VII INTERNATIONAL SEMINAR

BIOPOLYMERS AND SUSTAINBLE COMPOSITES

4-5 MARCH 2020

EXTRACTION AND PURIFICATION OF PHA: CONVERTING DIVERSE WASTE-DERIVED MATERIALS INTO USEFUL PRODUCTS

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BIOTREND SA

AIMPLAS

Advanced services

Process development and optimization

- Devise cultivation strategies aiming at maximizing productivities, yields and titers.
- Advanced process optimization strategies.
- Process integration with raw material pre-processing and with downstream processing.

Process scale-up, de-risking and validation

- 2L, 10L, 50L and 250L bioreactors available for thorough scale-up studies and process derisking.
- Connection to facilities with 1,000L to 200,000L fermentation capacity.

Real-life materials

Commercial sugars

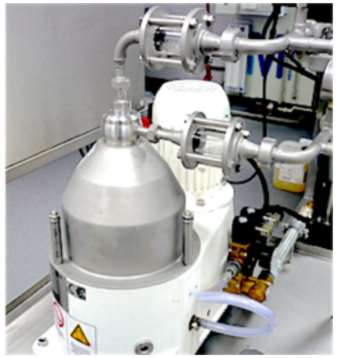
Agricultural hydrolysates

Crude glycerol

Confectionery waste

Pulp and paper waste

VFAs from AD



Pure Culture Fermentation → Extraction and purification → Bioplastics Chemicals Enzymes

Pure Culture Fermentation → Biomass Cell extracts

Real-life materials

BUGWORKERS

Fungus chain

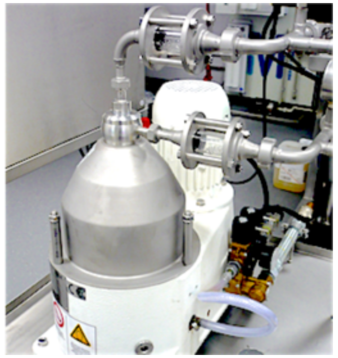
PHB 2Market

BRIGIT

Volatile

Commercial projects

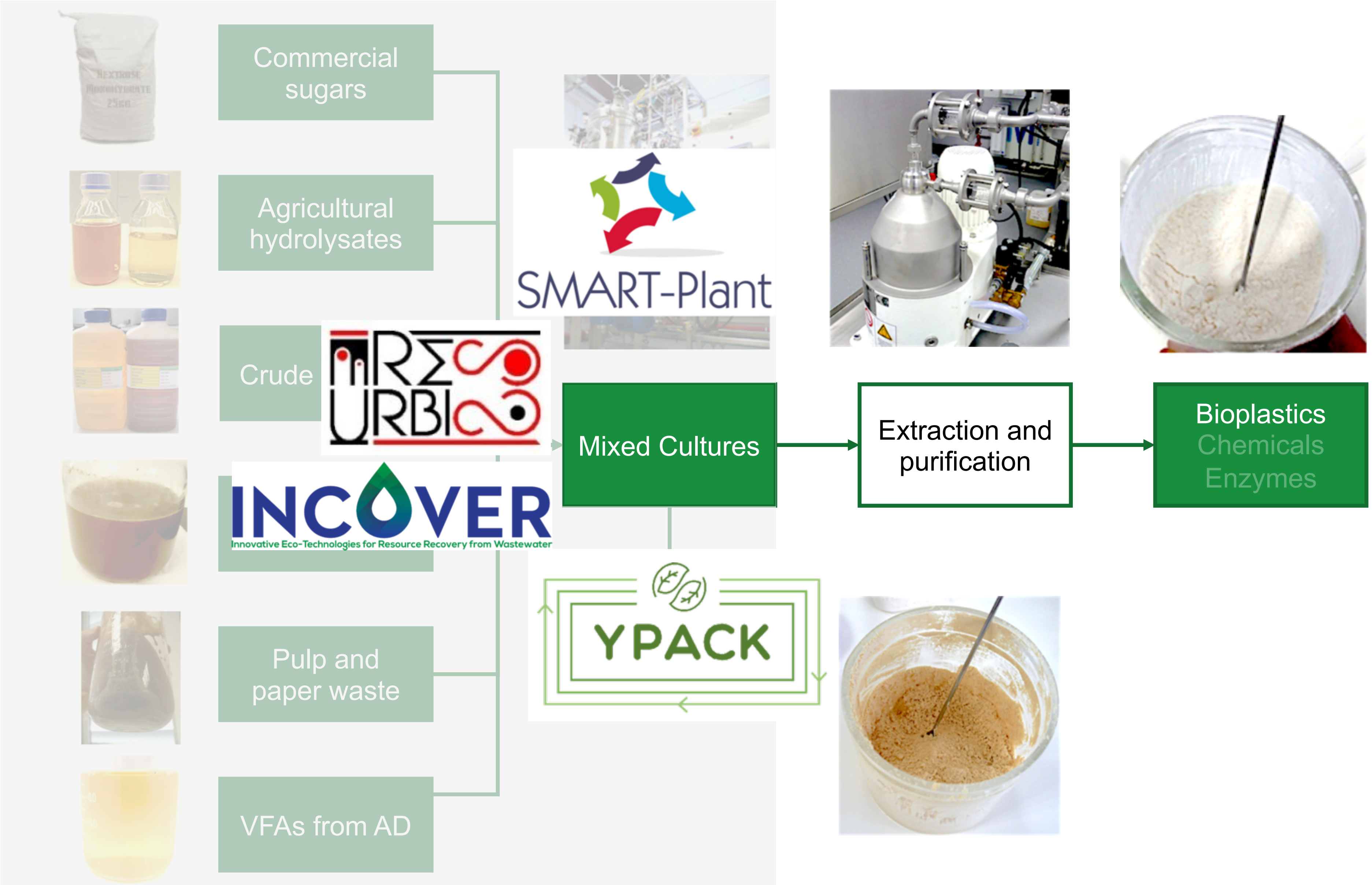
- Commercial sugars
- Agricultural hydrolysates
- Crude glycerol
- Confectionery waste
- Pulp and paper waste
- VFAs from AD



Pure Culture Fermentation → Extraction and purification → Bioplastics / Chemicals / Enzymes

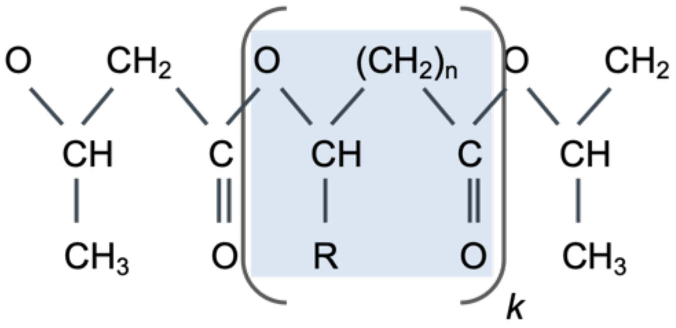
Pure Culture Fermentation → Biomass / Cell extracts

