, v/v) at room temperature. In these conditions, lipids recovery increased to 8.18 ± 0.67 g/100 g of DW (Meullemiestre *et al.*, 2016). In the same study, cold drying (−80 °C, −20 mbar, 48 h), freezing/defrosting (−20 °C, 24 h/4 °C, 12 h) and bead milling (4000 rpm, 30 min) were also tested as pretreatment techniques to improve lipids recovery from *Y. lipolytica*. The maximum lipid yield of 13.56 g/100 g of DW was obtained when cold drying under pressure was applied prior to the maceration step (Meullemiestre *et al.*, 2016).

4.5 Sub-critical water

Sub-critical water (SCW) treatment was also efficient for the recovery of lipids from *Y. lipolytica* (Tsigie *et al.*, 2012; Tran Nguyen *et al.*, 2013). It consists of using water as a solvent at high temperatures (between 100 °C and 374 °C) under high pressure to keep water in the liquid state. Under these conditions, the dielectric constant of water decreases which improves the extraction of hydrophobic compounds without using organic solvents (Shitu *et al.*, 2015). In a first study, Tsigie *et al.* (2012) applied SCW on *Y. lipolytica* biomass as a pretreatment step prior to maceration in a mixture of hexane:methanol (2:1, v/v) for 4 h. The amount of extracted neutral lipids increased from 23.21% w/w for untreated biomass to 42.69% w/w when the biomass was pretreated at 175 °C using 20 mL water for 20 min (Tsigie *et al.*, 2012). In another study, the pretreatment of *Y. lipolytica* biomass with SCW for 15 min at 175 °C caused cell walls' rupture and resulted in 10 times increase in the extractable neutral lipids compared to the untreated biomass (Tran Nguyen *et al.*, 2013).

Further cell disruption technologies for oil recovery from oleaginous microorganisms were developed by Koubaa *et al.* (2020). However, to date, there is no study regarding the membrane-based purification techniques of oil extracted from oleaginous yeasts including *Y. lipolytica*.

5 Conclusions

Single cell oils produced by oleaginous microorganisms are considered as potential alternatives to plant and animal oil for biodiesel production. The yeast *Y. lipolytica* presents a promising host for biodiesel production, due to its ability to accumulate high amounts of intracellular lipids under some cultivation conditions. The first crucial point for the commercial utilization of microbial lipids is the cost-effective production that depends on the yeast strain and the fermentation conditions. This review summarized the approaches that have been used to improve lipid production in *Y. lipolytica*. Higher lipid productivity in *Y. lipolytica* is achieved through metabolic engineering strategies including the regulation of endogenous genes and metabolic regulators, the expression of heterogeneous genes and the design and construction of alternative pathways. High lipid yields can also be obtained by using low-cost effective carbon and nitrogen sources and modifying fermentation conditions such as pH, temperature, aeration and cultivation mode. After the fermentation step, lipid extraction from biomass is a major issue that needs to be dealt with to improve the recovery yields. For this purpose, this review discussed the cell disruption technologies that have been used to optimize the recovery of lipids from *Y. lipolytica*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements. This work was performed within the project YaLiOl supported by the ANR grant "ANR-20-CE43-0007" of the French National Research Agency (ANR) in France.

References

Abghari A, Chen S. 2014. *Yarrowia lipolytica* as an oleaginous cell factory platform for production of fatty acid-based biofuel and bioproducts. *Front Energy Res* 2: 1–21. https://doi.org/10.3389/fenrg.2014.00021.

Abghari A, Chen S. 2017. Engineering *Yarrowia lipolytica* for enhanced production of lipid and citric acid. *Fermentation* 3(3). https://doi.org/10.3390/fermentation3030034.

Abghari A, Madzak C, Chen S. 2017.Combinatorial engineering of *Yarrowia lipolytica* as a promising cell biorefinery platform for the de novo production of multi-purpose long chain dicarboxylic acids. *Fermentation* 3(3). https://doi.org/10.3390/fermentation3030040.

Ali N, Zhang Q, Liu ZY, Li FL, Lu M, Fang XC. 2020. Emerging technologies for the pretreatment of lignocellulosic materials for bio-based products. *Appl Microbiol Biotechnol* 104(2): 455–473. https://doi.org/10.1007/s00253-019-10158-w.

Amalia L, Zhang YH, Ju YH, Tsai SL. 2020. Enhanced lipid production in *Yarrowia lipolytica* Po1g by over-expressing lro1 gene under two different promoters. *Appl Biochem Biotechnol* 191(1): 104–111. https://doi.org/10.1007/s12010-020-03226-9.

Araujo GS, Matos LJBL, Fernandes JO, *et al.* 2013. Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method. *Ultrason Sonochem* 20(1): 95–98. https://doi.org/10.1016/j.ultsonch.2012.07.027.

Athenstaedt K, Jolivet P, Boulard C, *et al.* 2006. Lipid particle composition of the yeast *Yarrowia lipolytica* depends on the carbon source. *Proteomics* 6(5): 1450–1459. https://doi.org/10.1002/pmic.200500339.

