Análise sitêmica e engenharia do metabolismo microbiano Biologia Sintética e Biologia de Sistemas.

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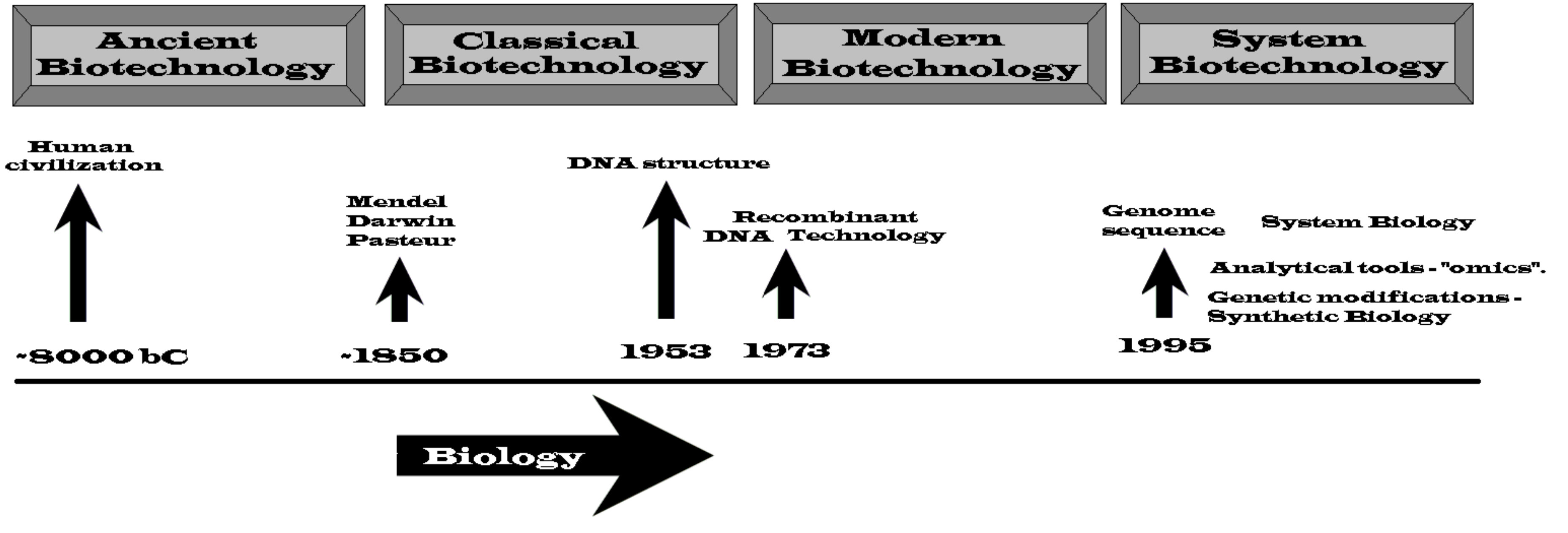
Biology and Biotechnology



ômicas

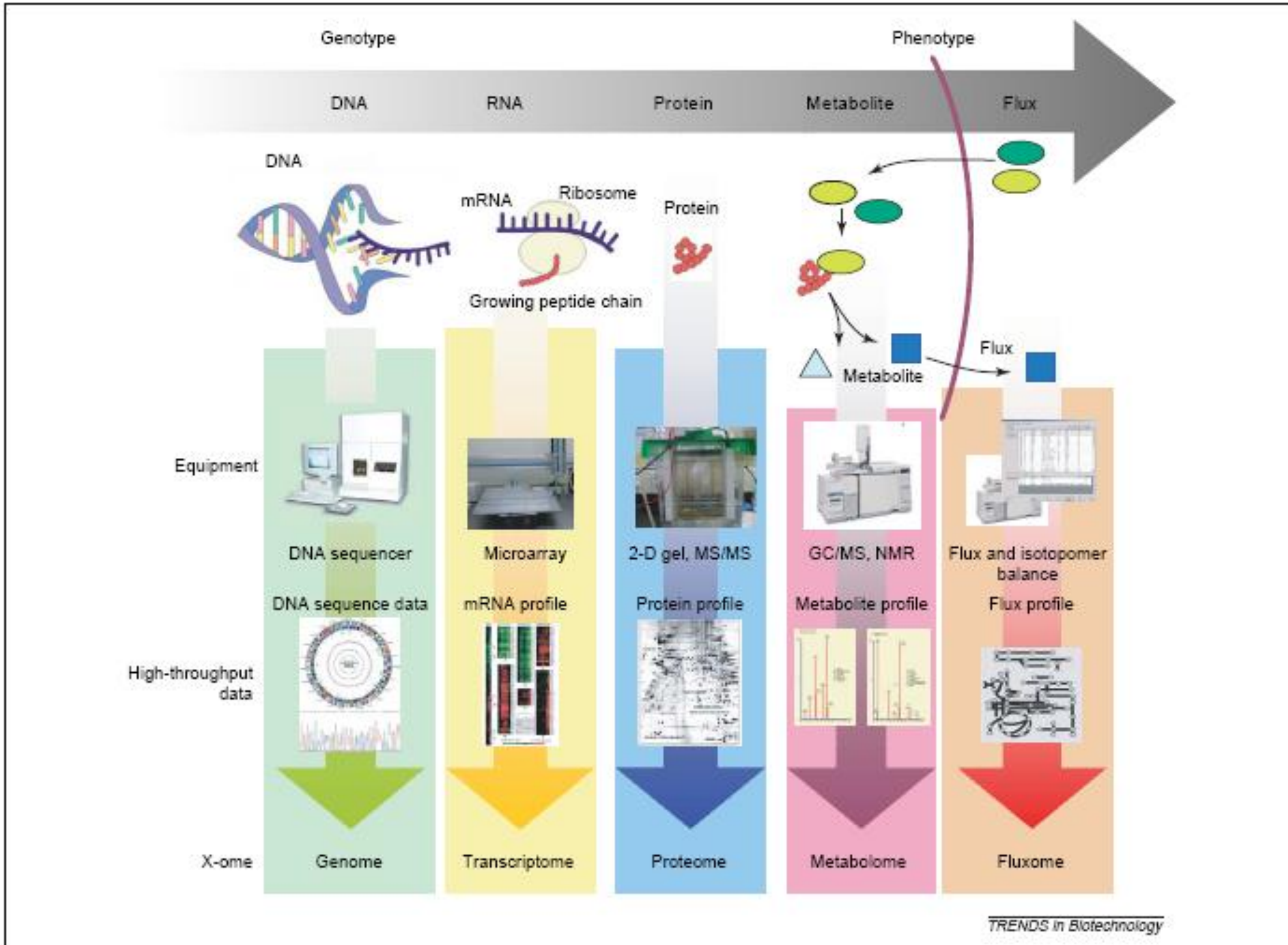
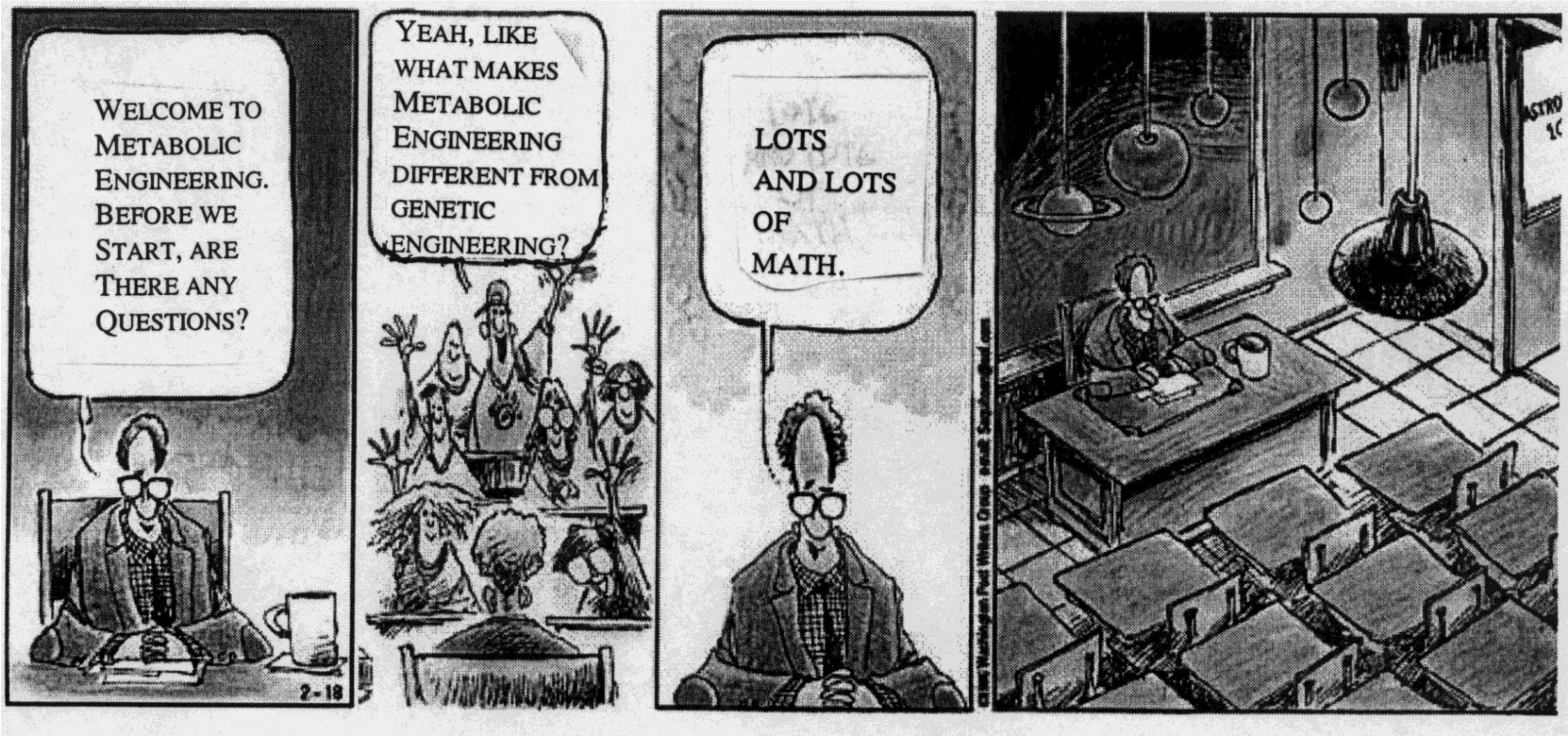


Figure 1. High-throughput omics research. Genomics advanced by the development of high-speed DNA sequencing is now accompanied by transcriptome profiling using DNA microarrays. Proteome profiling is joining the high-throughput race as 2D-gel electrophoresis combined with mass spectrography is advancing. 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...

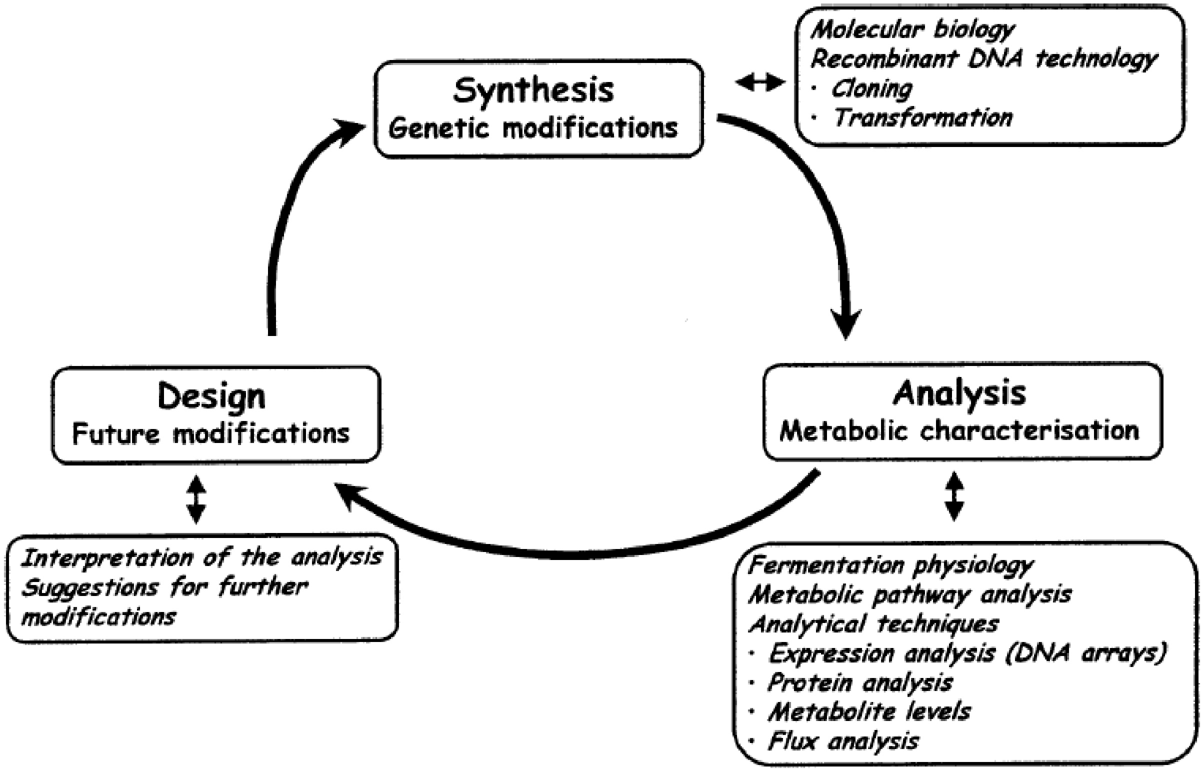
Toward a Science of Metabolic Engineering.
James E. Bailey Science, 252: 1668-1675.



