

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Production of Microbial Lipids from Lignocellulosic Biomass

---

Mirela Ivancic Santek, Suncica Beluhan and  
Bozidar Santek

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.74013>

---

## Abstract

The current industrial production of the biodiesel relies mainly on vegetable oils that could result in the shortage of edible oils in food markets and increase in their prices. Microbial lipids produced by oleaginous microorganism have attracted a lot of attention in the recent years as a source of high-value polyunsaturated acids as well as alternative feedstock for the production of biodiesel. However, the production of microbial oils faces a number of problems concerning the costs of lipid extraction, carbon source and operational cost for microbial cultivation in conventional stirred tank bioreactor which makes production economically unfeasible. Non-food feedstocks, lignocellulose biomass and different waste streams containing lignocellulose, are low-cost sources of renewable carbon that could significantly reduce the production cost of microbial lipids. This review analyses the current production of microbial lipids from lignocellulose feedstocks and gives an overview of the main stages in the process of lipid production, pretreatment and hydrolysis of the feedstock and microbial cultivation. Cultivation of oleaginous microorganisms has been conducted by submerged cultivation and solid state fermentation. Three process configurations have been used in the lipid production including, separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP). Implementing the biorefinery concept that includes co-production of different value-added products (polyunsaturated fatty acids, amino acids, lignin and pigments) could improve the feasibility of lipid production bioprocess.

**Keywords:** biodiesel, cellulase, lignocellulose, microbial lipids, value-added products

---

## 1. Introduction

Biodiesel is renewable, biodegradable and non-toxic transport fuel composed of fatty acid methyl esters. It is produced by transesterification of triacylglycerols with alcohol (mostly methanol and ethanol) in the presence of alkaline catalyst (e.g. sodium hydroxide). Common feedstocks for the production of biodiesel are different vegetable oils including rapeseed oil, palm oil, cooking oil, soybean oil and sunflower oil [1]. Production of biodiesel increased steadily in the last few years. In year 2016, the United States and Brazil were the world's biggest biodiesel producers with a production volume of around 5.5 and 3 billion liters, respectively [2]. All existing diesel engines, vehicles and infrastructure can run on pure biodiesel (B100) or blends with petroleum diesel fuel without any change [1]. Use of biodiesel has positive environmental impact, improves energy supply security, stimulates economic development and generates employment especially in the rural areas [3]. It reduces harmful emission characteristic for diesel exhaust such as particulate matter, carbon monoxide and total unburned hydrocarbons. Additionally, emission of toxic compounds including vapor-phase hydrocarbons from C1 to C12, aldehydes and ketones up to C8 and polyaromatic hydrocarbons and nitrated polyaromatic hydrocarbons are also decreased [4].

Microbial lipids are viewed as an alternative feedstock for the biodiesel production because fatty acid compositions of accumulated lipids are similar to vegetable oils currently used as feedstock for the production of first generation biodiesel. Microbial lipids are also known as single cell oils (SCO), and are produced by heterogeneous group of oleaginous microorganisms that include less than hundred species of different microbial species including yeasts, fungi, bacteria and algae [3, 4]. Oleaginous microorganisms have the ability to accumulate significant amounts of intracellular lipids (more than 20% of their dry weight), mostly triacylglycerols, under certain cultivation conditions. Yeast strains such as *Lipomyces starkeyi*, *Rhodospiridium toruloides*, *Rhodotorula graminis*, *Rhodotorula glacialis* and *Trichosporon oleaginosus* can accumulate intracellular lipids from 50 to 80% (w/w) under certain cultivated conditions [5–8]. The fatty acid composition of lipids depends on the microbial strain and the cultivation conditions used. The most common fatty acids are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids [9]. Microbial lipids of some oleaginous microorganisms are valuable source of polyunsaturated fatty acids that are used as additive for different food products and in nutraceuticals. Following omega-3 and omega-6 fatty acids are commercially produced using different wild-type and genetically modified oleaginous microorganisms such as  $\gamma$ -linolenic acid (GLA, C18:3, n<sup>-6</sup>) by *Mucor circinelloides*; dihomogamma-linoleic (DGLA) (20:3, n<sup>-6</sup>) by *Mortierella alpina* 1S-4; eicosapentaenoic acid (EPA) (20:5, n<sup>-3</sup>) by *Mortierella alpina* ST1358 and *Yarrowia lipolytica*; docosahexaenoic acid (DHA, 22:6, n<sup>-3</sup>) by *Cryptocodinium cohnii*, *Schizochytrium* and *Ulkenia* sp. and arachidonic acid (ARA, 20:4, n<sup>-6</sup>) by *Mortierella alpina* [10–17]. Microbial lipids from oleaginous yeast strains can be used as substitute for cocoa-butter and shea butter [18]. In comparison to vegetable oils, biodiesel production from microbial lipids have a number of advantages such as heterotrophic oleaginous microorganism grow much faster than the terrestrial crops; no need for arable land for cultivation; growth as well as cultivation does not depend on whether conditions and elimination of conflict between food and food supply chain [19]. Yeasts and fungi are favored oleaginous

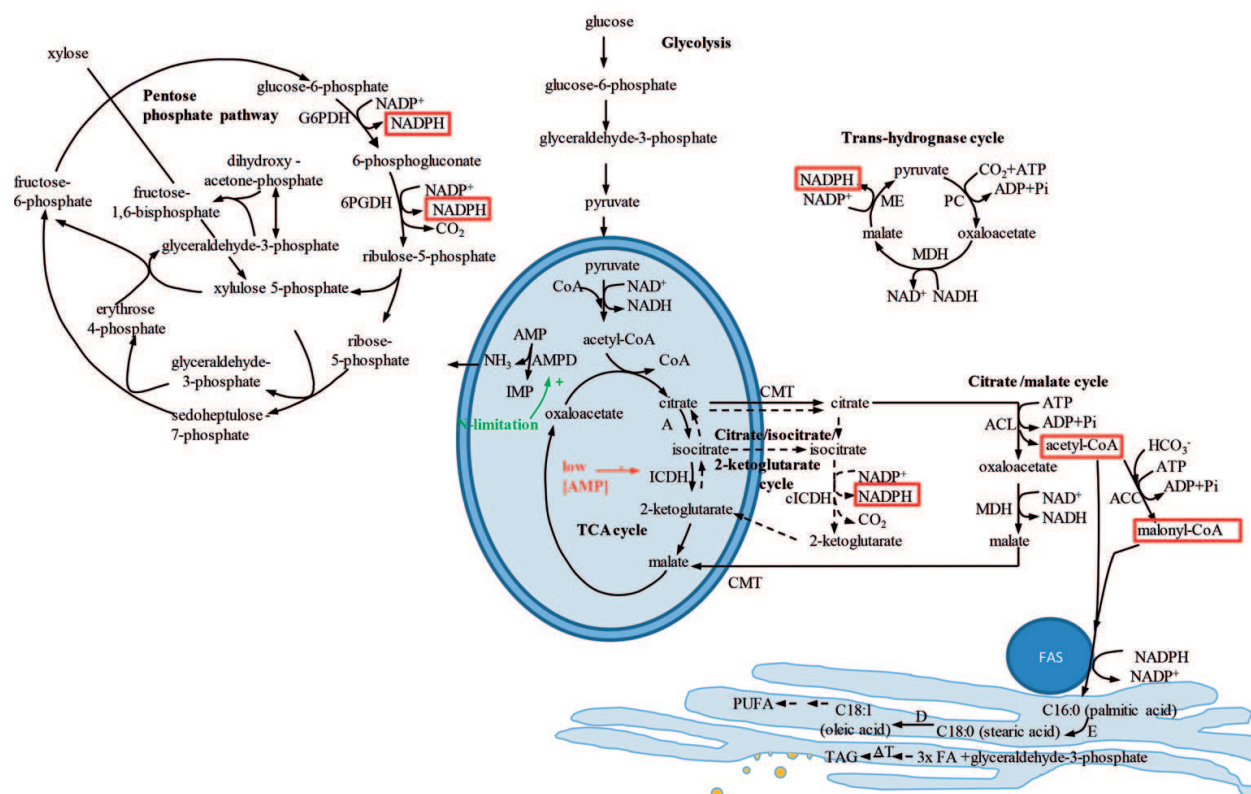
microorganism since they grow much faster than the microalgae. Unlike microalgae, they can use more diverse sugars and other carbon sources for their growth and lipid synthesis monosaccharides (glucose and xylose), amino sugars (N-acetylglucosamine), disaccharides (lactose, galactose, mannose, cellobiose and sucrose), alcohols (glycerol, ethanol and methanol), polysaccharides (starch and pectin) and organic acids (volatile fatty acids and acetic acid) [5, 8, 20–24].

In order to increase economic feasibility of the process production, different low-cost substrates have been used for the production such as crude glycerol, lignocellulose biomass (hydrolysate sweet sorghum bagasse, rice straw hydrolysate and corn stover hydrolysate), molasses waste, waste streams from food industry (whey permeate, olive pomace oil and olive oil mill wastewaters) and waste spent yeast from the brewing industry [5, 25–31].

## 2. Biochemistry of lipid accumulation

The fatty acid biosynthetic pathway in most of oleaginous microorganisms is similar to non-oleaginous microorganisms. Two features of oleaginous microorganisms make them an efficient producer of lipid such as ability to efficiently produce precursor acetyl-CoA and cofactor NADPH needed for fatty acid synthesis. Process of lipogenesis could be divided in two steps, synthesis of precursor acetyl-CoA followed by biosynthesis of triacylglycerols (**Figure 1**). Nitrogen starvation in the presence of excess of carbon sources triggers *de novo* synthesis of lipids in oleaginous microorganisms. Depletion of other media component like phosphorus or sulfur can efficiently induce lipogenesis [16]. Exhaustion of the nitrogen source induces a series of the consecutive biochemical reaction in the cell. The activity of AMP deaminase (AMPD) is upregulated. It cleaves the AMP to inosine monophosphate (IMP) and ammonia ions that cell can use as a nitrogen source. Consequently, concentration of AMP is reduced and the activity of NAD<sup>+</sup> (NADP<sup>+</sup>)-dependent isocitrate dehydrogenase (ICDH) is down-regulated. This enzyme in oleaginous microorganisms is allosterically regulated by its activator AMP. Isocitrate accumulates in mitochondria and isomerized to citrate by aconitase (A). Accumulated citrate is transported into the cytoplasm in exchange for malate (citrate/malate translocase, CMT). In the cytoplasm, ATP citrate lyase (found only in oleaginous microorganisms) converts citrate to acetyl-CoA and oxaloacetate [32]. The acetyl-CoA is used for fatty acid synthesis while oxaloacetate is converted to malate by malate dehydrogenase (MDH) and exported to mitochondria *via* CMT [16, 32].

The synthesis of lipids depends on efficient supply of NADPH, which is used for acetyl group reduction in the growing acyl chain. For the synthesis of 1 mol of a C18 fatty acid, 16 mol of NADPH is required. There is no unique metabolic route for generating NADPH in the oleaginous microorganism. Ratledge [33] described several routes for the synthesis of NADPH in the cytosol during lipogenesis. Transhydrogenase cycle which includes three enzymes pyruvate carboxylase (PC), MDH and malic enzyme (ME) has been proposed as a major route for the NADH production in the oleaginous microorganism. However, presence of ME in cytosol was not confirmed in some yeast species [16, 33]. In yeast *Y. lipolytica*, ME is located in the mitochondria and therefore cannot participate in the lipid synthesis [34].



