Puidukeemia ja biotöötluse tuumiklabor

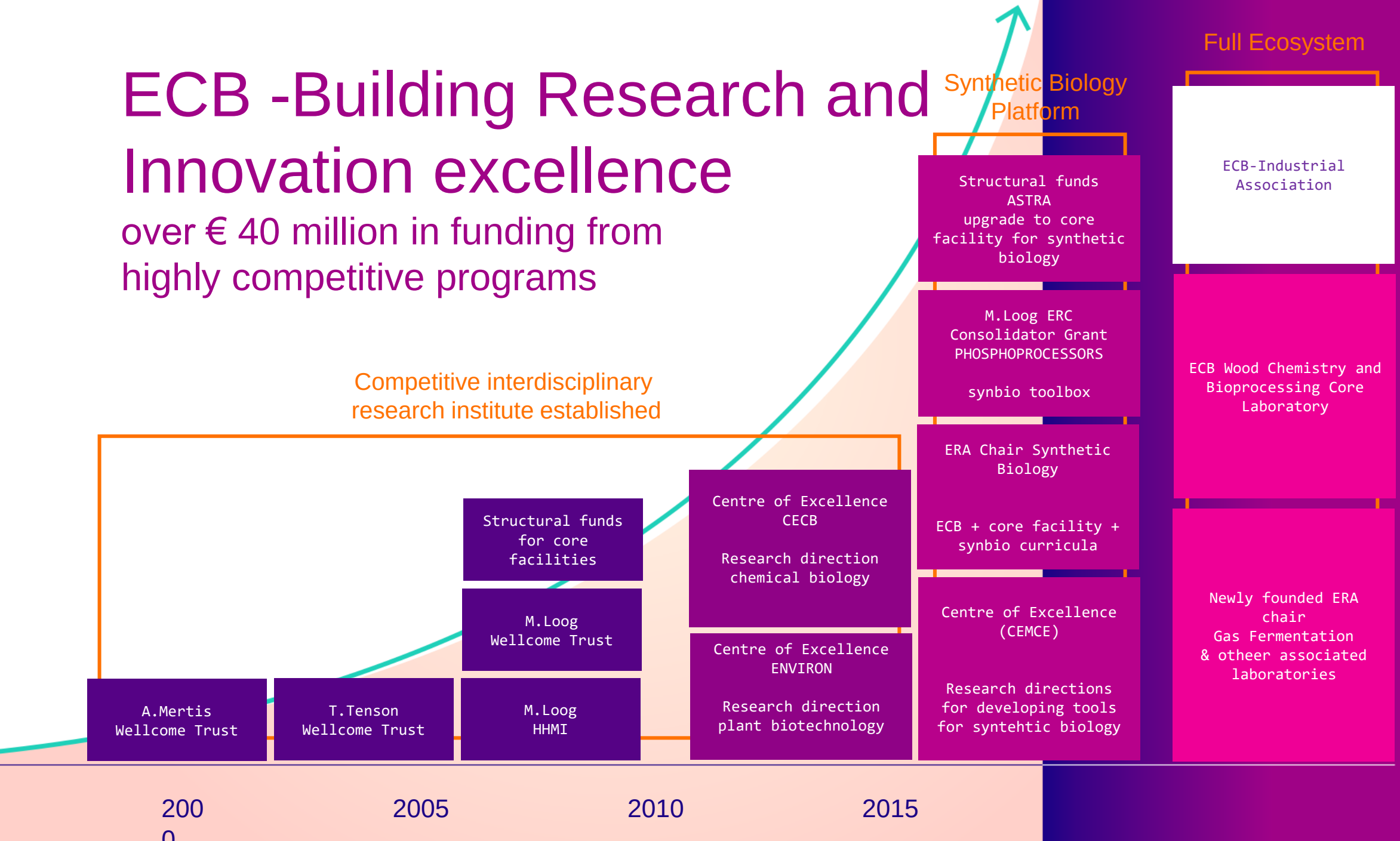
Mart Loog

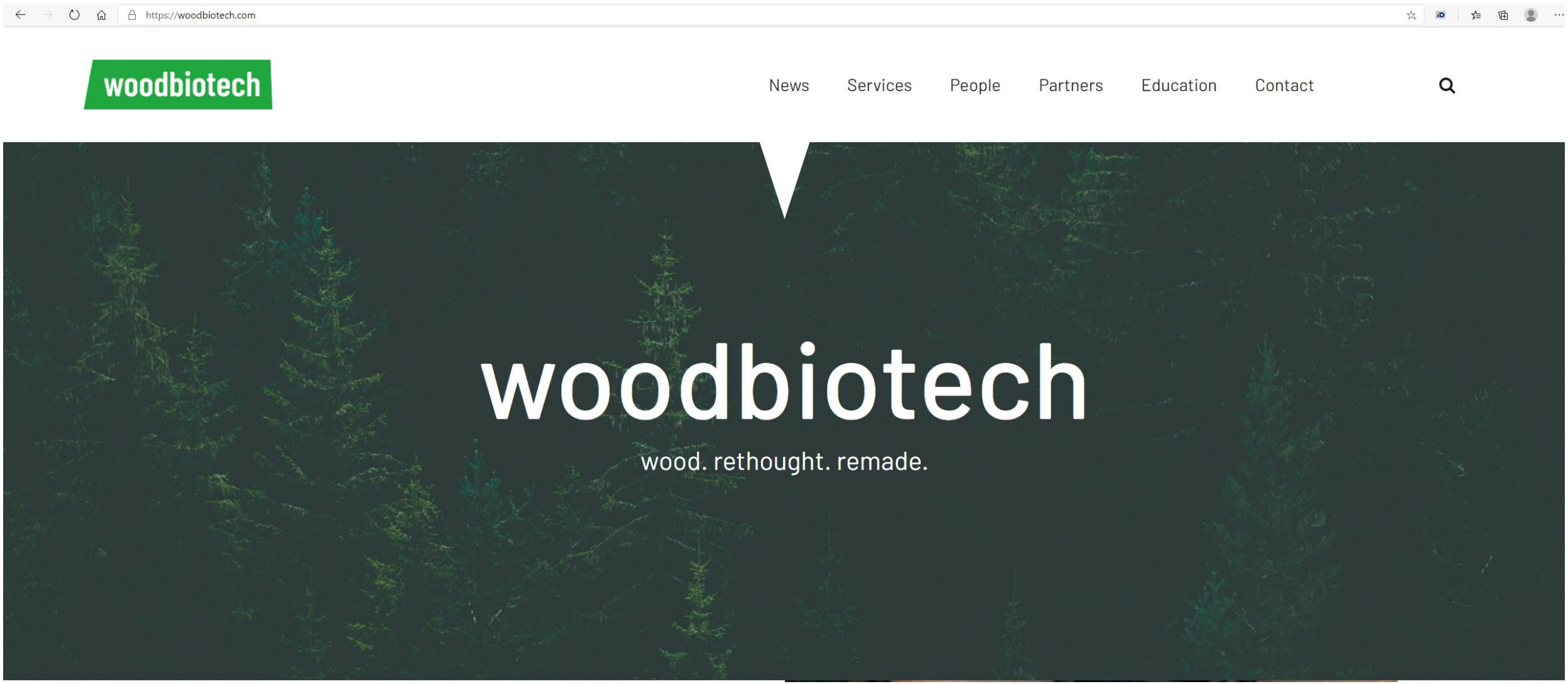
Estonian Centre for Biosustainability (ECB)

