บทความวิชาการ

The Production of Polyhydroxyalkanoates (PHAs) from Renewable Feedstock Derived from Various Wastes

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The development of biodegradable plastic has been contributing significantly towards the country's economy and decrease environmental problem. Poly(3-hydroxybutyrate) [P(3-HB)] and other polyhydroxyalkanoates (PHAs) have been drawing much attention in the past two decades. For the economical production of P(3-HB), various bacterial strain and new fermentation strategies were developed for the production of P(3-HB) with high concentration and production. However, the major barrier associated with the production of P(3-HB) is its high operation cost. To reduce the cost, several processed for P(3-HB) production from inexpensive substrate were also investigated. Currently the highest production of P(3-HB) was reported to a content of 81% of dry cell weight (DCW) from whey supplemented with citric acid by recombinant Escherichia coli. Nevertheless, the production of P(3-HB) production in the range of 7 – 80% of DCW with various wastes was also investigated. Utilization of waste as substrate was also developed not only for the efficient production of P(3-HB) but also for novel PHAs. Nowadays 90% of polyhydroxyhexanoate [P(HHx)] was produced by *Wauterisa* sp strain PZK cultivated under kraft cellulose mill effluent. With these entire advances, wastes from various industries are very interesting sources for PHAs production. This paper aims at understanding how waste from various sources may serve as a renewable feedstock for the biosynthesis of PHAs.

INTRODUCTION

Since the 2000s, Plastic waste has become a serious problem of contemporary life. Globally plastic waste accumulates in the environment at the rate of about 30 million tons per year. Several disadvantages of synthetic plastic such as that resistant to biodegradation in the nature as well as many toxins produced during the production and elimination process. This latter property, together with the fact that most common plastics are produced from fractions of non-renewable

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petroleum oil and plastic recycling offer limited possibilities, causes a great concern [1, 2]. Problems of global environment and solid waste management have generated interest in the development of eco-friendly biopolymer materials [3, 4, 5]. PHAs and blends of these are attractive substitutes for conventional petrochemical plastics since they have similar physical properties to commercial plastics, made from renewable resources and degradation by environment process within a period of one year. Moreover, PHAs are suitable for applications in several areas such as pharmaceutical and medical application. PHAs are naturally synthesized by a variety of different organisms including bacteria, yeast and plants using renewable resources [6, 7, 8]. PHAs are linear aliphatic polyesters composed of 3-hydroxy fatty acid monomers, produced in the nature under the nutrient restricted condition as a storage granules providing food, energy and reducing power [5, 9]. Nowadays, about 150 monomers of hydroxyalkanoic acids (HAs) have been identified as constituents of microbial polyester. However, the simplest and the most well known of the PHAs family is P(3-HB). It consists of only one type of monomer, 3-hydroxybutyrate (3-HB) [1, 10, 11].

The use of PHAs as substitutes for petroleumbased plastic has been restricted due to the high production cost of PHAs compared with conventional polymers. To produce large amount of PHAs, research and development for the PHAs production with high concentration from inexpensive substrate are emphasized [12, 13, 14]. Wastes and wastewater, low value source from various industries, showed the great potential supply of raw material to manufacture bioplastics, a higher value product.

1. Polyhydroxyalkanoates (PHAs)

PHAs are a class of natural polyesters that deposited intracellularly in the form of inclusion bodies("granules," visible as brilliant globules, 100-500 nm) in the phase contrast microscope and may account for up to 90% of DCW [15, 16]. They are one of the most fascinating and largest groups of thermoplastic polymers known. PHAs are accumulated as a carbon and energy storage material (Figure 1) in various microorganisms usually under the condition of limiting nutritional elements such as nitrogen, phosphorus, sulphate, oxygen, or magnesium in the presence of excess carbon source [1, 12]. PHAs are high-molar-mass polymers, usually in the range of 100 – 1,000 KDa [17], although P(3-HB) produced in recombinant *E. coli* has been reported to have ultra-high-molar-mass (M_n) of 20,000 KDa [18]. Molar mass depends on the microbial species and culture conditions such as pH and type and concentration of the carbon source [19].