**Figure 1.** Overview of major metabolic pathways involved in lipid synthesis. The precursors for fatty acid synthesis, acetyl-CoA, malonyl-CoA and NADPH, are highlighted (red rectangles). Green and red arrows indicate upregulation and downregulation of the key enzyme for lipid accumulation. Abbreviations used for enzymes and metabolic intermediates: 6PGDH: 6-phosphogluconate dehydrogenase; AT: acyltransferase, cytosolic; ACC: acetyl-CoA carboxylase; ACL: ATP:citrate lyase; AMPD: AMP deaminase; cICDH: NADP<sup>+</sup>-dependent isocitrate dehydrogenase; CMT: citrate-malate translocase; D: desaturase; E: elongase; FAS: fatty acid synthetase; G6PDH: glucose-6-phosphate dehydrogenase; ICDH: isocitrate dehydrogenase; MDH: malate dehydrogenase; ME: malic enzyme; PC: pyruvate carboxylase; FA: fatty acid; IMP: inosine monophosphate; PUFA: polyunsaturated fatty acid; TAG: triacylglycerol. Adapted from [16, 33].

Furthermore, expression of this enzyme is not changed upon limitation of the cell growth by nitrogen source [35]. Recent studies confirmed that primary source of NADPH for lipid synthesis in *Y. lipolytica* is the Pentose phosphate pathway [34, 36]. The NADPH is generated by enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). Additional NADPH could be also provided by the cytosolic NADP<sup>+</sup>-dependent isocitrate dehydrogenase (cICDH) present in some eukaryotic organisms (citrate/isocitrate/2-ketoglutarate cycle) [16, 32, 33].

The *de novo* fatty acid biosynthesis takes place in cytosol on a multifunctional enzyme complex called fatty acid synthetase (FAS). FAS is fed by three precursors needed for the fatty acid synthesis such as acetyl-CoA, malonyl-CoA and NADPH. Malonyl-CoA is synthesized by carboxylation of acetyl-CoA with the enzyme acetyl-CoA carboxylase (ACC). The end products are saturated fatty acids C16 (palmitic acid) or C18 (stearic acid) depending on the microorganism. Fatty acids are further elongated and desaturated by specific elongases (E) and desaturases (D) in the endoplasmic reticulum leading to fatty acids of different chain length and degree of unsaturation. The final step is triacylglycerol formation from glycerol-3-phosphate and fatty acids catalyzed by specific acyltransferases (AT). Neutral lipids including



triacylglycerols form lipid droplets on the lumenal and/or cytoplasmic side of the endoplasmic reticulum membrane [16].

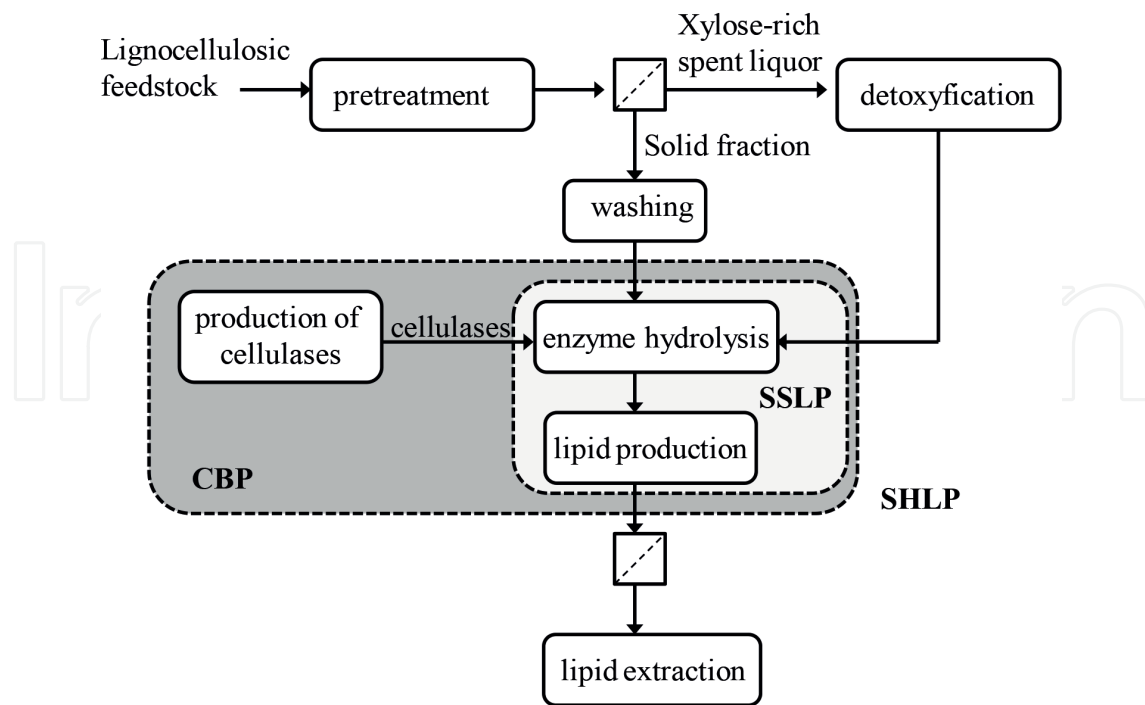
### **3. Lignocellulose biomass as carbon source for microorganism growth**

Lignocellulose is complex biopolymer composed of the polysaccharides (cellulose and hemicellulose), amorphous polymer lignin and a remaining smaller part including pectin, protein, extractives and ash. The structural carbohydrates, which accounts for approximately two thirds of the total dry weight of the lignocellulosic biomass, can be used as carbon source for microbial production of biofuels after hydrolysis to fermentable sugars. Composition of lignocellulosic biomass varies depending on the plant source. For example, the agriculture residues like rice, rye and wheat straw contains less cellulose (approximately 30%) than hardwood including poplar, pinewood and spruce (>40%) [37–42]. However, digestibility of carbohydrates in the native lignocellulosic biomass by cellulases is low due to its structural features. Structural features of lignocellulosic biomass are determined by its chemical composition (content of lignin, hemicellulose and acetyl groups bound to hemicellulose) and physical characteristics (accessible surface area, i.e., porosity, crystallinity and degree of cellulose polymerization, the physical distribution of lignin in the biomass matrix, pore volume and biomass particle size) [43]. Lignocellulosic biomass is subjected to pretreatment process which breaks down the native structure and exposes cellulose fibers to hydrolytic enzymes improving the yield of fermentable sugars. For the past three decades, various methods for the pretreatment of the lignocellulosic biomass have been developed. The pretreatment process is considered as one of the most expensive steps in the production of lignocellulosic biofuels. The estimated cost of pretreatment process in bioethanol production is approximately 30 US cent per gallon of ethanol [44]. The pretreatment processes are classified in the following groups: physical (milling, grinding, pyrolysis, extrusion and gamma-ray irradiation), chemical (alkali hydrolysis, dilute acid hydrolysis, organosolv process and oxidative delignification), physicochemical (steam explosion/autohydrolysis, ammonia fiber explosion, CO<sub>2</sub> explosion and SO<sub>2</sub> explosion), biological (biochemical degradation using white-, brow- and soft-rot fungi and lignin-degrading enzymes) and combination of these methods. During pretreatment process, a number of degradation products are formed: furan aldehydes (furfural and 5-hydroxymethyl furfural), aromatic compounds (vanillin, syringaldehyde and 4-hydroxybenzoic acid), aliphatic acids (acetic, formic and levulinic) and inorganic compounds [44–46]. During the pretreatment process at high temperature and pressure, hemicellulose is hydrolysed mainly to xylose and lesser extent to glucose. Furan and 5-hydroxymethyl furfural (HMF) are formed by dehydration of released xylose and glucose, respectively [45, 47, 48]. Acetic acid is formed by hydrolysis of acetyl groups in hemicellulose. Formic and levulinic acid are derived from furan aldehydes during prolonged exposure to high temperature in an acidic environment. Formic acid is formed by furfural and HMF degradation, while levulinic acid is generated from HMF. Concentration of HMF in lignocellulosic hydrolysate is much lower than the furfural due to limited hydrolysis of hexose from lignocellulosic biomass. The third group of degradation product includes diverse phenolic compounds which are derived from lignin

and extractive compounds present in the lignocellulosic biomass [45, 49–51]. The most common aromatic compounds in the lignocellulose acid hydrolysate are vanillin, syringaldehyde, 4-hydroxybenzoic acid, ferulic acid, etc. [45, 51]. Formation of degradation by-product strongly depends on the plant source and pretreatment process (temperature, pressure, reaction time and presence of catalyst) [46–48, 51].

#### 4. Production of microbial lipids from lignocellulose biomass

The bioconversion of lignocellulose to the microbial lipids includes following steps: pretreatment of lignocellulose biomass, hydrolysis of structural carbohydrates to fermentable sugars, microbial production of lipids and isolation and purification of the product. Since most of the oleaginous microorganisms lack cellulase and hemicellulase activity, structural polysaccharides in lignocellulosic biomass has to be hydrolysed to fermentable sugars (mainly xylose and glucose) which microorganism can use as a carbon source. The structural polysaccharides are hydrolysed using cellulolytic enzymes or thermochemical process conduct at elevated temperature in the presence of concentrate acid catalyst. Enzymatic hydrolysis is preferred over thermochemical route since the reaction is carried out under mild conditions (pH and temperature) in non-corrosive environment. Furthermore, inhibitors that could potentially inhibit the microorganism are not formed [65–67]. The major drawbacks of enzymatic hydrolysis are longer hydrolysis time, higher price of enzyme and inhibition by end products [67–70]. Production of the oleaginous lipids from lignocellulosic biomass is carried out using three process configurations such as separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP, **Figure 2**). The production of the lipids by SHLP involves two separate steps, enzymatic hydrolysis of lignocellulose followed by lipid production, while in SSLP these steps are integrated and carried out simultaneously in one vessel. In SHLP both steps are run under optimal conditions for microorganism (pH = 4.8–6.0, T = 25–30°C) and cellulases (pH = 4.5–6.0; T = 50–60°C) [25, 71, 72]. However, inhibition of cellulase by accumulated glucose and cellobiose decreases the yield of fermentable sugars. In SSLP, sugars released by hydrolysis are simultaneously assimilated by microorganism minimizing the inhibition effect by the end-product. Elimination of enzyme inhibition enhances the rate of carbohydrate hydrolysis and shortens the process time. Since the enzyme hydrolysis and microorganism growth are carried out in one vessel, the number of vessels needed for the process is reduced, decreasing the capital costs. The main disadvantage of SSLP in comparison to SHLP is the necessity of running the process at temperature favorable for the microbial growth (T = 30–32°C) which is usually suboptimal for the cellulase hydrolysis [67]. To compensate lower activity at the process temperature, enzyme loading is increased. Alternatively, lipids could be produced in a process known as ‘Consolidate bioprocessing’, which gain much attention in the production of lignocellulosic bioethanol [73]. CBP integrates cellulase production, carbohydrate hydrolysis and lipid production in one step. Besides high lipid productivity and titer, the industrially viable CBP-strain has to efficiently secrete cellulases for hydrolysis of carbohydrates. Suitable microorganism for the CBP could be isolated from nature or alternatively designed by genetic engineering using two strategies already used in development of CBP yeast strain for the lignocellulosic bioethanol production [74]. The first strategy includes a heterologous expression of the cellulose degrading genes in the oleaginous



**Figure 2.** Production of microbial lipids from lignocellulosic biomass by separate hydrolysis and lipid production (SHLP), simultaneous saccharification and lipid production (SSLP) and consolidate bioprocessing (CBP).

microorganism and the second strategy includes a metabolic engineering of cellulolytic microorganism for improved lipid accumulation.

