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ORIGINAL ARTICLE

Biovalorization of whole old oil palm trunk as low-cost nutrient sources for biomass and lipid production by oleaginous yeasts through batch and fed-batch fermentation

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Abstract
Biovalorization of whole old oil palm trunk (OPT) as low-cost nutrient sources for biomass and lipid production by oleaginous yeasts were intensively investigated. Oil palm sap (OPS) squeezed from OPT containing mainly glucose (52%), sucrose (28%), and arabinose (20%) was used directly while the residual OPT fiber was acid-hydrolyzed into fermentable sugars before use. The main sugars in OPT hydrolysate (OPTH) were arabinose (47%), xylose (35%), and glucose (18%). Six oleaginous yeasts including *Rhodotorula mucilaginosa* G43, *Candida tropicalis* X37, *Trichosporonoides spathulata* JU4-57, *Kluyveromyces marxianus* X32, *Yarrowia lipolytica* TISTR 5151, and *Yarrowia lipolytica* TISTR 5054 were screened. Among the strains screened, *R. mucilaginosa* G43 and *C. tropicalis* X37 gave the maximum lipid yields of 65–68 mg/g-sugar from OPS. When OPTH was used, *Y. lipolytica* TISTR 5054 grew best and produced higher yields of lipids (55–58 mg/g-sugar) than other strains. To manipulate the suitably high C/N ratio for lipid production, the fed-batch fermentations using various co-carbon sources were performed. Crude glycerol (CG), a byproduct from biodiesel production, was the best co-carbon source that could increase the lipid production up to 3.07 ± 0.03 g/L from OPS and 1.80 ± 0.06 g/L from OPTH. The preliminary mass balance revealed the practical biovalorization of whole felled OPT into yeast biomass and lipids that may greatly increase the competiveness of the bioenergy and palm oil industries.

Keywords Fed-batch fermentation · Lipids · Oil palm trunk · Oleaginous yeasts

1 Introduction

Biodiesel is broadly produced from plant oils; however, using plant oils as biodiesel feedstocks would compete with edible oils and cause increasing of food prices. Microbial lipids have gained increasing interest as renewable oil sources because they have similar composition to plant oils [1]. Microbial lipids are produced by oleaginous microorganisms including bacteria, yeasts, molds, and microalgae those are able to accumulate lipids > 20% of their biomass. Among these oleaginous species, yeasts are the most favorable oleaginous microorganism in terms of fast-growing rate, high cell density growth, high lipid content, and their ability to use low-cost fermentable substrates such as nutritional residues from agriculture and industries [2–5]. Most oleaginous yeasts naturally accumulate neutral lipids in form of triglycerides. Some of them show a remarkably high lipid content > 50%. Oleaginous yeasts accumulate lipids via two alternative pathways, namely de novo and ex novo lipid synthesis, using hydrophilic and hydrophobic substrates, respectively. Most well-known oleaginous yeasts contain fatty acids with C16–C18 range of carbon atom which are suitable for production of biodiesel with good fuel properties [4].

Recently, various low-cost substrates have been used to cultivate oleaginous microorganisms in order to reduce the production costs of lipids. Lignocellulosic biomass is one of potential substrates for lipid production due to its great availability in nature and renewable feature. Some lignocellulosic wastes such as rice straw, bagasse, corncob, and palm waste hydrolysate have been used for lipid production [2–5]. This

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could reduce the production costs of lipids and also help in solving environmental pollution. A number of oleaginous yeast species are capable of converting sugars derived from lignocellulosic wastes into lipids such as *Rhodotorula mucilaginosa*, *Candida tropicalis*, *Trichosporonoides spathulata*, and *Yarrowia lipolytica* [2–5]. In addition, it has been also reported that some yeasts are able to tolerate inhibitors in the hydrolysate and produced high amount of lipids. This tolerance property of the yeasts is beneficial for cost-effective lipid production from lignocellulosic hydrolysate without detoxification [5].

Oil palm is an important oil crop in Thailand with the plantation area more than 700,000 ha spreading mainly in the South. Usually, the palms give oil-containing fruits 3 years after being planted and in order to maintain oil productivity they need to be replanted at interval time of 20–25 years. Most of the old oil palm trees, around 17 million tons per year in Thailand [6], are cut down and burnt/left unused at the plantation site. Many attempts have been done to utilize the old oil palm trees in various purposes depending on their structure and compositions. Among them, oil palm trunk (OPT) is really soft and comprises sap up to 80% based on wet weight. It is therefore not suitable for making plywood. As the oil palm sap (OPS) contained various sugars and other nutrients [6], it is then expected to be used as low-cost nutrient sources for microorganisms.

The main aim of this study was to provide the practical procedures for biovalorization of whole OPT into yeast biomass and lipids which has not been done before. Therefore, the intensive investigation on the use of OPS and residual fiber after sap squeezing with the suitable yeasts as well as the process development to increase both biomass and lipids were performed. Six promising oleaginous yeasts those that have been recognized with their high growth rate and high lipid content [2–5] were screened and their culture conditions were optimized. The strategies to boost up lipid content by using co-carbon sources coupling with fed-batch fermentation were attempted. It should be noted that there was a limited number of research on the use of co-carbon source, especially coupled with fed-batch fermentation for yeast biomass and lipid production. The preliminary mass balance for prospect valorization of whole OPT into yeast biomass and lipids was also analyzed.

2 Materials and methods

2.1 Preparation of sap and hydrolysis of fiber from felled old oil palm trunk

Felled old OPT was provided by the Pure Parawood Co., Ltd. (Suratthanee Province, Thailand). The OPT was cut off into 2–3-cm thick slices and the OPS was squeezed by compressing machine, filtered using cheesecloth, and if necessary concentrated using evaporator (EYELA Rotary Evaporator N 1000, Tokyo Rikakikai, Japan) to obtain the desired sugar concentration. This OPS was used directly for yeast biomass and lipid production. The total sugar concentration of OPS was adjusted to 20 g/L and the pH was adjusted to 6.0. This OPS was used directly as a sole nutrient source for yeast cultivation. The fiber residues of the trunk after sap squeezing were hydrolyzed to fermentable sugars by hydrothermal-acid treatment using diluted sulfuric acid (0.5% w/v) at 120 °C for 60 min. The hydrothermal-acid treatment has been used for economically achieving high sugar production from lignocellulosic material [5]. This OPT hydrolysate (OPTH) was separated from the solid residues by filtration and used for yeast biomass and lipid production, and if necessary concentrated using evaporator.

