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Valorisation of sugar beet pulp for the production of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxy valerate

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|Objectives

- ❖ Development of Sugar Beet Pulp biorefinery
- ❖ Crude enzymes production via solid state fermentation (SSF)
- ❖ Sugar Beet Pulp hydrolysate as feedstock for PHB production
- ❖ PHBV production with different 3HV monomer content

|Poly(3-hydroxybutyrate) (PHB)

The transition to the circular bio-economy era requires technological breakthroughs in sustainable biorefinery development using **crude renewable resources, microbial bioconversions and recycling of biopolymers**

- **Member of polyhydroxyalkanoates** (PHAs)
- **Accumulated in intracellular granule**s by Gram+ and – microorganisms
- It is produced **under excess of carbon source and limited nutrient conditions** (N, P, O)
- Serve as a carbon and energy storage

+

- Similar properties with conventional polymers such as polypropylene and polyethylene.
- It is highly biodegradable, non-toxic, and biocompatible.

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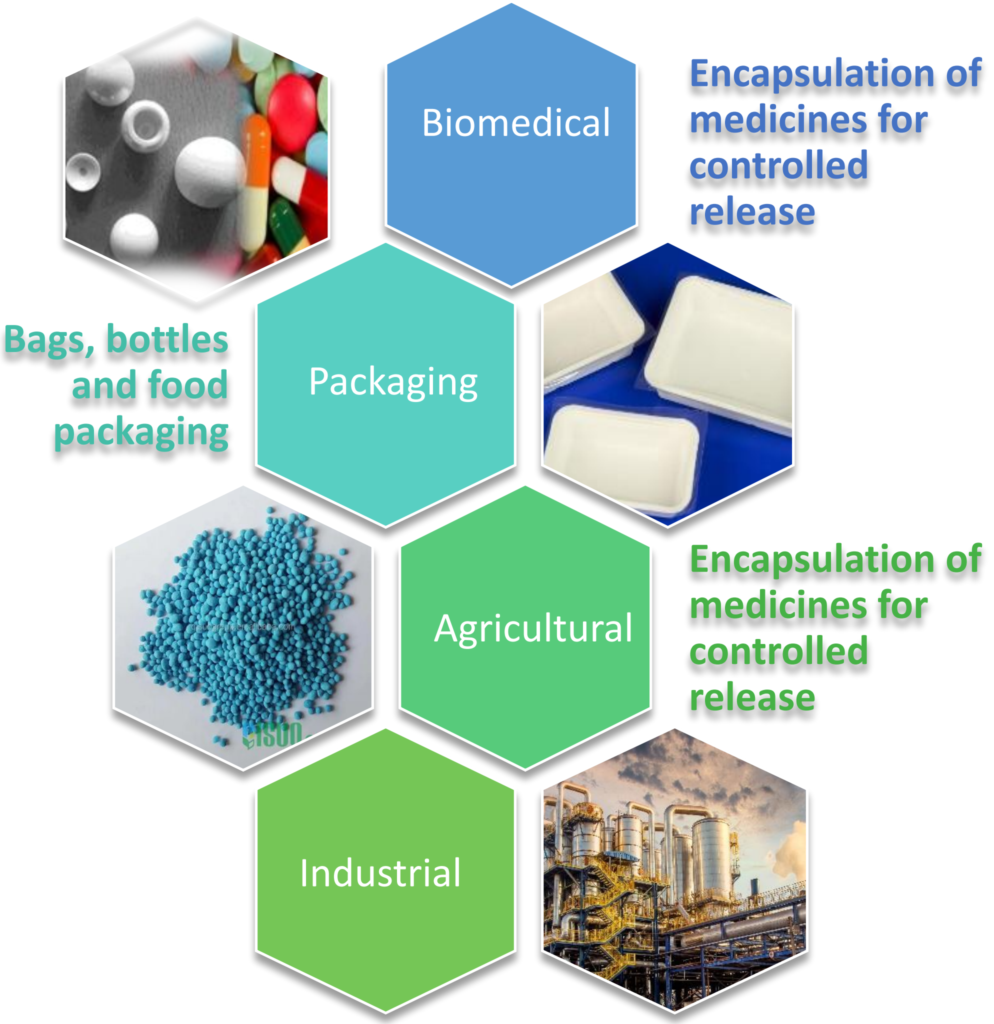
- high degree of crystallinity
- rigidity
- low elongation to break

Create difficulties in material processability

PHB applications



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|Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)

+

- Better physical properties
- its performance can vary when this polymer contains different proportions of the HV monomer
- Impact resistance
- Toughness
- Flexibility
- Low melting point

Due to its superior characteristics, PHBV is more attractive for both biomedical and food packaging applications

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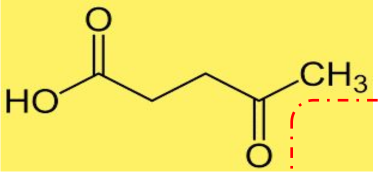
Limited use □ High production cost

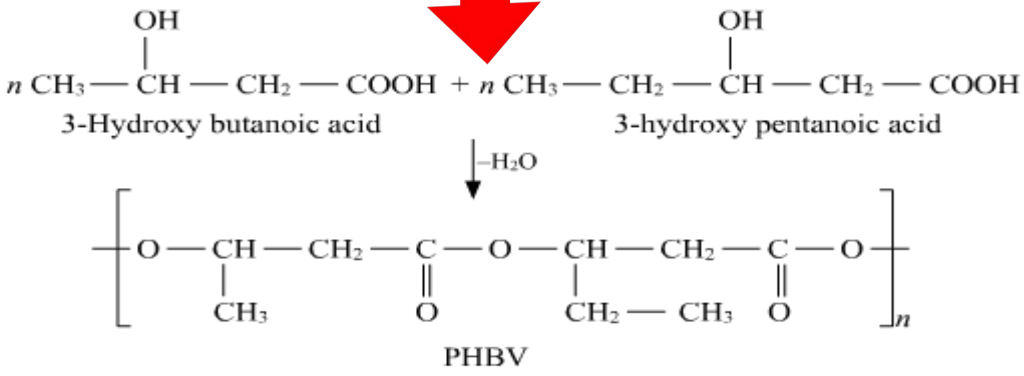
! The carbon source accounts for 28-50% of the total production cost

PHBV Synthesis

- 3-HV and 3-HB, the monomer units of PHBV are synthesized simultaneously in bacterial cytoplasm via two parallel pathways.
- Levulinic acid acts as a direct precursor that triggers the formation of 3-HV, in addition to 3-HB.



