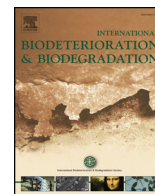




Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Enhanced valorization of industrial wastes for biodiesel feedstocks and biocatalyst by lipolytic oleaginous yeast and biosurfactant-producing bacteria



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ARTICLE INFO

Keywords:

Bacillus subtilis
Biosurfactant
Lipase
Microbial lipids
Yarrowia lipolytica

ABSTRACT

The lipolytic oleaginous yeast *Yarrowia lipolytica* and biosurfactant-producing bacteria *Bacillus subtilis* were used to improve the valorization of palm oil industrial wastes for lipids and lipases. Biosurfactant likely enhanced the performance of the yeast by modifying hydrophobic substrates and cell membrane permeability leading to an increase in substrate entry and also secretion of lipases. The secreted lipases and biosurfactant also synergistically enhanced the biodegradation of the wastes, especially the removal of hydrophobic compounds. The COD and oil removal were improved by 1.47 folds and 2.33 folds, respectively. Consequently, the yeast could grow better on the wastes and accumulate higher lipid content by 1.3–1.5 folds. The biosurfactant also positively affected saturated fatty acid contents in the yeast lipids which make them more suitable as biodiesel feedstocks with higher cetane number and better oxidative stability. This biological process not only improves the biodegradation of environmental pollution from industrial wastes but also lowers the production costs of lipids as biodiesel feedstocks and lipases as biocatalyst.

1. Introduction

Biodiesel has become one of promising biofuels due to the depletion and unstable price of fossil fuel (Li et al., 2008; Liang and Jiang, 2013). However, the use of plant oils as biodiesel feedstocks raise many concerns such as food starvation especially in the developing countries and other environmental problems relating to the use of arable land for oil crop production (Atabani et al., 2013). Recently, biodiesel production using microbial lipids has gained great attention because of their high productivity, less labor required, easy to scale up and short life cycle. The microorganisms that have lipid content higher than 20% of their dried biomass are defined as oleaginous species (Meng et al., 2009). *Yarrowia lipolytica* is one of the specific oleaginous yeasts that can assimilate both hydrophobic and hydrophilic substrates and convert them into lipids through “*ex novo*” and “*de novo*” synthesis pathways, respectively (Beopoulos et al., 2009; Papanikolaou and Aggelis, 2011). The *Y. lipolytica* lipids have been reported as suitable feedstocks for biodiesel production (Niehus et al., 2018; Louhasakul et al., 2019). In addition to lipids, this specific yeast also produces biotechnologically

valuable products such as specific lipases which have high potential as catalysts for enzymatic biodiesel production (Louhasakul et al., 2016; Darvishi et al., 2017). From these characteristics, the practical production of lipids and lipases by this yeast should be developed. Moreover, to be economically feasible the low-cost substrates such as industrial wastes should be used for their productions.

Palm oil mill effluent (POME) generated from palm oil extraction process contains high amount of organic and inorganic matters such as carbohydrate, protein, and mineral salts that stimulate microbial growth (Ugoji, 1997). Crude glycerol generated from transesterification of oils into biodiesel has also been considered as low-cost carbon sources showing greater degree of reduction than glucose fermentation (Garlapati et al., 2016). They are potentially suitable as fermentable substrates for microbial lipid and lipase production. However, to effectively utilize these wastes as low-cost substrates for the yeast the bioavailability and solubility of these wastes should be improved. Surface-active substances, namely surfactants, may alter physiological properties of substances and/or microorganisms by changing permeability and organization of cell membranes, promoting metabolite

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production, and stimulating respiration and cell growth (Benčekroun and Bonaly, 1992). The addition of surfactants is considered as a feasible approach to enhance the bioavailability, solubility and biodegradation of hydrocarbon substances through emulsifying function of the surfactants (Singh et al., 2007; Tian et al., 2016).

In general, the surfactants could form spherical micelles for enclosing hydrophobic substances and bilayer micelles for enclosing hydrophilic substances. These micelles then act in three different ways: roll-up, emulsification and solubilization (Mishra et al., 2009; Ceccarelli et al., 2012; Joshi-Navare and Prabhune, 2013). Guha and Jaffe (1996) had proposed a model for describing the biodegradation of substrate in micellar-phase as follows: (a) the substrate is first transported by filled micelles from bulk solution into the proximity of the cells; (b) the substrate is then delivered across cell membrane by the exchange of the filled micelles with the hemi-micellar layers around the cells; and (c) finally the substrate diffuses in the cells and is biodegraded. In cytoplasm, substrates and mixed micelles would be degraded by virtue of metabolic systems to obtain the necessary energy for cell growth and maintenance and to form intermediate metabolites such as the precursors for the synthesis of cellular materials including lipids (Papanikolaou and Aggelis, 2011).

