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Sustainable Production of Microbial Lipids from Lignocellulosic Biomass Using Oleaginous Yeast Cultures

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Abstract

Microbial lipids derived from oleaginous yeast could be a promising resource for biodiesel and other oleochemical materials. The objective of this study was to develop an efficient bioconversion process from lignocellulosic biomass to microbial lipids using three types of robust oleaginous yeast: *T. oleaginosus*, *L. starkeyi*, and *C. albidus*. Sorghum stalks and switchgrass were utilized as feedstocks for lipid production. Among oleaginous yeast strains, *T. oleaginous* showed better performance for lipid production using sorghum stalk hydrolysates. Lipid titers of 13.1 $g \cdot L^{-1}$ were achieved by *T. oleaginosus*, using sorghum stalk hydrolysates with lipid content of 60% ($wt \cdot wt^{-1}$) and high lipid yield of 0.29 $g \cdot g^{-1}$, which was substantially higher than the value reported in literature. Assessment of overall lipid yield revealed a total of 14.3 g and 13.3 g lipids were produced by *T. oleaginosus* from 100 g of raw sorghum stalks and switchgrass, respectively. This study revealed that minimization of sugar loss during pretreatment and selection of appropriate yeast strains would be key factors to develop an efficient bioconversion process and improve the industrial feasibility in a lignocellulose-based biorefinery.

Keywords

Trichosporon oleaginosus, Lipomyces starkeyi, Cryptococcus albidus, Lignocellulosic Hydrolysates, Sorghum Stalks, Switchgrass

1. Introduction

Microbial lipids are promising candidates for replacing traditional oil sources in the production of biodiesel, oleo-chemicals, and nutraceuticals, due to similar

chemical composition and energy value [1] [2]. A research estimated that cost of microbial lipids would be \$3.4 kg^{-1}, excluding a feedstock price, and \$5.5 kg^{-1}, including glucose as a feedstock [3], whereas cost of vegetable oil is \$1.5 - 3 kg^{-1} less [4]. A supply of low-cost carbohydrates for microbes is required for sustainable and cost-effective production of bio-based lipids.

Lignocellulosic biomass, such as agricultural residues and woody crops, is a strong alternative substrate for microbial lipid production due to their abundance, low-cost investment, and high content of polysaccharides (up to 75%) [5] [6]. More than 90% of global production of plant biomass is lignocellulosic biomass, which is composed of cellulose, hemicellulose, and lignin [7]. Recalcitrant lignocellulosic biomass is converted to monomer sugars via pretreatment and enzymatic hydrolysis [8]. Many pretreatment methods maximize exposure of carbohydrate polymers (cellulose and hemicellulose) with effective separation of the lignin portion, which is an interference biopolymer during bioconversion [9] [10]. Among many attempts, an alkaline pretreatment is known to efficiently remove lignin from plant cell wall structures [11]. Cellulose and hemicellulose are depolymerized to monosaccharides by synergetic actions of enzyme mixtures [12]. The most abundant monomer sugars derived from lignocellulosic biomass are D-glucose, since cellulose represents 70% of total plant cell walls, repeating the β-(1→4) glycosidic bond [13]. However, species of lignocellulosic-based monomer sugars depend on biomass types.

Several challenges remain for successful bioconversion of lignocellulosic biomass to microbial lipids. A broad array of monomer sugars is generated from lignocellulosic biomass including glucose, xylose, mannose, and arabinose. Typically, the ratio of hexoses to pentoses ranges from 1.5:1 to 3:1 [14]. However, some species of microbes only utilize limited types of monomer sugars as carbon sources. In addition, a number of by-products, such as furans, aldehydes, and organic acids, are generated during pretreatment and enzymatic hydrolysis [15] [16] [17] [18]. These compounds are known to inhibit microbes' growth and product formation during fermentation. Acetic acid, especially, is an inevitable compound, which are normally released from acetyl groups of hemicellulose during enzymatic hydrolysis [19]. Acetic acid adversely affects the integrity of the cell membrane by accumulating in deprotonated form [20].

Oleaginous yeast, which has an inherent ability to accumulate lipids from 20% to 70% as a percentage of cell dry weight, offers many advantages to overcome challenges associated with lignocellulose-based lipid production [21] [22]. Basidiomycetous yeast species such as *Cryptococcus albidus* and *Trichosporon oleaginosus* are known to enable use of a variety of carbon sources, and can be grown without supplemented costly nutrients [23] [24]. In addition, oleaginous yeast cultures are insusceptible to toxic compounds compared with bacteria. Previous studies reported some types of oleaginous yeast consumed weak acids, including acetic acid and formic acid [25] [26].