Microbial production of oleaginous lipids from lignocellulosic biomass is carried out either by submerged or by solid state cultivation.

#### 4.1. Submerged production of lipids

**Tables 1** and **2** summarize processes of lipid production by SHLP and SSLP in submerged culture. Most of the researches have been done in shake-flask cultures at 30°C, pH between 5 and 6, using 10% (v/v) of inoculum and buffer to maintain constant pH [57, 75–77]. The most favorable feedstocks for lipid production are agriculture waste, corn stover (stalks, leaves and cob) and corn cobs. Other lignocellulosic feedstocks used for lipid production include energy crops (*Panicum virgatum* and *Jerusalem artichoke*), forest residue (*Douglas fir*) and agriculture waste (sweet sorghum bagasse). The performance of the process depends on the cultivation mode (batch and fed-batch), the pretreatment method, method for carbohydrate hydrolysis, the substrate loading and type of microorganism. Acid and alkali pretreatments are the most often used methods for improving the digestibility of lignocellulose by cellulase [57, 75, 78–84]. Hydrolysis of structural polysaccharides is commonly carried out using enzymatic hydrolysis [27, 57, 75–77, 84–86]. The efficiency of cellulase hydrolysis mostly depends on pretreatment method, but also on used commercial cellulase. For efficient hydrolysis, at least 10 different enzymes are needed including enzymes from the glycoside hydrolase families 7 (CBHI, EGI), 6 (CBHII), 5 (EGII), 10 and 11 (xylanases) and 3 ( $\beta$ -glucosidases) as well as the acetyl xylan esterases. Commercial cellulase preparations are constantly improved and their prices are being reduced. Thus, Cellic CTech2 and Cellic CTech3 from Novozymes ([www.novozymes.com](http://www.novozymes.com))



Pretreatment process	Effect on lignocellulosic biomass	Disadvantage
Dilute acid hydrolysis	Hydrolysis of hemicellulose and amorphous cellulose, increase of crystallinity, increase of porosity of biomass [52–54]	Toxic and corrosive process, formation of inhibitors [40, 52, 55, 56]
Mild alkaline hydrolysis	Delignification, partial hydrolysis of hemicellulose, increase the surface area, reduction of degree of polymerization and crystallinity of cellulose [52, 53, 57–59]	Less corrosive and expressive process than dilute acid hydrolysis, formation of inhibitors, less efficient for feedstock with high lignin content [44, 52, 53, 58]
Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL)	Complete hemicellulose and minimal lignin removal, cellulose depolymerization [60]	
Ammonia fiber expansion (AFEX)	Depolymerization and deacetylation of hemicellulose, depolymerization and cleavage of lignin-carbohydrate bonds [63, 64]	Is not effective for biomass with higher lignin content, formation of some inhibitors [46, 61, 62]
Hydrothermal process	Partial hydrolysis of hemicellulose, redistribution of lignin in biomass, deacetylation of hemicellulose [56]	Does not use chemical catalyst, less corrosive, minimal formation of inhibitor [56]

**Table 1.** Overview of various pretreatment methods.

have improved cellobiohydrolases, endoglucanases,  $\beta$ -glucosidases and additional oxidative activity (auxiliary activity family 9, formerly known as GH61) for enhanced sugar yield especially at the high substrate loading [87, 88]. Spent liquors from acid pretreatment of lignocellulosic biomass are also used as a carbon source [57, 75, 78–84]. Unlike the enzymatic hydrolysate, spent liquor obtained by acid pretreatment of a lignocellulosic biomass contains lignocellulose-derived products that can inhibit microorganism growth and synthesis of product as well as the enzyme activity [47, 48].

Economically feasible process for industrial cellulosic lipid production requires high final lipid titer (**Table 2**). Most of the research has been done in batch SHLP using different oleaginous strains of yeasts. Concentration of lipid and productivity of batch SHLP process depends on lignocellulose feedstock, microorganism, pretreatment method, detoxification method and type of carbohydrate hydrolysis. As shown in **Table 2**, in most of the batch SHLP under optimized culture conditions, lipid concentration and lipid productivity was below 20 g/L and 0.15 g/L h, respectively.

Harde et al. [86] cultivated *Mortierella isabellina* on pretreated biomass and detoxified spent liquor obtained by SPORL pretreatment of *Douglas fir*. For lipid production, three strategies were investigated. First two strategies included separate processing of pretreated biomass and spent liquor. Lignocellulosic biomass was subjected to separate hydrolysis and lipid production and simultaneous saccharification and lipid production with prehydrolysis step. Third strategy included hydrolysis of whole lignocellulosic slurry, detoxification and lipid production (**Tables 2 and 3**). Lipid yield produced from whole lignocellulosic slurry was lower than those from other two strategies, where pretreated biomass and spent liquor were

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X <sup>a</sup> (g/L)	L <sup>b</sup> (g/L)	w <sub>L</sub> <sup>c</sup> (%)	Y <sub>L/S</sub> <sup>d</sup> (g/g)	Pr <sup>e</sup> (g/L/h)	Reference
Corn stover	<i>Trichosporon cutaneum</i> AS 2.571	H <sub>2</sub> SO <sub>4</sub> (0.1–1%, 140–180°C, 5–10 min)	Spent liquor from acid pretreatment	Batch	—	7.6	39	0.15	0.078	[78]
Sweet sorghum bagasse	<i>Cryptococcus curvatus</i> ATCC 20509	Microwave radiation (100°C, 4 min h)	Enzymatic hydrolysate of pretreated sweet sorghum bagasse (endoglucanase 778–1022 CMC U/g DM, β-glucosidase 126–186 pNG/g DM, xylanase 625–950 ABXU/g DM)	Batch	15.5	—	64	0.11 <sup>d*</sup>	—	[26]
Corn stover	<i>Mortierella isabellina</i> ATCC 42613	NaOH (1%, 121°C, 2 h)	Enzymatic hydrolysate of pretreated corn stover (26 FPU/g DM, substrate loading 5%)	Batch	10.9	2.48	29	—	0.027	[57]
		H <sub>2</sub> SO <sub>4</sub> (1%, 121°C, 2 h)	As above		14.1	4.78	34	—	0.05	[57]
Corn stover	<i>Trichosporon cutaneum</i>	Pre-soaking with H <sub>2</sub> SO <sub>4</sub> (190°C, 3 min)	Enzymatic hydrolysate of pretreated corn cobs (cellulase 7 FPU/g DM, substrate loading 10%)	Batch	—	0.97	—	—	0.014	[75]
Corn stover	<i>Cryptococcus curvatus</i>	Ionic liquid (1-ethyl-3-methylimidazolium acetate, 140°C, 1 h)	Enzymatic hydrolysate of pretreated corn stover (cellulase 10 FPU/g DM, cellobiase 20 CBU/g DM, xylanase 10 mg/g DM, substrate loading 5%)	Batch	16.5	7.2	43	0.138 <sup>d*</sup>	—	[79]
Jerusalem artichoke	<i>Cryptococcus</i> sp.	HNO <sub>3</sub> (0.57%, 117°C, 49 min)	Spent liquor from acid pretreatment	Batch	6.1	—	28	—	0.072	[80]
Corn cobs residue	<i>Trichosporon cutaneum</i> ACCC20271	-Unknown pretreatment conditions	Enzymatic hydrolysate of pretreated corn cob residue (cellulase 15 FPU/g DM)  substrate loading 15%)	Batch	38.4	12.3	32	0.131	0.047 <sup>f</sup>	[76]
Corn stover	<i>Cryptococcus curvatus</i> ATCC 20509	NaOH (0.5 M, 80°C, 75 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase: 20 FPU/g DM, β-glucosidase: 40 CBU/g DM, xylanase 140 U/g DM, 10% substrate loading)	Batch	27.7	—	44	0.155	0.156	[81]

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X <sup>a</sup> (g/L)	L <sup>b</sup> (g/L)	w <sub>L</sub> <sup>c</sup> (%)	Y <sub>L/S</sub> <sup>d</sup> (g/g)	Pr <sup>e</sup> (g/L/h)	Reference
Sweet sorghum stalks	<i>Lipomyces starkeyi</i> CBS 1807	No pretreatment	Enzymatic hydrolysate of sweet sorghum stalks cellulase (8 FPU/g, Celluclst 1.5 L: Novozyme 188 (β-glucosidase) at a1:5 (vol/vol)	Batch	6.4	—	29	0.077	0.033	[77]
Corn cobs	<i>Rhodotorula glutinis</i> CGMCC 2.703	Mixed acids (0.5% H <sub>2</sub> SO <sub>4</sub> + 1.5% H <sub>3</sub> PO <sub>4</sub> , 123°C)	Undetoxified spent liquor form acid pretreatment	Batch/65 h/ bioreactor cultivation	15.1	5.5	36	0.129	0.09	[82]
	As above	As above	As above	Fed-batch with constant C and N feed	75.4	30.6	39	0.146	0.15	[82]
	As above	As above	As above	Fed-batch with two stage feeding strategy (1st C + N-source, 2nd C-source)	70.8	33.5	47	0.159	0.17	[82]
Switchgrass	<i>Lipomyces tetrasporus</i> Y-11562	H <sub>2</sub> SO <sub>4</sub> (0.936%, 160°C, 15 min, 20% solids)	Undetoxified spent liquor form acid pretreatment	Batch	53.4	29.0	53	0.156	0.215	[83]
	<i>Lipomyces kononenkoae</i> Y-7042	As above	As above	As above	47.7	28.1	59	0.161	0.179	[83]
	<i>Rhodospiridium toruloides</i> Y-1091	As above	As above	As above	42.6	26.2	61	0.159	0.128	[83]
Corn stover	<i>Mortierella isabellina</i>	steam explosion (200°C, 7 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase 30 FPU/g, substrate loading 30%)	Batch	36.1	18.7	52	0.05 <sup>f</sup>	0.039	[85]
Douglas fir forest residue	<i>Mortierella isabellina</i> NRRL 1757	SO <sub>2</sub> (11 g/L, 140°C, 60 min)	Enzymatic hydrolysate of pretreated lignocellulose biomass (cellulase 14.6 FPU/g glucan, substrate loading 10%)	Batch	25.5	14.4	—	0.18	0.120 <sup>g</sup>	[86]
	As above	SO <sub>2</sub> (11 g/L, 140°C, 120 min)	As above	Batch	25.7	11.9	—	0.18	0.120 <sup>g</sup>	[86]

Feedstock	Microbial strain	Pretreatment	Cultivation media	Fermentation mode	X <sup>a</sup> (g/L)	L <sup>b</sup> (g/L)	w <sub>L</sub> <sup>c</sup> (%)	Y <sub>L/S</sub> <sup>d</sup> (g/g)	Pr <sup>e</sup> (g/L/h)	Reference
Corn stover	As above	SO <sub>2</sub> (11 g/L, 140°C, 60 min)	Detoxified spent liquor	Batch	16.05	8.4	—	0.18	0.050 <sup>h</sup>	[86]
	As above	SO <sub>2</sub> (11 g/L, 140°C, 120 min)	As above	Batch	11.6	7.7	—	0.16	0.046 <sup>h</sup>	[86]
	As above	SO <sub>2</sub> (11 g/L, 140°C, 60 min)	Detoxified enzymatic hydrolysate of whole pretreated slurry (cellulase 14.6 FPU/g glucan, substrate loading 10%)	Batch	35.4	18.55	—	0.17	0.086 <sup>i</sup>	[86]
	As above	SO <sub>2</sub> (11 g/L, 140°C, 120 min)	As above	Batch	38.2	17.6	—	0.18	0.081 <sup>i</sup>	[86]
	<i>Rhodospiridium toruloides</i>	NaOH (0.4%, 80°C, 2 h) and H <sub>2</sub> SO <sub>4</sub> (0.8%, 160°C, 10 min)	Enzymatic hydrolysate of pretreated corn stover (cellulase 40 mg protein/g cellulose- substrate loading 20%)	Batch	36.2	21.4	59	0.19	0.28	[84]
	As above	As above	As above	DO-stat fed-batch	42	25.2	60	0.23	0.33	[84]
	As above	As above	As above	Pulse fed-batch	43	26.7	62	0.24	0.35	[84]
	As above	As above	As above	Online sugar monitoring fed-batch	54	32	59	0.29	0.4	[84]

<sup>a</sup>X: Biomass concentration, g cell/L.  
<sup>b</sup>L: Lipid concentration, g lipids/L.  
<sup>c</sup>w<sub>L</sub>: Lipid content, g lipid produced/g dry cell weight.  
<sup>d</sup>Y<sub>L/S</sub>: Lipid yield, g lipid/g of consumed carbon source; <sup>d</sup>Y<sub>L/S</sub>: Lipid yield, g lipid/g pretreated lignocellulosic biomass.  
<sup>e</sup>Pr: Lipid productivity, g lipid produced/h L  
<sup>f</sup>Lipid productivity was calculated based on time for prehydrolysis (3 days) and fermentation (8 days): lipid concentration/time.  
<sup>g</sup>Lipid productivity, lipid concentration (L)/time of cultivation (216 h).  
<sup>h</sup>Lipid productivity, lipid concentration (L)/time of cultivation (120 h).  
<sup>i</sup>Lipid productivity, lipid concentration (L)/time of cultivation (168 h).