Several studies have shown the effective use of surfactants for improving cell growth and lipid accumulation of the oleaginous yeasts. Saenge et al. (2011a,b) have reported that the use of synthetic surfactants including Tween 20, Tween 80 and gum arabic, could improve lipid and carotenoid production by oleaginous yeast *Rhodotorula glutinis* TISTR 5159. The lipid content of yeast *Thraustochytrium aureum* was also significantly improved with the addition of Tween 80 (Taoka et al., 2011). Besides, the addition of surfactants also enhanced the extracellular lipase activity of the yeast *Y. lipolytica* ATCC 18942 (Dominguez et al., 2003). Several research groups reported that during the substrate is delivered across cell membrane the micelles may solubilize phospholipids on the membrane to form new mixed micelles and may even release membrane-bound components (Le Maire et al., 2000). This then possibly leads to an increase in membrane permeability and causes release of membrane-bound and intracellular enzymes. This phenomenon supports the increase in extracellular lipolytic activity and the decrease in membrane-bound lipolytic activity found in the microorganisms (Deive et al., 2009). However, the use of synthetic surfactants is costly and may not be feasible in industrial scale.

In comparison with synthetic surfactants, the biological compounds that exhibit high surface-active properties, namely biosurfactants are generally equally effective in terms of solubilization and emulsification. They are also considered to be biodegradable, less toxic, and more environmentally friendly than synthetic surfactants (Mulligan, 2009). Moreover, as they can be produced from low-cost substrates their production seems to be economically feasible. In the previous study, the lipopeptide type biosurfactant from *Bacillus subtilis* TD4 was produced from low-cost industrial wastes and it could exhibit better performance than the synthetic surfactants (Saimmai et al., 2012). In this study, the effective process for biodegradation of industrial wastes using lipolytic oleaginous yeast coupling with low-cost biosurfactant produced by bacteria was developed. The effects of this biosurfactant on yeast cell growth, secretion of lipolytic enzyme, fatty acid compositions of yeast lipids as well as biodegradation of pollutants in oily industrial wastes were evaluated.

2. Materials and methods

2.1. Microorganisms and media

The specific oleaginous yeast *Yarrowia lipolytica* TISTR 5151 was obtained from the Thailand Institute of Scientific and Technological Research. It was used as lipids and lipase producer in this study. The biosurfactant-producing bacteria *Bacillus subtilis* TD4 (Accession no. AB647203) from Enzyme Technology Laboratory, Faculty of Agro-

Industry, Prince of Songkla University, Thailand, was used as biosurfactant producer. YPD broth (pH 6.0) was used for preparation of yeast seed culture with the following composition: glucose, 40 g L⁻¹; peptone, 5 g L⁻¹; and yeast extract, 15 g L⁻¹. Nutrient broth (NB; pH 7.0) was used for preparation of bacteria seed culture with the following composition: beef extract, 1 g L⁻¹; yeast extract, 2 g L⁻¹; and peptone, 5 g L⁻¹. For low-cost production of biosurfactant, crude glycerol based medium was used with the following composition: crude glycerol, 20 g L⁻¹; and minimal salt based medium; monosodium glutamate, 1 g L⁻¹; K₂HPO₄, 0.8 g L⁻¹; KH₂PO₄, 0.2 g L⁻¹; CaCl₂, 0.05 g L⁻¹; MgCl₂, 0.5 g L⁻¹; FeCl₂, 0.01 g L⁻¹; and NaCl, 5.0 g L⁻¹.

The characteristics of *B. subtilis* TD4 biosurfactant were described previously in the study of Saimmai et al. (2012).

Palm oil mill effluent (POME) was collected from the primary wastewater treatment pond of palm oil mill in Surat Thani province, Thailand. Before use, the effluent was centrifuged at 6000 rpm for 30 min to remove suspended solid. After suspended solid removal, the chemical oxygen demand (COD), oil content, total nitrogen and pH of the effluent were: 44 g L⁻¹, 0.28 g L⁻¹, 1.2 g L⁻¹ and 4.9, respectively. Crude glycerol (CG) was collected from Biodiesel Plant at Faculty of Engineering, Prince of Songkla University, Thailand. The glycerol content in CG was approximately 47% (w/w) with the COD of 3448 g L⁻¹ and total nitrogen of 1.15 g L⁻¹, pH 10.27.

2.2. Culture conditions

The yeast inoculum was prepared by transferring the stock plate culture into Erlenmeyer flasks that contained 50 mL YPD medium (pH 6.0). The yeast culture was incubated at room temperature (30 ± 2 °C) and 140 rpm for 24 h before use as seed culture. Two hundred and 50 mL Erlenmeyer flasks contained 90 mL effluent with and without the addition of surfactant. The pH of the effluent was adjusted to 6.0 before sterilization. The cultures were inoculated with 10% seed culture (10⁷ cells mL⁻¹) and incubated at room temperature (30 ± 2 °C), 140 rpm for 72 h.