Ayadi I, Belghith H, Gargouri A, Guerfali M. 2018. Screening of new oleaginous yeasts for single cell oil production, hydrolytic potential exploitation and agro-industrial by-products valorization. In: *Process Safety and Environmental Protection*, Vol. 119. Institution of Chemical Engineers. https://doi.org/10.1016/j.psep.2018.07.012.

Bellou S, Makri A, Triantaphyllidou IE, Papanikolaou S, Aggelis G. 2014. Morphological and metabolic shifts of *Yarrowia lipolytica* induced by alteration of the dissolved oxygen concentration in the growth environment. *Microbiology (United Kingdom)* 160(Part 4): 807–817. https://doi.org/10.1099/mic.0.074302-0.

Bellou S, Triantaphyllidou IE, Mizerakis P, Aggelis G. 2016. High lipid accumulation in *Yarrowia lipolytica* cultivated under double limitation of nitrogen and magnesium. *J Biotechnol* 234: 116–126. https://doi.org/10.1016/j.jbiotec.2016.08.001.

Beopoulos A, Mrozova Z, Thevenieau F, *et al.* 2008. Control of lipid accumulation in the yeast *Yarrowia lipolytica*. *Appl Environ Microbiol* 74(24): 7779–7789. https://doi.org/10.1128/AEM01412-08.

Bhutada G, Kavšček M, Ledesma-Amaro R, *et al.* 2017. Sugar versus fat: Elimination of glycogen storage improves lipid accumulation in *Yarrowia lipolytica*. *FEMS Yeast Res* 17(3): 1–10. https://doi.org/10.1093/femsyr/fox020.

Blazeck J, Hill A, Liu L, *et al.* 2014. Harnessing *Yarrowia lipolytica* lipogenesis to create a platform for lipid and biofuel production. *Nat Commun* 5: 1–10. https://doi.org/10.1038/ncomms4131.

Bouras M, Chadni M, Barba FJ, Grimi N, Bals O, Vorobiev E. 2015. Optimization of microwave-assisted extraction of polyphenols from Quercus bark. *Ind Crops Prod* 77: 590–601. https://doi.org/10.1016/j.indcrop.2015.09.018.

Brabender M, Hussain MS, Rodriguez G, Blenner MA. 2018. Urea and urine are a viable and cost-effective nitrogen source for *Yarrowia lipolytica* biomass and lipid accumulation. *Appl Microbiol Biotechnol* 102(5): 2313–2322. https://doi.org/10.1007/s00253-018-8769-z.

Breil C, Abert Vian M, Zemb T, Kunz W, Chemat F. 2017. "Bligh and Dyer" and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of solvatation mechanisms and towards substitution with alternative solvents. *Int J Mol Sci* 18(4): 1–21. https://doi.org/10.3390/ijms18040708.

Carsanba E, Papanikolaou S, Erten H. 2018. Production of oils and fats by oleaginous microorganisms with an emphasis given to the potential of the nonconventional yeast *Yarrowia lipolytica*. *Crit Rev Biotechnol* 38(8): 1230–1243. https://doi.org/10.1080/07388551.2018.1472065.

Carsanba E, Papanikolaou S, Fickers P, Erten H. 2020. Lipids by *Yarrowia lipolytica* strains cultivated on glucose in batch cultures. *Microorganisms* 8(7): 1–14. https://doi.org/10.3390/microorganisms8071054.

Cheirsilp B, Louhasakul Y. 2013. Industrial wastes as a promising renewable source for production of microbial lipid and direct transesterification of the lipid into biodiesel. *Bioresour Technol* 142: 329–337. https://doi.org/10.1016/j.biortech.2013.05.012.

Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason Sonochem* 34: 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

Christophe G, Kumar V, Nouaille R, *et al.* 2012. Recent developments in microbial oils production: A possible alternative to vegetable oils for biodiesel without competition with human food? *Braz Arch Biol Technol* 55(1): 29–46. https://doi.org/10.1590/S1516-89132012000100004.

Chuck CJ, Lou-Hing D, Dean R, Sargeant LA, Scott RJ, Jenkins RW. 2014. Simultaneous microwave extraction and synthesis of fatty acid methyl ester from the oleaginous yeast *Rhodotorula glutinis*. *Energy* 69: 446–454. https://doi.org/10.1016/j.energy.2014.03.036.

Darvishi F, Fathi Z, Ariana M, Moradi H. 2017. *Yarrowia lipolytica* as a workhorse for biofuel production. *Biochem Eng J* 127: 87–96. https://doi.org/10.1016/j.bej.2017.08.013.

Datta A, Hossain A, Sanjay R. 2019. *Asian J Chem* 31(8): 1851–1858.

Deive FJ, Carvalho E, Pastrana L, Rúa ML, Longo MA, Sanroman MA. 2009. Strategies for improving extracellular lipolytic enzyme production by Thermus thermophilus HB27. *Bioresour Technol* 100(14): 3630–3637. https://doi.org/10.1016/j.biortech.2009.02.053.

Dobrowolski A, Mituła P, Rymowicz W, Mirończuk AM. 2016. Efficient conversion of crude glycerol from various industrial wastes into single cell oil by yeast *Yarrowia lipolytica*. *Bioresour Technol* 207: 237–243. https://doi.org/10.1016/j.biortech.2016.02.039.

