



**Presidencia
de la Nación**

Ministerio de
Ciencia, Tecnología
e Innovación Productiva



BIOECONOMÍA
ARGENTINA | 20
EL POTENCIAL DE LAS REGIONES | 15

Production of biodegradable plastics from bacterial cells

Prof^a. Dr^a. Luiziana Ferreira da Silva

Laboratory of Bioproducts  Laboratório de
Bioprodutos

Institute of Biomedical Sciences

University of São Paulo

Brazil



University of São Paulo

Overview Laboratory of Bioproducts

Impact of different metabolic networks

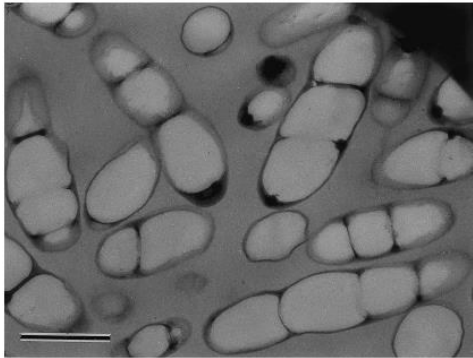
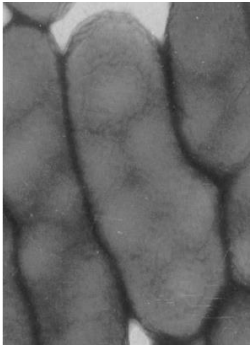
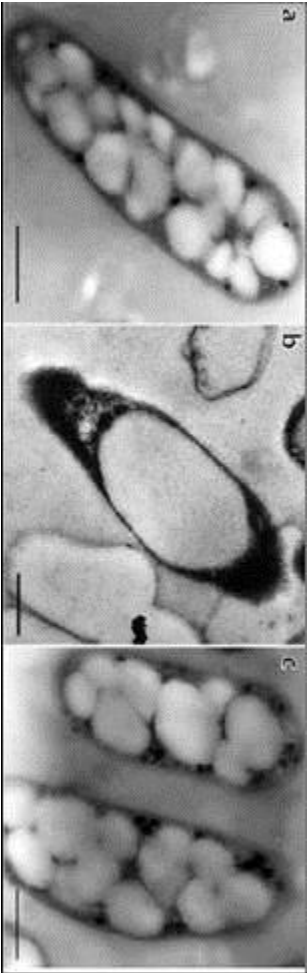
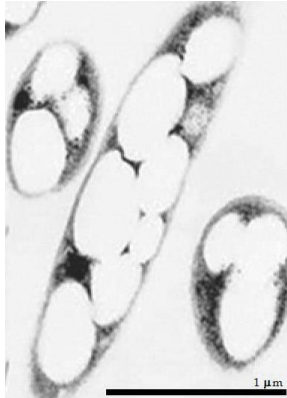
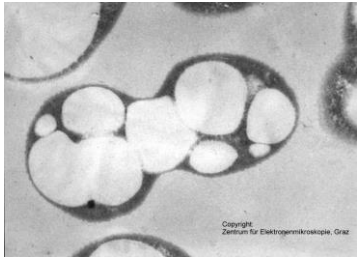


Sucrose
Glucose
Xylose
Glycerol
Fatty acids
Plant oils
Soybean molasses
Agro industrial residues
Others...

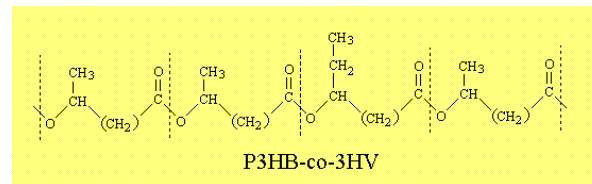
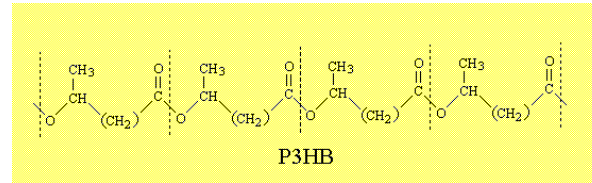
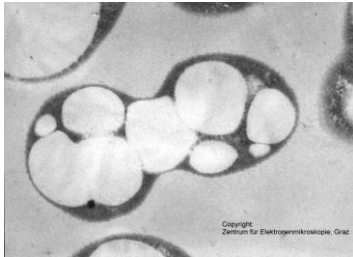
Pseudomonas sp.
B. sacchari
E. coli
Platforms for...

PHA – Biodegradable plastics
Rhamonolipids
1,3-Propanediol
Others...

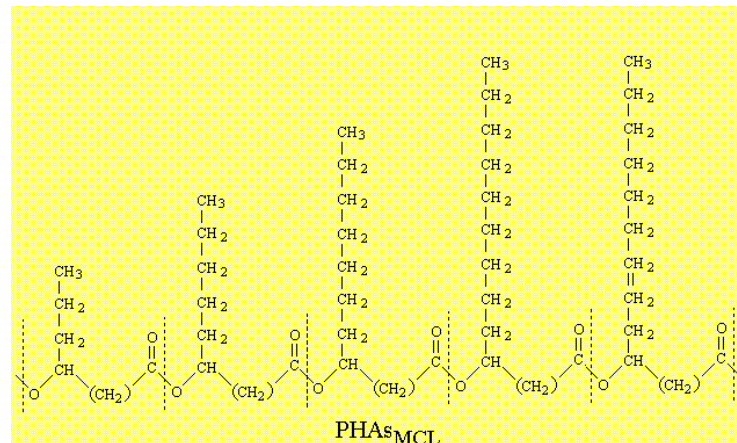
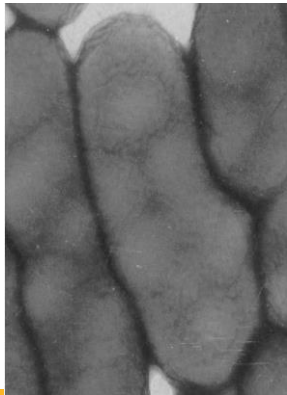
Polyhydroxyalkanoate (PHA) biodegradable & biocompatible polymers accumulated by bacteria



PHA are thermoplastic materials with variable monomer composition



C4 & C5
Short-chain length monomers
PHA scl



C6 & C12
Medium-chain length
monomers PHA mcl

Monomer composition is responsible for PHA properties and applications

Comparison of thermal and physical properties of different polymers.

PHA Polymer	T _M (°C)	T _G (°C)	Tensile strength (MPa)	Elongation to break (%)
P(3HB)	177	4	43	5
P(3HB-co-10%HV)	150	-	25	20
P(3HB-co-20%HV)	135	-	20	100
P(3HB-co-10%HHx)	127	-1	21	400
P(3HB-co-15%HHx)	115	0	23	760
P(3HB-co-17%HHx)	120	-2	20	850
P(3HB-co-19%HHx)	111	-4	-	-
Polipropileno	170	-	34	400
Poliestireno	110	-	50	-
PET	262	-	56	730
HDPE	135	-	29	-
LDPE	130	-30	10	620

3HB: 3-hydroxybutyrate; HV: 3-hydroxyvalerate; HHx: 3-hydroxyhexanoate, PET – polyethylene tereftalate, HDPE – high density polyethylene, LDPE – low density polyethylene, T_M – melting temperature de fusão; T_G glass transition temperature.

