EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/EA
EnvironmentAsia 4(2) (2011) 9-16

Optimal Condition of Fenton's Reagent to Enhance the Alcohol Production from Palm Oil Mill Effluent (POME)

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Abstract

Application of Fenton's reaction for a proper hydrolysis step is an essential and important step in obtaining a higher level of readily biodegradable sugars from palm oil mill effluent (POME) for improving the alcohol production by using immobilized *Clostridium acetobutylicum*. The objective of this research was, therefore, to investigate the optimum condition of Fenton's reaction in terms of COD: H_2O_2 ratios (w/w) and H_2O_2: Fe^{2+} ratios (molar ratio) used to oxidize carbohydrate and high molecular organic compounds into simple sugars, which are further fermented into alcohol. The experiments were carried out at H_2O_2: Fe^{2+} ratios (molar ratios) of 5, 10, 20, 30 and 40 and the COD: H_2O_2 ratios (w/w) of 50, 70, 100 and 130 (initial COD about 50,000 mg/L). The total sugar concentrations and organic compounds biodegradability (BOD_5/COD ratios) were also used for investigating suitable conditions for Fenton's reaction. The concentration of Fenton's reagent at H_2O_2:Fe^{2+} and COD:H_2O_2 ratio of 20 and 130 was identified as the optimum operating condition for the highest simple sugars of about 0.865% and BOD_5/COD ratios of 0.539. The alcohol productions were carried out in the continuous stirred tank reactors (CSTR) under an anaerobic continuous immobilization system. At a hydraulic retention time of 12 hours and POME pH of 4.8, the maximum total ABE concentration of 495 mg/L and the ABE yield of 0.236 grams of ABE produced/gram of reducing sugars were achieved at the mixed polyvinyl alcohol (PVA) and palm oil ash (POA) ratio of 10 : 3.

Keywords: palm oil mill effluent (POME); Fenton's reaction; acetone; butanol and ethanol production; biodegradability; color removal

1. Introduction

The palm oil industry has played an important role in Thailand's economy. In 2007, there were 36 wet-process crude palm oil factories in Thailand and the production of palm oil was estimated to be 6,390,000 tons and the planting area for the whole country was about 2,663,000 rai (OAE, 2007). The Thai Government aims to increase palm oil production in order to supply the biofuel industry because of a sharp increase in global oil prices, which has forced the concerned agencies to look for the alternative energy sources. In crude palm oil processing, a large quantity of water is required for the sterilization and clarification processes, resulting in a huge amount of palm oil mill effluent (POME) being released into the environment. POME is a thick, brownish liquid with a discharge, a temperature in the range of 80 to 90°C and consists of various suspended components including cell walls, organelles, short fibers, a spectrum of carbohydrates, ranging from hemicelluloses to simple sugar, a wide range of nitrogenous compounds from proteins to amino acids and free organic acids (Ugoji, 1997). POME contains useful materials used as biomass for bio-energy conversion via fermentation; it is used as a substrate to produce biofuel such as acetone, butanol and ethanol (ABE) by using solventogenic clostridia (Mun *et al.*, 1995). This is an alternative way to utilize waste from agricultural industry as substrate for renewable energy production since fermentation substrate is an important factor influencing the cost of alcohol production.

There are two forms of carbohydrates, insoluble and soluble carbohydrates, contained in POME. Ho and Tan (1983) did studies on the particulates of palm oil mill effluent and reported that the pentose found in POME was a building unit of insoluble carbohydrate. Water-soluble carbohydrates, in terms of glucose, reducing sugars and pectin, were also found in the soluble fraction of POME. However, the low concentrations of total soluble carbohydrate (0.390 g/100 ml POME) would restrict the usefulness of the soluble fraction of POME as a possible feedstock for substrate conversion via direct single-cell protein production (Ho *et al.*, 1984). The high molecular organic compounds such as cellulose, hemicellulose, starch etc. contained in POME were the least biodegradable of the substrate constituents. It is, therefore, necessary to hydrolyze palm oil mill effluent prior to processing it for ABE production by adding chemical and/or using advanced oxidation processes (AOPs) that utilize H_2O_2, O_3 or O_2 as the oxidant. These are very promising techniques for destruction of non-biodegradable organic compounds to biodegradable organic compounds and also for breaking down of high molecular organic compounds (Ghoreishi *et al*., 2007). Fenton's reaction is one of the most effective methods for the oxidation of organic pollutants.