The bacteria inoculum was prepared by transferring the stock agar plate culture into Erlenmeyer flasks that contained 45 mL NB medium (pH 7.0). The culture was incubated at room temperature (30 ± 2 °C) and 200 rpm for 24 h before use as seed culture. Two hundred and 50 mL Erlenmeyer flasks contained 90 mL crude glycerol based medium. The medium pH was adjusted to 7.0 and sterilized by autoclave. The cultures were inoculated with 6% seed culture (10⁷ cells mL⁻¹), shaken at 200 rpm and incubated at room temperature (30 ± 2 °C) for 54 h (Saimmai et al., 2012).

2.3. Analytical methods

Biomass were harvested by using centrifugation and dried at 60 °C to constant weight. The biosurfactant in bacterial culture supernatant was collected by adding ethyl acetate at a ratio of 1:1 (v/v). Then the mixture was shaken at 250 rpm and incubated at room temperature (30 ± 2 °C) for 15 min and centrifuged to collect the upper layer. The solvents were evaporated to recover the biosurfactant extracted. Surface tension of the biosurfactant was measured using Model 20 Tensiometer at 25 °C and critical micelle concentration (CMC) was determined by plotting the surface tension versus concentration of biosurfactant in the solution (Saimmai et al., 2012). Chemical oxygen demand (COD) and oil content in the wastes were determined according to the standard methods of APHA (2005). The pollutant removal efficiency, i.e. COD and oil, was calculated as follow:

$$\% \text{Pollutant removal} = [1 - (C_i/C_0)] \times 100 \quad (1)$$

where C₀ is the initial pollutant concentration and C_i is the final pollutant concentration in the wastes.

The yeast biomass were harvested by using centrifugation, washed and dried at 60 °C to constant weight. To extract the yeast lipids, a

mixture of chloroform:methanol (2:1, v/v) was added into the yeast biomass and sonicated for 1 h. Then, the solvents were separated by evaporator and the extracted lipids were weighed. The yeast lipids were transesterified by method of Jham et al. (1982) and the fatty acid compositions were analyzed by gas chromatography method. The gas chromatography (Hewlett Packard Plus 6850 series, Agilent, USA) equipped with specific capillary column (320 μm I.D., 0.25 film thickness, 30 m length) and flame ionization detector, was used. The detector temperature was 300 $^{\circ}\text{C}$. The column temperature was maintained at 210 $^{\circ}\text{C}$ for 12 min and then ramp up to 250 $^{\circ}\text{C}$ at a rate of 20 $^{\circ}\text{C}$ min^{-1} and held for 8 min. Extracellular and cell-bound lipase activities were determined using the modified procedure of Lee and Rhee (1993). One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed palm oil and released 1.0 μmole of free fatty acids (palmitic acid) per min at the specified condition. All experiments were performed in triplicates. The evaluation of statistical significances was performed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests ($P < 0.05$).

3. Results and discussion

3.1. Biosurfactant production and its properties

There are several suggested strategies to maximize the productivity of biosurfactants including the screening for overproducing strains and the optimization of medium components and environmental conditions (Satpute et al., 2008). Saimmai et al. (2012) have screened several biosurfactant-producing bacteria and successfully produced biosurfactant using minimal salt based medium added with crude glycerol as a low-cost medium (pH 7.0).

Biosurfactant produced by *B. subtilis* TD4 has been reported as lipopeptide type with the CMC of 12 mg L^{-1} . It exhibited higher oil recovery efficiency than synthetic surfactants (Saimmai et al., 2012). The minimal salt based medium added with crude glycerol from the previous study of Saimmai et al. (2012), was used for production of biosurfactant in this study. *B. subtilis* TD4 grew well and was able to produce biosurfactant at a concentration of $1180 \pm 60 \text{ mg L}^{-1}$. The surface activity of biosurfactant produced by *B. subtilis* TD4 was evaluated at various concentrations (Fig. 1). With increasing concentration of biosurfactant, the surface tension of water rapidly decreased from

