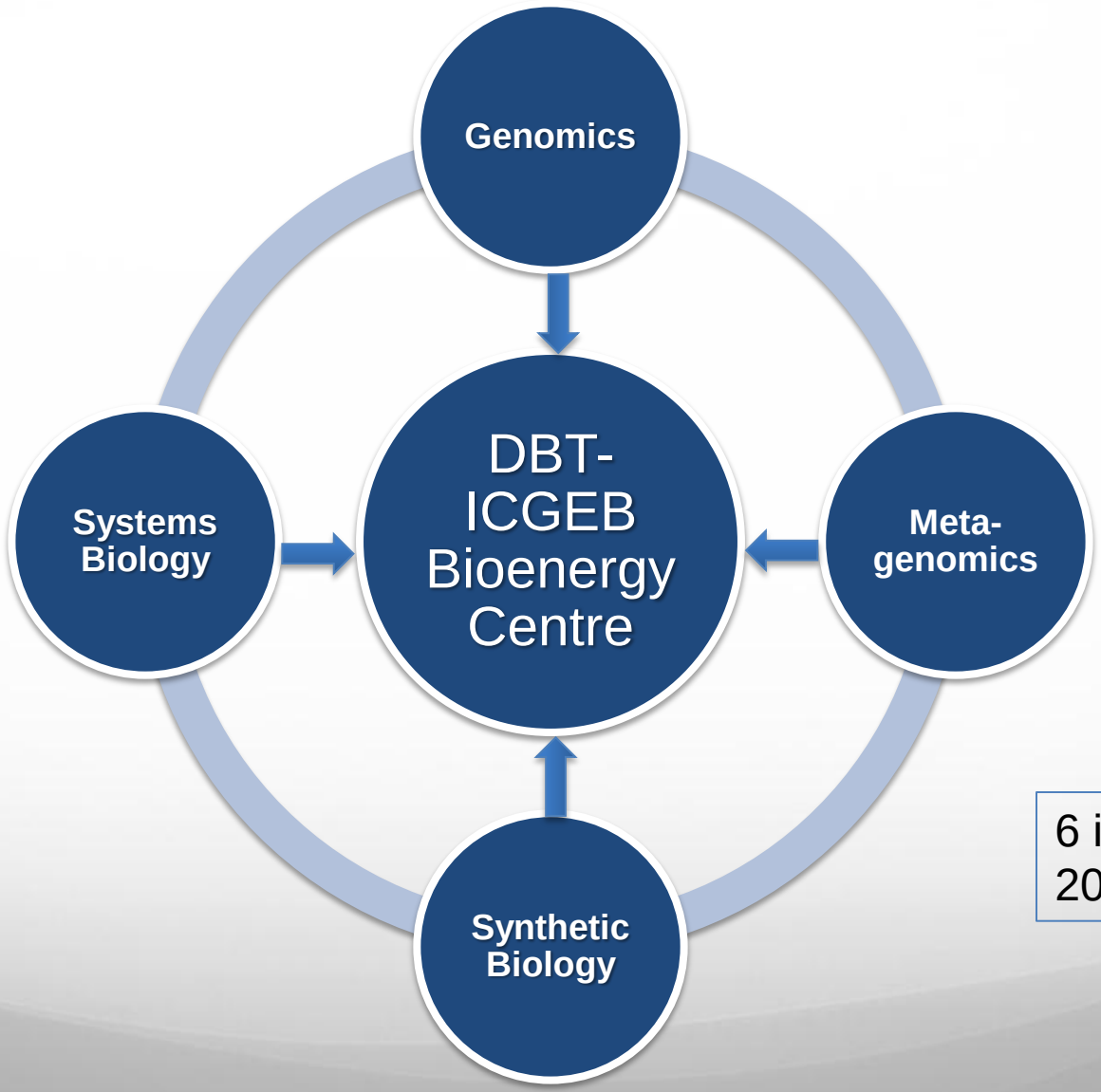
Molecular interventions towards bioenergy

Dr. Syed Shams Yazdani

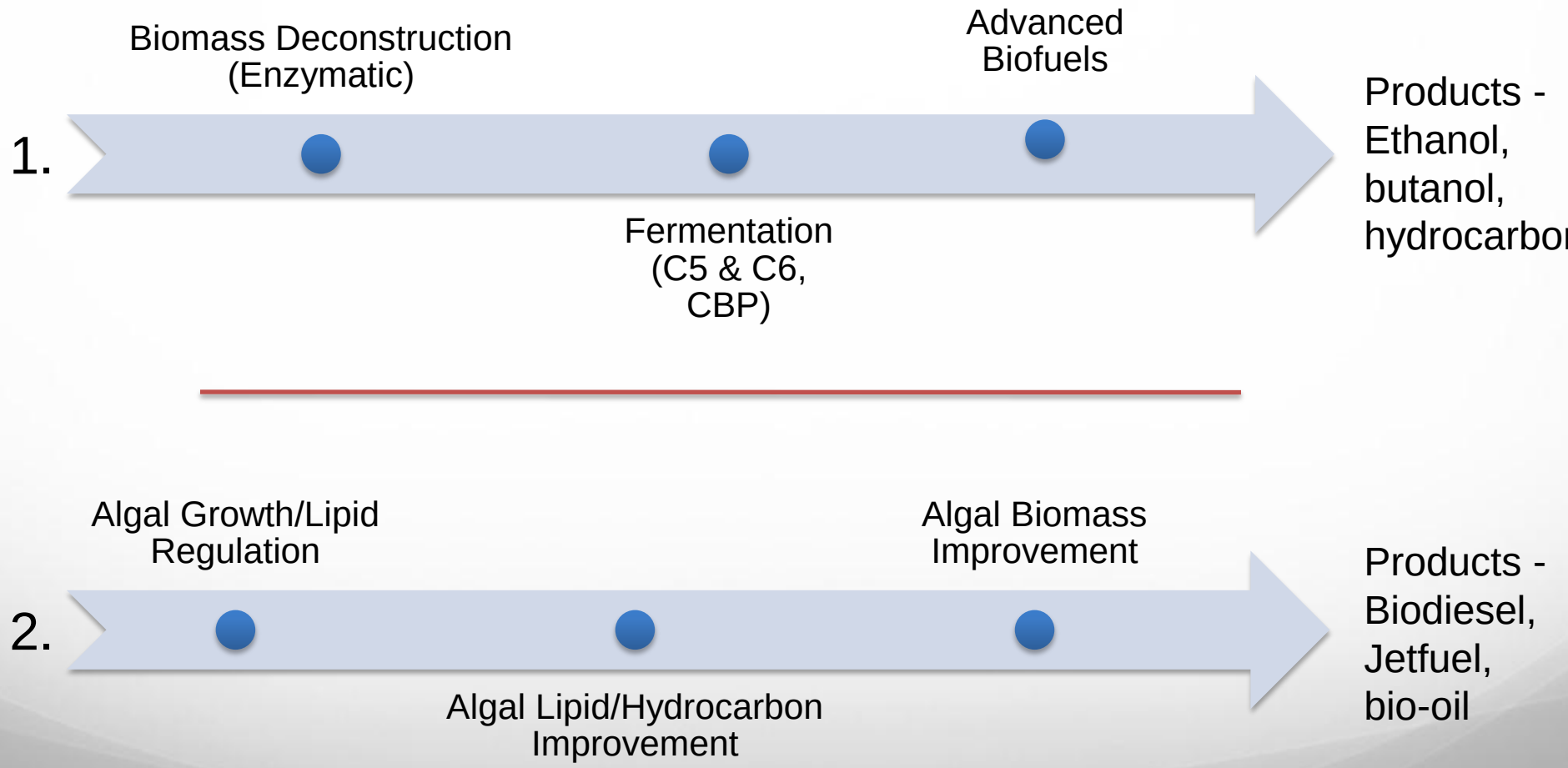
Synthetic biology and Biofuels Group
DBT-ICGEB Centre for Advanced Bioenergy Research
International Centre for Genetic Engineering and Biotechnology
New Delhi