The process of Fenton's reaction relies on the fact that iron-catalyzed H_2O_2 decomposition leads to the formation of hydroxyl radicals ($OH^{\bullet}$) that are capable of oxidizing many organic pollutants and converting them to lower molecular weight compounds and eventually to carbon dioxide and water (Ahmadi *et al*., 2008). The Fenton's reaction can be used to partially oxidize recalcitrant organics and convert them into more biodegradable compounds that could be easily utilized by bacteria for alcohol production. From the previous studies, Nadarajah *et al*. (2002) found that the Fenton's reagent at a concentration of 0.5% H_2O_2 and 10 mM Fe^{2+} (molar ratio, 15:1) was most effective in transforming anthracene (polycyclic aromatic hydrocarbons) at pH 4. In addition, Elmolla and Chaudhuri (2009) studied the effect of the photo Fenton process on biodegradability improvement of antibiotics wastewater. They found that the maximum BOD5/COD ratio was achieved at H_2O_2/COD (molar ratio) of 2:1, H_2O_2/Fe^{2+} (molar ratio) of 50:1.

This research aims to investigate the optimum operating condition of Fenton's reaction in terms of COD: H_2O_2 ratio (w/w) and H_2O_2: Fe^{2+} ratio (molar ratio) to increase the water-soluble carbohydrate which is the suitable substrate for alcohol production and also to improve organic compounds' biodegradability.

2. Materials and Methods

2.1. Palm oil mill effluent (POME)

The original POME was obtained from under the flow line of oil and grease traps of the palm oil industry, Chonburi, Thailand. The POME collected was stored at 4°C until further use. The characteristics of POME used in this study are presented in Table 1.

2.2. Experimental procedure for Fenton's reaction

This experiment was scheduled to investigate the influence of the COD: H_2O_2 ratio (w/w) and H_2O_2: Fe^{2+} ratio (molar ratio) on the efficiency of Fenton's reagent to produce water-soluble carbohydrates. The experiments were carried out using 1,000 ml beakers with a POME volume of 500 ml at the initial COD concentration of POME of about 50,000 mg/L. The pH was used at the natural actual pH of POME of about 4.5-4.8. Fenton reactions started with an addition of iron catalyst (as a solution of $FeSO_4.7H_2O$), and the H_2O_2 added slowly. The POME was continuously mixed (100 rpm) in a jar test apparatus at room temperature. After 2 hours, samples were then taken from the mixed solution and analyzed for Chemical Oxygen Demand (COD), 5-day Biological Oxygen Demand (BOD_5), color and total sugar concentrations. Using Fenton's reagent as an oxidant, the organic compounds in POME could be transformed to any kind of simple sugar, moreover, high molecular weight compounds could be converted to lower molecular weight compounds or non-biodegradable compounds converted to biodegradable organic compounds. However, it was necessary to use Fenton's reagent as little as possible to ensure no or less interference with the metabolism of microorganisms. The experiments were carried out at H_2O_2: Fe^{2+} ratio (molar ratios) of 5, 10, 20, 30 and 40 and the COD: H_2O_2 ratio (w/w) of 50, 70, 100 and 130 as described in Table 2. The required amount of H_2O_2 was then determined according to the initial COD (COD_0). The total sugar concentration and organic biodegradability (BOD_5/COD ratio) were used for investigating the optimum condition of Fenton's reaction.

