Microbial and Biocatalytic Production of Advanced Functional Polymers

Costas Kiparissides

Department of Chemical Engineering, Aristotle University of Thessaloniki, and Chemical Process Engineering Research Institute, Centre for Research and Technology Hellas

Thessaloniki, Greece

Biorefinica: International Symposium Biobased Products and Biorefineries,

Osnabruck, Germany, January 27-28, 2009

Bioproduction Objectives

- Development of an industrial R&D platform in sustainable production of functional biopolymers , chemical building blocks and biosurfactants.
- Use of renewable agricultural sources (e.g., corn, wheat, sugar beets) and wastes as raw materials and biological processes (fermentation, biocatalysis).
- Development of novel biocatalysts (enzymes) for higher product efficiency, improved stability, reduction of the number of conversion steps and replacement of difficult syntheses by simpler processes.
- Application of advanced modeling, on-line monitoring and control methodologies to bioprocesses (Digital Bioprobuction).

List of Partners

- **Industries & SMEs - 11**
  - KitoZyme SA (**KitoZyme**) – BELGIUM
  - Procter & Gamble (**P&G**) – BELGIUM
  - Tate and Lyle – FINLAND
  - Artes Biotechnology GmbH (**ARTES**) – GERMANY
  - FMC Biopolymer AS/Nova Matrix (**FMC Biopolymer**) – NORWAY
  - Companhia Petroquímica do Barreiro S.A. (**CPB**) – PORTUGAL
  - BIOMEDAL S.L. (**BIOMEDAL**) – SPAIN
  - BIOPOLIS S.L. (**BIOPOLIS**) – SPAIN
  - IMEnz Bioengineering BV (**IMEnz**) – THE NETHERLANDS
  - Ciba Specialty Chemicals PLC (**CIBA**) – UK
  - The Centre for Process Innovation (**CPI**) – UK

List of Partners *(cont'd)*

- **Research Institutions - 6**
  - Centre for Research and Technology Hellas / Chemical Process Engineering Research Institute (**CERTH/CPERI**) – GREECE
  - DWI an der RWTH Aachen (**DWI/RWTH**) – GERMANY
  - Instituto di Ricerca Protos (**PROTOS**) – ITALY
  - Consejo Superior De Investigaciones Científicas - Centro De Investigaciones Biológicas (**CSIC-CIB**) – SPAIN
  - Instituto de Ciencia e Tecnologia de Polimeros (**ICTPOL**) – PORTUGAL
  - Agrotechnology and Food Innovations (**A&F**) – THE NETHERLANDS

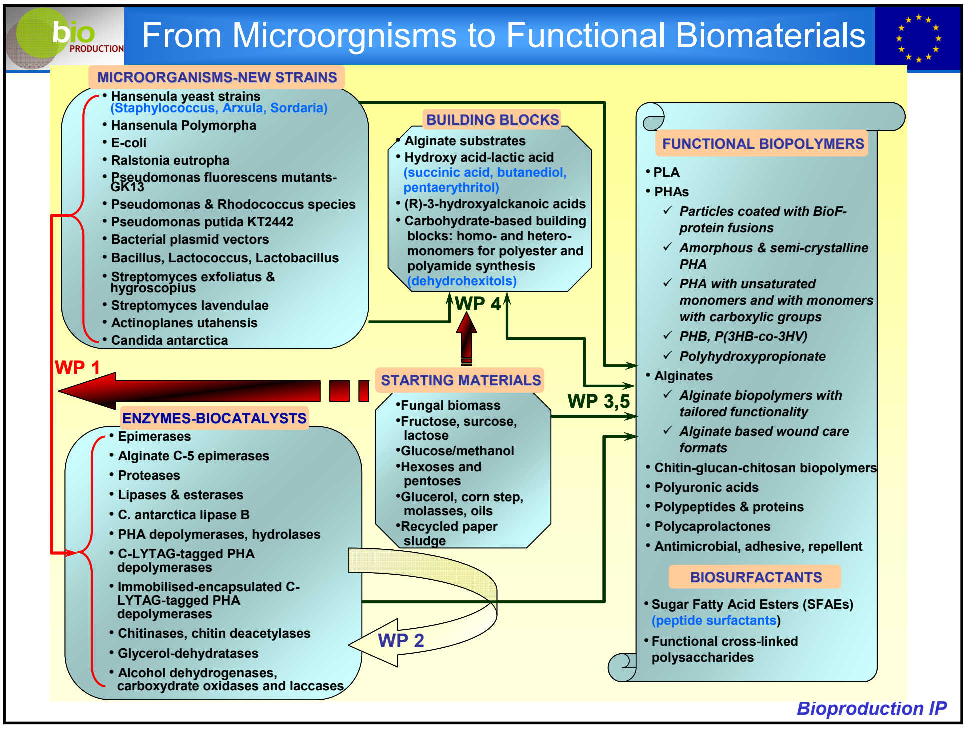
List of Partners *(cont'd)*

- **Universities - 8**
  - Institute of Chemical Technology Prague (**ICT Prague**) – CZECH REPUBLIC
  - Technical University of Denmark (**DTU**) – DENMARK
  - Université Louis Pasteur (**ULP**) – FRANCE
  - University of Stuttgart (**UST**) – GERMANY
  - Universidad del Pais Vasco (**UPV/EHU**) – SPAIN
  - Royal Institute of Technology (Kungliga Tekniska Högskolan) (**KTH**) – SWEDEN
  - University of Newcastle, Newcastle upon Tyne **(UNEW)** – UK
  - University of Liege, **(ULg)** – BELGIUM

Bioproduction IP

European Dimension of Partnership

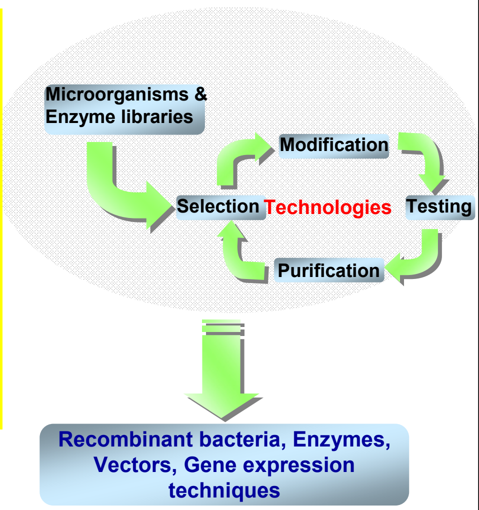
Bioproduction IP



Microorganisms-Biocatalysts

Objectives

- ***Selection*** **of high yield microorganisms and enzymes**
- ***Mutation*** **of bacteria strains for improved productivity of new compounds and/or enzyme generation with improved properties**
- ***Immobilization*** **of enzymes and microorganisms for increased operational stability, catalytic activity and efficient product separation/purification**
- ***Alternative Media*** **of high compatibility with substrates and product for maximum enzymic reaction routes**
- ***Production of Biocatalysts***

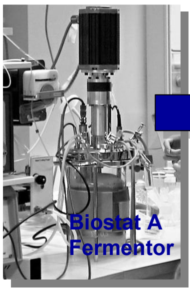


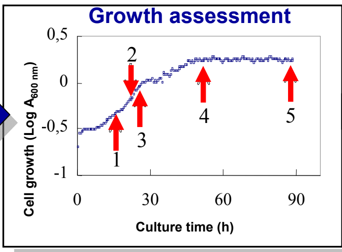
Bioproduction IP

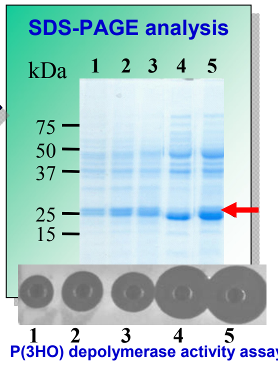
Production of Enzymes

Production, purification and characterization of prototype P(3HO) depolymerase from *P. fluorescens* GK13

Production







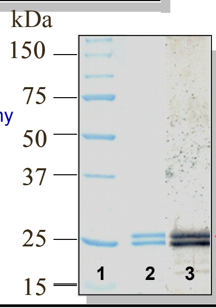
Purification

- **Step 1:** Hydrophobic interaction chromatography

The enzyme was adsorbed on Accurel MP1000 and eluted by 80% (v/v) isopropanol

- **Step 2:** Size-exclusion chromatography

Sephacryl S-200 (30 x 1.5 cm) equilibrated in 50 mM glycine-NaOH (pH 9.5) 0.15 M KCl in an ÄKTA equipment



