# Efficient Production of Lactate-based Polyesters from Renewable Biomass using Recombinant *Escherichia coli*

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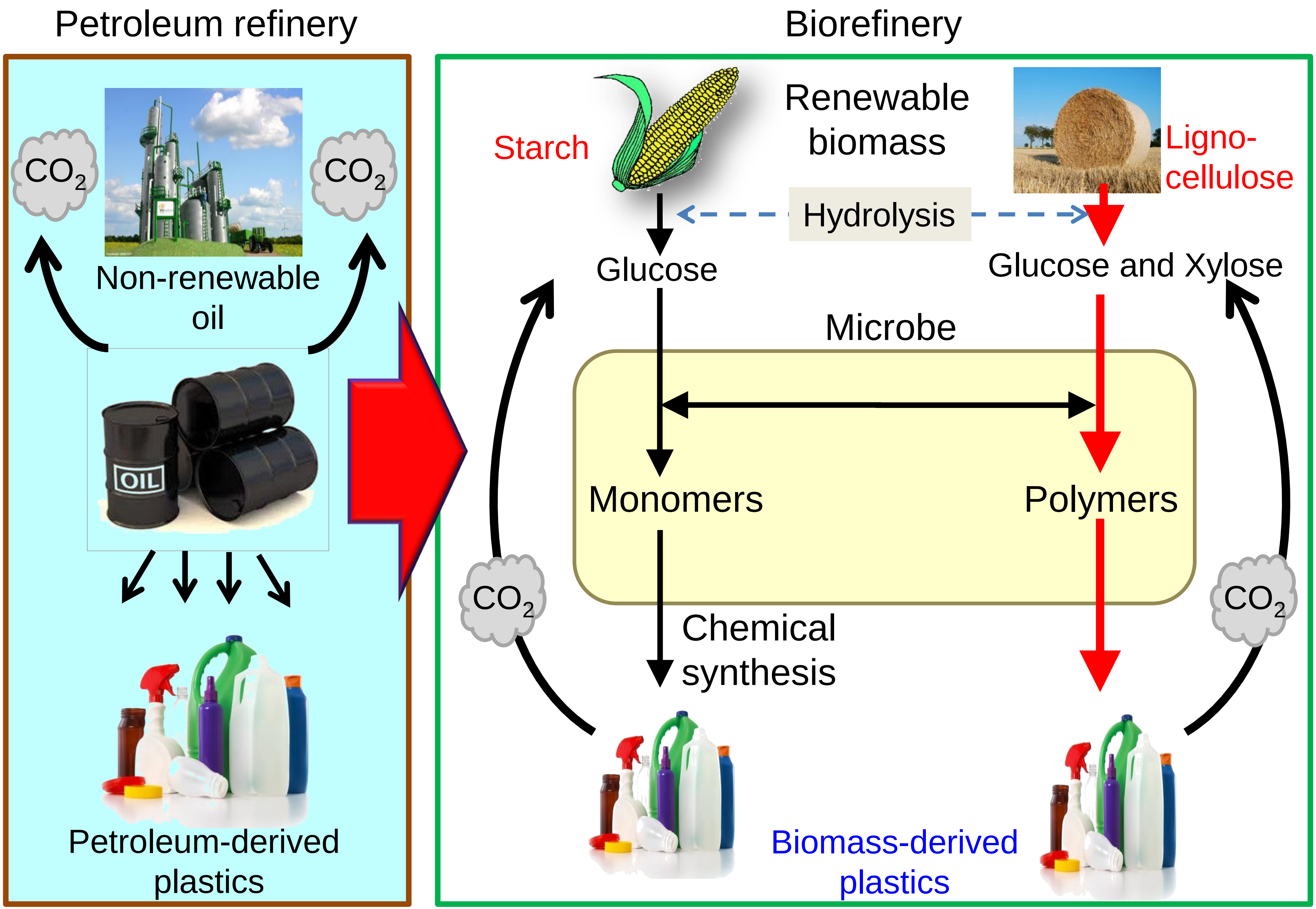
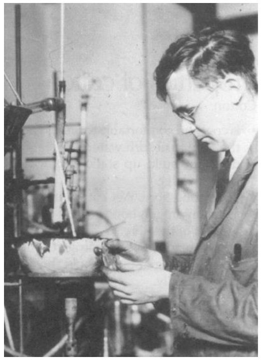
**3rd Annual Biosafety Conference, KICC-Nairobi, Kenya**

# Biorefinery for Bioplastic production



# Bioplastic production system



*Lactate polymer (3 step production)*

$CO_2 + H_2O$ → (Plant / Photosynthesis) → Sugar → (Microbes / Fermentation) → Lactate → (Metal catalyst / Chemical synthesis) → PLA

*Chemical Factory*

*Biopolymer (2 step production)*

$CO_2 + H_2O$ → (Plant / Photosynthesis) → Sugar → (Microbes / Fermentation) → PHA

*Microbial Factory*

↓ New biosynthetic process

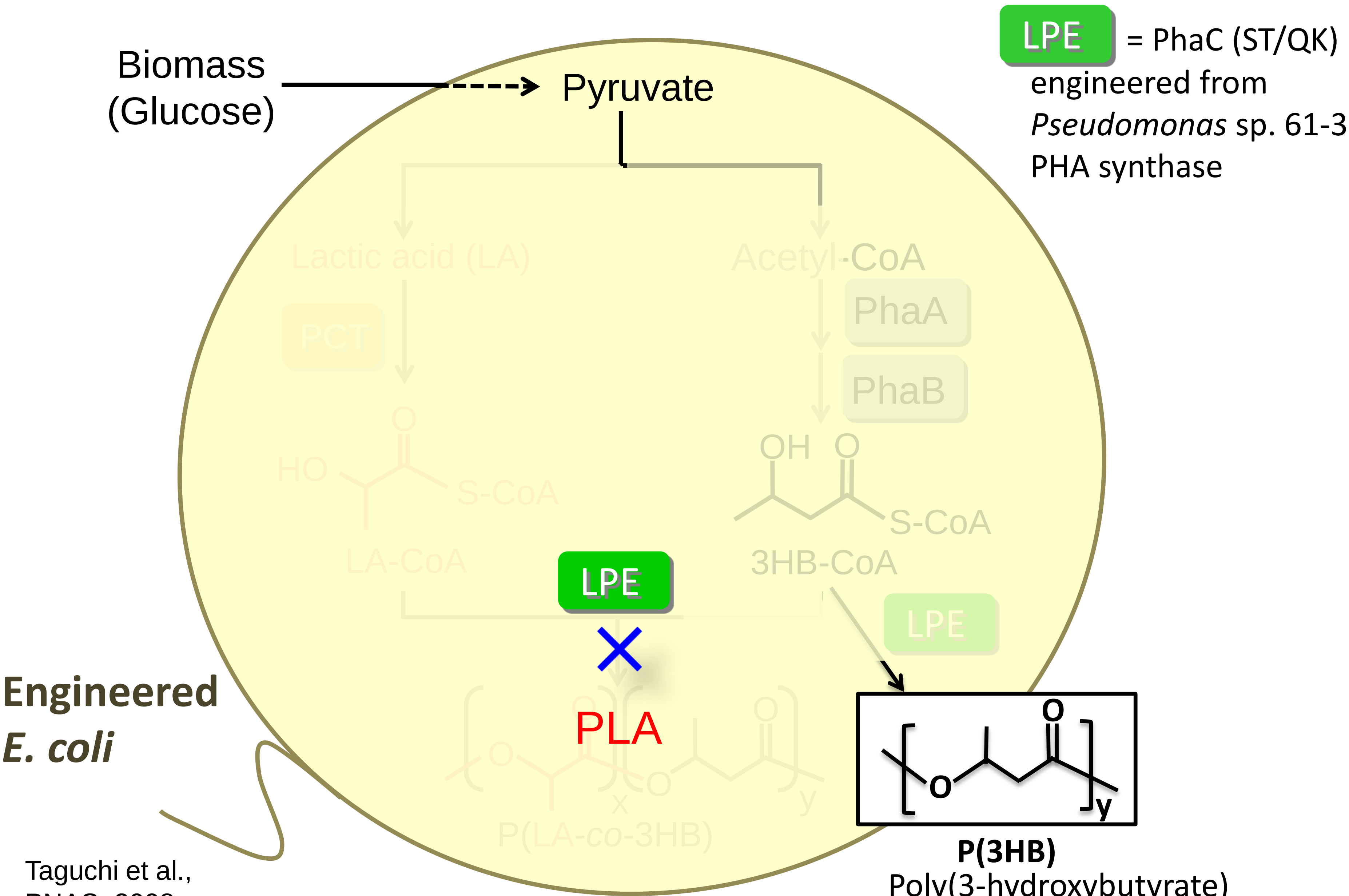
*Lactate-based polymer (2 step production)*

$CO_2 + H_2O$ → (Plant / Photosynthesis) → Sugar → (Microbes / Fermentation) → Lactate-based new polymers

