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Use of Immobilized *S. cerevisiae* in Beads Made from Polyvinyl Alcohol and Palm Oil Fuel Ash to Enhance Ethanol Production from a Distillery Wastewater

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Abstract

The purpose of this study was to use an immobilized yeast culture (*S. cerevisiae*) to explore the potential to produce ethanol by fermenting a distillery wastewater either in batch or continuous -flow reactors.The beads were made using a mixture of polyvinyl alcohol (PVA) and palm oil fuel ash (POFA)in order to enhance the beads' potential use. Results revealed that a 50% (v/v) cell concentration in the beads appeared to be the most suitable, while a PVA to POFA ratio of 10:3 (i.e. the highest POFA content investigated) was superior with respect to bead physical characteristics and performance, which was also validated by photomicroscopic images. The yeast cells were grown on both the outer and inner surface, especially at the cell concentration of 50% (v/v). Regarding the combined effect of PVA:POFA ratio and initial cell dry weight, a slight improvement in ethanol productivity (g/L/h) and ethanol yield (g/g) was observed with an increase in the POFA content and initial cell dry weight. Furthermore, ethanol productivity was significantly higher in a series of batch reactors operated at an HRT of 6 h compared to HRTs of 12 and 24 h; however, the effect

of HRT variation on ethanol yield was less pronounced. Lastly, the continuous-flow system was moderately more efficient to remove total sugar than the batch system of about 17.3%, operated at the same HRT of 6 h.

Keywords: distillery wastewater; ethanol production; yeast immobilization; polyvinyl alcohol; palm oil fuel ash

1. Introduction

Wastewater originating from distilleries can be a major source of water and soil pollution; therefore, proper treatment and disposal strategies are required to minimize its impact on the environment. The volume and characteristics of the wastewater generated depend upon the type of feedstock used (e.g. potato, wheat, cassava, etc.) and the nature of the industrial fermentation processes employed (Pant and Adholeya, 2007; Krzywonos et al., 2009; Sakdaronnarong et al., 2015). For instance, the production of 1 m^3 of ethanol requires 2.4 tons of cassava dry chips and generates 9.5 m^3 of distillery wastewater (Jakrawatana et al., 2016). Moreover, Neamhom (2017) reported that 2.84 tons of cassava dry chips were used as raw material to produce 1 m^3 of ethanol and generated wastewater of 14.4 m^3. In general, liquid wastes from bio-ethanol production facilities are characterized by a high organic matter and dissolved solids content and a low pH (Zhang et al., 2009). Consequently, the treatment and disposal of large quantities of liquid wastes can pose a serious challenge to ethanol-producing industries not only from a financial but also from an infrastructure and operation perspective (Moriizumi et al., 2012). A variety of physicochemical and biological treatment methods have been implemented for the treatment of distillery wastewater (also known as stillage), including adsorption (Mane et al., 2006), membrane filtration (Mohana et al., 2009), electrolysis (Yavuz, 2007), enzymatic treatment (Sangave and Pandit, 2006) as well as aerobic (Krzywonos et al., 2008) and anaerobic biological processes (Satyawali et al, 2008). In addition to being treated as a waste, distillery wastewater has been recently considered as a potential resource material in a number of applications including yeast cultivation, animal feedstock and fertilizer manufacturing (Krzywonos et al., 2009; Alotaibi et al., 2014). Distillery wastewater could also be further processed to generate useful end-products such as ethanol (Elemike et al., 2015).

Ethanol generation through the fermentation of non-food sources such as distillery wastewater has attracted widespread interest in the last decade since it can provide a viable alternative option as biofuel (Daylan and Ciliz, 2016). Microbial fermentation processes are typically affected by several factors, including temperature, pH, concentration and type of

substrate, nature of microorganisms, and culture media (Lin et al., 2012; Gumienna et al., 2014). Although yeasts (e.g. *Saccharomyces cerevisiae*) are the predominant microorganisms employed in industrial fermentation processes, certain bacterial species (e.g. *Zymomonas*) have been used as well (Behera et al., 2012).

The production of bioethanol can be accomplished either by free or immobilized cells. Compared to the use of free cells, immobilization offers several advantages, including enhanced productivity, increased operational stability and cell viability, as well as reduced risk of contamination (Chandel et al., 2007). Among the various immobilization technologies available, entrapment of cells within the polymeric matrices has been used widely for ethanol generation (Winkelhausen et al., 2010; Datta et al., 2013). Numerous natural and synthetic polymers have been utilized including agar-agar, Ca- or Na-alginate, gelatin, k-carrageenan, and polyvinyl alcohol (PVA) (Behera et al., 2010). In relation to other polymers, PVA offers several advantages such as low-cost, high durability and mechanical strength, as well as non-toxicity to microorganisms (Alenina et al., 2012). Furthermore, to improve the capacity of immobilized cell and cost effectiveness, the possibility of using POFA generated from burning of fiber and empty fruit brunches in a boiler binding with PVA to form the gel beads was considered. Since the feature of POFA has the properties appropriate to be used as an admixture to support and replace the percentage of PVA in gel bead, it reduced the capital and operating investment cost (Sinnaraprasat and Fongsatitkul, 2011).

The main objective of this study is therefore to investigate the potential of ethanol production during the fermentation of distillery wastewater using immobilized yeast (*S. cerevisiae*) entrapped in beads made from a PVA and POFA mixture. Specifically, the effect of PVA to POFA ratio and bead volume on cell immobilization as well as the role of hydraulic retention time (HRT) on ethanol production will be explored in detail.

2. Materials and methods

2.1 Feed characteristics

The wastewater used as feed was collected from the distillation process at a bioethanol production plant, located in Lopburi, Thailand, which used cassava dried chips as a raw material. Several important characteristics of the raw wastewater are shown in Table 1. Apart from the high content of total and soluble organic matter [expressed as total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD), respectively] which is typical for such complex liquid wastes, it is evident that carbohydrates are the major organic class present. Moreover, the untreated distillery wastewater exhibited a strong acidity and had a dark brown color. Prior to being fed into the reactors, the raw wastewater was diluted with tap water to obtain a TCOD concentration of about 50,000 mg/L for organic loading control and comparison among different runs.

2.2 Microorganisms and culture media

Ethanol fermentation was achieved by Saccharomyces cerevisiae species (TISTR 5339) purchased from the Thai Institute of Scientific and Technological Research (TISTR) as a freeze-dried culture. The microbial culture was first incubated at 30 °C for 1 to 3 days and then preserved on a sterilized yeast extract medium (which was replaced every six months) in 20 mL-test tubes at 4 °C until further use. The composition of the medium was 9 g/L glucose, 15 g/L $MgSO_4.7H_2O$, 2.5 g/L $(NH_4)_2SO_4$, 1 g/L yeast extract, 10 g/L KH_2PO_4, 5 g/L K_2HPO_4. The pH was maintained at 4.5 by addition of either 1 N NaOH or 1 N HCl, as required (Ghorbani et al., 2011). Sterilization of the medium was accomplished by autoclaving at 121 °C and 1 atm for 20 min. To ensure that cells were at the same growth stage throughout the experiment, the microorganisms were first inoculated in glass test tubes containing the same medium under sterile conditions using an incubator at 30 °C for 16 h, before each application.