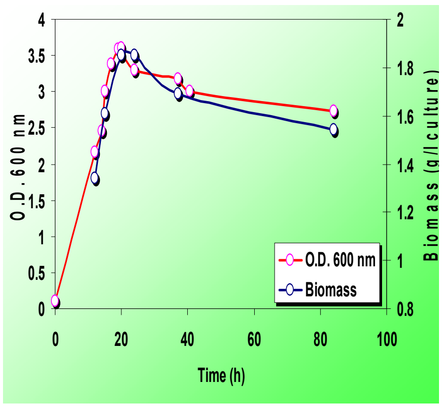
2 mg of pure enzyme was obtained from 1 liter of fermentation broth

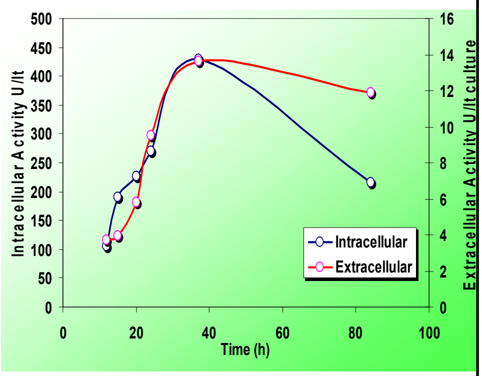
1. MW Markers
2. Coomassie Blue stain
3. Silver stain

Bioproduction IP

Production of Enzymes

Extracellular and Intracellular Production of Lipase from Thermus Thermophilus





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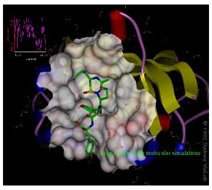
Enzyme Activity/Selectivity

Molecular Modelling

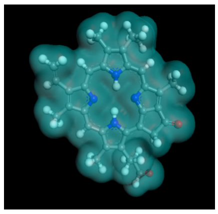
Force Field Description (Potential Energy Function)



MD Software Tools
- **ABINIT** (Density functional theory)
- **AMBER** (Molecular Mechanics)
- **CASTEP** (ab-initio)
- **Car-Parrinello MD** (Density functional theory)
- **CHARMM** (Molecular Mechanics)

- ✓ **Thermodynamic Properties & Parameters**
- ✓ **Enzyme Activity**
- ✓ **Enzyme Selectivity**
- ✓ **Free-Energy Profiles**

Objectives
- **Determination of information concerning *structural and dynamic properties* of biomolecules.**
- **Prediction of enzyme's *selectivity* and substrate *specificity*.**
- **Assessment of *theoretical tools* as replacement of costly and time consuming experiments.**



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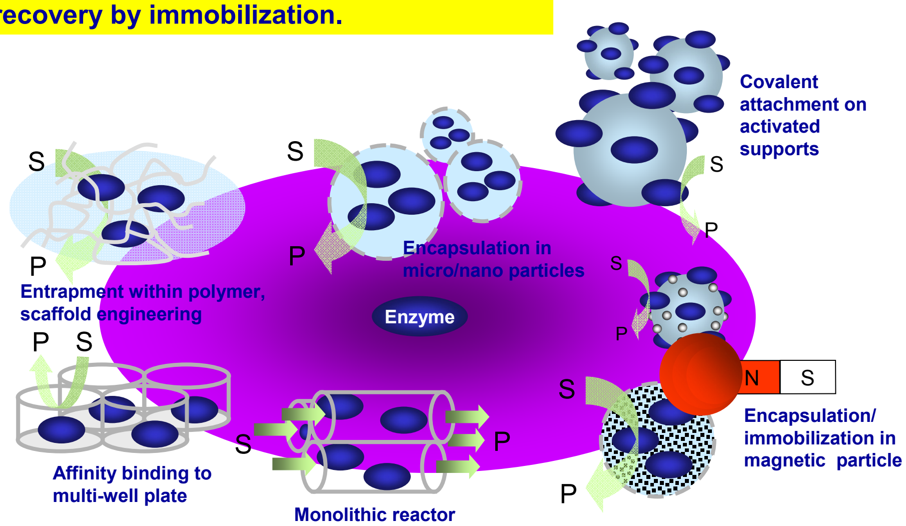
Enzyme Immobilization Strategies

Improving catalyst efficiency, stability and recovery by immobilization.



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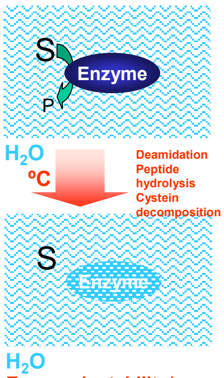
Enzymes in Non-conventional Media

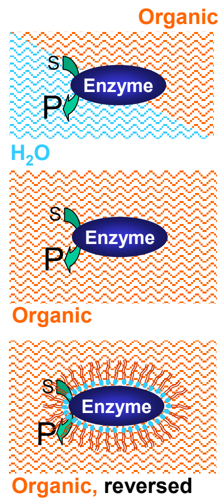
Improving catalyst efficiency and stability by modifying its molecular environment.



Enzyme instability/poor performance in aqueous media.

Medium engineering

- Solvent property screening tools.
- Atomistic models to predict enzyme and substrate stability in new solvent environment (composition, pH, ionic strength, temperature, etc.).
- Other solvent aspects (mass transfer limitations, surface tension, toxicity, flammability, waste disposal, cost, etc.).



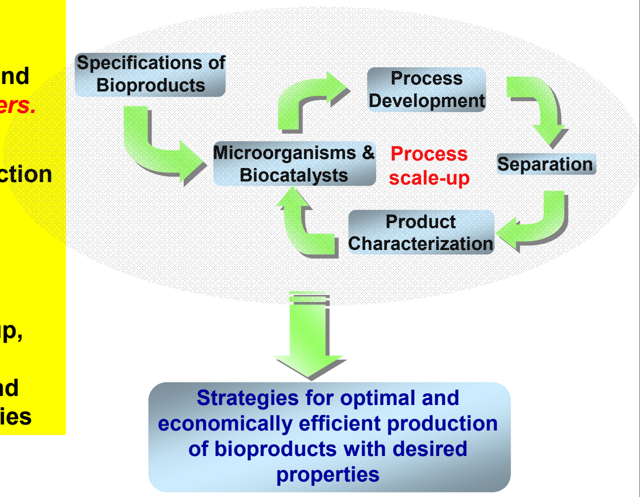
Organic, reversed micelles, nanoemulsions.

