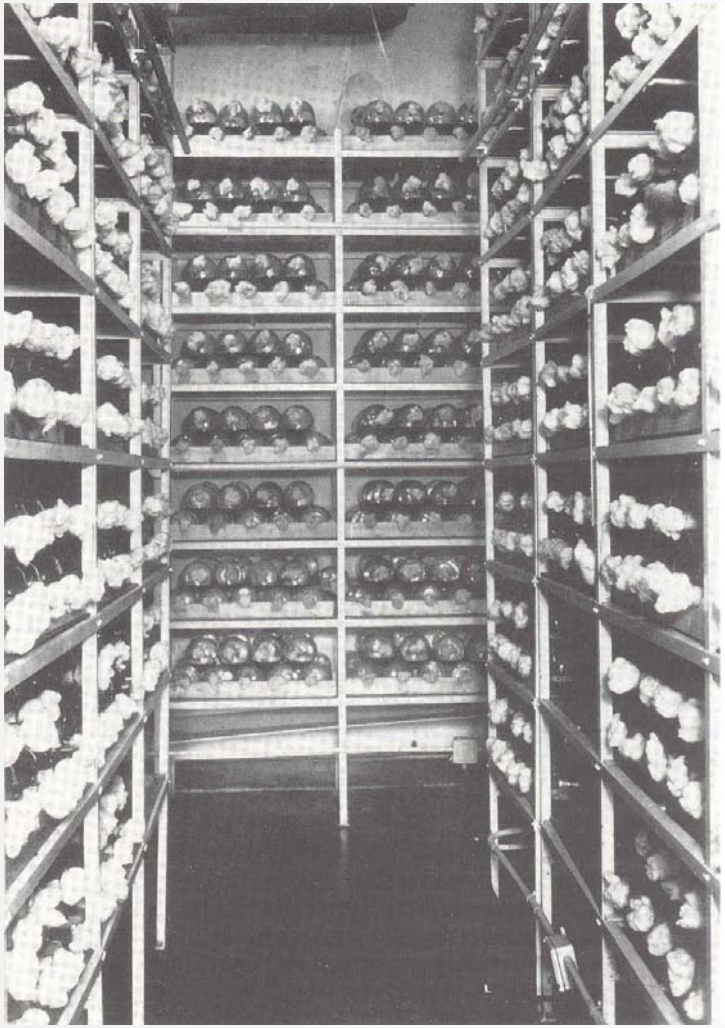
Thailand-EU collaboration on Industrial Biotechnology

Vitor Martins dos Santos

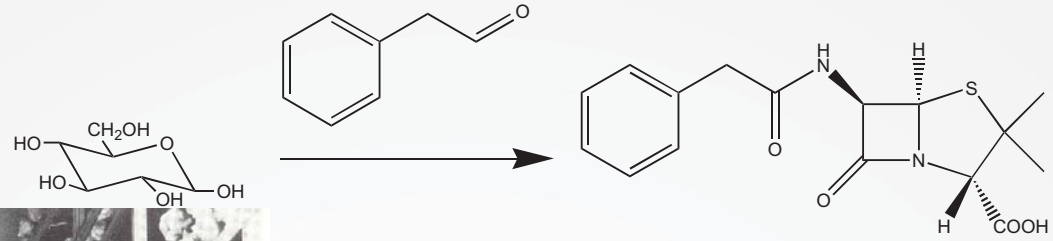
Wageningen University & Research, The Netherlands