2.2 Microorganisms and media

Six oleaginous yeasts including *Rhodotorula mucilaginosa* G43, *Candida tropicalis* X37, *Trichosporonoides spathulata* JU4-57, *Kluyveromyces marxianus* X32, *Yarrowia lipolytica* TISTR 5151, and *Yarrowia lipolytica* TISTR 5054 were obtained from the Bioprocess Engineering Laboratory (Faculty of Agro-Industry, Prince of Songkla University). The plate cultures incubated at 30 ± 2 °C for 24 h were transferred to 125 mL Erlenmeyer flasks containing 50 mL pre-culture medium. The pre-culture medium comprised of glucose 4%, peptone 0.5%, and yeast extract 1.5%, pH 6.0. The pre-cultures were incubated at 30 ± 2 °C on a shaking incubator (VRN-480, Gemmy orbital shaker, Taiwan) with shaking speed at 140 rpm for 24 h before use as the seed culture [7].

2.3 Selection of oleaginous yeasts

The initial sugar concentration of each medium was adjusted to be 20 g/L. This resulted in the diluted nitrogen in OPS medium but the C/N ratio was the same at 93. To increase the nitrogen concentration, the diluted OPS medium was added with ammonium sulfate at 0.5 g/L leading to the C/N ratio of 28. OPTH was added with ammonium sulfate at 0.5 g/L which corresponded to a C/N ratio of 40. The pH of the medium was adjusted to 6.0 before sterilization by using autoclave. A 24-h-old seed culture of the yeast (approximately 10^7 cells/mL) was inoculated and the cultures were incubated at 30 ± 2 °C on a shaking incubator with shaking speed at 140 rpm for 72 h. Each set of experiments were sampled every 12 h. The samples were centrifuged at a speed of 7000 rpm for 10 min to separate biomass and supernatant. The dry biomass and lipid content were determined. The biomass and lipid productions of the yeasts were compared.

2.4 Optimization of sugar concentration and fed-batch fermentation with various carbon sources

The effect of sugar concentration (20, 40, and 60 g/L) on biomass and lipid productions of the selected yeasts were investigated in batch fermentation. The fed-batch fermentation was initiated as the same as that for batch operation. Various co-carbon sources at 20 g/L were fed intermittently at 36, 48, and 60 h without discharging fermentation broth. Various co-carbon sources included concentrated OPS/OPTH, glucose, glycerol, and crude glycerol (a by-product from biodiesel plant). Time courses of yeast biomass and lipid production were determined. Crude glycerol used in this study contained 39–40% glycerol content with some impurities including potassium/sodium salts 4–5%, methanol < 3%, non-glycerol organic matters such as fatty acids from oil feedstocks < 7.5%.

2.5 Analytical methods

The yeast cells were recovered by centrifugation of culture broth at 7000 rpm for 10 min. The cell pellets were washed twice and then oven dried at 60 °C until constant weight [5]. The yeast biomass was determined gravimetrically. The dry biomass was used to extract lipids by added with chloroform:methanol (2:1) and the mixture was sonicated for 30 min. Solvent phase with extracted lipids was recovered by centrifugation. The extraction process was repeated twice. The extracted lipids were pooled together and the solvents were evaporated and the lipids were determined gravimetrically [5, 8]. Lipid content was expressed as percentage of gram lipid per gram dry biomass. The total sugar concentration was determined by the phenol–sulfuric method. The glycerol concentration was determined spectrophotometrically [5]. The lipid yield was calculated by dividing lipid production (mg/L) by consumed sugar (g/L). The lipid productivity was calculated by dividing maximum lipid production (mg/L) by fermentation time (h). OPT was analyzed for cellulose, hemicellulose, and lignin contents by the AOAC standard method. The sugar compositions and organic acids in OPS and OPTH was analyzed using high-performance liquid chromatography (HPLC) (Agilent technologies, Santa Clara, CA) equipped with an Animex HPX-87H (300 mm × 7.8 mm) column (Bio-Rad, Hercules, CA). The operation temperature was 45 °C and the mobile phase was 0.01 N of sulfuric acid. The pump rate was 0.6 mL/min. The sugar derivatives, namely furfural and 5-hydroxymethylfurfural (HMF) in the samples, were determined using spectrophotometer (2150-UV, UNICO, USA) with the maximum absorbance spectra of 276 and 282 nm, respectively. Their concentrations were calculated using standards of known concentrations [5]. All experiments were performed at least in triplicates. Analysis of variance (ANOVA) was performed to calculate significant differences in treatment means, and the least significant difference ($p \leq 0.05$) was used to separate means, using the SPSS software.

3 Results and discussion

3.1 The compositions of oil palm sap and oil palm trunk hydrolysate

The felled old OPT from the top of the apex down to 1 m with a diameter of 20 cm had a proximate volume of 0.0314 m^3 and weight of 32 kg. After squeezing, approximately 15 L of OPS was obtained. The compositions of the OPS were glucose, sucrose, and arabinose at concentrations of 19.39 ± 0.08 g/L, 10.45 ± 0.05 g/L, and 7.44 ± 0.07 g/L, respectively, corresponding to 52%, 28%, and 20% based on total sugar (Table 1). The total sugar and nitrogen concentration were 41.1 g/L and 0.44 g/L, respectively, which corresponded to a very high C/N ratio of 93. The pH of the OPS was 4.5. The similar sugar compositions have been reported by Komonkiat et al. [6] while Lokesh et al. [9] reported the different sugar compositions in which sucrose concentration was highest at 33.28 g/L followed by glucose at 16.01 g/L. The difference in composition and concentration of the OPS is due to the oil palm species, plant area, and the age of palm trees. The cellulose, hemicellulose, and lignin contents of residual fiber pulp were 37.93 ± 2.3% (w/w), 21.08 ± 0.8% (w/w), and 5.62 ± 1.9% (w/w), respectively (Table 1). The residual pulp was acid hydrolyzed using dilute sulfuric acid solution (0.5% w/v) with a solid loading of 10% to obtain OPTH before use. The sugar compositions of OPTH were arabinose, xylose, and glucose at concentrations of