Bioproduction IP

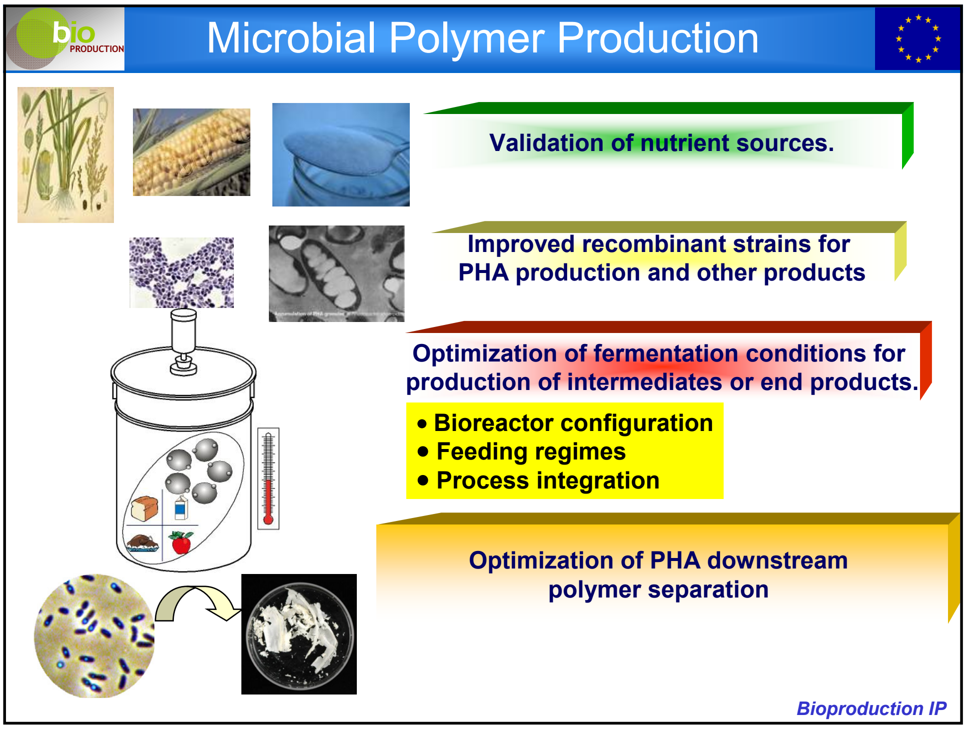
Production & Separation Systems

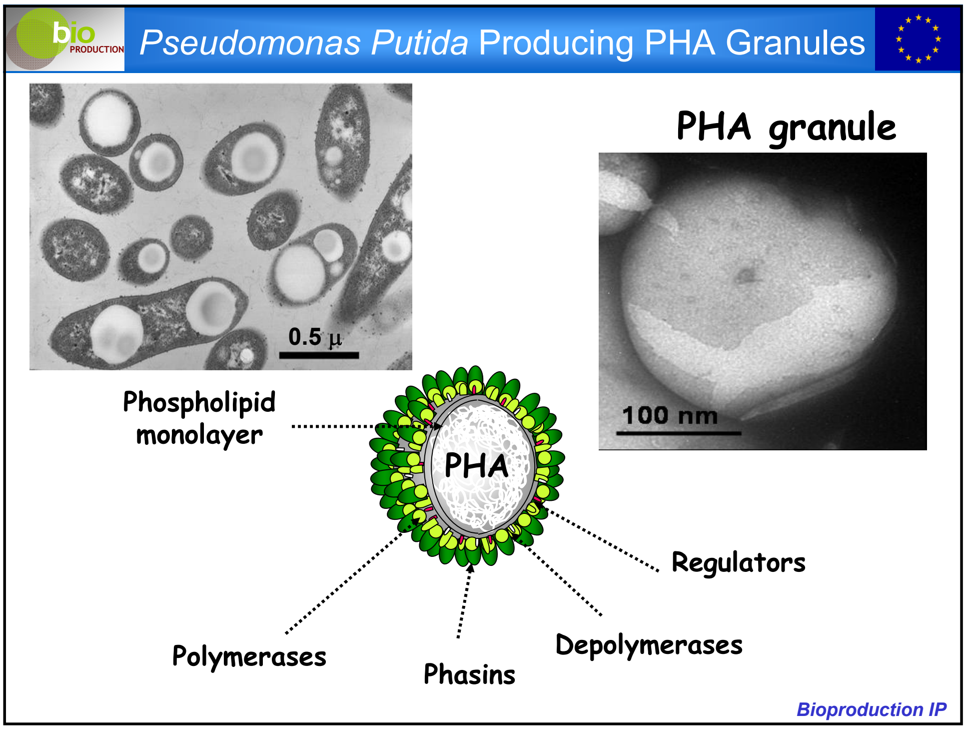
Objectives

- Cost effective in-vivo production, separation and purification of *biopolymers.*
- In-vitro or in-vivo production of *intermediates* from renewable sources.
- Emphasis on process intensification, scaling-up, optimization of the production procedure and product end-use properties

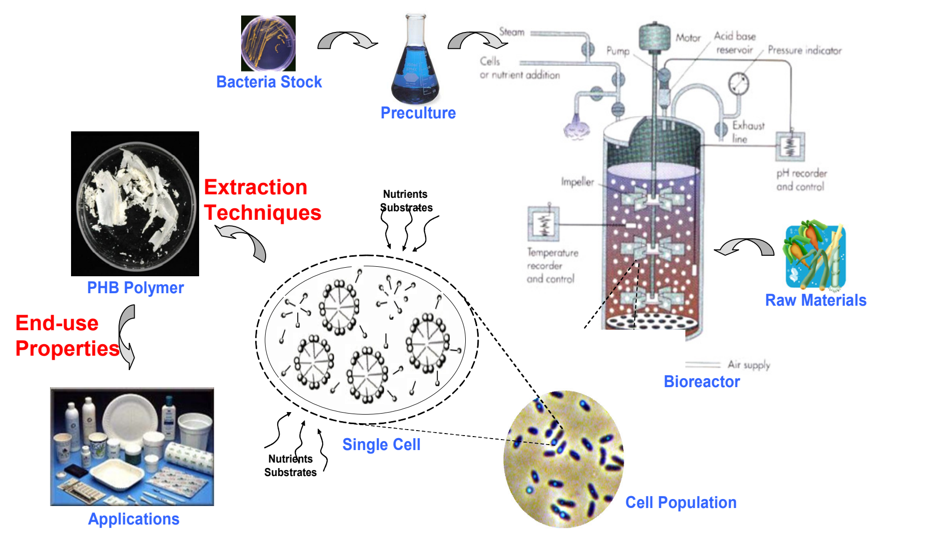


Bioproduction IP


bio PRODUCTION
Microbial Polymer Production
Validation of nutrient sources.
Improved recombinant strains for PHA production and other products
Optimization of fermentation conditions for production of intermediates or end products.
• Bioreactor configuration
• Feeding regimes
• Process integration
Optimization of PHA downstream polymer separation
Bioproduction IP

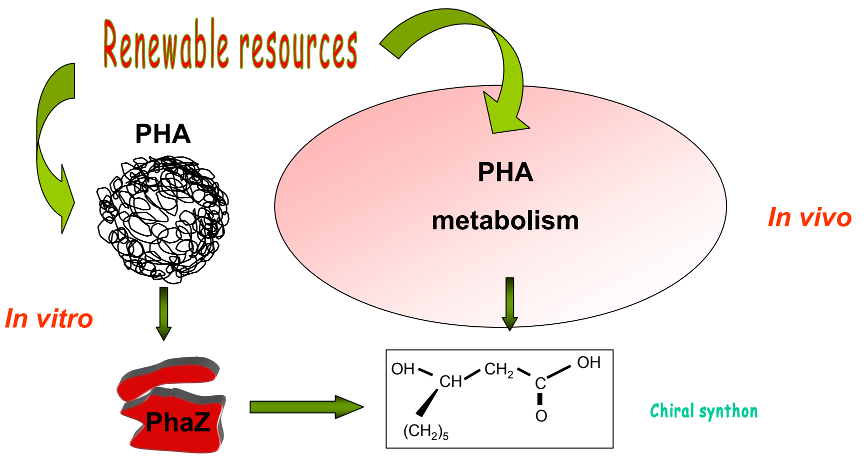

bio PRODUCTION
Pseudomonas Putida Producing PHA Granules
0.5 µ
PHA granule
100 nm
Phospholipid monolayer
PHA
Regulators
Polymerases
Phasins
Depolymerases
Bioproduction IP

Production Process-Flowchart



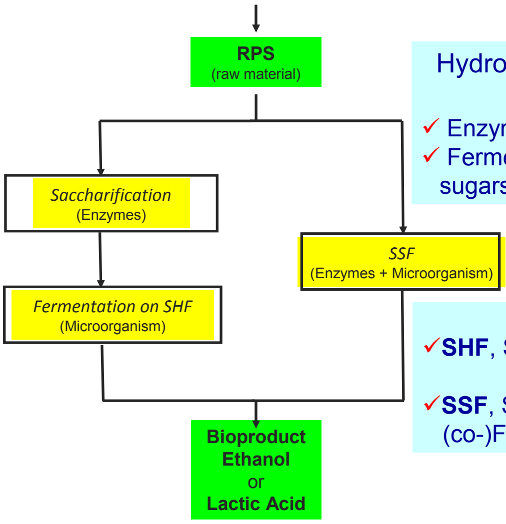
Building Blocks

Production of chiral hydroxyalkanoic acids from PHA



Production of Lactic Acid

Recycled Paper Sludge conversion process



Hydrolysis and downstream fermentation

- ✓ Enzymatic hydrolysis of Cellulose + Xylan
- ✓ Fermentation of C_5 (xylose) and C_6 (glucose) sugars

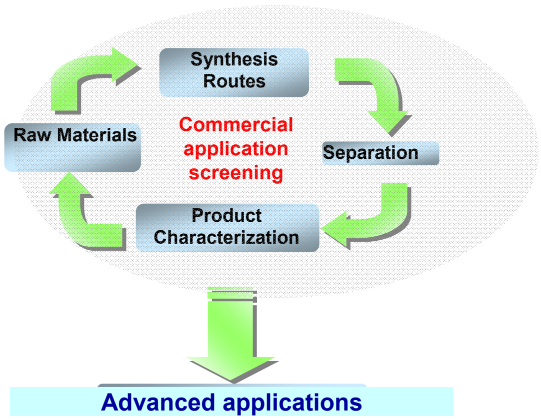
- ✓ **SHF**, Separate Hydrolysis and Fermentation
- ✓ **SSF**, Simultaneous Saccharification and (co-)Fermentation