*Xylose*

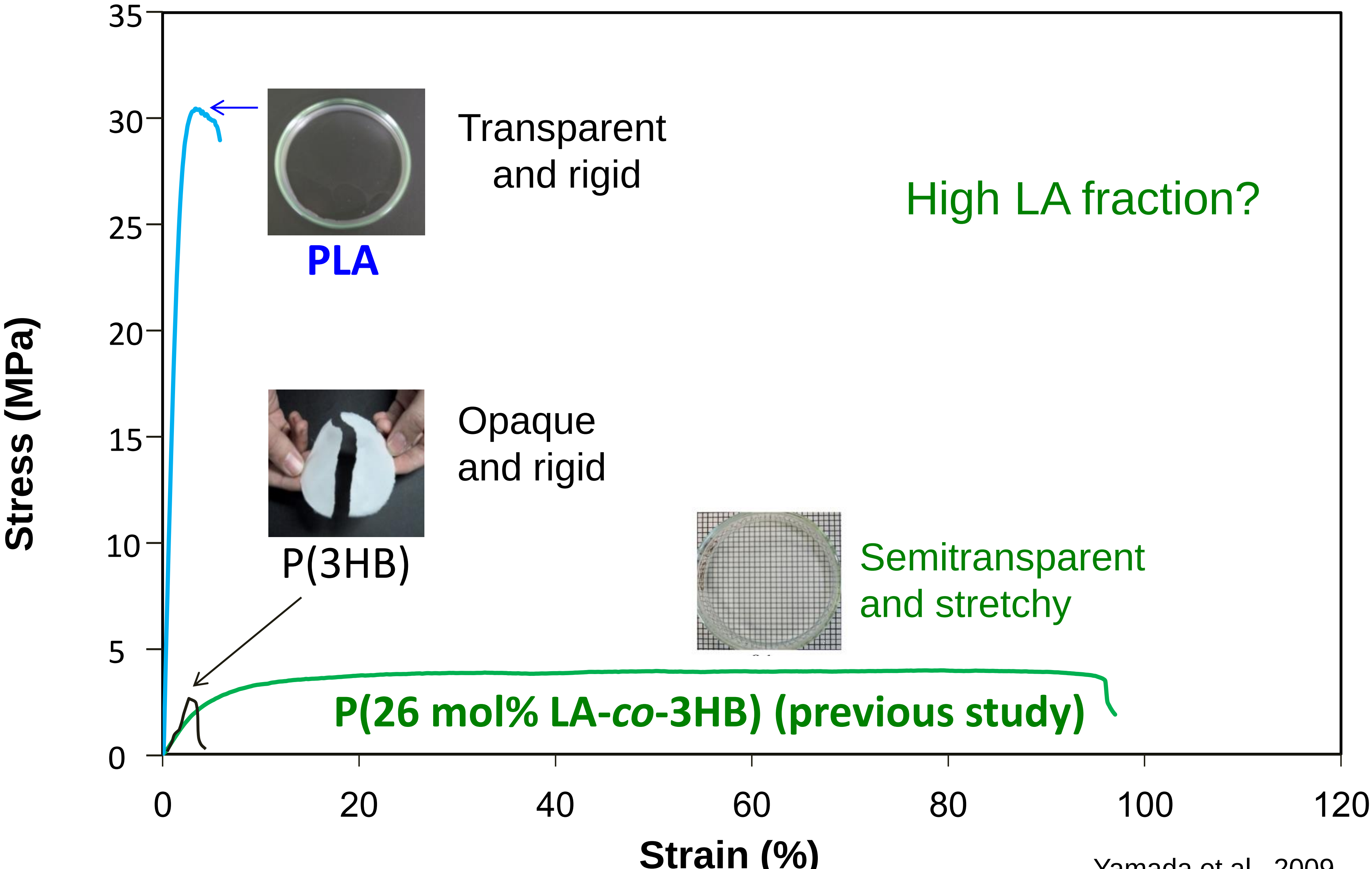
*New Microbial Factory*

# Microbial production of LA-based polymers




Taguchi et al., PNAS, 2008

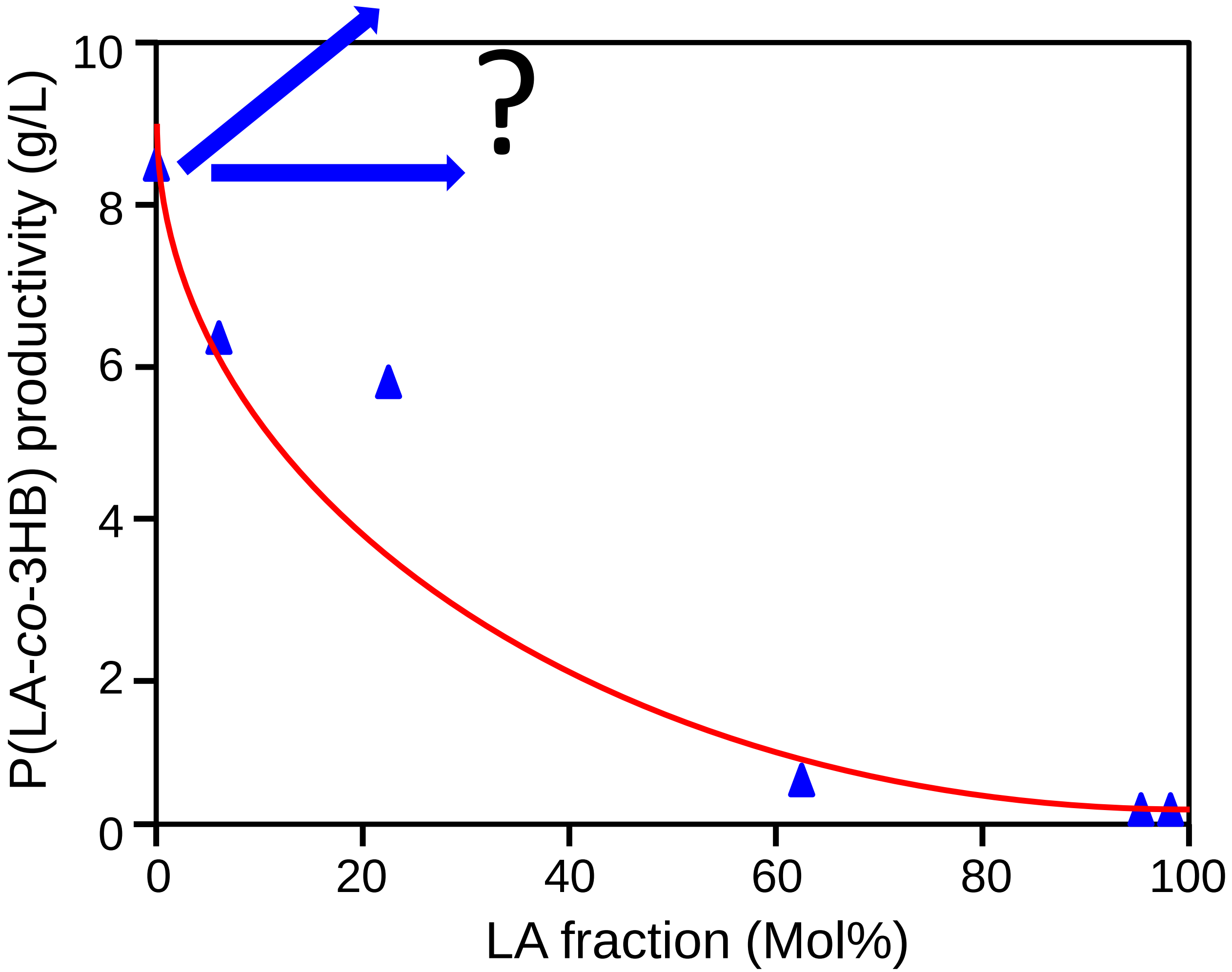
# Properties of LA-based polymers




Yamada et al., 2009

# Productivity of LA-based polymers

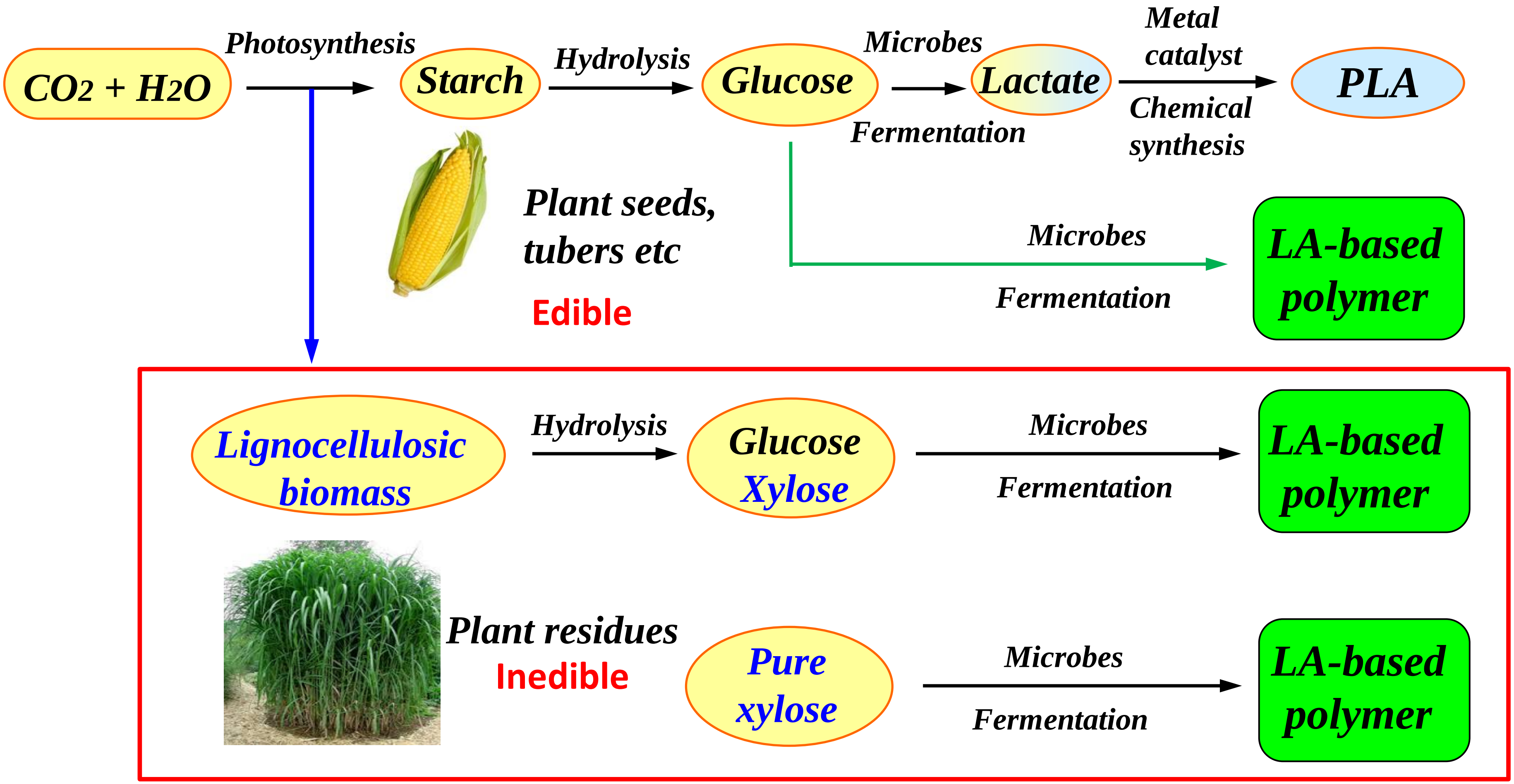
Inverse relationship between P(LA-*co*-3HB) productivity and LA fraction




Can LA-enriched P(LA-*co*-3HB) be produced at high productivity

Taguchi et al., 2008; Yamada et al., 2009; Shozui et al., 2011; Song et al., 2012.

# The present problems for LA-based polymer production

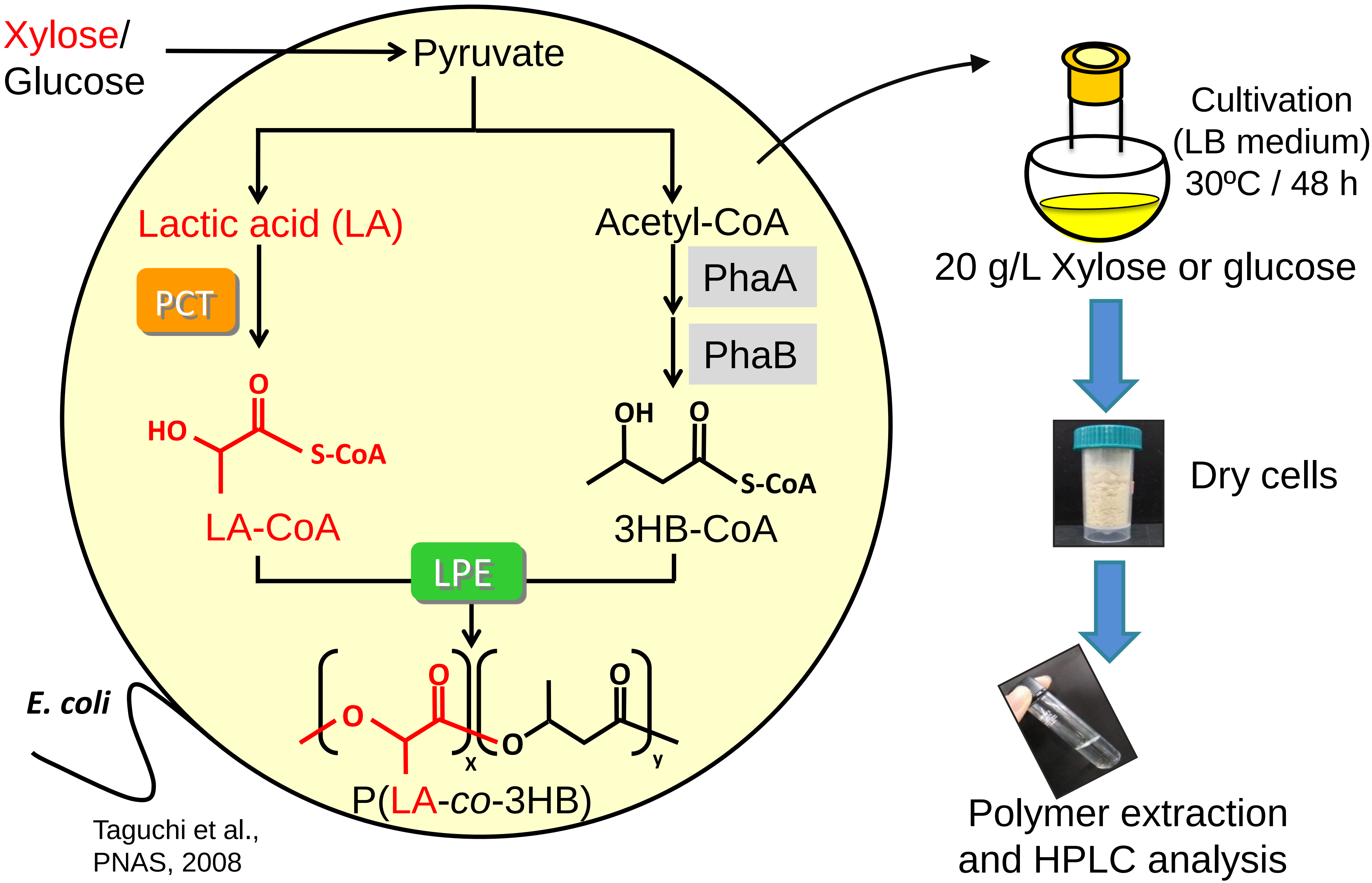



1. The kind of carbon sources used for polymer production
2. Polymer productivity
3. Regulation of LA fractions related to polymer property

