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The Optimization Conditions of Polyhydroxybutyrate Methyl Ester from Polyhydroxybutyrate via Acid-Catalyst

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Abstract

Recently, microbial polyhydroxyalkanoates (PHA) were proposed as an alternative biofuel, termed 3-hydroxyalkanoate methyl ester (3HAME). PHA is a strong candidate to make biofuels and be used in the fuel additive market today. However, an optimization study of 3HAME from PHA has never been reported. In this paper, 3HBME was produced from polyhydroxybutyrate (PHB) via acid catalyst. The effects of acid types and concentration, alcohol, reaction temperature and time were determined. The optimal conditions for 3HBME production were methanol with 10% (v/v) H_2SO_4 under a reaction temperature and a time of 67°C and 50 h, respectively. The highest yield of 3HBME (70.7%) was obtained under these optimal conditions. 3HBME was further characterized for chemical and fuel related properties following ASTM. The characteristics of 3HBME including acid value, free fatty acid, saponification and molecular mass were 2.16, 0.0098, 60.27 and 2792.434, respectively. The 3HBME properties were also compared with Thailand and ASTM standards. Almost all of the properties passed these standards.

Keywords: *Biofuel, Polyhydroxybutyrate, PHB, Hydroxybutyrate methyl ester, HBME*

1. Introduction

Biofuels are fuels derived from biomass. Biomass, organic matter taken from or produced by plants

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and animals, is gaining increased public and scientific attention. Fossil fuels are energy resources like coal, oil and natural gas that supply the vast majority of the world's energy needs [1]. Nowadays the world's fossil fuels will be running out in a few years. So, alternative renewable fuels such as biodiesel will be needed [2]. 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 [3]. In addition, extensive uses for biodiesel have created panic in the food and feed markets, leading to a food versus fuel debate [1].Therefore, the screening for novel types of biofuel together with developing the production processes is the practical way to reduce the aforementioned problems of biofuels. The novel biofuel termed hydroxyalkanoate methyl ester (3HAME) and polyhydroxybutyrate methyl ester (3HBME) derived from bacterial polyhydroxyalkanoates (PHA) and polyhydroxybutyrate (PHB) were reported to show that the PHA-based biofuel could be a strong candidate to replace current biofuels in the fuel additives market [4]. The production of 3HAME and 3HBME do not rely on food or feed. Therefore, 3HAME has become increasingly important because it is a microbes-based biofuel, petroleum independent and uses sustainable production methods [5]. When considering the production process, chemical structures and role as an energy carrier, it is easily found that 3HAME esters are similar to those of biofuels, particularly similar to biodiesel that is methyl esters of long-chain fatty acids. Consequently, the development of 3HAME as a sustainable fuel or fuel additive may contribute to the diversification of the biofuel or fuel additive market [6]. The objective of this present study aimed to optimize the conditions of 3HBME from PHB via acid-catalyst to be used as a new type of biofuel.

2. Research methodology

2.1 Strain and medium

Alcaligenes eutrophus TISTR 1107 were obtained from the Thailand Institute of Scientific and Technology Research (TISTR) and used as PHB-producing bacteria throughout this study. The culture of *A. eutrophus* was maintained on nutrient agar at 4^{o}C. The culture was prepared by growing the cell in 25 mL of nutrient broth in 100 mL conical flask on a rotary shaker (200 rpm) at 37°C for 24 h [7].

2.2 The production of PHB from molasses as a carbon source

10% of *A. eutrophus* was transferred to 5-L fomenter containing medium (3-L) supplemented with 60 grams of molasses as a carbon source. The culture was incubated up to 108 h under a rotary shaker (200 rpm). The experiments were maintained at 37°C (pH 7). Samples were taken after 108 h of cultivation to determine for growth (DCW) and PHB concentration. Afterward, PHB were extracted by solvent extraction with chloroform and utilized as substrate for biofuel production.

2.3 The production of biofuel (3HBME) from PHB via acid-catalyst

(a) The effect of acid-catalysed type

3.0 of grams PHB were dissolved in 40 mL of chloroform. Afterwards, 40 mL of acidic methanol including H_2SO_4, HCl and H_3PO_4 at a concentration of 10% (v/v) was added according to Wang et al [8]. The sample was collected for the chemical structures of 3HBME and fuel properties.

(b) The effect of acid-catalysed concentration

3HBME was operated under H_2SO_4 by varying the acid-catalyst at 5%, 10% and 15%, respectively. The sample was collected for the chemical structures of 3HBME and fuel properties.