Depending on the length of their monomers, three distinct groups of PHAs are extensively studied: the short chain length (scl-PHAs) such as P(3HB) and 3-hydroxyvalerate P(3-HV), the medium chain length (mcl-PHAs) containing monomer length of 6 to 14 carbon atoms and long chain length (lcl-PHAs), respectively. However, only scl-PHAs have properties close to conventional plastics while the mcl-PHAs are regarded as elastomers and rubbers. More than 100 different monomer units were found as constituents of PHAs, produced by Gram-positives and Gram-negative species [20]. The mechanical properties of PHAs is similar to those of polyethylene or polypropylene, with the additional advantage of being completely biodegradable, biocompatible and produced from renewable resources [11, 21].

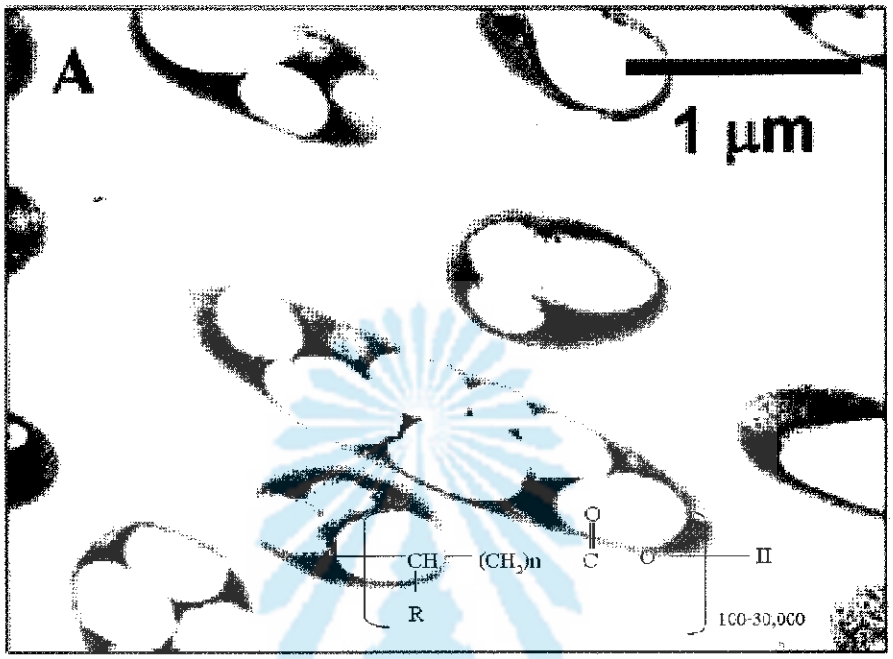

Figure 1 Chemical structure of polyhydroxyalkanaotes (PHAs).

n = 1	R = hydrogen	Poly(3-hydroxypropionate)
	R = methyl	Poly(3-hydroxybutyrate)
	R = ethyl	Poly(3-hydroxyvalerate)
	R = propyl	Poly(3-hydroxyhexanoate)
	R = pentyl	Poly(3-hydroxyoctanoate)
	R = nonyl	Poly(3-hydroxydodecanoate)
n = 2	R = hydrogen	Poly(4-hydroxybutyrate)
	R = methyl	Poly(4-hydroxyvalerate)
n = 3	R = hydrogen	Poly(5-hydroxyvalerate)
	R = methyl	Poly(4-hydroxydodecanoate)

Source: modified from [21].

2. Recent trends in the production of PHAs

Four commercial bands of PHAs are currently available including Biopol™ (copolymer of hydroxybutyrate (HB) and hydroxyvalerate (HV), Biomer™ (homopolymer of HB), Nodax™ [copolymer of HB and hydroxyhexanoate (HHx)] and Biocycle™ (homopolymer of HB, copolymer of HB and HV). All of these polymers are produced by wild type strains such as *Alcaligenes latus* (Biomer™) and *Burkholderia sacchari* (Biocycle™) in pure cultures or by using genetically modified strains

such as recombinant *E. coli* (Biopol™) or recombinant *Wautersia eutropha* (Nodax™) [22]. Although the production cost of PHAs are still limited. The cost difference between synthetic plastics and PHAs has been the main obstacle for its replacement in market [23]. Hence, there is a potential for widening the market for PHAs, provided that their cost decreases. Nowadays PHAs production is in the range of 10,000 – 50,000 tons per year. However, even the largest production capacity quoted above is small when compared to the whole plastics market, estimated at 150 million tons per year. Table 1 shows market prices of PHAs compared to conventional and petroleum-based polymer. The data indicated PHAs cost is 1 - 3 folds higher than synthetic polymer [24].

