INDUSTRIAL ENZYMES FOR CELLULOSIC SUGARS AND BEYOND

THE POWER AND SPEED OF TAILORED SOLUTIONS

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CTO
METGEN STRATEGIC FOCUS
Lignocellulosic biomass conversion in multiple segments

- **PULP AND PAPER**
  - Packaging, Tissue, Paper

- **RENEWABLE CHEMICALS**
  - Biobased plastics

- **BIOFUELS & ENERGY**
  - Energy & Liquid fuels

- **BIOMATERIALS**
  - Nanocellulose, Lignin-based polymers

- **WASTE WATER & SIDE STREAMS**
  - Clean water, biogas, Chemical building blocks
TARGETED MARKETS

Pulp, Tissue, and Packaging

Up to M€1.5 / mill through:
• Energy and cost savings
• Product quality & process improvements
• Sustainability improvements

Biorefineries and Renewable Chemicals

Making industry sustainable by:
• Lowering technology costs
• Lowering CAPEX
• Enabling new & better products
NEW BUSINESS DEVELOPMENT

Biogas

Improving biogas production by:
• Reducing inhibition of feedstock, waste and operational cost
• Better utilization of raw material
• Raw material substitution

Waste Water Treatment

Enhancing water treatment by:
• Reducing inhibitors, waste, and operational cost
• Lowering soluble phenols & hard COD
• Reducing micro pollutants
ENZINE® TECHNOLOGY PLATFORM
STRONG CHAIN OF COMPETENCE

• **FAST**: Industry leading short development cycle

• **FLEXIBLE**: Tailored enzyme design approach to meet challenging industrial conditions

• **ADAPTABLE**: Can produce large amounts of different enzymes, cost-effectively and supply globally

• **PROVEN**: Developed several novel enzymes from concept to validation at industrial scale and production
**METZYMES® COVER THE FULL VALUE-CHAIN IN BIOPROCESSING**

<table>
<thead>
<tr>
<th>Side streams and environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct enzymatic chemical conversions</td>
</tr>
<tr>
<td>Utilization of sugars</td>
</tr>
<tr>
<td>Hydrolysis</td>
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**Substrate**
- Various combinations of feedstock and pre-treatment
  - Softwood, hardwood, straw...
  - Steam explosion, acid treatment, extrusion...
  - Tailored enzymatic solutions are a MUST for processing biomass

**Sugar platform**
- High Yields
- Affordable sugars
- High solids %
- Lower capex
- Less inhibitors
- Valorization of lignin

**Fermentation**
- Less inhibitors
- Higher productivity
- Higher carbon yield

**Chemical building blocks**
- 5-HMF, FDCA
- LIGNIN MODIFICATION
- Fructose, xylitol, etc
- Streamlined processes
- Utilization of all sugars
- High conversion rates

**Water & biogas**
- Addressing lignin and phenolics
- Thorough utilization of nutrients
- Neutralizing toxins
- Less COD & waste
- Efficient processes

**LIGNO™**
- Substrate

**SUNO™**
- Sugar platform
- Fermentation

**PURECO™**
- Chemical building blocks

**FORICO™**
- Water & biogas

**FORCI™**
- Substrate
## Challenge
Fractionated technology offering makes it difficult to select and rely on unproven combination of processes.

## Market Opportunity
Enables cellulosic chemical production. First technology package already offered → path to advanced materials.

## Desired Effect
Affordable sugar platform, valorization of all fractions of biomass, and flexibility in changing end-products.

## Solution
MetZyme® is tailored to match the requirements of the pretreatment and the following processes, including end-product quality.

