เชื้อเพลิงชีวภาพจากพอลีไฮดรอกซีอัลคาโนเอต

Biofuel from Polyhydroxyalkanoates (PHAs)

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บทคัดย่อ

เชื้อเพลิงฟอสฟิลมีปริมาณที่จำกัดและเมื่อเผาไหม้จะส่งผลกระทบต่อสิ่งแวดล้อม ทำให้เกิดแรงกระตุ้นในการค้นหาเชื้อเพลิงชีวภาพทดแทน แบคทีเรียผลิตพอลีไฮดรอกซีอัลคาโนเอต (PHAs) ถูกเสนอเป็นเชื้อเพลิงชีวภาพทางเลือก ที่เรียกว่า ไฮดรอกซีอัลคาโนเอตเมทิลเอสเทอร์ (HAME) และไฮดรอกซีบิวทิเรตเมทิลเอสเทอร์ (HBME) ซึ่งมีคุณสมบัติใกล้เคียงกับไบโอดีเซล นอกจากนี้ HAME และ HBME เป็นมิตรต่อสิ่งแวดล้อม มีปริมาณออกซิเจนสูง แต่ไม่มีไนโตรเจนและกำมะถัน ซึ่งตรงกันข้ามกับปิโตรเลียมที่มีปริมาณออกซิเจนต่ำ แต่มีไนโตรเจนและกำมะถันสูง เมื่อเกิดการเผาไหม้อาจนำไปสู่มลภาวะต่อสิ่งแวดล้อม PHAs เป็นพอลีเอสเตอร์ที่ผลิตขึ้นตามธรรมชาติโดยแบคทีเรียหลายชนิด สามารถผลิตโดยแหล่งคาร์บอนหมุนเวียน ดังนั้นการพัฒนา HAME และ HBME เป็นเชื้อเพลิงอย่างยั่งยืนหรือเป็นสารเพิ่มคุณภาพในน้ำมันเชื้อเพลิง อาจนำไปสู่ความหลากหลายของตลาดเชื้อเพลิงชีวภาพหรือสารเติมแต่งในอนาคต บทความนี้จึงได้รวบรวมวิธีการผลิต คุณสมบัติต่าง ๆ ของเชื้อเพลิงชีวภาพจาก PHAs งานวิจัยที่เกี่ยวข้อง และข้อเสนอแนะเกี่ยวกับเชื้อเพลิงชีวภาพจาก PHAs ในอนาคต

คำสำคัญ: พอลิไฮดรอกซีอัลคาโนเอต (PHAs) เชื้อเพลิงชีวภาพ ไฮดรอกซีอัลคาโนเอตเมทิลเอสเทอร์ (HAME) ไฮดรอกซีบิวทิเรตเมทิลเอสเทอร์ (HBME)

Abstract

The limited reserves and negative environmental consequences of fossil fuel have spurred the search for renewable biofuels. Polyhydroxyalkanoates (PHAs) producing-bacteria was proposed as an alternative biofuel, termed as hydroxyalkanoate methyl ester (HAME) and hydroxybutyrate methyl ester (HBME) which are similar to biodiesel. In addition, HAME and HBME are environmental friendly fuel, have high oxygen content without nitrogen and sulfur. In contrast, petroleum contains very low oxygen high nitrogen and sulfur contents, which can lead to environmental pollutions when burning. PHAs are polyesters produced in nature by numerous bacterial, including through bacterial fermentation of renewable carbon sources. The utilization of PHAs as substrate for biofuel seems reasonable at first glance. Consequently, the development of HAME and HBME as sustainable fuel or fuel additives may contribute to the diversification of biofuel or fuel additive market. In this article, production, properties and research of biofuel from PHAs were reviewed. In addition, future work on biofuel from PHAs was also discussed.