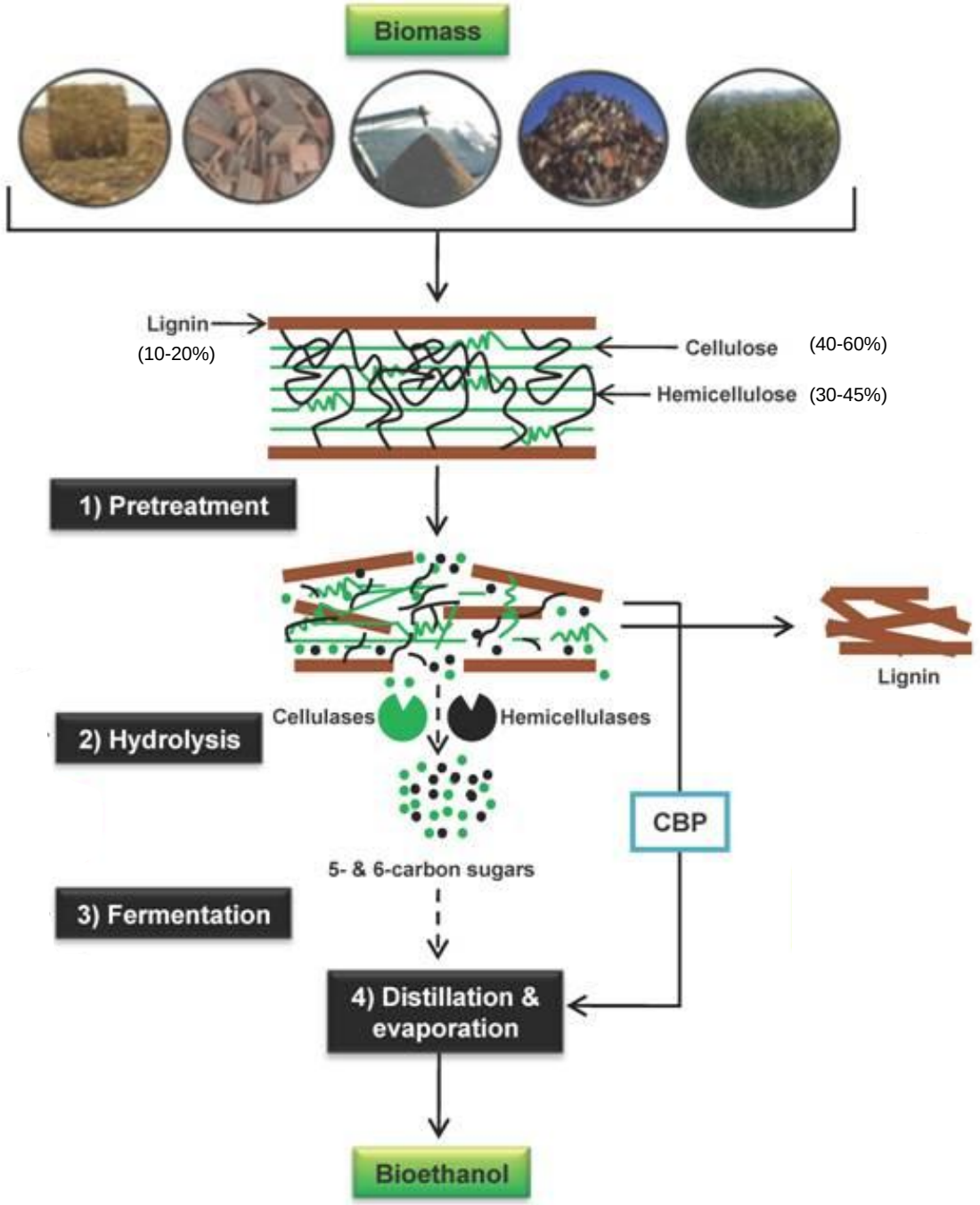
Four Pillars of Our Research

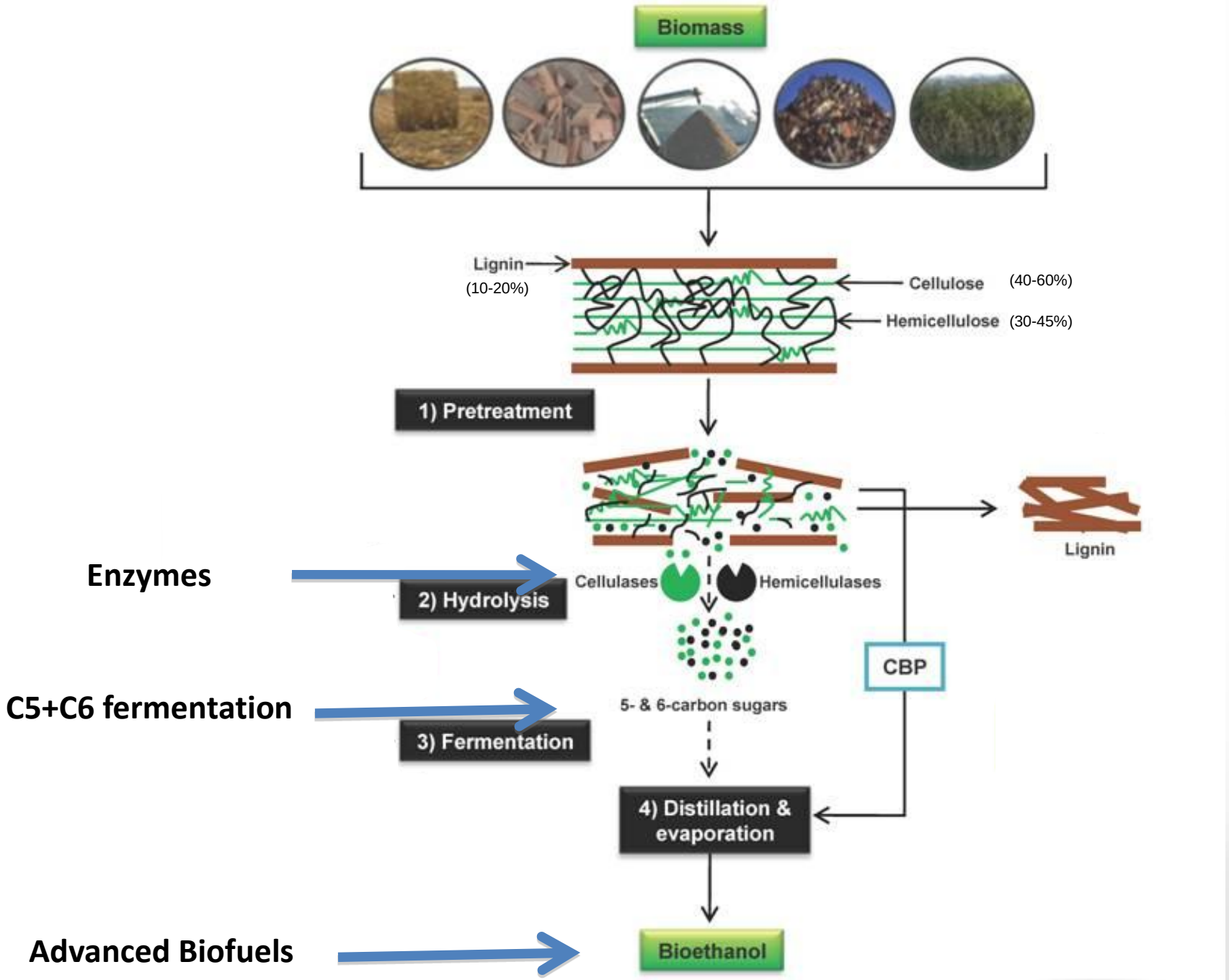


6 investigators
20 students

Research Theme at the Bioenergy Centre




Biomass
Lignin
(10-20%)
Cellulose
(40-60%)
Hemicellulose
(30-45%)
1) Pretreatment
Lignin
Cellulases
Hemicellulases
2) Hydrolysis
CBP
5- & 6-carbon sugars
3) Fermentation
4) Distillation & evaporation
Bioethanol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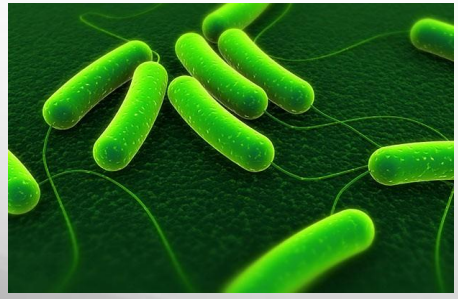
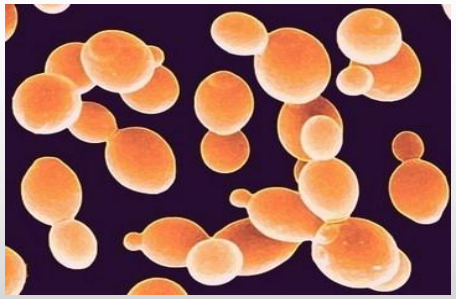
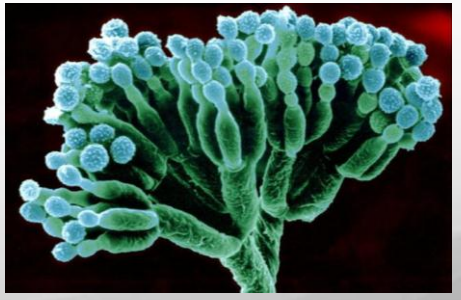
5- & 6-carbon sugars
3) Fermentation
4) Distillation & evaporation
Advanced Biofuels
Bioethanol

