Biodegradable polyesters from agro&industrial by-products

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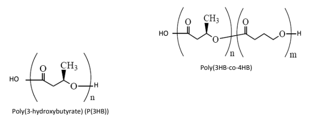
Introduction

Polyhydroxyalkanoates

- Polyhydroxyalkanoates (PHAs) are biodegradable, compostable polyesters synthesized by many microbial strains under unbalanced growth conditions [1] [2].
- Under appropriate feeding strategies, co-polymers with a wide range of properties can be produced, e.g. for agricultural, biomedical and packaging applications.
- The most common type of PHA is the homopolymer poly(3-hydroxybutyrate) (P(3HB)). Co-polymers such as P(3HB-co-4HB) exhibit higher elasticity, lower crystallinity and present more adequate properties for specific uses.



Objective

Conversion of waste glycerol and hydrolyzed straw into biodegradable, biocompatible polyesters through up-scalable, high productivity processes

Upgrading by-products

- Current biodiesel production is based on transesterification of vegetable oils generating FAMEs and a surplus of waste glycerol (GRP) (1 t of GRP for each 9 t of biodiesel).
- Today, approx. 120 plants produce up to 6 100 thousand tonnes of biodiesel annually in the EU, generating 600 thousand t of GRP [3].
- GRP can be used as the major C source for PHA production [4]-[6].
- Cellulose is the world's most abundant natural, renewable and biodegradable polymer.
- Lignocellulosic wastes are pretreated and then subjected to an enzymatic step to obtain an hydrolysate which can be used as C source by bacterial strains [7].

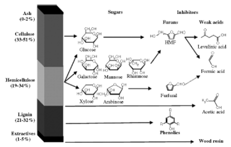


Figure 1. Average composition of lignocellulosic biomass and major derived hydrolysis products [7]

Methodology

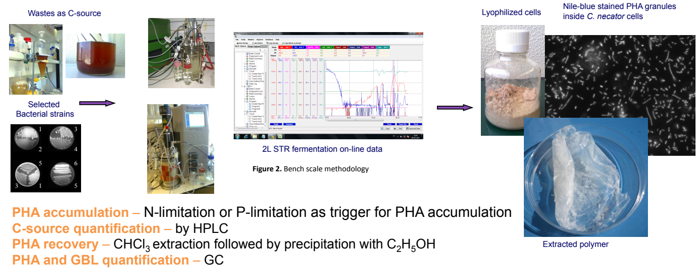


Figure 2. Bench scale methodology

- **PHA accumulation** – N-limitation or P-limitation as trigger for PHA accumulation
- **C-source quantification** – by HPLC
- **PHA recovery** – $CHCl_3$ extraction followed by precipitation with C_2H_5OH
- **PHA and GBL quantification** – GC
- **PHA characterization** – H^1-NMR, SEC, DSC

Results and Discussion

Waste glycerol

- *Cupriavidus necator* DSM 545 produced the homopolymer P(3HB) from GRP at a productivity of 1.1 $gL^{-1}.h^{-1}$ [8].
- Using γ-butyrolactone (GBL) as the precursor for 4HB monomers, *C. necator* DSM 545 cells were able to accumulate P(3HB-co-4HB) with different 4HB to 3HB ratios [9].
- Propionic acid (PA) was used as stimulator for 4HB incorporation, as suggested by Lee *et al.* 2000 [10]. PA considerably increased the 4HB ratio, but also acted as 3-hydroxyvalerate (3HV) precursor, resulting in the production of P(3HB-4HB-3HV).
- By manipulating the dissolved oxygen concentration (DOC) and cultivation time, 4HB molar percentages in the range 11.4 - 21.5 were attained. Terpolymers were obtained with 24.8% to 43.6% 4HB and 5.6% to 9.8% 3HV (see Table 1, Figure 1 and 2). Results indicate that a higher DOC favors PHA accumulation. Average MW varied between 5.5 x 10^5 Da and 1.37 x 10^6 Da with a PI from 2.6 to 4.0.

Table 1. PHA production - effect of DOC (%), PA and culture time on PHA productivity, concentration and composition [9].

Accum. conditions		PHA	Values at the time of max volumetric productivity					Values at the end of the cultivation				
DOC (%)	Supplements	type	$Prod_{Vol}$ $gL^{-1}h^{-1}$ [time (h)]	PHA gL^{-1}	PHA %	4HB %	3HV %	$Prod_{Vol}$ $gL^{-1}h^{-1}$ [time (h)]	PHA gL^{-1}	PHA %	4HB %	3HV %
20	GBL	P(3HB-4HB)	0.33 [29.3]	9.7	25.9	11.4	-	0.17 [64.3]	10.9	36.1	17.6	-
2	GBL	P(3HB-4HB)	0.15 [26.0]	4.0	14.8	12.3	-	0.06 [67.5]	4.2	17.9	21.5	-
20	GBL + PA	P(3HB-4HB-3HV)	0.29 [38.0]	10.8	29.5	26.2	9.8	0.25 [67.0]	16.7	36.9	43.6	6.0
2	GBL + PA	P(3HB-4HB-3HV)	0.35 [31.8]	11.0	25.0	30.6	6.7	0.21 [44.0]	9.3	18.1	24.8	5.6

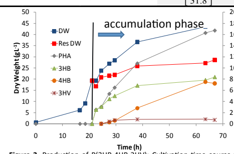


Figure 3. Production of P(3HB-4HB-3HV); Cultivation time course for the production of P(3HB-4HB) with the DOC controlled at 20%. Accumulation phase – 2 gL^{-1} of propionic acid were added at the beginning and GRP and GBL were continuously fed.

