Biomass Biorefinery Network

4th BBNet Conference

The future prospects for biorefining: Feedstocks, technologies and products

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Lignocellulosic feedstocks and the digestibility challenge

Leonardo Gomez
University of York
Centre for Novel Agricultural Products

BBNet conference 2025

Biorefining 101

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Centre for Novel
Agricultural Products

Biomass Feedstock

Pretreatment

Enzymatic hydrolysis

Fermentation

Product recovery

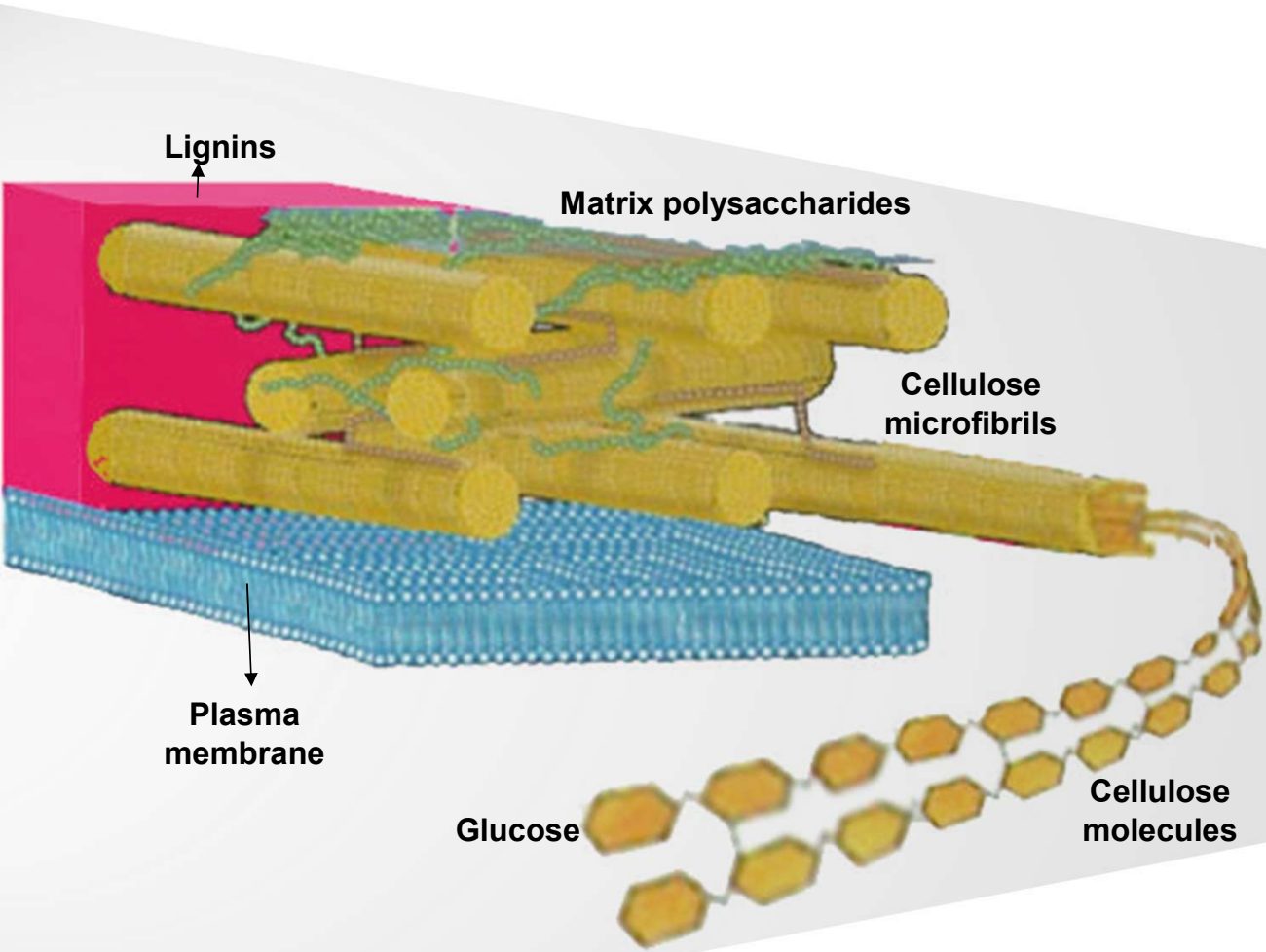
Biorefining 101

Biomass
Feedstock

Volume?
Price?
Logistics?

Quality?

Biomass feedstocks are essentially plant cell walls



Can we improve biomass to improve its uses?

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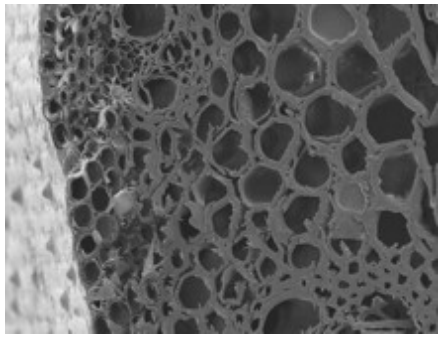
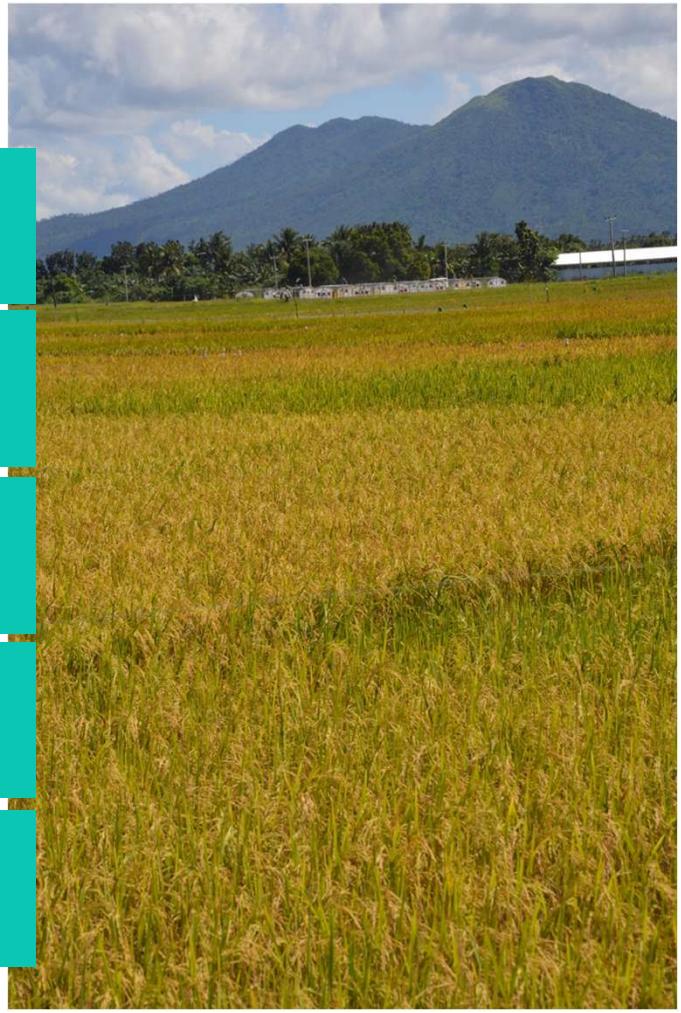
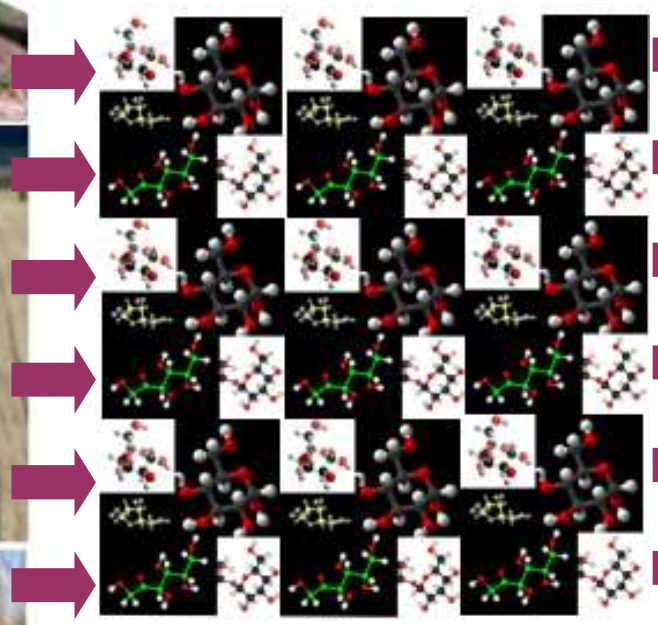


Changing plant characteristics

- Food
- Chemicals
- Liquid fuels
- Feed
- Fertilizers



THE RECALCITRANCE CHALLENGE



Chemicals

Fuels

Materials

Food

Feed

- Identify the molecular basis of recalcitrance to saccharification

Identifying the molecular basis of recalcitrance to saccharification

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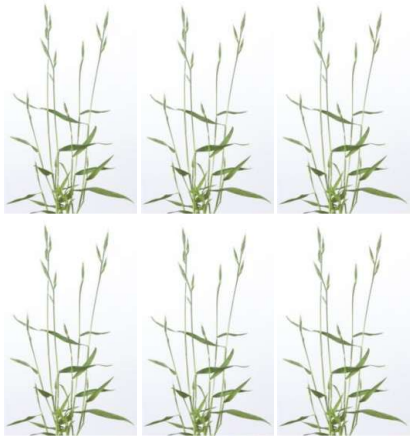
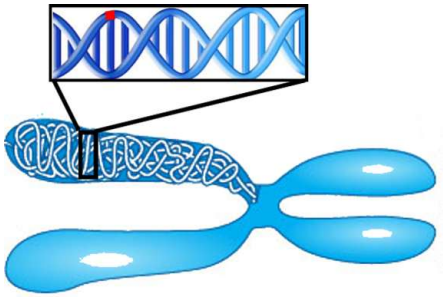
Screen plants for digestibility



Population

Identify plants with high digestibility



Map the mutation and improve biomass quality

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Saccharification mutants show a range of cell wall alterations

	sac1	sac2	sac3	sac4	sac5	sac6	sac7	sac8	sac9	sac10	sac11	sac12
lignin			↓↓↓*	↓↓↓↓*	↓↓↓*		↓	↓↓	↓↓*	↓	↓↓↓*	↓↓↓*
hemicellulose				↑↑*	↑↑*		↑↑↑*	↓				
crys cellulose	↓↓↓*			↓↓	↓↓	↓↓	↓↓↓↓*		↑		↓↓	↓↓
ferulic acid	↓↓↓*		↓	↓↓↓*		↓		↓↓			↓	
S:G ratio	↓↓↓↓*	↓		↓↓↓↓*		↓↓↓↓*	↓↓↓↓*	↑	↓↓↓*	↓	↓	
H monolignols	↑↑↑↑*	↑↑↑↑*	↑↑	↑↑↑↑*	↑↑	↑↑↑↑*	↑↑↑↑↑*	↓	↑↑↑↑↑*	↓↓	↑	

0 arrows: 0-5% increase/decrease
1 arrow: 5-10% increase/decrease
2 arrows: 10-20% increase/decrease
3 arrows: 20-30% increase/decrease
4 arrows: 30-40% increase/decrease

Plants with reduced crosslinking between polymers have higher saccharification

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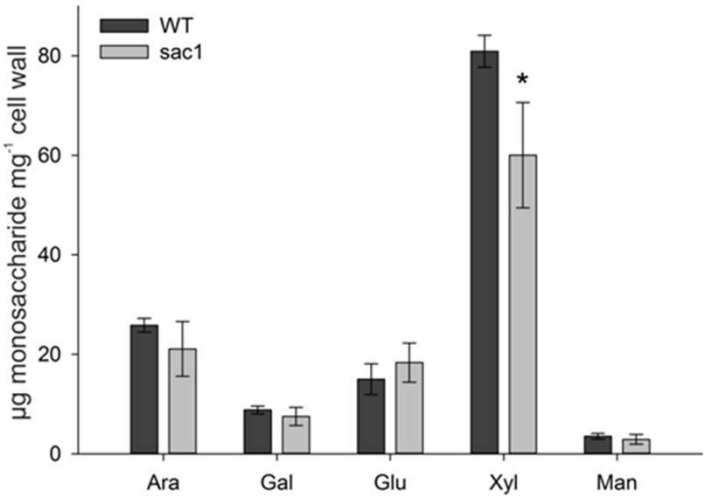
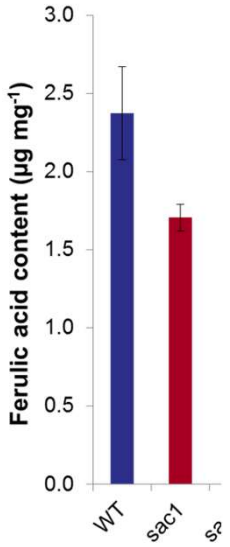
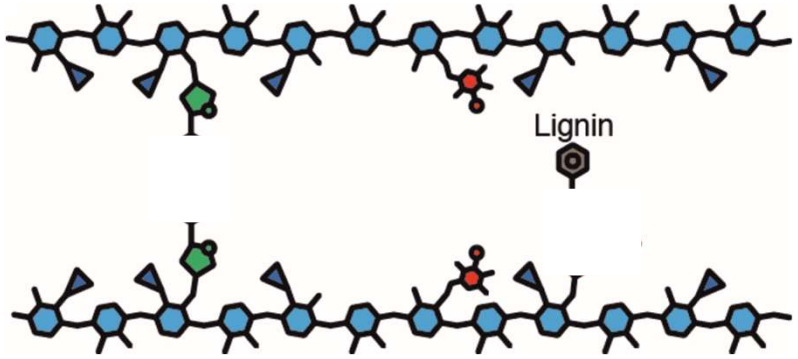
sac1 plants have a mutation in an orthologue of the rice *XAX1* gene

GT61 xylosyl transferase is required for xylan feruloylation

sac1 has slightly lower xylose and ferulic acid content







Plants with reduced crosslinking between polymers have higher saccharification

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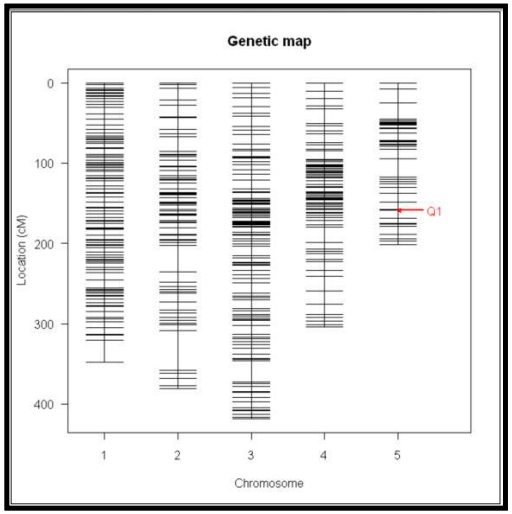
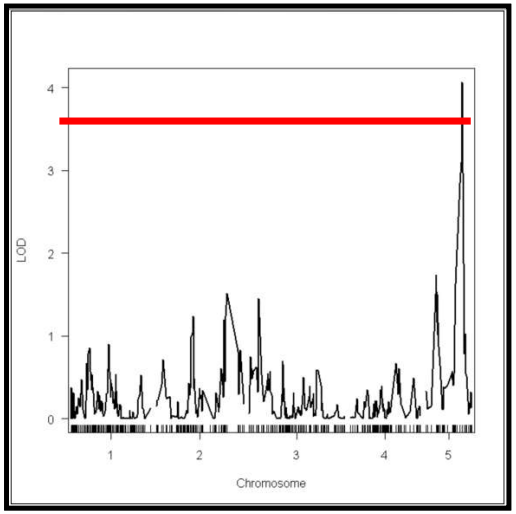
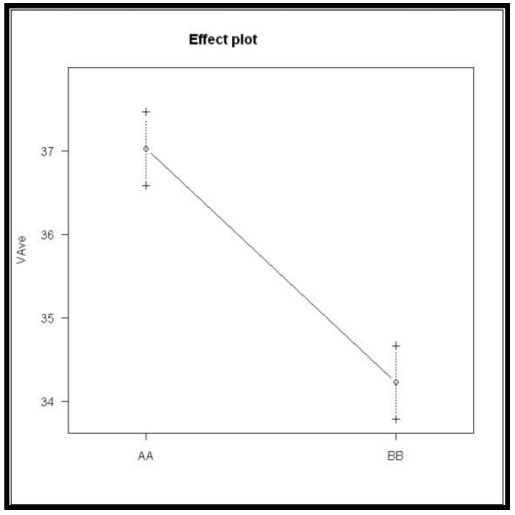
GT43/IRX14 modulate the recalcitrance of the cell walls







- QTL Analysis
  - Chromosome 5
  - LOD 5% = 3.03
  - Accounts for 11.83% of the genetic variance of the trait
  - Allele AA has an increase in digestibility when compared with allele BB

GT43/IRX14 modulate the recalcitrance of the cell walls

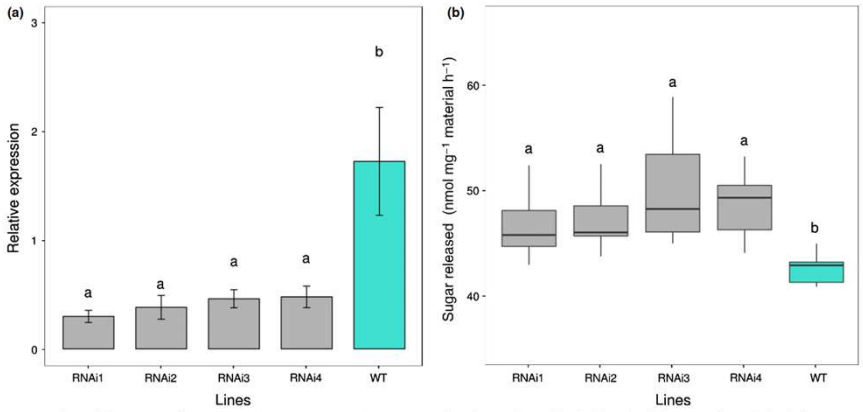
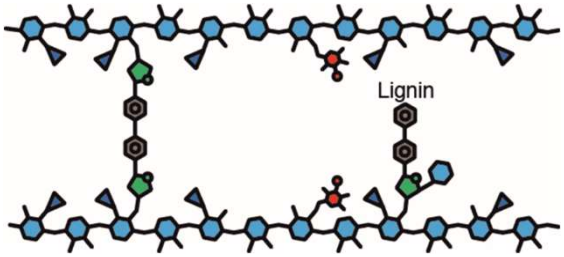
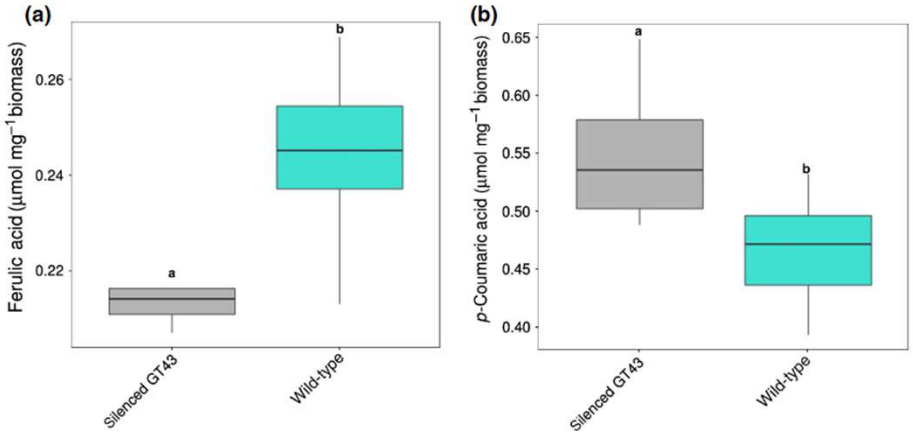


Fig. 5 Analysis of the RNA interference (RNAi) transgenic straw. (a) Transcript abundance indicated by the bar plot of the means for each level of independent variable of ANOVA showing ± SE. Tukey's honest significant difference (HSD) test indicates that those sharing the same letter are not significantly different. (b) Saccharification in silenced lines shown as boxplots to highlight the mean (line), 25th–75th percentile (box) and 10th–90th percentile (whiskers) of the glucose released for each genotype. Tukey's HSD test indicates that those sharing the same letter are not significantly different.