Real-life materials

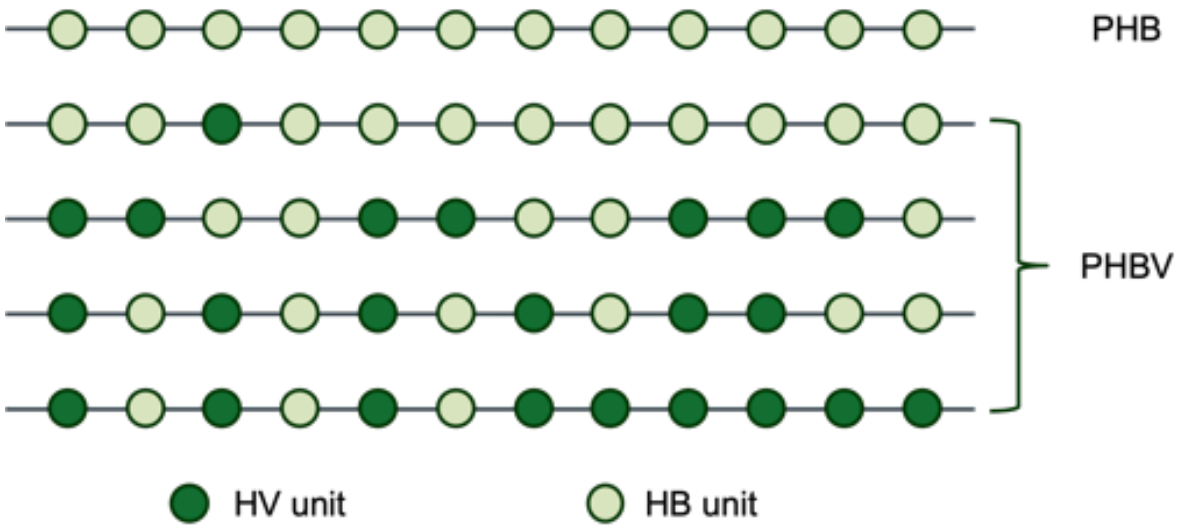


Motivation

Polyhydroxyalkanoates: biodegradable biopolyesters



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(5-hydroxyhexanoate)
n = 4	R = hexyl	Poly(6-hydroxydodecanoate)



Hompolymer

Co-polymer

Motivation

Commercial PHA production today

- Pure cultures
- Refined raw materials (sugars or oils), competing with food use
- Very limited material grades, particularly in scl-PHA (PHB and PHBV with very low HV incorporation) with limited ranges of properties and processing windows
- High production costs (raw materials and purification) limit market penetration, particularly if “drop-in” applications are envisaged
- Just a handful of “true” manufacturers exist

TianAn Biopolymer – Nature's Eco-Friendly Solution

BIOMER

danimer scientific – A Biotechnology Company

kaneka

Motivation

Research drivers

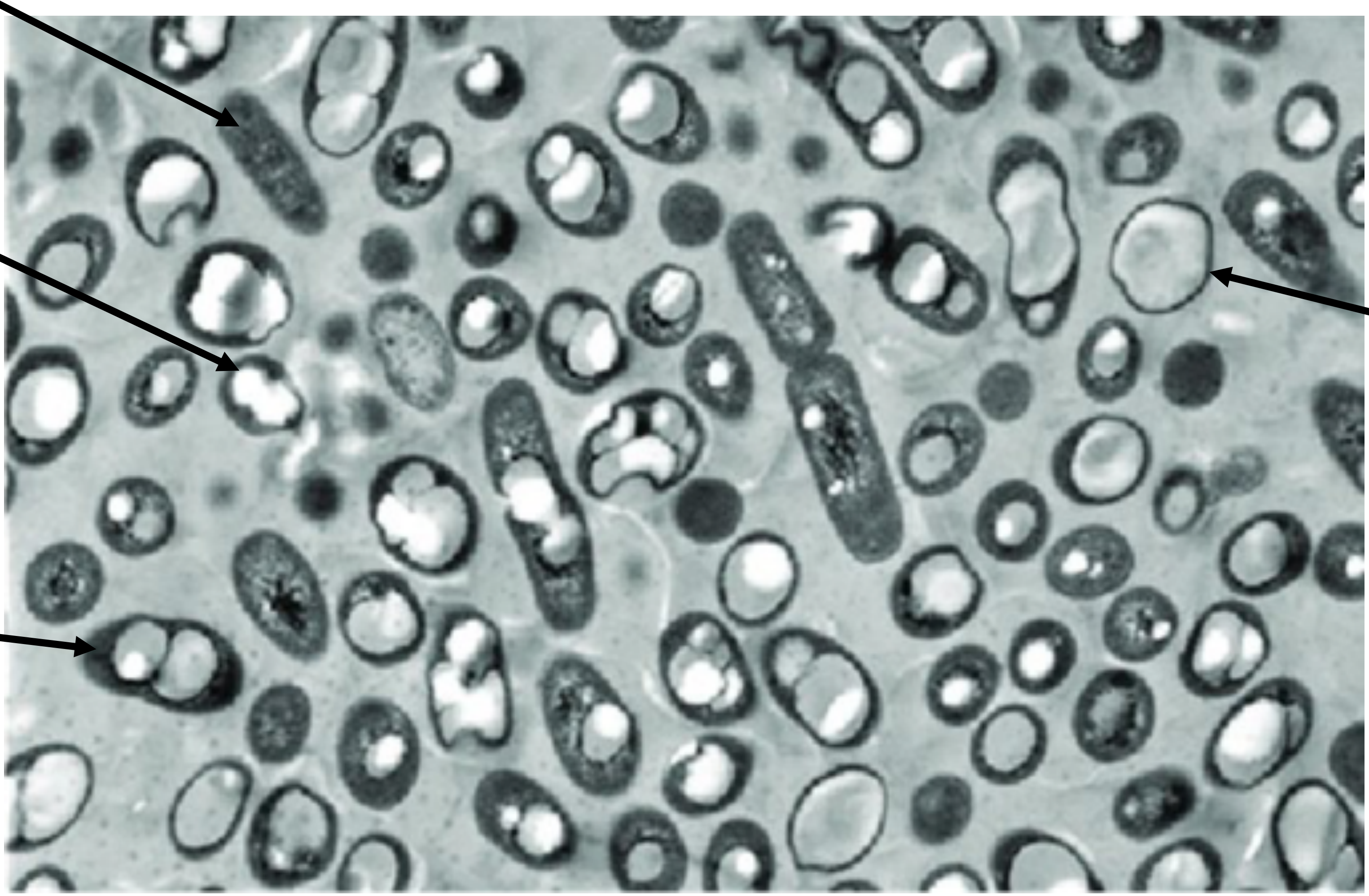
- *Use of industrial side-streams or wastes as raw material*
  - Cheaper raw materials, but…
  - Heterogenous and complex streams with varying composition