Table 1 Compositions of oil palm sap, residual fiber pulp and oil palm trunk hydrolysate

Feedstock	Composition	
Oil palm sap (OPS)	Glucose	19.39 ± 0.08 g/L
	Sucrose	10.45 ± 0.05 g/L
	Arabinose	7.44 ± 0.07 g/L
Residual fiber pulp	Cellulose	37.93 ± 2.3%
	Hemicellulose	21.08 ± 0.8%
	Lignin	5.62 ± 1.9%
Oil palm trunk hydrolysate (OPTH)	Arabinose	20.07 ± 0.74 g/L
	Xylose	14.81 ± 0.07 g/L
	Glucose	7.70 ± 0.05 g/L
	Furfural	1.56 ± 0.01 g/L
	5-Hydroxylmethylfurfural (HMF)	2.58 ± 0.05 g/L
	Acetic acid	1.71 ± 0.63 g/L

20.07 ± 0.74 g/L, 14.81 ± 0.07 g/L, and 7.70 ± 0.05 g/L, respectively, corresponding to 47%, 35%, and 18% based on total sugar. The concentrations of sugar derivatives, furfural, and HMF were 1.56 ± 0.01 g/L and 2.58 ± 0.05 g/L, respectively. The acetic acid, by-product from hemicellulose hydrolysis, was 1.71 ± 0.63 g/L (Table 1).

3.2 Screening of oleaginous yeasts cultivated in OPS and OPTH

Six oleaginous yeasts including *R. mucilaginosa* G43 (RM), *C. tropicalis* X37 (CT), *T. spathulata* JU4-57 (TS), *K. marxianus* X32 (KM), *Y. lipolytica* TISTR 5151 (YL5151), and *Y. lipolytica* TISTR 5054 (YL5054) were cultivated in three media including OPS as a sole nutrient source (C/N ratio of 93), OPS added with ammonium sulfate (C/N ratio of 28), and OPTH added with ammonium sulfate (C/N ratio of 40). The initial sugar concentration of each medium was adjusted to be 20 g/L. The results are shown in Fig. 1. All yeasts could grow on OPS without adding ammonium sulfate and gave the maximum biomass in the range of 6–9 g/L. Although *T. spathulata* JU4-57 grew best, but its lipid content was very low (less than 10%). The lipid contents of other yeasts were higher in the range of 10–18%. The lipid content trended to decrease with the prolonging cultivation time possibly due to the consumption of storage lipids after exhaustion of carbon sources. The consumption of storage lipids has also been observed in other oleaginous yeasts [5, 7, 10]. The addition of ammonium sulfate slightly increased biomass for *R. mucilaginosa* G43, *C. tropicalis* X37, and

