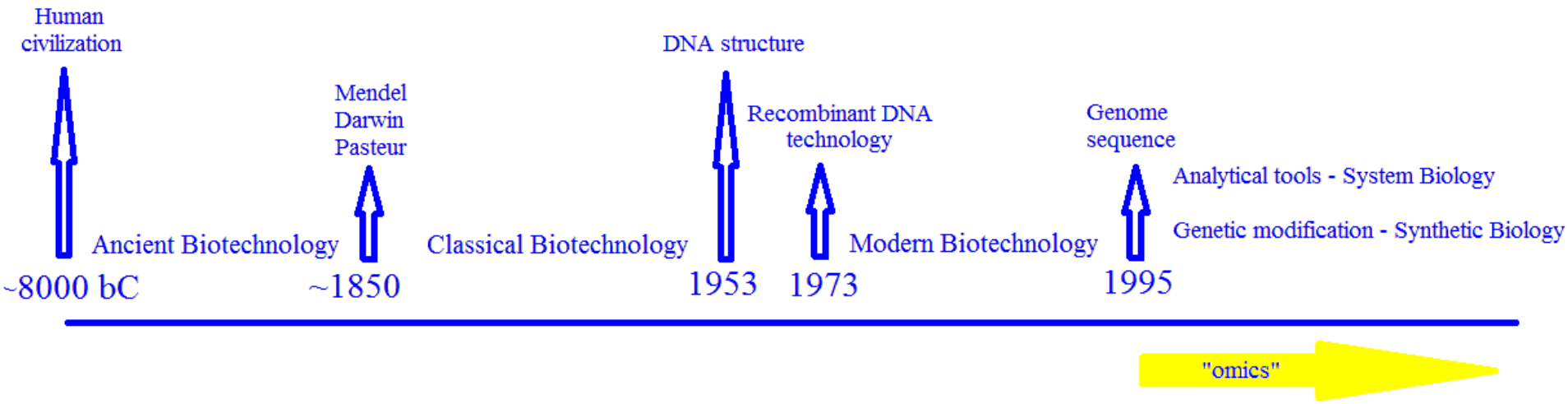
Metabolic fluxes analysis and development of bacterial processes for bio-based products.

José Gregório Cabrera Gomez
Department of Microbiology
Institute of Biomedical Sciences
University of São Paulo

DEPARTAMENTO DE
MICroBiologia
UNIVERSIDADE DE SÃO PAULO

Biology and Biotechnology



System Biology

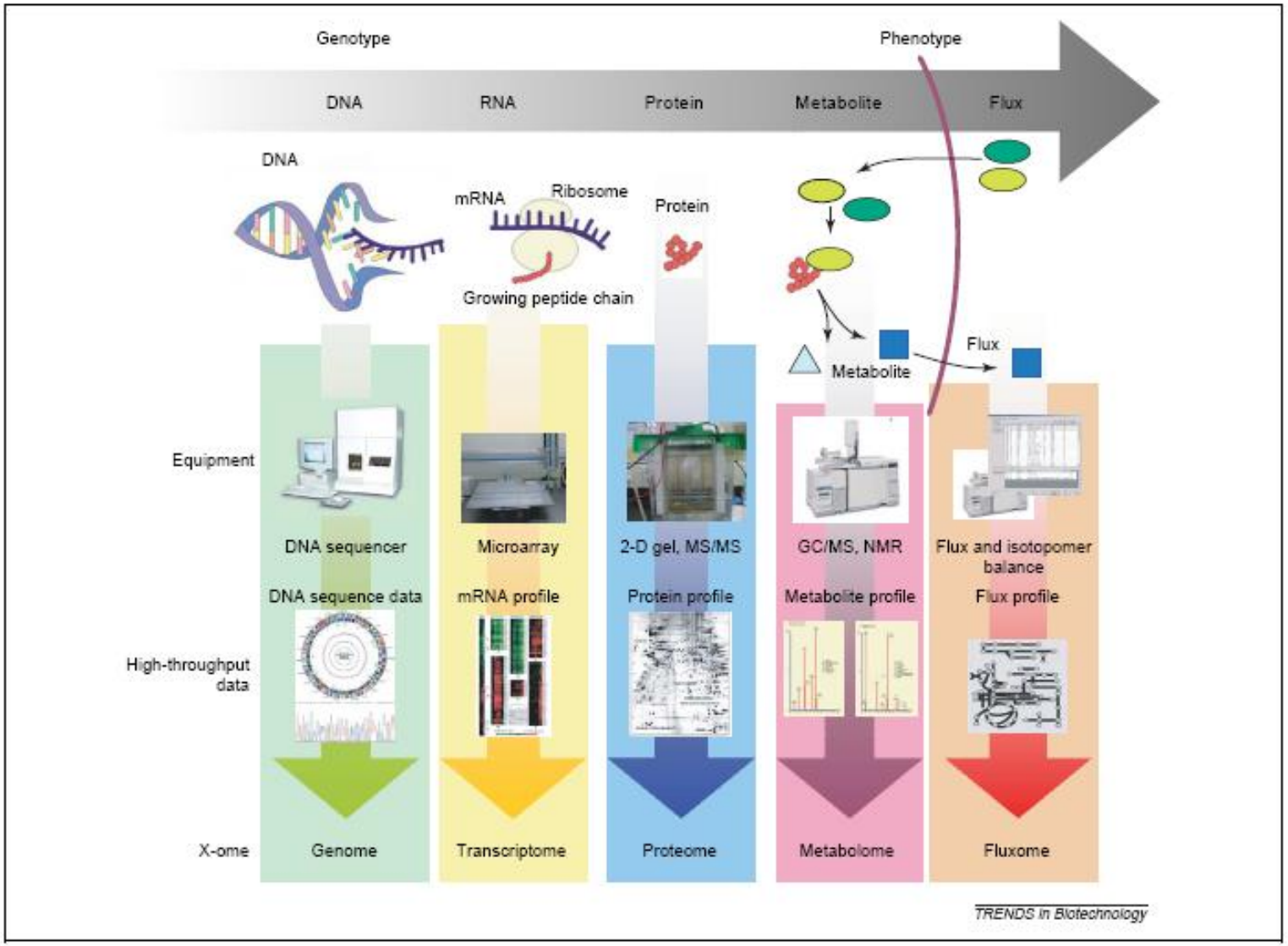
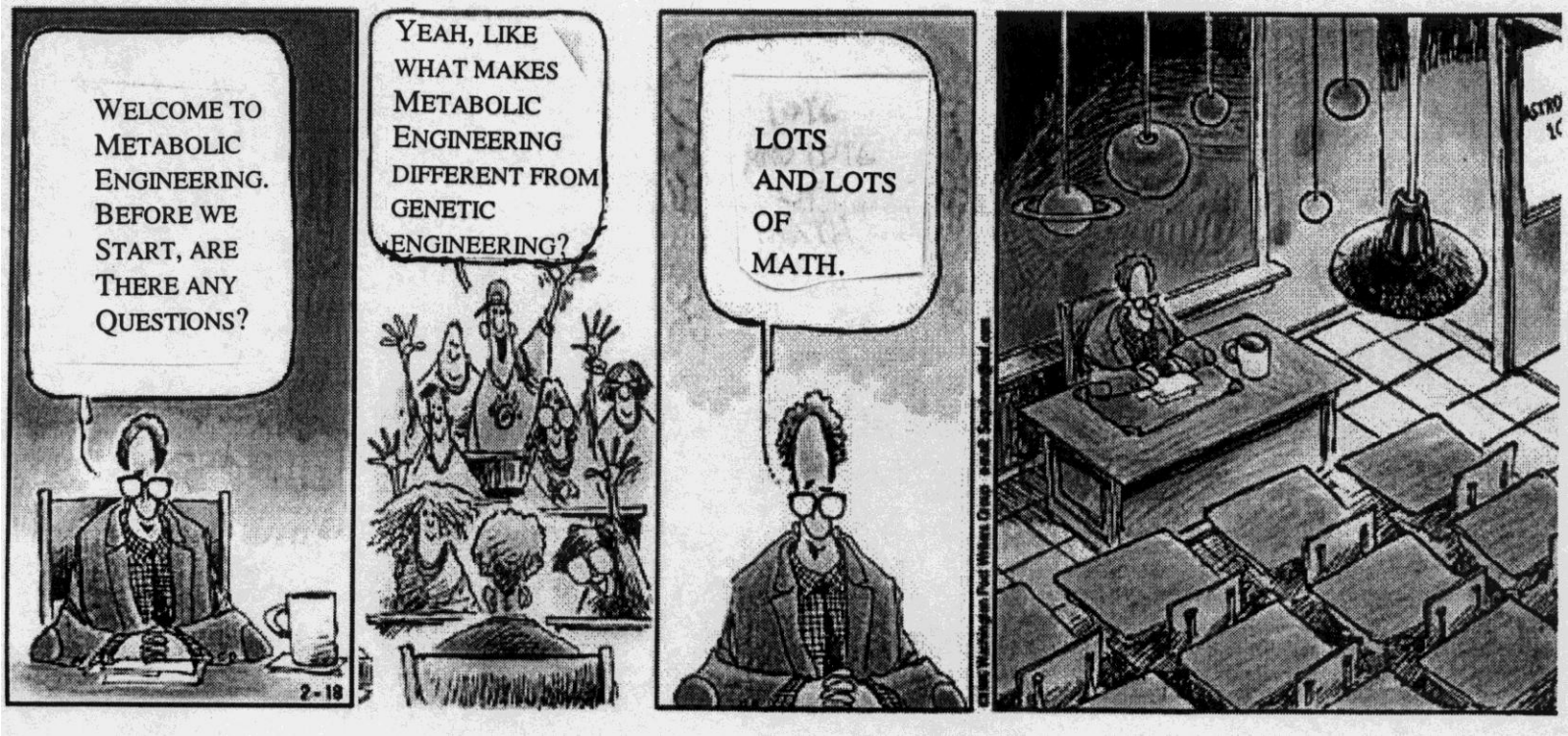