Identifying Quantitative Trait Loci for saccharification in rice

- 151 rice accessions from the Field Crops Research Institute (FCRI),Vietnam.
- 200 accessions from PhilRice, Philippines
- The samples are analysed across two seasons (2500 samples/season)

GWAS for Saccharification

Nguyen et al. Biotechnol Biofuels (2020) 13:165
https://doi.org/10.1186/s13068-020-01807-8

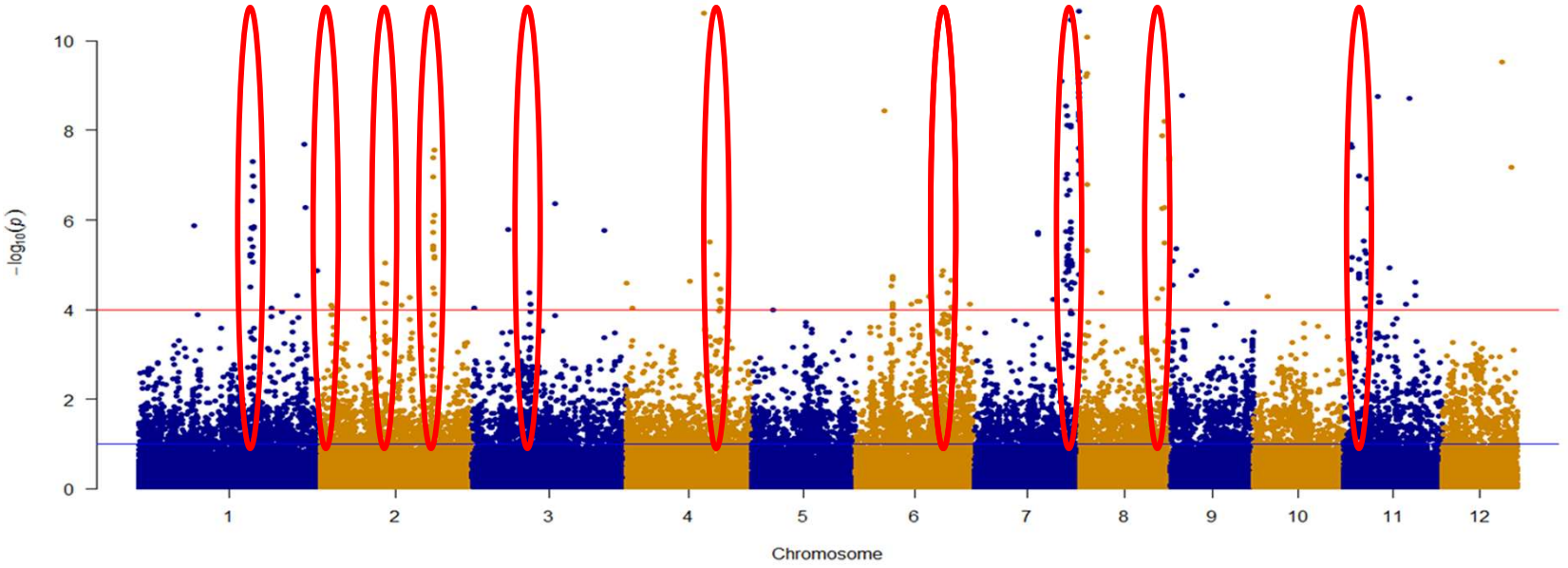
Biotechnology for Biofuels

RESEARCH Open Access

Association mapping identifies quantitative trait loci (QTL) for digestibility in rice straw

Duong T. Nguyen[1,7], Leonardo D. Gomez[2*], Andrea Harper[2], Claire Halpin[3], Robbie Waugh[3,4,5], Rachael Simister[2], Caragh Whitehead[2], Helena Oakey[3,5], Huong T. Nguyen[1], Tuat V. Nguyen[6], Tu X. Duong[1] and Simon J. McQueen-Mason[2*]

OsAT10



Reverse genetics: targeting lignin

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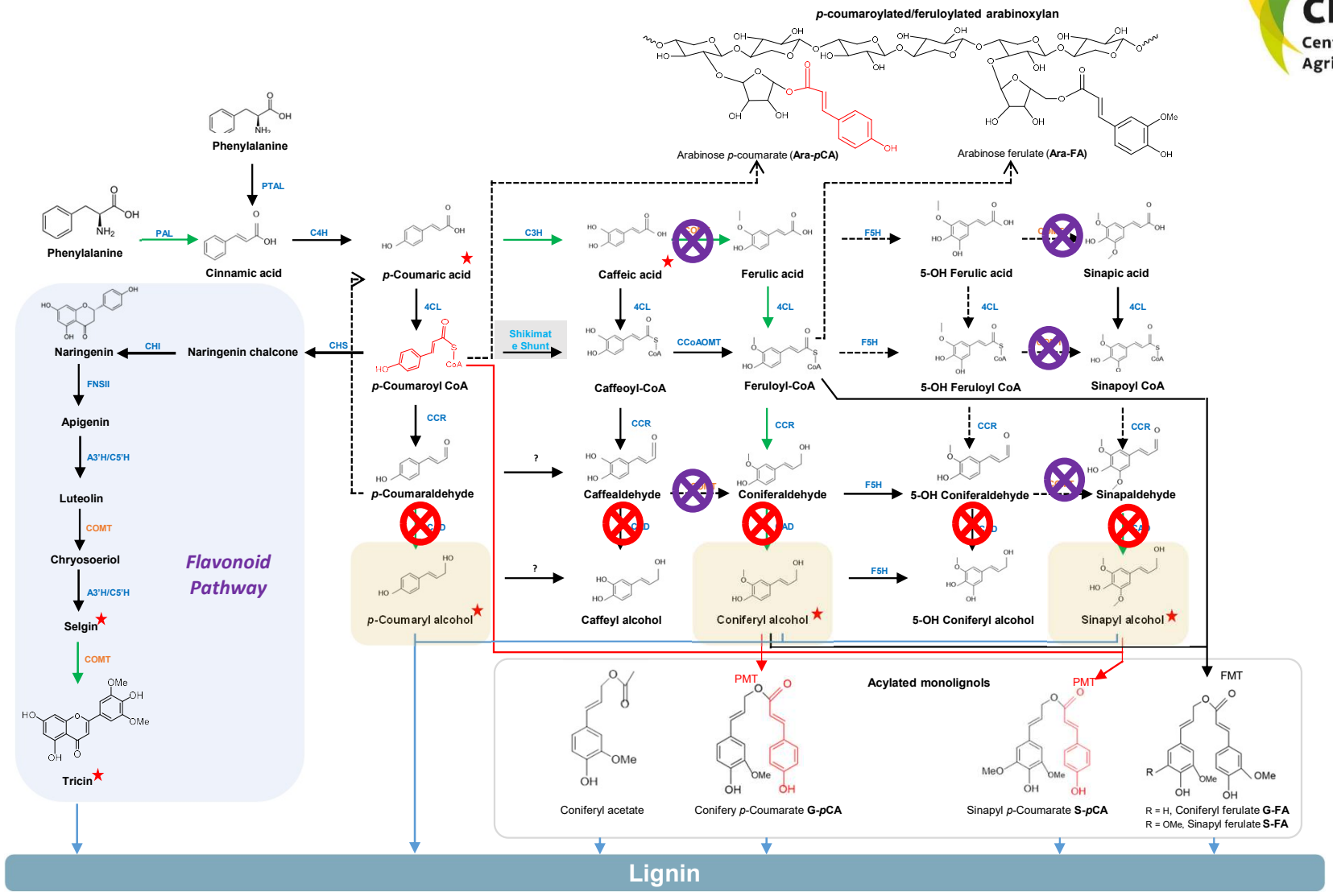

Reverse genetics: targeting lignin

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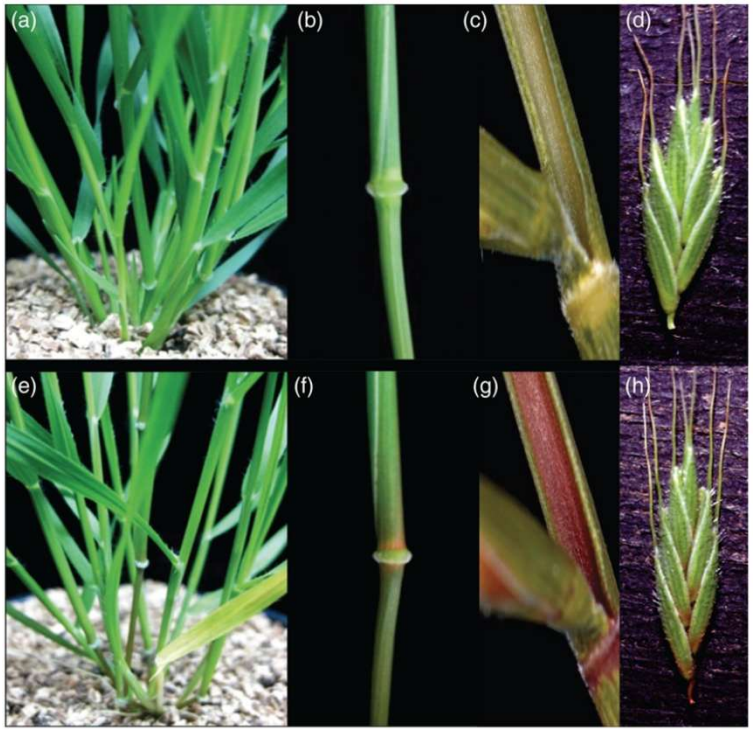
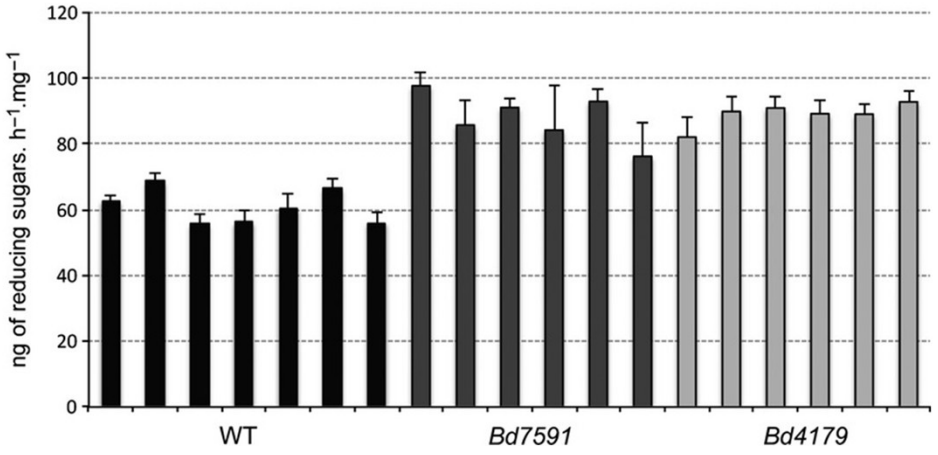


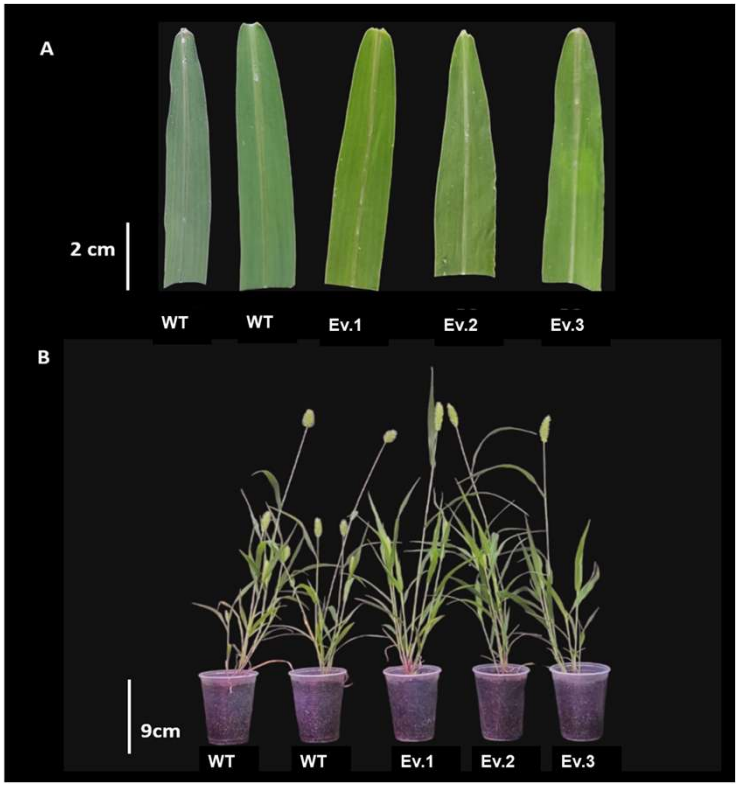
the plant journal
SEB
Original Article | Free Access

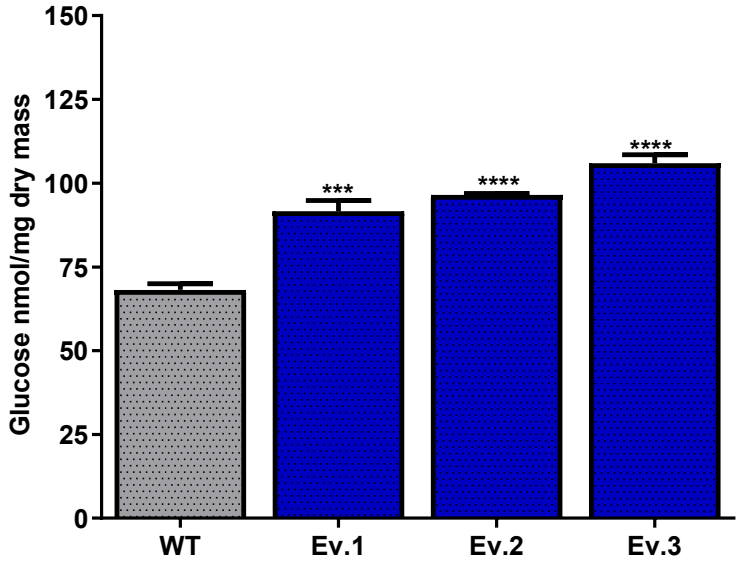
Disrupting the *cinnamyl alcohol dehydrogenase 1* gene (*BdCAD1*) leads to altered lignification and improved saccharification in *Brachypodium distachyon*



Reverse genetics: targeting lignin

CRISPR/Cas9 know out of ***COMT1*** in *Setaria viridis*

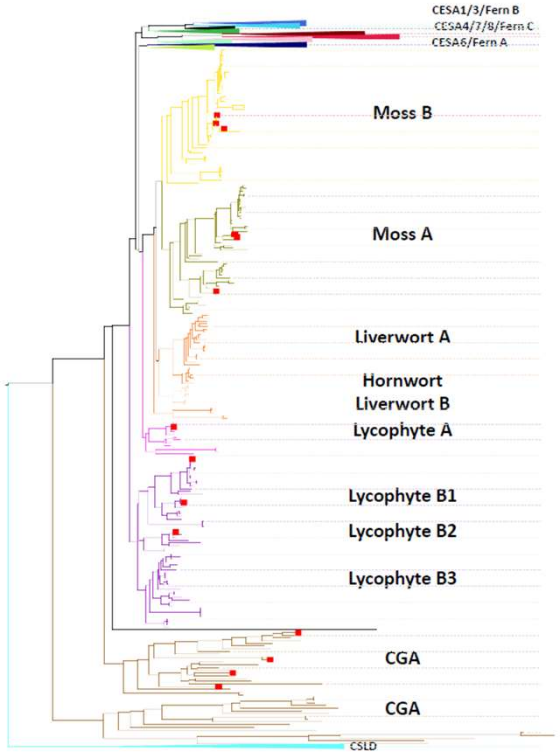


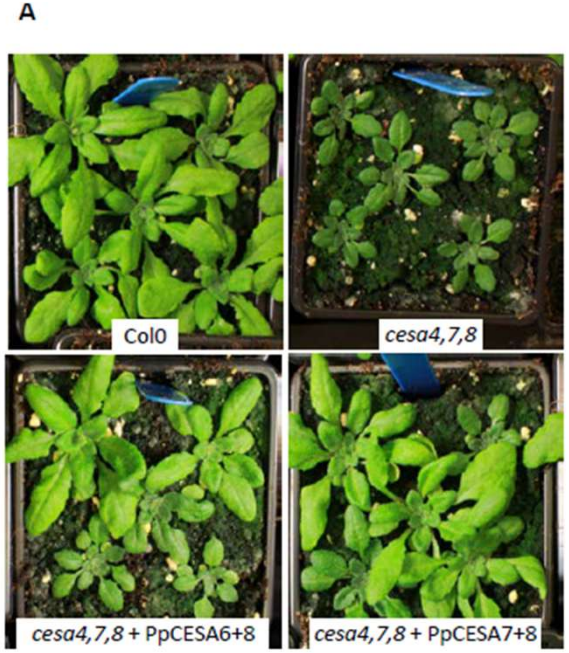


No pretreatment

Wagner Rodrigo de Souza (submitted)

Reverse genetics reducing cellulose crystallinity





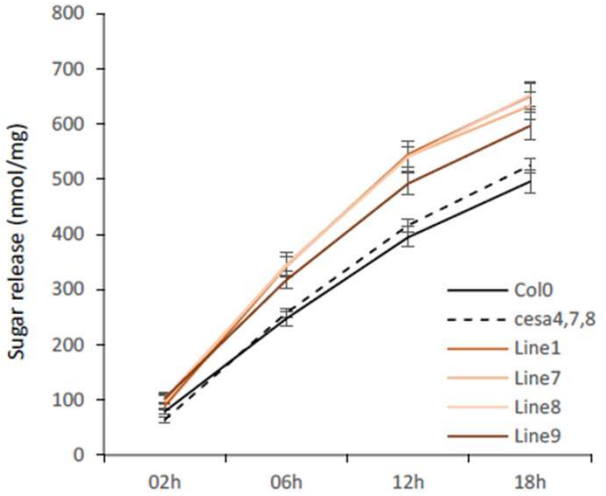


Figure 7. Digestibility assays of Arabidopsis *cesa4,7,8* triple mutant complemented with Physcomitrella genes PpCESA6 and PpCESA8.

Simon Turner, Manoj Kumar (submitted)

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Do engineered plants show differences under field conditions?

Do engineered plants show differences under field conditions?

frontiers | Frontiers in Plant Science

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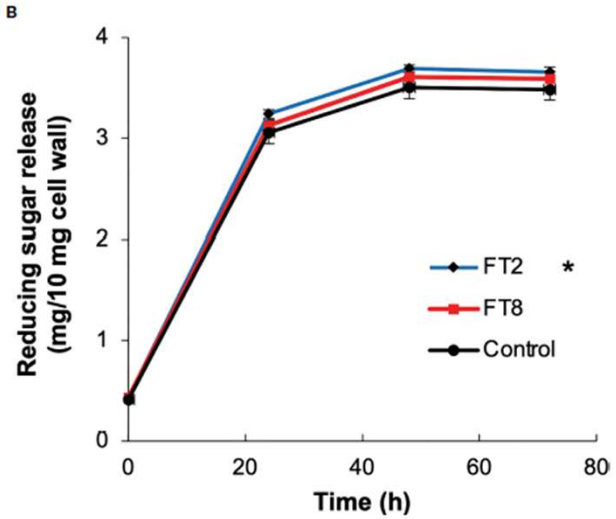
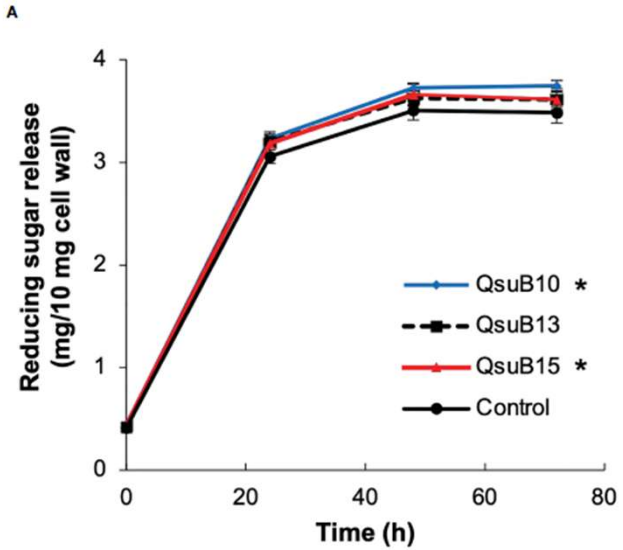
Field performance of switchgrass plants engineered for reduced recalcitrance

Aymerick Eudes[1,2], Chien-Yuan Lin[1,2], Christopher De Ben[1,3], Jasmine Ortega[1,2], Mi Yeon Lee[1,2], Yi-Chun Chen[1,2], Guotian Li[1,2,4], Daniel H. Putnam[1,5], Jenny C. Mortimer[1,2,5], Pamela C. Ronald[1,2,4], Corinne D. Scown[1,6,7] and Henrik V. Scheller[1,2,8]*





Do engineered plants show differences under field conditions?

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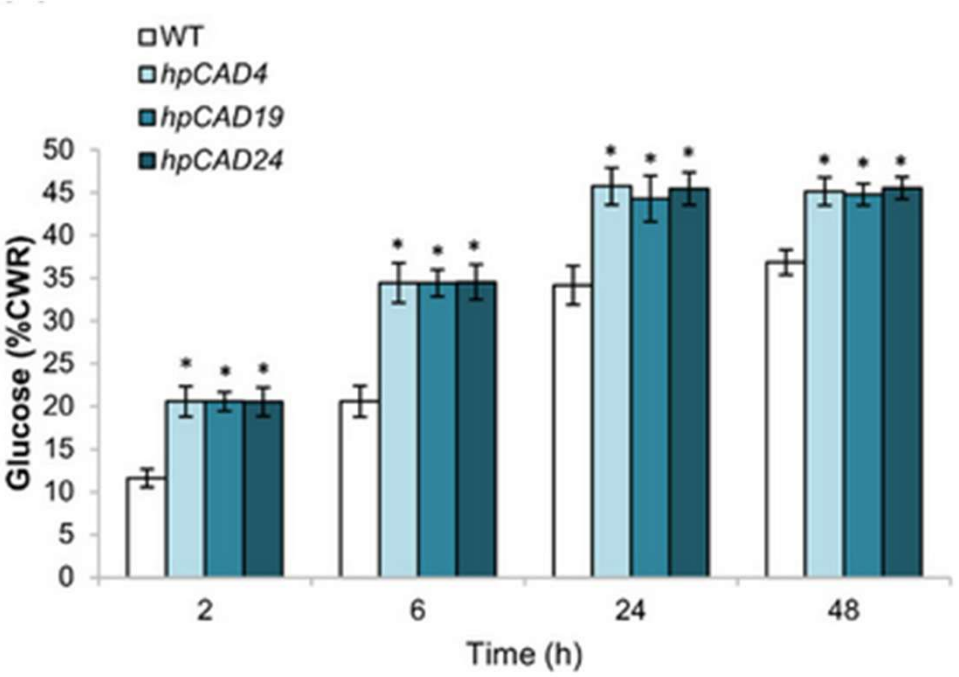
New Phytologist

Full paper | Free Access

Field and saccharification performances of poplars severely downregulated in *CAD1*

Barbara De Meester, Rebecca Van Acker, Marlies Wouters, Silvia Traversari, Marijke Steenackers, Jenny Neukermans, Frank Van Breusegem, Annabelle Déjardin, Gilles Pilate, Wout Boerjan

First published: 08 July 2022 | https://doi.org/10.1111/nph.18366 | Citations: 14



Do engineered plants show differences under field conditions?

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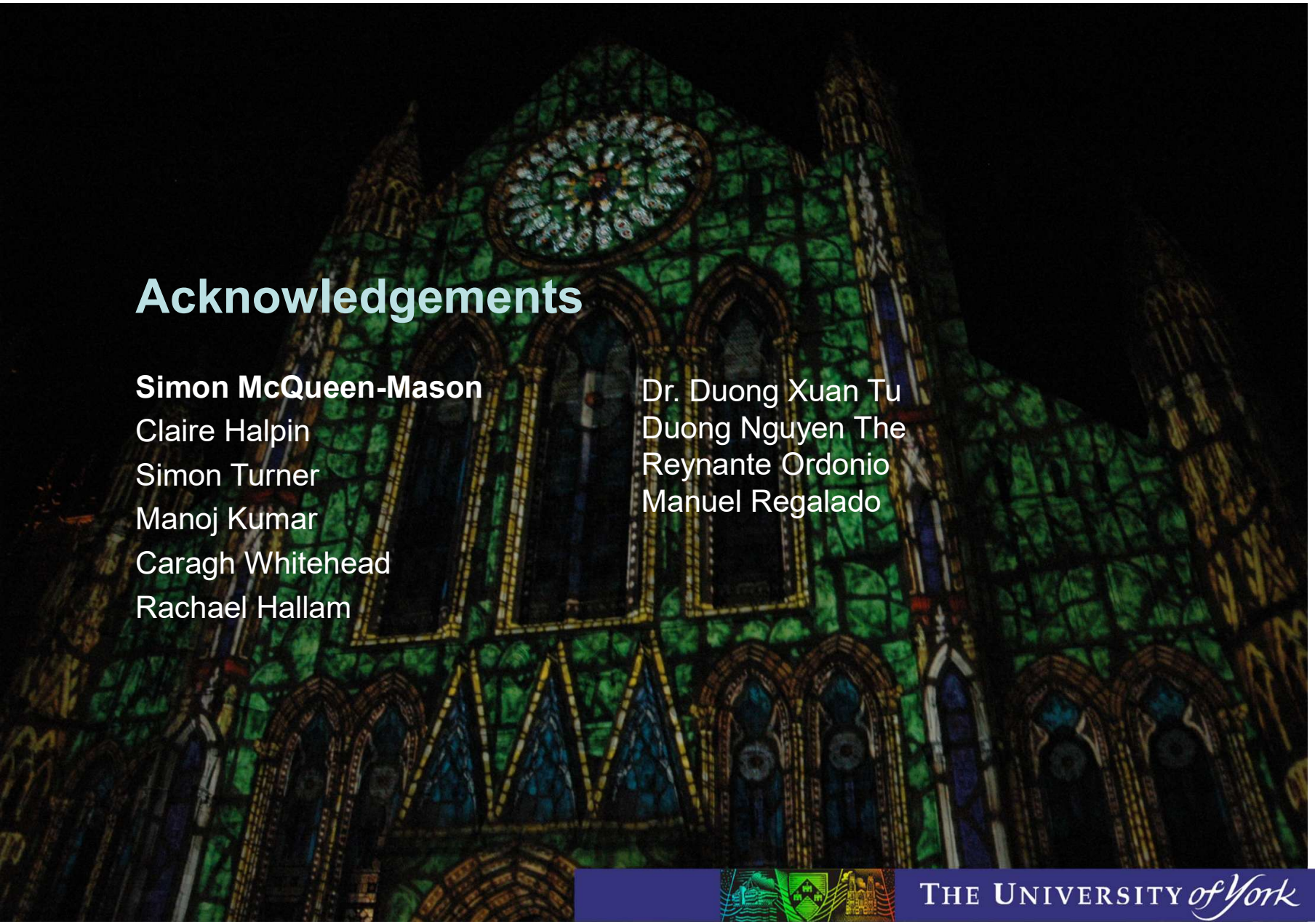
Plant/Crop	Genetic/Metabolic Target	Key Outcome
Switchgrass (QsuB and OsAT10 lines)	Reduced lignin and ferulic acid	Minimal saccharification or no improvement in the field
Aspen/Poplar (CAD downregulated)	Lower lignin	~27–29% increased saccharification with yield penalty
Aspen (Reduced xylan acetylation)	Lower xylan acetylation	Field-grown analysis supports improved glucose yield
Miscanthus	Natural variation/genotype performance	G×E interactions affect saccharification consistency

Challenges for biorefining optimised biomass

- **Need for multi-season field tests**– saccharification is a multigenetic trait that is affected by compensation effects and environmental changes
- **Process flexibility** – biorefineries can adapt with pretreatment/enzymes instead of relying on specialized feedstocks.
- **Regulation & public acceptance** – especially for GM trees and perennials.