2.3 Immobilization of Saccharomyces cerevisiae

The immobilized *S. cerevisiae* was prepared by using the PVA-boric acid method, at PVA (%w/v) and POFA (%w/v) ratios of 10:1, 10:2, and 10:3, based on previous work (Sinnaraprasat and Fongsatitkul, 2011). As mentioned in the Introduction, POFA was added to the mix as supporting material to enhance the performance of immobilized cells. Initially, 20 grams of PVA and 2 % (w/v) sodium alginate were dissolved in 140 mL of distilled water at 60 °C. The PVA-alginate solution was subsequently cooled to room temperature (30 to 35 °C) and mixed either with 60, 100 or 140 mL (i.e. 30, 50 or 70% v/v) of concentrated *S. cerevisiae*. The yeast culture was harvested at the exponential growth phase and the centrifuged cells were re-suspended. The yeast extract medium was centrifuged at 4,400 rpm for 20 min; afterwards it was mixed with the PVA solution and concentrated *S. cerevisiae* at a ratio of 70:30 (v/v) with the addition of 6 grams (3% w/v) of POFA, corresponding to PVA:POFA ratio of 10:3. The final concentration of the mixture was 10 % (w/v) PVA, 2% (w/v) sodium alginate, 3% (w/v) POFA, and 30, 50 or 70% (v/v) microbial cells in suspension. For the PVA:POFA ratios of 10:1 and 10:2, the amounts of POFA of 2 g and 4 g were added, respectively.

Immobilization of *S. cerevisiae* in a spherical form was achieved by using a syringe to transfer drops of the PVA-POFA and *S. cerevisiae* liquid mixed into a solution containing 2 % (w/v) calcium chloride ($CaCl_2$) and saturated boric acid (Leenen et al., 1996). In order to complete the gelation process, the beads were kept in a boric acid solution for 24 hours at 4 °C. Hardening of the PVA gel granules improves not only the gel strength but also the stabilization of entrapped bacteria cells (Tian et al., 2009). Afterwards the beads were washed thoroughly with deionized water several times and stored at 4 °C until further use. The average bead size

Table 1 Characteristics of the distillery wastewater

Parameters	Concentrations
Initial pH	3.0 - 3.5 ± 0.5
Fiber (mg/L)	832 ± 234
Total sugar (mg/L)	13,120 ± 4,310
Carbohydrate (mg L^{-1})	27,040 ± 1,290
Protein (mg/L)	8,008 ± 102
Ash (mg/L)	6,656 ± 122
Fat (mg/L)	2,392 ± 134
Moisture (%)	95.76 ± 12
TCOD (mg/L)	70,000 - 90,000 ± 5,500
SCOD (mg/L)	35,000 - 60,000 ± 4,200
BOD (mg/L)	20,000 - 35,000 ± 5,250

of PVA-POFA used in this study was about 4 to 5 mm in diameter. All chemical reagents used for immobilization were of laboratory grade.

2.4 Experimental set-up and operation

The potential of immobilized Saccharomyces cerevisiae to produce bioethanol from distillery wastewater was explored using batch and continuous-flow systems. Erlenmeyer flasks (250 mL volume) with working volume of 100 mL were used as batch reactors. Moreover, the continuous-flow, completely mixed anaerobic bioreactors were made from acrylic and had a working volume of 2.0 L (inner diameter of 11 cm and height of 32 cm) as depicted in Figure 1. The experiments were divided into four phases in order to explore the following issues:

2.4.1 Selection of cell concentration

Initially, a batch reactor was employed at a PVA:POFA ratio of 10:3, an initial cell weight of 0.45 g operated at an HRT of 24 h, using three different cell concentrations of 30, 50, and 70 % (v/v) in order to select a suitable and effective cell concentration for further investigation (N=12 in each condition).

2.4.2 Effect of PVA to POFA ratio and initial cell dry weight on cell immobilization

Experiments were conducted in a batch mode, using 250 ml flasks which were continuously mixed in a shaker at 100 rpm. Three different PVA:POFA ratios of 10:1, 10:2, and 10:3 and four initial cell dry weights of 0.45, 1.5, 1.8, and 2.1 g were employed with cell concentration

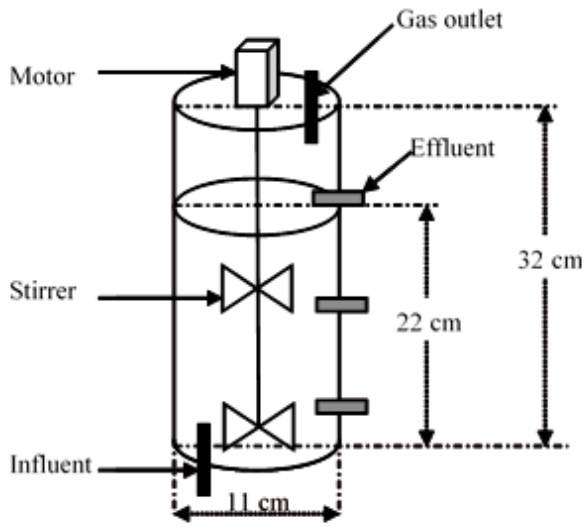


Figure 1. A schematic drawing of the experimental unit dimension

of 50% at an HRT of 24 hours. This was based on the results of the first phase (previous paragraph), which showedthat a cell concentration of 50% was the most effective one (N=12 in each condition).

2.4.3 Effect of HRTs on ethanol production

Batch reactors were operated at HRTs of 6, 12 and 24 hours under anaerobic conditions to investigate the effect of HRT. Three 250 mL flasks were connected in a series (designated as B1, B2 and B3, respectively), having an initial cell dry weight of 1.8 g and cell concentration of 50% (v/v), as per previous experimental work (i.e. subsections 2.4.1) and 2.4.2) and operated at a selected HRT. All reactors were continuously mixed in a shaker at 100 rpm. In order to better utilize the residual substrate, at the end of each HRT cycle the effluent (content) from the third reactor (B3) was decanted, the effluent from B2 was transferred to B3, the effluent from B1 was transferred to B2, while fresh distillery wastewater was added to B1. Such a procedure was repeated throughout this experimental phase (N=12 in each condition).