Levulinic Acid
(4-oxovaleric acid)



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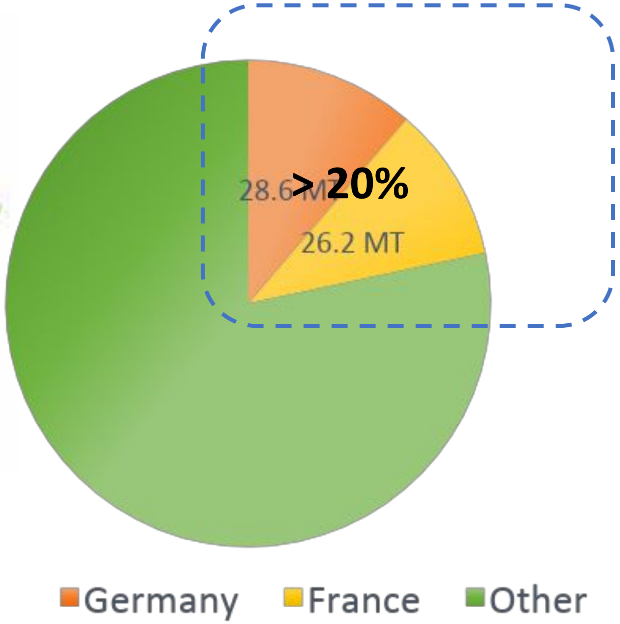
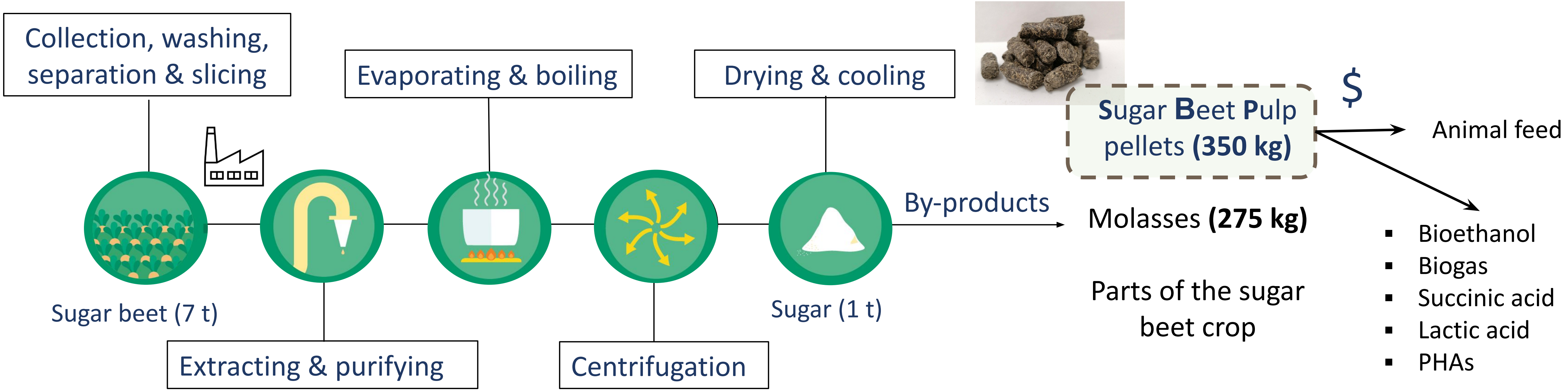
| Sugar Beet

- ✔ One of the most important agricultural crop
- ✔ Annual production: 253 million t of beets
- ✔ One of the highest volume vegetable wastes
- ✔ SBP is the main by-product of sugar industry
- ✔ 10.35 million t/y of SBP was produced in Europe
- ✔ 1 t of sugar beets yields to 50 kg dehydrated SBP





