Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol15-issue6-fulltext-1

RESEARCH ARTICLE

Application of palm pressed fiber as a carrier for ethanol production by *Candida shehatae* TISTR5843

Wiboon Riansa-ngawong[1,2] ✉ · Maneewan Suwansaard[3] · Poonsuk Prasertsan[1,4]

1 Prince of Songkla University, Faculty of Agro-Industry, Department of Industrial Biotechnology, Songkhla, Thailand

2 King Mongut's University of Technology North Bangkok, Faculty of Agro-Industry, Department of Agro-Industry Technology and Management, Prachinburi, Thailand

3 Bowling Green State University, Department of Biological Sciences, Bowling Green, Ohio, USA

4 Prince of Songkla University, Faculty of Agro-Industry, Palm Oil Products and Technology Research Center, Songkhla, Thailand

✉ Corresponding author: wiboon.007@gmail.com
Received April 25, 2012 / Accepted July 23, 2012
Published online: November 15, 2012
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Abstract Palm pressed fiber (PPF) is a clean and renewable lignocellulosic material. The PPF and delignified PPF (DPPF) were used as a carrier for immobilization of *Candida shehatae* TISTR5843 in bioethanol production. PPF was pre-treated by milling to obtain small particles, whereas DPPF was the delignification of PPF using $NaClO_2$. *C. shehatae* TISTR5843 was grown in modified yeast extract-malt (YM) medium at 30 ± 2ºC on an orbital shaker at 150 rpm for batch and repeated batch fermentation. In the batch system, immobilized cells on a small size, less than 0.5 mm, of DPPF (sDPPF) gave the maximum ethanol production of 11.5 g L^{-1} at 24 hrs cultivation period. The ethanol concentration and ethanol yield of sDPPF were 6.2% and 6.8% higher (ethanol production 11.5 g L^{-1}, ethanol yield 0.47 g g^{-1}) than those of free cells (ethanol production 10.8 g L^{-1}, ethanol yield 0.44 g g^{-1}) after 36 hrs of cultivation. In contrast, the small size of PPF (sPPF) was selected as a carrier in repeated batch fermentation for cost effectiveness. The ethanol productivity of immobilized yeast cells in repeated batch fermentations was 45.2-51.6% greater than that obtained from batch fermentations. The immobilized cells on sPPF improved the ethanol production and could be reused 4 times with retaining the activity of 93%. In conclusion, PPF is a potential carrier in the immobilization system. The pre-treatment of PPF increases the surface area that enhances cell adsorption and ethanol production by *C. shehatae* TISTR5843.

Keywords: *Candida shehatae*, ethanol production, immobilization, palm pressed fiber.

INTRODUCTION

Recently, alternative energies have been developed because of the shortage of fossil fuels worldwide. Bioethanol is considered as a source of energy as it's easy to combine with petroleum gasoline in engines. In Thailand, bioethanol derived from feedstock has been used as a gasoline blend in the amount of up to 10% by volume. The Ministry of Energy aims to blend 3 million litres per day of ethanol into gasoline by 2011 (Channukul, 2009). Bioethanol can be produced by either free or immobilized yeast cells. Several reports for the enhancement of ethanol production by immobilized yeast cells have been published (Krisch and Szajáni, 1997; Fujii et al. 1999; Kopsahelis et al. 2007; Yu et al. 2007; Chandel et al. 2009; Behera et al. 2010).

A carrier for cell immobilization of ethanol production can be divided into two types based on the sources (i) synthetic carriers such as gelatine, carrageen, Ca-alginate (Behera et al. 2010), agar-agar (Behera et al. 2010), polyurethane (Fujii et al. 1999) and ceramic beads or porous glass (Kourkoutas et al. 2006), and (ii) natural carriers such as chitosan (Fujii et al. 1999), sawdust, wood chip, rice husk,

rice straw, spent grain, delignified spent grain (Kopsahelis et al. 2007), apple pieces (Kourkoutas et al. 2006), sorghum bagasse (Yu et al. 2007) and watermelon pieces (Reddy et al. 2008). The benefits of natural immobilizing cell carrier are wide spread natural resources, cost effective, and easy to operate in bioprocess fermentation because of better operational stability, less contamination, easily separated in downstream process, less affected by inhibitory compounds and remains viable cells for several cycles of operations (Chandel et al. 2007; Reddy et al. 2008; Behera et al. 2010).