Opportunities?

- **Small biomass compositional changes can make a large difference in process viability**
- **Reducing the severity of pretreatment processes remains an industrial priority**



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BB Net Biomass Biorefinery Network
GCRF Global Challenges Research Fund
Newton Fund

THE UNIVERSITY of York

LIXEA

Leading the Biorefinery Revolution from Waste to Wealth

Economic biobased building blocks for the food, bio/chemicals and packaging industries from waste biomass

The Scale-up Challenge

15 October 2025

VISION - FROM WASTE TO WEALTH

BIO-BASED MARKET = € 80B

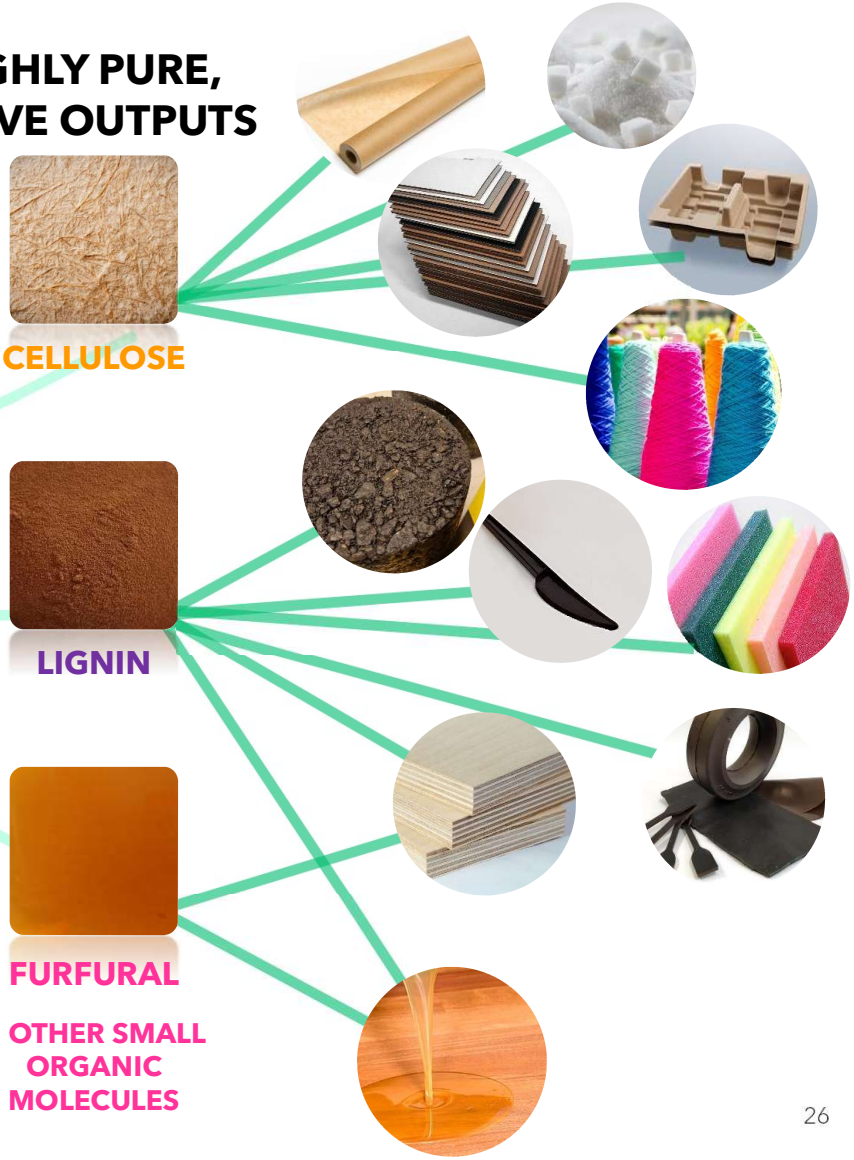
WASTE BIOMASS = 1.1 BT

PROCESS

- Flexible input
- Novel green solvent used
- Highly efficient
- Sustainable, low energy

LIXEA

HIGHLY PURE, NATIVE OUTPUTS



CELLULOSE

LIGNIN

FURFURAL

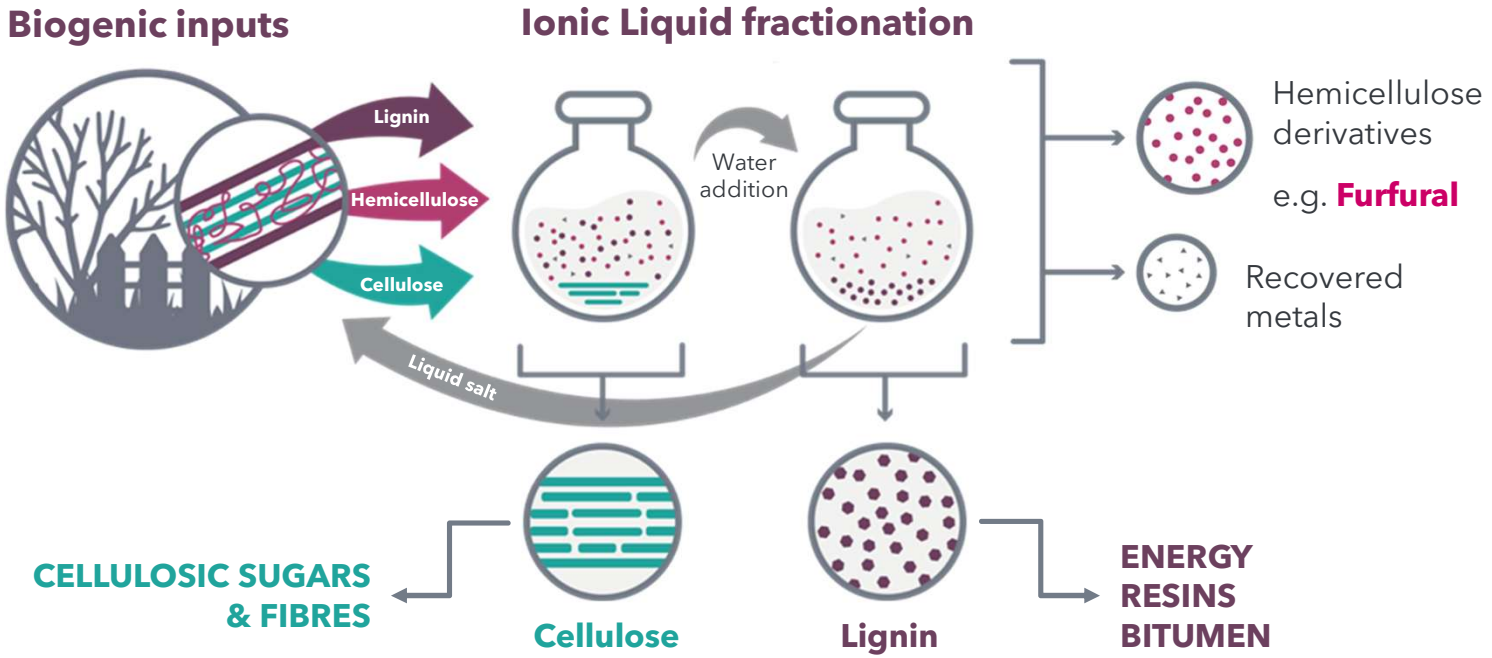
+ OTHER SMALL ORGANIC MOLECULES

CONVERTERS / CUSTOMERS

- DRIVEN BY COST
- Performance – drop in replacement
- New product range
- Sustainability

GROUNDBREAKING TECHNOLOGY

Lixea developed an **innovative biomass fractionation process** using environmentally friendly low-cost ionic liquids that allows separation of the components of wood and agricultural residues



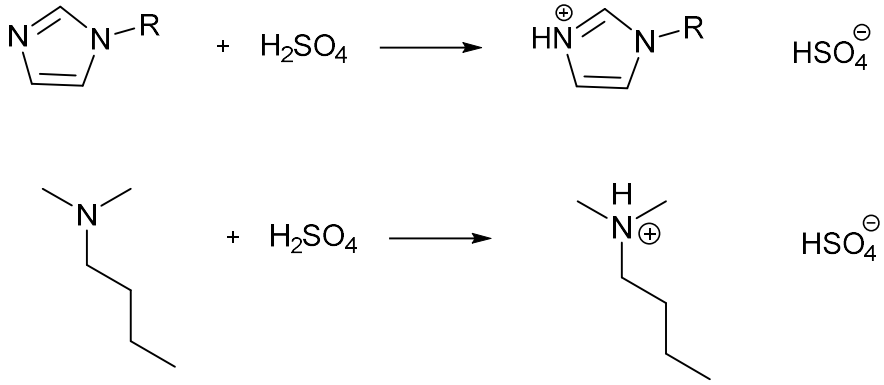
Produce bio-derived products sustainably at a low cost

Create revenue stream from unwanted waste

GREEN SOLVENT – THE HEART OF THE PROCESS

Ionic liquids are salts that melt below 100°C and are seen as **green solvents** due to low vapour pressure enabling better recyclability than volatile organic solvents

Lixea's ionic liquid is special for its exquisite ability to fractionate biomass and that it can be produced in a one-step reaction from widely available starting materials:



N,N-Dimethylbutylammonium Hydrogensulfate

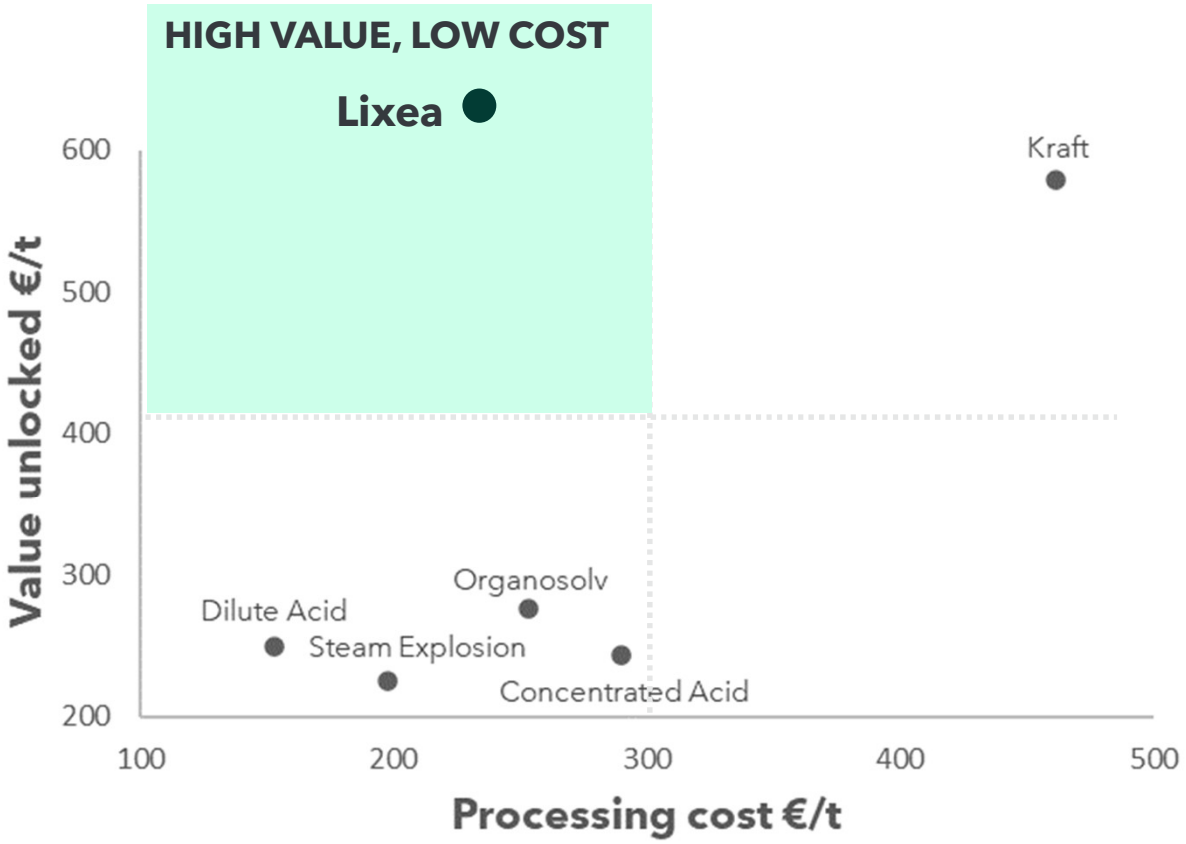
Cost: <EUR 2/kg (large-scale estimate)
Properties: Thermally stable, recyclable

COMPETITIVE EDGE

Feedstock agnostic

Compatible with **low-value waste** feedstocks

High efficiency - over 90% biomass valorisation and Mw fractionation of lignin



Compared across 300kt/y production scale

RAPID MARKET ENTRY

BUSINESS CASE STUDY EXAMPLE - COMMERCIAL PLANT
75 dry kt/y wheat straw feedstock

- Site 100% integrated with an industry partner
- Project financing: private equity investment – potential for EU and local grants

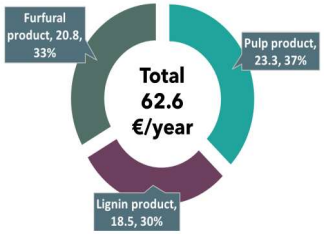
OPEX (in 2031)

Fixed cost €m/year	4.7
Variable cost €m/year	33.0
Total OPEX €m/year	**34.7**

CAPEX

ISBL (including 10% contingency) *€m total*	65.1
OSBL *€m total*	8.9
Total CAPEX *€m total*	**74.0**

Revenue by product (€m per year; in 2031)



2031 - first year at full production capacity

EBITDA (in 2031; €m/year)	25.0
IRR	20.0%
NPV (€m)	94.8

Calculated with 9.18% discount rate and 20-year plant life. IRR assumes 100% private equity investment, financing to be determined at a later stage.

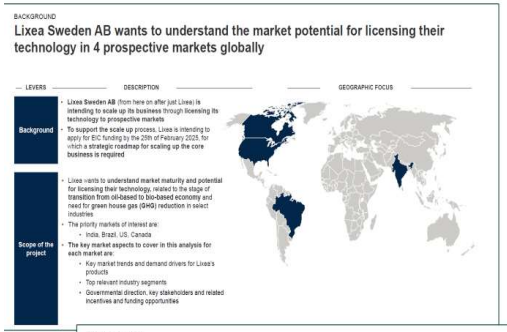
Green House Gas (GHG) Emission Avoidance (EA)

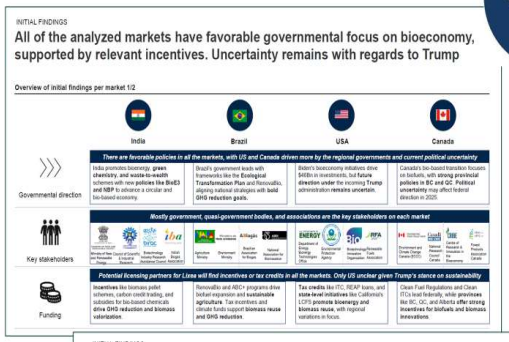
GHG from Lixea *megatonnes CO2 eq.*	0.64
GHG from replaced products *megatonnes CO2 eq.*	3.93
Absolute EA *megatonnes CO2 eq.*	**3.29**
Relative EA %	**84%**

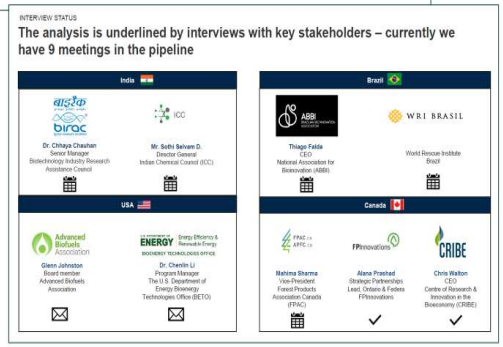
20 years of project duration

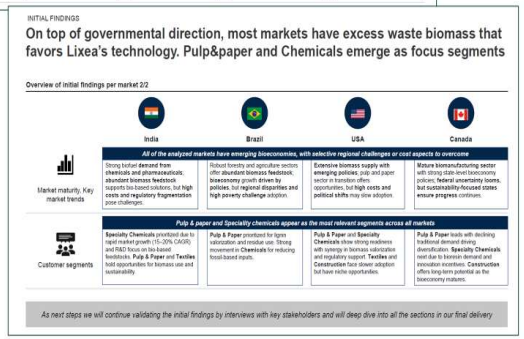
GHG calculation platform: EU Innovation Fund grant GHG calculation template

INTERNATIONAL MARKET ENTRY – Study by Business Sweden





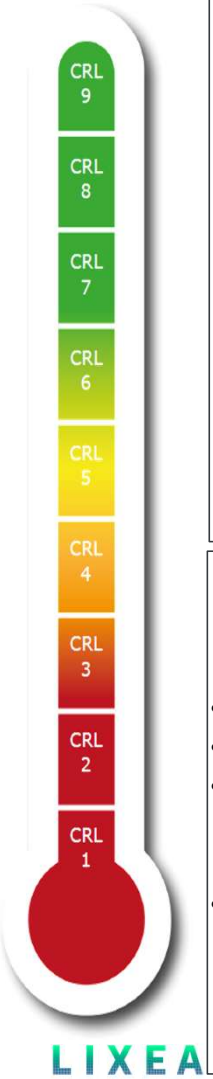




APPLICATION DEVELOPMENT AREAS

IN COLLABORATION WITH INDUSTRY PARTNERS

CRL = Customer Readiness Level



1, PULPING COMPANY

CELLULOSE

- No hemicellulose
- Tuneable lignin deposition
- High surface area for easy hydrolysis or defibrillation

2, MATERIAL COMPANY

LIGNIN

- Pleasant odour
- High purity
- In-situ fractionation - tuneable reactivity a hydrophobicity profi
- Very low ash and carbohydrate conter

3, COMMODITY CHEMICAL COMPANY

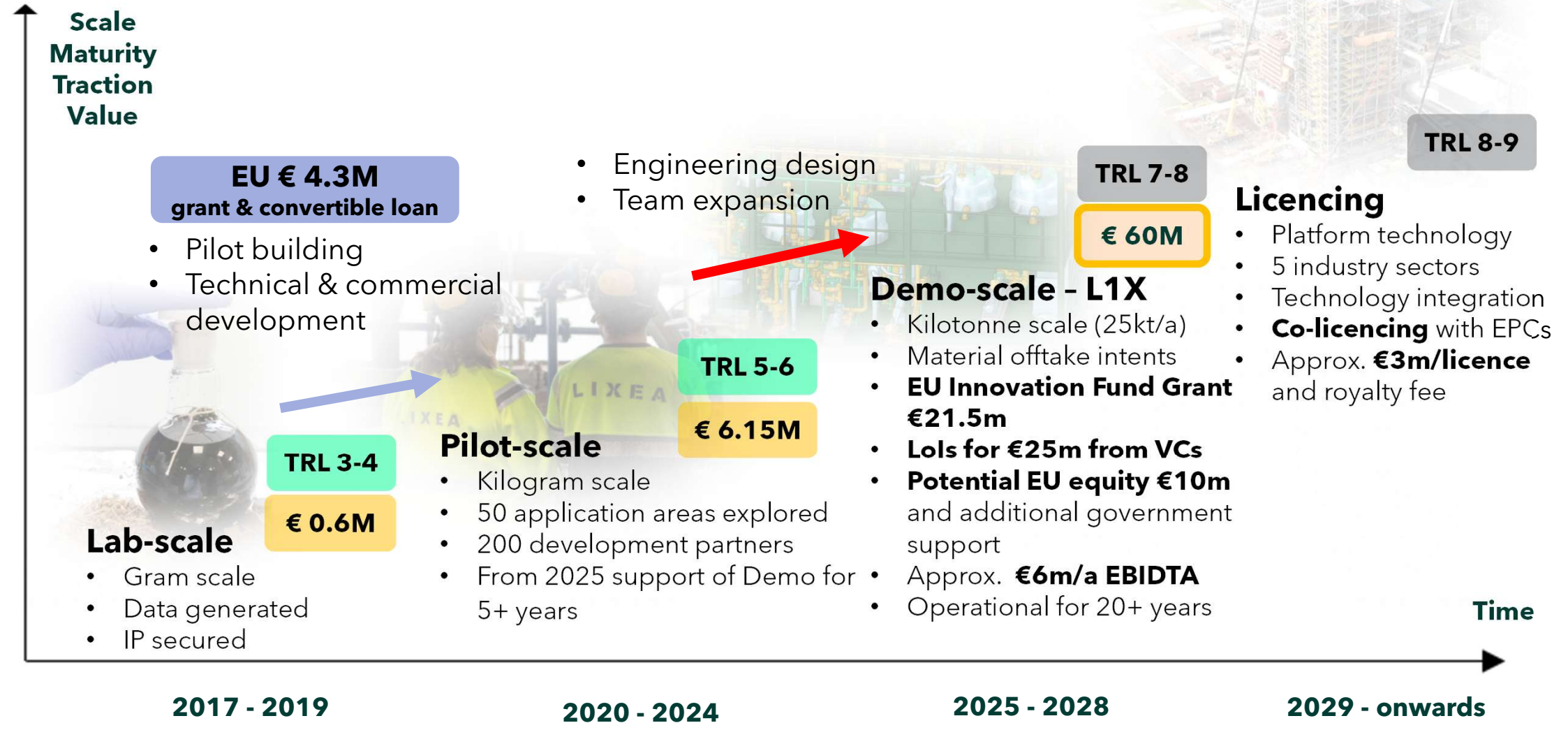
FURFURAL

Established application areas:

- Solvents
- Transportation fuels
- Lubricant
- Resins
- Food additive
- Fertilizer
- Pharmaceutical ingredient
- Platform chemical

LIXEA

TECHNICAL & COMMERCIAL DEVELOPMENT JOURNEY



STRONG VALUE PROPOSITION – Hybrid Business Model

- A **true platform technology** with a wide geographical relevance for waste biomass conversion

Plant Design	Mission	Value	Status	Market Entry
L1X Material	• High quality material production from waste biomass – outputs: cellulose, lignin, furfural • Focus: packaging, textile, (bio)chemical sectors	• Feedstock agnostic • Core technology owned by Lixea • Local feedstock & Local offtake • *Potential add-on: sugar production from cellulose for fermentation; hard carbon production from lignin for battery anode application*	• 25kt/a facility design in progress – supported by EU Innovation Fund - project budget: EUR 60m • Blueprint can be modified to meet the design variants of L2X, L3X, L4X licensing requirements	• Blueprint : 2026 • First L1X in operation: 2028 • L1X technology licensing: from 2028
L2X Bioethanol	• Biomass **PRETREATMENT** step - core technology is established & operated by partner company • Medium / High technology integration with existing host site	• Broadens feedstock options • Increased target (bioethanol / biogas) production efficiency (lignin removed) • New revenue stream – lignin & furfural production	• Feasibility and Tecno-economic Assessment (TEA) delivered for a US bioethanol company • Potential feasibility study in Canada • Many incentives to upgrade existing sites in Canada & US	• Based on L1X design, licensing discussions: from 2026
L3X Biogas			• Feasibility and TEA delivered for a Scandinavian biogas company • Europe is expanding its biogas production – many opportunities	
L4X Recycling	• Waste biomass **PREREATMENT** step - mixed wood / treated wood / contaminated / metal impregnated wood recycling	• Currently, no technology exist for contaminated wood processing • Interest in redirecting wood from burning; cost benefit against ash landfilling	• Assessed market potential – contaminated mixed construction waste and creosote treated wood – volumes are: 70-200kt/a depending on country and location • Main focus on Europe – working closely with specialty recycling facilities with suitable infrastructure	

TECHNOLOGY DEVELOPMENT

15 years of biomass research at Imperial College London

Delivered fundamental chemistry

- Proved process using a selection of biomass feedstocks
- Demonstrated key applications
- Cellulosic pulp hydrolysed and fermented
- High quality lignin produced

Established significant patent portfolio

Developed initial techno-economic model

Imperial College London

Successful initial scale-up at BBEPP (Belgium)

- Tested separation methods for lignin isolation
- Cellulose separation using industrially relevant equipment
- Obtained samples for product development tests
- De-risked and informed on focus areas for pilot plant

Bio Base Europe Pilot Plant

CURRENT PROCESSING SCALE - PILOT PLANT

Pilot Plant operating since Q2 2022 in Bäckhammar, Värmland (Sweden)

Scale: 20kg feedstock/batch

Deliver engineering data for technoeconomic models

Produce materials for commercial partners and for prototyping

Open Access Facility- industry driven commercial projects delivery

L1X PLANT - VISION

End-markets	EU market size 2022
LIGNIN	**€1.82 bn**
Phenol formaldehyde	€12.5bn
Bitumen	€50 bn
Bisphenol-A	€6.5 bn
Polyethylene	€98.3 bn
CELLULOSE PULP	**€60.6 bn**
PET Packaging	€50 bn
Polystyrene packaging	€6.5 bn
Kraft paper-based packaging	€98.3 bn
FURFURAL	**€51.4 bn**

LIXEA

Ref. Ares(2024)7537716 - 23/10/2024

STEP Seal | European Commission

The project proposal

101190975 — L1X

Unlocking a bio-based future by turning globally available waste biomass into renewable building blocks

by

LIXEA SWEDEN AB

submitted under the *Innovation Fund (INNOVFUND)* call

INNOVFUND-2023-NZT-PILOTS — Innovation Fund 2023 Net Zero Technologies – Pilots

was recognised as a **high-quality project** proposal in a highly competitive evaluation process and contributes to the Strategic Technologies for Europe Platform (STEP) objectives, following evaluation by an international panel of independent experts.