Metabolic Engineering

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.

Vallino & Stephanopoulos, 1992



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, 2008 FEMS Yeast Res.**

TABLE 1. Overview of reactions, metabolites, and ORFs in reconstructed metabolic networks[a]

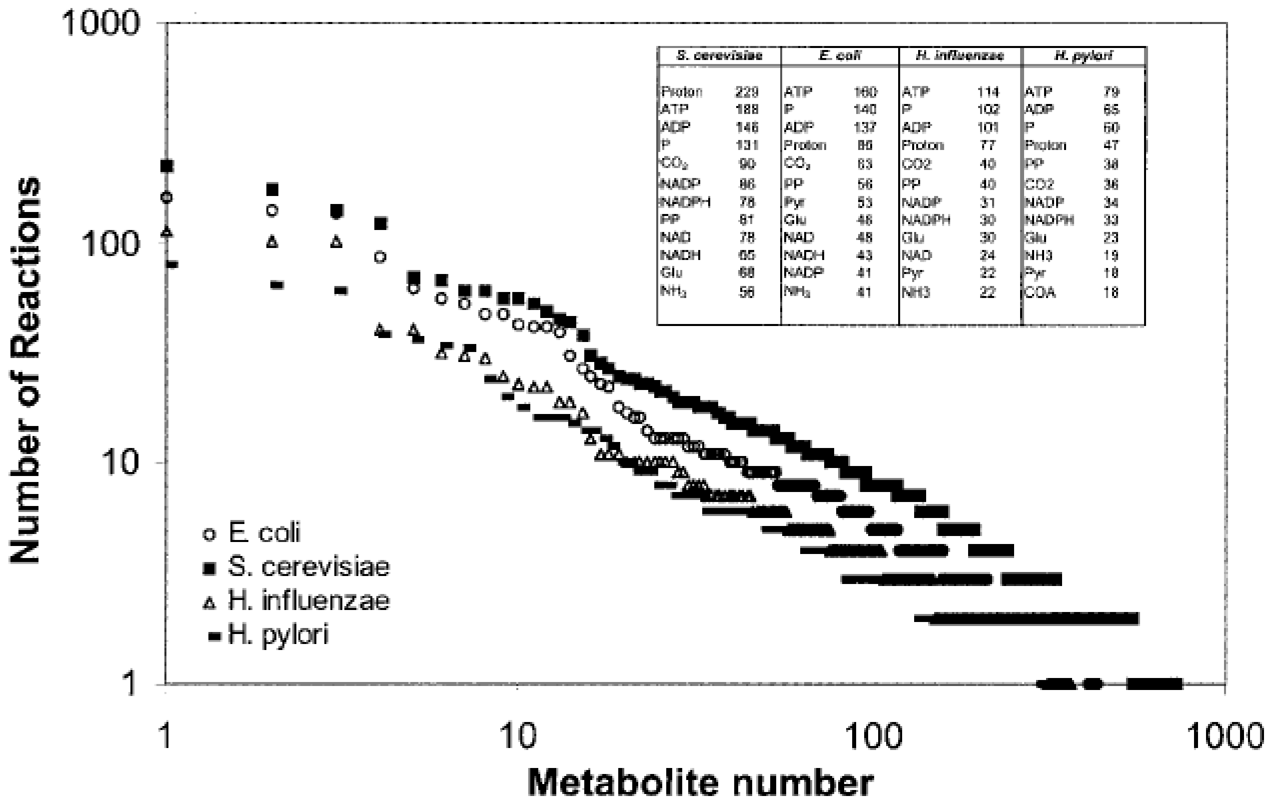
Organism	No. of reactions	No. of metabolites	No. of metabolic ORFs	Total no. of ORFs	% of ORFs involved in metabolism
H. pylori	444	340	268	1,638	16
H. influenzae	477	343	362	1,880	19
E. coli	720	436	695	4,485	15
S. cerevisiae	1,175	584	708	5,773	12[b]

[a] The reconstructed networks are described in references 6, 8, 17, and 18.
[b] The value is based on a recent gene count (3).

Table 3. Frequency of precursor metabolites and cofactors in a *Saccharomyces cerevisiae* genome scale model*

Precursor metabolite	No of reactions	Cofactor	No of reactions
Glucose-6P	16	ATP	188
Fructose-6P	18	ADP	146
Ribose-5P	20	NADH	65
Erythrose-4P	6	NAD^+	78
Glyceraldehyde-3P	13	NADPH	78
3-Phosphoglycerate	6	$NADP^+$	86
Phosphoenolpyruvate	12		
Pyruvate	27		
Acetyl-CoA	32		
2-Oxoglutarate	38		
Succinyl-CoA	3		
Oxaloacetate	12		

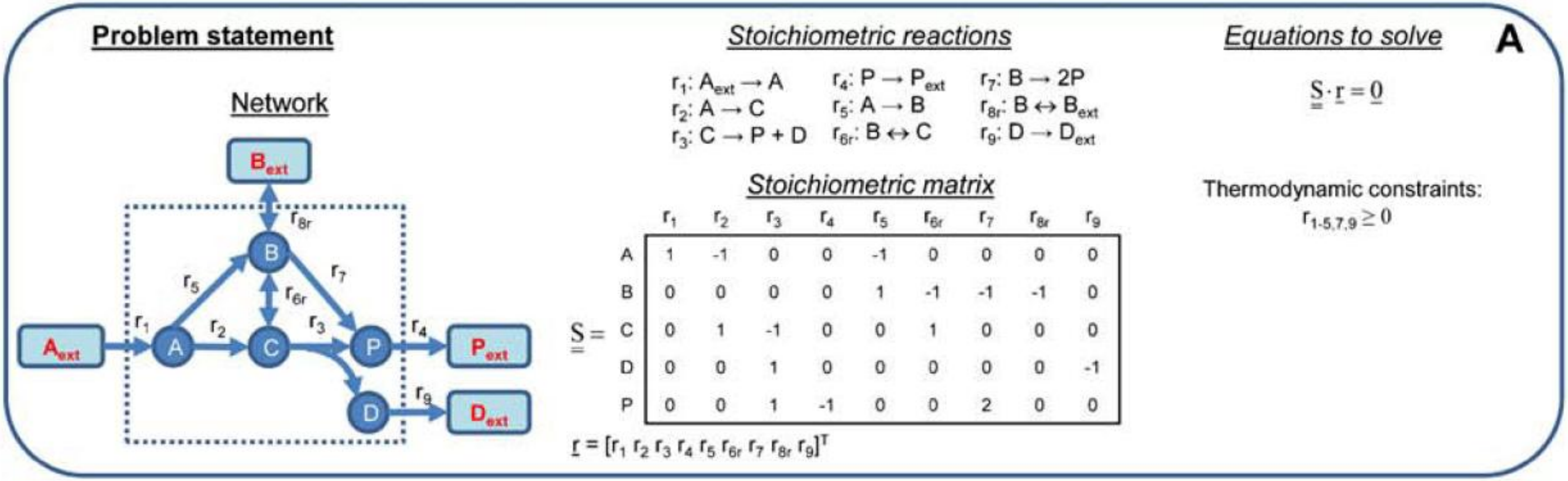
*The data are taken from the metabolic model developed by Forster *et al.* (2003).



S. cerevisiae		*E. coli*		*H. influenzae*		*H. pylori*	
Proton	229	ATP	160	ATP	114	ATP	79
ATP	188	P	140	P	102	ADP	65
ADP	146	ADP	137	ADP	101	P	60
P	131	Proton	86	Proton	77	Proton	47
CO_2	90	CO_2	63	CO2	40	PP	38
NADP	86	PP	56	PP	40	CO2	36
NADPH	78	Pyr	53	NADP	31	NADP	34
PP	81	Glu	48	NADPH	30	NADPH	33
NAD	78	NAD	48	Glu	30	Glu	23
NADH	65	NADH	43	NAD	24	NH3	19
Glu	68	NADP	41	Pyr	22	Pyr	18
NH_3	56	NH_3	41	NH3	22	COA	18

FIG. 1. Frequency plot of the number of reactions that each metabolite appears in for four different reconstructed metabolic networks. For each metabolic network the 10 metabolites that appear in the most reactions are listed. PP, pyrophosphate; COA, coenzyme A. The numbers in the box specify the numbers of reactions the 10 most frequently used metabolites participate in for the four different microorganisms.

analysis of cellular metabolism



	r_1	r_2	r_3	r_4	r_5	r_{6r}	r_7	r_{8r}	r_9
A	1	-1	0	0	-1	0	0	0	0
B	0	0	0	0	1	-1	-1	-1	0
C	0	1	-1	0	0	1	0	0	0
D	0	0	1	0	0	0	0	0	-1
P	0	0	1	-1	0	0	2	0	0

$$\frac{d}{dt}\underline{C} = \underline{\underline{S}} \times \underline{r} - \mu \times \underline{C},$$

μ.C (negligible)

dC/dt = 0 (steady state)

S.r = 0 (Eq 2)

ri $\geq$ 0 (Eq 3)

Tools for analysis of cellular metabolism can be grouped into three categories, all of them developed from the same mathematical model:

(1) Metabolic flux analysis,

(2) Flux balance analysis and

(3) Metabolic pathway analysis (Elementary mode analysis).

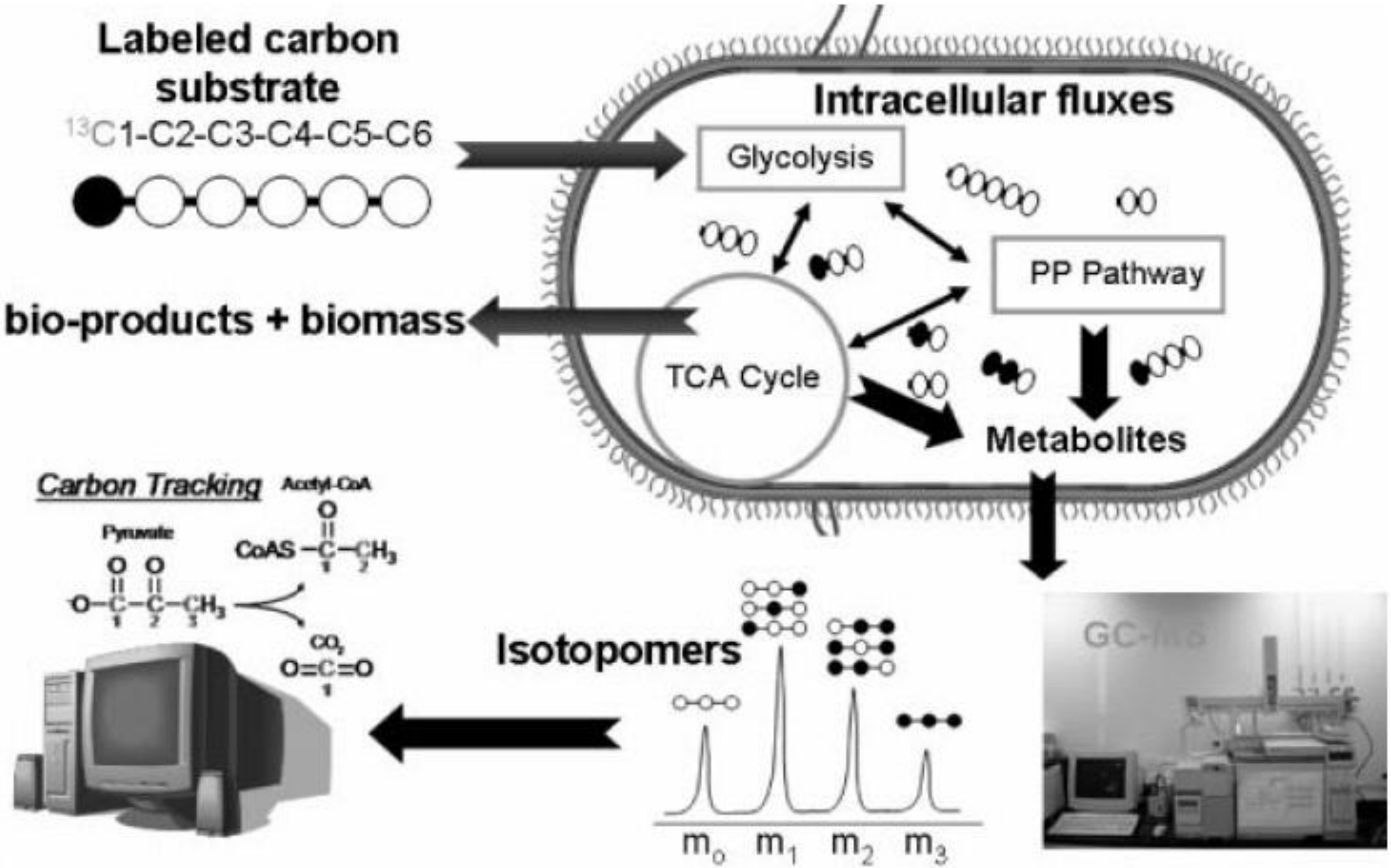
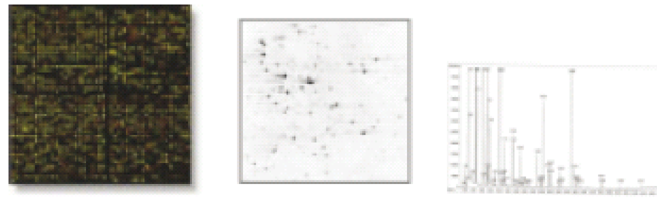