Bioproduction IP

Advanced Functional Biomaterials

Objectives

- ✓ ***Advanced functional biopolymers*** **with specific properties for selected applications**
- ✓ **Production of *biosurfactants-SFAEs***



Bioproduction IP

Sugar Fatty Acid Esters

Objectives

- SFAE that can be used as emulsifiers & surfactants
- Tailored properties via variation of hydrophilic moieties (carbohydrates, polysaccharides, ..) and hydrophobic chains
- Acceptable colour/odour profiles for use in consumer products

Lactic Acid Based Copolymers

Objectives

- Development of **processes** to produce **novel lactic acid based copolymers with improved properties** utilising biobased raw materials generated in the project
- Testing and characterisation of the produced materials
- Modeling/simulation of PLA formation
- **Novel functional copolymers** containing lactic acid
  - ✓ Improved properties compared to standard PLA
  - ✓ Comparable properties with traditional plastics for e.g. packaging applications
  - ✓ Materials with new properties for novel applications

Alginate Based Products

The main goals in the alginate part of the project are:

- ***Make alginate based products for wound care applications*** and evaluate the release of bioactive alginates and response to cellular biomarkers relevant to wound healing

- ***Production of alginate substrates*** by fermentation with available alginate over-producing ***Pseudomonas fluorescens*** strains.
- ***Find new mannuronan C-5 epimerases*** with tailored functionality by high throughput screening of an epimerase library generated by gene shuffling and error prone PCR
- Generation of ***yeast and bacterial strains that efficiently produces natural or engineered mannuronan C-5 epimerases*** in amounts sufficient to enable industrial epimerase production
- Develop an ***efficient and scalable fermentation and purification*** process for production of epimerases. Test novel reactor technology and "model assisted" optimization of the epimerase production in high cell density fermentations
- ***Produce test quantities of epimerases*** and use these for *in vitro* epimerization of alginate substrates to yield alginates possessing bioactivity

Fungal Biopolymers

Objectives

- Identify new functionalities to be added to fungal biopolymers
- Select chemical, enzymatic or blend modifications routes that follow the "non-polluting" process: industrial viability, vegetal based ingredients, avoid organic solvents, single step process.
- **Three functionalities were selected according to market needs:**
  - ✓ Water-soluble chitosan
  - ✓ Hydrophobic chitosan
  - ✓ Processable chitosan

Microbial Production of PHAs

Bioproduction IP

Production of Biopolymers

- Microbial Production of PHAs
  - ✓ Development of high productivity processes for 3HB homo-polymers and co-polymers with tailored molecular/mechanical properties from wild and modified strains using as carbon sources renewable and waste materials
  - ✓ Development and assessment of strategies for product separation/purification
- Digital Bioprocesses
  - ✓ Development of integrated metabolic/polymerization models
  - ✓ Algorithms for on-line adaptive metabolic flux analysis for biological systems with dynamic metabolic networks
  - ✓ Development of segregated population models for microbial cultures combined with multi-objective optimisation algorithms
  - ✓ Scaling-up of selected bioprocesses

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Microrganisms/Raw Materials/Products

Microorganisms

- **Genetically modified**
  - ***E.coli***
  - ***P. putida KTfadB***
- **Wild Strains**
  - ***P. putida* KT2442**
  - ***C. necator* DSM545**
  - ***H. Meditarranei***
  - ***A. latus***
- **Mixed Cultures**

Raw Materials

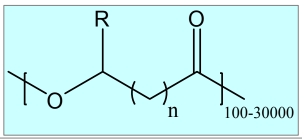
- **Pure substrates**
  - **Glycerol**
  - **Lactose**
  - **Sucrose**
  - **Octanoic acid**
- **Wastes/Surplus**
  - **Glycerol from Biodiesel**
  - **Fermented molasses**
  - **Cheese Whey**

Products

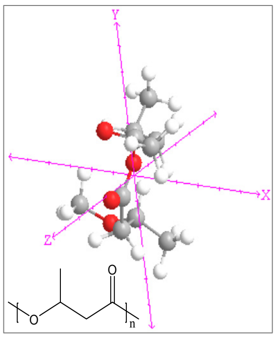
- **mcl-PHAs**
  - **P(3HHx)**
  - **Other**
- **scl- PHAs**
  - **PHB**
  - **P(3HB-co-3HV)**
  - **P(3HB-co-4HB)**

Polyhydroxyalkanoates (PHAs)

- Polyhydroxyalkanoates (PHAs) are completely biodegradable polyesters of hydroxyalkanoates that are synthesized by many bacteria. The molecular weight of these polymers is in the range of 200,000 – 3,000,000 Da and they are accumulated in the cells in the form of discrete granules as a carbon and energy storage material.

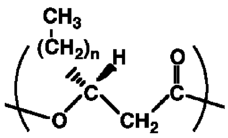


n =1	R =	hydrogen	poly (-3-hydroxypropionate)
		methyl	poly (-3-hydroxybutyrate)
		ethyl	poly(3-horoxyvalerate)
		propyl	poly (-3-hydroxyhexanoate)
		pentyl	poly (-3-hydroxyoctanoate)
		nonyl	poly (-3-hydroxydodecanoate)
n = 2	R =	hydrogen	poly (-4-hydroxybutyrate)
n = 3	R =	hydrogen	poly (-5-hydroxyvalerate)



- Polyhydroxybutyrate (PHB) was the first PHA to be discovered and is also the most widely studied and best characterized. It has mechanical properties very similar to conventional plastics, like propylene.

Research Objectives



- Composition
- Chain length distribution
- Mechanical properties
- Thermal properties

PRODUCT CHARACTERIZATION

Monitoring

Cost

CULTURE MEDIUM
CARBON SOURCE
Ps. putida KT2442

FERMENTATION → RECOVERY PURIFICATION → PHA PRODUCTION

Yield
Productivity

Yield
Productivity

- Operation mode: continuous, fed-batch
- Culture medium
- Limiting nutrient/nutrients:
- C limitation/C/N limitation
- Ambient variables:
- Oxygen concentration, temperature
- Fermentation time

- Disruption method: mechanical lysis, solvent extraction
- Solvent extraction: type of solvent, length, temperature
- Precipation method:Liquid/liquid, temperature

Bioproduction IP

Tailor-made PHAs

Tailor-made PHAs by fermentation technology:
The variability of bacterial PHAs produced by fermentation is extraordinary large (150 different monomers).

PHA production processes:

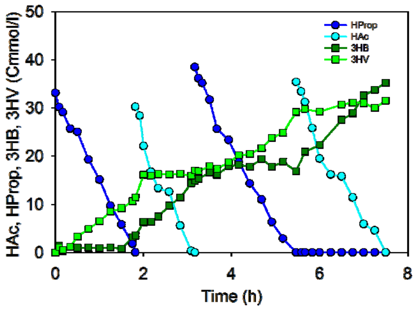
- Single, continuous or fed-batch mode or in two-stage fed-batch mode with usual productivities ranging between **0.5 and 5 g PHA/L•h** depending on strain and specifications of the productive procedure.
- Oxygen transfer rate becomes limiting at high cell densities and special reactor configurations are required.

Strain	Product	Cell concentration g/L	PHA concentration g/L	Overall productivity g/L·h
P. putida KT2442	mcl-PHA	141	72	1.90
C. necator	PHB	281	232	3.14
A. latus	PHB	111.7	98.7	4.94
Recombinant *E. coli*	PHB	194	141	4.6

PHAs-Mixed cultures

P(3HB-co-3HV) copolymers – Microstructure Analysis

- Batch experiments with alternated pulses of acetate and propionate and mixtures of acetate and propionate were performed.



- A copolymer P(3HB-co-3HV) was obtained and the 3HV fraction ranged from 34% to 78%, depending on the type and frequency of the substrate supplied.
- Monomers of 3HB produced with acetate pulses while production of 3HV was almost constant, the opposite was observed with propionate pulses.

- SEC analysis showed chromatograms with unimodal behaviour- copolymer;
- The molecular weights were in the range 0.4-1.6×10^6;
- One peak of Tg and Tm for the P(HB/HV) analysed ⇒ true copolymers obtained;
- T_m between 88.5ºC and 93.8ºC and Tg between -14.3ºC and -5.4ºC which are in accordance with the range of the HV proportion.

