



An overview of the production and prospect of polyhydroxyalkanoate (PHA)-based biofuels: Opportunities and limitations

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ABSTRACT

The significant challenges faced with the use of fossil fuel and its depleting effect have led to discovering several means of generating alternative fuels known as biofuels. Based on the renewable substrates used for biofuel production, different generations of biofuels have emerged. From these generations, microbial-based substrates, one of which is polyhydroxyalkanoate (PHA), are still relevant, as they do not compete with other valuable resources, such as food and farmland, vital to human survival. Currently, PHAs are used for many applications, most notably as bioplastics, drug delivery molecules, medical suture, food and feeds, and, recently, biofuel. The method used for converting PHA to biofuel had involved heating the PHA with sulphuric acid and methanol, which breaks the polymer up into its various constituent monomers and then transforms them into methyl esters. When comparing the manufacturing process, its chemical configurations and energy carrier role, PHA-based biofuels are predominantly biodiesel. As a renewable fuel, this fuel source's improvement may add to the sustainable energy and fuel additive market's growth. Consequently, this article reviews the advantages and limitations of PHA-based biofuel production, its physicochemical parameters, and the commercial use prospect.

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Introduction

Fossil fuel, a natural mineral resource, has proved its expediency to humanity in several ways, especially in transportation, engine ignition in industries, and power generation [1]. The outburst of the industrial revolution that orchestrated the world economy's continuous growth and a high energy matrix has brought to the limelight this non-renewable and natural resource [2]. However, this utility rate has led to the depletion of this resource concerning its high demand. Furthermore, is the fuel demand report given by IEA [3], showing a 20% demand increase for fuel in 2025 from the year 2019, with a policy momentum strategy giving more preface to clean renewable energy than other forms of energy. The high demand for fossil fuel has led to its unsustainability, production of the greenhouse effect, gas emission, production of toxic substances during

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degradation, increase CO₂ in the atmosphere, and recalcitrance of the fuel in the environment [4,5]. It also causes health hazards (such as black soot), water pollution, and the death of aquatic animals due to the spillage of crude fuel.

The scarcity of fuel, and other significant challenges, have led to economic inflation among nations [6]. The shortage of this product disrupts fuel's foreign exchange, thereby affecting essential valuable transactions necessary for the nation's economy. In overcoming these challenges, different generations of biofuel, based on substrates used, have evolved. This fuel is more stable and sustainable because of being produced from renewable resources [7]. These generations of biofuel grow from the use of agricultural products to the use of lignocellulose and microalgae substrates [8] and the use of novel PHA substrate [9], which is a renewable, biodegradable and microbial source [10].

The biodegradable, renewable and eco-friendly bio-based fuel has continuously gained more popularity in society. It increases research interest due to the increased crude oil price and the high carbon dioxide emission associated with fossil fuel use, which is of global concern [9]. The prospect of increased energy demand for both commercial and industrial use further enhanced research exploration [3]. Biofuel, even though, saves the problems caused by fossil fuel by reducing the greenhouse gas effect [11], still has high production cost [12,13]. It also creates panic in the food and feed market that led to the food versus fuel debates [14]. This invariably affects food security [15] by competing for resources used in food production, such as land, water, labour, and food crops.

Based on the increasing application of polyhydroxyalkanoate (PHA), its use as a substrate for biofuel production is currently understudied [7]. Biofuel has been produced from PHA by the esterification method using both acid- and base- catalytic approaches. This method involves changing the OH group of the PHA monomeric carboxylic group and replacing it with the OCH₂ present in the alcohol [16]. This process gives a methyl-ester compound that has similar physicochemical properties to biofuel, especially biodiesel and gasoline. Thus, using PHA-based biofuel as a fuel additive in agricultural-based biofuels will improve the produced agro-fuel features, thereby meeting the increasing demand for biofuel in the future. PHA-based biofuel has been chiefly produced from polyhydroxybutyrate (PHB), a short-chain length PHA [7,14,17], which could be the main reason why the octane and cetane numbers are low compared to other biofuels. Reports have shown that the chemical conversion of the esters group in a substrate to another ester having the properties of fuel always maintained the length of carbon of the side chain [18]. Moreover, the longer and more branched the carbon length, the more its cetane or octane number [19]. This significant deficiency could also limit its usage as a fuel additive in its blends with gasoline or diesel. An additional fuel additive with high cetane and octane number will have to be added to the blends, thereby increasing the cost. Also, the fuel of a low octane number can cause knocking of the gasoline engine, thus damaging the engine. Likewise, diesel fuel with low cetane will cause a delay in the engine's ignition, thereby affecting the engine's gas emissions and performance [20–22]. However, studies have shown that the generation of biofuel from long carbon length and saturated hydrocarbon substrates mainly produce high cetane and octane numbers [23]. Thus, producing biofuel with better physicochemical properties from PHA of long carbon length is possible.

Generations of biofuel

Fossil fuel supplies the vast world's energy need, including are coal, crude oil, and natural gas [12]. However, these fuels posed significant health and environmental challenges, which include; air and water pollution, diseases (such as black soothing), environmental degradation, increased fire explosion, and global warming due to high emission of CO₂ [24,25]. The development of biofuels such as ethanol, methanol, biogas/methane, biodiesel, n-butanol and acetone, obtained from plant and animal sources, serves as an excellent substitute for fossil fuel [26]. Because of its origin, biofuel is a renewable and sustainable energy source [27].