Enzyme Research

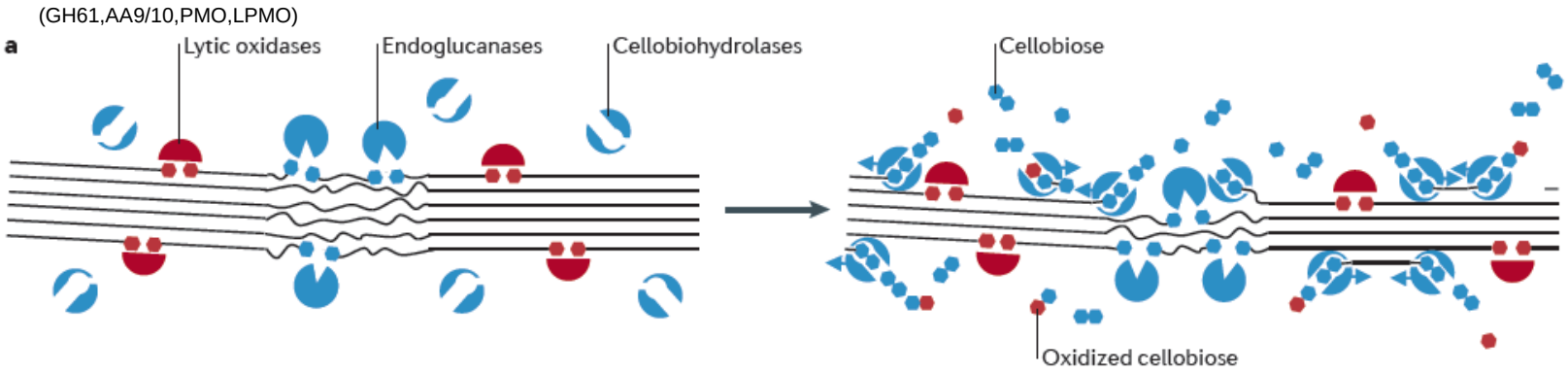
- Mainly to identify and design enzymes for efficient degradation of lignocellulosic biomass
- Source of enzymes are either free living fungi or microbes residing in insect guts that destroy plants
- Expression platforms are either native hosts or recombinant hosts such as *E. coli*, yeast or fungi