In this study, production of lignocellulose-based microbial lipids was investigated using three oleaginous yeast cultures: *Trichosporon oleaginosus*

ATCC20509, *Lipomyces starkeyi* ATCC 56304, and *Cryptococcus albidus* ATCC10672. Sorghum stalks and switchgrass, which are typical bio-energy crops, were utilized as sugar suppliers for microbial lipid production. In addition, fermentation performance of *T. oleaginosus*, *L. starkeyi*, and *C. albidus* were evaluated using sorghum stalks and switchgrass hydrolysates. To our knowledge, *C. albidus* ATCC 10672 has not previously been evaluated for lipid production using lignocellulosic hydrolysates. Also, overall yield of microbial lipids from raw biomass was studied to evaluate the lipid production process.

2. Materials and Methods

2.1. Lignocellulosic Biomass and Compositional Analysis

Sorghum stalks were obtained from Texas A&M University, College Station, Texas, and ground by Mesa Associate Inc., Knoxville, Tennessee. Switchgrass was obtained from the Kansas State University agronomy farm, Manhattan, Kansas, and ground at a size of less than 1 mm, using a Tomas-Wiley laboratory mill (Model 4). Biomass composition was determined following the protocol of NREL/TP-510-42618 [27].

2.2. Pretreatment and Enzymatic Hydrolysis of Lignocellulosic Biomass

A schematic diagram of the process for lignocellulosic hydrolysate preparation was shown in Figure 1.

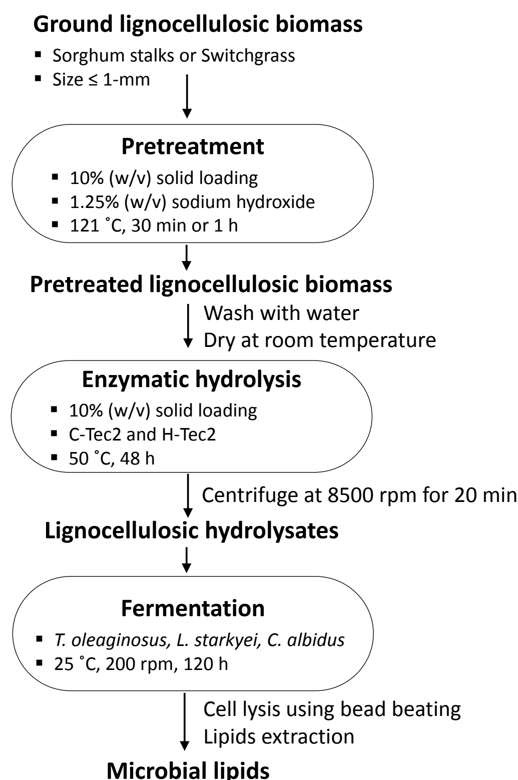

Figure 1. Schematic diagram of microbial lipid production from lignocellulosic biomass.

Pretreatment and hydrolysis conditions for each lignocellulosic biomass were optimized in our lab and it was followed in this study for lipid production [28]. The ground biomass was mixed with 1.25% ($w \cdot v^{-1}$) sodium hydroxide (NaOH), at the rate of 10% ($w \cdot v^{-1}$) solid loading, in a 500 mL flask for pretreatment. Sorghum stalks and switchgrass were pretreated at 121˚C for 30 min and 1 h, respectively. The pretreated biomass was washed with an abundant amount of water until the residue of NaOH was completely removed; about 2 L of water was used for 5 g of pretreated biomass. The pretreated biomass after washing process was dried at room temperature for five days. The pretreated biomass was mixed with 50 mM of a citrate buffer (pH 4.8), at the rate of 5% ($w \cdot v^{-1}$) solid loading, for the enzymatic hydrolysis. Commercial cellulolytic (Cellic C-Tec2) and hemicellulotyic (Cellic H-Tec2) enzymes, which were obtained from Novozymes Inc., Franklinton, North Carolina, were added into the pretreated biomass slurry at the rate of 5.4% and 0.6% ($w \cdot v^{-1}$) of biomass, respectively. Enzymatic hydrolysis was conducted in the shaking incubator (Innova 4300, New Brunswick Scientific, NJ) at 50˚C and 140 rpm for 48 h. The sorghum stalk and switchgrass hydrolysates, which were supernatant after enzymatic hydrolysis, were harvested via centrifugation (Sorvall Super T21, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 8500 rpm and 25˚C for 20 min.

2.3. Yeast Strains, Medium, and Culture Conditions

Trichosporon oleaginosus ATCC 20509, *Lipomyces starkeyi* ATCC 56304, and *Cryptococcus albidus* ATCC 10672 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), and cultivated in a yeast mold broth (YM broth, Difco, Detroit, MI, USA) at 25˚C and 200 rpm for 72 hr. All yeast cultures were preserved in YM agar plates at 4˚C, and transferred to fresh plates once a month.