The STEP (Sovereignty) Seal is a quality label awarded by the European Commission aimed at facilitating access to funding opportunities under other Union programmes covered by STEP. For further information, visit https://strategic-technologies.europa.eu/

Brussels, 22/10/2024

OUR FUTURE – DEMO PLANT "L1X" PLANS – Q1 2025 – Q2 2028

PROJECT SUMMARY - EU INNOVATION FUND GRANT

- **25kt/y hardwood** and **agriculture biomass** demo integrated with industry partner site in **Central Europe**
- **Project financing -** EU grant (Innovation Fund with GHG saving focus), match funding from VCs (EUR 25m Letters of Support received) , private equity, debt – currently, in the process of finalising these
- **Built a non-competing material offtake consortium (12kt/y cellulose and 6kt/y lignin)**- over 30 Letters of Support received

OPEX (in 2032)

Fixed cost €m/year	3.79
Variable cost €m/year	9.99
Total OPEX €m/year	**13.78**

CAPEX

ISBL (including 10% contingency) €m total	48.98
OSBL €m total	7.91
Total CAPEX €m total	**56.89**

LIXEA

Revenue by product (€m per year; in 2032)

2032 – first year at full production capacity

EBITDA (in 2032; €m/year)	6.0
IRR	13.9%
NPV (€m)	6.75

Calculated with 9.18% discount rate (WACC) and 20-year plant life. Project financing: eIF grant, private equity investment, and debt, with exact distribution finalized at a later date.

Green House Gas (GHG) Emission Avoidance (EA)

GHG from Lixea kilotonnes CO2 eq.	139
GHG from replaced products kilotonnes CO2 eq.	665
Absolute EA kilotonnes CO2 eq.	**526**
Relative EA %	**79%**

10 years of project duration

GHG calculation platform: IF grant GHG calculation template

17

CHALLENGES and IMPLEMENTATION GAPS

TECHNOLOGY

- Optimisation of an existing vs. Novel technology
- Need to reach relevant production scale
- Need to "speak" the industry language
- Risk mitigation is key

COMMERCIAL

- Business target – process or product company
- Customer trials / feedback are crucial
- Raw material security and offtakes are crucial
- Business model – material production vs. licensing
- World needs to be ready – including regulations

FINANCING / FUNDING

- Grants, Angels, VCs vs. cVCs vs. Industry
- Revenue generation key

LIXEA JOURNEY / STRATEGY

- ✓ 15 years of R&D - TRL 1–6
- ✓ Educating the world about ionic liquids
- ✓ Simple, scalable process with off the shelf units
- ✓ Working closely with EPC companies
- ✓ Became "experts" in 50 different application areas

- ✓ Made thousands of friends in this space – industry, commercial, startup
- ✓ Worked with over 200 companies around the world
- ✓ Delivered 7 Industry projects, currently 10 active ones
- ✓ Over 15 bespoke TEA model around the world
- ✓ Hybrid business model – material and technology platform
- ✓ World needs material and energy security – from local raw materials to local offtake

- ✓ Survival is key - grants are fantastic – local and EU
- ✓ Industry projects, joint development are essential
- ✓ Raised €7m – grants, equity, convertible, raising €40m
- ✓ Main focus on Industry and cVCs

OVERALL REFLECTIONS FOR STARTUPS

- ✓ Saving the world is not easy
- ✓ Disruptive, bold (and brave) ideas needed in this sector
- ✓ Need to be agile and ready to change strategy in a heartbeat
- ✓ Be open with your challenges
- ✓ Seek commercial discussions & collaborations as early as possible
- ✓ If you fail, fail quickly
- ✓ Everything takes longer than you think
- ✓ Cash (and any kind of revenue) is king
- ✓ Follow regulations & policies – local / EU / world
- ✓ Time needs to be right – some things are outside of your control
- ✓ Your BIGGEST asset is your Team

PEOPLE WHO MAKE THIS HAPPEN

Krisztina Kovacs-Schreiner
CEO

- Biochemical Engineer with over 10 years of experience in managing a portfolio of biomass to novel bio-materials development projects valued at €10m
- Experienced in business development, sales and developing ventures from idea to prototyping and field testing

Christer Gustavsson
Director

- Chemical Engineer with over 20 years of technical business development in the forest industry and energy sector
- As an investor, leading a portfolio of bioeconomy startups

Marcus Elmer
COO, Commercial Lead

- Mechanical Engineer with over 13 years of experience in product and business development with established networks in the forest & bioeconomy sectors
- Extensive knowledge in project management, sales, strategy and establishing early-stage start-ups

Janis Eglitis
Technical Lead

- Chemical Engineer with over 5 years of experience in scale-up and process design
- Experienced in Environment and Water consultancy, with a particular focus on process optimisation and support, technology evaluation, as well as scientific/quality strategy development.

LIXEA


LIXEA
Enabling future generations
to live sustainably in a clean world
krisztina@lixea.co
www.lixea.co

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SUCCESS FACTORS - EXECUTIVE SUMMARY

Opportunity

- Bio-based materials market growing to over **€77bn**
- Increased need of **locally sourced**, **secure raw material supply** with reliable quality and low cost

Problem

- Current alternatives to fossil-based products **are not cost effective** or **compete with food supply**
- Large amounts of **bio-wastes are not fully utilized**
- **Supply chains are broken** / potentially burdened by **tariffs** and other restrictions by **legislation**

Solution

- **Pilot-scale** confirmed, **patented**, viable technology
- Supported by **15 years** of research
- **7 years** of technical and commercial development
- Validated to TRL6 thanks to **€6.75m investment** (2017-2024)
- **7** outstanding, highly driven team of professionals with extensive technical and commercial experience and **4 external** expert advisors

Investment case

- Worked with over **200 enterprises on 50 different product applications**
- Over **10 industry driven projects** in pipeline
- Emerging interest from potential **technology licensees** in key industry sectors

- Scale of **opportunity is validated** by comprehensive techno-economic studies - **rapid industry entry is viable via technology co-licencing partners (EPCs)**

- **Significant return on investment** from this industry-disrupting technology

- **Demonstration Plant** investment of **€60m is derisked up to 60%** - utilizing **EU's Innovation Fund grant of €21.5m,** industry and local government contribution; along with debt and VC / PE support lined up for later stage

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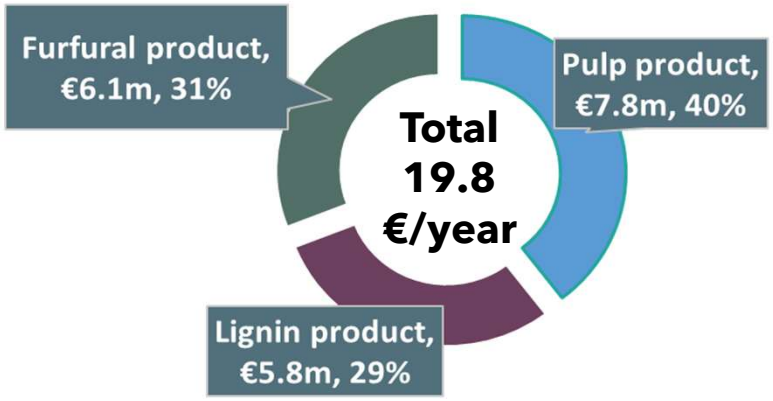

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LIXEA – TECHNOLOGY LICENSING - USPs

STARTING MATERIAL

- Highly effective on various feedstocks globally available

- Compatible with **low-value waste** feedstocks - not competing with food or resulting in deforestation

- Over 90% valorisation of biomass through **lignin, specialty chemicals and cellulose** products

PROCESS

- **Simple & scalable** – follows paper and pulping industry unit design, equipment available at scale

- Sustainable - low temperature (120-150C), low pressure and low water use

- Can be **implemented anywhere** in the world, cost effective due to **integrated design** & local biomass sourcing

OUTPUTS

- **Strong sustainability** angle – enable petrochemical replacement and is also moving away from virgin wood

- **High quality cellulose**, **fractionated lignin** with **unique properties** & high-value small molecules

STRONG BUSINESS CASE & SUPPORT

- **Protected technology** – over 10 patents & know-how

- Engaged with **150 organisations** (research and industry) – building blocks / product development – over **50 different application areas**

- Industry interest – **material offtake and technology licensing**

- EU industry strategy focusing on green transition (Green Deal) & **US – bio preferred** and Global Climate Action

TECHNOLOGY LICENCING PACKAGE

Our industry typical technology license package will comprise:

LICENCE AGREEMENT

- Right to use Lixea's proprietary technology under its exclusive patent rights

BASIC ENGINEERING AGREEMENT

- Dendronic® data package for Engineering, Procurement and Construction (EPC) contractor to design and build the plant
- Key data derived from pilot activities for licensee to operate the plant
- Cellulose and lignin product specifications

TECHNICAL SUPPORT

- Growing engineering team to provide
  - Engineering, procurement, commissioning support alongside EPC
  - Operational support

INTELLECTUAL PROPERTY

High quality portfolio of registered and non-registered IP rights to support licensing business model

TECHNOLOGY DATA

- Documented in series of controlled engineering memoranda
- Significant further data to be generated by pilot plant operation

10 + 4 PATENTS

- Exclusive license to three families of granted patents and pending applications covering the core chemistry
- Key markets protected

FREEDOM TO OPERATE

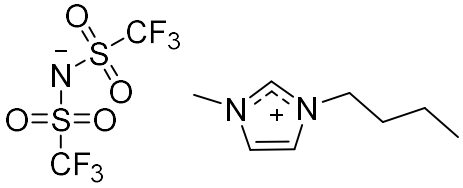
- Internal searches not identified any concerns to date

PORTFOLIO OF TRADEMARK RIGHTS

- LIXEA®
- DENDRONIC®

IONIC LIQUIDS - OVERVIEW

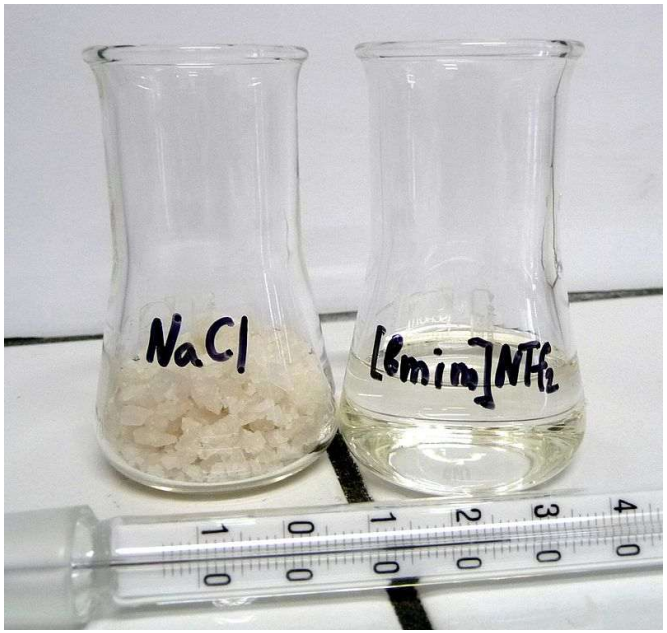
- **Salts melting below 100°C**
  - E.g. "[BMIM][NTf_2]": melting point -4°C
    vs. NaCl: table salt, melting point 801°C

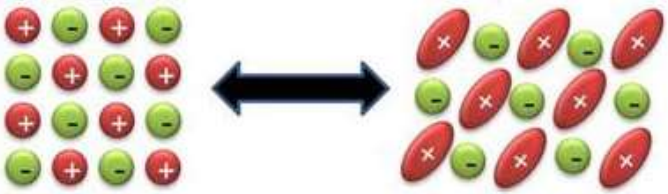


- Praised as **"green solvents"** due to low vapour pressure enabling better recyclability than volatile organic solvents

- **Tuneable properties**: many million different ionic liquids with different properties can be produced, e.g.:
  - Some ionic liquids are capable of dissolving cellulose, while others are not
  - Some ionic liquids have excellent thermal and/or chemical stability, while others do not
  - Some ionic liquids made from specialised chemicals and are expensive to produce

- **Lixea uses a selection of low-cost ionic liquids that can be produced easily at industry scale**






CNPEM
Biorenewables

CNPEM

Brazilian Center for Research in Energy and Materials

Synchrotron Source — LNLS sirius

Biosciences — LNBio

Nanotechnology — LNNano Brazilian Nanotechnology National Laboratory

Biorenewables — lnbr Brazilian Biorenewables National Laboratory

School of Sciences — ilum

Human Resources

Employees – 1.300
Students and PDs – 300
Trainees – 150

TOTAL | ~1.750
**July 2025*

530.000 m²

CNPEM is a non-profit social organization that operates four National Laboratories under a management contract with the **Ministry of Science, Technology and Innovation (MCTI)**

4th Generation Storage Rings in operation worldwide

APS (2025)

Energy	6.0 GeV
Circumference	1104 m
Natural emittance	**42 pm.rad**
Current (top-up)	200 mA

MAX IV (2016)

Energy	3.0 GeV
Circumference	528 m
Natural emittance	**330 pm.rad**
Current (top-up)	500 mA

SIRIUS (2020)

Energy	3.0 GeV
Circumference	518 m
Natural emittance	**250 pm.rad**
Current (top-up)	350 mA

Campinas

ESRF-EBS (2020)

Energy	3.0 GeV
Circumference	528 m
Natural emittance	**133 pm.rad**
Current (top-up)	200 mA

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO

GOVERNO FEDERAL BRASIL UNIÃO E RECONSTRUÇÃO

Orion

Laboratory complex for advanced pathogen research, which will include high (BSL-3) and maximum biological containment (BSL-4) facilities, unprecedented in Latin America.

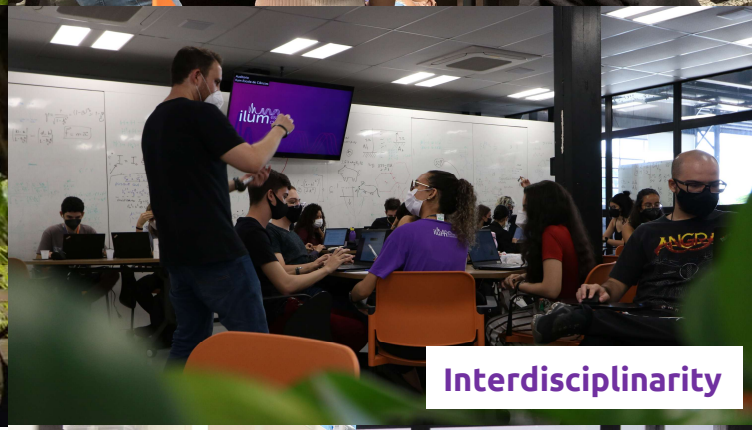
Orion: Maximum Biological Containment Laboratory Complex

Sirius

Orion will be the first structure of its kind in the world connected to a synchrotron light source, with three light lines.

NOVO PAC
DESENVOLVIMENTO E SUSTENTABILIDADE

Project-based Learning



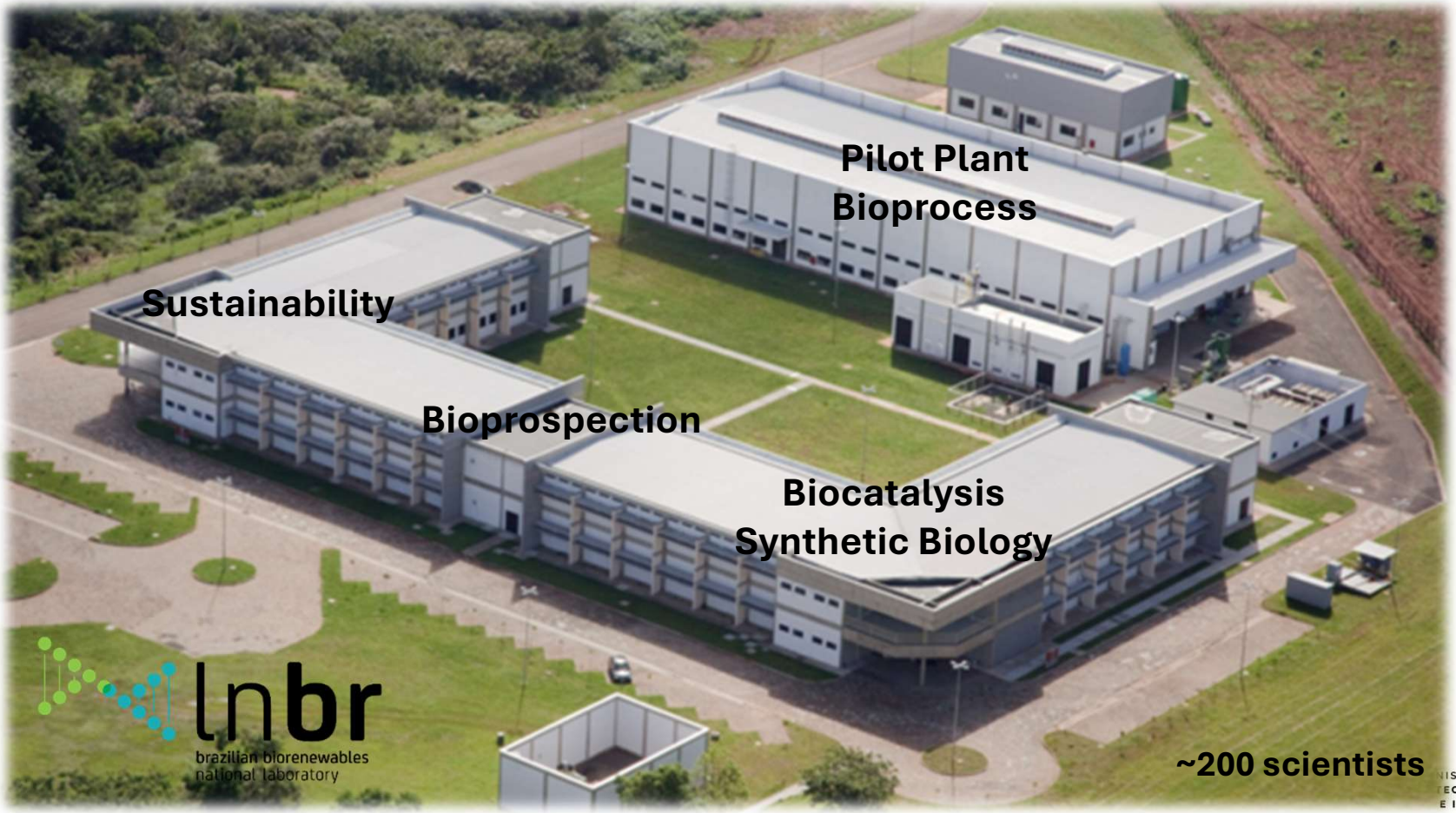
Interdisciplinarity

Experience in an advanced research environment

- **Free and 3 years long – with 40 students per year**
- Early entry into the world of scientific practice
- Hands-on activities and projects in CNPEM laboratories
- Support provided to students for full-time dedication to the program (housing, meals, transportation, psychological support)

CNPEM

Brazilian Biorenewables National Laboratory (LNBR)



MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO

GOVERNO FEDERAL
BRASIL
UNIÃO E RECONSTRUÇÃO

Mitigate fossil dependence and climate change

9,608 EJ

Oil extracted up to 2020

Oil proved reserves in 2024

9,150 EJ

(1.5 T barrels)

Yearly oil production (2024)

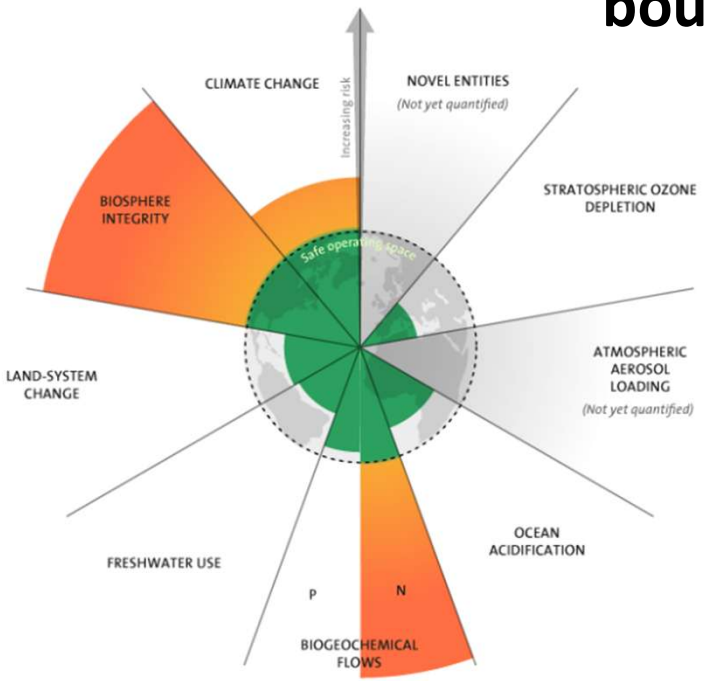
213 EJ (35 B barrels)