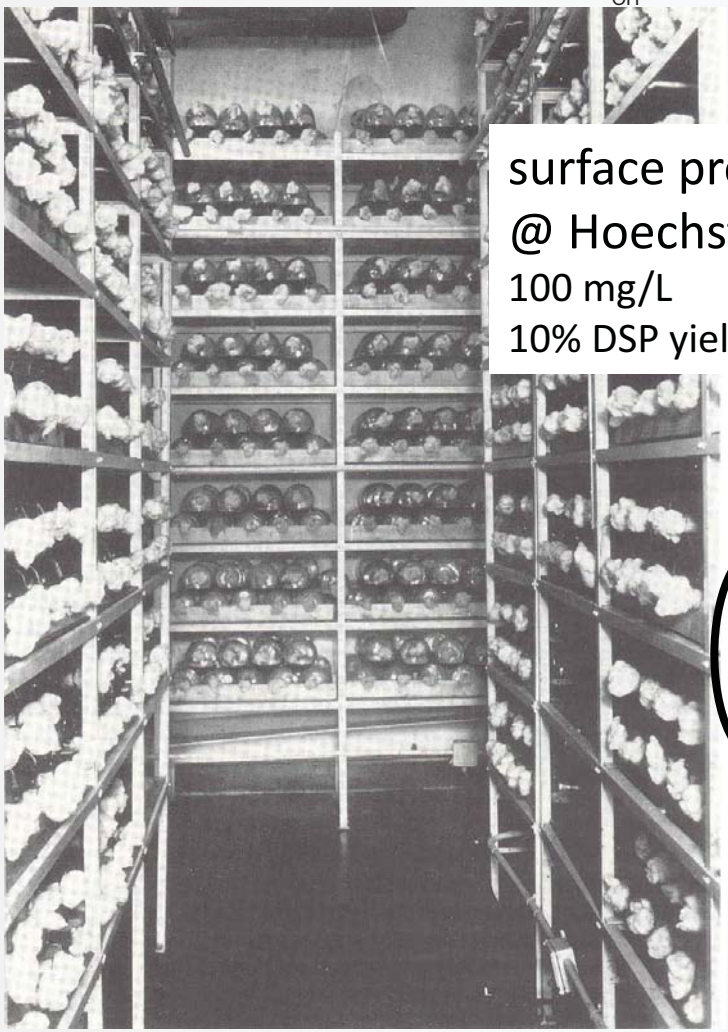
Wageningen University & Research

SPOT THE DIFFERENCE



Antibiotic Penincilin



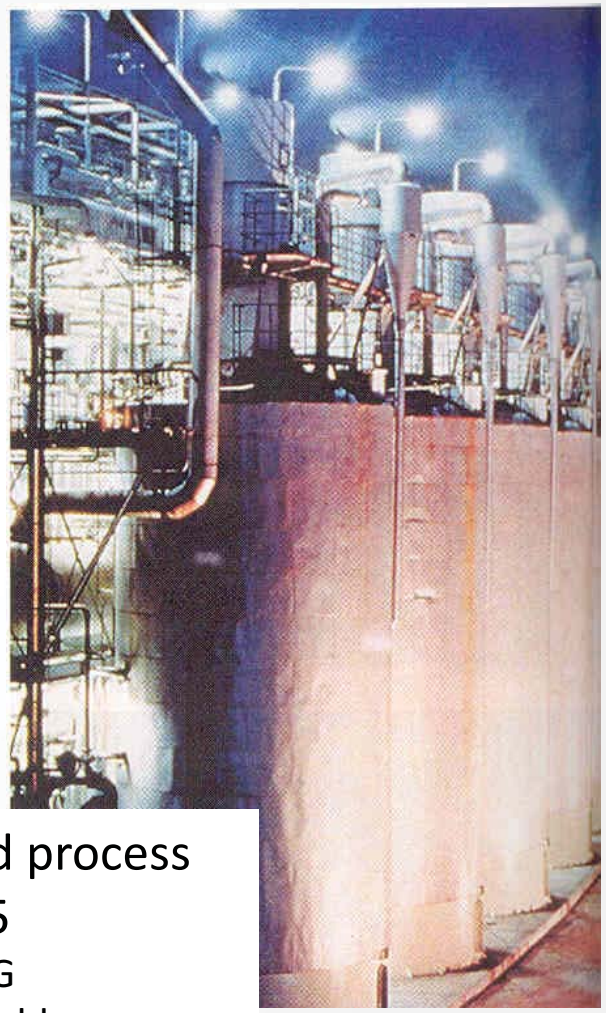


surface process
@ Hoechst, 1944
100 mg/L
10% DSP yield

UV mutagenesis

P. chrysogenum

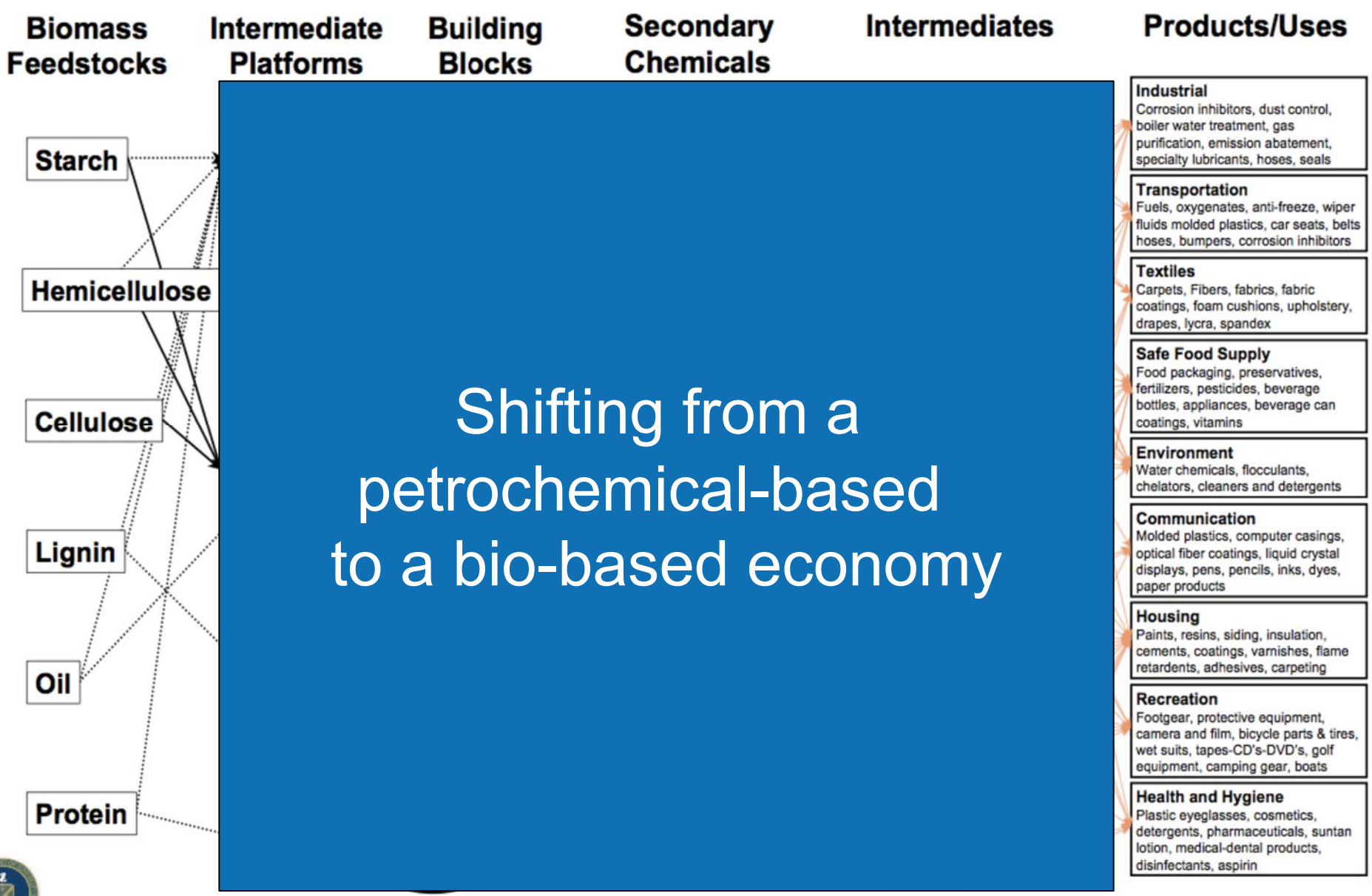
Selection



submersed process
DSM, 1995
> 50 g/L PenG
> 95% DSP yield

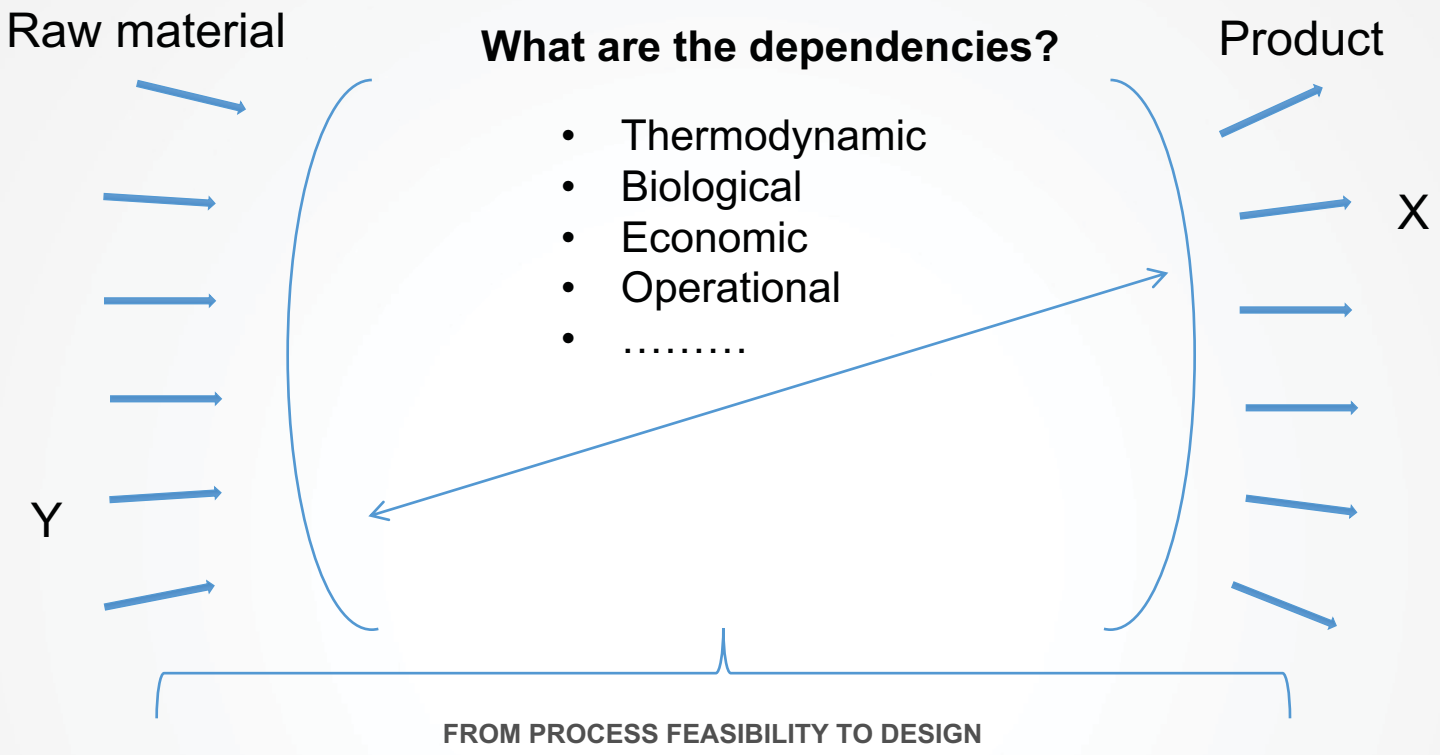
Courtesy S. Panke

Biobased Product Flow-chart for Biomass Feedstocks



Source: Report "Top Value Added Chemicals from Biomass" PNNL, DOE, 2004

A - How do we produce compound X (feasibly) ?

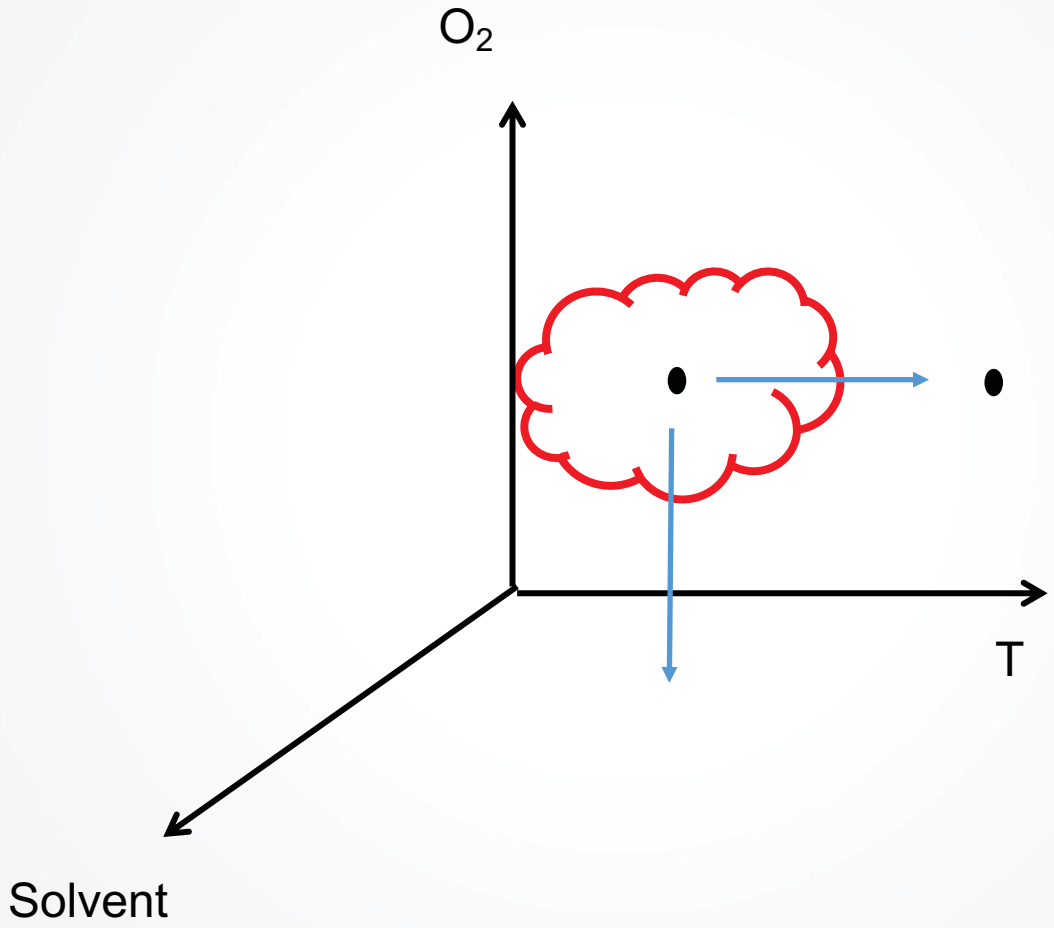


Some of the selection criteria for industrial application

- Scalable
- High titer, rates, yields (TRY), selectivity, flexibility
- Economically competitive over entire Lifecycle
- Reduced time-to-market

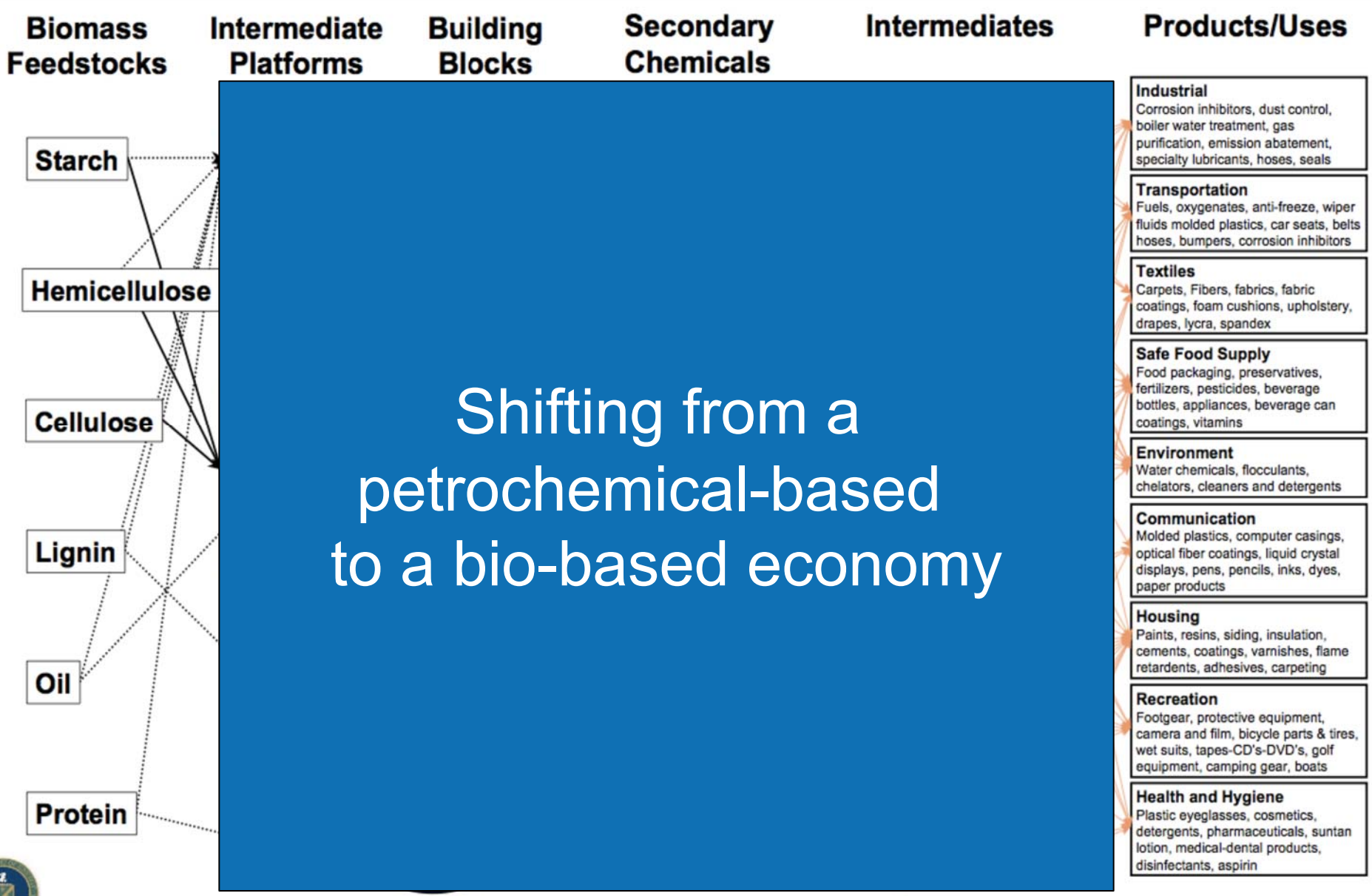
B - How do we increase industrial robustness?