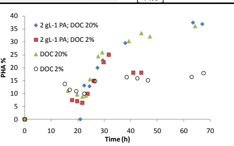


Figure 4. PHA (%) accumulated per total DW, by cells of *C. necator* DSM 545 fed with GRP and GBL in cultivations with the DOC controlled at 2 % or 20 % in the presence or absence of 2 gL^{-1} of PA.

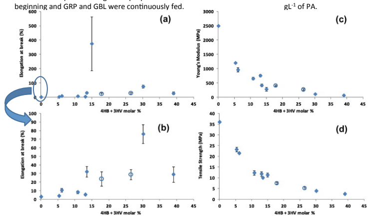


Figure 5. The effect of (4HB + 3HV) molar % on the terpolymer mechanical properties: (a) and (b) elongation at break plotted on two different scales, (c) Young's modulus and (d) tensile strength.

- The properties of the polymers considerably differ from those of the homopolymer P(3HB), even at low percent incorporation of 4HB monomers in the polyester chain [11].
- Evidence (obtained by DSC and SEC) demonstrates the biosynthesis of heterogeneous PHA blends composed by various fractions of different molar composition, instead of a narrow distribution of chains with similar composition [11].

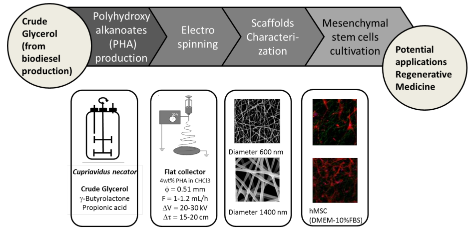


Figure 6. From P(3HB-4HB-3HV) to scaffolds for hMSC proliferation [12].

Wheat straw lignocellulosic hydrolysates

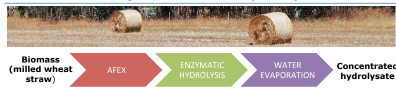


Figure 7. Main steps involved in hydrolysate production at **biorefinery.de GmbH**

Table 2. Preliminary strain selection.

Strain	Criteria: glucose	xylose	PHA	Risk	Comments
***Alcaligenes latus* DSM 1122**	+	-	+	1	Aggregates during the first stages of growth
Bacillus sp MA3.3	+	+	+	1	Not available
Burkholderia cepacia ATCC 17759	+	+	+	2	
***Burkholderia sacchari* DSM 17165**	+	+	+	1	High growth rates
***Cupriavidus necator* DSM 545**	+	-	+	1	High growth rates
Haloferax mediterranei	+	-	+	1	Costly growth medium and corrosion problems.
Methylobacterium extorquens	+	-	+	1	Low concentration of the optimal C sources in the hydrolysates
***Sphingopyxis macrogoltabida* LMG 17324**	+	-	+	1	Preferential uptake of oligosaccharides

Table 3. P(3HB) production from a sugar mixture simulating the hydrolysate (as control) and from a real hydrolysate (hydrolysate composition: 563 gL^{-1} glucose, 284 gL^{-1} xylose, 46 gL^{-1} arabinose).

C-source	$Y_{P(3HB)/S}$	$Prod_{vol}$ ($gL^{-1}h^{-1}$)	P (3HB) (%)	Dw (gL^{-1})	t (h)
Sugar mix	0.18	1.7	51	138	36.5
Hydrolysate	0.20	1.7	55	150	38.5

Table 4. PHA production from wheat straw hydrolysates (hydrolysate composition: 465 gL^{-1} glucose, 146 gL^{-1} xylose, 42 gL^{-1} arabinose).

Polymer	CDW (gL^{-1})	PHA (gL^{-1})	PHA (%)	$Prod_{vol}$ ($gL^{-1}h^{-1}$)
$P(3HB)_{6th}$	125	71	57	1.5
$P(3HB\text{-}co\text{-}6\%\ 4HB)_{6th}$	88	24	27	0.5

- A standardized fermentation protocol was successfully developed allowing for the testing of the supplied hydrolysates, as well as for further scale up of the process.
- Feedback from bench scale assays allowed for hydrolysates improvement by biorefinery.de GmbH.
- Feeding strategy for multiple carbon source consumption successfully implemented [13].
- Cell density and P(3HB) productivity were similar to those reached in control cultivations with mixtures of commercial sugars [13].
- Cell density and P(3HB-4HB) productivity using hydrolysates were lower than those reached in P(3HB) production cultivations due to inhibition caused by the precursor (GBL) [14].

Conclusions

- Waste glycerol was successfully used as an alternative C-source to produce PHAs.
- Integrating biopolyesters and biodiesel production can contribute to (i) reduce costs associated with the C-source for PHAs production (ii) the upgrade of the surplus glycerol generated in the biodiesel plants.
- P(3HB) and P(3HB-4HB) were successfully bioproduced from different batches of wheat straw hydrolysates.
- The attained P(3HB) and P(3HB-4HB) volumetric productivities are by far the highest ever achieved on agricultural waste hydrolysates and further optimization is under way (see poster Cesário *et al.* for more details!).

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Acknowledgements

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