Microorganism for Bioconversion of Sugar Hydrolysates into Lipids

Alessandro Luis Venega Coradini, Andréia Anschau, Annamaria Dória Souza Vidotti, Érika Marques Reis, Michelle da Cunha Abreu Xavier, Renato Sano Coelho, and Telma Teixeira Franco

Contents

Abstract Metabolic and genetic engineering and further other genomics, transcriptomics, and metabolomics tools still need to be further developed to provide more useful information and new ways on enhancing lipid production in oleaginous microorganisms, optimizing fatty acid (FA) profiles, enhancing lipid accumulation, and improving the use of low-cost raw materials as lignocellulosic hydrolysates. Several oleaginous organisms have been described as good lipid producers, being the fast ones the yeasts. However the kinetics for this production is much slower than that required for industrial processes, unless the products are

A.L.V. Coradini • A.D.S. Vidotti • É.M. Reis • M.d.C.A. Xavier • R.S. Coelho • T.T. Franco (✉)
School of Chemical Engineering, State University of Campinas, UNICAMP, 13083-970 Campinas, SP, Brazil
e-mail: franco@feq.unicamp.br

A. Anschau
Department of Food Engineering, State University of Midwestern of Paraná, UNICENTRO, 85040-080 Guarapuava, PR, Brazil

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sold at high competitive prices (fine chemicals, cosmetics, and food), covering the costs for the long residential bioreactor times.

Microalgal cultivation in heterotrophic systems is able to use organic carbon sources, sugars, or organic acids, and this cultivation mode offers some advantages over autotrophic cultivation including increased lipid productivity, besides good control of the cultivation process and low cost for harvesting the biomass, since higher cell density is obtained. However, the feasibility of large-scale cultures of microalgae in heterotrophic conditions is still limited by, among other things, the high cost of organic substrates used in this type of cultivation, unless urban/agricultural/industrial wastes are used as lignocellulose and wastewater and sewage are used as carbon or mineral sources.

1 Introduction

Lignocellulosic biomass is the most abundant raw materials on Earth and is a promising alternative energy source due to their abundance and renewable characteristics. Agro-industrial residues such as sugarcane bagasse (SCB), wheat straw, rice straw, and corn stover represent feedstocks for their conversion into value-added products (Chandel et al. 2012).

Microbial oils, also called single cell oils (SCO), are produced by some oleaginous microorganisms, such as yeast, fungi, bacteria, and microalgae, which are able to accumulate more than 20 % lipids (dry mass) (Ageitos et al. 2011; Karatay and Dönmez 2011). Different lignocellulosic biomasses have been used for microbial oil production, such as wheat straw, corncobs, rice straw, and SCB. SCB seems to be the most promising and competitive in sugarcane-producing lands, due to the availability of large amounts stored in the sugarcane mills after crushing/pressing (Matos 2012). SCB may be an attractive and promising feedstock for microbial oil production to reduce the costs of culture medium, which conventionally hampers the economic feasibility of SCO as alternative feedstock for biodiesel production (Anschau et al. 2014; Tsigie et al. 2011).

Two problems influence SCO production on lignocellulosic hydrolysates. Few oleaginous microorganisms are able to use xylose for SCO production, and as xylose is the second most abundant component of lignocellulose hydrolysates, substantial amounts of feedstock can be lost. Several strains of oleaginous microorganisms have their growth inhibited by small molecules from lignocellulose hydrolysates. Fortunately, it has been observed that some oleaginous strains are able to metabolize xylose for SCO production, without being so much inhibited by the growth inhibitors (Anschau et al. 2014).

Zhao (2005) described the possibility of SCO production from lignocellulose hydrolysates, the biomass-to-biodiesel three-step plan: lignocellulose biomass depolymerization into fermentable sugars, their conversion into microbial lipids by oleaginous microorganisms, and the chemical transformation of their lipids into biodiesel.

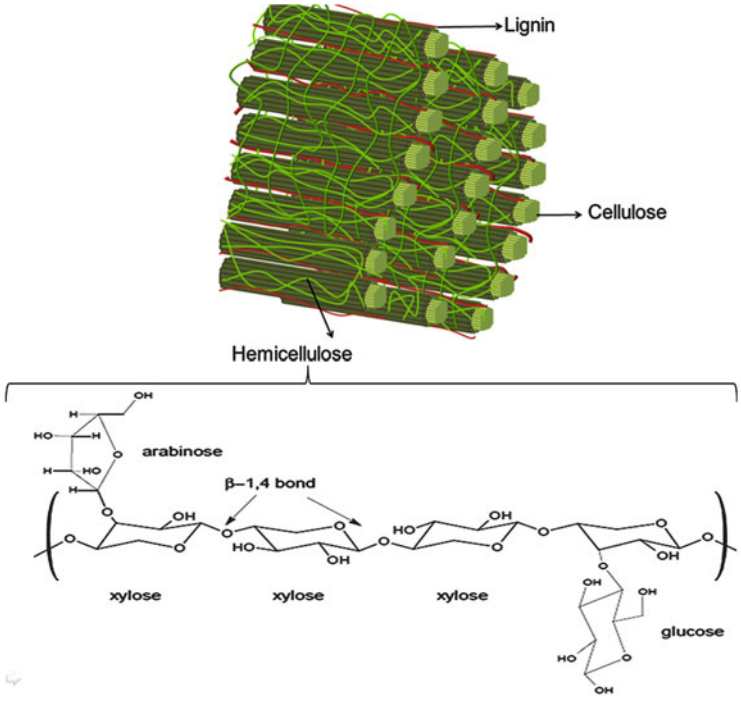

Fig. 1 Lignocellulosic biomass and hemicellulosic structures

2 The Lignocellulosic Biomass

Lignocellulose is a complex polymer of cellulose (40–80 %), hemicellulose (10–40 %), and lignin (5–25 %) (Chandel et al. 2012, Fig. 1). Cellulose, the main fraction of plant cell wall, is linear and crystalline and is a homopolymer of repeating units of glucose linked by β(1-4) glycosidic bonds. Hemicellulose is a highly branched heteropolymer composed of D-xylose, D-arabinose, D-glucose, D-galactose, and D-mannose (Fig. 2). Lignin, formed by polymerization of phenolic compounds, is hydrophobic in nature and is tightly bound to the cellulose and hemicellulose protecting them from microbial and chemical (Sarkar et al. 2012) and enzymatic action (Meng and Ragauskas 2014).

Xylose is the main carbohydrate present in the hemicellulose fraction (second most abundant component of lignocellulose, representing about 80 % of total sugars) (Girio et al. 2010). Potentially, the released carbohydrates, mono- and dimeric sugars, are substrates for the production of value-added products such as ethanol, xylitol, n-butanol, 2,3-butanediol, and lactic acid via microbial fermentation processes (Chandel et al. 2010, 2011, 2012).

The goal of pretreating vegetal biomasses is to remove or modify the lignin (delignification) and to reduce the crystallinity of cellulose (increasing the

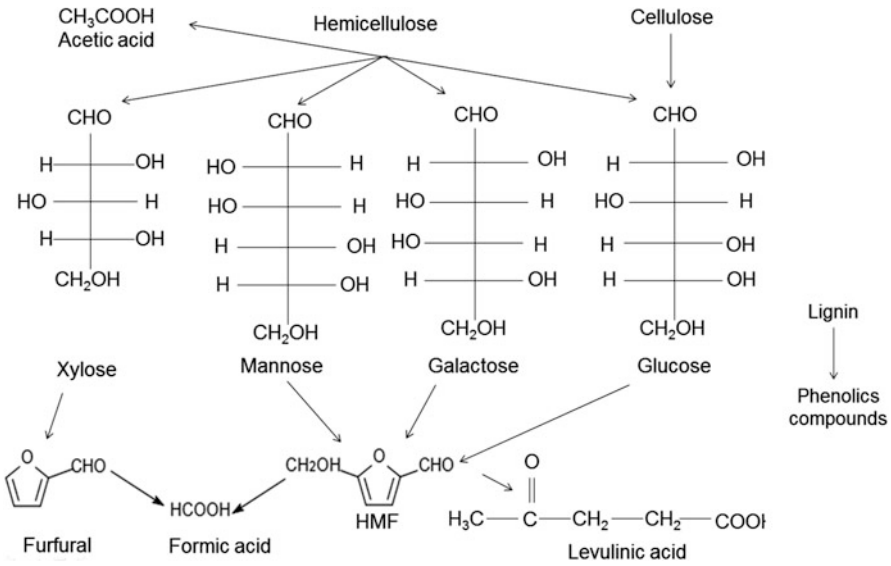

Fig. 2 Structural profile of inhibitors derived from lignocellulosic biomass (Chandel et al. 2011)

accessibility to enzymes, which convert carbohydrates into fermentable sugars). The pretreatment of lignocellulose is usually able to hydrolyze the hemicellulose fraction yielding xylose (mostly) and arabinose, mannose, galactose, and glucose in smaller relative concentrations, in addition to the small molecules, also known as microbial growth inhibitors (Meng and Ragauskas 2014; Girio et al. 2010).

There are different types of pretreatment methods such as steam explosion, gamma radiation, acid or alkali treatment, hydrothermal, ammonia, urea, hydrogen peroxide, solvents, milling, and fungal degradation (Girio et al. 2010; Chandel et al. 2010; Kumar et al. 2009; Moretti et al. 2014), being exclusively physical, chemical, physicochemical, biological, or a combination of mechanisms.

The choice of pretreatment depends on the biomass to be used, its composition, and by-products produced. Furthermore, pretreatments need to avoid sugar degradation and minimize the consequent formation of growth inhibitor molecules (Moretti et al. 2014) and significantly affect their costs.

Hydrochloric acid (HCl), phosphoric acid (H_3PO_4), and sulfuric acid (H_2SO_4) catalyze the hydrolysis of lignocellulosic materials and are used in two different combinations with the reactor's temperature, high concentrations of acid and low temperatures or diluted acid concentrations and high temperatures. In our laboratory, we hydrolyzed the SCB with 1.5 % (w/v) H_2SO_4, solid-to-liquid ratio (1:10) at 120 °C, for 20 min (Fig. 3). Xylose was the major product released (14.3 g/L) by SCB hemicellulosic hydrolysate (H–H) due to its high content of xylan. In addition, glucose and arabinose were found in low concentrations (1.6 and 2.3 g/L, respectively). Glucose was released from the cellulose and hemicellulose fractions, and the arabinose was from the depolymerization of arabino-xylan (a hemicellulosic

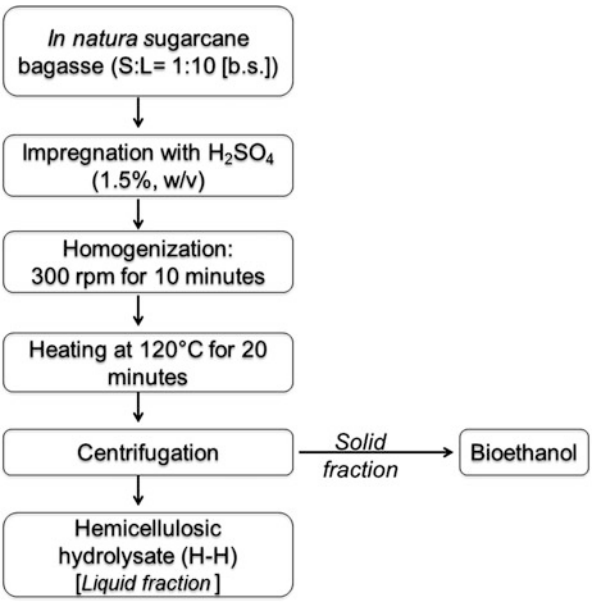

Fig. 3 Obtaining process of hemicellulosic hydrolysate (H–H). S: L = solid:liquid

heteropolymer that contains more xylose than arabinose). Usually, hexose fermenter microorganisms are unable to metabolize pentose sugars and do not tolerate the growth inhibitors generated from the biomass pretreatment (Carvalheiro et al. 2008). The full use of H–H must be understood as a way to reduce waste and to increase the efficiency of conversion of raw material into final products, mainly when value-added products that are obtained improve the economics of the process.