Sources: HOLMES, 1985; KING, 1982; DOI *et al.*, 1995; SUDESH *et al.*, 2000; PRADELLA, 2006; CARMINATTI *et al.*, 2006

Applications

Bucci, 2003

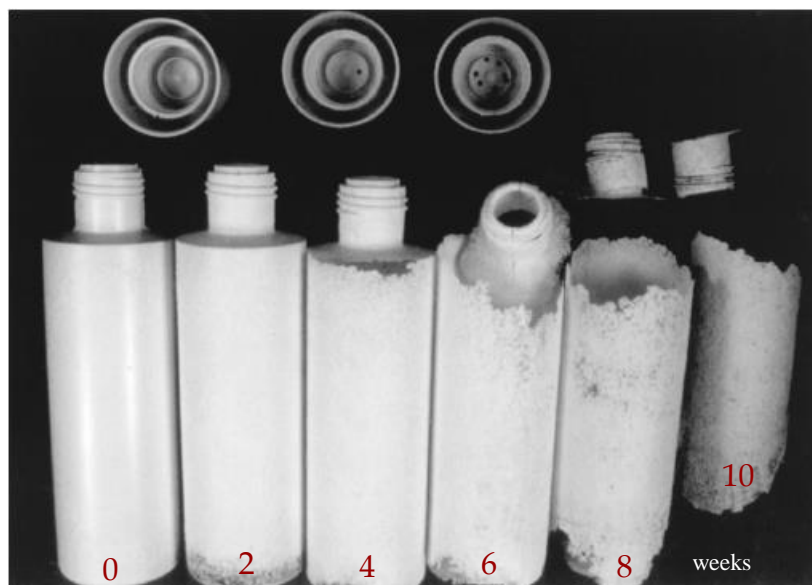
CARTÕES DERIVADOS DA CANA-DE-AÇÚCAR / CARDS MADE FROM SUGAR CANE

<p>Características:</p> <ul style="list-style-type: none"> • Recurso renovável anualmente; • Reciclável; • Reduzido uso de recursos fósseis; • Biodegradável; • Quando incinerado não libera gases tóxicos; • Cartão plástico produzido com PHB. <p>Principais aplicações:</p> <ul style="list-style-type: none"> • Cartões comerciais; • Cartões presentes; • Cartões pré-pagos. 	<p>Characteristics:</p> <ul style="list-style-type: none"> • Annually Renewable Resource; • Recyclable; • Reduces Fossil Resource Use; • Biodegradable through industrial composting; • When incinerated will burn cleanly; • Plastic card produced with PHB. <p>Main Applications:</p> <ul style="list-style-type: none"> • Commercial cards; • Gift cards; • Phone cards.
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BIOCYCLE



PHA are biodegradable materials



Madison & Huisman, 1999

PHA are biocompatible polymers

Applications

Bone tissue engineering

Cartilage Tissue Engineering

Drug delivery

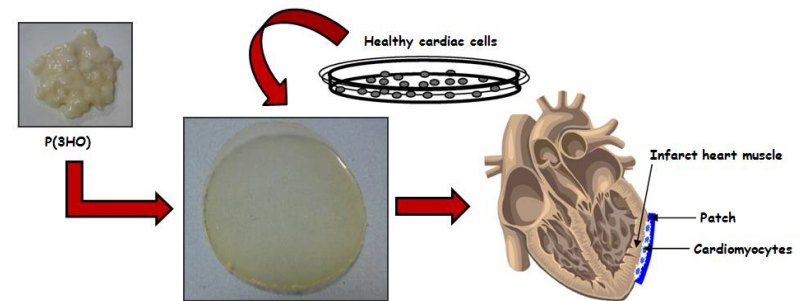
Medical device development:

Biodegradable drug eluting stents

Biodegradable nerve conduits

Skin Tissue Engineering/Wound Healing

Cardiac Tissue Engineering



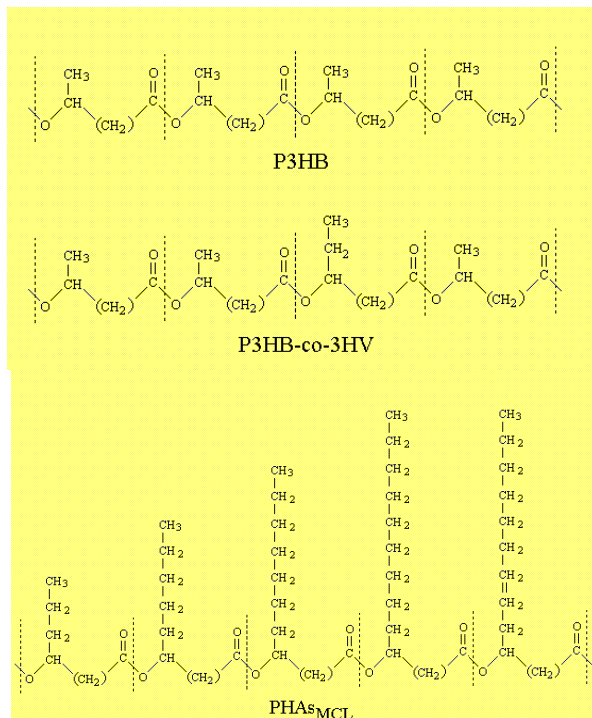
Recently reviewed @

by Dr Ipsita Roy
Imperial College London
Univ. Westminster



ISBP 2014 Brazil

PHA production integrated to a sugar and ethanol mill.



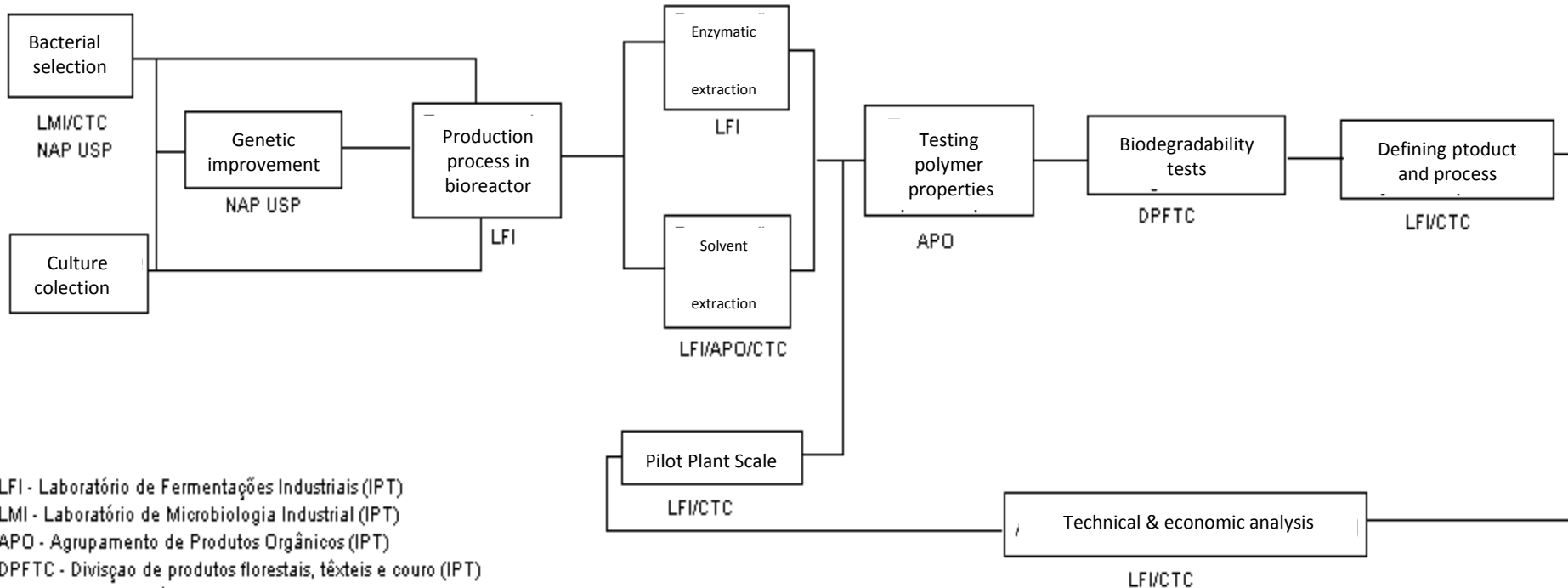
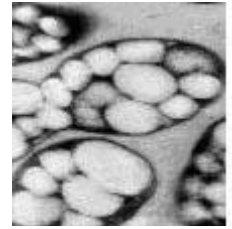
R. V. Nonato · P. E. Mantelatto · C. E. V. Rossell

