

Xylitol production from lignocellulosic hydrolysates

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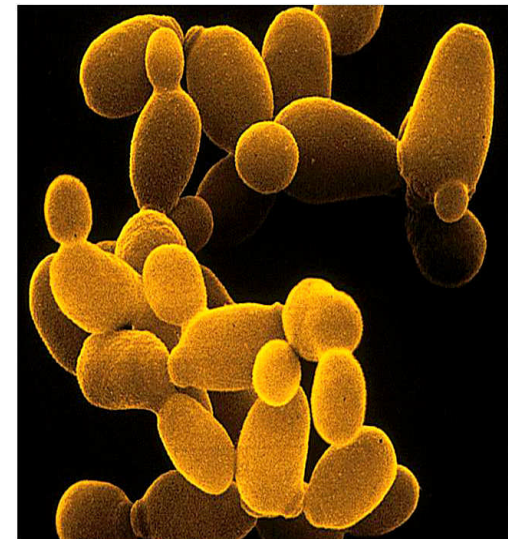
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Overview

- Current trends in biofuel production
- Needs for biorefinery/higher value products
- Why xylitol as a higher value product?
- Isolation of xylitol assimilation deficient mutant
- Its characterisation by fermentation studies
- Xylitol production from lignocellulosic hydrolysate
- Conclusion



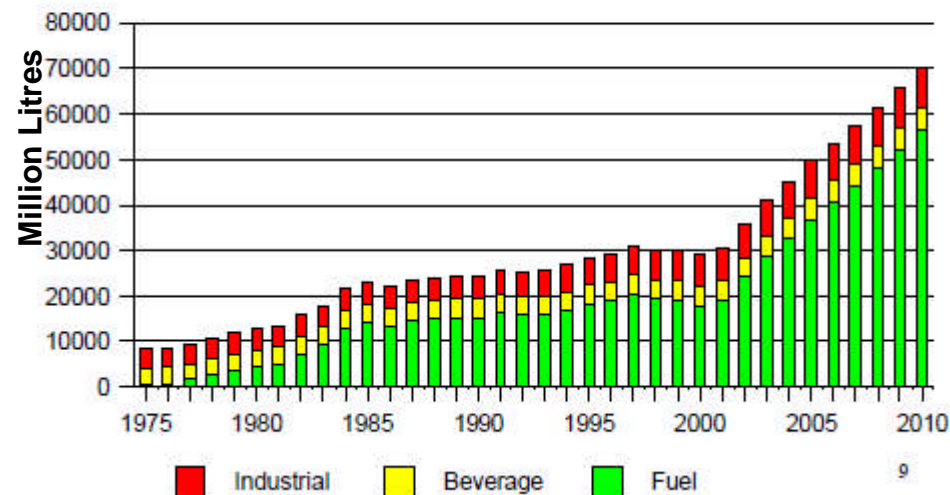
Biofuel production in global boom

Bioethanol in global boom (over decade)

- Energy security
- Environmental concerns associated with climate change



World Fuel Ethanol Ethanol production by type



Source from World Fuel Ethanol; Analysis and Outlook ,Christoph Berg and F. O. Lich(2010)

Current trends in bioethanol production

Current technology for bioethanol

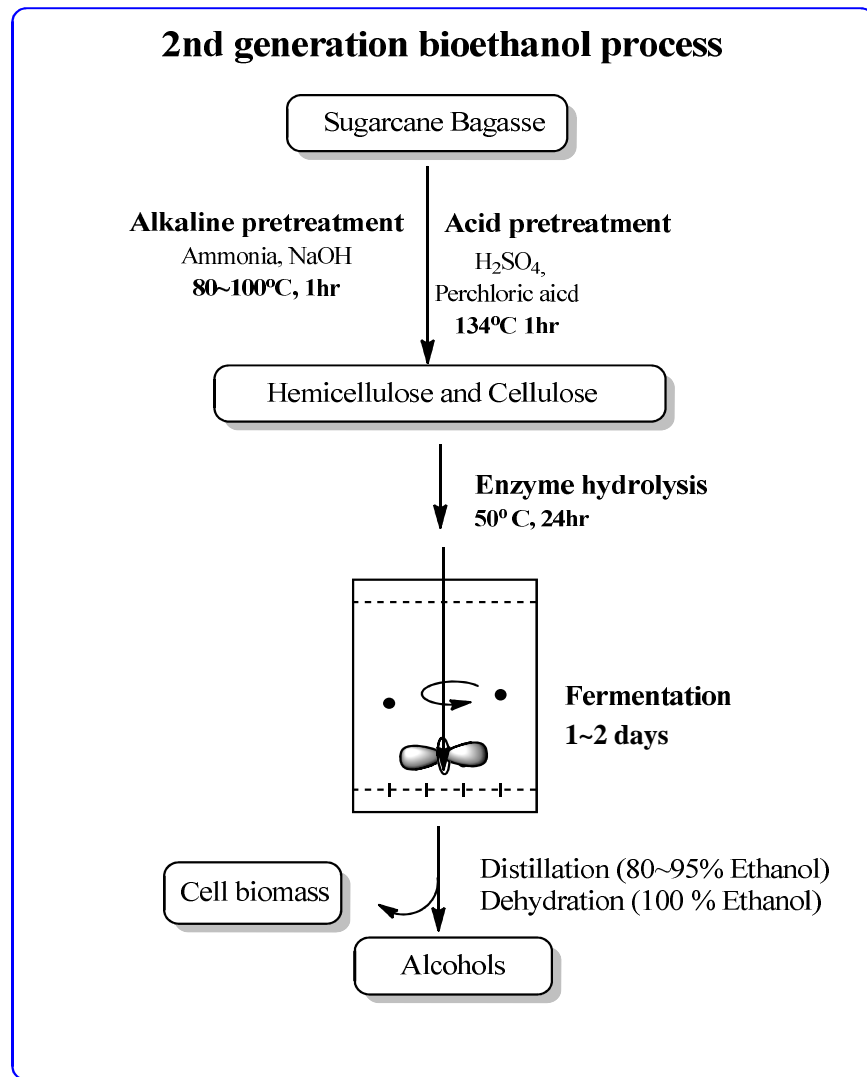
- Relies on food based substrate (sugarcane, corn, and cassava)
- Food vs energy competition