During acid hydrolysis of lignocellulosics, toxic inhibitors to the fermenting microorganism are generated in addition to the sugar monomers. These inhibitors include phenolic compounds, furans (furfural and 5-hydroxymethylfurfural (5-HMF)), and weak acids (acetic acid, formic, levulinic) (Fig. 2). Furfural and 5-HMF are formed by degradation of pentoses and hexoses, respectively. However, the HMF concentration tends to be smaller than furfural due to limited degradation of hexoses in acid saccharification. Acetic acid is formed by hydrolysis of acetyl groups from lignocellulose, as a result of deacetylation of acetylated pentosans, and its generation is mainly dependent on the temperature and residence time of pretreatment with diluted acid (Chandel et al. 2012; Kumar et al. 2009). The formation of the growth inhibitors during the acid hydrolysis depends on multiple factors, including the nature and composition of the lignocellulose, cell wall composition, thermochemical conditions, and reaction time of the hydrolysis (Stoutenburg et al. 2011; Palmqvist and Hahn-Hägerdal 2000a, b). These toxic compounds may affect microbial metabolism hindering the fermentation process (Sangeeta et al. 2014). Their toxicity is the major limiting factor to the bioconversion of lignocellulose, and often they need to be removed from the H–H prior to the

fermentation (Stoutenburg et al. 2011). Detoxification methods have been used to reduce the presence of growth inhibitors or to remove them in order to improve their fermentability and increase their biotechnological applications (Chandel et al. 2010; Palmqvist and Hahn-Hägerdal 2000a).

There are several detoxification methods such as physical, chemical, or biological and may be used individually or in combined form. These methods include activated charcoal treatment, neutralization, calcium hydroxide overliming, extraction with solvents, ion exchange resins, evaporation, membrane-mediated detoxification, enzymatic mediated using laccase, lignin peroxidase, in situ microbial detoxification, etc. (Ge et al. 2011; Chandel et al. 2012; Moretti et al. 2014), which will vary depending on the H–H, the type of microorganisms used in the fermentation, and their degree of tolerance to inhibitors. It is important to compare the different methods of treatment in order to select those that provide greater removal of inhibitors and lower reduction of sugar concentrations. For example, activated charcoal and alkali treatment with calcium oxide are chemical methods used to detoxify acid hydrolysates. Calcium oxide treatment causes precipitation of toxic compounds and instability of some inhibitors in high pH, removing furans and phenolic compounds efficiently with a marginal loss of sugars (Chandel et al. 2010). Activated carbon treatment by adsorption is capable of removing acetic acid, furans, phenolic and aromatic compounds, furfural, and HMF with high efficiency coupled with low cost (Chandel et al. 2010, 2011; Stoutenburg et al. 2011).

In our laboratory, we obtained a H–H with 3.6 g/L of acetic acid, 0.02 g/L of HMF, and 0.04 g/L of furfural. The pH was adjusted with CaO without marginal loss of sugars and slight reduction of inhibitors; therefore, the H–H detoxification was considered as unnecessary. The non-detoxified hydrolysate was used for lipid production by *Lipomyces starkeyi* without appreciable inhibition by the toxic compounds. Furthermore, the yeast consumed or bioreduced the inhibitors during fermentation suggesting that the strain could use these compounds as c Carbon and energy source. High tolerance of *L. starkeyi* is very interesting for the use of H–H since the detoxification step would not be required in the conditions tested.

Therefore, proper pretreatment methods can increase the relative concentrations of fermentable sugars after enzymatic saccharification, thereby improving the efficiency of the whole process. Enzymatic hydrolysis uses enzyme complexes to depolymerize complex carbohydrates into simple monomers and requires less energy and milder environmental conditions than acid hydrolysis. Enzymatic hydrolysis is carried out by cellulase and hemicellulase enzymes, which cleave the bonds of cellulose and hemicellulose, respectively. Enzymatic hydrolysis shows some advantages such as low toxicity and low corrosion compared to acid or alkaline hydrolysis and does not produce inhibitors (Sarkar et al. 2012). On the other hand, enzymes are costly, require longer time of hydrolysis, and have high loads which limit the economical feasibility of technological processes related to the lignocellulosic biomass. Therefore, an efficient pretreatment is to be selected to decrease cellulose crystallinity and to remove lignin to the maximum extent, so that hydrolysis time as well as cellulase loading will be minimized (Sarkar et al. 2012).

3 Microbial Oil Production from Lignocellulosic Biomass

3.1 Microbial Oil Production by Microalgae

Within the broad microbial diversity, microalgae have potential for lipid production, since many species can be induced to accumulate substantial quantities of lipids within the cell (Malcata 2011). The average lipid content of algal cells varies between 1 and 50 % of dry weight under certain conditions (Meng et al. 2009; Spolaore et al. 2006). Also already it has been known that microalgae could accumulate more lipids with stress treatments such as nitrogen deficiency (Illman et al. 2000). Coelho et al. (2014) working at fed-batch culture with *Chlorella*, during nitrogen-limited stage, obtained an increase of 38 % in lipid content (from 21 to 29 %) and 26 % in overall lipid productivity (from 1.25 to 1.58 g/L/day).

Microalgae are able to get energy from different metabolisms (Richmond and Qian 2004). In autotrophic metabolism, in the presence of light, organisms are able to convert CO_2 and water into biomass. Already heterotrophic systems are a different approach to microalgal biomass production with elimination of light requirement and CO_2 absorption.

In heterotrophic cultivation organic carbon sources are used, such as sugars or organic acids, as a source of carbon and energy. This cultivation mode offers several advantages over autotrophic cultivation including increased lipid productivity, besides good control of the cultivation process and low cost for harvesting the biomass because of higher cell density obtained in heterotrophic culture of microalgae. Cell densities as high as 80 g/L (Coelho and Franco 2013) and until 100 g/L (Zheng et al. 2013) have been achieved in heterotrophic systems using glucose as organic carbon source and different feeding strategies. The feasibility of large-scale cultures of microalgae in heterotrophic conditions is still limited by, among other things, the high cost of organic substrates used in this type of cultivation, unless wastes are used as carbon or mineral sources.

In heterotrophic culture, the biosynthesis of products is significantly influenced by medium nutrients, and carbon sources are the most important element for heterotrophic culture of microalgae in the production of lipids. Glucose is most commonly used as carbon source for heterotrophic cultures of microalgae, and far higher rates of growth are obtained with this substrate than with any other. Usually, glucose accounts for most of the medium cost of microalgal cultivation (it was estimated up to 80 %) (Li et al. 2007).

Corn powder hydrolysate has been used to replace glucose for developing a cheap medium for heterotrophic *Chlorella protothecoides* (Xu et al. 2006). Many additional options of organic carbon sources were tested for feasibility instead of glucose (Table 1).

Molasses, a by-product from sugar refinery, also can be a good alternative to glucose in promoting microalgal lipid accumulation. Vidotti et al. (2014) tested the hydrolyzed sugarcane molasses with two microalgal strains, and this substrate

Table 1 Substrates used for lipid production by *C. protothecoides strains*

Substrate	Lipids (%, w/w)	Lipid productivity (g/L/day)	References
Corn powder hydrolysate	55.3	NI	Xu et al. (2006)
Sugarcane juice hydrolysate	53	NI	Cheng et al. (2009a)
Sweet sorghum juice hydrolysate	52.5	0.5	Gao et al. (2010)
Jerusalem artichoke tuber hydrolysate	44	1.6	Cheng et al. (2009b)
Cassava hydrolysate	53	NI	Lu et al. (2009)
	26.5	0.4	Wei et al. (2009)
Waste molasses hydrolysate	57.6	5.5	Yan et al. (2011)

NI not informed

provided a similar cell growth as that observed when glucose was used in the cultivation of *Chlorella vulgaris* and *Scenedesmus bijugatus*.

Sugars released from lignocellulosic materials also are able to provide cheaper and sustainable carbon source for heterotrophic microalgal cultivation. However, besides the physical and chemical barriers already mentioned in this chapter, caused by the close association of the main components of lignocellulosic biomass, a variety of potential inhibitory degradation products are produced during pretreatment of lignocellulosic biomass, and all of these chemicals can potentially cause inhibition to algal growth (Liang 2013).

How microalgal species survive the presence of these toxic degradation by-products is unknown. In the literature, only the effect of acetate on microalgal growth has been evaluated (Perez-Garcia et al. 2011; Heredia-Arroyo et al. 2010, 2011; Yeh et al. 2012; Vidotti et al. 2013). Another important factor is the sugar composition present in the feedstock. As mentioned earlier, glucose is the most commonly used carbon source for heterotrophic cultures of microalgae, but microalgal species that can utilize xylose are few. At present, only one paper in the literature reported the xylose utilization by *Chlorella* strain, but only in the light or when glucose was present in the media (Hawkins 1999). On the other hand, Vidotti et al. (2014) tested xylose as carbon source for *Chlorella vulgaris* and *Scenedesmus bijugatus*; however none of the two strains were able to utilize this sugar.

To solve the xylose utilization issue, some directions can be taken: screening microalgal species that can grow on xylose as well as on glucose and genetically engineering strains to metabolize xylose. Moreover Liang (2013) mentions the possibility of coupling with other xylose utilizing microbial species, either yeast or bacteria in the same reactor or sequentially. In this way, glucose and xylose will both be consumed by different microorganisms, and different products can be resulted from the biomass hydrolysates. Recent research efforts have concentrated on the identification of microalgal strains capable of converting cellulosic feedstocks into oil (Chua and Somanchi 2012; Pourmir and Johannes 2011), but in light of the complexity involved in this application, few studies have been reported in the literature.