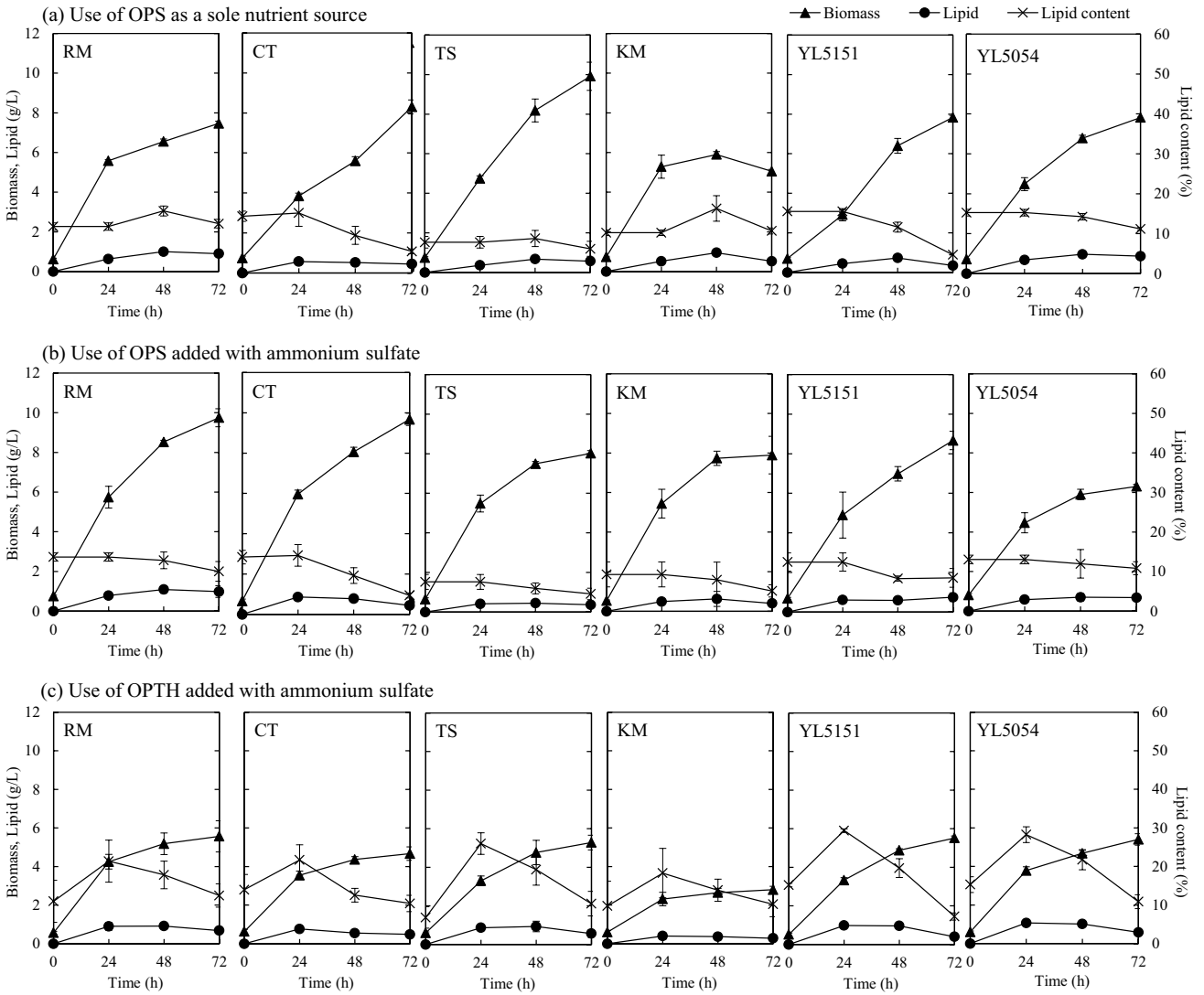


Fig. 1 Screening of oleaginous yeasts for cultivation in oil palm sap (OPS) as a sole nutrient source (**a**) and OPS added with ammonium sulfate (**b**) and oil palm trunk hydrolysate (OPTH) added with ammonium sulfate (**c**). RM: *R. mucilaginosa* G43, CT: *C. tropicalis* X37, TS: *T. spathulata* JU4-57 KM: *K. marxianus* X32, YL5151: *Y. lipolytica* 5151, YL5054: *Y. lipolytica* 5054

K. marxianus X32 (Fig. b). However, the lipid contents of all yeasts became lower compared to the use of OPS without addition of ammonium sulfate. It could be concluded that the yeasts tend to accumulate lipids at a lower content when using media with a lower C/N ratio. These results indicated that the higher C/N ratio, namely limited nitrogen source with excess carbon source, could promote more lipid accumulation in the yeasts than the lower C/N ratio [4, 11–13]. As most yeast could grow on OPS without addition of ammonium sulfate, the OPS was then used as a sole nutrient source without adding any other nutrients. Among the yeast screened, *R. mucilaginosa* G43 and *C. tropicalis* X37 were selected due to their high biomass and lipid content. Juanssilfero et al. [14] have reported the lipid production by the yeast *Lipomyces starkeyi* using OPS, but no reports about the comparison between different species of the yeasts and the use of residual OPT fiber after sap squeezing.

OPT fiber after sap squeezing could be potent sources of hemicelluloses and celluloses that could be acid-hydrolyzed into fermentable sugars, namely OPTH. OPTH was diluted to obtain a sugar concentration of 20 g/L and added with 0.5 g/L ammonium sulfate before use for yeast cultivation. The biomass and lipid productions of the oleaginous yeasts using OPTH are shown in Fig. 1c. Although the sugar concentrations in the OPS and OPTH were the same at 20 g/L, the growth and lipid production of the yeasts cultivated in the OTPH were much lower possibly due to the selectivity toward sugars and the possible presence of inhibitory compounds. Most yeast prefers glucose rather than arabinose and xylose [5, 14]. Moreover, the presence of sugar derivatives such as furfural and HMF might be another reason for the lower biomass production in OPTH. It has been reported that furfural at a concentration > 0.096 g/L and HMF at a concentration > 1.85 g/L did inhibit the growth and lipid production of *Rhodosporidium toruloides* Y4 yeast [15]. It was also shown that these inhibitors likely suppressed growth more severely than lipid synthesis. The concentrations of these inhibitors depend on the source of biomass and the conditions of hydrolysis. Their concentrations in the hydrolysates may range from 0.5 to 11 g/L [16]. These inhibitors have been believed to negatively affect cell walls/membranes and also inhibit RNA synthesis in the microorganisms [17, 18]. In this study, the concentration of HMF in the hydrolysate after dilution to obtain 20 g/L sugar was lower than the previously reported inhibitory levels. But the concentration of furfural was still higher than those previously reported as inhibitory levels (> 0.2 g/L). Therefore, the inhibitory effect on yeast cell growth might be because of the presence of furfural. There are several methods to reduce the concentration of the inhibitors such as evaporation, overliming, and adsorption by activated carbon [5, 17]. However, these methods are costly and time-consuming, therefore the selection of the yeasts those could be tolerant to these inhibitors should be selected. In this study, *R. mucilaginosa* G43 and *Y. lipolytica* TISTR 5054 was selected to be cultivated in OPTH due to its higher biomass and lipid production compared to other yeasts indicating its selectivity toward arabinose and xylose in the OPTH.

3.3 Effect of sugar concentration in OPS

To improve lipid production by *R. mucilaginosa* G43 and *C. tropicalis* X37, the suitable sugar concentration was determined. The concentration of sugar was varied at 20, 40, and 60 g/L. As shown in Fig. 2a, with increasing the sugar concentration up to 40 g/L *R. mucilaginosa* G43 grew better and gave higher biomass and lipid productions. However, with higher sugar concentration up to 60 g/L *R. mucilaginosa* G43 grew slower possibly due to the substrate inhibition at high concentration [5]. The maximum biomass production at sugar concentration of 40 g/L and 60 g/L were not significantly different. They were 8.50 ± 0.95 g/L and 8.62 ± 0.45 g/L, respectively, at 72 h. It should be noted that the lipid content became higher with increasing sugar concentration and this increased sugar concentration seemed to alleviate the degradation of storage lipids. These results imply that the lipid content could be increased by maintaining high carbon source concentration in the growth medium and/or manipulating the suitably high C/N ratio [19, 20]. Similarly, the biomass of *C. tropicalis* X37 increased gradually with increasing sugar concentration and reached the maximum value of 9.13 ± 0.05 g/L at 60 g/L sugar concentration (Fig. 2b). The lipid content of *C. tropicalis* X37 was also increased with increasing sugar concentration. At this sugar concentration, the maximum lipid yields by two yeast strains were 65–68 mg/g-sugar from OPS. The residual sugar concentration became higher when using higher initial sugar concentration. The sugar consumption by *R. mucilaginosa* G43 when using 20, 40, and 60 g/L sugar concentration were 14.29 ± 0.83 g/L, 18.30 ± 1.07 g/L, and 20.13 ± 2.02 g/L, respectively and those for *C. tropicalis* X37 were 15.94 ± 0.69 g/L, 20.54 ± 0.51 g/L, and 32.05 ± 0.63 g/L, respectively.