7.7 EJ

Only 40-50 years left

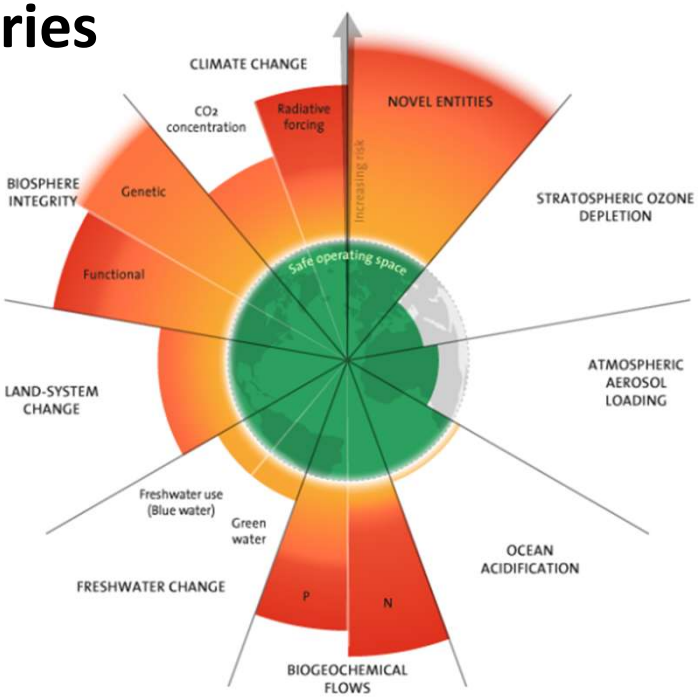
Planetary boundaries

2009



7 boundaries assessed,
3 crossed

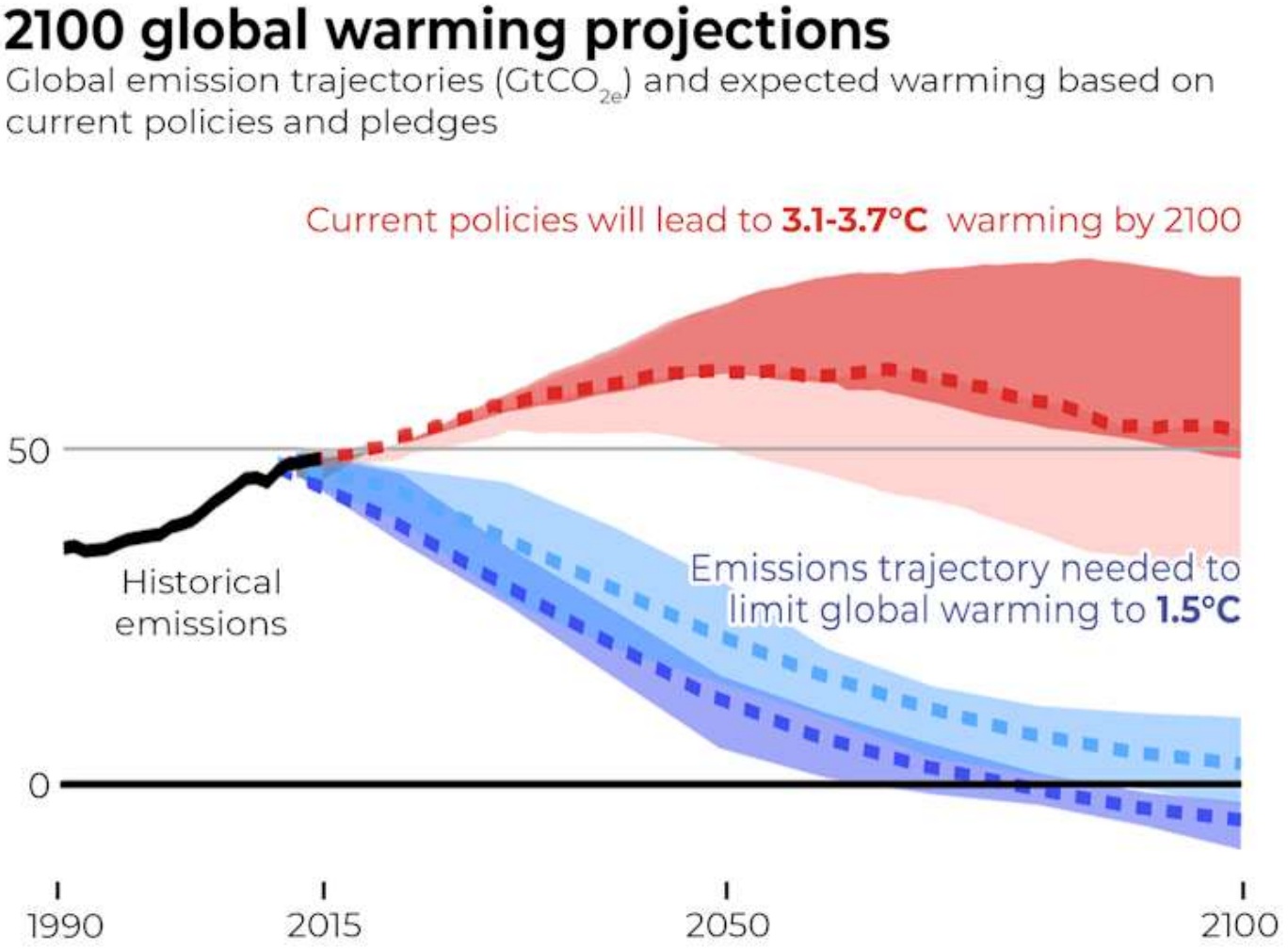
2025



9 boundaries assessed,
7 crossed

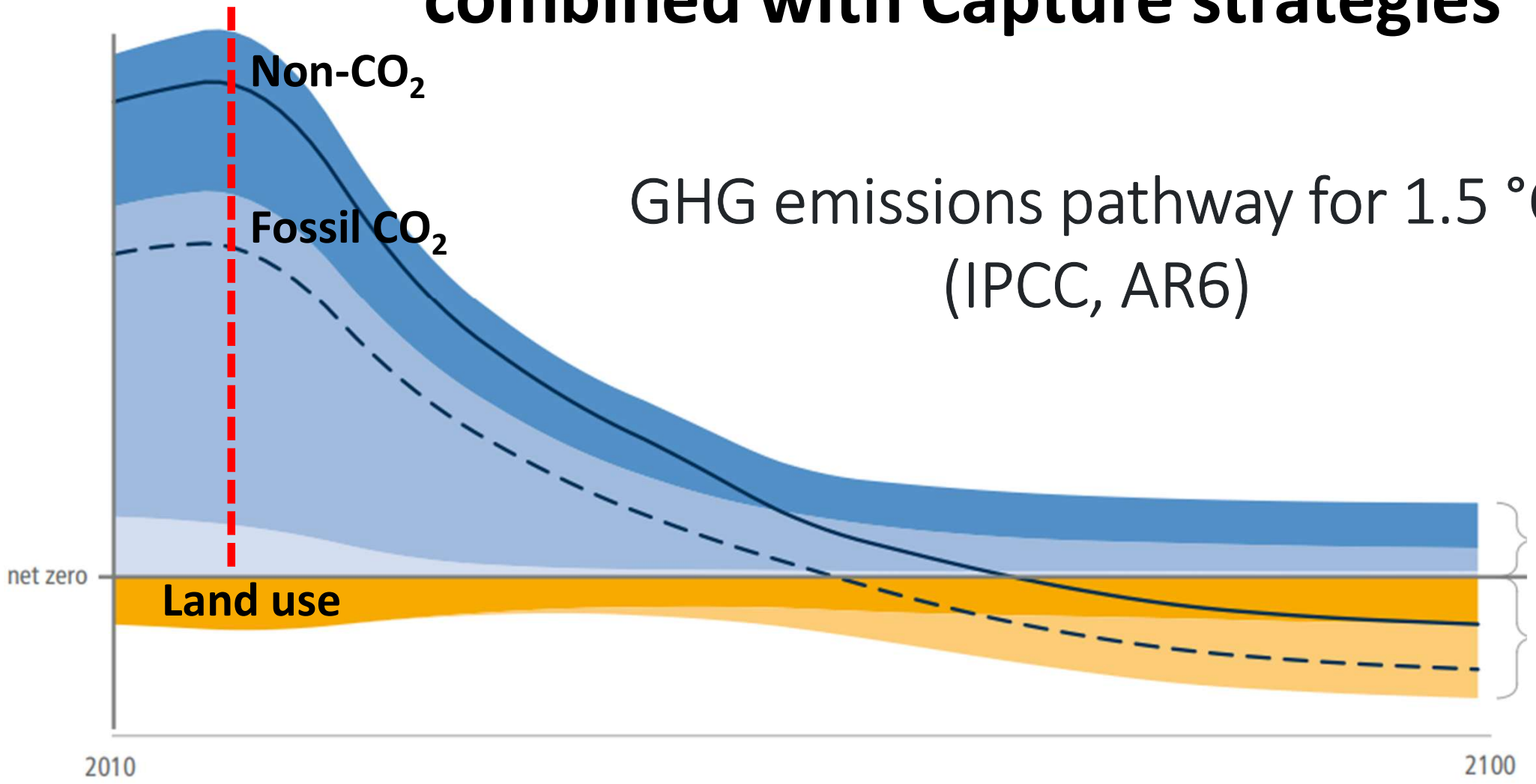
Based on data from ourworldindata.org

Climate Change Projections

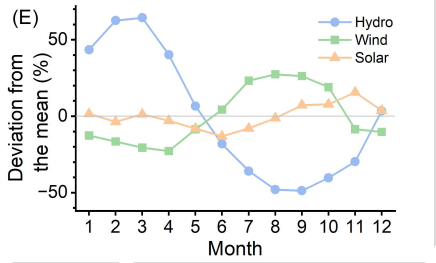


Carbon Emission Reduction must be combined with Capture strategies

GHG emissions pathway for 1.5 °C (IPCC, AR6)



Unique features that pose Brazil as a born leader of the transition to a sustainable Bioeconomy



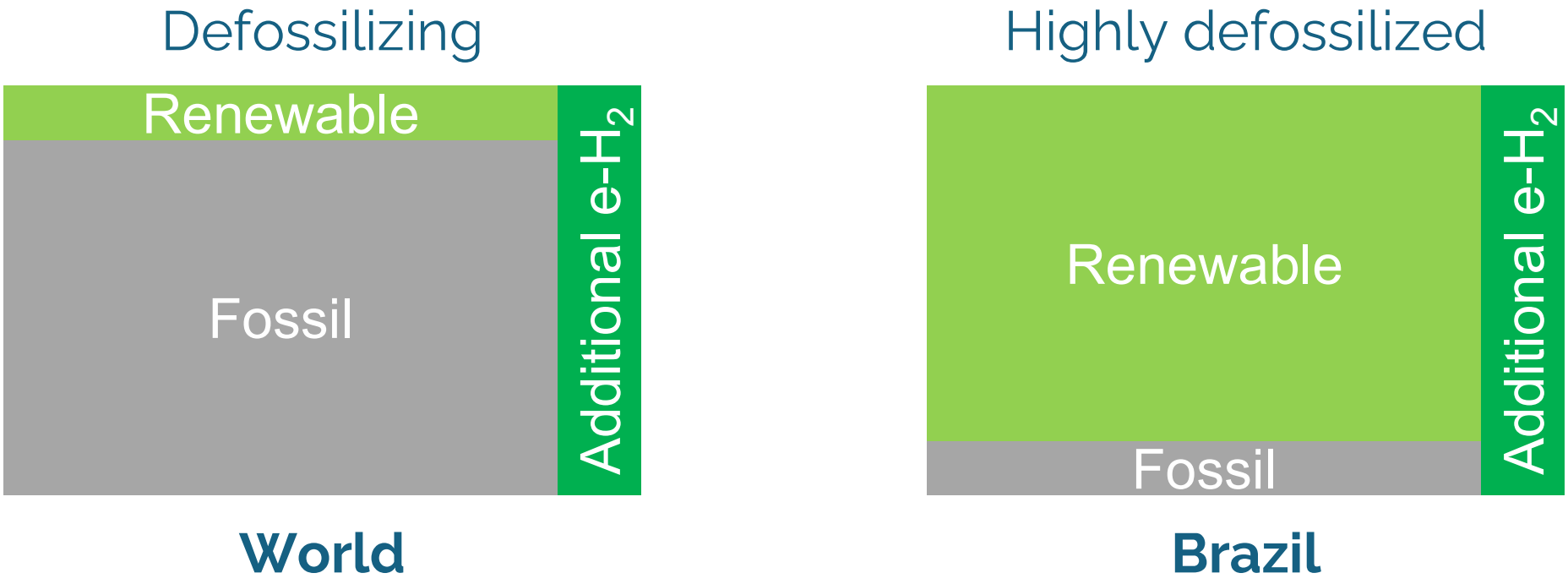
Lignocellulosic biomass

Renewable Electricity

Degraded pasturelands

Biodiversity

Brazil has a highly defossilized energy matrix

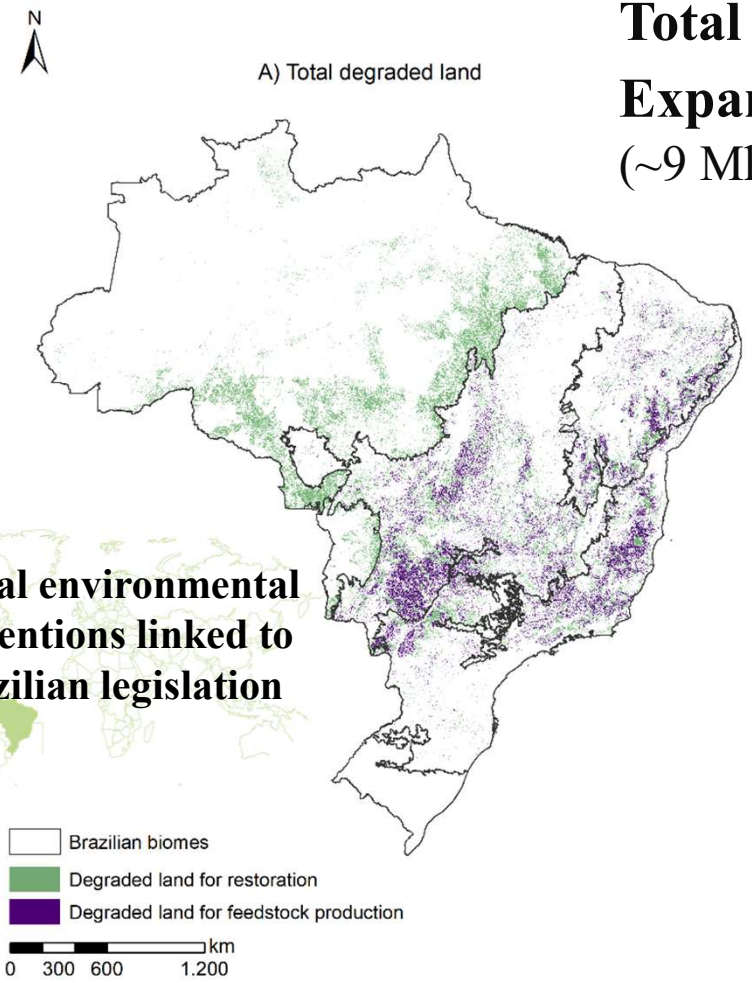


The potential of degraded lands for bioenergy

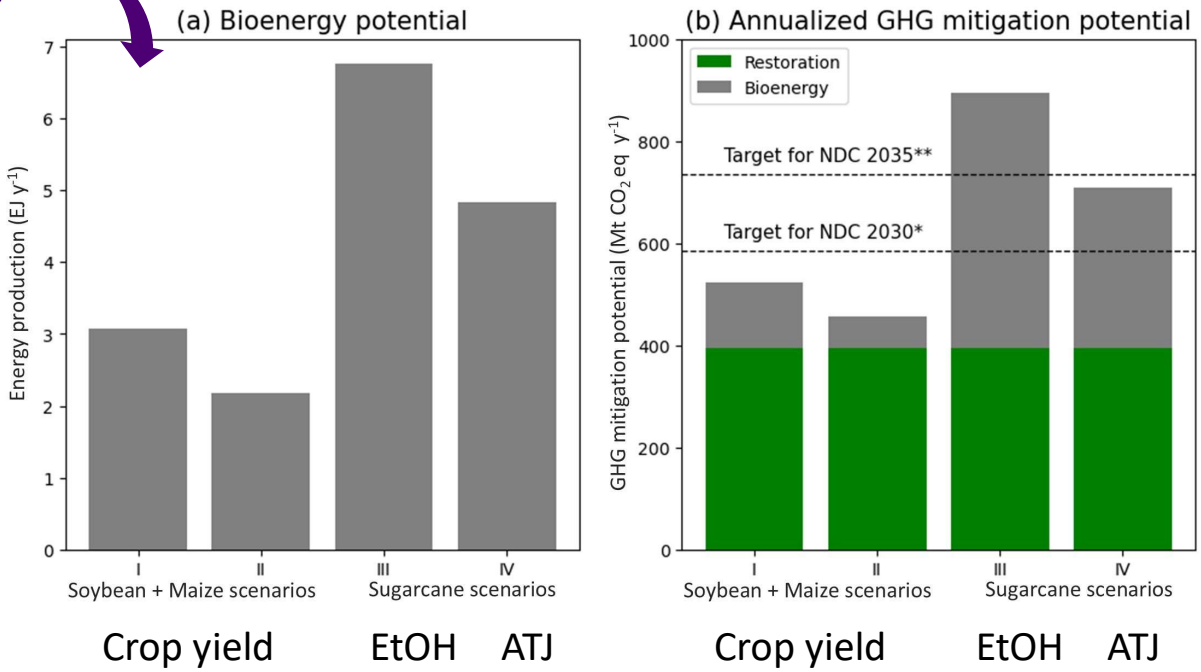
Total degraded area = 100 Mha

Expandable area with C sequestration = 36 Mha = 6.8 EJ y^{-1}

(~9 Mha is the current sugarcane area in Brazil)



Global environmental conventions linked to Brazilian legislation



Padgurschi *et al.* submitted

Unique features that place Brazil as a born leader of the transition to a sustainable Bioeconomy

Source: FAO, 2022; IBA, 2019; CONAB 2022

Consolidated industry produces

CAPTIVE BIOMASS,

essential for the lignocellulosic biorefineries

Conversion rate → 300 L / tonne

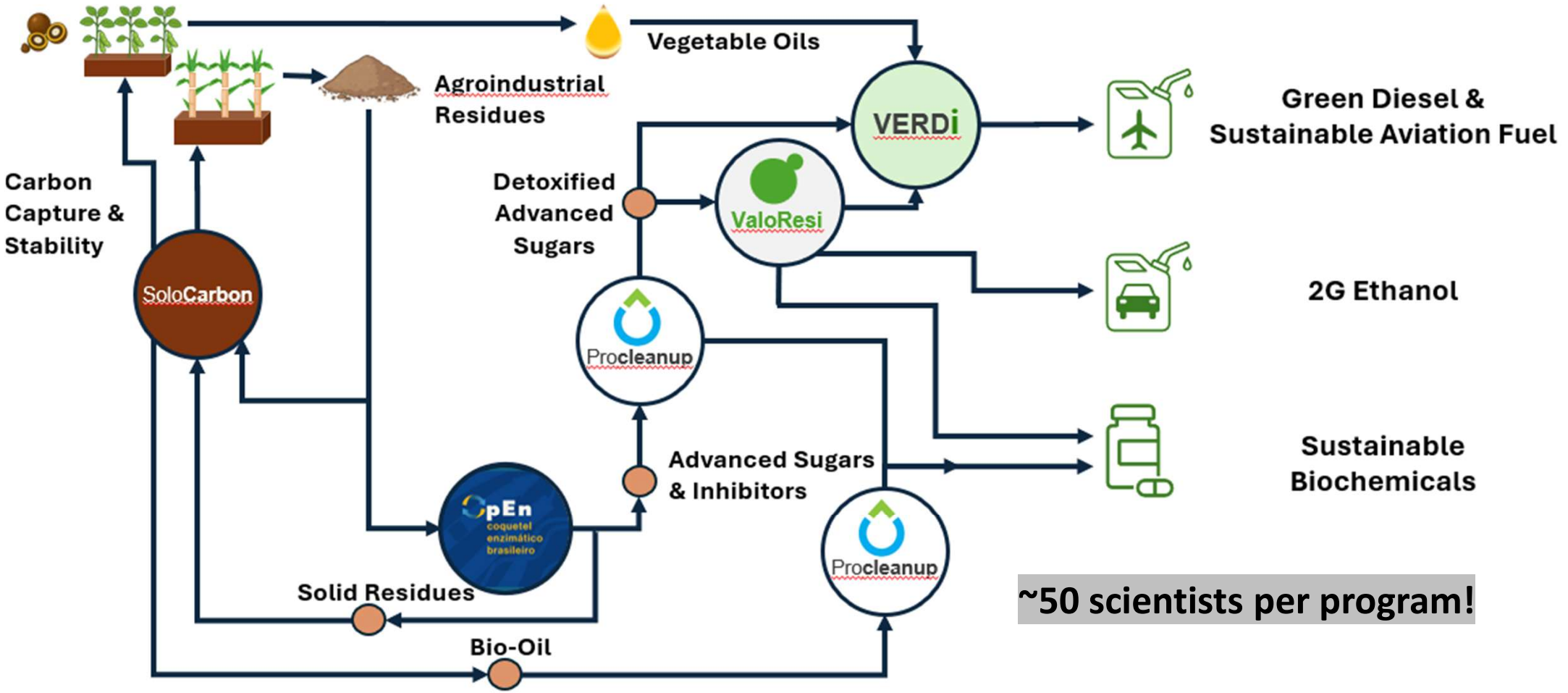
135 Bi/L = 3.0 EJ (potential)

(36 Bi/L = 0.75 EJ corn & sugarcane)

World = 4.7 EJ (Brasil = 1.02 EJ)

COP30 proposal: 4x more biofuels in 2035

INTEGRATION OF LNBR/CNPEM BIOTECHNOLOGICAL PLATFORMS FOR ZERO-RESIDUE LIGNOCELLULOSIC BIOREFINERIES



~50 scientists per program!