Integrated production of biodegradable plastic, sugar and ethanol

Appl Microbiol Biotechnol (2001) 57:1–5
 DOI 10.1007/s002530100732

MINI-REVIEW

A green cycle for simultaneous poly 3-hydroxybutyric acid, sugar and ethanol production



LFI - Laboratório de Fermentações Industriais (IPT)
 LMI - Laboratório de Microbiologia Industrial (IPT)
 APO - Agrupamento de Produtos Orgânicos (IPT)
 DPFTC - Divisão de produtos florestais, têxteis e couro (IPT)
 CTC - Centro Tecnológico Copersucar
 NAP - USP Instituto de Ciências Biomédicas (USP) e Universidade Federal da Paraíba

Esquema geral de projeto de pesquisa e desenvolvimento de tecnologia de produção de PHA (Gomez *et al.*, 1993)

Process transfer to industry

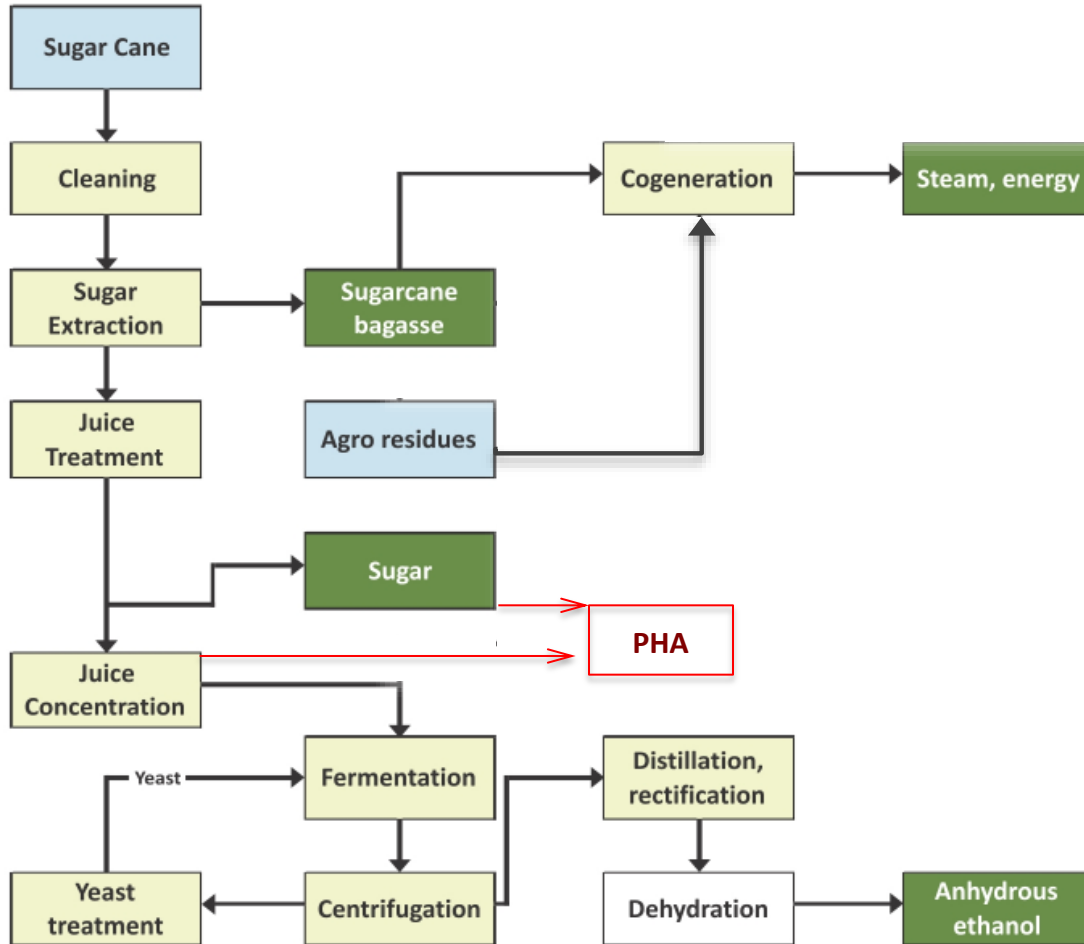
Table 1 Worldwide PHA producing and researching companies

Company	Types of PHA	Production scale (t/a)	Period	Applications
ICI, UK	PHBV	300	1980s to 1990s	Packaging
Chemie Linz, Austria	PHB	20–100	1980s	Packaging & drug delivery
btF, Austria	PHB	20–100	1990s	Packaging & drug delivery
Biomers, Germany	PHB	Unknown	1990s to present	Packaging & drug delivery
BASF, Germany	PHB, PHBV	Pilot scale	1980s to 2005	Blending with Ecoflex
Metabolix, USA	Several PHA	Unknown	1980s to present	Packaging
Tepha, USA	Several PHA	PHA medical implants	1990s to present	Medical bio-implants
ADM, USA (with Metabolix)	Several PHA	50 000	2005 to present	Raw materials
P&G, USA	Several PHA	Contract manufacture	1980s to 2005	Packaging
Monsanto, USA	PHB, PHBV	Plant PHA production	1990s	Raw materials
Meridian, USA	Several PHA	10 000	2007 to present	Raw materials
Kaneka, Japan (with P&G)	Several PHA	Unknown	1990s to present	Packaging
Mitsubishi, Japan	PHB	10	1990s	Packaging
Biocycles, Brazil	PHB	100	1990s to present	Raw materials
Bio-On, Italy	PHA (unclear)	10 000	2008 to present	Raw materials
Zhejiang Tian An, China	PHBV	2000	1990s to present	Raw materials
Jiangmen Biotech Ctr, China	PHBHHx	Unknown	1990s	Raw materials
Yikeman, Shandong, China	PHA (unclear)	3000	2008 to present	Raw materials
Tianjin Northern Food, China	PHB	Pilot scale	1990s	Raw materials
Shantou Lianyi Biotech, China	Several PHA	Pilot scale	1990s to 2005	Packaging and medical
Jiang Su Nan Tian, China	PHB	Pilot scale	1990s to present	Raw materials
Shenzhen O'Bioer, China	Several PHA	Unknown	2004 to present	Unclear
Tianjin Green Bio-Science (+DSM)	P3HB4HB	10 000	2004 to present	Raw materials & packaging
Shandong Lukang, China	Several PHA	Pilot scale	2005 to present	Raw materials & medical

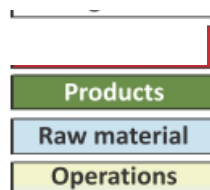
First generation ethanol (1G)

Currently produced from sugar or starch-based raw materials

1st generation PHA



Captions



Second generation ethanol (2G)