Palm pressed fiber (PPF) is a renewable and low cost lignocellulosic material. It is a solid waste extracted from oil palm's empty fruit bunch through decortation process in palm oil industries located in Malaysia, Indonesia and Thailand (Shinoj et al. 2011). The fibres are clean, non-carcinogen and free from pesticide. In 2010, the production of PPF in Thailand amassed large quantities equalling 1.08 million ton per year, which was converted from 5.3 million ton oil palm fruit per year using a factor of 0.12 as described in Prasertsan and Sajjahulnukit (2006). Technology for utilization of PPF in bioethanol production has been developed (Riansa-ngawong and Prasertsan, 2010; Riansa-ngawong and Prasertsan, 2011). However, the remaining PPF and delignified PPF after hydrolysis were found in a significant quantity. To address this concern, we aim to investigate the use of PPF as a carrier for improvement of ethanol production in batch and repeated batch fermentation.

MATERIALS AND METHODS

Raw material pre-treatment

The palm pressed fiber (PPF) that was used in the experiment was graciously provided by Thai Tha Lo & Oil, Co., Ltd., Suratthanee Province, Thailand. The provided PPF was then sun-dried, and milled by the method described in Riansa-ngawong and Prasertsan (2010); Riansa-ngawong and Prasertsan (2011). Briefly, PPF particles were screened by passing through the mesh sieve (Fritsch 35 mesh ASTM analytical sieve, Germany) to obtain the diameter of < 0.5 mm for small PPF particles (sPPF) after the milling process. The oversize 0.5-20 mm, called large PPF particles (lPPF) were trapped above the screen. In delignification process, PPF to sodium chlorite ($NaClO_2$) in the ratio of 10:1 (w/w) was soaked in 0.01% acetic acid solution at 70ºC for 1 hr and repeated 3-4 times. The delignified PPF were then separated by centrifugation at 5,720 x *g* for 20 min (Hettich Zen Trifugen Universal 32R, Germany) and washed twice with warm water (< 60ºC to prevent autohydrolysis of C5 sugars), then centrifuged again, and finally incubated at 45ºC overnight.

Microorganism and growth medium

Candida shehatae TISTR5843, obtained from Thailand Institute of Scientific and Technological Research (TISTR), was maintained on yeast extract-malt (YM) agar slant. The YM medium contained (g L^{-1}): glucose, 10.0; peptone, 5.0; malt extract, 3.0; yeast extract, 3.0; and agar, 25.0 (Chandel et al. 2007; Lebeau et al. 2007). The culture was stored at 4 ± 0.5ºC and subcultured every 2 weeks.

Starter culture preparation with immobilized yeast cells

A loop of *C. shehatae* TISTR5843 was inoculated into 50 mL modified YM medium (Chandel et al. 2007; Lebeau et al. 2007) containing (g L^{-1}) glucose, 25; malt extract, 3; yeast extract, 3; KH_2PO_4, 10 and $(NH_4)_2SO_4$, 5 in a 150 mL Erlenmeyer flask. The medium was adjusted to a pH of 5 before sterilization because of its optimal pH in the previous study (unpublished data). The flask was incubated on an orbital shaker (150 rpm) for 24 hrs at room temperature (30 ± 2ºC). The starter culture of immobilized *C. shehatae* TISTR5843 on PPF and DPPF was prepared by inoculating 20 mL of culture broth (0.80 g cells L^{-1}, corresponding to the OD at 600 nm of 0.5) into 200 mL modified YM medium containing 100 g L^{-1} PPF or DPPF and then incubated under the same condition for 18 hrs. The immobilized system was detected by a scanning electron microscope (SEM) (FEI Quanta 400, Czech Republic) (Yu et al. 2007).