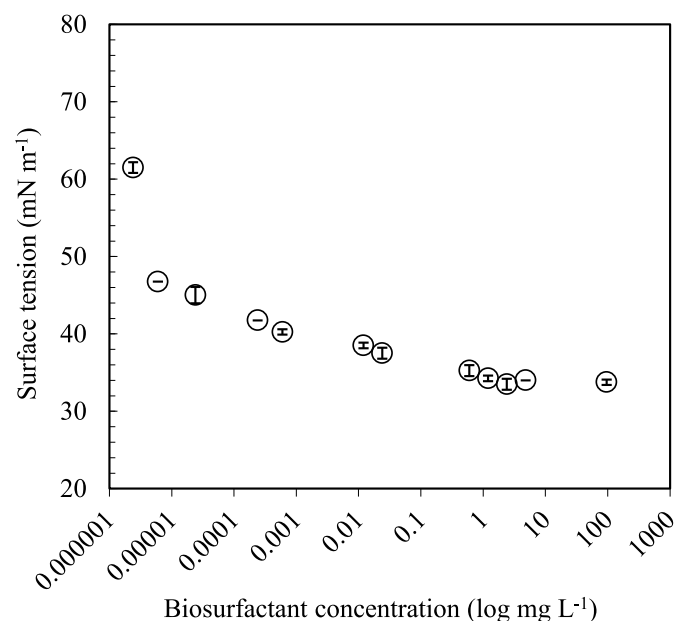


Fig. 1. Surface tension of biosurfactant produced by *B. subtilis* TD4. Data are expressed as means of triplicate experiments and their standard deviations.

70.25 to 34 mN m^{-1} likely due to the formation of micelles (Pacwa-Płociniczak et al., 2011). Most of surfactants also decrease surface tension of water from 72 to 30 mN m^{-1} (Desai and Banat, 1997). There was no further change in surface tension at biosurfactant concentration higher than 1.2%. Generally, the surface tensions at CMC of various biosurfactants have been reported to be in the range of 20–35 mN m^{-1} (Santos et al., 2016). Nogueira Felix et al. (2019) reported that the CMC of biosurfactant produced by *B. subtilis* was 12.5 mg L^{-1} showing the capacity of decreasing the surface tension of water to 31.8 mN m^{-1} , while Hentati et al. (2019) reported higher CMC of biosurfactant from *B. stratosphericus* FLU5 of 50 mg L^{-1} .

3.2. Effect of biosurfactant addition on the yeast cell growth and production of lipids and lipases

In this study, *Y. lipolytica* TISTR 5151 was cultivated in POME added with 2% crude glycerol. As the effluent contained varieties of hydrophobic and hydrophilic substrates, it was expected that the use of surfactants might facilitate the substrate assimilation by the yeast and enhance not only cell growth but also production of lipids and lipases. There have been few works regarding the use of synthetic surfactants for improvement of lipid production by the yeasts but no any report available for the use of biosurfactants. As biosurfactants are more environmental friendly than the synthetic surfactants and can be produced using low-cost production medium (Saimmai et al., 2012), it was added into the yeast culture media. The results are compared with those using synthetic surfactants i.e. Tween 20 and Tween 80 (Fig. 2, Table 1). The biosurfactant and synthetic surfactants were added at their CMC. The effects of surfactant addition on production of cell-bound and extracellular lipases are shown in Fig. 3.

In the medium without surfactant addition, the yeast biomass and lipids reached $3.14 \pm 0.26 \text{ g L}^{-1}$ and $2.04 \pm 0.01 \text{ g L}^{-1}$, respectively (Fig. 2a). When the surfactants were added into the medium, the yeast grew better and accumulated higher lipids (Fig. 2b–d). The final biomass and lipid production of the culture with biosurfactant were $4.83 \pm 0.11 \text{ g L}^{-1}$ (1.53 folds increase) and $2.54 \pm 0.02 \text{ g L}^{-1}$ (1.25 folds increase), respectively. Likewise, cell-bound lipase was produced along with the cell growth and reached the highest level of $186.9 \pm 15 \text{ U g-dried cells}^{-1}$ (or $586.8 \pm 41 \text{ U L}^{-1}$ in total) at 48 h of the cultivation time. However, the lipase production dropped after the cell growth ceased (Fig. 3a). It should be noted that there was no extracellular lipase activity observed in the medium without the addition of surfactants (Fig. 3a). Interestingly, the extracellular lipase activities were detected when the surfactants were added (Fig. 3b–d). The extracellular lipase activities reached $8439 \pm 623 \text{ U L}^{-1}$ with the addition of biosurfactant followed by the addition of Tween 80 ($7677 \pm 533 \text{ U L}^{-1}$) and Tween 20 ($5658 \pm 128 \text{ U L}^{-1}$).

As the surfactants have the ability to solubilize phospholipids on the membrane (Le Maire et al., 2000), it was possible that the addition of surfactants might stimulate a partial disruption of the cell membrane, leading to a release of both cell-bound and intracellular lipases. This phenomenon then led to an increase in extracellular lipase activity in the culture medium. Deive et al. (2009) have studied the effect of surfactants on lipase production by *Thermus thermophilus* HB27. They also found that with the addition of surfactants the extracellular activity drastically increased while the membrane-bound activity decreased. They therefore demonstrated that the surfactants might help to release of this enzyme from the cell membrane. The results in this study were also consistent with those reported by Saenge et al. (2011a,b) who found that *Rhodotorula glutinis* TISTR 5159 grew better and accumulated lipids at a higher content with the addition of surfactants. It has been reported that the positive effect of surfactants was possibly due to the increase in permeability of cell membrane leading to the increase in uptake of essential nutrients from the medium (Taoka et al., 2011). However, for other species like *Rhodospiridium toruloides* AS 2.1389 there was no significant effect of surfactant on cell growth and lipid