2.4. Fermentation Conditions

Starter cultures of all yeast strains were begun by inoculating a single colony from a YM agar plate. *T. oleaginosus*, *L. starkeyi*, *and C. albidus* were grown in a YM broth at 25˚C and 200 rpm for 12 h, and cells were transferred into a 500 mL shake flask containing 100 mL of fermentation media. Sorghum stalks and switchgrass hydrolysates, containing a total of 50 $g \cdot L^{-1}$ sugars, were utilized as carbon sources for lipid production. Nitrogen source of yeast extract (0.33 $g \cdot L^{-1}$) and peptone (1 $g \cdot L^{-1}$) was supplemented into the fermentation media. Fermentation was carried out at 25˚C and 200 rpm for 120 h in a shaking incubator (Innova 4300, New Brunswick Scientific, NJ).

2.5. Analysis of Sugars and Organic Acids

Dry-cell weight (DCW) was used to determine cell concentrations. Cell pellets were washed with water two times, dried at 80˚C overnight, and measured for weight. Sugars and organic acid concentrations were analyzed via a high-performance liquid chromatography (HPLC; Shimadzu Scientific Instruments, Inc.,

Columbia, MD, USA) equipped with a refractive index detector (RID) and a Rezex ROA organic acid column (150 × 7.8 mm, Phenomenex Inc., Torrance, CA, USA). Oven temperature was kept at 80˚C, and 0.005 N sulfuric acid was utilized as a mobile phase, with a pumping rate of 1.0 $mL \cdot min^{-1}$.

2.6. Yeast Cell Lysis and Lipid Extraction

Yeast cells were harvested via centrifugation (Sorvall Super T21, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 8500 rpm for 20 min. Cells were washed two times with water and concentrated to 10^9 cells mL^{-1}. The concentrated cells were preserved at −80˚C for one day prior to lipid extraction. Thawed cell pellets (0.5 mL) were transferred into a 2.5 mL polypropylene microvial, followed by adding 0.5 mL of methanol, 0.5 mL of chloroform, and 1 mL of 0.5 mm cubic zirconia beads. Bead beating was performed using a bead-beater homogenizer (Mini-Beadbeater-24, BioSpec Products, Inc., Bartlesville, OK, USA) in 45 sec intervals, with a cooling of 10 min on ice repeated six times. Lipid extraction was conducted by following a modified Bligh and Dyer method [29]. The cell lysate after bead beating was transferred into a 7 mL Kimax tube, and chloroform: methanol:water were added with a ratio of 1:2:0.8, respectively. Tubes containing cell lysate mixtures were vortexed and centrifuged at 4000 rpm for 20 min. The lipid layer (bottom layer) of the mixture was transferred into a clean tube using a Pasteur pipette, and 1 mL of chloroform was added into the mixture followed by vortexing and centrifugation. Lipid extraction was repeated three times and the combined lipid layers were filtered using PTFE filters with 0.22 μM pore size. The filtrates were washed two times with a 1 M potassium chloride solution, followed by drying under nitrogen gas at 40˚C until 1 ml of mixture was left in the Kimax tube. The residue was transferred into a glass vial and dried down under nitrogen gas, again to completely remove chloroform and measure the lipid weight. After determination of lipid weights, 1 mL of chloroform was added into each glass vial and kept at −80˚C for further compositional analysis of the lipids. Lipid content in the yeast cells was determined by dividing weights of lipids from yeast cells by weights of concentrated cells. Cell weight was determined by measuring DCW of the concentrated cell.

2.7. Analysis of Fatty Acid Composition

Fatty acids in the lipids were converted to fatty acid methyl esters (FAMEs) via a transesterification for compositional analysis. Lipid samples were transferred into a 7 mL Kimax tube with 25 nmol of internal standard (pentadecanoic acids) and the chloroform was evaporated under nitrogen gas at 40˚C. For transesterification, 1 mL of methanolic hydrochloric acid (3 M) was added into each tube and incubated at 78˚C for 30 min in the heating block. After cooling down the samples, 2 ml of water were added, followed by 1.6 mL of chloroform and 0.4 mL of hexane. The layers were then separated via centrifugation at 4000 rpm for 5 min. The lower layer was transferred into a clean Kimax tube and the organic phase was dried down under nitrogen gas. One hundred μL of hexane were

added to solubilize FAMEs, and then transferred into a glass vial. FAMEs were analyzed by injecting 1 μL of the sample into a gas chromatograph (GC-2014, Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a flame-ionization detector (FID) and an aqueous-stable polyethylene glycol capillary column (Zebron ZB-Wax$_{plus}$ 30 m × 0.25 mm × 0.25 μm, Phenomenex, Torrance, CA, USA). The initial oven temperature of 160˚C was gradually increased to 200 ˚C at a rate of 5 ˚C min^{-1}, and detector temperature was 250˚C. The FAME mixture (Supelco, 37 component FAME mix) was utilized as an external standard to identify fatty acid composition in the lipids.