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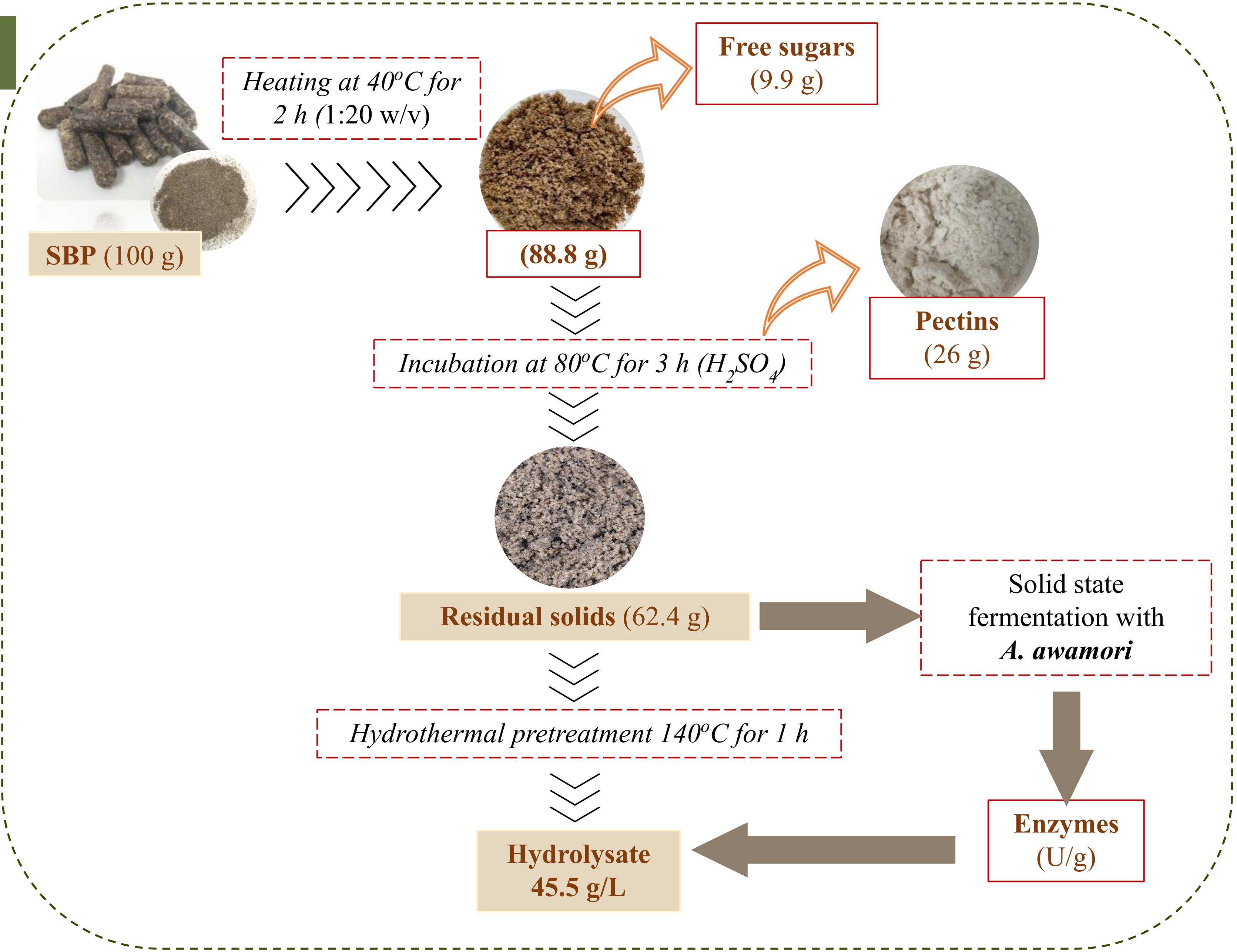
|SBP Biorefinery

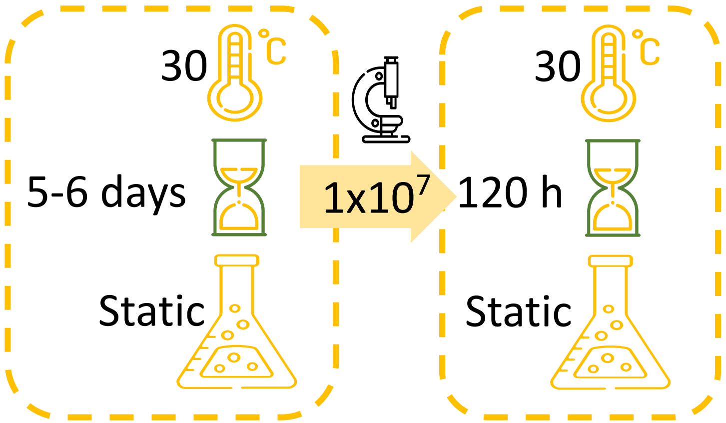
Composition of Sugar beet pulp pellets (% dry basis)		
Free sugars	10.9 ± 1.7	
Sucrose	9.0 ± 0.7	1.0 – 8.0
Glucose	1.5 ± 0.8	
Fructose	0.4 ± 0.3	
Ash content	3.7 ± 0.6	2.4 – 4.0
Protein Content (N x 6.25)	9.1 ± 0.1	5.9 - 11.4
Pectin (uronic acid)	19.1 ± 0.9	13.5 - 22.8
Lipid Content	0.9	1.5
Glucan	27.9 ± 4.9	14.0 - 25.5[1]
Xylan	3.1 ± 0.2	25.0 -36.6[2]
Galactan	5.5 ± 0.2	
Mannan	4.3 ± 0.9	
Arabinan	12.0 ± 4.1	
Lignin	2.3 ± 0.3	1.2 - 2.0
Phenolics (mg GAE)	205.7 ± 22.2	
REFERENCE	**This work**	**Literature**