## Status and next steps
Letter of Intent signed with collaboration partner, a common technology offering to build a new 100 000 ton ethanol facility.
METZYME® SUNO™ - STRONG SOLUTION FOR HYDROLYSIS AND SACCHARIFICATION
STREAMLINED CHEMO-ENZYMATIC ROUTE TO PLATFORM CHEMICALS

**BIOCONVERSION**

Glucose → MetZyme® PURECO™ → Isomerase → Fructose

**CHEMICAL CONVERSION**

Fructose → Dehydration → HMF Oxidation → HMF

Redacted
YIELD INCREASE WITH METGEN’S PROCESS

Process yields by unit operation and overall for single pass of raw material

**Conventional process**

- Glucose 100%
- Isomerization to Fructose *Yield 45%
- Separation of sugars Yield 85%
- Dehydration of Fructose to HMF **Yield 62%
- Oxidation to FDCA Yield 95%

Cumulative Yield: 45% 38% 22% 20%

**MetGen’s process**

- Glucose 100%
- Bioconversion to [Redacted] Yield 95%
- No sugar separation needed
- Dehydration of [Redacted] to HMFA **Yield 92%
- Oxidation to FDCA 95%

Cumulative Yield: 95% 87% 83%

*Recovery of glucose after separation excluded, however, potential increase to process yield remains limited
**Technical assumption on maximum yields for dehydration is based on known furan yields in literature
**ACTION OF LACCASE ON LIGNIN**

**ACTIVATION OF LIGNIN STRUCTURE**

- Electron withdrawal from subunit in lignin
- Increased reactivity

**Bond Cleavage**
- Electron withdrawal from subunit in lignin
- Increased reactivity

**Modification**
- Change in functional group
- Acetylation and demethylation

**Coupling**
- Attachment of phenylpropanoids and/or Mediators

**Depolymerization**
- Consecutive cleavage
- Decrease in average molecular weight & release of lignin substructures

**Grafting**
- Single attachment for changes in properties (e.g. Solubility)

**Polymerization**
- Continuing attachment
- Increase in average molecular weight & capture of lignin substructures

### Challenge
Average mill spends €20 million/year on energy, refining is 50% of the electricity bill

### Solution
Reduce energy consumption by treating wood chips with MetZyme® LIGNO™

### Results
20% less electricity consumption and final product strength increased 10-15 %

### Value Proposition
Net Savings to Mill operator 1.5 M€ / year

### Reference
Industrial Mill trials
DELIGNIFICATON OF KRAFT PULP

**Challenge**
Cost and environmental aspects of bleaching chemicals
No sustainable alternative exists due to high pH and temperature

**Market Opportunity**
Higher yield, Less bleaching chemicals.
For pulp mill +2% yield means >10M€ production increase

**Desired Effect**
 Obtaining Kappa, viscosity, and brightness targets with higher yield

**Solution**
Novel and unique MetZyme® LIGNO™ with operational optimum of pH 10-11 and thermostability up to 80 °C

**Status**
Patent filed, lab and pilot experiments are on-going
**METZYMÉ® LIGNO™ FOR BIOREFINERIES:**
**LIGNIN MODIFICATION / VALORIZATION**

**Challenge:** Economics of modern Biorefineries require commercial lignin utilization (other than burning)

**Market Opportunity:** Economically viable drop-in substitute for oil-based chemicals in binders, fillers, foams, plasticizers, coatings, etc. More defined aromatic compounds open up new chemistries.

**Desired Effect:** Affordable lignin fractionation process for smaller molecules in water-soluble conditions

**Solution:** Novel and unique MetZyme® LIGNO™ with operational optimum of pH 10-11 and thermostability up to 80 °C

**Status and next steps:** Lab test completed, Patents in preparation. Demo scale next.
LIGNIN FRACTIONING

MetZyme® LIGNO™ fractionates the bulk of industrial lignin at fully soluble conditions @ pH 10.5 – *in water without a mediator*
# LIGNIN POLYMERIZATION

**Challenge**

Hemicellulose is often after pre-treatment contaminated with lignosulfonates or other monomeric lignols rendering sugar unusable for fermentation.

**Market Opportunity**

Increases the overall economics of biorefineries, enables further valorization of C5 sugars to renewable chemicals.

**Desired Effect**

Filtration is easily and energy efficient, C5 sugars are purified and valorized.

**Solution**

MetZyme® LIGNO™ polymerizes the lignin-based monomeric and colloidal particles.

