Engineering *E. coli* biocatalysts for consolidated biofuel production from hemicellulose

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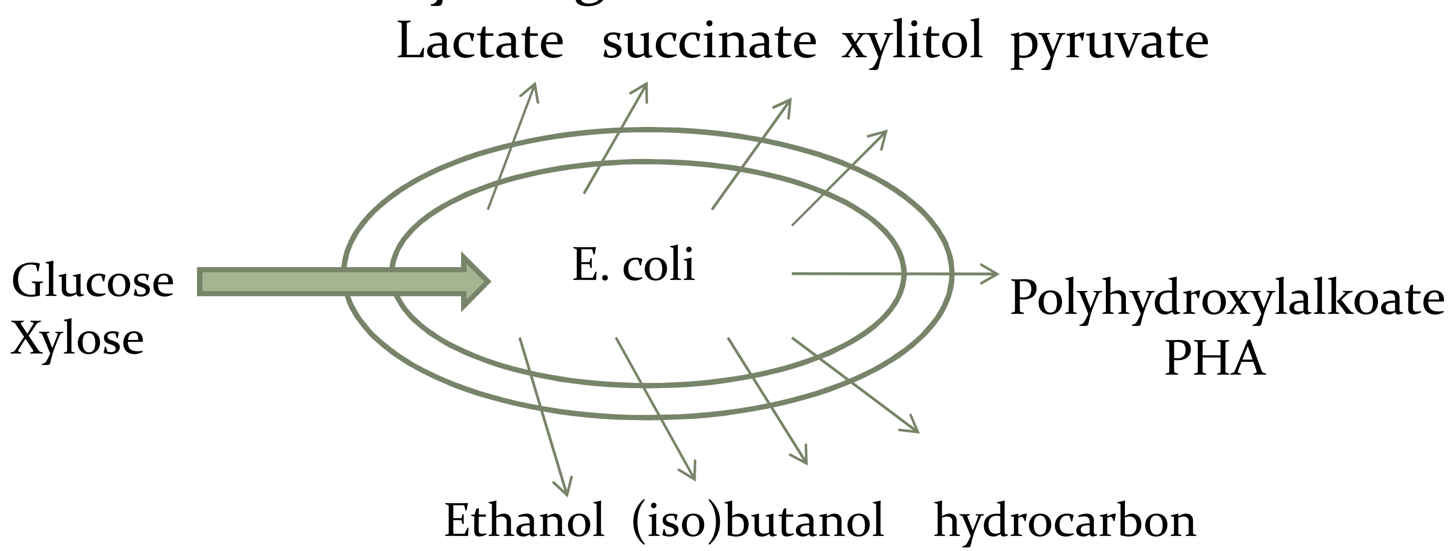
Bioenergy III,
Canary Island, May 2011

E. coli as biocatalyst for bioenergy and biorefinery

- Metabolic engineering has been successful in making E. coli to churn out anything desirable

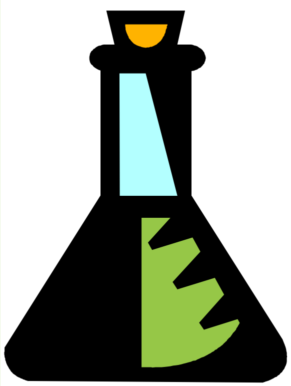


- Need to take on polymeric substrates (cellulose/hemicellulose) to reduce cost

Consolidated Production for cost reduction

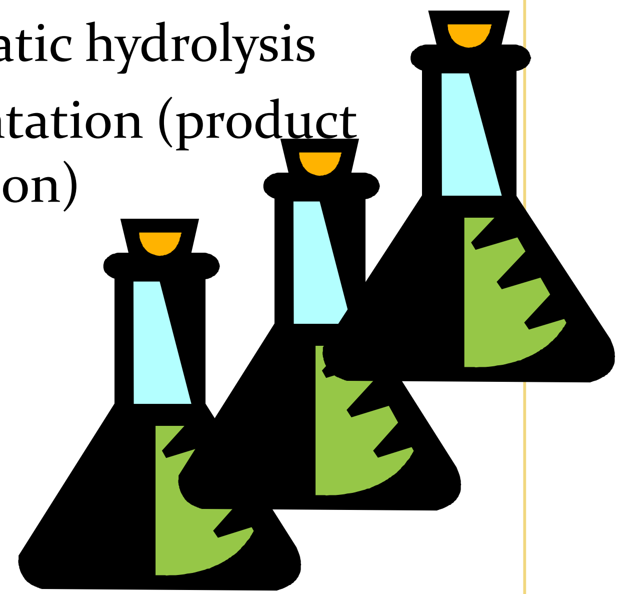
Consolidated

- Single step accomplished in a reactor as single unit operation
- Requires biocatalyst to multitask