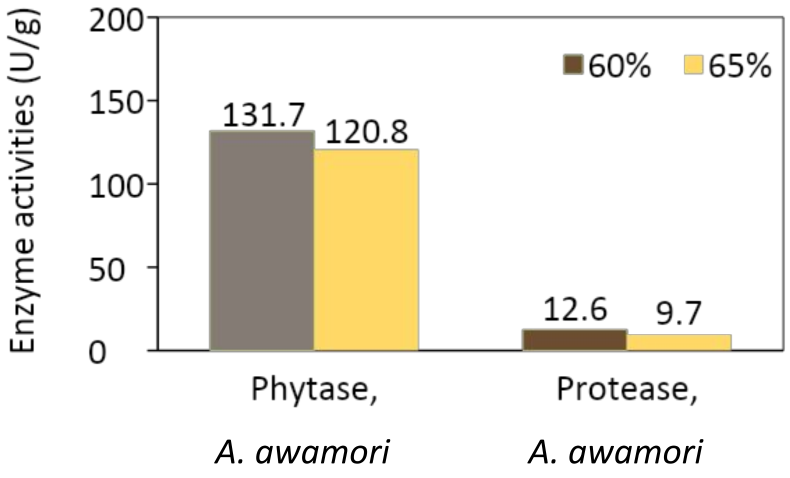
[1]cellulose, [2]hemicellulose

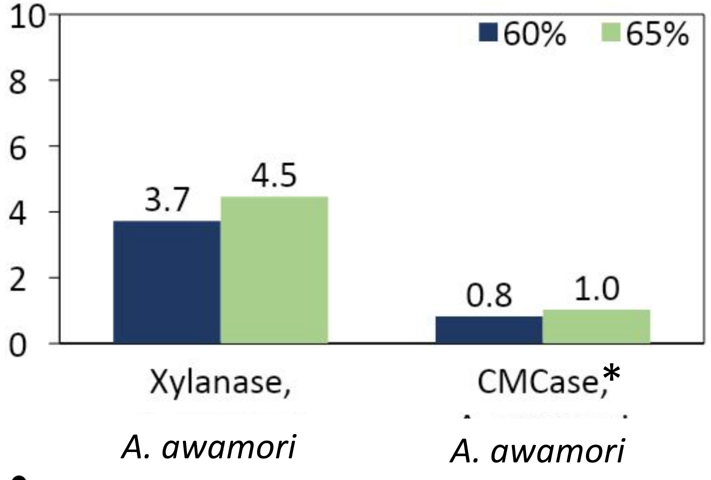


|Production of crude enzymes during solid state fermentations by *Aspergillus awamori* cultivated on a mixture of sugar beet pulp (SBP) & sunflower meal (2:1)

INOCULUM **FERMENTATION**





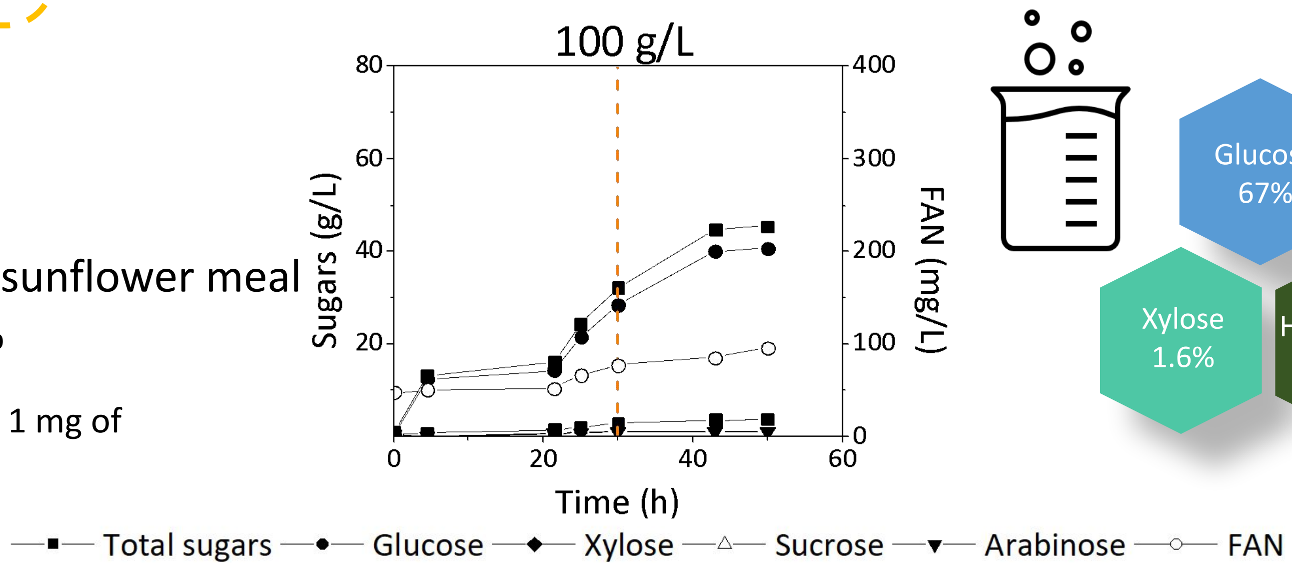


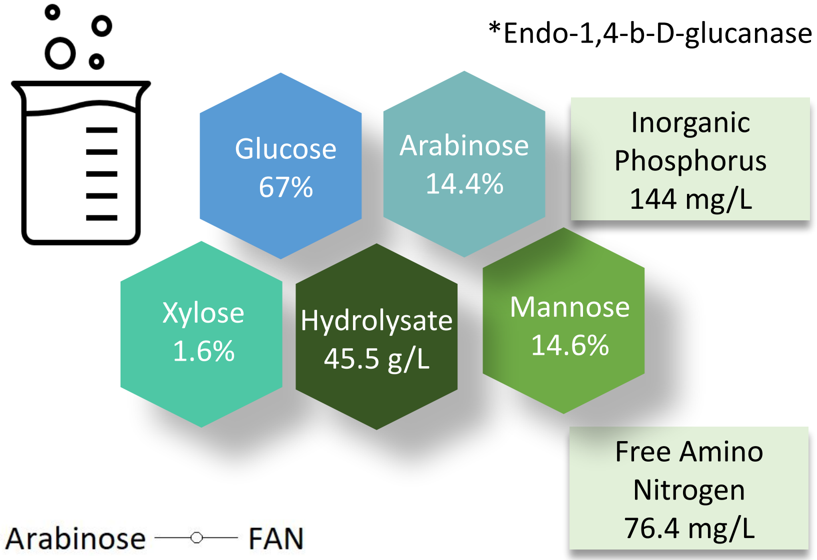
*Endo-1,4-b-D-glucanase

EXPERIMENTAL DESIGN

- *Aspergillus awamori*
- SBP free of sugars and pectin & sunflower meal
- Moisture content: 60% and 65%