# Aim of this study

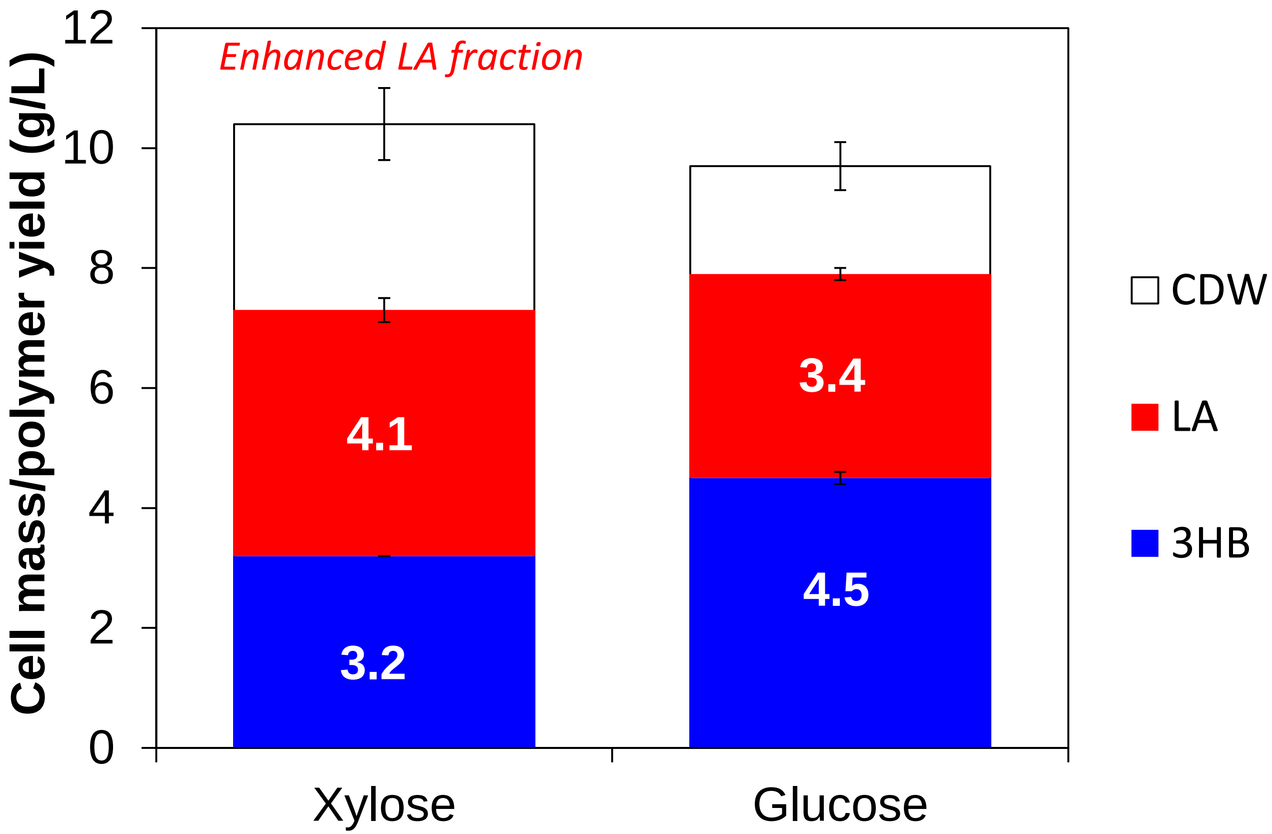
The aim of this research was to produce a variety of LA-based polymers in order to elucidate the relationship between LA fraction and polymer properties as well as enhanced polymer productivity.

# Which is better, glucose or xylose?

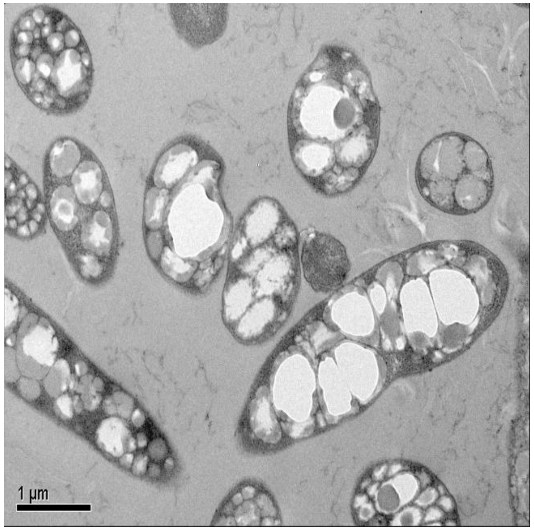



Taguchi et al.,
PNAS, 2008

# Xylose produces LA-enriched P(LA-*co*-3HB) than glucose




P(60 mol% LA-*co*-3HB) P(47 mol% LA-*co*-3HB)

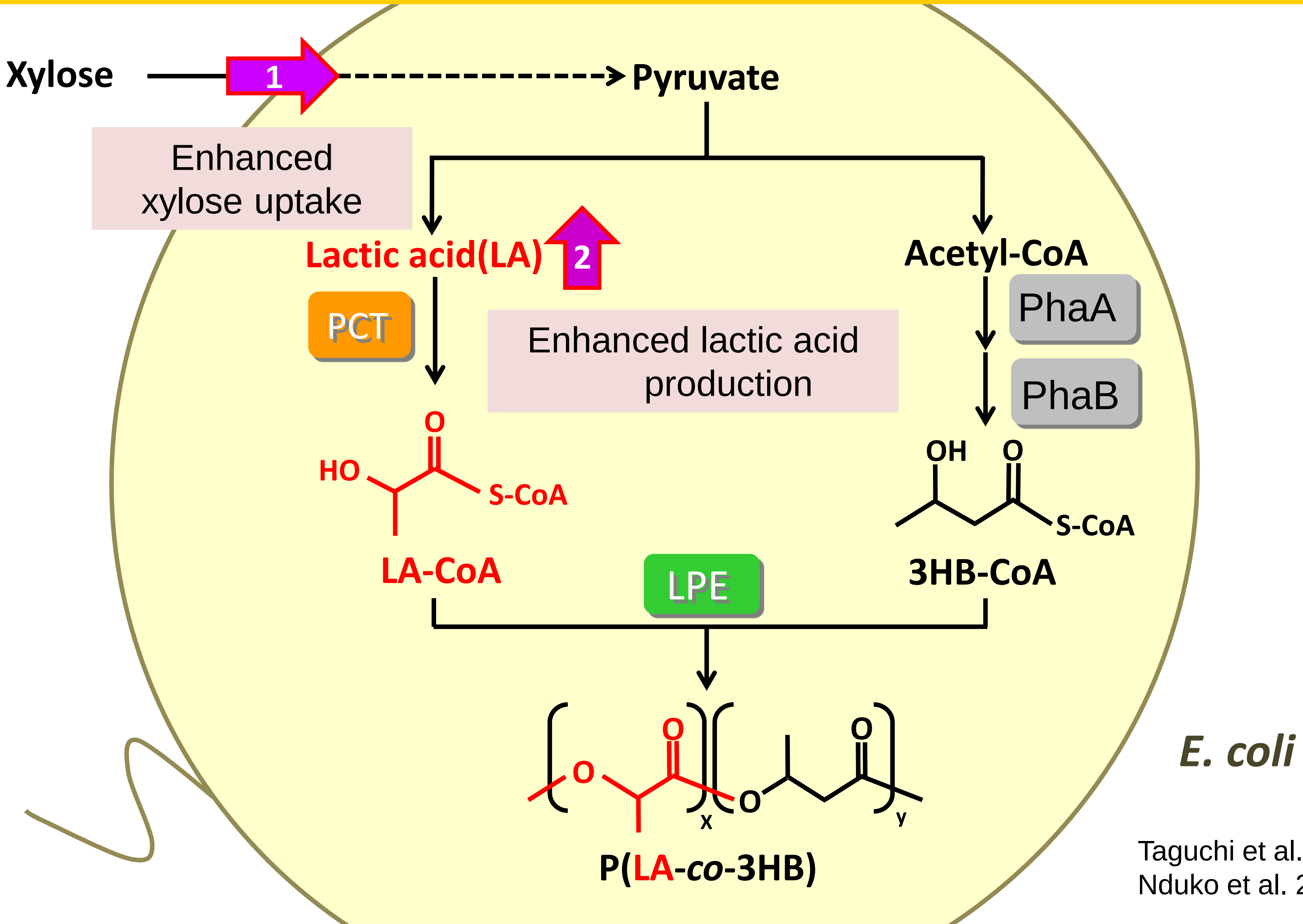



TEM image of *E. coli* accumulating P(LA-*co*-3HB)