Figure 1. High-throughput omics research. Genomics advanced by the development of high-speed DNA sequencing is now accompanied by transcriptome profiling using DNA microarrays. Proteome profiling is joining the high-throughput race as 2D-gel electrophoresis combined with mass spectrography is advancing. Metabolome profiling is also rapidly advancing with the development of better GC/MS, LC/MS and NMR technologies. Isotopomer profiling followed by challenging with isotopically labeled substrate allows determination of flux profiles in the cell (fluxome).

Metabolic engineering is the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology. The opportunity to introduce heterologous genes and regulatory elements distinguishes metabolic engineering from traditional genetic approaches to improve strains.

... An interactive cycle of a genetic change, an analysis of the consequences, and the design of a further change...

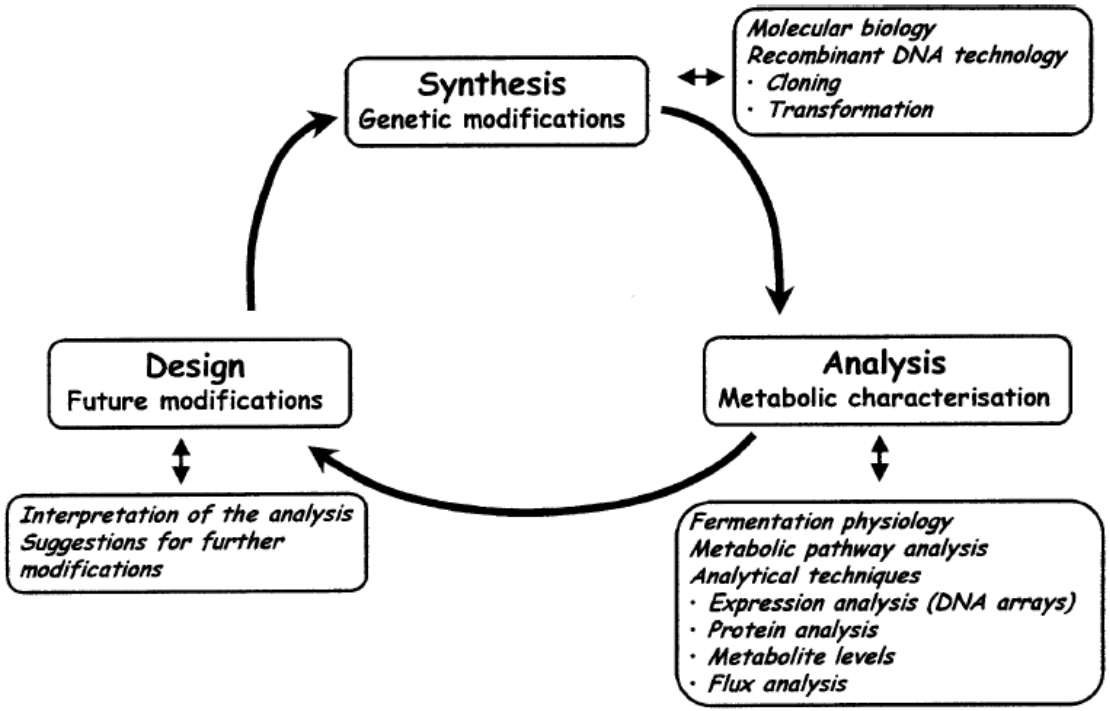
Toward a Science of Metabolic Engineering.
James E. Bailey Science, 252: 1668-1675.



Metabolic Engineering

Metabolic engineering is an enabling science, and distinguishes itself from applied genetic engineering by the use of advanced analytical tools for identification of appropriate targets for genetic modifications and possibly even the use of mathematical models to perform *in silico* design of optimized cell factories.

Nielsen & Jewett, 2007 FEMS Yeast Res.

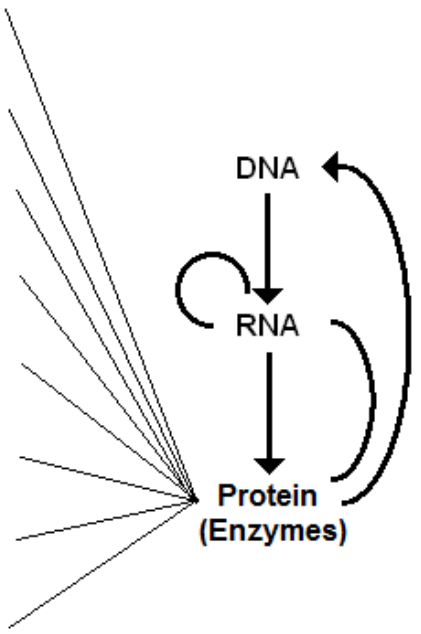


Metabolic Engineering

The knockout or overexpression of genes, usually used in Genetic Engineering, frequently does not result in product yield improvements due a resistance in the metabolism. Therefore, a better knowledge of the metabolism is needed to promote metabolism engineering as a whole to improve biotechnological processes.

1	3	3	2	1	4	1	V1
2	0	3	0	1	2	3	V2
2	0	2	2	1	3	1	V3
3	2	4	1	0	3	0	V4
2	0	2	3	0	2	2	V5
1	2	1	3	3	2	1	V6
3	0	1	0	3	0	1	V7
4	2	1	0	2	2	1	V8

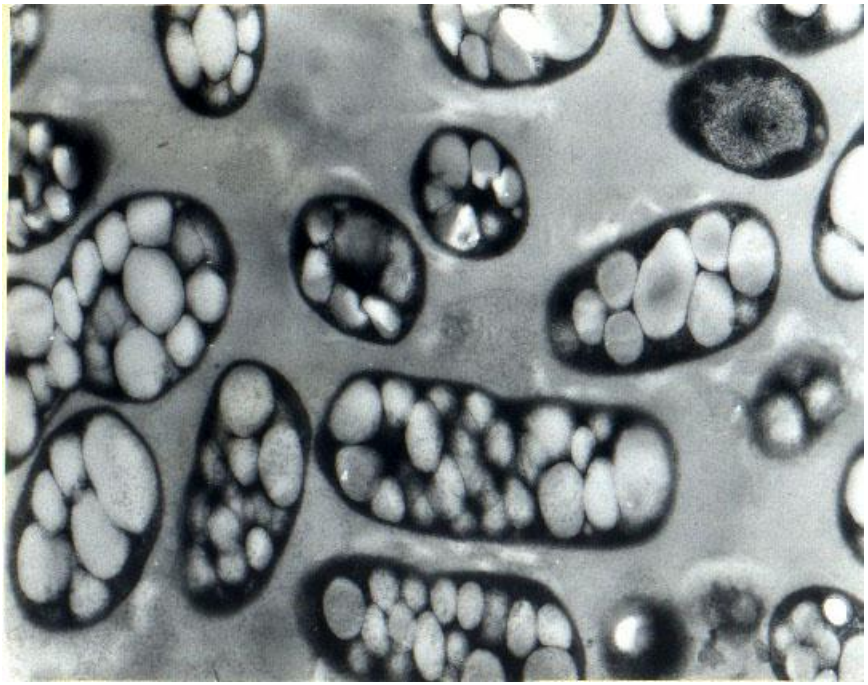
Metabolic level regulation

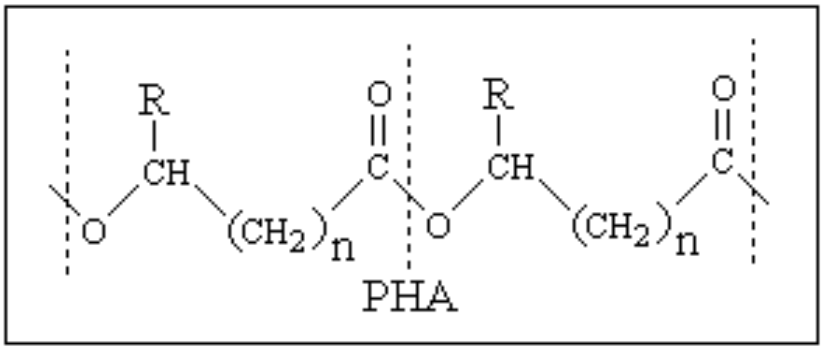


Hierarquical level regulation

Polyhydroxyalkanoates (PHA)

A family of polyesters accumulated by bacteria.