Sustainable technology for bioethanol production

- Using renewable lignocellulosic biomass

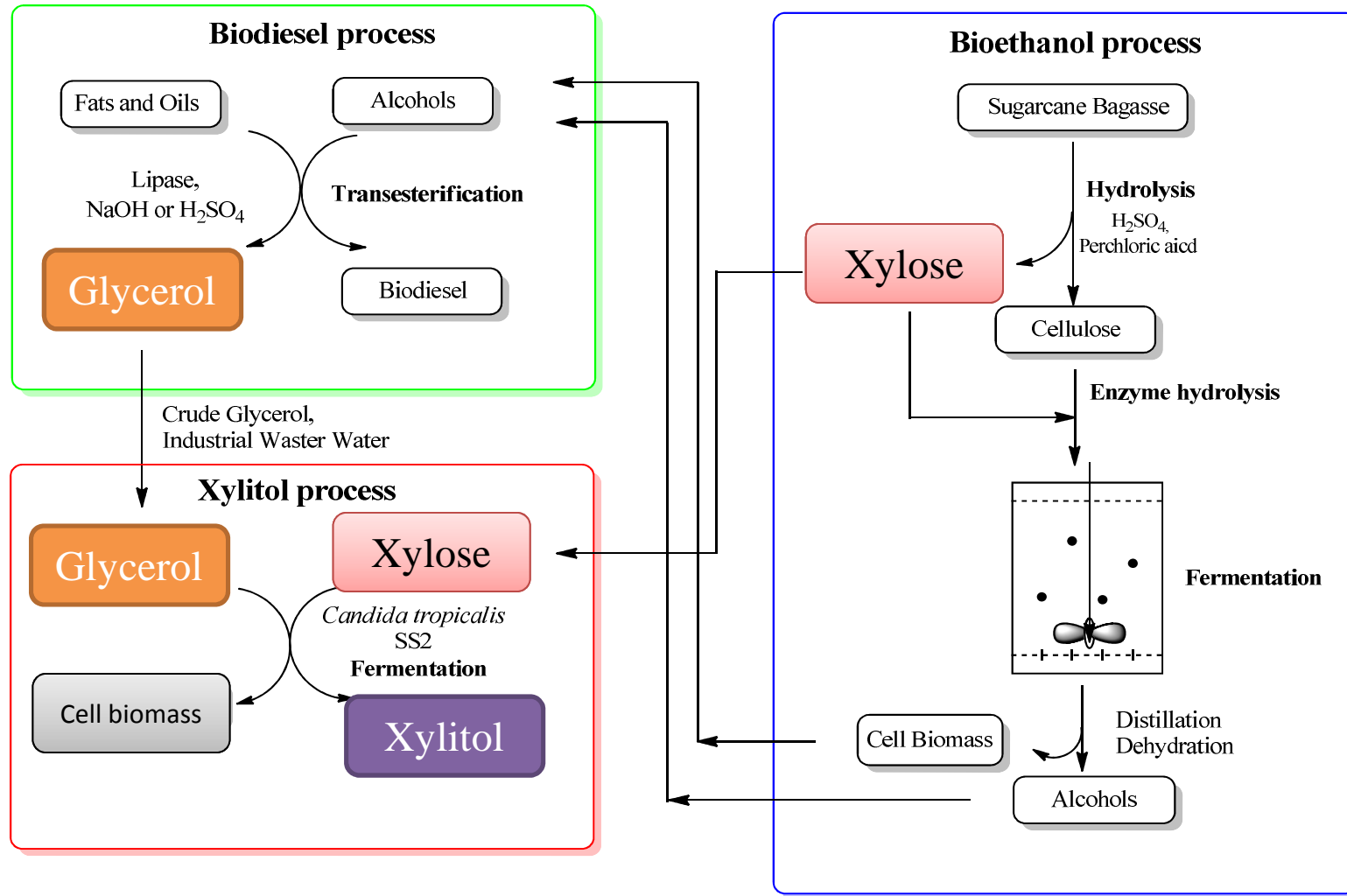


Biochemical process for 2nd generation bioethanol

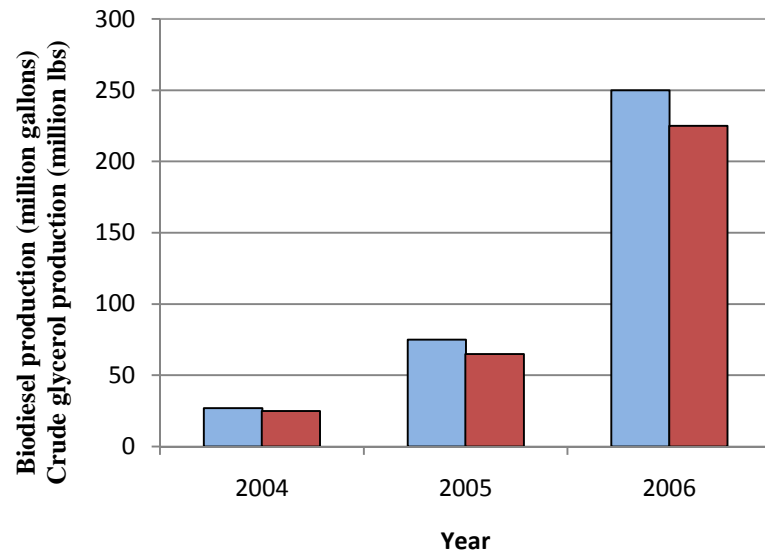


- Biomass recalcitrance
- High energy input
- Enzyme production cost
- Higher production cost compared to gasoline or diesel

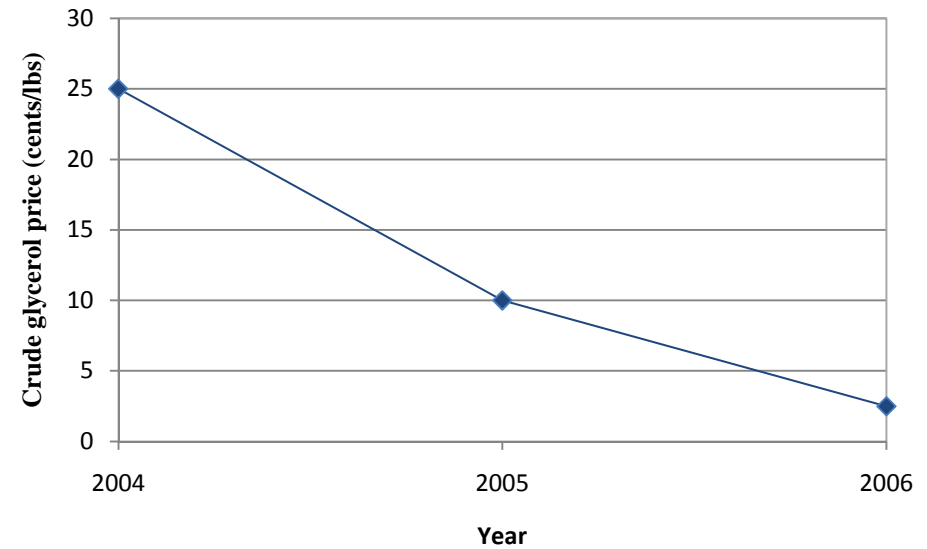
Biorefinery concept



Biodiesel production and glycerol price



■ Biodiesel production (million gallons)
■ crdue glycerol (80%) production (million lbs)



◆ crdue glycerol (80%) price (cents/lbs)

Source adapted from Yazdani and Gonzalez (2007) *Current Opinion in Biotechnology*