Keywords: Polyhydroxylakanoates (PHAs), Biofuel, Hydroxyalkanoate Methyl Ester (HAME), Hydroxybutyrate Methyl Ester (HBME)

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Introduction

Polyhydroxyalkanoates (PHAs) are bio-polyesters synthesized by bacteria as intracellular storage reserves of carbon and energy [1]. PHAs have recently attracted great deal of industrial attention as promising biomaterials due to their excellent biodegradability, biocompatibility and capability of being produced from renewable resources. Microbial PHAs were proposed for the first time as a new type of biofuel, termed hydroxyalkanoate methyl ester (HAME) and hydroxybutyrate methyl ester (HBME) [2]. The PHAs based biofuel could provide a strong candidate for current biofuels or fuel additives market. When considering the production process, chemical structures and role as energy carrier, it is easily found that HAME and HBME are similar to those of biofuels, particularly similar to biodiesel that is methyl esters of long-chain fatty acids. HAME and HBME are very different from petroleum. Petroleum contains very low oxygen high nitrogen and sulfur contents, which can lead to environmental pollutions upon burning. In contrast, HAME and HBME have high oxygen content without nitrogen and sulfur. Because it has been commonly recognized that oxygenated additives can decrease exhaust smoke for diesel, reduce the ignition delays, and shorten the combustion duration and engine emissions of smoke, HC and CO can also be reduced [3]. In this article, production, properties and research of biofuel from PHAs was reviewed. In addition, future work on biofuel from PHAs was also discussed.

Polyhydroxyalkanoates (PHAs)

PHAs is a family of naturally-occurring biopolyesters synthesized by various microorganisms. First discovered by Lemogine in 1926, PHAs has since attracted much commercial and research interests due to its biodegradability, biocompatibility, chemical-diversity, and its manufacture from renewable carbon resources [4]. A PHAs molecule is typically made up of 600 to 35,000 (R)-hydroxy fatty acid monomer units. Each monomer unit harbors a side chain (R) group which is usually a saturated alkyl group but can also take the form of unsaturated alkyl groups, branched alkyl groups, and substituted alkyl groups although these forms are less common [5]. Depending on the total number of carbon atoms within a PHAs monomer, PHAs can be classified as either short-chain length PHAs (scl-PHAs; 3 to 5 carbon atoms), medium-chain length PHAs (mcl-PHAs; 6 to 14 carbon atoms), or long-chain length PHAs (lcl-PHAs; 15 or more carbon atoms). About 150 different PHAs monomers have been identified and this number continues to increase with the introduction of new types of PHAs through the chemical or physical modification of naturally-occurring PHAs. These features gave rise to diverse PHAs properties which can be tailored for various applications ranging from biodegradable packaging materials to medical products. PHAs is also considered as pharmaceutically-active compound and currently investigated as potential anti-HIV drugs, anti-cancer drugs, antibiotics, etc. [4, 6].

Energy Derived from Biofuels

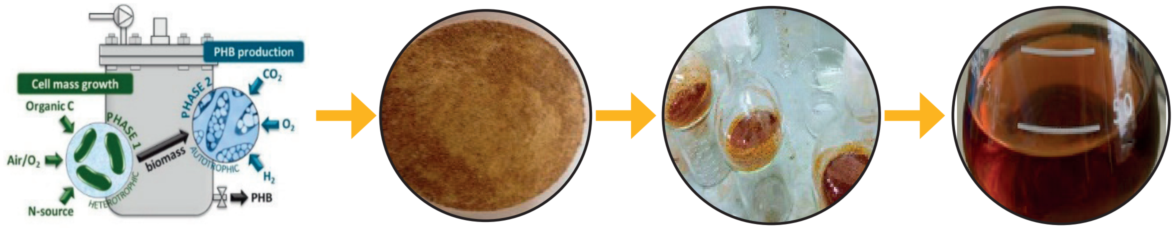
Bioenergy is energy derived from biofuels. Biofuels are fuels produced directly or indirectly from organic material biomass including plant materials and animal waste [7] (Table 1). However, most people associate biofuel with liquid biofuels (bioethanol, biodiesel and straight vegetable oil). In this review the term "biofuels" refers to liquid biofuels.