PHA production integrated to a sugar and etanol mill.

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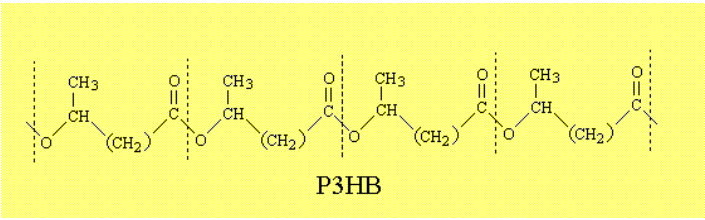
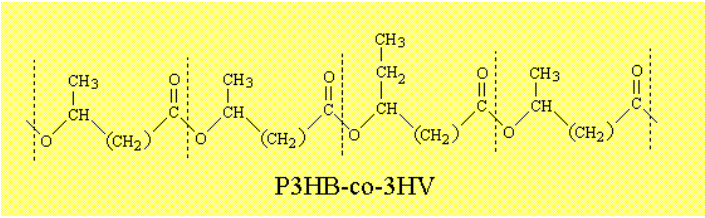




R. V. Nonato · P. E. Mantelatto · C. E. V. Rossell

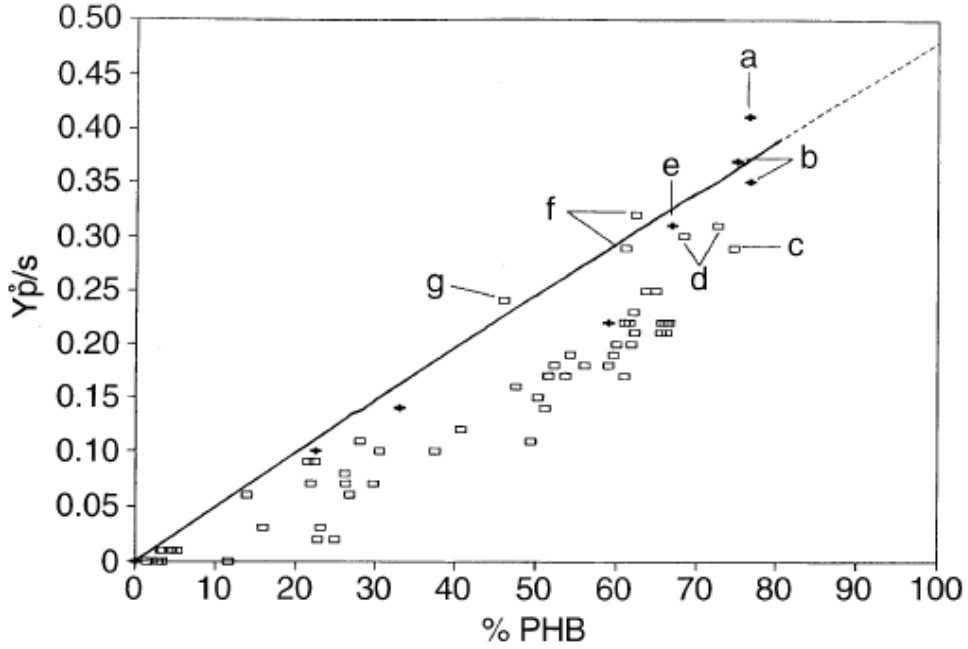
Integrated production of biodegradable plastic, sugar and ethanol

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

P3HB production from sugarcane carbohydrates



$$Y^{O}_{P/C} = \frac{PHB}{PHB\left(\frac{1}{Y^{T}_{P/C}} - \frac{1}{Y_{X/C}}\right) + \frac{100}{Y_{X/C}}}$$

Fig. 1 Relation between $Y^{O}_{P/C}$ and poly-(3-hydroxybutyrate) (*PHB*) content for different strains isolated from soil (□) or obtained from the culture collection (+) when glucose plus fructose was used as the carbon source. The line represents the values expected when $Y^{T}_{P/C} = 0.48$ g/g and $Y_{X/C} = 0.50$ g/g. Points related to strains *A. latus* DSM 1123 (*a*), *A. eutrophus* DSM 545 (*b*), IPT-101 (*c*), IPT-083 (*d*), *A. eutrophus* DSM 428 (*e*), IPT-086 (*f*), and IPT-055 (*g*) are indicated

P3HB-*co*-3HV production from carbohydrates and propionic acid

Strains	CDW (g/l)	Residual carbohydrates (%)	PHA			
			CDW %	3HB (mol%)	3HV (mol%)	$Y_{3HV/PROP}$ (g/g)
A. eutrophus DSM 545	3.92	0.0	71.4	96.1	3.9	0.13
A. latus DSM 1123	0.95	101.8	14.6	55.0	45.0	0.07
P. cepacia DSM 50181	3.35	1.9	38.4	97.3	2.7	0.04
IPT-040	3.77	1.7	32.3	97.1	2.9	0.05
IPT-044	3.92	1.7	51.1	97.1	2.9	0.07
IPT-045	3.73	0.0	49.4	96.2	3.8	0.08
IPT-048	2.97	0.0	44.3	96.2	3.8	0.06
IPT-055	4.27	72.2	1.5	100.0	0.0	0.00
IPT-056	3.60	31.3	30.9	98.5	1.5	0.02
IPT-076	5.06	1.9	56.8	97.1	2.9	0.10
IPT-083	4.89	5.2	56.8	96.9	3.1	0.10
IPT-086[a]	2.06	75.4	39.0	89.9	10.1	0.09
IPT-098	5.90	0.0	17.7	94.7	5.3	0.07
IPT-101	2.98	41.8	32.3	95.4	4.6	0.05

[a] Fructose instead of glucose was supplied

Maximum theoretical yield = 1.35 g/g

P3HB-*co*-3HV production from carbohydrates and propionic acid

Efficiency of *B. sacchari* mutants in converting propionic acid to 3HV units.

Strain	Phenotype	Results		
		3HB mol%	3HV mol%	$Y_{3HV/prp}$ (g/g)[c]
IPT 101[d]	wild type	93.8	6.2	0.10
IPT 183	I	84.1	15.9	0.34
IPT 185	II	82.6	17.4	0.35
IPT 190	III	80.1	19.9	0.37
IPT-195	IV	39.0	61.0	0.81
IPT 196	IV	33.2	66.8	0.78
IPT 189	IV	44.7	55.3	0.81
IPT 189	feeding strategies of suc/prp			1.34

Silva *et al.*, 2000

Rocha *et al.*, 2008

Use of sugarcane bagasse hydrolysate do produce PHA

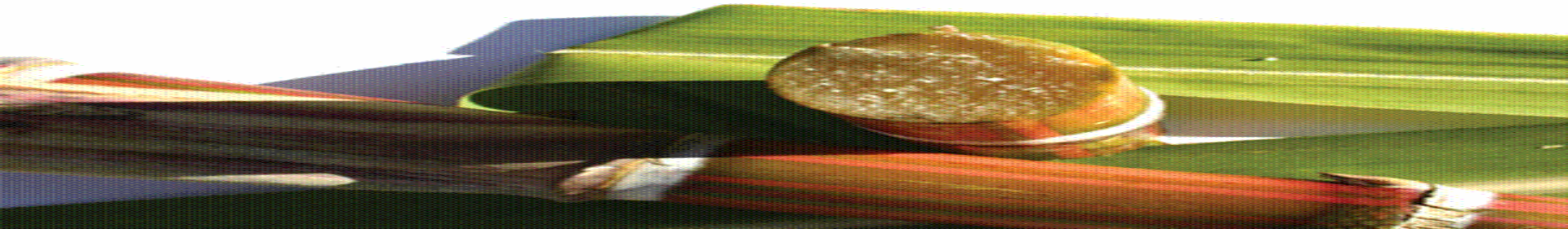
Strain	Carbon source	CDW (g l $^{-1}$)	P3HB (%)	μ_{Xrgmax} (h^{-1})	μ_{Pmax} (h^{-1})	$Y_{P3HB/S}$ (g g $^{-1}$)	P_{P3HB} (g l $^{-1}$ h $^{-1}$)
Pseudomonas pseudoflava ATCC 33668	Glucose	3.5	22.8	0.58	0.11	0.04	0.080
P. pseudoflava ATCC 33668	Xylose	4.0	27.5	0.13	0.03	0.04	0.031
B. cepacia ATCC 17759	Xylose	7.5	45	0.22	0.07	0.11	0.10
B. cepacia	Xylose		48.8	–	–	0.11	–
Escherichia coli TG1 (pSYL107)[a]	Xylose	4.75	35.8	–	–	0.097	0.028
E. coli r TG1 (pSYL107)[a]	Xylose + CSH	3.76	64.0	–	–	0.188	0.040
E. coli TG1 (pSYL107)[a]	Xylose + SH	5.95	73.9	–	–	0.226	0.070
B. sacchari IPT 101	Sugarcane bagasse hydrolysate	4.4	62	0.24	0.16	0.39	0.11
B. cepacia IPT 048	Sugarcane bagasse hydrolysate	4.4	53	0.36	0.08	0.29	0.09
B. sacchari IPT 101	Xylose + glucose	60	58	0.25	0.03	0.22	0.47
B. cepacia IPT 048	Xylose + glucose	57	57	0.28	0.06	0.19	0.46

