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Polyhydroxyalkanoates production from waste biomass

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Abstract. Polyhydroxyalkanoates (PHAs) is a group of biopolymers that are extensively researched for such purpose due to the biocompatibility with mammal tissue and similar properties with conventional plastic. However, commercialization of PHA is impended by its high total production cost, which half of it are from the cost of pure carbon source feedstock. Thus, cheap and sustainable feedstocks are preferred where waste materials from various industries are looked into. This paper will highlight recent studies done on PHA production by utilizing crop and agro waste material and review its potential as alternative feedstock.

1. Introduction
Polyhydroxalkanoate (PHA) is one of the most researched bioplastic in hope to replace conventional plastic of today. This biopolymer is a group of naturally-occuring biopolysters produced by microorganisms under imbalanced growth condition due to carbon source surplus and/or limitation of essential nutrient such as phosphorus and nitrogen [1]. Classification of PHAs depends on the number of carbon atoms present in their monomers as short-chain-length PHAs (scl-PHA; three to five C-atoms), medium-chain-length PHAs (mcl-PHA; with six or fourteen C-atoms) and long-chain-length PHAs (lcl-PHA; more than 14 carbon atoms) [1].

Currently, PHA and its co-polymers have been researched for its potential applications in various industry and fields, for example, poly-(3-hydroxybutyrate) (P3HB) is a homopolymer of PHAs group that are toxicologically safe and exhibit high biocompatibility with mammalian cells, making them suitable for medical applications and food industry [1,2,3]. Additionally, the thermoplastic properties of P3HB and its biodegradability, without generation of toxic by-products, makes it a potential sustainable alternative to conventional plastics [2,3]. Unfortunately, PHA production cost remains substantially expensive (about USD8–11 per kg) compared to other polymers, and has been one of the main obstacle in exploitation of PHA as commercial commodity [2].

Most of the carbon sources used for conventional PHA production are of pure raw material, comprising of pure carbohydrates (glucose, sucrose, maltose, starch), fatty acids and its derivatives, methanol and alkanes [2]. In terms of the production cost, carbon source for microbial growth attributed to 50% of the total costs [3], thus it is desirable to find cheap alternative carbon feedstocks in order to improve sustainability of PHA production while obtaining commercial viability through

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fermentation. As such, inexpensive carbon sources such as agro-industrial by-products and agricultural residues have been reported as potential alternative substrates. Therefore, this paper will review on the current advances involving utilization of biomass from various waste from agriculture, agro-industrial practice and domestic waste for PHA production as well as the challenges and opportunities for commercialisation.

2. PHA Production from Biomass

2.1. Sugar Rich Feedstock
Sugar producing plant such as maple tree, which is known for its sweet sugar sap, has been studied as carbon feedstock for PHA production. Yezza et al. employed maple sap as a carbon source to Alcaligenes latus (now known as Azohydromonas lata) for the production of poly-beta-hydroxybutyrate (PHB) and was able to produce up to 78% of this bioplastic monomer [4]. However when feedstock is varied, for example conversion of sugarbeet juice using the same microbe A. lata, Wang et al. reported lower PHA yield of 66% compared to pure sucrose which gave 80.0% of PHA [5]. This suggests that PHA yield depends on the total chemical composition of the sap/juices used. In another study, Suvannasing et al. utilized juice from pineapple for the production of PHB using isolated Bacillus sp. 5V13 from agriculture soil. The study showed that 56% of PHB could be produced from pineapple juice compared to pure glucose and sucrose (18.5% and 29%) [6].

In a relatively new sugar rich feedstock, Zahari et al. showed that oil palm frond can be simply compressed to obtain their plant juice or sap, which contains high glucose of 53.95 g/l that constitute about 70% of its total sugar content [7]. The study reported a PHA yield of 30.5 g/l, comprising of 75 wt% of the biomass dry weight.

Besides virgin feedstocks, sugar rich by-products are also readily available as raw material for bioconversion into high value products. Molasses is a by-product of sugar manufacturing industries and is currently being utilized as a carbon source for industrial scale fermentations due to its abundance and low price. It is estimated that 12 million ton of beet molasses and 39 million ton of cane molasses are readily available for further conversion [4]. In a study by Albuquerque et al., a three-step fermentation strategy was proposed for cane molasses fermentation to produce PHA. The molasses were fermented to organic acids in the first step followed by PHA accumulation, and then finally, batch fermentation for the production of PHA, where about 30% cell dry weight (CDW) of PHA is produced. [8]. Similarly, soy molasses has also shown positive conversion to PHA resulting in 5-17% of mcl-PHAs and lcl-PHAs [11]. A study done by Obruca et al. using waste rapeseed oil with precursor propanol is able to produce up to 76% PHA and 8% 3-hydroxyvalerate (3-HV) in co-polymer [12]. Another study using same carbon source showed that the high nitrogen content in heated oil due to thermal degradation was able to increase cell growth and eventually increase PHA yield [13].