In the ongoing thrive on replacing coal and petroleum, the International Energy Agency (IEA) has proposed a plan to ensure biofuel's adequate production by 2030, which will meet the consumers' energy demand [28]. The European Union (EU) also depends on sustainable fuel from a renewable source and electric vehicles to supply at least 14% of the energy needed for transportation by 2030 [29]. Furthermore, the US Renewable Fuels Standard has requested the distributors of fuel that by 2022, the amount of biofuel blended with fossil fuel should have increased to 36 billion gallons annually [30]. In realizing the medium-term climate policy plan of 2017, the National Energy and Climate Strategy agency in Finland, has drawn actions on how to face out the use of petroleum energy to increase dependence on renewable energy sources and reduce greenhouse gas emissions to a bearable point. According to the plan, out of the energy consumed in the transportation sector, biofuel will share not less than 30% of the total energy consumption. In comparison, there will be a 10% increase in its blend with light fossil fuel used in machinery [31]. China likewise has targeted an increase of 13 billion litres of bioethanol and 2.3 billion litres of biodiesel per annual with effect from the year 2020 [32], while South Africa blending plan will significantly increase the demand for biofuel given the high transport sector of the country [33]. Therefore, the need to enhance research and development that will suitably meet this demand is rising.

Biofuels are non-polluting, locally available, accessible, sustainable and reliable fuel obtained from renewable sources [34]. They are generally referred to as liquid fuels produced from several materials of biological origin, such as plant and animal materials [35–37]. Biofuel includes biodiesel, fuel ethanol, and other types of fuels generated from biomass and mainly used for automotive, thermal and power generation as described in the Nigerian biofuels policy and incentive docu-

ment. The biofuels must also meet the expected quality conditions specified by the Standards Organisation of Nigeria (SON), Department of Petroleum Resources (DPR) and any other competent government agency [38].

First generation (1G) biofuel, as reported by Antoni *et al.* [39], has captured about 90% of the current biofuel. These biofuels, such as bioethanol fermented from corn, and biodiesel esterified from edible vegetable oils or animal fats, are vastly replacing petroleum fuel. However, first generation biofuel would require extensive farmland and crops, which otherwise would have been used for food and feeds production, thereby competing with the world food supply and causing economic and ethnic problems [40,41]. Also, cultivating food crops for biofuel production consumes large amounts of water, fertilizer and pesticides, which burdens the environment. The use of extensive farmland will likewise cause an increase in the clearing process and pollution associated with agricultural harvesting and processing [42].

Second generation (2G) biofuels are mass-produced from a broad group of feedstock, ranging from lignocellulose to community solid waste and animal fat [35]. Generally, 2G biofuels are obtained from non-edible feedstock such as wood and its waste, non-food crops, animal fats, and waste cooking oil [43]. This generation of biofuel has some additional benefits more than first generation biofuel. It may be used at the various fraction in diesel engines with no alteration in properties and about 15-20% more effective than gasoline engines. This generation of biofuel can overcome the ethical, cost-effective and ecological trials without impeding our food value and generating stress on land use since it is non-edible, eco-friendly and can be cultivated on marginal land [44]. However, some factors directly affect the fuel properties of these biofuels. These fundamental elements are fatty acid configuration and quality of the feedstock, manufacturing and refining procedure and downstream processing of the biofuel. Standard properties are essential to be sustained before biofuel is used as engine fuel. Furthermore, 2G biofuels are not being manufactured industrially, as the machinery used to convert lignocellulose materials into sugars is expensive and requires the use of superior enzymes [1]. Likewise, large biomass wastes are generated after the separation of biofuel from lignocellulose, which requires a different anaerobic fermentation process, thereby increasing the total cost of biofuel production [45].

Third generation (3G) biofuel is a microbial-based biofuel. Microalgae are predominantly used to produce these 3G biofuels [46,47]. An example of microalgae primarily used for biofuel is cyanobacteria. It has a very distinguishing growing biomass paralleled to conventional lignocellulose. The dynamic energy index, low cost, biodegradable and unlimited feedstock are a few of the remarkable benefits which mark microalgae as a unique biomass source for biofuel production [48]. The entire favourable property which facilitates algae to be used as a 3G biofuel is their high oil content. Compared to conventional crops, microalgal biomass yields 15-300 more oil for manufacturing biodiesel [35]. Recently, biofuel from methyl esterification of polyhydroxyalkanoate, which is also a microbial substrate-based biofuel, is being considered [49]. Although this has not been entirely accepted, researches are ongoing to make it industrial important. PHA as a substrate for biofuel production has some advantages over agricultural feedstock, including its sustainable production from a renewable source, independence from a petroleum source. It also reduces the dependence on fossil fuel and, more importantly, stops the food and land versus fuel crisis due to its microbial origin [9]. Despite these multiple advantages, 3G biofuel's chief drawback is its high predictable cost and dependence on fossil fuels in production steps, raising ecological concerns [37,50,51].

The generation of fuel via genetic engineering of algae is deliberated under the fourth generation biofuel term. The biomass supplies in the fourth generation (4G) biofuel originates from both microalgae and macroalgae, which are eukaryotic organisms [52]. The 4G biofuels are the newly developed generational biofuels produced using modern methods such as low pressure or geo-synthesis, inventive biochemistry, low-temperature electrochemical and petroleum-hydro-processing processes. These methods use seized carbon from the atmosphere to generate biofuels [35]. Unlike the second and third generation biomass, fourth generation biofuels take up the feedstock while refining and converting it to biofuel. The feedstock of 4G biofuel is ecological CO₂, H₂O and heat energy. The summary of the substrates use for biofuel production and the challenges faced in each generations is as shown in Fig. 1.