Batch and repeated batch fermentation

In batch fermentation, a 200 mL working volume of fresh fermentation medium was inoculated with 10% (v v^{-1}) of immobilized yeast cells (immobilized carrier concentration of 100 g L^{-1}). The initial cells

concentrations of free and immobilized cells inoculated into media were determined by mass balance. Cell growth was determined as dry cell weight (DCW) as modified from Fujii et al. (1999). The cells concentration was measured at 600 nm absorbance, and then converted to dry cell concentration using a relationship curve between absorbance values and dry cell concentrations. The immobilized cells on the carrier were also measured as DCW. However, the dry weight obtained during cultivation was the combined weight of carrier and cells. Thus, the cells weight was calculated using the equation below.

$$\text{Cell weight (g L}^{-1}\text{)} = \text{Total weight} - \text{Carrier weight}$$

[**Equation 1**]

After cultivation, the carrier and cells were centrifuged at 12,134 x *g* (Juan Centrifuge A14, France) to remove the fluid, and then incubated in a hot air oven at 103ºC for 24 hrs. The dry weight of yeast cells was calculated using the Equation 1. The culture was incubated on an orbital shaker (150 rpm) at room temperature (30 ± 2ºC) for all experiments. The culture broth of batch experiment was decanted after 48 hrs fermentation. In repeated batch experiments, the culture medium was removed and replaced with the new 200 mL of modified YM medium at 24, 48 and 72 hrs cultivation period (Kopsahelis et al. 2007). The ratio of the carrier to the medium was kept constant at 1:2 (w v^{-1}). A 5 mL culture broth was collected to determine ethanol concentration, glucose consumption, and cell growth.

Analytical methods

Ethanol production was determined by Gas Chromatography (HP 6850, USA) equipped with flame ionized detector (FID). A 30 m x 0.25 mm x 0.25 mm capillary column packed with crossbond-acid-deactivate carbowax polyethyleneglycol (Restek Stabilwax-DA, USA) was used. The operational condition was run as followed. Flow rate of helium was 1.2 ml min^{-1}. The temperatures of the injection port and the detection port were 230ºC and 250ºC, respectively. The injection volume was 1 ml. The initial temperature of the oven was 70ºC for 1 min followed with a ramp of 20ºC min^{-1} to the final temperature of 180ºC and then hold for 2 min (Suwansaard et al. 2009). Residual glucose was measured by High Performance Liquid Chromatography (Agilent 1100, USA)-refractive index detector (RID) using a Zorbax NH_2 column (4.6 x 250 mm, 5-Micron, Agilent, USA). The operational condition was run as described by Rahman et al. (2006). Briefly, aqueous acetonitrile (75%, v v^{-1}) was used as a mobile phase with flow rate of 0.7 ml min^{-1} and oven temperature was maintained at 50ºC.

The structure and immobilization property of lPPF, sPPF, and sDPPF were studied by SEM (FEI Quanta400, Czech Republic). The carriers were firstly soaked in 3.5% glutaraldehyde for 6 hrs, and then dried out by serial treatment of 50, 70, 90, 95 and 100% ethanol, followed by incubation overnight in a desiccator for the removal of moisture (Yu et al. 2007).

Kinetic parameters and statistical analysis

The cell yield (*Yx/s*, g g^{-1}) was calculated by the ratio between cell concentration (g L^{-1}) and glucose consumed (g L^{-1}). The ethanol yield (*Yp/s*, g g^{-1}) was calculated by the ratio between ethanol produced (g L^{-1}) and glucose consumed (g L^{-1}). The substrate uptake rate (*Qs*, g L^{-1} h^{-1}) was calculated by the ratio between glucose consumed (g L^{-1}) and fermentation time (hr). The ethanol productivity (*Qp*, g L^{-1} h^{-1}) was calculated by the ratio between ethanol produced (g L^{-1}) and fermentation time (hr). The glucose consumption was calculated as shown in Equation 2.

$$\textit{Glucose consumption}\ (\%) = \frac{\textit{Glucose consumption}\ (gL^{-1})}{\textit{Glucose contentin medium}\ (gL^{-1})} \times 100$$

[**Equation 2**]

All experiments were studied in triplicate. Statistical values were analyzed using ANOVA (SPSS statistic software version 17, USA).

DOI: 10.2225/vol15-issue6-fulltext-1

RESULTS

Immobilization of yeast cells on PPF and DPPF

PPF is fibrous and porous (Figure 1a), and after size reduction by a milling process, the surfaces area increased in sPPF as well as in delignified sPPF (Figure 1b and 1c). The yeast cells were observed on the surface of lPPF, sPPF, and sDPPF (Figure 1d and 1f). However, the changing structure of sPPF and sDPPF has increased in the surface area resulting in the increase of microbial population of 0.52-0.58 g DCW g carrier $^{-1}$ (Figure 1e-1f).