Tehnoloogiainstituut, Tartu Ülikool

ECB -Building Research and Innovation excellence

over € 40 million in funding from highly competitive programs

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Education
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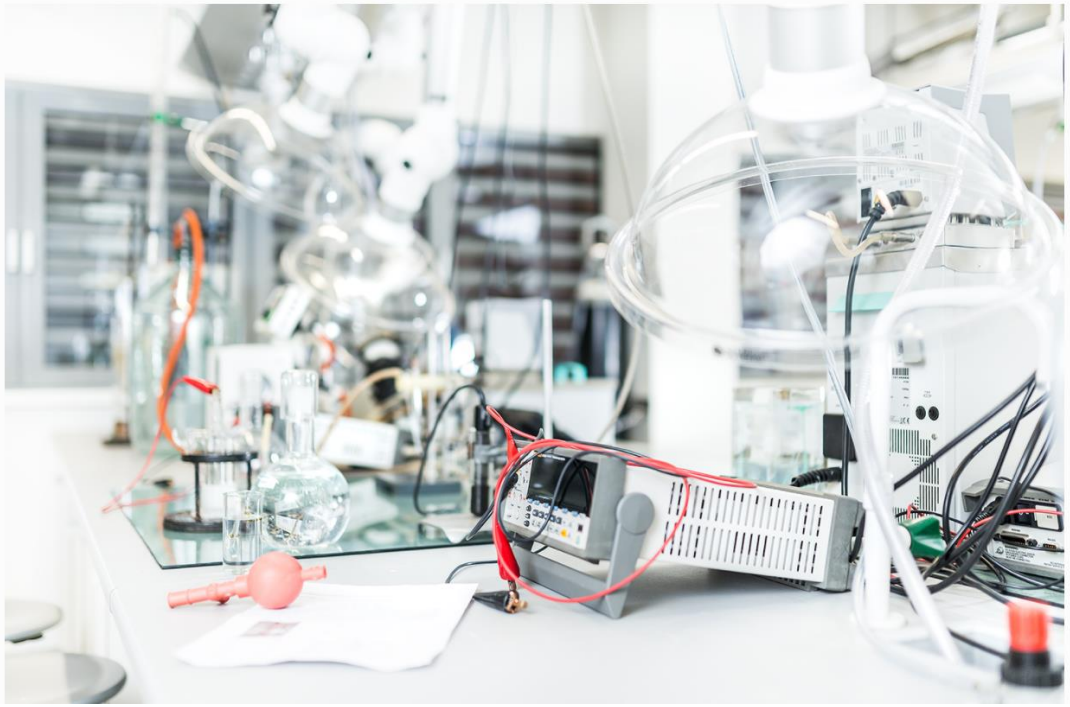
Associated laboratories



- Laboratory of systems and synthetic biology - **Prof. Mart Loog** (www.looglab.com)
- The laboratory for novel bio-based chemicals and polymers - **Dr. Lauri Vares, Prof. Patric Jannasch**
- Laboratory of Synthetic Biology and Bioprocess optimization - **Dr. Petri-Jaan Lahtvee**
- Laboratory of Gas Fermentation Technologies - **Dr. Kaspar Valgepea**
- Chair of Organic Chemistry - **Prof. Jaak Järv, Dr. Siim Salmar**

Synthetic biology in Estonia:
a historical opportunity

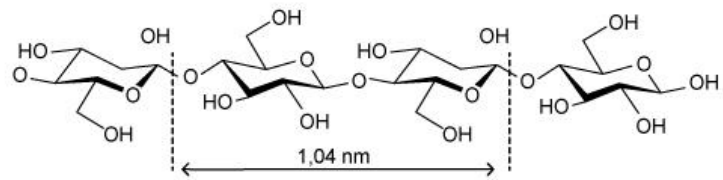
Local substrate

Value added chemicals

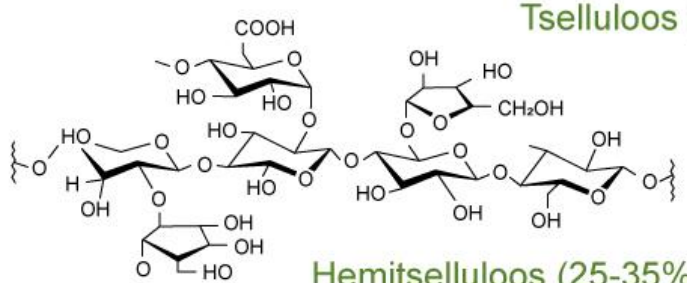
Atmosfääriline CO_2

fotosüntees

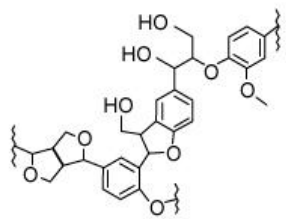
Puit



Tselluloos (40-50%)



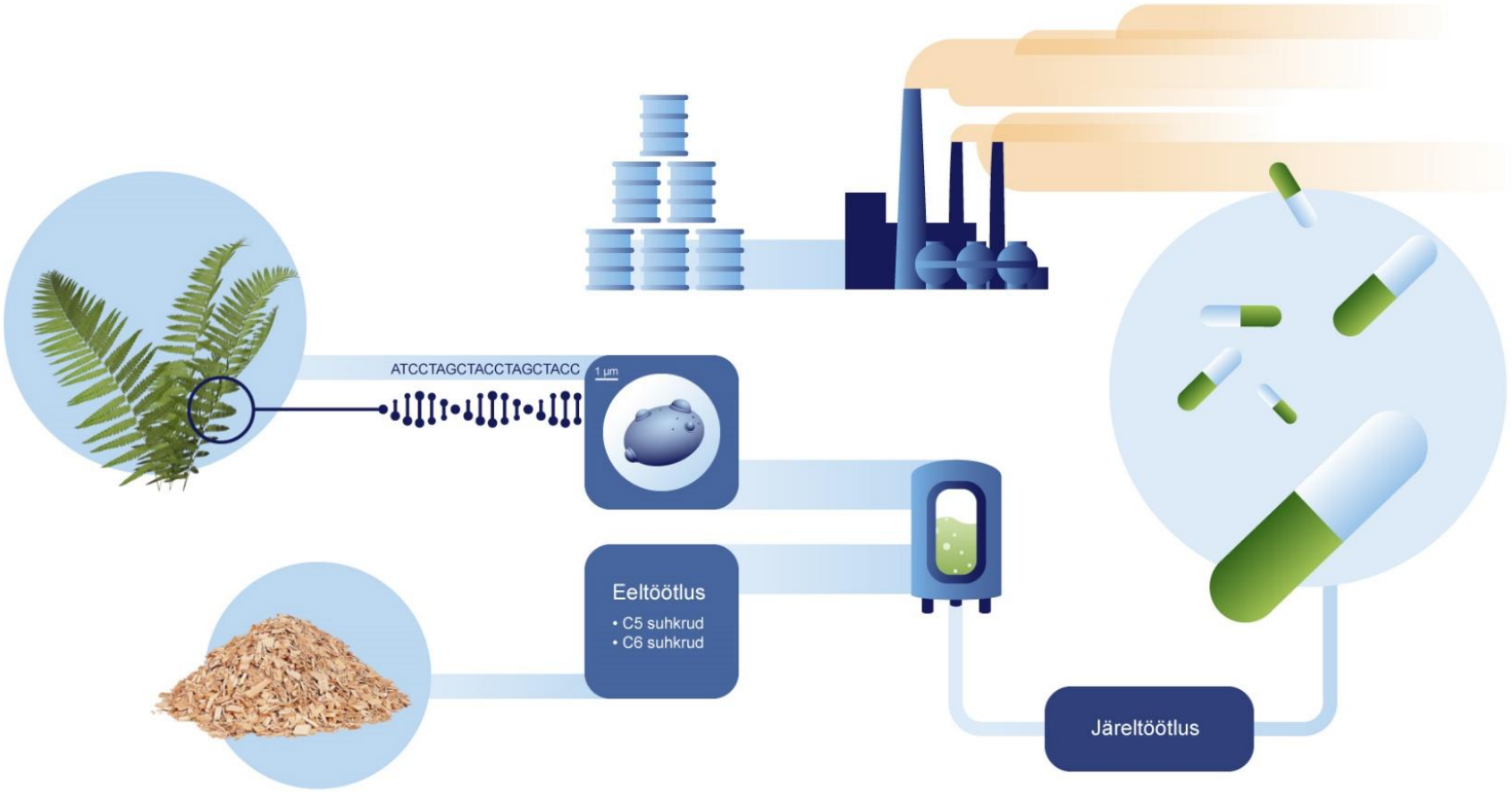
Hemitselluloos (25-35%)