[a]Recombinant strain

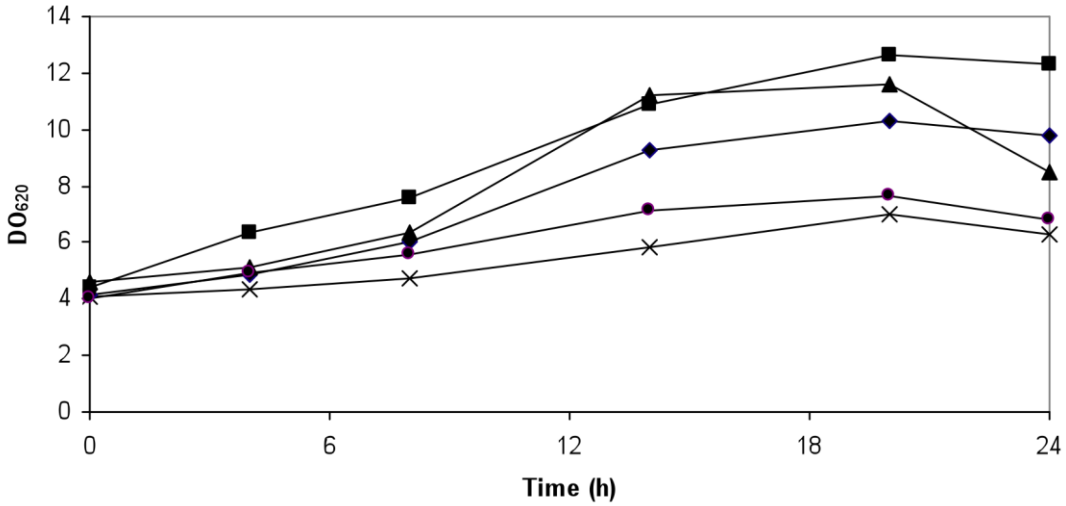
High PHB content

Low productivity

Detoxification of hydrolysate needed



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

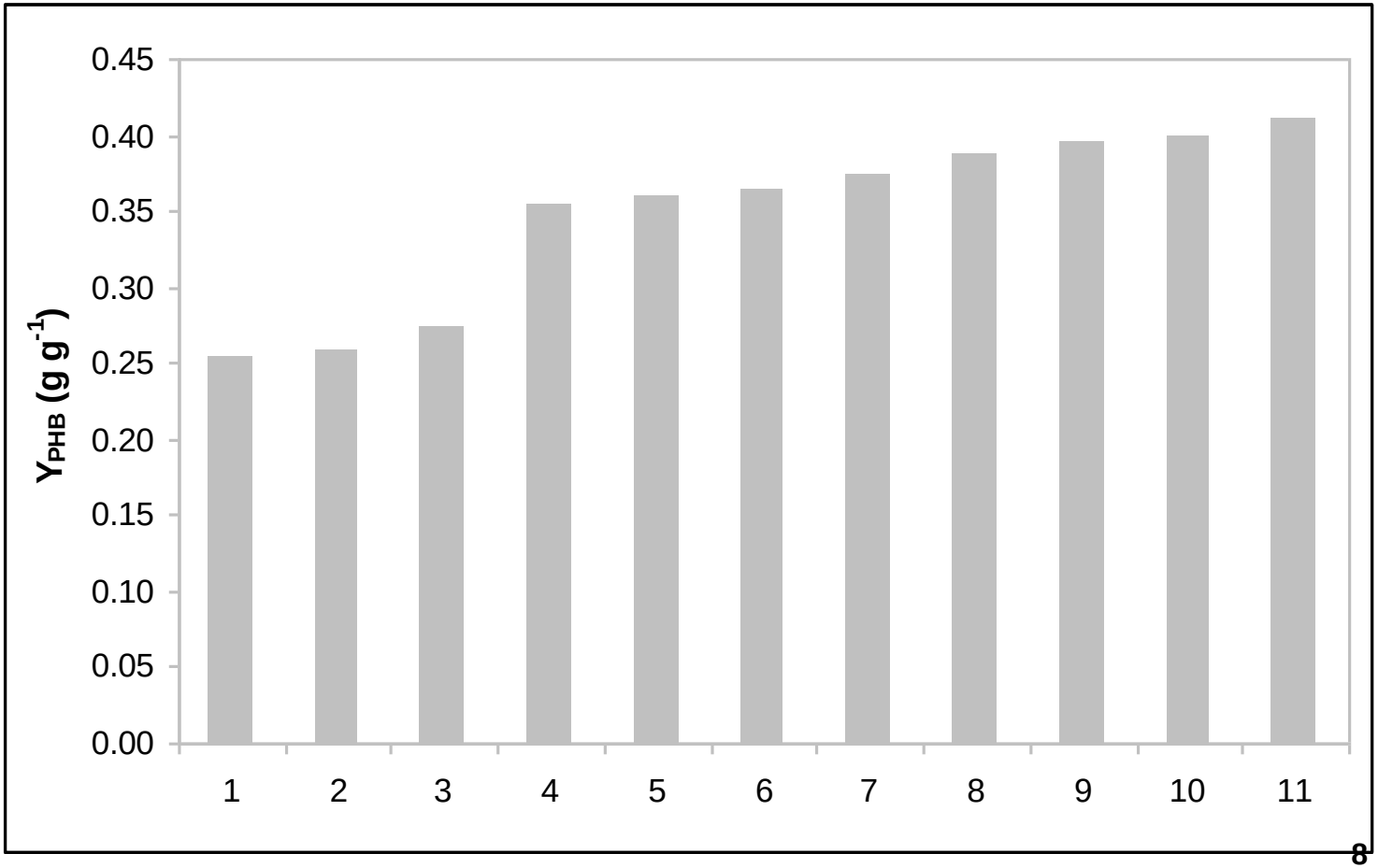


— Growth experiment with F24 in mineral media with xylose (10 g l^{-1}) and individual compounds: (■) 2.5 g l^{-1} of acetic acid, (▲) 1.25 g l^{-1} of formic acid, (♦) control experiment only with xylose, (●) 0.5 g l^{-1} of HMF, and (x) 0.5 g l^{-1} of furfural.

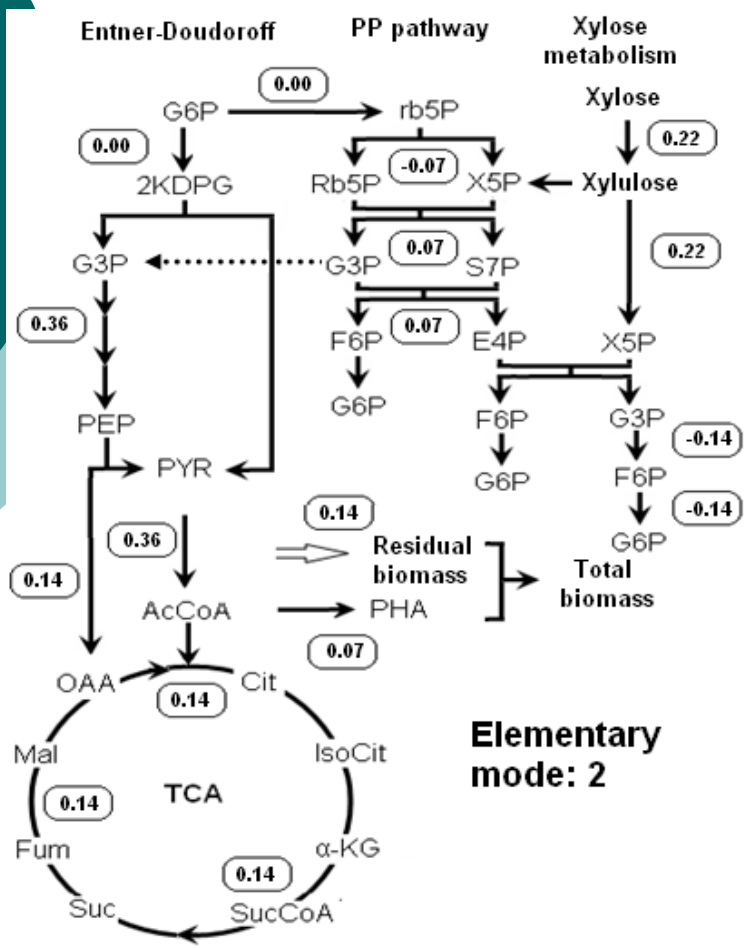
Seed	Formic acid	Acetic acid	Furfural	HMF	Xylose	Cell density	%PHA
0	1.57	1.97	0.46	0.15	17.65	0	0
0.5	0.14	0.43	0.13	0.03	11.18	2.3	37.45
1.0	0.02	0.02	0.17	0.03	12.13	2.89	42.15
1.5	0.03	0.14	0.21	0.01	5.23	3.75	42.15
3.2	0.07	0.00	0.04	0.00	4.78	7.18	32.35
6.5	0.00	0.00	0.07	0.02	2.31	10.48	35.72