Donot F, Fontana A, Baccou JC, Strub C, Schorr-Galindo S. 2014. Single cell oils (SCOs) from oleaginous yeasts and moulds: Production and genetics. *Biomass Bioenergy* 68(04): 135–150. https://doi.org/10.1016/j.biombioe.2014.06.016.

Drévillon L, Koubaa M, Vorobiev E. 2018. Lipid extraction from *Yarrowia lipolytica* biomass using high-pressure homogenization. *Biomass Bioenergy* 115: 143–150. https://doi.org/10.1016/j.biombioe.2018.04.014.

Drévillon L, Koubaa M, Nicaud JM, Vorobiev E. 2019. Cell disruption pre-treatments towards an effective recovery of oil from *Yarrowia lipolytica* oleaginous yeast. *Biomass Bioenergy* 128: 105320. https://doi.org/10.1016/j.biombioe.2019.105320.

Dulermo T, Nicaud JM. 2011. Involvement of the G3P shuttle and B-oxidation pathway in the control of TAG synthesis and lipid accumulation in *Yarrowia lipolytica*. *Metab Eng* 13(5): 482–491. https://doi.org/10.1016/j.ymben.2011.05.002.

Dulermo T, Tréton B, Beopoulos A, Gnankon APK, Haddouche R, Nicaud JM. 2013. Characterization of the two intracellular lipases of *Y. lipolytica* encoded by TGL3 and TGL4 genes: New insights into the role of intracellular lipases and lipid body organisation. *Biochim Biophys Acta – Mol Cell Biol Lipids* 1831(9): 1486–1495. https://doi.org/10.1016/j.bbalip.2013.07.001.

Dulermo R, Gamboa-Meléndez H, Dulermo T, Thevenieau F, Nicaud JM. 2014. The fatty acid transport protein Fat1p is involved in the export of fatty acids from lipid bodies in *Yarrowia lipolytica*. *FEMS Yeast Res* 14(6): 883–896. https://doi.org/10.1111/1567-1364.12177.

Enamala MK, Enamala S, Chavali M, *et al.* 2018. Production of biofuels from microalgae – A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. *Renew Sustain Energy Rev* 94: 49–68. https://doi.org/10.1016/j.rser.2018.05.012.

Fabiszewska AU, Zieniuk B, Kozłowska M, *et al.* 2021. Studies on upgradation of waste fish oil to lipid-rich yeast biomass in *Yarrowia lipolytica* batch cultures. *Foods* 10(2): 1–15. https://doi.org/10.3390/foods10020436.

Fickers P, Destain J, Thonart P. 2009. Improvement of *Yarrowia lipolytica* lipase production by fed-batch fermentation. *J Basic Microbiol* 49(2): 212–215. https://doi.org/10.1002/jobm.200800186.

Fontanille P, Kumar V, Christophe G, Nouaille R, Larroche C. 2012. Bioconversion of volatile fatty acids into lipids by the oleaginous yeast *Yarrowia lipolytica*. *Bioresour Technol* 114: 443–449. https://doi.org/10.1016/j.biortech.2012.02.091.

Friedlander J, Tsakraklides V, Kamineni A, *et al.* 2016. Engineering of a high lipid producing *Yarrowia lipolytica* strain. *Biotechnol Biofuels* 9(1): 1–12. https://doi.org/10.1186/s13068-016-0492-3.

Fukuda R. 2013. Metabolism of hydrophobic carbon sources and regulation of it in n-alkane-assimilating yeast *Yarrowia lipolytica*. *Biosci Biotechnol Biochem* 77(6): 1149–1154. https://doi.org/10.1271/bbb.130164.

Gálvez-López D, Chávez-Meléndez B, Vázquez-Ovando A, Rosas-Quijano R. 2019. The metabolism and genetic regulation of lipids in the oleaginous yeast *Yarrowia lipolytica*. *Braz J Microbiol* 50 (1): 23–31. https://doi.org/10.1007/s42770-018-0004-7.

Gao R, Li Z, Zhou X, Cheng S, Zheng L. 2017. Oleaginous yeast *Yarrowia lipolytica* culture with synthetic and food waste-derived volatile fatty acids for lipid production. *Biotechnol Biofuels* 10(1): 1–15. https://doi.org/10.1186/s13068-017-0942-6.

Gao R, Li Z, Zhou X, Bao W, Cheng S, Zheng L. 2020. Enhanced lipid production by *Yarrowia lipolytica* cultured with synthetic and waste-derived high-content volatile fatty acids under alkaline conditions. *Biotechnol Biofuels* 13(1): 1–16. https://doi.org/10.1186/s13068-019-1645-y.

Ghogare R, Chen S, Xiong X. 2020. Metabolic engineering of oleaginous yeast *Yarrowia lipolytica* for overproduction of fatty acids. *Front Microbiol* 11. https://doi.org/10.3389/fmicb.2020.01717.

Gimpel JA, Henríquez V, Mayfield SP. 2015. In metabolic engineering of eukaryotic microalgae: Potential and challenges come with great diversity. *Front Microbiol* 6: 1–14. https://doi.org/10.3389/fmicb.2015.01376.

Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. 2008. Oxidative stress and covalent modification of protein with bioactive aldehydes. *J Biol Chem* 283(32): 21837–21841. https://doi.org/10.1074/jbc.R700019200.