FIGURE 2. Protocol for ^{13}C-based flux analysis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

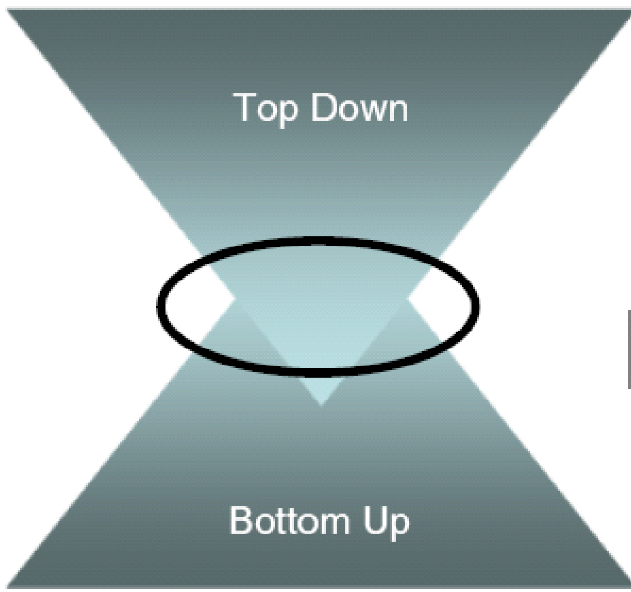
Metabolic Engineering



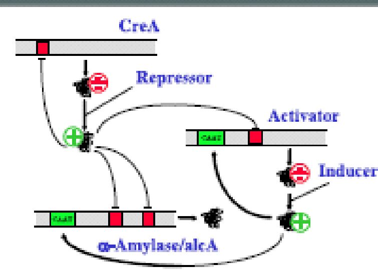
High level models

↕

Low level models



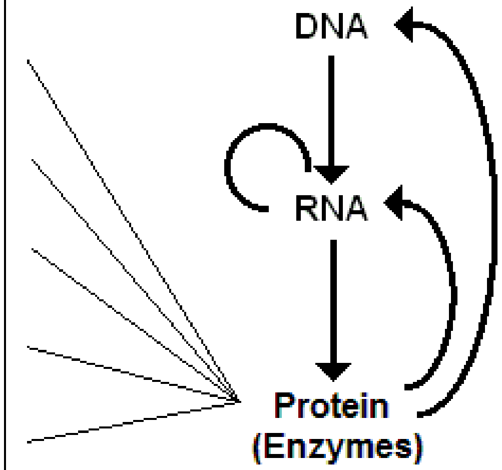
Global analysis (omics)
↓
Data analysis (bioinformatics)
↓
Reduction of dimensionality
↓
System description
↑
Reaction/process kinetics
↑
Molecular interactions



Metabolic Engineering

-1	1	0	0	-1	1	0	0
0	-1	1	0	0	0	0	0
0	0	-1	1	0	0	0	0
0	0	0	-1	1	-1	1	0
0	0	-1	0	1	-1	0	1

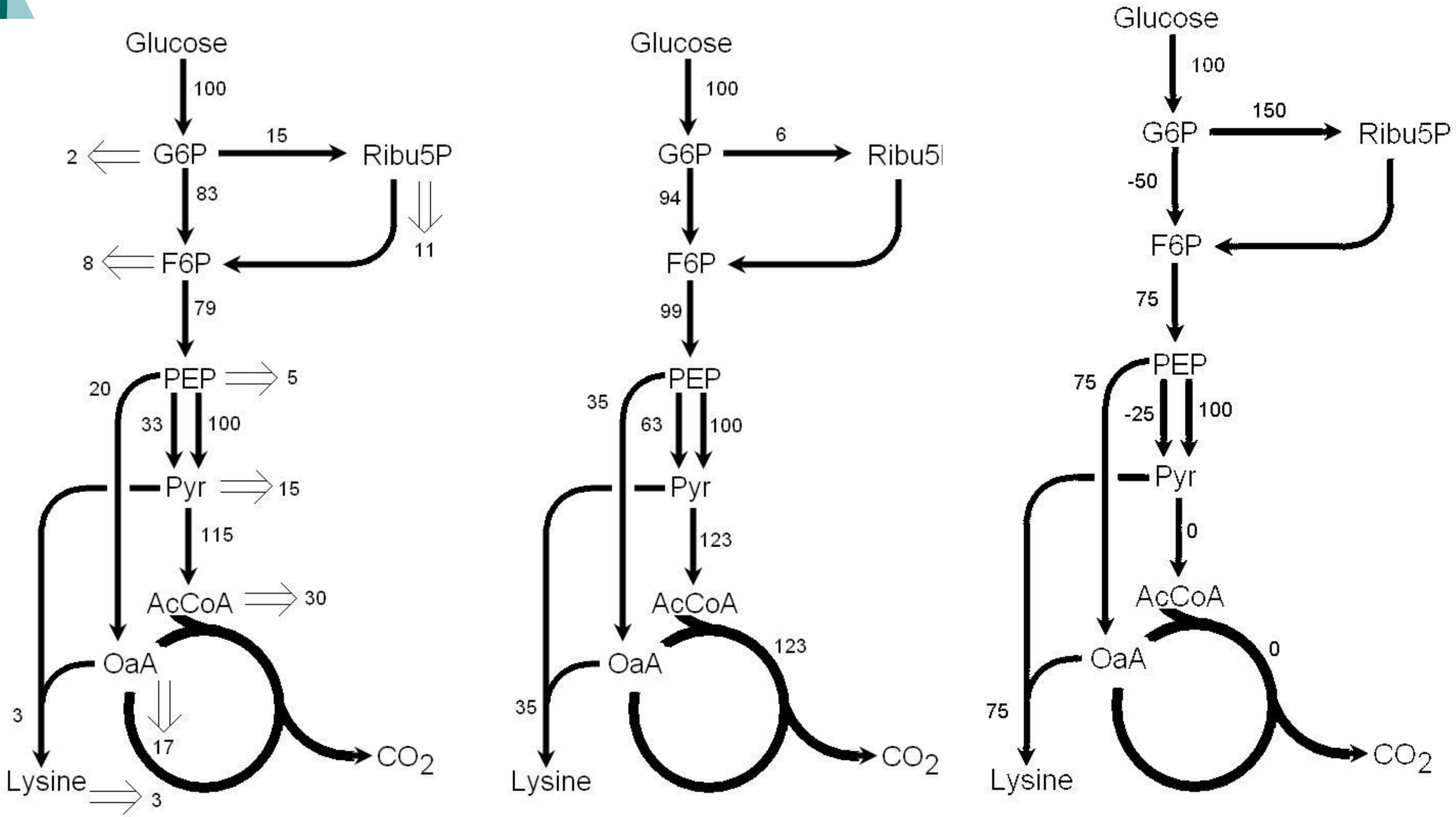
V1
V2
V3
V4
V5



Metabolic level regulation

Hierarquical level regulation

Fluxos metabólicos

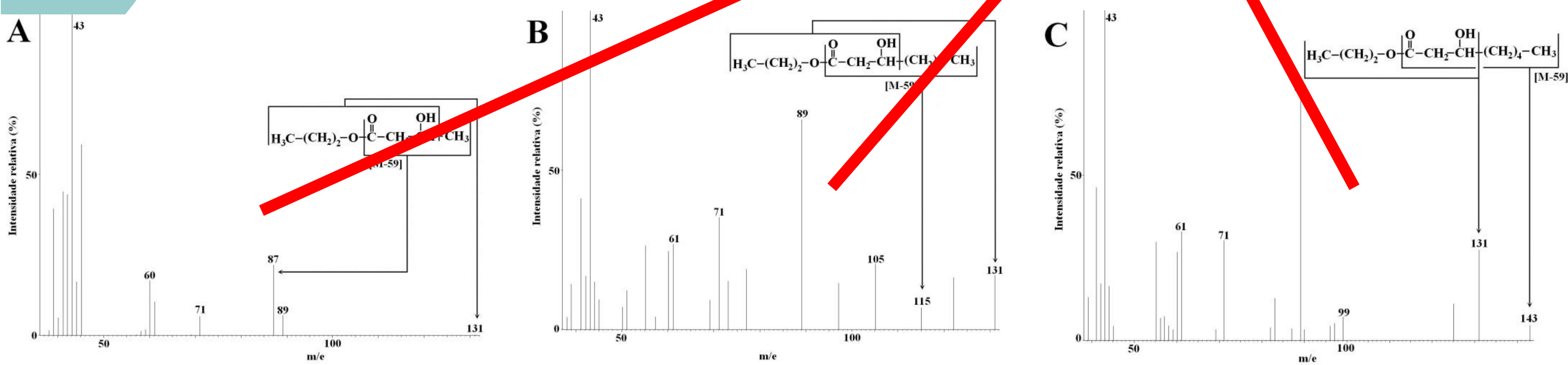


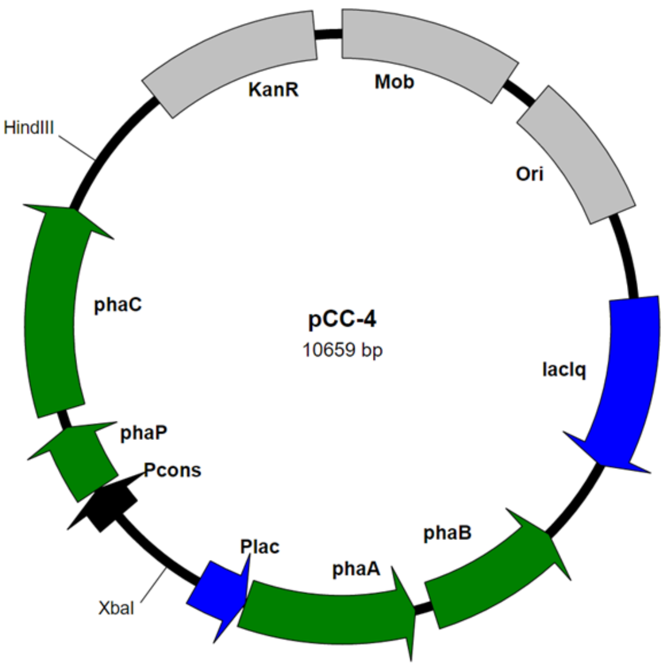
Pseudomonas sp. abrigando genes de *R. eutropha*

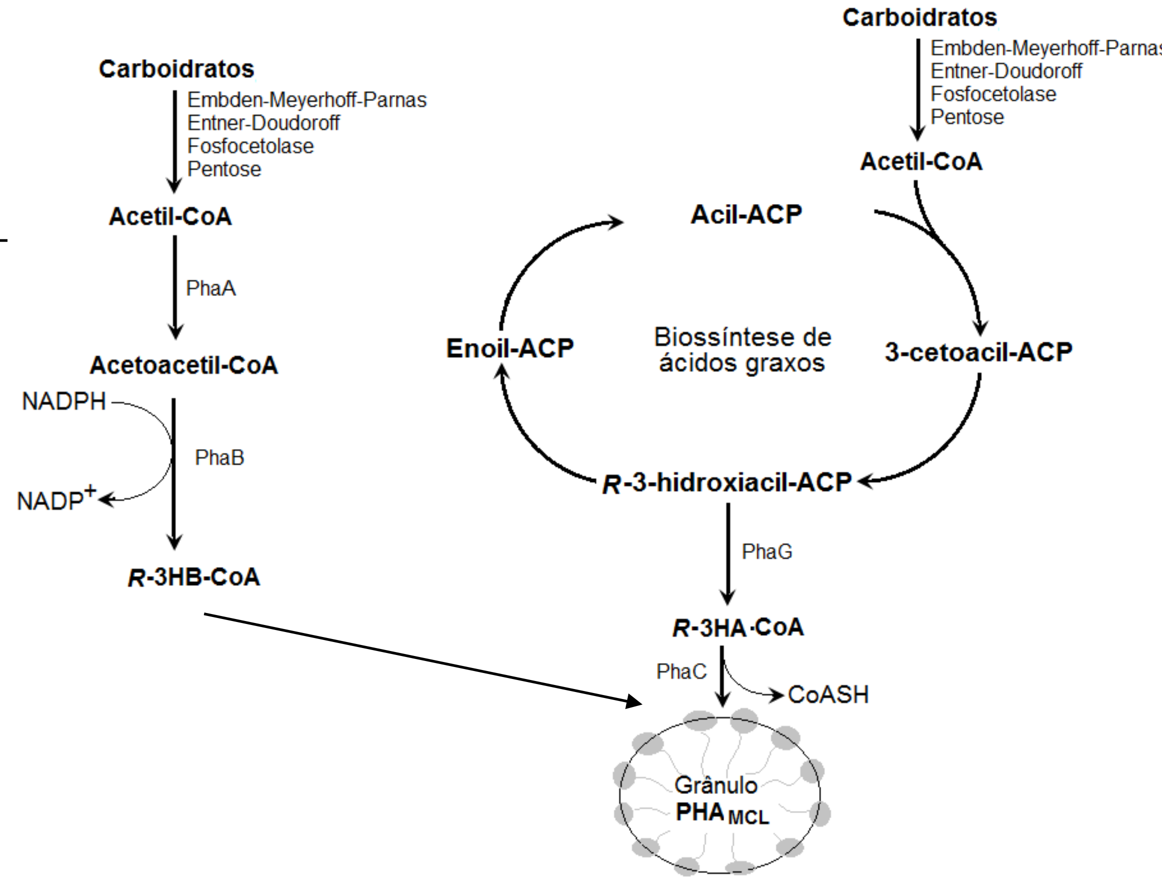
Tabela 3. Composição de PHA produzido. Análise de células liofilizadas e polímero purificado.