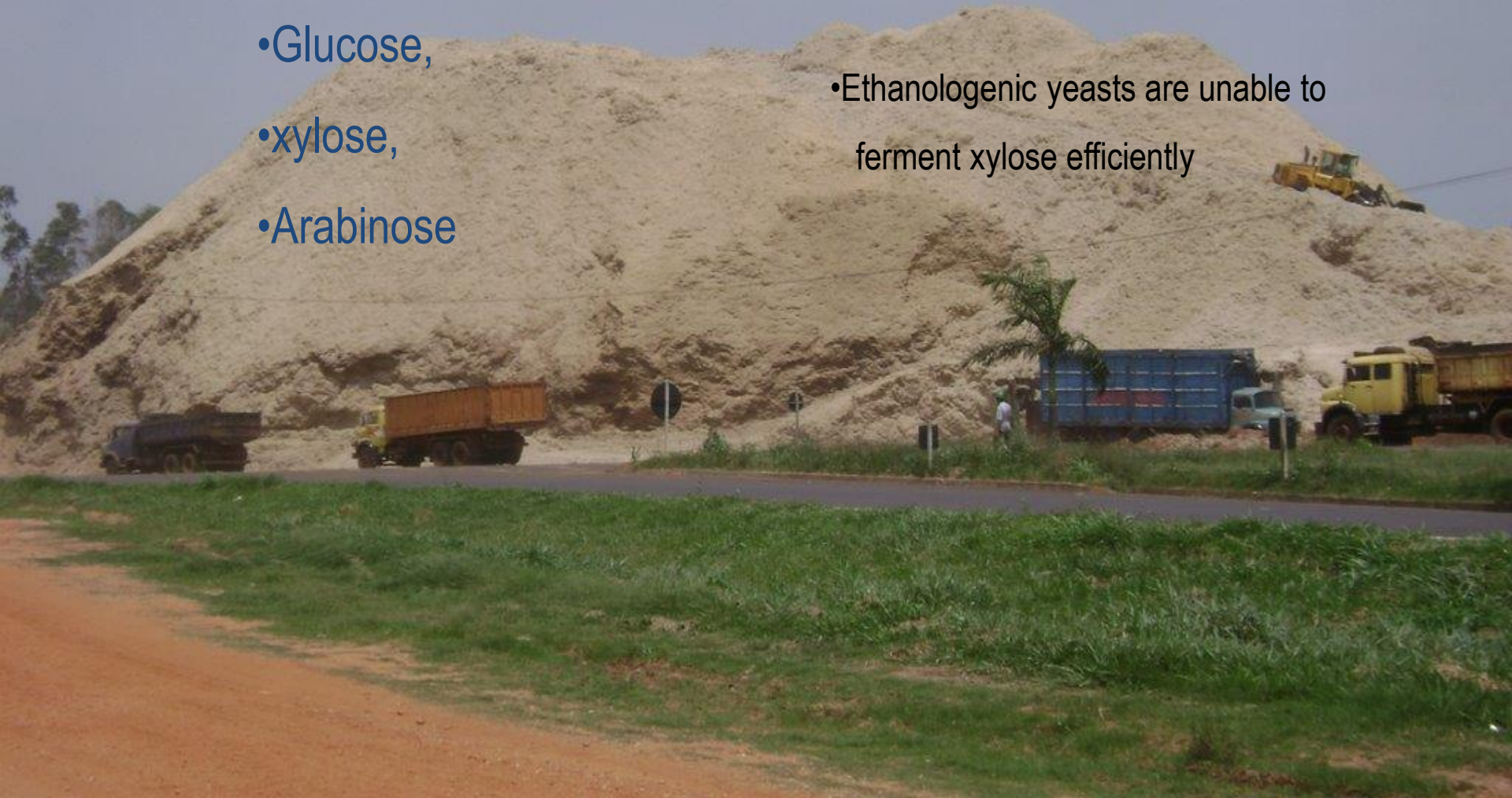


Over 40,000,000 tons bagasse and sugarcane leaves per season
source UNICA

Releasing sugars from bagasse

- Chemical or enzymatic hydrolysis
- Sugar mixtures available for fermentation
 - Glucose,
 - xylose,
 - Arabinose

• Ethanologenic yeasts are unable to ferment xylose efficiently





Scenario in Brazil

Ethanol 2G from ligno & hemicellulose

- Xylose & arabinose available in large amounts
 - Perspective 10^7 ton/year of C5 available*
- Use of xylose to generate ethanol and other products

Pradella, 2012, Pereira et al., 2013





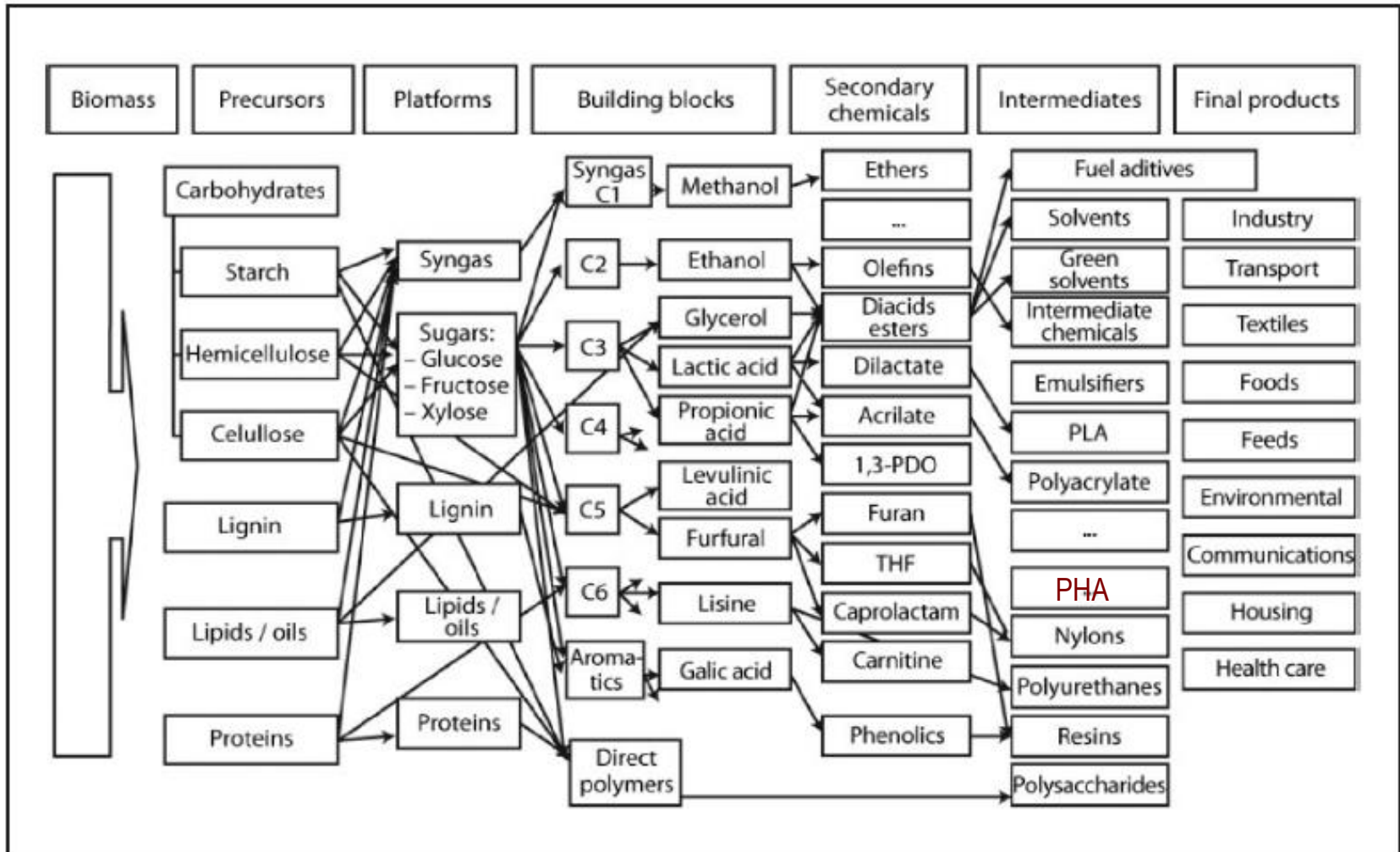
Opportunity to expand bioplastics industry



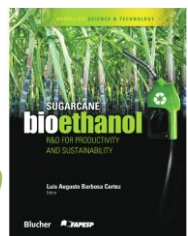
- Sugarcane is an environmentally sustainable feedstock for bioplastics
- Integration do sugar and ethanol mills
- Green plastics
- PHA
- PLA
- Others



