Accelerated Development of Biobased Processes
New Developments in Platform Technology

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Biotechnology is delivering advantages today
Offers solutions to consumer demand for more sustainable products

Chemical producing organisms engineered by Genomatica
More than Bugs: A practical look at developing commercially-advantaged bioprocesses, addressing Development cost & timelines | Production economics

1) Integrated approach to bioprocess development
2) Innovations – chemicals, feedstocks, timelines
3) Directions for future process technology developments
Integrated bioengineering platform
Drives high impact innovation, products, solutions

Systems Engineering Mindset:
• computer-aided design for biology (since 2000)
• enables focused efficient experimentation
• integrated process engineering
• results in rapid cost-effective development

Low molecular weight molecules
- Intermediate Chemicals
- Specialty Chemicals
Understanding the complex metabolic network is critical throughout the entire R&D process

The Engineering Blueprint

- In silico high throughput screening and prioritization of pathways
- Prioritization of strain designs and metabolic engineering strategies
- Global context for “omics” data analysis and fermentation/media optimization
SimPheny platform for metabolic engineering
Modeling and simulation, fully integrated with experimental capabilities
Computational Technologies at the Core
SimPheny, Pathway Predictor, Custom LIMS tools drive R&D approaches

Systems-Based Approach
Rapid omics data analysis
Extraction of information

High Throughput Computational Tools

- Metabolic Flux
- Gene KO Strategies
- Lower By-Products

- Adaptive Evolution
- Simulated Phenotypes
- Growth-Coupled Prodn

- Metabolic Models
- Novel Pathways
- Engineering Blueprints

- Assessing Risk
- Creating IP
- Evaluating Opportunities

Integrated R&D Workflow
Path to a production strain (non-natural chemicals)

Strain design guided by process design

Bio-Pathway
Identification and Pathway Engineering

Metabolic Network
System Design and Engineering

Commercial Strain
Efficient Chemical Production

Platform Tools for Strain Engineering

- Computational Modeling and Simulation
- Systems Biology-based Engineering
- Protein Engineering
- High Throughput Cloning/Screening
- in vitro Transcription-Translation (TX-TL)
Path to a Commercial Process Technology
Strain design guided by process design

Strain Engineering to Produce Chemical

Fermentation Development

Recovery/Purification

Optimize sugar to chemical flux

Optimize fermentation conditions

Optimize recovery and purification

Purified Chemical Meet industry specs
**Techno-economic analysis**
Providing the framework for guiding R&D decisions

Definition: **Combined (simultaneous) process modeling and economic evaluation**

- **Assess potential of new opportunities**
- **Identify high-impact areas for optimization**
- **Compare economics of design alternatives**
- **Provide metrics for tracking performance**
Rigorous focus on cost advantage
Production cost advantage vs. best available technology (BAT)

What is our current advantage or disadvantage?

What are the contributions of each of the key elements of the cost stack for each process?

Where are feedstock pricing trends heading?
What are the pricing scenarios?

How significant does cost change with various GENO approaches?

How frequently are we advantaged vs. disadvantaged?
(over the prior 3 years and future 1 year of pricing)
What scenarios produce advantaged and 25% advantage?
Integrated and iterative development workflow
Design, build, test, learn cycle

- **Optimize recovery & economics**
- **Implement strain designs**
- **Fermentation**
- **Optimize organism**
- **Modeling & Simulation**
- **Learn**
- **Design**
- **Test**
- **Build**

Recovery & Purification
High purity Industry specs

Optimize fermentation economics

Feedstock
Pentose Phosphate Pathway
Chemical Pathway

Genomatica sustainable chemicals
Continuous stream of innovations
Solving new problems, reducing costs, faster time to market

Novel Pathways & Feedstocks

Downstream Purification

Reliable scale-up

Faster Development
## Intermediate and specialty chemical IP portfolio
Platform generating expansive pathway & process IP; +600 patents/filings