**Table 2.** Production of lipids by separate hydrolysis and lipid production (SHLP) from lignocellulosic hydrolysate.

processed separately. Despite the lower process efficiency, this approach is attractive from the economic point of view since it reduces the operational and capital costs for lipid production. Development of the strain with high tolerance toward inhibitors in spent liquor could reduce the number of steps in production and production cost. Harde et al. [86] developed sulfite tolerant strain of *M. isabellina* by gradual adaptation of the strain to inhibitors from spent liquor. The sulfite-adapted strain was able to grow in the presence of 2.0 g/L of sulfite in synthetic media and spent liquor [86].

Slininger et al. [83] designed two step screening assay for the detection of the highly productive yeast strains with high tolerance to lignocellulose-derived inhibitors. Growth media contained undetoxified enzyme hydrolysate of corn stover pretreated by ammonia fiber expansion (AFEX) and acid pretreated switchgrass. Three yeast strains, *Lipomyces tetrasporus*, *Lipomyces kononenkoae* and *Rhodospiridium toruloides* were identified. Yeast strains were able to grow on the undetoxified switchgrass hydrolysate and accumulate 25–30 g/L lipids at the rate of 0.128–0.215 g/L h with lipid yield of 0.156–0.161 g/g of consumed substrate [83]. Those values are the highest values reported in literature for batch cultivation of oleaginous microorganisms using lignocellulosic hydrolysate. Contrary to expectation, performance of the isolated oleaginous yeasts was significantly better than other used yeasts in SHLP with detoxified spent liquor (**Table 2**). Some oleaginous microorganisms show high tolerance to most of lignocellulose-derived inhibitors. Indeed, yeast strain *R. toruloides* tolerates acetate, 5-hydroxymethylfurfural and syringaldehyde at concentrations below 70, 14.7 and 12 mM, respectively. Negligible effect on growth and lipid production showed the presence vanillin and *p*-hydroxybenzoate at concentrations below 10 mM. The strongest inhibitory effect on growth and lipid accumulation had furfural. At concentration of 1 mM, biomass and lipid concentrations dropped by 45.5 and 26.5% [89].

Fed-batch mode of cultivations in production of microbial lipids has already been proved to be superior to batch cultivation. High cell and lipid concentration of 106.5 and 71.9 g/L (67.5%), respectively, were obtained in pilot scale fed-batch in a 15 L stirred tank bioreactor cultivation by yeast *R. toruloides* using glucose as a carbon source with the productivity of 0.54 g/L h [90]. Fei et al. [84] applied fed-batch cultivation mode to improve the efficiency of lipid production by *R. toruloides* using lignocellulosic hydrolysate. Different feeding strategies of the culture were investigated including dissolved oxygen-stat (DO-stat) feeding mode, pulse feeding mode and online sugar control mode. All three fed-batch strategies improved processes performance in comparison to the batch cultivation in terms of cell concentration, lipid yield and process productivity. The highest lipid yield of 0.29 g/g and lipid productivity 0.4 g/(L h) was obtained using the online sugar control feeding mode. Those values are the highest reported in the literature obtained by using concentrated enzymatic hydrolysate of lignocellulose biomass. This study represents major breakthrough in the research of lipid production from lignocellulosic biomass that could improve feasibility of the bioprocess. However, production of concentrated lignocellulosic hydrolysate (~ 550 g/L) used in research relies on the cost-intensive evaporation [84].

Therefore, developing new methods for preparation of concentrated lignocellulosic hydrolysate could improve the process economics. Fed-batch cultivation was applied in process of the



lipid production by yeast *R. glutinis* using the undetoxified spent liquor from acid pretreatment of corn cobs as a carbon source [83]. In this study, the lipid productivity was remarkably improved using two feeding strategies regarding the dynamics of nitrogen supplementation. Since yeast *R. glutinis* showed high tolerance toward inhibitors, a corn cob acid hydrolysate was used without detoxification. First strategy included feeding with concentrated undetoxified spent liquor (790.2 g/L xylose and 40.5 g/L glucose) supplemented with the nitrogen source. The second strategy included feeding of the culture for the first 80 h of cultivation with concentrated undetoxified spent liquor supplemented with nitrogen source and afterwards only with the carbon source. The highest biomass concentration of 75.4 g/L was obtained using first feeding strategy, while second feeding strategy resulted with the highest lipid concentration of 33.5 g/L, which is the highest value of lipid concentration reported in literature for culture grown on lignocellulose hydrolysate [82].

Still most of the studies on lipid production have been done by SHLP using hydrolysate of lignocellulosic biomass as a carbon source. Research by Gong et al. [79, 81] showed that the efficiency can be improved by integrating the enzyme hydrolysis and microbial process applying SSLP (**Table 3**). Two SSLP processes were conducted in cultivation media with and without the addition of nitrogen source. In cultivation media-containing alkaline pretreated corn stover without nitrogen, cells did not grow due to the lack of nitrogen and carbon sources was used for lipid production. To obtain high lipid productivity, the culture media was inoculated at high inoculum size (7.2 g/L), while control culture supplemented with nitrogen was inoculated at average inoculum size (10% v/v). The highest lipid productivity of 0.195 g/L h was obtained in SSLP without nitrogen source and this is the highest value reported for the SSLP using the lignocellulose as a carbon source. In comparison to the SHLP using the same pretreated lignocellulosic biomass (**Table 2**), the productivity of SSLP was improved and loading of cellulase and xylanase was reduced for 50%, while  $\beta$ -glucosidase was not used. The major disadvantage of this strategy is increased cost for cultivation of larger quantities of inoculum using enriched growth media [81]. Gong et al. [79] applied similar strategy using the corn stover pretreated with ionic liquids as a carbon source. However, lower lipid yields of 0.125 and 0.135 g/g DM were obtained for SSLP and SHLP, respectively [79]. SSLP was also conducted with fungus *M. isabellina* using pretreated *Douglas fir* forest residue but without success. After prehydrolysis step, fungus was able to grow in semi-solid media obtaining 17.0 g/L of lipids. However, the productivity of lipid synthesis with fungus was half of those obtained in SHLP with detoxified enzymatic hydrolysate of whole pretreated *Douglas fir* [86].

All cultivations were carried out at substrate loading of 10% (w/w) or lower suggesting possible problems with the enzyme hydrolysis and microorganism growth at higher substrate loadings. The product titer could be improved by increase of the substrate loading conducting so-called high-gravity fermentations which have been successfully applied in the bioethanol production from starch and lignocellulosic feedstock by simultaneous hydrolysis and fermentation. Significant savings in energy input, decrease of waste discharge, distillation costs and capital costs increased the competitiveness of the process [91]. However, running the SSLP under high-gravity conditions imposes a number challenges with respect to the lignocellulose-derived inhibitors and mixing and mass transfer in cultivation broth. Due to high substrate loading, the concentration of inhibitory by-products are increased and consequently lead to the

Substrate	Microbial strain	Pretreatment	Enzyme hydrolysis	Fermentation mode/time/ note	L <sup>a</sup> (g/L)	Y <sub>L/S</sub> <sup>b</sup> (g/g)	Pr <sup>c</sup> (g/L/h)	Reference
Corn stover	<i>Trichosporon cutaneum</i>	Pre-soaking with H <sub>2</sub> SO <sub>4</sub> (190°C, 3 min)	Prehydrolysis for 6 h, cellulase 7 FPU/g DM, substrate loading 10%	Batch/80 h/bioreactor	3.03	—	0.042	[75]
Corn stover	<i>Cryptococcus curvatus</i>	Ionic liquid (1-ethyl-3-methylimidazolium acetate, 140°C, 1 h)	Cellulase 4 FPU/g DM, cellobiase 8 CBU/g DM, xylanase 5 mg/g DM, substrate loading 5%	Batch/2 days/ no nitrogen source	6.0	0.112	0.125 <sup>d</sup>	[79]
Corn stover	<i>Cryptococcus curvatus</i> ATCC 20509	NaOH (0.5 M, 80°C, 75 min)	Cellulase 10 FPU/g DM, xylanase 80 U/g DM, substrate loading 10%	Batch/3 days/	11.9	0.129	0.168	[81]
	<i>Cryptococcus curvatus</i> ATCC 20509	As above	As above	Batch/3 days/ high inoculums concentration of 7.2 g/L, media without nitrogen source	15.9	0.159	0.195	[81]
Douglas fir forest residue	<i>Mortierella isabellina</i> NRRL 1757	SO <sub>2</sub> (11 g/L, 140°C, 60 min)	Prehydrolysis for 24 h, cellulase 14.6 FPU/g glucan, substrate loading 10%	Batch/168 h	17.0	0.21 <sup>b*</sup>	0.101 <sup>e</sup>	[86]
	<i>Mortierella isabellina</i> NRRL 1757	SO <sub>2</sub> (11 g/L, 140°C, 120 min)	As above	Batch/ 168 h	11.7	0.18 <sup>b*</sup>	0.070 <sup>e</sup>	[86]

<sup>a</sup>L: Lipid concentration, g lipids/L.

<sup>b</sup>Y<sub>L/S</sub>: Lipid yield, g lipid/g pretreated lignocellulosic biomass; <sup>b\*</sup> Y<sub>L/S</sub>: Lipid yield, g lipid/g theoretical sugar yield from pretreated biomass.

<sup>c</sup>Pr: Lipid productivity, g lipid produced/h L.

<sup>d</sup>Lipid productivity, lipid concentration (L)/time of cultivation (48 h).

<sup>e</sup>Lipid productivity, lipid concentration (L)/time of cultivation (168 h).