Classification of biofuel based on use

Biofuels are mainly classified into primary biofuel and secondary biofuel [1]. The primary biofuels are natural biofuels mainly utilized directly from their crude form for heating, cooking, and producing electricity. In contrast, secondary biofuels are the processed form of primary biofuels manufactured in solid, liquid or gaseous forms. Primary biofuels include landfill gas, pellets, fuel-wood, crop remains, and wood chips [37]. Examples of secondary biofuels include bioethanol, biodiesel, and biogas used in vehicles and various industrial processes [1]. Based on natural resources and biological methods used for manufacturing, secondary biofuels are further categorized into the four biofuel generations (as discussed in section 2.0)

PHA-based biofuel

PHA-based biofuel is a microbial substrate-based biofuel produced from PHA via methyl esterification process with the use of methanol and in the presence of a catalyst. This novel biofuel, designated as 3-hydroxybutyrate methyl ester (3HBME) and 3-hydroxyalkanoate methyl ester (3HAME), currently are the two reported PHA-based biofuel. These are strong candidates that could stand as alternatives for developing biofuels in the fuel additives market [7]. This biofuel production is

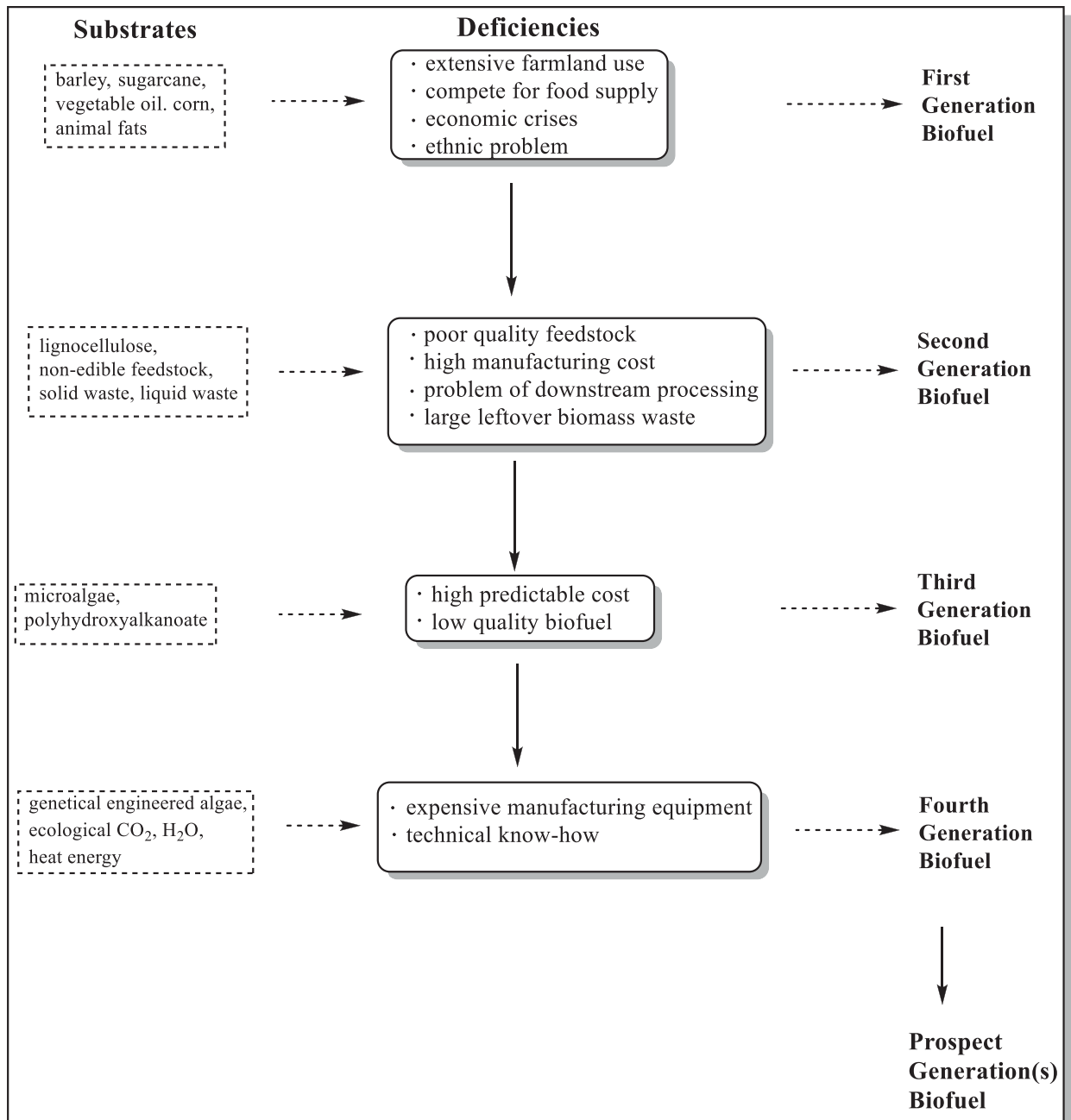


Fig. 1. Summary of the substrates use for biofuel production and the challenges faced in each generations.

non-dependent on petroleum, uses maintainable manufacturing methods and does not depend on feed or food [7]. When comparing the manufacturing process, its chemical configurations and energy carrier role, 3HAME and 3HBME esters are related to biofuels, particularly biodiesel. The improvement of 3HAME as a renewable fuel and fuel additive may add to the biofuel and fuel additive market's growth. However, the octane and cetane numbers, which are fundamental properties of any fuel to prevent knocking of the engines and delay autoignition when this fuel is used, are very low in the laboratory produced PHA-based biofuel [14,17,49]. Nevertheless, different carbon length PHAs have been produced, as shown in Fig. 2, which project promising good biofuel properties when longer carbon length PHAs are used for biofuel production. Accordingly, an increase in the cetane number observed in biodiesel has been reported to be dependent on the long straight chain length, a decrease in the amount of the double bonds of unsaturated fatty acid esters, and an increase in the molecular weight of the alcohol used for conversion [19].