Table 1 Types of biofuel for energy

Biofuels	Gaseous	Methane gas and Hydrogen gas
	Solid	Fuel wood, Charcoal, Bagasse and Sawdust
	Liquid	Methanol, Ethanol, Biodiesel and HAME/HBME

Biofuels from PHAs

The limited reserves and negative environmental consequences of fossil fuel have spurred the search for renewable biofuels. Till now, several substitution biofuels from biomass have been researched and used in some domains in our life, including hydrogen, methane/biogas, methanol, ethanol, biodiesel, *n*-butanol, acetone and others [7]. Among all of these biofuels, biodiesel and ethanol are exploited most intensively. However, extensive uses of biodiesel and ethanol have created panic in the food and feed market, leading to “food vs fuel” controversy [8]. At the same time, high production costs also restrict their large-scale application in industry and transportation. Recently, a novel biofuel termed hydroxyalkanoate methyl ester (HAME) and hydroxybutyrate methyl ester (HBME) derived from bacterial PHAs (Figure 1) was reported. HAME and HBME can be produced from residual substrates in wastewater of food processing industries or even household wastewater.



Fermentation of PHAs   PHAs   Esterification of PHAs   HAME (Biofuel)

Figure 1 Biofuel from polyhydroxyalkanoates (PHAs)

The production of HAME obtained from esterification of PHAs could be biofuel (Figure 2). When considering the HAME and HBME properties showed the possible ability to be utilized as a novel biofuel or a fuel additive, the fuel properties similar to biodiesel.

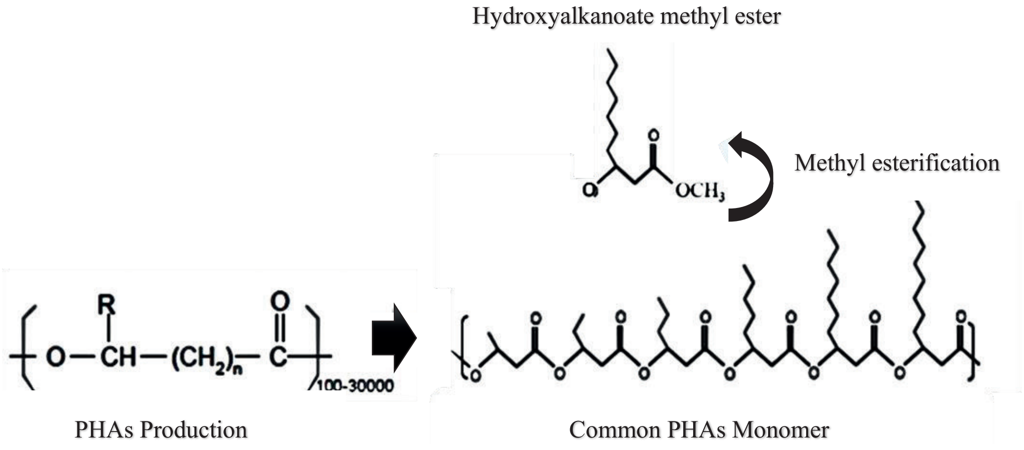
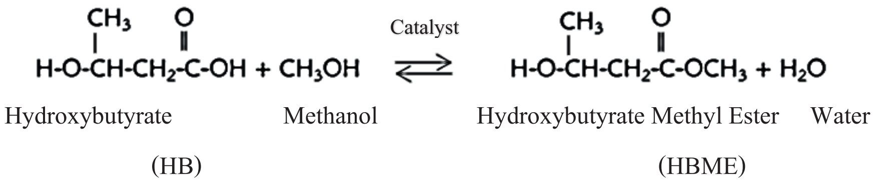


Figure 2 The production of hydroxyalkanoate methyl ester (HAME) [3]

Preparation of HAME and HBME were obtained via acid-catalyzed hydrolysis of PHAs (Figure 3). The most commonly used catalyst for the HBME production is sulfuric acid (H_2SO_4). Usually, the yield reaches an optimal value when the catalyst (H_2SO_4) concentration reaches 10 % (v/v) with methanol under a reaction temperature of 67 °C to 100 °C and a time for 50 h to 60 h. The highest yield of HBME (44.4 % to 70.7 %) was obtained (Table 2).



$$\mathrm{H{-}O{-}\overset{\overset{CH_3}{|}}{C}H{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}OH + CH_3OH \xrightleftharpoons{Catalyst} H{-}O{-}\overset{\overset{CH_3}{|}}{C}H{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}OCH_3 + H_2O}$$

