Polyhydroxyalkanoate production from palm oil factory wastes and its application for 3-hydroxyalkanoate methyl esters as biofuels

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Abstract:
Polyhydroxyalkanoate (PHA) synthesized and stored in the various microorganism cell cytoplasms. In this study, the palm oil factory wastes; liquid after squeezing the empty fruit branch (LEFB), palm oil mill effluent (POME) and biogas tank effluent (BTE), was used as substrate for PHA production. The result showed that POME gave the highest dry cell weight (DCW) of 2.97 g/L and PHA production of 2.27 g/L. Then POME was selected for the large amount of PHA production in 5 L aeration reactor and then acid hydrolysis for 3-Hydroxyalkanoate methyl esters (3HAME) production. The 44.4% of 3HAME content was obtained. The properties of 3HAME has met the regulation standard of ASTM D6751-07 with a heating value, methyl ester content, flash point and pour point of 32.9 KJ/g, 96.8%, 132°C and 7°C, respectively. Therefore, the 3HAME from extracted PHA using POME as a sole carbon source has a feasibility to be an alternative biodiesel fuel.

Keywords: *3-hydroxyalkanoate methyl esters; 3HAME; polyhydroxyalkanoate; PHA; PHA accumulating bacteria; biodiesel*

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1. Introduction

Now a day, a world fossil fuel will be running out in a few years. So, the alternative renewable fuel such biodiesel will be needed. Biodiesel is normally made from vegetable oils, animal fats and many other sources. However, the use of food crops for biodiesel and other renewable fuels may be an uneconomical long term solution (Patzek and Pimentel, 2005). Polyhydroxyalkanoate (PHA) has attracted much attention in recent years due to their varied properties (thermoplastic and elastomeric), biocompatibility and biodegradability (Keshavarz and Roy, 2010). Moreover, PHA can be also applied in the biodiesel production via acid-catalyzed hydrolysis (Zhang et al., 2009). However, the major obstacle to wide acceptance of PHA is their high cost, which is 10 times higher than those of synthetic plastics due to the high cost of raw materials and product recovery. Therefore, the cheap substrate from the waste of palm oil factory was used for PHA production in this study. Furthermore, the produced PHA was then used for 3-Hydroxyalkanoate methyl esters (3HAME) production and the feasibility of 3HAME as a biodiesel fuel was investigated.

2. Materials and Methods

2.1 Substrate selection for PHA production

An inexpensive substrate from palm oil industry such as liquid after squeezing the empty fruit branch (LEFB), palm oil mill effluent (POME) and biogas tank effluent (BTE) was considered as substrate for PHA production using PHA accumulating bacteria. The characteristics of the inexpensive substrate, including pH, chemical oxygen demanded (COD) (Open reflux method), were determined using procedures outline in Standard Methods (APHA, 1995). Moreover, the determination of total carbon and nitrogen by CHN analyzer, total phosphorus by inductively couple plasma-optical emission spectrometry (ICP-OES) and Volatile fatty acid (VFA) and long chain fatty acid (LCFA) by gas chromatograph with flame ionization detector (GC-FID) was analyzed (Table 1). The experiment was conducted in 250 mL flask under aerobic condition. The isolated PHA accumulating bacteria from POME (Junpadit and Boonsawang, 2011) was grown in nutrient rich (NR) medium for inoculum preparation. The 10% of inoculum with $OD_{660\ nm}$ of 0.5 were transferred to the inexpensive substrate. PHA production was carried out at pH and agitation rate of 7 and 180 rpm, respectively, and at ambient temperature for 42 hr. After 42 hr, the samples were taken for DCW, PHA, PHA content and monomer composition of 3-hydroxyalkanoate (3HA) analysis.

Table 1 The characteristics of palm oil factory wastes as a carbon sources.

Parameters	Values		
	LEFB	POME	BTE
Color	dark yellow	dark brown	light brown
pH	4.88	4.10	8.10
COD (g/L)	154.12	67.09	4.74
Total carbon (g/L)	-[a]	19.25	-[a]
Total nitrogen (g/L)	2.26	1.13	0.41
Total phosphorus (g/L)	0.26	0.22	0.02
Volatile fatty acid (g/L)	-[a]	4.35	1.39
Long chain fatty acid (g/L)	-[a]	0.89	0.26

Note: [a] not detect

2.2 PHA production in 5 L aeration bioreactor

The selected inexpensive substrate was then used for PHA production in a 5 L aeration bioreactor (4 L working volume) for further biodiesel production. The 10% of the PHA accumulating bacteria grown in NR medium ($OD_{660\ nm}$ of 0.5) were transferred to the selected inexpensive substrate. PHA production was carried out at pH, agitation rate, C/N ratio, aeration rate and phosphorus addition of 7, 180 rpm, 10, 1 vvm and 0.1 g/L, respectively, and at ambient temperature for 42 hr. For the C/N ratio and the phosphorus addition were adjusted using $(NH_4)_2SO_4$ and KH_2PO_4, respectively. After 42 hr, the samples were also taken for DCW, PHA, PHA content and monomer composition of 3HA analysis.