As a result, much of the literature has focused on reducing the cost of production of PHAs. With this global aim, different approaches have been adopted: the used of various fermentation strategies [13, 14, 25, 26], development of more efficient recovery processes [27, 28], use of recombinant DNA technology and metabolic engineering [29, 30], production of PHAs in transgenic plants [31], process integration with other products [32] and use of inexpensive carbon sources as raw materials [33, 34, 35]. The cost contribution of the carbon source per kilogram of produced P(3HB) based on the theoretical yields estimated indicated that fermentation based on pure glucose, the substrate cost amounts to €1.02/kg P(3HB), whereas for crude carbon sources such as chesses whey the substrate contribution lower to €0.17/kg P(HB). It becomes clear that, if based on expensive substrates such as glucose, even the most efficient processes will not enable PHAs to compete on the basis of price with petroleum-based polymers [24]. Taking into consideration that PHAs content and productivity are usually lower for bacteria grown

Table 1 Market price of bio-based polymers and conventional, petroleum-based polymers (December, 2009).

Polymer	**Producer/Company**	**Market price (€/kg)**
P(3HB)	Biomer™ (Germany)	12
P(3HB-co-3HV)	Metabolix (USA)	10 – 12
Modified starch polymer	Novamount (Italy)	2.5 – 3.0
Polylactic acid (PLA)	Cargill Dow (USA)	2.2 – 3.4
Polypropylene (PP)		0.74
High-density polyethylene (HDPE)		0.78
Low-density polyethylene (LDPE)		0.74
Polyvinyl chloride (PVC)		0.70
Polystyrene (PS)		0.70
Polyethyleneterphtalate (PET)		0.81

Source: [24, 36]

in crude, inexpensive substrate [24, 27], the development of efficient processes based on crude carbon sources, such as agro-industrial by products and waste, remains a challenge to be pursued.

3. The production of PHAs from various wastes

PHAs productivity, PHAs content of the biomass, PHAs yield on the carbon used, cost of raw materials and the recovery methods are the main problems preventing the commercial application of PHAs [27]. Most importantly, as the process is scaled-up, the raw materials contribute an increasing fraction of overall manufacturing costs and these costs of raw materials are dominated by the carbon source [27]. Therefore, the economic feasibility of bulk PHAs production is intrinsically coupled to developing efficient biotechnological processes from inexpensive carbon sources. Additionally, the use of waste products as carbon sources presents the advantage of simultane-ously enabling a decrease in disposal costs and the production of value-added products. The production of PHAs by different inexpensive carbon sources using various microorganisms is summarized in Table 2.

Table 2 Examples of PHAs production from various wastes

Type of wastes	Microorganisms	Fermentation condition	Type of PHAs	PHAs content (%)	References
Pea shell slurry	*Bacillus cereus*	Shaken flask	P(3HB)	41	[36]
Saccharified waste	*Rastonia eutropha* NCIMB 11599	NG[a]	P(3HB)	46	[37]
Mollasses	*Bacillus* sp.	Shaken flask	P(3HB)	7.92	[38]
Peach pulp				7.78	
Malt waste	Activated sludge	Sequence batch reactor	P(3HB-co-HV)	70	[39]
Hydrolyzed whey	Osmophillic organism	42 L bioreactor	P(3HB-co-HV)	49.6	[40]
Beet molasses	*B. megaterium*	NG[a]	P(3HB)	52	[41]
Malt waste	Activated sludge	Fed-batch	P(3HB)	69	[42]
Fermented organic waste	*R. eutropha* TF 93	Batch	P(3HB-co-HV)	40	[43]
Whey	Recombinant *E. coli*	Fed-batch (2.5L)	P(3HB)	80	[44]

Table 2 (cont.)