Biodiesel and glycerol

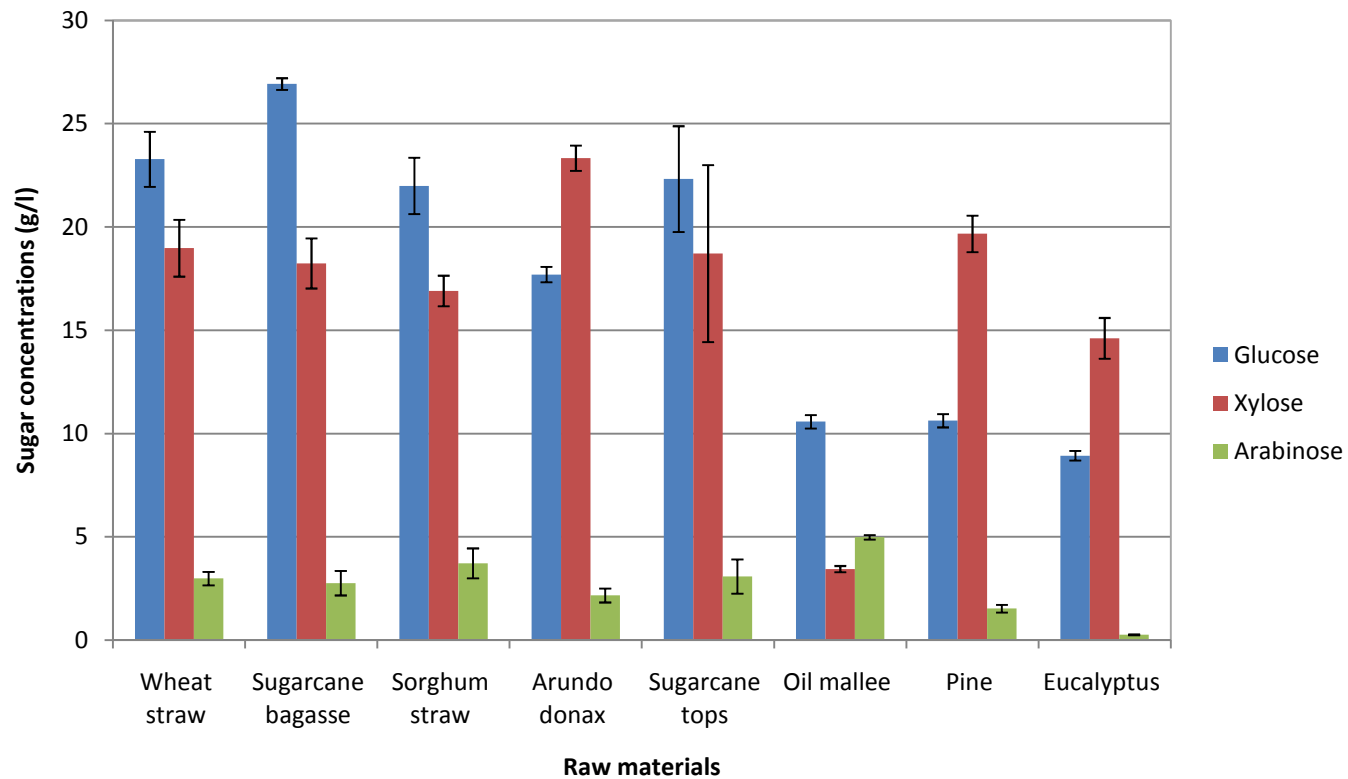


→ Biodiesel Phase

→ Glycerol Phase

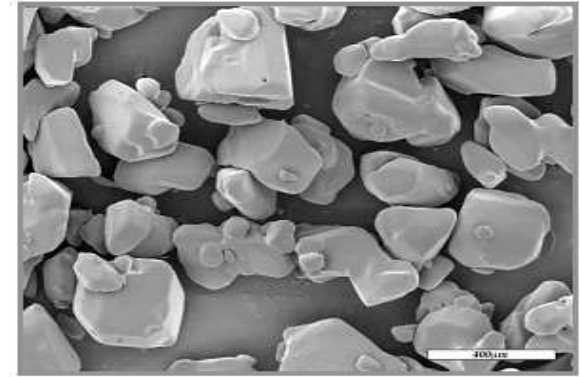


Suitability test of raw materials for 2nd generation bioethanol process



Jeon et al. (2010, Letters in Applied Microbiology, **51**: 518–524)

Why xylitol ?

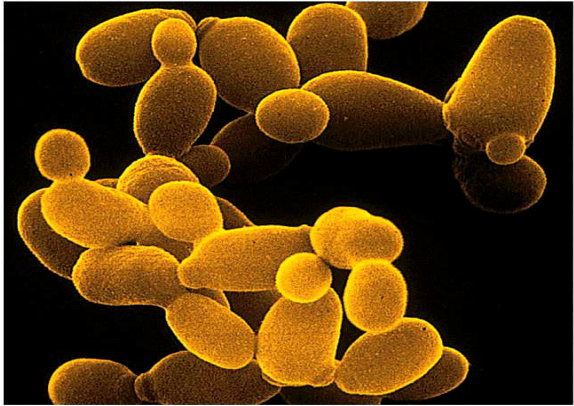
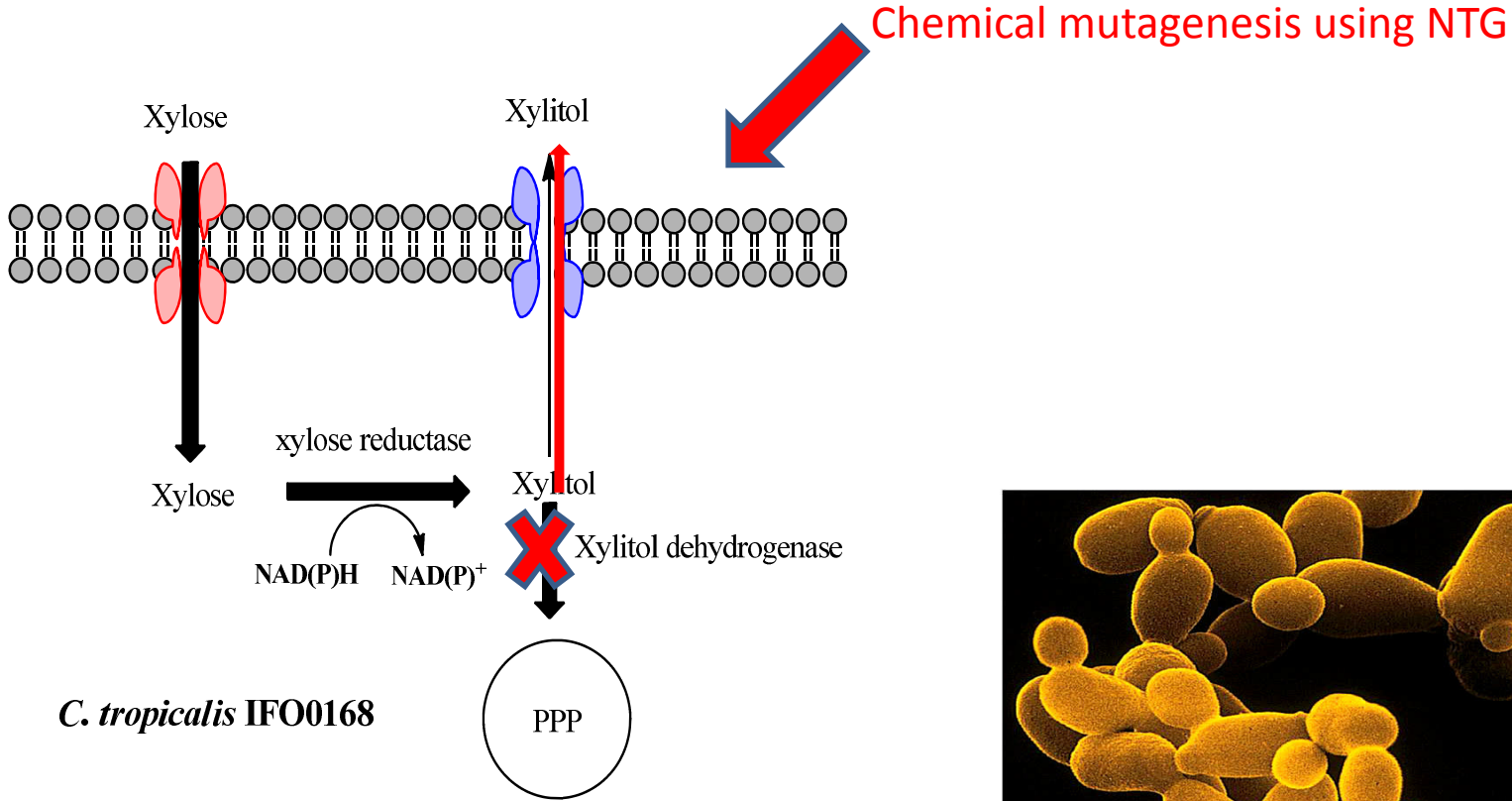