2.4.4 Ethanol production in continuous-flow bioreactors

Three continuous-flow bioreactors were operated in series (designated as R1, R2 and R3, respectively) to explore ethanol production taking into account the "best performance" conditions during the previous three phases [i.e. cell concentration 50% (v/v), PVA:POFA ratio 10:3, an initial cell dry weight of 1.8 g, and HRT 6 h] (N=12 in each condition). Figure 2 displays a schematic representation of the three

reactor system in a series. Initially the reactors were sterilized in a 50% (v/v) ethanol solution for 24 h, rinsed with sterile distilled water and purged with sterile filtered nitrogen gas for 1 h to ensure that anaerobic conditions are maintained within the reactors (Qurishi and Maddox, 1995). Subsequently, the reactor was filled with 1 L of distillery wastewater having a COD of approximately 50,000 mg/L and inoculated with immobilized S. cerevisiae in PVA:POFA gel beads for 3 days to allow for acclimation of the immobilized cells. Once ethanol production was detected, the distillery wastewater was continuously fed into the reactors by a peristaltic pump to achieve a working volume of 2 L. The initial average cell concentration in the reactor was about $2.1x10^8$ CFU/ mL. Mixing was provided by a two-blade paddle rotating at 100 rpm. Steady-state operation was determined by checking the stability of alcohol production.

2.5 Analytical methods

Influent and effluent samples were taken on a daily basis. TCOD, SCOD, biological oxygen demand (BOD5) and pH were analyzed by standard methods (APHA, 2005). Total sugars including fructose, glucose, galactose, sucrose, maltose and lactose were determined by high performance liquid chromatography (HPLC) using an ASP-2-Hypersil column and refractive index (RI) detection with 75:25 (% v/v) acetonitrile/water as mobile phase at the flow rate of 1.0 mL/min. Before injection to the HPLC, the samples were centrifuged and filtered through the GF-C membrane; subsequently, appropriate aliquots were taken from the filtrate into 0.2μm cellulose acetate membrane. Ethanol was analyzed by gas chromatography equipped with an HP-INNOWAX column (30 m x 0.25 mm x 0.25 μm, Agilent Technology) and a flame ionization detector (FID). The temperature of the injector and FID were 220 °C and 270 °C, respectively. Helium was used as a carrier gas with a flow rate of 1 mL/min. A 1 μL of injection volume and split sample ratio of 30:1 were used. Ethanol productivity was calculated as ethanol concentration in g/L divided by the fermentation time (h). Ethanol yield was calculated as g of ethanol produced per g of sugar utilized (expressed in g/g).The cell dry weight measurement was obtained by pipetting 5 mL of well mixed broth *S. cerevisiae* on to a pre-dried Whatman GF/C. The filter and solids were replaced in the drying oven, and dried to constant weight (24 hours at 90 °C). When dry, the filters were allowed to cool to room temperature in a desiccator and then reweighed (Stone et al., 1992). Moreover, to determine the specific growth rate, the initial weight of the beads before and after operated in the fermentation system for 20 days were analyzed.

The surface and cross-sectional morphology of the beads were observed using scanning electron microscopy (SEM). Bead samples were prepared in four steps as follows: i) freeze in 2.5% glutaraldehyde (in 0.1M phosphate buffer

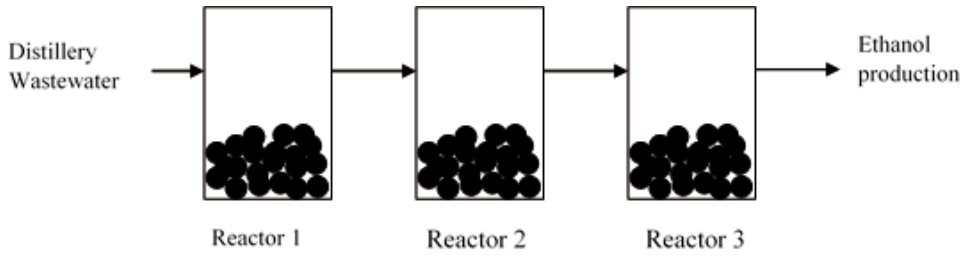


Figure 2. Schematic diagram of three reactors connected in series under anaerobic condition

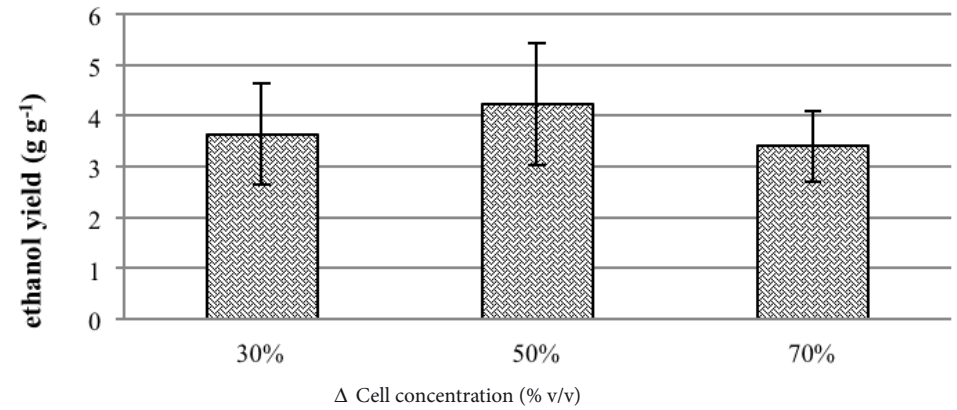
pH 7.2) for 2 h, ii) rinse sample twice with 0.1 M phosphate buffer at pH 7.2, iii) wash with distilled water for 10 min, and iv) dehydrate with ethanol concentrations of 30%, 50%, 70%, 95%, and 100%, respectively, at 10 min, (3 times for 5 min each). Samples were dried at critical point employing a critical point dryer (Quorum, model K850, UK), sputtered with gold under ion sputter (Balzers, model SCD 040, Liechtenstein), and photographed. The coated specimens were examined under a SEM using a JOEL JSM-5410LV microscope (Tokyo, Japan). Further details on all experimental methods are available elsewhere (Khongkkeam, 2014).

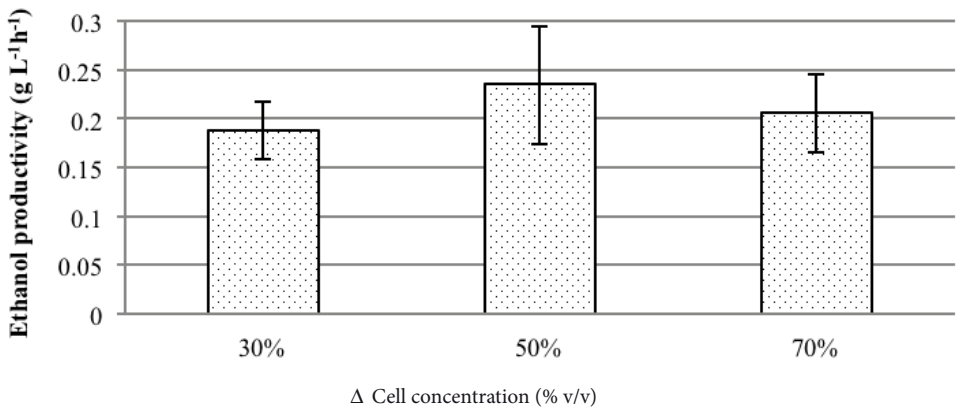
3. Results and discussion

3.1 Selection of cell concentration

The results summarized in Figure 3 indicate that an increase in the cell concentration from 30 to 50% (v/v) improved bead cell dry weight, ethanol productivity (expressed a volumetric rate) and ethanol yield (expressed as g ethanol produced per g of total sugar consumed). However, all three performance parameters declined when the cell concentration increased further to 70 % ($p<0.05$, $N=12$). This can be attributed to the fact that as the concentration of the entrapped cells inside the bead increases, beyond a certain value, the specific growth rate is reduced since cells have limited space to grow.