Table 1. Chemical composition of palm oil mill effluent (POME)

Parameters	Concentration (g/L)	
	Range	Mean±S.D.
Fiber	0.70-2.30	1.50±0.8
Total sugar	1.50-3.00	2.29±0.67
Carbohydrate	28.10-30.30	29.20±1.1
Protein	4.20-6.00	5.10±0.9
Ash	6.50-9.70	8.10±1.6
Fat	6.00-7.20	6.60±0.6
COD	60.00-90.00	73.00±12.17
BOD	20.00-37.50	28,785±5.47
Suspended solid	18.50-25.00	21.21±2.31

2.3. Experimental procedure for alcohol production

The POME obtained from the optimum condition of Fenton's reaction was used as a substrate for alcohol production (acetone, butanol, ethanol (ABE)) by using immobilized *Clostridium acetobutylicum* TISTR 1462 (DSM 4685). The bacterium was purchased from the Thailand Institute of Scientific and Technological Research (TISTR) and it was cultivated in anaerobic conditions in a Reinforced Clostridial Medium (RCM) for 48 h at 37°C. Liquid medium of RCM was used

Table 2. The experimental conditions of Fenton's reaction

Group	Experimental conditions	$H_2O_2:Fe^{2+}$	$COD:H_2O_2$
1	1	5	50
	2	5	70
	3	5	100
	4	5	130
2	5	10	50
	6	10	70
	7	10	100
	8	10	130
3	9	20	50
	10	20	70
	11	20	100
	12	20	130
4	13	30	50
	14	30	70
	15	30	100
	16	30	130
5	17	40	50
	18	40	70
	19	40	100
	20	40	130

for inoculum in anaerobic conditions for 48 h at 37°C. The immobilized *Clostridium acetobutylicum* TISTR 1462 was prepared by using polyvinyl alcohol (PVA) mixed with palm oil ash (POA) under the PVA-boric acid method at the PVA (%w/v) and POA (%w/v) ratios of about 10:3, 12.5:3 and 15:3, respectively. The 20, 25 and 30 grams of PVA and 2% (w/v) sodium alginate were dissolved in 140 ml of distilled water at a temperature of 60ºC. The solution was cooled down to room temperature and mixed with 60 ml (30% v/v) of concentrated *C. acetobutylicum* TISTR 1462 and then 6 grams (3% w/v) of palm oil ash was gently added. The mixtures were dropped into saturated boric acid and $CaCl_2$ (2% w/v) solution and kept for 24 hours at 4ºC to form spherical beads. The beads were washed with deionized water several times and then stored at 4ºC until further use. The alcohol productions have been carried out in the continuous stirred tank reactors (CSTR) having a working volume of 2 liters in an anaerobic continuous operation system. The immobilized cell beads of about 200 ml (10% v/v) were inoculated in a bioreactor and acclimatized for 2-3 days in order to recover the activities after the cell was immobilized. Once solventogenesis occurred, the raw POME was continuously fed via a peristaltic pump. Determination of the steady state condition of the system was made by checking the stability of alcohol production.

2.4. Analytical procedures

The samples were analyzed for Chemical Oxygen Demand (COD), 5-day Biological Oxygen Demand (BOD_5) following the methods as described in the Standard Methods for the Examination of Water and Wastewater 21st edition (APHA, 2005). The total sugar concentrations 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 being injected into the HPLC, the samples were prepared by a centrifuge and filtered through the GF-C filter membrane and then appropriate aliquots were taken from the filtrate into 0.2 µm cellulose acetate membrane. The concentrations of ABE were 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 220ºC and 270°C, respectively. Helium was used as a carrier gas with a flow rate of 1ml/min. 1 µl of injection volume and split sample ratio of 30:1 were used. The color was determined by the American Dye Manufacturers Institute (ADMI) method, the light scan absorbance from 400 to 700 nm using Shimadzu UV-160A, UV-visible recording spectrophotometer.