Günerken E, D'Hondt E, Eppink MHM, Garcia-Gonzalez L, Elst K, Wijffels RH. 2015. Cell disruption for microalgae biorefineries. *Biotechnol Adv* 33(2): 243–260. https://doi.org/10.1016/j.biotechadv.2015.01.008.

Hapeta P, Rakicka M, Dulermo R, *et al.* 2017. Transforming sugars into fat–lipid biosynthesis using different sugars in *Yarrowia lipolytica*. *Yeast* 34(7): 293–304. https://doi.org/10.1002/yea.3232.

Harzevili FD. 2014. Biotechnological applications of the Yeast *Yarrowia lipolytica*. Springer International Publishing, pp. 1–16. https://doi.org/10.1007/978-3-319-06437-6.

Imatoukene N, Koubaa M, Perdrix E, Benali M, Vorobiev E. 2020. Combination of cell disruption technologies for lipid recovery from dry and wet biomass of *Yarrowia lipolytica* and using green solvents. *Process Biochem* 90: 139–147. https://doi.org/10.1016/j.procbio.2019.11.011.

Jeevan Kumar SP, Banerjee R. 2019. Enhanced lipid extraction from oleaginous yeast biomass using ultrasound assisted extraction: A greener and scalable process. *Ultrason Sonochem* 52: 25–32. https://doi.org/10.1016/j.ultsonch.2018.08.003.

Karamerou EE, Webb C. 2019. Cultivation modes for microbial oil production using oleaginous yeasts – A review. *Biochem Eng J* 151: 107322. https://doi.org/10.1016/j.bej.2019.107322.

Kavšcek M, Bhutada G, Madl T, Natter K. 2015. Optimization of lipid production with a genome-scale model of *Yarrowia lipolytica*. *BMC Syst Biol* 9(1): 1–13. https://doi.org/10.1186/s12918-015-0217-4.

Khoomrung S, Chumnanpuen P, Jansa-Ard S, *et al.* 2013. Rapid quantification of yeast lipid using microwave-assisted total lipid extraction and HPLC-CAD. *Anal Chem* 85(10): 4912–4919. https://doi.org/10.1021/ac3032405.

Khot M, Raut G, Ghosh D, Alarcón-Vivero M, Contreras D, Ravikumar A. 2020. Lipid recovery from oleaginous yeasts: Perspectives and challenges for industrial applications. *Fuel* 259: 116292. https://doi.org/10.1016/j.fuel.2019.116292.

Kim M, Park BG, Kim EJ, Kim J, Kim BG. 2019. In silico identification of metabolic engineering strategies for improved lipid production in *Yarrowia lipolytica* by genome-scale metabolic modeling. *Biotechnol Biofuels* 12(1): 1–14. https://doi.org/10.1186/s13068-019-1518-4.

Koubaa M, Imatoukene N, Drévillon L, Vorobiev E. 2020. Current insights in yeast cell disruption technologies for oil recovery: A review. *Chem Eng Process – Process Intensif* 150: 107868. https://doi.org/10.1016/j.cep.2020.107868.

Koutinas AA, Chatzifragkou A, Kopsahelis N, Papanikolaou S, Kookos IK. 2014. Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. *Fuel* 116: 566–577. https://doi.org/10.1016/j.fuel.2013.08.045.

Krzyczkowska J, Kozłowska M. 2017. Effect of oils extracted from plant seeds on the growth and lipolytic activity of *Yarrowia lipolytica* yeast. *JAOCS – J Am Oil Chem Soc* 94(5): 661–671. https://doi.org/10.1007/s11746-017-2975-1.

Kumar LR, Yellapu SK, Tyagi RD, Drogui P. 2020. Purified crude glycerol by acid treatment allows to improve lipid productivity by *Yarrowia lipolytica* S KY7. *Process Biochem* 96: 165–173. https://doi.org/10.1016/j.procbio.2020.06.010.

Kuttiraja M, Dhouha A, Tyagi RD. 2018. Harnessing the Effect of pH on Lipid Production in Batch Cultures of *Yarrowia lipolytica* SKY7. *Appl Biochem Biotechnol* 184(4): 1332–1346. https://doi.org/10.1007/s12010-017-2617-y.

Lazar Z, Liu N, Stephanopoulos G. 2018. Holistic approaches in lipid production by *Yarrowia lipolytica*. *Trends Biotechnol* 36(11): 1157–1170. https://doi.org/10.1016/j.tibtech.2018.06.007.

Ledesma-Amaro R, Dulermo T, Nicaud JM. 2015. Engineering *Yarrowia lipolytica* to produce biodiesel from raw starch. *Biotechnol Biofuels* 8 (1): 1–12. https://doi.org/10.1186/s13068-015-0335-7.

Ledesma-Amaro R, Dulermo R, Niehus X, Nicaud JM. 2016. Combining metabolic engineering and process optimization to improve production and secretion of fatty acids. *Metab Eng* 38: 38–46. https://doi.org/10.1016/j.ymben.2016.06.004.

Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. 2008. Biofuels from microalgae. *Biotechnol Progress* 24(4): 815–820. https://doi.org/10.1021/bp.070371k.