Space limitation also affects the specific substrate utilization rate because the diffusion of substrate and other nutrients is progressively reduced as well (Pramanik and Khan, 2009). In addition,to showing the best performance, the inoculum of 50% (v/v) also resulted in minimal splitting of gel beads during mixing. Electronic microscopic observations of immobilized *S. cerevisiae* (both of the outer and inner surface) provided further supporting evidence (Figure 4, c and d) regarding the behavior of the immobilized cells as a function of cell concentration.





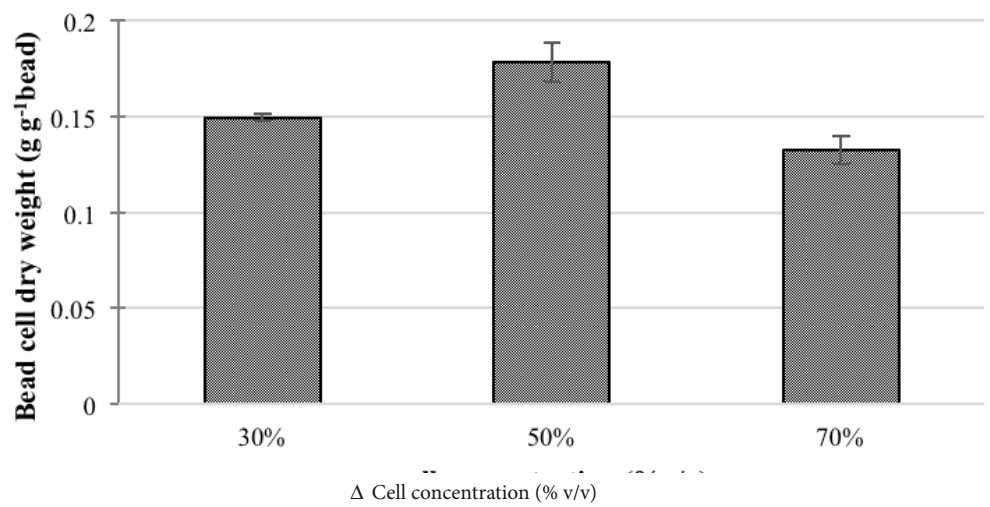


Figure 3. Ethanol yield, ethanol productivity, and bead cell dry weight, at HRT 24 h and PVA:POFA of 10:3 in batch reactor ($p<0.05$, N=12)

3.2 *Effect of PVA to POFA ratios and initial cell dry weight on cell immobilization*

Regarding the three initial PVA:POFA ratios investigated, results indicated that at the highest POFA content (a ratio 10:3) ($p<0.05$, N=12), the physical characteristics of the beads formed were superior since they were highly flexible, hard, and stable. In contrast, at the lowest POFA content (a ratio 10:1) the beads were easy to break, and exhibited a low level of stability. A summary of the physical properties of the beads is shown in Table 2. Moreover, a PVA:POFA ratio of 10:3 resulted in better performance compared with ratios of 12.5:3 and 15:3 (Sinnaraprasat and Fongsatitkul, 2011), with respect to cell concentration and specific growth rate, which is due to the larger surface area available for bacterial entrapment. Electronic photomicroscope images illustrate that highly porous and vacuous structures were predominant at the PVA:POFA ratio of 10:3 (Figure 5, e and f). It is also obvious that the yeast cells were grown on the outer and inner surfaces of the PVA:POFA beads after operated for 20 days. The overall bead structure is porous enough to allow the entrance of yeast cells during the immobilization process and also facilitate efficient diffusion of substrate and micronutrients during fermentation (Pramarik and Khan, 2009). The addition of POFA as an admixture is to provide support material, which may also result in several other benefits including an increase in the overall surface area in the gel beads, improvement in mechanical strength and stability, increase in pore size to allow better yeast adhesion, and reduction in capital and operating costs (Najafpour et al., 2004). Furthermore, Fujii et al. (1999) suggested that the surface characteristics of the carrier including pore size, water content and hydrophilicity are considered to be crucial in influencing the efficiency of adsorption, the behavior of the immobilized yeast cells and their productivity.

Concerning the combined effect of PVA:POFA ratio and initial cell dry weight on ethanol productivity and ethanol yield ($Y_{P/S}$), it was noticed in general a slight improvement in both parameters with an increase in the POFA content and initial cell dry weight (Figure 6). The highest ethanol productivity was obtained at an initial cell dry weight of 2.1 g and a PVA:POFA ratio of 10:3; however, the highest initial cell dry weight generated consistently the best results, regardless of the PVA:POFA ratio. Although the highest ethanol yield was obtained at an initial cell dry weight of 1.8 gat a PVA:POFA ratio of 10:3, the highest cell dry weight of 2.1 g was associated, in all other cases, with the best performance. Overall, an increase in initial cell dry weight in the reactor resulted in an increase in ethanol yield and productivity which suggest a more efficient use of the reactor's capacity.