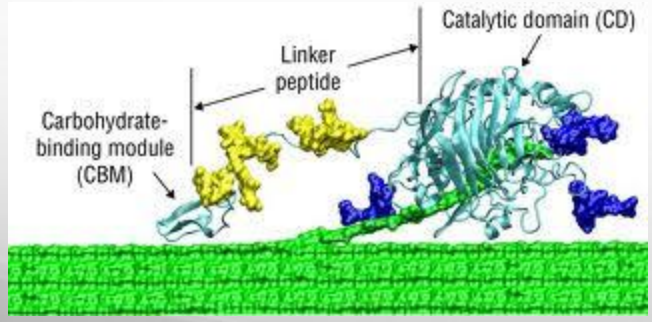


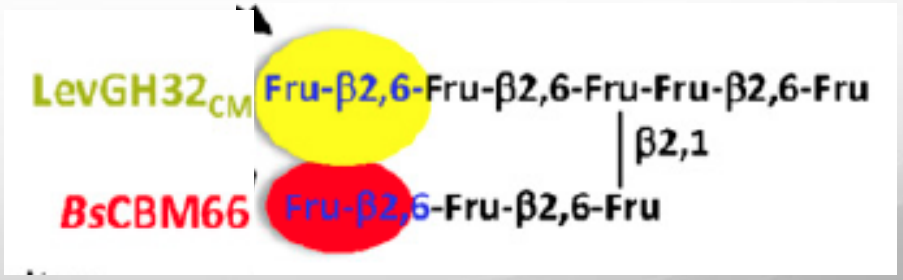




Cellulolytic enzymes and CBMs







From Literatures

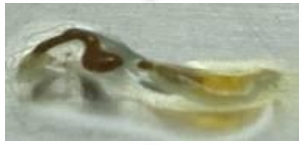
Identifying novel cellulolytic enzymes

Cotton Bollworm

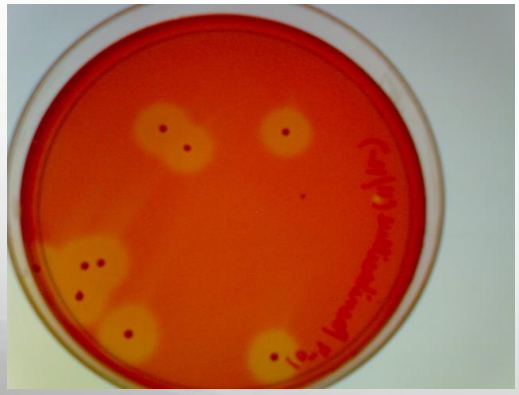
Rice Stem Borer

Termite

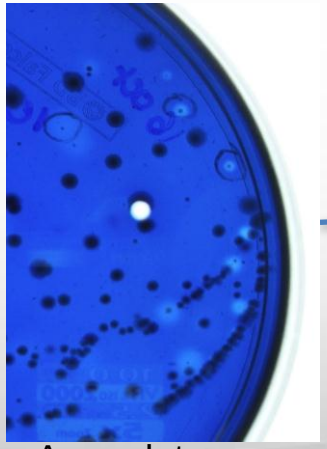
Pillbug



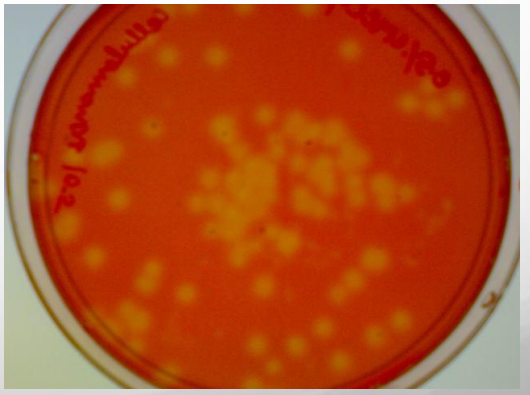
Gut screened
for cellulase producer



Strain I

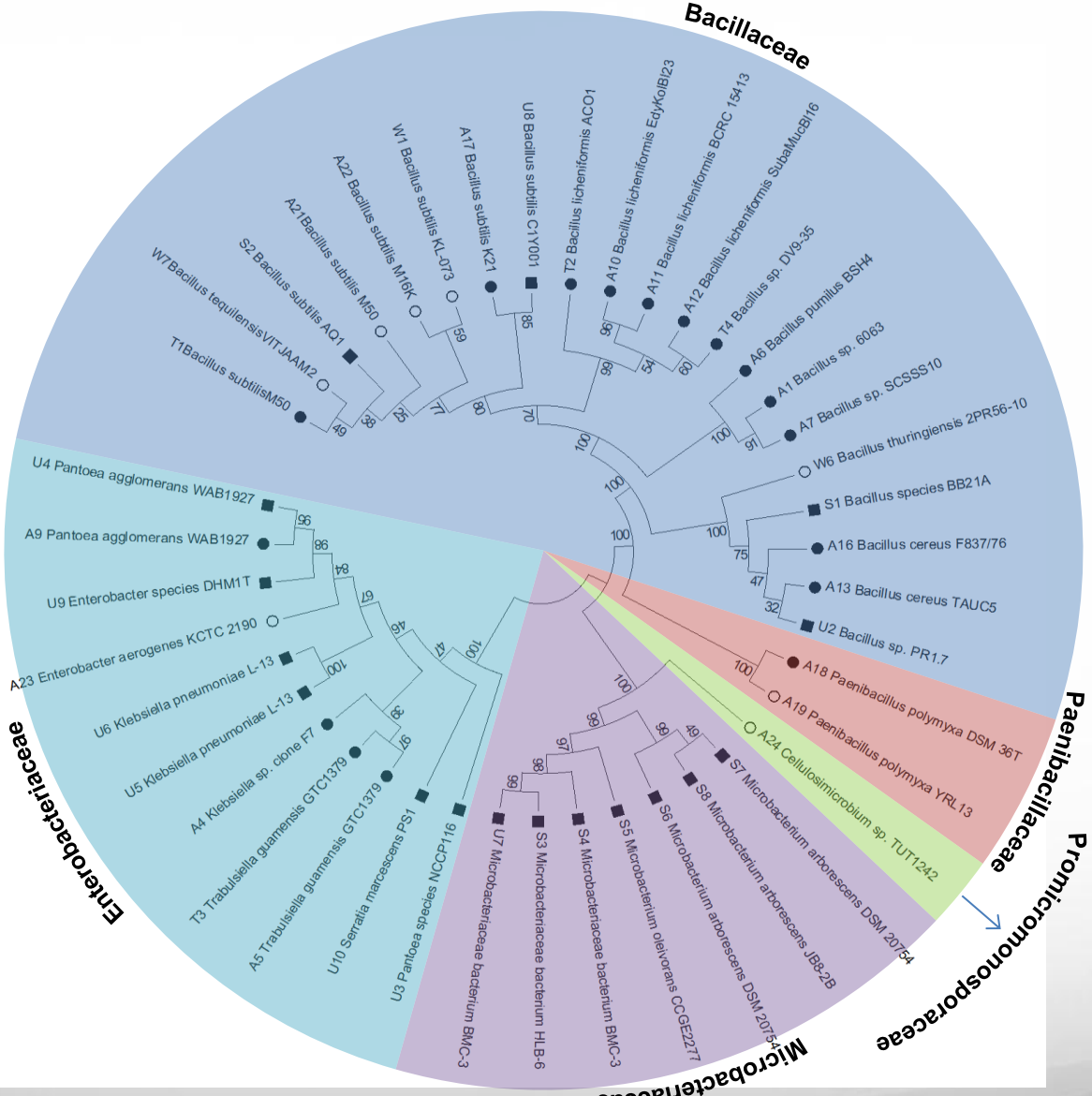


Agar plate



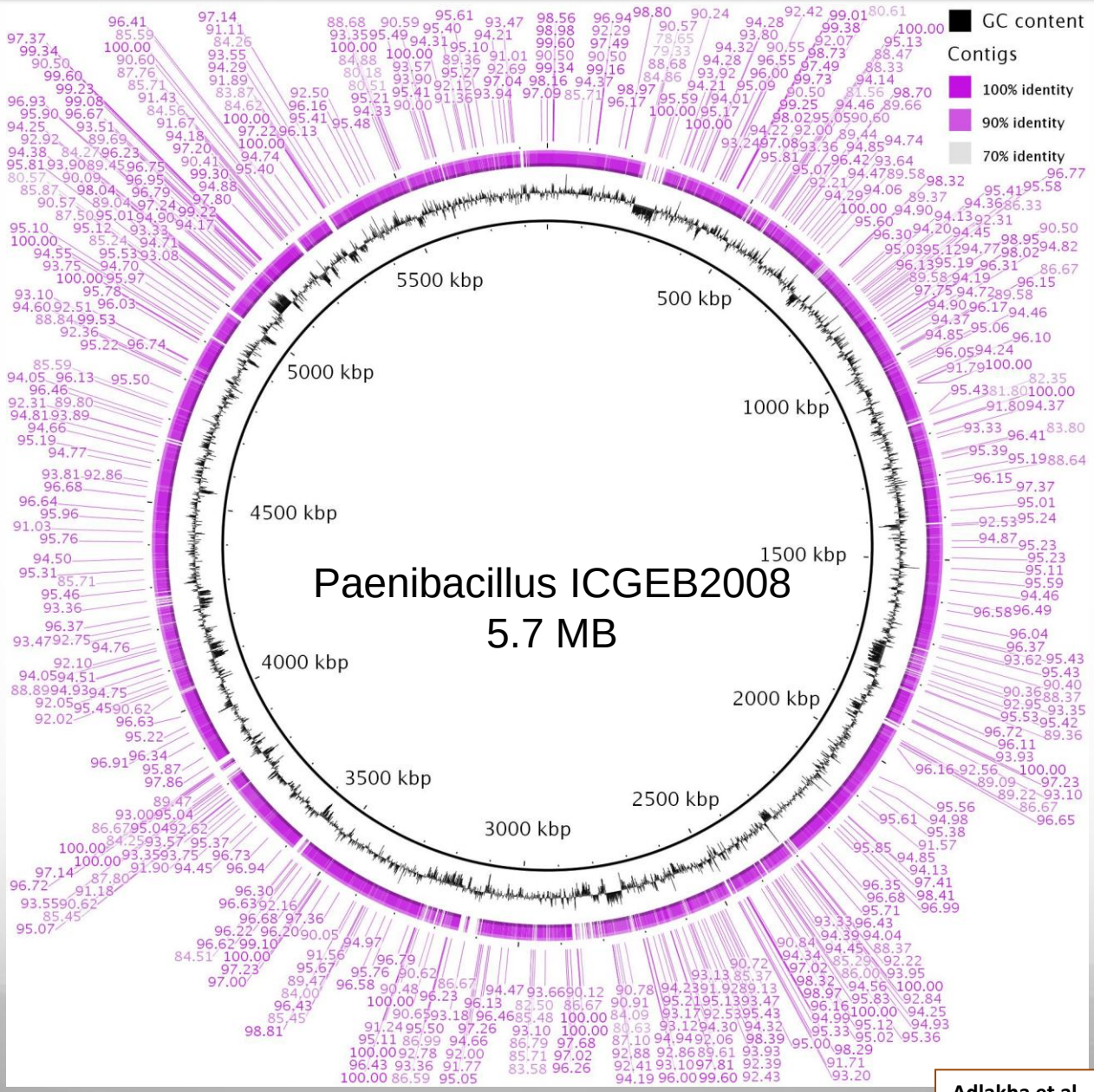
Strain II

Phylogeny of Gut Cellulolytic Microbes



Bashir et al., 2013, Nature Scientific Reports

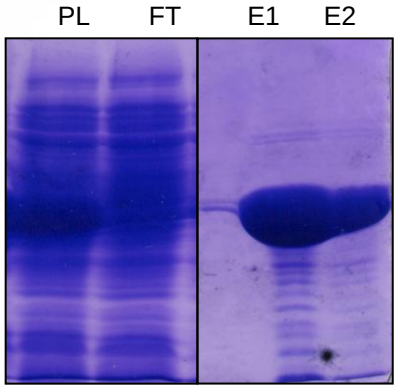
Genome Sequencing of *Paenibacillus* ICGEB2008



Adlakha et al., 2012, Genome Announcement

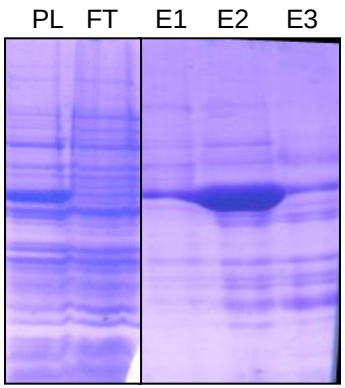
Novel cellulolytic enzymes overexpressed with high specific activity

Endocellulase – 41 kDa



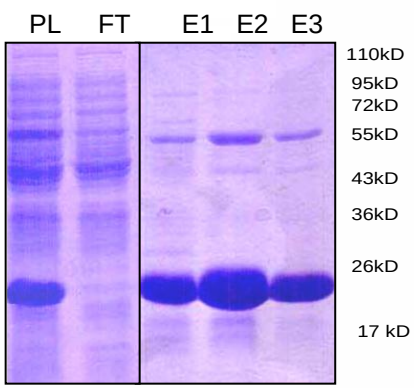
pH optima ~ 6-7
Temp optima ~50 ^{o}C

β-glucosidase – 51 kDa



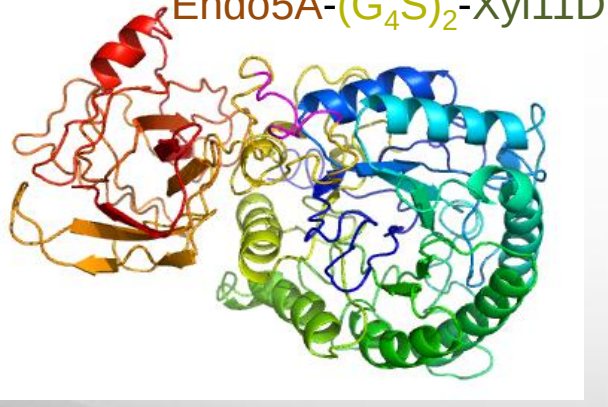
pH optima ~ 6
Temp optima ~50 ^{o}C

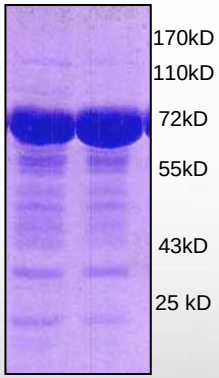
Xylanase – 21 kDa



pH optima ~ 6-7
Temp optima ~50 ^{o}C

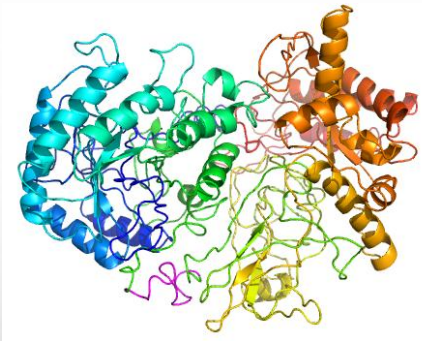
Endo5A-$(G_4S)_2$-Xyl11D

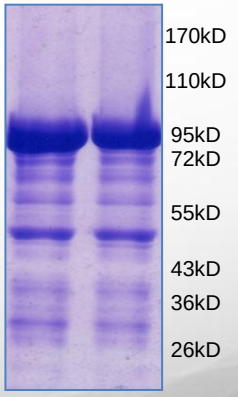




Adlakha et al. Applied and Environmental Microbiology. July 2011
Yazdani et al. Patent Application no. 2071/DEL/2010