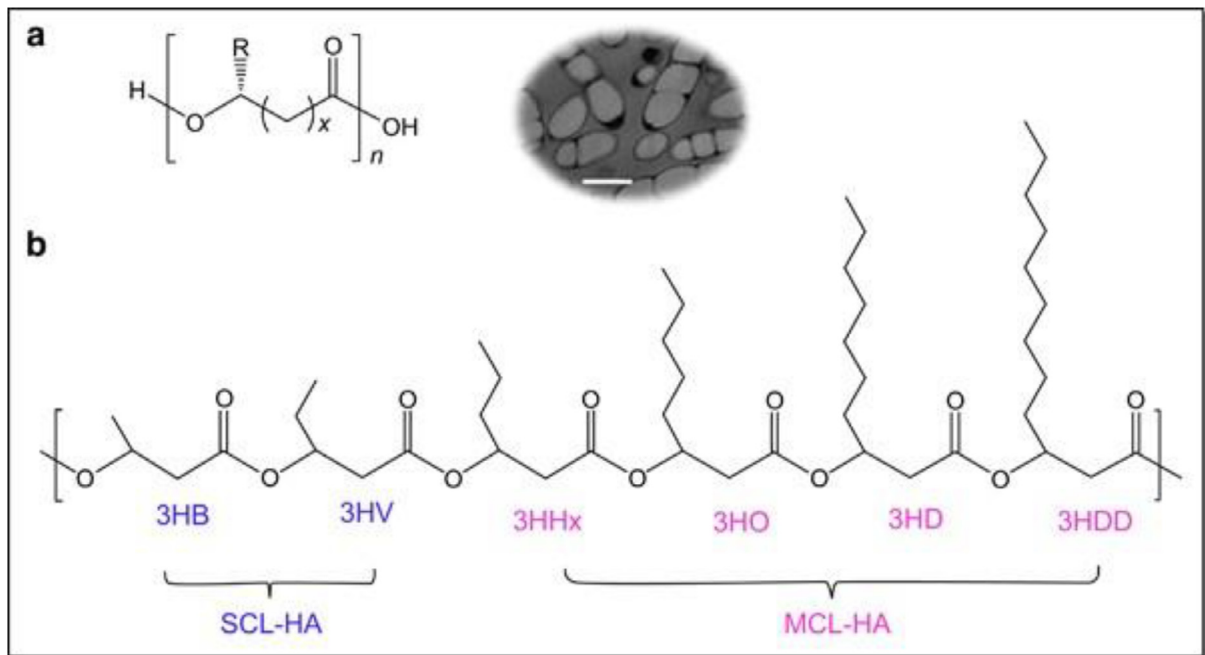


Fig. 2. (a) Basic fundamental structure of PHA, (b) different PHA structural backbone with their side chain [53].

Production of PHA-based biofuel

Economy of the process

PHA and its monomers are known to be degradable both through chemical and biological means, leading to energy generation [54,55]. However, why waste the energy contained in the PHA and the time taken for degradation when this polymer can be converted through methyl esterification into another value-added product that has close chemical properties as fuel. At present, biofuel from PHA does not require highly purified PHA to generate it [55].

The possibility of producing biofuel from PHA was first reported by Zhang *et al.* [49], where 3-hydroxybutyrate methyl ester (3HBME) and 3-hydroxyalkanoate methyl ester (3HAME) were generated from PHB and medium chain length PHA (mcl-PHA), respectively. Ever since then, minor research works have been ongoing to check for their fuel-related properties and also to improve the fuel quality and reduce the cost of production. The combustion heats of 3HBME and 3HAME, as reported by Zhang *et al.* [49], are 20 kJ/g and 30 kJ/g, respectively, and when 10% of each biofuel was blended with ethanol (which has a combustion heat of 27 kJ/g), the heat increased to 30 kJ/g and 35 kJ/g respectively. They also observed the blending of these biofuel components to n-propanol, n-butanol and fossil fuels (gasoline and diesel), having a reduced combustion heat when compared to the original state. Their research also estimated that this biofuel's production cost was around the US \$1,200/ton.

Wang and co-workers also studied the physicochemical and fuel-related properties of HBME for the possibility of using it as a gasoline additive. The study analysed the effect of various volume of HBME blended with 97# gasoline (gasoline of 97 Research Octane Number), as compared with ethanol when used as a fuel additive [17]. The hydroxybutyrate methyl ester (HBME) was produced using PHB as substrate. The conversion of PHB to HBME occurs when methanol is added to the already chloroform-dissolved PHB in the presence of H_2SO_4 , which acts as an acid catalyst. The physicochemical and fuel-related properties of HBME, such as flash point, oxygen content, distillation ranges at different points, octane number, cetane number, boiling and freezing temperature were analysed according to the American Society for Testing and Materials (ATSM) methods. It was observed that the flashpoint, freezing and boiling points and oxygen content of HBME were higher than ethanol, which symbolizes excellent fuel properties when it was considered as a fuel additive. Nevertheless, the octane and cetane numbers were still low, and there were high water content and density that can cause phase separation when blended.

Sangkharak *et al.* [14] studied biofuel production from PHB and its characterization and degradation rate at different environmental conditions. The PHB used was isolated from a mutant *Bacillus licheniformis* M2-12 cultured on a PHA-producing medium, containing 3% palm oil mill effluent serving as the only carbon substrate, and kept in a 5 L glass fermenter with a rotation speed of 150 rpm at 37 °C for 60 h, with the pH of the medium maintained at 7.0. After converting PHB to HBME, they observed a high degradation rate of 30-90% of the biofuel over 52 weeks when stored under 30-40 °C. Thus, as the temperature of storage increased, the acid content used to check the purity of the HBME content also increased [14]. They also reported the drawback encountered using an acid catalyst to esterify PHB, which include high temperature, longer re-

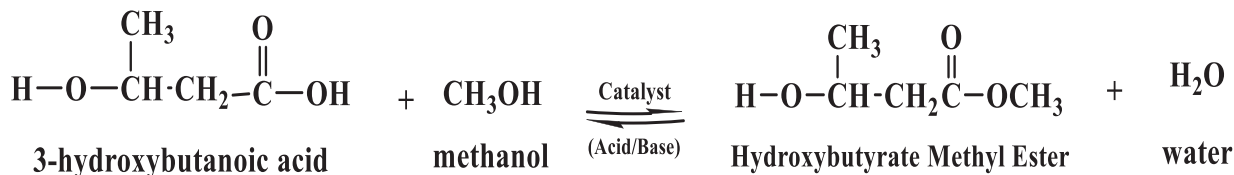


Fig. 3. Esterification of PHB by methanol in the presence of both acid and alkaline catalysts to produce HBME [55].

tention time, and causing undesired corrosion of the equipment. However, to solve the longer retention time, they adopted both acid catalyst (which serves as pretreatment) and alkali catalyst reaction for the methyl esterification process [14].