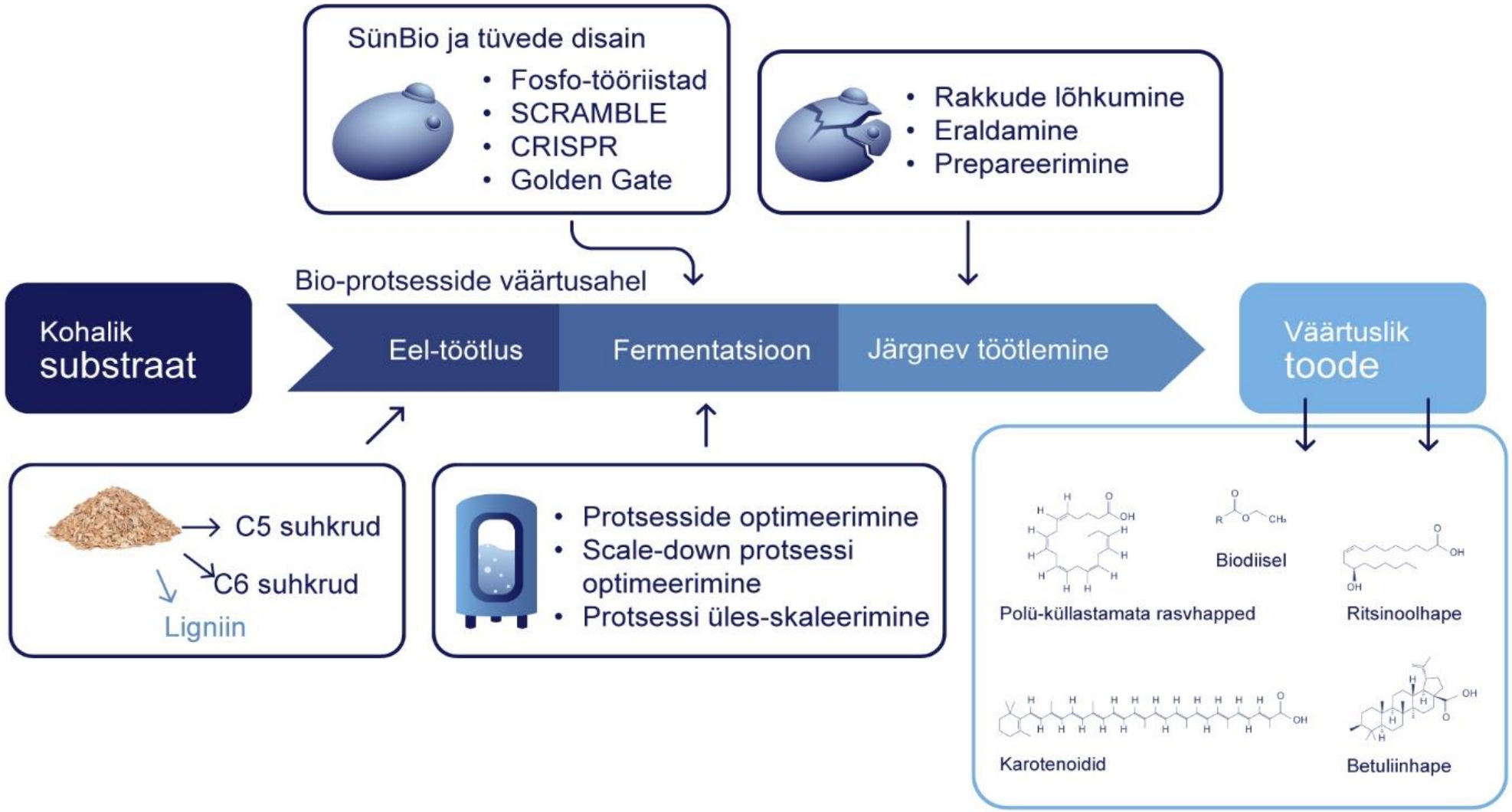
Ligniin (15-20%)

Rakuvabrikud ja biotöötlus

Traditsiooniline keemiatööstus



Rakuvabrikud ja biotöötlus


SünBio ja tüvede disain
• Fosfo-tööriistad
• SCRAMBLE
• CRISPR
• Golden Gate
• Rakkude lõhkumine
• Eraldamine
• Prepareerimine
Bio-protsesside väärtusahel
Kohalik substraat
Eel-töötlus
Fermentatsioon
Järgnev töötlemine
Väärtuslik toode
C5 suhkrud
C6 suhkrud
Ligniin
• Protsesside optimeerimine
• Scale-down protsessi optimeerimine
• Protsessi üles-skaleerimine
Biodiisel
Polü-küllastamata rasvhapped
Ritsinoolhape
Karotenoidid
Betuliinhape

SCOTT BOTTOMS

UNIVERSITY OF TARTU

EDUCATION

- Purdue University
  BSc. Microbiology
- University of Wisconsin
  MSc. Bacteriology
- Technical University of Munich
  PhD Candidate. Biotechnology

SELECT PUBLICATIONS

- Chemical genomic guided engineering of gamma-valerolactone tolerant yeast. **Microbial Cell Factories 2018, 12:17(1)5.** DOI: 10.1186/s12934-017-0848-9
- Mechanism of imidazolium ionic liquids toxicity in *Saccharomyces cerevisiae* and rational engineering of a tolerant, xylose-fermenting strain. **Microbial Cell Factories 2016, 15:17.** DOI: 10.1186/s12934-016-0417-7
- Inhibition of microbial biofuel production in drought-stressed switchgrass hydrolysate. **Biotechnology for Biofuels 2016, 9:237**. DOI: 10.1186/s13068-016-0654-0
- SynergyScreen, an R package for design and analysis of compound synergy screens. **F1000Research 2016, 5(ISCB Comm J):1886.** DOI: 10.7490/f1000research.1112669.1
- Diverse lignocellulosic feedstocks can achieve high field-scale ethanol yields while providing flexibility for the biorefinery and landscape-level environmental benefits. **GCB Bioenergy 2018; 1–16.** DOI: 10.1111/gcbb.12533
- Identifying and engineering ancient variants of enzymes using Graphical Representation of Ancestral Sequence Predictions (GRASP). (Pre-print 2020: DOI: 10.1101/2019.12.30.891457 Under review)

PATENT / INVENTION

- "Recombinant Yeast Having Increased Tolerance to Ionic Liquids and Methods of Use". **United States Patent Application:** US20160333362A1**.** Publication Date: November 17, 2016.

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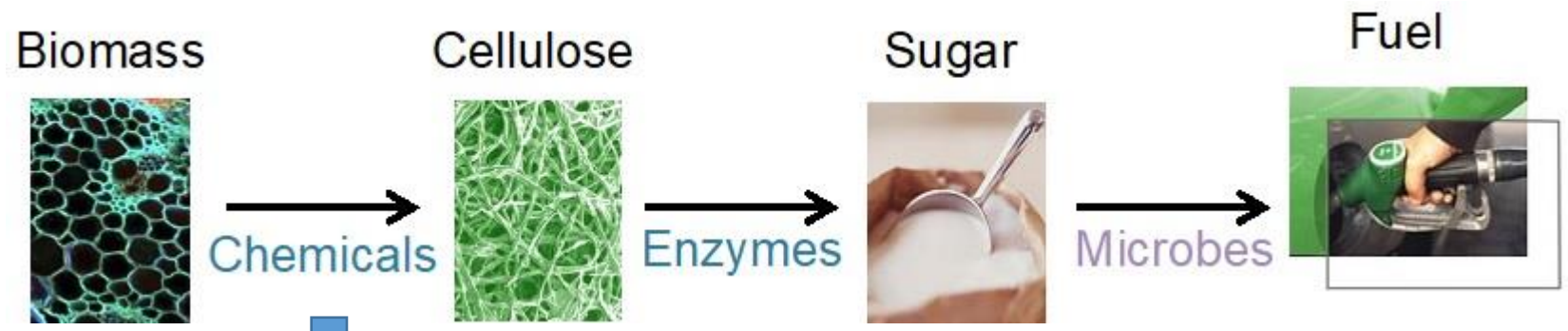
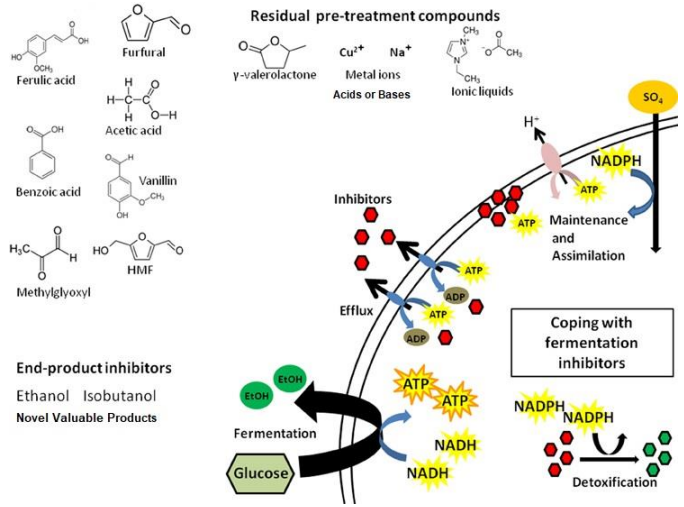
From Forest to Valuable Products