Traditional

- Multi-step accomplished as separate unit operations
  - Enzyme production
  - Enzymatic hydrolysis
  - Fermentation (product formation)

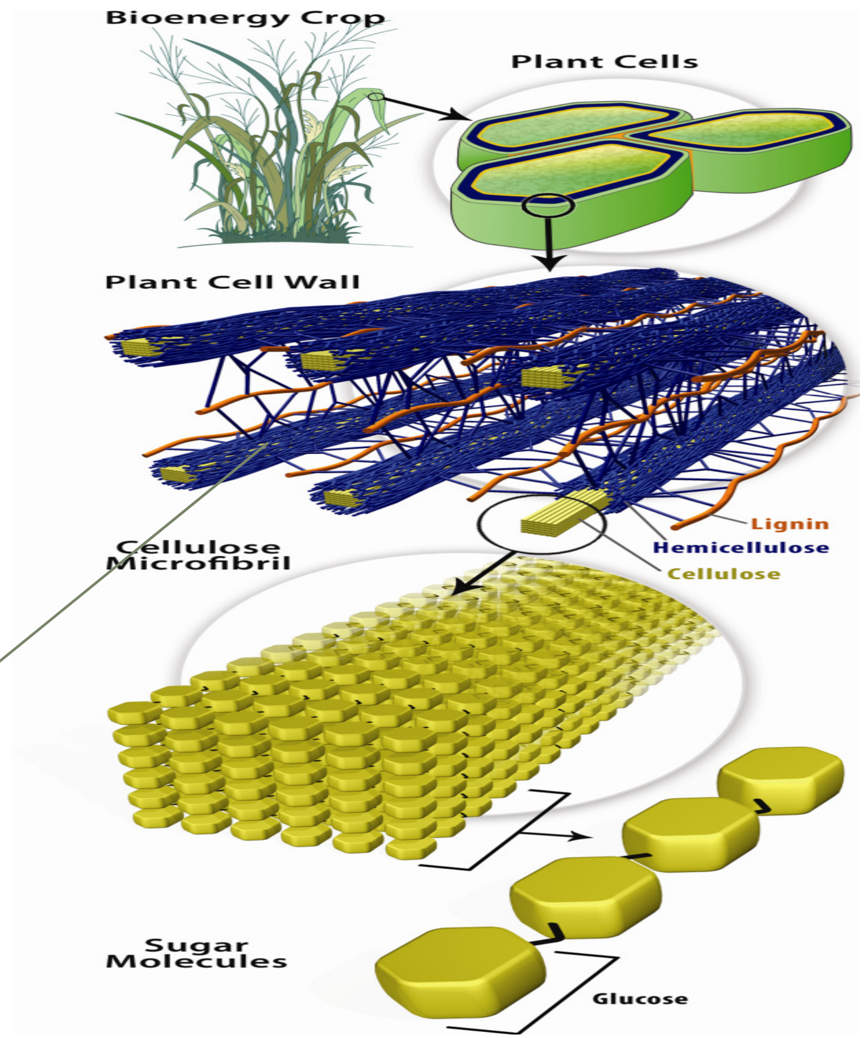


Could this be done?