Table 3. The COD concentrations vs. reaction time at different $COD:H_2O_2$ ratios

$H_2O_2:Fe^{2+}$ ratio	$COD:H_2O_2$ ratio	Mean ±S.D. of COD concentrations (g/L)				
		influent	30min	60min	90min	120min
20	10	53.00±0.5	44.80±0.8	44.00±1.0	43.20±0.2	45.60±0.6
20	30	53.00±1.0	44.00±0.5	45.60±0.6	48.80±1.0	46.40±0.9
20	50	53.00±1.0	51.60±0.6	52.00±1.0	51.20±0.3	51.40±0.4
20	70	53.76±0.76	52.72±0.72	52.40±0.6	53.68±0.68	53.56±0.66

3. Results and Discussion

This research was conducted to determine the optimum condition of Fenton's reaction in terms of COD: H_2O_2 ratio (w/w) and H_2O_2: Fe^{2+} ratio (molar ratio) to increase the water-soluble carbohydrate, especially total sugar, which is a suitable substrate for alcohol production and also to improve the biodegradability of organic compounds. Moreover, during this process, the color of POME was also reduced. Therefore, the parameters of COD and total sugar concentrations, BOD_5/COD ratios and color removal efficiency were measured so as to evaluate the optimum condition of Fenton's reaction. In addition, the alcohol production from POME was also investigated.

3.1. COD concentration

COD concentration is the prime indicator used for determining the range of H_2O_2 and Fe^{2+} concentrations to design the experimental conditions to optimize the operating conditions of Fenton's reaction. Based on the initial experiment, at constant ratio of COD:H_2O_2 equal to 10, the oxidation reactions with Fe^{2+} as a catalyst were observed, using the H_2O_2:Fe^{2+} ratio of 5 and 20. It was found that at a low H_2O_2:Fe^{2+} ratio, the COD concentration decreased more than at a high H_2O_2:Fe^{2+} ratio. The results were nearly the same as those of El-Gohary *et al*., (2008), with a slight improvement in the quality of the wastewater produced by increasing the dose of Fe^{2+}. However, the objective of this study was to use Fenton's reagent to hydrolyze high molecular organic compounds into smaller organic compounds or biodegradable compounds not to fully convert them to CO_2 and water. Therefore, the higher ratio of H_2O_2:Fe^{2+} was used in this study.

The experiments were further performed with the H_2O_2:Fe^{2+} ratio of 20 and the COD:H_2O_2 ratios of 10, 30, 50 and 70. The results of COD concentrations are presented in Table 3. It indicates that if high a COD:H_2O_2 ratio is used, the COD concentrations would not decrease, whereas if a lower COD:H_2O_2 ratio is used, the COD concentrations would decrease. The COD concentrations at high COD:H_2O_2 ratios (50 and 70) did not change too much as only a small amount of H_2O_2 reacted with the organic compounds. Therefore,

Table 4. The mean value of any kind of sugar and total sugar concentrations at different H_2O_2:Fe^{2+} and COD:H_2O_2 ratios

H_2O_2:Fe^{2+} ratio	COD:H_2O_2 ratio	Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)	Total sugar (%)
Raw POME		-	0.167	-	-	0.167
5	50	0.09	0.094	0.082	-	0.266
5	70	0.115	0.125	0.101	-	0.341
5	100	0.184	0.217	0.146	-	0.547
5	130	0.223	0.252	0.154	0.096	0.725
10	50	0.196	0.227	0.191	0.087	0.701
10	70	0.237	0.259	0.145	0.092	0.733
10	100	0.249	0.283	0.147	0.096	0.775
10	130	0.233	0.285	0.208	0.105	0.831
20	50	0.22	0.266	0.199	0.1	0.785
20	70	0.261	0.308	0.151	0.098	0.818
20	100	0.22	0.264	0.197	0.1	0.781
20	130	0.244	0.297	0.214	0.11	0.865
30	50	0.217	0.254	0.141	0.074	0.686
30	70	0.22	0.257	0.145	0.084	0.706
30	100	0.251	0.297	0.156	0.073	0.777
30	130	0.228	0.274	0.204	0.103	0.809
40	50	0.245	0.288	0.18	0.077	0.79
40	70	0.22	0.254	0.166	-	0.64
40	100	0.241	0.282	0.178	0.072	0.773
40	130	0.248	0.292	0.183	-	0.723

Remark: The S.D. of each value was less than 0.05

the high COD:H_2O_2 ratios were carried out in further experiments to investigate the optimum condition of Fenton's reaction.