- Natural sweetener
- Anti-cariogenic properties
- Benefits used in chewing gums and toothpaste
- No side effects compared to artificial sweeteners viz, Saccharin, Aspartame etc.
- Non-nutritive
- Benefits as an alternative sweetener for diabetics
- Potential for higher value product association with ethanol

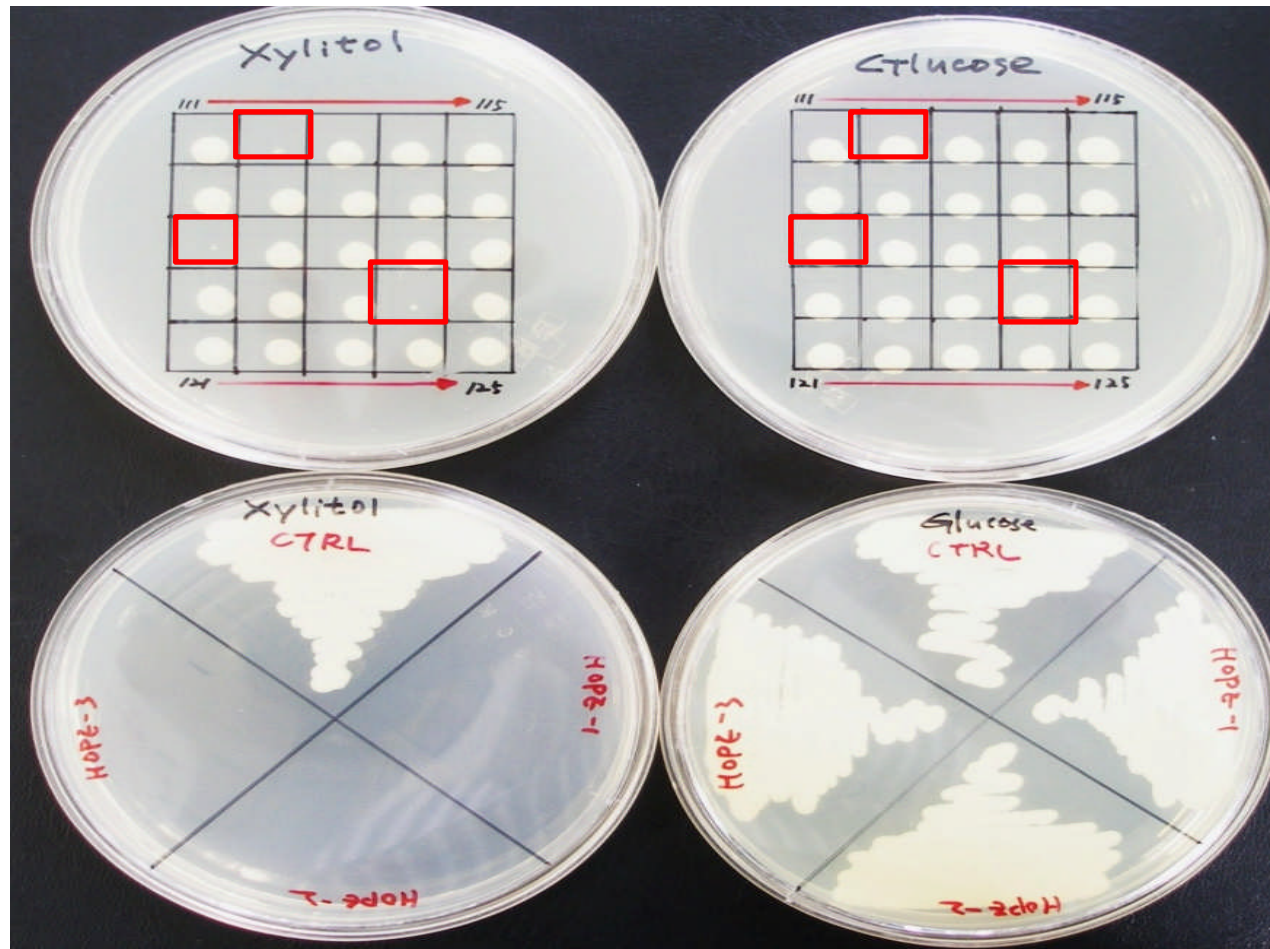
Current xylitol production

- Chemical process, hydrogenation of xylose derived from hemicellulose with an active Ni catalyst
- Major expensive cost associated with xylose purification
- Temperature (130~150°C) and pressure (2~20 bar)
- Current market capacity
20,000 ~40, 000 tonne per annum (Werpy *et al.*, 2004 and Granström *et al.*, 2007)
- Alternative process under being developed
-Using mutant yeast strains and/or controlling environmental conditions such as dissolved oxygen
- Characteristics of current biological process
-Xylitol yield: 70 ~85 %; Controlled oxygen; Low productivity

Chemical mutagenesis to enhance xylitol production by xylitol assimilation deficiency