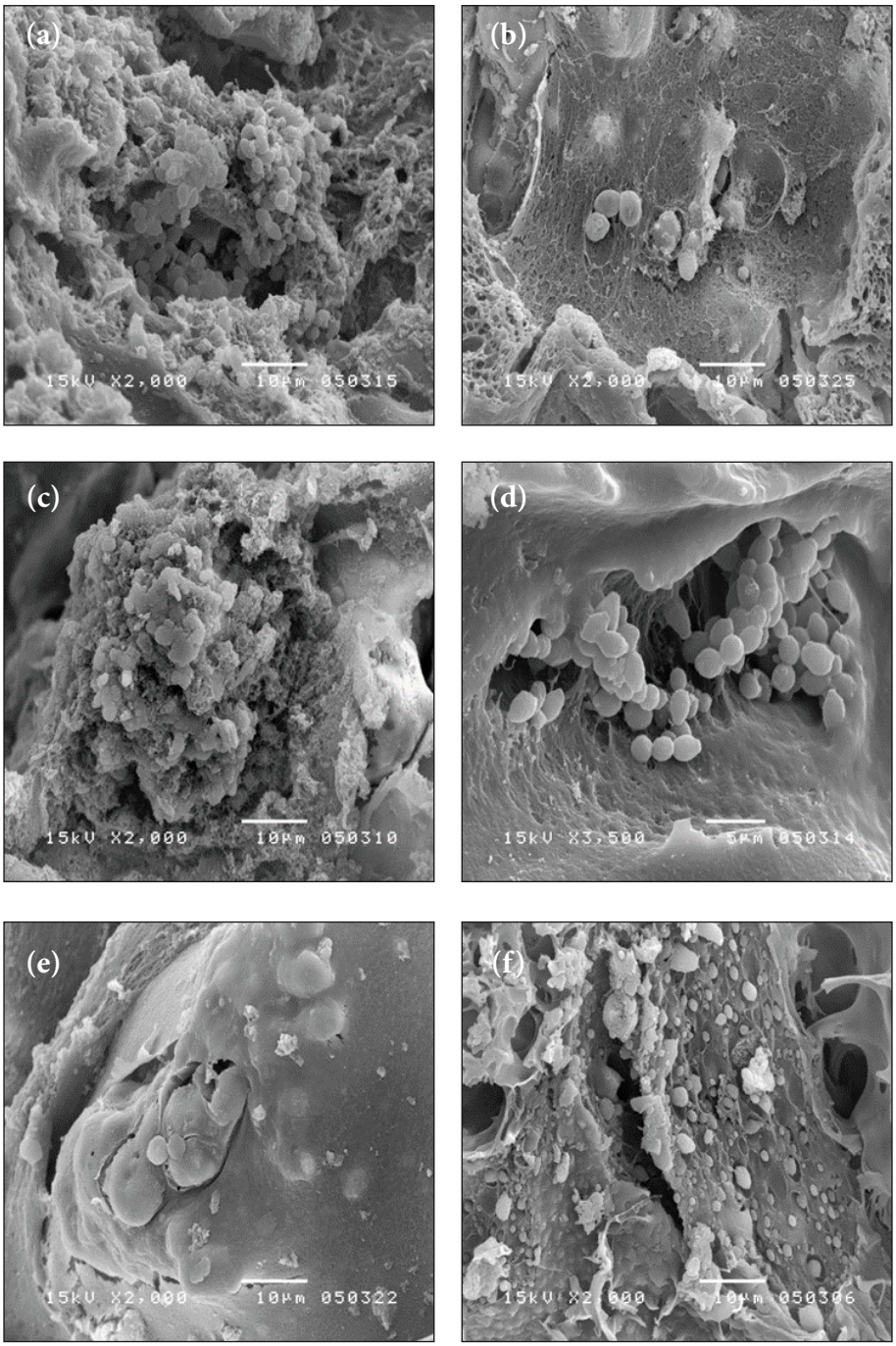


Figure 4. Electronic photomicroscope with magnification X2,000 of immobilized *S. cerevisiae* beads: (a) outer surface, (b) inner surface bead at cell concentration of 30% (v/v); (c) outer surface, (d) inner surface bead at cell concentration of 50% (v/v); (e) outer surface, (f) inner surface bead at cell concentration of 70% (v/v)

Table 2 Physical properties and appearance of *S. cerevisiae* beads

Physical parameter	PVA:POFA ratio of 10:1 (%w/v)	PVA:POFA ratio of 10:2 (%w/v)	PVA:POFA ratio of 10:3 (%w/v)
- Alginate content (%wt.)	2	2	2
- Diameter (mm)	5-5.5	4.8-5	4.9-5
- Physical appearance	Inflexible and easy to break	Moderately flexible	Highly flexible and not easy to break
- Stability	Not stable	Reasonably stable	Very stable

3.3 Effect of HRT

Fermentation of the distillery wastewater at varying HRTs of 6, 12, and 24 h resulted in successful ethanol production, which reached its maximum concentration in the first batch reactor in the series (B1), regardless of the variation in HRT (Table 3). At the lowest HRT of 6 h, the ethanol concentration was practically the same in all three reactors (i.e. no further net production was observed), while at longer HRTs a sharp reduction in ethanol concentration was observed in reactors B2 and B3 as compared to reactor B1, possibly due to its conversion to other soluble products such as volatile fatty acids in a strong acidic environment (pH of 3.2 ± 0.2) (Thuvander, 2012). Furthermore, the ethanol concentrations in reactors B2 and B3 at an HRT of 6 h were significantly higher than the corresponding values at HRT 12 and 24 h ($p<0.05$, $n=12$). Rao et al. (2007) have reported that ethanol was the main product during the fermentation of a brewery wastewater, at an HRT of 6 h. With respect to total sugar profile, it is evident that there is a steady utilization of total sugar in all three reactors in the series, which is more pronounced at longer HRTs, eventually exceeding the 80 % level at an HRT of 24 h (Table 3). It has been reported that sugar consumption by immobilized *S. cerevisiae* is sensitive to HRT variation and increases with an increase in HRT since the microorganisms are given a longer time to assimilate the available substrate (Ghorbani et al., 2011). In this study, it is also noteworthy to mention that the net sugar conversion to ethanol (expressed as a percentage) is higher at the shortest HRT and diminishes steadily at longer HRTs.

Experimental data depicted in Figure 7 indicate that ethanol productivity was significantly higher (at the 95 % confidence level) at an HRT of 6 h than that estimated at longer HRTs.

A similar observation was made by Sinnaraprasat and Fongsatitkul (2011) who reported that the highest ethanol productivity was obtained at a 6 h HRT. In addition, results regarding ethanol yield reveal a better performance at the shortest HRT investigated, which is evident in the latter part of the series