3.2. Total sugar concentrations

Total sugar concentrations including fructose, glucose, galactose, sucrose, maltose and lactose were analyzed in order to observe the depolymerization of the carbohydrate polymer (cellulose, hemicelluloses and starch) into simple sugar using Fenton's reagent. The results showed that the total sugar concentrations were slightly increased by increasing the H_2O_2:Fe^{2+} ratio from 5, 10, 20, 30 and 40 and COD:H_2O_2 ratio from 50,70, 100 and 130 as shown in Table 4. This indicated that if an adequate Fenton's reagent concentration was used, the organic compounds, especially carbohydrates, would be oxidized to any kind of sugar. Whereas, if too high a H_2O_2 concentration was used,, most organic compounds would be oxidized to CO_2.

The purpose of using Fenton's reagent in this study was to oxidize carbohydrate and high molecular organic compounds to any kind of simple sugar (glucose, fructose, maltose, etc.). Previous work has shown that typical H_2O_2:Fe^{2+} from 2:1 to 300:1 have been used for the transformation of various hydrocarbons. Nonetheless, in order to avoid the high chemical doses required for complete oxidation, only partial oxidation was desired to provide more soluble organic compounds (Nadarajah *et al*., 2002). Therefore, less Fenton's reagent should be used but enough to sufficiently oxidize carbohydrates into simple sugar; however, the amount of Fenton's reagent used should not be in excess to the point that it oxidizes simple sugar to CO_2. Hence, the condition of Fenton's reaction at H_2O_2:Fe^{2+} ratio of 20 and COD:H_2O_2 ratio of 130 that obtained the highest total sugar about 0.865% was selected for use in further experiments in ABE production.

3.3. BOD_5/COD ratios (biodegradability)

The BOD_5/COD ratio is one of the indicators to determine the optimum condition of Fenton's reaction since the BOD_5/COD ratio constituted an appropriate measure of the biodegradability of treated wastewater (Chamarro *et al*., 2001). In this research, biodegradability of the treated POME was slightly increased from 0.40 to 0.539 as shown in Table 5. The high BOD_5/COD ratios were obtained from the H_2O_2:Fe^{2+} ratio of 20 at all COD:H_2O_2 ratios and the highest of BOD_5/COD ratio was achieved at H_2O_2:Fe^{2+} and COD:H_2O_2 ratio of 20 and 130, respectively. The increase in BOD_5/COD ratio

Table 5. The BOD_5/COD ratios and total sugar concentrations at different H_2O_2:Fe^{2+} and COD:H_2O_2 ratios

H_2O_2:Fe^{2+} ratio	COD:H_2O_2 ratio	BOD5/COD ratios	Total sugar (%)
Raw POME		0.400	0.167
5	50	0.397	0.266
5	70	0.397	0.341
5	100	0.418	0.547
5	130	0.450	0.725
10	50	0.459	0.701
10	70	0.464	0.733
10	100	0.467	0.775
10	130	0.472	0.831
20	50	0.530	0.785
20	70	0.530	0.818
20	100	0.534	0.781
20	130	0.539	0.865
30	50	0.494	0.686
30	70	0.498	0.706
30	100	0.472	0.777
30	130	0.507	0.809
40	50	0.502	0.790
40	70	0.495	0.640
40	100	0.495	0.773
40	130	0.494	0.723