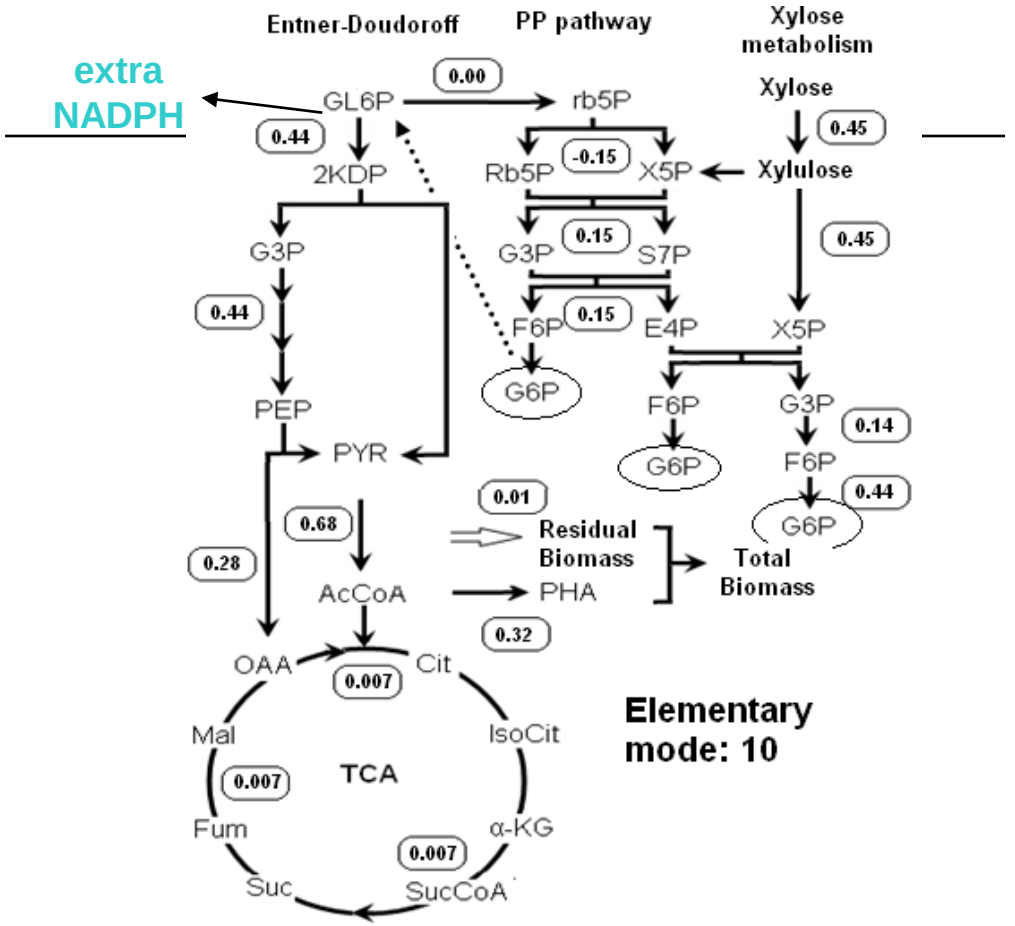
Effect of the inoculum size (g l^{-1}) on utilization of hydrolysates (g l^{-1}), cell growth (g l^{-1}) and PHA biosynthesis (% of PHA of the cell dry weight) in hydrolysate medium after 48 hours

Elementary mode analysis



Elementary mode analysis



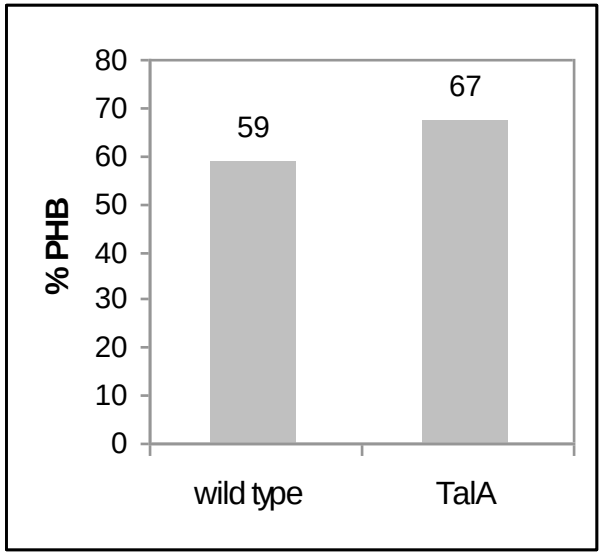


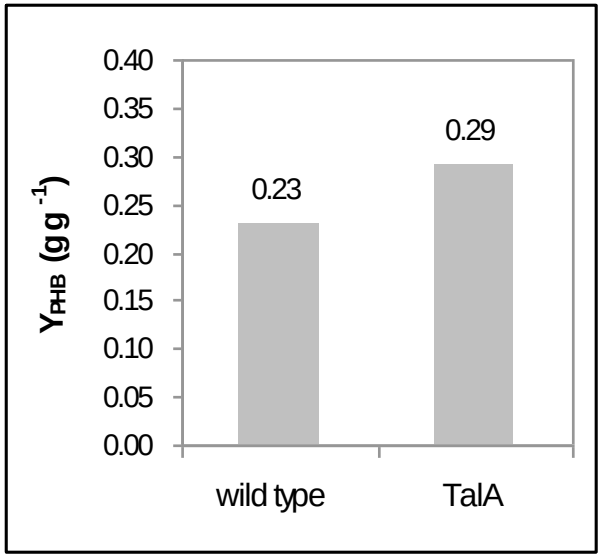
$Y_{PHB} = 0.25$

$Y_{PHB} = 0.40$

Metabolic engineering:
talA overexpression

- Effect of *talA* overexpression on %PHB and Y_{PHB} in experiments with minimal mineral media supplemented with xylose (Xyl):





Average from at least two independent experiments for each test, with standard deviation lower than 0.01 for Y_{PHB} and lower than 1.5 for %PHB

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PHA production integrated to a sugar and etanol mill.

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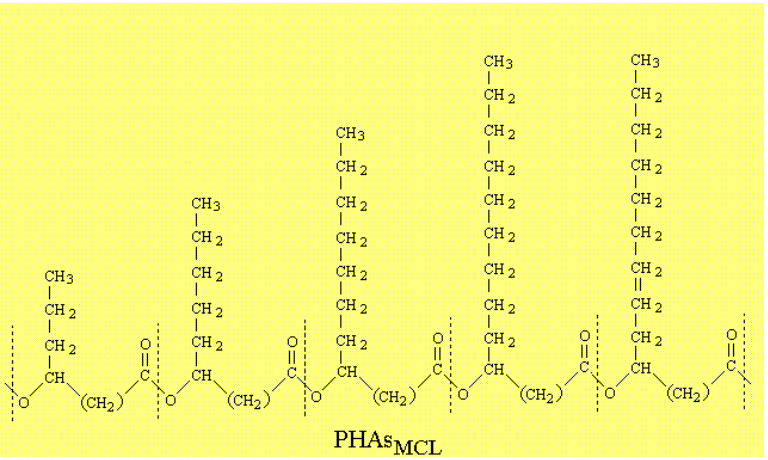

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Integrated production of biodegradable plastic, sugar and ethanol

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

MINI-REVIEW

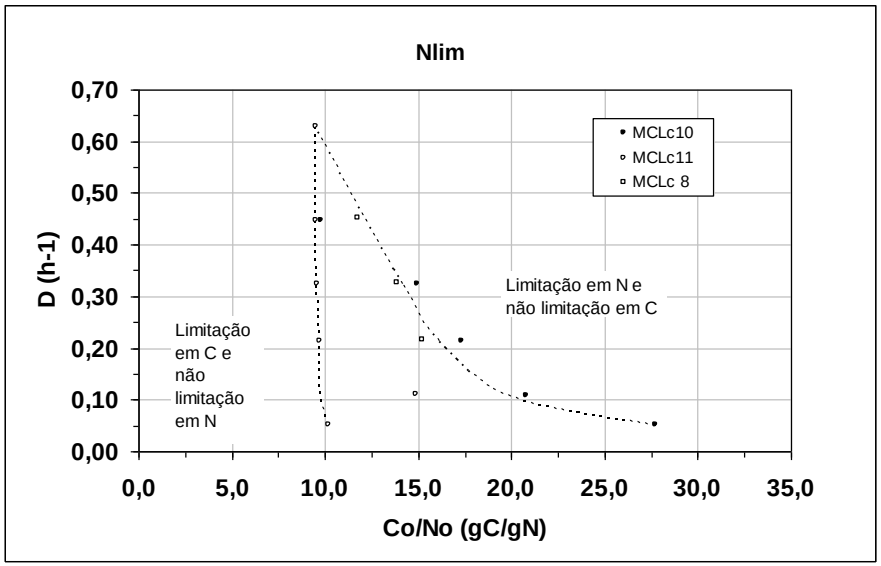
A green cycle for simultaneous poly 3-hydroxybutyric acid, sugar and ethanol production