2.8. Statistical Methods

SAS software (SAS v9.4, SAS institute, Cary, NC, USA) was used to analyze all data by performing PROC GLM for the least-significant difference (LSD) test at a 95% confidence level ($P < 0.05$).

3. Results and Discussion

3.1. Sugar Recoveries from Sorghum Stalks and Switchgrass

The composition of ground sorghum stalks and switchgrass is shown in Figure 2. Sorghum stalks had a higher content of lignin (20%) compared with switchgrass (16.9%). Sorghum stalks contained three types of polysaccharides including 28.4% glucan, 19.4% xylan, and 1.7% arabinan. Switchgrass structure was 35% glucan and 29% xylan, containing higher amounts of total polysaccharides compared with sorghum stalks. Sorghum stalks and switchgrass were deconstructed using a 1.25% (w·v^{-1}) sodium hydroxide solution, following the optimized conditions in the previous study [30]. Pretreated biomass totals of 58.6 g and 58.4 g were obtained from sorghum stalks and switchgrass, respectively. Alkaline pretreatment was utilized to effectively eliminate lignin compounds without significant loss of polysaccharides [11].

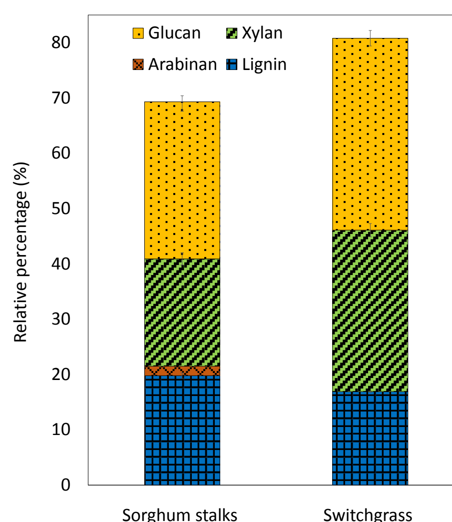

Figure 2. Composition of lignocellulosic biomass. The data shows average value of triplicate experiments and error bars representing sample standard deviation.

After saccharification of each pretreated biomass, sugar recoveries from lignocellulosic biomass were investigated in **Figure 3**. Also, maximum theoretical yields of sugar recoveries were determined using a conversion factor of 0.9 from glucose to glucan, and 0.88 from xylose (arabinose) to xylan (arabinan) [27]. **Figure 3(a)** shows sugar yields released from 100 g of each lignocellulosic biomass. Totals of 29.8 g·glucose, 17.8 g xylose, and 1.7 g arabinose were released from 100 g of raw sorghum stalks. This was 94%, 81%, and 89% of maximum theoretical yields (TY) for glucose, xylose, and arabinose, respectively. Similar amounts of fermentable sugars were achieved from sorghum stalks and switchgrass, although they had a different content of polysaccharides. Total sugar yield from 100 g of switchgrass was 34 g of glucose and 15 g of xylose. Sugar recoveries from raw switchgrass were 88% and 45% of maximal TY for glucose and xylose, respectively. Even though switchgrass content showed higher amounts of polysaccharides, lower sugar recovery was obtained due to hemicellulose loss during pretreatment [31]. Xylose recovery was substantially low because harsher conditions were applied for pretreatment of switchgrass compared with sorghum stalks. Sugar yield from 100 g of each pretreated biomass is shown in **Figure 3(b)**. Sugar yields of glucose, xylose, and arabinose from 100 g of pretreated sorghum stalks were 51 g, 30 g, and 2.9 g, respectively. Whereas, 58 g of glucose and 26 g of xylose were released from 100 g of switchgrass. In spite of higher content of xylan in switchgrass, xylose yield from pretreated biomass was lower than from sorghum stalks. This also reflects significant loss of hemicellulose during pretreatment. Total sugar yields from pretreated sorghum stalks and switchgrass were similar.