3.2 Microbial Oil Production by Bacteria

The advantages of using bacteria for lipid production are obvious since these microorganisms show usually higher growth rates than yeast and fungi and can be more easily genetically manipulated. Prokaryotes have fewer genes allocated all in one chromosome, and the lower complexity of genome demands less complexity of control mechanisms. However, while triacylglycerols (TAG) can be found in most eukaryotic organisms, they are rarely found in bacteria. Extractable lipids in bacteria are usually in the form of specialized lipids such as poly(3-hydroxybutiric acid) or other poly-hydroxyalkanoates. Only a few species of actinomycetes group such as *Rhodococcus* and *Mycobacterium* can synthesize and accumulate TAGs (Alvarez and Steinbuchel 2002). Among these, the genus *Rhodococcus* is frequently studied for its high capacity of accumulating lipids while growing in different substrates. *Rhodococcus opacus* PD630 is usually selected as the model microorganism for bacterial lipid accumulation. It was found to accumulate over 70 % of its cell dry weight (CDW) in FAs under nitrogen limitation condition (Alvarez et al. 2000) and grow at specific growth rates of 0.1–0.2 h^{-1} with glucose as carbon source (Xiong et al. 2012; Baboshin and Golovleva 2005).

Rhodococcus opacus PD630 was shown to grow well in the presence of lignocellulosic biomass pretreatment by-products such as furfural, 5-hydroxymethyl furfural (5-HMF), vanillin, vanillic acids, and *trans*-p-coumaric acid (TPCA), which are known for inhibiting microbial growth. In addition, the strain could use vanillin, vanillic acid, and TPCA for growth and lipid accumulation in the absence of other carbon sources (Table 2). Furfural and 5-HMF were also fully degraded but not as sole carbon sources. The strain could grow in corn stover, sorghum, and grass hydrolysates while accumulating TAGs (Wang et al. 2014).

Xylose is one of the substrates that cannot be metabolized by *Rhodococcus* species. However the metabolic pathway of D-xylose utilization was introduced into strains *Rhodococcus opacus* PD630 and *Rhodococcus jostii* RHA1. The heterogeneous expression of genes xylA (xylose isomerase) and xylB (xylulokinase) from *Streptomyces lividans* TK23 allowed the recombinant *R. opacus* PD630 and *R. jostii* RHA1 to grow on xylose and accumulate 68.3 and 52.5 % of lipids, respectively. However, the specific growth rate on xylose (0.07 h^{-1}) was 63 % lower than the observed for glucose (0.19 h^{-1}) (Xiong et al. 2012).

Regarding cellulose direct utilization, *Rhodococcus opacus* PD630 was engineered to express six cellulase genes from *Cellulomonas fimi* ATCC 484 (cenABC, cex, and cbhA) and *Thermobifida fusca* DSM43792 (cel6A) which enabled the strain to degrade cellulose into cellobiose. The modified strain was able to hydrolyze 9.3 % of cellulose provided, and a second strain engineered to utilize cellobiose could accumulate 15 % of lipids from the hydrolyzed cellulose (Hetzler et al. 2013).

Lignin is usually considered to be a bottleneck in lignocellulosic biomass bioconversion. It represents 10–25 % of lignocellulosic feedstocks, and few microorganisms are capable of metabolizing it, thus limiting its utilization as fuel for

Table 2 Oleaginous bacteria used for lipid production from different substrates

Microorganism	Substrate	Lipids (%)	Major FA (%, w/w)	References
Recombinant *R. opacus* PD630	Xylose	68.3	C16:0 (21.54 %), C17:0 (19.62 %)	Xiong et al. (2012)
R. opacus PD630	Corn stover, sorghum, grass hydrolysates	36	N.A.	Wang et al. (2014)
Recombinant *R. jostii* RHA1	Xylose	52.5	C17:0 (22.39 %), C17:1 (19.37 %)	Xiong et al. (2012)
R. opacus PD630	Lignin model compounds	18.2	C16:0 (27.9 %), C18:1 (14.2 %)	Kosa and Ragauskas (2012)
R. opacus DSM 1069	Lignin model compounds	11.4	C16:0 (22.9 %), C17:1 (14.5 %)	Kosa and Ragauskas (2012)
R. opacus PD630	Glucose	38	C16:0 (27.7 %), C18:1 (24.7 %)	Kurosawa et al. (2010)
Gordonia sp. DG	Orange waste	50	C22:0 (>30 %), C18:3 (>10 %)	Gouda et al. (2008)

Major *FA* the concentration of only the two major fatty acids is shown. *NA* not available data

energy cogeneration or bioconversion through pyrolysis. Recently lignin model compounds (4-HBA, VanA, and SyrA) were found to be directly metabolized by *R. opacus* DSM1069 and PD630 to lipid production (11.44 and 18.22 % of dry cell weight, respectively) (Kosa and Ragauskas 2012).

3.3 Microbial Oil Production by Fungi

Filamentous fungi usually grow at lower growth rates, yields, and productivities than yeasts. However some of these organisms are capable of producing lipids containing more than 70 % of polyunsaturated fatty acids (PUFAs) such as arachidonic acid and γ-linolenic acid. The composition of the lipids produced depends directly on the culture medium composition and may change during cultivation.

It is well known that lipid synthesis and accumulation occur under nitrogen limitation conditions. Interestingly arachidonic acid synthesis seems to occur after carbon source starvation (Nie et al. 2013). However, under carbon-depleted conditions, the concentration of total FAs remains constant, but a rapid increase in arachidonic acid content is observed while at the same time other FAs such as oleic and stearic acids are consumed. It was suggested that saturated FAs and oleic acid are converted to linolenic and γ-linolenic acids and subsequently to arachidonic acid (Eroshin et al. 2002). However if no carbon limitation is induced,

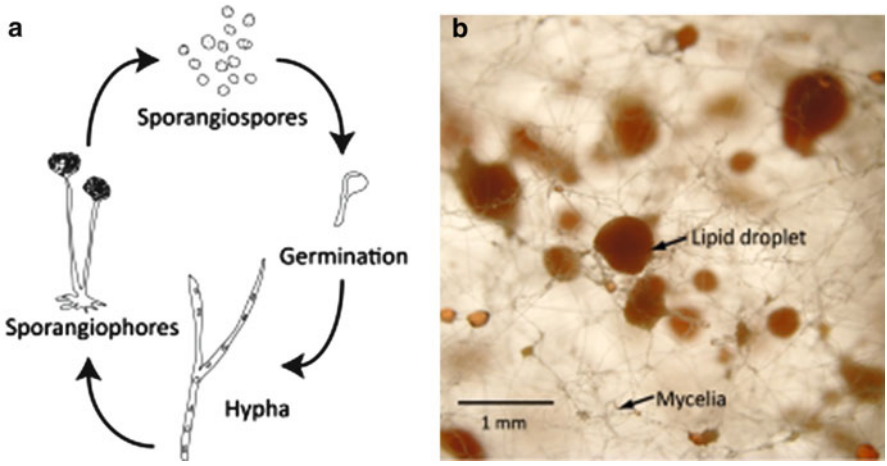

Fig. 4 *Mortierella alpina* (**a**) asexual lifecycle of the fungus. Haploid cells form sporangiophores, and sporangiospores germinate to hypha. (**b**) Fungal culture grown on PDA plate stained with 0.5 % triphenyl tetrazolium chloride. Lipid droplets are stained *brown* (Wang et al. 2011)

lipid composition is mainly composed by saturated and monounsaturated FAs as observed by Stressler et al. (2013) and Nie et al. (2013).

Strains of the genus *Mortierella* (Fig. 4) are regarded as some of the most productive organism for PUFA synthesis, with the capacity of accumulating 20–70 % of total lipids (Table 3) containing mostly PUFA (Sakuradani 2010). These fungi can grow at high growth rates of up to 0.566 h^{-1} when grown in non-limiting conditions and yield lipid productivities as high as 0.785 g/(L h) (Economou et al. 2011a).

Arachidonic acid is already commercially produced from *Mortierella* strains by Cargill Alking Bioengineering in Wuhan, China; by Nippon Suisan Kaisha in Tokyo, Japan; and by Martek Biosciences (DSM) in Columbia, USA (Tyburczy et al. 2011). These fungi are also one of the few highly productive oleaginous microorganisms that can grow well using xylose as sole carbon source (Huang et al. 2013).

Mortierella isabellina could grow on both C5 (arabinose, ribose, xylose, and mannose) and disaccharides (sucrose and cellobiose). When grown in the presence of model lignocellulosic inhibitor compounds, phenolic compounds were highly inhibitory, while furfural, 5-HMF, acetic acid, formic acid, and levulinic acid showed limited inhibition. Surprisingly acetic and formic acid improved lipid production by twofold as compared with that observed for the control. When grown on wheat straw, hydrolysate up to 53 % total lipids was obtained with a lipid yield of 16.8 % from the carbon sources (Zeng et al. 2013).

The oleaginous fungi *Mucor circinelloides* was grown on corn ethanol stillage resulting in 52 % total lipids. When the culture was supplemented with glycerol at the stationary phase, an increase of 46–61 % in lipid content was observed.

Table 3 Oleaginous fungi used for lipid production from different substrates

Microorganism	Substrate	Lipids (%, w/w)	Major FA (%)	References
Mortierella isabellina	Wheat straw hydrolysate	53	C18:1 53 %, 11.6 % PUFA	Zeng et al. (2013)
Mortierella alpina	Glucose	49	C20:4 38 %, 69 % PUFA	Stressler et al. (2013)
Mortierella isabellina	Corn stover, switchgrass, *Miscanthus,* and giant reed hydrolysates	35	N.A.	Ruan et al. (2013)
Mortierella alpina	Glucose (three stage fermentation)	64	C20:4 44.3 %, C18:0 16.5 %	Nie et al. (2013)
Mortierella alpina	Wheat straw hydrolysate	16.8	C18:1 49.9 %, C16:0 26.19 %	Zeng et al. (2013)
Aspergillus oryzae	Potato processing wastewater	40	C18:1 30.3 %, C18:19.3 %	Muniraj et al. (2013)
Mortierella isabellina	Xylose	64.3	C18:1 56.2 %, C16:0 24.9 %	Gao et al. (2013)
Mortierella isabellina	Rice hull hydrolysate	64.3	C18:1 50.7 %, C16:0 22.6 %	Economou et al. (2011b)
Mucor circinelloides	Ethanol corn stillage	61	C18:2 50 %, C18:1 29.6 %	Mitra et al. (2012)
Mucor circinelloides	Glucose	19.9	C18:1 37 %, 18.5 %	Vicente et al. (2009)
Cunninghamella echinulata	Xylose	65.5	C18:3 17 %	Fakas et al. (2009)

Major *FA* the concentration of only the two major fatty acids is shown
NA data not available

3.4 Microbial Oil Production by Yeast

Among fungi, oleaginous yeasts are distinguished by their capacity to accumulate high concentrations of lipids. Species such as *Rhodosporidium toruloides* and *Lipomyces starkeyi* have been found to accumulate lipids at around 60 and 70 % of dry cell weight (Shi et al. 2011). Microbial fermentation for producing biodiesel from lignocellulosic hydrolysates is receiving increasing attention; however, oleaginous yeasts that can utilize lignocellulosic hydrolysates are few (especially for acid lignocellulosic hydrolysates to accumulate lipids) and still at its initial stages (Table 4). Therefore, it is necessary to discover oleaginous strains that have high lipid yield on lignocellulosic biomass hydrolysates.