Varying yields, polymer content and composition

Massive challenges for purification

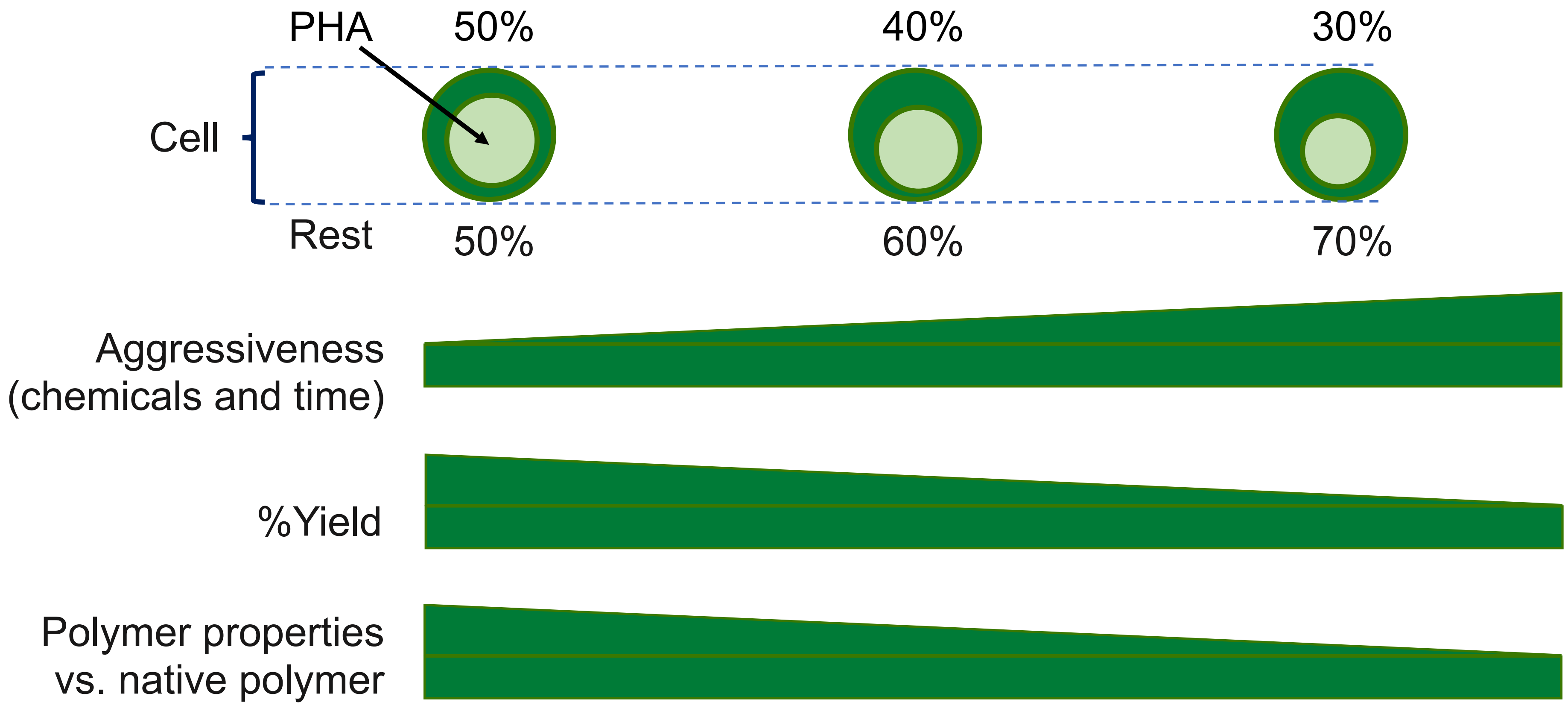
- *Cost-competitive purification processes, preferably aqueous-based*
  - Application at decentralised locations (including fairly low scale)
  - Complying with existing regulatory landscape and permits
  - Use standard and readily available equipment, preferably equipment routinely used on-site, not requiring special training for the plant operators

PHA in the biomass



biotrend
experts in bioprocessing

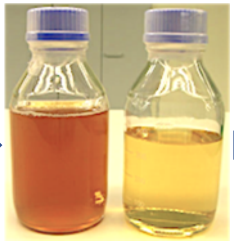
PHA in the biomass

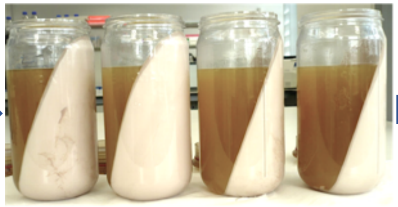


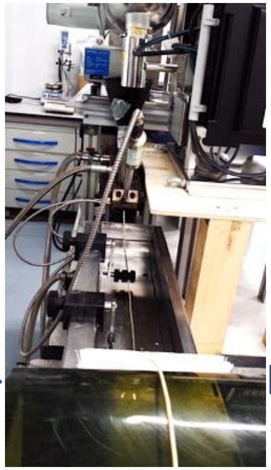
Pure culture 1

Wheat straw hydrolysate | Pure culture | Acid digestion

- Dry biomass: 130 g/L
- PHB concentration: 71 g/L
- PHB content: 56%






BUGWORKERS

AIMPLAS PLASTICS TECHNOLOGY CENTRE

Cesário et al. (2014) New Biotechnology, Volume 31, 104-113

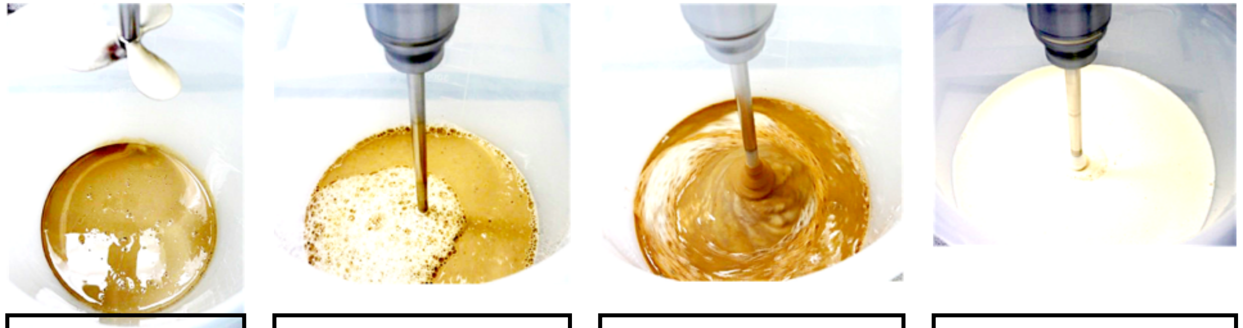
	Mw, kDa	Tm, ºC	ΔHm, J/g	Tc, ºC	ΔHc, J/g
Commercial PHB	739	176	107	52	19
Chloroform extraction	784	179	97		
Acid digestion	674	175	88	72	35