Making a biocatalyst more industriophile



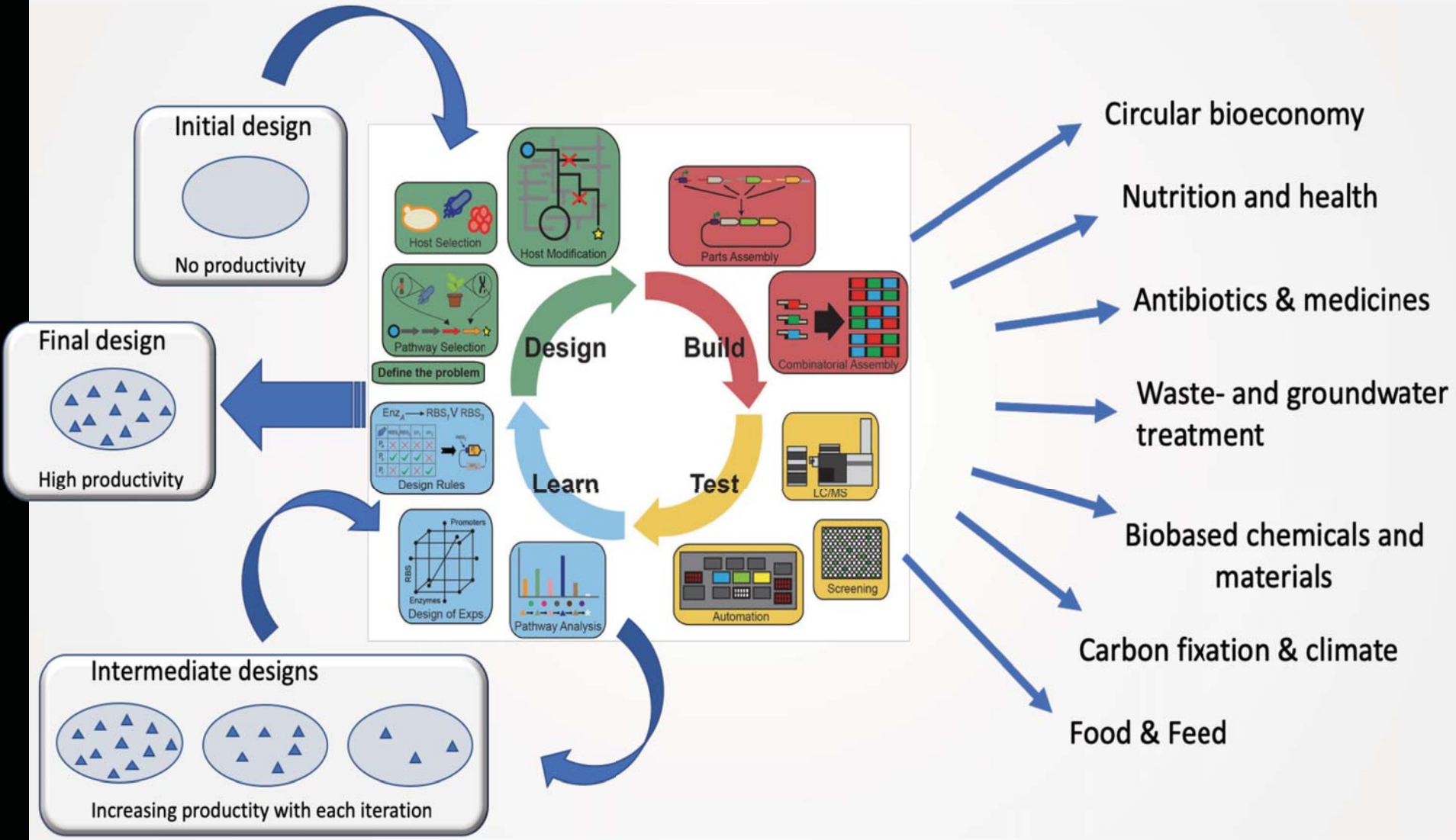
Can we "bioprospect" traits that enable this?

Biobased Product Flow-chart for Biomass Feedstocks



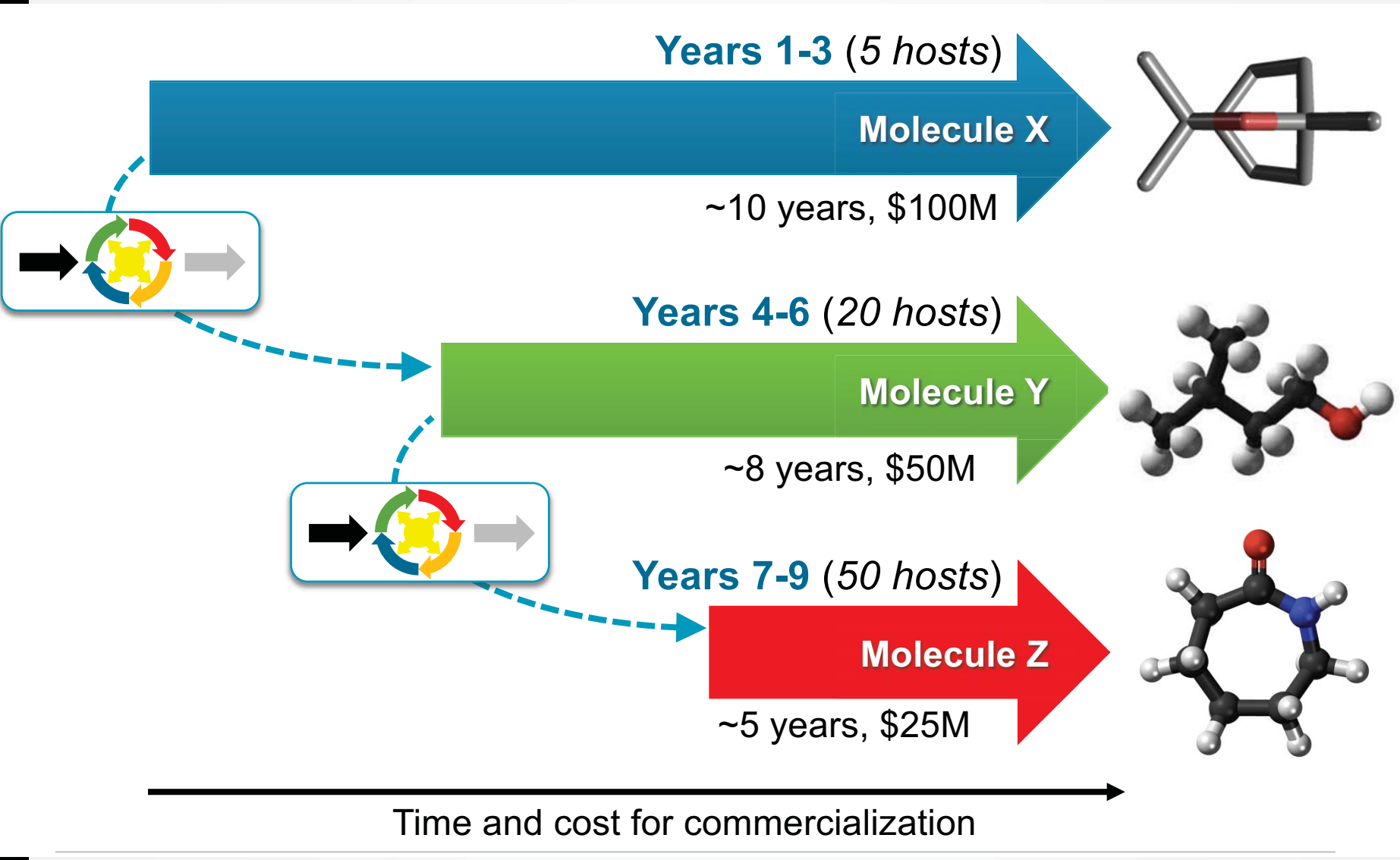
Source: Report "Top Value Added Chemicals from Biomass" PNNL, DOE, 2004

Systematic workflow for engineering cell factories



Adapted from Petzold et al, 2015

Reducing costs and time-to-market to enable transition



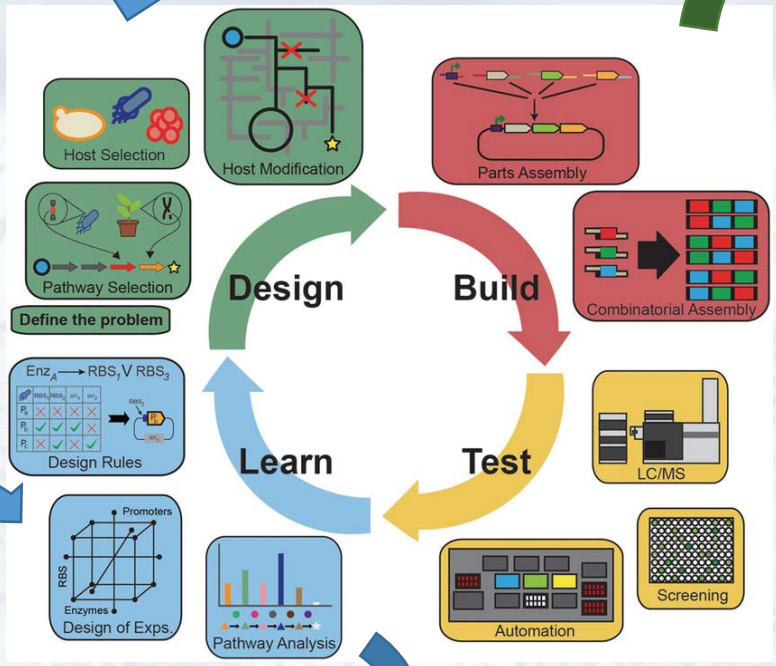
Adapted from ABF, 2019

Workflow for scalable cell factories

Initial design

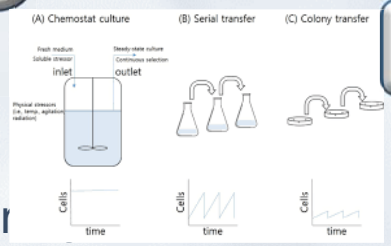
No productivity

Bioprospecting



Intermediate designs

Increasing productivity with each iteration



Lab evolution

Higher productivity

Increased robustness

Scale-up/scale-down

Microbial Oils, Fragrances, Polymers Applications eg:

Safety-by-design

Wageningen Univer

Pseudomonas putida as cell factory for tailored biocatalysis

Pseudomonas putida KT2440

•Paradigm of ubiquitous copiotrophic soil bacteria

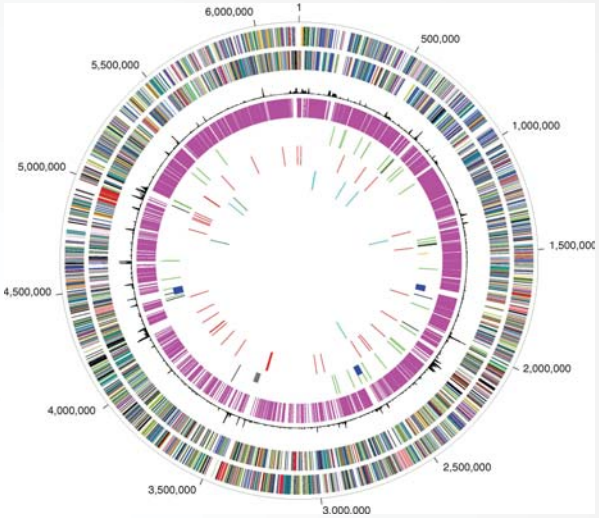
•Great metabolic diversity

•HV1 safety strain

•Preferred host for the design of novel degradation/biocatalytic processes

•Large range of applications:

Agricultural, environmental, industrial



•Biotransformation of a wealth of chemicals, in particular aromatic and aliphatic derivatives

•Resistance to many stresses (eg. solvents)

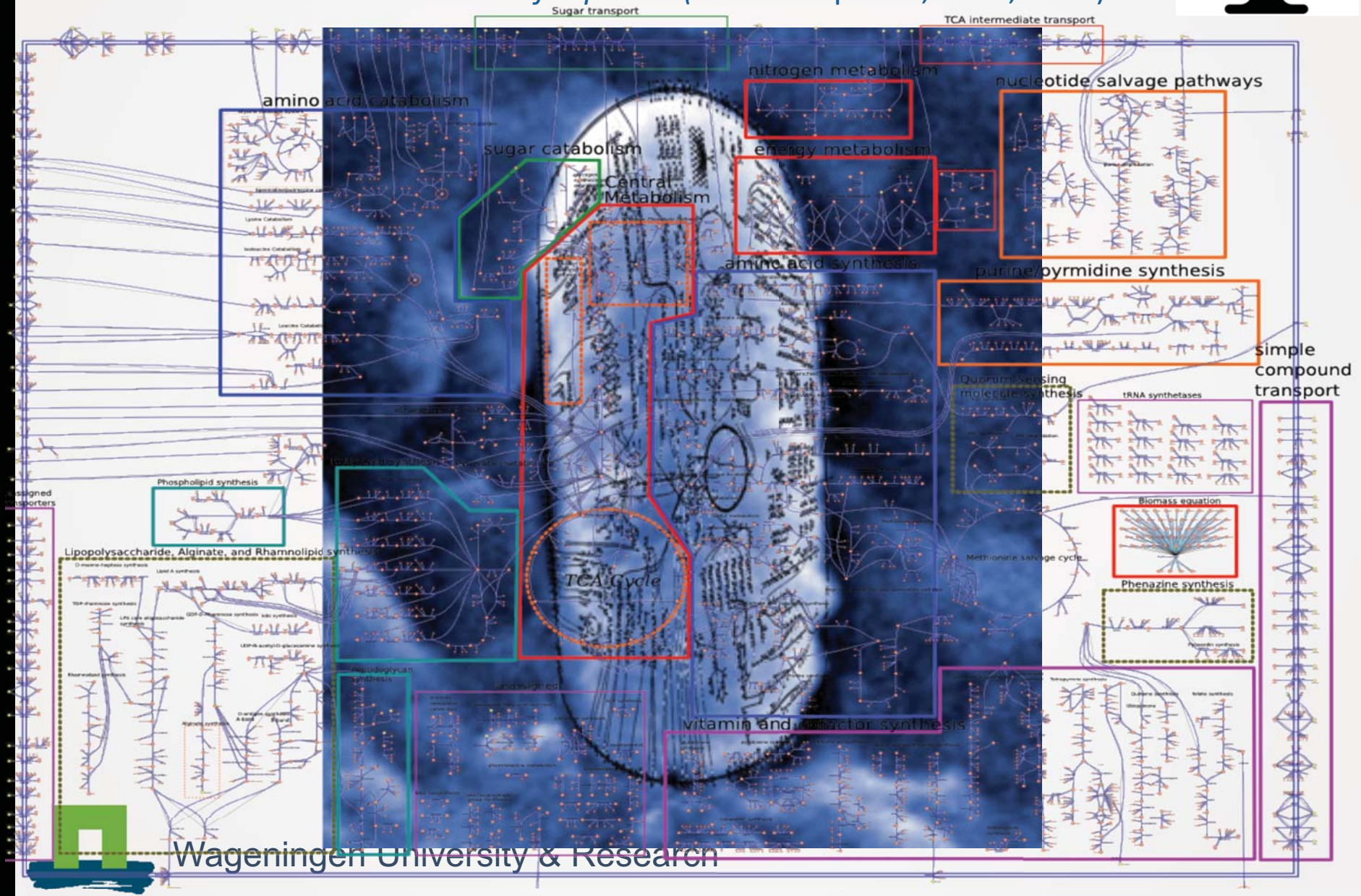
•Genetically amenable for engineering

•Excellent host for heterologous expression

•High oxidative capabilities

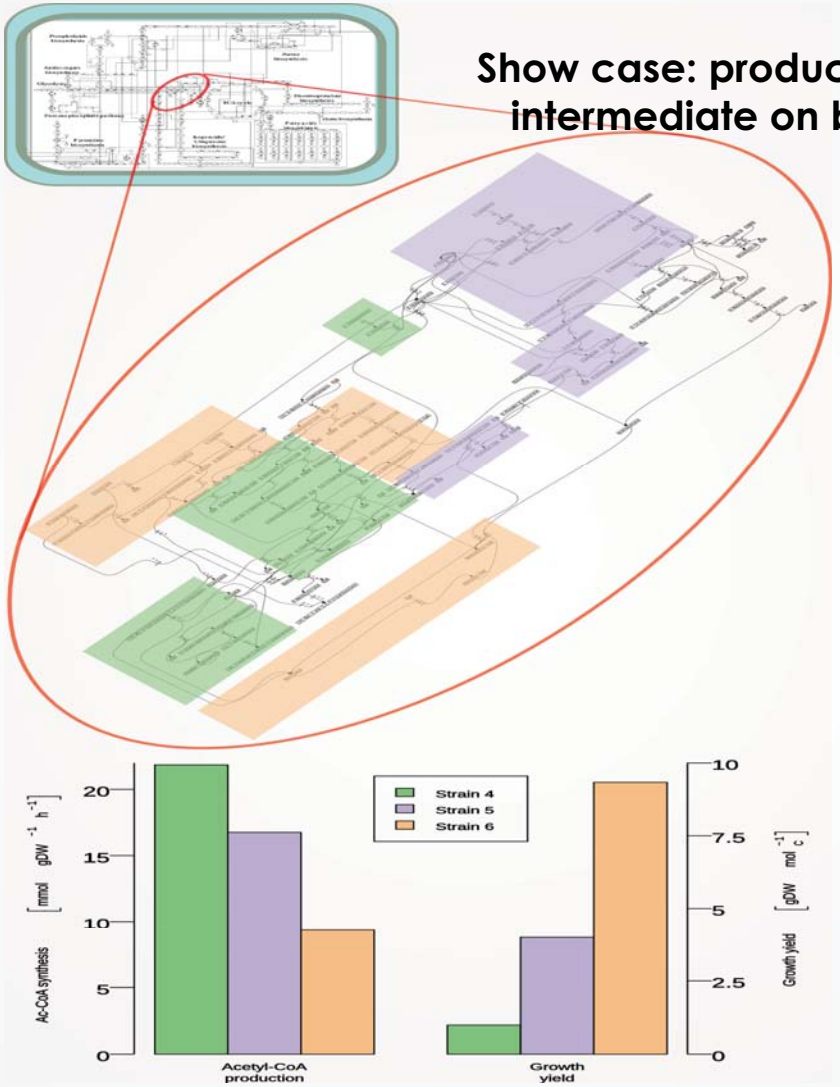
Design – Genome-scale metabolic modeling

*Genome-scale model of P. putida (*PLoS Comp. Biol, 2008, 2015)