* U = the amount of enzyme that releases 1 mg of substrate per minute





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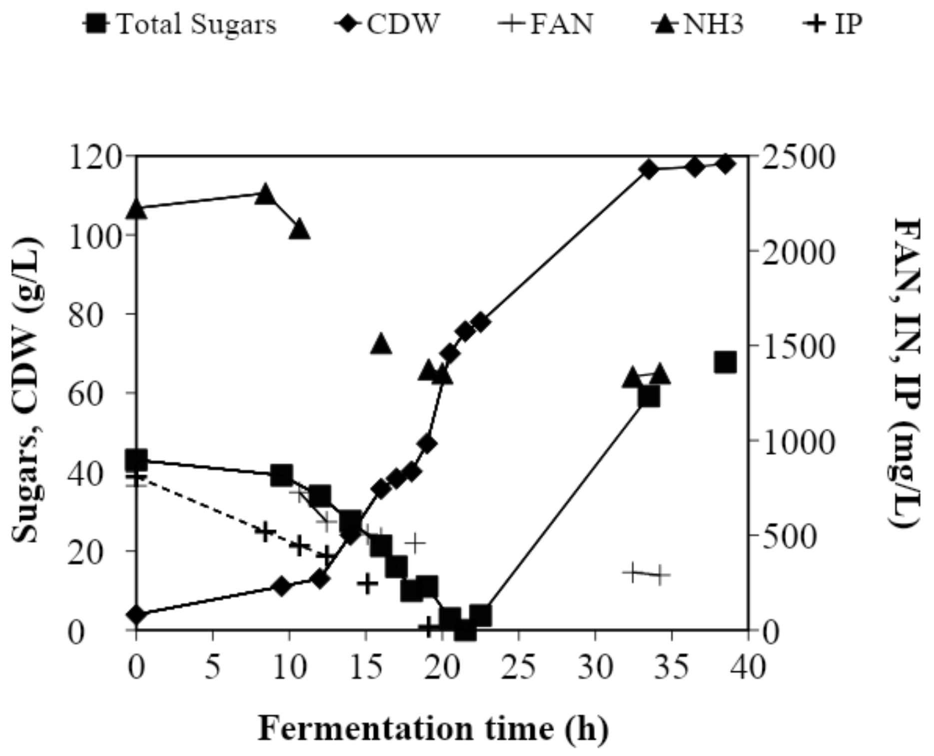
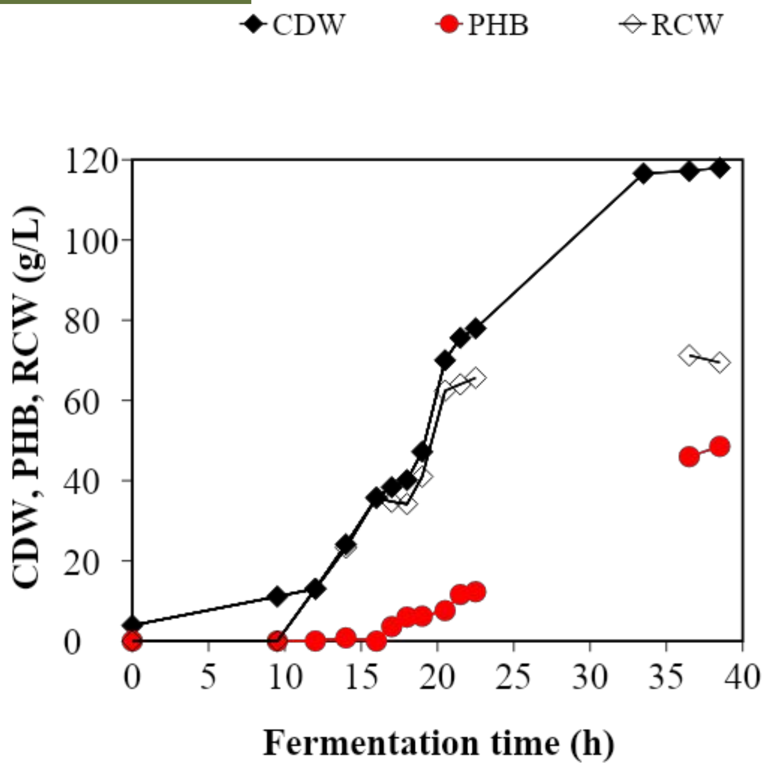
|Bioreactors Set up

	Paraburkholderia sacchari DMSZ 17165
Carbon Source	SBP hydrolysate, Commercial mix sugars 40 g/L Levulinic Acid
Inorganic Phosphorus	800(mg/L)
Working Volume	1 L
pH	6.8 (28% NH_4OH και 2 M HCl)
Temparature	30°C
Agitation	1200 rpm
Ventilation	2.5 vvm
Minerals (g/L)	$(NH_4)_2SO_4$, 4.0 g; KH_2PO_4, 3.0 g; citric acid, 1.7 g; EDTA, 40 mg; trace elements solution, 10 mL; $MgSO_4 \cdot 7H_2O$, 1.2 g. **Trace element (g/L):** $FeSO_4 \cdot 7H_2O$, 10 g; $ZnSO_4 \cdot 7H_2O$, 2.25 g; $CuSO_4 \cdot 5H_2O$, 1 g; $MnSO_4 \cdot 4H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 2 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.23 g; $(NH_4)_6Mo_7O_{24}$, 0.1 g; 35% HC1 10 mL

|Fermentation with SBP hydrolysate for PHB production

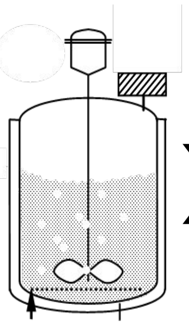




Time (h)	CDW (g/L)	PHB (%)	**PHB g/L**	Yield (g PHB / g consumed sugars)	Productivity (g/L/h)
38.5	118	41.1	**48.54**	0.28	1.26

- **CDW**: cell dry weight
- **RCW**: residual cell weight
- **IN:** Inorganic nitrogen
- **IP**: Inorganic phosphorus

|Feeding Strategy with Levulinic Acid supplementation



Case Study 1

- Addition of 8 g Levulinic Acid
- After 24 h of fermentation
- 1 g / h

Case Study 2

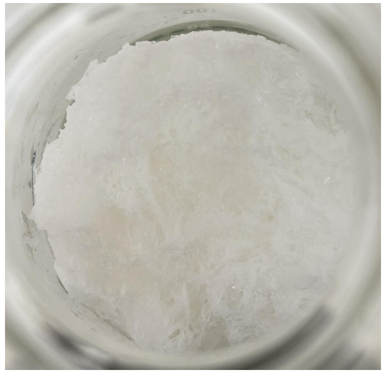
- Addition of 13 g Levulinic Acid
- After 24 h of fermentation
- 1 g / 30 min