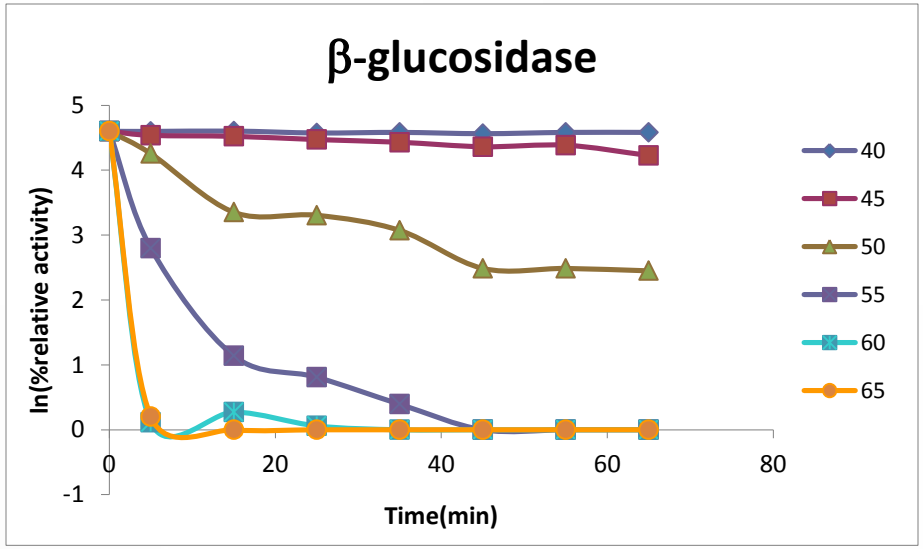
Endo5A-$(G_4S)_3$-Gluc1C



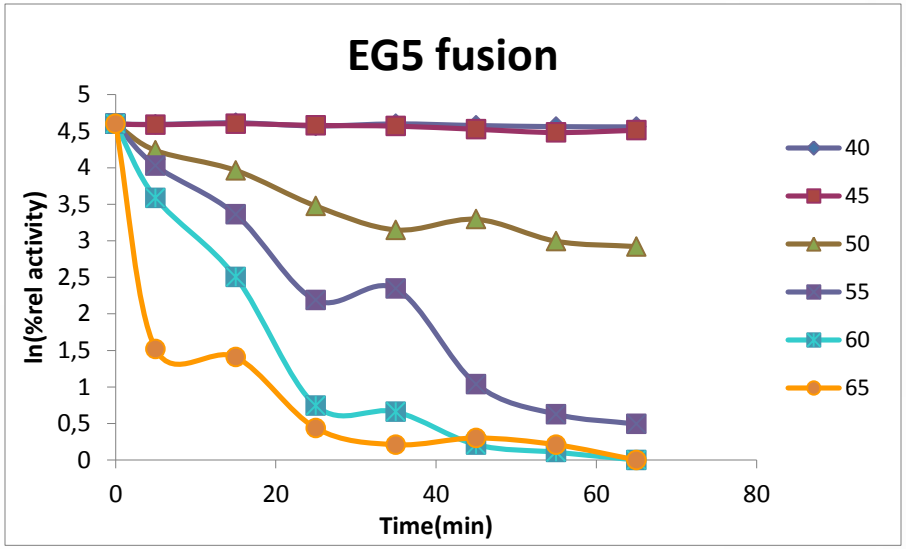


Adlakha et al. Applied and Environmental Microbiology. Oct 2012
Yazdani et al. Patent Application no. 2695/DEL/2012

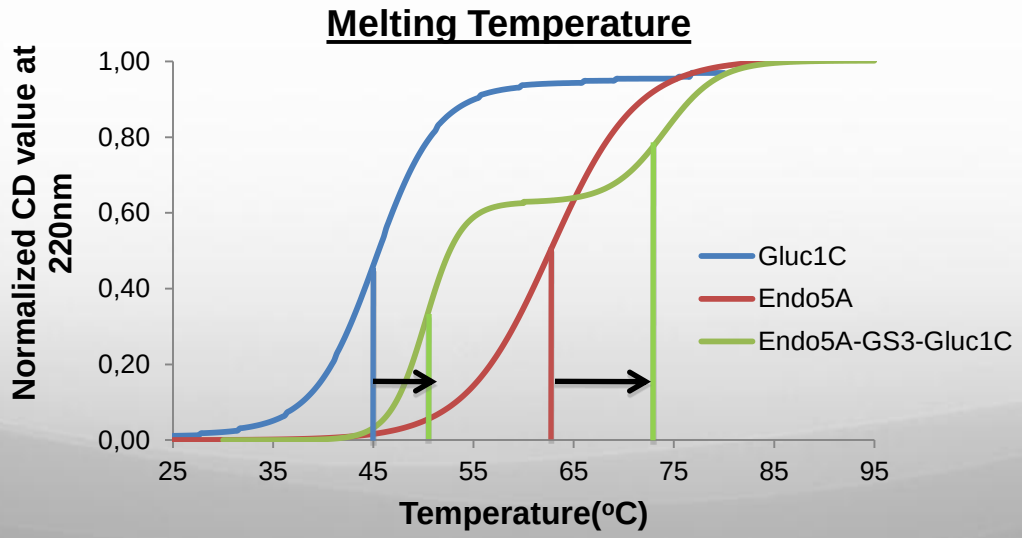
Fusion enhances thermostability



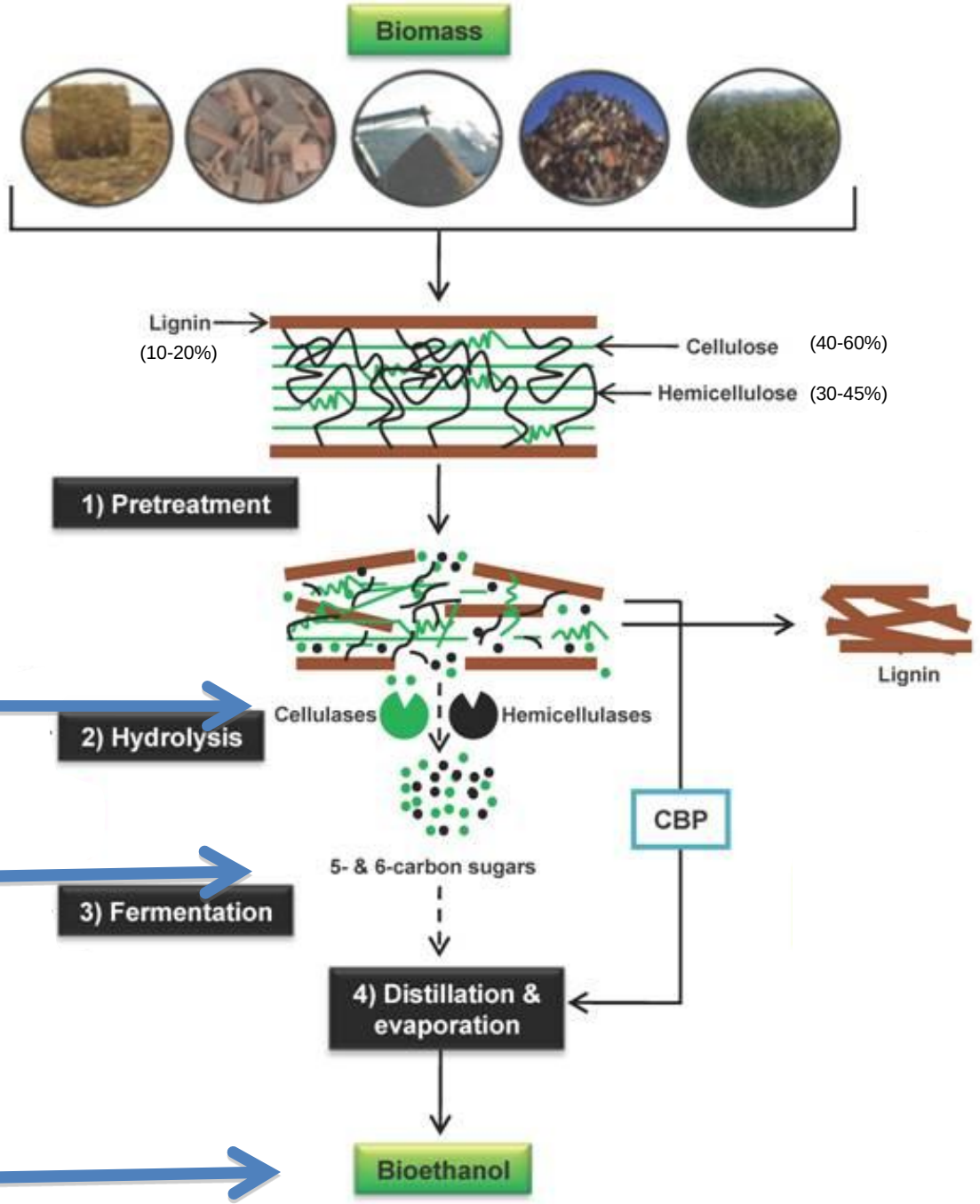
Half life of activity at 55 ºC – 1.9 min



Half life of activity at 55 ºC – 7.5 min



Secondary structure more stable in fusion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5- & 6-carbon sugars
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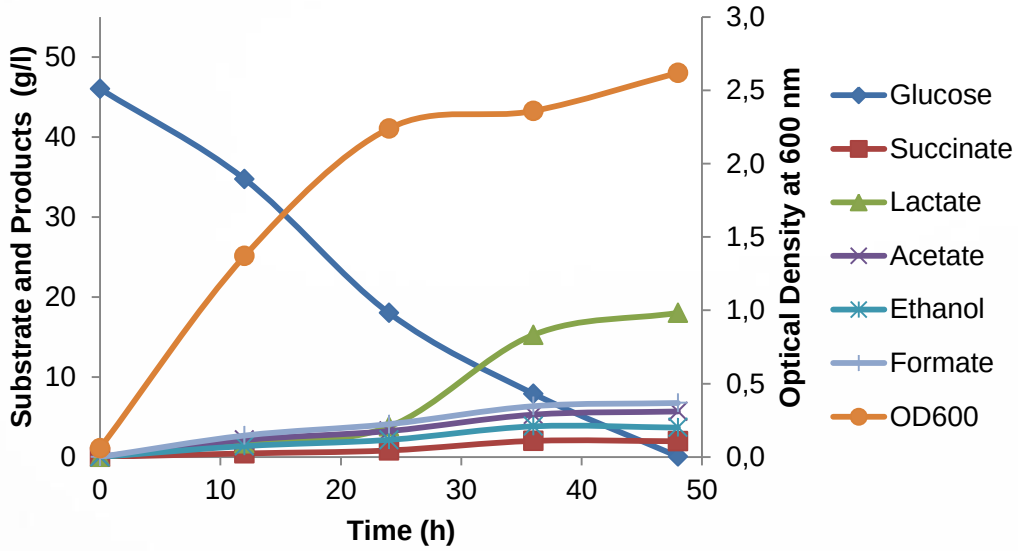
4) Distillation & evaporation
Advanced Biofuels
Bioethanol

