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# Perspectives on the production of polyhydroxyalkanoates in biorefineries associated with the production of sugar and ethanol

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ABSTRACT

Polyhydroxyalkanoates (PHA) are biodegradable and biocompatible bacterial thermoplastic polymers that can be obtained from renewable resources. The high impact of the carbon source in the final cost of this polymer has been one of the major limiting factors for PHA production and agricultural residues, mainly lignocellulosic materials, have gained attention to overcome this problem. In Brazil, production of 2nd generation ethanol from the glucose fraction, derived from sugarcane bagasse hydrolysate has been studied. The huge amounts of remaining xylose will create an opportunity for the development of other bioprocesses, generating new products to be introduced into a biorefinery model. Although PHA production from sucrose integrated to a 1G ethanol and sugar mill has been proposed in the past, the integration of the process of 2G ethanol in the context of a biorefinery will provide enormous amounts of xylose, which could be applied to produce PHA, establishing a second-generation of PHA production process. Those aspects and perspectives are presented in this article.



## 1. The potential of polyhydroxyalkanoates (PHA) as bioplastics

PHA represent a large family of thermoplastic polymers accumulated as intracellular granules by a wide variety of bacteria as carbon and energy storage compounds [1]. Poly-3-hydroxybutyrate (P3HB) and the copolymer poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate (P3HB-*co*-3HV) are the most studied PHA [2]. Interest on PHA has greatly increased during the past decade because, besides being biodegradable and biocompatible, they can be obtained from renewable resources [3].

P3HB and other PHA productions have been explored since 1980 and since then many enterprises and researching companies have invested to improve its production in a commercially viable way [4,5]. However, they are still more expensive than conventional petrochemical polymers [6]. A number of studies have been performed to estimate the cost of PHA production [3,7–10]. Various factors involved in the production are mentioned as important to be improved to reduce the elevated costs of industrial production [4]. The expense associated to the carbon source to feed PHA-accumulating bacteria has been considered as one of the major limiting factors for polymers production, accounting for up to 50% of the overall production costs [11], decreasing to approximately 30% when PHA production is integrated to a sugarcane mill [10]. This can be attributed to the use of noble carbon sources to feed PHA-producing bacteria, namely pure carbohydrates, alkanes and fatty acids [6]. Therefore, the use of less expensive, locally available and renewable carbon sources or by-products would be of economic interest. Thus glycerol, molasses, cheese whey, agricultural and forest biomass residues have been considered as alternative carbon sources to produce PHA [6,12–15]. Also strategies intended to enhance the sustainability of PHA production processes have been proposed [15,16].

### 1.1. Use of low-cost sugars from biomass as raw materials to produce PHA

Carbon sources derived from sugarcane and hydrolysates have been tested for the production of PHA since de decade of 1990

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**Table 1**
Data of PHA production using lignocellulosic hydrolysates or main sugars present on hydrolysates reported in literature.

| Strain | Carbon source | PHA | CDW (g/l) | PHA content (% CDW) | $Y_{PHA/C}$ (g/g) | $\mu_{Xmax}$ (g/g h) | $q_{PHB\ max}$ (g/g h) | Productivity (g PHA/L h) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Pseudomonas pseudoflava ATCC* 33668 | Xylose | P3HB | 4.0 | 27.5 | 0.04 | 0.13 | 0.03 | 0.03 | [24] |
| *Burkholderia cepacia ATCC* 17759 | Xylose | P3HB | 7.5 | 45.0 | 0.11 | 0.22 | 0.07 | 0.10 | [25] |
| *Escherichia coli* TG1 (pSYL107) | Xylose | P3HB | 4.8 | 35.8 | 0.10 | – | – | 0.03 | [26] |
| *Escherichia coli* TG1 (pSYL107) | Xylose + cotton seed hydrolysate | P3HB | 3.8 | 64.0 | 0.19 | – | – | 0.03 | [26] |
| *Escherichia coli r* TG1 (pSYL107) | Xylose + soybean hydrolysate | P3HB | 6.0 | 73.9 | 0.23 | – | – | 0.07 | [26] |
| *Burkholderia sacchari* IPT101 | Sugarcane bagasse hydrolysate | P3HB | 4.4 | 62.0 | 0.39 | 0.24 | 0.16 | 0.11 | [14] |
| *Burkholderia sacchari* IPT101 | Xylose + glucose | P3HB | 60.0 | 58.0 | 0.22 | 0.25 | 0.03 | 0.47 | [14] |
| *Burkholderia cepacia* IPT048 | Sugarcane bagasse hydrolysate | P3HB | 4.4 | 53.0 | 0.29 | 0.36 | 0.08 | 0.09 | [14] |
| *Burkholderia cepacia* IPT048 | Xylose + glucose | P3HB | 57.0 | 57.0 | 0.19 | 0.28 | 0.06 | 0.46 | [14] |
| *Burkholderia cepacia*[a] | Aspen wood hydrolysate and levulinic acid | P(HB-co-3HV) | 5.0 | 40.0 | – | – | – | – | [11] |
| *Burkholderia cepacia*[a] | Maple wood hydrolysate and levulinic acid | P(HB-co-3HV) | 4.1 | 39.0 | – | – | – | – | [11] |
| *Halomonas boliviensis* | Wheat bran hydrolysate and potato waste biodigested | P3HB | 6.6 | 43.0 | 0.39 | – | – | 0.14 | [27] |
| *Burkholderia sacchari* IPT 101[a] | Xylose | P3HB | 5.5 | 58.1 | 0.23 | 0.32 | – | 0.07 | [28] |
| *Baccilus* sp. *M.A. 3.3* | Xylose | P3HB | 5.8 | 62.2 | 0.24 | – | – | 0.06 | [29] |
| *Burkholderia sacchari* LFM 828 PTS$^-$ glu$^+$[a] | Xylose | P3HB | 2.9 | 42.5 | 0.09 | 0.08 | – | 0.03 | [28] |
| *Burkholderia sacchari* LFM 828 PTS$^-$ glu$^+$[a] | Xylose + yeast extract | P3HB | 5.3 | 50.0 | 0.17 | 0.35 | – | 0.07 | [28] |
| *Burkholderia sacchari* LFM 828 PTS$^-$ glu$^+$[a] | Xylose + glutamate | P3HB | 5.5 | 48.1 | 0.15 | 0.40 | – | 0.05 | [28] |
| *Burkholderia sacchari* DSM 17165 | Wheat straw hydrolysate | P3HB | 100–145 | 45–72 | 0.16–0.22 | 0.31 | – | 1.3–1.6 | [6] |
| *Burkholderia* sp. *F24* | Sugarcane bagasse hydrolysate | P3HB | 25.8 | 44.0 | 0.21 | 0.12 | 0.03 | 0.29 | [30] |