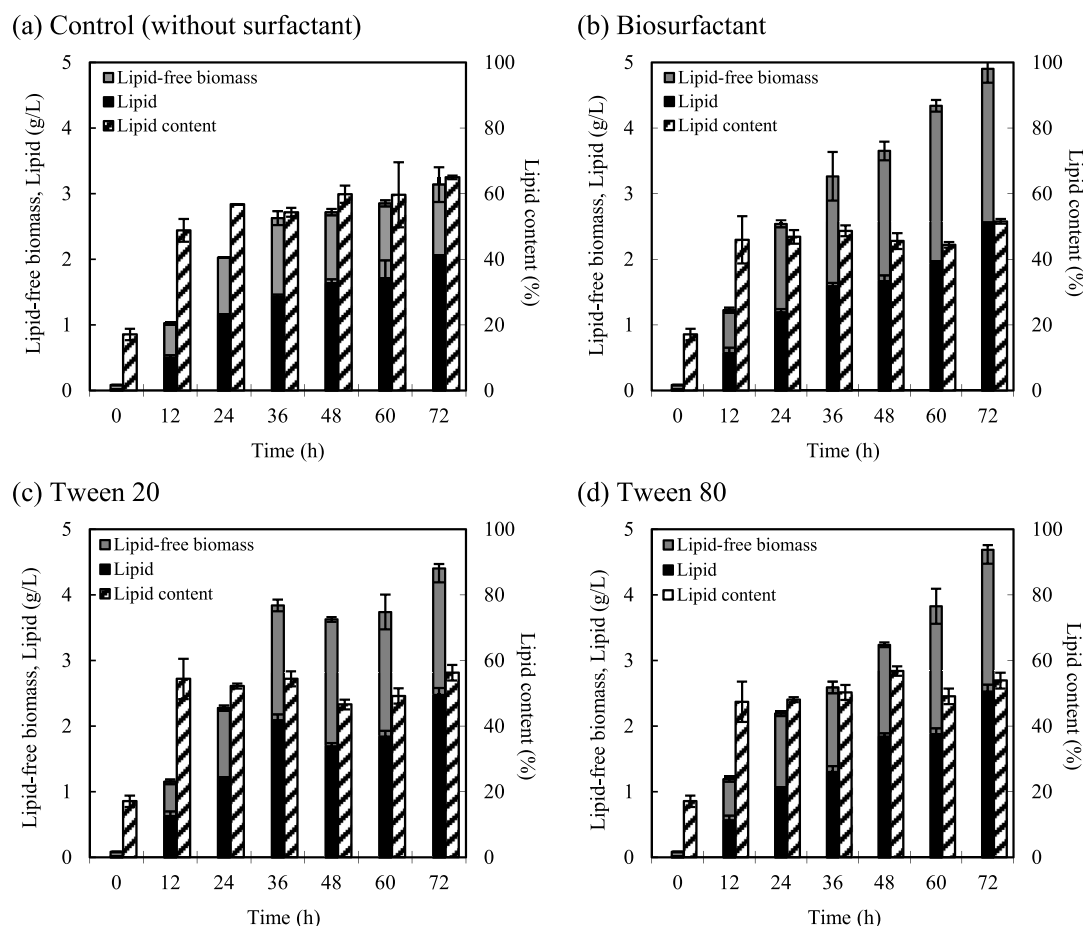


Fig. 2. Effect of surfactant addition on the growth and lipid production of *Y. lipolytica* TISTR 5151 cultivated in palm oil mill effluent added with 2% crude glycerol. Data are expressed as means of triplicate experiments and their standard deviations.

production even at an increased surfactant concentration up to 0.2%w/v (Xu et al., 2016).

3.3. Effect of biosurfactant on removal of pollutants

The effect of biosurfactant on COD and oil removal from the effluent was investigated and compared with those of synthetic surfactants (Table 1). The biosurfactant and synthetic surfactants were added at their critical micelle concentrations. Among the surfactants tested, the addition of biosurfactant gave the highest COD removal of $88.35 \pm 1.21\%$ while there was no improvement when adding synthetic surfactants. The oil removal from the effluent was increased from $34.72 \pm 1.96\%$ up to $71.41 \pm 1.69\%$, $75.61 \pm 1.61\%$ and $76.06 \pm 7.54\%$ when the culture was added with biosurfactant, Tween 20 and Tween 80, respectively. As a result, with the addition of biosurfactant, the COD and oil removal were improved by 1.47 folds and

2.33 folds, respectively. It has been reported that synthetic and nature surfactants have the ability to increase the solubility of hydrocarbon compounds in water likely by reducing the interfacial tensions of oil and water and the viscosity of the oil (Al-Sabagh, 2000; Liu et al., 2004; Chu, 2003; Pekdemir et al., 2005). This then increased the affinity of microorganisms to hydrophobic surfaces of the substrates. As the yeast in this study had the ability to produce lipases, the secretion of lipases might also help to increase the solubility of the substrates. Moreover, the secreted lipases and biosurfactant might synergistically enhance the biodegradation of the wastes, especially the removal of hydrophobic compounds.