Bioproduction IP

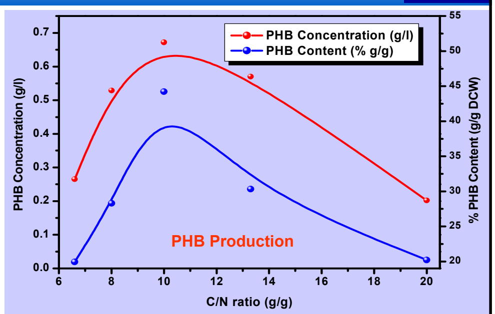
PHB-Medium Composition

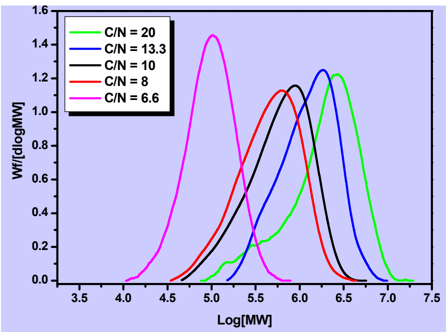
✓ Study of the influence of the initial C/N ratio on the PHB production by *Alcaligenes Latus*

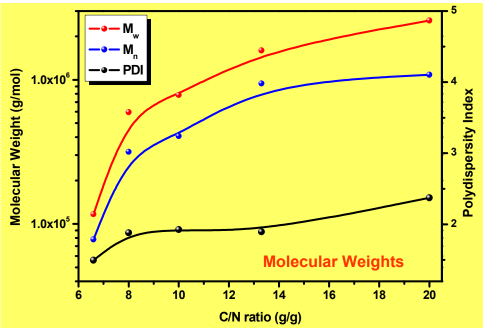
Batch Experiments

Same initial sucrose concentration (20 g/l) and different ammonium sulphate initial loadings (from 1 to 3 g/l).

Cultivation up to the stationary phase.

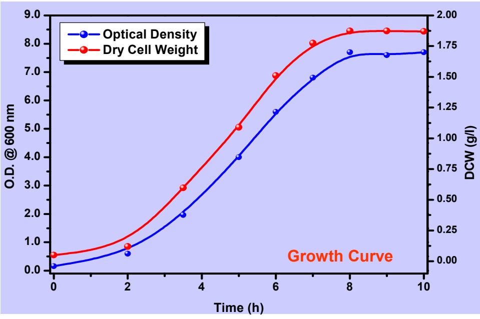


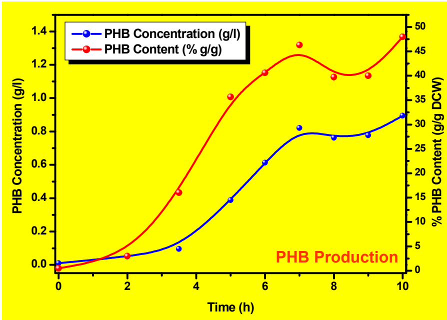


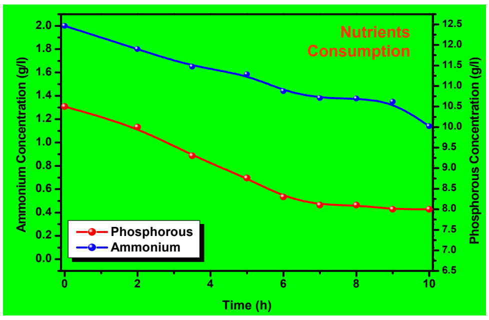


PHB-Kinetic Studies

✓ ***Microbial PHB production by Alcaligenes Latus in a 2 lt. Flask***







<u>Molecular Properties</u>

M_w = 1,073,000 Da

M_n = 373,000 Da

PD = 2.87

<u>Thermal Properties</u>

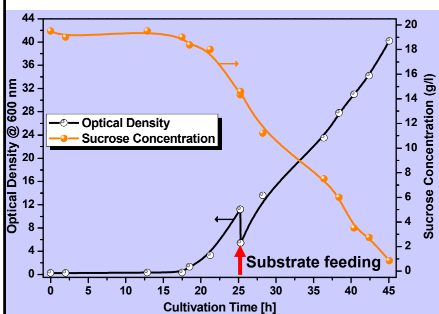
Degree of Crystallinity = 66%

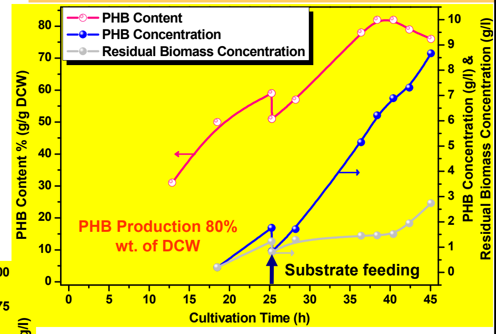
T_m = 176 ^oC

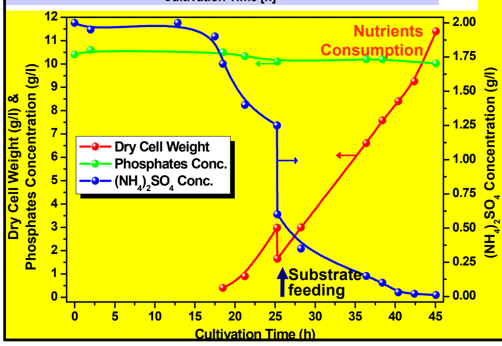
Bioproduction IP

PHB Kinetic Studies - Fed-Batch

✓ ***Microbial PHB production by A. Latus in a 2 lt. Flask Fed-Batch***







<u>Molecular Properties</u>

M_w = 436,300 Da

M_n = 199,100 Da

PD = 2.19

<u>Thermal Properties</u>

Degree of Crystallinity = 63.24%

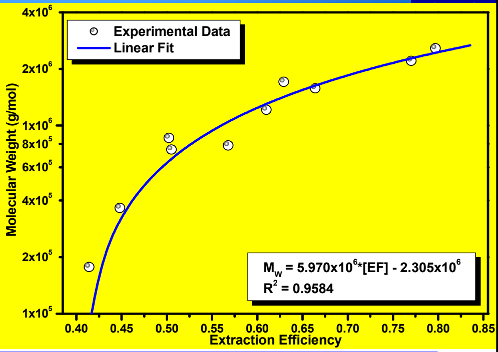
T_m = 175.71 ^oC

Bioproduction IP

PHB Processing

✓ Study of the efficiency of the product recovery method depending on polymer Mw

- Fermentation of *A. latus in* flasks and bioreactors
- Recovery Method: Cell disruption with sonication-$CHCl_3$-based PHB extraction
- M_W is the weight average molecular weight and [EF] the extraction efficiency ratio



Content of Precipitated PHB (%)	PHB Content Determined by GC-MS/FT-IR Analysis (%)	Efficiency of the Recovery Method	Molecular Weight of Recovered Polymer (g/mol)
7.35	11.68	0.63	1,710,000
13.03	31.46	0.41	177,000
14.73	32.88	0.45	365,300
15.00	29.70	0.51	745,000
15.40	28.30	0.54	595,800
16.10	20.20	0.79	2,576,000
18.88	37.58	0.50	860,000
22.37	36.67	0.61	1,213,000
25.30	38.10	0.66	1,576,100
37.01	48.05	0.77	2,210,000

on IP

PHB-Downstream Processing

✓ Effect of the product recovery method on the polymer MWD

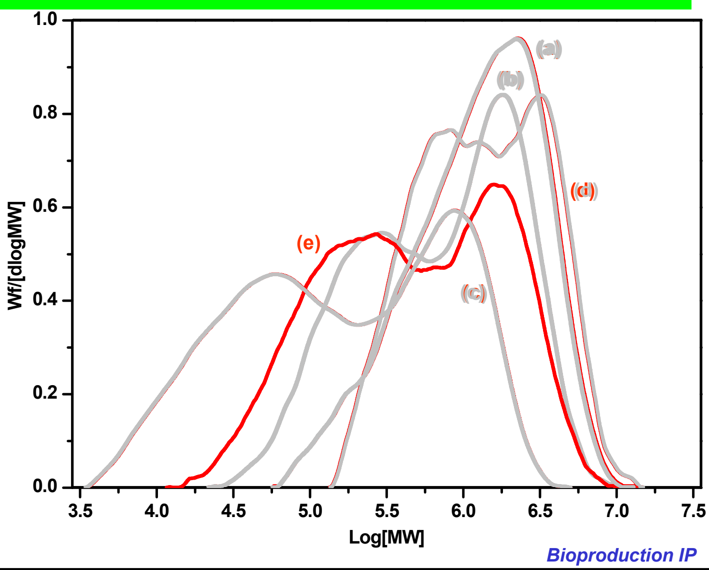
(a) Sonication:
40% amplitude 30 min
$M_w = 1.8 \cdot 10^6$