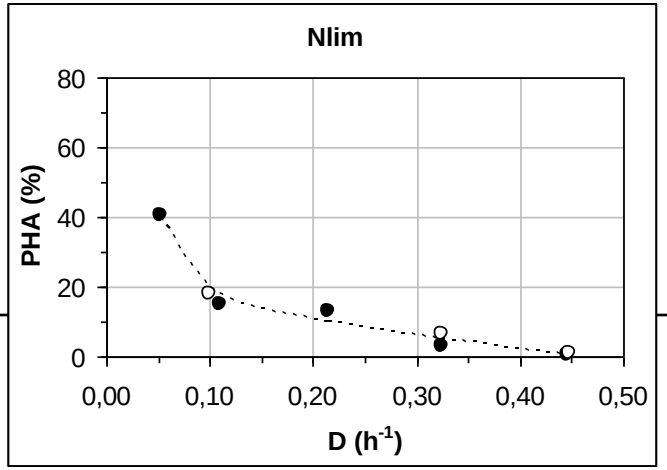
$$Y^{G}_{PHA/G} = \frac{\%PHA}{\frac{100}{Y_{Xr/G}} - \frac{\%PHA}{Y_{Xr/G}} + \frac{\%3HHx}{Y_{3HHx/G}} + \frac{\%3HO}{Y_{3HO/G}} + \frac{\%3HD}{Y_{3HD/G}} + \frac{\%3HDd}{Y_{3HDd/G}}}$$

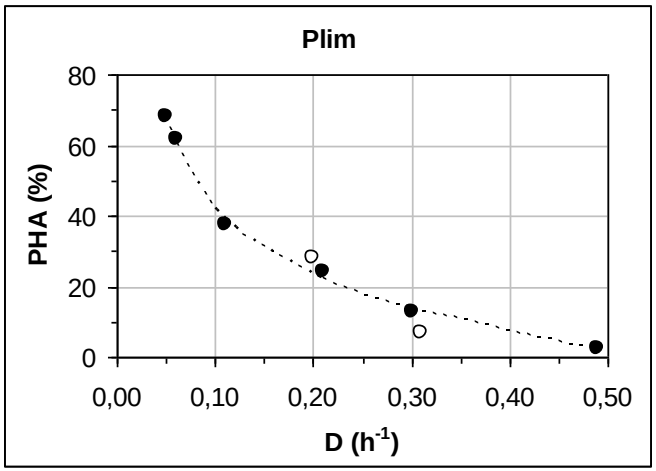
Table 1. Production of PHA_{MCL} from carbohydrates by some sugarcane soil isolates.

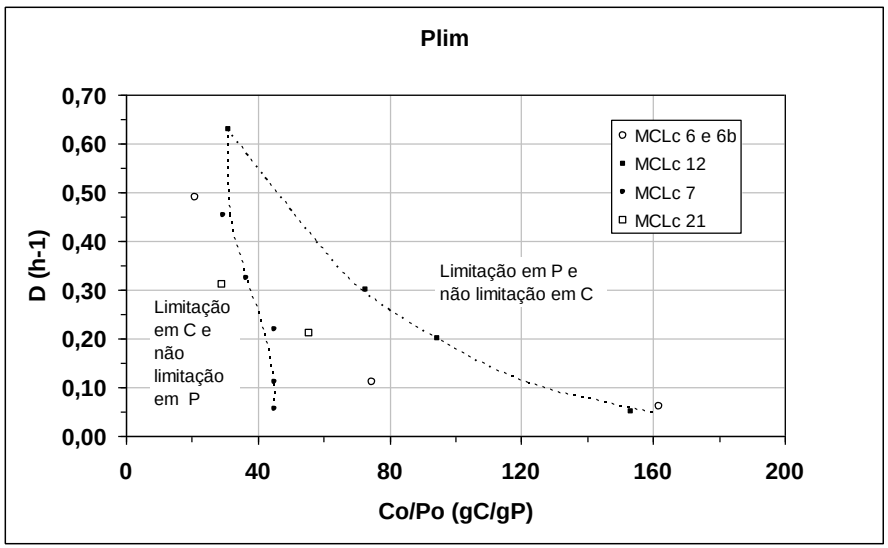
Bacterial strain	CDW (g/L)	PHA composition (mol%)				PHA (%CDW)	$Y^{G}_{PHA/G}$ (g/g)	$\%Y_{MAX}$
		3HHx	3HO	3HD	3HDd			
KT2440	3.96	3.25	12.48	79.88	4.39	48.52	0.127	60.0
LFM046	3.72	5.01	21.85	71.83	1.31	60.51	0.161	62.9
LFM047	2.21	1.72	22.88	61.81	13.58	13.99	0.023	34.3
LFM050	2.50	0.92	15.98	67.75	15.35	18.55	0.037	41.9
LFM065	4.06	0.00	8.88	87.81	3.30	30.52	0.084	60.6

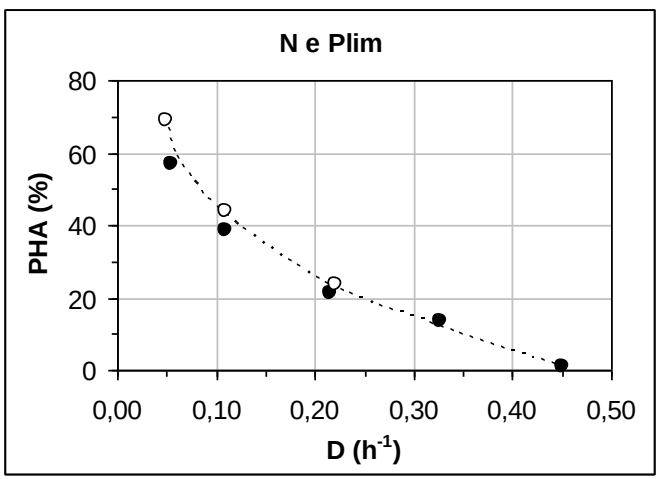
CDW – Cell dry weight 3HHx - 3-hydroxyhexanoic acid 3HO - 3-hydroxyoctanoic acid
3HD - 3-hydroxydecanoic acid 3HDd - 3-hydroxydodecanoic acid $Y^{G}_{PHA/G}$ – global PHA yield from glucose
$\%Y_{MAX}$ - percentual of the maximum theoretical yield.


Nlim
D (h-1)
Co/No (gC/gN)
MCLc10
MCLc11
MCLc 8
Limitação em C e não limitação em N
Limitação em N e não limitação em C