3.4 Fed-batch fermentation using OPS added with various co-carbon sources

The fed-batch culture method was performed to reduce the inhibitory effect of high sugar concentration. The additional sole carbon source was intermittently added to keep C/N ratio at a high level to promote lipid production in the yeasts. Figure 3 shows the time-course of biomass, lipid, and lipid content of the selected *R. mucilaginosa* G43 and *C. tropicalis* X37 cultivated in OPS with initial sugar concentration of 40 g/L and fed intermittently with 20 g/L various co-carbon sources at 36, 48, and 60 h. Various carbon sources include

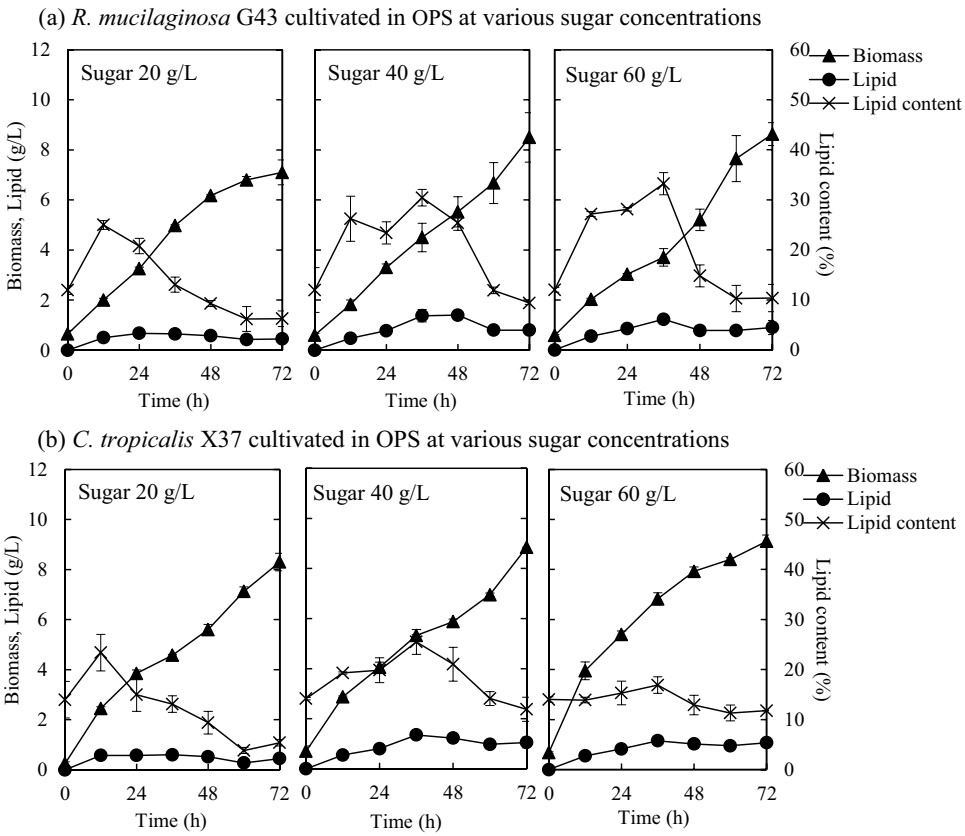


Fig. 2 Effect of sugar concentration on biomass and lipid production of **a** *R. mucilaginosa* G43 and **b** *C. tropicalis* X37 cultivated in oil palm sap (OPS). The cultures were incubated at room temperature (30 ± 2 °C) and 140 rpm for 72 h

concentrated OPS, glucose, glycerol, and crude glycerol. The cultures were incubated at room temperature (30 ± 2 °C) and 140 rpm for 72 h. Compared with batch fermentation (Fig. 2), the fed-batch fermentation was more suitable to enhance lipid production (Fig. 3). There was no significant difference in biomass production when fed with glucose and glycerol compared to that fed with concentrated OPS. But the lipid production by both yeasts was more improved with the addition of glucose and glycerol as co-carbon sources. These results indicate that the addition of only carbon source (glucose or glycerol) is more suitable for lipid accumulation to keep high C/N ratio than the addition of concentrated OPS containing nitrogen source. Therefore, OPS can be used as low-cost nutrient source for biomass production during the first period and feeding with pure carbon source can be used to increase the lipid content during fed-batch fermentation in the latter period.

In this study, the fed-batch fermentation of both yeasts fed with crude glycerol gave higher biomass than that fed with pure glycerol. It was possible that some impurites such as salts and fatty acids from oil feedstocks in the crude glycerol might stimulate the growth of the yeasts [21, 22]. Although crude glycerol has been used as a carbon source for lipid production by the yeasts, its impurites are crucial factor determining the yeast cell growth as well as lipid accumulation [23]. Although there was no significant difference between lipid production in the fed-batch using pure glycerol and crude glycerol for *R. mucilaginosa* G43, the fed-batch fermentation with crude glycerol enhanced both biomass and lipid production for *C. tropicalis* X37. Jeevan Kumar et al. [24] reported the use of crude glycerol with the optimal C/N ratio of 105 added with 1.52 g/L $MgSO_4$ and 4.55 mM $FeSO_4$ for biomass and lipid production by *Trichosporon shinodae*. The yeast accumulated lipids at 49.85 ± 0.8% (w/w). Gong et al. [25] revealed the synergetic effects of glucose and glycerol on lipid production by *Cutaneotrichosporon oleaginosum*. The synergistic effects might be an increase in substrate transportation efficiency through simultaneous assimilation of dual carbon sources. In this study, *R. mucilaginosa* G43 gave the highest lipid production and lipid content of 2.65 ± 0.86 g/L and 54.64 ± 0.80%, respectively, with the addition of pure glycerol. While *C. tropicalis* X37 gave the highest biomass of 9.83 ± 0.10 g/L and the highest lipid production of 3.07 ± 0.03 g/L with the addition of crude glycerol. The lipid productivity calculated using the maximum lipid production at 48 h was 63.96 ± 0.63 mg/L h. It could then be concluded that the fed-batch fermentation with glycerol as a co-carbon source could avoid the substrate inhibition and enhance the production of lipids. The results shown in Fig. 3 also indicate that the addition of co-carbon sources during fed-batch fermentation could alleviate the degradation of storage lipids in the cells.