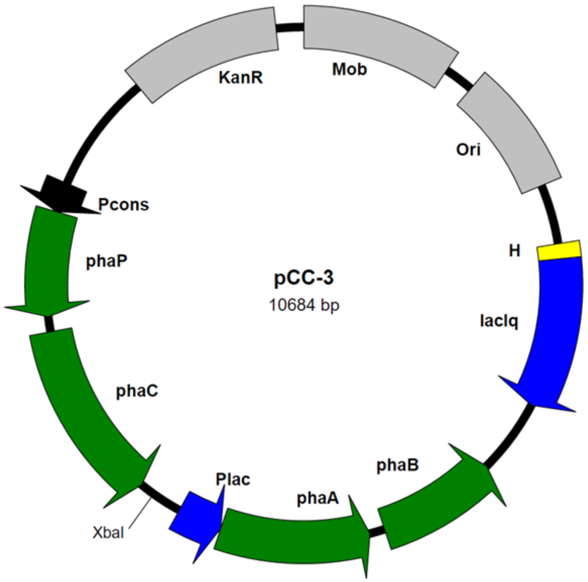
Linhagens recombinantes	Fonte de carbono	Material	PHA (mol%)				
			3HB	3HHx	3HO	3HD	3HDd
***Pseudomonas* sp. LFM046 pBBR1MCS-2**	Octanoato	Cel. Liof.	0,71	13,69	73,72	9,95	1,34
		Polímero	0,86	14,35	75,14	9,65	Tr
Pseudomonas sp.* LFM046 pBBR1MCS-2::*phaB	Octanoato	Cel. Liof.	0,00	15,13	57,02	12,10	15,76
		Polímero	2,75	20,52	74,99	1,73	Tr
***Pseudomonas* sp. LFM461 pBBR1MCS-2**	Glicose	Cel. Liof.	0,00	0,00	0,00	47,99	52,01
		Polímero	-	-	-	-	-
Pseudomonas sp.* LFM461 pBBR1MCS-2::*phaC	Glicose	Cel. Liof.	91,59	4,10	3,28	1,03	Tr
		Polímero	92,38	4,33	3,29	0,00	0,00

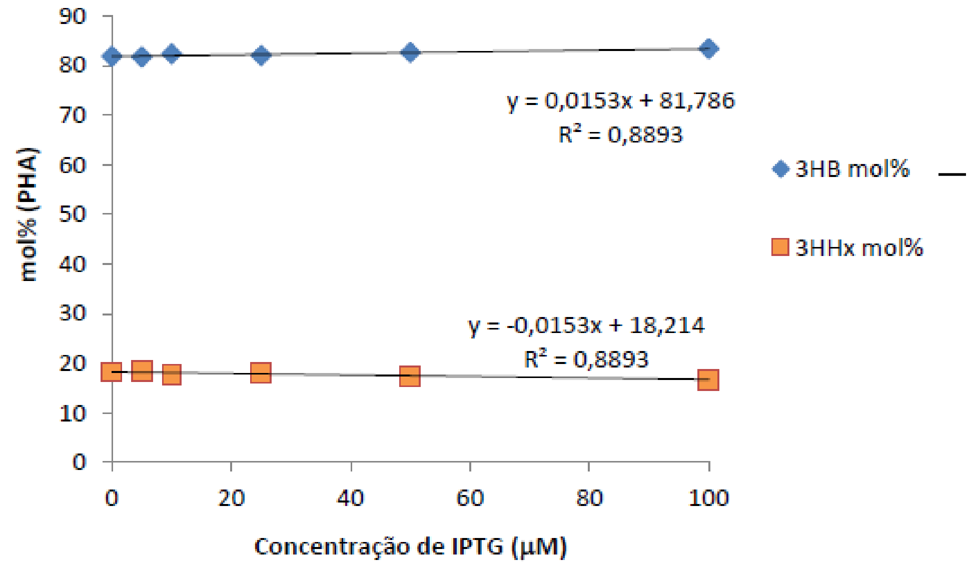
3HB – 3-hidroxibutirato
3HHx – 3-hidroxihexanoato
3HO – 3-hidroxioctanoato
3HD – 3-hidroxidecanoato
3HDd – 3-hidroxidodecanoato
Polímero – polímero purificado.
Cel. Liof. – células liofilizadas

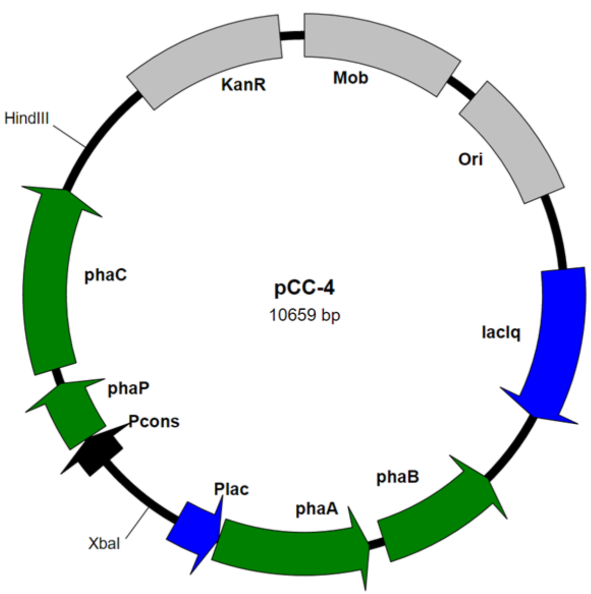


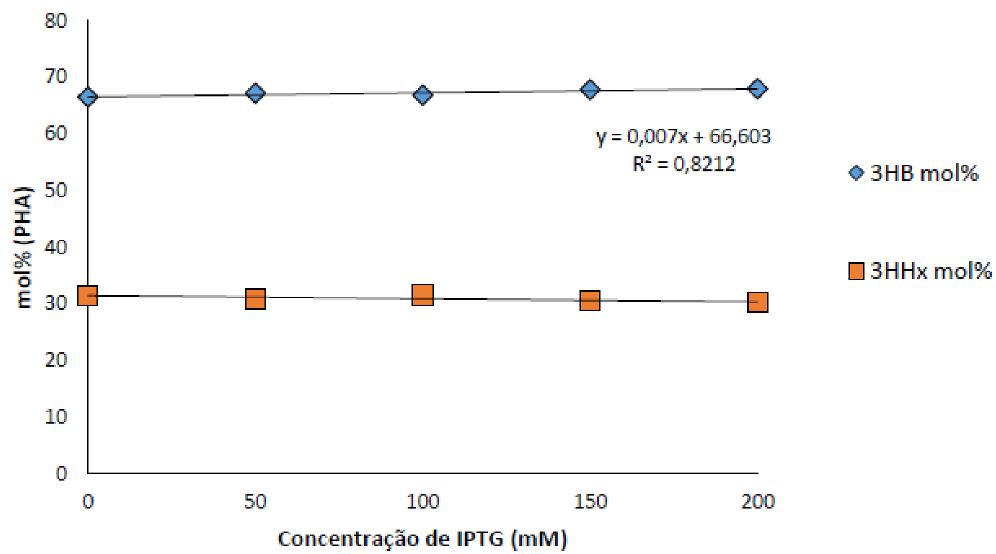

KanR
Mob
HindIII
Ori
phaC
pCC-4
10659 bp
lacIq
phaP
Pcons
Plac
phaB
phaA
XbaI


Carboidratos
Embden-Meyerhoff-Parnas
Entner-Doudoroff
Fosfocetolase
Pentose
Acetil-CoA
PhaA
Acetoacetil-CoA
NADPH
PhaB
NADP+
R-3HB-CoA
Carboidratos
Embden-Meyerhoff-Parnas
Entner-Doudoroff
Fosfocetolase
Pentose
Acetil-CoA
Acil-ACP
Enoil-ACP
Biossíntese de ácidos graxos
3-cetoacil-ACP
R-3-hidroxiacil-ACP
PhaG
R-3HA-CoA
PhaC
CoASH
Grânulo
PHA MCL

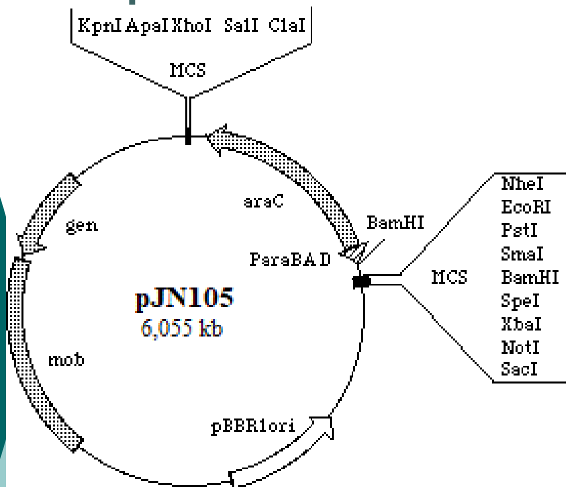

KanR
Mob
Ori
Pcons
H
phaP
pCC-3
10684 bp
lacIq
phaC
Plac
phaA
phaB
XbaI

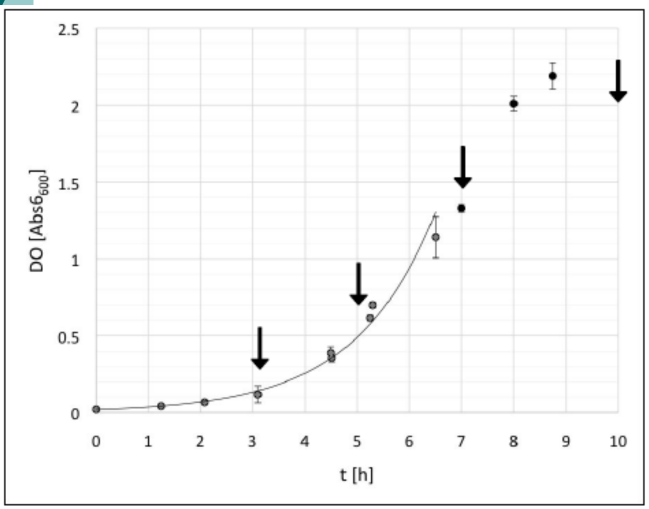

y = 0,0153x + 81,786
R² = 0,8893
y = -0,0153x + 18,214
R² = 0,8893
mol% (PHA)
Concentração de IPTG (µM)
3HB mol%
3HHx mol%