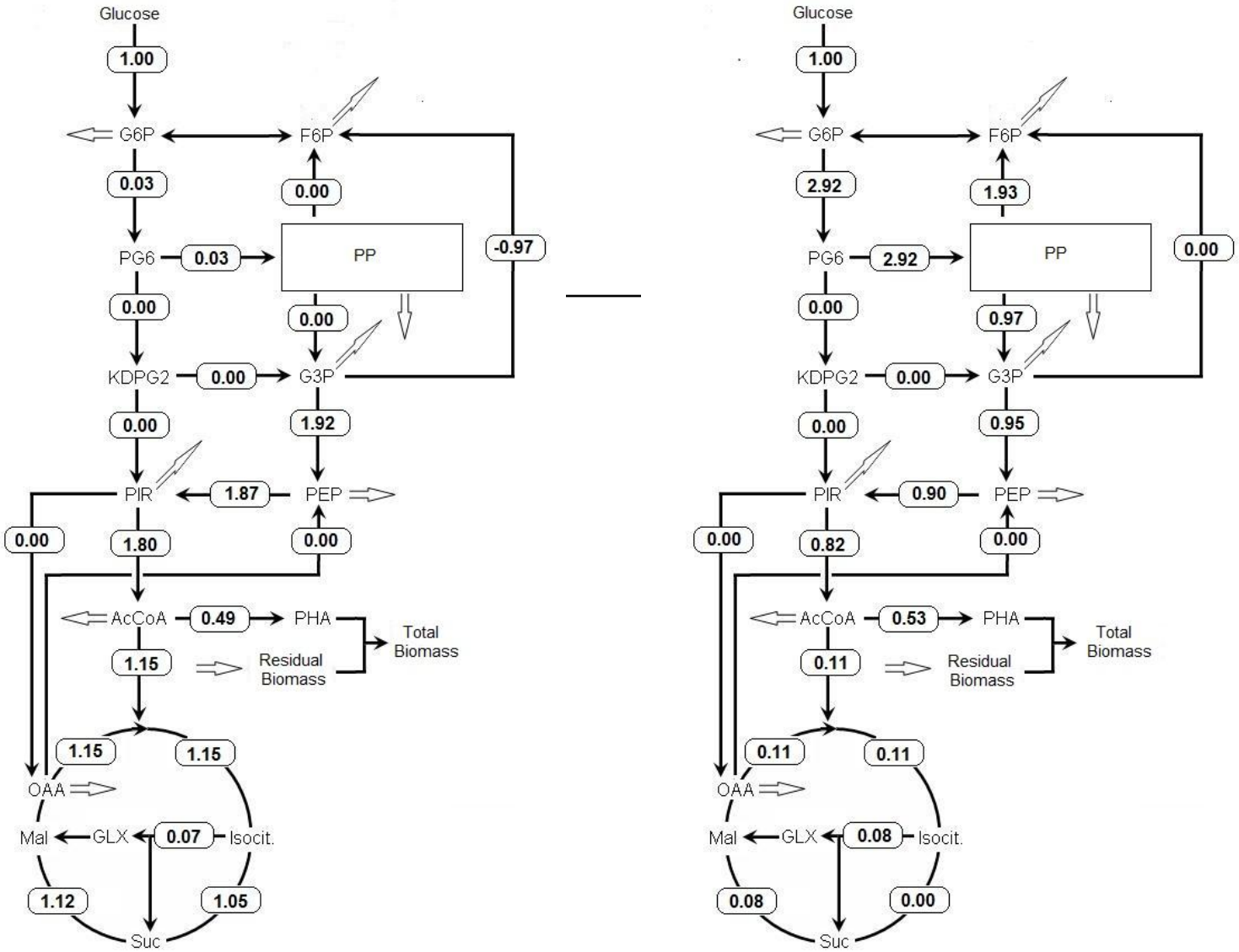

Nlim
PHA (%)
D (h-1)


Plim
PHA (%)
D (h-1)


Plim
D (h-1)
Co/Po (gC/gP)
MCLc 6 e 6b
MCLc 12
MCLc 7
MCLc 21
Limitação em P e não limitação em C
Limitação em C e não limitação em P


N e Plim
PHA (%)
D (h-1)

Metabolic fluxes analysis

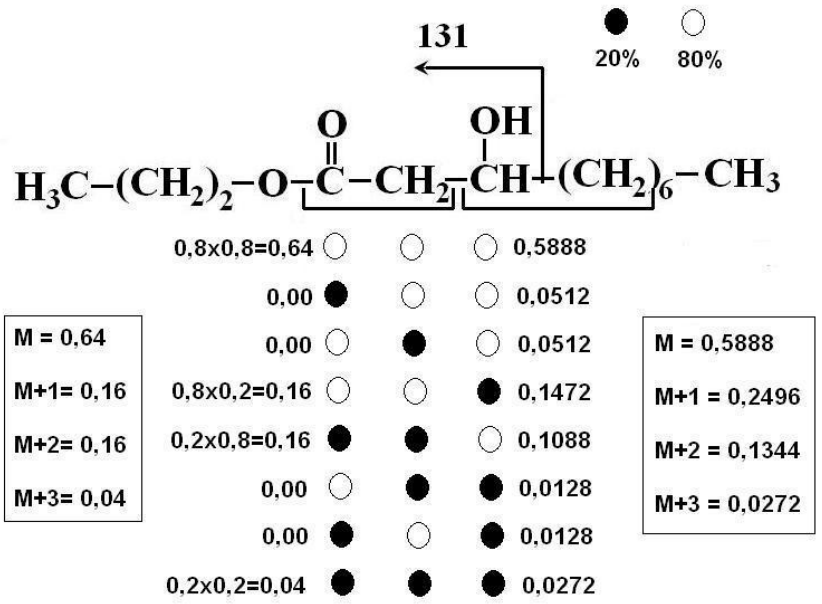


Fluxes distribution for PHA production by *Pseudomonas* sp. from glucose.

Metabolic fluxes analysis

Theoretical and experimental isotopomers distribution on 3HA in PHA_{MCL}

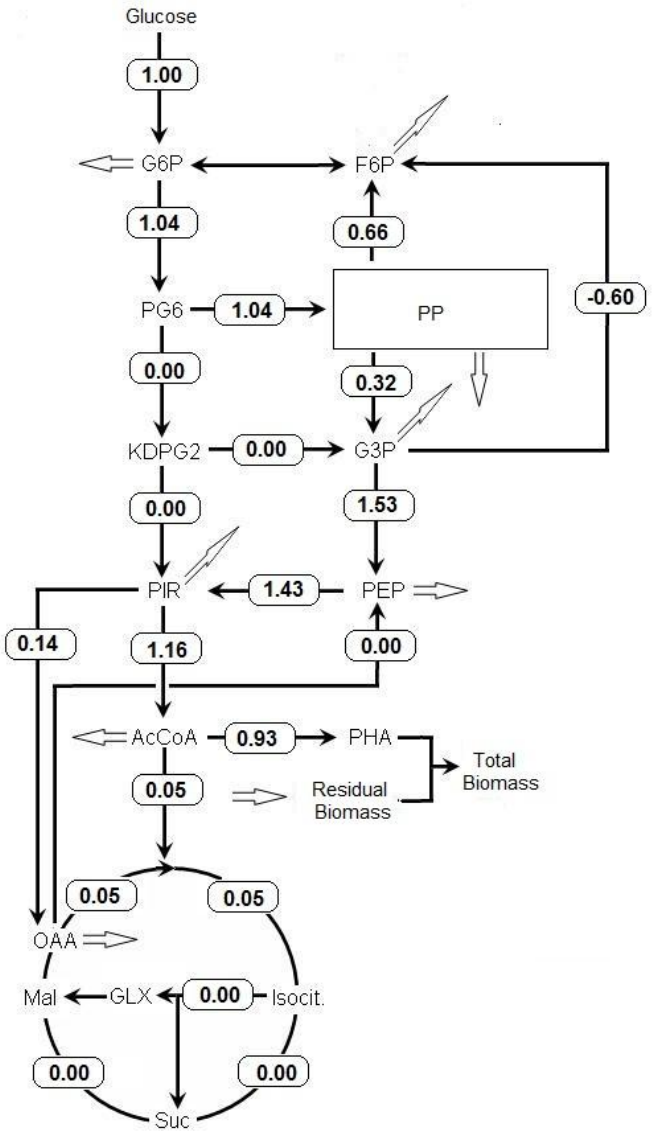
	Theoretical values		Experimental values	
	EMP, ED or PP+EMP	PP+ED	N limited	P limited
M	0.64	0.59	0.58±0.02	0.60±0.02
M+1	0.16	0.25	0.22±0.01	0.22±0.01
M+2	0.16	0.13	0.15±0.01	0.14±0.01
M+3	0.04	0.03	0.04±0.01	0.04±0.01

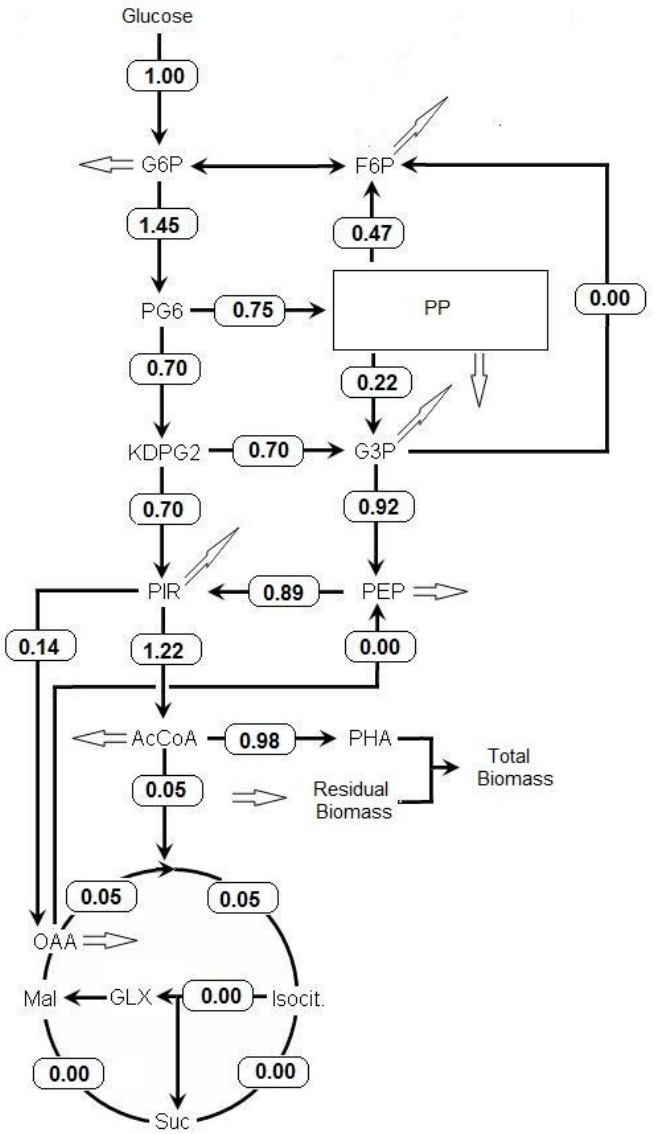


ED, EMP or PP+EMP

PP+ED (PP+??) .