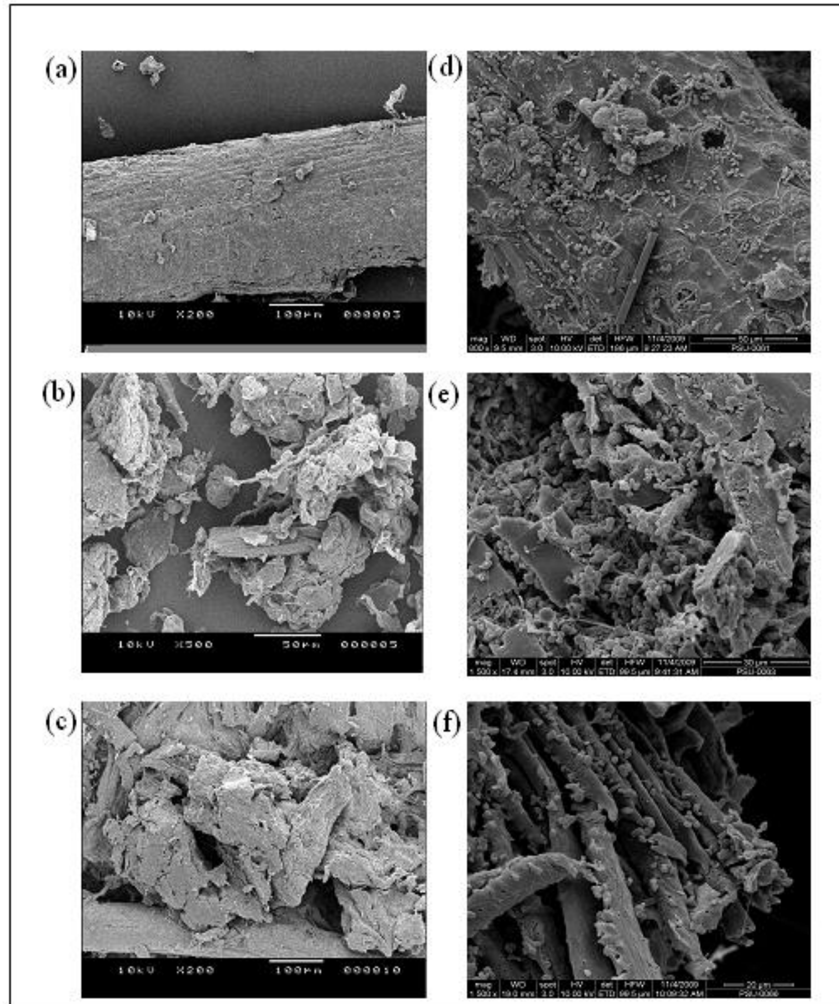

Fig. 1 Scanning electron micrograph of palm press fiber, PPF, (in figures a-c) and immobilized *C. shehatae* TISTR5843 cells on PPF (in figures d-f) after 18 hrs cultivation. In detail: (a) is lPPF at magnification 200-fold; (b) is sPPF at magnification 500-fold; (c) is sDPPF at magnification 350-fold; (d) is immobilized yeast cells on lPPF at magnification 1,000-fold; (e) is immobilized yeast cells on sPPF at magnification 1,500-fold and; (f) is immobilized yeast cells on sDPPF at magnification 1,500-fold.

Kinetics analysis of ethanol production in batch fermentation by free and immobilized cells

The values of the kinetics in batch fermentation were determined (Table 1). The maximum ethanol production (P_{max}) of immobilized yeast cells on sPPF, sDPPF, and lPPF was observed at 24 hrs cultivation period with the values of 11.3, 11.5, and 10.7 g L^{-1}, respectively (Figure 2 and Table 1). The P_{max} values of immobilized cells on sPPF and sDPPF increased by 4.2 and 6.2%, respectively

compared to those of free cells which was measured at 10.8 g L^{-1}, but not for lPPF which the results were not significantly different (*P < 0.05*). The substrate uptake rate (*Qs*) of immobilized cells on sPPF and sDPPF were 5.2 and 5.4% higher, respectively than those of free cells whereas the ethanol yields (*Yp/s*) were 4.5 and 6.8% higher, respectively. Correspondingly, the ethanol productivity (*Qp*) and sugar consumption of immobilized cells (0.45-0.47 g ethanol L^{-1} h^{-1} and 96.5-97.4 %) were higher than those of free cells (0.34-0.42 g ethanol L^{-1} h^{-1} and 92.0-96.4 %). All cell concentrations rapidly increased within 24 hrs cultivation period and slightly increased thereafter due to the depletion of the carbon source (Figure 2c).