Choonut *et al.* [7] reported the optimization study of 3-Hydroxybutyrate Methyl Ester (3HBME) produced from PHB isolated from *Alcaligenes eutrophus*, which were cultivated on molasses, via acid catalyst. Their findings show that optimal conditions for 3HBME production were methanol with 10% (v/v) H_2SO_4 under a reaction temperature of 67 °C and time of 50 hours to give the highest yield of 70.7% HBME. Furthermore, Junpadit *et al.* [56] estimated the HBME obtained from PHB to have a 96.8% methyl ester content, 32.9 kJ/g heating value, viscosity at 20 °C to be 5.3 mm^2/s , 132 °C flashpoint, and pour point temperature to be -7 °C, which are within the confined standard limit set by ASTM for excellent fuel properties.

Recently, Sangkharak and co-workers have produced this type of biofuel from a co-polymer (3HB-co-3HV), and the fuel-related properties were partially characterized [10]. They extracted this polymer from *Bacillus thermoamylovorans* using waste cooking oil as the sole carbon source. These authors outlined the optimal conditions that gave HAME the highest yield to be the use of methanol solvent with 15% (v/v) H_2SO_4 and the reaction setup for 60 h at an optimum temperature of 65 °C. These conversion conditions gave a yield of 74.4% HAME containing 97% methyl ester. The authors evaluated density (at 15 °C), dynamic viscosity (at 40 °C) and cetane number to be 860 g/cm^3 , 4 °C and 25, respectively. These properties meet with the ASTM standard except for the low cetane number, which, although it is higher than the formerly generated HBME, but still not up to the minimum limit of excellent fuel properties. Their result also proved the possibility of producing biofuel with a high cetane number using longer carbon chain length PHA [10].

Conversion route of PHA to biofuel

The conversion of PHA to biofuel has been done through the esterification procedure, as shown in Fig. 3, which is the interchange of the hydrogen of a carboxylic group with the organic group of an alcohol. The PHA monomer contained carbonyl group ($\text{C}=\text{O}$), whose OH (hydroxyl) group are replaced by the OR' of the alcohol used. This chemical reaction is frequently catalysed by adding a base or acid catalyst. The reaction may also be carried out using enzymes (biocatalysts), predominantly lipases.

The biofuel, hydroxylalkanoates methyl ester (HAME), generated from PHA, has been produced using both acid- and alkali-catalysts. The result of biofuel's characteristics produced from these two different catalysts is the same, except for the volume/quantity of biofuel obtained from the constant amount of PHB [14]. The production process involves dissolving the dry PHA in chloroform solvent (shown in Fig. 4) in the ratio of 3:40, respectively. The equal amount of methanol (or ethanol), which is the digesting solvent containing H_2SO_4 or KOH (sometimes, NaOH is used), is added to the dissolved solution. This is refluxed at a temperature of about 67 °C for 50-100 h to allow for complete esterification of PHA to biofuel. The acid or base used is known to serve as the catalyst that would enable the esterification process to occur; otherwise, it may take a long time. After that, the mixture was then allowed to cooled and mixed with distilled water. After stirring vigorously and continually for about 10 min, this suspension, as reported by Keunun *et al.* [9], gives room for water and organic phase separation. Sangkharak and co-workers also showed in their study that the collected organic phase could be evaporated in a vacuum to get rid of chloroform, water and some remaining methanol [10]. This leave behind the crude HBME, a clear mahogany liquid with an ester smear that can be purified under vacuum distillation [14,17,49].

The yield of methyl esterification is influenced by types of catalysts and solvent used, amount of alcohol, reaction temperature and time, mixing intensity [55].

Characterization of the chemical structure of PHA-based biofuel has been widely done using Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) and Attenuated Total Reflectance- Fourier Transform Infrared (ATR-FTIR). Choonut *et al.* [7] reported the structure's spectra analysis using ATR-FTIR, with O-H stretching having a wavelength of 2924.70 cm^{-1} , 2855.06 cm^{-1} peaks recorded for C-H stretching, 1745.17 cm^{-1} for $\text{C}=\text{O}$ and peak between 1246.73 cm^{-1} for C-O-C stretching [7]. This result synchronized with the report of Wang *et al.* [17] when NMR was used.

The physicochemical properties of PHA based biofuel

To be considered fuel and fuel additive, several physicochemical components of HBME have been provided for evaluation purposes. This includes dynamic viscosity, flashpoint, density, ignition point, carbon residue, cold filter plugging point, ash content, freezing point, cetane number, water content, octane number and distillation ranges [14,17,49]. To provide a thriving comparison with other fuels, HBME has been compared with gasoline, ethanol, and diesel [17]. It was shown that HBME had comparable or even improved properties as a fuel additive when equated with ethanol in terms of dynamic viscosity, oxygen