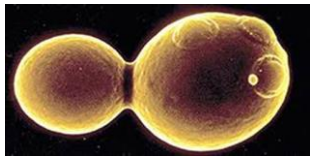
UNIVERSITY OF TARTU
1632



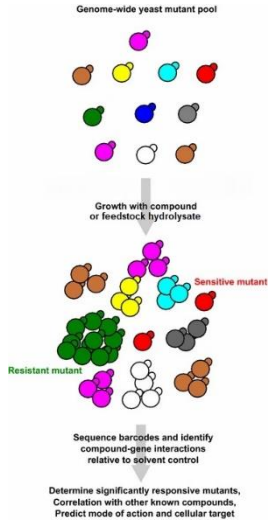
Lignin Monomer Inhibitors



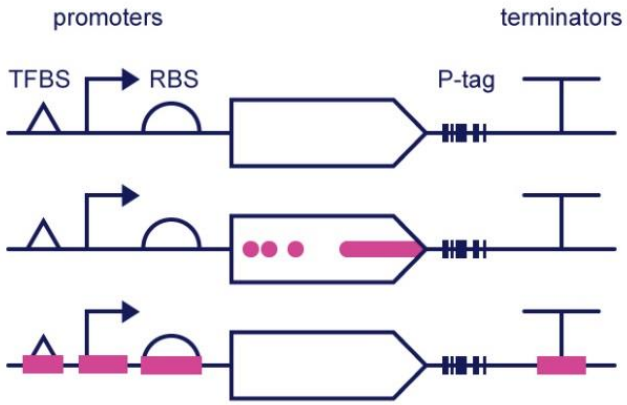
Piotrowski et al., 2015 (modified)

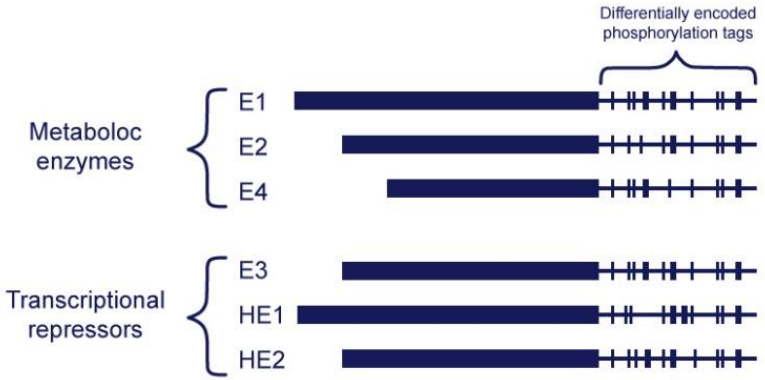


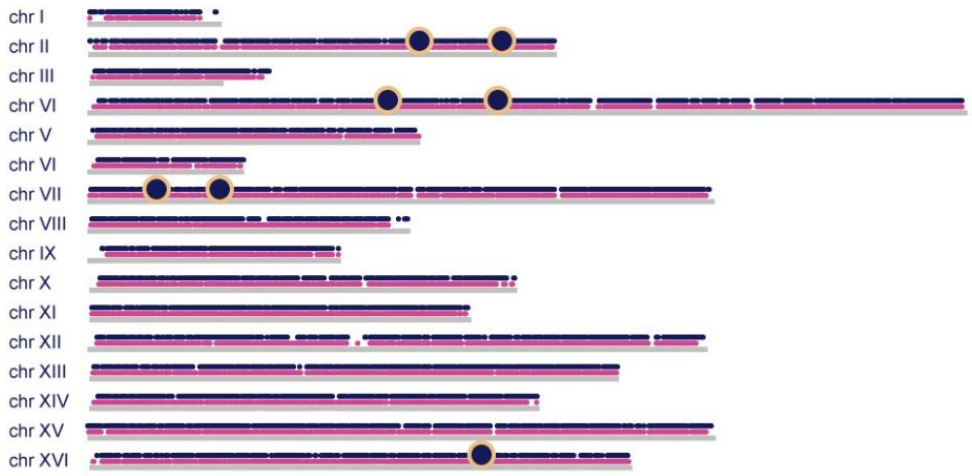
- Challenge to Using Lignocellulosic Feedstocks
  - Cellular inhibition by lignin monomers
- Microbial Strain Development is Essential
  - The Chemical Genomics Approach

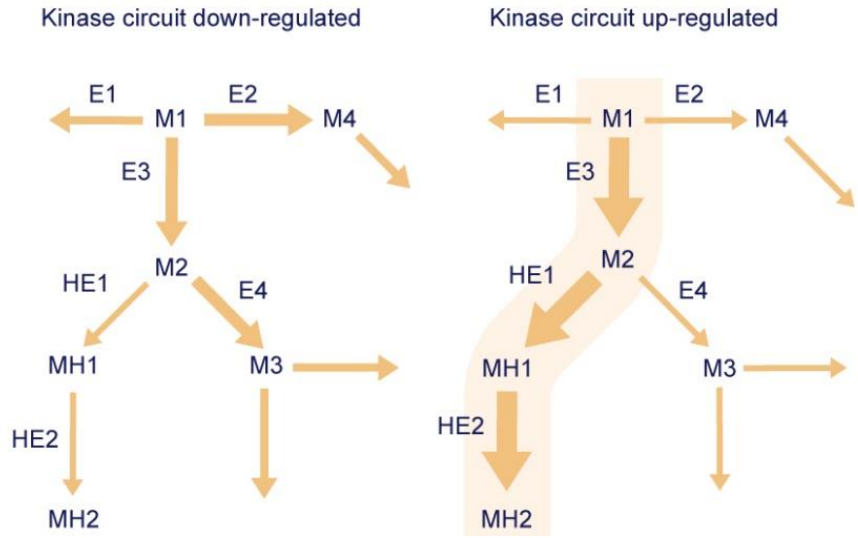


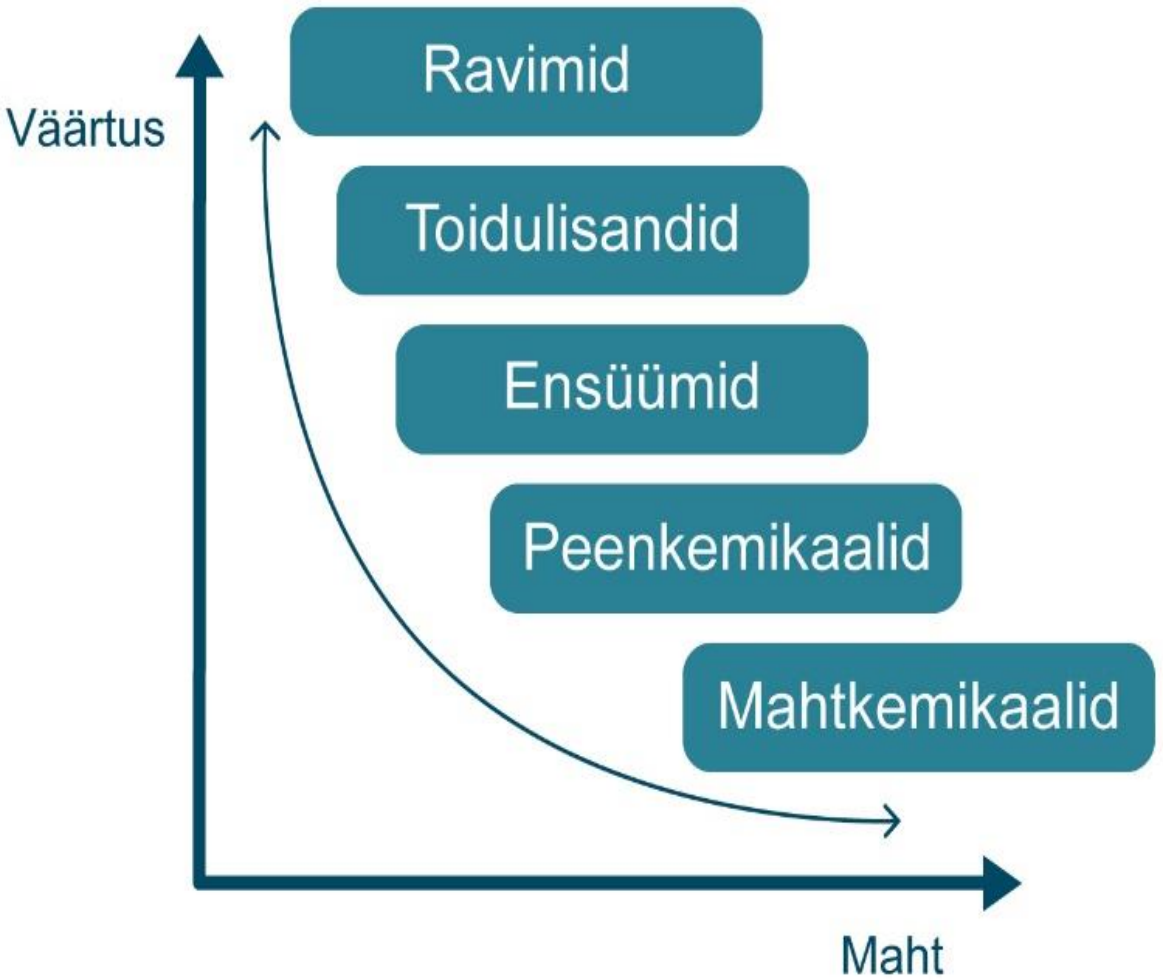
Bottoms et al., 2016


promoters
terminators
TFBS
RBS
P-tag


Differentially encoded phosphorylation tags
Metaboloc enzymes
E1
E2
E4
Transcriptional repressors
E3
HE1
HE2


chr I
chr II
chr III
chr VI
chr V
chr VI
chr VII
chr VIII
chr IX
chr X
chr XI
chr XII
chr XIII
chr XIV
chr XV
chr XVI