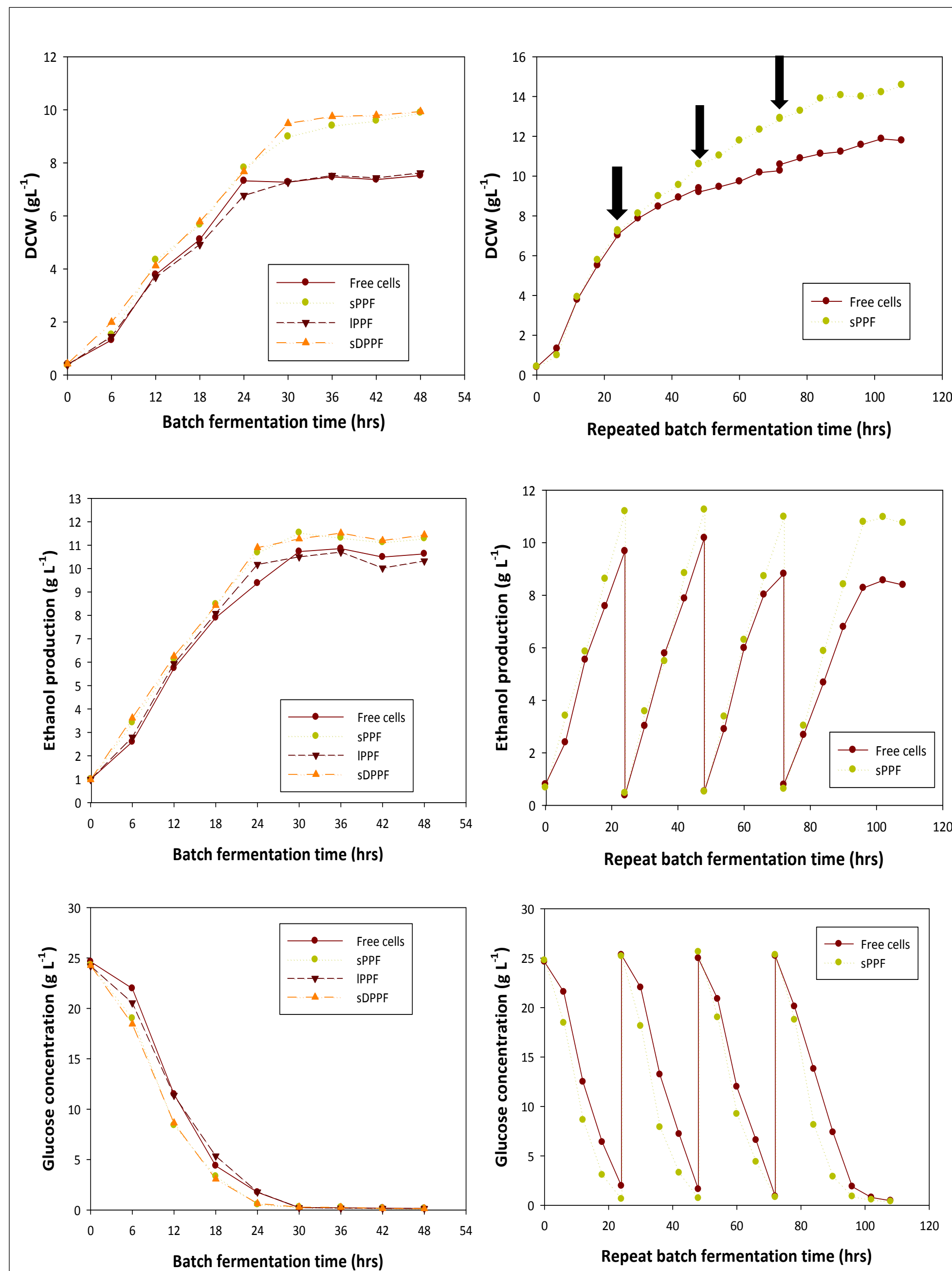

Fig. 2 Performances of ethanol production by free cells and immobilized cells of *C. shehatae* TISTR5843 in batch fermentation and in repeated fermentation by replacing the medium at 24, 48 and 72 hrs (arrows). In detail: (a) and (d) are cell growth; (b) and (e) are ethanol production and; (d) and (f) are glucose consumption. All cases were performed in triplicate; values varied less that 10%.