**Status and next steps**

Pilot with collaboration partner.
Renewable Materials – Nanocellulose

### Challenge
Current process requires 10x energy of TMP refining to achieve cellulose nanocrystals (CNC)
Enzymatic solutions convert pulp to sugar and reduce yield

### Market Opportunity
250M€ global market by 2019 **(MarketsandMarkets.com research)**
Nanocellulose has incredible potential in material industry:
Photonics, composites, medical, modifications, films, foams

### Desired Effect
Energy saving in refining and higher dry-matter content

### Solution
Pure MetZyme® PURECO™ endoglucanase only fibrillates but do not cut the fibre to sugars

### Status and next steps
Lab trials ongoing, pilot scale with collaboration partner next, BIOFOREVER
CLEAN FRACTIONS HAVE PLENTY OF USES

CLEAN LIGNIN:
- Foams: polyurethanes, expanded polystyrene, starch
- Biopolymers: resins, polyols, binders
- Packaging materials: fillers, biodegradable components
- Activated carbon

**CARBON CONVERSION**

<table>
<thead>
<tr>
<th>100%</th>
<th>35%</th>
<th>50%</th>
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<tbody>
<tr>
<td>Sugar</td>
<td>Lignin</td>
<td>WASTE LIGNIN TO REPLACE LOW-VALUE OIL AND GAS</td>
</tr>
<tr>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td></td>
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</tbody>
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**TARGET**
- 65% HIGH QUALITY SUGAR FOR SPECIALTY CHEMICALS

**CONVENTIONAL**
- 50% LOW VALUE SUGAR FOR FUEL ETHANOL

**Sustainable Packaging Market**

![Graph showing the growth of the sustainable packaging market from 2008 to 2018](image)
ADDRESSING LIGNIN ENABLES ENTIRE INDUSTRIES

• BIOREFINERIES
  – Cellulosic sugar platform cost is lowered through valorization of lignin
  – New sugar-based chemistries are enabled together with lignin valorization
  – More thorough purification of other streams can be achieved by addressing lignin

• BIOGAS
  – Lignin also inhibits anaerobic fermentation processes
  – Potential to increase productivity and reduce waste

• WATER TREATMENT
  – Microbial water treatment is inhibited by phenolic content
  – Addressing lignin provides up to 20% improvement in efficiency
  – Decreased COD, VFA, and waste

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>COD (mg/L)</th>
<th>Lignin (mg/L)</th>
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<tbody>
<tr>
<td>Untreated</td>
<td>8555</td>
<td>187.7</td>
</tr>
<tr>
<td>MetZyme®</td>
<td>1941</td>
<td>109.4</td>
</tr>
<tr>
<td>FORICO™</td>
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ON-GOING H2020 DEVELOPMENT PROJECTS

36 PARTNERS IN 6 EU PROJECTS WITH TOTAL OF 36.3 M€
METGEN’S TOTAL EU PROJECT FINANCING: 4.6 M€

- **Soft & Hard Wood**: Glucose Isomerase + Hydrolysis Enzymes
- **Forestry Feedstocks**: Inhibitor Resistant Glucose Isomerase
- **2nd Gen Biorefinery Lignin**: Solvent Tolerant Industrial Laccase
- **Straw & Grass**: Detoxification + Lignolytic Enzymes + Hydrolysis Solution
- **Biowaste**: Detoxification + Lignolytic Enzymes + Hydrolysis Solution
- **Renewable Plastic**
- **Chemical Building Blocks**
- **Marine Diesel**
- **Butanol**
- **Sugar Platform**
ACKNOWLEDGMENT OF PUBLIC EU H2020 FUNDING

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CONCLUSIONS

METGEN HAS A UNIQUE TECHNOLOGY PLATFORM AND THE TEAM TO SUPPORT UNPARALLELED SPEED-TO-MARKET:

MetGen Team and ENZINE™ will:
• address the LIGNOCELLULOSIC market with full product portfolio,
• change the future of bio-refining and
• pave the way to renewable chemicals via enzymatic routes,
• open up new opportunities in water treatment and biogas sectors.
BIO-BASED INDUSTRY REVOLUTION REQUIRES ONLY ONE MORE THING: COLLABORATION!

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