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butadiene</td>
<td>● Synthetic rubbers, resins, latex</td>
</tr>
<tr>
<td>Propylene</td>
<td>● Injection molding, fibers, film and sheet</td>
</tr>
<tr>
<td>1,4-butanediol (BDO)</td>
<td>● High performance polymers, solvents, fibers</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>● Solvent, intermediate for other chemicals</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>● Polyester fibers, resins, films</td>
</tr>
<tr>
<td>Terephthalic Acid</td>
<td></td>
</tr>
<tr>
<td>Adipic Acid</td>
<td>● Nylon fibers, engineering resins</td>
</tr>
<tr>
<td>Hexamethylenediamine (HMDA)</td>
<td></td>
</tr>
<tr>
<td>Caprolactam</td>
<td></td>
</tr>
<tr>
<td>Cyclohexanone</td>
<td>● Key intermediate to caprolactam and adipic acid</td>
</tr>
<tr>
<td>Aniline</td>
<td>● Polyurethane foams, elastomers, coatings</td>
</tr>
<tr>
<td>Methyl Ethyl Ketone (MEK)</td>
<td>● Solvent</td>
</tr>
<tr>
<td>Fatty Alcohols</td>
<td>● Surfactants, fuels</td>
</tr>
<tr>
<td>Acrylic Acid</td>
<td>● Coatings, detergent, superabsorbent polymers</td>
</tr>
<tr>
<td>Acrylate Esters</td>
<td>● Surface coatings, textiles, plastics</td>
</tr>
<tr>
<td>Methyl Methacrylate</td>
<td>● Surface coatings, resin</td>
</tr>
</tbody>
</table>

132 Issued or Allowed Patents

491 Pending Patents
Driving Technology & Commercial Innovations

Integrated Bioengineering Platform

1) Process Development & Commercialization
2) Accelerated Organism Development
3) Expanded Feedstock Enablement
4) Rapid Pathway Prototyping
BDO Commercialization Journey and Success
5 years from concept to commercial production

GENO BDO™ Process

- 2008: BDO producing organisms
- 2009: Purified BDO
- 2010: Piloting
- 2011: Shipping tons at a time
- 2011: Integrated demo plant
- 2012/13… Commercialization
- Commercial-scale

3,000L
13,000L
Complete chemical manufacturing process
process technology developed and scaled-up; commercially licensed

GENO BDO™ Process Flow Diagram
for polymer-grade BDO, 99.8% purity

- Fermentation
- Cell Separation
- Salt Separation
- Water Evaporation
- BDO Purification

Sugars (dextrose, sucrose)

well-established commercial fermentation configuration

standard equipment for all unit operations
Commercial-scale production
2,500 tons in five weeks, Loudon-TN, produced Q4’12
Scalable performance: lab-to-commercial scale
highly predictable scale-up/down (100,000 fold)

- Fermentation performance across lab, pilot, and demo scales is highly consistent, enabling rapid lab-to-commercial scale development path
- Average commercial-scale performance over ~50 campaign fermentations equivalent to demonstration-scale performance for same strain (+/-2%)
- Low variability in performance across ~50 campaign fermentations, indicates process robustness and predictability
Competitive economics even at $50 oil
GENO BDO vs. best available petroleum-based, 50KT scale

CME Corn Price

N.A. Butane Price

Equivalent Cost Line
25% Cost Advantaged Line

trailing (-3 yr average price)
current price
future (+1 yr average price)
× trailing monthly average price for -3 yr
× future monthly average price for +1 yr

GENO ≥ 0%
Advantaged Zone

GENO ≥ 25%
Advantaged Zone
First Bio-BDO plant under construction in Italy by Novamont
BASF licenses the GENO BDO™ process
“exceptionally advanced and reliable” process technology

#1 chemical company
#1 BDO producer
535KT BDO capacity

PRESS RELEASE – November 27, 2013
BASF produces first commercial volumes of butanediol from renewable raw material

PRESS RELEASE – March 5, 2015
BASF now offers bio-based PolyTHF
- Properties identical to conventional PolyTHF
- Extends range of products based on renewable raw materials
- Opportunity for customers to test new market segments
Technology & Commercial Innovations

1) Process Development & Commercialization
2) Accelerated Organism Development
3) Expanded Feedstock Enablement
4) Rapid Pathway Prototyping
Designing the optimal BDO producing organism
Metabolic Engineering of *E. coli* for direct production of 1,4-BDO

**Carbohydrates**

- six-carbon sugars (e.g. dextrose)
- disaccharides (e.g. sucrose)
- five-carbon sugars (e.g. xylose)