Kinetics analysis of immobilization for ethanol production in the repeated batch fermentation

To increase the ethanol productivity and retain the ethanol yield of recycling the cells, repeated batch fermentation were conducted. The free cells and the immobilized cells were recycled into a fresh medium every 24 hrs for four repeated batch experiments (Figure 2). The ethanol productivity (*Qp*) of free cells and immobilized cells in repeated batch fermentation increased in the range of 13.3-40.0% and 45.2-51.6%, respectively (Table 2 and Figure 2d-2f) compared to those of free and immobilized cells in batch process (Table 1). The ethanol yield (*Yp/s*) of immobilized cells decreased 6.4% at the forth cycle (0.44 g ethanol g glucose^{-1}) whereas the ethanol yield of free cells (0.42 g ethanol g glucose^{-1}) decreased 9.52% in the third cycle (0.38 g ethanol g glucose^{-1}) and 14.28% in the forth cycle (0.36 g ethanol g glucose^{-1}).

DISCUSSION

The characteristics of the individual fiber depends on the constituent, the fibrillar structure and lamellae matrix. The fiber is composted of numerous elongated fusiform fiber cells that taper toward each end. The fiber cells are linked together by means of middle lamellae, which consists of hemicellulose, lignin and pectin (Joseph et al. 1999). Lignin fills the spaces in the cell wall between cellulose, hemicellulose, and pectin components. The lignin in the fibers changes its structure, properties and morphology (Joseph et al. 1999). The PPF in this experiment was found to have 32% cellulose, 23.8% hemicellulose, and 17.2% lignin, respectively (Riansa-ngawong and Prasertsan, 2010). The principle of immobilization using natural carrier is based on passive adhesion to the surface (Yu et al. 2007). After delignification that causes 50% removal (Riansa-ngawong and Prasertsan, 2010), the remaining holocellulose, which is a mixture of cellulose and hemicellulose, shows deforming rigid structure, which increased the opportunity for cell adsorption between crystalline structures of cellulose (Figure 1c). Genisheva et al. (2011) explained that yeast cells were favoured to adhere on specific regions of natural supports such as cellulose than smooth structure. The porous structures between crystalline and amorphous of holocellulose help solutes transportation and gas transportation between medium phase and structure matrix (Sokolnicki et al. 2006). The changing structures of sPPF and sDPPF increased mass transportation which resulted in the increase of microbial population. The higher cell densities (Figure 1e and 1f) were responsible for advances in the immobilization system as demonstrated in the increase of the substrate uptake rate, glucose consumption, ethanol productivity, and ethanol yield (Table 1). The most commonly used immobilization systems of living cells are the entrapment and the adhesion. The advantages of the adhesion technique are its simplicity and reducing mass transfer problem associated with the entrapped cells in the carriers which have insufficient space for living cells that could lead to cell breakdown and leakage to the medium (Nussinovitch et al. 1994; Behera et al. 2010). Additionally, the pre-treatment of natural support was not necessary for cell adsorption. The ethanol yield of *Saccharomyces cerevisiae* immobilized on grape seeds treated with citric aciddecreased 23% (0.51 to 0.39 g g glucose^{-1}) (Genisheva et al. 2011).

Ethanol yield, which is a measurement of how much substrate is converted into ethanol, is a criteria to evaluate ethanol production. It is well known that 0.51 g ethanol is produced from 1 g glucose. However, the carbon flow in the cells is also used for biomass production. Therefore, the theoretical ethanol yield is approximately 0.46-0.48 g ethanol g glucose^{-1} (Kopsahelis et al. 2007). The ethanol yields of free and immobilized cells from this study in batch fermentation were 0.44 g ethanol g glucose^{-1} and 0.44-0.47 g ethanol g glucose^{-1}, respectively. The immobilized cells on sDPPF gave 6.8% higher ethanol yield than that of free cells. The result of this study was 10.6% higher than those reported by Martini et al. (2010) whose results immobilized *Saccharomyces cerevisiae* on rice hulls for ethanol production (Table 3). Generally, the performance of ethanol production by immobilized cells adhesion onto the surface of the carrier is much better than free cells because the immobilization system protects the cells from inhibition of ethanol by biofilm formation (Yao et al. 2011). The adhesion genes of biofilm formation in yeast are activated by the change in the substrate levels, pH or ethanol levels (Verstrepen and Klis, 2006) which leads to greater ethanol tolerance, yeast cells survival and ethanol productivity. Moreover, the immobilization system remains a much higher option for living cells over several cycles of operations (Figure 2d) (Chandel et al. 2007; Reddy et al. 2008; Behera et al. 2010).