Engineering microbe for converting C5/C6 sugars to ethanol

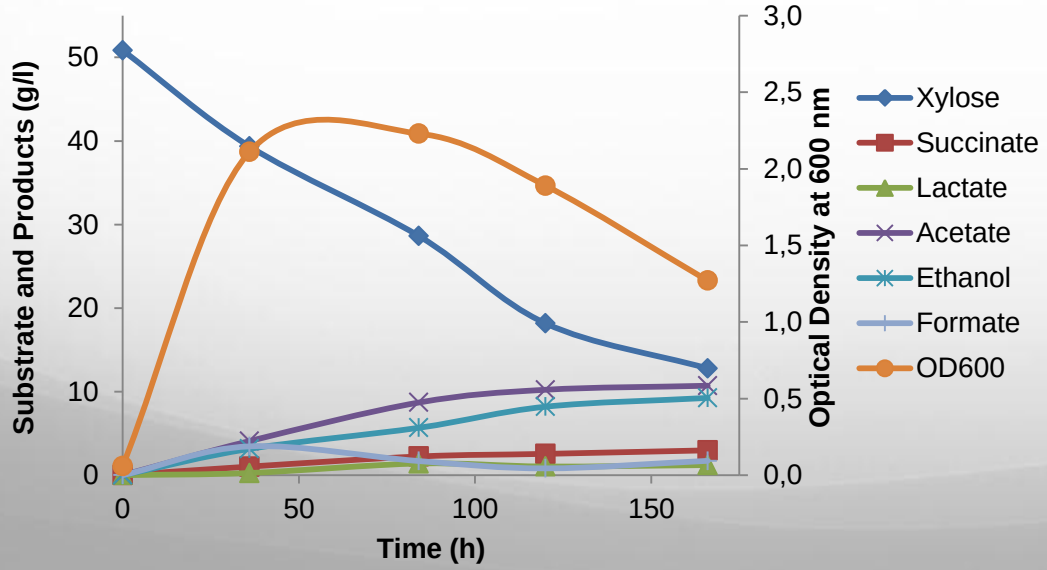
- Up to 30% pentose sugars in agricultural residues
- Traditional yeast, *Saccharomyces cerevisae,* cannot utilize pentose sugars
- *E. coli* could ferment all pentose and hexose sugars present in the lignocellulosic biomass
- It, however, produces various competing products under anaerobic condition

Competing co-products of *E. coli* under fermentative condition



Max Ethanol Productivity = 0.14 g/l/h

Ethanol Yield = 0.08 g/g glucose

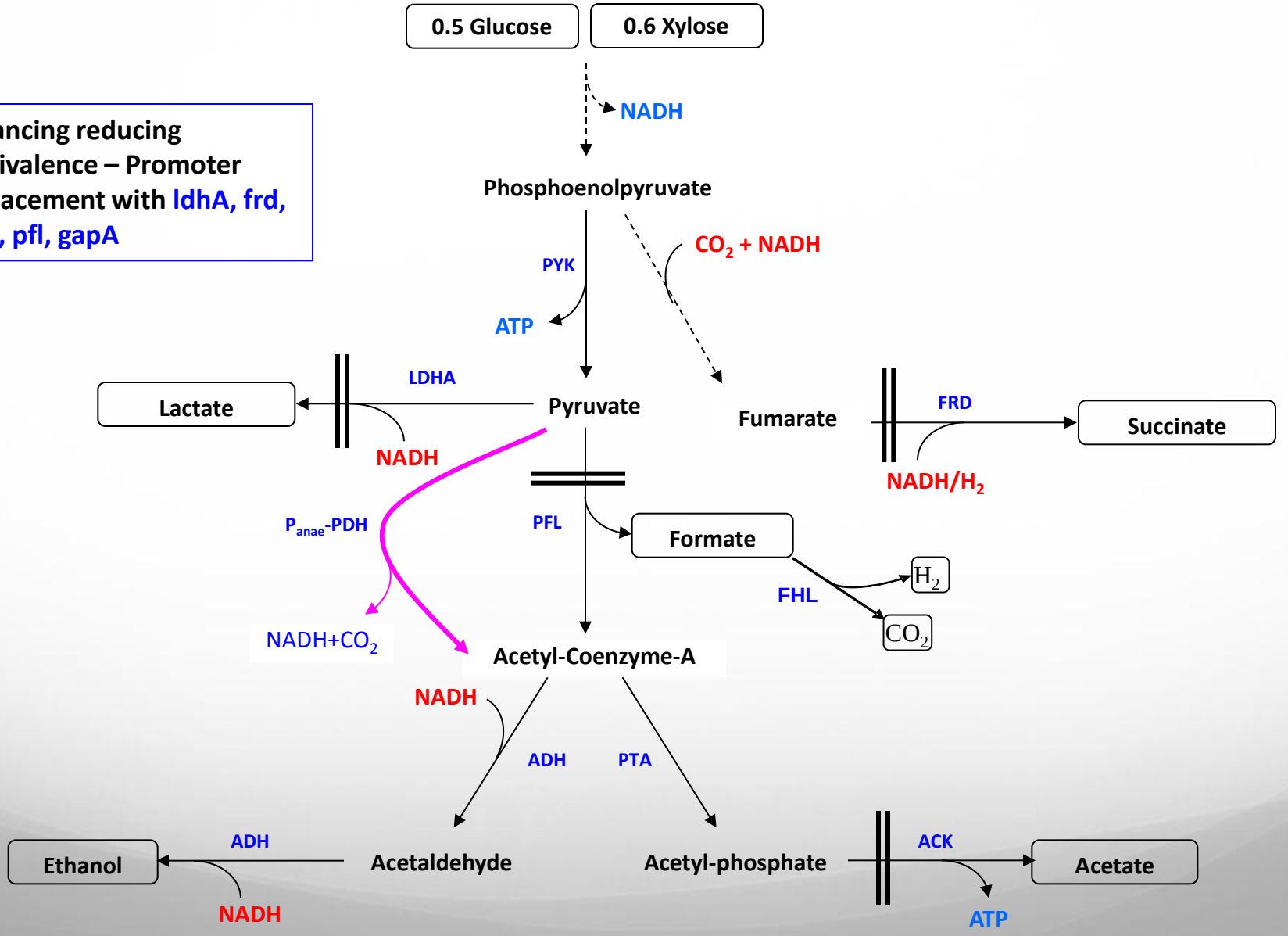


Max Ethanol Productivity = 0.09 g/l/h

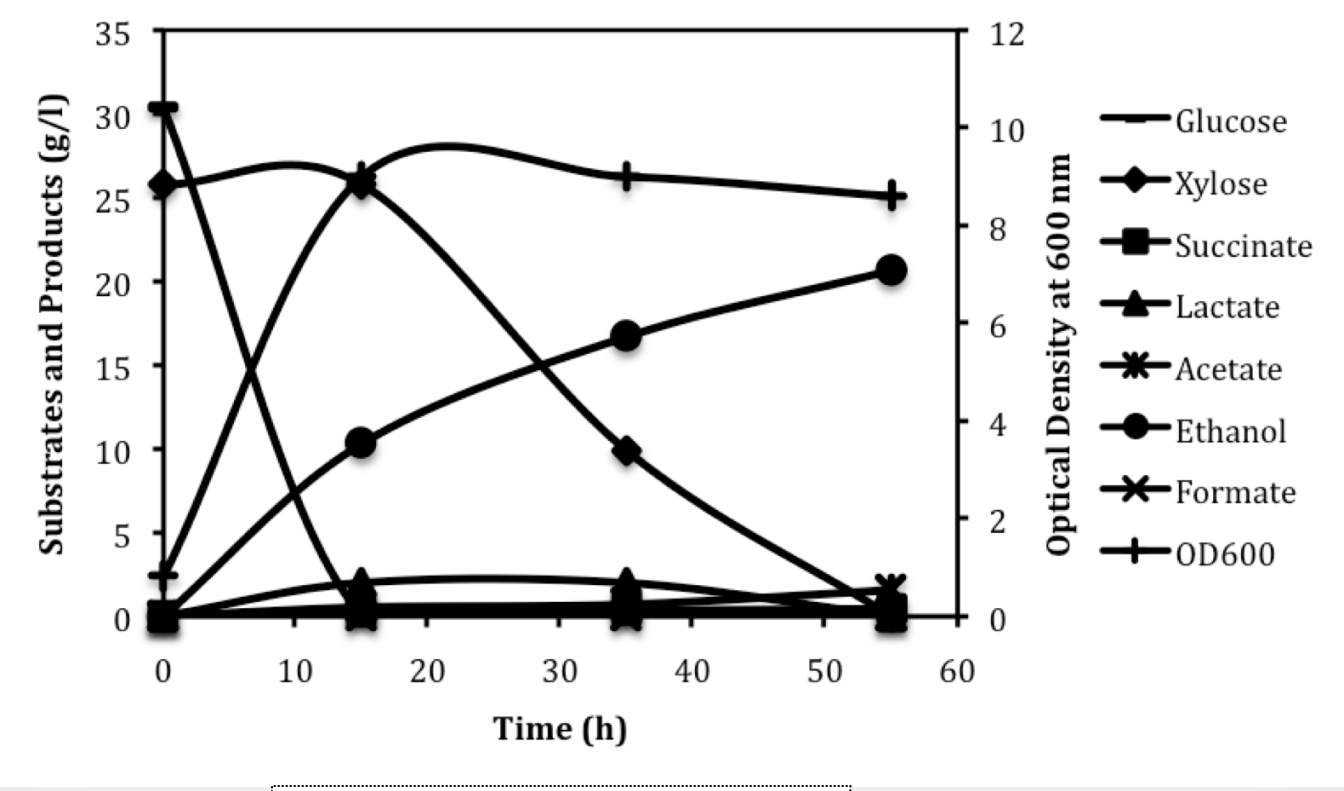
Ethanol Yield = 0.24 g/g xylose

Engineering *E. coli* for ethanol production

Balancing reducing equivalence – Promoter replacement with ldhA, frd, adh, pfl, gapA