Xylose is superior to glucose in synthesizing LA-enriched copolymers

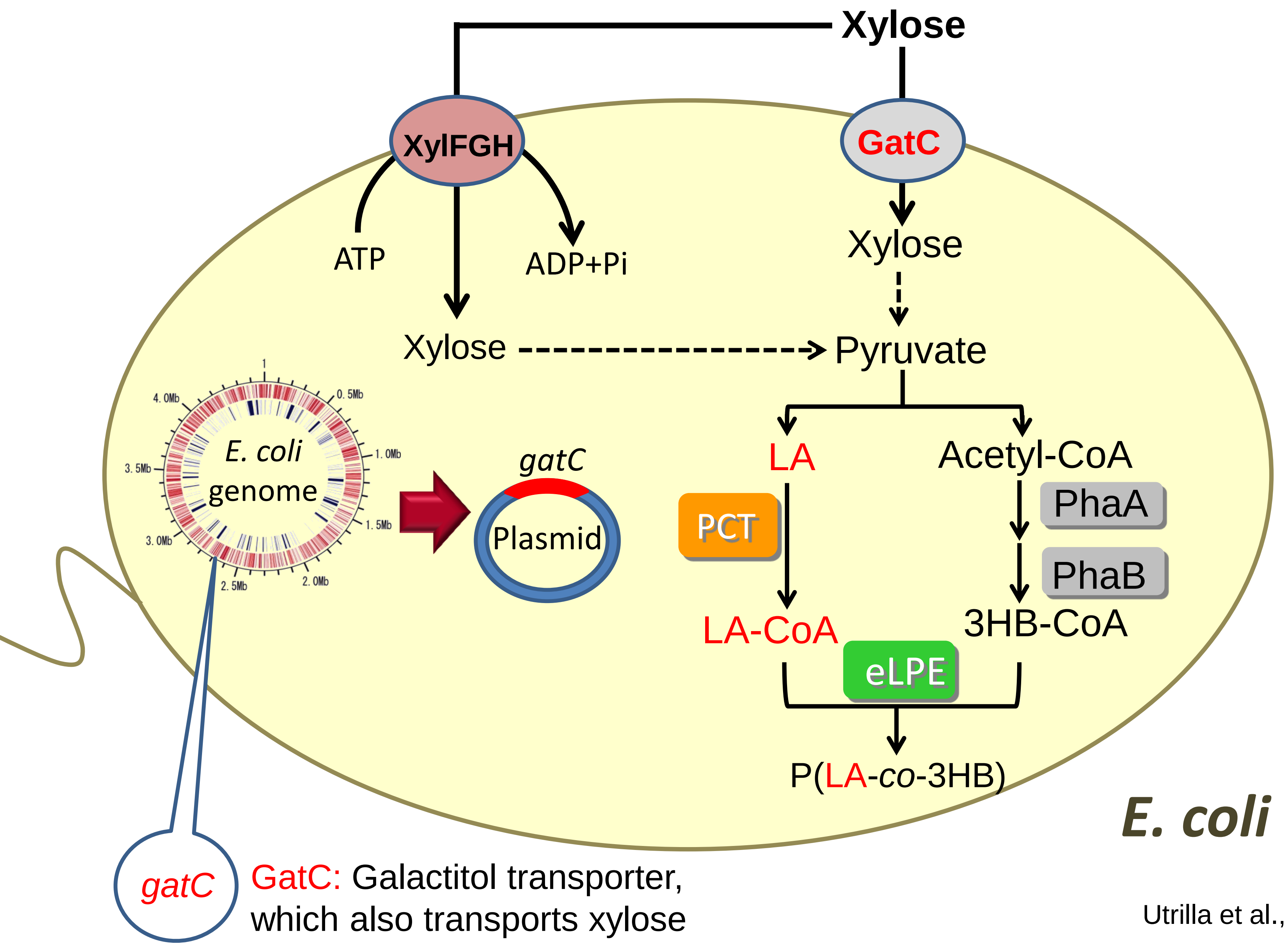
Nduko et al., *Metab. Eng*. 2013

# Strategies for increasing production and LA fraction of P(LA-*co*-3HB)




Taguchi et al. 2010
Nduko et al. 2013

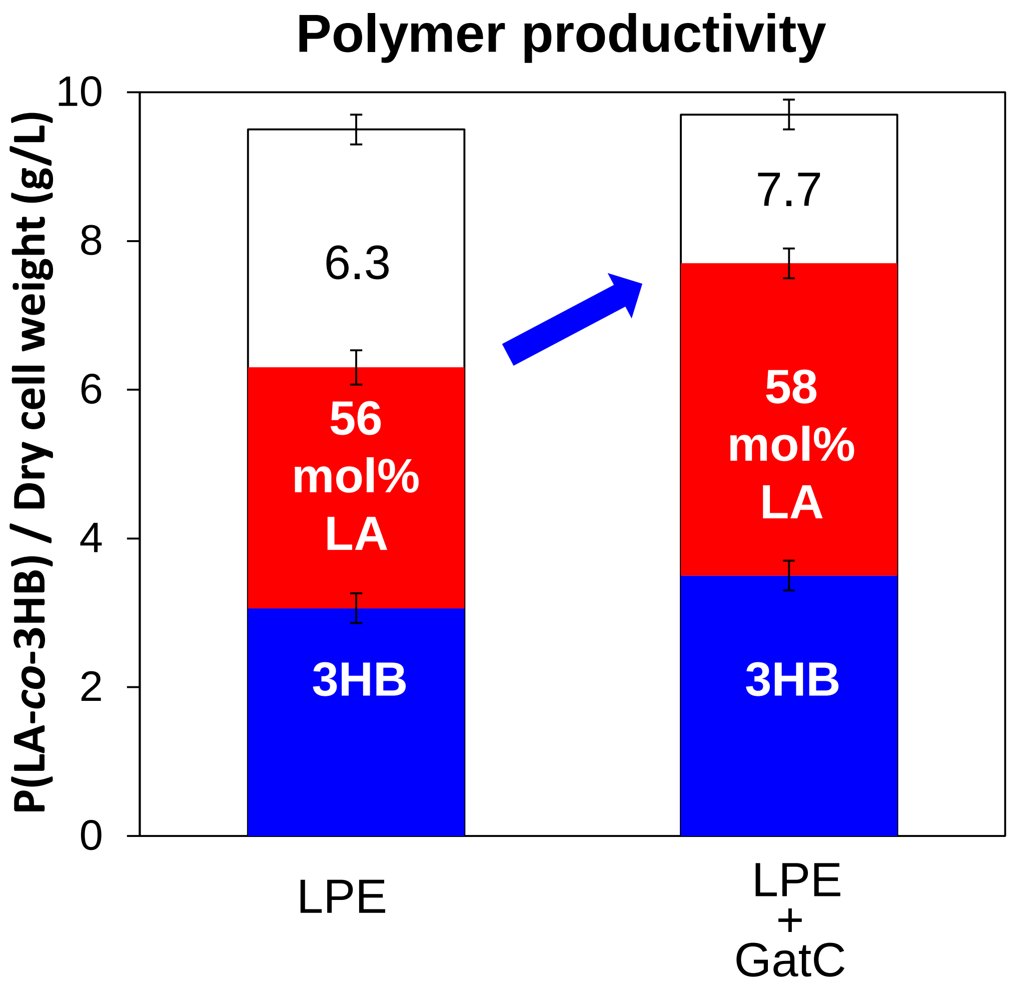
# Enhanced xylose uptake for P(LA-*co*-3HB) production




GatC: Galactitol transporter, which also transports xylose

Utrilla et al., 2012

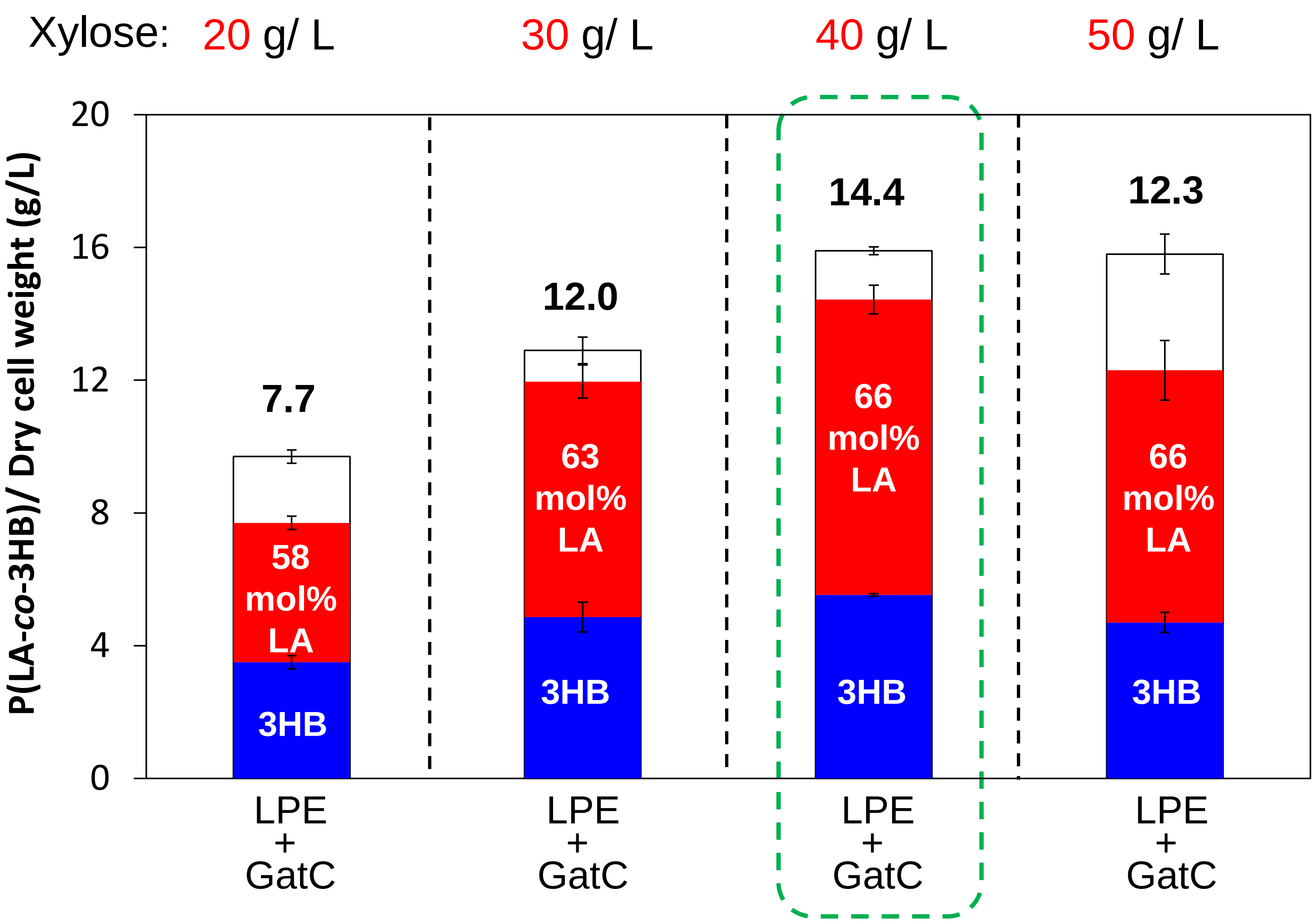
# GatC enhanced production of P(LA-*co*-3HB)