Pure culture 2

SSL | Pure culture | Alkaline + bleach digestion

- Dry biomass: 28 g/L
- PHB concentration: 12 g/L
- PHB content: 43%

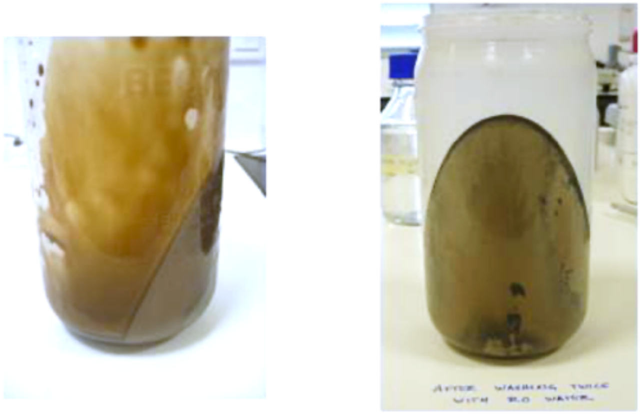
BRIGIT



Slurry | Surfactant | NaOH | Bleach

	Mw, kDa	Tm, ºC	ΔHm, J/g
Commercial PHB	739	176	107
Sugars | Alkaline + bleach	1278	174	98
SSL | Alkaline + bleach	625	174	85

Recovered biomass **Washed biomass**



- Lignosulphonates
- Cellular material
  - N (proteins)
  - P (DNA, RNA, nucleotides)
  - PHB

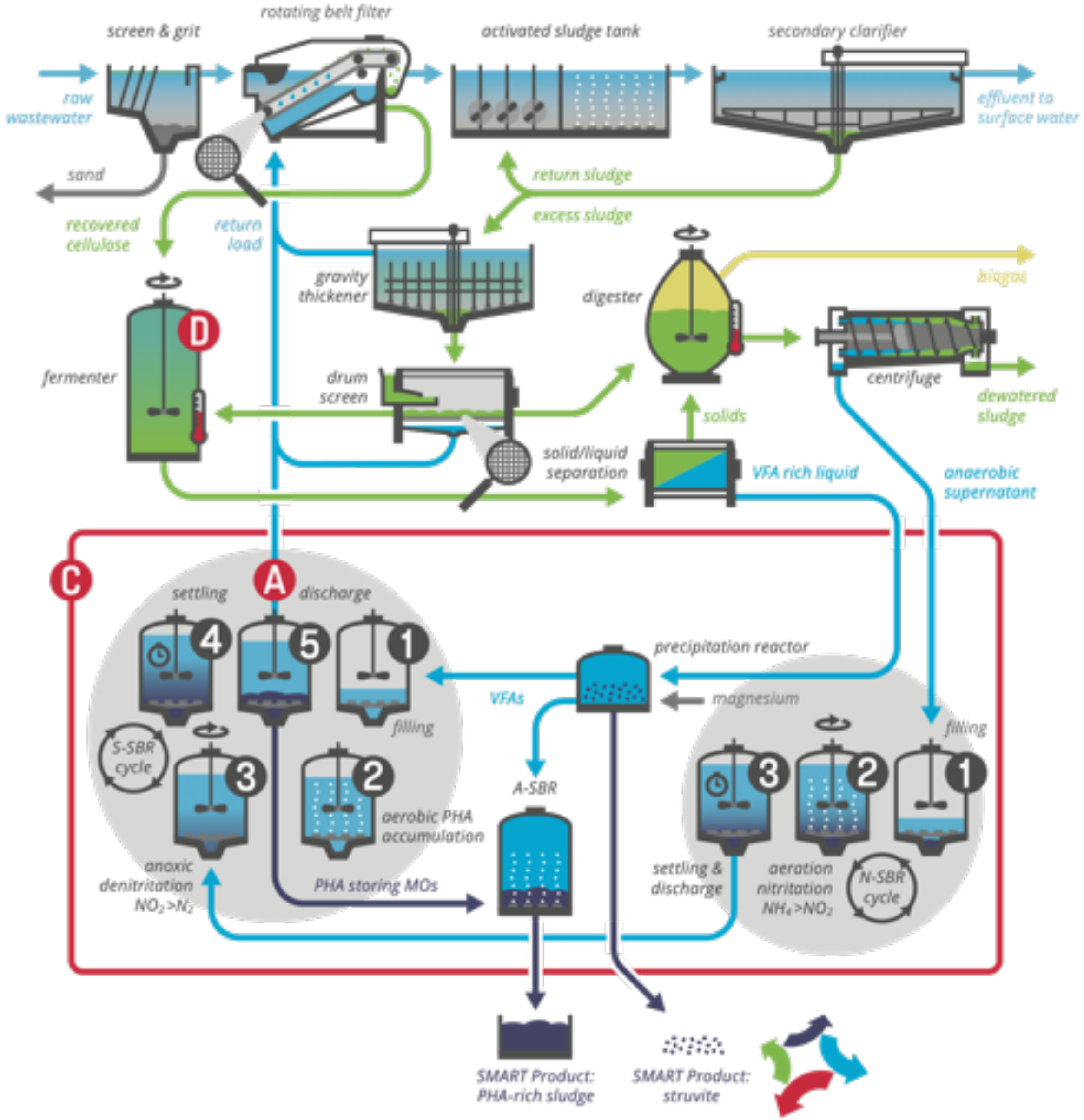
Daren Labs & Scientific Consultants Ltd.