Biorefinery



Kamm et al., 2006, Franco & Garzón In: Cortez, 2010



Biorefinery

Ethanol and sugar mills

Current

- Include a number of processes already used since 19th century
- Some processes already integrated

Challenges

- Integration of different technologies
 - Metabolic engineering
 - Full knowledge of the raw material (biomass)
 - Addition of new products (intermediates and final)
- Evaluation of social and environmental impacts

sustainability

Bottlenecks/obstacles

Ethanol 1G

- The potential of ethanol 1G is far from being exhausted
- Productivity gains in the same cultivated area (yield/ha yield/sugarcane ton) – new varieties, GMOs
- Geographical expansion (other areas and other countries)

Ethanol 2G & biorefineries

- Hydrolysis of lignocellulose & hemicellulose to release sugars: toxic compounds are also released
- Development of (micro) organisms and processes to transform sugars into bioproducts

Contributions to the process

J Ind Microbiol Biotechnol (2004) 31: 245–254
 DOI 10.1007/s10295-004-0136-7

ORIGINAL PAPER

L. F. Silva · M. K. Taciro · M. E. Michelin Ramos
 J. M. Carter · J. G. C. Pradella · J. G. C. Gomez

Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolysate

Table 5 Comparison of the present data with data reported in the literature about P3HB production from cellulose hydrolysates or sugars obtained from the hydrolysis of these materials. *CS* Cotton seed hydrolysate, *SH* soybean hydrolysate, $\mu_{X_{rmax}}$ maximum

specific growth rate of residual cells (non-polymer material), μ_{Pmax} maximum specific polymer accumulation rate, P_{P3HB} polymer productivity, $Y_{P3HB/S}$ polymer yield from the carbon source

Strain	Carbon source	CDW (g l ⁻¹)	P3HB (%)	$\mu_{X_{rmax}}$ (h ⁻¹)	μ_{Pmax} (h ⁻¹)	$Y_{P3HB/S}$ (g g ⁻¹)	P_{P3HB} (g l ⁻¹ h ⁻¹)	Reference
<i>Pseudomonas pseudoflava</i> ATCC 33668	Glucose	3.5	22.8	0.58	0.11	0.04	0.080	[2]
<i>P. pseudoflava</i> ATCC 33668	Xylose	4.0	27.5	0.13	0.03	0.04	0.031	[2]
<i>B. cepacia</i> ATCC 17759	Xylose	7.5	45	0.22	0.07	0.11	0.10	[20]
<i>B. cepacia</i>	Xylose		48.8	–	–	0.11	–	[30]
<i>Escherichia coli</i> TG1 (pSYL107) ^a	Xylose	4.75	35.8	–	–	0.097	0.028	[12]
<i>E. coli r</i> TG1 (pSYL107) ^a	Xylose + CSH	3.76	64.0	–	–	0.188	0.040	[12]
<i>E. coli</i> TG1 (pSYL107) ^a	Xylose + SH	5.95	72.0	–	–	0.226	0.070	[12]
<i>B. sacchari</i> IPT 101	Sugarcane bagasse hydrolysate	4.4	62	0.24	0.16	0.39	0.11	Present paper
<i>B. cepacia</i> IPT 048	Sugarcane bagasse hydrolysate	4.4	53	0.36	0.08	0.29	0.09	Present paper
<i>B. sacchari</i> IPT 101	Xylose + glucose	60	58	0.25	0.03	0.22	0.47	Present paper
<i>B. cepacia</i> IPT 048	Xylose + glucose	57	57	0.28	0.06	0.19	0.46	Present paper

^aRecombinant strain

High PHB content

Low productivity

Detoxification of hydrolysate needed

Screening of bacteria to produce polyhydroxyalkanoates from xylose

Mateus Schreiner Garcez Lopes · Rafael Costa Santos Rocha ·
Sandra Patricia Zanotto · José Gregório Cabrera Gomez ·
Luiziana Ferreira da Silva

Cloning and overexpression of the xylose isomerase gene from *Burkholderia sacchari* and production of polyhydroxybutyrate from xylose

Can. J. Microbiol. 55: 1012–1015 (2009)

Mateus Schreiner Garcez Lopes, José Gregório Cabrera Gomez, and Luiziana Ferreira Silva

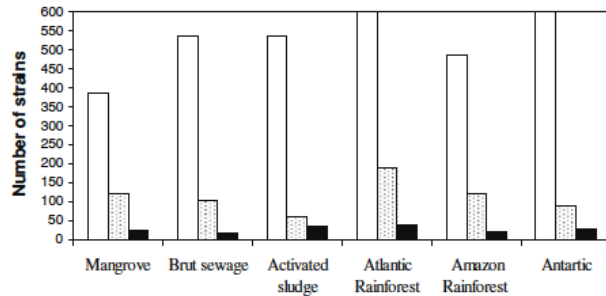
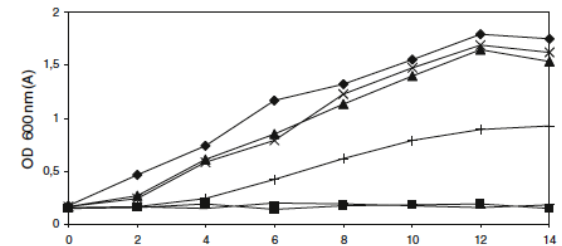


Table 1. Specific xylose isomerase activities (assayed as described in Shamanna and Sanderson (1979)) in wild-type *Burkholderia sacchari* IPT101, *B. sacchari* IPT101 harboring pBBR1MCS-2 used as control, and *B. sacchari* IPT101 harboring pBBR1xylA (LFM900).

Strain	Activity (U·mg protein ⁻¹)
<i>B. sacchari</i> IPT101	1.46
<i>B. sacchari</i> :: pBBR1MCS-2	1.32
<i>B. sacchari</i> pBBR1xylA	2.44

Note: One unit of isomerase activity is defined as the amount of crude enzyme required to produce 1 μmol product·min⁻¹.

Fig. 2. Growth experiments in mineral media (based on Rocha et al. 2008) with xylose as carbon source (2 g·L⁻¹): ♦, *Burkholderia sacchari* IPT101 wild type; ×, *B. sacchari* harboring pBBR1MCS-2 used as control; ▲, *B. sacchari* LFM900 harboring pBBR1MCS-2 with *xylA* cloned (pBBR1xylA); ■, *B. sacchari* xyl⁻ IPT536, IPT101 mutant unable to grown in xylose; –, *B. sacchari* xyl⁻ IPT536 harboring pBBR1MCS-2 used as control; +, *B. sacchari* xyl⁻ IPT536 harboring pBBR1xylA.



Performance in sugar mixtures (bagasse hydrolysate main sugars)

Strain	Sugar	CDW (g l ⁻¹)	Sugar (g l ⁻¹)	PHB (%)	Time (h)	Y _{HB} (g g ⁻¹)	P _{HB} (g l ⁻¹ h ⁻¹)
<i>Bu.sacchari</i>	Glu	6.37	14.07	63.14	36	0.29	0.11
<i>Busacchari</i>	Xyl	5.53	12,37	58.07	48	0.26	0.07
<i>Bu.sacchari</i>	Glu+Xyl	5.82	12.42	53.42	36	0.25	0.09
<i>Bu.sacchari</i>	Glu+Xyl+Ara	5.72	12.26	47.49	36	0.22	0.08
MA 3.3	Glu	5.76	14.58	62.15	36	0.25	0.10
MA 3.3	Xyl	5.54	14.97	64.36	60	0.24	0.06
MA 3.3	Glu+Xyl	3.86	10.19	38.16	24	0.14	0.06
MA 3.3	Glu+Xyl+Ara	3.99	14.50	39.89	48	0.11	0.03

Cell dry weight (CDW), PHB content of the cell dry weight (%PHB) PHB yield from carbon source (Y_{PHB/C}), and PHB volumetric productivity (P_{PHB})

Bacillus megaterium

- P_{PHB} is 40% lower in xylose than in glucose

-In sugar mixtures parameters were lower: %PHB, Y_{PHB/C} e P_{PHB}.