**Table 3.** Production of lipids by simultaneous saccharification and lipid production (SSLP) from lignocellulosic hydrolysate.

decrease or complete inhibition of growth and product accumulation along with their enzyme activity. Furthermore, increased viscosity of the lignocellulose slurry prohibits the efficient mixing, decreasing the heat and mass transfer (substrate, enzyme and oxygen) in bioreactor. Increasing the stirring rate in a conventional stirred tank bioreactor provides even mixing directly around the impeller, while the solid substrate settles down to the bottom and toward to the bioreactor's wall. To avoid the above motioned problems, cultivation should start with lower substrate loadings. The substrate should be gradually fed keeping the viscosity of culture broth sufficiently low (fed-batch cultivation). Kinetics of substrate additions depends on the activity of cellulolytic enzymes and substrate consumption by working microorganism and it should be experimentally optimized. Gradual addition of substrate should enable the working microorganism to adapt to increasing inhibitors concentrations and convert some of them to less toxic compounds (furfural and HMF into less toxic compounds such as furfuryl alcohol and 2,5-bis-hydroxymethylfuran, respectively) [92]. Using this strategy Elliston et al. [93] produced 11.6% (vol/vol) ethanol from waste paper in a bioreactor with high shear mixing. Gradual addition of substrate resulted in cumulative substrate loading of 65% [93].

#### 4.2. Production of lipids by solid state fermentation

Solid state fermentation offers a number of advantages over submerged cultivation in the production of microbial biomass and specific products of microbial metabolism. This technique of cultivation has been successfully used for the production of food (fermented sausages and sea food), products of microbial metabolism including antibiotics, gibberellinic acid, aflatoxines, pigments, alkaloids, organic acids and plant growth factors, enzymes, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compounds, etc. [94–96]. The major benefit of the solid state cultivation is higher bioprocess productivity, lack of catabolic repression, higher product concentration and low water and energy demands. In comparison to submerged culture, the risk of contamination is decreased due to lower water content in growth media. Furthermore, in comparison to the submerged culture, the product isolation is simpler and also less cost effective. The major drawback of solid state fermentation includes engineering problems with control of process parameters (temperature, water content, pH, substrate and oxygen concentration, etc.) and the scale-up of process to industrial size [96].

Several researches on lipid production by solid state fermentation using lignocellulose biomass have been described in literature (Table 4). Production of lipids by this type of cultivation depends on oleaginous microorganism's ability to hydrolyse the carbohydrates from lignocellulosic biomass to fermentable sugars. This bioprocess of lipid production is also called consolidate process (CBP). Desirable characteristics of CBP-strain are efficient lipid accumulation, high lipid productivity, high cellulase and hemicellulase activity and the ability to grow on insoluble substrate in the absence of free water. The oleaginous microorganisms used in the submerged production of lipid are not able to grow on the solid substrate or secrete cellulase and hemicellulase. Several fungi strains were isolated with 20–35% (w/w) of accumulated lipids in cell dry weight. Low lipid yield in solid state fermentation is a consequence of insufficient cellulolytic activity of isolated CBP-strains and low efficiency of lipid accumulation [97]. The cellulolytic activity in submerged cultivation was between 10 and 20 FPU/g and 4–15 FPU/g of dry matter of lignocellulosic biomass in SHLP and SSLP, respectively (Tables 2 and 3). The

unrestricted carbon source supply is required for the efficient growth and lipid accumulation. Therefore, enhancement of the cellulase activity in cultivation media was recognized as crucial for the improvement of bioprocess performance. Enhancement of cellulase activity was obtained by the optimization of moisture content of solid substrate, cultivation temperature, addition of complex substrates (e.g. wheat bran) and addition exogenous cellulase [97, 98]. The most promising CBP-strain for solid state cultivation is fungus *A. tubingensis* TSIP9 with high cellulase activity and moderate lipid content of 20.5% [99, 100]. Different modes of the solid state fermentation were applied to improve the lipid yield including batch, fed-batch and batch with repeated substrate replacement. Simple strategy of substrate addition in fed-batch cultivation (0.0719 g/g DM) did not improve the lipid yield in comparison to the batch cultivation (0.0799 g/g of substrate dry matter). The batch cultivation with repeated substrate replacement was the most efficient strategy for the production of lipids on the solid substrate. Repeated cycles of the batch cultivations with replacement of 90% fermented substrate with fresh one shortened the process time in comparison to the batch cultivation. Furthermore, cleaning and sterilization of the bioreactor between the batches and inoculum preparation was avoided that additionally saved the time, energy as well as labor [99]. Regardless the fermentation mode, the bioprocess efficiency of solid state fermentations was lower than in the submerged culture (Tables 2 and 3). Lipid yields in solid state fermentations were at least two times lower than the submerged cultures. In addition to strain characteristic, significant impact on process efficiency have concentration gradients of hydrogen ions, oxygen, fermentable sugars, products of metabolism formed in the layer of solid substrate during cultivation that inhibited growth of microorganism and cellulase activity.

## 5. Future perspective of lipid production from lignocellulose biomass

Microbial lipids are promising feedstock for biodiesel production, but the development of lipid production is still far from ready to be commercialized. The process of microbial lipid production is still uncompetitive with agricultural production of vegetable oils which market price is significantly lower. Techno-economic study of biodiesel production with *R. toruloides* ( $\mu = 0.54$  g/L h and  $Y_{L/S} = 0.23$  g/g) using glucose as a carbon source pointed out the main obstacles in commercialization of this process. Estimated costs for biodiesel production and microbial lipids using glucose as a carbon source are US\$5.9/kg biodiesel and US\$5.5 / kg lipids, respectively. The glucose cost accounts for 80% of the raw material used for production of biodiesel and for approximately 35% of the overall cost of biodiesel produced. Furthermore, the main generators of capital and energy costs are connected to production of microbial lipids using stirred tank bioreactors [103]. The production cost could be reduced by using low-cost substrates such as lignocellulose instead of glucose. The replacement of glucose with the lignocellulose feedstock as a carbon source reduces the cost for the raw material but also brings number issues including technical problems connected to complexity of process production and high capital costs. Due to the lack of investor interests and government assistance, the progress in development of this technological process is still very slow. The production of microbial lipid could become more economically feasible, if the biorefinery concept of co-production of different value-added products is applied. To obtain additional

Substrate	Microbial strain	Pretreatment	Fermentation mode/ time/note	Enzyme activity	$Y_{LS}$ (g/g) <sup>a</sup>	Reference
Wheat straw and wheat bran mixture	<i>Microsphaeropsis</i> sp.	Steam exploded (121°C, 1 h)	Batch (75% moisture, 10 days, 27°C ratio of wheat straw to wheat bran 4:1 g/g)	Cellulase 0.31–0.54 FPU/g DM <sup>b</sup>	0.024–0.042 <sup>e</sup>	[97]
	<i>Sclerocystis</i> sp.	As above	As above	Cellulase 0.34–0.52 FPU/g DM <sup>b</sup>	0.019–0.028 <sup>b</sup>	[97]
	<i>Phomopsis</i> sp.	As above	As above	Cellulase 0.32–0.56 FPU/g DM <sup>b</sup>	0.021–0.027 <sup>b</sup>	[97]
	<i>Cephalosporium</i> sp.	As above	As above	Cellulase 0.39–0.58 FPU/g DM <sup>b</sup>	0.026–0.034 <sup>b</sup>	[97]
	<i>Nigrospora</i> sp.	As above	As above	Cellulase 0.069 FPU/g DM <sup>b</sup>	0.023 <sup>e</sup>	[97]
Wheat straw and wheat bran mixture	<i>Microsphaeropsis</i> sp.	Steam exploded (15% water, 1.5 MPa, 10 min)	Batch (75% moisture, 30°C, 10 days, 27°C, ratio of wheat straw to wheat bran 4:1 g/g)	Cellulase: 0.32 FPU/g DM <sup>c</sup>	0.042 <sup>c</sup>	[98]
	<i>Microsphaeropsis</i> sp.	As above	As above	Addition of exogenous cellulase 10 FPU/g DM <sup>c</sup>	0.074 <sup>c</sup>	[98]
	<i>Microsphaeropsis</i> sp.	As above	Batch (75% moisture, 30°C, 10 days, 27°C, ratio of wheat straw to wheat bran 9:1 g/g)	Addition of exogenous cellulase 10 FPU/g DM <sup>c</sup>	0.08 <sup>c</sup>	[98]
Rice straw and wheat bran	<i>Colletotrichum</i> sp.	—	Batch	Cellulase 1.84 FPU/g DM	0.0682	[101]
	<i>Colletotrichum</i> sp.	—	Batch	+ Exogenous cellulase 10 FPU/g DM	0.0843	[101]
	<i>Alternaria</i> sp.	—	Batch	Cellulase 1.21 FPU/g DM	0.0603	[101]
	<i>Alternaria</i> sp.	—	Batch	+ exogenous cellulase 10 FPU/g DM	0.0817	[101]
Wheat straw and wheat bran mixture	<i>Aspergillus oryzae</i> A-4	Acid (0.7% H <sub>2</sub> SO <sub>4</sub> , 121°C, 1 h)	Batch (50–80% moisture, 6 days, 30°C, weight ration of wheat straw to wheat bran 2:8 g/g)	Cellulase: 1.69 FPU/g DM <sup>d</sup>	0.06287	[102]
Palm pressed fiber and palm empty fruit bunches	<i>Aspergillus</i> <i>tubingensis</i> TSIP9	Acid (0.5% H <sub>2</sub> SO <sub>4</sub> , 121°C, 1 h)	Batch, (65% moisture, 28°C 5 days)	Cellulase: 26.1 U/g DM <sup>b</sup> xylanase 59.3	0.0885	[100]



Substrate	Microbial strain	Pretreatment	Fermentation mode/ time/note	Enzyme activity	Y <sub>LS</sub> (g/g) <sup>a</sup>	Reference
Palm empty fruit bunch and palm kern cake	<i>Aspergillus tubingensis</i> TSIP9	Alkali (10% NaOH, 100°C, 15 min)	Batch (28°C, 5 days)	Cellulase: 11.1 U/g DM	0.0799	[99]
	<i>Aspergillus tubingensis</i> TSIP9	As above	Fed- batch (28°C, 12 days)	Cellulase: 19.0 U/g DM xylanase: 65.6 U/g DM	0.0719	[99]
	<i>Aspergillus tubingensis</i> TSIP9	As above	Repeated-batch (28°C, 12 days/ substrate was added every 3 days)	Cellulase: 18.4 U/g DM xylanase: 119 U/g DM) <sup>c</sup>	0.0919	[99]

<sup>a</sup>Y<sub>LS</sub>: Lipid yield, g produced lipid/g of dry matter of pretreated lignocellulosic biomass.  
<sup>b</sup>Cellulase activity was determined on 6th and lipid content on 10th day of cultivation.  
<sup>c</sup>Cellulase activity on 10th and lipid content on 9th day of cultivation.