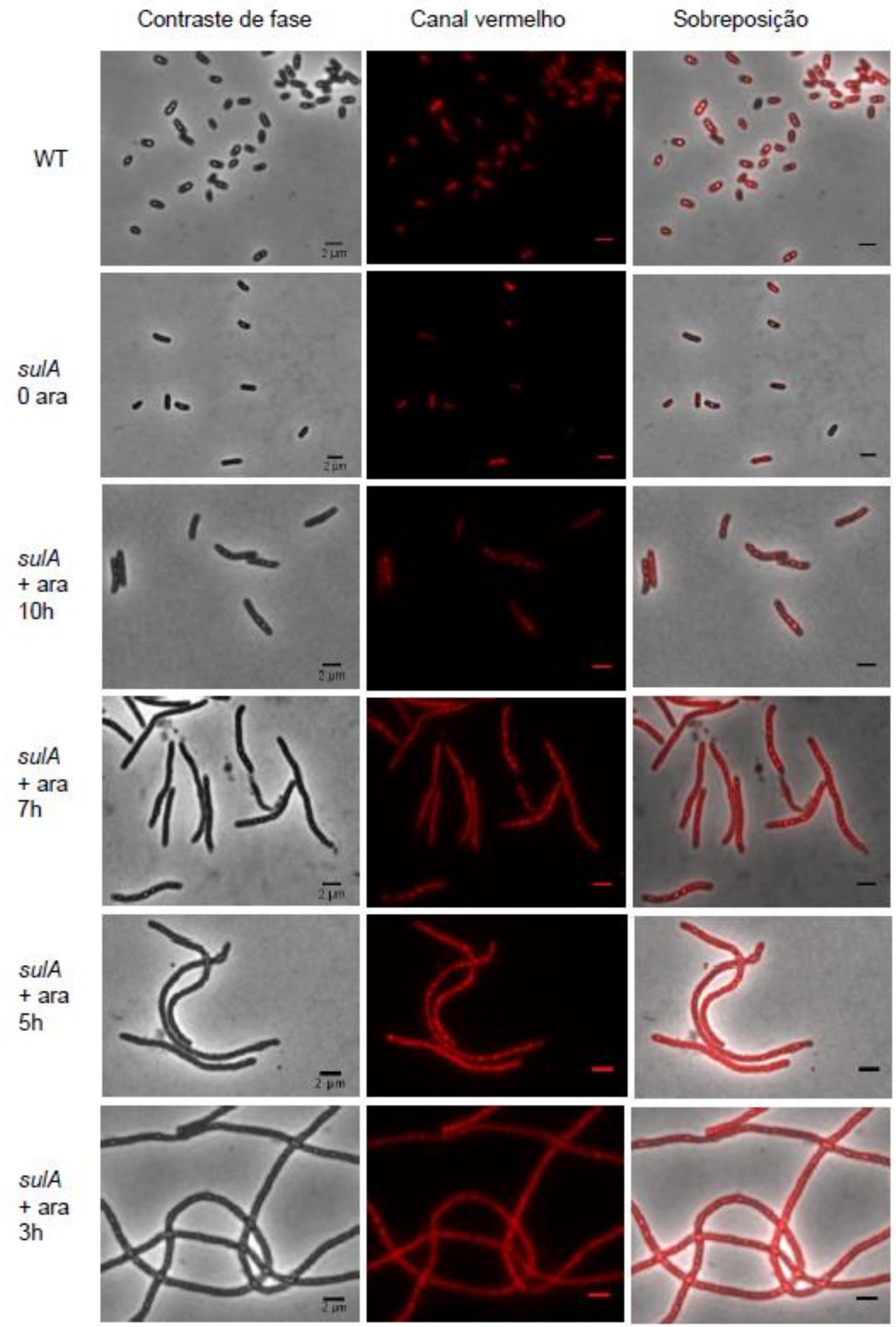

KanR
Mob
HindIII
Ori
phaC
pCC-4
10659 bp
lacIq
phaP
Pcons
Plac
phaA
phaB
XbaI


y = 0,007x + 66,603
R² = 0,8212
mol% (PHA)
Concentração de IPTG (mM)
3HB mol%
3HHx mol%

Pseudomonas sp. LFM046 pJN105::*sulA*

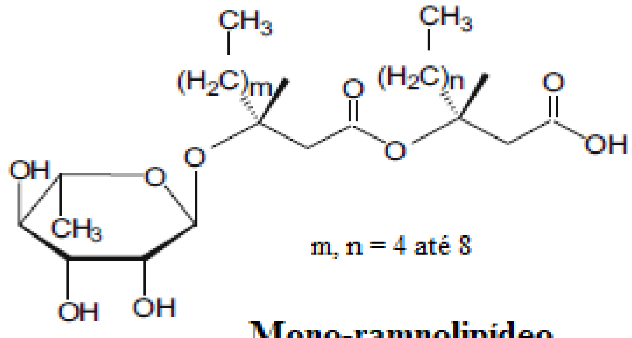




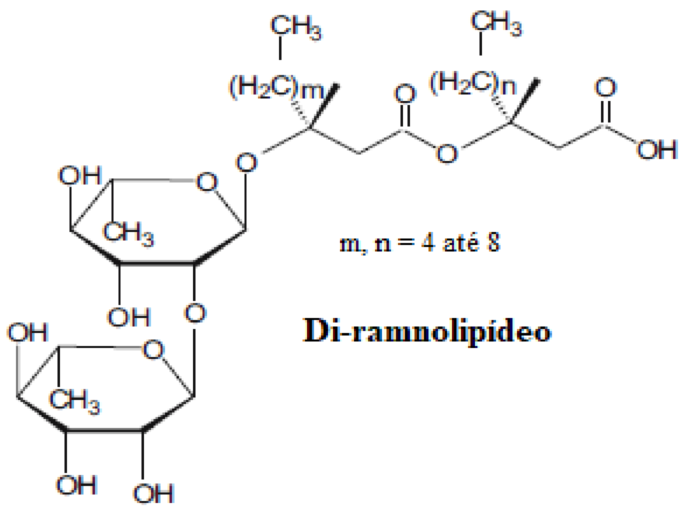


Imagens de microscopia de cultivos de *Pseudomonas* sp. LFM046:pJN105*sulA* com adição de L-arabinose em diferentes tempos de cultivo. As amostras foram retiradas após 72h de cultivo em meio para acúmulo de PHA com adição de L-arabinose (ara) 20 mM após 3h, 5h, 7h e 10h de cultivo ou sem adição, como indicado na figura. As amostras foram coradas com o corante de PHA Nile Red e analisadas em microscópio de fluorescência. Barra de escala de 2 μm.

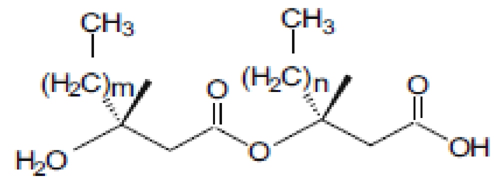
Ramnolipídeos



Mono-ramnolipídeo



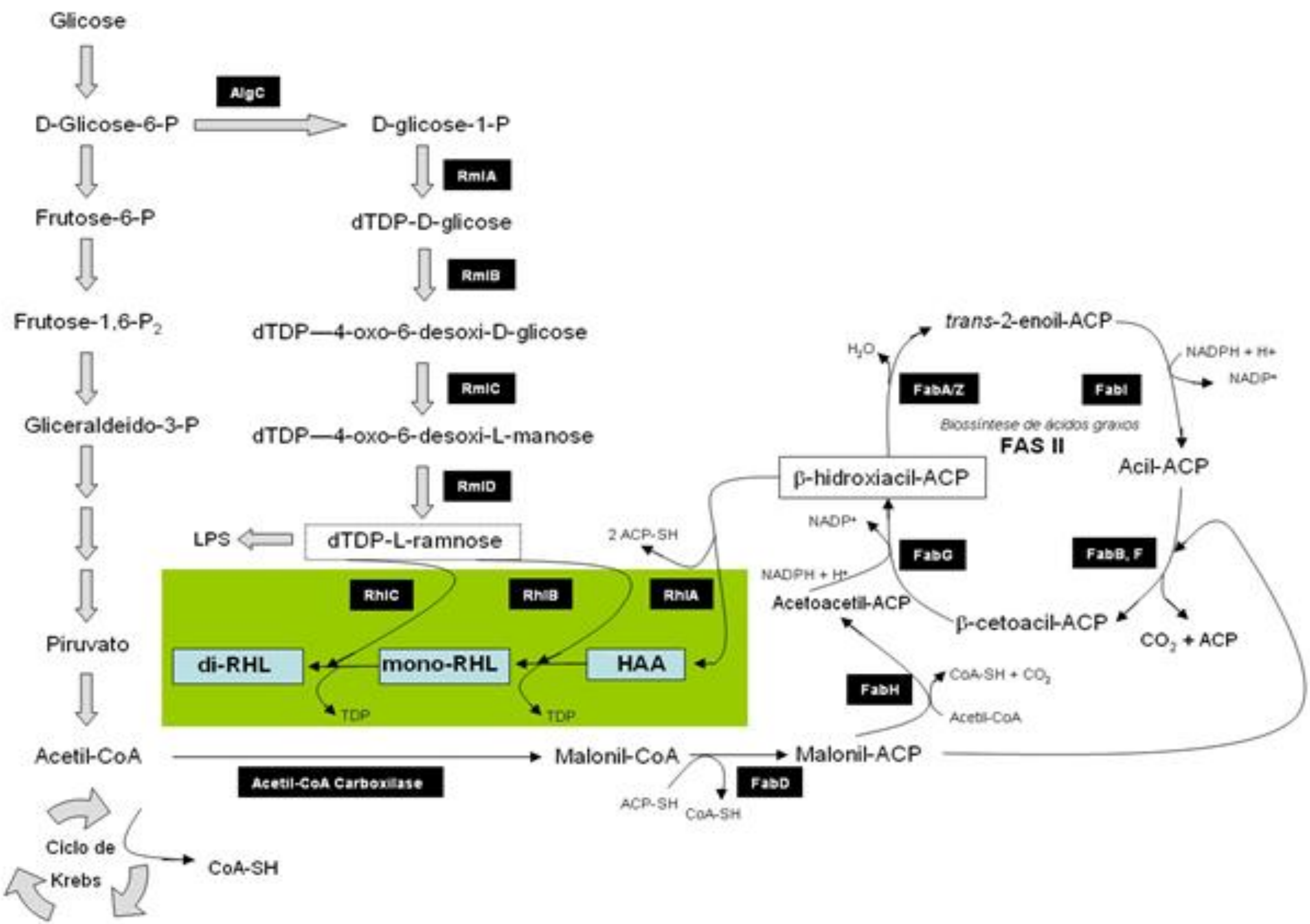
Di-ramnolipídeo



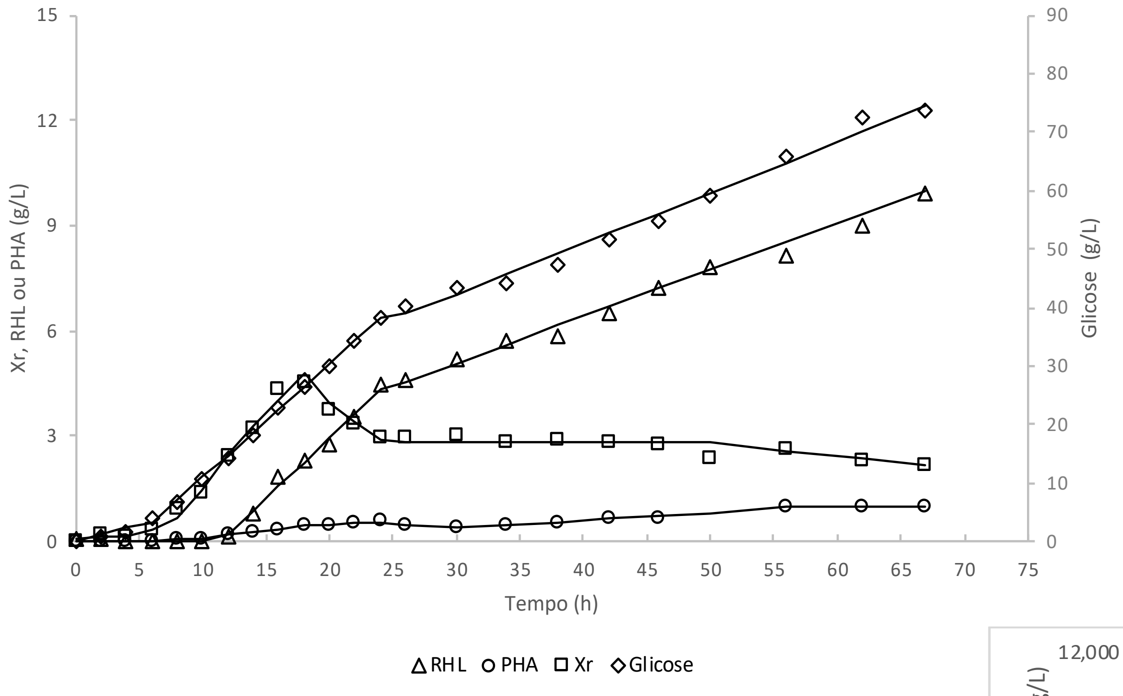
m, n = 4 até 8

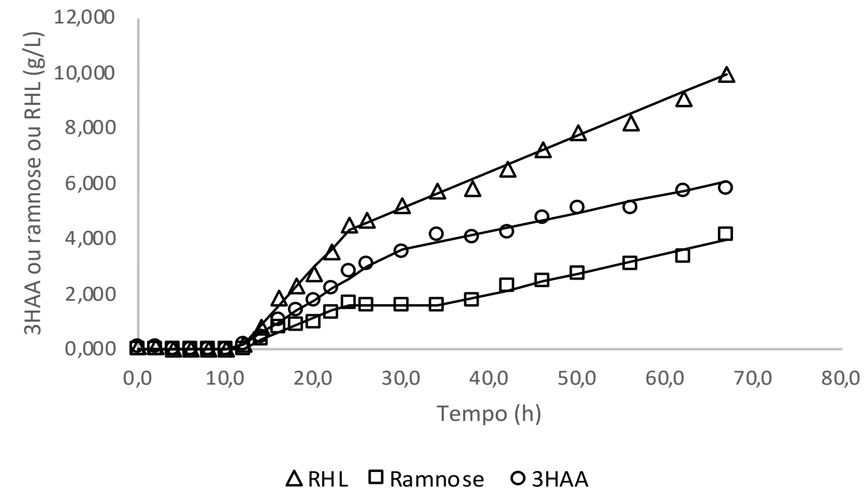
3-(3-hidroxialcanoiloxi)alcanoato (HAA)