To date, SCO production from lignocellulosic biomass was usually carried out through a batch fermentation mode. It is possible that using a fed-batch or continuous fermentation mode could fulfill the high-cell-density cultivation on lignocellulosic hydrolysates, and this is undoubtedly beneficial for the industrialization of SCO production (Huang et al. 2013). Anschau et al. (2014) indicated the possibility

Table 4 Microbial oil production on lignocellulosic hydrolysates

Lignocellulosic biomass	Strains	Lipids (%)	References
Sugarcane bagasse	*Lipomyces starkeyi*	26.7	Anschau et al. (2014)
	Lipomyces starkeyi	18.5	Aristizabal et al. (2012)
	Yarrowia lipolytica	58.5	Tsigie et al. (2011)
	Trichosporon fermentans	15.8 g/L 59.5	Huang et al. (2012b) Huang et al. (2011)
Sweet sorghum bagasse	*Cryptococcus curvatus*	64	Liang et al. (2012)
Corncob	*Trichosporon cutaneum*	45.4	Chen et al. (2013)
	Trichosporon dermatis	40.1	Huang et al. (2012a)
Rice straw	*Trichosporon fermentans*	40.1	Huang et al. (2009)
Wheat straw	*Cryptococcus curvatus* *Rhodotorula glutinis* *Rhodosporidium toruloides* *Lipomyces starkeyi* *Yarrowia lipolytica*	33.5 25.0 24.6 31.2 4.6	Yu et al. (2011)

of the transformation of lignocellulosic materials into biodiesel studying the lipid production by *L. starkeyi* DSM 70296 using different feeding strategies. Glucose and xylose were used for batch, fed-batch, repeated fed-batch, and continuous cultures, and hemicellulose hydrolysate (H–H) was tested at continuous culture. High cell mass (~85 g/L) and lipid (~40 g/L) concentrations were achieved through both fed-batch and repeated fed-batch cultivations using glucose:xylose (30:70), with a maximum specific growth rate of 0.07 h^{-1}. The kinetic profile obtained from a continuous cultivation with H–H was similar to that obtained from cultivation in a synthetic medium. This may be explained by the fact that the hydrolysate contained some protein and microelements, which could promote the yeast growth and lipid accumulation. At a dilution rate of 0.03 h^{-1} with H–H, the cultivation yielded cell mass and lipid concentrations of 13.9 g/L and 3.7 g/L (26.7 %), with a maximum specific growth rate at batch step of 0.089 h^{-1}. Arabinose was not consumed throughout the cultivation. The inhibitor concentrations (acetic acid, furfural, and HMF) were reduced during the batch stage and the first residence times of the continuous cultivation, indicating that this yeast can use these compounds as carbon sources. This is of particular interest because it is possible that *L. starkeyi* is highly tolerant to inhibitors, which may allow a broader study of H–H from SCB in fermentation processes. Continuous cultivations at 0.03 h^{-1} using glucose:xylose (30:70, w/w) and H–H from SCB presented the highest yields and productivities, indicating that this approach is the most appropriate for scale-up (Fig. 5).

The same strain (*L. starkeyi* DSM 70296) was adapted by evolutionary engineering in culture medium containing SCBH (H–H) (Aristizabal et al. 2012). The H–H was obtained after seven sequential extraction steps of bagasse previously pretreated by steam explosion. The yeast was adapted by evolutionary engineering in culture medium containing increasing concentrations of H–H. As a result, a yeast adapted to culture medium containing 30 % of H–H was obtained, which showed higher cell concentration (9.79 g/L) with lower lipid content (18.5 %) compared to

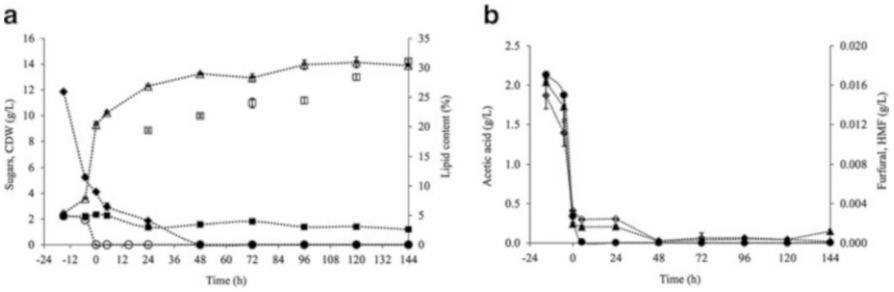

Fig. 5 (**a**) CDW (△), lipid content (□), xylose (♦), glucose (○), arabinose (■), (**b**) acetic acid (●), furfural (▲), and HMF (◊) concentrations during continuous cultivation at 0.03 h^{-1} with H–H

not adapted strain (5.21 g/L of cells with 31.5 % of lipids) with a maximum specific growth rate of 0.039 h^{-1}.

The possibility of utilizing detoxified sugarcane bagasse hydrolysate (DSCBH) was investigated as an alternative carbon source to culture *Y. lipolytica* Po1g for microbial oil and biodiesel production (Tsigie et al. 2011). Compared with (DSCBH) medium, growth in the non-detoxified sugarcane bagasse hydrolysate (NDSCBH) medium was limited because of the presence of inhibitors (furfural and HMF).

Cryptococcus curvatus has great potential in fermenting unconditioned hydrolysates of sweet sorghum bagasse (Liang et al. 2012). During yeast fermentation, glucose and xylose were consumed simultaneously, while cellobiose was released from the residual bagasse. Surprisingly, the cellobiose utilization rates were much higher than those on glucose and xylose. It seems that *C. curvatus* could engulf cellobiose rapidly, but then it had to spend more energy on digesting this disaccharide, which resulted in the lowest cell yield among all of the sugars tested.

Trichosporon fermentans was proved to be able to use sulfuric acid-treated sugarcane bagasse hydrolysate as substrate to grow and accumulate lipid (Huang et al. 2012b). Although the volumetric productivity of *T. fermentans* on bagasse hydrolysate is not as high as that of *Y. lipolytica* on industrial fats, the higher lipid concentration and moderate volumetric productivity make oleaginous yeast *T. fermentans* very promising for lipid production from abundant and inexpensive lignocellulosic materials. The effects of five representative aldehydes in lignocellulosic hydrolysates on the growth and the lipid accumulation of oleaginous yeast *T. fermentans* were also investigated (Huang et al. 2011). There was no relationship between the hydrophobicity and the toxicity of aldehyde, and 5-hydroxymethylfurfural was less toxic than aromatic aldehydes and furfural. Binary combination of aromatic aldehydes caused a synergistic inhibitory effect, but a combination of furan and aromatic aldehydes reduced the inhibition instead. Furthermore, the inhibition of aldehydes on cell growth was more dependent on inoculum size, temperature, and initial pH than that on lipid content.

The inhibitory effect of lignocellulose degradation products on the oleaginous yeast fermentation was carefully investigated by Chen et al. (2009). Preliminary

screening was carried out in the minimum nutritious medium without adding any expensive complex ingredients and then was carried out in the lignocellulosic hydrolysate pretreated by dilute sulfuric acid. Seven typical lignocellulose degradation products formed in various pretreatments and hydrolysis processing were selected as the model inhibitors, including three organic acids, two furan compounds, and two phenol derivatives. The inhibition of the degradation compounds on the cell growth and the lipid productivity of the selected oleaginous yeasts were examined. Acetic acid, formic acid, furfural, and vanillin were found to be the strong inhibitors for the fermentation of oleaginous yeasts, while levulinic acid, 5-hydroxymethylfurfural, and hydroxybenzaldehyde were relatively weak inhibitors. *T. cutaneum* 2.1374 was found to be the most adopted strain to the lignocellulose degradation compounds.

In summary, these works showed the great potential of SCO production from lignocellulosic biomass. The use of lignocellulosic hydrolysates as substrates could serve as the basis for the industrialization of SCO production. However, many problems in this process still exist that must be solved.

4 Fatty Acid Composition in Microbial Lipids

Microbial oils may have great potential for biodiesel production due to FA composition. Microorganisms also produce edible oils rich in PUFA for dietary supplements and infant nutrition applications (Ratledge 2004; Cahoon and Schmid 2008). It is possible to observe that the FA profile produced by microorganisms is quite similar to vegetable oils (Table 5) and can thus be used as a substitute for biofuels.

According to Table 5, most PUFAs produced by fungi, algae, and bacteria have been proposed as producers of certain PUFAs. Yeasts are not potential producers of PUFAs. The number of unsaturations does not have an effect only on the values of viscosity and density of biodiesel but also on the oxidative stability (Lobo et al. 2009). Most biodiesel fuels present significant amounts of esters of oleic, linoleic, or linolenic acids, influencing the oxidative stability of the fuel (Knothe 2005). Biodiesel presents portability, greater renewability, higher combustion efficiency, lower sulfur and aromatic content, and higher cetane number than diesel oil (Balat 2011). It is recommended that these fuels meet the biodiesel standards of US ASTM D 6751 and European EN 14214 (Ashraful et al. 2014).

When the extraction process is used for oils, it seeks to achieve the following objectives: (1) to obtain a fat or oil uninjured and free from undesirable impurities, (2) obtain the best possible performance without changing the cost of litigation, and (3) produce a minimum of waste with the greatest possible value. The choice of the optimal extraction method for large-scale oil must meet certain criteria: the ease with which the cell breaks down, the cost of the method, rate of cell disruption, and so on (Hulteberg et al. 2008).

Table 5 Fatty acid composition of some microorganisms

Microorganism	References	Relative average fatty acids (%, w/w)					
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Microalgae	Meng et al. (2009)	12–21	55–57	1–2	58–60	4–20	14–30
C. vulgaris	Wynn and Ratledge (2005)	16	11.9	2	58	9	14
Yeast	Meng et al. (2009)	11–37	1–6	1–10	28–66	3–24	1–3
Lipomyces starkeyi	Anschau et al. (2014)	33.2	2.6	8	54.4	1.3	0.5
Rhodotorula glutinis	Alvarez and Steinbuchel (2002)	36	1	3	47	8	–
Fungi	Meng et al. (2009)	7–23	1–6	2–6	19–81	8–40	4–42
M. circinelloides	Vicente et al. (2009)	20	2.3	2	37	14.3	18.5
Bacteria	Meng et al. (2009)	8–10	10–11	11–12	25–28	14–17	–
R. opacus	Alvarez and Steinbuchel (2002)	25.7	9.5	3.5	22.0	–	–
Vegetable oils							
Palm oil	Liu and Zhao (2007)	33.0	0.1	4.7	55.1	1.6	–
Soybean	Balat (2011)	11.9	0.3	4.1	23.2	54.2	6.3
Canola	Haagenson et al. (2010)	3.9	–	1.1	64.4	20.4	9.6

Tables 6 contains, respectively, the properties of FAMES collected from the literature and the results of the prediction of quality parameters of oleaginous yeast and microalgae (Lacerda et al. 2013) compared with the American, European, and Brazilian standards. The values of heat of combustion (HC), index of oxidative stability (OSI) and iodine value (II) are out of limits imposed by these standards, however, the values of density (ρ), viscosity (u) and cetane number (CN) are within it. Even though the indexes are slightly out of bounds, the biodiesel obtained from Chlorella vulgaris and *Lipomyces starkeyi* may still be used as fuel.