observed after oxidation with the lower doses of H_2O_2 using Fenton's reagent lead to wastewater containing organics that were considerably more biodegradable than those contained in raw wastewater (Barbusinski and Filipek, 2001). These results were similar to those of Ahmadi *et al*., (2008); the increase in biodegradability was more significant with the lower initial concentration of H_2O_2. Moreover, Mater *et al*. (2007) indicated that low reagent concentrations (1% H_2O_2 and 1 mM Fe^{2+}) were sufficient to start the degradation process, which could be continued using microorganisms. An excess of H_2O_2 appeared to cause a detrimental effect on the microorganism (Oliva *et al*., 2005). To ensure the extendable conversion of residue organic compounds to carbon dioxide, the amount of hydrogen peroxide present in the system must be determined. From the hydrogen peroxide determination, the results showed that there were no hydrogen peroxide residues at high COD:H_2O_2 ratios (i.e., low hydrogen peroxide concentration). Fe used in Fenton's reaction was used as a catalyst, and it is a widely available and nontoxic element (Zazo *et al*., 2005). Moreover, it can only accelerate the oxidation rate. A small amount of Fe was used in this study, so the residual Fe should not affect the system operating system. The increase in BOD_5/COD ratios indicated the opportunity of the Fenton's reagent to breakdown the complicated compounds (higher molecular organic compounds) and convert them into simple biodegradable forms (glucose, fructose, maltose, etc.).

Moreover, the dark brown color of palm oil mill effluent consisted of many organic compounds such as anthrocyanin and carotene pigment that was extracted from fresh fruit bunches in the sterilization process. Moreover, it included polyphenol compounds, tannin, polyalcohol and melanoidin. The wastewater from the sterilization process contained pectin and polyphenol of about 5.7 and 2.0 g/L, respectively (Hwang *et al*., 1978). In Fenton's reaction, the removal of color may have occurred as well. Nevertheless, the dark brown color in POME cannot be degraded during only the anaerobic condition. The Fenton's reaction is a very promising technique for the destruction of non-biodegradable chromophore to biodegradable organic compounds and breakdown of high molecular organic compounds (Ghoreishi *et al*., 2007).

As shown in Fig. 1, the results showed that almost all of the color unit of the wastewater, after reacted with Fenton's reagent, reduced except at the ratio of H_2O_2:Fe^{2+} and COD:H_2O_2 equal to 5 and 130. At this ratio, the color unit was higher than that of the raw wastewater. Consequently, this could be described by Mijangos' (2006) study of changes in solution color during phenol oxidation by Fenton's reagent, that during the first steps of the reaction, phenol was degraded to dihydroxylated rings (catechol, resorcinol, and hydroquinone). These aromatic intermediates generated higher colored compounds such as ortho- and para-benzoquinone. On the other hand, the dihydroxylated rings could react with their own quinines to generate charge transfer complexes (quinhydrone), compounds which took on a dark color at low concentrations (Mijangos *et al*., 2006). Under Fenton's reaction, the ferrous ions reacted with hydrogen peroxide initially generating a large amount of hydroxyl radicals that gave rise to fast destruction of the aromatic rings. However, the color units were slightly reduced after they reacted with these Fenton's reagent concentrations. This was most likely due to using low H_2O_2 and Fe^{2+} concentrations.