PHB Biosynthesis in Catabolite Repression Mutant of *Burkholderia sacchari*

Mateus Schreiner Garcez Lopes · Guillermo Gosset ·
Rafael Costa Santos Rocha · José Gregório Cabrera Gomez ·
Luiziana Ferreira da Silva

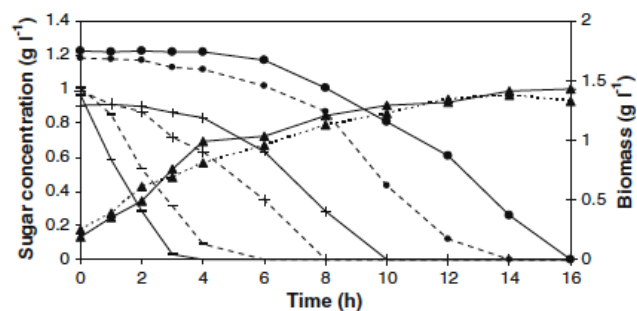
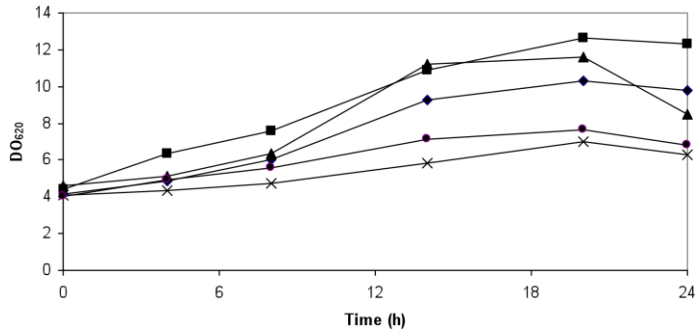


Fig. 3 Growth experiments in triple sugar mixtures with wild type *B. sacchari* IPT101 (solid line) and *B. sacchari* LFM828 PTS⁻ glucose⁺ (dotted line): (filled triangle) biomass, (minus) glucose, (plus) arabinose, and (filled circle) xylose

Improving simultaneous
consumption of different sugars
from bagasse

Polyhydroxyalkanoate biosynthesis and simultaneous remotion of organic inhibitors from sugarcane bagasse hydrolysate by *Burkholderia* sp.

Mateus Schreiner Garcez Lopes ·
 José Gregório Cabrera Gomez · Marilda Keico Taciro ·
 Thatiane Teixeira Mendonça · Luiziana Ferreira Silva



Growth experiment with F24 in mineral media with xylose (10 g l⁻¹) and individual compounds: (■) 2.5 g l⁻¹ of acetic acid, (▲) 1.25 g l⁻¹ of formic acid, (◆) control experiment only with xylose, (●) 0.5 g l⁻¹ of HMF, and (x) 0.5 g l⁻¹ of furfural.

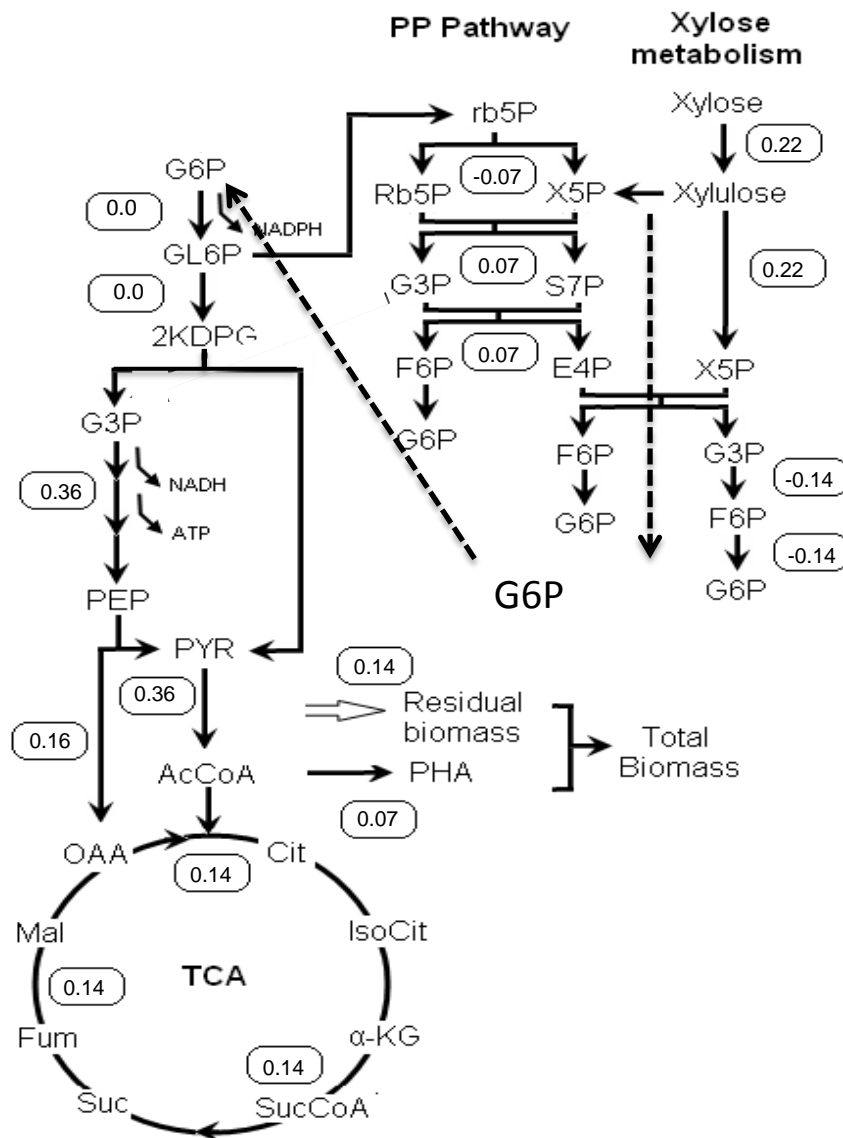
Isolate F24 (*Burkholderia* sp) can use toxic compounds from sugarcane hydrolysate

Seed	Formic acid	Acetic acid	Furfural	HMF	Xylose	Cell density	%PHA
0	1.57	1.97	0.46	0.15	17.65	0	0
0.5	0.14	0.43	0.13	0.03	11.18	2.3	37.45
1.0	0.02	0.02	0.17	0.03	12.13	2.89	42.15
1.5	0.03	0.14	0.21	0.01	5.23	3.75	42.15
3.2	0.07	0.00	0.04	0.00	4.78	7.18	32.35
6.5	0.00	0.00	0.07	0.02	2.31	10.48	35.72

Effect of the inoculum size (g l⁻¹) on utilization of hydrolysates (g l⁻¹), cell growth (g l⁻¹) and PHA biosynthesis (% of PHA of the cell dry weight) in hydrolysate medium after 48 hours