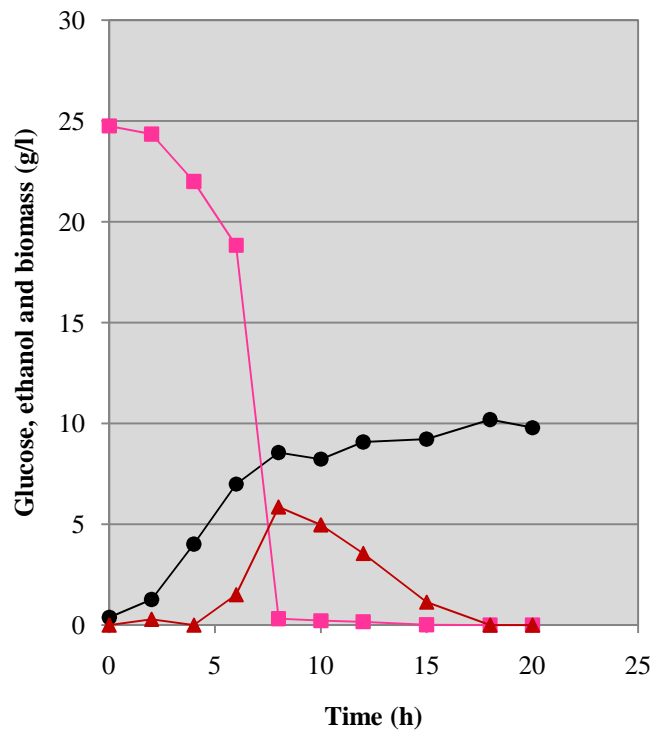


Screening of xylitol assimilation deficient mutant of *Candida tropicalis* SS2

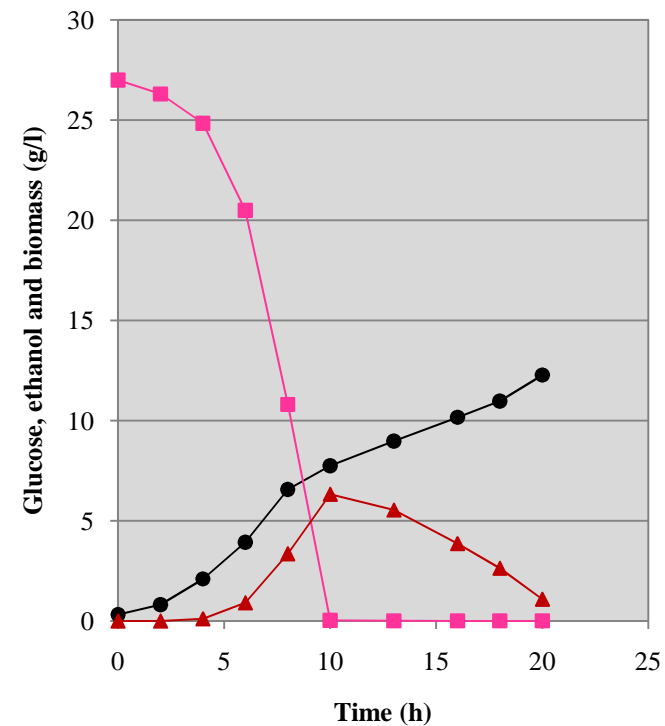


Growth on glucose

C. tropicalis IFO0618



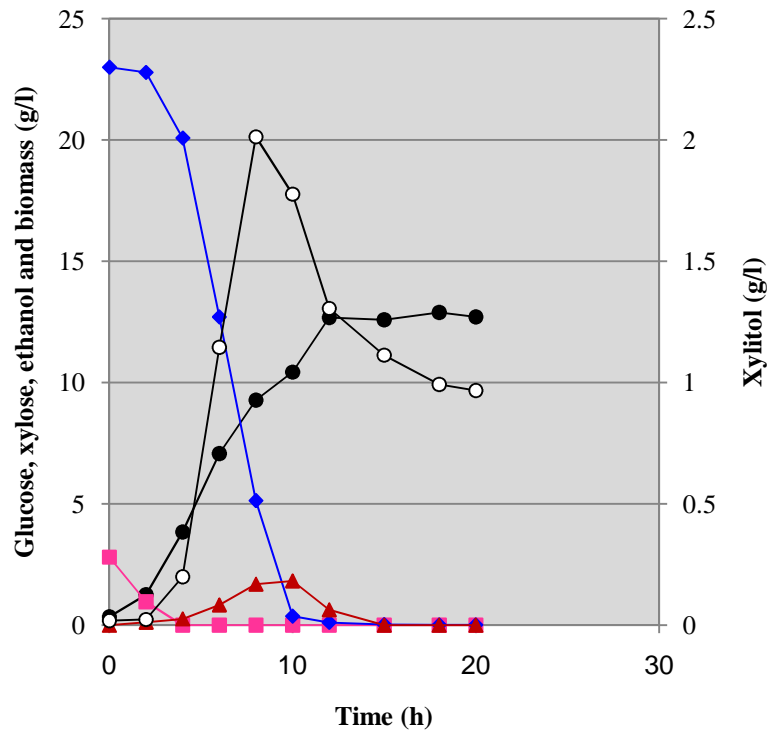
C. tropicalis SS2



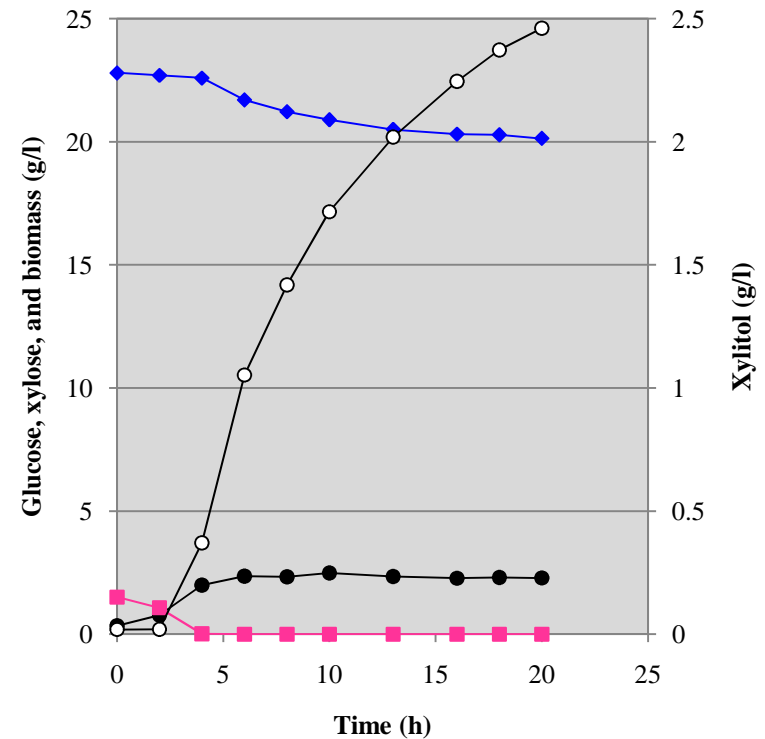
Fermentation profiles on the semi-synthetic medium containing 25 g/l glucose. Symbols: ■, glucose; ▲, ethanol; ●, biomass

Growth on xylose

C. tropicalis IFO0618



C. tropicalis SS2



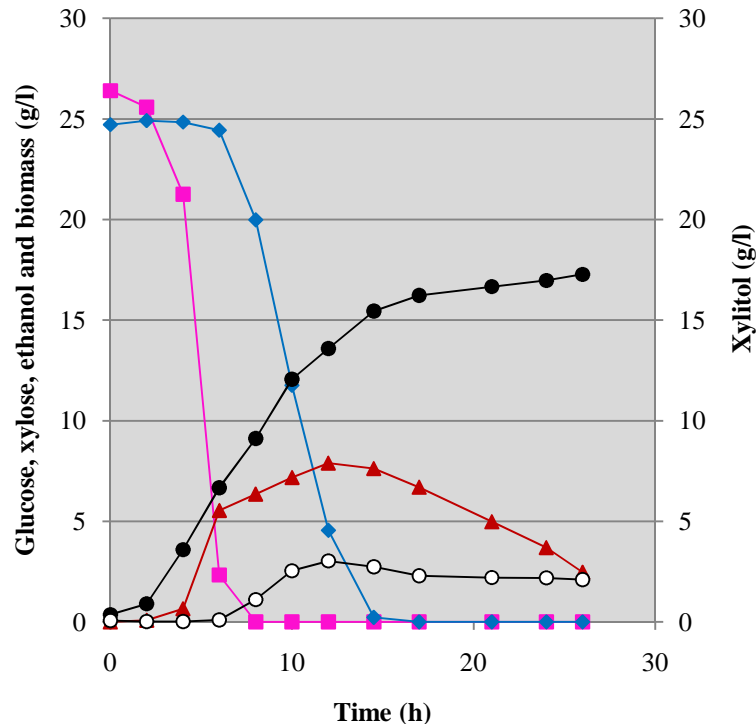
Fermentation profiles on the semi-synthetic medium containing 25 g/l xylose.