Crude glycerol gained more interest as feedstock due to its large quantities as by-product in various industrial processes, particularly in biodiesel production. Crude glycerol from soy-based biodiesel has been studied by utilizing Pseudomonas corrugata 388 and Pseudomonas oleovorans NRRL B-14682 resulting in accumulation of mcl-PHAs [14]. A study by Mozumder et al. yielded the highest PHA content up to 63% by using crude glycerol via a three step fermentation process [15]. In an attempt to
reduce production cost further, a study utilizing crude glycerol combined with meat and bone meals as a nitrogen source was done and it resulted in 5.9 g l$^{-1}$ PHAs and a co-polymer PHBV without addition of precursor in the media [16].

Another interesting alternative carbon source is wastewater from industrial practices such as biodiesel wastewater, food processing waste effluent, municipal wastewater and brewery waste effluent [17]. In the first step, organic carbon sources from wastewater are converted into volatile fatty acid in aerobic activated sludge, followed by PHAs conversion by mixing cell cultures in the second step. Generally, PHA concentrations from wastewater are low in current studied settings, with only around 50% production of PHA [17, 18]. Martinez et al. managed to accumulate 55% PHA content with 11% of HV when pure Cupriavidus necator is fed with pre-treated olive mill wastewater, without synthetic precursor under acidogenic condition [19]. However, another study by Khardenavis et al. reported high PHA yield of 67% from mixed microbial culture (MMC) by using rice grain distillery wastewater as carbon source [20]. The high yields were only achieved after adding di-ammonium hydrogen phosphate (DAHP) as extra nitrogen source.

**Figure 1.** Various feedstock and their respective pre-processing technology prior to microbial PHA

### 2.3. Lignocellulosic Biomass

Agricultural practices often produces abundant lignocellulosic biomass. Utilization of non-food lignocellulosic materials for PHA production is preferable over food crops. Usually, lignocellulosic materials need to be pre-treated and undergo hydrolysis to convert to sugar monomers. In a study by Cesario et al., PHA production from wheat straw hydrolysate is able to accumulate up to 72% CDW through fed-batch fermentation using Burkholderia sacchari DSM17165 [21]. It was also stated that poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV production by utilizing the same carbon source can also be achieved using propionic acid as a co-substrate. Most research on utilizing lignocellulose as carbon feedstock centre around the conversion of the monomer sugars from hemicelluloses.

Other agro industrial residues such as inexpensive mixture of extruded rice bran and wheat bran also has been studied by using haloarchaen Haloferax mediterranei, which resulted in 56% of PHA content [22]. While the resulting PHA is still relatively low, it is important to note that extraction using archaea is inexpensive and safe due to the lack of chemicals used for the separation and purification of the PHA accumulated in the haloarchaea species.
Therefore, the production of PHA depends on many factors, which is the feedstock quality, microbes used, nutrients and pre-processing technology. The PHA production via microbial fermentation of various feedstocks and their pre-processing technology is summarized in figure 1 and table 1 below.

### Table 1. PHA production in terms of cell dry weight percentage (%CDW) from microbial fermentation of various waste biomass

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Microorganism</th>
<th>PHA Content, Cell Dry Weight (%CDW)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy molasses</td>
<td><em>Pseudomonas corrugata.</em></td>
<td>5.00-17.00</td>
<td>[9]</td>
</tr>
<tr>
<td>Molasses (sugarcane)</td>
<td><em>Bacillus cereus</em> SPV</td>
<td>61.07</td>
<td>[10]</td>
</tr>
<tr>
<td>Crude glycerol</td>
<td><em>Cupriavidus necator DSM545</em></td>
<td>62.70</td>
<td>[15]</td>
</tr>
<tr>
<td>Wheat straw hydrolysate</td>
<td><em>Burkholderia sacchari DSM 17165</em></td>
<td>72.00</td>
<td>[21]</td>
</tr>
<tr>
<td>Extruded rice bran and wheat bran</td>
<td><em>Haloferax mediterranei</em></td>
<td>56.00</td>
<td>[22]</td>
</tr>
<tr>
<td>Rice grain distillery wastewater with nutrient added</td>
<td><em>Activated sludge (MMC)</em></td>
<td>67.00</td>
<td>[20]</td>
</tr>
<tr>
<td>Olive Mill Wastewater</td>
<td><em>Cupriavidus necator DSM 545</em></td>
<td>55.00 (11% 3HV co-polymer)</td>
<td>[19]</td>
</tr>
<tr>
<td>Waste rapeseed oil</td>
<td><em>Cupriavidus necator H16</em></td>
<td>76.00 (8% 3-HV co-polymer)</td>
<td>[12]</td>
</tr>
<tr>
<td>Pineapple juice</td>
<td><em>Bacillus sp.</em></td>
<td>48.89</td>
<td>[6]</td>
</tr>
<tr>
<td>Sugarbeet Juice</td>
<td><em>Azohydromonas lata</em></td>
<td>65.60</td>
<td>[5]</td>
</tr>
<tr>
<td>Maple Sap</td>
<td><em>Azohydromonas lata</em></td>
<td>77.60</td>
<td>[4]</td>
</tr>
<tr>
<td>Oil Palm Frond Juice</td>
<td><em>Cupriavidus necator NCIMB 11599</em></td>
<td>75.00</td>
<td>[7]</td>
</tr>
</tbody>
</table>