Bio-based Flame Retardant

Mixed cultures

PHA production from wastewater treatment

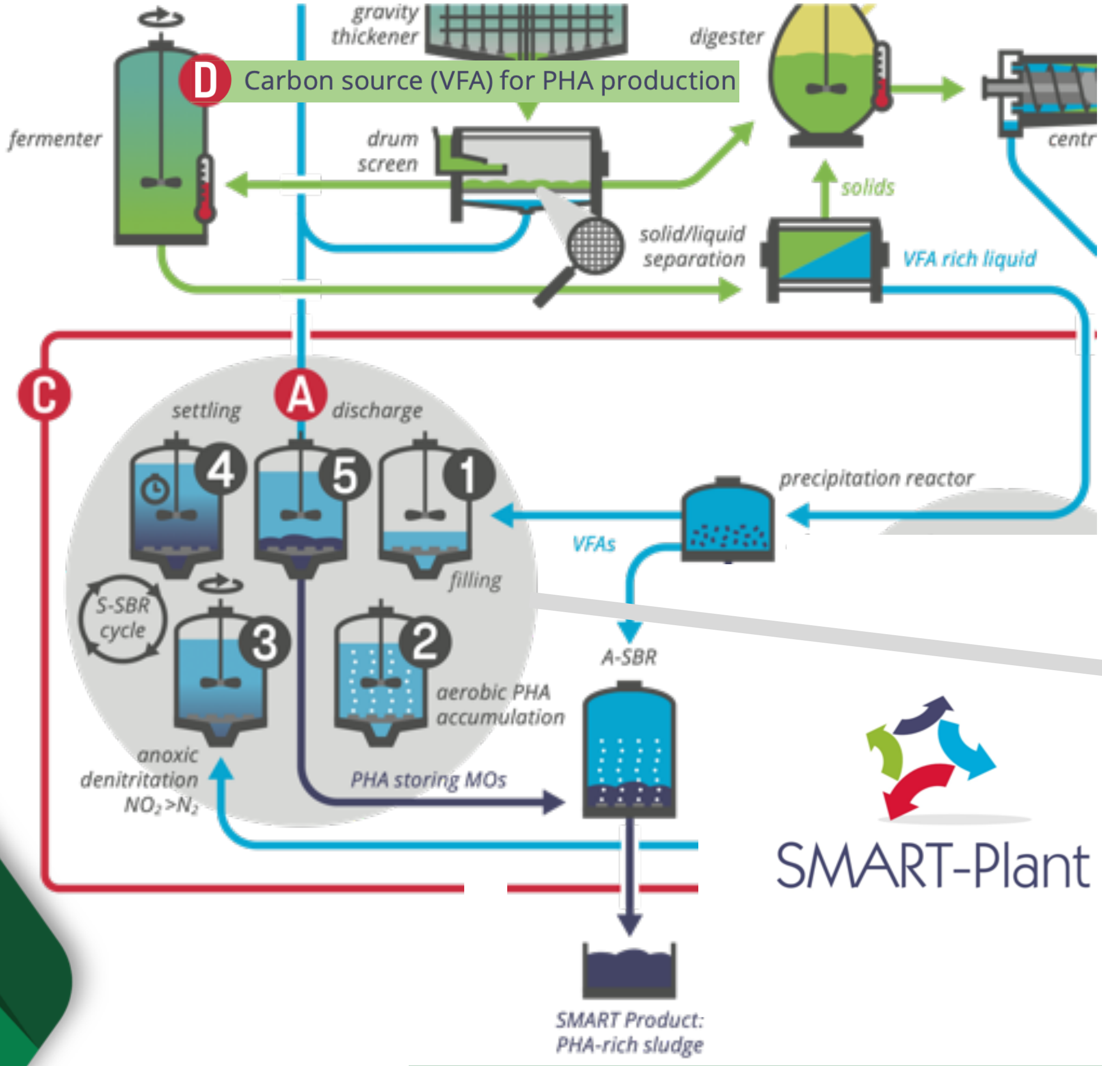
SMART-Plant

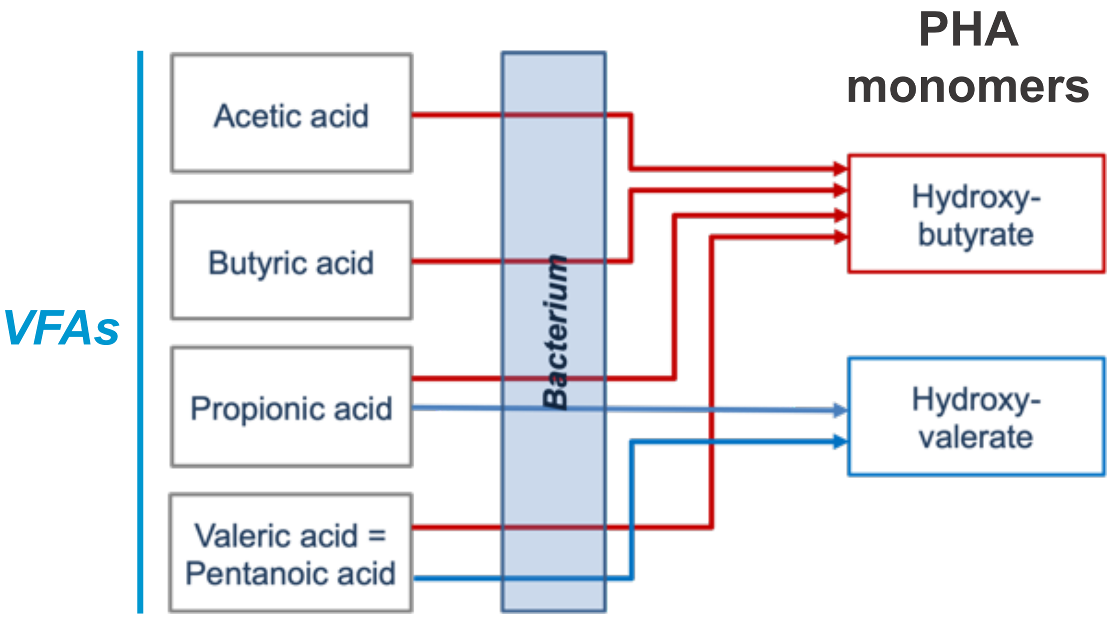


www.smart-plant.eu

Mixed cultures

PHA production from wastewater treatment

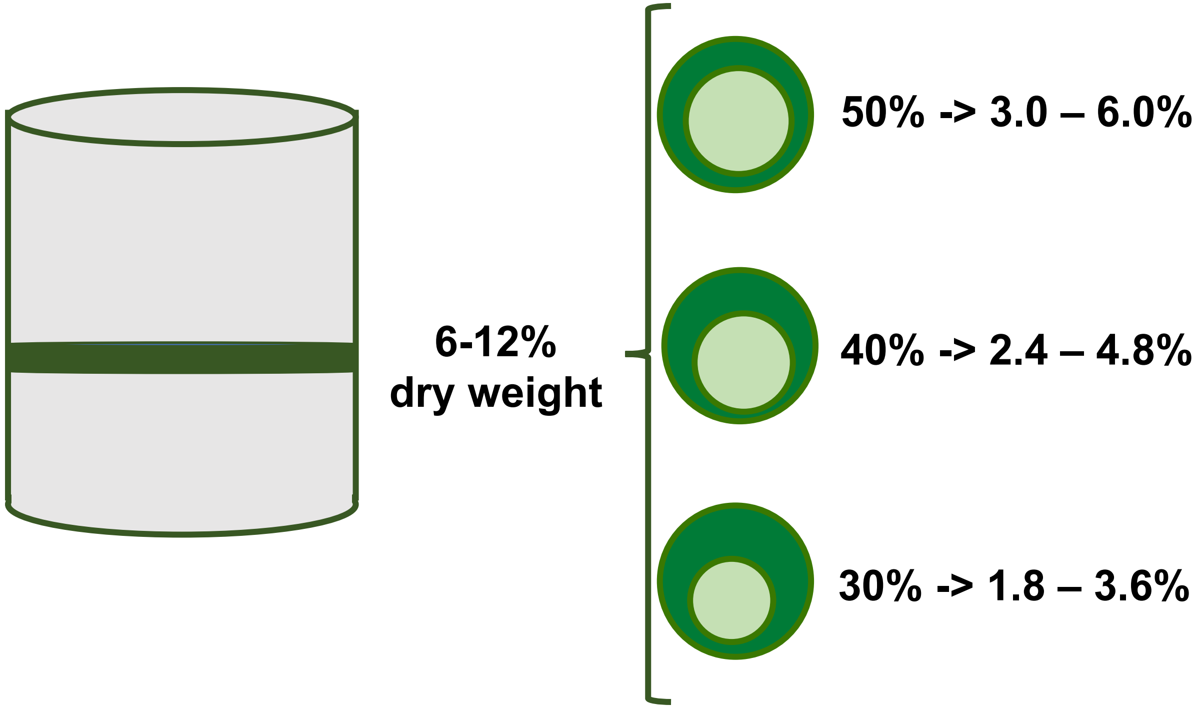




Pilot plant at the Carbonera WWTP (Italy)