CNPEM

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO

GOVERNO FEDERAL BRASIL UNIÃO E RECONSTRUÇÃO

A “zero-residue” biorefinery model for Brazil

Fully integrates conventional mills

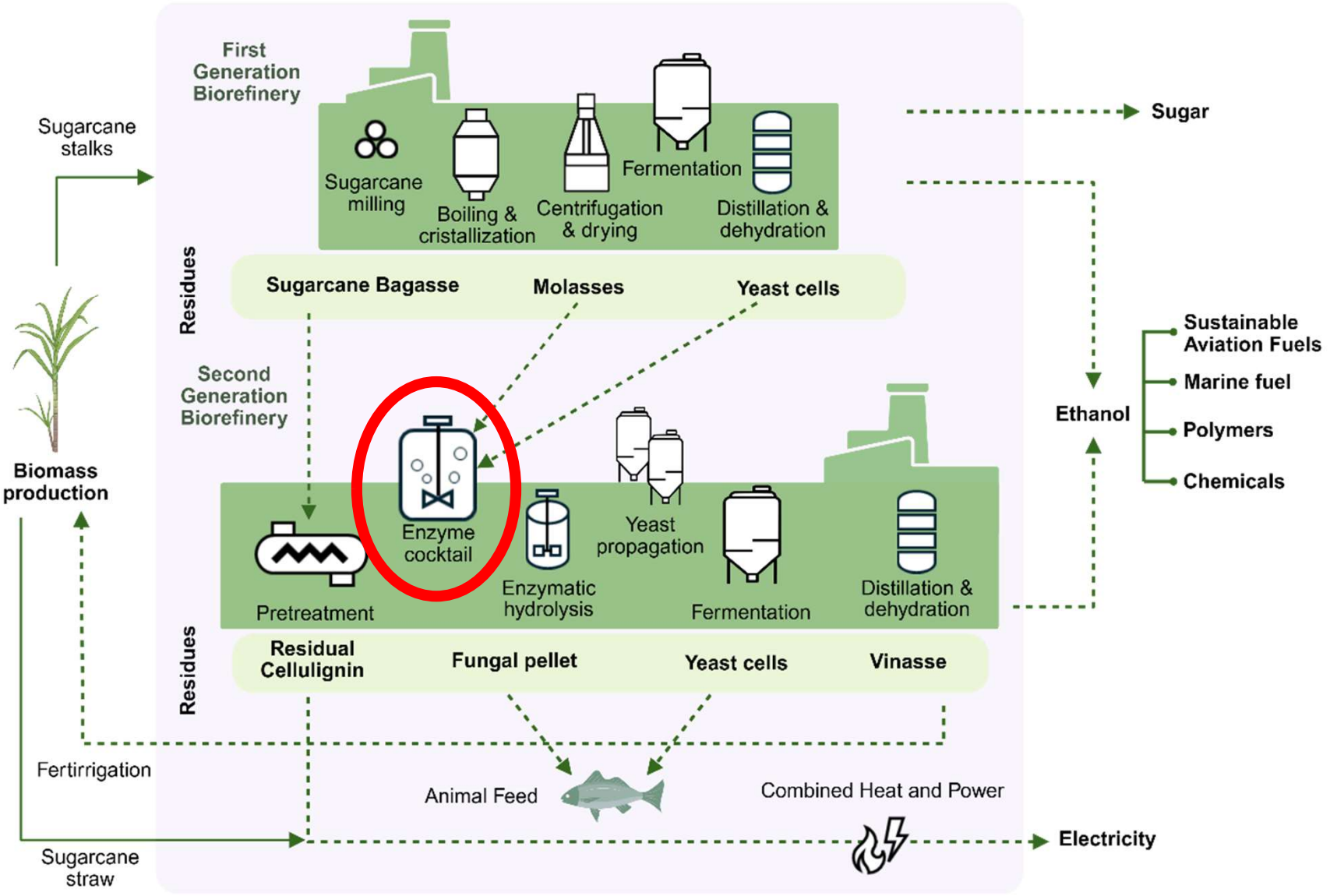
75% lower emissions
(gasoline)

All solid and liquid streams valorized

Food & Feed
Alternative proteins

Carbon Efficiency
350 kg/input-carbon ton

270 kg/input
Conventional 1G mills



Mandelli *et al.* submitted

Brazilian Enzyme Cocktail Platform

OpEn (Open-access Enzyme Cocktail)

OpEn
coquetel
enzimático
brasileiro

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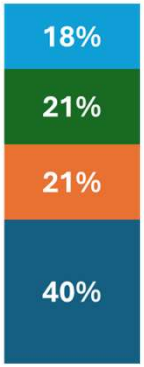
CNPEM/MCTI Technological Platforms

OpEn – Brazilian Open-Access Enzyme Cocktail

Enzyme cocktail: a key biotechnological input for biorefineries



- Other inputs
- Feedstock
- Enzymes
- CAPEX

Estimated Brazilian Market
5 Bi USD/year

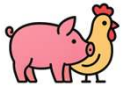
Value-added input for different production chains

2G ethanol

57% ethanol without agricultural expansion (16 Bi L/year)

1.5G ethanol

1.5 Bi L/year ethanol



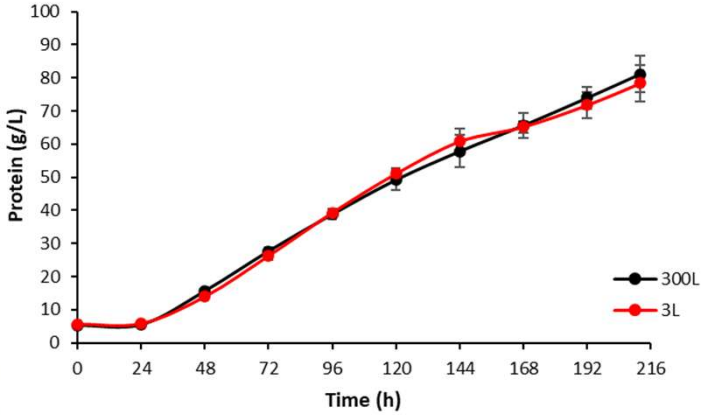
Animal nutrition

Tailored cocktail as additive / residual cells as protein source

Fully validated under industrially relevant conditions during 5 years

Bioprocess development resulted in <u>100% reproduction</u> of bench results in a 300 L pilot-plant bioreactor <u>without strain degeneration</u>

50% lower GHG emissions with on-site production



@65 g/L.7d – 2.75-3.10 USD/Kg
@100 g/L.7d – 3.40-3.80 USD/Kg

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OpEn – Brazilian Open-Access Enzyme Cocktail

Transforming OpEn in a national community-engaging platform

- Free distribution for universities and non-profit research institutions
- 80 versions available
  - Tailored to:
    - “split-fraction” PT technology
    - “whole-slurry” PT technology
    - sugarcane bagasse
    - sugarcane straw
    - corn stover
    - eucalyptus residues
    - Animal feed
    - Cellulose functionalization

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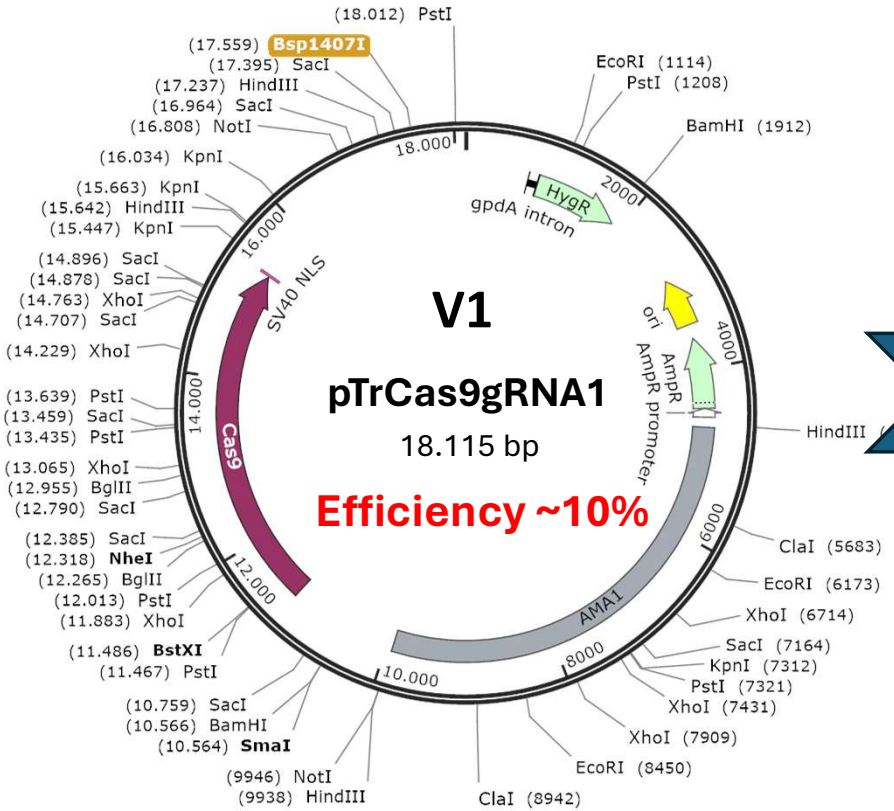
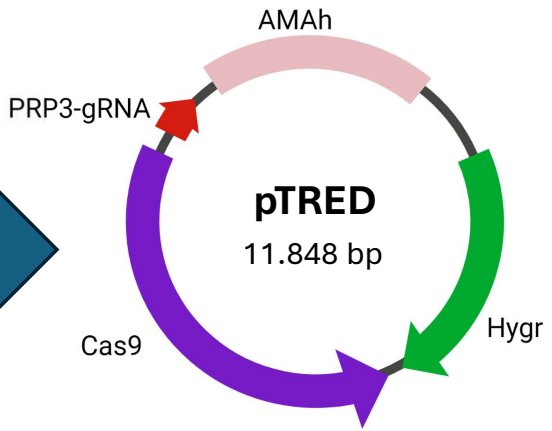
Developing tools for Trichoderma engineering

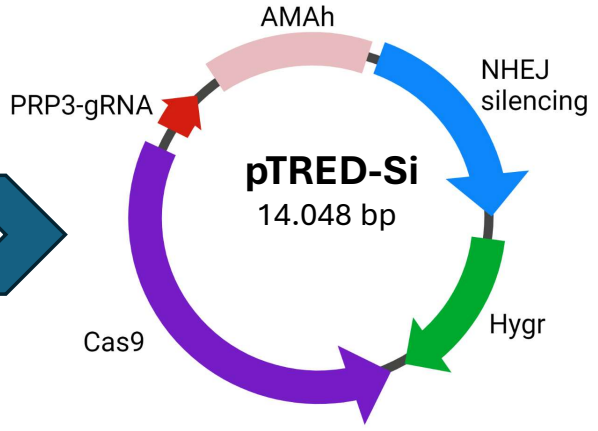




Efficiency ~20%

V2

Silencing NHEJ pathway by producing dsRNA



Efficiency ~80%

V3

Unpublished results, patent filed

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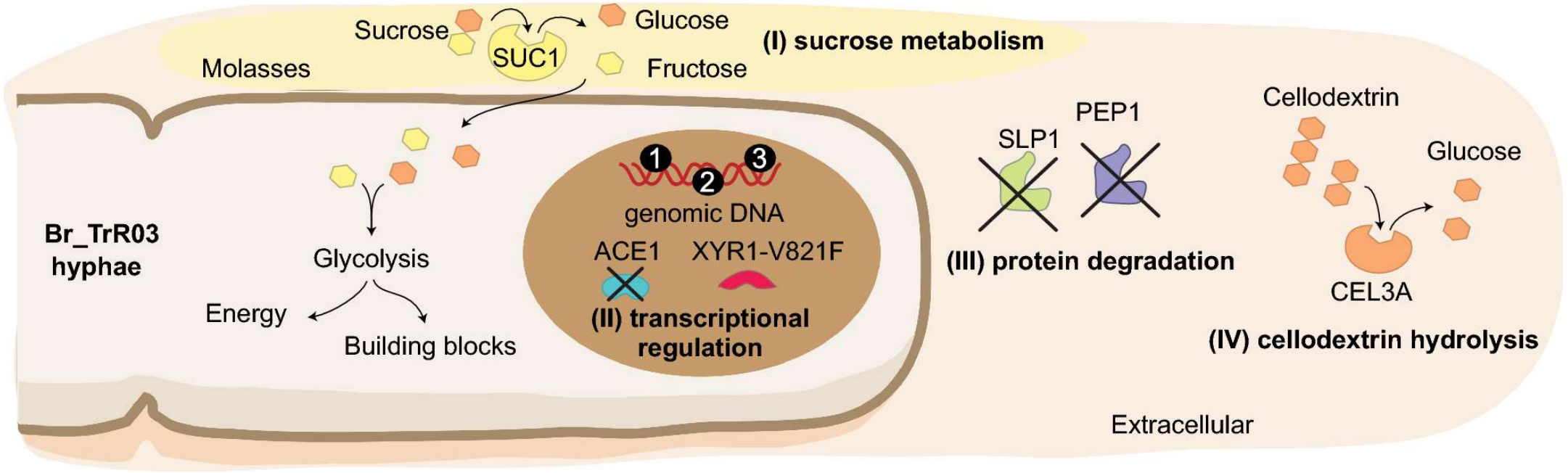
The genetic basis for obtaining the OpEn technology



Biotechnology for Biofuels. ***2020****, 93, 13*

The engineered strain still has room for improvements

Major goal: To translate fundamental discoveries from biodiversity into improved saccharification efficiency

Screening approach:

Saccharification assays that closely mimic industrial conditions (reducing sugar release)

- **biomass pretreated in pilot plant reactors**
- **high total solid contents (no free water!)**

Criteria for target selection from metagenomic libraries:

Unclassified sequences with remote identity (<20% SeqID) to CAZymes

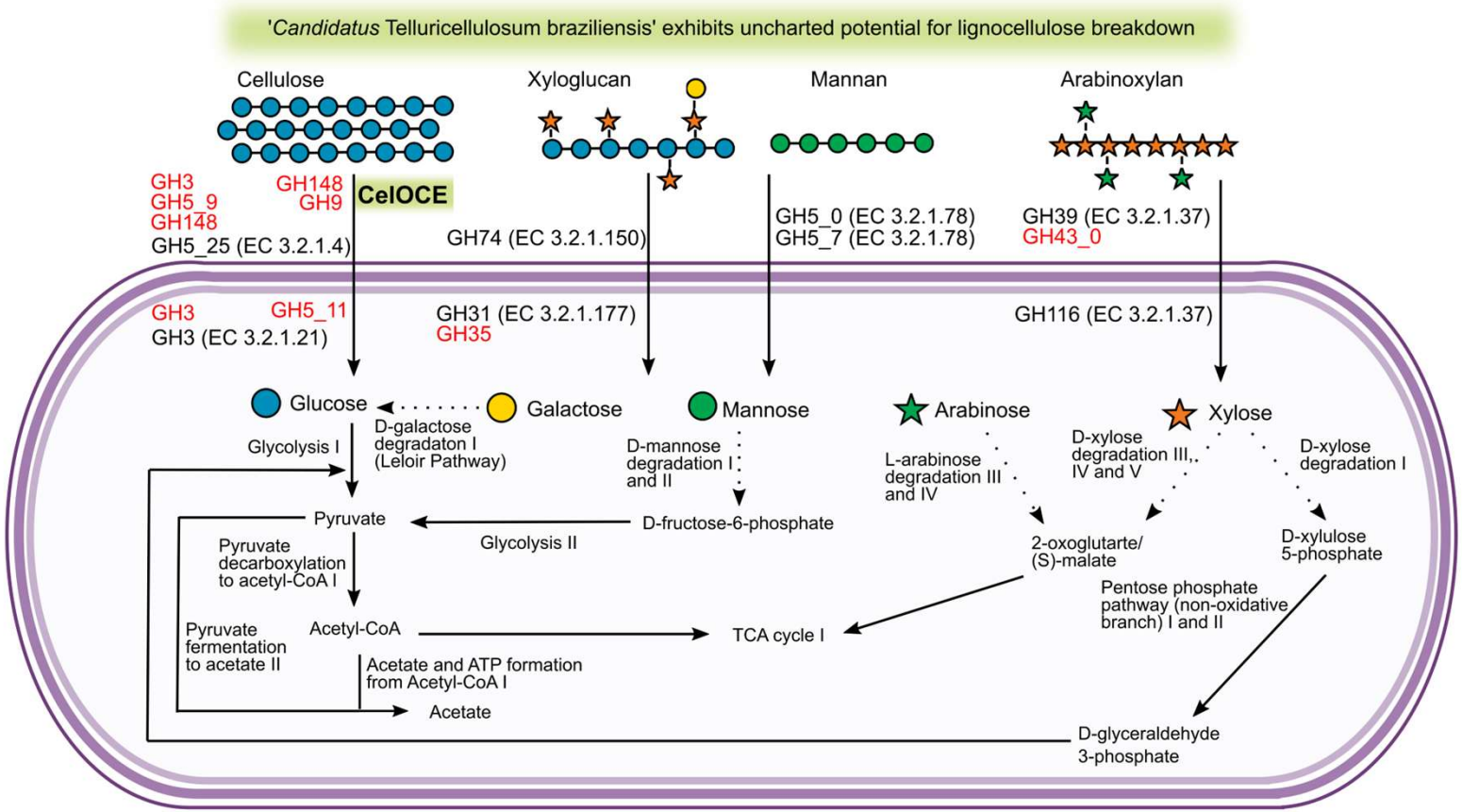
Fused to other CAZy-annotated proteins

Colocalized in MAGs with CAZymes

Co-expressed CAZymes in the presence of lignocellulosic materials

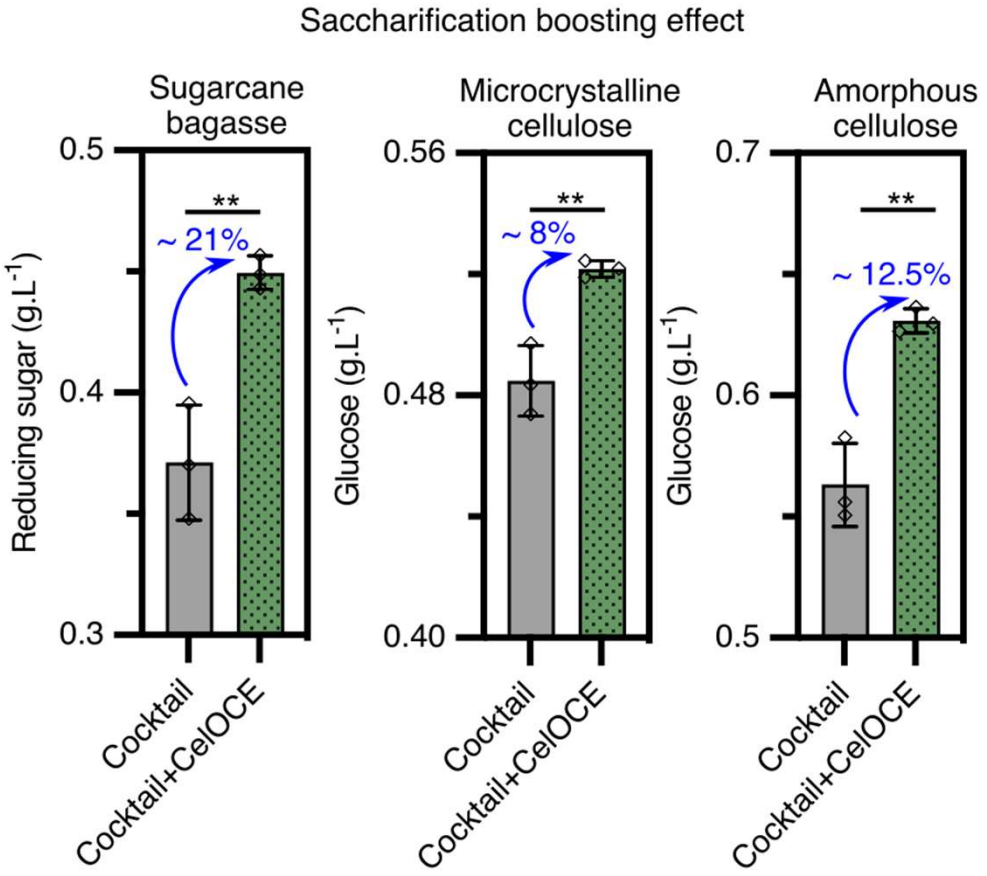
Manual curation and structural inspection of AI-based models

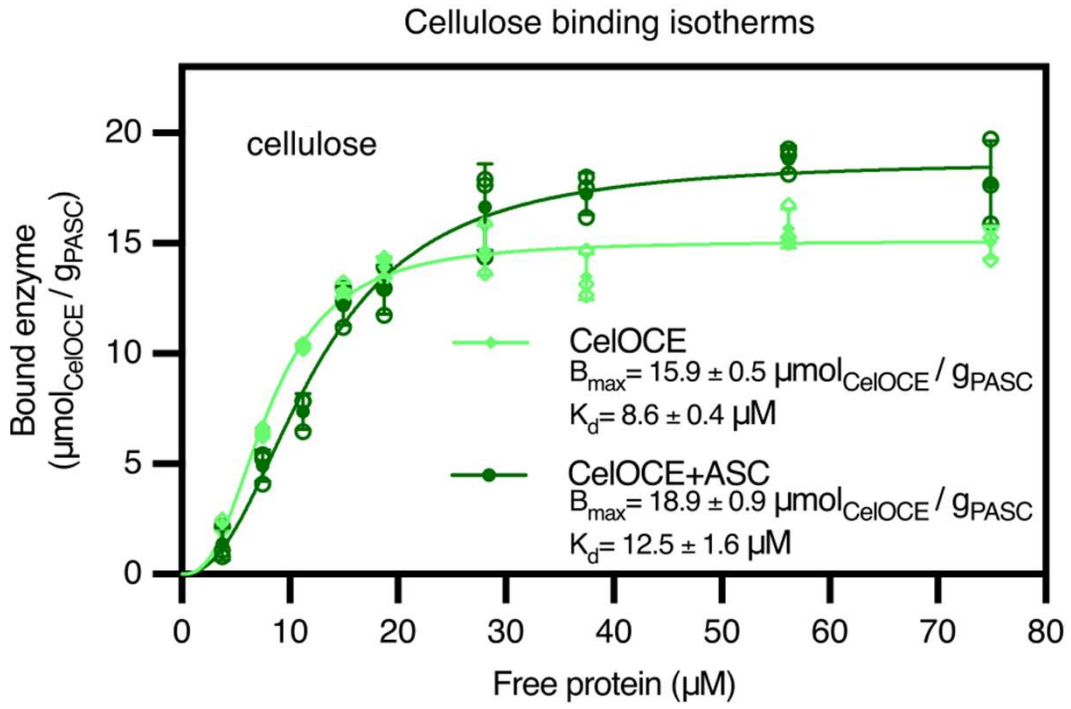
Uncharacterized phylum with uncharted potential for lignocellulose breakdown



Nature, 2025

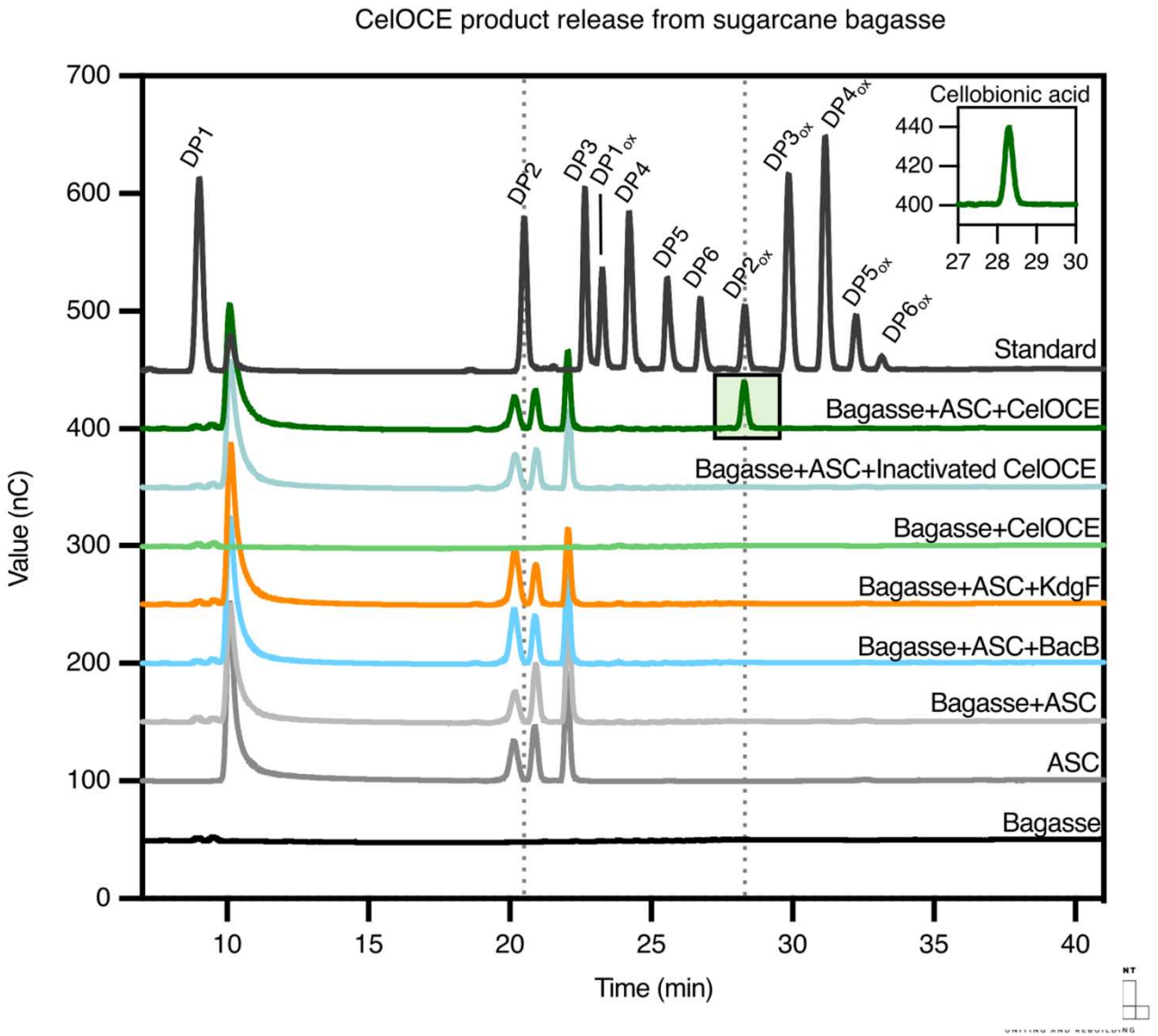
Mining the genome of this bacterium led to the discovery of a *T. reesei* secretome booster that binds to cellulose