3.2. Microbial Lipid Production from Lignocellulosic Hydrolysates

Sorghum stalks and switchgrass hydrolysates were utilized as feedstocks for lipid production using *T. oleaginosus, L. starkeyi, and C.albidus*. Both lignocellulosic

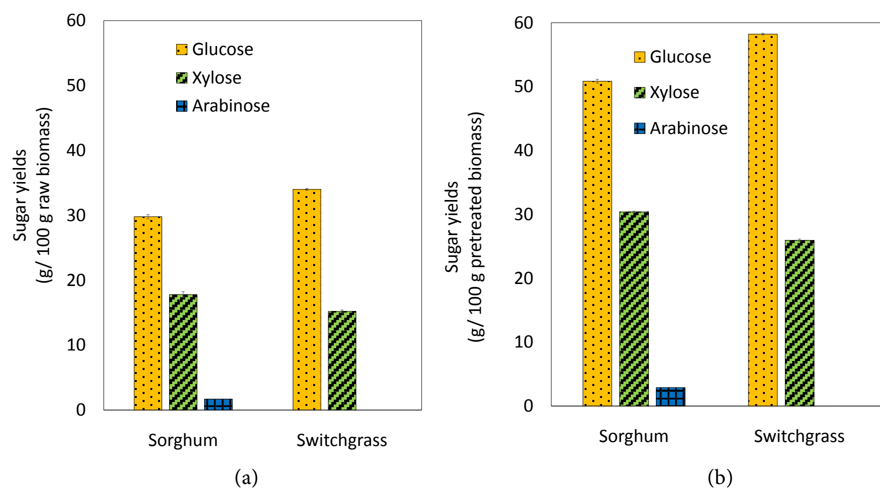

Figure 3. Sugar yield from (a) raw lignocellulosic biomass; (b) pretreated lignocellulosic biomass. The data show average value of triplicate experiments and error bars representing sample standard deviation.

hydrolysates contained acetic acid and citric acid as byproducts of enzymatic hydrolysis process. Acetic acid is normally released from acetylated hemicellulose [19]. The reason why citric acid was contained in the hydrolysates seemed that citric acid was included during enzymatic hydrolysis process to maintain pH. Figure 4 shows the fermentation profile of each oleaginous yeast using sorghum stalks and switchgrass hydrolysates.

Sugar consumption rate of *T. oleaginosus* was the fastest, compared with the other two strains. *T. oleaginosus* consumed all sugars in sorghum stalks and switchgrass hydrolysates at 72 h. *L. starkeyi* consumed all glucose in the biomass hydrolysates at 72 h, and started using xylose. *C. albidus* slowly consumed only glucose for 120 h. Citrate utilization was only observed by *T. oleaginosus*. *T. oleaginosus* consumed a total of 6 g·L^{-1} citrate in both biomass hydrolysates after all glucose was utilized at 48 h. *L. starkeyi* and *C. albidus* did not use citrate as nutrients. Instead of utilization, citrate accumulation was observed by *C. albidus* during lipid production. A total of 3 g·L^{-1} of citric acid was produced as a