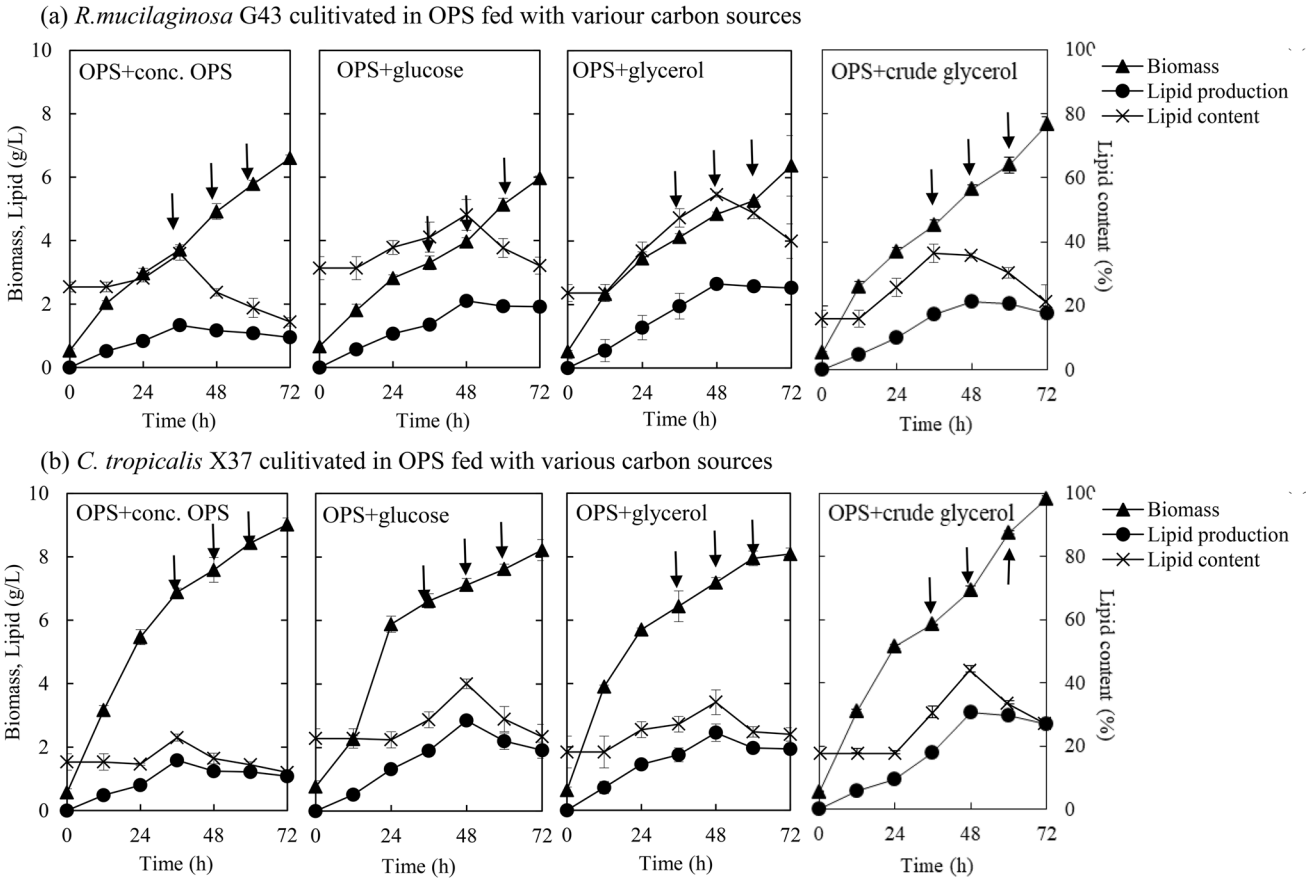


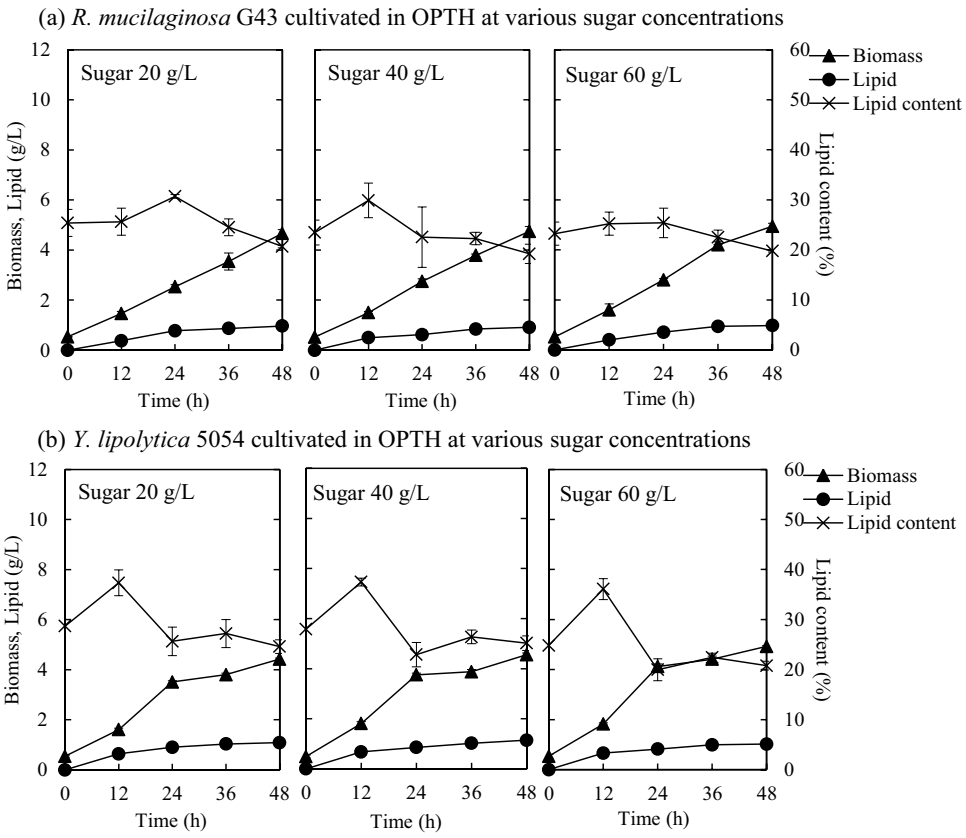
Fig. 3 Fed-batch cultivation of **a** *R. mucilaginosa* G43 and **b** *C. tropicalis* X37 trop in oil palm sap (OPS) at initial sugar concentration of 40 g/L and added with various carbon sources including concentrated OPS, glucose, glycerol, and crude glycerol at 24, 36, 48, and 60 h. The cultures were incubated at room temperature (30 ± 2 °C) and 140 rpm for 72 h. Arrows indicate feeding time