Li Q, Bai Z, O'Donnell A, Harvey LM, Hoskisson PA, McNeil B. 2011. Oxidative stress in fungal fermentation processes: The roles of alternative respiration. *Biotechnol Lett* 33(3): 457–467. https://doi.org/10.1007/s10529-010-0471-x.

Liu L, Pan A, Spofford C, Zhou N, Alper HS. 2015a. An evolutionary metabolic engineering approach for enhancing lipogenesis in *Yarrowia lipolytica*. *Metab Eng* 29: 36–45. https://doi.org/10.1016/j.ymben.2015.02.003.

Liu L, Markham K, Blazeck J, *et al.* 2015b. Surveying the lipogenesis landscape in *Yarrowia lipolytica* through understanding the function of a Mga2p regulatory protein mutant. *Metab Eng* 31: 102–111. https://doi.org/10.1016/j.ymben.2015.07.004.

Lopes M, Gomes AS, Silva CM, Belo I. 2018. Microbial lipids and added value metabolites production by *Yarrowia lipolytica* from pork lard. *J Biotechnol* 265: 76–85. https://doi.org/10.1016/j.jbiotec.2017.11.007.

Lopes M, Miranda SM, Alves JM, Pereira AS, Belo I. 2019. Waste cooking oils as feedstock for lipase and lipid-rich biomass production. *Eur J Lipid Sci Technol* 121(1): 1–9. https://doi.org/10.1002/ejlt.201800188.

Louhasakul Y, Cheirsilp B, Intasit R, Maneerat S, Saimmai A. 2020. Enhanced valorization of industrial wastes for biodiesel feedstocks and biocatalyst by lipolytic oleaginous yeast and biosurfactant-producing bacteria. *Int Biodeterior Biodegrad* 148: 104911. https://doi.org/10.1016/j.ibiod.2020.104911.

Magdouli S, Guedri T, Tarek R, Brar SK, Blais JF. 2017. Valorization of raw glycerol and crustacean waste into value added products by *Yarrowia lipolytica*. *Bioresour Technol* 243: 57–68. https://doi.org/10.1016/j.biortech.2017.06.074.

Magdouli S, Brar SK, Blais JF. 2018. Morphology and rheological behaviour of *Yarrowia lipolytica*: Impact of dissolved oxygen level on cell growth and lipid composition. *Process Biochem* 65: 1–10. https://doi.org/10.1016/j.procbio.2017.10.021.

Martins FF, Ferreira TF, Azevedo DA, Coelho MAZ. 2012. Evaluation of crude oil degradation by *Yarrowia lipolytica*. *Chem Eng Trans* 27: 223–228. https://doi.org/10.3303/CET1227038.

Mat Aron NS, Khoo KS, Chew KW, Show PL, Chen WH, Nguyen THP. 2020. Sustainability of the four generations of biofuels – A review. *Int J Energy Res* 44(12): 9266–9282. https://doi.org/10.1002/er.5557.

Mathiazhakan K, Ayed D, Tyagi RD. 2016. Kinetics of lipid production at lab scale fermenters by a new isolate of *Yarrowia lipolytica* S KY7. *Bioresour Technol* 221: 234–240. https://doi.org/10.1016/j.biortech.2016.09.015.

Meng X, Yang J, Xu X, Zhang L, Nie Q, Xian M. 2009. Biodiesel production from oleaginous microorganisms. *Renew Energy* 34 (1): 1–5. https://doi.org/10.1016/j.renene.2008.04.014.

Meullemiestre A, Breil C, Abert-Vian M, Chemat F. 2016. Microwave, ultrasound, thermal treatments, and bead milling as intensification techniques for extraction of lipids from oleaginous *Yarrowia lipolytica* yeast for a biojetfuel application. *Bioresour Technol* 211: 190–199. https://doi.org/10.1016/j.biortech.2016.03.040.

Mlíčková K, Luo Y, D'Andrea S, Peč P, Chardot T, Nicaud JM. 2004. Acyl-CoA oxidase, a key step for lipid accumulation in the yeast *Yarrowia lipolytica*. *J Mol Catal B: Enzym* 28(2-3): 81–85. https://doi.org/10.1016/j.molcatb.2004.01.007.

Moreno LB. 2018. Cellular ecophysiology of microbe: Hydrocarbon and lipid interactions. In: *Cellular Ecophysiology of Microbe: Hydrocarbon and Lipid Interactions*. https://doi.org/10.1007/978-3-319-50542-8.

Morin N, Cescut J, Beopoulos A, *et al.* 2011. Transcriptomic analyses during the transition from biomass production to lipid accumulation in the oleaginous yeast *Yarrowia lipolytica*. *PLoS ONE* 6(11). https://doi.org/10.1371/journal.pone.0027966.

Nicaud J-M. 2012. *Yarrowia lipolytica*. *Yeast* 29: 409–418. https://doi.org/10.1002/yea.

Niehus X, Crutz-Le Coq AM, Sandoval G, Nicaud JM, Ledesma-Amaro R. 2018. Engineering *Yarrowia lipolytica* to enhance lipid production from lignocellulosic materials. *Biotechnol Biofuels* 11(1): 1–10. https://doi.org/10.1186/s13068-018-1010-6.

Papanikolaou S, Aggelis G. 2002. Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. *Bioresour Technol* 82(1): 43–49. https://doi.org/10.1016/S0960-8524(01)00149-3.