Kinase circuit down-regulated
Kinase circuit up-regulated
E1
M1
E2
M4
E3
M2
HE1
E4
MH1
M3
HE2
MH2


Väärtus
Ravimid
Toidulisandid
Ensüümid
Peenkemikaalid
Mahtkemikaalid
Maht


Väärtus
Betuliinhape
Polü-küllastamata rasvhapped
Karontenoidid
Ritsinoolhape
Biodiisel
Maht

Shahab *et al.*, *Science* **369**, eabb1214 (2020) 28 August 2020

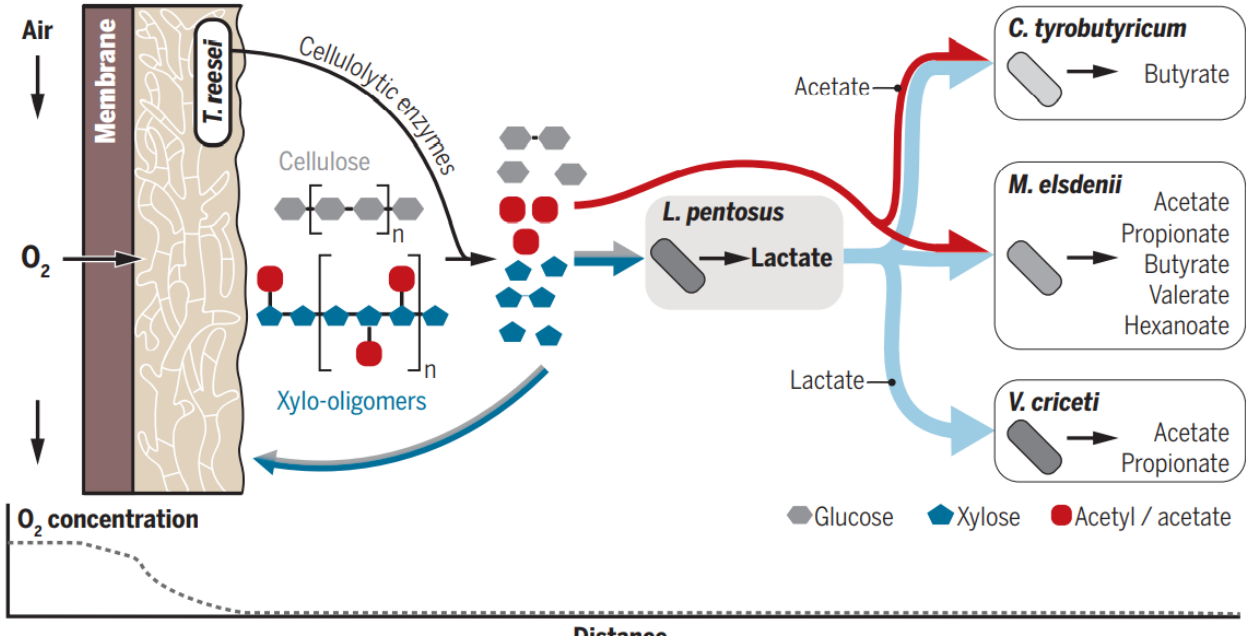
RESEARCH ARTICLE

MICROBIAL ENGINEERING

A heterogeneous microbial consortium producing short-chain fatty acids from lignocellulose

Robert L. Shahab[1,2], Simone Brethauer[2], Matthew P. Davey[3], Alison G. Smith[3], Silvia Vignolini[4], Jeremy S. Luterbacher[1], Michael H. Studer[2]*

Microbial consortia are a promising alternative to monocultures of genetically modified microorganisms for complex biotransformations. We developed a versatile consortium-based strategy for the direct conversion of lignocellulose to short-chain fatty acids, which included the funneling of the lignocellulosic carbohydrates to lactate as a central intermediate in engineered food chains. A spatial niche enabled in situ cellulolytic enzyme production by an aerobic fungus next to facultative anaerobic lactic acid bacteria and the product-forming anaerobes. *Clostridium tyrobutyricum*, *Veillonella criceti*, or *Megasphaera elsdenii* were integrated into the lactate platform to produce 196 kilograms of butyric acid per metric ton of beechwood. The lactate platform demonstrates the benefits of mixed cultures, such as their modularity and their ability to convert complex substrates into valuable biochemicals.



196 kilograms of butyric acid per metric ton of beechwood.

A membrane-aerated bioreactor featuring an oxygen gradient (depicted at the bottom) allows for the consolidated bioprocessing of lignocellulose to short-chain fatty acids by a heterogeneous consortium consisting of an aerobic, cellulolytic fungus growing as a biofilm in the oxygenreplete spatial niche, a facultative anaerobic lactic acid bacterium to funnel the carbohydrate mixture to lactate, and a lactate-consuming obligate anaerobic bacterium for product formation.

LETTER

https://doi.org/10.1038/s41586-019-0978-9

Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

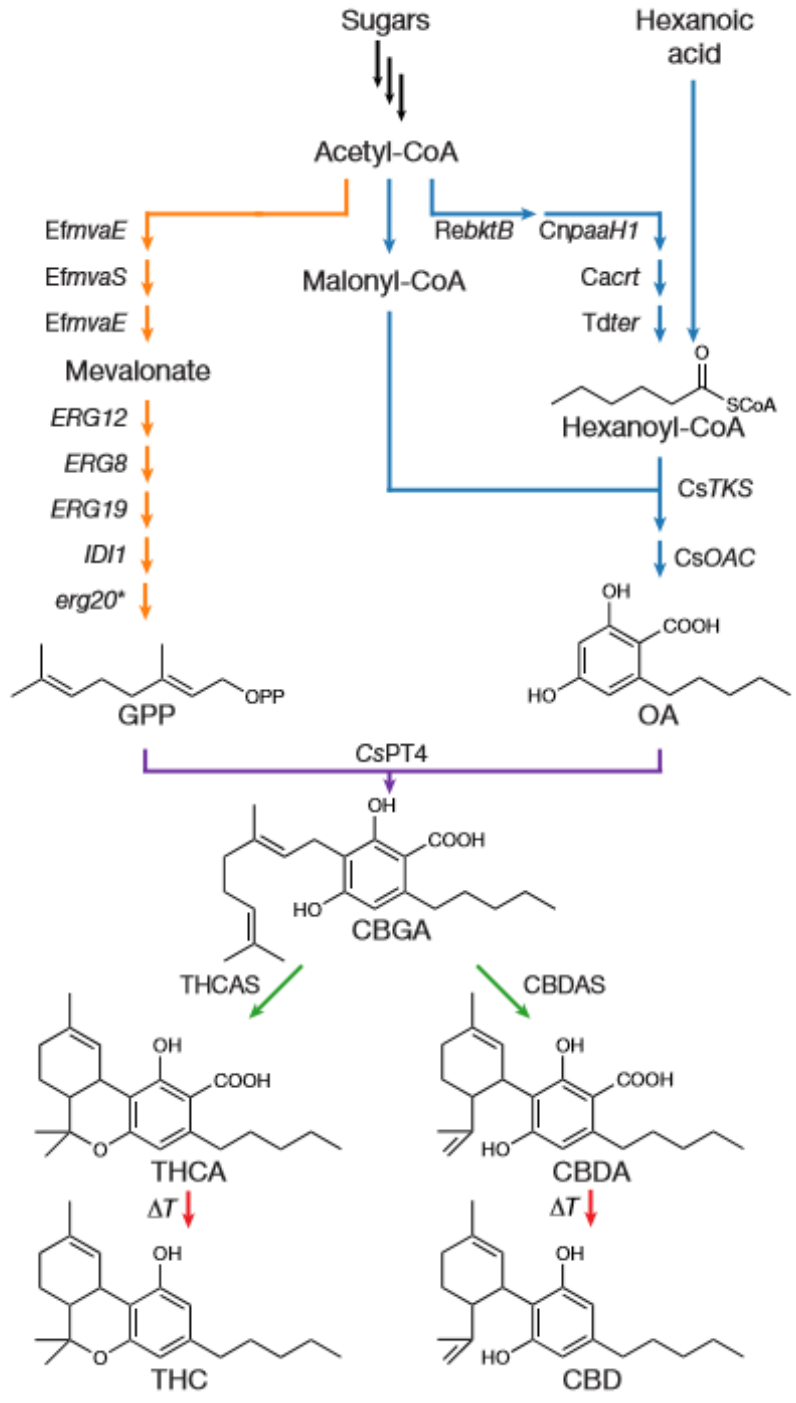
Xiaozhou Luo[1,15], Michael A. Reiter[1,2,15], Leo d'Espaux[3,12], Jeff Wong[3,12], Charles M. Denby[1,13], Anna Lechner[4,5,14], Yunfeng Zhang[1,6], Adrian T. Grzybowski[1], Simon Harth[3], Weiyin Lin[3], Hyunsu Lee[3,7], Changhua Yu[3,5], John Shin[3,4], Kai Deng[8,9], Veronica T. Benites[3], George Wang[3], Edward E. K. Baidoo[3], Yan Chen[3], Ishaan Dev[3,4], Christopher J. Petzold[3] & Jay D. Keasling[1,3,4,5,10,11]*