3.5 Effect of sugar concentration in OPTH and fed-batch fermentation

The concentration of sugars in OPTH was varied at 20, 40, and 60 g/L and used as a carbon source for the selected *R. mucilaginosa* G43 and *Y. lipolytica* 5054 (Fig. 4). There was no significant difference in biomass and lipid production by *R. mucilaginosa* G43 using three different sugar concentrations. While the biomass and lipid production by *Y. lipolytica* 5054 slightly increased with increasing sugar concentration. *Y. lipolytica* TISTR 5054 produced lipids with the yields of 55–58 mg/g-sugar. There was also no significant difference in the lipid contents at three different sugar concentrations for both strains. The fed-batch fermentation for *Y. lipolytica* 5054 was performed using additional various co-carbon sources (Fig. 5). Compared to the intermittent addition of concentrated OPTH with sugar concentration of 20 g/L at 36, 48, and 60 h, the addition of glucose, glycerol, and crude glycerol at the same concentration and time gave higher biomass and lipid production. The lower biomass production when adding with concentrated OPTH was likely due to the high concentration of toxic compounds as mentioned above. Interestingly, the lipid production by both yeasts was more improved with the addition of glycerol and crude glycerol as co-carbon sources. The higher biomass obtained when using crude glycerol was possibly due to some nutritional compounds in the crude glycerol that might stimulate the growth of the yeasts as mentioned above. *Y. lipolytica* 5054 gave the highest biomass and lipid production of 9.63 ± 0.09 g/L and 1.80 ± 0.07 g/L, respectively, with the addition of crude glycerol. The lipid productivity calculated using the maximum lipid production at 60 h was 30.0 ± 1.0 mg/L h.

3.6 Preliminary mass balance analysis

A preliminary mass balance analysis was performed for the production of yeast lipids from felled OPT using oleaginous yeasts those are suitable for fermentation of OPS and OPTH (Fig. 6). The experimental data from the previous section was used to estimate the yeast lipid production from 100 kg OPT. The 100 kg OPT was separated into 46.9 L



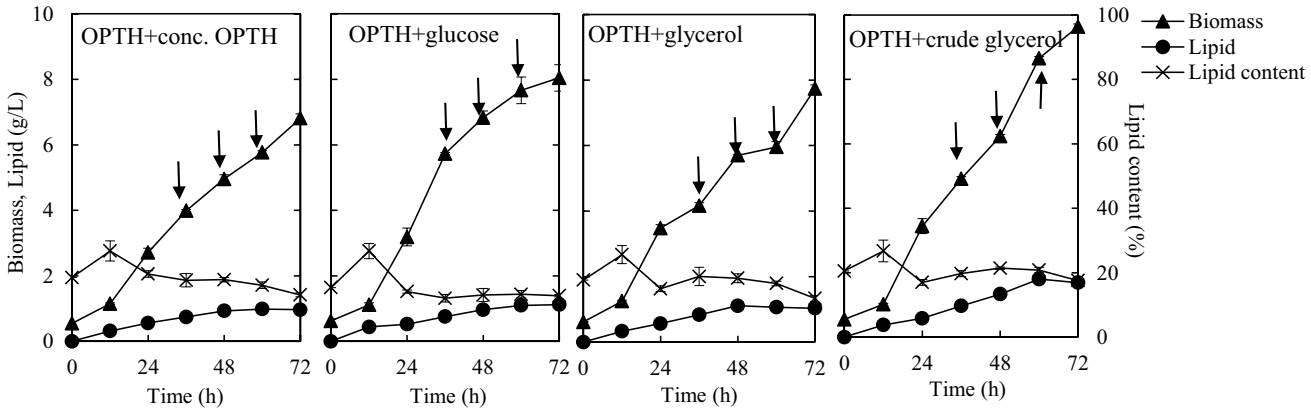


Fig. 4 Effect of sugar concentration on biomass and lipid production of **a** *R. mucilaginosa* G43 and **b** *Y. lipolytica* 5054 cultivated in oil palm trunk hydrolysate (OPTH). The cultures were incubated at room temperature (30 ± 2 °C) and 140 rpm for 48 h

Fig. 5 Fed-batch cultivation of **a** *Y. lipolytica* 5054 in oil palm trunk hydrolysate (OPTH) at initial sugar concentration of 40 g/L and added with various carbon sources including concentrated OPTH, glucose, glycerol, and crude glycerol at 24, 36, 48, and 60 h. The cultures were incubated at room temperature (30 ± 2 °C) and 140 rpm for 72 h. Arrows indicate feeding time

OPS and 53.1 kg wet residual OPT fiber. The sugar contents in 46.9 L OPS were glucose 0.909 kg (19.39 g/L × 46.9 L), sucrose 0.490 kg (10.45 g/L × 46.9 L), and arabinose 0.349 kg (7.44 g/L × 46.9 L). The 46.9 L OPS was inoculated with 0.02 kg yeast *C. tropicalis* and after 36 h batch fermentation, crude glycerol was intermittently added as co-carbon source (20 g/L × 3 times × 46.9 L = 2.814 kg in total) during fed-batch fermentation. Based on the above experimental data, 46.9 L OPS would yield yeast biomass 0.461 kg (9.83 g/L × 46.9 L) and yeast lipids 0.144 kg (3.07 g/L × 46.9 L). The spent medium could be reused by adding more co-carbon source.