Papanikolaou S, Aggelis G. 2010. *Yarrowia lipolytica*: A model microorganism used for the production of tailor-made lipids. *Eur J Lipid Sci Technol* 112(6): 639–654. https://doi.org/10.1002/ejlt.200900197.

Papanikolaou S, Chevalot I, Komaitis M, Aggelis G, Marc I. 2001. Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. *Antonie van Leeuwenhoek Int J Gen Mol Microbiol* 80(3-4): 215–224. https://doi.org/10.1023/A:1013083211405.

Papanikolaou S, Chevalot I, Komaitis M, Marc I, Aggelis G. 2002a. Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. *Appl Microbiol Biotechnol* 58(3): 308–312. https://doi.org/10.1007/s00253-001-0897-0.

Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I. 2002b. *Yarrowia lipolytica* as a potential producer of citric acid from raw glycerol. *J Appl Microbiol* 92(4): 737–744. https://doi.org/10.1046/j.1365-2672.2002.01577.x.

Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I. 2003. Accumulation of a cocoa-butter-like lipid by *Yarrowia lipolytica* cultivated on agro-industrial residues. *Curr Microbiol* 46(2): 124–130. https://doi.org/10.1007/s00284-002-3833-3.

Papanikolaou S, Chevalot I, Galiotou-Panayotou M, Komaitis M, Marc I, Aggelis G. 2007. Industrial derivative of tallow: A promising renewable substrate for microbial lipid, single-cell protein and lipase production by *Yarrowia lipolytica*. *Electron J Biotechnol* 10(3): 425–435. https://doi.org/10.2225/vol10-issue3-fulltext-8.

Papanikolaou S, Chatzifragkou A, Fakas S, *et al.* 2009. Biosynthesis of lipids and organic acids by *Yarrowia lipolytica* strains cultivated on glucose. *Eur J Lipid Sci Technol* 111(12): 1221–1232. https://doi.org/10.1002/ejlt.200900055.

Paschalidou A, Tsatiris M, Kitikidou K. 2016. Energy crops for biofuel production or for food? – SWOT analysis (case study: Greece). *Renew Energy* 93: 636–647. https://doi.org/10.1016/j.renene.2016.03.040.

Patel A, Mikes F, Matsakas L. 2018. An overview of current pretreatment methods used to improve lipid extraction from oleaginous microorganisms. *Molecules* 23(7). https://doi.org/10.3390/molecules23071562.

Patrignani F, Vannini L, Gardini F, Guerzoni ME, Lanciotti R. 2011. Variability of the lipolytic activity and volatile molecules production by a strain of *Yarrowia lipolytica* in pork fat and its dependence on environmental conditions. *Meat Sci* 89(1): 21–26. https://doi.org/10.1016/j.meatsci.2011.03.015.

Poli JS, Dallé P, Senter L, *et al.* 2013. Fatty acid methyl esters produced by oleaginous yeast *Yarrowia lipolytica* QU21: an alternative for vegetable oils. *Braz J Biosci* 1985: 203–208.

Poli JS, da Silva MAN, Siqueira EP, Pasa VMD, Rosa CA, Valente P. 2014. Microbial lipid produced by *Yarrowia lipolytica* QU21 using industrial waste: A potential feedstock for biodiesel production. *Bioresour Technol* 161(2014): 320–326. https://doi.org/10.1016/j.biortech.2014.03.083.

Poontawee R, Limtong S. 2020. Feeding strategies of two-stage fed-batch cultivation processes for microbial lipid production from sugarcane top hydrolysate and crude glycerol by the oleaginous red yeast *Rhodosporidiobolus fluvialis*. *Microorganisms* 8(2): 151. https://doi.org/10.3390/microorganisms8020151.

Qiao K, Imam Abidi SH, Liu H, *et al.* 2015. Engineering lipid overproduction in the oleaginous yeast *Yarrowia lipolytica*. *Metab Eng* 29: 56–65. https://doi.org/10.1016/j.ymben.2015.02.005.

Qiao K, Wasylenko TM, Zhou K, Xu P, Stephanopoulos G. 2017. Lipid production in *Yarrowia lipolytica* is maximized by

engineering cytosolic redox metabolism. *Nat Biotechnol* 35(2): 173–177. https://doi.org/10.1038/nbt.3763.

Qin L, Liu L, Zeng AP, Wei D. 2017. From low-cost substrates to single cell oils synthesized by oleaginous yeasts. *Bioresour Technol* 245: 1507–1519. https://doi.org/10.1016/j.biortech.2017.05.163.

Rakicka M, Lazar Z, Dulermo T, Fickers P, Nicaud JM. 2015. Lipid production by the oleaginous yeast *Yarrowia lipolytica* using industrial by-products under different culture conditions. *Biotechnol Biofuels* 8(1): 1–10. https://doi.org/10.1186/s13068-015-0286-z.

Ratledge C. 2014. The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: A reappraisal and unsolved problems. *Biotechnol Lett* 36(8): 1557–1568. https://doi.org/10.1007/s10529-014-1532-3.