Co-Fermentation of Glucose and Xylose by SSY10

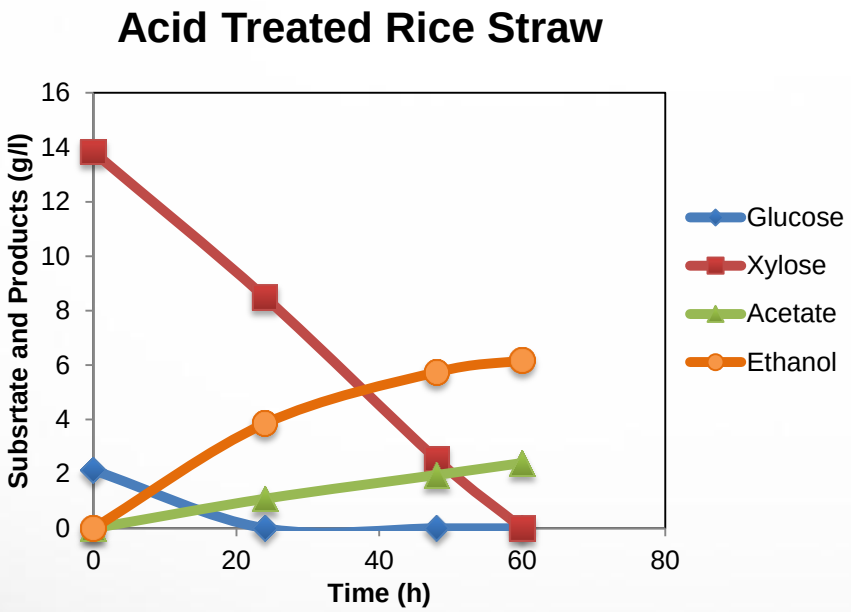


Ethanol Productivity = 0.7 g/l/h

Ethanol Yield = 0.43 g/g glucose

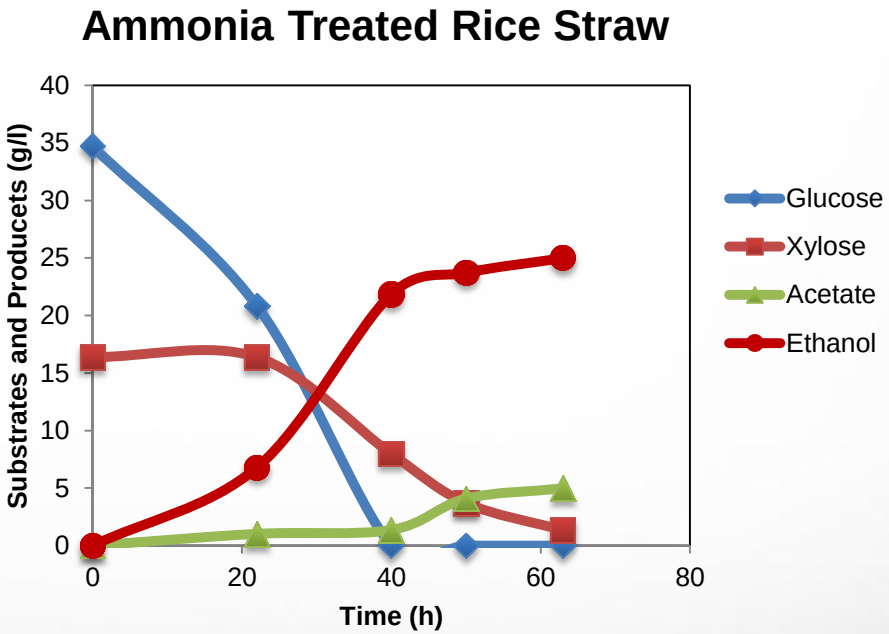
Munjal et al. Microbial Cell Factories. Nov 2012
Yazdani et al. Patent Application no. 2696/DEL/2012
PCT/IN2013/000535