**1,4-Butanediol**

- 21 enzyme steps
- 35 gene modifications
- 6 heterologous genes
BDO pathway and process
Engineering central metabolism and heterologous pathways to BDO

E. coli

Sugars → Glycolysis → TCA Cycle → BDO Pathway → BDO

C₆H₁₂O₆ + 0.5 O₂ → C₄H₁₀O₂ + 2 CO₂ + H₂O
max yield = 1 mol/mol (0.50 g/g, 67 C-mol %)

Sugar
Glycolysis, Oxidative TCA

succinyl-CoA → succinate → 4-hydroxybutyrate → 4-hydroxybutyryl-CoA → 4-hydroxybutyraldehyde → 1,4-butanediol

ATP = 0
NAD(P)H = +1

ATP via oxidative phosphorylation

1,4-butanediol

Lowering by-products and increasing rate
Engineering of the BDO pathway enzymes and central metabolism

Reducing by-products:
1. Increases yield, rate
2. Lowers separations costs

Strategies implemented:
• CO₂ – sucCD deletion
• Glutamate - sucAB-lpdA OE
• Acetate - ack/pta OE
• GBL – novel lactonase OE
• EtOH - ppc OE & ald-specificity
• 4-HB – next major challenge...

1,4-BDO
Continuous BDO organism improvement
Exceeded all commercialization targets, further space for improvement

- **Titer** > 140 g/L
- **Rate** > 3.0 g/L/hr
- **Yield** > 100%

\( Y^* = \% \) of commercial yield target
Systems-based strain engineering
Uniquely integrated computational and experimental capabilities

SimPheny Lab
LIMS

Parent strain

Omics data
Systems analysis

Product
Feedstock
Organism & Tools

Select

Transporter
engineering

Regulatory
network engineering

By-product deletions

Pathway engineering

Iterative
Strain
Engineering

Data

Engineered
strains

Fermentation
development/
scale-up

SimPheny®
Computational Technologies

Bioinformatics,
Metagenomics,
Protein Engineering

High Throughput
Cloning

High Throughput
Screening

13C-Fluxomics
Metabolomics
Proteomics
Transcriptomics
Genomics
1,3-BDO pathway and process – not 1,4 BDO 2.0
New pathway, new organism, new fermentation, but leveraging knowledge

1,4-BDO vs. 1,3-BDO Project

Similarities
- Theoretical yield
- Purification process
- Some enzyme classes

Differences
- Pathway modifications
- Central metabolism design
- Number/types of byproducts
- Fermentation process
Platform driving ever-faster milestones
2.5X faster to hit milestone compared to commercial BDO program

Titer

Time (months)

1,3-BDO program

10 mos

54 g/L

1,4-BDO program

27 mos

51 g/L

10 mos

54 g/L

15 mos

16 g/L

16 g/L

50 g/L
Enzyme Engineering in 1,3-BDO
Tightening substrate specificity to remove ethanol as a byproduct

![Biomass Scurpas](image)

**Discovery and screening of ALD collection**

**Structure guided directed evolution**

**Improved Enzyme Specificity**

16-fold improvement in specificity, effectively removing ethanol as a byproduct
Technology & Commercial Innovations

1) Process Development & Commercialization
2) Accelerated Organism Development
3) Expanded Feedstock Enablement
4) Rapid Pathway Prototyping
Advancing Prospects for Biomass Feedstocks
Setting clear specification for pretreatment technology providers

- Integrated approach (feedstock + strain + down stream process + final product application)
- Modified dextrose based strain to co-utilization C5 and C6 sugars, reduce diauxic growth
- Differential responses observed due to variation in biomass hydrolysate components
- Qualified 4 pretreatment technology providers out of 12 on quality (not cost)

Table: Relative Performance

<table>
<thead>
<tr>
<th></th>
<th>Titer</th>
<th>Rate</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysate B</td>
<td>88%</td>
<td>100%</td>
<td>91%</td>
</tr>
<tr>
<td>vs. Refined Dextrose</td>
<td></td>
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</tr>
</tbody>
</table>
Qualifying pretreatment technologies
Setting clear specifications for input quality to enable cost benchmarking

2.5X Product Cost

REFINED CORN DEXTROSE  BIOMASS HYDROLYSATE A  BIOMASS HYDROLYSATE B

- Downstream Materials
- Consumables
- Utilities
- Waste Disposal
Technology & Commercial Innovations