Cannabis sativa **L. has been cultivated and used around the globe for its medicinal properties for millennia[1]. Some cannabinoids, the hallmark constituents of *Cannabis*, and their analogues have been investigated extensively for their potential medical applications[2]. Certain cannabinoid formulations have been approved as prescription drugs in several countries for the treatment of a range of human ailments[3]. However, the study and medicinal use of cannabinoids has been hampered by the legal scheduling of *Cannabis*, the low in planta abundances of nearly all of the dozens of known cannabinoids[4], and their structural complexity, which limits bulk chemical synthesis. Here we report the complete biosynthesis of the major cannabinoids cannabigerolic acid, Δ[9]-tetrahydrocannabinolic acid, cannabidiolic acid, Δ[9]-tetrahydrocannabivarinic acid and cannabidivarinic acid in *Saccharomyces cerevisiae*, from the simple sugar galactose. To accomplish this, we engineered the native mevalonate pathway to provide a high flux of geranyl pyrophosphate and introduced a heterologous, multi-organism-derived hexanoyl-CoA biosynthetic pathway[5]. We also introduced the *Cannabis* genes that encode the enzymes involved in the biosynthesis of olivetolic acid[6], as well as the gene for a previously undiscovered enzyme with geranylpyrophosphate:olivetolate geranyltransferase activity and the genes for corresponding cannabinoid synthases[7,8]. Furthermore, we established a biosynthetic approach that harnessed the promiscuity of several pathway genes to produce cannabinoid analogues. Feeding different fatty acids to our engineered strains yielded cannabinoid**

of hexanoyl-CoA[11]. To increase the supply of hexanoyl-CoA, we fed the yCAN01 strain with 1 mM hexanoic acid, which can be converted to hexanoyl-CoA by an endogenous acyl activating enzyme (AAE), and observed a sixfold increase in olivetolic acid production (1.3 mg l^{-1}). A known byproduct of TKS, hexanoyl triacetic acid lactone (HTAL)[6], was also detected (Extended Data Fig. 1).

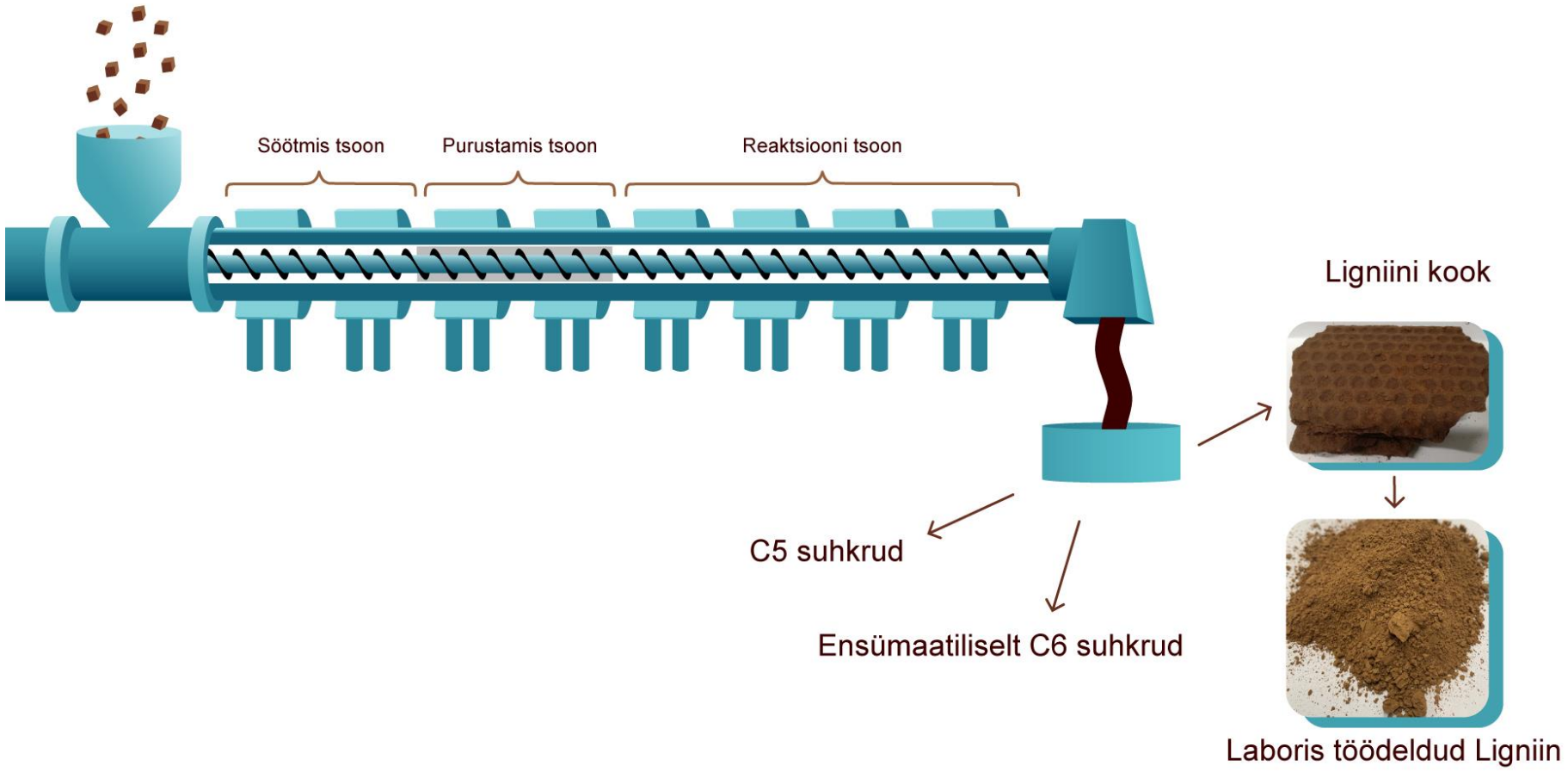
To optimize the conversion of hexanoic acid to hexanoyl-CoA, we introduced *Cs*AAE1, an AAE from *Cannabis* that is thought to catalyse this step in planta, into yCAN01[12]. When fed with 1 mM hexanoic acid, the resulting strain (yCAN02) showed a twofold increase in olivetolic acid titre (3.0 mg l^{-1}) compared with yCAN01 (Fig. 2a). To produce hexanoyl-CoA from galactose and complete the olivetolic acid pathway, we introduced a previously reported hexanoyl-CoA pathway into yCAN01[5]. The resulting strain (yCAN03) produced 1.6 mg l^{-1} olivetolic acid (Fig. 2a).