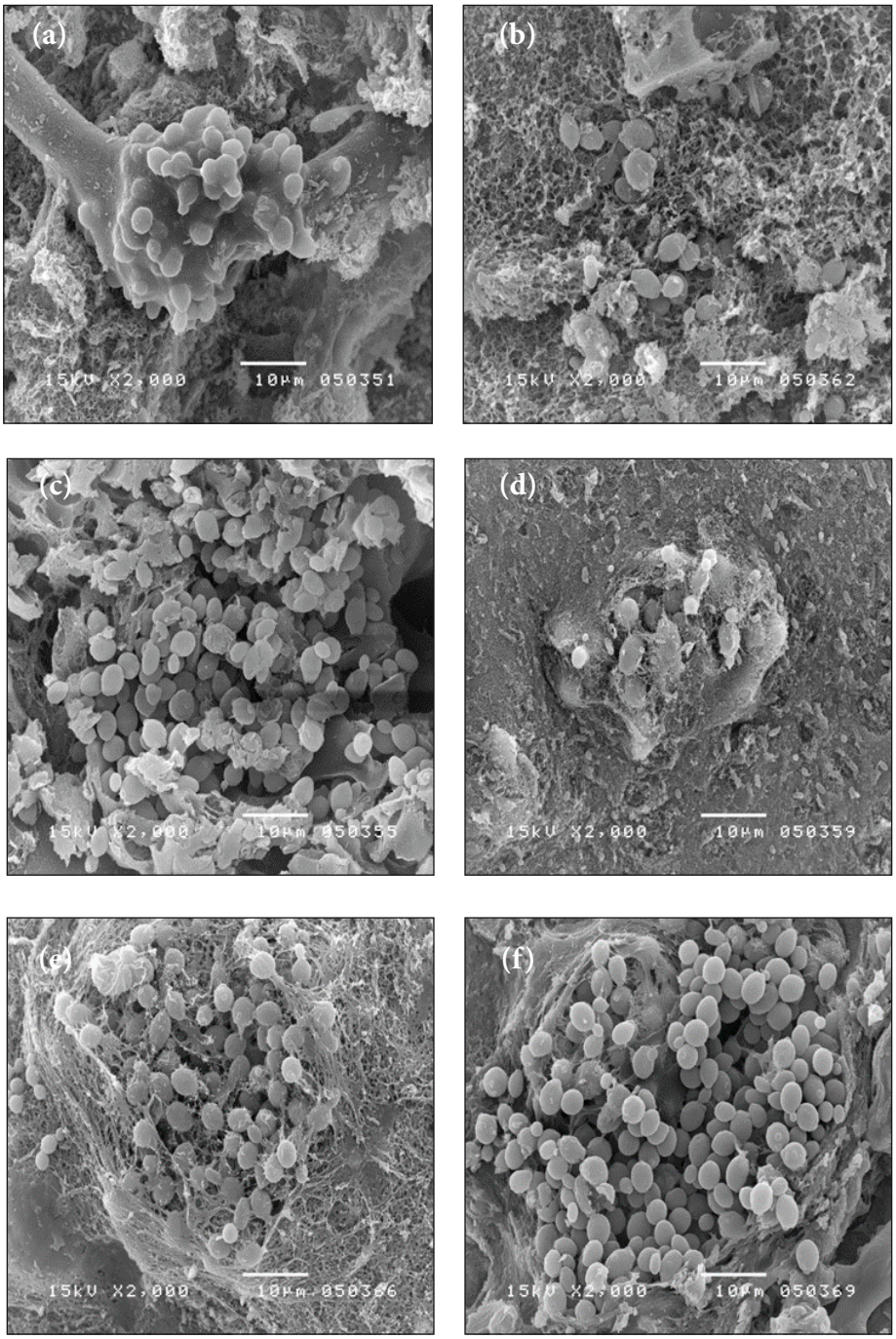


Figure 5. Electronic photomicroscope with magnification X2,000 of immobilized *S. cerevisiae* beads at cell concentration of 50% (v/v): (a) outer surface, (b) inner surface bead at PVA:POFA 10:1; (c) outer surface, (d) inner surface bead at PVA:POFA 10:2; (e) outer surface, (f) inner surface bead at PVA:POFA 10:3

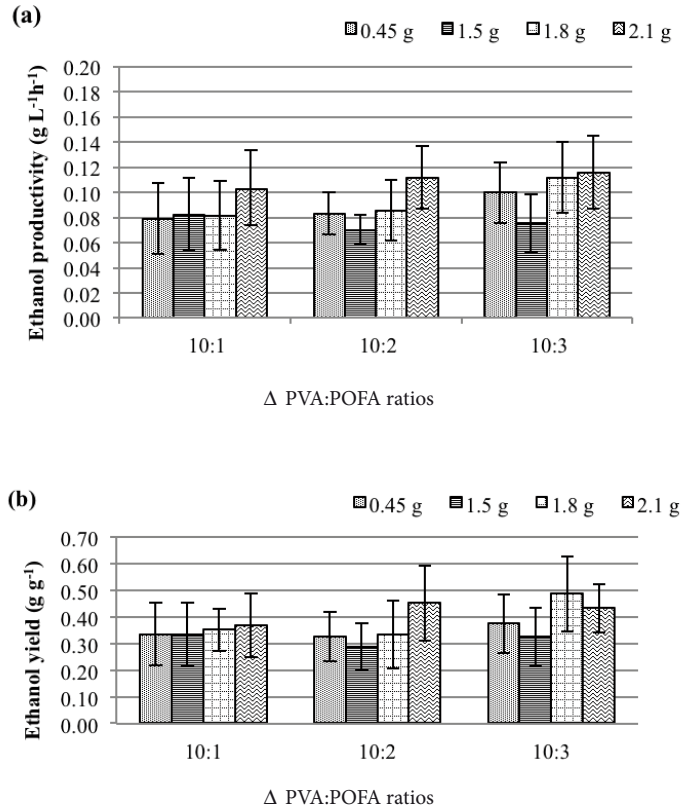


Figure 6. Ethanol productivity and yield as a function of initial cell dry weight of 0.45 g, 1.5 g, 1.8 g and 2.1 g under PVA: POFA ratios of 10:1, 10:2 and 10:3 ($p<0.05$, N=12)

Table 3 Influent and effluent ethanol and total sugar concentrations as a function of HRT in a series of batch reactors (B1 to B3) ($p<0.05$, N=12)

Parameter	Influent (mg/L)	Effluent (mg/L)								
		HRT = 6 h			HRT = 12 h			HRT = 24 h		
		B1	B2	B3	B1	B2	B3	B1	B2	B3
Ethanol	189 ± 15	1187 ± 40	1096 ± 78	1186 ± 50	1092 ± 157	714 ± 28	215 ± 28	1049 ± 61	698 ± 86	697 ± 130
Total sugar	13885 ± 1330	7653 ± 547	5010 ± 457	3447 ± 817	5056 ± 181	3985 ± 258	2785 ± 191	3902 ± 186	3426 ± 129	2297 ± 182
Tot. sugar (% removal)		44.9	63.9	75.2	63.6	71.3	79.9	71.9	75.3	83.5
Net sugar conversion (%)		16.0	10.2	9.56	10.2	5.30	0.23	8.61	4.87	4.38

(i.e. reactors B2 and B3). Purwadi and Taher zadeh (2008) reported that volumetric ethanol productivity and ethanol yield in both single and serial bioreactor experiments increased with an increase in the dilution rate (i.e. decrease in HRT). Regarding the bead cell dry weight, the highest values ranging from 0.13 to 0.18 g/g bead were obtained in the first reactor of the series (B1), regardless of the HRT, and decreased as the wastewater was further processed in the other two batch reactors. Overall, higher ethanol concentration and ethanol productivity are associated with a higher cell dry weight value which is an indication of the ability of the immobilized yeast to convert the substrate into the target product

3.4 Ethanol production in continuous bioreactors

The results from using continuous-flow reactors to produce ethanol have been summarized in Table 4. It is apparent that both ethanol productivity and ethanol yield reached a maximum in the first reactor in the series (R1) and then declined steadily as the wastewater was further processed in reactors R2 and R3.This indicated that ethanol was converted to other soluble products under strong acidic conditions (pH of 3.2 ± 0.1) as similar to pH of the influent and fermentation system. Similar to the behavior of the batch reactors in a series, the ethanol yield in the continuous-flow system declined sharply from the first to the last reactor of the sequence, indicating conversion of ethanol to other products.