GatC overexpression was effective in enhancing P(LA-*co*-3HB) productivity

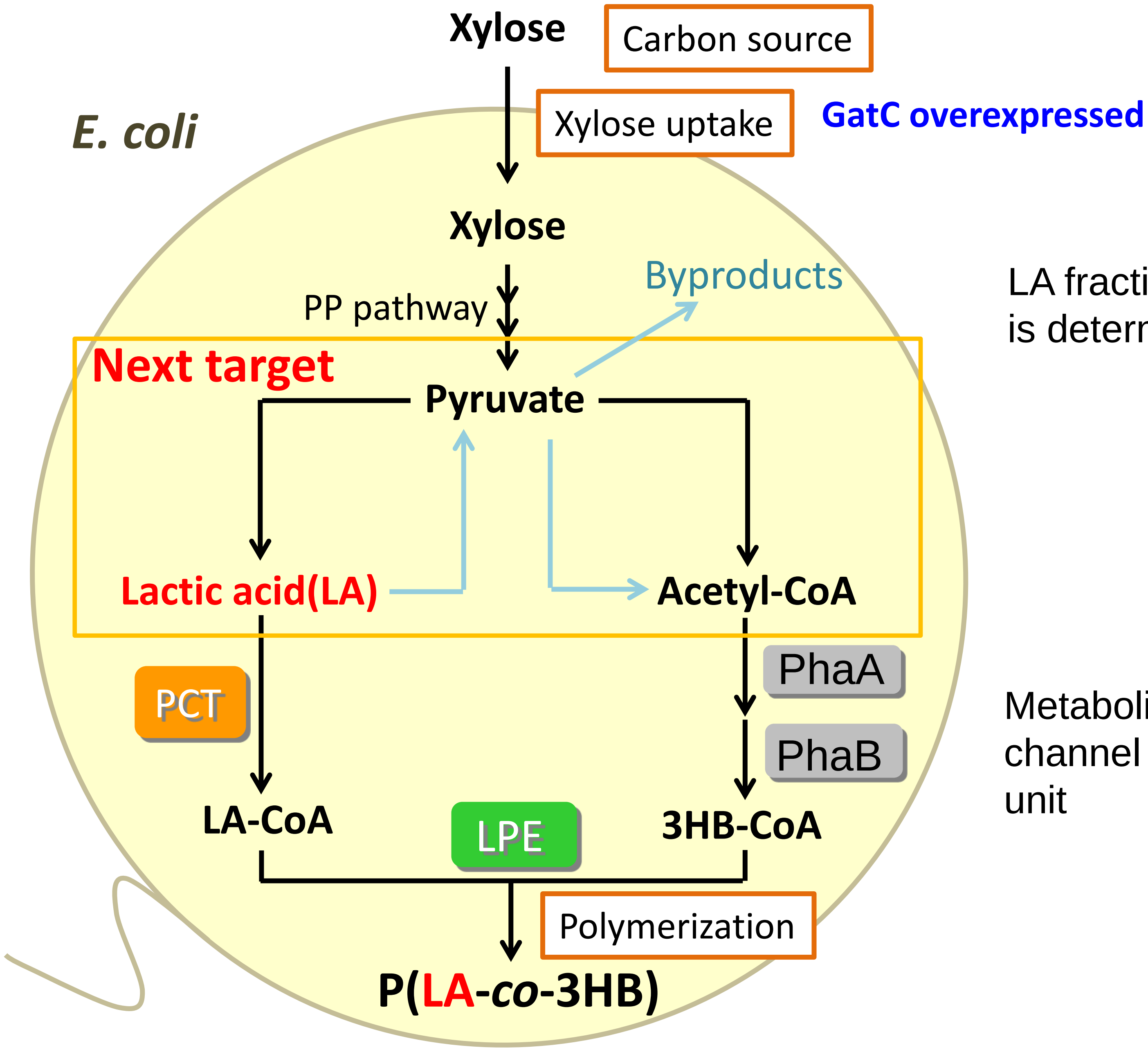
Nduko et al., 2014

# Xylose concentration-dependent increase of polymer production




Nduko et al., 2014

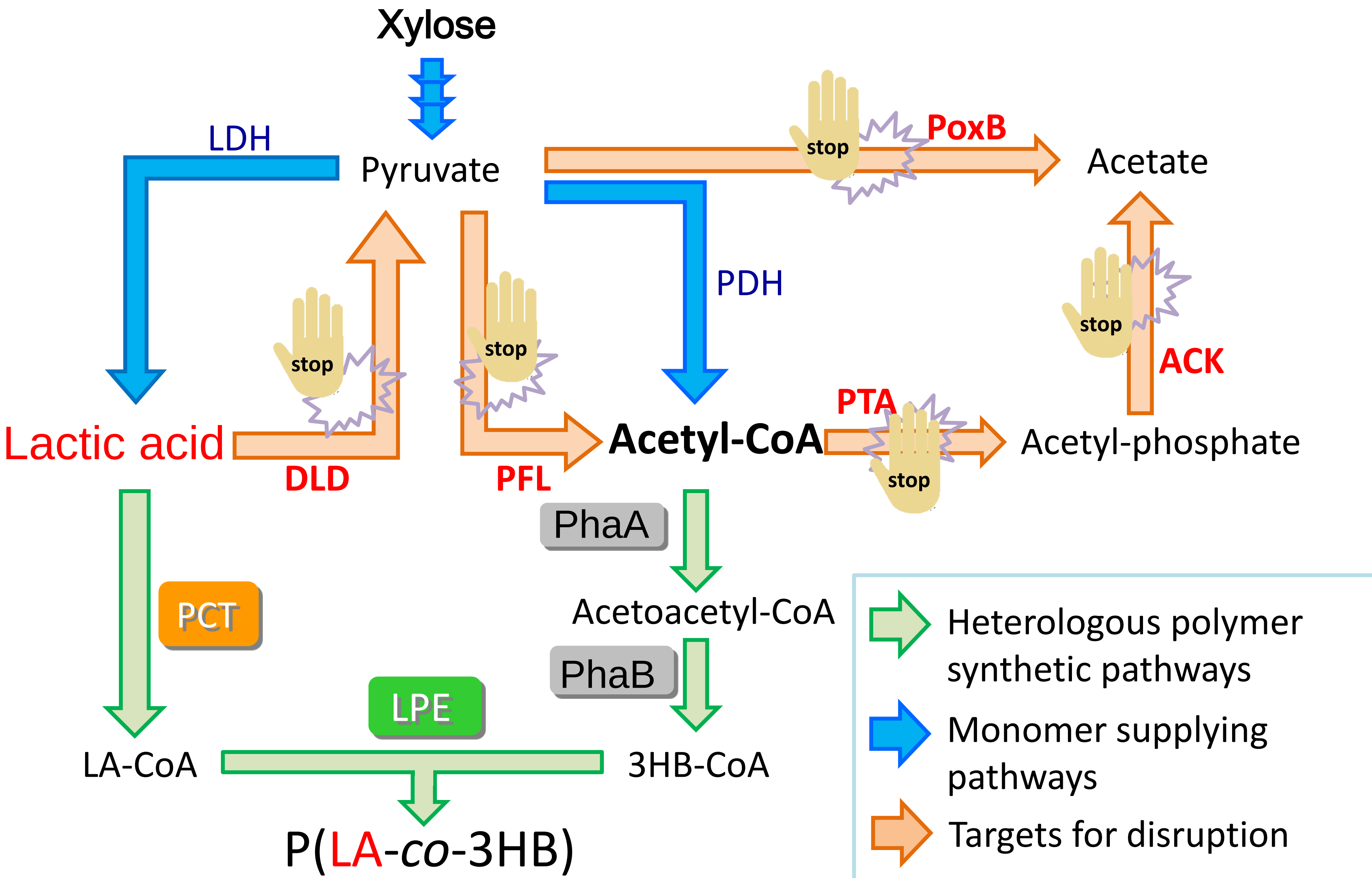
# How to further increase the LA fraction?




LA fraction in P(LA-*co*-3HB) is determined by carbon fluxes

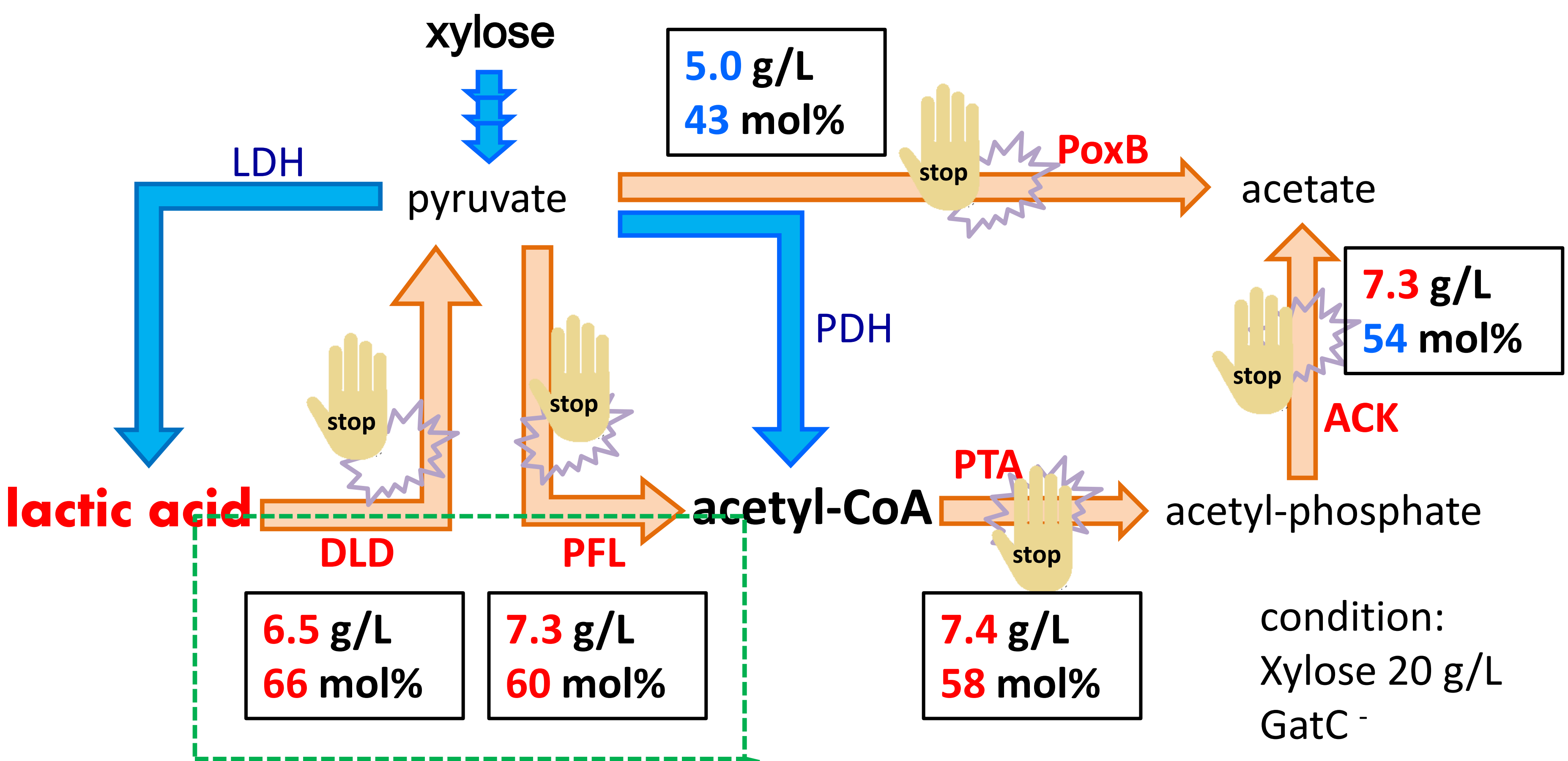
Metabolic engineering to channel carbon flux to LA unit

# Metabolic engineering for enhancing LA fraction




Zhou et al. , 2012

# Up or down?




**Parent strain**

| | |
|---|---|
| production | 6.3 g/L |
| LA fraction | 56 mol% |

**Red number: Increase**

**Blue number: Decrease**

**DLD+PFL knockout**

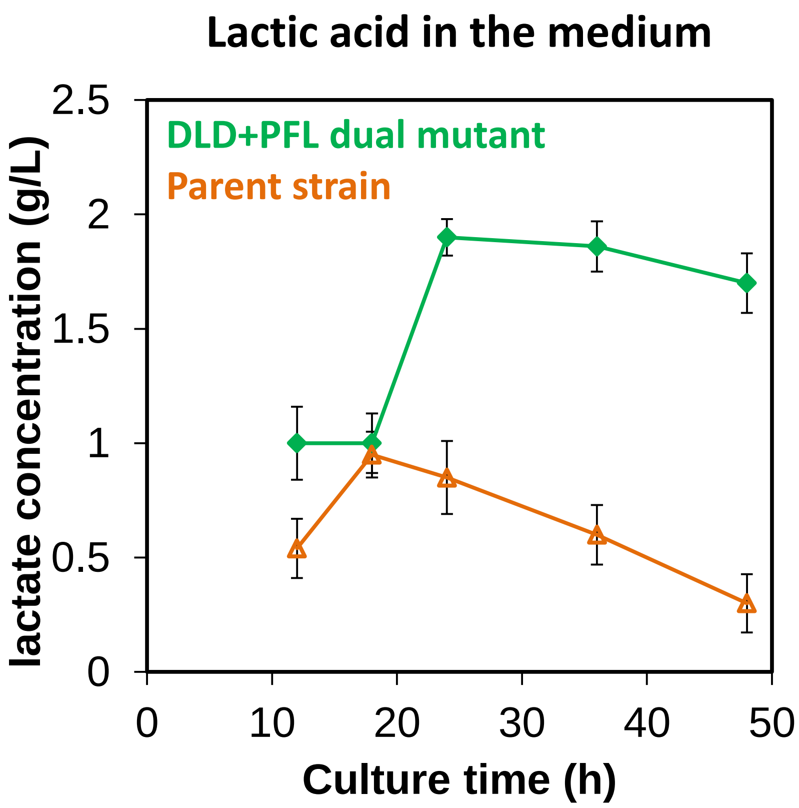
4.5 g/L

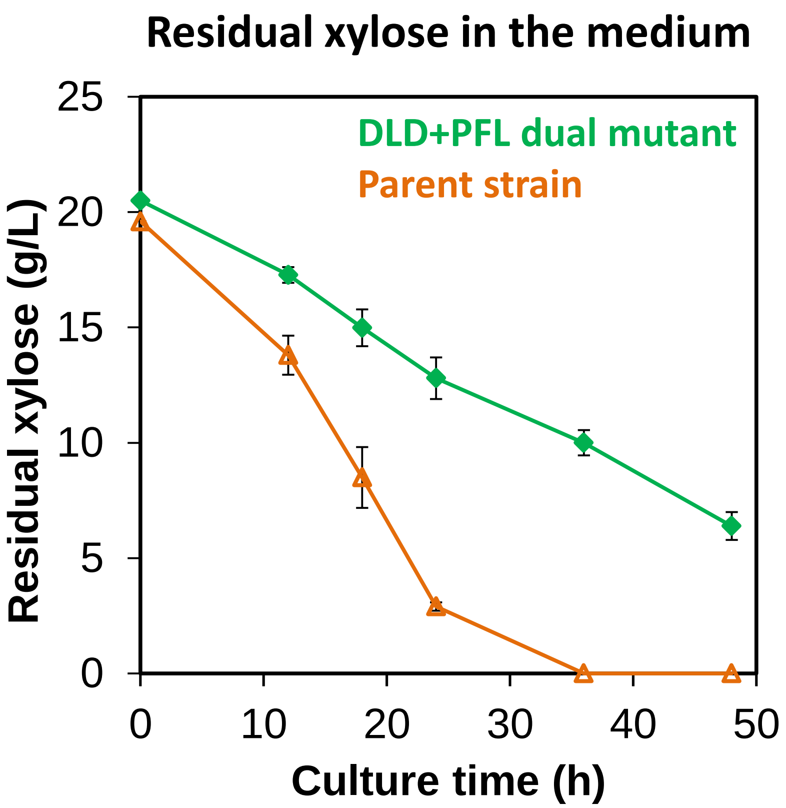
71 mol%

Nduko et al., 2014

# Time course analysis of the dual mutant

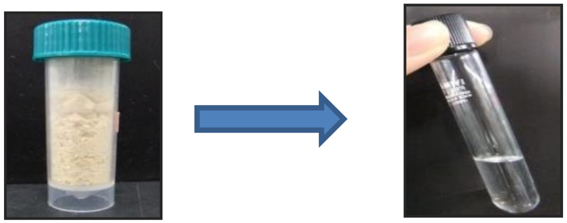
## Supernatant analysis for parent and mutated strains