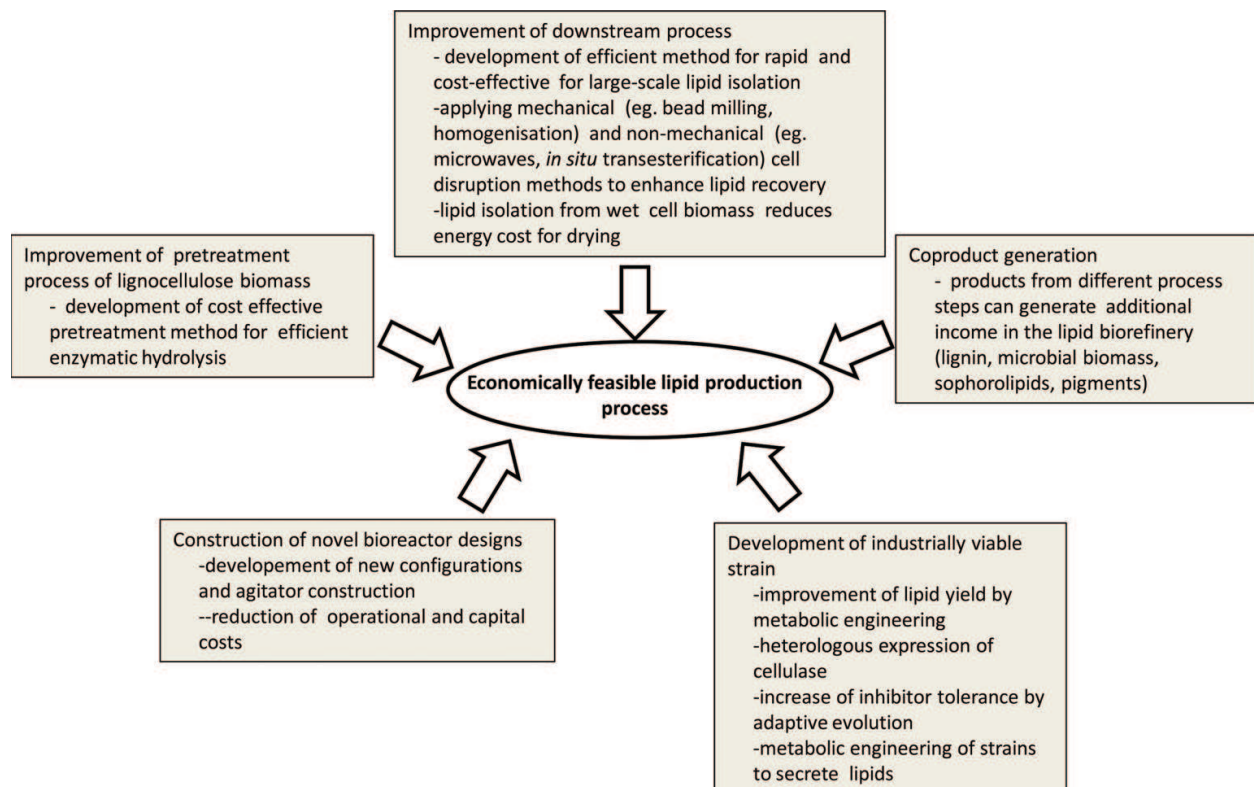
**Table 4.** Production of lignocellulosic lipids by solid state fermentation.

income, microbial fatty acids should be fractionated depending on their price; low value fatty acid should be used for biodiesel production, while high value (GLA, DHA and ARA) should be used as food supplement and in production of nutraceuticals [11–18]. Therefore, oleaginous microorganism with high content of unsaturated fatty acids such as fungus *Mortierella* sp. would be favorable for process of microbial lipids production. Other value-added products such as pigments or sophorolipids could also give additional revenue. Oleaginous yeast such as *R. glutinis*, *R. rubra* and *S. ruberrimus* accumulate valuable pigments,  $\beta$ -carotene, torulene and astaxanthin [13, 104–107]. Sophorolipids can be used as biosurfactants instead of classical chemical-derived surfactants in cosmetics, food, cleaning and petroleum industry. Unlike chemical surfactants, sophorolipids are biodegradable and also have interesting biological activities including anti-microbial, anti-cancer, anti-HIV, anti-inflammatory and anti-viral activities [108–110]. Lignin is by-products generated during pretreatment that should be separated and sold. In biorefinery, lignin can be converted to heat and power for the processing steps. Building blocks derived from lignin can be used for production of vanillin, carbon fiber, bio-oil, resin, adhesives, polymer fillers, coating agents, bioplastics, paints, soil amendment, slow nitrogen release fertilizers, rubbers, elastomers and microbial agents. Proteins from lignocellulosic biomass and microbial biomass after lipid isolation could also be used as animal feed or after acid hydrolysis to amino acids could be used as building blocks for the synthesis of different chemicals [111–114]. Significant influence on production cost of biodiesel has process of lipid recovery from cell biomass. Lipid isolation on laboratory scale is based on laborious and expensive isolation protocols that include cell harvesting by centrifugation, energy-intensive step of biomass drying and lipid extraction using an organic solvent.

Therefore, a new cost-effective method for isolation of lipids from wet cell biomass is needed to improve the competitiveness of the process [115].

## 6. Conclusions

The current production of microbial lipids from lignocellulose biomass faces a number obstacles associated with low lipid yield of producing strains, low tolerance of microbial strains to lignocellulose-derived inhibitors, insufficiently high substrate concentration in lignocellulose hydrolysate and high costs of product isolation. In order to reduce production cost and improve feasibility of the bioprocess, research efforts must be focused on: (1) optimization of oleaginous microorganism applying genetic engineering methods and adaptive evolution to obtain higher lipid concentrations and tolerance to inhibitors from pretreatment process, (2) new effective method of pretreatment and hydrolysis of lignocellulosic biomass that provide high concentration of fermentable sugars in growth media, (3) novel innovative designs of bioreactor should improve the productivity of the process and reduce the production cost, (4) optimization of lipid isolation from wet cell biomass and (5) generation of valued-added products that could provide additional income and improve economic feasibility of the bioprocess (Figure 3).



**Figure 3.** Strategies for improvement of lipid production process.

## Acknowledgements

This work was financially supported by Phoenix Grant Agreement (No. 690925 - Phoenix) and the Croatian Science Foundation under the project “Sustainable production of bioethanol and biochemicals from agricultural waste lignocellulosic raw materials (No. 9158)”.

## Author details

Mirela Ivancic Santek\*, Suncica Beluhan and Bozidar Santek

\*Address all correspondence to: mivancicsantek@pbf.hr

Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia

## References

- [1] Agarwal AK. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. *Progress in Energy and Combustion Science*. 2007;**33**(3):233-271. DOI: 10.1016/j.pecs.2006.08.003
- [2] The statistics portal. Available from: <https://www.statista.com/statistics/271472/bio-diesel-production-in-selected-countries/> [Accessed: 2017-11-26]
- [3] Ewing M, Msangi S. Biofuels production in developing countries: Assessing tradeoffs in welfare and food security. *Environmental Science and Policy*. 2009;**12**(4):520-528. DOI: 10.1016/j.envsci.2008.10.002
- [4] Ratledge C, Wilkinson SG. *Microbial Lipids*. London: Academic press; 1988. pp. 23-53
- [5] Galafassi S, Cucchetti D, Pizza F, Franzosi G, Bianchi D, Compagno C. Lipid production for second generation biodiesel by the oleaginous yeast *Rhodotorula graminis*. *Bioresource Technology*. 2012;**111**:398-403. DOI: 10.1016/j.biortech.2012.02.004
- [6] Amaretti A, Raimondi S, Sala M, Roncaglia L, De Lucia M, Leonardi A, Rossi M. Single cell oils of the cold-adapted oleaginous yeast *Rhodotorula glacialis* DBVPG 4785. *Microbial Cell Factories*. 2010;**9**(1):73. DOI: 10.1186/1475-2859-9-73
- [7] Zhang J, Fang X, Zhu XL, Li Y, Xu HP, Zhao BF, Zhang XD. Microbial lipid production by the oleaginous yeast *Cryptococcus curvatus* O3 grown in fed-batch culture. *Biomass and Bioenergy*. 1906-1911;**35**(5). DOI: 10.1016/j.biombioe.2011.01.024
- [8] Zhang G, French WT, Hernandez R, Hall J, Sparks D, Holmes WE. Microbial lipid production as biodiesel feedstock from N-acetylglucosamine by oleaginous microorganisms. *Journal of Chemical Technology and Biotechnology*. 2011;**86**(5):642-650. DOI: 10.1002/jctb.2592
- [9] Xue Z, Sharpe PL, Hong SP, Yadav NS, Xie D, Short DR, Pollak DW. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. *Nature Biotechnology*. 2013;**1**(8):734-740. DOI: 10.1038/nbt.2622

- [10] Tang X, Chen H, Chen YQ, Chen W, Garre V, Song Y, Ratledge C. Comparison of biochemical activities between high and low lipid-producing strains of *Mucor circinelloides*: An explanation for the high oleaginicacy of strain WJ11. PLoS One. 2015;**10**(6): e0128396. DOI: 10.1371/journal.pone.0128396
- [11] Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Science and Nutrition. 2014;**2**(5):443-463. DOI: 10.1002/fsn3.121
- [12] Bellou S, Triantaphyllidou IE, Aggeli D, Elazzazy AM, Baeshen MN, Aggelis G. Microbial oils as food additives: Recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Current Opinion in Biotechnology. 2016;**37**:24-35. DOI: 10.1016/j.copbio.2015.09.005
- [13] Schneider T, Graeff-Hönniger S, French WT, Hernandez R, Merkt N, Claupein W, Pham P. Lipid and carotenoid production by oleaginous red yeast *Rhodotorula glutinis* cultivated on brewery effluents. Energy. 2013;**61**:34-43. DOI: 10.1016/j.energy.2012.12.026
- [14] Okuda T, Ando A, Negoro H, Muratsubaki T, Kikukawa H, Sakamoto T, Ogawa J. Eicosapentaenoic acid (EPA) production by an oleaginous fungus *Mortierella alpina* expressing heterologous the  $\Delta 17$ -desaturase gene under ordinary temperature. European Journal of Lipid Science and Technology. 2015;**117**(12):1919-1927. DOI: 10.1002/ejlt.201400657
- [15] Ling X, Guo J, Liu X, Zhang X, Wang N, Lu Y, Ng IS. Impact of carbon and nitrogen feeding strategy on high production of biomass and docosahexaenoic acid (DHA) by *Schizochytrium* sp. LU310. Bioresource Technology. 2015;**184**:139-147. DOI: 10.1016/j.biortech.2014.09.130
- [16] Ratledge C. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie. 2004;**86**(11):807-815. DOI: 10.1016/j.biochi.2004.09.017
- [17] Li X, Lin Y, Chang M, Jin Q, Wang X. Efficient production of arachidonic acid by *Mortierella alpina* through integrating fed-batch culture with a two-stage pH control strategy. Bioresource Technology. 2015;**181**:275-282. DOI: /10.1016/j.biortech.2015.01.009
- [18] Wei Y, Siewers V, Cocoa NJ. Butter-like lipid production ability of non-oleaginous and oleaginous yeasts under nitrogen-limited culture conditions. Applied Microbiology and Biotechnology. 2017;**101**(9):3577-3585. DOI: 10.1007/s002563-017-8126-7
- [19] Sitepu IR, Garay LA, Sestric R, Levin D, Block DE, German JB, Boundy-Mills KL. Oleaginous yeasts for biodiesel: Current and future trends in biology and production. Biotechnology Advances. 2014;**32**(7):1336-1360. DOI: 10.1016/j.biotechadv.2014.08.003
- [20] Papanikolaou S, Galiotou-Panayotou M, Fakas S, Komaitis M, Aggelis G. Lipid production by oleaginous *Mucorales* cultivated on renewable carbon sources. European Journal of Lipid Science and Technology. 2007;**109**(11):1060-1070. DOI: 10.1002/ejlt.200700169
- [21] Meesters PAEP, Huijberts GNM, Eggink G. High-cell-density cultivation of the lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source. Applied Microbiology and Biotechnology. 1996;**45**(5):575-579. DOI: 10.1007/s002530050731