### 3. Challenges and Opportunities

Cellulose and hemicellulose from lignocellulosic materials are both excellent non-food carbon source to be used in different biological processes after hydrolysis to monomeric sugars. However, the process is cost inefficient due to natural lignin component that is recalcitrant to degradation. Various pre-treatments are required to loosen up the lignocellulose structure followed by utilization of enzymes or special microbial strains to convert the holocellulose into sugar feedstock which contributed to additional cost [18]. Furthermore, hydrolysis of lignocellulose waste by chemical and/or enzymatic treatments unfortunately releases several inhibitory compounds, such as phenolic, furans and organic acids which can have negative effect to microbial fermentation. To solve this, an adapted
tolerant strain of *C.s necator* is engineered, which can grow in diluted bagasse hydrolysates and efficiently reduce acetic acid, formic acid, furfural and acid soluble lignin while producing PHAs [23]. The polyesters produced were mainly PHB (57% on CDW), but also included PHBV. *C. necator* strain mainly utilizes glucose as carbon source leaving potential down streaming of the unutilised sugar components in the treated hydrolysates in the production of biofuels and other chemical.

As for the usage of wastewater for PHA production, it inevitably causes the formation of non-storing PHA population which may adversely affect production of the biopolymer. In a study by Marang *et al.* using a mixed culture dominated by *Methylobacillus flagellatus*, an obligate methylotroph, that cannot store PHA, and *Plasticicumulans acidivorans*, a known PHA producer, has lowered the PHA content from 80 to 66% wt, due to the presence of the non-storing population [25]. The fermentation was enriched with acetate and methanol, as they reduced the effect of methylotroph *M. flagellatus* on the PHA production [25].

Usage of virgin oils such as soybean produces higher PHA compared to waste oils, which may have been due to the presence of impurities in the waste oils [18]. However, the usage of edible oils for PHAs production is no longer considered cost effective due to increase in price. Thus the focus is on non-edible oils and waste cooking oils. Crude glycerol from biodiesel is one of the feedstock that cannot be used as raw material for drug, food and pharmaceutical industries due to the presence of major impurities [15]. Refining crude glycerol is costly, thus the biological conversion of crude glycerol to higher value chemicals, such as PHA, is more desirable. Unfortunately, it is important to note that utilization of glycerol can affect the quality of the polymer by reducing its molecular mass, thus changing the properties of the PHA [15].

Conversely, PHA production using sugar based crops generate higher yield, depending on the sap and strain type used as shown in Table 1. Plant sap or juice consists of high sugar content and as well as minerals such as potassium, nitrogen and other which are essential for PHA accumulation and microbial growth [5, 4, 7]. The surplus of sugar and presence of mineral make the plant juice or sap as potential natural media for microbial fermentation. This is supported by a study by Zahari *et al.* which showed that the PHA extracted from oil palm frond juice produced relatively high molecular mass; an important factor in determining PHA’s physicochemical and mechanical properties [7, 25]. The only drawback from using plant saps and juices as carbon feedstock is the negative view on using potential sugar crops for food.

4. Conclusion

PHA differs from conventional plastics as it is non-toxic and biodegradable where the end degradation products are mostly carbon dioxide and water. Current PHA production is still hindered by raw material as carbon feedstock and downstream-processing cost. Various waste biomass from agro-industry and municipal wastes have been looked into as cheap alternative carbon source. However, it has been highlighted in this paper that each alternative feedstock has its challenges towards commercialization of PHA. However, sugar based carbon source produced notably higher PHA in term of CDW, compared to other feedstock and not requiring pre-treatment prior to bioconversion of PHA. The production of PHA may turn to be cost efficient when alternative or underutilized waste or crops are used to make non-edible sugars for PHA fermentation. More study on alternative sugar resources are needed to investigate this notion.

5. References

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