2.3 3HAME production from PHA

3HAME was produced via an acid hydrolysis of PHA according to Zhang et al. (2009). The properties of 3HAME was determined according to the American Society of Testing and Materials (ASTM) standard (D6751-07) (ASTM International, 2008) and compared with the biodiesel standards of Thailand’s regulations and ASTM D6751-07.

3. Results and Discussion

3.1 Substrate selection for PHA production

LEFB, POME and BTE as substrate for PHA production were investigated. POME gave the highest dry cell weight (DCW) and PHA of 2.97 and 2.27 g/L, respectively. It might be due to the high fatty acid content (VFA and LCFA) in POME. Fatty acid was degraded by β-oxidation, which is the main metabolic route for the PHA synthesis. In addition, POME gave the highest PHA monomer composition containing the 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxy-hexanoate (3HHx), and 3-hydroxydecanoate (3HD) of 534, 552, 488 and 41 mg/L, respectively (Table 2). Therefore, POME was selected for further experiment.

3.2 PHA production in a 5 L aeration bioreactor

The large amount of PHAs was produced in a 5 L aeration bioreactor using POME as a sole carbon source for further biodiesel production. DCW and PHA of 3.20 and 2.09 g/L, respectively, were obtained with 3HB, 3HV, 3HHx and 3HD of 174, 277, 208 and 17.5 mg/L, respectively (Table 3). According to the different values of the PHA monomer content from Section 3.1, it was due to the variation of POME characteristics collected from the different sampling period time.

Table 2 Content and composition of PHA from the isolated PHA accumulating bacteria at 42 hr

Carbon sources	DCW (g/L)	PHA (g/L)	PHA content % of DCW	Monomer composition of 3HA (mg/L)				
				3HB (C_4)	3HV (C_5)	3HHx (C_6)	3HO (C_8)	3HD (C_{10})
LEFB	0.96	0.34	35.42	75.54	118.85	147.93	n/a	04.22
POME	2.97	2.27	76.43	534.24	551.57	488.06	n/a	40.97
BTE	1.74	1.09	62.64	154.44	145.37	240.18	n/a	10.55

Note: n/a; not available

Table 3 Content and composition of PHA from the isolated PHA accumulating bacteria in 5 L bioreactor using POME as a sole carbon sources

DCW (g/L)	PHA (g/L)	PHA content % of DCW	Monomer composition of 3HA (mg/L)				
			3HB (C_4)	3HV (C_5)	3HHx (C_6)	3HO (C_8)	3HD (C_{10})
3.20	2.09	65.31	173.94	276.93	208.04	n/a	17.50

Note: n/a; not available

3.3 Technical feasibility of 3HAME production from PHA

The PHA was acid hydrolyzed for 3HAME production using H_2SO_4. 3HAME content of 44.4% was then achieved. The obtained 3HAME has met the regulation standard of ASTM D6751-07 with a heating value, methyl ester content, flash point and pour point of 32.9 KJ/g, 96.8%, 132°C and 7°C, respectively (Table 4).

Table 4 Some important fuel properties of 3HAME in this study compared with the biodiesel standards of Thailand's regulations and ASTM D6751-07

Property	Test method	Thailand's regulations[a]	ASTM D6751-07	3HAME in this study
Methyl ester content (%)	GC-FID	≥ 96.5	-[b]	96.8
Heating value (kJ/g)	Calorific method	-[b]	31.5	32.9
Viscosity at 40°C (mm^2/s)	ASTM-D445	3.5 to 5.0	1.9 to 6.0	05.3
Flash point (°C)	ASTM-D930	≥ 120	130 to 170	132
Pour point (°C)	ASTM-D970	-[b]	-15 to 10	007

Note: [a] standard specification for biodiesel fuel 2014 from Department of Energy Business, Ministry of Energy, Thailand
[b] not defined

4. Conclusion

PHA production using POME as a sole carbon source gave the highest DCW and PHA of 3.20 and 2.09 g/L, respectively. The 3HAME content of 44.4% was obtained from the acid hydrolysis of PHA produced from POME. The extracted 3HAME from PHA has the properties following ASTM standard and has a feasibility to be used as an alternative biodiesel fuel.

5. References

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