Cannabigerolic acid (CBGA)—the precursor to Δ[9]-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and numerous other cannabinoids—is produced from olivetolic acid and the mevalonate-pathway intermediate geranyl pyrophosphate (GPP) by a geranylpyrophosphate:olivetolate geranyltransferase (GOT). GOT activity was detected in *Cannabis* extracts two decades ago[13], and a *Cannabis* GOT (*Cs*PT1) was patented ten years later[14]. To test *Cs*PT1 in vivo, we constructed a GPP-overproducing strain (yCAN10) with an upregulated mevalonate pathway[15] and a mutant version of the endogenous farnesyl pyrophosphate synthase ERG20 (ERG20(F69W/N127W) that preferentially produces GPP over FPP[16]. However, we

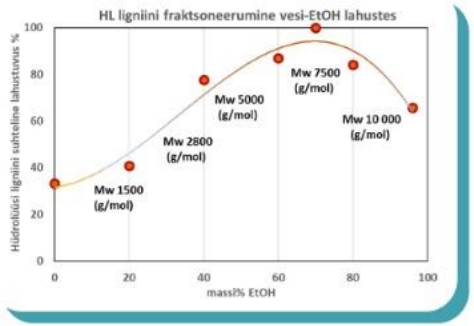


Ligniini väärindamine

Graanul Invest: BBI-JU SWEETWOODS project



Hüdrolüüsi Ligniin



Alus-happekatalüüs

Vesi-orgaanilised lahustid

Ultrahelitehnoloogia, mehhanokeemia, voogkeemia TF.

Lahustamine, fraktsioneerimine, depolümerisatsioon

Erinevate omadustega ligniini lahused (pH2-13)

Erineva lahustuvus-omadustega ligniinid

Erineva molekulmassiga ligniini fraktsioonid

Nanoligniin

Naturaalne S-vaba puhas ligniin

TF kondensaat

Keemiline töötlus,süntees, puhastamine

Ensümaatiline töötlus

Bioloogiline töötlus

Vahud-liimid, plastikud/polümeerid
polüuretaanid
polüfenoolid
polüepoksiidid
Biolagunevad materjalid

Dispergandid
Puidukaitse/immutusvahendid
Kosmeetika
UV-kaitse, antibakteriaalsed omadused, antiooksüdandid
Tehnilised adsorbendid, tardained, klombistuvad agendid

Biokemikaalid
Biokütused
Aromaatsed ligniini derivaadid

Ligniini depolümeriseerimine

Hüdrotermiline lõhustamine

Ligniin

Kontsentreerimine

Hüdrogeenimine ja polümeriseerimine

Hüdrogeenimine

Aurustamine

Adipiinhape

Polükondensatsioon

Nailon-6,6

Bio- ja järeltöötlus

Fermentatsioon

Biomassi kogumine

Biomassi eraldamine

Vaakumfiltreerimine

Aktiivsöega de-koloreerimine

Sadestamine happega

Lüofiliseerimine

Mükoonhape

Gaasfermentatsioon

https://gasfermtech.ee

GasFermTEC

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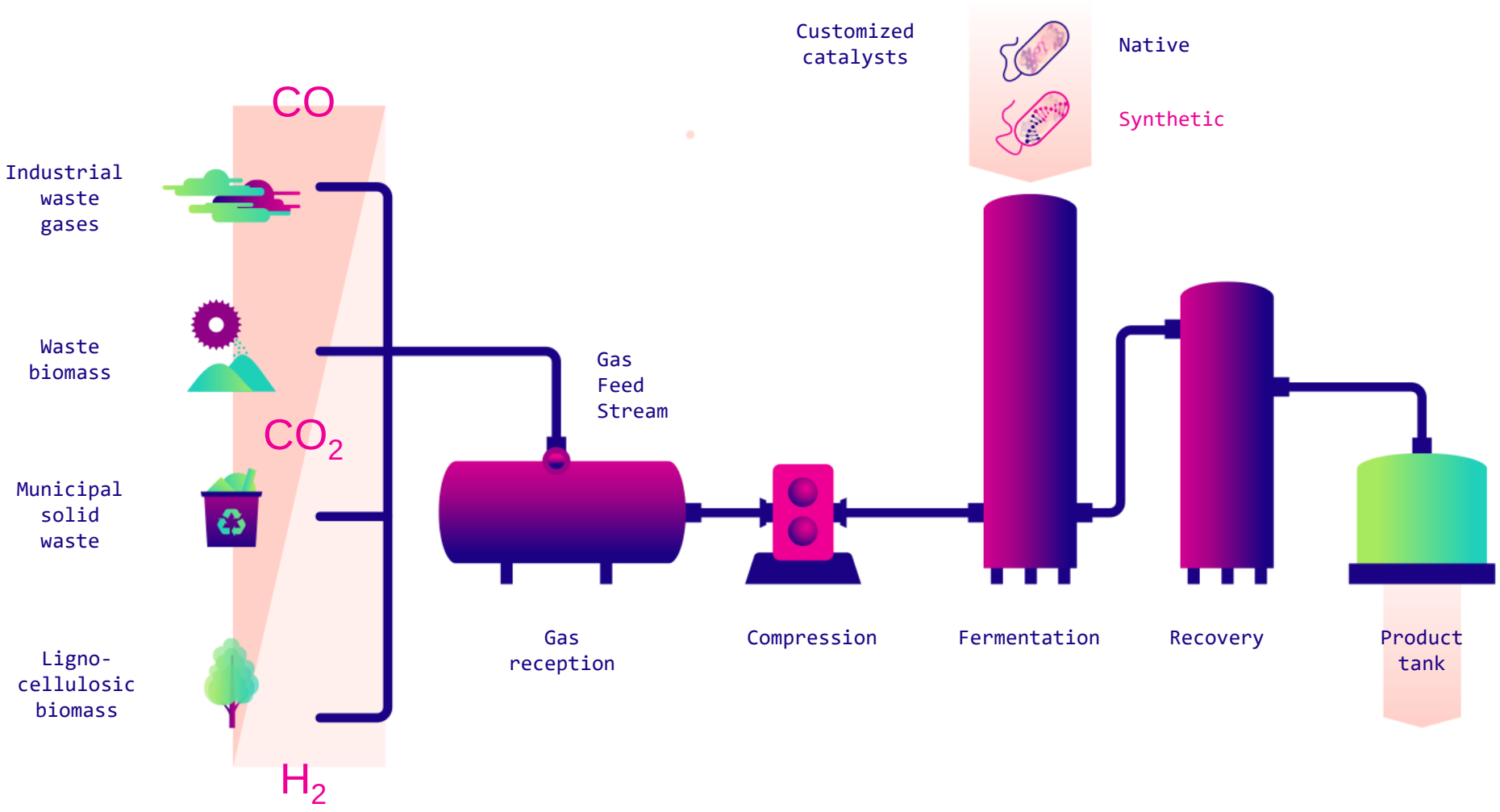
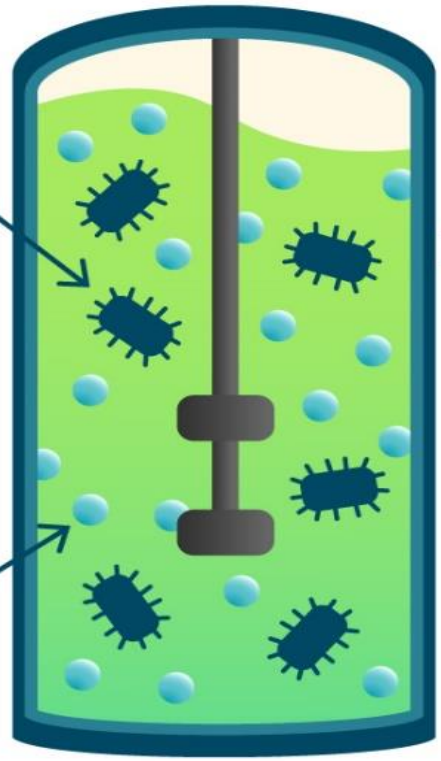
Gaasfermentatsioon