Biodiesel produced by *Lipomyces starkeyi* has the advantage of being deficient in of C18:3, which is limited to 12 % by EN 14214. *Chlorella vulgaris* has an advantage if compared to mitigation of carbon dioxide associated with the production of biofuel. In both cases, the FA profile showed compounds commonly found in biodiesel. The results of the prediction quality are classified into three available standards, demonstrating the potential of *C. vulgaris* and *L. starkeyi* oil as raw materials for biodiesel production (Lacerda et al. 2013).

Yeasts have several difficulties for lipid extraction, including the presence of a thick cell wall that renders the yeast cells resistant to many solvents, as well as the possible presence of lipases in their cell extracts, and most of the neutral lipids are intracellularly stored in lipid bodies. However, lipid bodies also contain other lipophilic compounds, in particular aromatic compounds, which are difficult to remove during lipid purification (Ageitos et al. 2011). Table 7 shows the profile of TAG for some lipid extraction methods from *L. starkeyi*.

Table 6 Comparison of the results with the limits set by the rules

	ρ (kg m^{-3})	υ (mm^2 s)	ΔHC (kJ g^{-1})	OSI (h)	CN	II (gI2/100 g)	% C18:3
Chlorella vulgaris[a]	863	3.9	39.2	13.8	53	97	12.72
Lipomyces starkeyi[a]	864	4.4	39.7	12.5	63	49	0.00
ASTM D6751	–	1.9–6.0	–	–	Min 47	–	–
EN 14214/ EN14213	860–900	3.5–5.0	35	Min 6 e 4	Min 51	Max 120/130	12
ANP 255/2003	–	–	–	–	Min 45	–	–

ρ density, υ viscosity, ΔHC heat of combustion, *OSI* index of oxidative stability, *CN* cetane number, II iodine value
[a]Results from Lacerda et al. (2013)

Table 7 Lipid extraction methods from *L. starkeyi*

Solvents	Polarity	Extraction efficiency (%)	TAG (%)	DAG (%)	MAG (%)
Soxhlet					
Hexane	0.1	5.06	64.14	20.64	15.20
Chloroform:Methanol (2:1)	4.4	95.41	60.71	20.14	19.13
Ethanol	4.3	65.05	35.93	18.66	45.39
Butt					
Hexane	0.1	56.06	58.47	14.93	26.58
Chloroform:Methanol (2:1)	4.4	90.32	47.50	10.03	42.46
Ethanol	4.3	66.40	45.97	19.46	34.56
Cell hydrolysis					
Hexane	0.1	89.32	81.03	10.10	8.9

TAG triacylglycerols, *DAG* diacylglycerol, *MAG* monoacylglycerol. Adapted from Reis et al. (2012)

Glycerides are desirable for biodiesel production mainly for alkaline transesterifications (Chisti 2007). Acylglycerols generally have a lower degree of unsaturation than other lipid fractions (i.e., polar lipids) and produce FAME with higher oxidation stability (Halim et al. 2012).

From a technological point of view, the TAG profile represents a key to the understanding of the several physical properties of an oil or fat. The ability to change the physical properties of fats has attracted great interest in recent years. Information on the TAG profile is needed to control these processes (Buchgraber et al. 2004).

The microbial oil production process requires four main steps: cell disruption, oil extraction, separation of value-added compounds (PUFAs), and esterification/

cracking of lipids. The improvement of each of these steps demonstrates the use and application of these lipids for biofuels, food and cosmetic industries, jet fuels, health supplements, and PUFA.

5 Genetic and Metabolic Engineering to Enhance Microbial Lipids

In the last years metabolic and genetic engineering has established itself as an enabling technology for biofuel development. Those tools are able to modify microorganisms aiming to improve their natural features and even introduce new characteristics. Metabolic engineering emerged approximately 15 years ago as a distinct field that is differentiated from genetic engineering by its focus on the properties of biosynthetic and metabolic pathways in their entirety, instead of single genes and enzymes (Liang and Jiang 2013). Oleaginous microorganisms able to use lignocellulosic hydrolysates as some yeasts, molds, bacteria, and microalgae can be engineered to be a lipid platform production.

As was presented in this chapter, only few microorganisms are able to convert sugars, from lignocellulosic materials, into lipids. However, these native microorganisms, which are derived from environmental isolates, are often distinct from the microorganisms that are traditionally used and that have been proved to be effective in industrial processes. Furthermore, these isolated strains typically suffer from a lack of genetic and molecular biology traditional tools and therefore require much scientific investment to transform them into modifiable production platforms. Nevertheless, the innate capacity and potential of these cells—which may be able to use recalcitrant substrates or withstand toxic products—is immense (Alper and Stephanopoulos 2009).

To overcome this lack of traditional genetic and metabolic tools, that can be used to genetically manipulate those strains, other genetic approaches as site-direct and random mutagenesis, as made for yeast *Lipomyces starkeyi* (Tapia et al. 2012), which presented a productivity increase of 15.1 % in biomass and 30.7 % in lipid productivity, and microalga *Isochrysis affinis galbana* (Bougaran et al. 2012) resulted in a 1.8-fold increase for neutral lipid productivity, could be important to enhance their natural capacity to storage lipids. However, it is necessary to understand the metabolic pathways linked to lipid material metabolism, in order to select the genes and the best approach to metabolic engineering.

Oleaginous microorganisms do not have high oil levels under balanced nutrient conditions; however, when a given substrate is limiting—typically nitrogen—they channel carbon sources into lipid formation. It has been shown that phosphorus limitation has some advantages; for example, the nitrogen content of the substrate biomass does not influence lipid accumulation. FA synthesis is the first step of lipid accumulation. Subsequently, phospholipid (PL) and triacylglycerol (TAG) (also known as “neutral lipid”) synthesis results in membrane and reserve lipid accumulation, respectively. Conversion of these lipids into FA short-chain alcohol esters

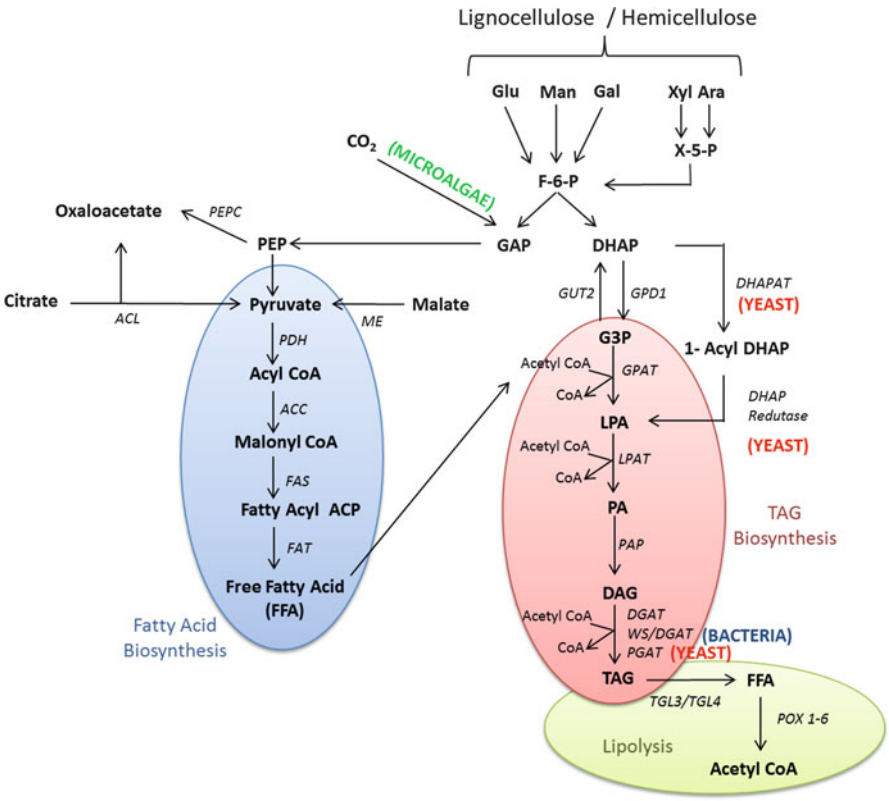

Fig. 6 The fatty acid and TAG biosynthesis pathway in microorganisms. For microalgae, both inorganic carbon (CO_2) and organic carbon sources (glucose) can be utilized for lipid production. For yeasts, de novo formation of LPA can occur either through the G3P or DHAP pathways. In yeasts, the DGAT and PDAT catalyze TAG formation. In *Acinetobacter calcoaceticus* ADP1 (bacteria), WS/DGAT exhibits the DGAT activity. *GAP* glyceraldehyde 3-phosphate, *DHAP* dihydroxyacetone phosphate, *PEP* phosphoenolpyruvate, *ACP* acyl-carrier protein, *FFA* free fatty acid, *G3P* glycerol-3-phosphate, *LPA* lysophosphatidate, *PA* phosphatidate, *DAG* diacylglycerol, *CDPDAG* CDP-diacylglycerol, *TAG* triacylglycerol, *PDH* pyruvate dehydrogenase, *PEPC* phosphoenolpyruvate carboxylase, *ME* malic enzyme, *ACL* ATP citratelyase, *ACC* acetyl-CoA carboxylase, *MAT* malonyl-CoA: ACP transacetylase, *FAS* fatty acid synthetase, *FAT* acyl-ACP-thioesterase, *GPAT* glycerol-3-phosphate acyltransferase, *LPAT* lysophosphatidate acyltransferase, *PAP* phosphatidic acid phosphatase, *DGAT* diacylglycerol acyltransferase, *WS/DGAT* wax ester synthase/acyl-CoA-to-diacylglycerol acyltransferase, *PDAT* phospholipid: diacylglycerol acyltransferase, *DHAPAT* DHAP acyltransferase, *GPD1* and *GUT2* encoding glycerol 3-phosphate dehydrogenase, *TGL3* and *TGL4* encoding triacylglycerol lipases, *POX1–6* encoding the six acyl-CoA oxidases [Adapted from Liang and Jiang (2013)]

[mainly methanol (FAME) and ethanol (FAEE)] is the final step in biodiesel production (Kosa and Ragauskas 2011). The enzymes and steps that lead to TAG accumulation in eukaryotes and prokaryotes microorganism, using as carbon sources sugars from lignocellulosic biomass, are shown in Fig. 6.

The genetic engineering of oleaginous microorganism is focused in enhancing the FFA and TAG biosynthesis by overexpressing main enzymes linked to those metabolic routes, as ACC1, FAT, ME, and ACL (free FA biosynthesis) and GPAT, LPAT, GPD1, and GUT2 (TAG biosynthesis), or partially blocking competing pathways as lipolysis. Furthermore a multigene transgenic approach can be used in order overexpressing more than one key enzyme in the TAG pathway to enhance lipid biosynthesis as showed in Table 8.