Mixed cultures

Very diluted material, even after centrifugation



Need to clean and digest ~ 94% - 98.2% of the material

Mixed cultures

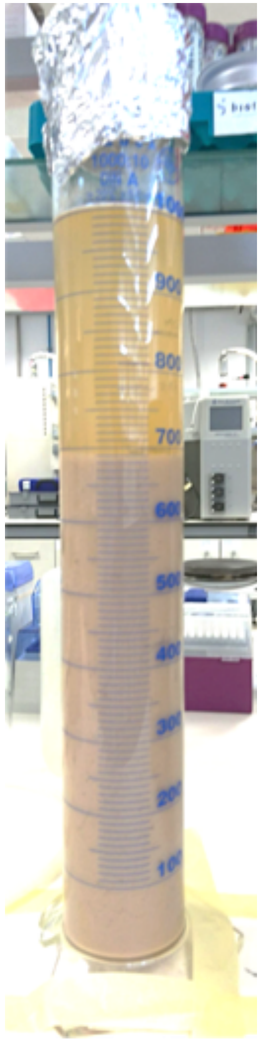
Very diluted material, even after centrifugation

biotrend
experts in bioprocessing

PHBV

Mixed cultures

Significant specification variations of the mixed culture

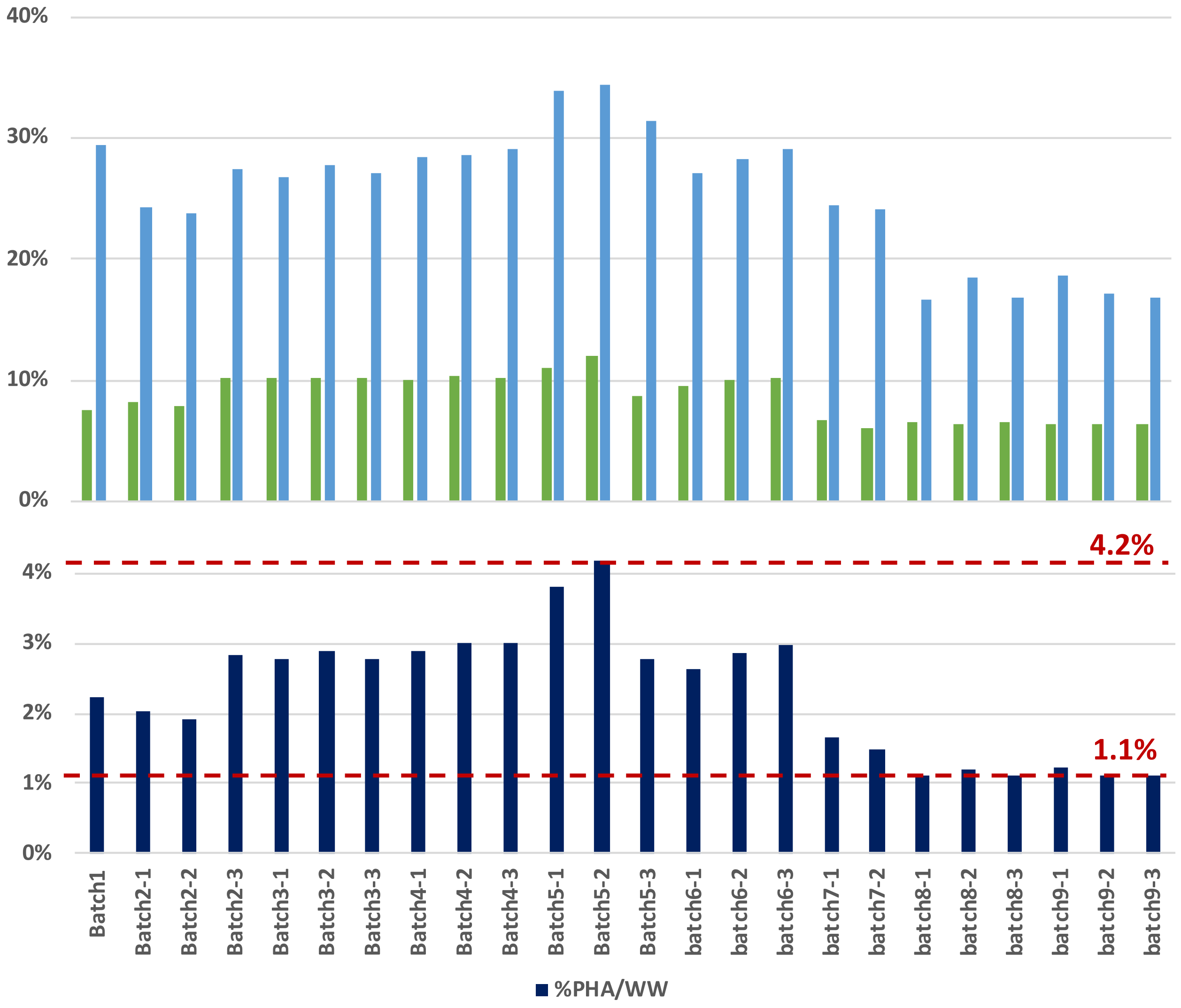


SMART-Plant

Mixed cultures

Significant specification variations of the mixed culture

DS: Dry solids
%PHA/DS



PHA extraction

Benchmark protocols

- Use of organic solvents, particularly chlorinated such as chloroform
- Use of bleach = sodium hypochlorite (NaClO)
    - When used on organic samples may generate trihalomethanes, particularly chloroform, and haloacetic acids.
    - Typical smell detected on the resulting polymer powder (relevant for processing and final use).
    - Residual chlorine will cause significant corrosion issues to processing equipment, particularly above PHA melting temperature.