(b) Sonication:
50% amplitude 30 min
$M_w = 1.2 \cdot 10^6$

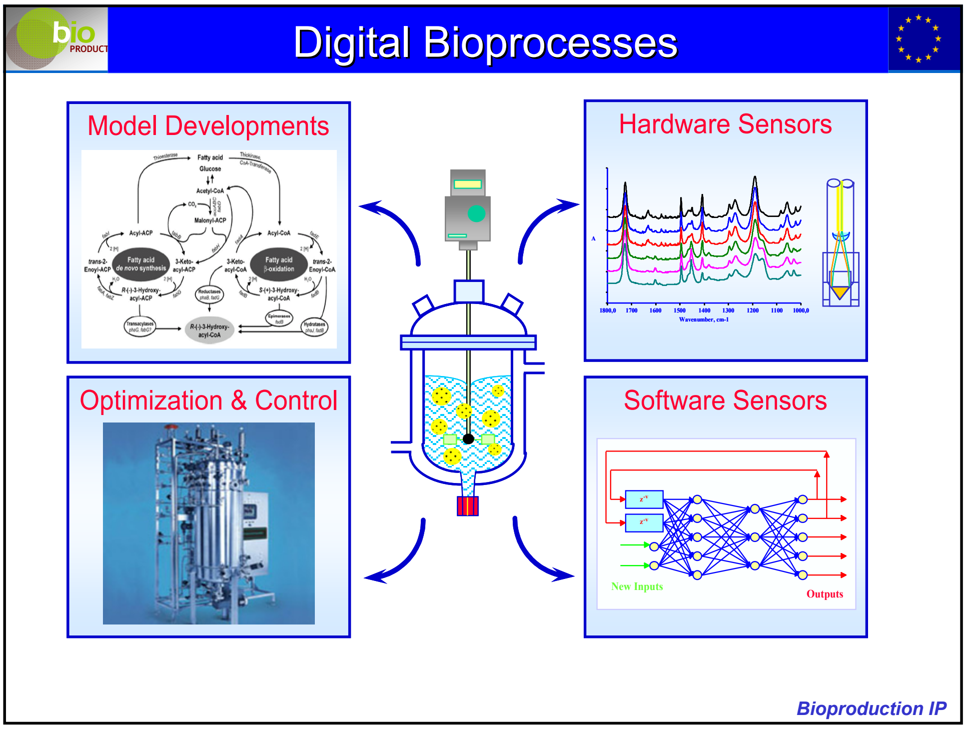
(c) Sonication:
50% amplitude 40 min
$M_w = 0.5 \cdot 10^6$

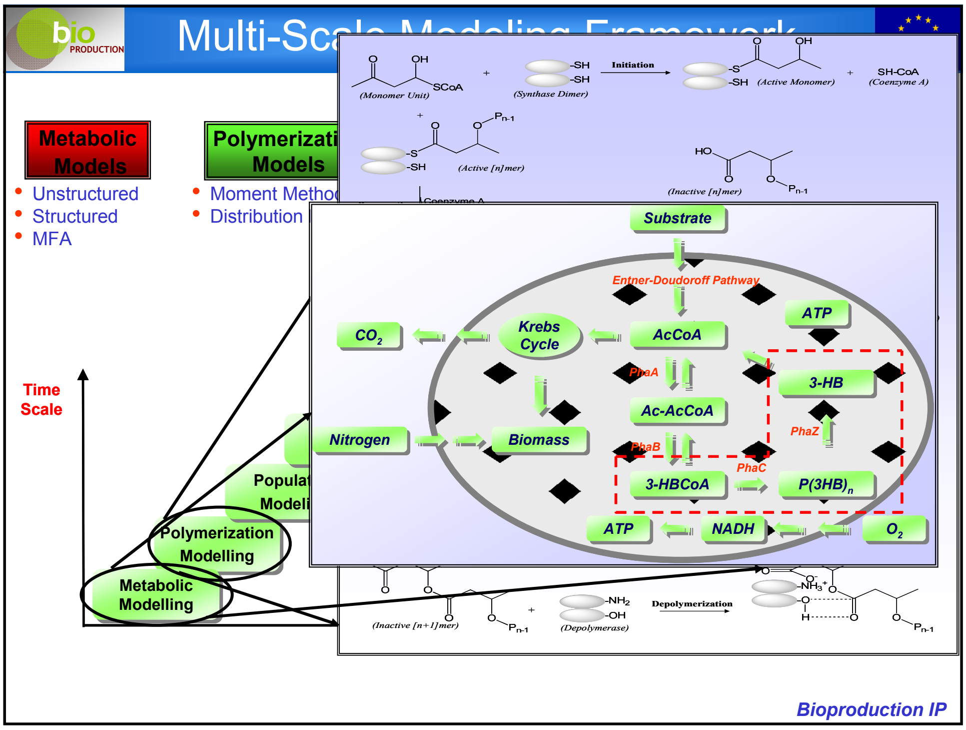
(d) Reflux:
65 °C 20 hr
$M_w = 2.0 \cdot 10^6$

(e) Reflux:
65 °C 40 hr
$M_w = 1.0 \cdot 10^6$



Bioproduction IP


Digital Bioprocesses
Model Developments
Hardware Sensors
Optimization & Control
Software Sensors
New Inputs
Outputs
Bioproduction IP


Multi-Scale Modeling Framework
Metabolic Models
Unstructured
Structured
MFA
Polymerization Models
Moment Method
Distribution
Time Scale
Polymerization Modelling
Metabolic Modelling
Substrate
Entner-Doudoroff Pathway
ATP
CO2
Krebs Cycle
AcCoA
PhaA
3-HB
Ac-AcCoA
PhaZ
Nitrogen
Biomass
PhaB
PhaC
3-HBCoA
P(3HB)n
NADH
O2
Initiation
(Monomer Unit)
(Synthase Dimer)
(Active Monomer)
SH-CoA
(Coenzyme A)
(Active [n]mer)
(Inactive [n]mer)
(Inactive [n+1]mer)
(Depolymerase)
Depolymerization
Bioproduction IP

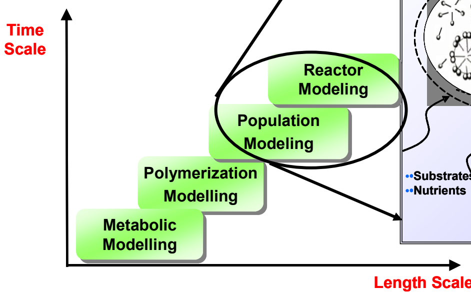
Multi-Scale Modeling Framework

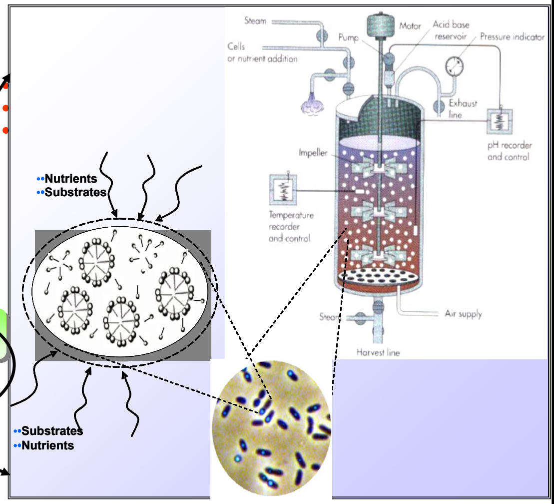
Metabolic Models

- Unstructured
- Structured
- MFA

Polymerization Models

- Moment Methods
- Distribution Methods



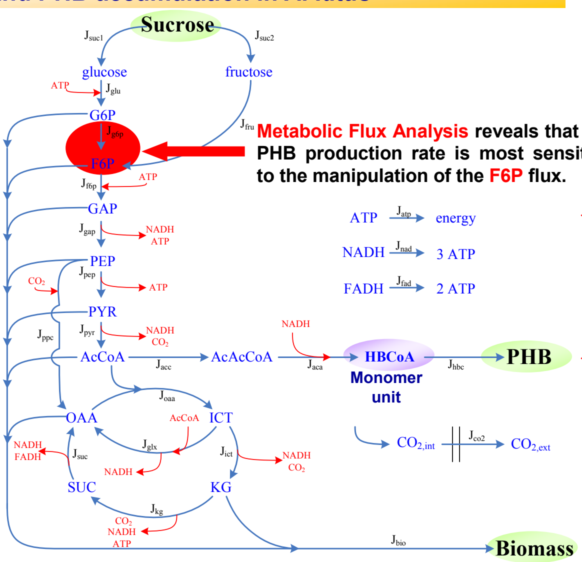


Bioproduction IP

Cell-Level: Metabolic Flux Analysis

Complete metabolic pathway of biomass growth and PHB accumulation in *A. latus*

"Bacteria Fermentation Culture for Synthesis of PHA Biopolymers as Intracellular Product"



Metabolic Flux Analysis reveals that the PHB production rate is most sensitive to the manipulation of the F6P flux.