The immobilized cells on sDPPF and sPPF in batch fermentation gave insignificant different ethanol yields (0.47 and 0.46 g ethanol g glucose^{-1}, respectively) ($P < 0.05$), but there were significantly different ethanol yield in lPPF because of its less surface area. Therefore, the sPPF was chosen as a

carrier in repeated batch fermentation because the delignifinication process was not required as it was not healthy for the environment or a cost effective process. The glucose consumption, ethanol productivity and ethanol yields of immobilized cells on sPPF in repeated batch fermentation was higher than that of free cells (Table 2) because the immobilization system gave higher cell densities per unit bioreactor volume (Figures 2a and 2d), which leads to high volumetric productivity, shorter fermentation times and elimination of non-productive cell growth phases resulting in the increasing substrate uptake and the yield improvement (Kourkoutas et al. 2004). In the batch system, the cells and ethanol production increased rapidly at 24 hrs cultivation period together with the dramatic decrease in glucose concentration in the medium (Figures 2a-2c). Based on this time point in batch system which gave the highest ethanol concentration and glucose concentration, the culture medium in the repeated batch fermentations was removed and replaced with the new modified YM medium at 24, 48 and 72 hrs cultivation period to retain ethanol yield and ethanol productivity (Figures 2e and 2f).

In comparison, ethanol production of immobilized cells in this study gave much better results than ethanol production by *S. cerevisiae* adhered on spent grain and delignified spent grain in four repeated batch process which the ethanol productivity and ethanol yield decreased 22.8% and 15.0%, respectively (Kopsahelis et al. 2007). Moreover, the ethanol yield (0.44-0.47 g g^{-1} substrate) of *C. shehatae* TISTR5843 adhered on PPF materials were similar to those ethanol yields by *S. cerevisiae* immobilized on wild sugarcane (0.43 g g^{-1} substrate) (Chandel et al. 2009), sugar beet pulp (0.446 g g^{-1} substrate) (Vučurovic and Razmovski, 2012) and thin-shell silk cocoon (0.43-0.48 g g^{-1} substrate) (Rattanapan et al. 2011) (Table 3). Some of the researches suggested that a suitable natural support should be (i) a coarse surface area that increased the cells adhesion (Yu et al. 2010; Genisheva et al. 2011), (ii) the ultrafine and porous networks that allowed product leakage into the medium, the nutrients transportation to the yeast cells (Yao et al. 2011) and (iii) the protection of detached cells into the medium resulting from agitation (Genisheva et al. 2011). Additionally, *C. shehatae* is capable of xylose consumed (Delgenes et al. 1996) which was the main composition of lignocellulosic hydrolysate (Riansa-ngawong and Prasertsan, 2010; Riansa-ngawong and Prasertsan, 2011). To use lignocellulosic hydrolysate, the phenomena of ethanol production in co substrate, glucose and xylose, by the immobilized *C. shehatae* TISTR5843 on sPPF should be further investigated.

CONCLUDING REMARKS

PPF, an abundant agro-industrial waste in southern Thailand, was a good carrier for immobilization of *C. shehatae* TISTR5843. The pre-treatments of PPF by size reduction and delignification increased surface area which enhanced cell adsorption and ethanol production. The immobilized yeast cells of this study demonstrated an efficient ethanol production, high immobilized cells concentration and the good operational stability without decreased its activity within 4 cycles. The advantages of PPF immobilization system are low carrier cost, free from pesticide fiber, protection of the cells from shear stress, easily separated in downstream process, and remains viable cells for several cycles of operations. The further improvement of ethanol production should be the investigation of immobilized cells in the continuous process and use PPF hydrolysate as a nutrient source in a pilot scale of ethanol production.