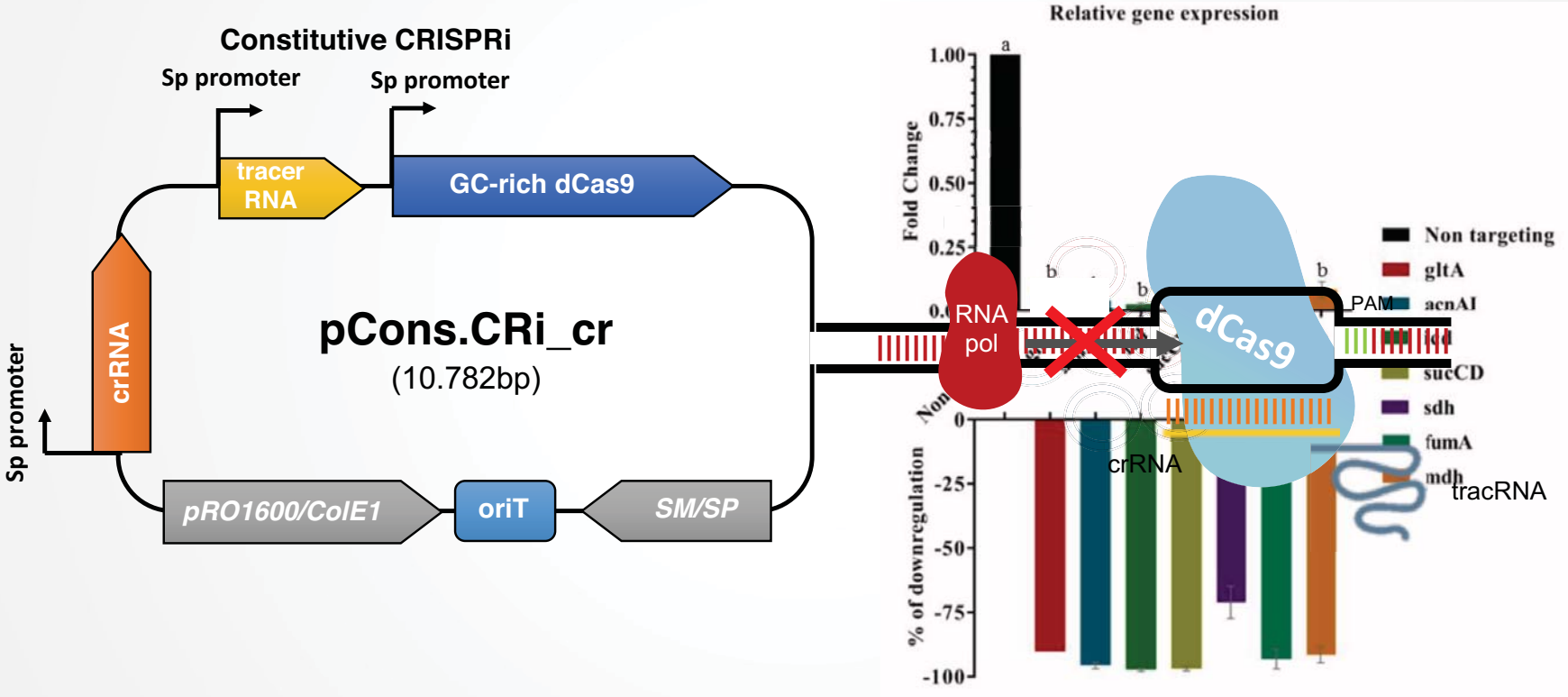
Design

Show case: production of cis,cis muconate, intermediate on bio-production of nylon

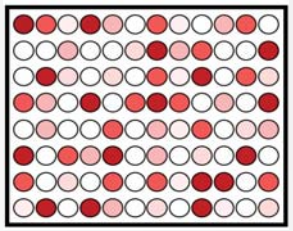


PLoS Comp. Biol, 2008

Build - CRISPRi library



Build - Experimental setup

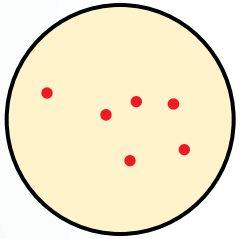


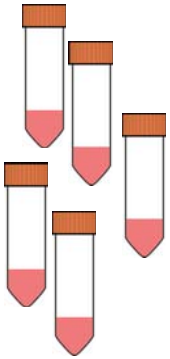
Transformation

Check for recombinations of crRNA

Overnight culture in M9 + glucose

Screening at 340nm



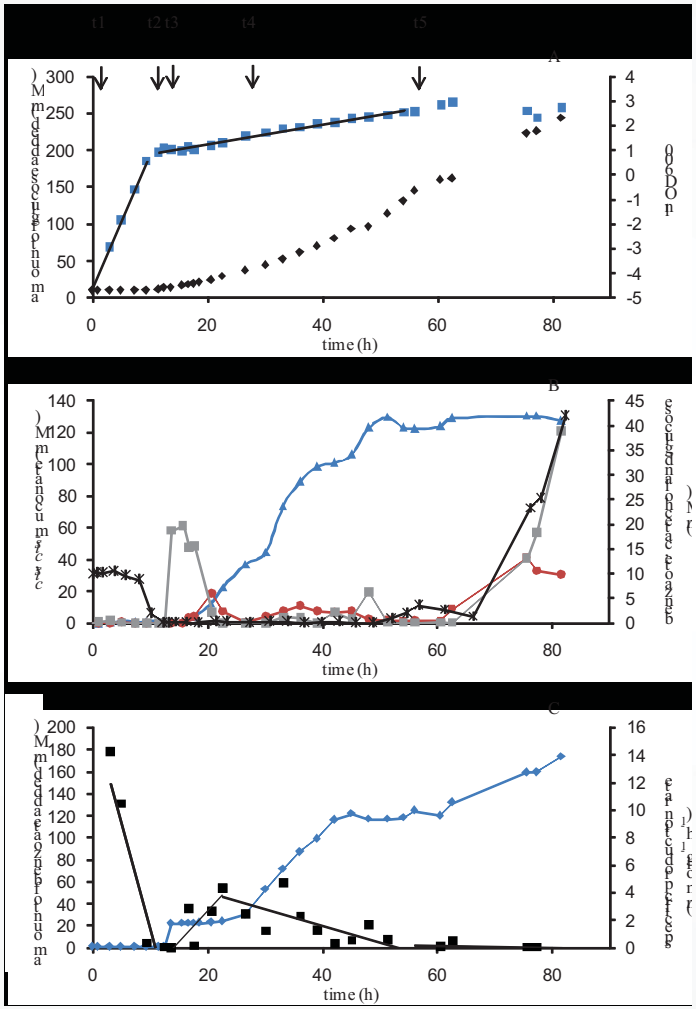


CRISPRi library

Test - Bioreactor operation

Test pH-stat fed-batch process to accumulate *cis*, *cis*-muconate

specific production rate EIGHT times higher than those thus far reported!



■ ln OD600
♦ amount of glucose added (mM)

▲ cis, cis-muconate (mM)
■ benzoate (mM)
● catechol (mM)
× glucose (mM)

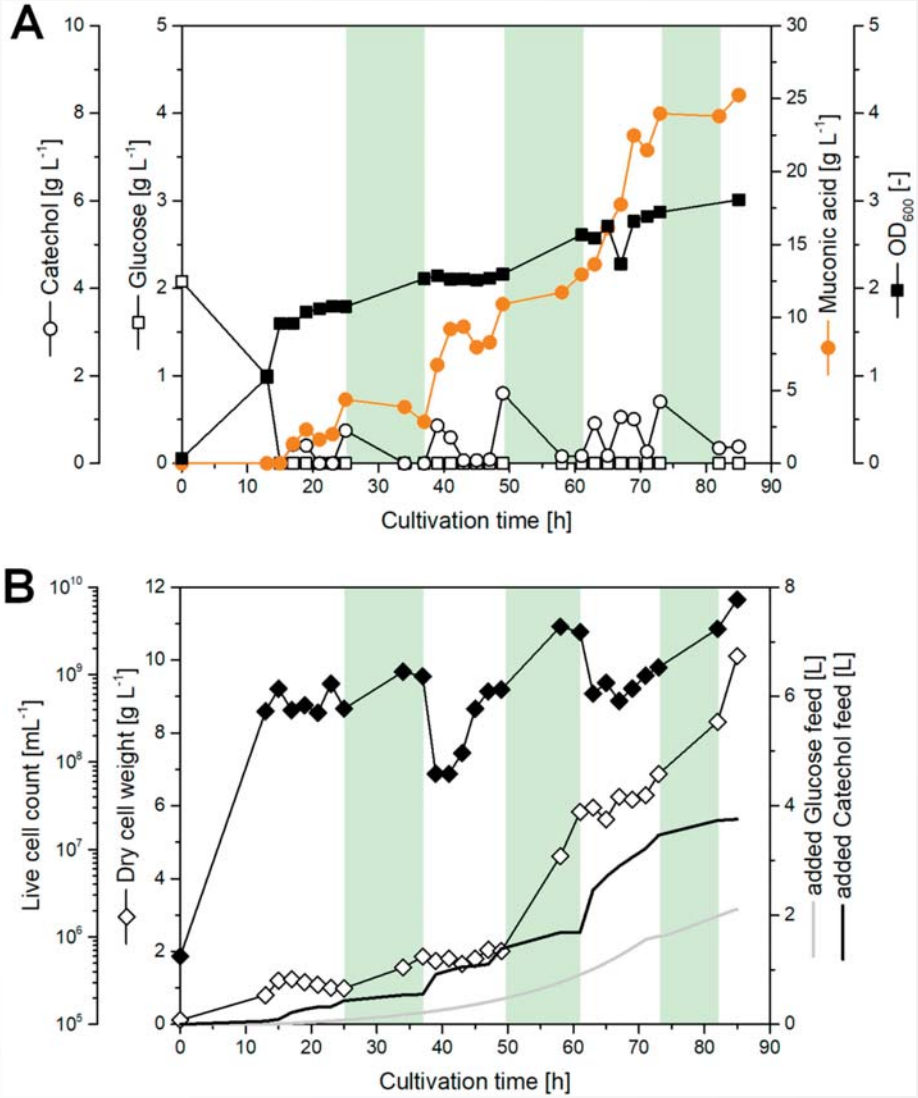
♦ amount of benzoate added
■ specific production rate (mmol g^{-1} h^{-1})

van Duuren JB, et al. . Biotechnol Prog. 2012

Scaling-up

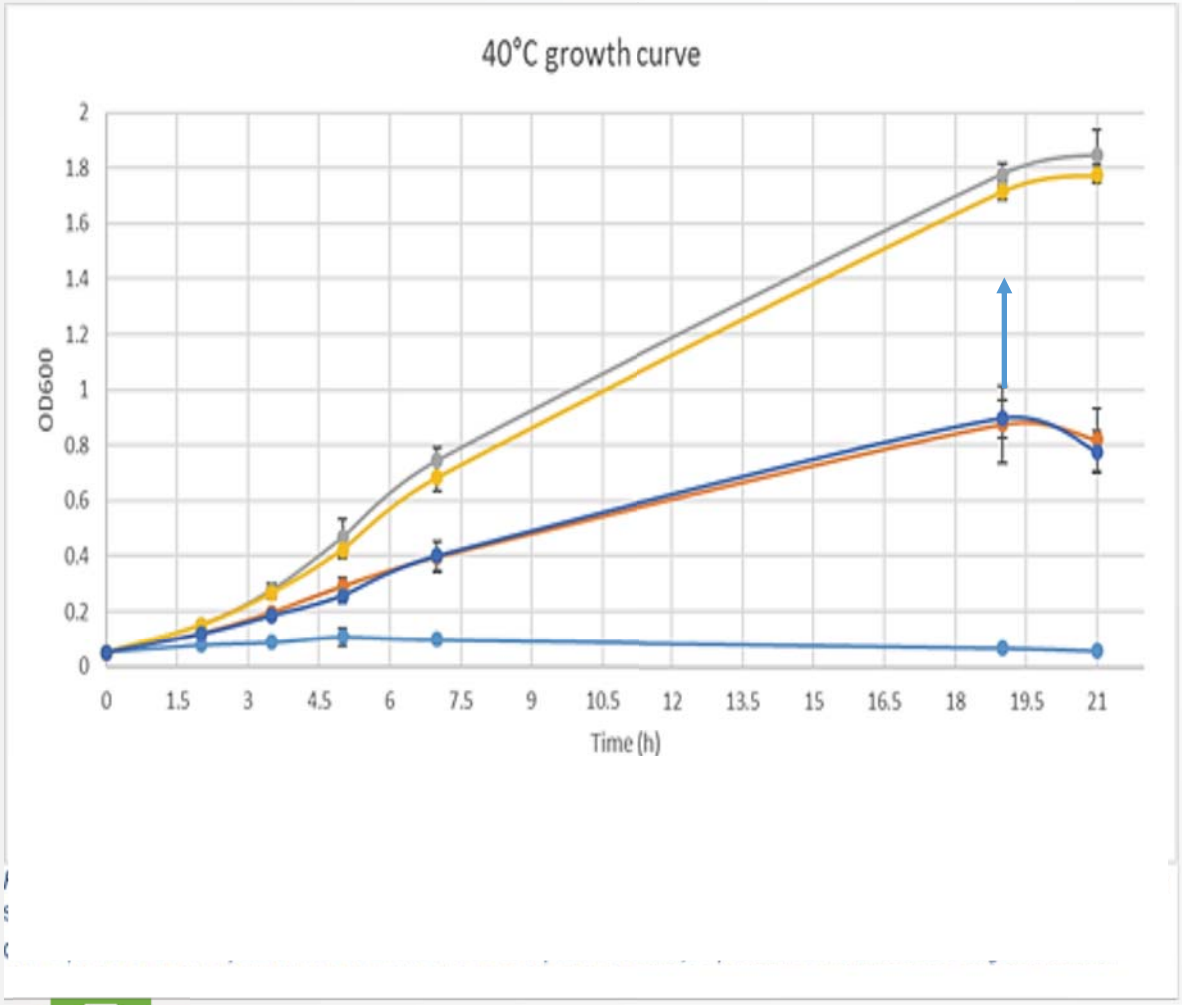
Demonstration of technical feasibility by pilot-scale production of *cis,cis*-muconic acid from catechol using in an advanced fed-batch process with transient regeneration phases.