- ✓ **Metabolism of sucrose towards the accumulation of PHB through Glucolysis as energy carbon storage and biomass growth through Krebs Cycle**
- ✓ **The PHB production rate and the biomass growth rate are functions of *sucrose diffusion* from the culture medium through the cell membrane and its *assimilation rate* inside the cell.**

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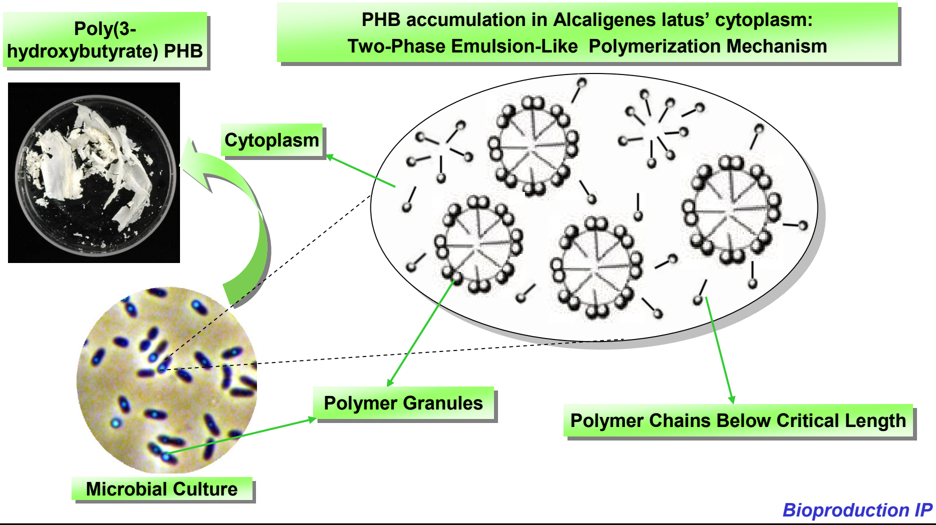
Intracellular PHB Accumulation in *A. Latus*

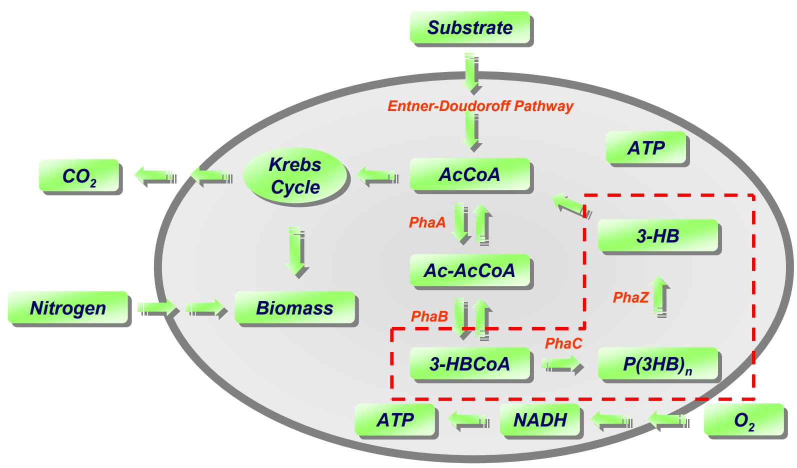
"Bacteria Fermentation Culture for Synthesis of PHA Biopolymers as Intracellular Product"



Bioproduction IP

Model Integration at the Cell Level

Metabolic pathway of synthesis and degradation of P(3HB).



The activated monomer 3-HBCoA constitutes the link between the two models.

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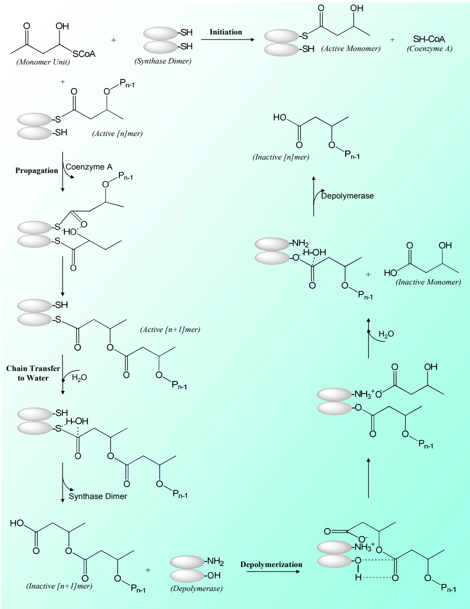
Polymerization Mechanism



Polymerization Model

Initiation

$$E-SH + M-SCoA \xrightarrow{k_m} ESH-M \xrightarrow{k_i} P_1-ES + SH-CoA$$

Propagation

$$P_n-ES + M-SCoA \xrightarrow{k_m} P_n-ES-M \xrightarrow{k_p} P_{n+1}-ES + SH-Co$$

Termination with H_2O

$$P_n-ES + H_2O \xrightarrow{k_t} D_n + E-SH$$

Depolymerization

$$D_n + E-OH \xrightarrow{k_d} D_{n-1} + D_1 + E-OH$$

Assumptions

- Polymerase (PhaC), depolymerase (PhaZ) and water concentrations are constant throughout the course of polymerization.
- Cell death occurring after a certain period of time (i.e. 30h) causes polymer degradation to gradually decrease.
- Kinetic rate constants are independent of chain length.

Bioproduction IP

Formulation of Differential Equations

Polymerization Model

Live polymer chains of length n

$$\frac{d[P_n]}{dt} = k_i[\text{E-SH-M}]\delta(n-1) - k_m[P_n][M] + k_p\left[P^*_{n-1}\right]\mathcal{H}(n-1) - k_t[P_n][H_2O]$$

Intermediate polymer chains of length n

$$\frac{d[P^*_n]}{dt} = k_m[P_n][M] - k_p[P^*_n]$$

Dead polymer chains of length n

$$\frac{d[D_n]}{dt} = k_t[P_n][H_2O] - k^*_d[D_n]\mathcal{H}(n-1) + k^*_d\sum_{n=2}^{\infty} D_n\delta(n-1) + k^*_d[D_{n+1}]$$

Monomer

$$\frac{d[M]}{dt} = J_M(t) - k^*_m[M] - k_m[M]\sum_{n=1}^{\infty}[P_n]$$

Monomer-Synthase Complex

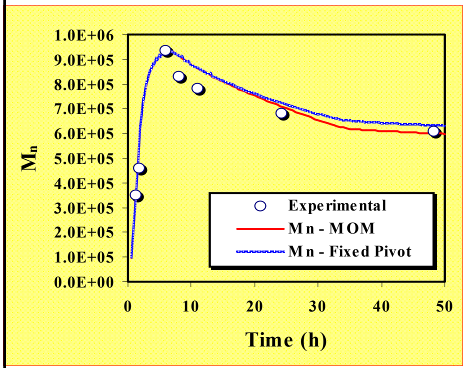
$$\frac{d[\text{E-SH-M}]}{dt} = k^*_m[M] - k_i[\text{E-SH-M}]$$

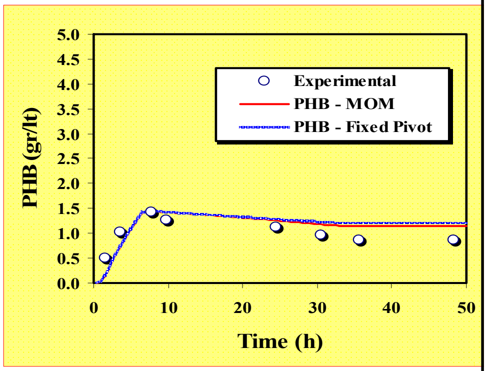
Metabolic Model

$$J_M(t) = g(\mathbf{k},\mathbf{Y},\mathbf{C},\mathbf{J},t)$$

Bioproduction IP

Model Tuning (Fructose 5 g/l)





- The polymerization model was solved with two different numerical methods.
- Parameters are estimated using the General Regression Software (GREG).
- The model is able to predict the evolution of M_n and polymer yield.
- There is a very good agreement between numerical methods.