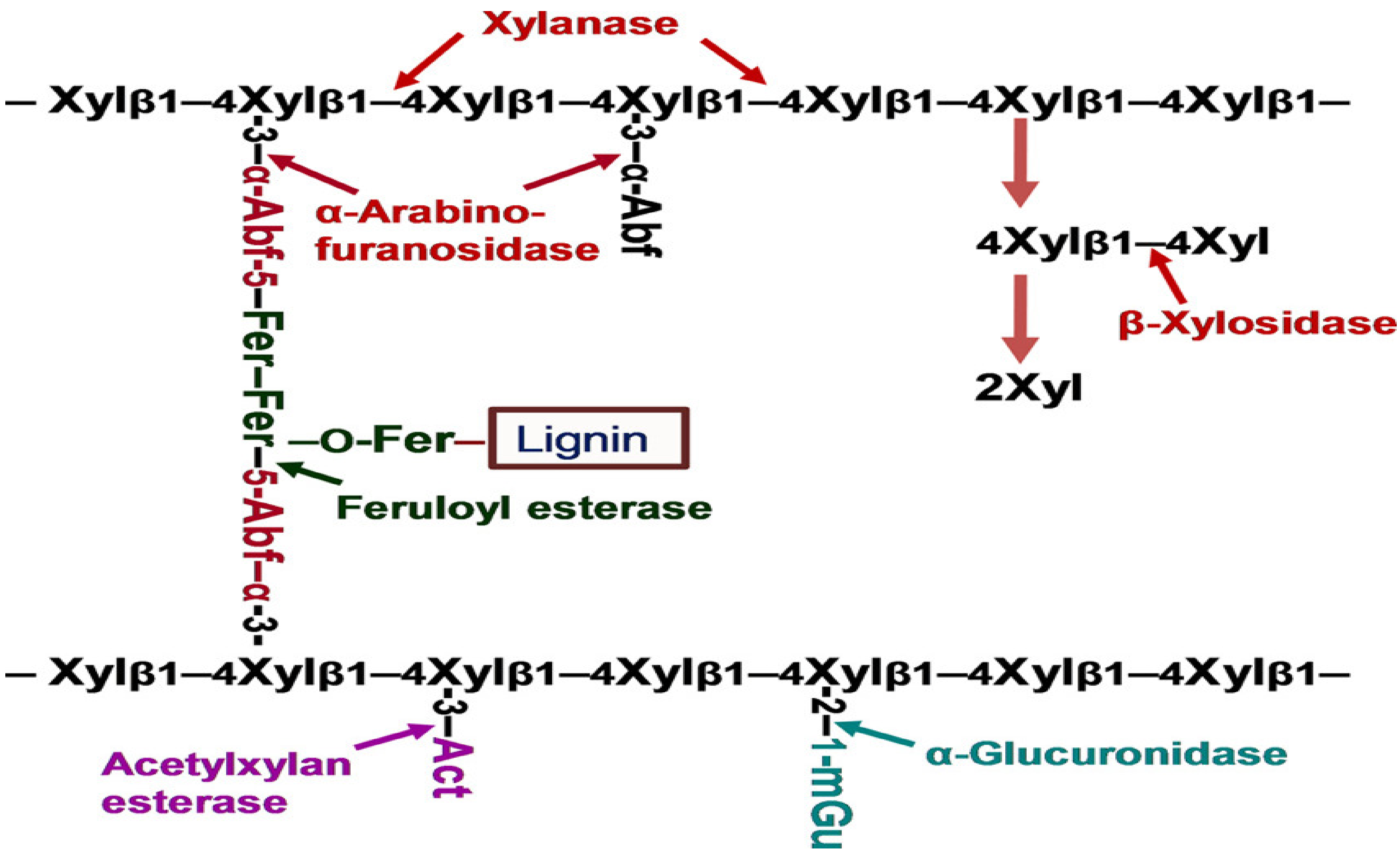
Objectives:

Demonstrate a binary strategy to achieve direct fermentation of xylan to ethanol

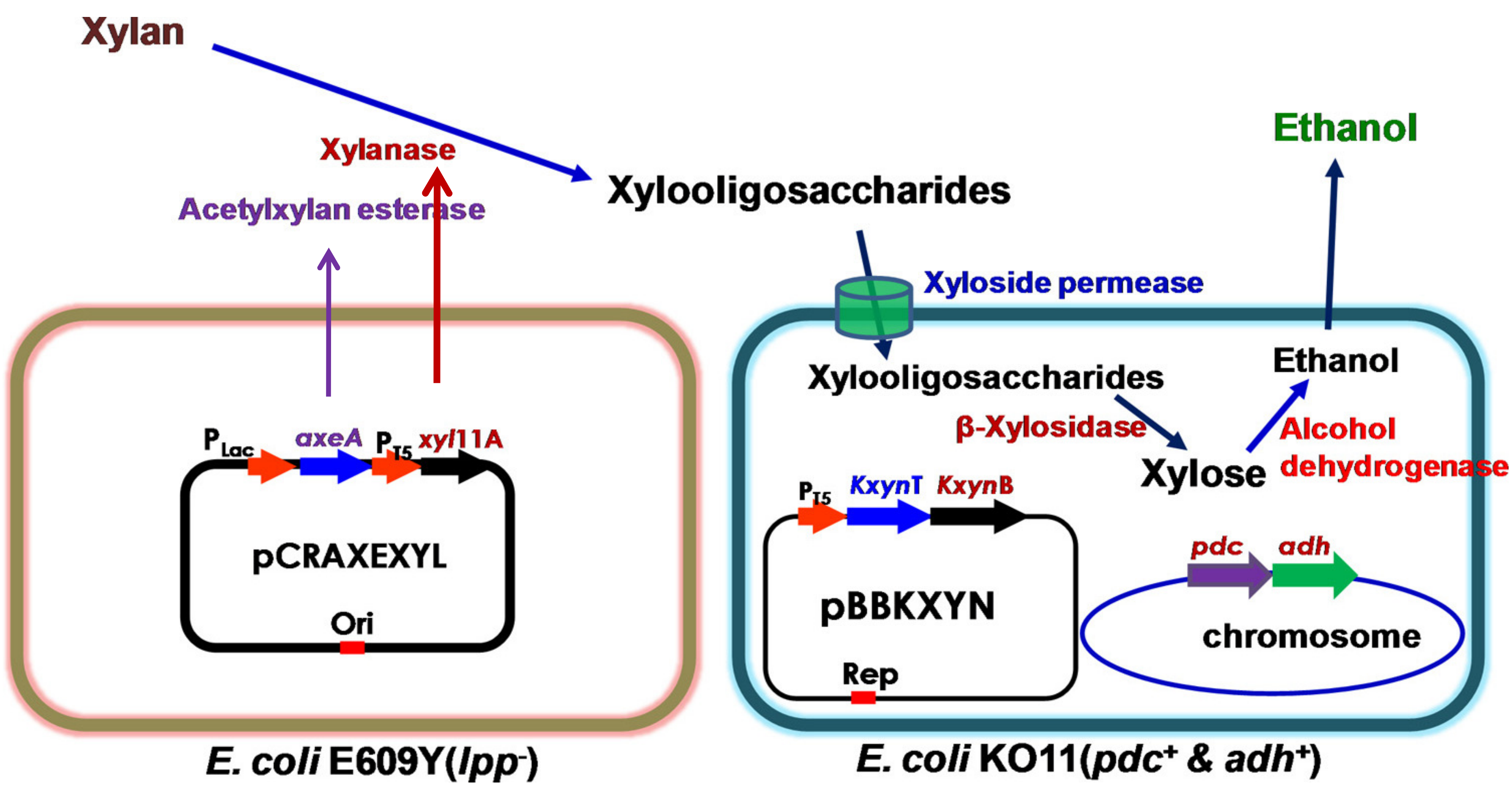
Hemicellulose: 20-40% of lignocellulose



Six types of enzymes are needed for complete hydrolysis of xylan

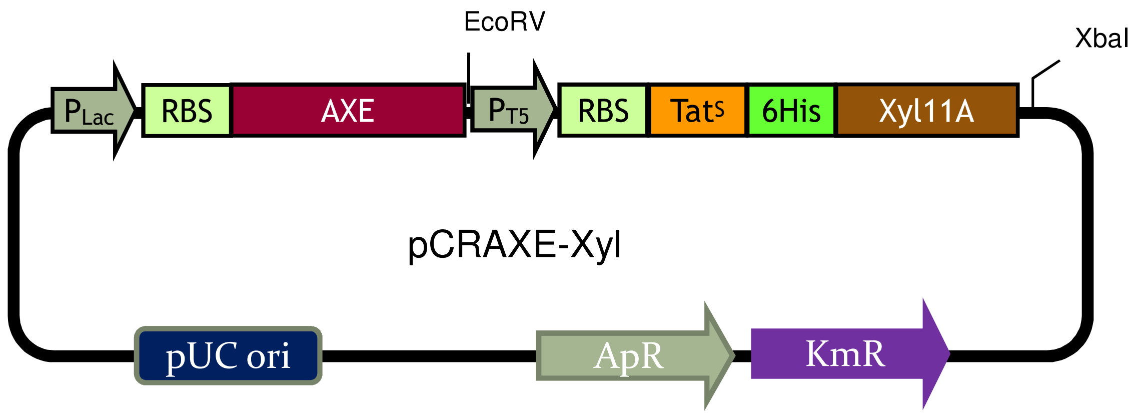


First design of the binary system



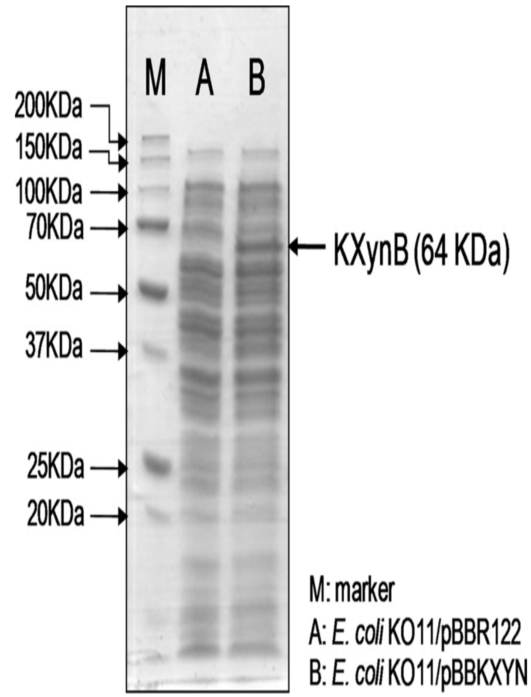
1st strain as hemicellulase producer/secretor

Enzyme activity (U/ml)	pCRAXE-T5Xyl		
	Total	Extracellular	Secretion percentage (%)
Xylanase	0.28	0.26	93
Acetylxylan esterase (AXE)	2.76	2.62	95