Case Study 3

- Addition of 22 g Levulinic Acid
- After 24 h of fermentation
- 3 g / h

Case Study 4

- Addition of 28 g Levulinic Acid
- After 24 h of fermentation
- 2 g / 30 min



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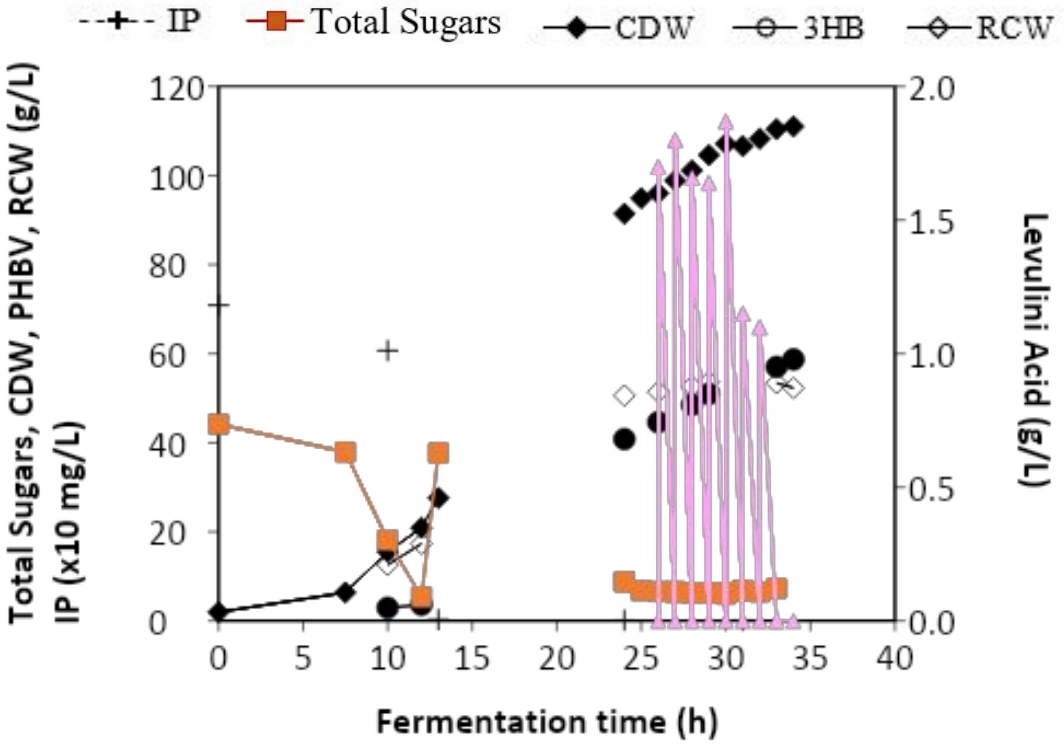
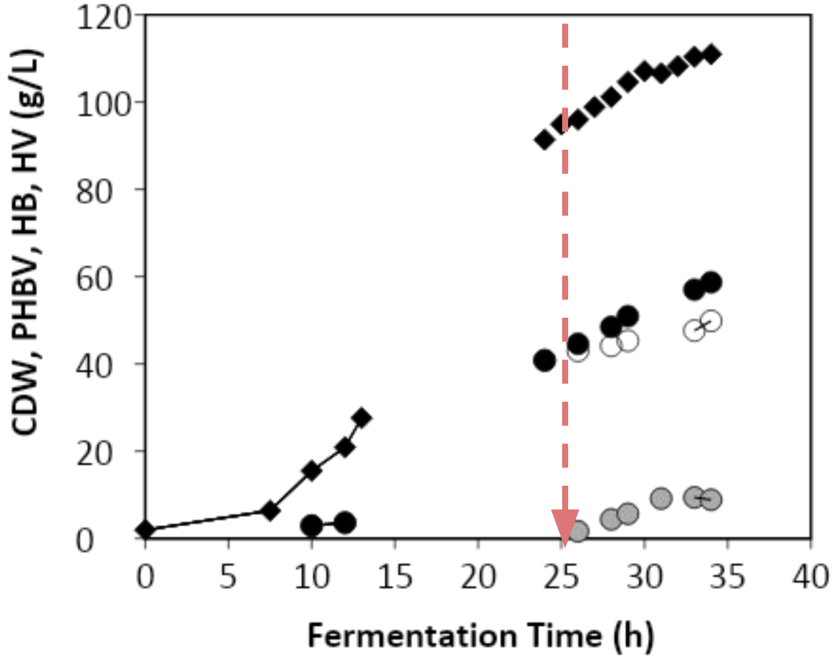
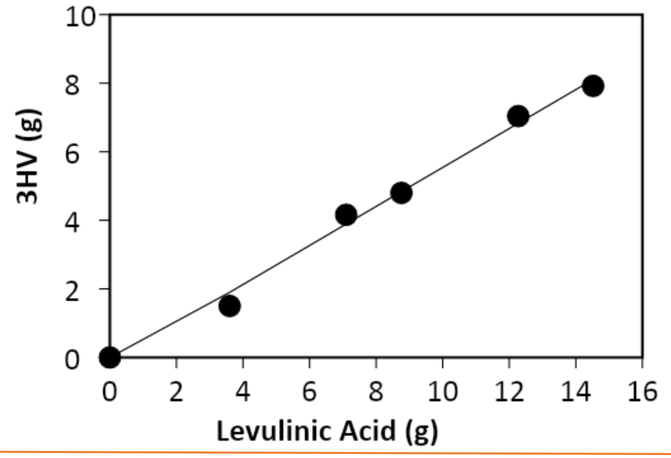
|Fermentations with mixed sugars simulating hydrolysate for PHBV production