Efficient fermentation of hemicellulosic sugars is critical for the bioconversion of lignocellulosics to lipids. While most hexoses are readily phosphorylated as soon as they enter the cell, hemicellulosic sugars must go through several biochemical steps before phosphorylation. Eukarya and bacteria use two distinct pathways each for the assimilation of D-xylose and L-arabinose (Van Vleet and Jeffries 2009) as showed in Fig. 7.

6 Future Perspectives

Only few studies about oleaginous microorganisms have used metabolic and genetic engineering tools to improve metabolism of sugars: oleaginous fungi (*Mortierella isabellina*) and yeasts (as *Lipomyces starkeyi* and *Rhodotorula glutinis*) have a good capacity to assimilate those sugars, and also, majority of autotrophic microalgae are used to accumulate lipids. The lack of traditional genetic and metabolic tools for those nonconventional species, as discussed before, also contributes to the small number of works in this area. However some studies have used heterologous pathways to introduce xylose assimilation capacity in wild strains that accumulate good amounts of lipids but are not able to metabolize xylose. Bacteria *Rhodococcus* sp., which is known as high lipid production strain, was engineered to express heterogeneously two well-selected genes, xylA, encoding xylose isomerase, and xylB, encoding xylulokinase from *Streptomyces lividans* TK23, under the control of the tac promoter with an *Escherichia coli-Rhodococcus* shuttle vector. Lipid produced from xylose by recombinants of *R. jostii* RHA1 and *R. opacus* PD630 carrying xylA and xylB represented up to 52.5 and 68.3 % of the CDW, respectively (Xiong et al. 2012). Microalgae are also engineered to metabolize xylose as described by Solazyme patent (Chua and Somanchi 2012).

Metabolic and genetic engineering and further other genomics, transcriptomics, and metabolomics tools provide more information and new ways on enhancing lipid production in oleaginous microorganisms, optimizing FA profiles, enhancing lipid accumulation, and improving the use of low-cost raw materials as lignocellulosic hydrolysates.

Table 8 Metabolic engineering strategies employed to enhance lipid biosynthesis in oleaginous organisms

Genes (enzymes)	Source—species	Receiver—species	Note	References
FFA biosynthesis				
accA, accB, accC, accD, (ACC), tesA (thioesterase I)	*E. coli* (BL21) (bacteria)	*E. coli* (BL21) (bacteria)	6× fatty acid synthesis	Davis et al. (2000)
Acc1 (ACC)	*Cyclotella cryptica* (algae)	*Cyclotella cryptica* (algae)	2–3× ACC activity, no change in lipid content	Dunahay et al. (1996), Dunahay et al. (1995)
Acc1 (ACC)	*Yarrowia lipolytica* (yeast)	*Yarrowia lipolytica* (yeast)	2× lipid content	Tai and Stephanopoulos (2013)
Acc1 (ACC)	*Mucor rouxii* (fungi)	*Hansenula polymorpha* (yeast)	+40 % fatty acid content	Ruenwai et al. (2009)
FAT	*Ricinus communis* (plant)	*E. coli* ML103 (bacteria)	>2.0 g/L fatty acid content	Zhang et al. (2011)
Malic enzyme (ME)	*Mortierella alpina* and *Mucor circinelloides* (fungi)	*Mucor circinelloides* (fungi)	2.5× lipid accumulation	Zhang et al. (2007)
ACL	*Aspergillus oryzae* (fungi)	*Aspergillus oryzae* (fungi)	1.7× fatty acid content	Tamano et al. (2013)
TAG biosynthesis				
ΔGUT2	*Y. lipolytica* (yeast)	*Y. lipolytica* (yeast)	3× lipid content	Beopoulos et al. (2008)
GPD1	*Y. lipolytica* (yeast)	*Y. lipolytica* (yeast)	1.5× TAG content	Dulermo and Nicaud (2011)
ΔGUT2,GPD1	*Y. lipolytica* (yeast)	*Y. lipolytica* (yeast)	5.6× TAG content	Dulermo and Nicaud (2011)
DGAT	*Arabidopsis* (plant)	Yeast	200–600× DGAT activity	Jako et al. (2001)
Blocking competing pathways				
DAGPase	*Chlamydomonas* (microalgae)	*Chlamydomonas* (microalgae)	10× TAG content	Li et al. (2010)
ΔTGL3, ΔTGL4 (TAG lipases)	*Y. lipolytica* (yeast)	*Y. lipolytica* (yeast)	+ Lipid production	Dulermo and Nicaud (2011)
Multigene approach				
ACP, KAS, FAT	*Haematococcus pluvialis* (microalgae)	*Haematococcus pluvialis* (microalgae)	+ fat acid synthesis	Lei et al. (2012)
POX1-6 (AOXs), MFE1, GPD1, DGUT2	*Y. lipolytica* (yeast)	*Y. lipolytica* (yeast)	+ lipid accumulation	Dulermo and Nicaud (2011)

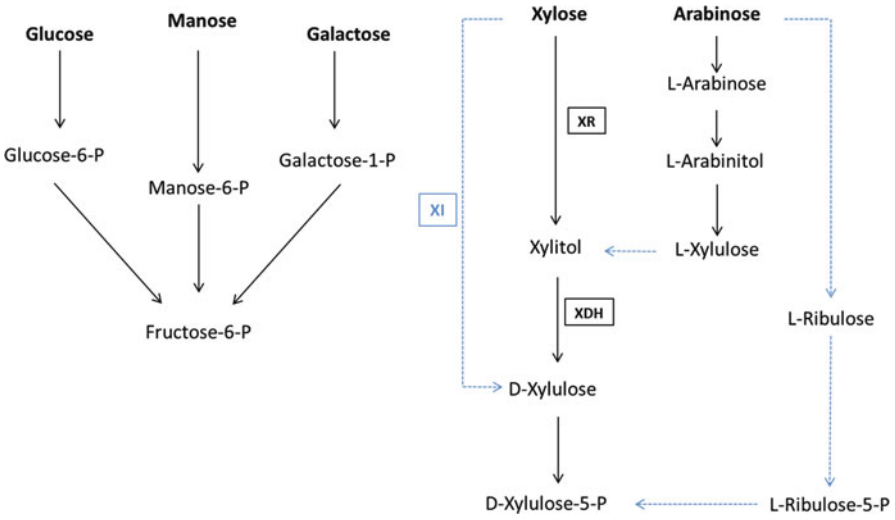

Fig. 7 Hexose and pentose pathways for Eukarya and Bacteria. Mannose, glucose, and galactose are quickly phosphorylated after uptake in the cell. Pentoses are assimilated by yeast (*solid lines*) through an oxidoreductase pathway, whose bottleneck to xylose assimilation is the imbalance redox generated by xylose reductase (XR) and xylitol dehydrogenase (XDH) distinct cofactor preference. The same does not occur in bacteria, once single step xylose assimilation is done by enzyme xylose isomerase (XI). *Blue dotted lines* represent pentose assimilation pathways for Bacteria

References

Ageitos JM, Vallejo JA, Veiga-Crespo P, Villa TG (2011) Oily yeasts as oleaginous cell factories. Appl Microbiol Biotechnol 90(4):1219–1227. doi:10.1007/s00253-011-3200-z

Alper H, Stephanopoulos G (2009) Engineering for biofuels: exploiting innate microbial capacity or importing biosynthetic potential? Nat Rev Microbiol 7(10):715–723. doi:10.1038/nrmicro2186

Alvarez HM, Kalscheuer R, Steinbuchel A (2000) Accumulation and mobilization of storage lipids by *Rhodococcus opacus* PD630 and *Rhodococcus ruber* NCIMB 40126. Appl Microbiol Biotechnol 54(2):218–223

Alvarez HM, Steinbuchel A (2002) Triacylglycerols in prokaryotic microorganisms. Appl Microbiol Biotechnol 60(4):367–376. doi:10.1007/s00253-002-1135-0

Anschau A, Xavier MCA, Hernalsteens S, Franco TT (2014) Effect of feeding strategies on lipid production by *Lipomyces starkeyi*. Bioresour Technol 157:214–222. doi:10.1016/j.biortech.2014.01.104

Aristizabal RVS, Franco TT, Caruso CS (2012) Adaptation of *Lipomyces starkeyi* DSM 70296 by evolutionary engineering in culture medium containing sugarcane bagasse hydrolysate. Paper presented at the 24th interamerican congress of chemical engineering, Uruguay

Ashraful AM, Masjuki HH, Kalam MA, Rizwanul Fattah IM, Imtenan S, Shahir SA, Mobarak HM (2014) Production and comparison of fuel properties, engine performance, and emission characteristics of biodiesel from various non-edible vegetable oils: a review. Energy Convers Manag 80:202–228. doi:10.1016/j.enconman.2014.01.037

Baboshin MA, Golovleva LA (2005) The relative value of redox potential in *Rhodococcus rhodochrous* cells as a function of their growth rate. Microbiology 74(1):118–119

Balat M (2011) Potential alternatives to edible oils for biodiesel production–a review of current work. Energy Convers Manag 52(2):1479–1492. doi:10.1016/j.enconman.2010.10.011

Beopoulos A, Mrozova Z, Thevenieau F, Le Dall MT, Hapala I, Papanikolaou S, Chardot T, Nicaud JM (2008) Control of lipid accumulation in the yeast *Yarrowia lipolytica*. Appl Environ Microbiol 74(24):7779–7789. doi:10.1128/aem.01412-08

Bougaran G, Rouxel C, Dubois N, Kaas R, Grouas S, Lukomska E, Coz J, Cadoret J (2012) Enhancement of neutral lipid productivity in the microalga *Isochrysis affinis galbana* (T-Iso) by a mutation selection procedure. Biotechnol Bioeng 109(11):2737–2745. doi:10.1002/bit.24560/abstract

Buchgraber M, Ulberth F, Emons H, Anklam E (2004) Triacylglycerol profiling by using chromatographic techniques. Eur J Lipid Sci Technol 106(9):621–648. doi:10.1002/ejlt.200400986

Cahoon EB, Schmid KM (2008) Metabolic engineering of the content and fatty acid composition of vegetable oils. In: Bohnert HJ, Nguyen H, Lewis NG (eds) Bioengineering and molecular biology of plant pathways, vol 1. Pergamon, New York, pp 161–200. doi:10.1016/s1755-0408(07)01007-7

Carvalheiro F, Duarte LC, Gírio FM (2008) Hemicellulose biorefineries: a review on biomass pretreatments. J Sci Ind Res 67(11):849–864

Chandel AK, Antunes FAF, Arruda PV, Milessi TSS, Silva SS, Almeida Felipe MG (2012) Dilute acid hydrolysis of agro-residues for the depolymerization of hemicellulose: state-of-the-art. In: da Silva SS, Chandel AK (eds) D-Xylitol: fermentative production, application and commercialization. Springer, Berlin, pp 39–61. doi:10.1007/978-3-642-31887-0_2

Chandel AK, Silva SS, Singh OV (2011) Detoxification of lignocellulosic hydrolysates for improved bioethanol production. In: Bernardes MAS (ed) Biofuel production–recent developments and prospects. InTech, Rijeka, pp 225–246. doi:10.5772/16454