1) Process Development & Commercialization
2) Accelerated Organism Development
3) Expanded Feedstock Enablement
4) Rapid Pathway Prototyping
TX-TL Technology Implementation
Successfully adapted for industrial metabolic engineering

Enables substantial reduction in resources and cost for pathway building and optimization

- Cell-free system allowing for **rapid expression and activity screening** without the need for plasmid based cloning and **in vivo** propagation. (linear DNA)
- No cell wall or cellular viability allows for a **flexible platform for constructing and characterizing complex biochemical systems and pathways**
- Suitable for **screening toxic** metabolites and proteins.
- Can **optimize pathway flux** by directing resources to user defined objectives.
- **Central metabolism, oxidative phosphorylation**, and **protein synthesis** can be co-activated.
TX-TL: Rapid Expression Screening
1,4-BDO pathway expression validated using linear DNA

- Validated expression of 4 out of 5 BDO pathway genes.

- Expression of Cat2 not detected (as expected; not detected in *in vivo* experiments via SDS-PAGE).

**Expression Validation: Caliper-LabChip**
TX-TL: Pathway Demonstration

1,4-BDO production validated in wild-type and advanced strain extracts

Parts Library

Wild-type and Engineered Variants (*)

Linear DNA

TX-TL BL21 Extract Energy Solution/AAs

1,4-BDO, GBL and 4HB Production in TX-TL (BL21)

1,4-BDO and 4HB Production in TX-TL (Advanced Strain)
TX-TL: Optimizing Pathways/Bottlenecks
Optimizing 1,4-BDO production

Parts Library

Wild-type and Engineered Variants (*)

HT Cloning

sucD +
4hbD +
Cat2 +

Promoter Strength

ADH ♦
ALD ♦

Linear DNA (equal molar)
TX-TL BL21 Extract

Energy Solution/AAAs

Promoter
Strength

ADH ♦

[ADH]

[GBL]

[4HB]

0.1 2.6 3.6 1.9 1.2 0.4 0.3 0.7

[ADH]

[GBL]

[4HB]

0.0 2.7 4.9 0.6 0.4 0.5 1.9

ADH Promoter Strength

ALD Promoter Strength
Establishing polyamide intermediate pathways
TX-TL rapidly enabling C6 intermediate & specialty chemicals opportunities

Single Pathway

Multiple Products

Acetyl-CoA + Succinyl-CoA
\[ \text{HO}_2\text{C} - \text{CO} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{CO} - \text{OH} \]
Levulinic Acid

3-Oxoadipyl-CoA
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{C} - \text{CO} - \text{S} - \text{CoA} \]
Adipic Acid

Adipyl-CoA
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
6-Aminocaproic Acid

6-Aminocaproic Acid
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{C} - \text{NH}_2 \]
Caprolactam

Caprolactam
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{C} - \text{NH}_2 \]
Hexamethylenediamine

Caprolactone
\[ \text{HO}_2\text{C} - \text{C} - \text{S} - \text{CoA} \]
\[ \text{HO}_2\text{C} - \text{C} - \text{NH}_2 \]
Hexanediol
Feedstock & Product Diversification Strategy
providing feedstock optionality and flexibility customized to the chemical

- Starch Crops
- Sugar Crops
- Biomass
- Waste
- Natural Gas

Fermentable Sugars
Fermentable C1’s

Biotechnology Enabled Conversion

BDO
More than Bugs: A practical look at developing commercially-advantaged bioprocesses, addressing Development cost & timelines | Production economics

1) Integrated approach to bioprocess development
2) Innovations – chemicals, feedstocks, timelines
3) Directions for future process technology developments
Biotechnology for sustainable chemicals
Harness new feedstocks; better way to make the exact same chemicals

- Hydrocarbon Volatility
- Capital Intensive
- Environmentally Challenged

- Feedstock Diversification
- Differentiated Products
- Sustainable Footprint
our core purpose

Lead the irresistible transition to sustainable materials through our technology and, united with industry leaders, make our world a better place.

our core values

we are real
Results count. Commitments count. Integrity and honesty are absolutes.

we are innovative
We invent, experiment and create—across our entire business. We seek out and embrace differences, to help us think differently.

we are united

we are relentless
We don’t give up. We strive for excellence. Our passion flows from our shared vision.