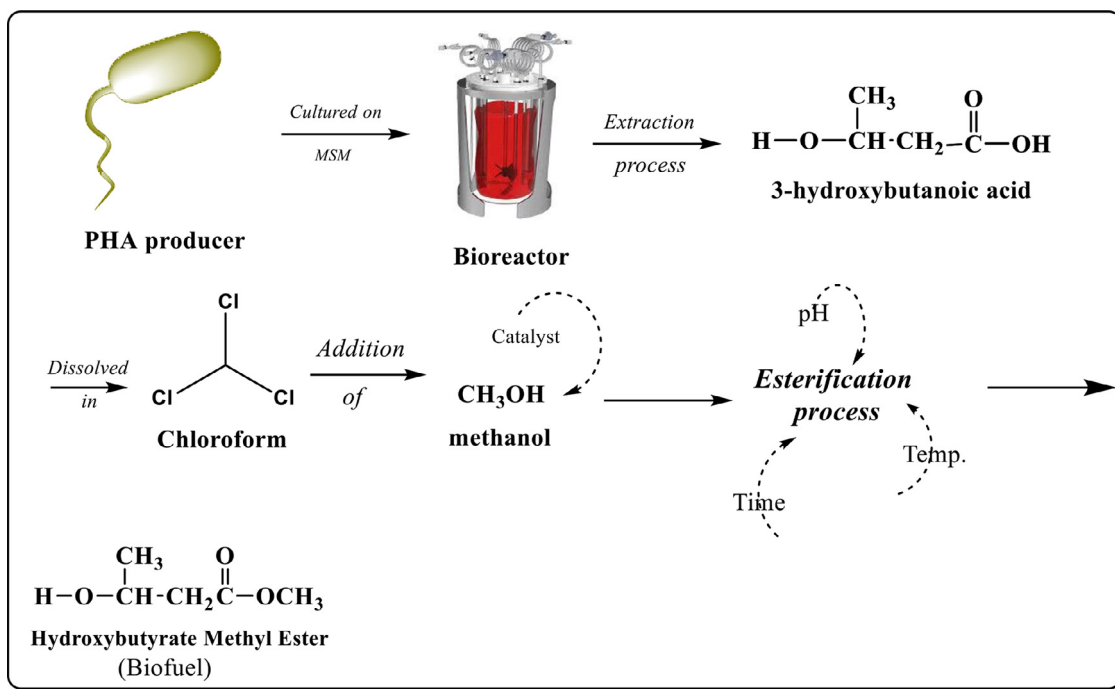


Fig. 4. Conversion of polyhydroxyalkanoates to biofuel.

content, boiling point and flashpoint. HBME flash point, ignition point and oxygen content were 68.5 °C, 80 °C and 41%, respectively [14]. This high flash point and ignition point will allow easy transportation of the fuel and reduce the danger of explosion when stored under room temperature due to its high boiling point of 163 °C. However, the disadvantage seen in its blend is a phase separation which can occur when the fuel is kept at low temperature, and this may be due to the higher density value of HBME [7]. Also, the cetane number and research octane number of HBME is lower than diesel and gasoline, respectively; hence, other additives that increase octane and cetane numbers needed to be added to the blend [17].

Factors that can affect the effective use of PHA based biofuel

High temperature

Moderately high temperature is needed to allow the conversion of the monomers into methyl ester biofuel. This is primarily encountered in the esterification process of converting oil or any other polyoxoester substrates into methyl ester [57]. For this process to occur using chemicals, high energy input must meet up with the enthalpy energy and ultimately convert the substrate's fatty acid, such as the PHA monomers, into methyl esters. This is to prevent the further accumulation of free fatty acids and soap formation in the fuel, which are also difficult to separate [58]. A high temperature setup is also needed to enhance the esterification reaction rate [10]. However, the temperature used must be below the alcohol's boiling temperature to prevent its evaporation [59]. For 15 g of PHA to be converted to biofuel, the reaction mixture will have to be held up to about 67 °C before a considerable higher yield will be produced [49]. This may inflate the final cost of producing HAME.

Longer retention time

The use of acid catalyst has been widely used for the esterification and transesterification processes of biofuel production, especially biodiesel production [60]. The advantage is mainly attributed to the high methyl esters content and purity of the produced biofuels, with little or no soap formation [61]. The result is similar in converting PHA to biofuel using an acid catalyst [10,55]. However, the reaction process is slower when compared with the base catalyzed esterification process [60,62]. For complete esterification of PHA to occur, the time taken is about 50-60 hours which will invariably slow down the production process. This prolonged time may also be due to the limiting maximum energy input due to the boiling point of the alcohol used. However, this drawback is being overcome when Sangkharak *et al.* [14] employed the use of both acid- and alkali-catalysis, where the acid catalyst serves as a pretreatment agent, and the reaction proceeded with alkali-catalyst, serving as a promoter that hastens the high performance of the production process [63,64].

High degradation rate at room temperature

The storing and handling procedures for biofuel should not be overlooked, and careful measures must be followed to ensure that the properties followed the specific guidelines of standard measurement [65]. When biofuel is stored under high temperature above the optimum, there is an increase in the acid content of the fuel, indicating the degradation rate of the fuel. The rate at which the acid content increases also depends on the storage duration and the containing material [66–68]. Sangkharak *et al.* [14], in their study of the degradation rate of HBME at various environmental conditions, observed that when this biofuel was stored at 30–40 °C for over 52 weeks, the degradation rate increases to 30–90%, leading to the increased acidic content of the fuel. This can be disadvantageous for the use of the fuel in a warm temperate region. Pattamaprom and co-workers have also observed this high degradation of biodiesel obtained from palm oil due to high storage temperature [69]. Further is the study conducted by Nizam *et al.* [70], evaluating the effect of long term storage on the significant properties (such as acid value, kinematic viscosity, density) of biodiesel transesterified from palm oil. However, the use of antioxidant by these researchers slows down the oxidation process, thereby helping to sustain the quality of the fuel [70]. This is one of the main disadvantages of biofuel in comparison to diesel. Diesel is stable at an increasing temperature in contrast to biodiesel. This is probably because of the low oxygen content in diesel preventing the oxidation process (that leads to the increase of acid content) from occurring [71].

Undesired corrosion of the equipment

The use of an acid catalyst yielded high biofuel when compared with a base catalyst. This benefit is due to the acid resistance to hydrolysis, thereby favouring the forward reaction of the conversion process [10]. Nevertheless, the acid also causes undesirable corrosion of the equipment used for methyl esterification [14]. Corrosion and degradation of the bioreactor equipment and storing materials are frequently encountered in the biodiesel industry due to the catalyst and chemical reagents used. This has mostly led to an increased capital cost of setting up the production process, as non-corrosive and degradable-resistance materials will have to be used and replaced regularly [72]. Nevertheless, biocatalyst such as lipase have advantages over this demerit nature of the chemical catalyst. However, the high cost of the enzyme from a high production cost still pose another cost problem for biodiesel production [73]. This situation can also impede the production of PHA-based biofuel.