2nd strain to convert oligosaccharides to ethanol

Enzymes	Activity (U/L)
β-Xylosidase (β-Xyl)	1,616.5±17.02
α-Arabinofuranosidase	46.30±6.64



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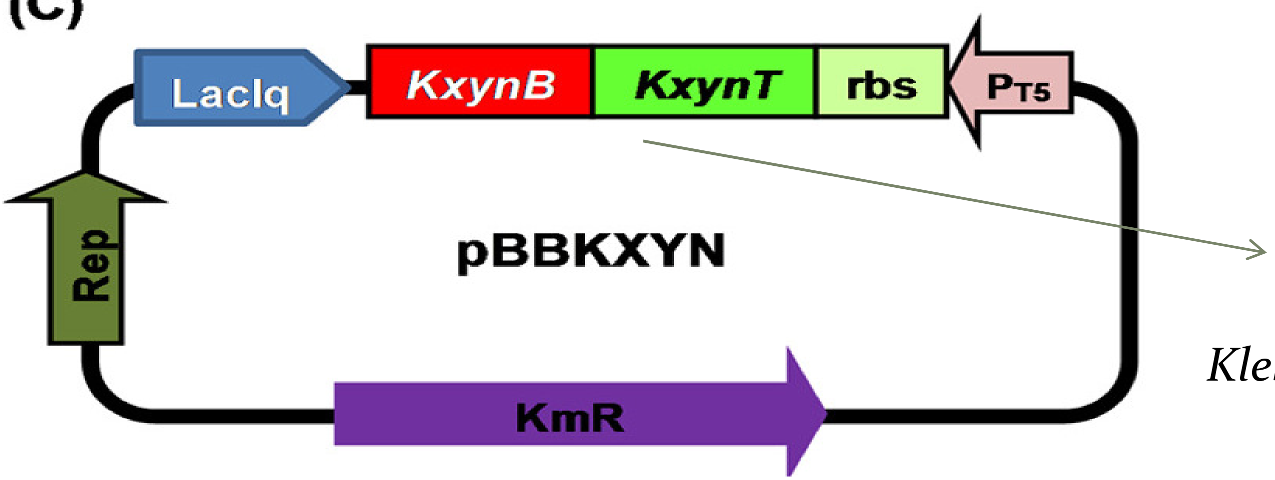


From
Klebsiella pneumoniae

2^{nd} strain: converting xylooligosaccharide to ethanol

	0 h		48 h	
	KO11	KO11/ pBBKXYN	KO11	KO11/ pBBKXYN
Cell growth(A_{600})	1±0.08	1±0.11	3.02±13	4.74±0.35
Reducing Sugar (g/L)	1.74±0.13	1.74±0.15	4.39±0.25	1.49±0.17
Ethanol (g/L)	N.D.	N.D.	1.30±0.12	2.91±0.18