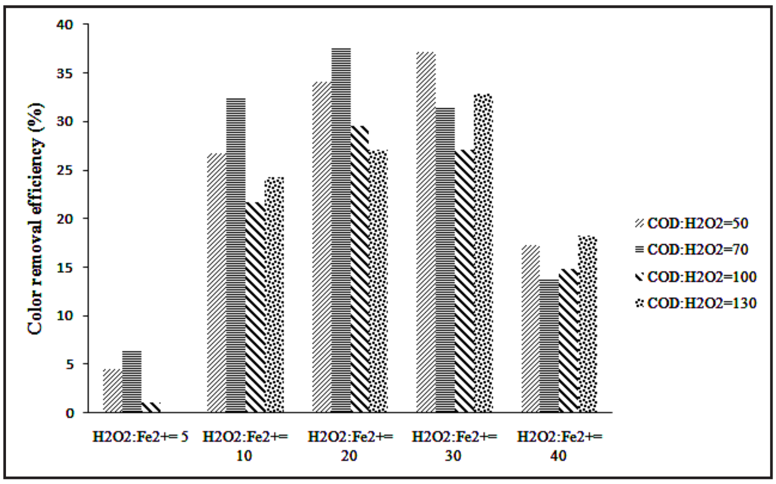

Figure 1. The mean value of color removal efficiency after reacted with Fenton's reagent at different H_2O_2:Fe^{2+} and COD:H_2O_2 ratios

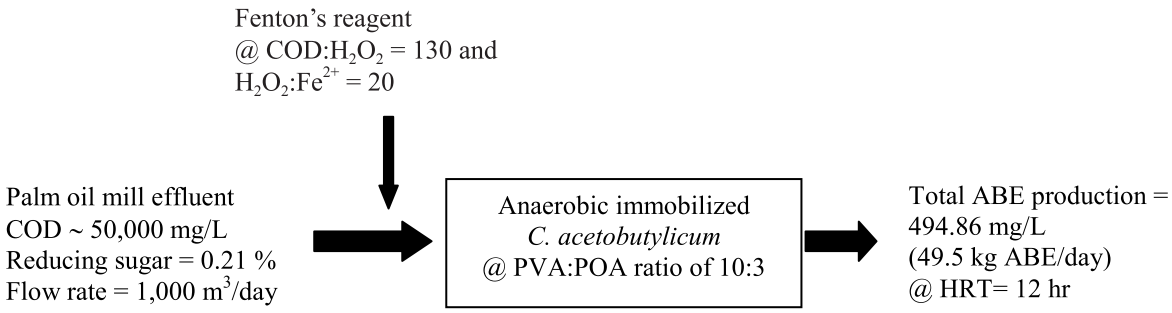

Figure 2. The process summary of ABE production from palm oil mill effluent

3.5. Alcohol production

The POME obtained from the optimum condition of Fenton's reaction was used as a substrate for alcohol production by using immobilized *Clostridium acetobutylicum* TISTR 1462 (DSM 4685). As illustrated in Fig. 2, the results showed that at hydraulic retention time of 12 hours and a POME pH of 4.8, the maximum total ABE concentrations at PVA and POA ratios of about 10.0:3, 12.5:3 and 15.0:3 were 494.86, 445.96 and 417.36 mg/L or equivalent to 49.5, 44.5 and 41.7 kg ABE/day (with the plant flow rate of about 1,000 m^3/day), respectively. The ABE productivities were 0.041, 0.037 and 0.034 g/L-hr and the ABE yields (grams of ABE produced per grams of total sugar) were 0.236, 0.212 and 0.198, respectively. The ABE yields in this study were similar to the yields in studies of solvent fermentation from palm oil mill effluent using *C. acebutylicum* in an oscillatory flow bioreactor by Takriff *et al*. (2009). They found that the ABE yields when using oscillation frequencies (Hz) of 0, 0.45 and 0.78 were 0.125, 0.163 and 0.454, respectively. The results of this research indicated that raw POME is a viable substrate for alcohol production because it consists mainly of a mixture of carbohydrate and sufficient nutrients that can be utilized by *Clostridium acetobutylicum* in alcohol fermentation. These research results should be applied in the actual palm oil industry (possible pilot scale level), leading to the 3-E concept: Environmentally (waste utilization), Economically (returned benefit of alcohol production), and finally Engineering sound (technically viable immobilization and fermentation techniques).

Acknowledgment

This research work was fully supported by the grant from the Office of the Higher Education Commission (Strategic Scholarships Frontier Research Network), Thailand. The authors would also like to thank Eric Curkendall from the Mahidol University Office of International and Public Relations for his help editing this article.

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Received 30 January 2011
Accepted 16 April 2011

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