Higher density value of the HBME

The density value of HBME observed by Wang and co-workers was 1.0443 kg/m³ [17], which is higher when compared with gasoline, diesel, and ethanol. This might lead to phase separation, which may occur when its mixtures are kept below 30 °C or mixed with water. Although recently produced PHA-based biofuel has a density value analogous to diesel [10]. However, studies still need to be done to ensure a stable density value of the biofuel to the final selling point. This is likewise important as a higher density value (that is, the amount of mass per volume) of the fuel can lead to the fuel flow's resistance, thereby leading to the emission of more gas particles [74].

Low octane number associated with HBME

To be considered a fuel additive, the HBME octane number is lesser when compared with gasoline [17]. Therefore, when blends with gasoline, it may decline the octane number of the mixtures. Octane number (octane rating) is a standard used to measure gasoline's capability to withstand compression. A high octane number is a critical property in petrol for engines as a low octane number can cause knocking in cars or any other engines when used [75]. Thus, an engine of higher compression potential will require gasoline with a higher octane rating and vice-versa. Fundamentally, the fuel of high octane number and sensitivity properties, together with high latent heat of vaporization, exhibits strong resistance to engine knocking [76]. Nevertheless, an octane rating higher than the required for a particular engine will slow down the engine performance [77], likewise affecting the thermal efficiency and brake power, as studied by Alahmer and Aladayleh [78]. In this instance, additive(s) to raise the octane number may be necessary when HBME is used as an additive, thereby increasing the cost [17].

Low cetane numbers associated with HBME

The cetane number (CN) observed in PHA based biofuel is lesser than one [14,17,49]. Cetane Number of fuel is the extent of the ignition quality of fuel. In simple terms, CN evaluates the delay in the switch of fuel injection into the combustion/ignition chamber and the starting of self-ignition (auto-ignition). Engines that use diesel depend on this self-ignition (without a spark), so the fuel should be capable of auto combustion. Cetane number is an essential property for qualifying biodiesel's value [79]. Hypothetically parallel to the octane number in gasoline, it is the main gauge for biodiesel ignition in a combustion cylinder. An increased cetane number denotes short ignition delay time and further complete combustion of the fuel-charge in the combustion cylinder [23]. This results in the smooth running and improved quality of the fuel with negligible pollutant emissions in the atmosphere. A low CN observed in HBME can lead to incomplete combustion, which may raise the NO_x emissions causing engine knock [80].

Also, the minimum CN for biodiesel as specified in the EN 14214 and ASTM D6751 is 51 and 47, respectively, while the minimum for seasonal diesel (otherwise known as conventional diesel) and No. 1 Diesel is 40 as prescribed in ASTM D975. 3HBME do not meet up with any of these standards. The CN of biodiesel was reported by Yang *et al.* [81] to be mainly dependent on the chain length and the degree of unsaturation of fatty acid esters, which contain double bonds. An increase

in chain length and a decrease in the number of double bonds will increase the biodiesel CN [23,81,82]. This could be the main reason for the low CN observed in HBME, as reported. In this regard, 3HBME alone may not be suitable for use as a blending agent for other fuels because it may lower the mixture's cetane number, thereby causing an undesirable property on it.

Different reaction technologies

To select the type of reaction technology used for biofuel (particularly biodiesel) production, consideration are predominantly given to the chemical configuration of the feedstock [83]. The choice of esterification, transesterification, or both reaction technologies employed is of critical important as it determine the yield, quality and cost value of the produced biofuel [84]. Esterification process is essentially considered if the feedstock contain high percentage (>2%) of free fatty acid (FFA) content. This reaction process ensures the reduction of the FFA content, thereby preventing soap formation that mainly attribute to the high production cost [85]. Conversely, increase and high quality biodiesel esters are produced during transesterification process, but the FFA content must be less than 2%. However, the polyester nature of PHA (containing different monomeric units) will affect the type of reaction procedure used per time as the FFA percentage content vary for each produced PHA. The effect will routinely impede the rate of production and quality of the PHA-based biofuel as analysis of the FFA content will have to be checked before the substrate is used for production.

Advantages of PHA-based biofuel

Utilize low purified PHA

The possibility of obtaining PHA from cheap or no cost substrates such as activated sludge or nutrient-rich wastewater reduces the cost of production of PHA and makes PHA-based biofuel look very promising. Also, since this biofuel production does not need a highly purified PHA, the cost will be further reduced [86].

High flashpoint of the produced PHA-based biofuel

The flashpoints of HBME produced using acid- and base catalyzed processes, as reported by Sangkharak and co-workers, are 68.5 and 67.8 °C, respectively [14]. Flashpoint, which is one of the specific properties expected to be seen in fuel, is the less temperature at which an explosive material can turn to vapour to form a flammable blend in the air in the presence of a spark. It is so essential since it helps describe the fuel fire threats during production, storage and transportation of the fuel so that it can be securely handled [87]. The minimum flash point of diesel at the production facility is expected to be higher, usually about 45 - 65 °C, and 43 °C at the terminals, to assure a flashpoint of 40 °C at the sale point. HBME well fulfils this requirement. Flashpoint also determines the contamination of the fuel that may arise during the movement of fuel from the fuel plant to the customer. Also, HBME has a flashpoint considerably higher than gasoline and ethanol. Hence, it can advance the fuel-transport security when mixed with gasoline paralleled to ethanol-gasoline blend.

Moderate viscosity of the produced PHA-based biofuel

The specification of the viscosity of fuel differs according to the locality where it will be used. A well moderate viscosity fuel is essential for fuel quality as an inappropriate fuel viscosity can affect the gear train and wear of the fuel pump [88]. The dynamic viscosity of HBME is found to be 3.768 mm²/s [17]. This moderate value makes it suitable that it can be used as biodiesel.