Sagnak R, Cochot S, Molina-Jouve C, Nicaud JM, Guillouet SE. 2018. Modulation of the Glycerol Phosphate availability led to concomitant reduction in the citric acid excretion and increase in lipid content and yield in *Yarrowia lipolytica*. *J Biotechnol* 265: 40–45. https://doi.org/10.1016/j.jbiotec.2017.11.001.

Sara M, Brar SK, Blais JF. 2016. Lipid production by *Yarrowia lipolytica* grown on biodiesel-derived crude glycerol: Optimization of growth parameters and their effects on the fermentation efficiency. *RSC Adv* 6(93): 90547–90558. https://doi.org/10.1039/c6ra16382c.

Sarantou S, Stoforos NG, Kalantzi O, Papanikolaou S. 2021. Biotechnological valorization of biodiesel-derived glycerol: Trials with the non-conventional yeasts *Yarrowia lipolytica* and *Rhodosporidium* sp. *Carbon Resourc Convers* 4: 61–75. https://doi.org/10.1016/j.crcon.2020.12.006.

Seip J, Jackson R, He H, Zhu Q, Hong SP. 2013. Snf1 is a regulator of lipid accumulation in *Yarrowia lipolytica*. *Appl Environ Microbiol* 79(23): 7360–7370. https://doi.org/10.1128/AEM.02079-13.

Sekova VY, Dergacheva DI, Isakova EP, Gessler NN, Tereshina VM, Deryabina YI. 2019. Soluble sugar and lipid readjustments in the *Yarrowia lipolytica* yeast at various temperatures and ph. *Metabolites* 9(12). https://doi.org/10.3390/metabo9120307.

Shitu A, Izhar S, Tahir TM. 2015. Sub-critical water as a green solvent for production of valuable materials from agricultural waste biomass: A review of recent work. *Global J Environ* 1(3): 255–264.

Silverman AM, Qiao K, Xu P, Stephanopoulos G. 2016. Functional overexpression and characterization of lipogenesis-related genes in the oleaginous yeast *Yarrowia lipolytica*. *Appl Microbiol Biotechnol* 100(8): 3781–3798. https://doi.org/10.1007/s00253-016-7376-0.

Singh A, Olsen SI, Nigam PS. 2011. A viable technology to generate third-generation biofuel. *J Chem Technol Biotechnol* 86(11): 1349–1353. https://doi.org/10.1002/jctb.2666.

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

Tan T, Lu J, Nie K, Deng L, Wang F. 2010. Biodiesel production with immobilized lipase: A review. *Biotechnol Adv* 28(5): 628–634. https://doi.org/10.1016/j.biotechadv.2010.05.012.

Taoka Y, Nagano N, Okita Y, Izumida H, Sugimoto S, Hayashi M. 2011. Effect of Tween 80 on the growth, lipid accumulation and fatty acid composition of Thraustochytrium aureum ATCC 34304. *J Biosci Bioeng* 111(4): 420–424. https://doi.org/10.1016/j.jbiosc.2010.12.010.

Tchakouteu SS, Kalantzi O, Gardeli C, Koutinas AA, Aggelis G, Papanikolaou S. 2015. Lipid production by yeasts growing on biodiesel-derived crude glycerol: Strain selection and impact of substrate concentration on the fermentation efficiency. *J Appl Microbiol* 118(4): 911–927. https://doi.org/10.1111/jam.12736.

Thiru M, Sankh S, Rangaswamy V. 2011. Process for biodiesel production from *Cryptococcus curvatus*. *Bioresour Technol* 102 (22): 10436–10440. https://doi.org/10.1016/j.biortech.2011.08.102.

Tran Nguyen PL, Go AW, Huynh LH, Ju YH. 2013. A study on the mechanism of subcritical water treatment to maximize extractable cellular lipids. *Biomass Bioenergy* 59: 532–539. https://doi.org/10.1016/j.biombioe.2013.08.031.

Tribst AAL, Franchi MA, Cristianini M. 2008. Ultra-high pressure homogenization treatment combined with lysozyme for controlling Lactobacillus brevis contamination in model system. *Innovat Food Sci Emerg Technol* 9(3): 265–271. https://doi.org/10.1016/j.ifset.2007.07.012.

Tsigie YA, Wang CY, Truong CT, Ju YH. 2011. Lipid production from *Yarrowia lipolytica* Po1g grown in sugarcane bagasse hydrolysate. *Bioresour Technol* 102(19): 9216–9222. https://doi.org/10.1016/j.biortech.2011.06.047.

Tsigie YA, Huynh LH, Ahmed IN, Ju YH. 2012. Maximizing biodiesel production from *Yarrowia lipolytica* Po1g biomass using subcritical water pretreatment. *Bioresour Technol* 111: 201–207. https://doi.org/10.1016/j.biortech.2012.02.052.

Wang ZP, Xu HM, Wang GY, Chi Z, Chi ZM. 2013. Disruption of the MIG1 gene enhances lipid biosynthesis in the oleaginous yeast *Yarrowia lipolytica* ACA-DC 50109. *Biochim Biophys Acta – Mol Cell Biol Lipids* 1831(4): 675–682. https://doi.org/10.1016/j.bbalip.2012.12.010.