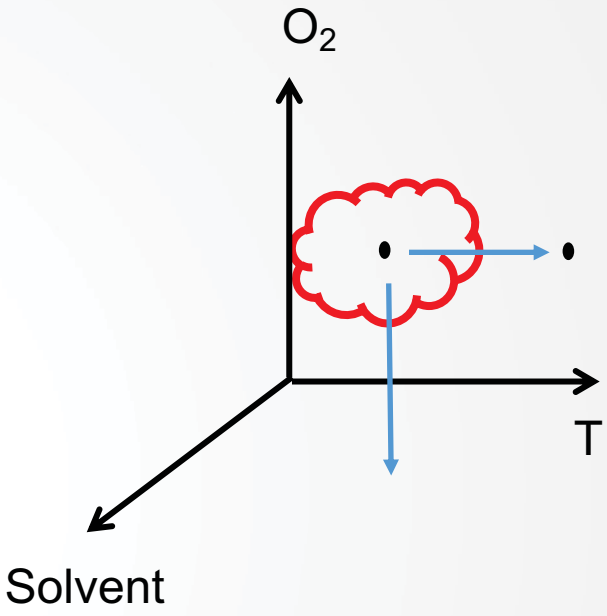
The process was conducted in a 50-L fermentation volume and included pilot-scale downstream purification of the product to 95% purity at the kilogram scale.



Kohlstedt M., et al., Metabolic Engineering v47 (2018 05 01): 279-293, C. Wittmann's Lab

Making *P. putida* more industriophile





Patent application

Thailand 4.0 & Bio-based industry

National agricultural area reformation strategic plan

Reduce rice production

Increase crops for bio-industry feedstock

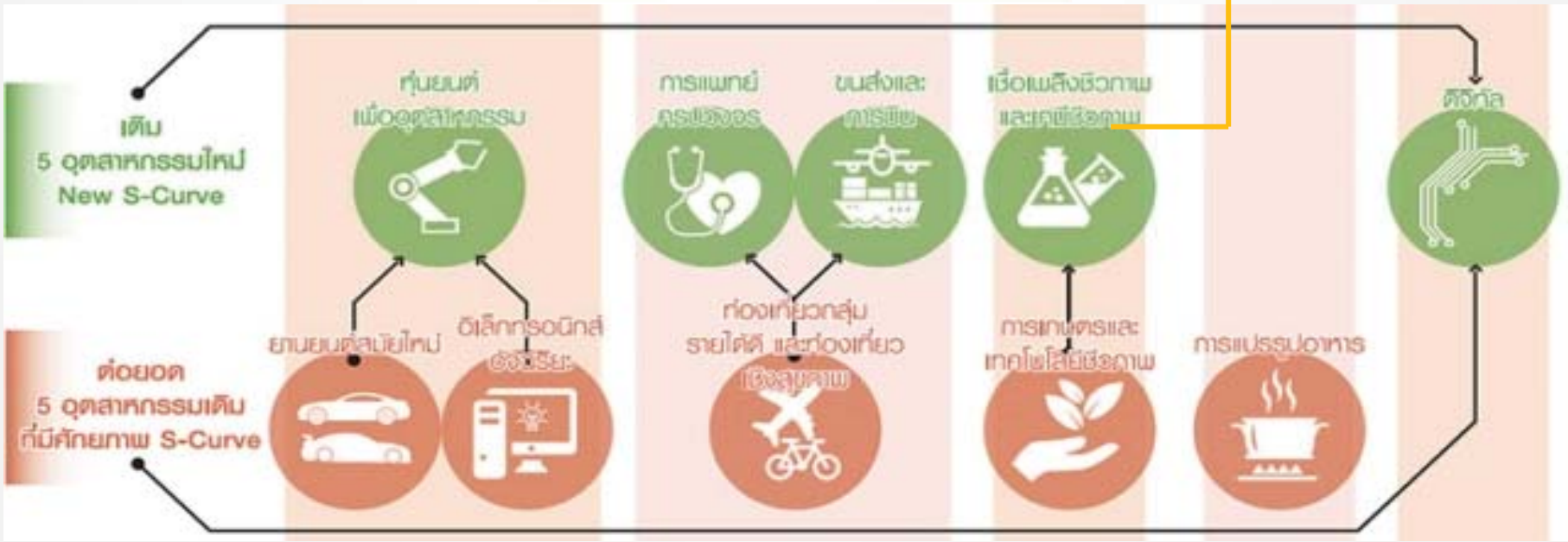
Sugarcane from 104→182 Mt/year (2567)

Cassava to 43→55 Mt/year (2564)

Benefit Millions people in agricultural sectors

Bioenergy& Biofuels
Biochemicals
Bioplastics
Bio(food/feed) ingredients
Biopharmaceuticals

PPP

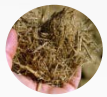


Courtesy Dr. Verawat Champreda

Reformation of Bio-refinery and Bio-industry

More 1st G feedstock

8.8 → 16.8 Mt sugar/year
5.3→ 7.3 Mt starch/year
8→ 9.5 Mt chip/year



More 2nd G feedstock

53 Mt bagasse/year
4.9 Mt cassava pulp/year

Multi-disciplinary valorization
& Waste management NEEDED

However…

EV car
(finding mor  e electricity resources**?**)

Local biofuel industry will soon be reformed to integrated biorefinery with more product spectrum to increase competitiveness and fully utilize existing and future facilities

Maximized utilization

- Biofuels
- Biochemicals
- Biocomposites
- Biospecialties

Zero-waste process
Sustainability & competitiveness

Value extraction from biomass + indirect impact on waste management

Courtesy Dr. Verawat Champreda

Sustainable starting materials for a broader product spectrum

- **300,000 M THB/year**
Sugarcane

2nd Gen

1st Gen

Cellulosics

Sugars
Starch

Cassava

- **100,000 M THB/y**

Biorefinery

Microbial bank
Biotechnological resources

Bio-based industry

Renewable feedstock + Biodiversity → Bio-products

Bio-specialties

High

Bio-pharma

Specialty chemicals

Enzymes

Biocontrol agents

Value

Commodity chemicals

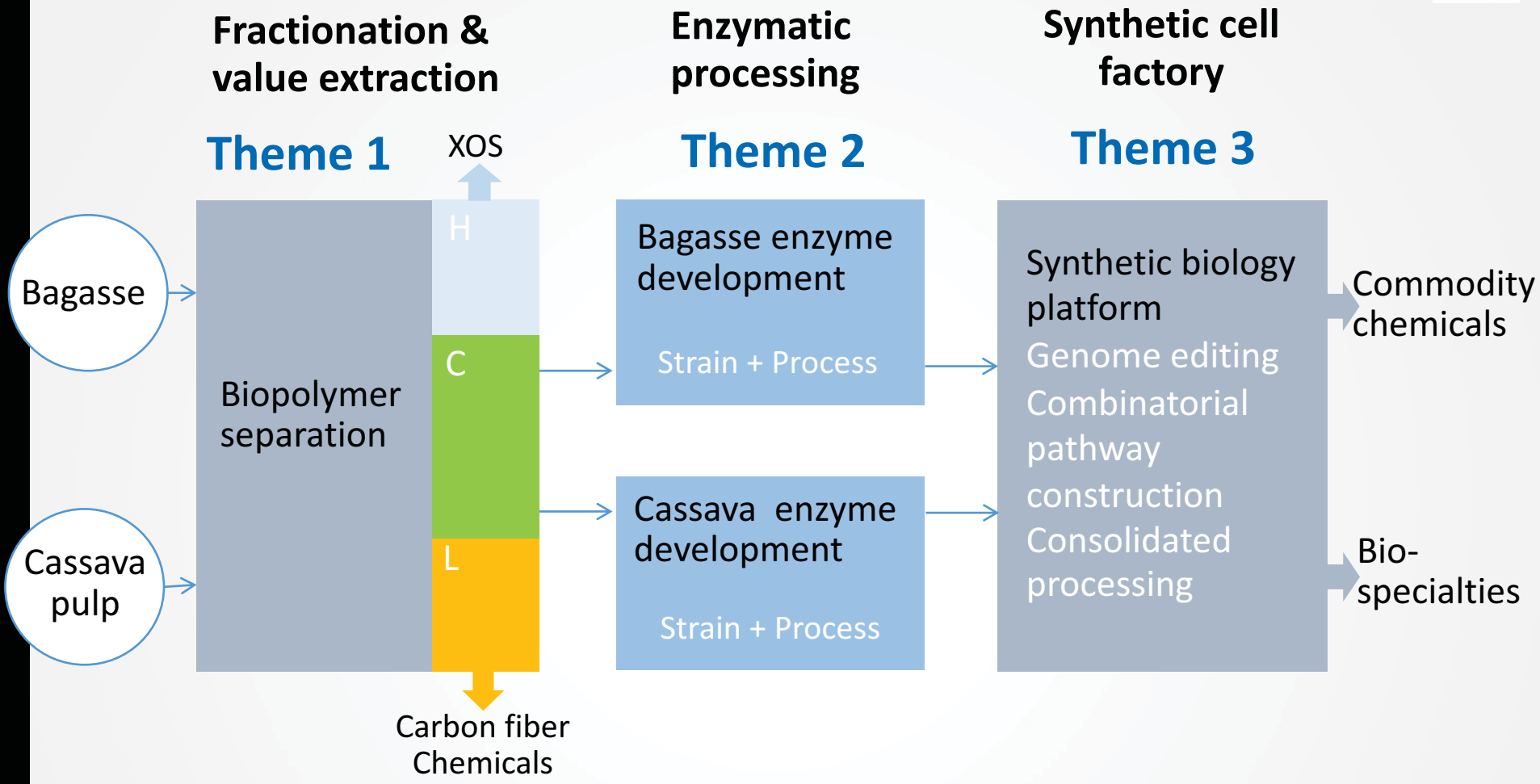
Liquid biofuels

Energy

Low

Conventional biorefinery

BIOTEC biorefinery roadmap



Task	Up-scaling g to kg	Enz efficiency/productivity	Platform technology
Tech	Hydrothermal/Organosolv	Strain (MT/Rec)/Bioprocess	Genome design/editing
Grant	Integrated biomaterial/STI*	BG: Industry/CP: Platform	Platform bio-based
Partner	Industrial partners	Industrial partners	WAGENINGEN UR For quality of life