Type of wastes	Microorganisms	Fermentation condition	Type of PHAs	PHAs content (%)	References
Paper mill wastepaper	Activated sludge	Batch	P(3HB-co-HV)	48	[45]
Raw rice grain-based distillery spentwash	Activated sludge	Shaken flask	P(3HB)	42.3	[5]
Anaerobic wastewater	Activated sludge	Batch	P(3HB)	58	[46]
Kraft cellulose mill effluent	*Sphingopyxis chilensis* S37	Shaken flask	P(3HB)	80	[47]
	Wauterisa sp. PZK		P(HHx)	90	
Acidified wastewater	*Alcaligenes eutrophus*	Fed-batch (2L)	P(3HB-co-HV)	60	[48]
Municipal wastewater	Activated sludge	Batch	P(3HB)	21	[49]
Municipal wastewater + acetate				31	
Olive oil wastewater	*Pseudomonas putida* KT2442	NG[a]	PHAs	3.59	[50]
Swine waste liquor	*Azotobacter vinelandii UWD*	NG[a]	P(3HB-co-HV)	34	[51]
Alcoholic distillery wastewater	*Actinobacillus* sp EI-9	NG[a]	P(3HB)	42	[52]
Beet molasses (1% w/v)	*B. cereus* M5	Batch	P(3HB)	73.8	[53]

Table 2 (cont.)

Type of wastes	Microorganisms	Fermentation condition	Type of PHAs	PHAs content (%)	References
Beet molasses (5% w/v)	*A. vinelandii* UWD	Fed-batch	P(3HB)	38.5	
Beet molasses				66	
Beet molasses + valerate			P(3HB-co-HV)	59 -71	
Beet molasses + salts + trace metals	Recombinant *E. coli*	Fed-batch	P(3HB)	80	
Sugarcane liquor	*P. fluorescens*	Batch	P(3HB)	70	[21]
Sugarcane liquor	*Bhurkolderia* sp. and *C. necator*	Fed-batch	P(3HB)	65 – 70	
Soluble starch	*A. chroococcum*	Fed-batch	P(3HB)	46	
Soluble starch	*B. cereus*	Batch	P(3HB)	48	
Wheat hydrolysate	*C. necator*	Fed-batch	P(3HB)	70	
Bagasse hydrolysate	*B. sacchari* IPT101	Batch	P(3HB)	62	
Bagasse hydrolysate	*B. cepacia* IPT048	Batch	P(3HB)	53	
Bagasse hydrolysate	*C. necator*	Batch	PHAs	65	
Xylose + soybean hydrolysate	Recombinant *E. coli*	Batch	P(3HB)	73.9	

Table 2 (cont.)

Type of wastes	Microorganisms	Fermentation condition	Type of PHAs	PHAs content (%)	References
Whey + citric acid	Recombinant *E. coli*	Batch	P(3HB)	81.3	
Olive oil mill waste Corn oil waste Palm oil waste	Recombinant *C. necator*	Batch	P(3HB-co-3HHx)	76 – 81	[24]
Soybean oil	*C. necator*	Fed-batch	P(3HB)	72 – 76	
	Recombinant *C. necator*		P(3HB-co-3HHx)	71 – 74	
Residual oil	*C. necator*	Batch	P(3HB)	19.7	
		Fed-batch		41.3	
	P. oleovorans	Batch	P(3HB-co-3HHx-co-3HO-co-3HD-co-3HDD)	17.3	
		Fed-batch		38.9	
Tallow waste	*P. resinovorans*	Batch	PHAs	15	
Whey + casamino acids	*P. hydrogenovora*	Fed-batch	P(3HB)	12	
Whey + casamino acids + valerate		Fed-batch	P(3HB-co-HV)	12	
Whey + corn steep liquor	Recombinant *E. coli*	Fed-batch	P(3HB)	72.9	
Wheat based biorefinery	*C. necator*	Batch	P(3HB)	60	

Table 2 (cont.)