Wang G, Li D, Miao Z, Zhang S, Liang W, Liu L. 2018. Comparative transcriptome analysis reveals multiple functions for Mhy1p in lipid biosynthesis in the oleaginous yeast *Yarrowia lipolytica*. *Biochim Biophys Acta – Mol Cell Biol Lipids* 1863(1): 81–90. https://doi.org/10.1016/j.bbalip.2017.10.003.

Wang ZP, Wang QQ, Liu S, Liu XF, Yu XJ, Jiang YL. 2019. Efficient conversion of cane molasses towards high-purity isomaltulose and cellular lipid using an engineered *Yarrowia lipolytica* strain in fed-batch fermentation. *Molecules* 24(7). https://doi.org/10.3390/molecules24071228.

Wang J, Ledesma-Amaro R, Wei Y, Ji B, Ji XJ. 2020. Metabolic engineering for increased lipid accumulation in *Yarrowia lipolytica* – A review. *Bioresour Technol* 313. https://doi.org/10.1016/j.biortech.2020.123707.

Wasylenko TM, Ahn WS, Stephanopoulos G. 2015. The oxidative pentose phosphate pathway is the primary source of NADPH for lipid overproduction from glucose in *Yarrowia lipolytica*. *Metab Eng* 30: 27–39. https://doi.org/10.1016/j.ymben.2015.02.007.

Wei S, Jian X, Chen J, Zhang C, Hua Q. 2017. Reconstruction of genome-scale metabolic model of *Yarrowia lipolytica* and its application in overproduction of triacylglycerol. *Bioresour Bioprocess* 4(1). https://doi.org/10.1186/s40643-017-0180-6.

Xie D. 2017. Integrating cellular and bioprocess engineering in the non-conventional yeast *Yarrowia lipolytica* for biodiesel production: A review. *Front Bioeng Biotechnol* 5. https://doi.org/10.3389/fbioe.2017.00065.

Xu P, Qiao K, Stephanopoulos G. 2017. Engineering oxidative stress defense pathways to build a robust lipid production platform in *Yarrowia lipolytica*. *Biotechnol Bioeng* 114(7): 1521–1530. https://doi.org/10.1002/bit.26285.

Xua P, Qiao K, Ahn WS, Stephanopoulos G. 2016. Engineering *Yarrowia lipolytica* as a platform for synthesis of drop-in transportation fuels and oleochemicals. *Proc Natl Acad Sci USA* 113(39): 10848–10853. https://doi.org/10.1073/pnas.1607295113.

Xue J, Balamurugan S, Li T, *et al.* 2021. Biotechnological approaches to enhance biofuel producing potential of microalgae. *Fuel* 302: 121169. https://doi.org/10.1016/j.fuel.2021.121169.

Yan FX, Dong GR, Qiang S, Niu YJ, Hu CY, Meng YH. 2020. Overexpression of △12, △15-Desaturases for Enhanced Lipids Synthesis in *Yarrowia lipolytica*. *Front Microbiol* 11: 1–11. https://doi.org/10.3389/fmicb.2020.00289.

Yap BHJ, Dumsday GJ, Scales PJ, Martin GJO. 2015. Energy evaluation of algal cell disruption by high pressure homogenisation. *Bioresour Technol* 184: 280–285. https://doi.org/10.1016/j.biortech.2014.11.049.

Yook S Do Kim J, Gong G, *et al.* 2020. High-yield lipid production from lignocellulosic biomass using engineered xylose-utilizing *Yarrowia lipolytica*. *GCB Bioenergy* 12(9): 670–679. https://doi.org/10.1111/gcbb.12699.

Zhang Y, Adams IP, Ratledge C. 2007. Malic enzyme: The controlling activity for lipid production? Overexpression of malic enzyme in Mucor circinelloides leads to a 2.5-fold increase in lipid accumulation. *Microbiology* 153(7): 2013–2025. https://doi.org/10.1099/mic.0.2006/002683-0.

Zhang H, Zhang L, Chen H, *et al.* 2014. Enhanced lipid accumulation in the yeast *Yarrowia lipolytica* by over-expression of ATP: Citrate lyase from Mus musculus. *J Biotechnol* 192(Part A): 78–84. https://doi.org/10.1016/j.jbiotec.2014.10.004.

Zhang H, Kang X, Xiao N, *et al.* 2019a. Intracellular expression of Vitreoscilla haemoglobin improves lipid production in *Yarrowia lipolytica*. *Lett Appl Microbiol* 68(3): 248–257. https://doi.org/10.1111/lam.13111.

Zhang S, Jagtap SS, Deewan A, Rao CV. 2019b. pH selectively regulates citric acid and lipid production in *Yarrowia lipolytica* W29 during nitrogen-limited growth on glucose. *J Biotechnol* 290: 10–15. https://doi.org/10.1016/j.jbiotec.2018.10.012.

Zieniuk B, Fabiszewska A. 2019. *Yarrowia lipolytica*: a beneficious yeast in biotechnology as a rare opportunistic fungal pathogen: A mini review. *World J Microbiol Biotechnol* 35(1): 1–8. https://doi.org/10.1007/s11274-018-2583-8.

Cite this article as: El Kantar S, Khelfa A, Vorobiev E, Koubaa M. 2021. Strategies for increasing lipid accumulation and recovery from *Y. lipolytica*: A review. *OCL* 28: 51.