Symbols: ■, glucose; ◆, xylose; ▲, ethanol; ○, xylitol; ●, biomass

Growth on Glucose/Xylose

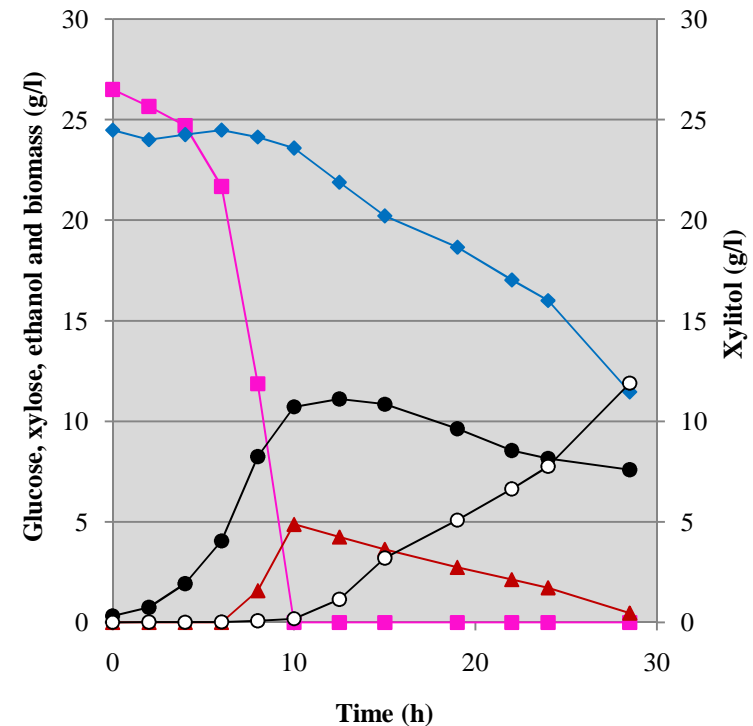
$$Y_{p/s} = 0.12 \text{ g g}^{-1}$$

C. tropicalis IFO0618



$$Y_{p/s} = 0.93 \text{ g g}^{-1}$$

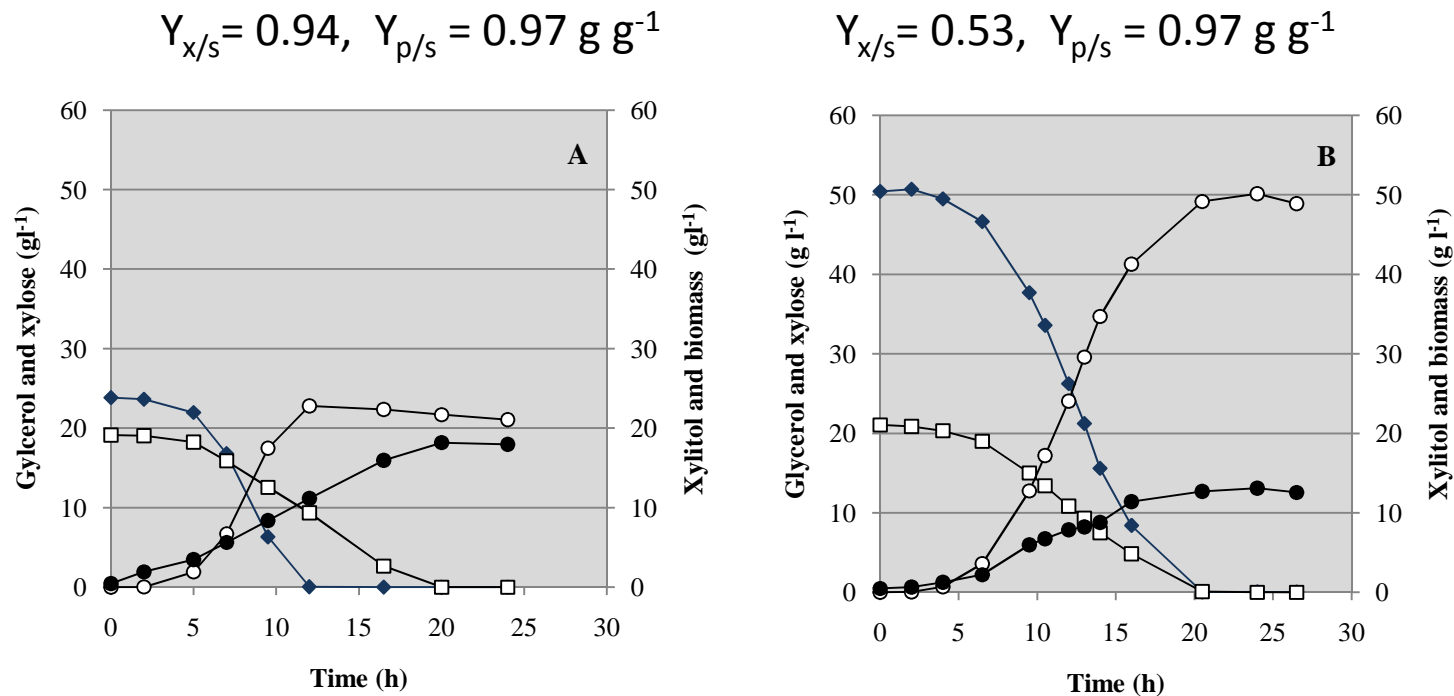
C. tropicalis SS2



Fermentation profiles on the semi-synthetic medium containing 25 g/l glucose and 25 g/l xylose.

Symbols: \blacksquare , glucose ; \blacklozenge , xylose; \blacktriangle , ethanol; \circ , xylitol; \bullet , biomass

Growth of SS2 on Glycerol and Xylose



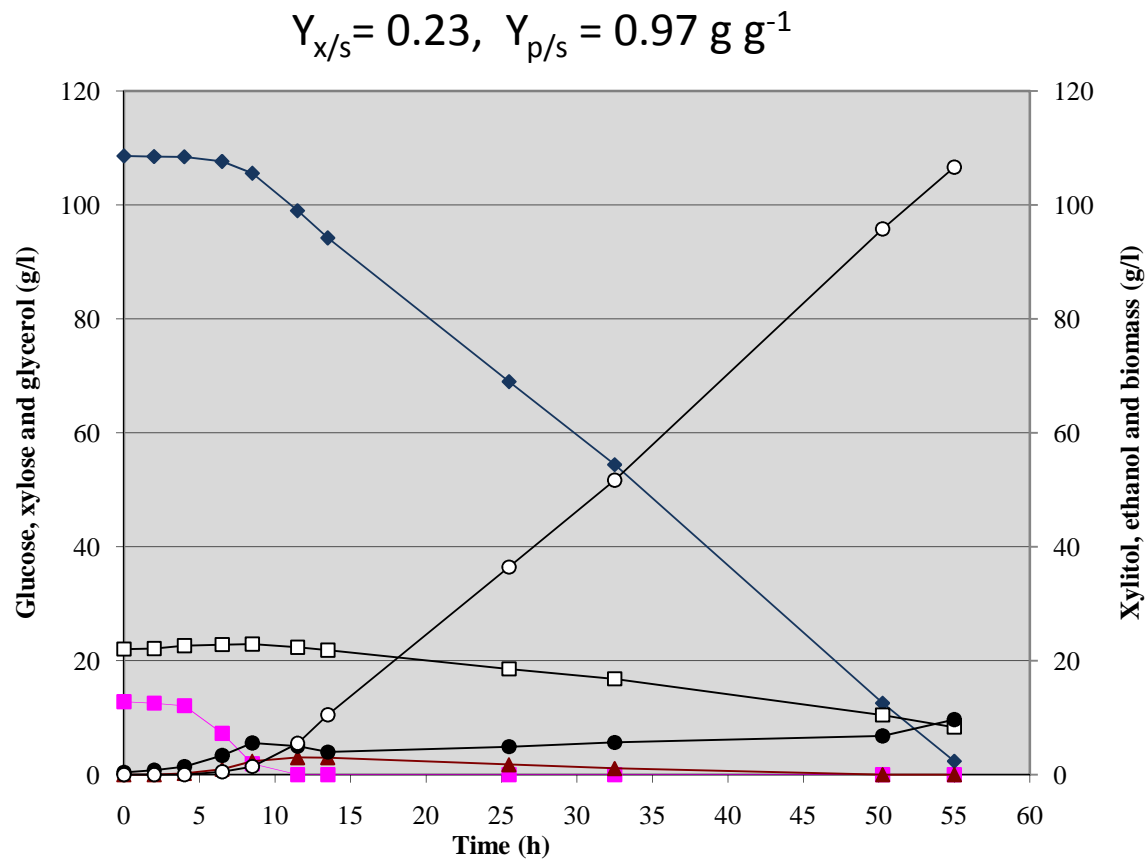
Fermentation profiles of *C. tropicalis* SS2 for xylitol production on the semi-synthetic medium containing glycerol and xylose as substrates.