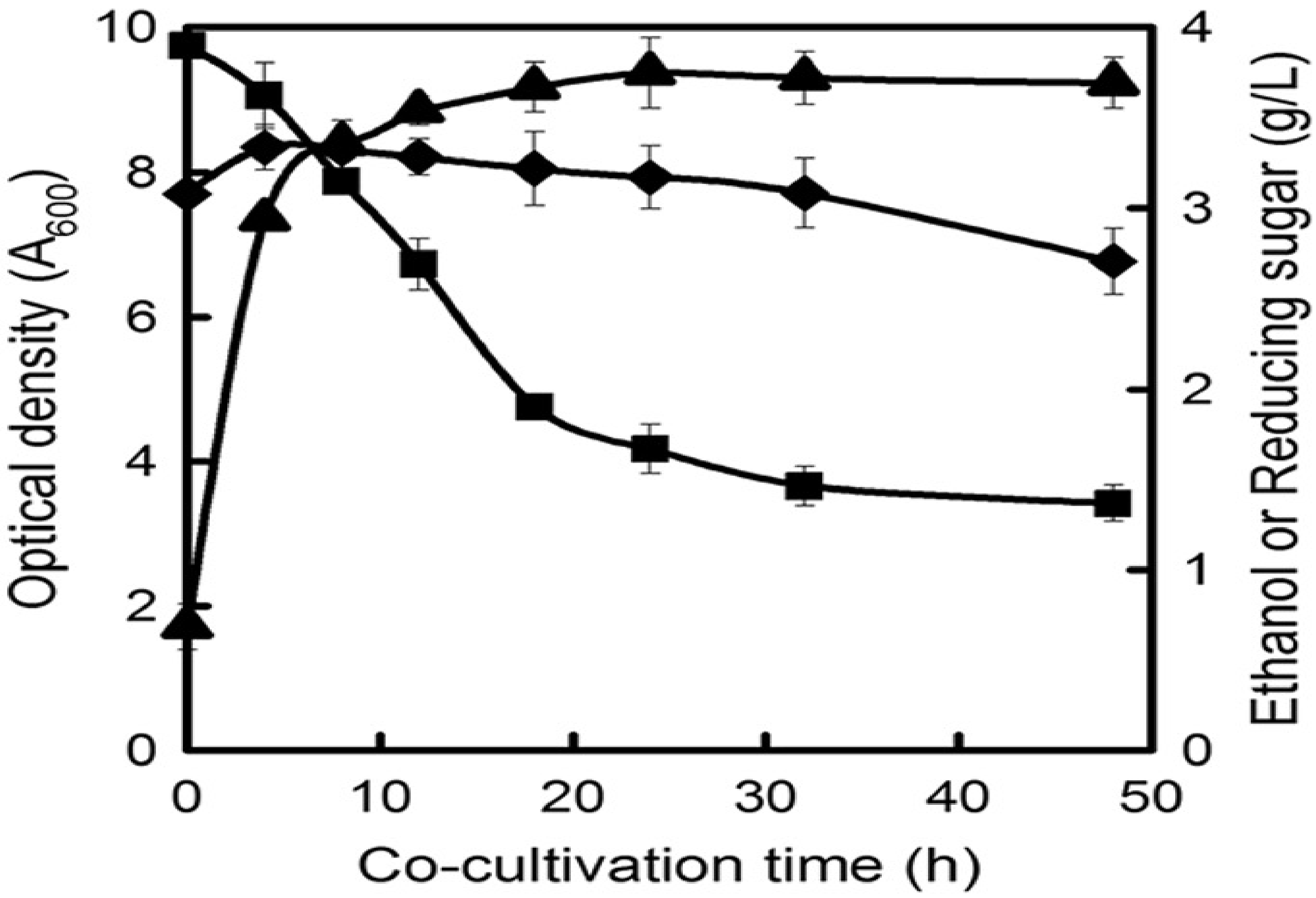
Binary vs. Single strain vs. acid hydrolysis

	24 h fermentation		
	Residual reducing sugar(g/L)	Ethanol (g/L)	Ethanol yield (%)
Binary	1.71±0.06	2.84±0.13	**54.5**
Single strains	0.84±0.07	1.95±0.14	**37.6**
Acid hydrolysate	1.16±0.06	4.38±0.11	**84.1**