Courtesy Dr. Verawat Champreda

Synthetic biology platform development

Synthetic biology platform

- Multi-omics
- Genome wide modeling
- Gene editing
- Bio-process opt: High solid/ SSCF

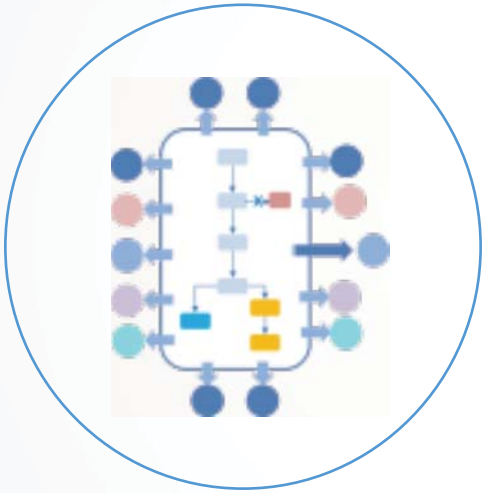
Platform host

S T I R

Phenotypic & Genomics

Pathway/ Genetic element

Genomic Metagenomics (rare/unique envi)

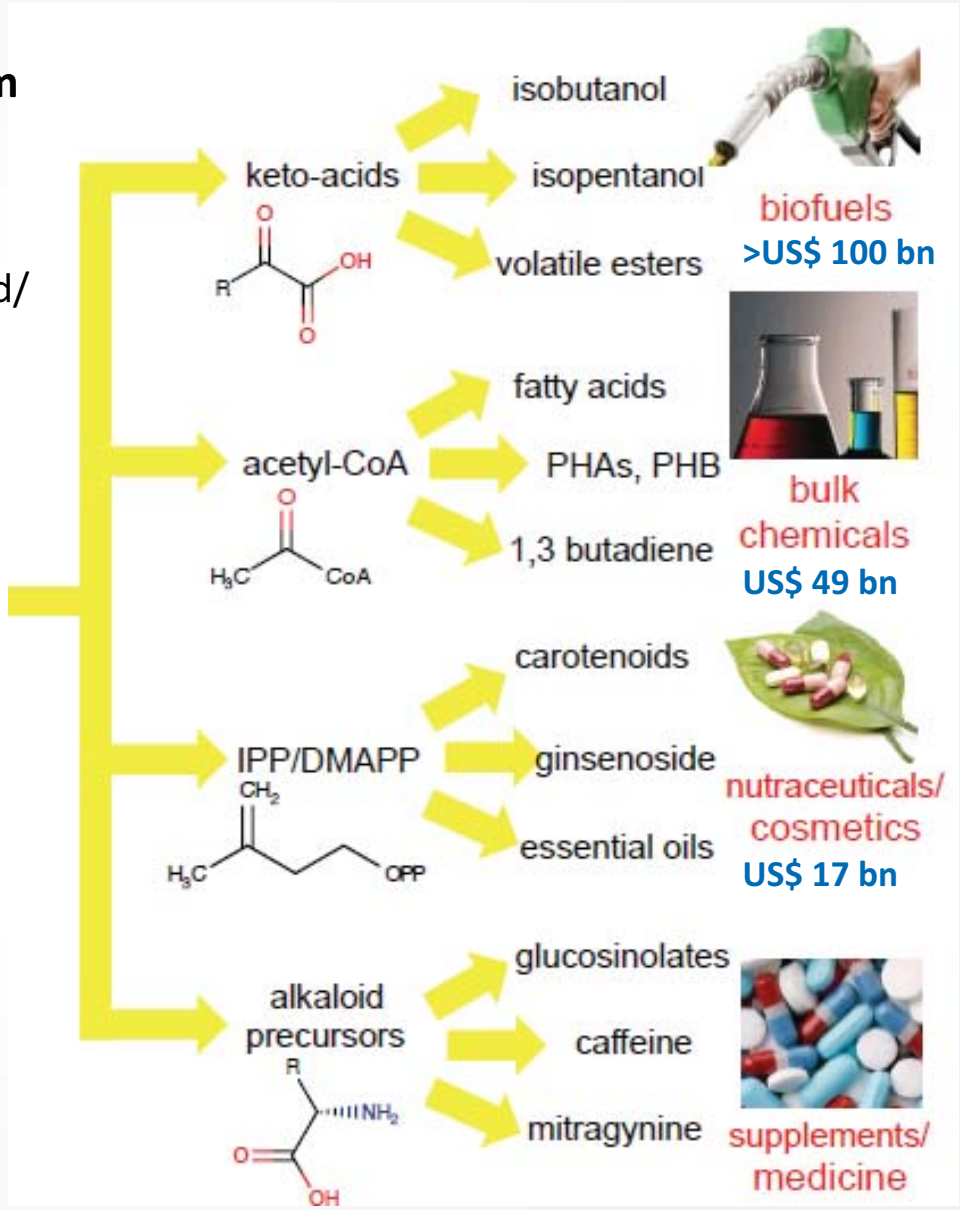


Cell factory

Yeast platform

INDUSTRIOPHILE

WAGENINGEN UNIVERSITY & RESEARCH



Contributors: BBR (BMCF, BIS), MBB (MENZ, MBIA), GTU, STU, FBU, MMU

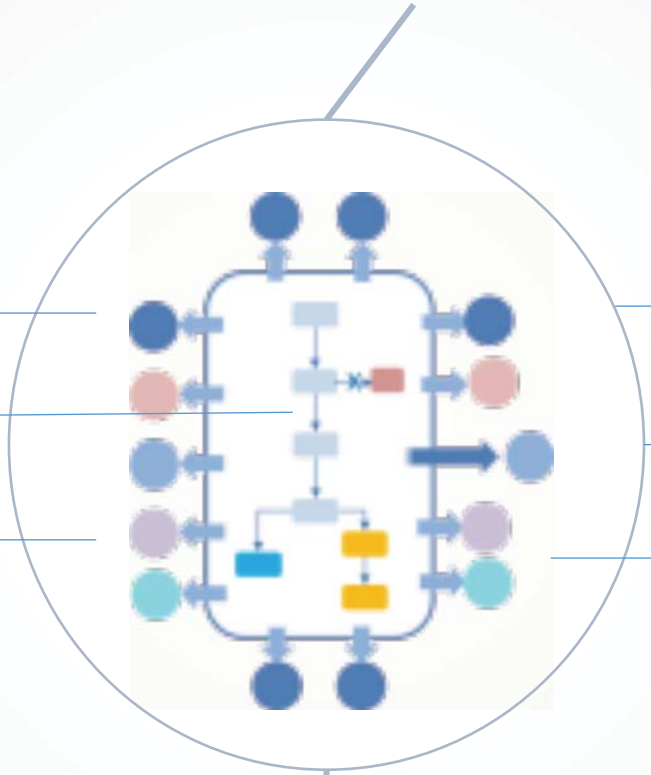
Courtesy Dr. Verawat Champreda

BIOTEC-WUR collaboration on Industrial Biotechnology

Host strain selection and improvement

Bioinformatic tools for genome modeling and editing

Synthesis pathway: Design and construction



Pathway design & flux analysis

Enzyme on substrate hydrolysis

Strain construction

BIOTEC staff (current 2 +1)

Researcher exchange 1/ year

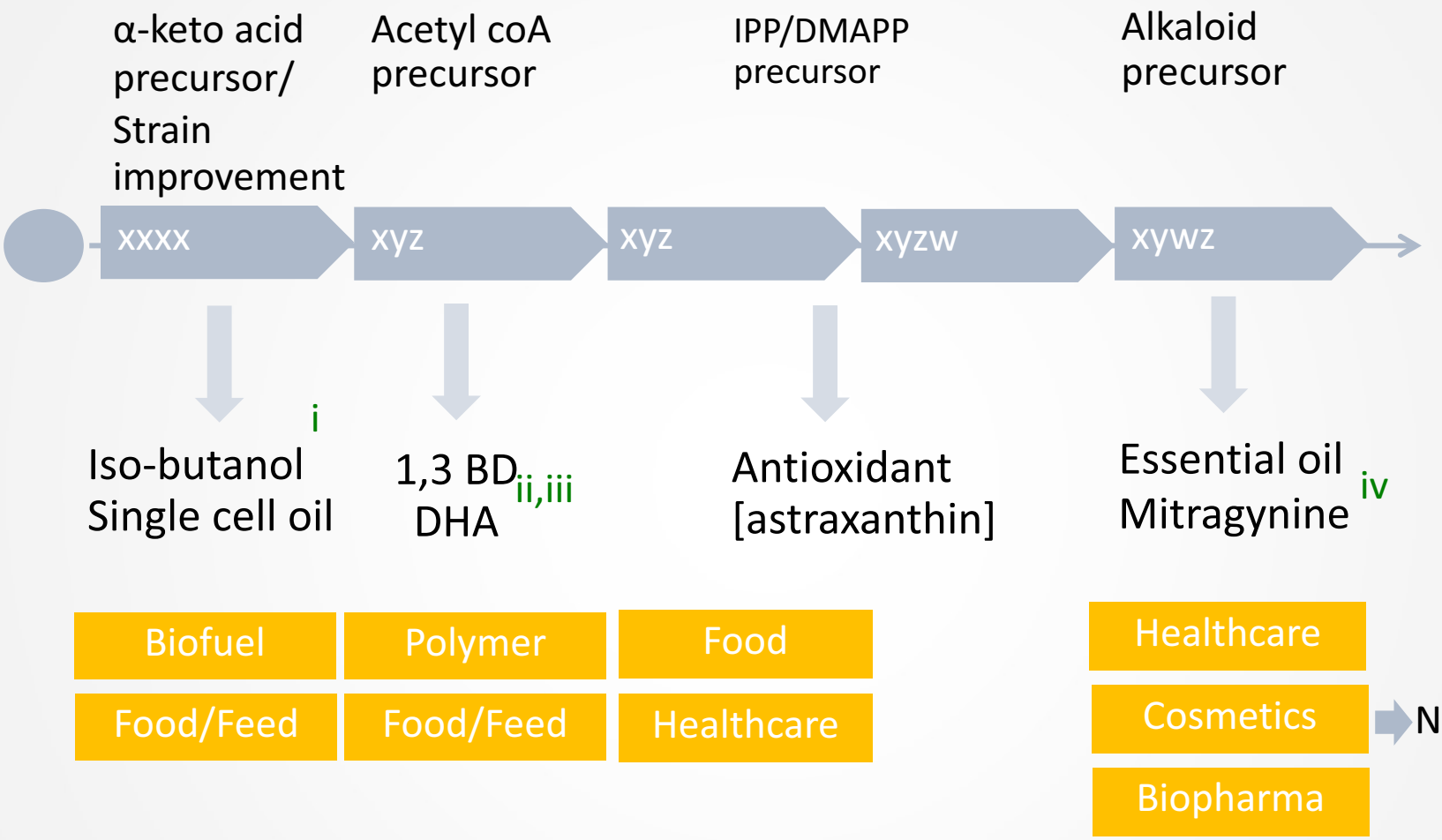
Ph.D student @WGN

- Yeast (1st INDUSTRIOPHILE)
- Fungi

Short tem training

Courtesy Dr. Verawat Champreda

Cell factory product pipeline



IND	Plastic monomer/polymer
IND	Ethanol/ biofuels
IND	Biofuels/Biochemicals/Food ingredients