A. For medium containing 20 g l^{-1} glycerol and 25 g l^{-1} xylose;

B. 20 g l^{-1} glycerol and 50 g l^{-1} xylose.

Symbols: □, glycerol; ◆, xylose; ○, xylitol; ●, biomass

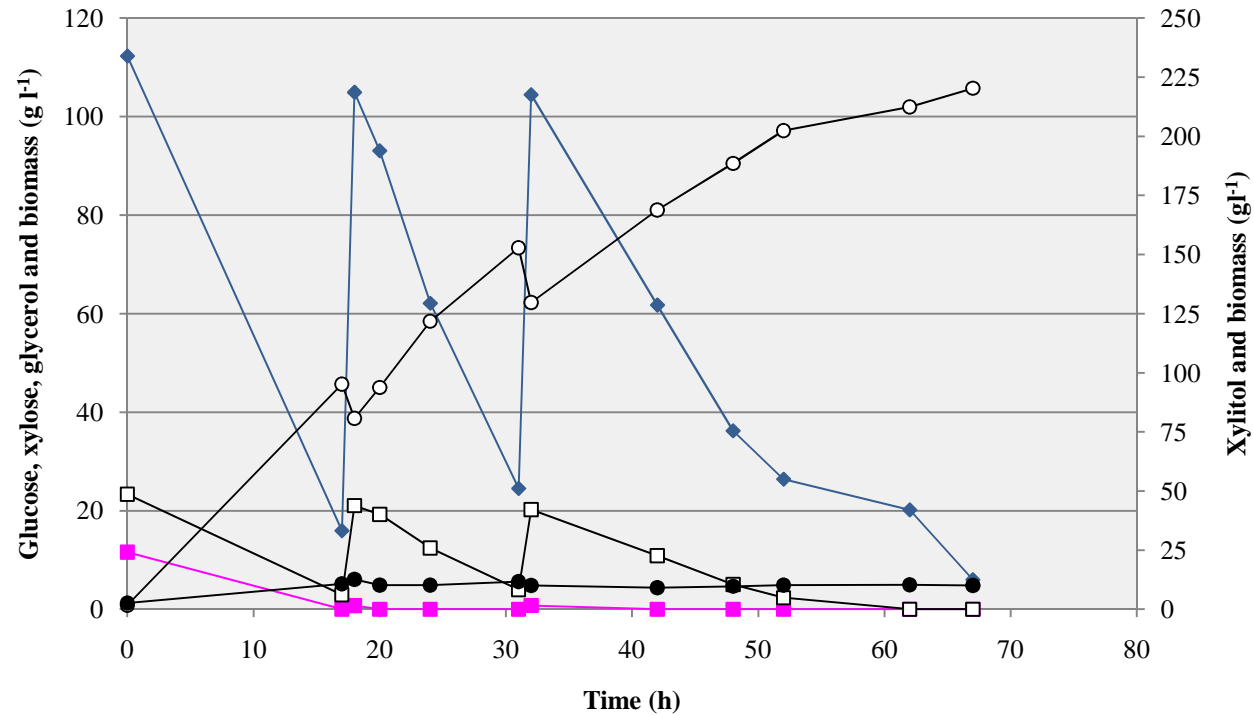
Xylitol Production of SS2



Fermentation profiles on the semi-synthetic medium containing 10g/l glucose, 20 g/l glycerol and 110 g/l xylose .
Symbols: ■, glucose; ◆, xylose; , glycerol ; , xylitol; ●, biomass

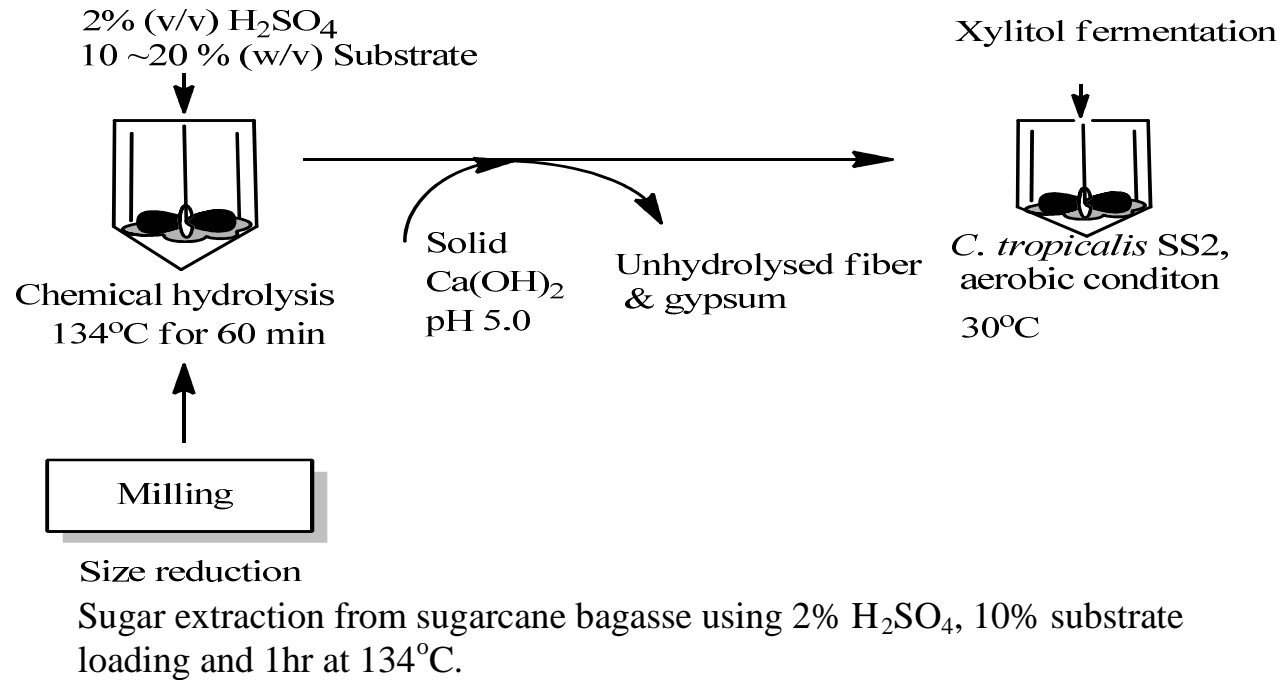
Fed batch fermentation profile of SS2 using xylose and glycerol

$$Y_{p/s}=0.93 \text{ g g}^{-1}, \text{ Productivity} = 3.3 \text{ g l}^{-1} \text{ h}^{-1}$$