Chandel AK, Singh OV, Venkateswar Rao L (2010) Biotechnological applications of hemicellulosic derived sugars: state-of-the-art. In: Singh OV, Harvey SP (eds) Sustainable biotechnology: renewable resources and new perspectives. Springer, Dordrecht, pp 63–81. doi:10.1007/978-90-481-3295-9_4

Chen X, Li Z, Zhang X, Hu F, Ryu DD, Bao J (2009) Screening of oleaginous yeast strains tolerant to lignocellulose degradation compounds. Appl Biochem Biotechnol 159(3):591–604. doi:10.1007/s12010-008-8491-x

Chen XF, Huang C, Yang XY, Xiong L, Chen XD, Ma LL (2013) Evaluating the effect of medium composition and fermentation condition on the microbial oil production by *Trichosporon cutaneum* on corncob acid hydrolysate. Bioresour Technol 143:18–24. doi:10.1016/j.biortech.2013.05.102

Cheng Y, Lu Y, Gao C, Wu Q (2009a) Alga-based biodiesel production and optimization using sugar cane as the feedstock. Energy Fuels 23(8):4166–4173

Cheng Y, Zhou W, Gao C, Lan K, Gao Y, Wu Q (2009b) Biodiesel production from *Jerusalem artichoke* (*Helianthus tuberosus* L.) tuber by heterotrophic microalgae *Chlorella protothecoides*. J Chem Technol Biotechnol 84(5):777–781

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306. doi:10.1016/j.biotechadv.2007.02.001

Chua P, Somanchi A (2012) Genetically engineered microorganisms that metabolize xylose. USA Patent WO/2012/154626, 15.11.2012

Coelho RS, Franco TT (2013) High biomass productivity from substrate sensitive microalgae. In: Paper presented at the 3rd international conference on algal biomass, biofuels and bioproducts, Toronto

Coelho RS, Vidotti ADS, Reis EM, Franco TT (2014) High cell density cultures of microalgae under fed-batch and continuous growth. Chem Eng Trans 38:313–318. doi:10.3303/CET1538053

Davis MS, Solbiati J, Cronan JE Jr (2000) Overproduction of acetyl-CoA carboxylase activity increases the rate of fatty acid biosynthesis in *Escherichia coli*. J Biol Chem 275(37):28593–28598. doi:10.1074/jbc.M004756200

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

Dunahay TG, Jaruis EE, Roessler PG (1995) Genetic transformation of the diatoms *Cyclotella cryptica* and *Navicula saprophila*. J Phychol 31:1004–1012

Dunahay TG, Jarvis EE, Dais SS, Roessler PG (1996) Manipulation of microalgal lipid production using genetic engineering. Appl Biochem Biotechnol 57/58(8):223–231

Economou CN, Aggelis G, Pavlou S, Vayenas DV (2011a) Modeling of single cell oil production under nitrogen limited and substrate inhibition conditions. Biotechnol Bioeng 108 (5):1049–1055. doi:10.1002/bit.23026

Economou CN, Aggelis G, Pavlou S, Vayenas DV (2011b) Single cell oil production from rice hulls hydrolysate. Bioresour Technol 102(20):9737–9742. doi:10.1016/j.biortech.2011.08.025

Eroshin VK, Dedyukhina EG, Satroutdinov AD, Chistyakova TI (2002) Growth-coupled lipid synthesis in *Mortierella alpina* LPM 301, a producer of arachidonic acid. Microbiology 71 (2):169–172

Fakas S, Papanikolaou S, Batsos A, Galiotou-Panayotou M, Mallouchos A, Aggelis G (2009) Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina*. Biomass Bioenerg 33(4):573–580. doi:10.1016/j.biombioe.2008.09.006

Gao CF, Zhai Y, Ding Y, Wu QY (2010) Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*. Appl Energy 87(3):756–761. doi:10.1016/j.apenergy.2009.09.006

Gao D, Zeng J, Zheng Y, Yu X, Chen S (2013) Microbial lipid production from xylose by *Mortierella isabellina*. Bioresour Technol 133:315–321. doi:10.1016/j.biortech.2013.01.132

Ge JP, Cai BY, Liu GM, Ling HZ, Fang BZ, Song G, Yang XF, Ping WX (2011) Comparison of different detoxification methods for corn cob hemicelluose hydrolysate to improve ethanol production by *Candida shehatae* ACCC 20335. Afr J Microbiol Res 5(10):1163–1168

Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R (2010) Hemicelluloses for fuel ethanol: a review. Bioresour Technol 101(13):4775–4800. doi:10.1016/j.biortech.2010.01.088

Gouda MK, Omar SH, Aouad LM (2008) Single cell oil production by *Gordonia sp* DG using agro-industrial wastes. World J Microbiol Biotechnol 24(9):1703–1711. doi:10.1007/s11274-008-9664-z

Haagenson DM, Brudvik RL, Lin H, Wiesenborn DP (2010) Implementing an in situ alkaline transesterification method for canola biodiesel quality screening. J Am Oil Chem Soc 87 (11):1351–1358. doi:10.1007/s11746-010-1607-9

Halim R, Danquah MK, Webley PA (2012) Extraction of oil from microalgae for biodiesel production: a review. Biotechnol Adv 30(3):709–732. doi:10.1016/j.biotechadv.2012.01.001

Hawkins RL (1999) Utilization of xylose for growth by the eukaryotic alga, *Chlorella*. Curr Microbiol 38(6):360–363

Heredia-Arroyo T, Wei W, Hu B (2010) Oil accumulation via heterotrophic/mixotrophic *Chlorella protothecoides*. Appl Biochem Biotechnol 162(7):1978–1995. doi:10.1007/s12010-010-8974-4

Heredia-Arroyo T, Wei W, Ruan R, Hu B (2011) Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. Biomass Bioenerg 35(5):2245–2253. doi:10.1016/j.biombioe.2011.02.036

Hetzler S, Bröker D, Steinbüchela A (2013) Saccharification of cellulose by recombinant *Rhodococcus opacus* PD630 strains. Appl Environ Microbiol 79(17):5159–5166

Huang C, Chen XF, Xiong L, Chen XD, Ma LL (2012a) Oil production by the yeast *Trichosporon dermatis* cultured in enzymatic hydrolysates of corncobs. Bioresour Technol 110:711–714. doi:10.1016/j.biortech.2012.01.077

Huang C, Chen XF, Xiong L, Chen XD, Ma LL, Chen Y (2013) Single cell oil production from low-cost substrates: the possibility and potential of its industrialization. Biotechnol Adv 31 (2):129–139. doi:10.1016/j.biotechadv.2012.08.010

Huang C, Wu H, Li R-F, Zong M-H (2012b) Improving lipid production from bagasse hydrolysate with *Trichosporon fermentans* by response surface methodology. New Biotechnol 29 (3):372–378. doi:10.1016/j.nbt.2011.03.008

Huang C, Wu H, Liu Q-P, Li Y-Y, Zong M-H (2011) Effects of aldehydes on the growth and lipid accumulation of oleaginous yeast *Trichosporon fermentans*. J Agric Food Chem 59 (9):4606–4613. doi:10.1021/jf104320b

Huang C, Zong MH, Wu H, Liu QP (2009) Microbial oil production from rice straw hydrolysate by *Trichosporon fermentans*. Bioresour Technol 100(19):4535–4538. doi:10.1016/j.biortech.2009.04.022

Hulteberg C, Karlsson HT, Børresen BT, Eklund MSH (2008) Biodiesel production from microalgae–a feasibility study. Lund University and StatoilHydro ASA, Oslo

Illman AM, Scragg AH, Shales SW (2000) Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme Microb Technol 27(8):631–635

Jako C, Kumar A, Wei Y, Zou J, Barton DL, Giblin EM, Covello PS, Taylor DC (2001) Seed-specific over-expression of an arabidopsis cDNA encoding a diacylglycerol acyltransferase enhances seed oil content and seed weight. Plant Physiol 126(2):861–874

Karatay SE, Dönmez G (2011) Microbial oil production from thermophile cyanobacteria for biodiesel production. Appl Energy 88(11):3632–3635. doi:10.1016/j.apenergy.2011.04.010

Knothe G (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 86(10):1059–1070. doi:10.1016/j.fuproc.2004.11.002

Kosa M, Ragauskas AJ (2011) Lipids from heterotrophic microbes: advances in metabolism research. Trends Biotechnol 29(2):53–61. doi:10.1016/j.tibtech.2010.11.002

Kosa M, Ragauskas AJ (2012) Bioconversion of lignin model compounds with oleaginous *Rhodococci*. Appl Microbiol Biotechnol 93(2):891–900. doi:10.1007/s00253-011-3743-z

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 48(8):3713–3729

Kurosawa K, Boccazzi P, de Almeida NM, Sinskey AJ (2010) High-cell-density batch fermentation of *Rhodococcus opacus* PD630 using a high glucose concentration for triacylglycerol production. J Biotechnol 147(3–4):212–218. doi:10.1016/j.jbiotec.2010.04.003

Lacerda LMCF, Reis EM, Anschau A, Piovani MR, Franco TT (2013) Biodiesel production from yeast and microalgae. Paper presented at the 19th national symposium of bioprocesses, Foz do Iguaçú

Lei A, Chen H, Shen G, Hu Z, Chen L, Wang J (2012) Expression of fatty acid synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under different stressors. Biotechnol Biofuels 5(1):18. doi:10.1186/1754-6834-5-18

Li X, Xu H, Wu Q (2007) Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. Biotechnol Bioeng 98 (4):764–771. doi:10.1002/bit.21489

Li Y, Han D, Hu G, Dauvillee D, Sommerfeld M, Ball S, Hu Q (2010) Chlamydomonas starchless mutant defective in ADP-glucose pyrophosphorylase hyper-accumulates triacylglycerol. Metab Eng 12(4):387–391. doi:10.1016/j.ymben.2010.02.002

Liang MH, Jiang JG (2013) Advancing oleaginous microorganisms to produce lipid via metabolic engineering technology. Prog Lipid Res 52(4):395–408. doi:10.1016/j.plipres.2013.05.002

Liang Y (2013) Producing liquid transportation fuels from heterotrophic microalgae. Appl Energy 104:860–868. doi:10.1016/j.apenergy.2012.10.067

Liang Y, Tang T, Siddaramu T, Choudhary R, Umagiliyage AL (2012) Lipid production from sweet sorghum bagasse through yeast fermentation. Renew Energy 40(1):130–136. doi:10.1016/j.renene.2011.09.035

Liu B, Zhao Z (2007) Biodiesel production by direct methanolysis of oleaginous microbial biomass. J Chem Technol Biotechnol 82(8):775–780. doi:10.1002/jctb.1744

Lobo IP, Ferreira SLC, Cruz RSD (2009) Biodiesel: quality parameters and analytical methods. Quim Nova 32(6):1596–1608

Lu Y, Zhai Y, Liu M, Wu Q (2009) Biodiesel production from algal oil using cassava (*Manihot esculenta crantz*) as feedstock. J Appl Phycol 22(5):573–578. doi:10.1007/s10811-009-9496-8