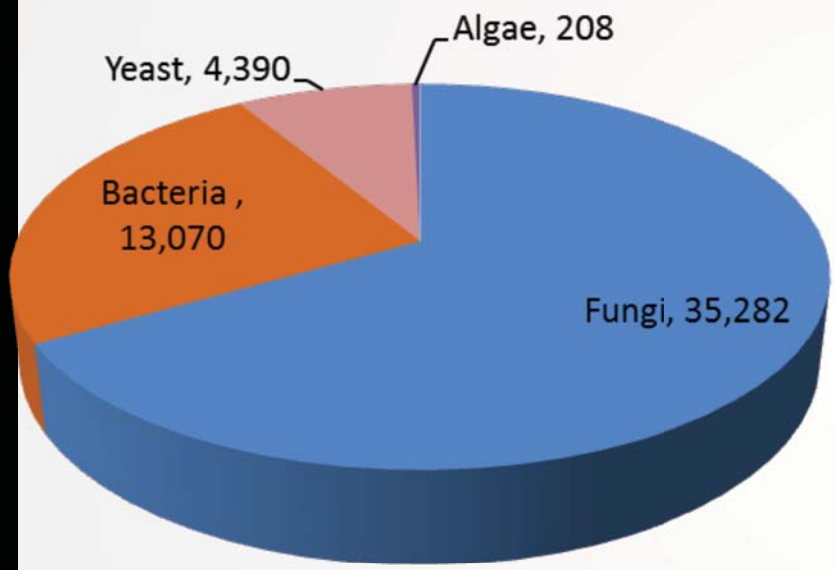
IND	Plastic monomer/polymer
IND	Biofuels/Food ingredients
SMEs	Food/healthcare/cosmetics

Courtesy Dr. Verawat Champreda

Aligning with existing BIOTEC-WGN Research plan Step 1 screening of

BIOTEC Culture collection

196 strains



Field isolation

482 strains

Distilleries/ Agri. Landfill/waste/ traditional fermented food and drink/ soil etc.

Courtesy Dr. Verawat Champreda

STEP I (Industriophilic trait identification)

- **Objective**
  - To screen for potential yeast strains capable of withstand common stresses and inhibitors encountered during bio-ethanol fermentation.
  - To examine the ability of yeast strains for sugar assimilation as well as the potential for DNA transformation.

- **Expected outcomes**
  - At least 300 samples of natural yeasts will be collected and screened for potential yeast strains.
  - The potential yeast strains for DNA transformation will be examined for further applications.
  - Yeast catalog

Courtesy Dr. Verawat Champreda

BIOTEC-WGN Research plan

To develop a yeast system based on new isolated yeast strain with modified pathway and enzyme display for consolidated bioprocessing

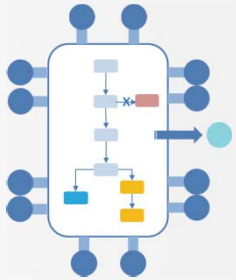
- Grant: Platform BIOTEC/CPM
- Researcher exchanges
- Potential for international grant application

Pathway engineering
[Weerawat]
INV yeast (commercial strain)

Cell surface display yeast II
[Surisa]
BH-38-3 [BIOTEC strain]

SSF Bio-process
[Pornkamol]

Genomic study



2016 2017 2018 2019 2020

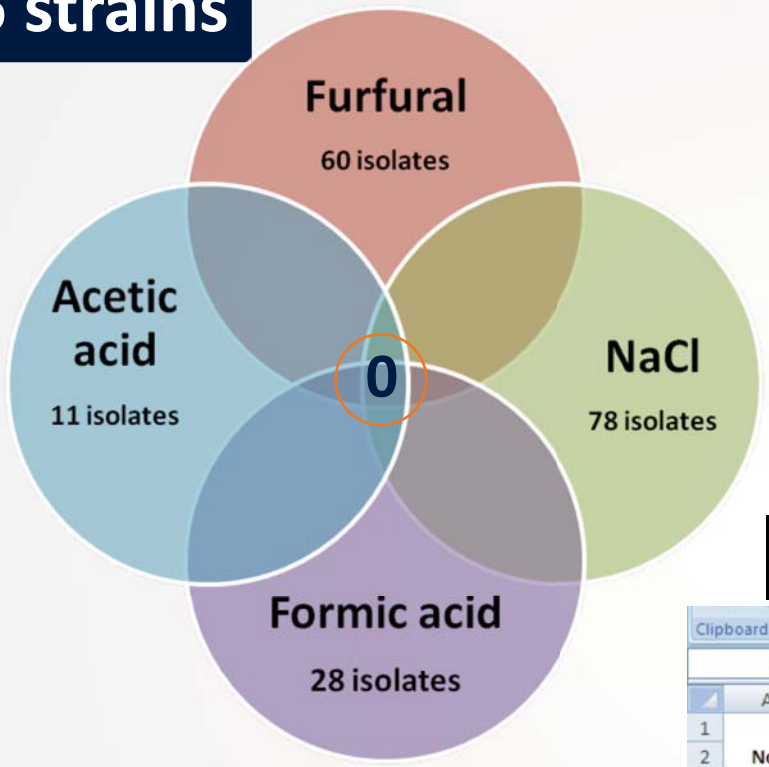
Yeast screening
[Warasirin]
- Multi-C
- Inhibitor tolerant
- High T

NSTDA CPM/Platform [Collaboration with WGN]
Yeast synthetic biology platform
- Modelling/Flux analysis/Genome/Transcriptome
- Pathway engineering/Enzyme display

Courtesy Dr. Verawat Champreda

BIOTEC Culture collection

196 strains



I. Inhibitor tolerance

	A	B	C	D	E	F
1			Inhibitor			
2	No.	Strain	(0.8-1%)AA	(0.2-0.4%)FA	(0.2-0.4%)FF	(2M) NaCl
3	1	33134				NaCl
4	2	54648			FF	
5	3	15178			FF	
6	4	32878				
7	5	59282				NaCl
8	6	50634				NaCl
9	7	59276	AA			
10	8	15186	AA	FA		
11	9	32835				
12	10	32873				
13	11	42664				
14	12	TBRC980				
15	13	4317				
					FF	
						NaCl +/-

Sheet2 Sheet3 Sheet4 Sheet5 Sheet6 TBRC_1

II. Sugar assimilation

	A	B	G	H	I	J	K	L	M
1			Sugar assimilation						
2	No.	Strain (BCC)	Glu	Suc	Cel	Xyl	Man	Lac	Organism Name
3	1	33134	+++	+++	++	++	+++	++	Trichosporon asahii Akagi ex Sugit
4	2	54648	+++	+++	+	++	+++	+	Kluyveromyces siamensis
5	3	15178	+++	+++	+	++	+++	+	Ogataea philodendri
6	4	32878	+++	+++	++	++	+++	+	Ogataea polymorpha
7	5	59282	+++	+++	++	++	+++	++	Dekkera bruxellensis
8	6	50634	+++	+++	+	++	+++	+	Ogataea philodendri
9	7	59276	+++	+++	+	+	+++	+	Dekkera bruxellensi
10	8	15186	+++	+++	+	+	+++	+	Zygosaccharomyces sp.
11	9	32835	+++	+++	++	++	+++	+	Pichia kudriavzevii
12	10	32873	+++	+++	++	++	+++	+	Ogataea polymorpha
13	11	42664	+++	+++	+	++	+++	+	Ogataea wangdongensis
14	12	TBRC980	+++	+++	++	+	+++	+	Kluyveromyces lactis
15	13	4317	+++	+++	+	+/-	+++	+	Unidentified yeast
16	14	15056	+++	+++	+	+/-	+++	+	Kluyveromyces sp.
17	15	TBRC650	+++	+++	++	++	+++	+	Ogataea nakhonphanomensis

Sheet1 Sheet2 Sheet3 Sheet4 Sheet5 Sheet6 TBRC_1 TBRC_2 TBRC_3

Courtesy Dr. Verawat Champreda

Research plan-step II (Trait detection)

Yeast phenotype catalog

Based on desirable traits for bio-ethanol fermentation

Genetic modification potential

New generation cell factory

Trait identification

Genome sequencing and trait analysis

- using **semantic technologies** SAPP (WUR) and
- simplified domain scale models gapfilled with the **Biogrowmatch** algorithm (uses phenotype data as input, developed at SSB)

- Transcriptomics / metabolomics when necessary

Courtesy Dr. Verawat Champreda

Research plan-step III (Trait portability)

From step II Design of Defined synthetic Genetic building blocks (WUR, Biotec)

Based on desirable traits e.g for bio-ethanol fermentation, improved furfural resistance etc

Strain improvement of Existing industrial strains & Promising new strains

Courtesy Dr. Verawat Champreda

Summary

- Integrated engineering approaches essential to unlock the true potential of Industrial Biotechnology
- Expand product portfolio & industrial robustness
- Very fruitful collaboration between BIOTEC and Wageningen University synergizing on complementary expertise
- Boosting the use of Thai natural resources
- Academic – Industrial partnerships

Acknowledgments:

Wageningen
University & Research

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BIOTEC
a member of NSTDA

Verawat Champreda

Warasirin Sornlek

Thanaporn Laothanachareon

Wasin Poncheewin

Sutipa Tanapongpipat

Lily Eurwilaichitr

Tanaporn Uengwetwanit

Wanilada Rungrassamee

Nitsara Karoonuthaisiri