Tian et al. (2016) have reported the effect of natural and synthetic surfactants on crude oil biodegradation by indigenous strains. They illustrated that surfactant supplementation at the concentration about 0.1 and 0.2 CMC did improve the degradation rate of crude oil. However, when using higher concentration above 1 CMC the degradation

Table 1

Effect of surfactants at critical micelle concentration on the performance of *Y. lipolytica* TISTR 5151.

Conditions	Cell-bound lipase (U/L)	Extracellular lipase (U/L)	COD removal (%)	Oil removal (%)
Control	361 ± 19^b	- ^a	68.15 ± 1.82^b	34.72 ± 1.96^b
Biosurfactant	555 ± 11^a	8439 ± 1172^a	88.35 ± 1.21^a	71.41 ± 1.69^a
Tween 20	365 ± 44^b	5658 ± 285^b	50.00 ± 5.44^c	75.61 ± 1.61^a
Tween 80	491 ± 39^a	7677 ± 1563^a	51.79 ± 2.53^c	76.06 ± 7.54^a

The maximum lipase observed at 48 h.

Values are means \pm SD. Data are expressed as means of triplicate experiments and their standard deviations and different letters in the same column indicate significant difference ($p < 0.05$).

^a Not detect.

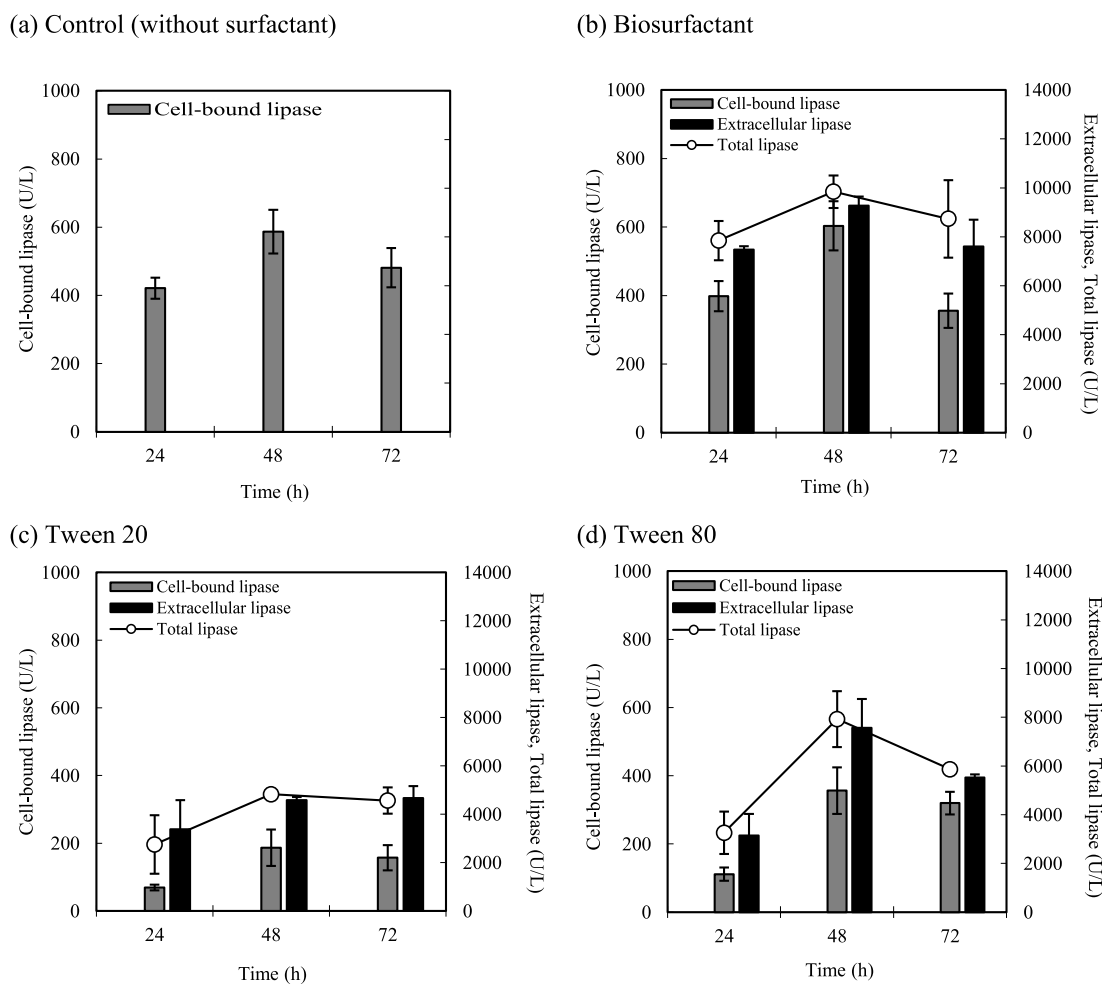


Fig. 3. Effect of surfactant addition on lipase production of *Y. lipolytica* TISTR 5151 cultivated in palm oil mill effluent added with 2% crude glycerol. Data are expressed as means of triplicate experiments and their standard deviations.

rate decreased from 50.5% to 28.9%. Although surfactants can increase the solubility of hydrophobic compounds, too high concentrations of surfactants could be toxic to the microorganisms and they might even reduce the adhesion of microorganisms to hydrophobic surfaces (Laha and Luthy, 1992; Rosenberg and Rosenberg, 1995).

3.4. Effect of biosurfactant concentration on the performance of the yeast

As shown in the previous section, there were significant improvements of yeast growth, lipid and lipase production as well as COD and oil removal by the addition of biosurfactant. The sorption of surfactants to microbial cells are believed to be an important mechanism that could help the cells for the uptake of carbon compounds, especially hydrophobic compounds (Wick et al., 2002). The sorption of surfactants depends on the nature of the cell surfaces and also the surfactant concentration