Elementary Mode analysis





Optimal fluxes distribution for PHA production by *Pseudomonas* sp. from glucose.

PHA - properties

Table 3
Comparison of PHA polymers with common plastics in properties

Sample	Melting temperature (°C)	Glass-transition temperature (°C)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation to break (%)
P(3HB)	180	4	3.5	40	5
P(3HB-*co*-20 mol% 3HV)	145	−1	0.8	20	50
P(3HB-*co*-6 mol% 3HA)[a]	133	−8	0.2	17	680
Polypropylene	176	−10	1.7	38	400
Low-density polyethylene	130	−30	0.2	10	620

[a] 3HA units: 3-hydroxydecanoate (3 mol%), 3-hydroxydodecanoate (3 mol%), 3-hydroxyoctanoate (<1 mol%), 3-hydroxy-*cis*-5-dodecenoate (<1 mol%).

Recombinant Pseudomonas sp.

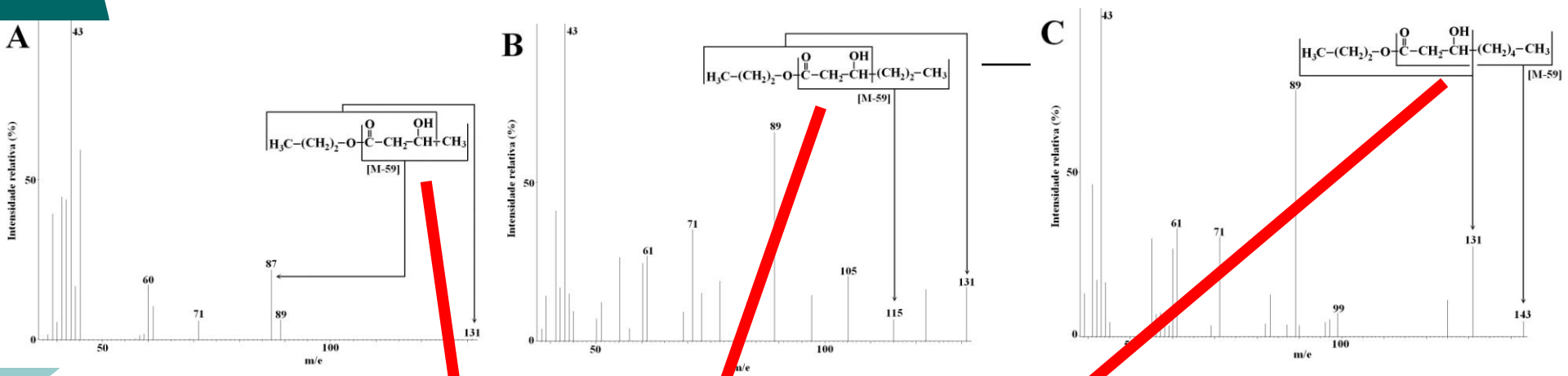
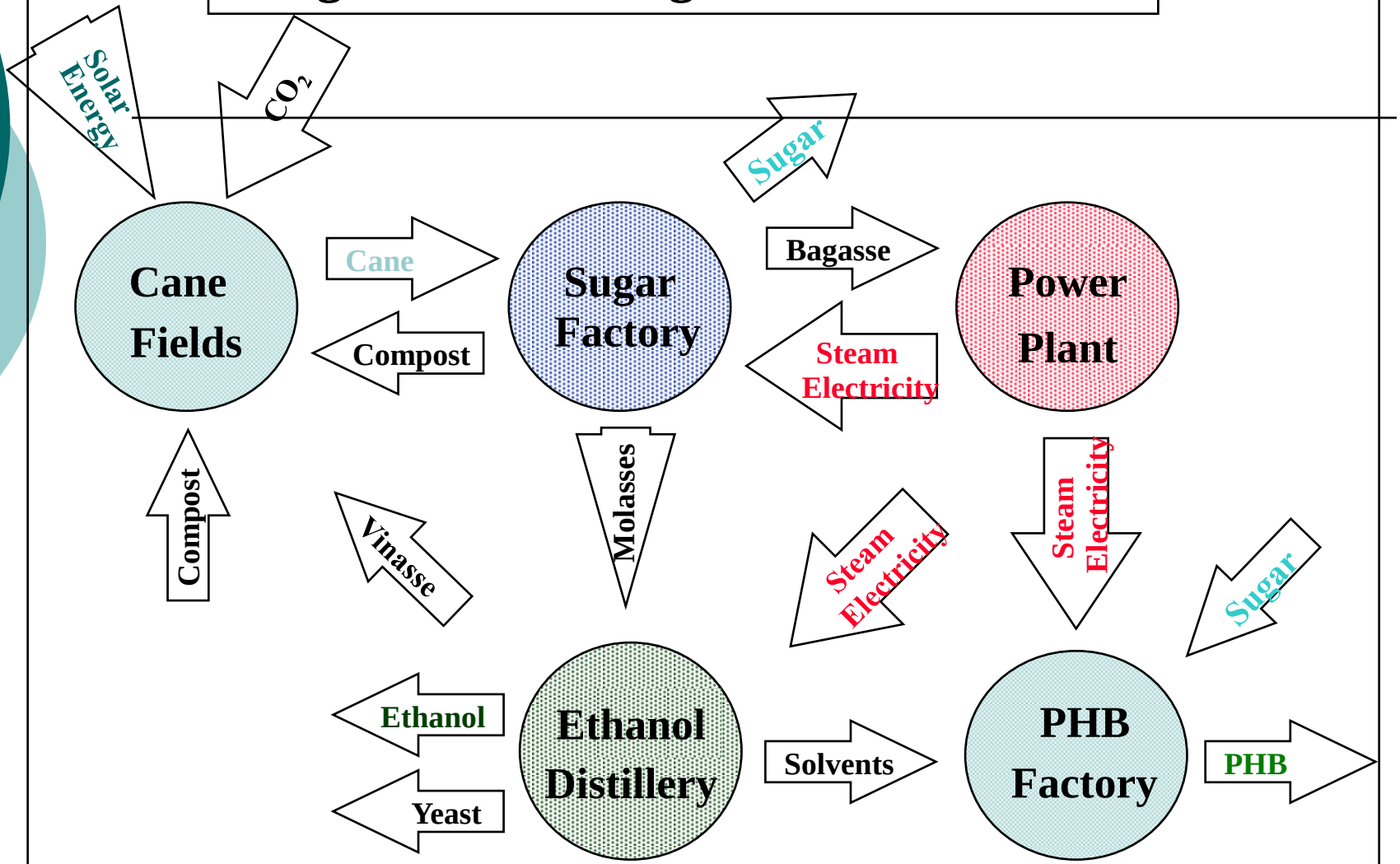


Table 4. Composition of PHA produced by *Pseudomonas* sp. LFM461 harboring the plasmid pBBR1MCS-2::*phaC* and purified.

Samples	PHA (mol%)				
	3HB	3HHx	3HO	3HD	3HDd
1	92,38	4,33	3,29	0,00	0,00
2	93,25	3,60	2,88	0,27	0,00
3	92,83	4,35	2,82	0,00	0,00
Average	92,82	4,09	3,00	0,09	0,00
± Standard Deviation	±0,44	±0,43	±0,26	±0,16	±0,00

3HB – 3-hydroxybutyric acid; 3HHx – 3-hydroxyhexanoic acid; 3HO – 3-hydroxyoctanoic acid; 3HD – 3-hydroxydecanoic acid; 3HDd – 3-hydroxydodecanoic acid.

Integrated PHB, Sugar and Ethanol Mill



Team and Financial Support

jgcgomez@usp.br

Dr. J.Gregório C. Gomez
Dr. Luiziana F. Silva
Dr. José Geraldo Pradella (CTBE)
Dr. Marilda Keico Taciro
Dr. Ruben Sanchez (UENF)
Dr. Rafael C. S. Rocha
Dr. Mateus Lopes
Dr. Rogério S. Gomes
Dr. Sonia R Silva-Queiroz
Dr. Erica M. Pereira
Dr. Nuri A. Merchan-Castellanos
MSc. Thatiane T. Mendonça
MSc. Tatiana Strelec
MSc. Renata M. Peixoto
MSc. Tatiana S. Matsuda
MSc. Gil Raicher
MSc. A. Carolina S. Cintra

Johana K. Bocanegra-Rodriguez
Daniela C. P. Lício
Y. Paola Galindo-Rozo
Karen L. Almeida
Romine K.B. Freire
Karina Chouman
Liege A. Kawai
Nathália G.F. Machado
Amanda B. Flora
Mariana N. Limas
Estela A. Brandi
Aelson L. Santos

FAPESP

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Conselho Nacional de Desenvolvimento Científico e Tecnológico

CAPES

CYTED
CIENCIA Y TECNOLOGÍA PARA EL DESARROLLO

DEPARTAMENTO DE MICROBIOLOGIA
UNIVERSIDADE DE SÃO PAULO

Centro Argentino Brasileño de Biotecnología
CBAB
Centro Brasileiro-Argentino de Biotecnologia

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