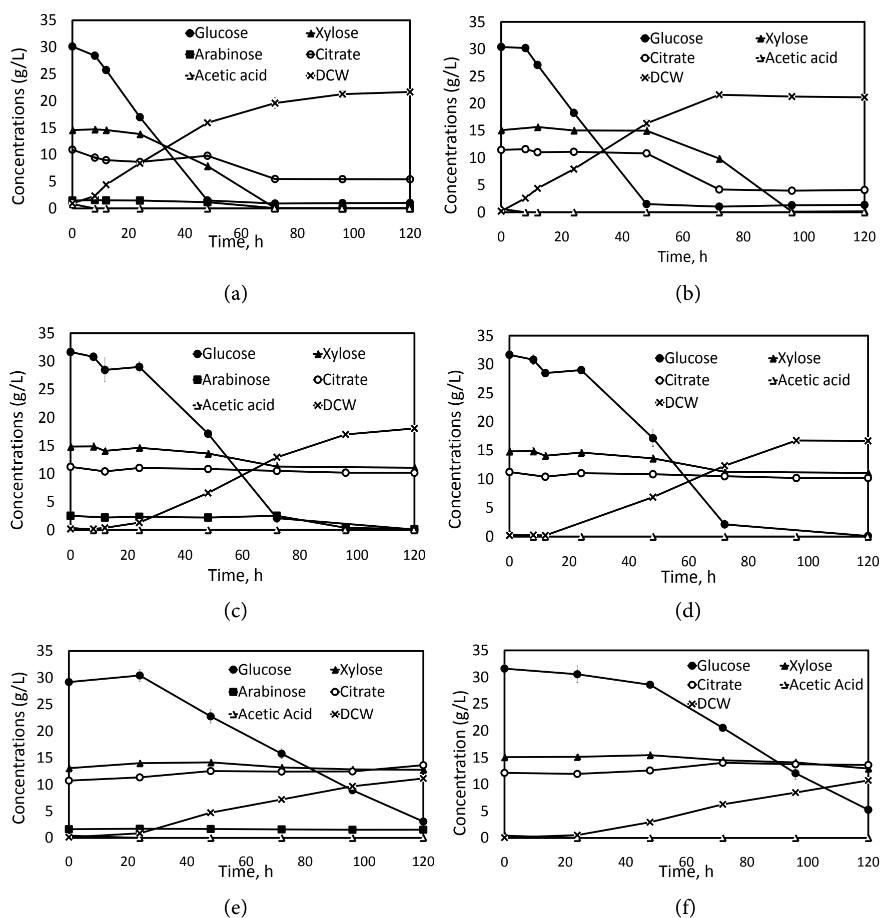

Figure 4. Fermentation profile during lipid production by (a) *T. oleaginosus* using sorghum stalk hydrolysates; (b) *T. oleaginosus* using switchgrass hydrolysates; (c) *L. starkeyi* using sorghum stalk hydrolysates; (d) *L. starkeyi* using switchgrass hydrolysates; (e) *C. albidus* using sorghum stalk hydrolysates; (f) *C. albidus* using switchgrass hydrolysates. The data show average value of triplicate experiments and error bars representing sample standard deviation.

secondary metabolite. It is known that citric acid is an important substrate of ATP citrate lyase (*ACL*) to improve lipid accumulation in oleaginous yeast; *ACL* enzymes have responsibility to increase cytosolic Acetyl-CoA pool which is major substrate for lipid synthesis [32] [33] [34]. Therefore, it is assumed that consumption of citrate might play important roles for high lipid production of *T. oleaginosus*. As was our expectation, all strains utilized acetic acid as nutrients. Sorghum stalks and switchgrass hydrolysates contained 0.5 $g \cdot L^{-1}$ acetic acid and completely consumed by all yeast strains. Acetic acid consumption by oleaginous species was also consented with other studies [35] [36]. **Table 1** shows fermentation performance of oleaginous yeast during lipid production using both lignocellulosic hydrolysates. *T. oleaginosus* showed the best performance of lipid production among oleaginous yeast strains. *T. oleaginosus* accumulated a total of 60% and 58% of lipids using sorghum stalk and switchgrass hydrolysates, respectively. Lipid content using sorghum stalk hydrolysates did not significantly differ from that using switchgrass hydrolysates at a 95% confident level. Similar levels of DCW (about 21 $g \cdot L^{-1}$) were achieved from sorghum stalk and switchgrass hydrolysates. Although utilized sugar concentration of *T. oleaginosus* was higher in the switchgrass hydrolysates, higher lipid concentrations (13 $g \cdot L^{-1}$) were attained in the sorghum stalk hydrolysates.

A high lipid yield of 0.29 $g \cdot g^{-1}$ was obtained by *T. oleaginosus* using sorghum stalk hydrolysates. This product yield was a close value to the economically feasible lipid yield suggested by Lennen and F.Pfleger; 0.3 - 0.4 $g \cdot g^{-1}$ would be theoretical limit to replace current petrochemical technologies [37]. *L. starkeyi* also produced higher concentrations of lipids with higher lipid content in the sorghum stalk hydrolysates compared with switchgrass hydrolysates, and it was statistically different value at 95% confident level. However, lower lipid yield was

Table 1. Fermentation parameters of oleaginous yeast during lipid production

	[a]Lipid content (%)	Lipid concentration ($g \cdot L^{-1}$)	[b]Lipid yield ($g \cdot g^{-1}$)	Dry-cell weight ($g \cdot L^{-1}$)	Sugar consumption ($g \cdot L^{-1}$)
		Sorghum stalk hydrolysates			
T. oleaginosus	60 ± 2.5^{A}	13.1 ± 0.7^{A}	0.29 ± 0.0^{A}	21.7 ± 0.3^{A}	45 ± 0.7^{B}
L. starkeyi	44 ± 2.0^{B}	7.9 ± 0.3^{C}	0.16 ± 0.0^{C}	18.1 ± 0.1^{B}	48 ± 0.7^{A}
C. albidus	$42 \pm 2.0^{B,C}$	4.6 ± 0.2^{E}	0.17 ± 0.0^{D}	11.1 ± 0.1^{D}	27 ± 0.6^{E}
		Switchgrass hydrolysates			
T. oleaginosus	58 ± 2.6^{A}	12.3 ± 0.2^{B}	0.27 ± 0.0^{B}	21.1 ± 0.6^{A}	46 ± 1.1^{B}
L. starkeyi	39 ± 0.1^{C}	6.5 ± 0.3^{D}	0.17 ± 0.0^{D}	16.6 ± 0.4^{C}	38 ± 0.9^{C}
C. albidus	44 ± 0.0^{B}	4.7 ± 0.1^{E}	0.16 ± 0.0^{C}	10.7 ± 0.3^{D}	29 ± 1.4^{D}