Fluxes leading to: $Y_{xyl/HB} = 0.25 \text{ g g}^{-1}$

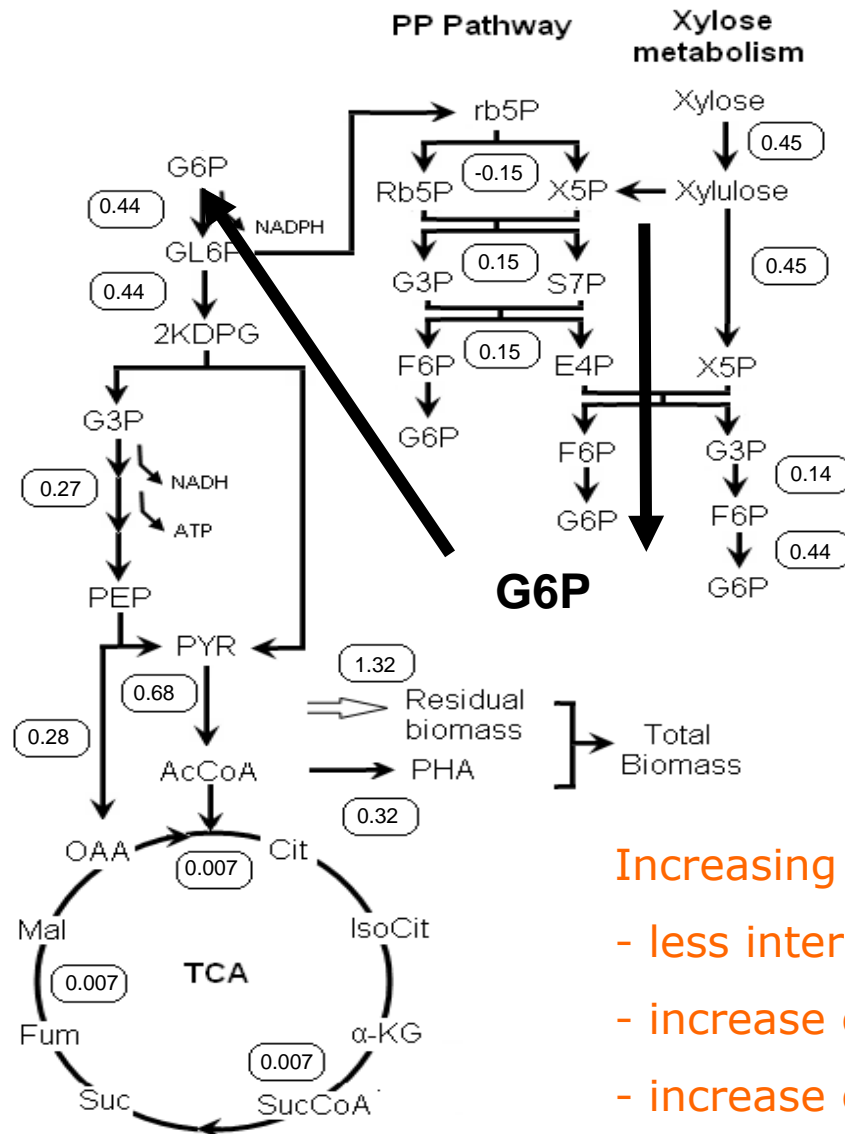
Metabolic flux analysis as a tool for bacterial improvement



NADPH needed for PHA production in *B. sacchari*

Fluxes resulting on: $Y_{\text{Xyl}/\text{HB}} = 0.41 \text{ g g}^{-1}$

Metabolic flux analysis as a tool for bacterial improvement



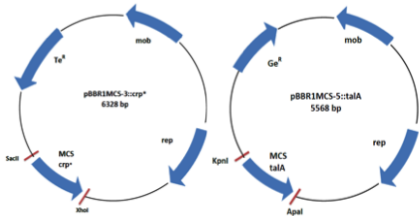
talA

Increasing fluxes on PPP:

- less intermediate accumulation;
- increase on NADPH yield;
- increase on PHB yield;

Metabolic engineering of *Burkholderia sacchari* for improved production of bio-based products from xylose

Linda Guaman, M. Schreiner, J. Cabrera, L.F. da Silva, M. Keico



Plasmid maps of pBBR1MCS-3::crp* and pBBR1MCS-5::talA respectively.

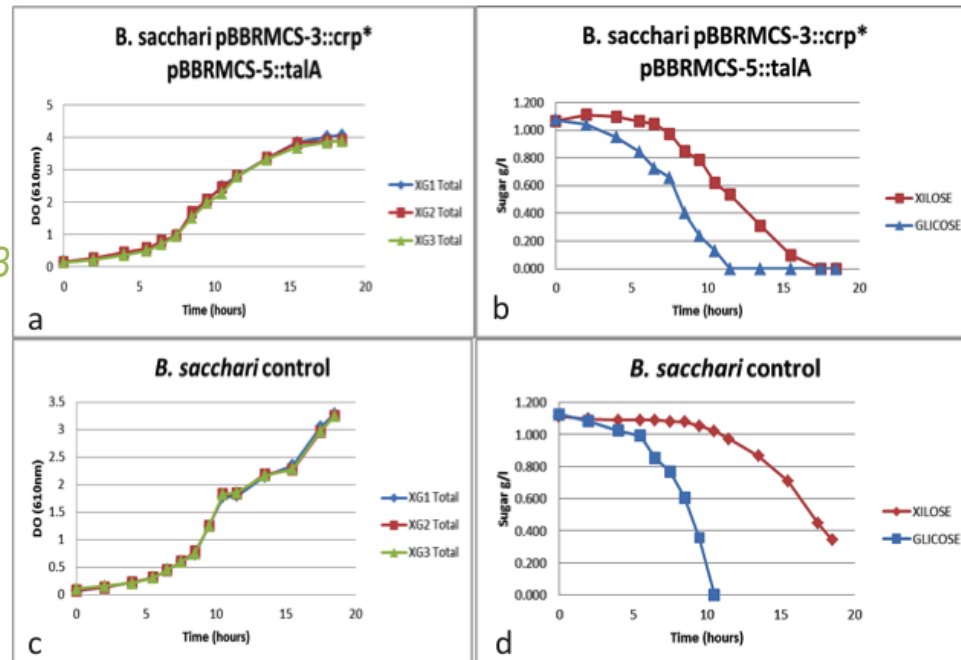


Fig 6. a-c: Bacterial growth curve. b-d: Sugar consumption

15% increase μ_{max}
 35% reduction on lag phase
 simultaneous consumption of xylose and glucose
 40% productivity CDW.



Metabolic engineering of *Burkholderia sacchari* for improved production of bio-based products from xylose

Linda Guaman, M. Schreiner, J. Cabrera, L.F. da Silva, M. Keico

Strain	Carbon Source	PHA Composition	CDW (g l ⁻¹)	%PHB	PHB g/l	Time (h)	Reference
<i>B. sacchari</i> IPT101	Xilose	PHB	4.16	58.07	2.42	48h	Lopes et al., 2009
<i>B. sacchari</i> IPT101	Glicose e xilose	PHB	5.82	53.42	3.11	48h	Lopes et al., 2009
<i>B. sacchari</i> LFM 1103 [#]	Xilose	PHB	7.1	53.71	3.81	48h	This work
<i>B. sacchari</i> LFM 1103 [#]	Glicose e xilose	PHB	7	60.12	4.21	48h	This work
<i>B. sacchari</i> LFM 1105 [¥]	Xilose	PHB	4.12	55.07	2.27	48h	This work
<i>B. sacchari</i> LFM 1105 [¥]	Glicose e xilose	PHB	4.81	52.83	2.54	48h	This work

recombinants harboring *crp** and *talA* increased polymer accumulation

**Expanding the spectrum of carbohydrate utilization by *Pseudomonas* sp.
LFM046: Xylose utilization** E.R. Oliveira, L.P. Guamán, Luiziana F. Da Silva, J.G.
Gomez, M.K. Taciro

*Introducing the ability to consume xylose and sugars from biomass
to other industrially interesting bacteria*

1 2 3

1 2 3

ORIGINAL ARTICLE

Exploring the potential of *Burkholderia sacchari* to produce polyhydroxyalkanoates

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Modulation of monomer composition of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) P(3HB-co-3HHx) produced by *Burkholderia sacchari*