- [22] Wu S, Hu C, Zhao X, Zhao ZK. Production of lipid from N-acetylglucosamine by *Cryptococcus curvatus*. European Journal of Lipid Science and Technology. 2010;**112**(7): 727-733. DOI: 10.1002/ejlt.201000005
- [23] Fei Q, Chang HN, Shang L. Exploring low-cost carbon sources for microbial lipids production by fed-batch cultivation of *Cryptococcus albidus*. Biotechnology and Bioprocess Engineering. 2011;**16**(3):482-487. DOI: 10.1007/s12257-010-0370-y
- [24] Fei Q, Chang HN, Shang L, Kim N, Kang J. The effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. Bioresource Technology. 2011;**102**(3):2695-2701. DOI: 10.1016/j.biortech.2010.10.141
- [25] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A. Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. The Plant Journal. 2008;**54**(4):621-639. DOI: 10.1111/j.1365-313X.2008.03492.x
- [26] Liang Y, Tang T, Siddaramu T, Choudhary R, Umagiliyage AL. Lipid production from sweet sorghum bagasse through yeast fermentation. Renewable Energy. 2012;**40**(1): 130-136. DOI: 10.1016/j.renene.2011.09.035
- [27] Huang C, Zong MH, Wu H, Liu QP. Microbial oil production from rice straw hydrolysate by *Trichosporon fermentans*. Bioresource Technology. 2009;**100**(19):4535-4538. DOI: 10.1016/j.biortech.2009.04.022
- [28] Gouda MK, Omar SH, Aouad LM. Single cell oil production by *Gordonia* sp. DG using agro-industrial wastes. World Journal of Microbiology and Biotechnology. 2008;**24**(9):1703. DOI: 10.1007/s11274-008-9664-z
- [29] Zhu L, O'Dwyer JP, Chang VS, Granda CB, Holtzapple MT. Structural features affecting biomass enzymatic digestibility. Bioresource Technology. 2008;**99**(9):3817-3828. DOI: 10.1016/j.biortech.2007.07.033
- [30] Cheng MH, Walker TH, Hulbert GJ, Raman DR. Fungal production of eicosapentaenoic and arachidonic acids from industrial waste streams and crude soybean oil. Bioresource Technology. 1999;**67**(2):101-110. DOI: 10.1016/S0960-8524(98)00113-8
- [31] Zhang X, Yan S, Tyagi RD, Surampalli RY, Valéro JR. Lipid production from *Trichosporon oleaginosus* cultivated with pre-treated secondary wastewater sludge. Fuel. 2014;**134**: 274-282. DOI: 10.1016/j.fuel.2014.05.089
- [32] Ratledge C, Wynn JP. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Advances in Applied Microbiology. 2002;**51**:1-52
- [33] Ratledge C. The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: A reappraisal and unsolved problems. Biotechnology Letters. 2014;**36**(8): 1557-1568. DOI: 10.1007/s10529-014-1532-3
- [34] Zhang H, Zhang L, Chen H, Chen YQ, Ratledge C, Song Y, Chen W. Regulatory properties of malic enzyme in the oleaginous yeast, *Yarrowia lipolytica*, and its non-involvement in lipid accumulation. Biotechnology Letters. 2013;**35**(12):2091-2098. DOI: 10.1007/s10529-013-1302-7



- [35] Morin N, Cwscut J, Beopoulos A, Lelandas G, Le Berre V, Uribelarrea JL, Nicaud JM. Transcriptomic analysis during the transition from biomass production to lipid accumulation in the oleaginous yeast *Yarrowia lipolytica*. PLoS One. 2011;**6**(11):e27966. DOI: 10.1371/journal.pone.0027966
- [36] Tai M, Stephanopoulos G. Engineering the push and pull of lipid biosynthesis in oleaginous yeast *Yarrowia lipolytica* for biofuel production. Metabolic Engineering. 2013;**15**:1-9. DOI: 10.1016/j.ymben.2012.08.007
- [37] Ballesteros I, Negro MJ, Oliva JM, Cabañas A, Manzanares P, Ballesteros M. Ethanol production from steam-explosion pretreated wheat straw. Applied Biochemistry and Biotechnology. 2006;**130**(1-3):496-508. DOI: 10.1385/ABAB:130:1:496
- [38] Chen WH, Pen BL, Yu CT, Hwang WS. Pretreatment efficiency and structural characterization of rice straw by an integrated process of dilute-acid and steam explosion for bioethanol production. Bioresource Technology. 2011;**102**(3):2916-2924. DOI: 10.1016/j.biortech.2010.11.052
- [39] García-Cubero MT, González-Benito G, Indacochea I, Coca M, Bolado S. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw. Bioresource Technology. 2009;**100**(4):1608-1613. DOI: 10.1016/j.biortech.2008.09.012
- [40] Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial Engineering Chemistry Research. 2009;**48**(8):3713-3729. DOI: 10.1021/ie801542g
- [41] Du B, Sharma LN, Becker C, Chen SF, Mowery RA, van Walsum GP, Chambliss CK. Effect of varying feedstock-pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates. Biotechnology and Bioengineering 2010; **107**(3): 430-440. DOI: 10.1002/bit.22829
- [42] Shafiei M, Karimi K, Taherzadeh MJ. Pretreatment of spruce and oak by N-methylmorpholine-N-oxide (NMMO) for efficient conversion of their cellulose to ethanol. Bioresource Technology. 2010;**101**(13):4914-4918. DOI: 10.1016/j.biortech.2009.08.100
- [43] Zhu LY, Zong MH, Wu H. Efficient lipid production with *Trichosporon fermentans* and its use for biodiesel preparation. Bioresource Technology. 2008;**99**(16):7881-7885. DOI: 10.1016/j.biortech.2008.02.033
- [44] Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology. 2004;**96**(6):673-686. DOI: 10.1016/j.biortech.2004.06.025
- [45] Jönsson LJ, Alriksson B, Nilvebrant NO. Bioconversion of lignocellulose: Inhibitors and detoxification. Biotechnology for Biofuels. 2013;**6**(1):16. DOI: 10.1186/1754-6834-6-16
- [46] Jørgensen H, Kristensen JB, Felby C. Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. Biofuels, Bioproducts and Biorefining. 2007;**1**(2):119-134. DOI: 10.1002/bbb.4

- [47] Ishizawa CI, Davis MF, Schell DF, Johnson DK. Porosity and its effect on the digestibility of dilute sulfuric acid pretreated corn stover. *Journal of Agricultural and Food Chemistry*. 2007;**55**(7):2575-2581. DOI: 10.1021/jf062131a
- [48] Palmqvist E, Hahn-Hägerdal B. Fermentation of lignocellulosic hydrolysates. I: Inhibition and detoxification. *Bioresource Technology*. 2000;**74**(1):17-24. DOI: 10.1016/S0960-8524(99)00161-3
- [49] Sjöström E, Alén R, editors. *Analytical Methods in Wood Chemistry, Pulping and Papermaking*. Springer Science & Business Media; 2013. pp. 37-146
- [50] Rowell RM, editor. *Handbook of Wood Chemistry and Wood Composites*. Taylor Francis: CRC press; 2005. pp. 9-75
- [51] Jönsson LJ, Martín C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*. 2016;**199**:103-112. DOI: 10.1016/j.biortech.2015.10.009
- [52] Yang B, Wyman CE. Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*. 2008;**2**(1):26-40. DOI: 10.1002/bbb.49
- [53] Balat M, Balat H, Öz C. Progress in bioethanol processing. *Progress in Energy and Combustion Science*. 2008;**34**(5):551-573. DOI: 10.1016/j.pecs.2007.11.001
- [54] Mood SH, Golfeshan AH, Tabatabaei M, Jouzani GS, Najafi GH, Gholami M, Ardjmand M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pre-treatment. *Renewable and Sustainable Energy Reviews*. 2013;**27**:77-93. DOI: 10.1016/j.rser.2013.06.033
- [55] Kumar R, Mago G, Balan V, Wyman CE. Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *Bioresource Technology*. 2009;**100**(17):3948-3962. DOI: 10.1016/j.biortech.2009.01.075
- [56] Hu F, Ragauskas A. Pretreatment and lignocellulosic chemistry. *Bioenergy Research*. 2012;**5**(4):1043-1066. DOI: /10.1007/s12155-012-9208-0
- [57] Ruan Z, Zanotti M, Wang X, Ducey C, Liu Y. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. *Bioresource Technology*. 2012;**110**:198-205. DOI: 10.1016/j.biortech.2012.01.053
- [58] Hendriks ATWM, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*. 2009;**100**(1):10-18. DOI: 10.1016/j.biortech.2008.05.027
- [59] Galbe M, Zacchi G. Pretreatment of lignocellulosic materials for efficient bioethanol production. In: Olsson L, editor. *Biofuels Ed., Advanced in Biochemical Engineering/ Biotechnology*. Springer Berlin Heidelberg; 2007. pp. 41-65. DOI: 10.1007/10\_2007\_070
- [60] Zhu JY, Pan XJ, Wang GS, Sulfite GR. Pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresource Technology*. 2007;**100**(8):2411-2418. DOI: 10.1016/j.biortech.2008.10.057
- [61] Balan V, Sousa LDC, Chundawat SP, Marshall D, Sharma LN, Chambliss CK, Dale BE. Enzymatic digestibility and pretreatment degradation products of AFEX-treated hardwoods (*Populus nigra*). *Biotechnology Progress*. 2009;**25**(2):365-375. DOI: 10.1002/btpr.160

- [62] McMillan JD. Pretreatment of lignocellulosic biomass. In: Himmel ME, Baker JO, Overend RP, editors. *Enzymatic Conversion of Biomass for Fuels Production*. ACS Symposium Series; 1994. p. 292-324. DOI: 10.1021/bk-1994-0566.ch015
- [63] Mes-Hartree M, Dale BE, Craig WK. Comparison of steam and ammonia pretreatment for enzymatic hydrolysis of cellulose. *Applied Microbiology and Biotechnology*. 1988;**29**(5):462-468. DOI: /10.1007/BF00269069
- [64] Gollapalli LE, Dale BE, Rivers DM. Predicting digestibility of ammonia fiber explosion (AFEX)-treated rice straw. *Applied Biochemistry and Biotechnology*. 2002;**98**(1-9):23-35. DOI: 10.1385/ABAB:98-100:1-9:23
- [65] Taherzadeh MJ, Karimi K. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*. 2007;**2**(3):472-499
- [66] Taherzadeh MJ, Karimi K. Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources*. 2007;**2**(4):707-738. DOI: 10.15376/biores.2.4.707-738
- [67] Olofsson K, Bertilsson M, Lidén GA. Short review on SSF—an interesting process option for ethanol production from lignocellulosic feedstocks. *Biotechnology for Biofuels*. 2008;**1**(1):7. DOI: 10.1186/1754-6834-1
- [68] Sheehan JS, Outlook HME. For bioethanol production from lignocellulosic feedstocks: Technology hurdles. *Agro Food Industry Hi-Tech*. 2001;**12**(5):54-57
- [69] Wayman CE. *Handbook of Bioethanol: Production and Utilization*. Taylor & Francis: Washington, DC; 1996
- [70] Linde M, Galbe M, Zacchi G. Simultaneous saccharification and fermentation of steam-pretreated barley straw at low enzyme loadings and low yeast concentration. *Enzyme and Microbial Technology*. 2007;**40**(5): 1100-1107. DOI: 10.1016/j.enzmictec.2006.08.014
- [71] Sigma-Aldrich. Available from: <https://www.sigmaaldrich.com/catalog/product/sigma/c2730?lang=en&region=HR> [Accessed: 2017-11-24]
- [72] Zeng J, Zheng Y, Yu X, Yu L, Gao D, Chen S. Lignocellulosic biomass as a carbohydrate source for lipid production by *Mortierella isabellina*. *Bioresource Technology*. 2013;**128**:385-391. DOI: 10.1016/j.biortech.2012.10.079
- [73] Xu Q, Singh A, Himmel ME. Perspectives and new directions for the production of bioethanol using consolidated bioprocessing of lignocellulose. *Current Opinion in Biotechnology*. 2009;**20**(3):364-371. DOI: 10.1016/j.copbio.2009.05.006
- [74] Lynd LR, Van Zyl WH, McBride JE, Laser M. Consolidated bioprocessing of cellulosic biomass: An update. *Current Opinion in Biotechnology*. 2005;**16**(5):577-583. DOI: 10.1016/j.copbio.2005.08.009
- [75] Liu W, Wang Y, Yu Z, Bao J. Simultaneous saccharification and microbial lipid fermentation of corn stover by oleaginous yeast *Trichosporon cutaneum*. *Bioresource Technology*. 2012;**118**:13-18. DOI: 10.1016/j.biortech.2012.05.038
- [76] Gao Q, Cui Z, Zhang J, Bao J. Lipid fermentation of corncob residues hydrolysate by oleaginous yeast *Trichosporon cutaneum*. *Bioresource Technology*. 2014;**152**:552-556. DOI: 10.1016/j.biortech.2013.11.044