and the changes of surface properties are significant at surfactant concentrations not higher than CMC (Neu, 1996). Therefore, the effects of biosurfactant concentration at the different levels were tested. The results are shown in Fig. 4a–b. When biosurfactant concentration was increased up to 0.012% and 0.12% the final biomass obtained were 3.74 ± 0.05 and 3.84 ± 0.02 g L⁻¹, respectively. The activity of extracellular lipases also increased up to 7332 ± 244 and 8467 ± 199 U L⁻¹, while there was no significant improvement in lipid production (Fig. 4b). Several kinds of surfactants such as PEG-200, Triton X-100 and Tween 80 were added in the culture of *Y. lipolytica*

ATCC 18942. It was found that these surfactants did not improve lipase production by this yeast (Dominguez et al., 2003). While the addition of Tween 80 positively affected the growth of *Y. lipolytica* NICM 3639 and the yeast could produce a higher extracellular lipase of 2.8 U mL⁻¹ but with a lower activity of cell-bound lipase. An increase in Tween 80 concentration up to 2% w/v did increase the production of extracellular lipase up to 15,200 U L⁻¹ (Yadav et al., 2011). This study has shown that with the addition of biosurfactant, the lipolytic *Y. lipolytica* TISTR 5151 could grow better and present high activity of both cell-bound and extracellular lipases.

3.5. Fatty acid compositions of the yeast lipids

It has been reported that with the addition of surfactant the microorganisms might modify their fatty acid composition to maintain the fluidity of cell membranes (Kaczorek et al., 2013). The fatty acid compositions of yeast lipids are shown in Table 2. The main fatty acids in yeast lipids were palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids. Of these, palmitic and oleic acids are most abundant which are similar to the typical fatty acids found in vegetable oils and animal fats (Hoekmana et al., 2012). It was found that the addition of surfactants changed the ratio of saturated to unsaturated fatty acids in the yeast lipids. The saturated and unsaturated fatty acid contents of the yeast lipids without the addition of surfactant were 45.80% and 54.21%, respectively. With the addition of biosurfactant,