Malcata FX (2011) Microalgae and biofuels: a promising partnership? Trends Biotechnol 29 (11):542–549. doi:10.1016/j.tibtech.2011.05.005

Matos ITSR, Cassa-Barbosa LA, Neto PQC, Filho SA (2012) Cultivation of *Trichosporon mycotoxinivorans* in sugarcane bagasse hemicellulosic hydrolyzate. Electron J Biotechnol 14 (1):1–7. doi:10.2225/vol14-issue1-fulltext-2

Meng X, Ragauskas AJ (2014) Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates. Curr Opin Biotechnol 27C:150–158. doi:10.1016/j.copbio.2014.01.014

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

Mitra D, Rasmussen ML, Chand P, Chintareddy VR, Yao L, Grewell D, Verkade JG, Wang T, van Leeuwen JH (2012) Value-added oil and animal feed production from corn-ethanol stillage using the oleaginous fungus *Mucor circinelloides*. Bioresour Technol 107:368–375. doi:10.1016/j.biortech.2011.12.031

de Moretti MMS, Bocchini-Martins DA, Nunes CCC, Villena MA, Perrone OM, da Silva R, Boscolo M, Gomes E (2014) Pretreatment of sugarcane bagasse with microwaves irradiation and its effects on the structure and on enzymatic hydrolysis. Appl Energy 122:189–195. doi:10.1016/j.apenergy.2014.02.020

Muniraj IK, Xiao L, Hu Z, Zhan X, Shi J (2013) Microbial lipid production from potato processing wastewater using oleaginous filamentous fungi *Aspergillus oryzae*. Water Res 47 (10):3477–3483. doi:10.1016/j.watres.2013.03.046

Nie ZK, Deng ZT, Zhang AH, Ji XJ, Huang H (2013) Efficient arachidonic acid-rich oil production by *Mortierella alpina* through a three-stage fermentation strategy. Bioprocess Biosyst Eng. doi:10.1007/s00449-013-1015-2

Palmqvist E, Hahn-Hägerdal B (2000a) Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. Bioresour Technol 74(1):17–24

Palmqvist E, Hahn-Hägerdal B (2000b) Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresour Technol 74(1):25–33

Perez-Garcia O, Escalante FM, de Bashan LE, Bashan Y (2011) Heterotrophic cultures of microalgae: metabolism and potential products. Water Res 45(1):11–36. doi:10.1016/j.watres.2010.08.037

Pourmir A, Johannes T (2011) Engineering the microalgae strain *Chlamydomonas reinhardtii* for xylose utilization. Paper presented at the AIChE annual meeting, Minneapolis

Ratledge C (2004) Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie 86(11):807–815. doi:10.1016/j.biochi.2004.09.017

Reis EM, Anschau A, Franco TT (2012) Lipid extraction from *Lipomyces starkeyi*. Paper presented at the 26th interamerican congress of chemical engineering, Montevideo

Richmond A, Qian H (2004) Handbook of microalgal culture: biotechnology and applied phycology. Wiley, Oxford

Ruan ZH, Zanotti M, Zhong Y, Liao W, Ducey C, Liu Y (2013) Co-hydrolysis of lignocellulosic biomass for microbial lipid accumulation. Biotechnol Bioeng 110(4):1039–1049. doi:10.1002/bit.24773

Ruenwai R, Cheevadhanarak S, Laoteng K (2009) Overexpression of acetyl-CoA carboxylase gene of *Mucor rouxii* enhanced fatty acid content in *Hansenula polymorpha*. Mol Biotechnol 42(3):327–332. doi:10.1007/s12033-009-9155-y

Sakuradani E (2010) Advances in the production of various polyunsaturated fatty acids through oleaginous fungus *Mortierella alpina* breeding. Biosci Biotechnol Biochem 74(5):908–917. doi:10.1271/bbb.100001

Sangeeta MS, Pande M, Rani M, Gakhar R, Sharma M, Rani J, Bhaskarwar AN (2014) Alternative fuels: an overview of current trends and scope for future. Renew Sustain Energy Rev 32:697–712. doi:10.1016/j.rser.2014.01.023

Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: an overview. Renew Energy 37(1):19–27. doi:10.1016/j.renene.2011.06.045

Shi S, Valle-Rodriguez JO, Siewers V, Nielsen J (2011) Prospects for microbial biodiesel production. Biotechnol J 6(3):277–285. doi:10.1002/biot.201000117

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101(2):87–96. doi:10.1263/jbb.101.87

Stoutenburg RM, Perrotta JA, Nakas JP (2011) Overcoming inhibitors in a hemicellulosic hydrolysate: improving fermentability by feedstock detoxification and adaptation of *Pichia stipitis*. J Ind Microbiol Biotechnol 38(12):1939–1945. doi:10.1007/s10295-011-0981-0

Stressler T, Eisele T, Rost J, Haunschild E, Kuhn A, Fischer L (2013) Production of polyunsaturated fatty acids by *Mortierella alpina* using submerse and solid state fermentation. Chem Ing Technol 85(3):318–322

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

Tamano K, Bruno KS, Karagiosis SA, Culley DE, Deng S, Collett JR, Umemura M, Koike H, Baker SE, Machida M (2013) Increased production of fatty acids and triglycerides in *Aspergillus oryzae* by enhancing expressions of fatty acid synthesis-related genes. Appl Microbiol Biotechnol 97(1):269–281. doi:10.1007/s00253-012-4193-y

Tapia EV, Anschau A, Coradini AL, Franco TT, Deckmann AC (2012) Optimization of lipid production by the oleaginous yeast *Lipomyces starkeyi* by random mutagenesis coupled to cerulenin screening. AMB Express 2(1):64–71. doi:10.1186/2191-0855-2-64

Tsigie YA, Wang C-Y, Chi-Thanh T, Ju Y-H (2011) Lipid production from *Yarrowia lipolytica* Po1g grown in sugarcane bagasse hydrolysate. Bioresour Technol 102(19):9216–9222. doi:10.1016/j.biortech.2011.06.047

Tyburczy C, Brenna ME, DeMari JA, Kothapalli KS, Blank BS, Valentine H, McDonough SP, Banavara D, Diersen-Schade DA, Brenna JT (2011) Evaluation of bioequivalency and toxicological effects of three sources of arachidonic acid (ARA) in domestic piglets. Food Chem Toxicol 49(9):2320–2327. doi:10.1016/j.fct.2011.06.033

Van Vleet JH, Jeffries TW (2009) Yeast metabolic engineering for hemicellulosic ethanol production. Curr Opin Biotechnol 20(3):300–306. doi:10.1016/j.copbio.2009.06.001

Vicente G, Bautista LF, Rodriguez R, Gutierrez FJ, Sadaba I, Ruiz-Vazquez RM, Torres-Martinez S, Garre V (2009) Biodiesel production from biomass of an oleaginous fungus. Biochem Eng J 48(1):22–27. doi:10.1016/j.bej.2009.07.014

Vidotti ADS, Coelho RS, Franco LM, Franco TT (2014) Miniaturized culture for heterotrophic microalgae using low cost carbon sources as a tool to isolate fast and economical strains. Chem Eng Trans 38:325–330. doi:10.3303/CET1438055

Vidotti ADS, Lacerda LMCF, Franco TT (2013) Substrate inhibition of *Chlorella sp*. by acetate in heterotrophic culture. Paper presented at the 3rd international conference on algal biomass, biofuels and bioproducts, Toronto

Wang B, Rezenom YH, Cho K-C, Tran JL, Lee DG, Russell DH, Gill JJ, Young R, Chu K-H (2014) Cultivation of lipid-producing bacteria with lignocellulosic biomass: effects of inhibitory compounds of lignocellulosic hydrolysates. Bioresour Technol 161:162–170. doi:10.1016/j.biortech.2014.02.133

Wang L, Chen W, Feng Y, Ren Y, Gu Z, Chen H, Wang H, Thomas MJ, Zhang B, Berquin IM, Li Y, Wu J, Zhang H, Song Y, Liu X, Norris JS, Wang S, Du P, Shen J, Wang N, Yang Y, Wang W, Feng L, Ratledge C, Zhang H, Chen YQ (2011) Genome characterization of the oleaginous fungus *Mortierella alpina*. PLoS ONE 6(12):e28319. doi:10.1371/journal.pone.0028319

Wei A, Zhang X, Wei D, Chen G, Wu Q, Yang ST (2009) Effects of cassava starch hydrolysate on cell growth and lipid accumulation of the heterotrophic microalgae *Chlorella protothecoides*. J Ind Microbiol Biotechnol 36(11):1383–1389. doi:10.1007/s10295-009-0624-x

Wynn JP, Ratledge C (2005) Oils from microorganisms. In: Shahidi F (ed) Bailey's industrial oil and fat products, vol 3, 6th edn, Edible oil and fat products: specially oils and oil products. Wiley, Hoboken, pp 121–153

Xiong X, Wang X, Chen S (2012) Engineering of a xylose metabolic pathway in *Rhodococcus* strains. Appl Environ Microbiol 78(16):5483–5491. doi:10.1128/AEM.08022-11

Xu H, Miao X, Wu Q (2006) High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J Biotechnol 126(4):499–507. doi:10.1016/j.jbiotec.2006.05.002

Yan D, Lu Y, Chen YF, Wu Q (2011) Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production. Bioresour Technol 102 (11):6487–6493. doi:10.1016/j.biortech.2011.03.036

Yeh K-L, Chen C-Y, Chang J-S (2012) pH-stat photoheterotrophic cultivation of indigenous *Chlorella vulgaris* ESP-31 for biomass and lipid production using acetic acid as the carbon source. Biochem Eng J 64:1–7. doi:10.1016/j.bej.2012.02.006

Yu X, Zheng Y, Dorgan KM, Chen S (2011) Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid. Bioresour Technol 102 (10):6134–6140. doi:10.1016/j.biortech.2011.02.081

Zeng J, Zheng Y, Yu X, Yu L, Gao D, Chen S (2013) Lignocellulosic biomass as a carbohydrate source for lipid production by *Mortierella isabellina*. Bioresour Technol 128:385–391. doi:10.1016/j.biortech.2012.10.079

Zhang X, Li M, Agrawal A, San KY (2011) Efficient free fatty acid production in *Escherichia coli* using plant acyl-ACP thioesterases. Metab Eng 13(6):713–722. doi:10.1016/j.ymben.2011.09.007

Zhang X, Li M, Wei D, Wang X, Chen X, Xing L (2007) Disruption of the fatty acid Delta6-desaturase gene in the oil-producing fungus *Mortierella isabellina* by homologous recombination. Curr Microbiol 55(2):128–134. doi:10.1007/s00284-006-0641-1

Zhao B (2005) Toward cheaper microbial oil for biodiesel oil. J Chin Biotechnol 25:8–11

Zheng Y, Li T, Yu X, Bates PD, Dong T, Chen S (2013) High-density fed-batch culture of a thermotolerant microalga *Chlorella sorokiniana* for biofuel production. Appl Energy 108:281–287. doi:10.1016/j.apenergy.2013.02.059