The data represent average value of triplicate experiments ± sample standard deviation. Values with the same letters, in superscripts, within the same column, are not significantly different at a 95% confidence level. [a]Lipid content was defined as weight of extractable lipid relative to weight of dry cell mass. [b]Lipid yield was calculated by dividing amount of lipids by amount of sugar consumed.

obtained in the sorghum stalk hydrolysates. It was because of that fewer amounts of sugars were consumed in the switchgrass hydrolysates. Results of statistical analysis showed that lipid accumulation of *C. albidus* was similar with *L. starkeyi* in the sorghum stalks hydrolysates, but *C. albidus* produced the lowest concentration of lipids in both biomass hydrolysates. This was because lower amounts of DCW were obtained using both biomass hydrolysates. These results demonstrated that both lipid content and DCW were important factors to achieve high titers of lipids by oleaginous yeast, because lipids are intracellular products.

Figure 5 shows the composition of fatty acids produced by oleaginous yeast cultures using sorghum stalks and switchgrass hydrolysates. To identify fatty acid profile of lipids, fatty acids were methylated to FAMEs via transesterification process and analyzed by injecting them into GC and GC/MS. Different species of fatty acids were produced by *T. oleaginosus, L. starkeyi, and C. albidus*. Major fatty acids of *T. oleaginosus* was oleic acid (C18:1), and this result was consistent with previous studies [27] [28]. Myristic acid (C14) was only produced by *T. oleaginosus,* but the amount was marginal. The most abundant fatty acid of *L. starkeyi* was also oleic acid, accounting for more than 60%. Other studies also reported that *L. starkeyi* contented relatively high levels of oleic acid (up to 70%),

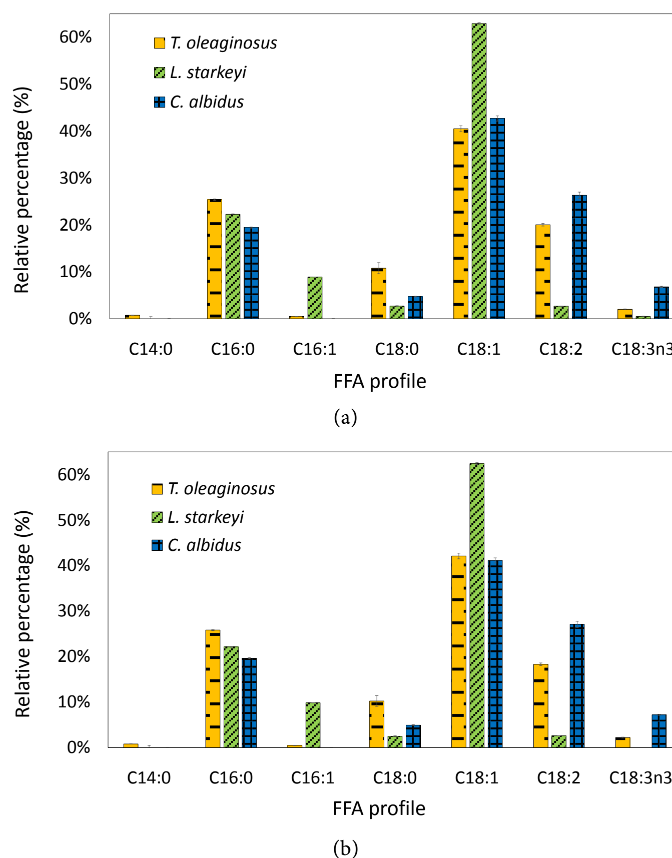

Figure 5. Composition of fatty acid produced from (a) sorghum stalk hydrolysates; (b) switchgrass hydrolysates. The data show average value of triplicate experiments and error bars representing sample standard deviation.

which is preferable in the oleochemical industry [39] [40]. Oleic acid was a major fatty acid for all yeast strains because most yeast species include a Δ9 desaturase, which incorporates a double bond at Δ9 position of stearic acid or palmitic acid [4]. *T. oleaginosus* and *C. albidus* produced relatively higher levels of linoleic acid (C18:2n6) and linolenic acid (C18:3n3) compared with *L. starkeyi*. In addition, *L. starkeyi* did not produce linolenic acid. In the fatty acid elongation cycle, oleic acid can be further desaturated to linoleic acid and linolenic acid by Δ^{12}desaturase and ω^3 desaturase, respectively [4]. It was assumed that *L. starkeyi* does not have ω^3 desaturase, and Δ^{12} desaturase enzyme activity would be insignificant. Therefore, the highest amount of oleic acid, which is a substrate of both desaturase enzymes (Δ^{12} desaturase and ω^3 desaturase), was contented in the *L. starkeyi*. Also, it is anticipated that a desaturase enzyme produced by *T. oleaginosus* or *C. albidus*, can be utilized to develop microbial strains for polyunsaturated fatty acid production.