Ramnolipídeos

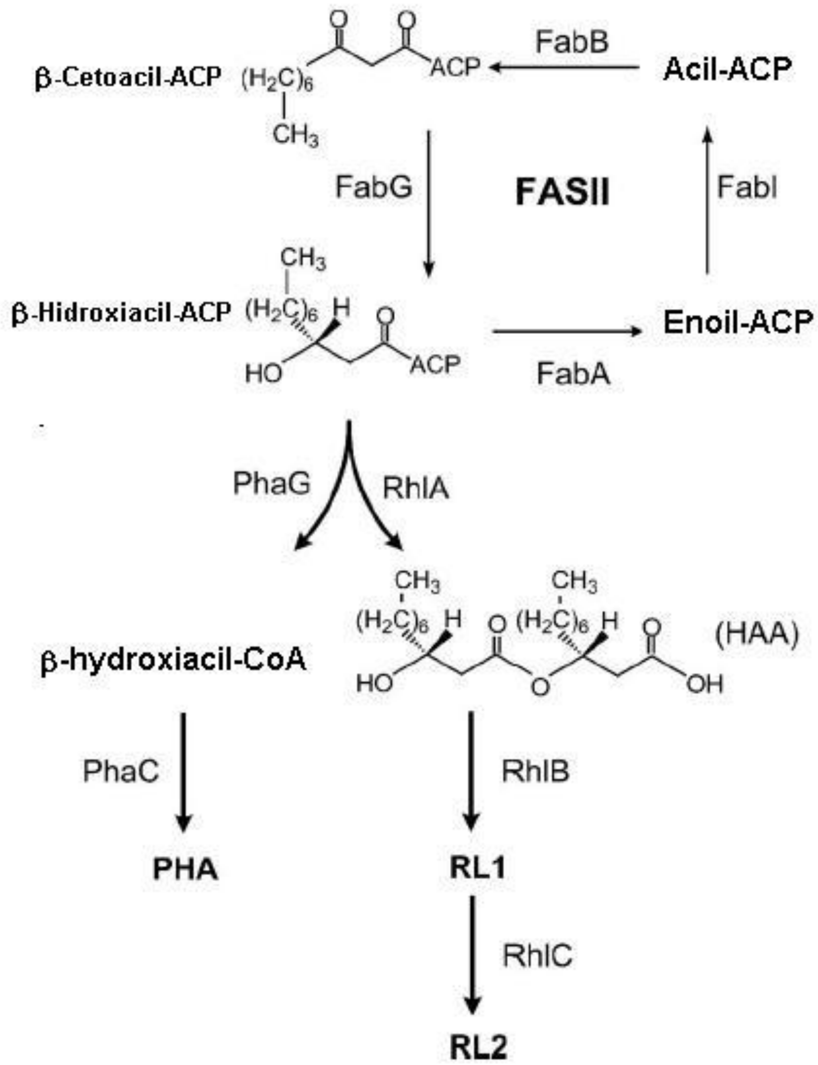


Ramnolipídeos - Bioprocesso





Ramnolipídeos



Ramnolipídios composição.

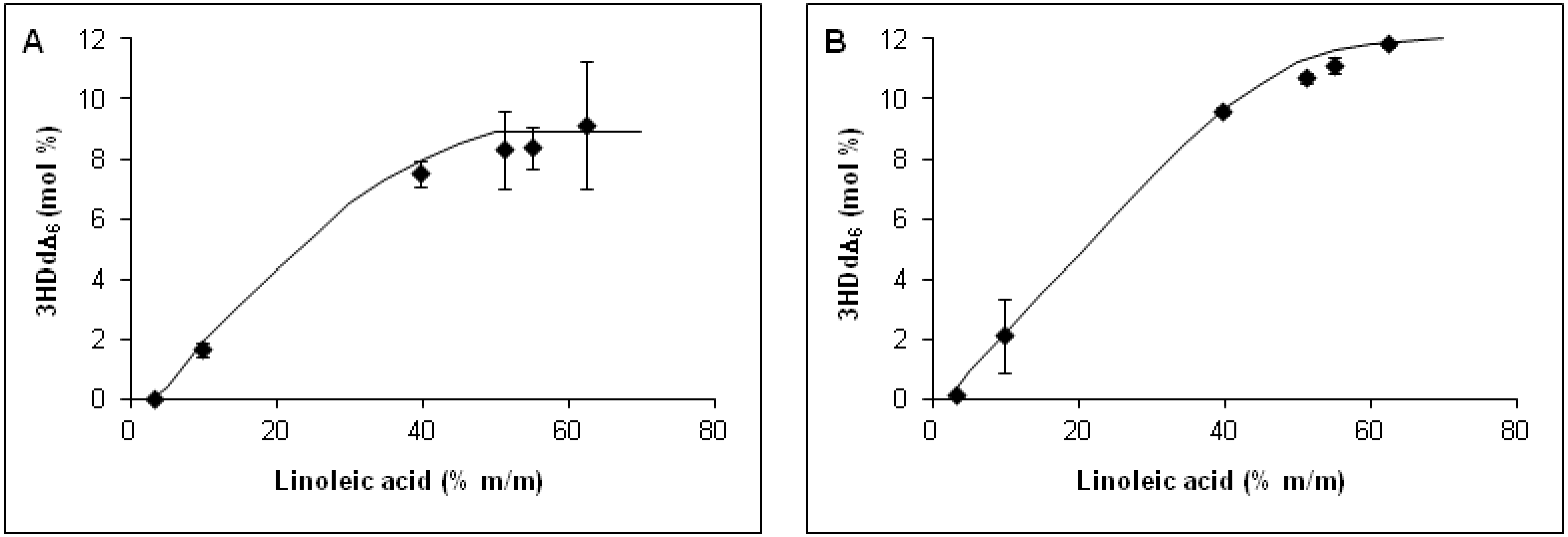
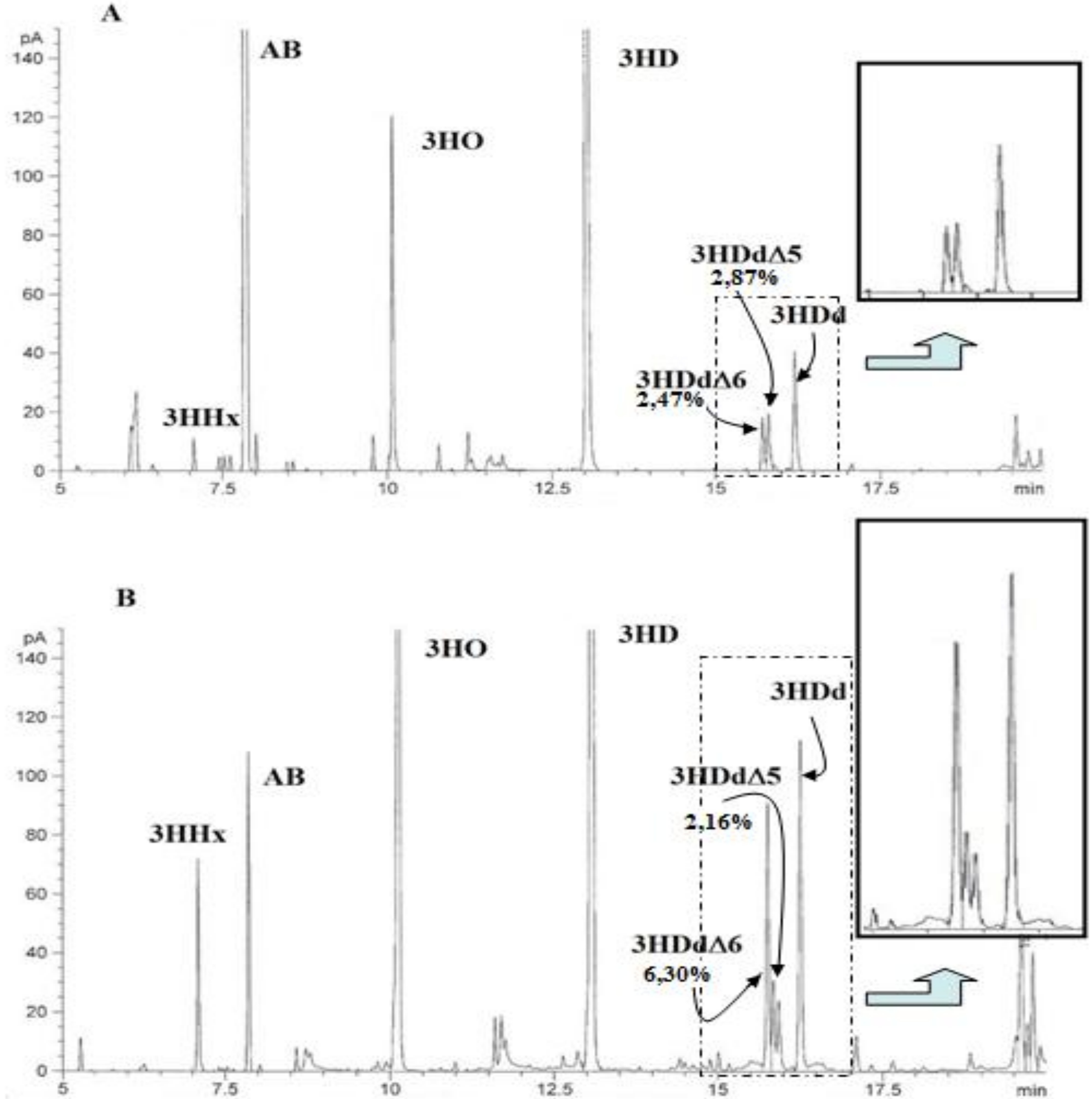


Figure 2. Relationship between linoleic acid fraction in plant oils supplied and 3-hydroxy-6-dodecenoic acid ($3HDdD_6$) detected in rhamnolipids (A) or polyhydroxyalkanoates (B) produced by bacterial strain RMP1315.

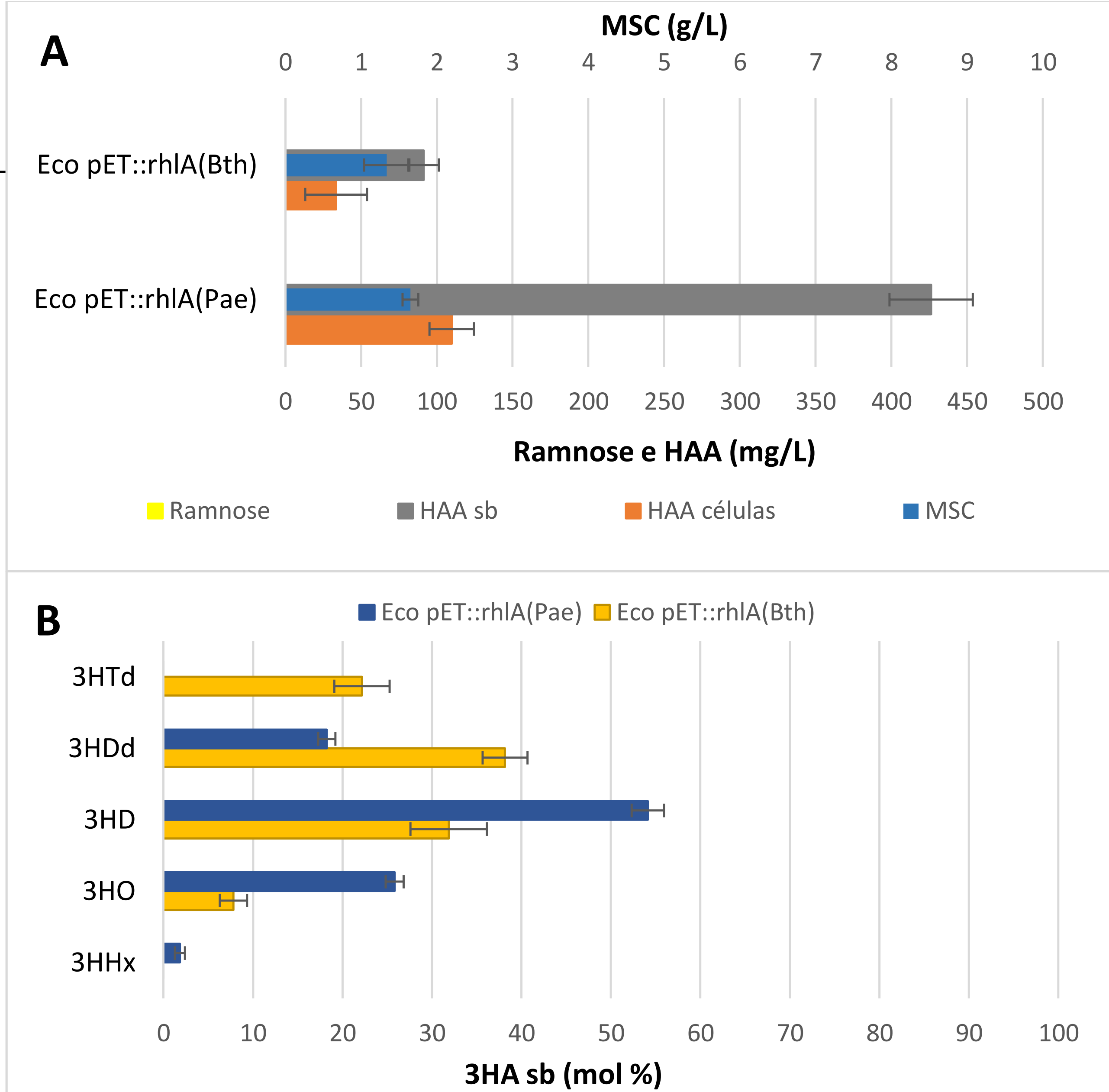


A
pA
140
120
100
80
60
40
20
0
5
7.5
10
12.5
15
17.5
min
AB
3HD
3HO
3HHx
3HDdΔ5
2,87%
3HDd
3HDdΔ6
2,47%
B
3HO
3HD
AB
3HHx
3HDd
3HDdΔ5
2,16%
3HDdΔ6
6,30%