Type of wastes	Microorganisms	Fermentation condition	Type of PHAs	PHAs content (%)	References
Olive oil mill waste Corn oil waste Palm oil waste	*C. necator*	Batch	P(3HB)	79 – 81	

NG[a] = Not Given

The production of PHAs from glycerol waste

As aforemention, several reports on the production of PHAs are from cheap carbon sources by wild-type and recombinant PHAs producer. However, the PHAs concentration and PHAs content obtained were considerably lower than those obtained using purified carbon substrates [12].

Glycerol is a main by-product of biodiesel production generated from the tranesterification process. An annual production of biodiesel is approximately 150 million gallon per year; an amount of 50 million Kg of crude glycerol is generated. However, glycerol which obtain from biodiesel process is impure and of low economic value. With the even-growing production of biodiesel and by-product glycerol, it has been suggested that the open market value of crude glycerol may be eventually stabilize at low price of $0.05/lb, but the cost to refine this crude glycerol will cost approxi-mately $0.20/lb [36]. Although pure glycerol is an important industrial feedstock with found in various application such as food, drug, cosmetic and tobacco industries. But, purification is costly and often out of the range of economical feasibility for small and medium sized plants. The alternative uses for the by-product glycerol from biodiesel will need to be found.

Converting glycerol into value-added products provides an alternative for glycerol disposal and for its surplus problems. Though thermo-chemical processes, glycerol can be converted into propylene glycol and acetol. It can also serve as carbon source in fermentation processes to produce various products such as 1,3 propanediol, lipid and pigment. Anaerobic fermentation of glycerol by *E. coli* also generates a mixture of products such as ethanol, succinate, acetate, lactate and hydrogen [54]. Interesting the production of PHAs from low cost glycerol was also reported [34, 55]. However, only little information has been published. Besides some data of the taxonomic description of the genus *Methylobacterium* processes were patented for the production of PHB by *M. rhodesianum* MB 126 and *R. eutropha* DSM 11348 [56]. *M. extorquens* and *R. eutropha* produced P(3HB) during cultivation on a mineral medium containing glycerol [57].

PHAs production in waste glycerol and commercial glycerol was investigated. The results indicated that maximum P(3HB) concentrations achieved in the waste glycerol (30% DCW) were

approximately the half of those obtained in commercial carbon source (62% DCW). However, optimization of the time point to impose nitrogen depletion to induce PHAs accumulation in the waste glycerol cultivations provided 50% P(3HB) content and resulted in a 30% increase in productivity (1.1 g/L/h), as compared to the productivity (0.84 g/L/h) that had been initially obtained [56]. A wide type highly osmophilic microorganism grown on another glycerol-rich waste from biodiesel industry, supplemented with yeast extract and peptone produced a PHAs concentration of 16.2 g/L. When the expensive nitrogen sources (yeast extract and peptone) were replaced by hydrolyzed meat-and-bone meal (MBM), PHAs production decreased to 5.9 g/L. Despite this, an interesting finding was that the strain investigated was able to produce a P(3HB-co-HV) containing 8 – 10% (w/w) of 3HV unit directly from the glycerol rich medium, without any need for precursors such as proionic or valeric acid [34]. *Jatropha* biodiesel byproduct was also studied as carbon source for PHAs production by *B. sonorensis* and *Halomonas hydrothermalis*. Both bacteria utilized *Jatropha* biodiesel byproduct containing crude glycerol for growth and PHB biosynthesis and accumulated PHB up to 71.8 and 78%, respectively [58].

4. Conclusion and future outlook

Biodegradable polymers and especially PHAs have rapidly gained interest both in research and industry. Although their manufacturing costs today are still too high to compete with conventional and petroleum-based polymers, advance in biotechnological processes using inexpensive carbon sources combined with the long-term increasing trend of oil prices will certainly improve PHAs competitiveness and make a broad use of these biopolymers possible in the future. The production of PHAs from cheap raw material including a variety of waste and by products has been explored using bacteria. However, the potential for PHAs production seem to be limited by the consistency and reliability of the raw material. Therefore, storage issue and the correct balance of the ingredients will need to be carefully scrutinized [1, 59]. The chance to increase PHAs yield and productivity as well as PHAs variety and ease of polymer recovery will depend on the successful discovery of cheap raw materials which can offer microorganism to produce high PHAs with a variety of PHAs monomer. This will further decrease the limited of PHAs application.

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