Polydroxyalkanoate, PHA; poly-3-hydroxybutyrate, P3HB; cell dry weight, CDW; polymer yield on carbon source, $Y_{PHA/C}$; maximum specific growth rate, $\mu_{Xmax}$ and specific polymer accumulation rate, $q_{PHB\ max}$.
[a] Shaken-flask culture.

[7,10,13,18–23]. Different results on PHA production from lignocellulosic hydrolysates and main sugars present on hydrolysates reported in literature are summarized in Table 1.

Most of the experiments reported on Table 1 were exploratory. Two groups performed bioreactor experiments and reached high cell densities [6,14]. Interestingly both tested the same *Burkholderia sacchari* strain and media based on hydrolysates of agricultural residues (Table 1). Strain DSM17165 is a wild type *B. sacchari* able to grow on xylose as single carbon source and also in sugar mixtures as previously demonstrated [14,28,30].

Silva et al. [14] adopted three approaches to study the conversion of sugarcane bagasse derivatives to produce PHA: (a) screening of soil bacteria able to grow and to accumulate P3HB from xylose and from hydrolysates, (b) evaluation of detoxification procedures for bagasse hydrolysates and (c) bioreactor cultures for the development of high-cell-density protocols. Among 55 strains previously selected as good P3HB producers from sucrose, glucose and/or fructose [17], 54 were able to grow and accumulate P3HB also from xylose, an expected result, since xylose is the second most abundant sugar in nature. Three of them were selected based on the higher efficiency in converting xylose to P3HB (above 65% of the maximum theoretical yield). They were then evaluated for the ability to utilize bagasse hydrolysate after different detoxification procedures. *B. sacchari* was the strain showing the best performance when the hydrolysate submitted to three detoxification steps was supplied. Bioreactor experiments were supplied either with sugarcane bagasse hydrolysate or with mixtures of xylose and glucose in concentrations mimicking the hydrolysate composition. Higher polymer contents and yields were observed under phosphate limitation. Productivity values reached up to 0.47 g l$^{-1}$ h$^{-1}$. P3HB yield from carbon source was significantly higher when hydrolysate was supplied, indicating that other non-measured carbon sources from the hydrolysate were also efficiently utilized by *B. sacchari*.

Cesário et al. [6] achieved promising results for an industrial process using lignocellulosic hydrolysates from wheat straw. High cell density up to 145 g/L with 72% polymer content was obtained, resulting on productivity values of 1.6 g/L h (Table 1). Results obtained with *B. sacchari* confirm that this is a very promising industrial strain.

Usually, in microbial processes supplied with mixtures of glucose and xylose, either on hydrolysates or on combinations of analytical grade carbon sources, glucose is consumed faster than xylose. One possible explanation for this behavior is the carbon catabolite repression (CCR), a phenomenon mediated by the phosphotransferase system (PTS) in Gram-negative bacteria [31] and by the catabolite control protein CcpA in Gram-positives [32]. Another possibility is that xylose is less efficiently used by the bacteria due to low gene expression or enzymes activity. Whatever the phenomenon involved, it may result in the accumulation of xylose in the medium during a fed-batch process, leading to possible inhibitory effects. The overexpression of genes involved on xylose metabolism has resulted on increase of the rate of xylose consumption, a result compatible with the second hypothesis [33–35].

To avoid the accumulation of xylose in a fed-batch process, Cesário and coworkers [6] applied a feeding regime based on the measurement of a decrease on metabolic activity. The decrease of metabolic activity due to the total consumption of glucose did