Figure 3 Synthesis of HBME from polyhydroxybutyrate (PHB) using esterification [9]

Table 2 Optimal condition of hydroxybutyrate methyl ester (HBME) production via acid-catalyzed hydrolysis of polyhydroxyalkanoates (PHAs)

Methods of HAME Production			**% HBME**	**References**
Acidic Methanol	**Temperature (°C)**	**Time (h)**		
methanol with 15 % (v/v) H_2SO_4	100	60	65.0	[9]
			44.4	[10]
methanol with 10 % (v/v) H_2SO_4	67	60	46.7	[11]
			68.0	[12]
methanol with 10 % (v/v) H_2SO_4	67	50	70.7	[13]

Factors Affected on HAME and HBME Production

There are four primary factors affecting the yield of HAME and HBME, i.e. type of acid-catalyzed, catalyst concentration, type of alcohols, reaction time and reaction temperature.

1. Type of acid-catalyzed and concentration

For acid-catalyzed systems, sulfuric acid (H_2SO_4) has been the most investigated catalyst, but other acids, such as hydrochloric acid (HCl), phosphoric acid (H_3PO_4), and organic sulfonic acids [10]. In a pioneering work, Wang *et al*. [8] and Zhang *et al*. [3] examined the esterification of PHAs with methanol, using H_2SO_4 as the catalyst. In addition, Choonut *et al*. [10] study, the effect of acid-catalyzed type (H_2SO_4, HCl and H_3PO_4) on HBME production. The results indicated that the system containing H_2SO_4 as an acid-catalyst gave the highest yield. Sangkharak *et al*. [11] found that high HBME recovery percentage (68 %) and purity (> 95 %) were obtained by acid-catalysis using methanol with H_2SO_4.

Catalyst concentration can affect the yield of the HBME product. As mentioned before, the most commonly used catalyst for the reaction is sulfuric acid (H_2SO_4). Because, upon mixing H_2SO_4 with methanol a small amount of water will be produced, this will affect the product yield because of the hydrolysis reaction [12]. This is the reason why the catalyst should be added into the CH_3OH first and then mixed with the PHAs. As the catalyst concentration increases the conversion of polyesters and the yield of HAME and HBME increase. This is because an insufficient amount of catalysts result in an incomplete conversion of the polyesters into the methyl ester. Usually, the yield reaches an optimal value when the catalyst (H_2SO_4) concentration reaches 10 % (v/v) and then decreases a little with a further increase in catalyst concentration. Choonut *et al*. [10] found that high HBME recovery percentage (70.7 %) were obtained by acid-catalysis using methanol with 10 % (v/v) H_2SO_4 under a reaction temperature at 67 °C for 67 h. In addition, Zhang *et al*. [3] and Junpadit *et al*. [13] the preparation of HBME from PHB was addition of methanol with 15 % H_2SO_4 under 100 °C for 60 h, gave the yield of HBME 65.0 % and 44.4 %, respectively. Canakci and Gerpen [14] also reported that the completeness of ester formation increases when increasing the acid catalyst amount. A further complication of working with high acid catalyst concentration becomes apparent during the catalyst neutralization process, which precedes product separation [15]. However, an acid catalyst at a higher concentration does not increase the reaction products.

2. Type of alcohols

Alcohols used in acid-catalyzed esterification have included methanol, ethanol, propanol and butanol [10, 16]. Methanol and ethanol are used most frequently in laboratory research. As already mentioned, the low cost of methanol makes and its physical and chemical advantages, it the first choice for the reaction. Choonut *et al*. [10] study, the effect of type of alcohols (methanol and ethanol) on HBME. The PHB was acid-catalyzed for HBME production using methanol with 10 % H_2SO_4, the highest yield. Canakci and Gerpen reported that longer chain alcohols have longer reaction times when the same reaction temperature is used [14].The addition of one methylene group (CH_2) to the alcohol doubles the reaction time. However, the level of water in an alcohol is crucial for its successful application in the production of methyl esters [17].