Financial support: This research was financially supported by The Joint Graduate School of Energy and Environment (JGSEE/THESIS/137), King Mongkut's University of Technology Thonburi, and Palm Oil Products and Technology Research Center (POPTEC/002/2550), Prince of Songkla University, Thailand.

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How to reference this article:

RIANSA-NGAWONG, W.; SUWANSAARD, M. and PRASERTSAN, P. (2012). Application of palm pressed fiber as a carrier for ethanol production by *Candida shehatae* TISTR5843. *Electronic Journal of Biotechnology*, vol. 15, no. 6. http://dx.doi.org/10.2225/vol15-issue6-fulltext-1

Note: Electronic Journal of Biotechnology is not responsible if on-line references cited on manuscripts are not available any more after the date of publication. Supported by UNESCO / MIRCEN network.

Tables

Table 1. Growth and fermentation kinetics of free and immobilized cells by *C. shehatae* TISTR5843 adhered on various supports in batch fermentation.

	Free cells	Immobilized cells on sPPF	Immobilized cells on sDPPF	Immobilized cells on IPPF
Maximum ethanol production (P_{max}, g ethanol L^{-1})	10.8[a]	11.3[b]	11.5[b]	10.7[a]
Maximum cells concentration (X_{max}, g DCW L^{-1})	7.52[b]	9.89[a]	9.93[a]	7.62[b]
Specific growth rate (μ, h^{-1})	0.09	0.09	0.08	0.08
Cell yield (Yx/s, g DCW g $glucose^{-1}$)	0.31	0.40	0.40	0.31
Ethanol yield (Yp/s, g ethanol g $glucose^{-1}$)	0.44[a]	0.46[b]	0.47[b]	0.44[a]
Substrate uptake rate (Qs, g glucose L^{-1} h^{-1})	0.95	1.00	1.00	0.93
Ethanol productivity (Qp, g ethanol L^{-1} h^{-1})	0.30	0.31	0.32	0.30
Glucose consumption (%)	98.2	99.1	99.0	98.0

[a] and [b] are significant difference at $p < 0.05$. All cases were performed in triplicate; values varied less that 10%.

Table 2. Fermentative kinetics of free and immobilized cells by *C. shehatae* TISTR5843 adhered on sPPF in four repeated batch fermentation.

	Free cells				Immobilized cells on sPPF			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Maximum ethanol production (P_{max}, g ethanol L^{-1})	9.7	10.2	8.8	8.3	11.2	11.3	11.0	10.8
Ethanol yield (Yp/s, g ethanol g $glucose^{-1}$)	0.42	0.42	0.38	0.36	0.46	0.47	0.44	0.44
Substrate uptake rate (Qs, g glucose L^{-1} h^{-1})	0.94	0.99	1.00	0.97	1.00	1.02	1.03	1.02
Ethanol productivity (Qp, g ethanol L^{-1} h^{-1})	0.40	0.42	0.37	0.34	0.47	0.47	0.46	0.45
Glucose consumption (%)	92.0	93.6	96.4	92.5	97.4	97.1	96.8	96.5

All cases were performed in triplicate; values varied less that 10%.

Table 3. Comparison of ethanol production by cells immobilization on the various natural carriers.

Immobilization carriers	Yeast stain	Process of fermentation	Ethanol yield (g g substrate^{-1})	References
Wild sugarcane	*Saccharomyces cerevisiae* VS3	Batch	0.43	Chandel et al. 2009
Corn cobs Grape stems Grape skins Grape seeds	*Saccharomyces cerevisiae*	Batch	0.40 0.38 0.50 0.39	Genisheva et al. 2011
Rice hull	*Saccharomyces cerevisiae*	Repeated batch	0.32	Martini et al. 2010
Sugar beet pulp	*Saccharomyces cerevisiae* DNT	Repeated batch	0.446	Vučurovic and Razmovski, 2012
A thin-shell silk cocoon	*Saccharomyces cerevisiae* M30	Repeated batch	0.43-0.48	Rattanapan et al. 2011
		Continuous	0.48	
Palm pressed fiber	*Candida shehatae* TISTR5843	Repeated batch	0.44-0.47	This study

All cases were performed in triplicate; values varied less that 10%.

DOI: 10.2225/vol15-issue6-fulltext-1