Nature, 2025

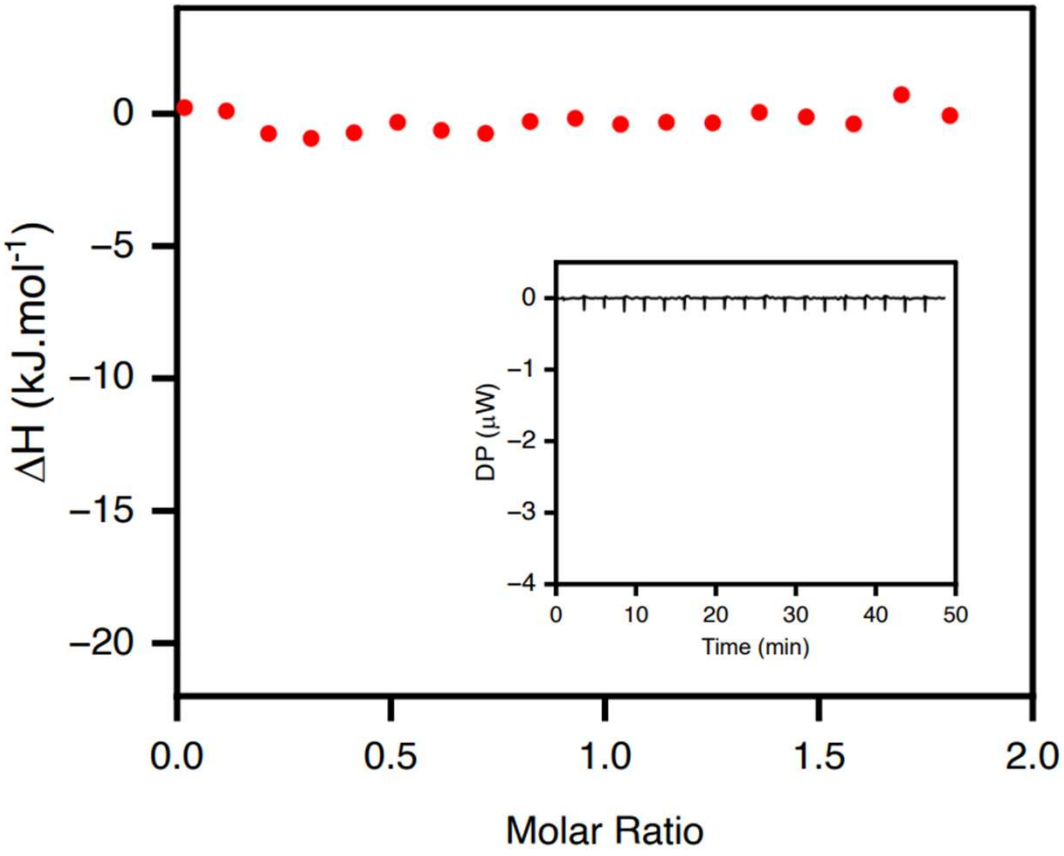
This protein releases cellobionic acid from lignocellulose



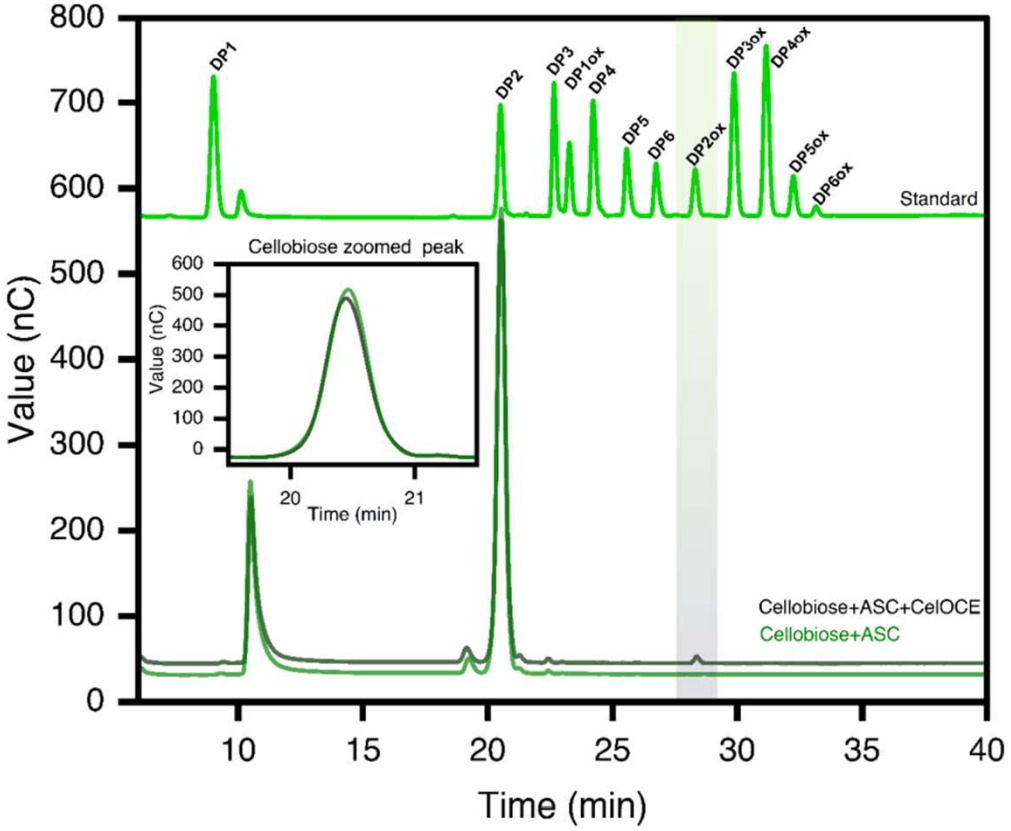
Nature, 2025

Is it a novel type of cellobiose dehydrogenase?

Isothermal Titration calorimetry



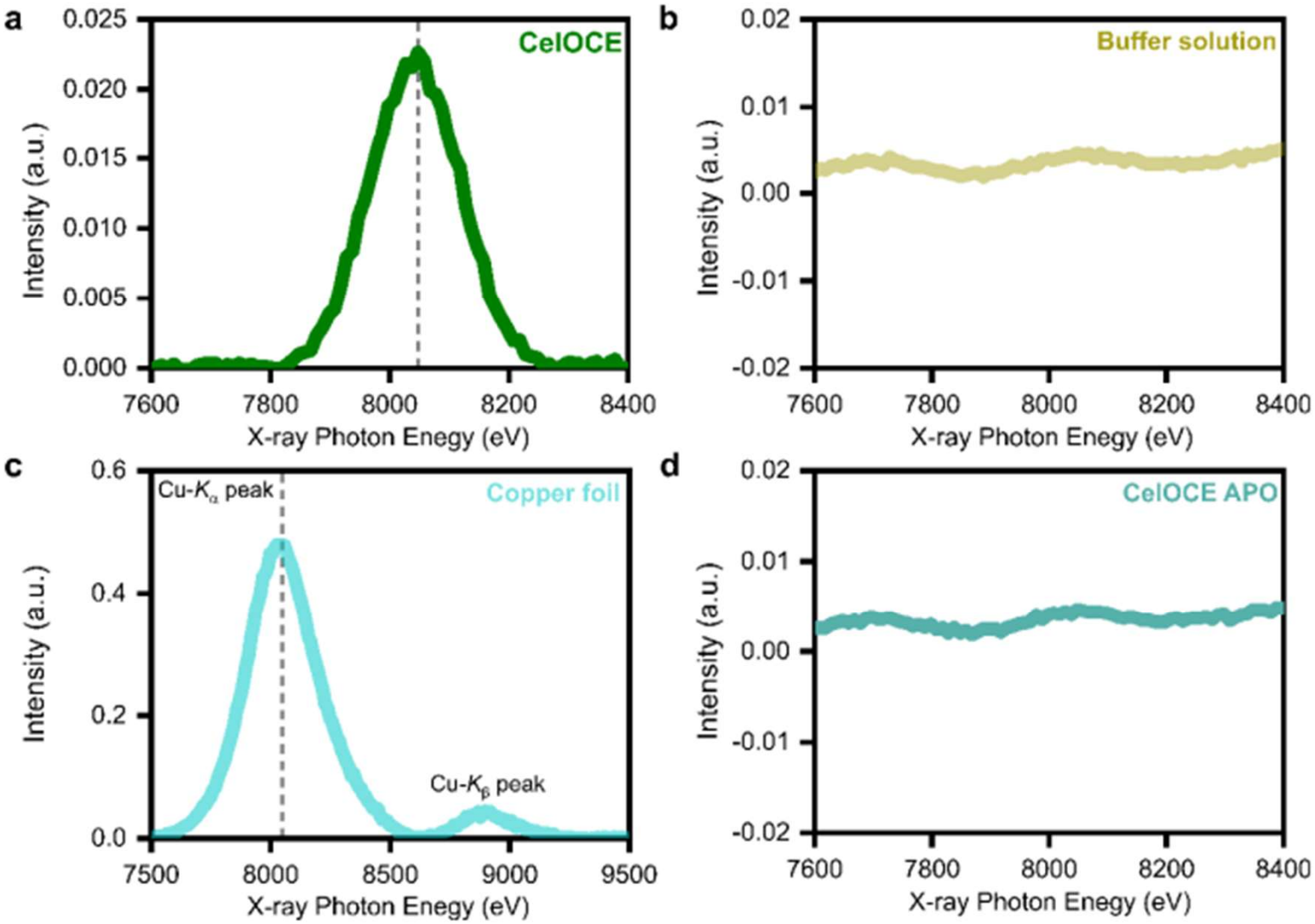
HPLC-PAD



Nature, 2025

It is a copper enzyme!

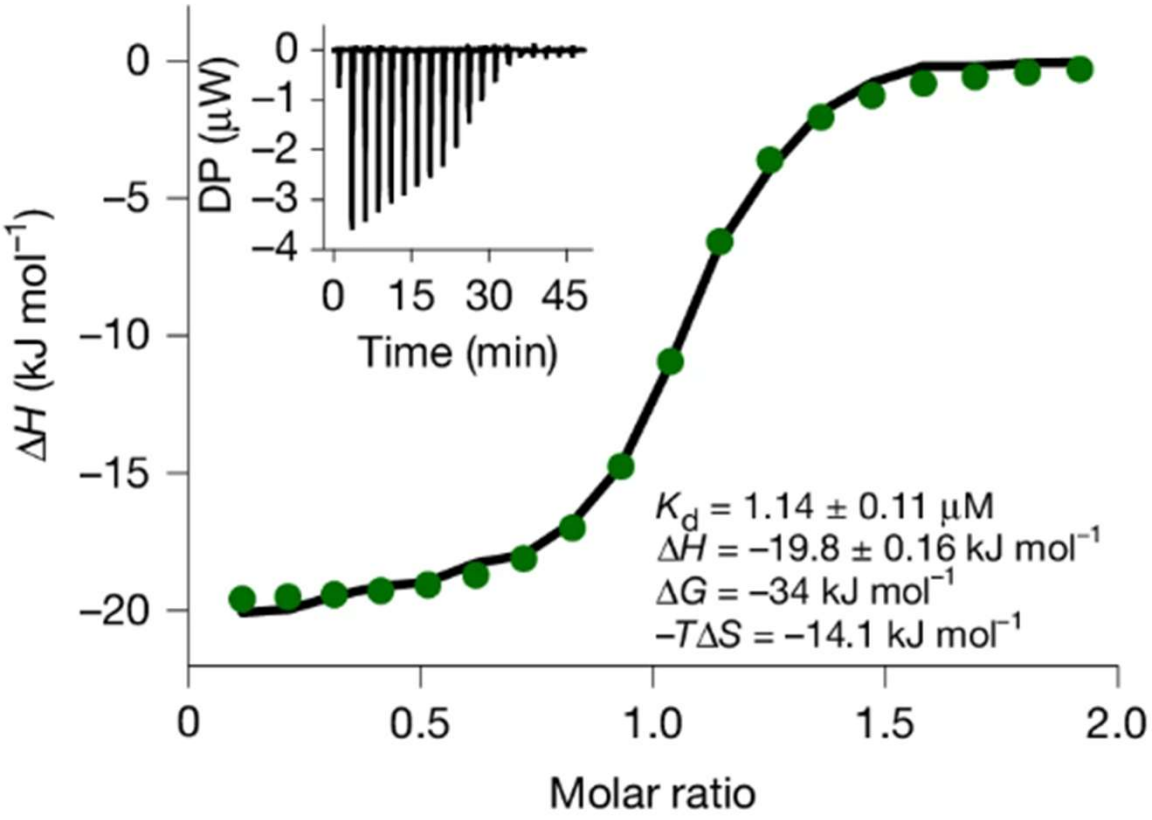
Synchrotron X-ray fluorescence spectroscopy (XRF) for accurate identification of copper binding to CelOCE

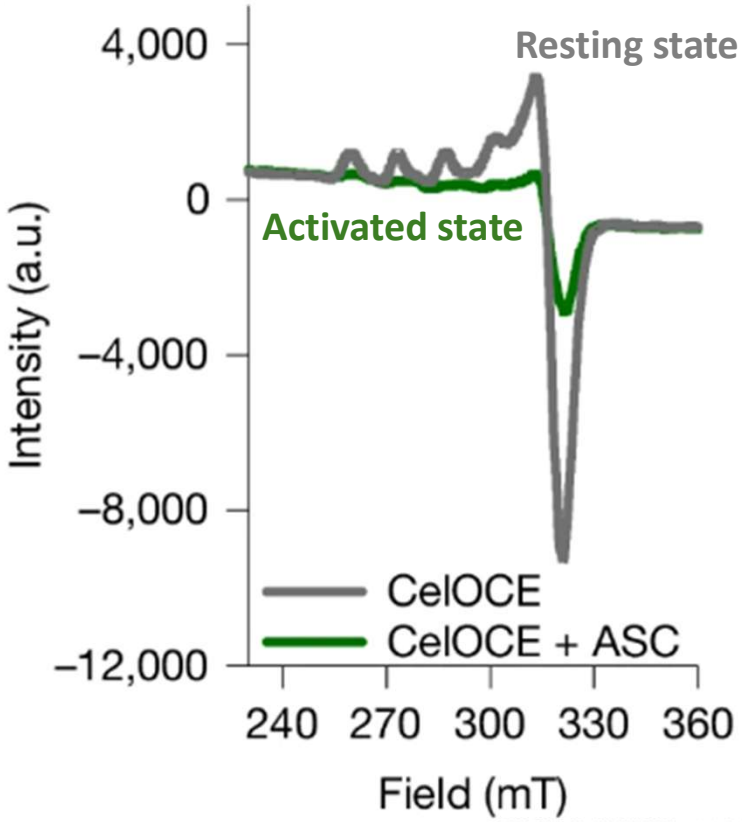


Nature, 2025

It is a copper enzyme!

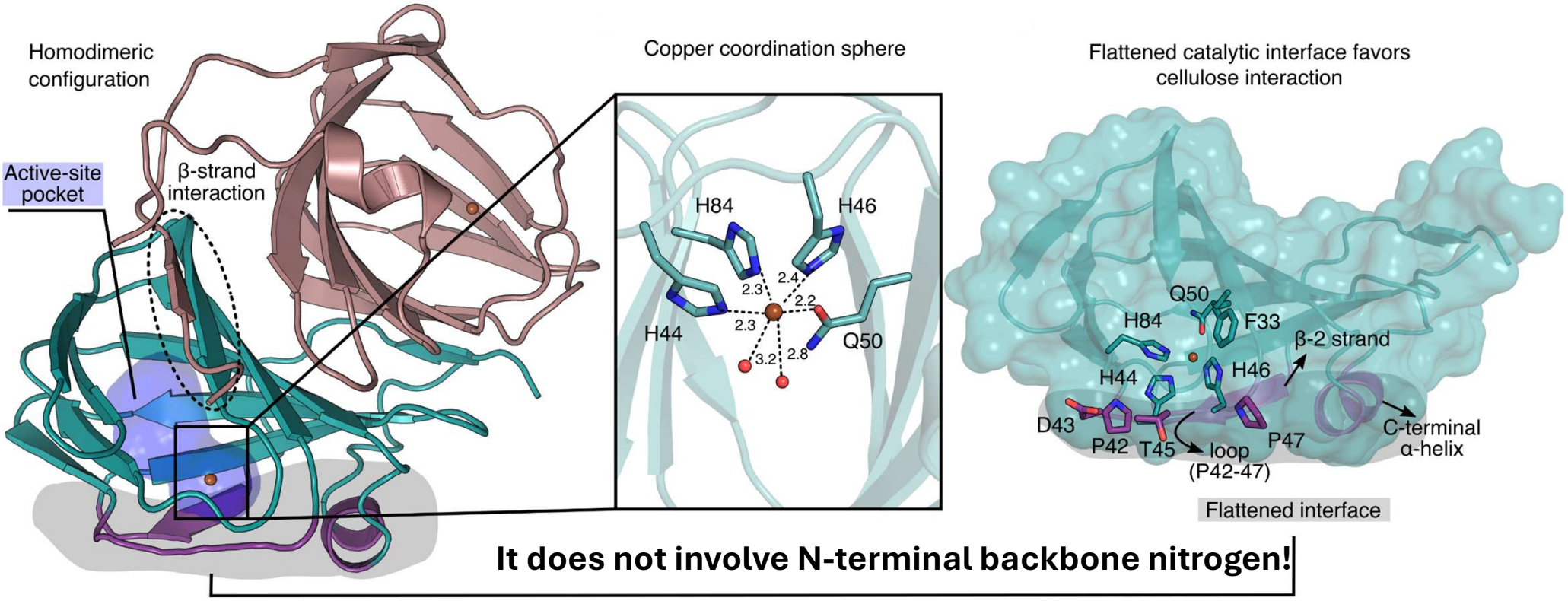
ITC data for copper binding to CelOCE and EPR spectra of CelOCE in the absence (grey line) and presence (green line) of reductant (ASC)





Nature, 2025

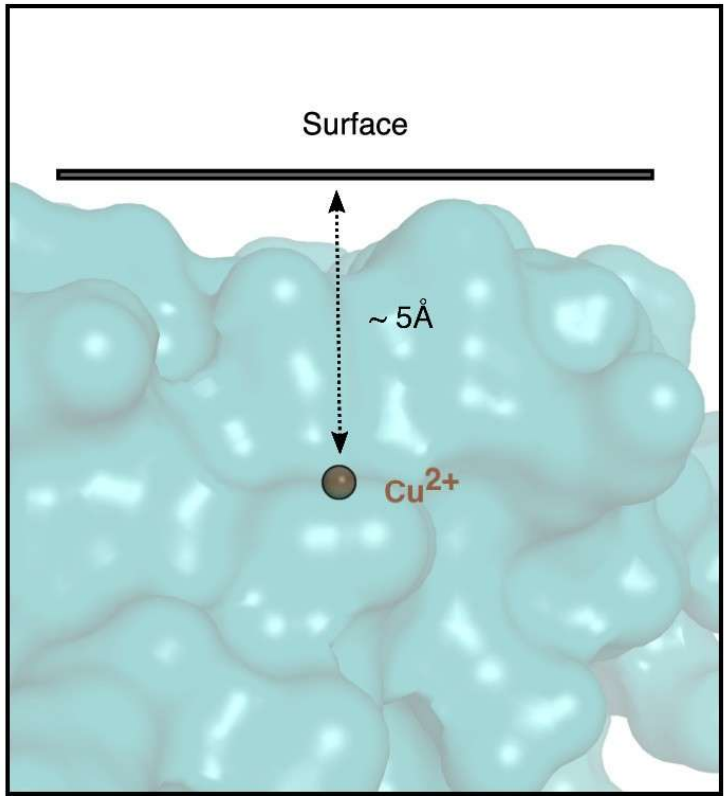
Crystal structure reveals a novel copper coordination sphere and a pocket-like active site

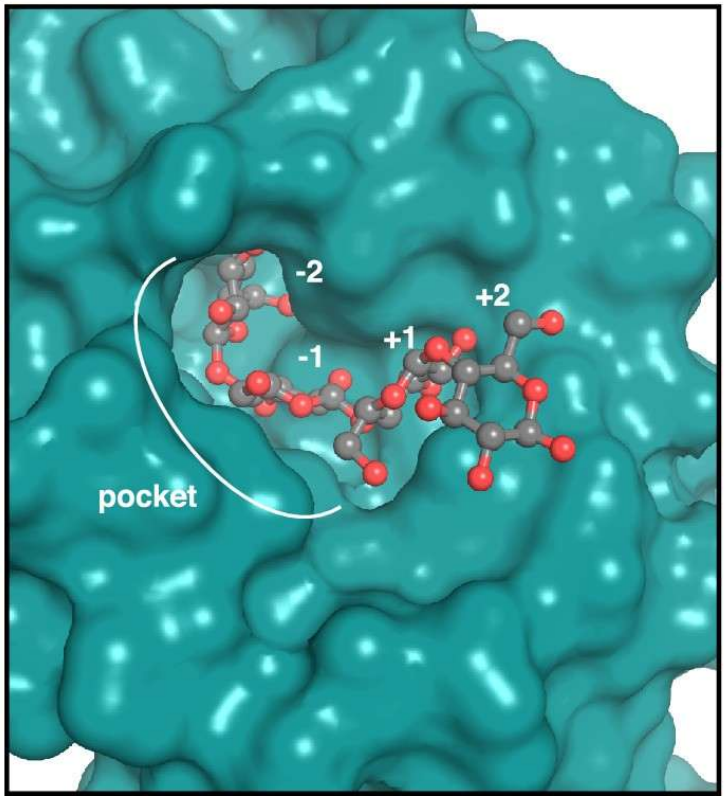


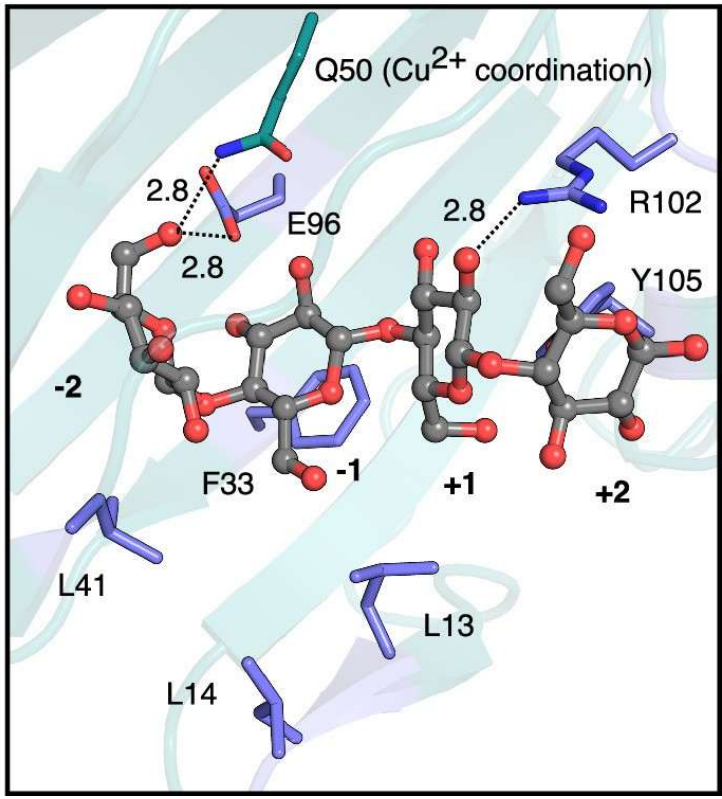
It does not involve N-terminal backbone nitrogen!