13 g Levulinic Acid





13 g LA

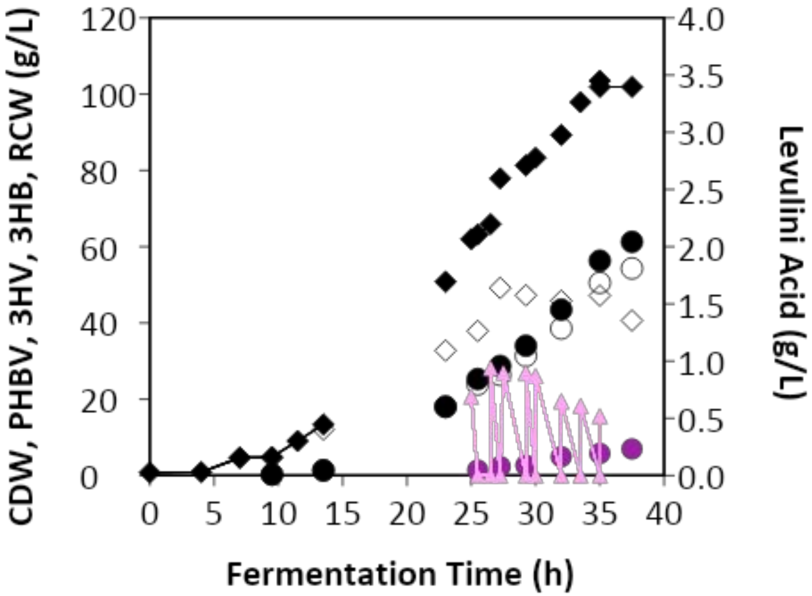


PHBV 58.7 (g/L) 3HB (g/L)	3HV (g/L)	CDW (g/L)	Yield (g PHBV/g consumed carbon*)	Productivity (g/L/h)	Levulinic acid consumed (g)	3HV/PHBV
49.8	8.86	111	0.29	1.72	13	15%