3.3. Lipid Yield from Lignocellulosic Biomass

Our bioconversion process of lignocellulose-based microbial lipid production was evaluated by calculating the overall yield of lipid from raw sorghum stalks and switchgrass (**Figure 6**). Type of lignocellulosic hydrolysate did not considerably affect lipid yields whereas species of yeast strains and their fermentation performance directly affected total lipid yield from sorghum stalks and switchgrass. It was due to that similar amount of sugars (about 49 g) were recovered from 100 g of both biomasses, and similar fermentation performance was obtained from both biomass hydrolysates.

The highest lipid yield was achieved by *T. oleaginosus* from both lignocellulosic biomasses, since *T. oleaginosus* showed the best fermentation performance among other yeast strains during lipid production. *T. oleaginosus* produced 8% higher amounts of lipids from sorghum stalks containing a 14% lower con-

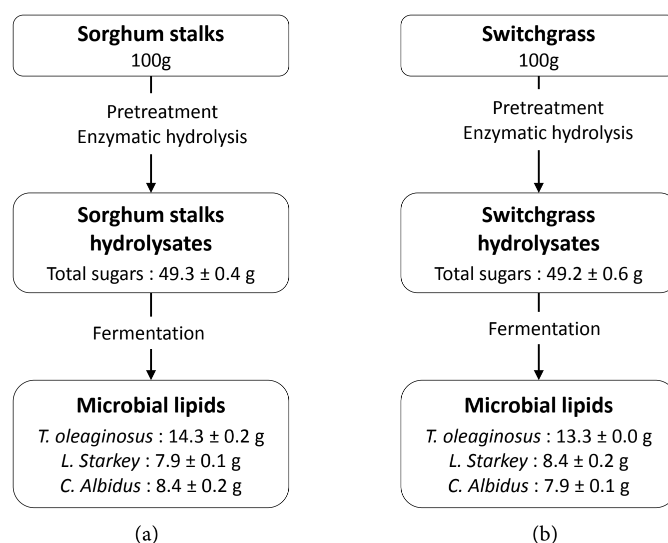

Figure 6. Lipid yields from (a) sorghum stalks; (b) switchgrass. The data represent average value of triplicate experiments ± sample standard deviation.

tent of polysaccharides compared with switchgrass. This might be due to a significant hemicellulose loss during pretreatment of switchgrass. It showed another key factor to attaining high lipid yields from biomass was to maximize sugar recoveries during pretreatment and enzymatic hydrolysis for sugar production.

Lipid yields obtained by *C. albidus* and *L. starkeyi* were not substantially different because they showed similar fermentation performance during lipid production. *C. albidus* produced higher amounts of lipids from sorghum stalks compared with switchgrass, although low-lipid concentrations were obtained during fermentation. It was due to that higher product yield was achieved during fermentation using sorghum stalk hydrolysates. Whereas, lower amounts of lipids were obtained by *L. starkeyi* using sorghum stalks compared with switchgrass, even though higher lipid concentrations and contents were attained during fermentation of *L. starkeyi* using sorghum stalk hydrolysates. This was because lower sugar consumptions and product yields were observed by *L. starkeyi* in sorghum stalk hydrolysates. To sum up these results, maximization of sugar recoveries during sugar production, and selection of proper microbial strains for lipid production, were key factors to achieve high yields of microbial lipids from lignocellulosic biomass.

4. Conclusion

Microbial lipid production from lignocellulosic biomass such as sorghum stalks and switchgrass was investigated using three oleaginous yeast strains; *T. oleaginosus*, *L. starkeyi and C. albidus*. High-sugar recoveries (89% of TY) from sorghum stalks were obtained via an alkaline pretreatment whereas total sugar recoveries from switchgrass were 67% of the TY. *T. oleaginosus* showed the best fermentation performance using both biomass hydrolysates among oleaginous yeast cultures. Lipid titers of 13.2 $g \cdot L^{-1}$ and lipid yield of 0.29 $g \cdot g^{-1}$ were achieved by *T. oleaginosus* using sorghum stalk hydrolysates. Results of overall lipid yield assessment revealed that a key matrix to improve industrial feasibility of bioconversion for lignocelluose-based microbial lipid production is maximal recovery of fermentable sugars from raw biomass and strain development to attain better fermentation performance.

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