3. Reaction time

The conversion rate of fatty acid esters increases with reaction time [18]. At the beginning, the reaction is slow due to the mixing and dispersion of alcohol into the PHAs. After a while, the reaction proceeds very fast. Normally, the yield reaches a maximum at a reaction time of < 90 min, and then remains relatively constant with a further increase in the reaction time [19]. Moreover, excess reaction time will lead to a reduction in the product yield due to the backward reaction of transesterification, resulting in a loss of esters [20]. Choonut *et al*. [10] study, the effect of times (10, 20, 30, 40, 50, 60 and 70 h) on HBME production. HBME production increased from 12.8 % to 70.7 % when the reaction time was increased from 10 h to 50 h, under optimum condition (methanol with 10 % H_2SO_4 at 67 °C). It is clear that long times are required for high conversion rates using an acid catalyst. Feedman *et al*. [18] reported that the methanolysis of soybean oil in the presence of H_2SO_4 with alcohol at 65 °C for 50 h to reach complete conversion of the soybean oil (> 99 %).

4. Reaction temperature

Temperature clearly influences the reaction and yield of the HAME and HBME product. A higher reaction temperature results in an increased reaction rate, and a shortened reaction time. However, reaction temperature increases beyond the optimal level, the yield of the HBME product decreases because a higher reaction temperature accelerates the saponification reaction of polyesters. Zhang *et al*. [3] and Junpadit *et al*. [13] the preparation of HBME from PHB was addition of acidic methanol under 100 °C for 60 h, gave the highest yield of HBME 65.0 % and 44.4 %, respectively. Choonut *et al*. [10] and Sangkharak *et al*. [11] found that high HBME 70.7 % and 68.0 % of optimal conditions (methanol with H_2SO_4) under a reaction temperature at 67 °C. The reaction temperature must be less than the boiling point of alcohol in order to ensure that the alcohol will not leak out through vaporization [20]. Depending on the condition used, the optimal temperature ranges from 67 °C to 100 °C.

Characterization for HBME Physicochemical and Fuel Related Properties.

The Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy (ATR-FTIR) spectrum was used for evaluate the HBME structure and the results are shown in Table 3.

Table 3 Description of the main ATR-FTIR peaks in the spectra of HBME

Assignment (stretching)	**Wavenumber (cm^{-1}) of HAME and HBME**			
	1[a]	**2[b]**	**3[c]**	**4[d]**
O-H	2924	3390	3454	1045
C-H	2855	2954-2842	2960-2860	2928
C=O	1745	1713	1740	1720
C-O-C	1246	1155-1041	1260-1160	1129

[a]Data obtained from Choonut *et al*. [10]; [b]Data obtained from Sangkharak *et al*. [11]; [c]Data obtained from Zhang *et al*. [3]; [d]Data obtained from Kueanun *et al*. [21].

The HBME showed the intense absorption characteristic for ester carbonyl (C=O) stretching groups. The spectra indicated groups of O-H, C=O, C-O-C, and C-H. The intense band at 1745 cm^{-1} represented the presence of aliphatic carbonyl (C=O) group of R-CO-A in HBME [10]. Silverstein *et al*. [22] also reported the C=O absorption is very strong in the IR at 1750-1735 cm^{-1}; in addition, C-O stretching bands are observed in the range of 1100-1300 cm^{-1}. The spectrum of the HBME revealed 3 major peaks of C-O-C stretching at 1155-1041 cm^{-1}, C-H banding at 2954-2842 cm^{-1} and C=O stretching at 1713 cm^{-1} [11]. ATR-FTIR spectrum of the PHB sample revealed 3 major peaks of C-O-C stretching at 1260-1160 cm^{-1}, C-H banding at 2960-2860 cm^{-1} and C=O stretching at 1750-1735 cm^{-1}. However, the major difference of the FTIR spectra HBME was in the band of C=O stretching located. The PHB polymer showed the intense absorption characteristic for ester carbonyl (C=O) stretching groups in comparison with the HAME. The spectra indicated groups of O-H, C=O, C-O-C, and C-H. According to ATR-FTIR analysis, spectra of HBME were almost similar (Figure 4). The fuel properties of HBME were indicated in Table 4.