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Figure 3 – Composition of PHA accumulated from glucose (10 g.l⁻¹) and hexanoic acid (0-1.5 g.l⁻¹) by *B. sacchari* wild type (A) and recombinants (B) (*phaPCJ* - left - and *phaCJ* - right)

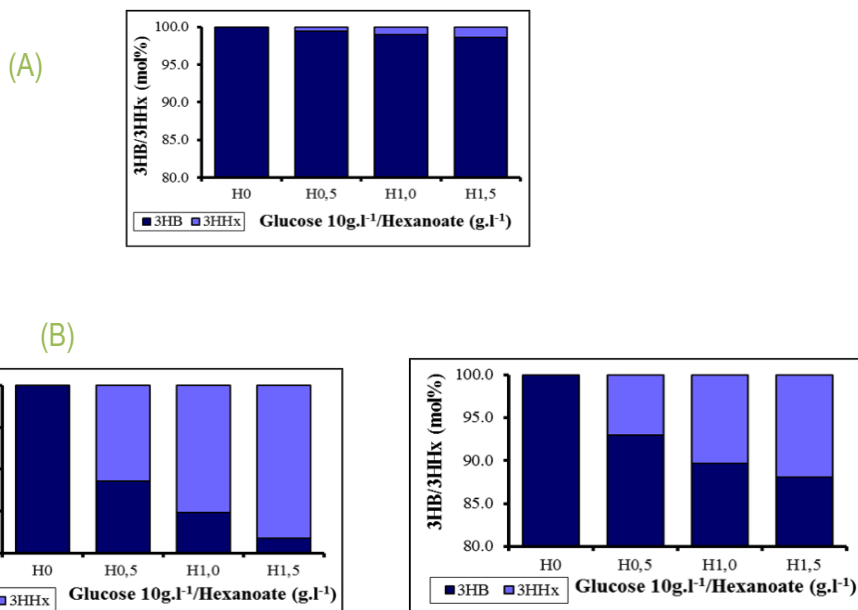
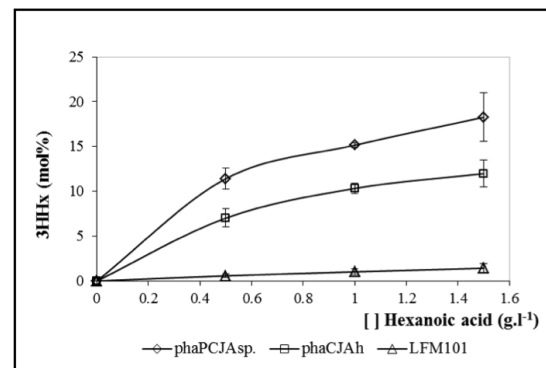


Figure 4 – Correlation of hexanoic acid concentration provided and 3HHx molar fraction obtained in experiments with *B. sacchari* strains (wild type and recombinants)



Other approaches

Integration of bagasse enzymatic hydrolysis to the biorefinery

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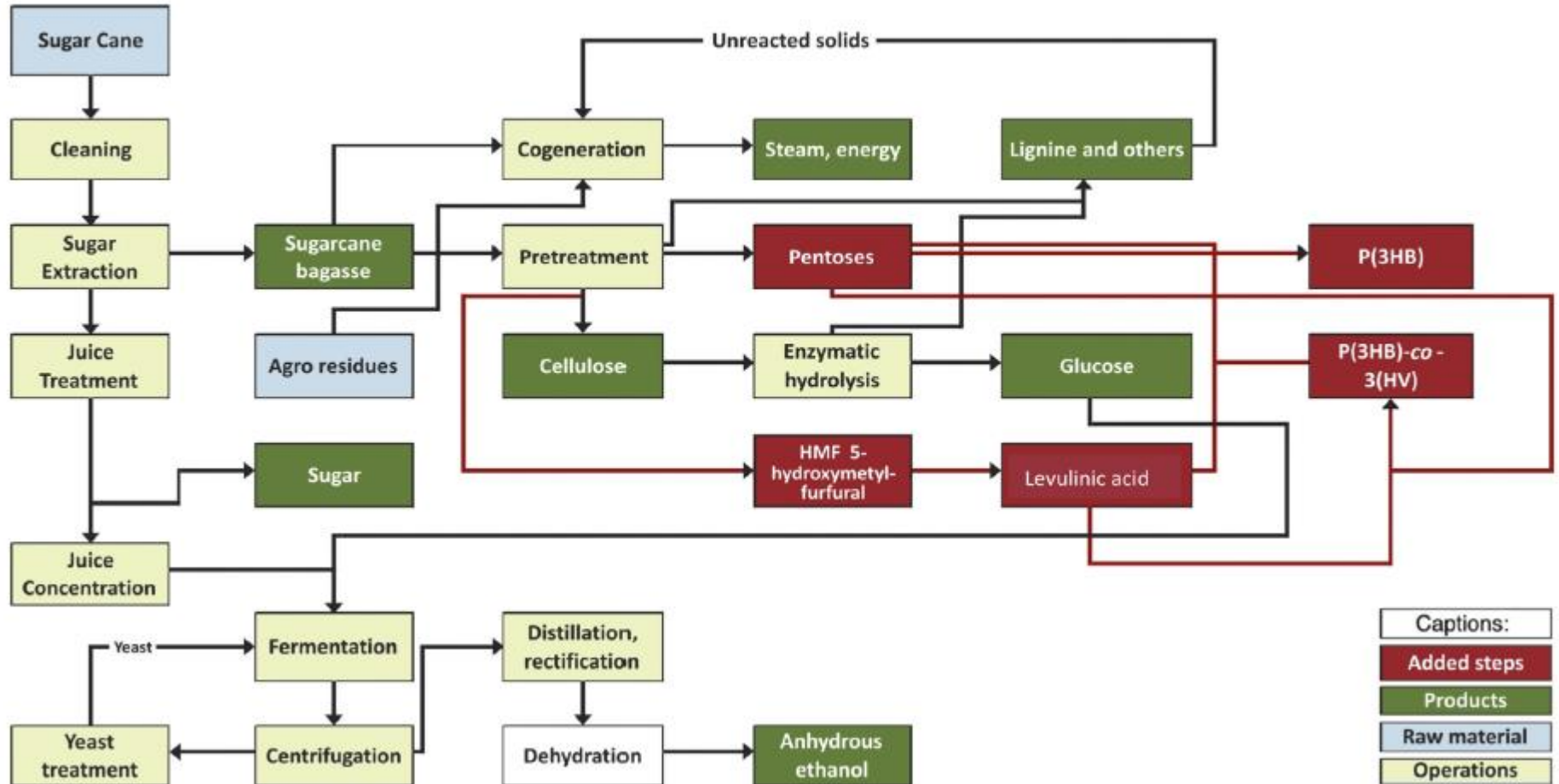
Cellulase On-Site Production from Sugar Cane Bagasse Using *Penicillium echinulatum*

**Beatriz Merchel Piovesan Pereira • Thabata Maria Alvarez •
Priscila da Silva Delabona • Aldo José Pinheiro Dillon •
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Biopolymer production integrated to a sugar and ethanol mill

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Perspectives and challenges

- *The production of lignocellulosic hydrolysates of sugarcane bagasse, usable by microorganisms, is still a challenge, as well as obtaining ethanologenic yeast or other microorganisms efficient in transforming the resulting hydrolysate in commercially attractive products.*
- **Important issues**
 - *Use of other residues as raw materials*
 - *Other biorefinery models*
 - *Interdisciplinary work*
 - *Interaction with industries*
 - *discussion forums involving different areas*
 - *Government policies and support*

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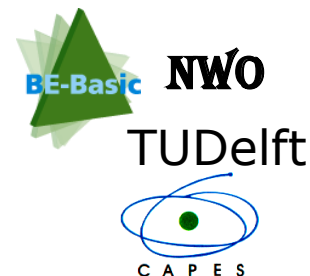
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