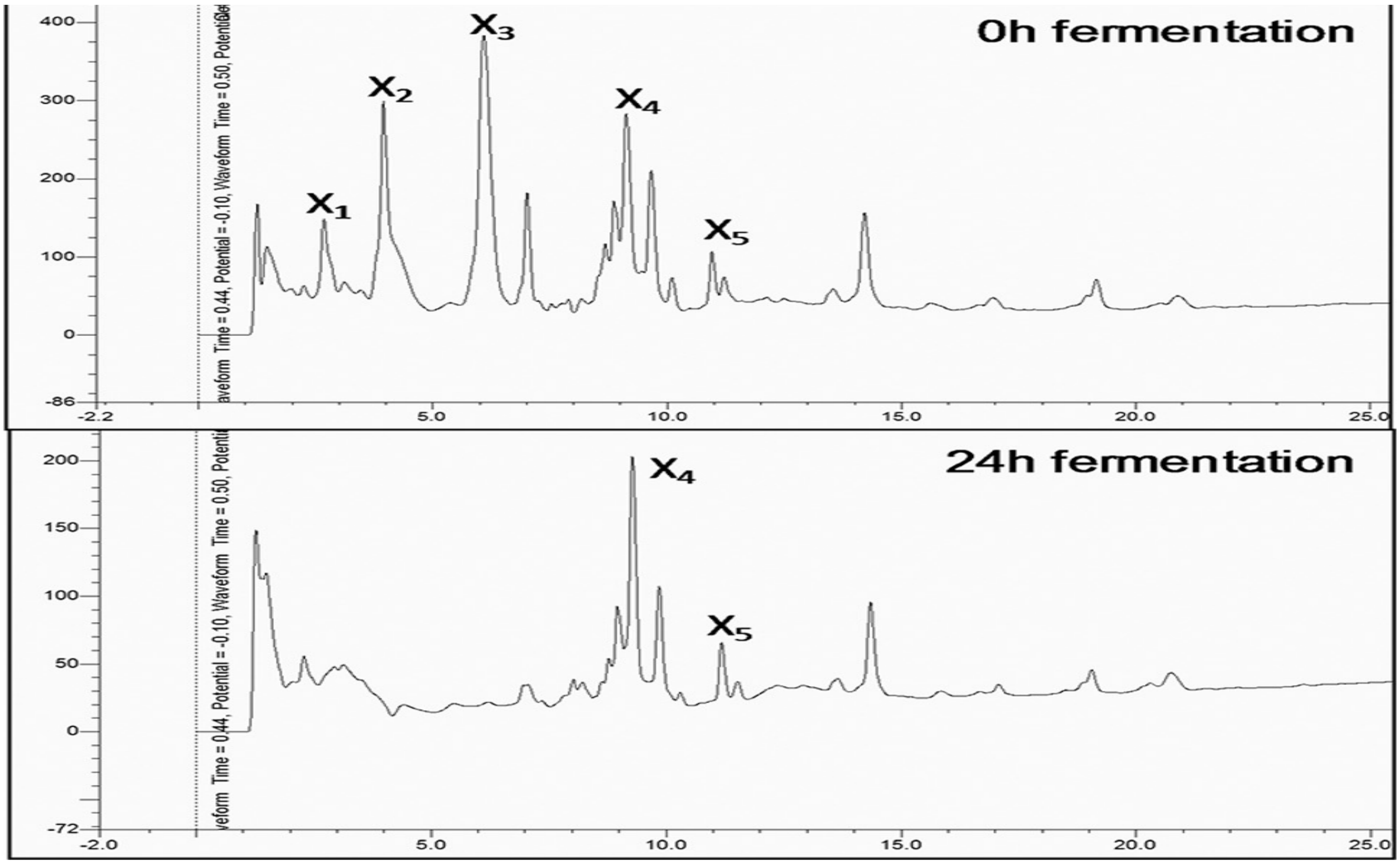
Improvement on ethanol yield

Case	After 36h induction		After 24h fermentation	
	Reducing sugar (g/L)	Hydrolysis yield (%)	Ethanol (g/L)	Ethanol yield (%)
A(3 enzymes)	3.35±0.11	32.7	2.84±0.13	**54.5**
B	3.35±0.13	32.7	3.29±0.12	**63.1**
C	3.87±0.12	37.8	3.11±0.14	**59.6**
D	3.92±0.06	38.3	3.22±0.13	**61.7**
E (6 enzymes)	3.95±0.25	38.6	3.71±0.27	**71.1**

Xylan to ethanol



Why not 100% yield?