With respect to the total sugar removal, the continuous-flow system was moderately more efficient than the batch one, operated at the same HRT of 6 h. Although the cell dry weight followed a similar trend, diminishing steadily from R1 to R3, the respective values were much higher (more than double) than those estimated for the corresponding batch system (as per previous paragraph) which implies that bead under continuous-flow conditions were able to sustain a larger amount of biomass.

4. Conclusions

Overall, results indicated that the distillery wastewater used had the potential to generate ethanol under the conditions investigated. Moreover, agricultural waste (palm oil fuel ash) can be utilized as supporting material for yeast cell immobilization. Ethanol formation was affected by cell concentration, reaching a maximum value at the 50 % (v/v) level. Concerning the bead physical characteristics and performance, a PVA to POFA ratio of 10:3 proved to be the most effective one. Indicating that addition of POFA gave the suitable property of cell beads. In addition, a moderate improvement in ethanol productivity of 0.118 g/L/h and ethanol yield of 0.49 g/g was observed with an increase in the amount of POFA added and the initial cell dry weight. An increase in initial cell dry weight to 2.1 g resulted in an increase in ethanol production which suggests a more efficient use of the reactor's capacity. The variation in HRT studied had a more noticeable

effect on ethanol productivity than ethanol yield in a series of batch reactors. At an HRT of 6 h, total sugar removal was slightly higher in the continuous-flow system compared to the corresponding batch reactor series.

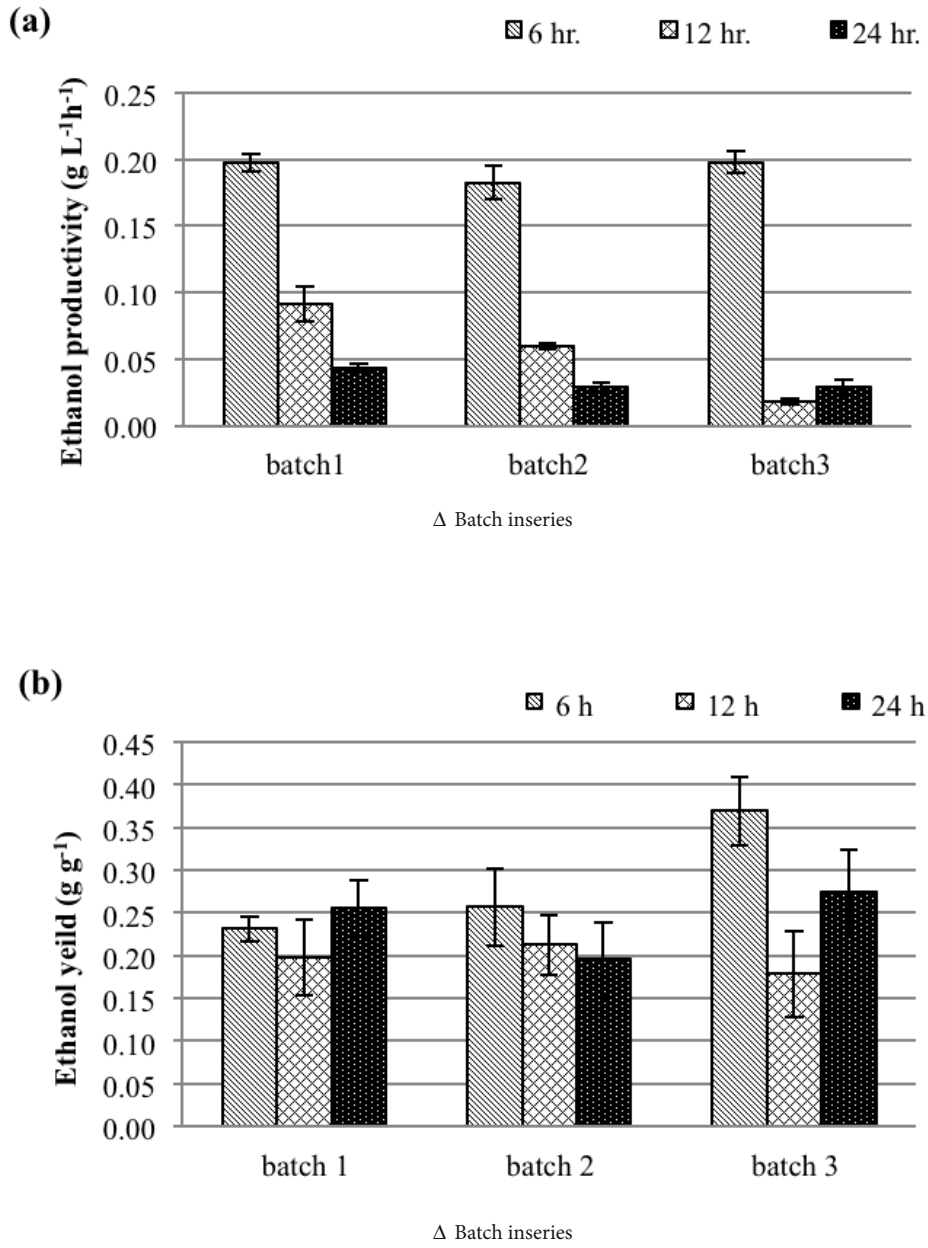


Figure 7. Ethanol productivity and ethanol yield at HRTs of 6, 12, and 24 hours under batch in series ($p<0.05$, N=12)

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References

Pant P, Adholeya A. Biological approaches for treatment of distillery wastewater: A review. Bioresource Technology 2007;98 (12):2321-34.

Krzywonos M, Cibis E, Miśkiewicz T. Ryznar-Luty A. Utilization and biodegradation of starch distillery wastewater (distillery wastewater). Electronic Journal of Biotechnology 2009;12(1):1-12.

Sakdaronnarong C, Ittitanakam A, Tanubumrungsuk W, Chaithong S, Thanosawan S, Sinbuathong N, Jeraputra C. Potential of lignin as a mediator in combined systems for biomethane and electricity production from ethanol distillery wastewater wastewater. Renewable Energy 2015;76:242-8.

Jakrawatana N, Pingmuangleka P, Gheewala SH. Material flow management and cleaner production of cassava processing for future food, feed and fuel in Thailand. Journal of Cleaner Production 2016; 134:633-641

Neamhom T. Evaluation of renewable energy potentials from sugarcane and tapioca productions based on a carbon balanced model: a case study in Thailand. Ph.D. Thesis, Department of Sanitary Engineering, Mahidol University, Bangkok, Thailand. 2016.