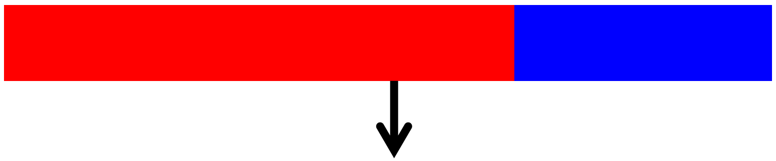

Higher lactic acid production affects xylose uptake

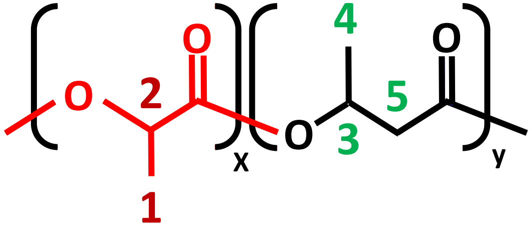
# $^{1}$H NMR analysis of P(51 mol% LA-*co*-3HB)



Cells accumulating P(LA-*co*-3HB)

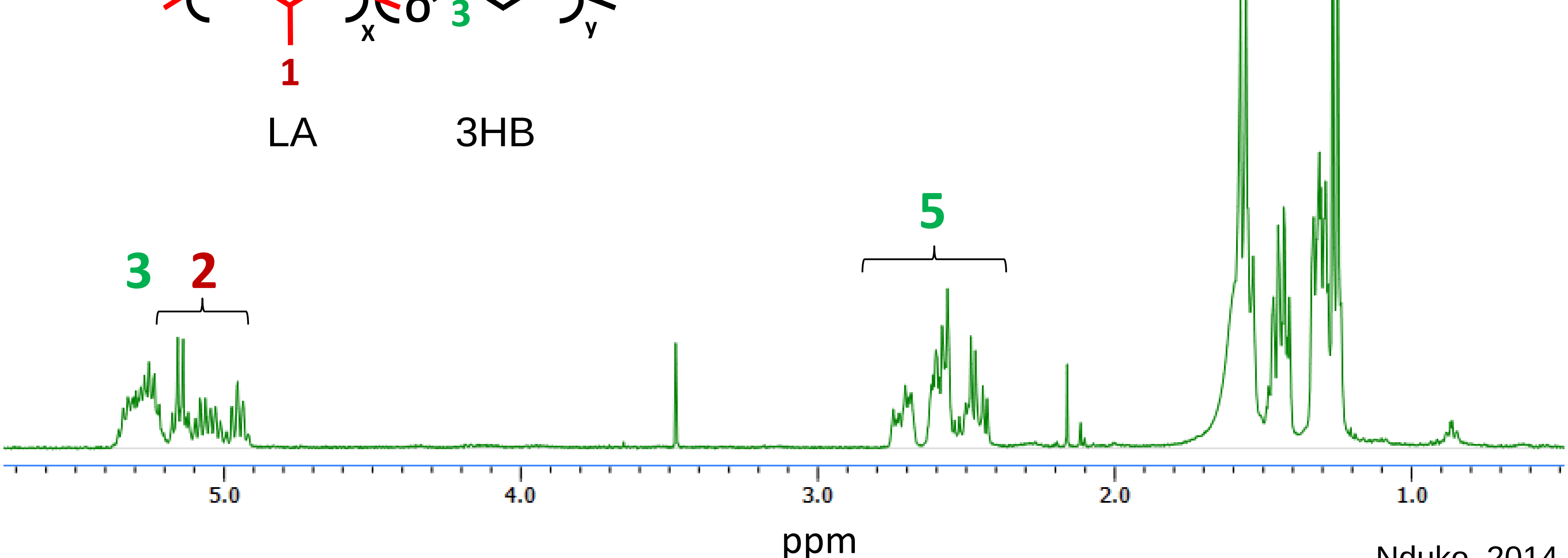
Polymer extracted




LA

3HB

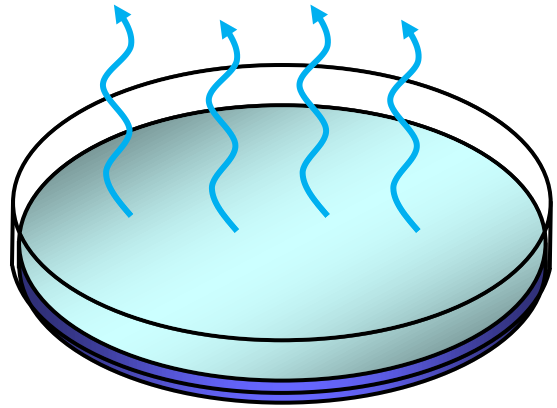



Nduko, 2014

# Mechanical property analysis: method

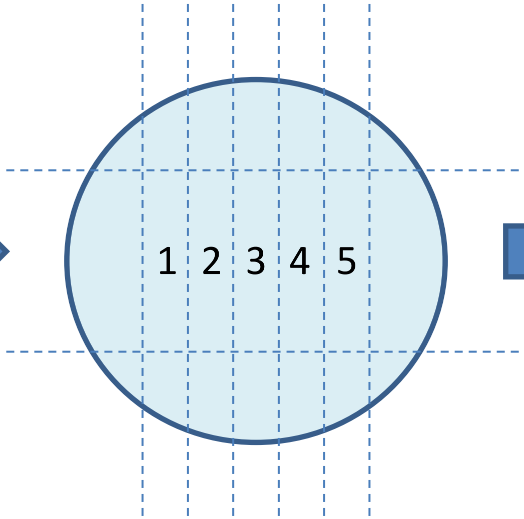
Conventional

Polymer extracted

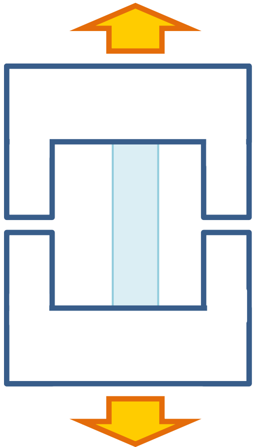


Evaporation of solvent


Polymer film formed

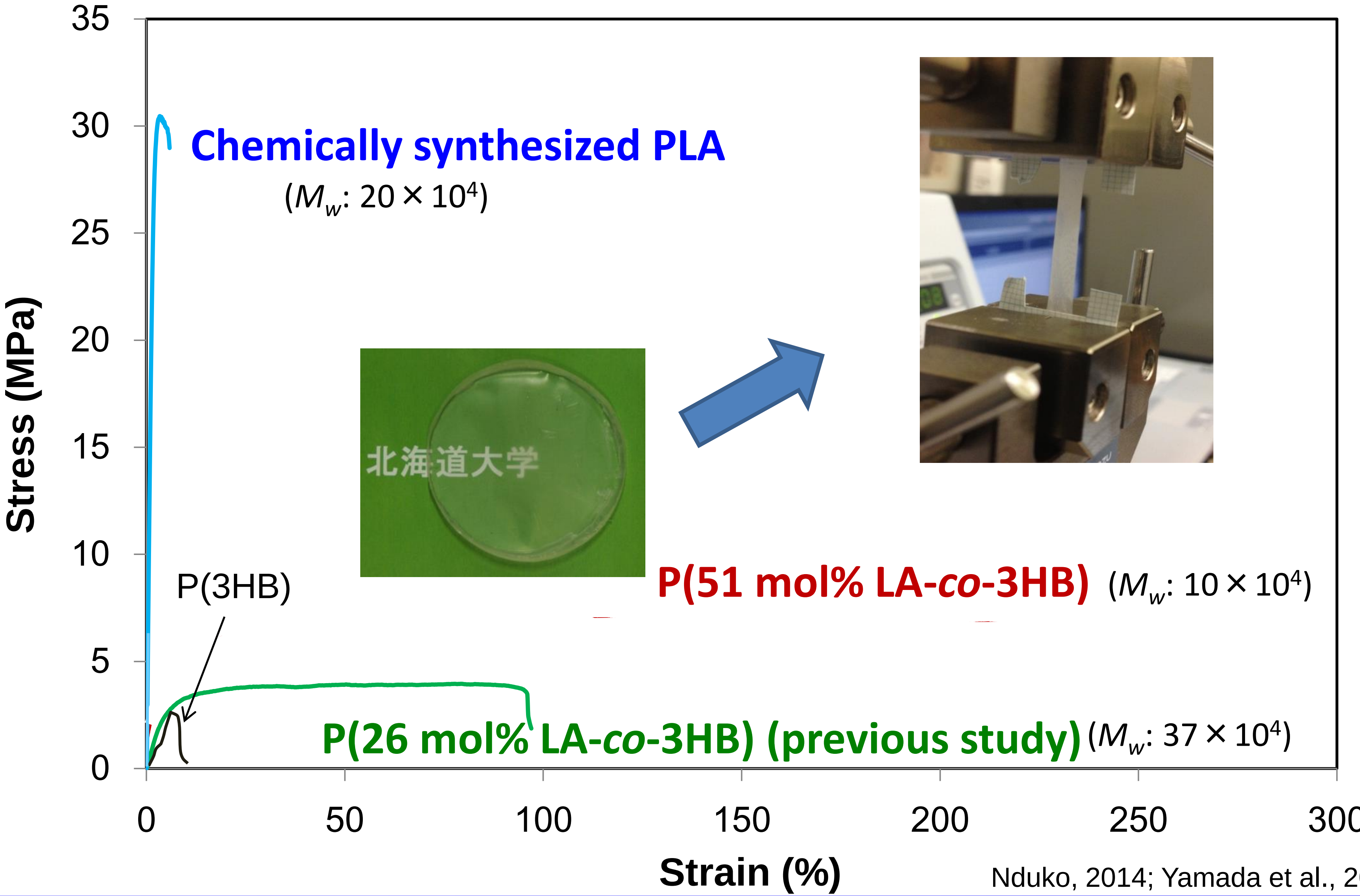



Cut



Property analysis

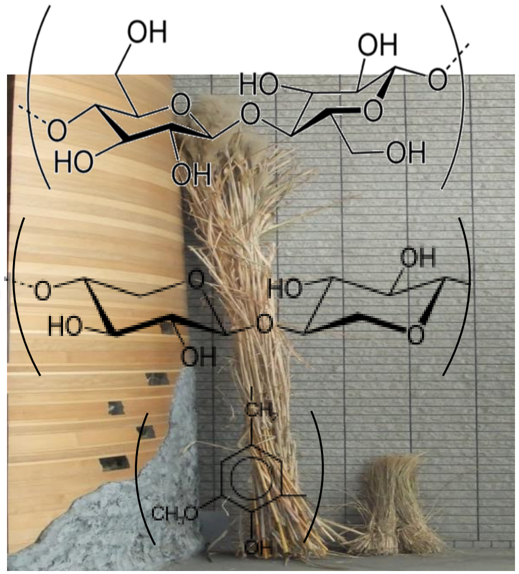
# P(51 mol% LA-*co*-3HB) exhibited stretchy property