High oxygen content of HBME

Biofuel of higher oxygen content has the advantage of emitting reduced particulate matters in the environment [89]. Hypothetically, HBME has a higher oxygen content when compared with ethanol, gasoline, and diesel [49], which contributes to the complete combustion of fuels when mixed with gasoline and diesel, resulting in the decline release of CO and hydrocarbon compounds (HC).

Favourable boiling and freezing point

HBME has a boiling point of about 163 °C [17]. This is higher when equated with the boiling point of gasoline and ethanol, which helps reduce the dangers of a flare-up when kept at room temperature. Likewise, HBME has a comparable freezing point (-46 °C) with gasoline and diesel [17]. Therefore, it is suitable for use in areas where gasoline or diesel is used.

Moderate combustion heat

Zhang et al. [49] reported that the combustion heats of 3HBME and 3HAME are 20 and 30 kJ/g, respectively. When 10% of 3HBME and 3HAME was blended with ethanol (with a combustion heat of 27 kJ/g), the heat increased to 30 and 35 kJ/g, respectively. While the addition of these biofuels to n-propanol, n-butanol, diesel and gasoline lead to an insignificant reduction of their combustion heat compared to their original combustion heat. When the combustion heat of gasoline and diesel is reduced, it aids in the fuel transportation, as it reduces the risk of corrosion and fire outbreak.

Lack of sulphur and Nitrogen content

These elements in fuel have a significant impact on the performance and emissions of engines. These can increase the particular matters emitted by the engine when in use, thereby increasing environmental pollution [90]. The presence of excess sulphur in the fuel can cause corrosion due to the combustion of the element to SO_2 , which is further oxidized to SO_3 . When this react with water, it becomes H_2SO_4 , which settle on the metals in the engine to cause corrosion, failure and even damage to the engine parts. These elements in the human environment can also cause breathing problems, leading to health disease and eventually death [91]. However, biofuel generated from PHA does not show traces of nitrogen and sulphur contents, making it an ethical and environmentally friendly fuel.

Prospect of PHA-based biofuel

PHA-based biofuel shows a promising future to be used as biofuel. However, the economy, ethical, ecological, and engineering aspects of PHA production have to be adequately addressed to consider a sustainable generation of biofuel on a large scale [92]. Modern synthetic biology will allow the engineering of PHA-producing host [93] for an improved result. This will subsequently meet the necessities for ultra-high PHA production with controlled flocculation properties and high cell biomass on a cheap substrate, which is well needed to optimize biofuel's economic production [93].

PHA-based biofuel can fit into biorefining, which is an ideal approach for large scale sustainable use of biomass in the bioeconomy, ensuing a cost-competitive coproduction. Rodriguez-Perez *et al.* [94], in their study of PHA production, explained that to moderate the price of producing PHA, the incorporation of PHA synthesis into other industrialized processes and using waste effluent as a source of organic carbon to the microorganisms are considered as next research exploration. The possibility of obtaining PHA from cheap or no cost substrates such as activated sludge or nutrient-rich wastewater and waste hydrocarbon sources reduces the cost of production of PHA and makes PHA-based biofuel looks very promising. Also, since this biofuel production does not need highly purified PHA, the price will be further reduced [86]. Integration of PHA-based biofuel production into manufacturing lines of industries such as biodiesel, sugar and wood industries with carbon-rich waste may increase the need for human power, thereby contributing to job creation in these areas [95].

The prospect of PHA-based biofuel also relies on the fact that there will be an increase in biopolymer-based plastic and other refined PHA value products, which, after use, can cause waste into the environment. However, why waste the energy in these products and the time to degrade them when it can be transformed into another useful product such as biofuel [86].

The current limitation of PHA based biofuel revolved around the sustainable production of PHA, which serves as its substrate. Another limitation is the availability of large biomass for PHA production. In reducing PHB cost and the influence of the culture waste on the environment, recycling of cultivated medium to be reused by the PHA producing organisms has been demonstrated [9]. This will lead to increased biomass, higher yield of PHB, and subsequently reduce biofuel production cost. Currently, the yields of HBME from the wild microbes are inadequate enough to be cost-effective compared with petroleum-derived fuels. Hence, synthetic biology and metabolic engineering technologies are being engaged to increase production and produce entirely new fuels with better properties [96]. Knowledge of the role of genes used for bacterial lipid absorption, buildup, and processing are critical reflections for optimizing and large-scale production of biofuel from waste effluents and other complex lipid-rich feedstock [97].

Conclusion

Scientists around the world have demonstrated intensive and progressive efforts in the production of biofuels with the major aims of increasing production yield at a reduced price, and ensuring improved quality products. Variables such as the feedstock oils, catalyst type, dissolving solvents, reaction technology type, time and temperature variables have majorly been applied to achieve these significant objectives. Springing from being used as bioplastic to more expedient application in the medical and biomedical sectors, PHAs are finding their way into the energy generation sector, where they can serve as a microbial renewable substrate for fuel generation. The high demand for biofuel to complement the non-renewable fossil fuel makes further research attention a necessity in this area. The recently and upcoming PHA-based biofuel is an important aspect that can contribute to fuel accessibility and also serve as a fuel additive to other generated fuel. PHA-based biofuel also shows great potential in contributing positively to meet fuel demand. Additionally, PHA-based biofuel has higher oxygen content and low sulphuric content when compared with its contemporary fossil fuel. This will allow a reduced accumulation of particulate matter in the environment where this biofuel will be used. Nevertheless, the low cetane number and octane number associated explicitly with these produced biofuels should not be overlooked, as they contribute significantly to the fuel quality. The complexity of its mass per volume in the already generated PHA-based biofuel will cause retarded movement of the fuel in a working engine. It can also result in phase separation if the biofuel is left for an extended period. Besides, the high degradation rate encountered when this fuel is kept at room temperature makes it further challenging to be used in warm regions. Hence, there is a need to explore still and improve biofuel production from PHA substrate, considering the cost and quality of the biofuel production.

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