PHA extraction

Alternative protocol developed by Biotrend

- *In situ* production of reactive species that degrade cellular biomass
- Depending on the biomass, no surfactant is required
- Significant reduction in amounts of chemicals is possible
- No “swimming pool” smell
- Room temperature digestion
- Easy implementation in stirred vessels and solid-liquid separations

PHA extraction

Alternative protocol developed by Biotrend

- Process needs to be tuned depending on the PHA-containing biomass

SMART-Plant

	Batch	%PHA	%HB	%HV	Pellet DW/WW (g/g)
Acetic acid	Batch A, 01/03	53.4	100	0	0.16
Sludge	Batch B, 02/04	36.4	74	21	0.18
Sludge	Batch C, 26/04	35.1	-	-	0.20
Sludge	Batch D, 10/05	32.3	59	41	-

PHA extraction

Effect of the process on the polymer

- Materials:
  - Nova: Fruit waste
  - U.Roma: MWWTP

Protocol	Biomass	GC Purity (%)	TGA PHA content (%)	GPC Mw (kDa)
Bleach	U. Nova	102	100	366
	U. Roma	85	86	217
Modified bleach protocol	U. Nova	101	98	405
	U. Nova	103	100	414
1st gen Biotrend	U. Nova	96	87	458
2nd gen Biotrend	U. Nova	100	95	366
		92	94	327

PHA extraction

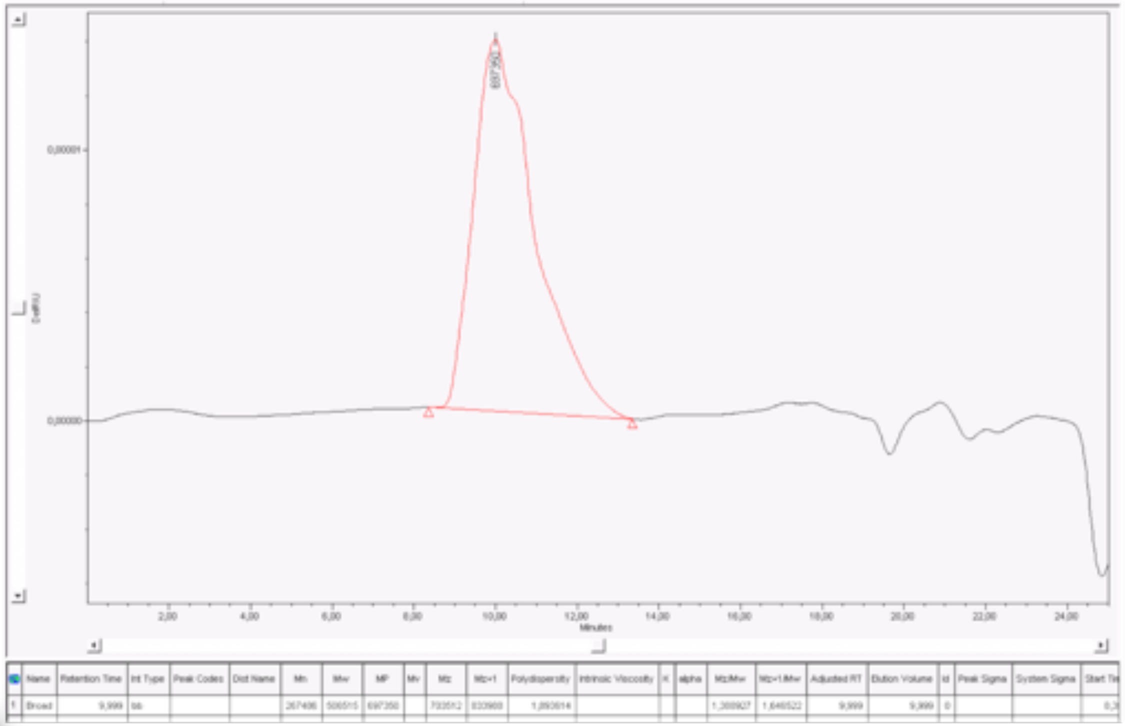
Effect of the process on the polymer

$CHCl_3$	Extracted	Decrease	Method
MW (Da)	MW (Da)		
336	235	30%	Bleach
389	241	38%	1st gen Biotrend
397	309	22%	1st gen Biotrend/controlled
336	308	8.3%	Biotrend 50% Reagents
336	309	8.1%	Biotrend 25% Reagents

Effect of the process on the polymer

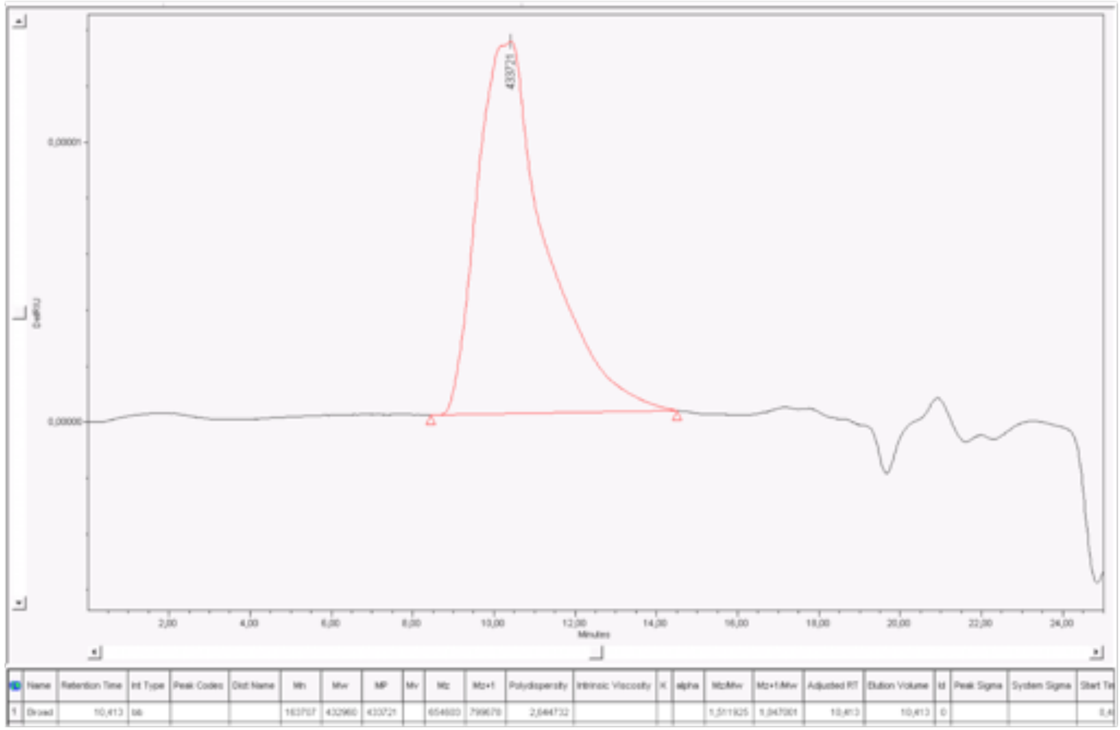
GPC

Native polymer, recovered using $CHCl_3$



Mn	Mw	Mp	Mw/Mn
267486	506515	697350	1.89

After Biotrend extraction



Mn	Mw	Mp	Mw/Mn
163707	432960	433721	2.64

PHA extraction

Effect of the process on the polymer

	Native polymer			After extraction	
	HB wt.%	HV wt.%		HB wt.%	HV wt.%
ACC53	88%	12%		86%	14%
ACC55	66%	34%		69%	31%
ACC57	37%	63%		39%	61%
ACC64	77%	23%		77%	23%
ACC71	64%	36%		62%	38%