Nduko, 2014; Yamada et al., 2011

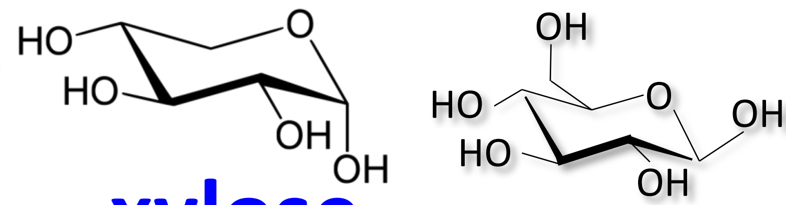
# Conclusion

$CO_2$



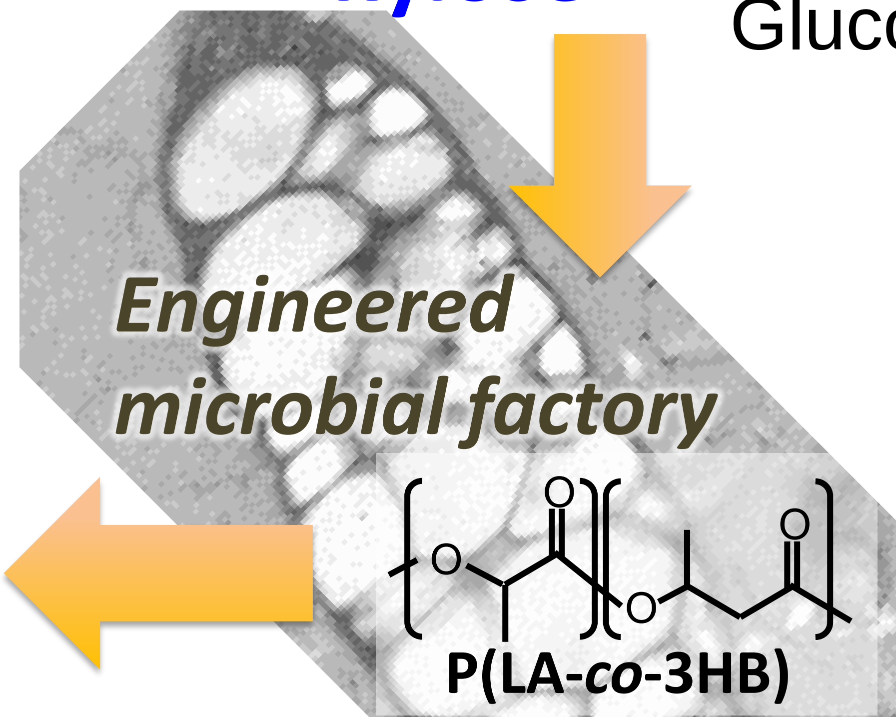

Lignocellulosic biomass

*First utilization of xylose for LA polymer production*



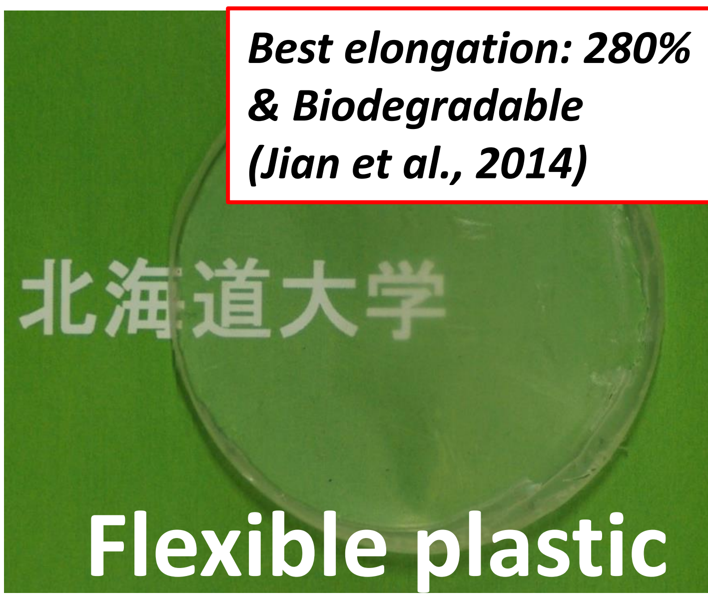

xylose

Glucose




*The champion productivity: 14 g/L*
*LA fraction of 71 mol% attained*

*Best elongation: 280%*
*& Biodegradable*
*(Jian et al., 2014)*



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for financial support

Egerton University

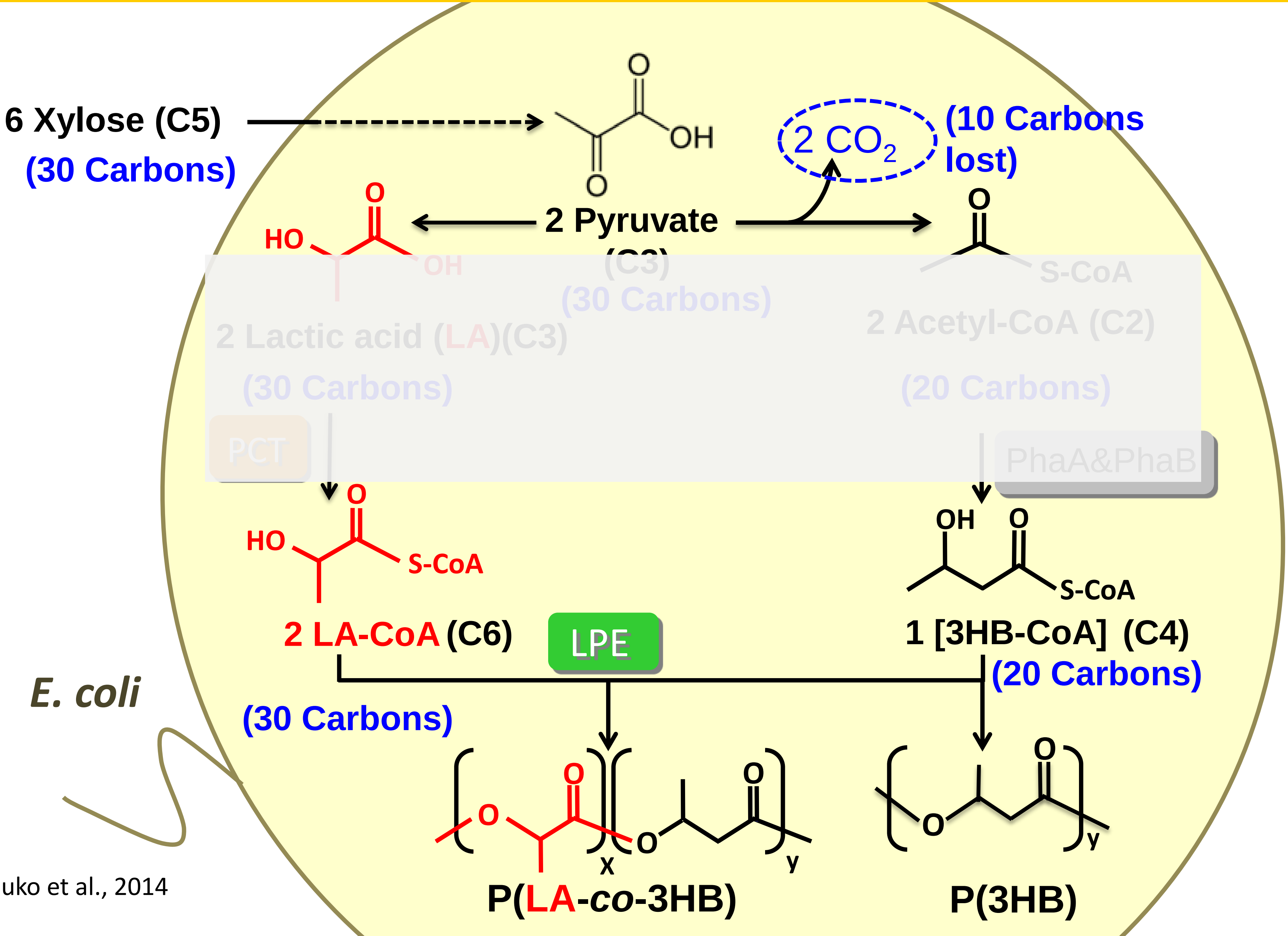
For financial support

# Prof. Taguchi Lab. Molecular Bioengineering, Hokkaido Univ., Japan

Thank you for your kind attention!
ご清聴ありがとうございました

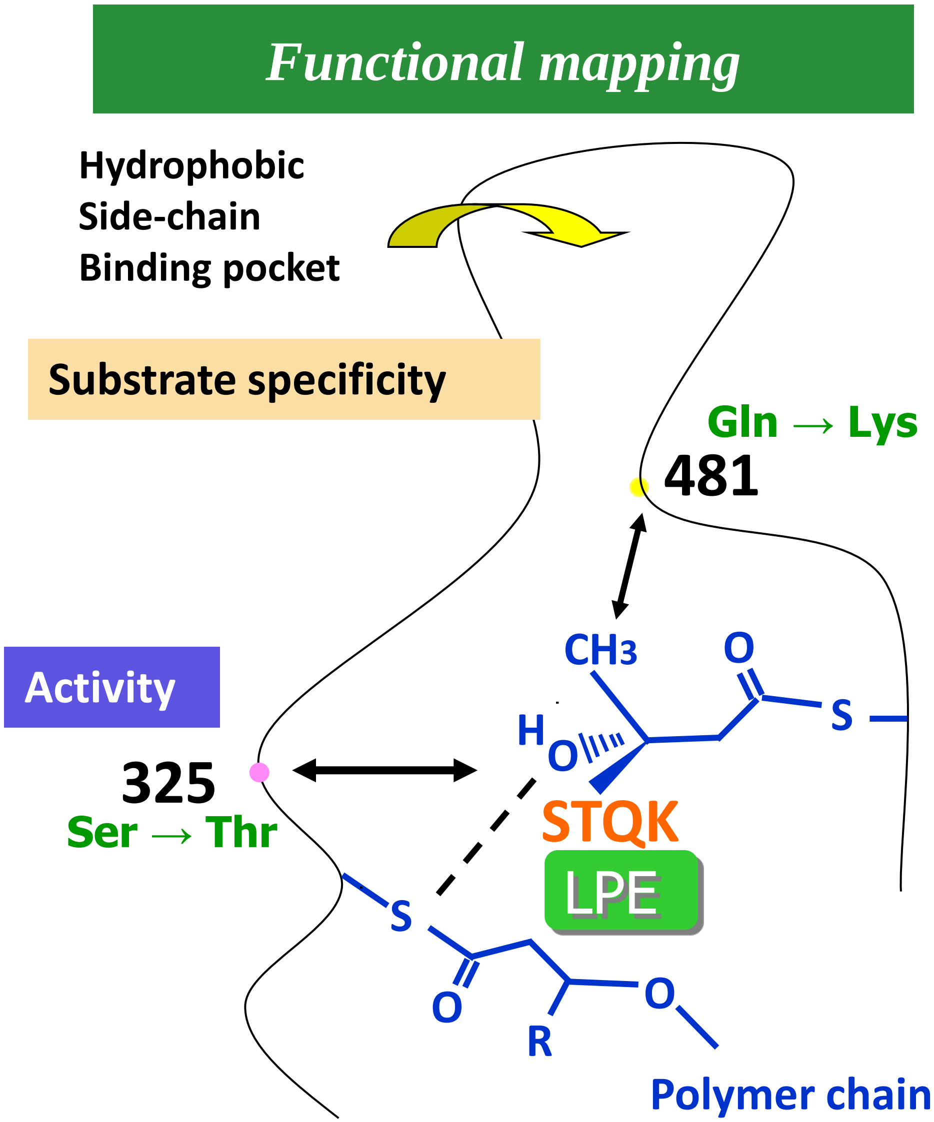
| No. | Monomer composition (mol%) | | Molecular weights | Thermal properties | |
|---|---|---|---|---|---|
| | LA | 3HB | $M_w$ ($\times 10^3$) | $T_g$ (ºC) | $T_m$ (ºC) |
| 1[a] | 0 | 100 | 700 | -7 | 176 |
| 2 | 43 | 57 | 102 | 18 | 107 |
| 3 | 541 | 46 | 114 | 25 | n.d |
| 4 | 56 | 44 | 89 | 22 | n.d |
| 5 | 58 | 42 | 50 | 26 | n.d |
| 6 | 60 | 40 | 34 | 22 | n.d |
| 7 | 66 | 34 | 36 | 29 | n.d |
| 8 | 71 | 27 | 38 | 29 | n.d |
| 9[a] | 100 | 0 | 200 | 60 | 153 |