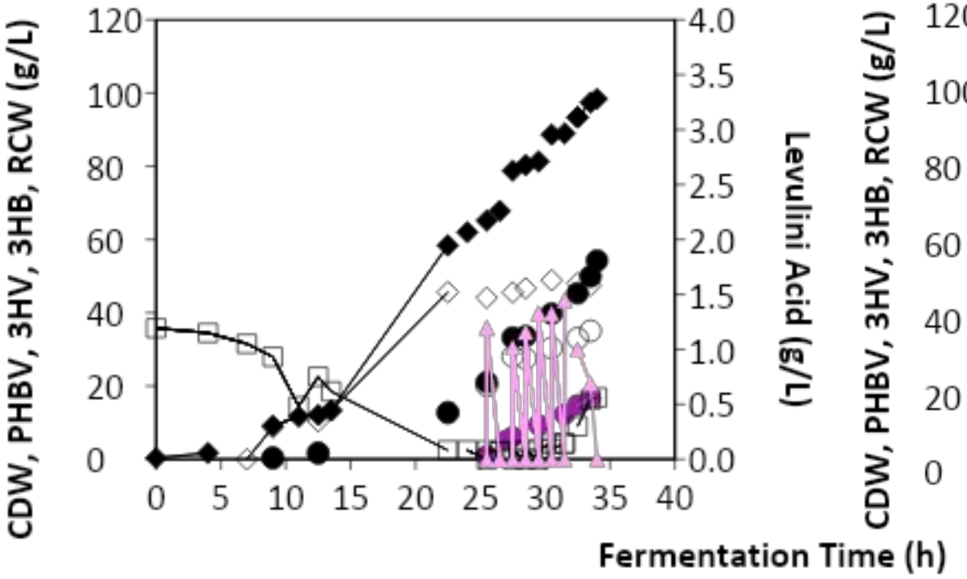
*Including sugars and Levulinic Acid

|Fermentations with mixed sugars simulating hydrolysate for PHBV production

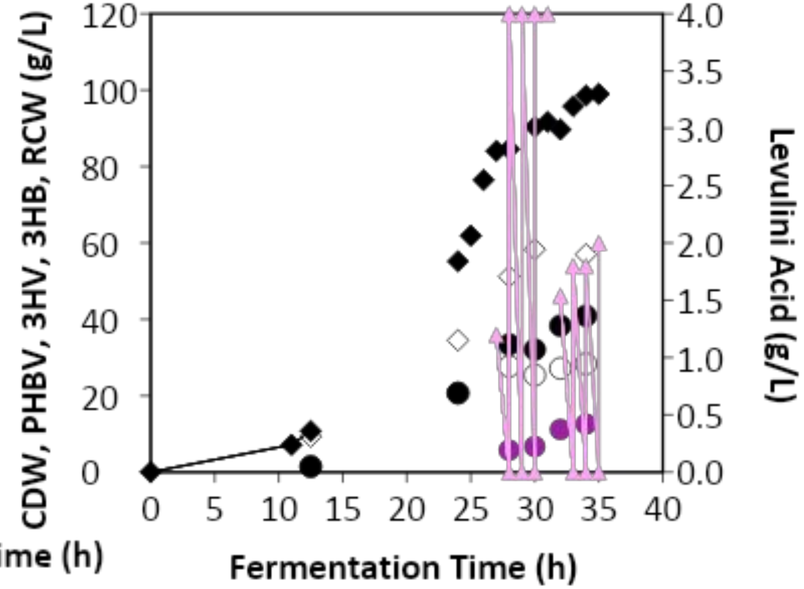
10 g Levulinic Acid



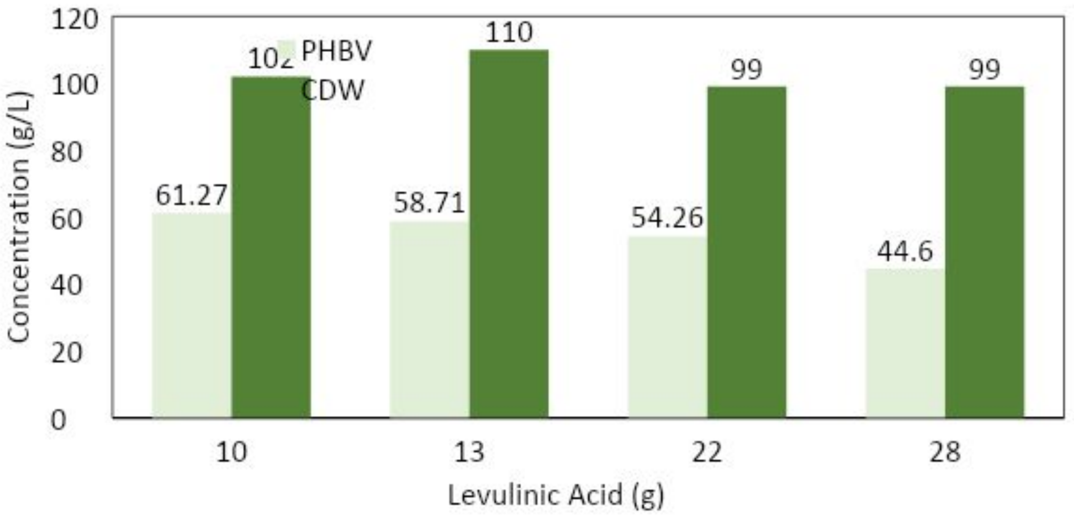
22 g Levulinic Acid

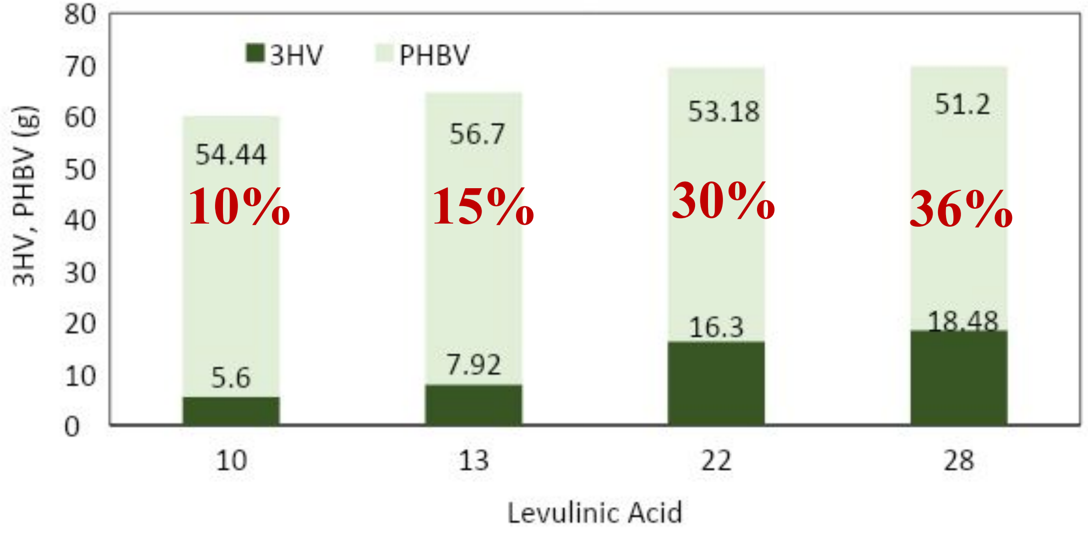


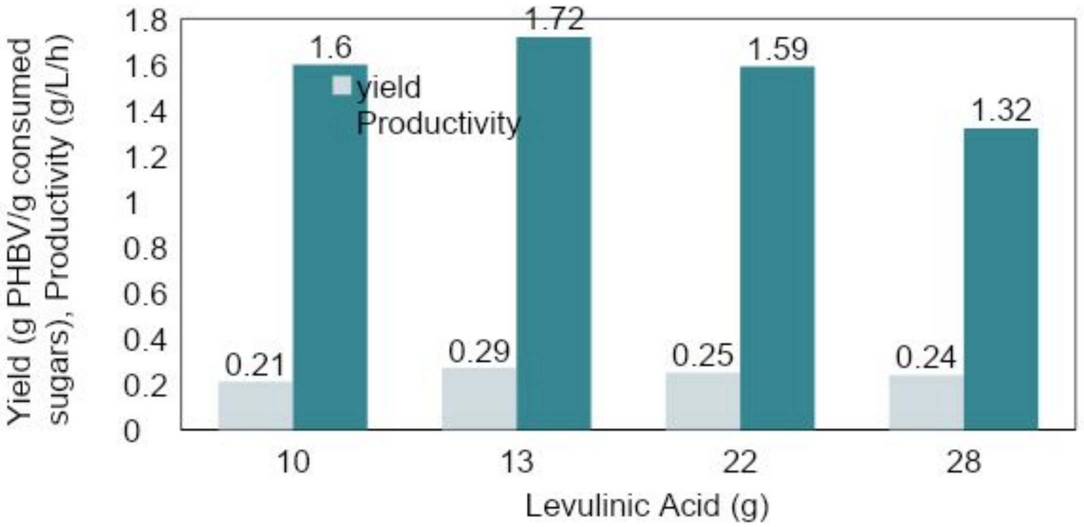
28 g Levulinic Acid



|Fermentation Results







- ✔ Increasing levulinic acid consumption leads to increased 3HV accumulation
- ✔ Based on the experimental results presented, the preferred 3HV content can be controlled during fermentation

Conclusions

- Crude enzymes were produced via solid state fermentation with *Aspergillus awamori*
- SBP hydrolysate was efficiently valorized as carbon source for PHB with of total dry weight of 118 g/L and PHB accumulation of 42% (w/w)
- The total amount of LA consumed demonstrate significant role in final 3HV accumulation

Thank you for your attention !

This work was supported by the project "Valorisation of sugar beet cultivation residues and by-products of sugar manufacturing process for the production of bio-based and biocomposite biodegradable packaging materials – Beet2Bioref" (MIS 5069983) which is implemented under the Action "Research - Create - Innovate", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

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