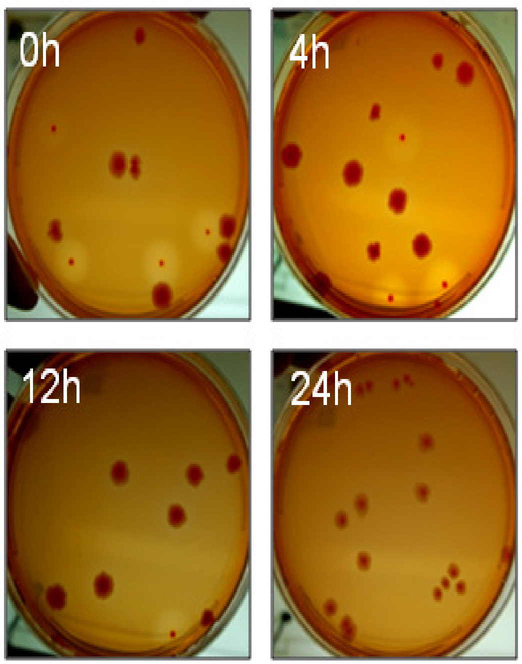


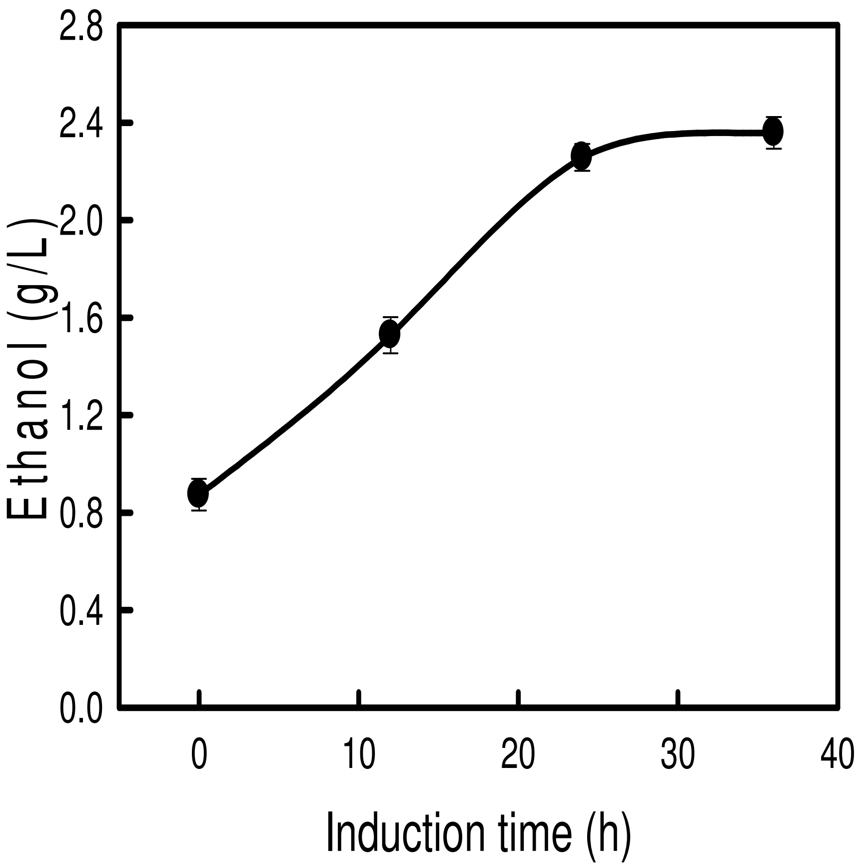
Xylooligosaccharides with 4 or more xylose units were not utilized

Conclusions

- Binary strategy is a viable option for consolidated bioprocessing of lignocellulose.
- Modular design allows easy extension to other biofuel and biorefinery products.
- Inclusion of all enzymes necessary to de-polymerize lignocellulose and judicious division of labor are important to achieve high yield.
- Hydrolysis of xylan to smaller units outside the cells or identifying a transporter for uptake larger xylooligosaccharides is important for further improvement.

Co-cultivation of two strains





Acknowledgements

Shara McClendon

Hyun-Dong Shin

Trinh Vo

NSF USDA NASA GEORGIA RESEARCH ALLIANCE AIR PRODUCTS