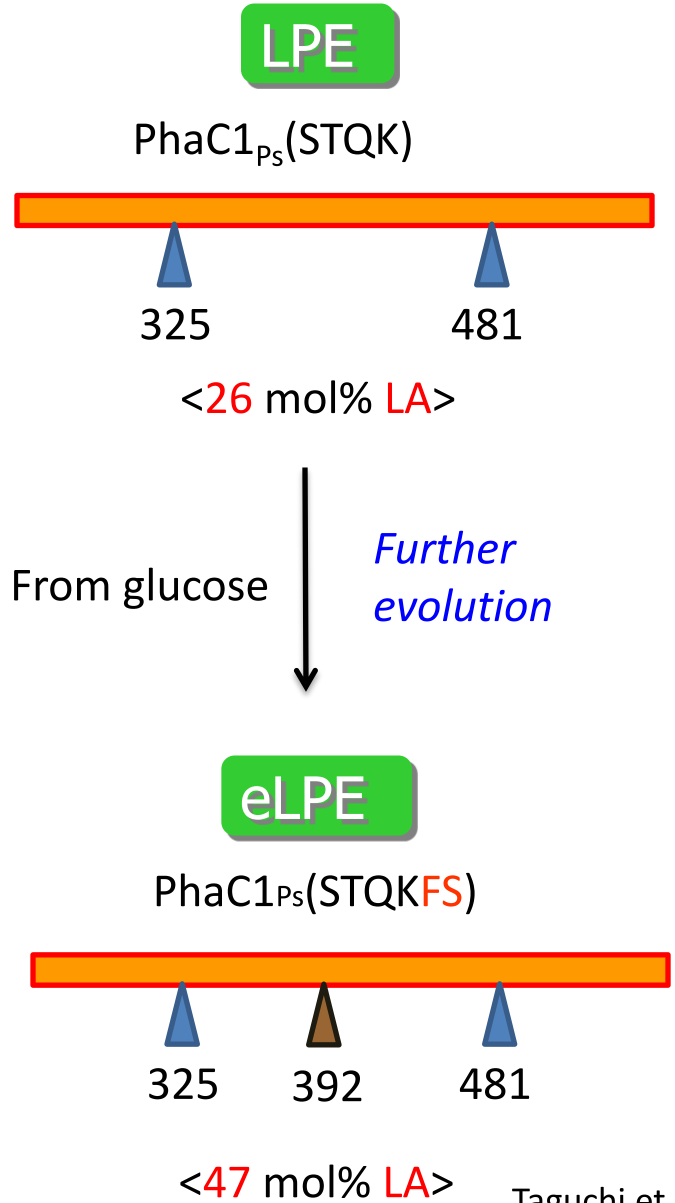
# In fact, theoretically reasonable!




Nduko et al., 2014

# Further evolution of LPE

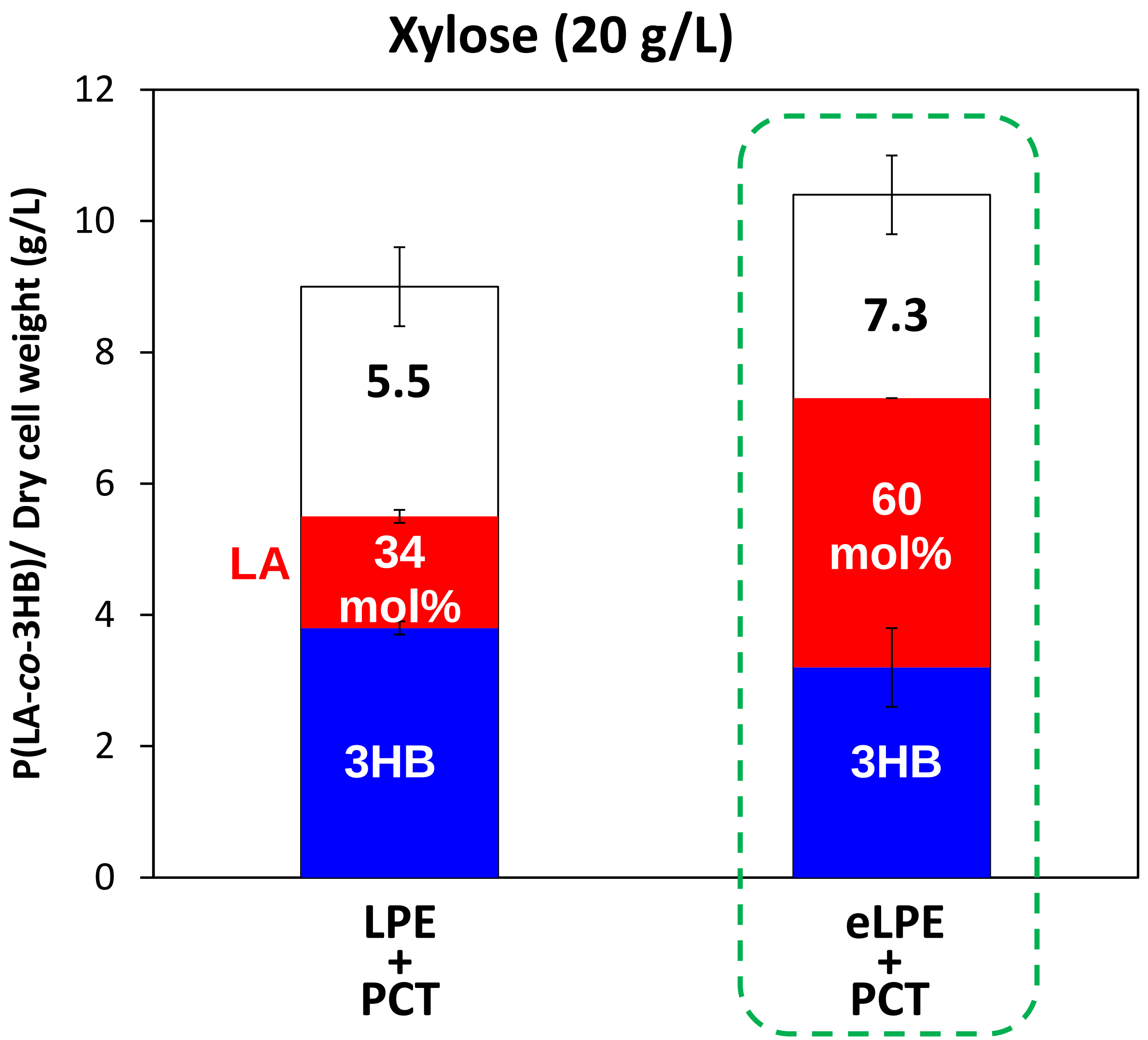



Taguchi et al., 2002
Yamada et al., 2010


*(1) K. Takase et al.: Biomacromolecules, 2004*
*(2) S. Taguchi and Y. Doi: Macromol. Biosci., (Review) 2004*

# Evolved LPE improves LA fraction



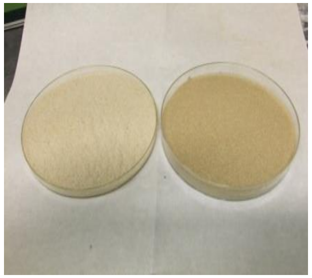

Evolved LPE (eLPE) was effective for improving LA fractions in the polymer

Nduko et al., 2013

# Enzymatic hydrolysis

*Miscanthus giganteus*
*ジャンボススキ*

*Milling*



Small particles

*Delignification*

NaOH

$NaClO_2$

Cellulose and hemicellulose

Cellulose and hemicellulose

***Enzymatic hydrolysis***

Cellulase

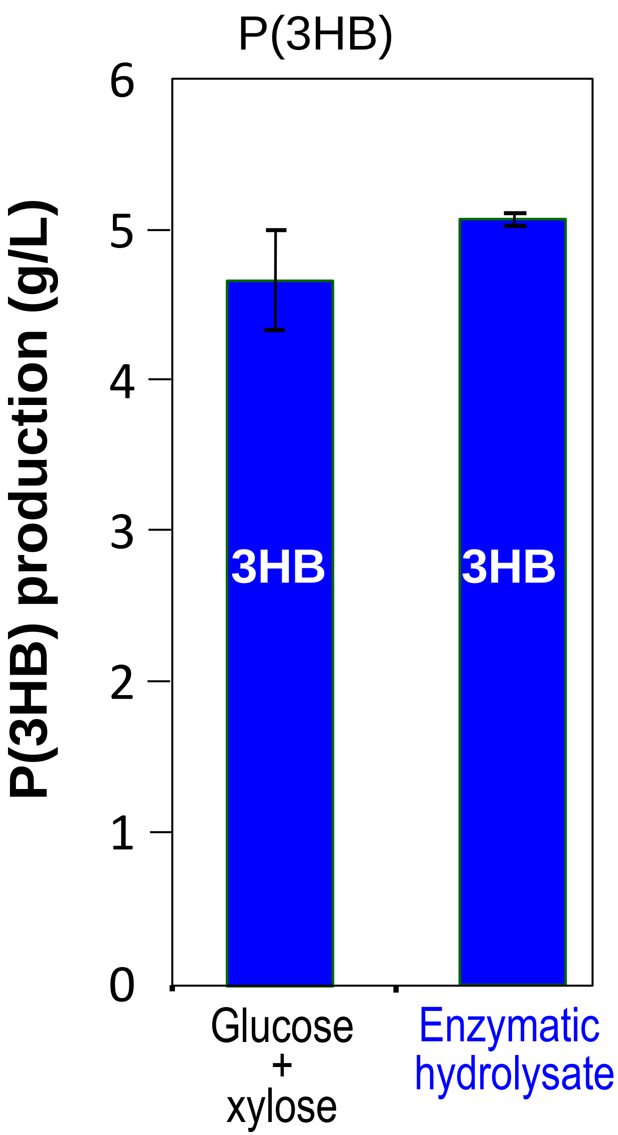
50℃ /
4 days

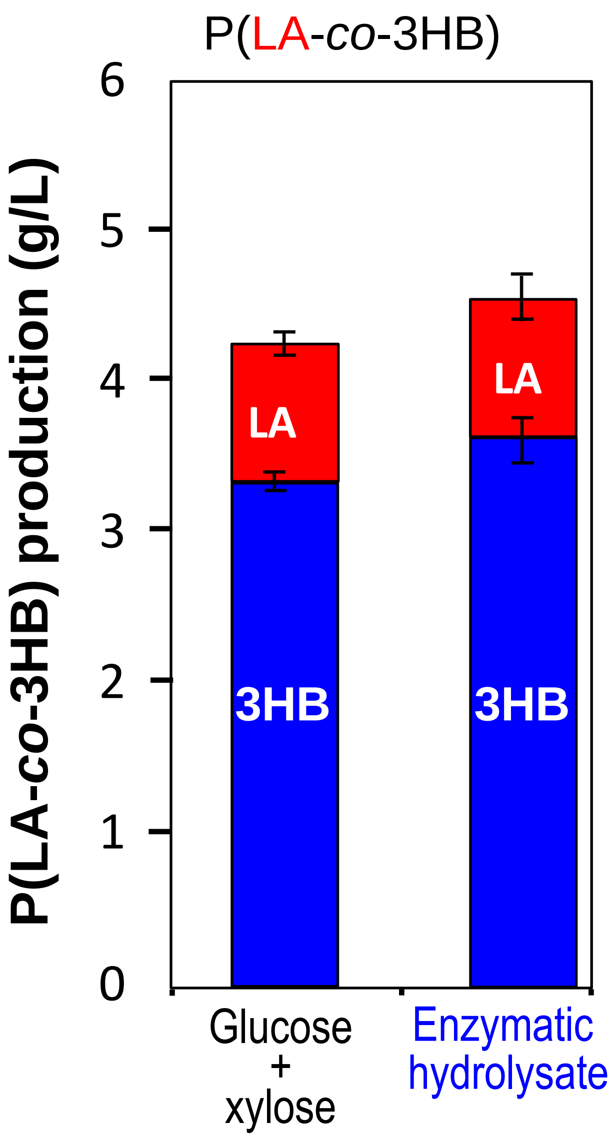
Enzymatic hydrolysate
(Glucose & xylose)

*Polymer production*

Cell harvesting & polymer analysis

# Polymer production from enzymatic hydrolysate




Enzymatic hydrolysate was effective as a carbon source for polymer production

# Xylose concentration-dependent increase of polymer production