Nature, 2025

Pocket-like active site can accommodate a disaccharide agreeing with detected product

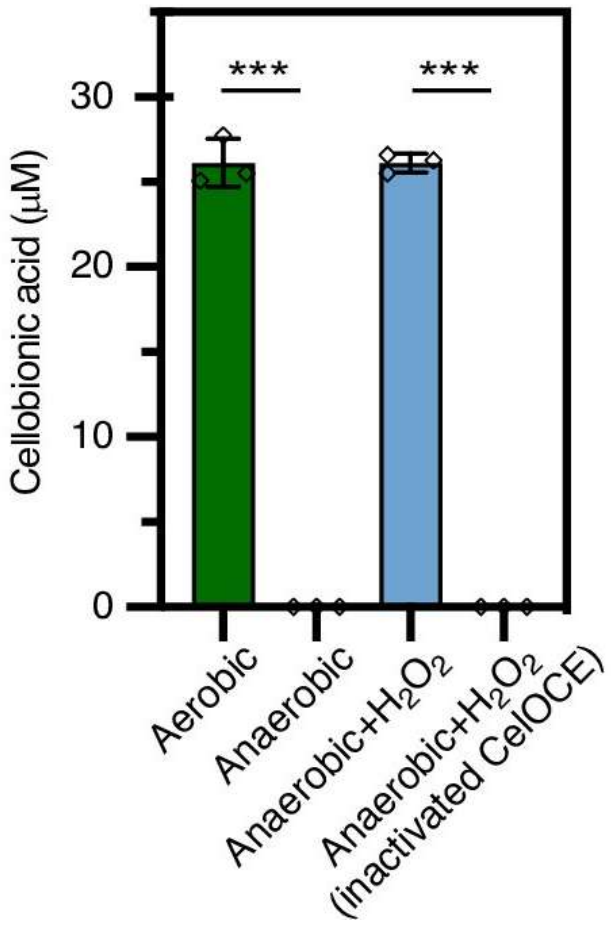


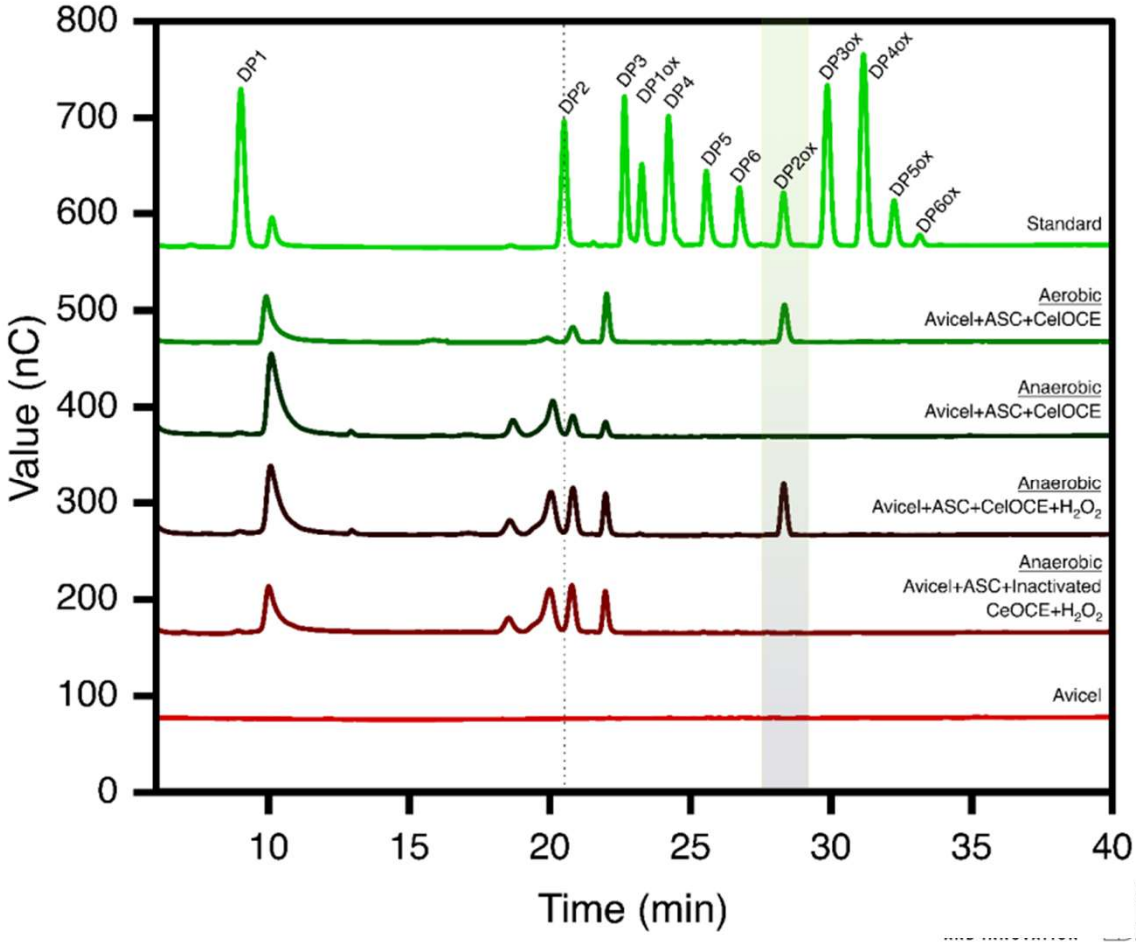




Nature, Accepted

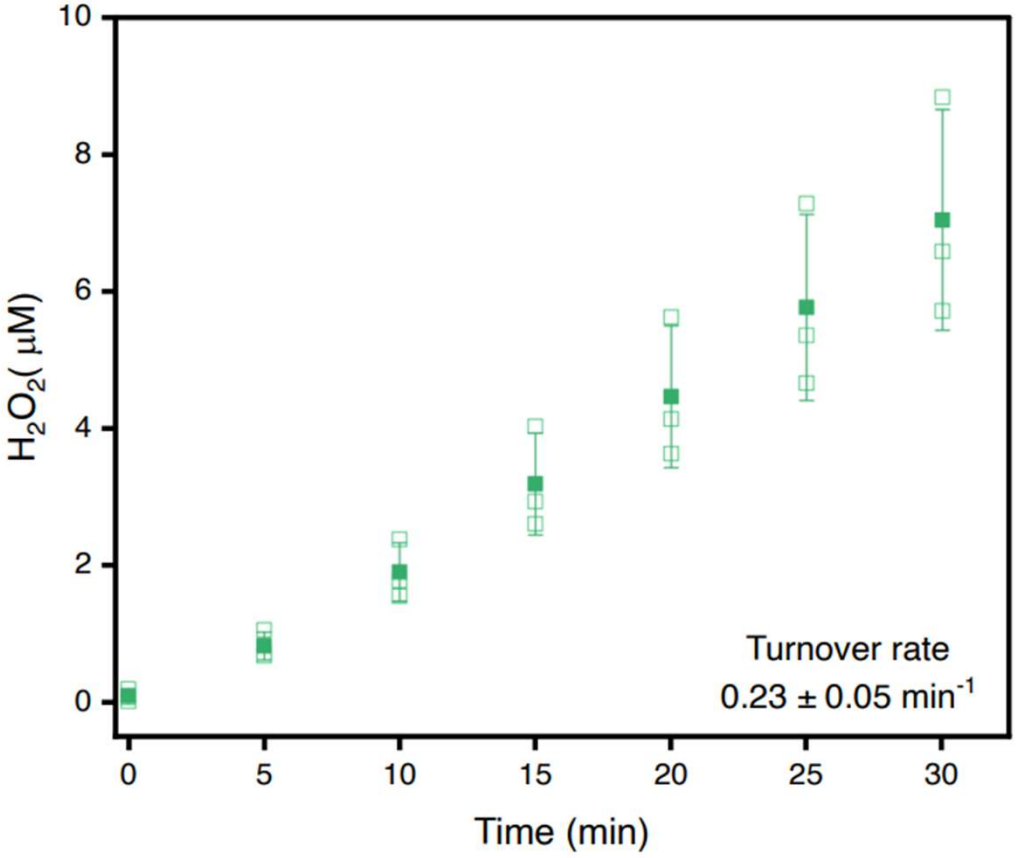
Activity under anaerobic conditions indicates that CelOCE may not an oxygenase!





Nature, 2025

CelOCE is able to produce H_2O_2 at a similar rate of cellobionic acid release from cellulose



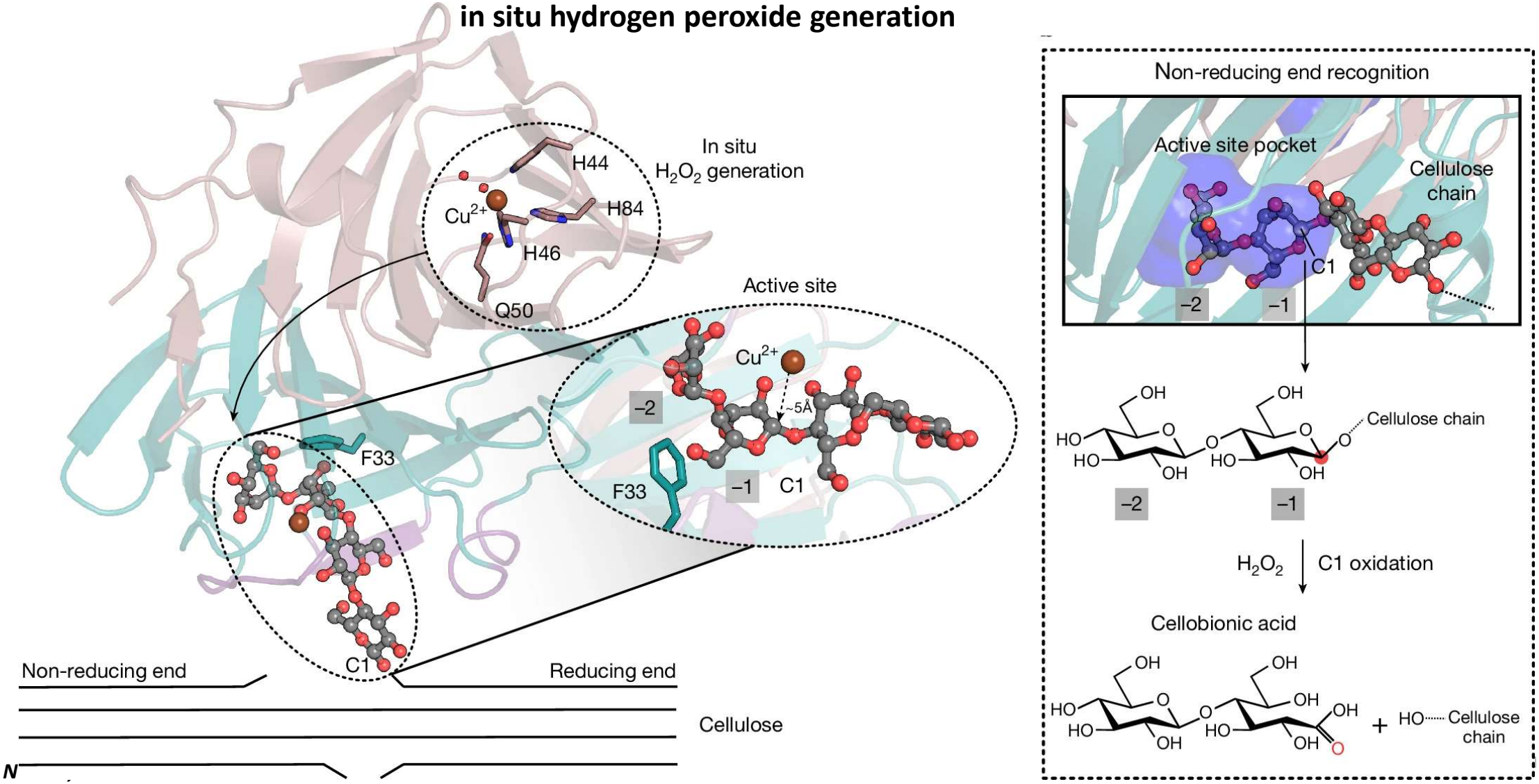
Nature, 2025

Comparison of turnover rates of CelOCE and LPMOs

Enzyme (conc.)	Avicel (g.L^{-1})	Oxygen source	Turnover (min^{-1})
CelOCE (1 µM)	1	O_2	0.05
SscLPMO10B (0.5 µM)	10	O_2	0.12
TbAA10-CBM2 (1 µM)	0,3	O_2	0.1
ScLPMO10C (2 µM)	10	O_2	0.05
AA10_07 (1 µM)	10	O_2	0.06

Proposed action mechanism

in situ hydrogen peroxide generation



Brazilian Enzyme Cocktail Platform

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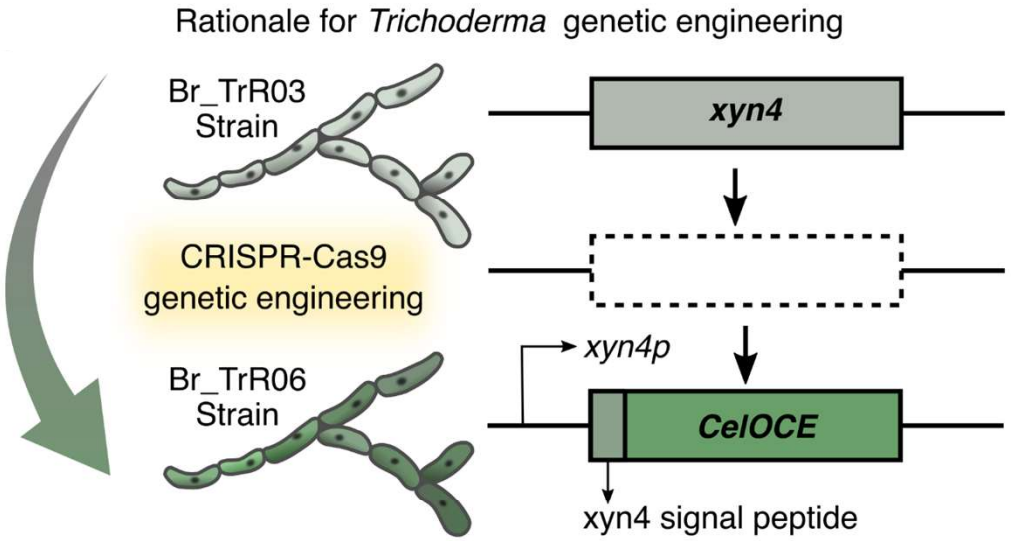
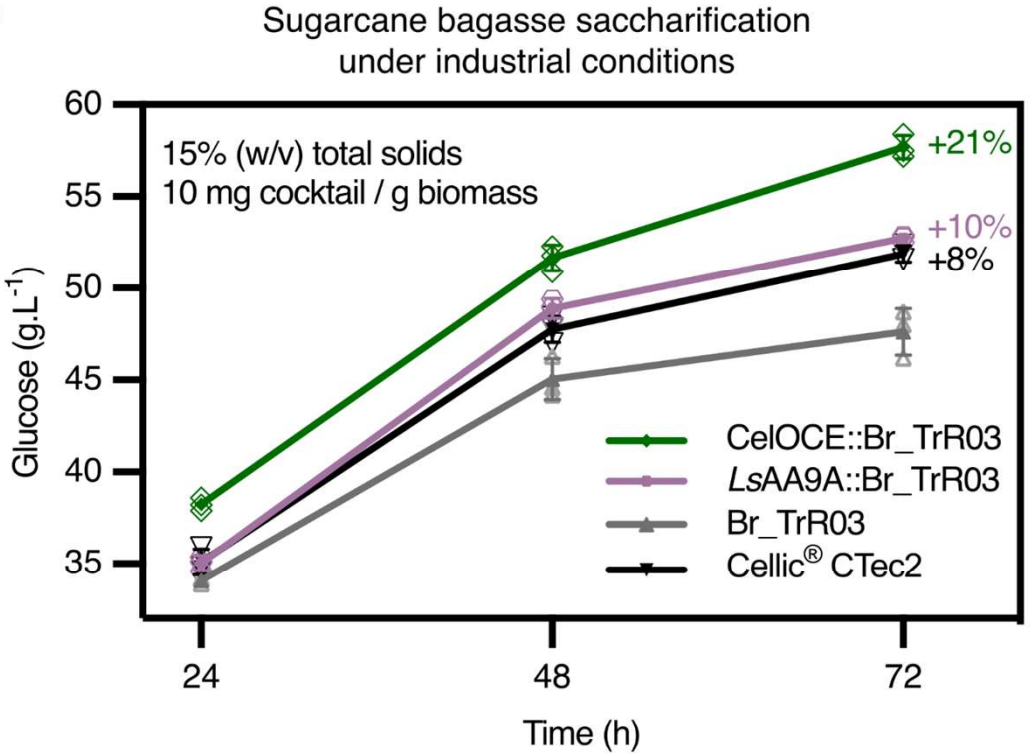
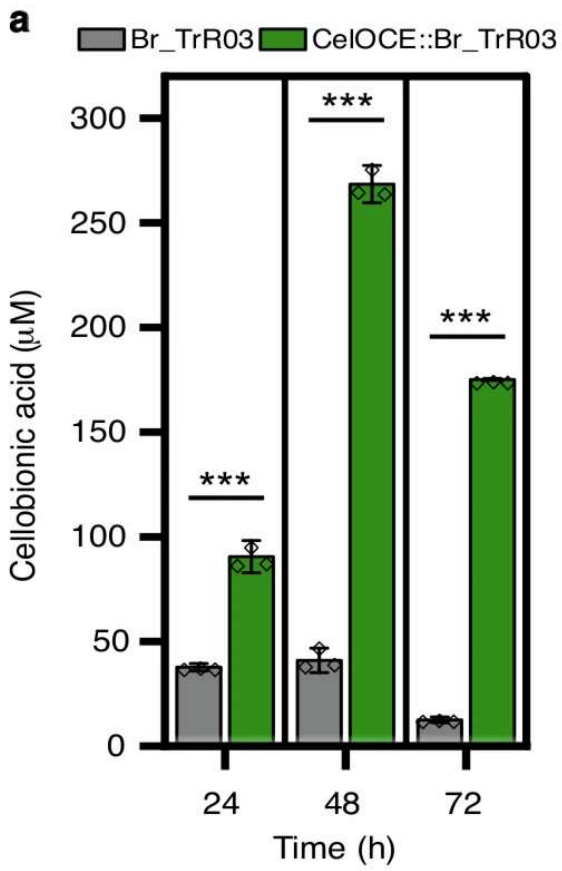
CelOCE co-expression in the OpEn platform boosts glucose release from pretreated biomass

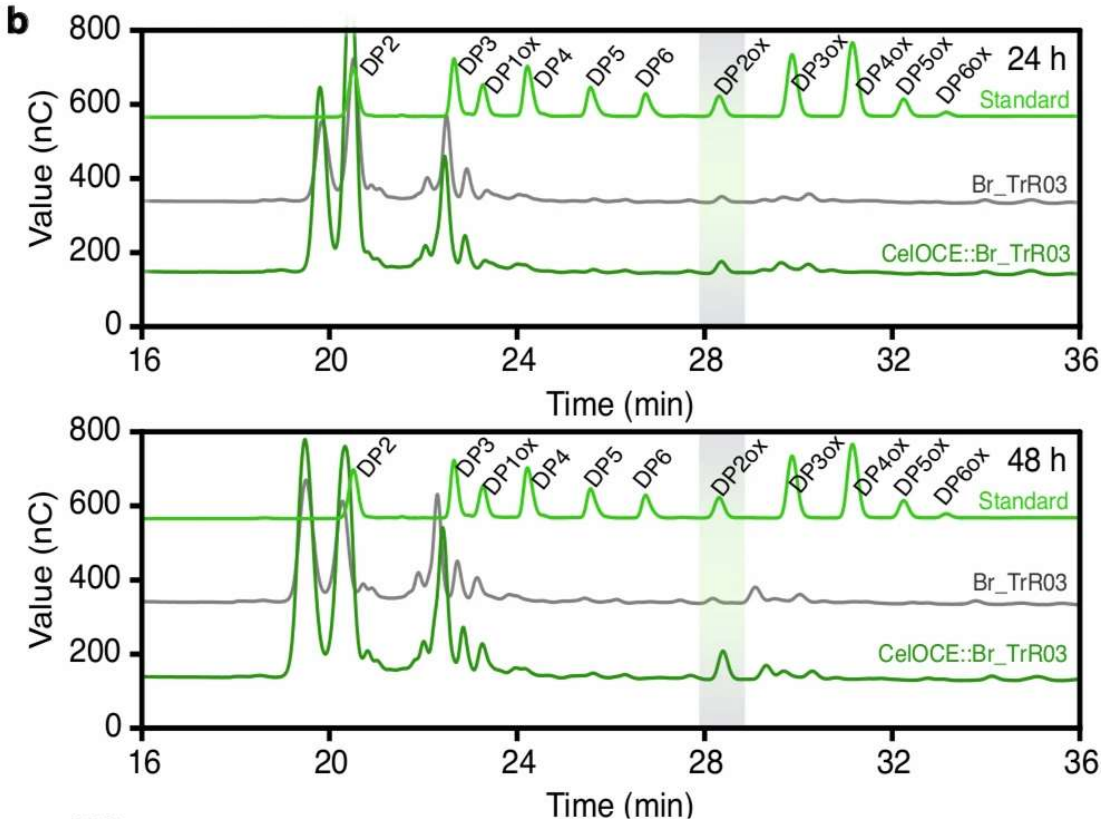




Nature, 2025

Cellobionic acid production by the secretome of engineered strains





Nature, 2025

Concluding remarks

LNBR/CNPEM research aims the development of zero-residue lignocellulosic biorefineries

Nature found different ways to cope with cellulose recalcitrance previously thought to be restricted to the LPMO scaffold

Open questions

What is the molecular basis for cellulose interaction?

What is the mechanism of synergism/potentiation with cellulases?

What is the functional and mechanistic diversity among orthologs?

Nature, 2025

Thank you!

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