Second generation lignocellulosic ethanol



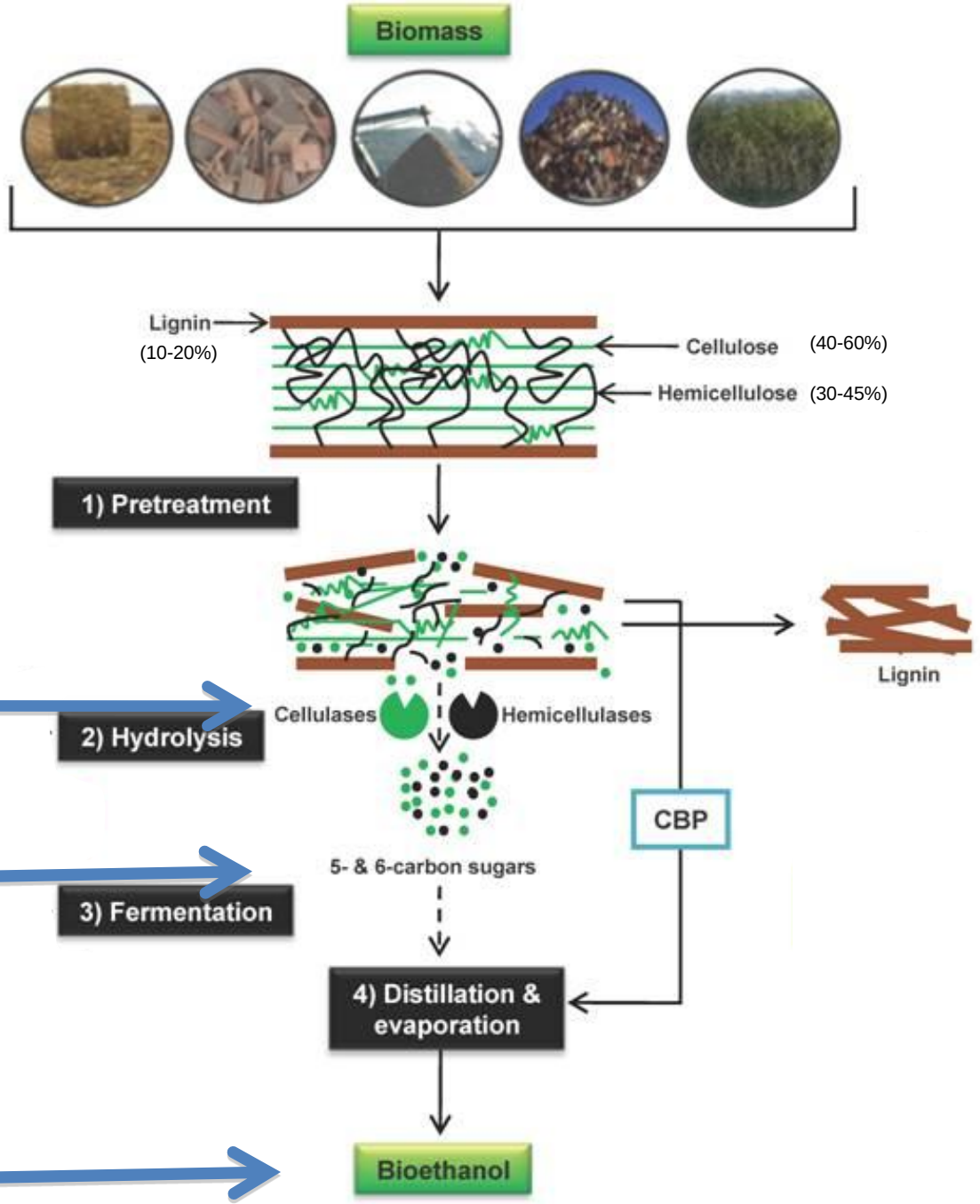
Ethanol Yield = 0.4 g/g

Pre-treated Biomass from IOCL



Ethanol Yield = 0.5 g/g

Pre-treated Biomass from IGL/ICT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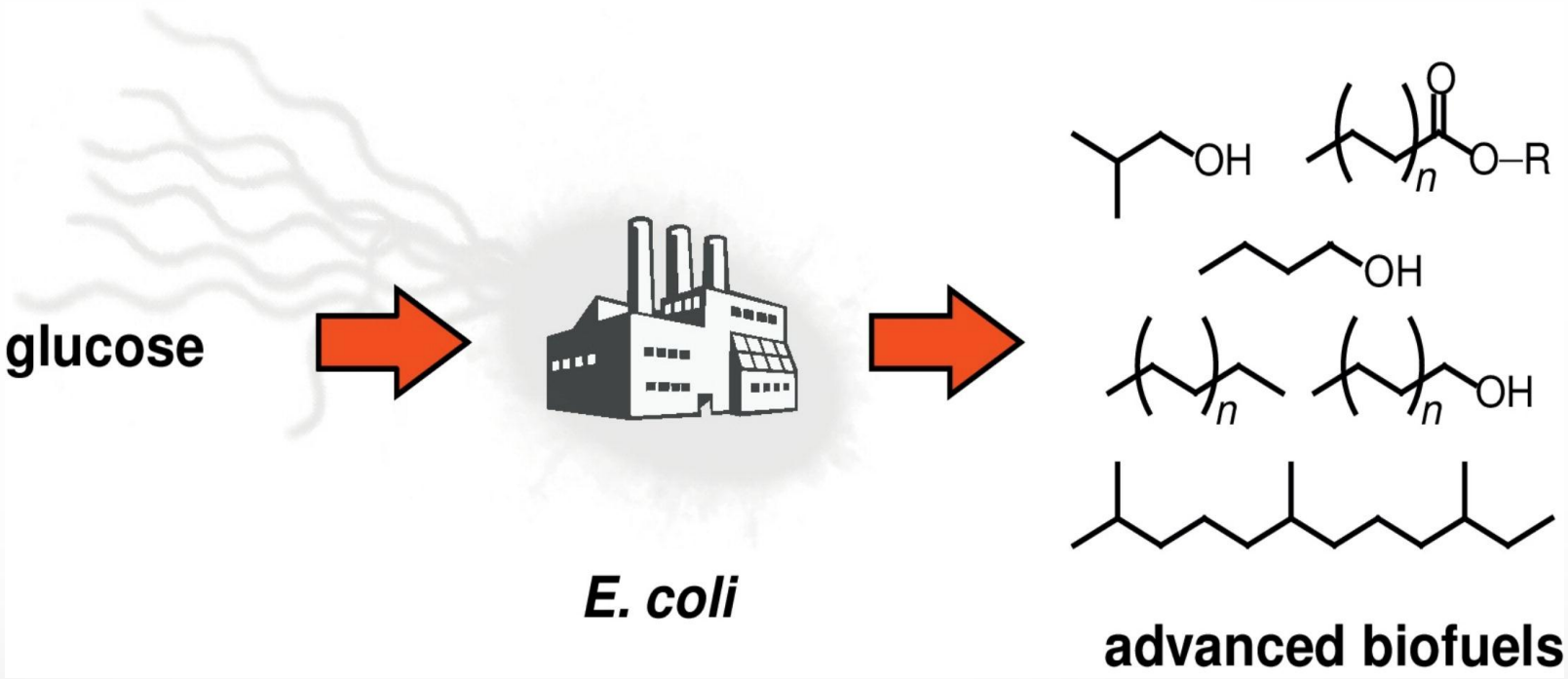
Advanced Biofuels



Current Opinion in Chemical Biology Volume 17, Issue 3 2013 472 - 479

nature Vol 451|3 January 2008|doi:10.1038/nature06450

LETTERS

Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels

Shota Atsumi[1], Taizo Hanai[1] & James C. Liao[1,2]

Science
AAAS

Microbial Biosynthesis of Alkanes
Andreas Schirmer, *et al.*
Science **329**, 559 (2010);
DOI: 10.1126/science.1187936

PNAS

Synthesis of customized petroleum-replica fuel molecules by targeted modification of free fatty acid pools in *Escherichia coli*

Thomas P. Howard[a], Sabine Middelhaufe[a], Karen Moore[a], Christoph Edner[a], Dagmara M. Kolak[a], George N. Taylor[a], David A. Parker[a,b], Rob Lee[a,b], Nicholas Smirnoff[a], Stephen J. Aves[a], and John Love[a,1]

[a]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom; and [b]Biodomain, Shell Technology Centre Thornton, Chester CH1 3SH, United Kingdom

Edited by Alexis T. Bell, University of California, Berkeley, CA, and approved March 15, 2013 (received for review September 13, 2012)

Mattam and Yazdani *Biotechnology for Biofuels* 2013, **6**:128
http://www.biotechnologyforbiofuels.com/content/6/1/128

Biotechnology for Biofuels

RESEARCH Open Access

Engineering *E. coli* strain for conversion of short chain fatty acids to bioalcohols

Anu Jose Mattam and Syed Shams Yazdani*

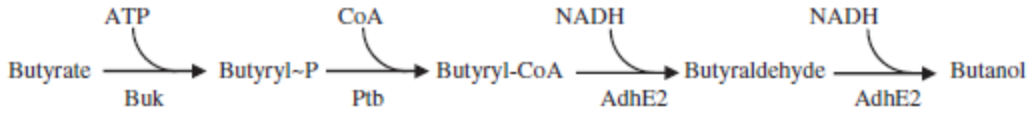
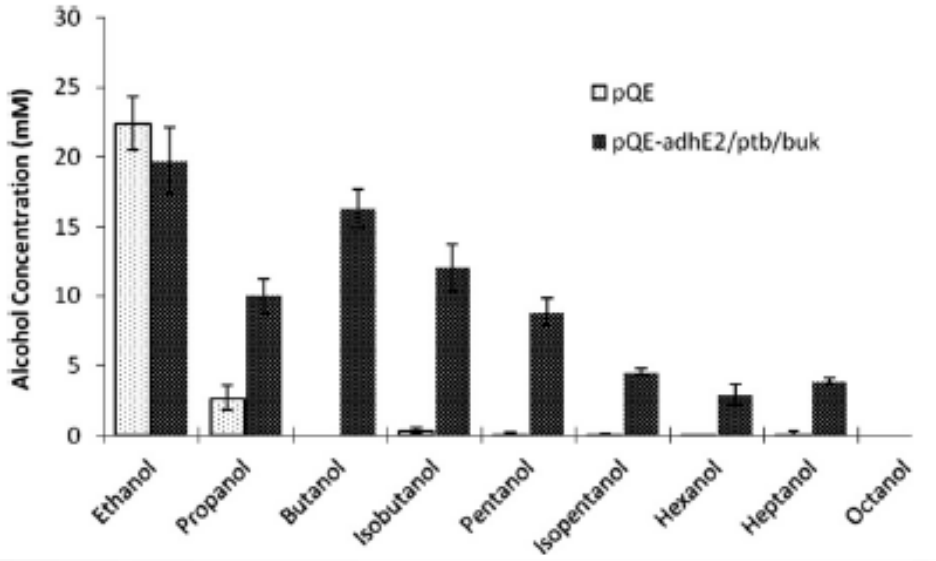
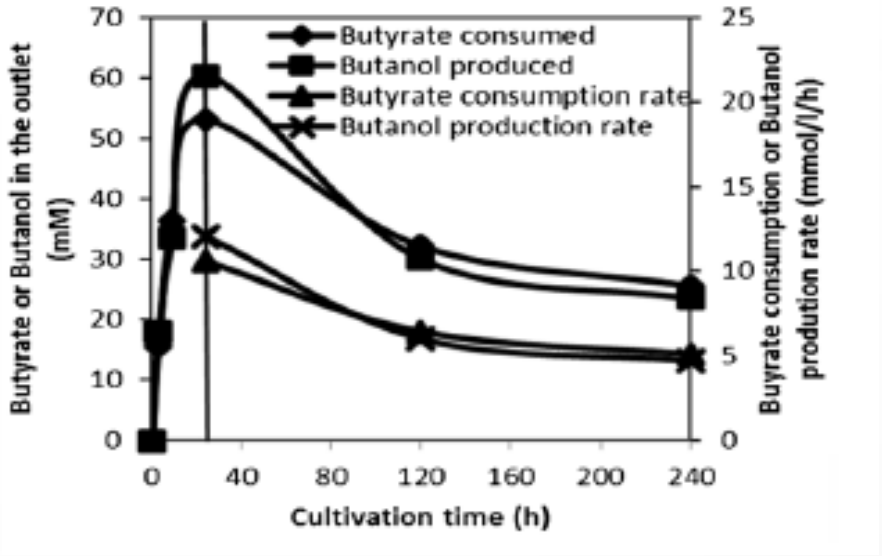


Figure 1 Metabolic pathway of *Clostridium acetobutylicum* engineered in *E. coli*. Abbreviations: Buk – butyrate kinase, Ptb – phosphotransbutyrylase, AdhE2 – aldehyde-alcohol dehydrogenase.

Engineering *E. coli* for short chain alcohols





Production of C3-C7 alcohol

Continuous Production of Butanol

Mattam et al. Biotechnology for Biofuels. Sept 2013

Acknowledgment

- ICGEB Team
  - Nidhi, Research Associate
  - Neha, Graduate Student
  - Anu, Graduate Student
  - Zia, Graduate Student
  - Zeenat, Junior Res Fellow
  - Vamsi, Junior Res Fellow
  - Tabinda, Junior Res Fellow
  - Kamran, Junior Res Fellow

- Collaborators
  - Insect Resistance Group, ICGEB
  - DBT-ICT Centre for Energy Bioscience, Mumbai
  - DBT-IOC Centre for Advanced Bioenergy Research, Faridabad

- Funding
  - DBT, Govt of India
  - DST, Govt of India

Thank You