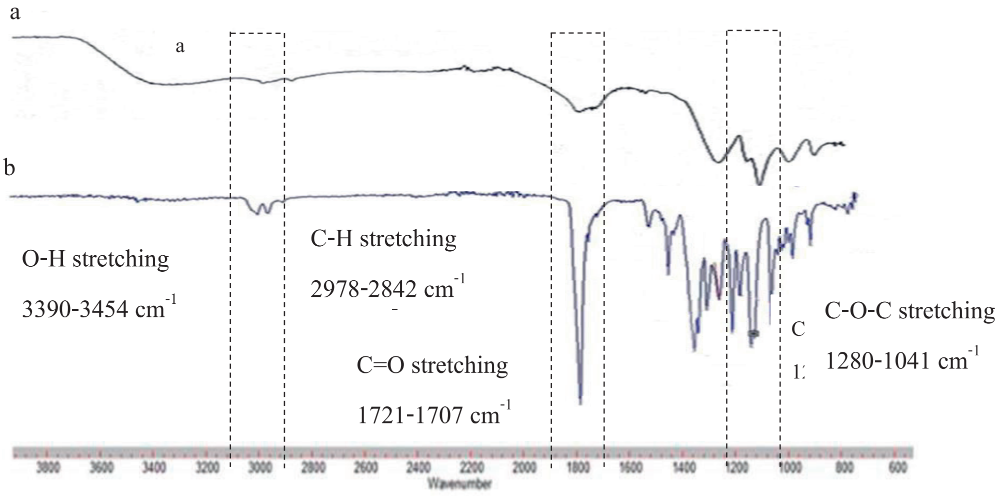


Figure 4 FTIR spectra of hydroxybutyrate methyl ester (HBME) (a) and polyhydroxybutyrate (PHB) (b) [11]

Table 4 The fuel related properties of HBME

Property	**Thailand's**	**ASTM**	**HBME**^{a}	**HBME**^{b}	**HBME**^{c}
Methyl ester content (%)	≥ 96.5	not defined	96.8	no data	no data
Heating value (kJ/g)	not defined	31.5	32.9	25.1	21.1
Viscosity at 20 °C (mm^2/s)	3.5 to 5.0	1.9 to 6.0	5.3	4.0	3.8
Flash point (°C)	not defined	130 to 170	132	68.5	51.0
Pour point (°C)	≥ 12	-15 to 10	-7	no data	no data

[a] Data obtained from Junpadit *et al.* [13]; [b] Data obtained from Sangkharak *et al.* [11]; [c] Data obtained from Zhang *et al.* [3]

Almost all of the properties of HBME passed these standards of Thailand's and American Society for Testing and Materials (ASTM). Therefore, HBME revealed the possible ability to utilize HBME as a novel biofuel or fuel additive and has a feasibility to be used as an alternative biodiesel fuel [13, 21].

Conclusion and Future Direction

The rise in biofuel demand also increases, limited reserves and negative environmental consequences of fossil fuel have spurred the search for renewable. The application of polyhydroxyalkanoates (PHAs) as a source of biofuel looks very promising since it does not require highly purified PHAs, and thus, the PHAs can possibly be obtained from activated sludge or nutrient-rich wastewater, which does not compete with human or animals for food, resulting in cost reduction. The utilization of PHAs as a novel substrate for biofuel shows great promise. The recent development of PHAs production from open and continuous mixed cultures will allow the PHAs to be produced with a very low cost for the biofuel applications. After using as bioplastics from PHAs, can be methyl esterified to become biofuels, which further extends the PHAs application value.

Upcoming research should focus on improving the PHAs production in activated sludge or on using wastewater as a fermentation medium to grow high PHAs, producing mixed cultures. Because the application of PHAs does not require highly purified PHAs, the production process appears to be much simpler. In addition, improving the HAME production using different PHAs monomers may contribute to the great potential of HAME as a new type of biofuel. PHB belongs to short chain length PHAs (scl-PHAs) containing only hydroxybutyrate (HB) monomers. It is important to exploit more new biofuels; also, it is equally important to improve the quality of biofuels. The development of HAME into biolubricants or fuel additives may contribute to the diversification of biofuel or fuel additive market.

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