rhlA

linhagens / linhagens	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
A *P.aeruginosa* LFM634	100%																
B *P.aeruginosa* ATCC14886	100%	100%															
C *P.aeruginosa* LESB58	100%	100%	100%														
D *P.aeruginosa* M18	100%	100%	100%	100%													
E *P.aeruginosa* DK2	99%	99%	99%	99%	100%												
F *P.aeruginosa* PAO1	99%	99%	99%	99%	99%	100%											
G *P.aeruginosa* PA7	96%	96%	96%	96%	95%	95%	100%										
H *P.fluorescens* SBW25	65%	65%	65%	65%	65%	65%	65%	100%									
I *P.poae* RE1_1_14	62%	62%	62%	62%	62%	62%	64%	90%	100%								
J *P.chlororaphis* AGH13750	63%	63%	63%	63%	63%	63%	63%	60%	59%	100%							
K *B.mallei* ATCC23344	48%	48%	48%	48%	48%	48%	49%	43%	45%	45%	100%						
L *B.pseudomallei* 1710b	48%	48%	48%	48%	48%	48%	48%	43%	45%	45%	99%	100%					
M *B.thailandensis* E264	48%	48%	48%	48%	48%	48%	48%	42%	44%	45%	97%	97%	100%				
N *B.oklahomensis* C6786	46%	46%	46%	46%	46%	46%	47%	42%	42%	44%	93%	93%	92%	100%			
O *B.glumae* PG2	45%	45%	45%	45%	45%	45%	46%	42%	42%	43%	79%	79%	80%	78%	100%		
P *B.gladioli* BSR3	44%	44%	44%	44%	44%	44%	45%	42%	41%	43%	79%	79%	79%	80%	88%	100%	
Q *B.cenocepacia* J2315	44%	44%	44%	44%	44%	44%	45%	42%	42%	42%	76%	76%	77%	75%	75%	76%	100%

rhlA


A
MSC (g/L)
0 1 2 3 4 5 6 7 8 9 10
Eco pET::rhlA(Bth)
Eco pET::rhlA(Pae)
0 50 100 150 200 250 300 350 400 450 500
Ramnose e HAA (mg/L)
Ramnose
HAA sb
HAA células
MSC
B
Eco pET::rhlA(Pae)
Eco pET::rhlA(Bth)
3HTd
3HDd
3HD
3HO
3HHx
0 10 20 30 40 50 60 70 80 90 100
3HA sb (mol %)

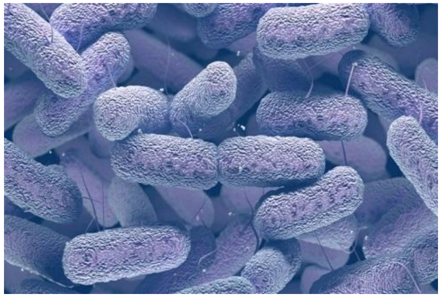
Produção de 1,3-propanodiol

Vantagens:
Fonte renovável, limpa e de baixo custo

Processo biotecnológico:

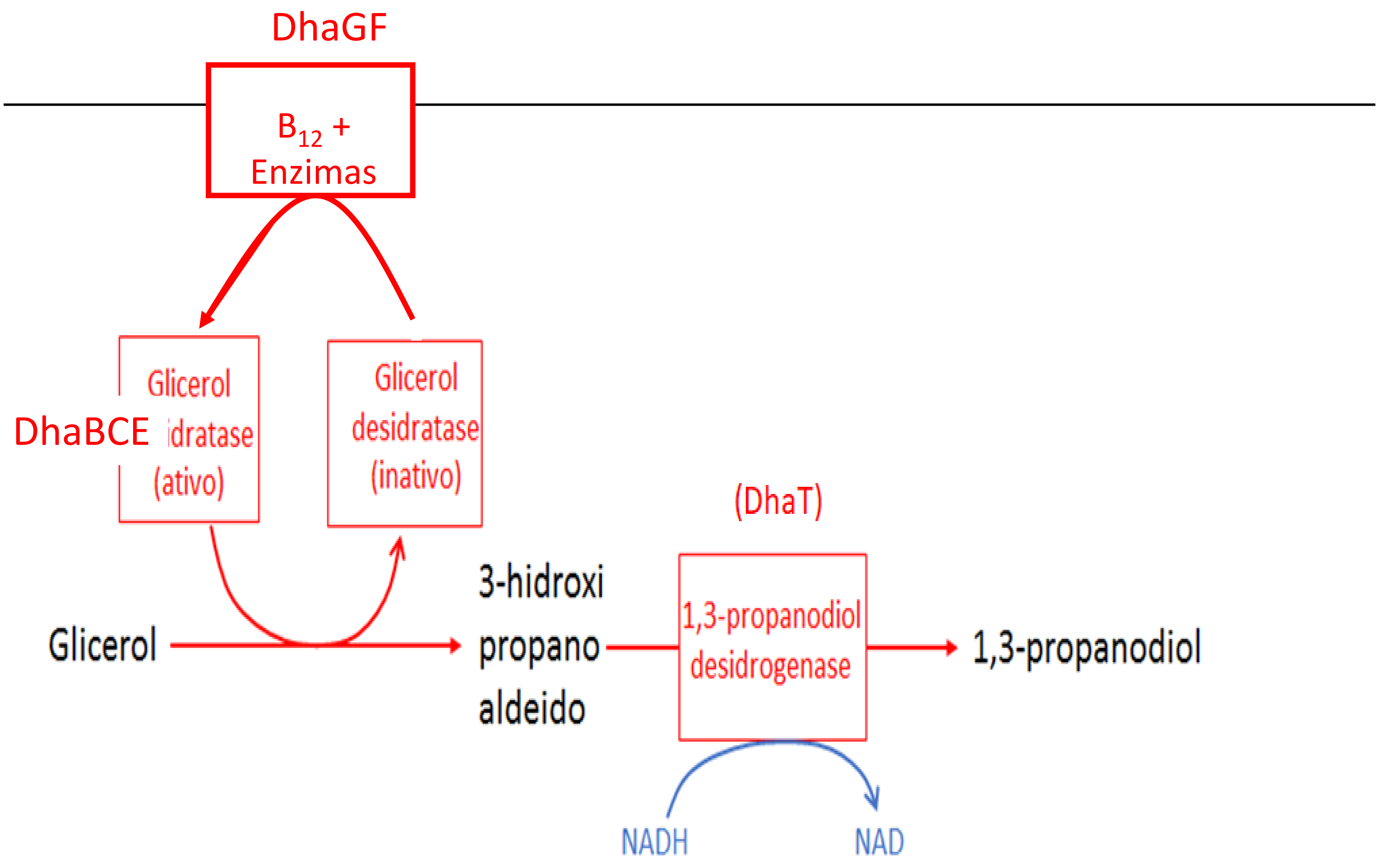
- Produção de propanodiol a partir do glicerol, por exemplo

- Bactérias produtoras como: *Klebsiella pneumoniae*, *Citrobacter freundii* e *Clostridium butyricum*