The 53.1 kg wet residual OPT fiber (35.6 kg based on dry mass) were cellulose 13.5 kg (37.93% × 35.6 kg), hemicellulose 7.5 kg (21.08% × 35.6 kg), and lignin (5.62% × 35.6 kg) 2.0 kg. Residual OPT fiber was added with 338 L of dilute

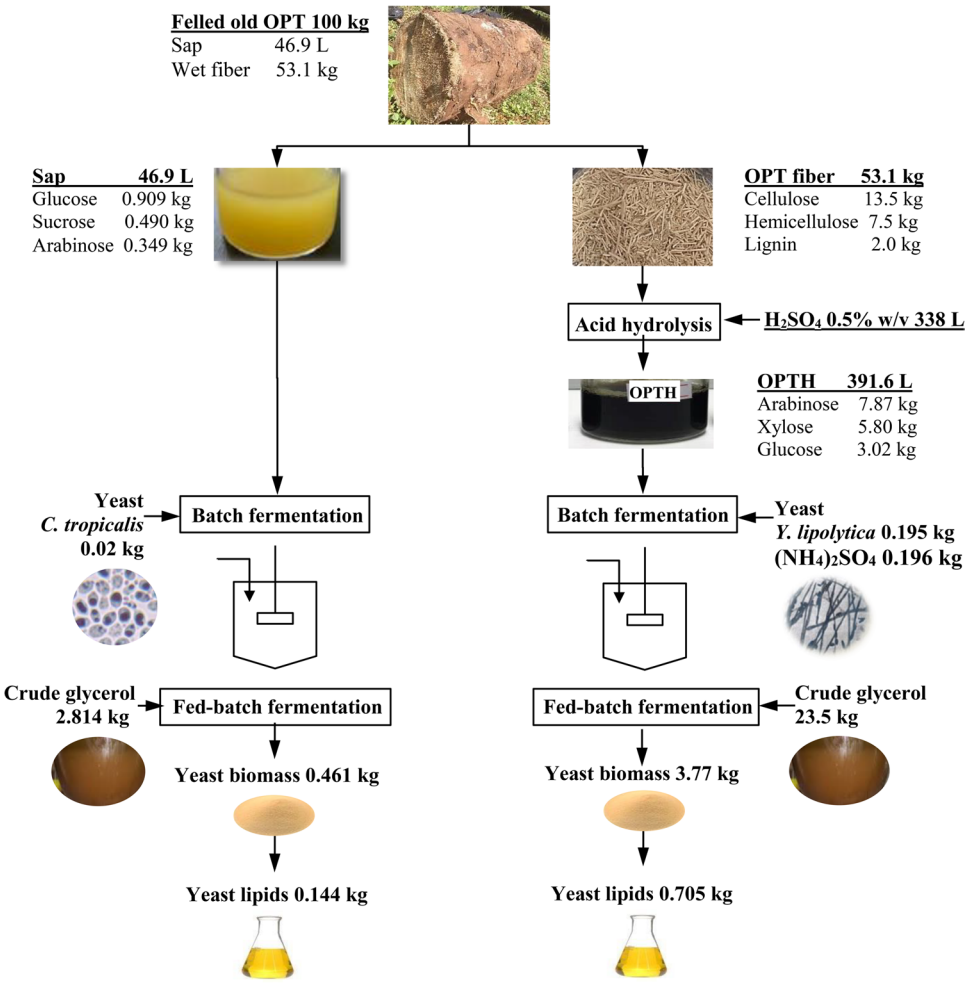


Fig. 6 Preliminary mass balance analysis of yeast lipids production from felled old oil palm trunk

sulfuric acid (0.5%, w/v) and hydrolyzed to obtain OPTH with fermentable sugars including arabinose 7.86 kg (20.07 g/L × 391.6 L), xylose 5.80 kg (14.81 g/L × 391.6 L), and glucose 3.02 kg (7.70 g/L × 391.6 L). The 391.6 L OPTH was inoculated with 0.195 kg yeast *Y. lipolytica* and 0.196 kg ammonium sulfate after 36 h batch fermentation crude glycerol was intermittently added as co-carbon source (20 g/L × 3 times × 391.6 L = 23.5 kg in total) during fed-batch fermentation. Based on the above experimental data, 391.6 L OPTH would yield yeast biomass 3.77 kg (9.63 g/L × 391.6 L) and yeast lipids 0.705 kg (1.80 g/L × 391.6 L). Finally, 100 kg felled old OPT could be valorized into yeast biomass 4.231 kg (0.461 kg + 3.77 kg) and yeast lipids 0.849 kg (0.144 kg + 0.705 kg).

In addition, to improve the lipid content of the yeasts, the nitrogen limitation coupled with fed-batch fermentation should be applied [7, 20, 26]. The addition of surfactants might also be another strategy to increase the lipid content of the yeasts as these surfactants can increase the substrate availability and membrane permeation [11]. It should be noted that the fatty acid composition of the selected yeast strains in this study have been reported to contain C16–C18 as the predominant fatty acids. These kinds of fatty acids can be promising feedstocks for production of biodiesel with high ignition quality, more complete combustion, and high oxidative stability [4, 11]. Based on the environmental friendly concept, the fermentation broth after harvesting of yeast cells might be used as fertilizer or feedstocks for biogas production [27].

4 Conclusions

This study is the first report on the valorization of whole felled old oil palm trunk into yeast biomass and lipids by cultivation of the suitable oleaginous yeasts. The sap squeezed from the trunk could be used directly while the residual fiber was acid-hydrolyzed into fermentable sugars before use as a carbon source. The strategies using co-carbon sources coupling with fed-batch fermentation successfully boost up the biomass and lipid production. The preliminary mass balance revealed the prospect practical valorization of oil palm trunk into yeast biomass and lipids. The proposed integrated processes for valorization of whole felled

old oil palm trunk into yeast biomass and lipids may greatly increase the competiveness of the bioenergy and palm oil industries.

Author contribution Benjamas Cheirsilp: conceptualization, methodology, funding acquisition, writing—review and editing. Rawitsara Intasit: investigation, data curation, writing—original draft preparation. Yasmi Louhasakul: investigation, data curation.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

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