- [77] Matsakas L, Sterioti AA, Rova U, Christakopoulos P. Use of dried sweet sorghum for the efficient production of lipids by the yeast *Lipomyces starkeyi* CBS 1807. *Industrial Crops and Products*. 2014;**62**:367-372. DOI: 10.1016/j.indcrop.2014.09.011
- [78] Hu C, Wu S, Wang Q, Jin G, Shen H, Zhao Z K. Simultaneous utilization of glucose and xylose for lipid production by *Trichosporon cutaneum*. *Biotechnology for Biofuels*. 2011;**4**(1):25. DOI: 10.1186/1754-6834-4-25
- [79] Gong Z, Shen H, Wang Q, Yang X, Xie H, Zhao Z K. Efficient conversion of biomass into lipids by using the simultaneous saccharification and enhanced lipid production process. *Biotechnology for Biofuels*. 2013;**6**(1):36. DOI: 10.1186/1754-6834-6-36
- [80] Sung M, Seo YH, Han S, Han JI. Biodiesel production from yeast *Cryptococcus* sp. using Jerusalem artichoke. *Bioresource Technology*. 2014;**155**:77-83. DOI: 10.1016/j.biortech.2013.12.024
- [81] Gong Z, Shen H, Yang X, Wang Q, Xie H, Zhao Z K. Lipid production from corn stover by the oleaginous yeast *Cryptococcus curvatus*. *Biotechnology for Biofuels*. 2014;**7**(1):158. DOI: 10.1186/s13068-014-0158-y
- [82] Liu Y, Wang Y, Liu H, Zhang JA. Enhanced lipid production with undetoxified corncob hydrolysate by *Rhodotorula glutinis* using a high cell density culture strategy. *Bioresource Technology*. 2015;**180**:32-39. DOI: 10.1016/j.biortech.2014.12.093
- [83] Slininger PJ, Dien BS, Kurtzman CP, Moser BR, Bakota EL, Thompson SR, Sousa LDC. Comparative lipid production by oleaginous yeasts in hydrolyzates of lignocellulosic biomass and process strategy for high titers. *Biotechnology and Bioengineering*. 2016;**113**(8):1676-1690. DOI: 10.1002/bit.25928
- [84] Fei Q, O'Brien M, Nelson R, Chen X, Lowell A, Enhanced DN. Lipid production by *Rhodosporidium toruloides* using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. *Biotechnology for Biofuels*. 2016;**9**(1):130. DOI: 10.1186/s13068-016-0542-x
- [85] Fang H, Zhao C, Chen S. Single cell oil production by *Mortierella isabellina* from steam exploded corn stover degraded by three-stage enzymatic hydrolysis in the context of on-site enzyme production. *Bioresource Technology*. 2016;**216**:988-995. DOI: 10.1016/j.biortech.2016.06.051
- [86] Harde SM, Wang Z, Horne M, Zhu JY, Pan X. Microbial lipid production from SPORL-pretreated Douglas fir by *Mortierella isabellina*. *Fuel*. 2016;**175**:64-74. DOI: 10.1016/j.fuel.2016.02.023
- [87] Harris PV, Xu F, Kreel NE, Kang C, Fukuyama S. New enzyme insights drive advances in commercial ethanol production. *Current Opinion in Chemical Biology*. 2014;**19**:162-170. DOI: 10.1016/j.cbpa.2014.02.015
- [88] Cannella D, Chia-wen C H, Felby C, Jørgensen H. Production and effect of aldonic acids during enzymatic hydrolysis of lignocellulose at high dry matter content. *Biotechnology for Biofuels*. 2012;**5**(1):26. DOI: 10.1186/1754-6834-5-26
- [89] Hu C, Zhao X, Zhao J, Wu S, Zhao ZK. Effects of biomass hydrolysis by-products on oleaginous yeast *Rhodosporidium toruloides*. *Bioresource Technology*. 2009;**100**(20):4843-4847. DOI: 10.1016/j.biortech.2009.04.041



- [90] Li Y, Zhao ZK, Bai F. High-density cultivation of oleaginous yeast *Rhodospiridium toruloides* Y4 in fed-batch culture. *Enzyme and Microbial Technology*. 2007;**41**(3):312-317. DOI: 10.1016/j.enzmictec.2007.02.008
- [91] Koppram R, Tomás-Pejó E, Xiros C, Olsson L. Lignocellulosic ethanol production at high-gravity: Challenges and perspectives. *Trends in Biotechnology*. 2014;**32**(1):46-53. DOI: /10.1016/j.tibtech.2013.10.003
- [92] Liu ZL. Genomic adaptation of ethanologenic yeast to biomass conversion inhibitors. *Applied Microbiology and Biotechnology*. 2006;**73**(1):27-36. DOI: 10.1007/s00253-006-0567-3
- [93] Elliston A, Collins SR, Wilson DR, Roberts IN, Waldron KW. High concentrations of cellulosic ethanol achieved by fed batch semi simultaneous saccharification and fermentation of waste-paper. *Bioresource Technology*. 2013;**134**:117-126. DOI: i.org/10.1016/j.biortech.2013.01.084
- [94] Pandey A. Solid-state fermentation. *Biochemical Engineering Journal*. 2003;**13**:81-84. DOI: 10.1016/S1369-703X(02)00121-3
- [95] Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I-bioprocesses and products. *Process Biochemistry*. 2000;**35**(10):1153-1169. DOI: 10.1016/S0032-9592(00)00152-7
- [96] Hölker U, Höfer M, Lenz J. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. *Applied Microbiology and Biotechnology*. 2000;**64**(2):175-186. DOI: 10.1007/s00253-003-1504-3
- [97] Pen XW, Chen HZ. Microbial oil accumulation and cellulase secretion of the endophytic fungi from oleaginous plants. *Annals of Microbiology*. 2007;**57**(2):239-242. DOI: 10.1007/BF03175213
- [98] Peng X, Chen H. Single cell oil production in solid-state fermentation by *Microsphaeropsis* sp. from steam-exploded wheat straw mixed with wheat bran. *Bioresource Technology*. 2008;**99**(9):3885-3889. DOI: 10.1016/j.biortech.2007.08.015
- [99] Cheirsilp B, Kitcha S. Solid state fermentation by cellulolytic oleaginous fungi for direct conversion of lignocellulosic biomass into lipids: Fed-batch and repeated-batch fermentations. *Industrial Crops and Products*. 2015;**66**:73-80. DOI: 10.1016/j.indcrop.2014.12.035
- [100] Kitcha S, Cheirsilp B. Enhanced lipid production by co-cultivation and co-encapsulation of oleaginous yeast *Trichosporonoides spathulata* with microalgae in alginate gel beads. *Applied Biochemistry and Biotechnology*. 2014;**173**(2):522-534. DOI: 10.1007/s12010-014-0859-5
- [101] Dey P, Banerjee J, Maiti MK. Comparative lipid profiling of two endophytic fungal isolates—*Colletotrichum* sp. and *Alternaria* sp. having potential utilities as biodiesel feedstock. *Bioresource Technology*. 2011;**102**(10):5815-5823. DOI: 10.1016/j.biortech.2011.02.064
- [102] Hui L, Wan C, Hai-Tao D, Xue-Jiao C, Qi-Fa Z, Yu-Hua Z. Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. *Bioresource Technology*. 2010;**101**(19):7556-7562. DOI: 10.1016/j.biortech.2010.04.027



- [103] Koutinas AA, Chatzifragkou A, Kopsahelis N, Papanikolaou S, Kookos IK. Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production. *Fuel*. 2014;**116**:566-577. DOI: 10.1016/j.fuel.2013.08.045
- [104] Johnson EA. Biotechnology of non-*Saccharomyces yeasts*—The basidiomycetes. *Applied Microbiology and Biotechnology*. 2013;**97**(17):7563-7577. DOI: 10.1007/s00253-013-5046-z
- [105] Alipour S, Habibi A, Taavoni S, Varmira K.  $\beta$ -carotene production from soap stock by loofa-immobilized *Rhodotorula rubra* in an airlift photobioreactor. *Process Biochemistry*. 2017;**54**:9-19. DOI: 10.1016/j.procbio.2016.12.013
- [106] Cardoso LAC, Jäckel S, Karp SG, Framboisier X, Chevalot I, Marc I. Improvement of *Sporobolomyces ruberrimus* carotenoids production by the use of raw glycerol. *Bioresource Technology*. 2016;**200**:374-379. DOI: 10.1016/j.biortech.2015.09.108
- [107] Mata-Gómez L C, Montañez J C, Méndez-Zavala A, Aguilar C N. Biotechnological production of carotenoids by yeasts: An overview. *Microbial Cell Factories*. 2014;**13**:12. DOI: 10.1186/1475-2859-13-12
- [108] Van Bogaert IN, Zhang J, Soetaert W. Microbial synthesis of sophorolipids. *Process Biochemistry*. 2011;**46**(4):821-833. DOI: 10.1016/j.procbio.2011.01.010
- [109] Shoeb E, Akhlaq F, Badar U, Akhter J, Imtiaz S. Classification and industrial applications of biosurfactants. *Academic Research International*. 2013;**4**(3):243
- [110] Vaishnav P, Demain AL. Unexpected applications of secondary metabolites. *Biotechnology Advances*. 2011;**29**(2):223-229. DOI: 10.1016/j.biotechadv.2010.11.006
- [111] Doherty WO, Mousavioun P, Fellows CM. Value-adding to cellulosic ethanol: Lignin polymers. *Industrial Crops and Products*. 2011;**33**(2):259-276. DOI: 10.1016/j.indcrop.2010.10.022
- [112] Zhang YHP Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. *Journal of Industrial Microbiology and Biotechnology*. 2008;**35**(5):367-375. DOI: /10.1007/s10295-007-0293-6
- [113] Holladay J E, White J F, Bozell J J, Johnson D. Top Value Added Chemicals from Biomass-Volume II, Results of Screening for Potential Candidates from Biorefinery Lignin (No. PNNL-16983). Pacific Northwest National Lab.(PNNL), Richland, WA (United States); National Renewable Energy Laboratory (NREL), Golden, CO (United States). Available from: <https://www.energy.gov/sites/prod/files/2014/03/f14/pnnl-16983.pdf> [Accessed: 2017-12-02]
- [114] Liu W, Zhou R, Goh HLS, Huang S, Lu X. From waste to functional additive: Toughening epoxy resin with lignin. *ACS Applied Materials Interfaces*. 2014;**6**(8):5810-5817. DOI: 10.1021/am500642n
- [115] Dong T, Knoshaug EP, Pienkos PT, Laurens LM. Lipid recovery from wet oleaginous microbial biomass for biofuel production: A critical review. *Applied Energy*. 2016;**177**:879-895. DOI: 10.1016/j.apenergy.2016.06.002