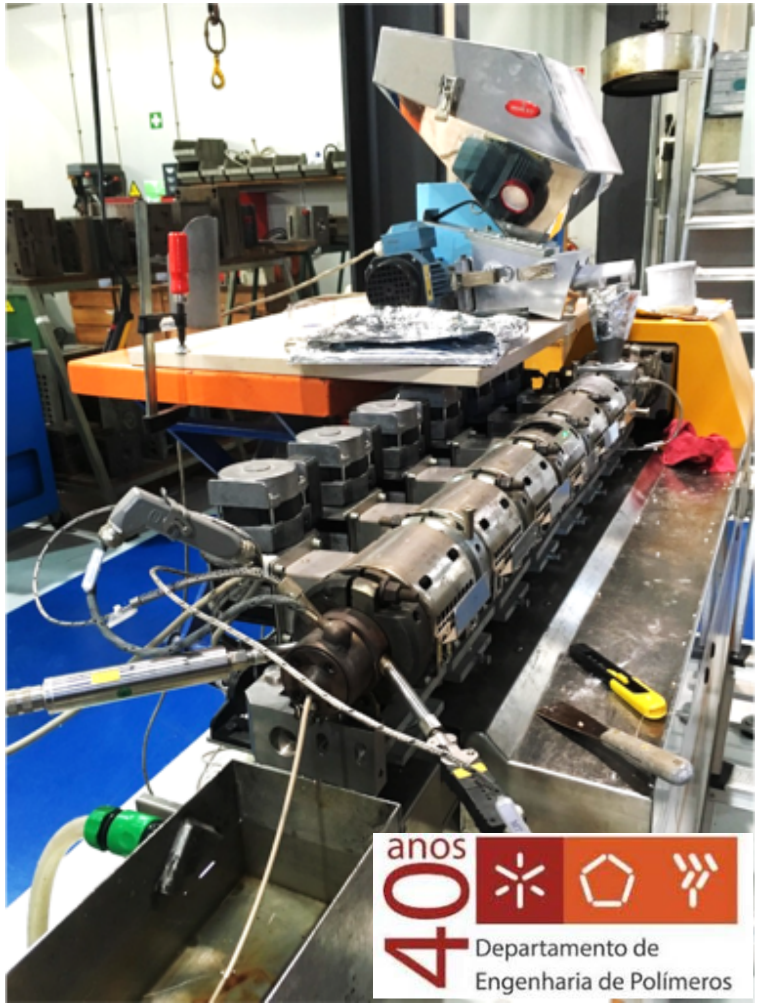
YPACK

Processability

Effect of the process on the polymer

- No changes in the monomeric composition of the polymer
- Preservation of the MW achieved.
- Conditions can be tuned to modulate the MW of the polymer
- Low molecular weight / low viscosity material obtained used as adhesive in multilayer packaging
- kg-scale pellet production successful
- Yields > 85%

CSIC
iata
Instituto de Agroquímica y Tecnología de Alimentos
Bioinicia
YPACK



Scale-up

- Scale-up successful
- Transition towards continuous operation
- Shorter residence times and lower chemicals consumption possible in larger scale
- Towards >100 kg polymer production target

INCOVER
Innovative Eco-Technologies for Resource Recovery from Wastewater
RECO URBIO
SMART-Plant
YPACK

1: Membrane housing
2: Feed tank
3: Permeate tank
4: Pump

1: Digestion vessel
2: Reagent IBC
3: Tangential flow membrane unit
4: Permeate tank

Aqueous extraction of PHAs

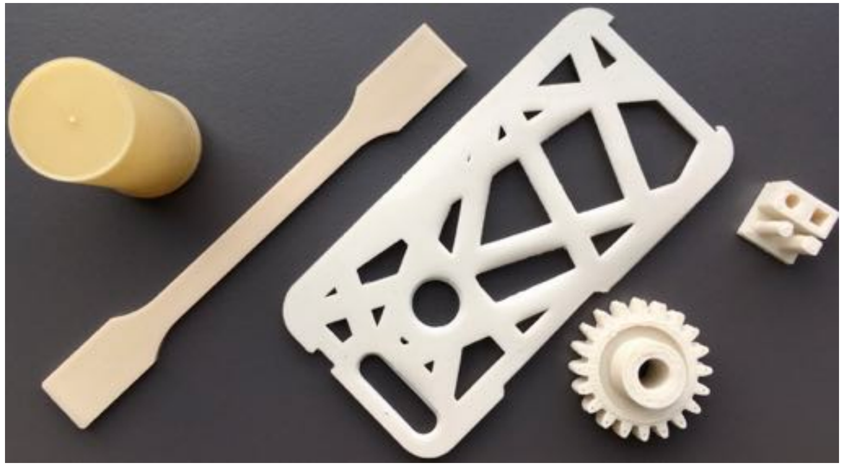
Biomass sources:

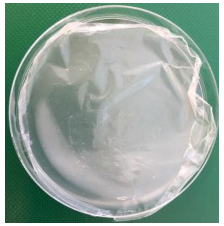
- *Pure cultures:*
  - Fermented on refined sugars
  - Fermented on residues, including lignocellulosic hydrolysates, side-streams of the pulp and paper industry, glycerol from the biodiesel industry, VFAs from anaerobic digestion

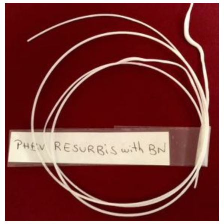
- *Mixed cultures:*
  - Municipal waste treatment, cheese manufacturing waste, fruit processing waste

Application validation:

- Melt extrusion
- Casting
- 3D printing
- Preserves MW
- White product







Aqueous extraction of PHAs

On going work:

- Stream recycle within the PHA purification process
- Continuous operation for CAPEX and OPEX reduction
- Integration opportunities of the purification process with the PHA-containing biomass producing operation (ex. water, energy)
- Full characterization of the aqueous stream with the digested biomass and proper recycle, reuse or disposal

biotrend
experts in bioprocessing

Acknowledgements

Upstream partners:

- U. of Verona
- U. Nova de Lisboa
- U. Roma La Sapienza
- U. Ca Foscari of Venice
- Innoven SRL
- Avecom
- Aqualia
- Biorefinery.de
- Borregaard
- Portucel/Soporcel
- Sniace
- Monaghan Bio
- Commercial partners

Downstream partners:

- AIMPLAS
- Brunel University
- Ecodek
- Fraunhofer-ICT
- Bioinicia
- IATA/CSIC
- U. Minho
- Sabio Materials
- Mi-Plast
- INRA
- Daren Labs
- AIMEN
- U. Liége

@Biotrend:

- Hugo Almeida
- João Cavalheiro
- Joshua Anjos
- Marina Pires
- Diogo Figueira

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