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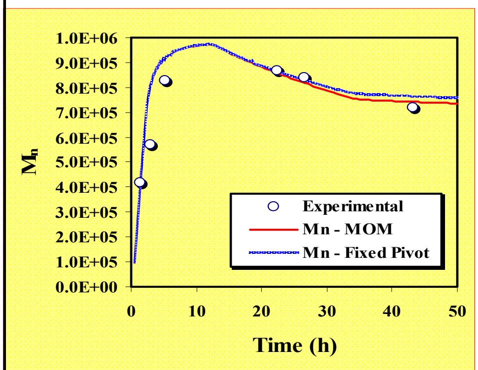
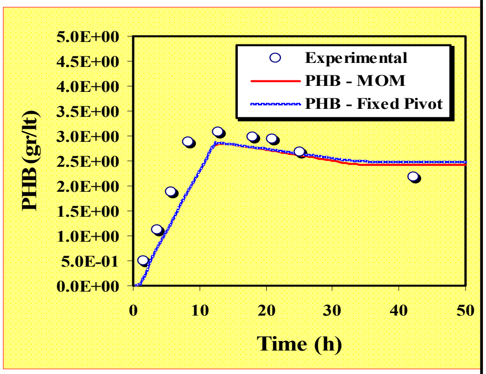
Model Validation (Fructose 10 g/l)



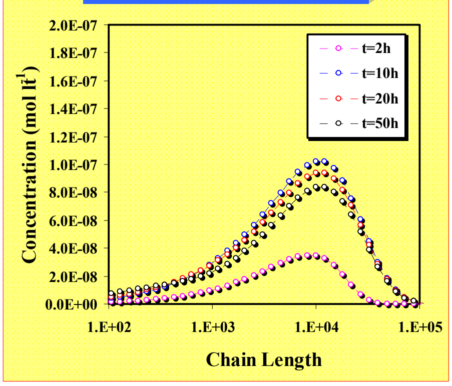


- The model is able to predict the evolution of M_n and polymer yield.
- There is a very good agreement between numerical methods.
- In batch cultures a maximum M_n and polymer yield are achieved and subsequently there is a decrease to a steady-state value.

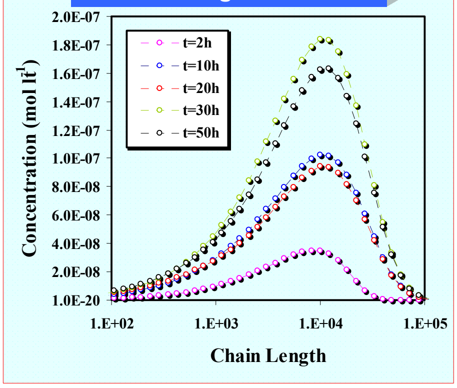
Bioproduction IP

Investigation of MWD Evolution

Fructose 5 g/l Batch



Fructose 5 g/l Fed-batch



- Chain length distributions reflect the behavior of M_n and polymer yield.

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The Population Balance Equation (PBE)

✓The evolution of the cell mass distribution under the combined action of cell growth and division is described by the following equation:

$$\frac{\partial n(m,t)}{\partial t}+\frac{\partial}{\partial m}\left[G(m,S)n(m,t)\right]=2\int_{m}^{\infty}\Gamma(m',S)P(m,m')n(m',t)dm'-\Gamma(m,S)n(m,t)$$

$n(m,t)$: number of cells with mass between $[\mathbf{m},\mathbf{m}+\mathbf{dm}]$ per unit biovolume at time t, $\mathbf{g}^{-1}\cdot\mathbf{m}^{-3}$.

$G(m,S)$: growth of cells with mass **m**, **g/s**.

$\Gamma(m,S)$: division rate (intensity) of cells with mass **m**, $\mathbf{s}^{-1}$.

$P(m,m')$: partitioning function, i.e., probability that a mother cell with mass **m′** will give birth to a daughter cell with mass **m**, $\mathbf{m}^{-3}$.

✓The PBE is coupled to the mass balance for the substrate concentration.

$$\frac{dS}{dt}=\frac{1}{Y}\int_{0}^{\infty}G(m,s)n(m,t)dm$$

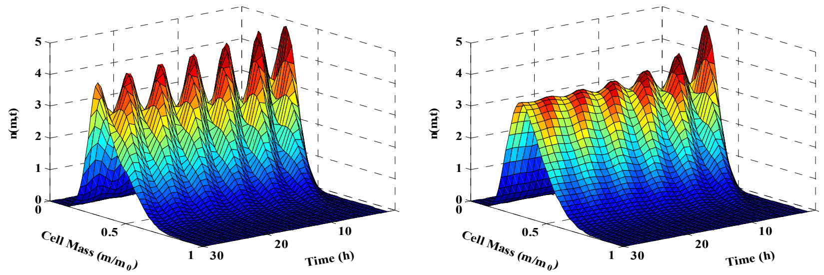
Y: yield coefficient (g substrate/g biomass)

Bioproduction IP

Constant Substrate Concentration

Case I: Linear Growth - Equal partitioning

Case II: Linear Growth – Unequal Partitioning

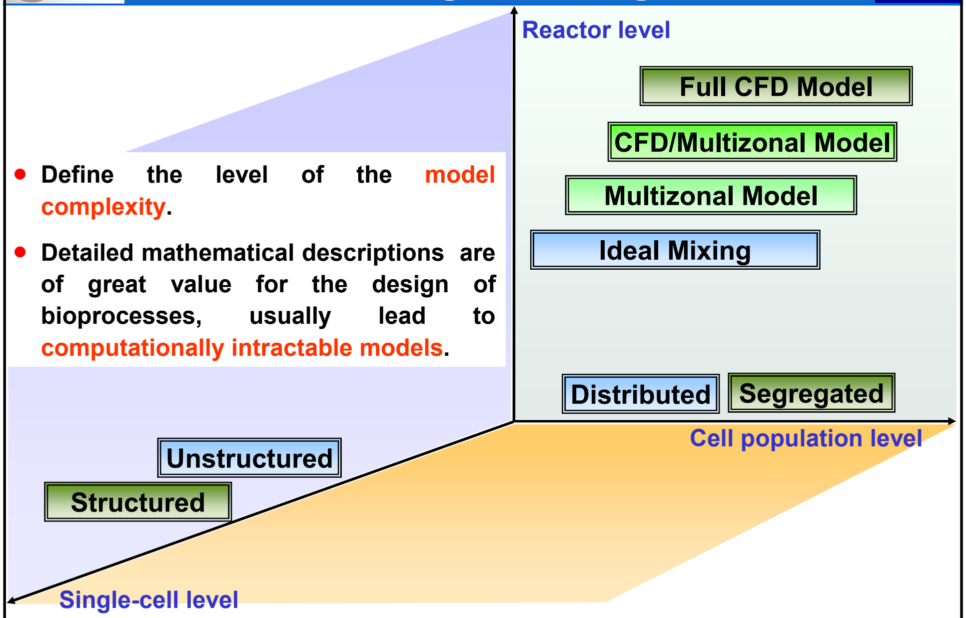


- ✓ For linear cell growth rate and equal partitioning the cell mass distribution exhibits a **periodic behavior** with a frequency equal to the cell doubling time T_d=5h.
- ✓ For linear cell growth rate and unequal partitioning a state of **balanced growth** is gradually achieved.

Bioproduction IP

Modelling Challenges

- Define the level of the **model complexity**.
- Detailed mathematical descriptions are of great value for the design of bioprocesses, usually lead to **computationally intractable models**.



Bioproduction IP

Conclusions

- The microbial PHA production process is a multi-parametric system
- The physiological state of the culture is determinant for both the quality and the quantity of the produced biopolymers
- Exhaustive investigation and mapping of the influence of all the operating parameters on both the physiological state of the culture and the productivity and quality of the biopolymers is necessary
- A model-based optimization of these production processes and simulation of the biopolymer molecular properties will enhance their competitiveness and will assist their economic viability.
- Operating profiles, product recovery techniques, and reactor configuration were explored through an experimental- and a model-based approach
- The conceptual framework for the development of integrated metabolic, polymerization and population models at a reactor level accounting for heat and mass transfer phenomena was presented
- An integrated metabolic/polymerization model was developed based on experimental data from fermentation of Alcaligenes species.