Zhang W, Xiong R, Wei G. Biological flocculation treatment on distillery wastewater and recirculation of wastewater. Journal of Hazard Materials 2009;172(2-3):1252-7.

Moriizumi Y, Suksri P. Hondo H, Wake Y. Effect of biogas utilization and plant co-location on life-cycle greenhouse gas emissions of cassava ethanol production. Journal of Cleaner Production 2012;37:326-34.

Mane JD, Modi S, Nagawade S, Phadnis SP, Bhandari VM. Treatment of spentwash using chemically modified bagasse and color removal studies. Bioresource Technology 2006;97(14):1752-5.

Mohana S, Acharya BK, Madamwar D. Distillery spent wash: Treatment technologies and potential applications. Journal of Hazard Materials2009;163(1):12-25.

Yavuz Y. EC and EF processes for the treatment of ethanol distillery wastewater. Separation and-PurificationTechnology 2007;53(1):135-140.

Sangave PC, Pandit AB. Enhancement in biodegradability of distillery wastewater using enzymatic pretreatment. Journalof Environmental Management 2006;78(1):77-85.

Krzywonos M, Cibis E, Mifskiewicz T, Kent CA. Effect of temperature on the efficiency of the thermo- and mesophilic aerobic batch biodegradation of high strength distillery wastewater (potato distillery wastewater). Bioresource Technology 2008;99(16):7816-24.

Satyawali Y, Balakrishnan M. Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. Journalof Environment Management 2008;86(3):481-97.

Alotaibi K, Schoenau JJ, Hao X. Fertilizer potential of thin distillery wastewater from wheat-based ethanol production. BioEnergy Research 2014;7(4):1421-9.

Elemike EE, Oseghale OC, Okoye AC. Utilization of cellulosic cassava waste for bio-ethanol production. Journal of Environmental Chemical Engineering 2015;3(4):2797-800.

Daylan B, Ciliz N. Life cycle assessment and environmental life cycle costing analysis of lignocellulosic bioethanol as an alternative transportation fuel. Renewable Energy 2016;89:578-87.

Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H. Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. Biomass &Bioenergy 2012;47:395-401.

Gumienna M, Szambelan K, Jeleń H, Czarnecki Z. Evaluation of ethanol fermentation parameters for bioethanol production from sugar beet pulp and juice. Journal of the Institute of Brewing 2014; 120(4):543-9.

Behera S, Mohanty RC, Ray RC. Ethanol fermentation of sugarcane molasses by Zymomonasmobilis MTCC 92 immobilized in Luffacylindrica L. sponge discs and Ca-alginate matrices. Brazilian Journal of Microbiology 2012;43(4):1499-1507.

Chandel AK, Chan ES, Rudravaram R, Narasu ML, Rao LV, Pogaku R. Economics and environmental impact of bio-ethanol production technologies: an appraisal. Biotechnology Molecular Biology Reviews 2007;2(1):14-32.

Winkelhausen E, Velickova E, Amartey SA, Kuzmanova S. Ethanol production using immobilized Saccharomyces cerevisiae in lyophilized cellulose gel. Applied Biochem istry and Biotechnology 2010;162(8):2214-20.

Datta S, Christena LR, Rajaram YRS. Enzyme immobilization: an overview on techniques and support materials. 3 Biotech 2013;3(1): 1-9.

Behera S, Kar S, Mohanty RC, Ray RC. Comparative study of bio-ethanol production from mahula (Madhucalatifolia L.) flowers by Saccharomyces cerevisiae cells immobilized in agar agarandCa-alginate matrices. Applied Energy 2010;87(1):96-100.

Alenina KA, Aleskerova LE, Kascheyeva PB, Ismailov, AD. The poly(vinyl alcohol) -immobilized photobacteria for toxicology monitoring, Engineering 2012;5(10B): 118-120.

Ghorbani F, Younesi H,Esmaeili Sari A, Najafpour G. Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by Saccharomyces cereviciae. Renewable Energy 2011;36(2):503-9.

Sinnaraprasat S, Fongsatitkul P. Optimal condition of Fenton's reagent to enhance the alcohol production from palm oil mill effluent (POME). EnvironmentAsia 2011;4(2):9-16.

Leenen EJTM, Santos VAPD, Grolle KCF, Tramper J, Wijffels RH. Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment, Water Research 1996;30 (12):2985-2996.

Tian X, Liao Q, Liu W, Wang YZ, Zhu X, Li J, Wang H. Photo-hydrogen production rate of a PVA-boric acid gel granule containing immobilized photosynthetic bacteria cells. International Journal of Hydrogen Energy 2009;34(11):4708-4717.

Qurishi, N. and Maddox, I.S. Continuous production of acetone-butanol-ethanol using immobilized cell of Clostridium acetobutylicum and intrigration with product removal by liquid-liquid extraction.Journal of fermentation and bioengineering 1995;80(2):185-189.

APHA. Standards Methods for examination of water and wastewater. 22th ed. APHA, WPCF, Washington DC, USA. 2012.

Stone KM, Roche FW, Thornhill NF. Dry weight measurement of microbial biomass and measurement variability analysis. Biotechnology Techniques 1992;6(3): 207-212.

Khongkhaem P. Utilization of Fenton's reaction and immobilization of Saccharomyces cerevisiae to produce ethanol from ethanol wastewater. M.Sc. Thesis, Department of Sanitary Engineering, Mahidol University, Bangkok, Thailand. 2014.

Pramanik S, Khan E. Effects of cell entrapment on growth rate and metabolic activity of pure cultures commonly found in biological wastewater treatment. Biochemical Engineering Journal 2009:46(3):286-93.

Najafpour G, Younesi H, Syahnida K, Ismail K. Ethanol fermentation in an immobilized cell reactor using Saccharomyces cerevisiae. Bioresource Technology 2004;92 (3):251-60.

Fujii N, Sakurai A, Onjoh K, Sakakibara M. Influence of surface characteristics of cellulose carriers on ethanol production by immobilized yeast cells. Process Biochemistry 1999;34(2):147-52.

Thuvander J. Continuous ethanol fermentation using membrane bioreactors (MBR). Master Thesis, Department of Chemical Engineering, Lund University, Lund, Sweden, 2012.

Rao, K., Chaudhari, V., Varanasi, S., and Kim, D. S. Enhanced ethanol fermentation of brewery wastewater using the geneti cally modified strain E. coli KO11. Applied Microbiology and Biotechnology 2007;74(1):50-60.

Ghorbani, F., Younesi, H., Sari, A. E., Najafpour, G. Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by Saccharomyces cerevisiae. Renewable Energy,2011; 36(2): 503-509.

Purwadi R, Taherzadeh MJ. The performance of serial bioreactors in rapid continuous production of ethanol from dilute-acid hydrolyzates using immobilized cells. Bioresource Technology 2008; 99(7): 2226-33.