Gaasfermentatsioon



Rakuvabrikud

Bioreaktor

Sisend
- CO
- CO
- H_2

Väljund
- 2,3-Butaandiool
- Etanool
- Atsetaat
- Biomass

Gaasimullid

Karakteristikud
- kontentratsioon
- saagis
- tootlikkus

Optimeemimine
- pH
- gaasi-vedelikuülekanne
- Rakkude kasvukiirus

Kaspar Valgepea, PhD

Group Leader/Senior Research Fellow

NEWS

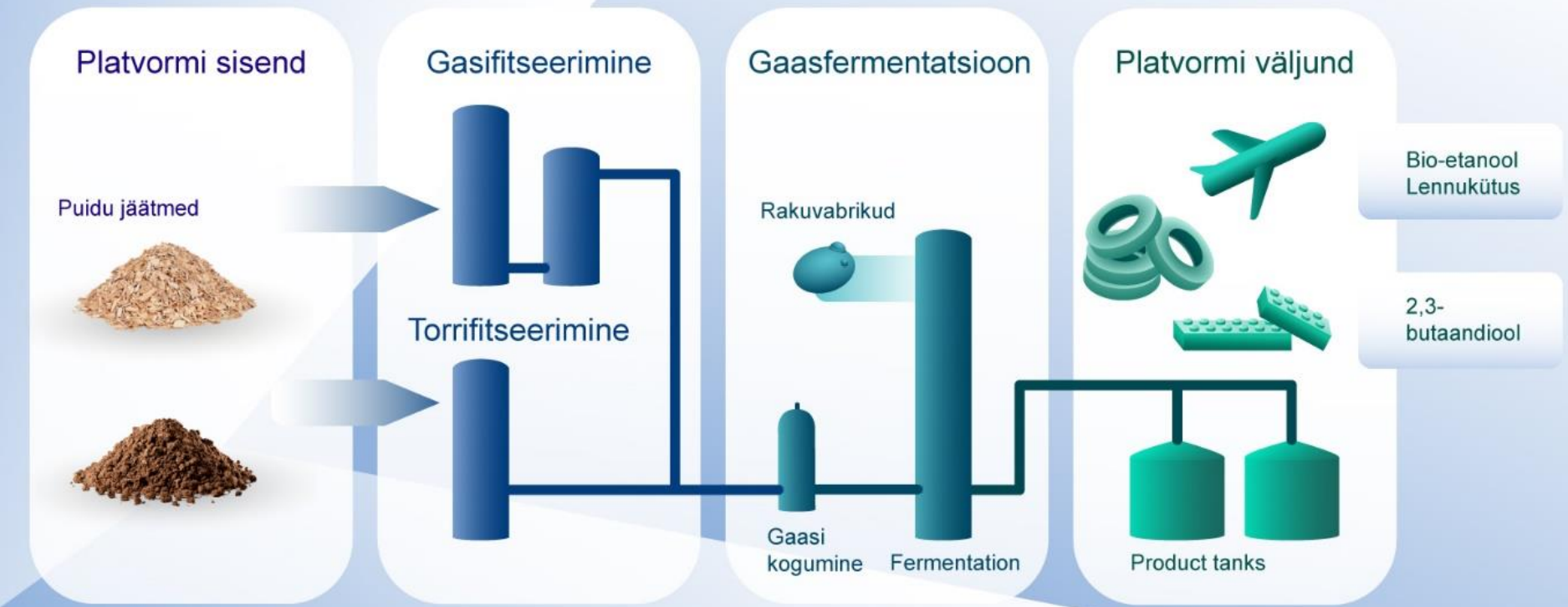
- Research article published from the ERA Chair and collaborators in Proceedings of the National Academy of Sciences (PNAS)
- Research article published from the ERA Chair and collaborators in Frontiers in Bioengineering and Biotechnology
- Start-up of the state-of-the-art gas fermentation facility at GasFermTEC
- Preprint from the ERA Chair and

Gas fermentation platform at ECB

LanzaTech

UNIVERSITY OF TARTU

Integreeritud gasifitseerimise ja gaasfermentatsiooni platvorm



Demonstrated range of chemistries and functional groups directly from gas

	Acids						Alcohols		Diols			Aromatics	Dienes	Esters	Ketones	Terpenes
	Carboxylic	Dicarboxylic	Hydroxy	Dihydroxy	Keto	Amino	Linear	Branched	1,2-	1,3-	2,3-					
C2	Acetic						Ethanol		MEG							
C3			Lactic	3-hydroxypropionic		Alanine	n-propanol	Isopropanol	1,2-PDO (R,S,mix)	1,3-PDO (R,S,mix)					Acetone	
C4	Butyric	Succinic	2-HB	3-HB (R,S,mix)	4-HB	2-HIBA	n-butanol	2-butanol		1,3-BDO (R,S,mix)	2,3-BDO (RR,meso,mix)		Butylene		Acetoin MEK	
C5			Citramalic		Ketoglutaric	Methionine Valine							Isoprene			
C6+			C6-C14 Hydroxyacids PHB		Mevalonic C6-C14 Alkylmalates	Leucine Isoleucine	linear branched C6-C14 Alcohols					PHAB Salicylic Aromatic Compound	FAEE FABE			Farnesene

Commercial | **Pilot** | **Labscale** | **PoC** | **Modelled** >500 molecules

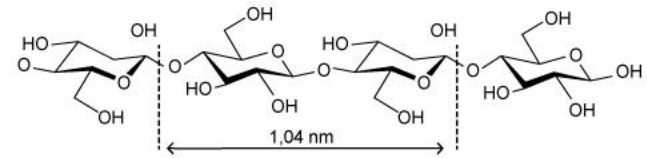
LanzaTech

Uute (puidu)bio-põhiste kemikaalide ja polümeeride süntees

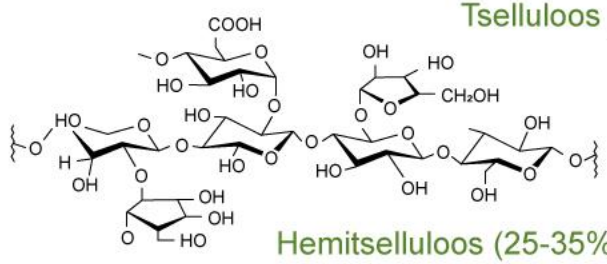
Atmosfääriline CO_2

fotosüntees

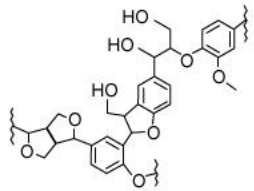
Puit



Tselluloos (40-50%)



Hemitselluloos (25-35%)



Ligniin (15-20%)

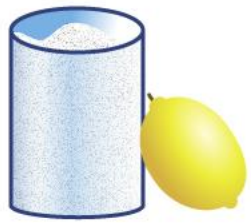
kemikaalid/monomeerid

Peenkemikaalid
(ravimid, toidulisandid, kosmeetika jt)

plastid/materjalid

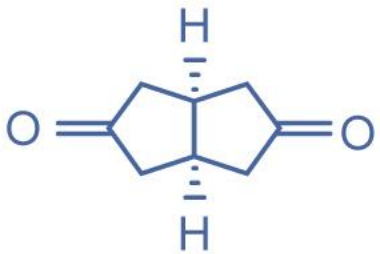
C6 suhkrud

fermentatsioon



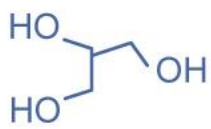
Sidrunhape (ca 0,6€/kg)

Bitsükliline diketoon

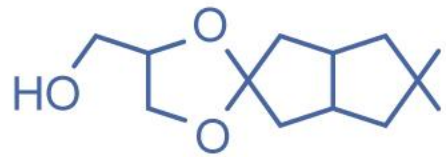


4

Hiljuti meie poolt väljatöötatud meetodiga (patenditaotlus GB2002792.6)



glütseriin (<1€/kg)



5

TMP (saadaval ka biotoormes)

6

- Polükarbonaadid
- Polüestrid
- Polüamiid
- Polüuretaanid

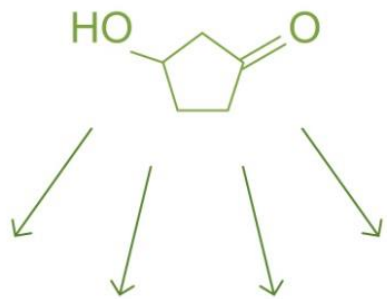
Valik uuritavaid spiro-mono-meere ja nendest tehtavaid polümeere/materjale

A

C5 suhkrud puidust

furfuraal
(platvormkemikaal)

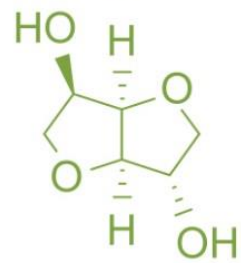
mikrolaineahi



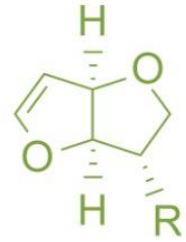
Erinevad derivatiseerimise ja polümeriseerimise võimalused

B

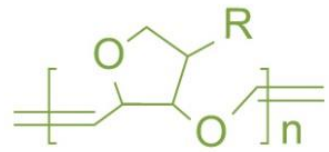
C6 suhkrud puidust



Vares, et al. JOC 2016, 7510.



Grubbs katalüsaator
(ringi avamise polüm.)



Eeldatavalt täielikult biolagunev polümer

Synthetic biology in Estonia:
a historical opportunity

Local substrate

Value added chemicals