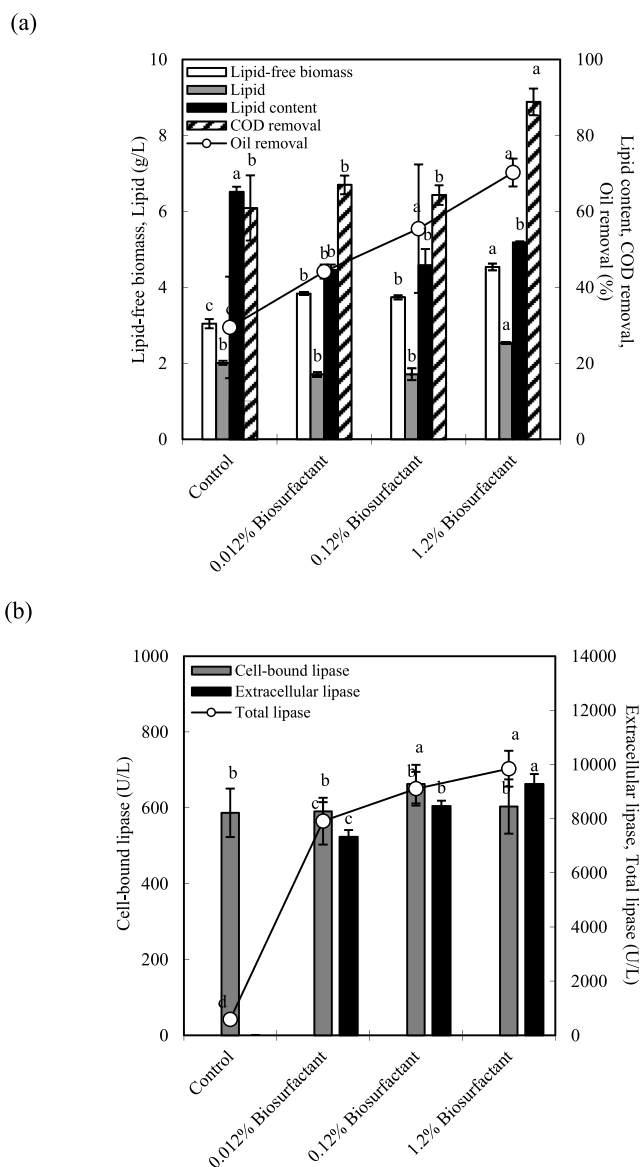


Fig. 4. Effect of biosurfactant concentration on the growth, lipid production, lipase production and the consumption of COD and oil of *Y. lipolytica* TISTR 5151. Data are expressed as means of triplicate experiments and their standard deviations and different letters in the same bar indicate significant difference ($p < 0.05$).

the content of saturated fatty acids slightly increased up to 49.64% while that of unsaturated ones decreased to 50.36%. While the addition of Tween 20 and Tween 80 increased the content of saturated fatty acids up to 83.35% and 77.16%, respectively. It has been reported that the activities of enzymes involving in lipid synthesis, especially via *de novo* pathway changed significantly in the presence of surfactant. Especially, the activity of carnitine acetyltransferase involving in translocation of lipid precursors within and between cellular compartments increased seven fold in the presence of Tween 80 (Antononkov and Hiltunen, 2012; van Rossum et al., 2016). In addition, the surfactant also likely limited the desaturation and elongation of fatty acids and led to an increased content of saturated fatty acids in the microbial lipids (Wynn and Ratledge, 2000). The lipids with high content of saturated fatty acids are preferred as feedstocks for biodiesel. The derived biodiesel would have high cetane number, short ignition delay time, and high oxidative stability (Knothe and Razon, 2017).

Table 2
Fatty acid composition (%) of lipid of *Y. lipolytica* TISTR 5151.

Parameters	Relative amount of total fatty acids (%)			
	Control	Biosurfactant	Tween 20	Tween 80
Lauric acid (C12:0)	0.12	0.09	0.60	n.d.
Myristic acid (C14:0)	0.93	0.41	1.13	0.56
Palmitic acid (C16:0)	36.88	34.86	73.02	65.14
Palmitoleic acid (C16:1)	2.62	n.d.	n.d.	n.d.
Stearic acid (C18:0)	6.09	5.72	6.16	5.15
Oleic acid (C18:1)	40.62	39.78	9.37	11.24
Linoleic acid (C18:2)	8.58	6.08	1.78	2.14
Arachidic acid (C20:0)	0.71	2.84	0.55	n.d.
Eicosenoic acid (C20:1)	n.d.	1.05	0.32	n.d.
Erucic acid (C22:1)	2.39	3.45	5.19	9.45
Lignoceric acid (C24:0)	0.41	4.47	1.27	6.13
Others	0.66	1.25	0.62	n.d.
Saturated fatty acids	45.80	49.64	83.35	77.16
Unsaturated fatty acids	54.21	50.36	16.66	22.83

n.d.: not detected.

4. Conclusions

The lipolytic oleaginous yeast *Yarrowia lipolytica* and biosurfactant-producing bacteria *Bacillus subtilis* effectively valorized the palm oil industrial wastes into lipids and lipases. The biosurfactant produced by bacteria could increase the bioavailability of hydrophobic substrates in the wastes and also permeability of yeast cell membrane leading to more secretion of lipases. The secreted lipases and biosurfactant synergistically enhanced the biodegradation of the wastes, nutrient uptake rate, cell growth and lipid production of the yeast. The biosurfactant also increased the content of saturated fatty acids in the yeast lipids and make them more suitable as biodiesel feedstocks. This promising strategy may greatly contribute to the effective biodegradation of industrial wastes and also the low-cost production of biodiesel feedstocks and biocatalyst.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was financially supported by Prince of Songkla University Grant No. AGR590148S-1. The first author was supported by Graduate School of Prince of Songkla University and the program Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree from the Office of the Higher Education Commission. The second author was supported by Thailand Research Fund under Grant No. RTA6280014.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ibiod.2020.104911>.

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