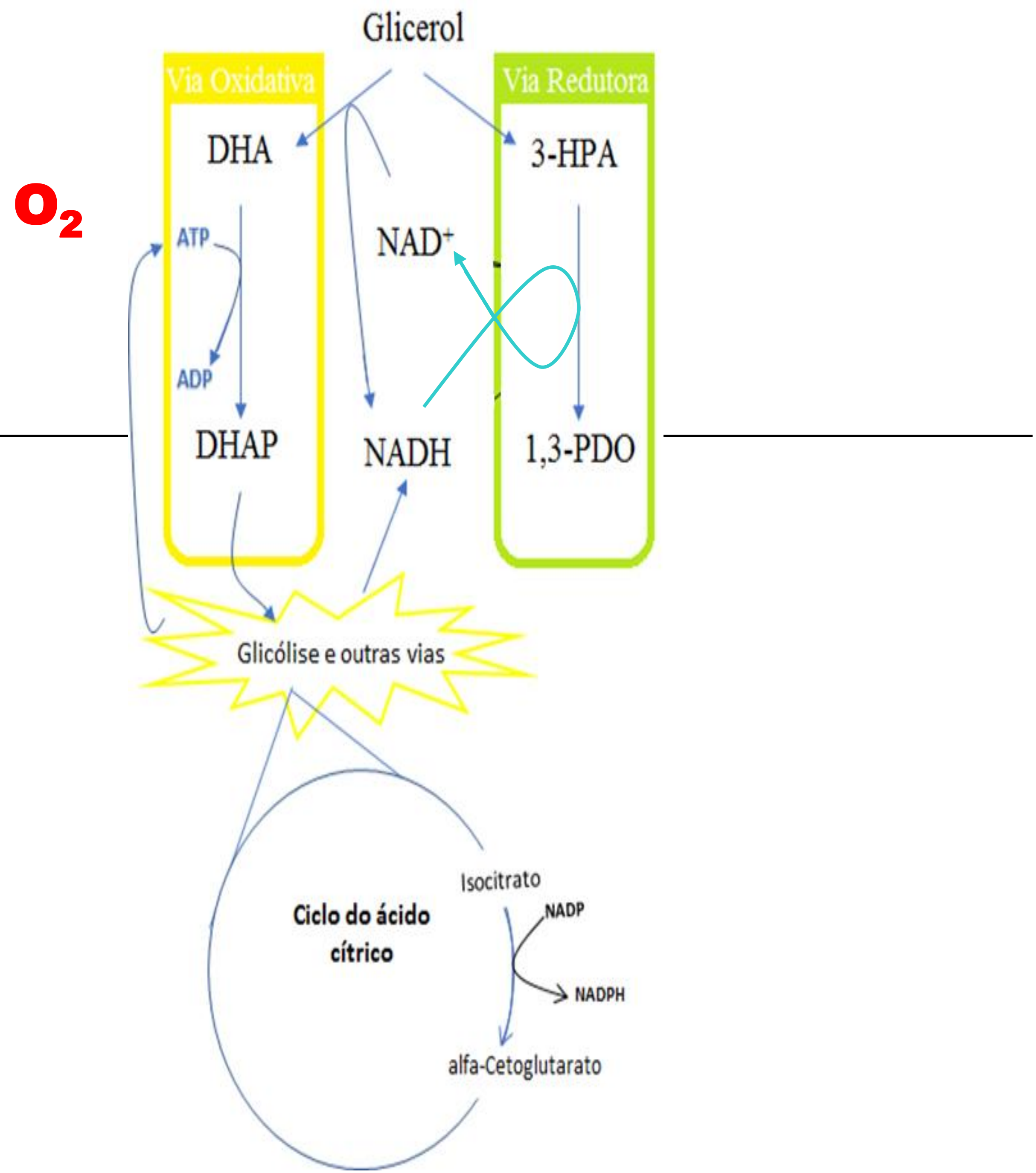


Produção de 1,3-PDO por bactérias

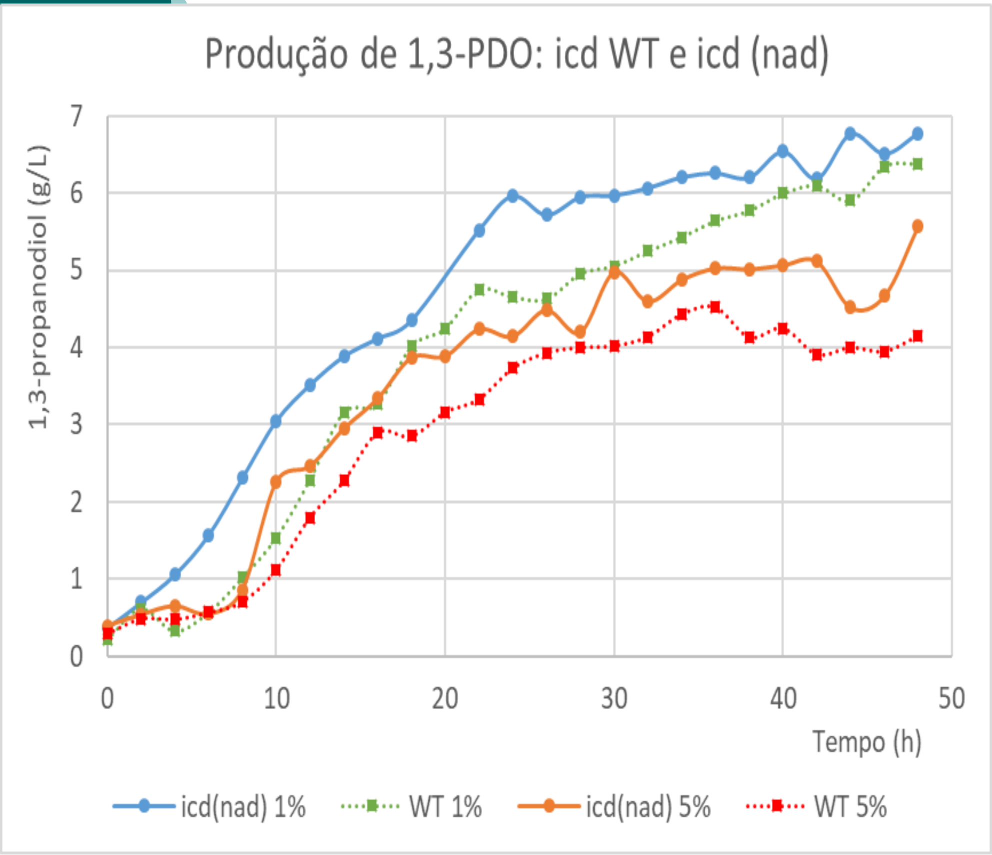
- *Klebsiella pneumoniae*

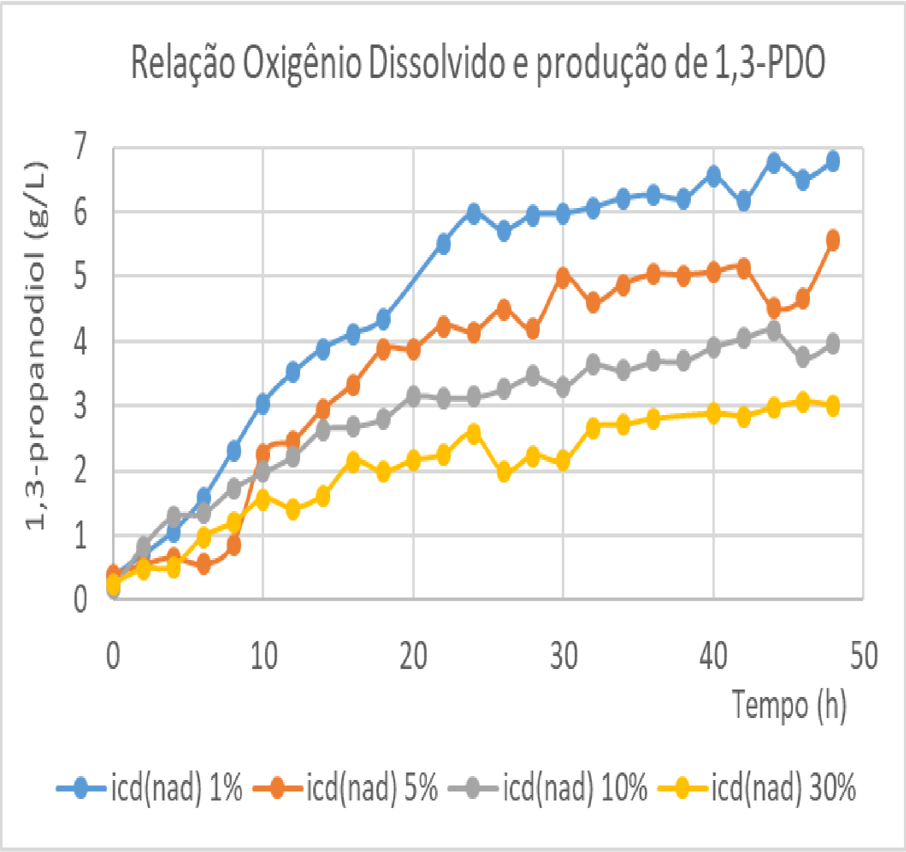


Balanço NAD/NADH
Respiração/Fermentação



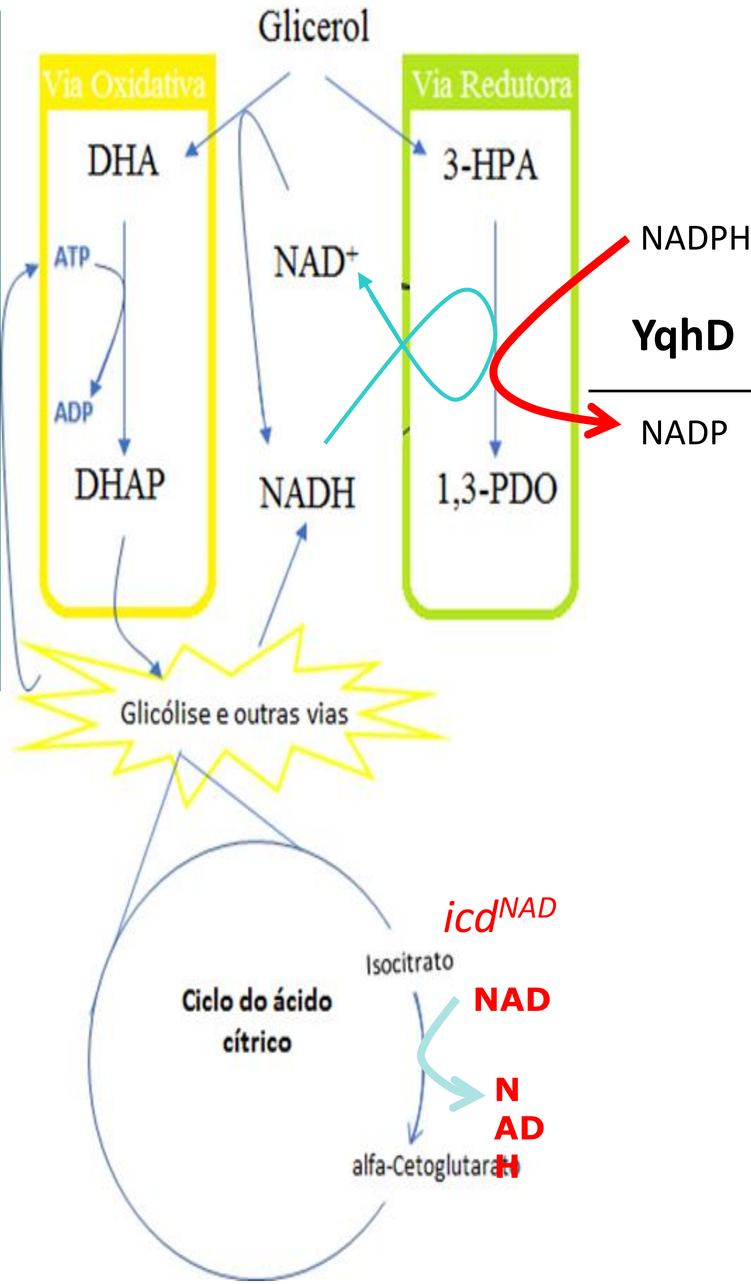
- *E. coli* transformada capaz de produzir 1,3-PDO



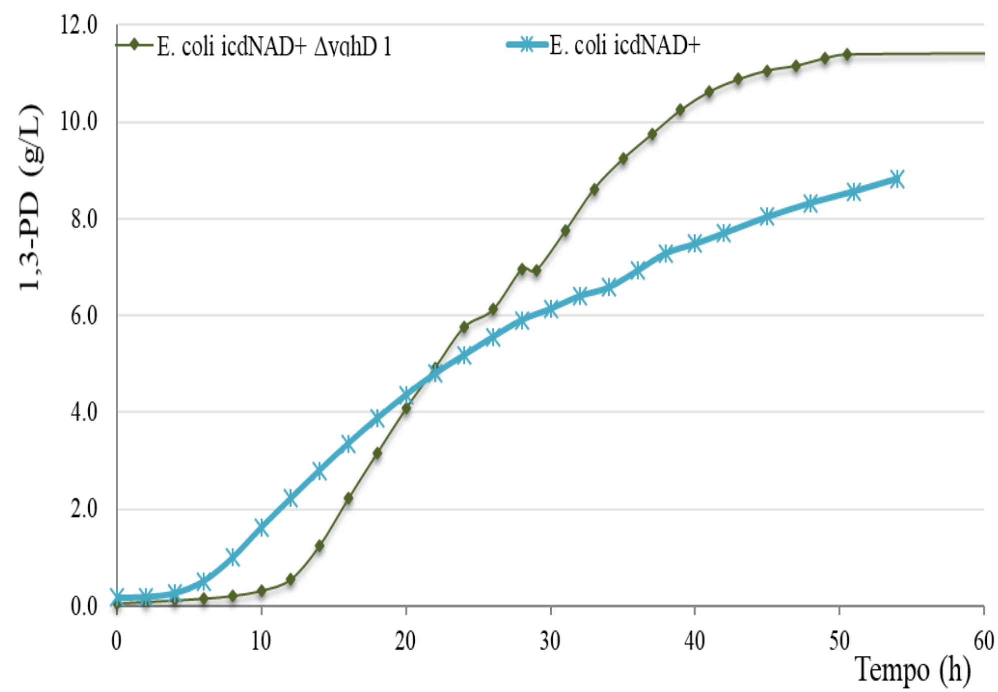


Trabalhos anteriores

- O gene *yqhD* de *E. coli* é homólogo ao *dhaT*
- No entanto, YqhD é NADPH dependente.



E. coli MG1655 *icd*^{NAD} Δ*yqhD* + pBBR1::*dha*



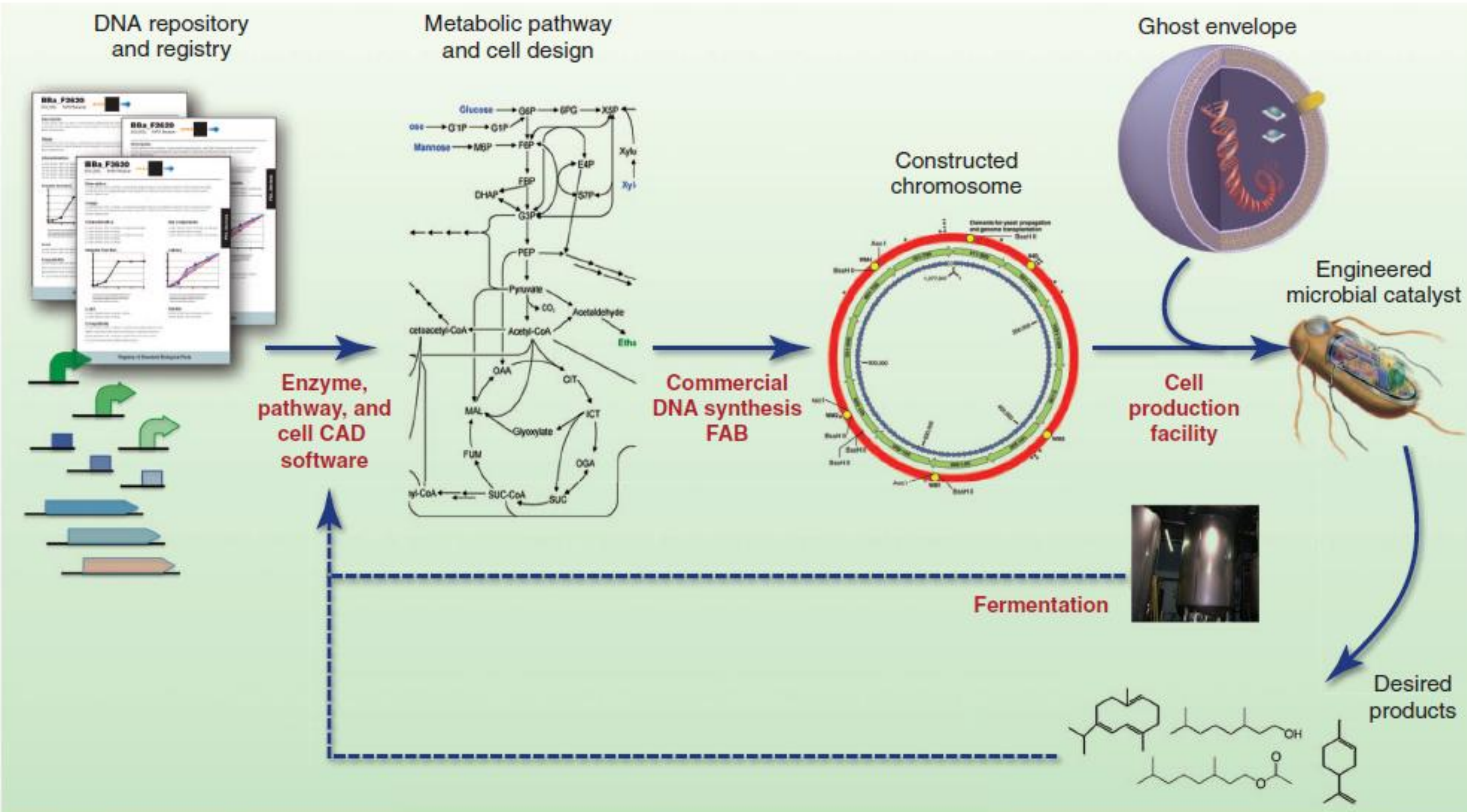


Fig. 3. The future of engineered biocatalysts. Pathways, enzymes, and genetic controls are designed from characteristics of parts (enzymes, promoters, etc.) by means of pathway and enzyme CAD software. The chromosomes encoding those elements are synthesized at a FAB and incorporated into a ghost envelope to obtain the new catalyst. The design of the engineered catalyst is influenced by the desired product and the production process.

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