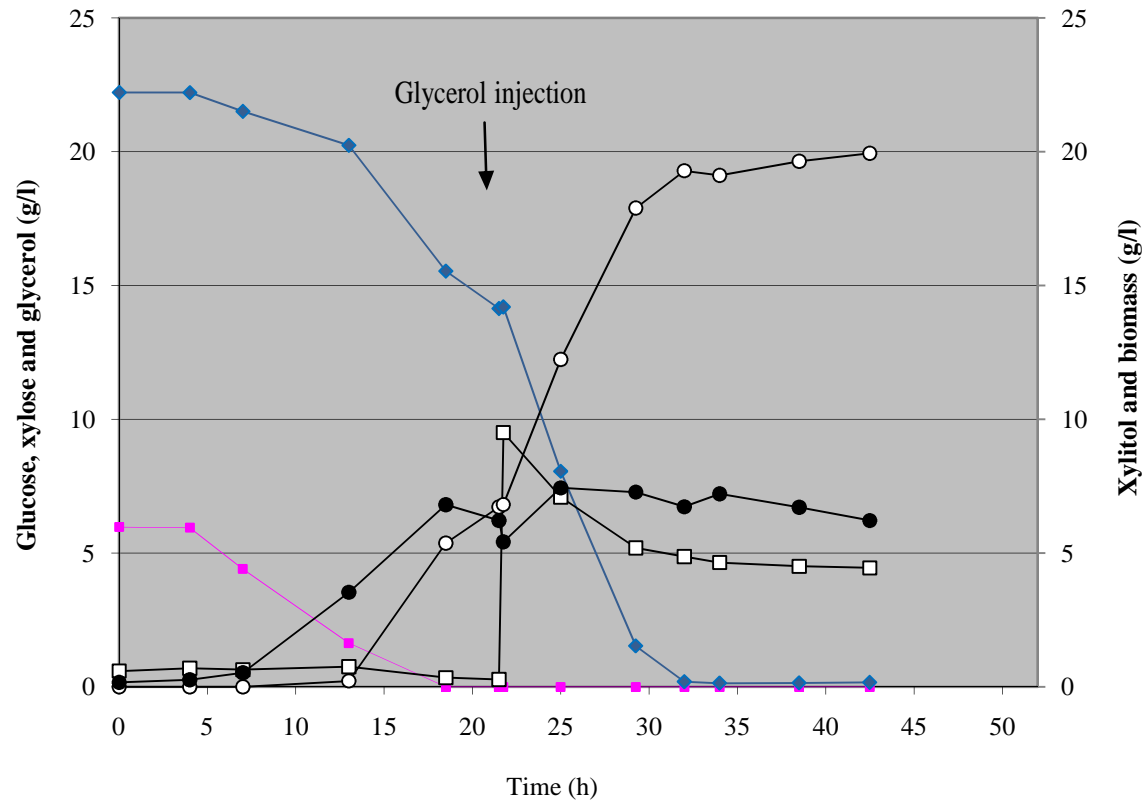
Fed-batch fermentation profile under the environmental conditions at pH=4.5, 30° C, aeration rate=1vvm, and 500rpm Symbols: ■, glucose; ◆, xylose; □, glycerol ;○, xylitol; ●, biomass.

Acid bagasse hydrolysate (10% substrate loading)



	Sugar compounds (g/l)		
	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)
Sugarcane Bagasse	5.4	21	3

Xylitol production using 10% sugar cane bagasse with glycerol feeding

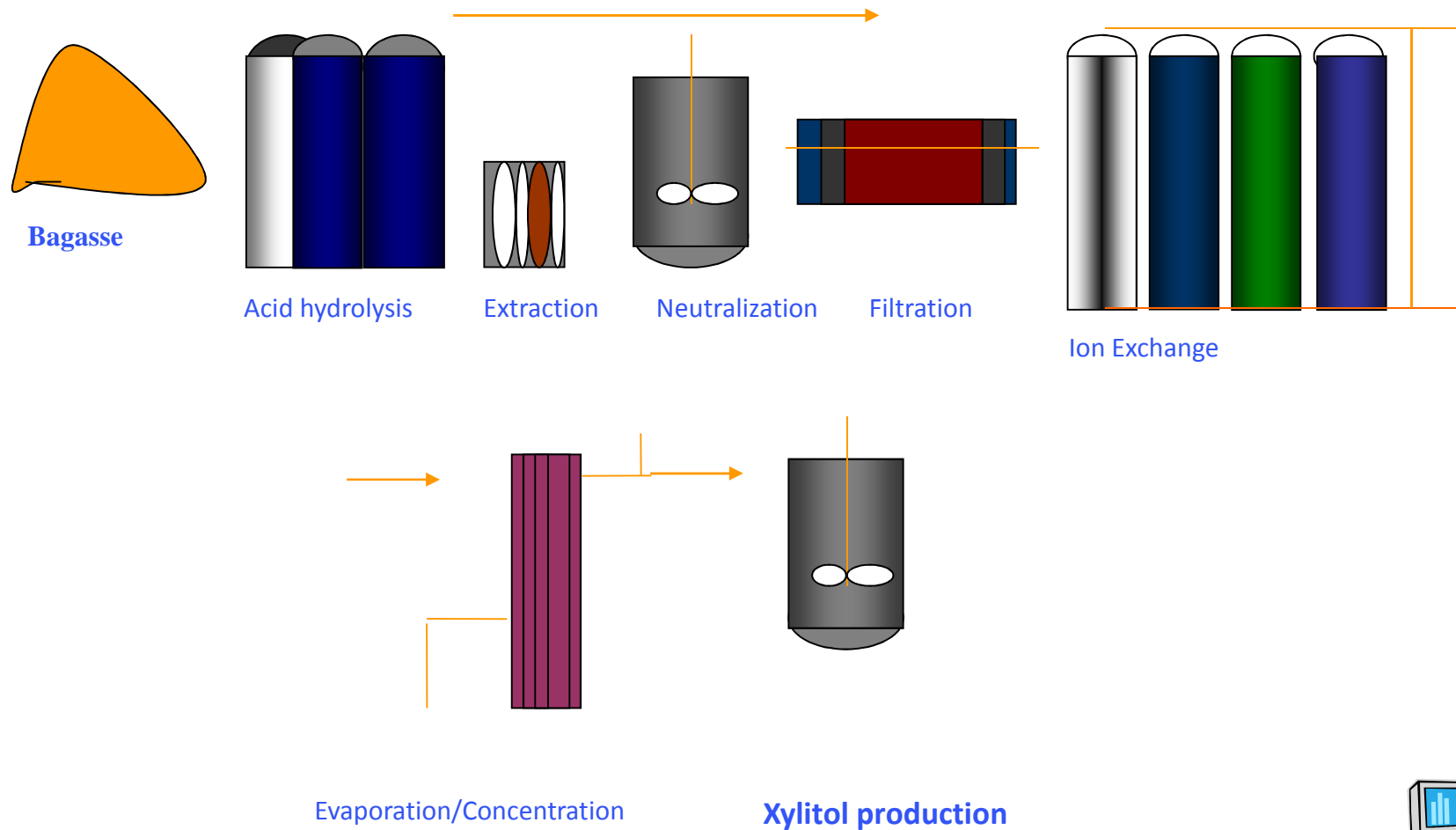


$$Y_{p/s} = 0.90 \text{ g g}^{-1}$$

Fermentation profile of *C. tropicalis* SS2 in the medium containing acid-hydrolysate of sugarcane bagasse with glycerol injection

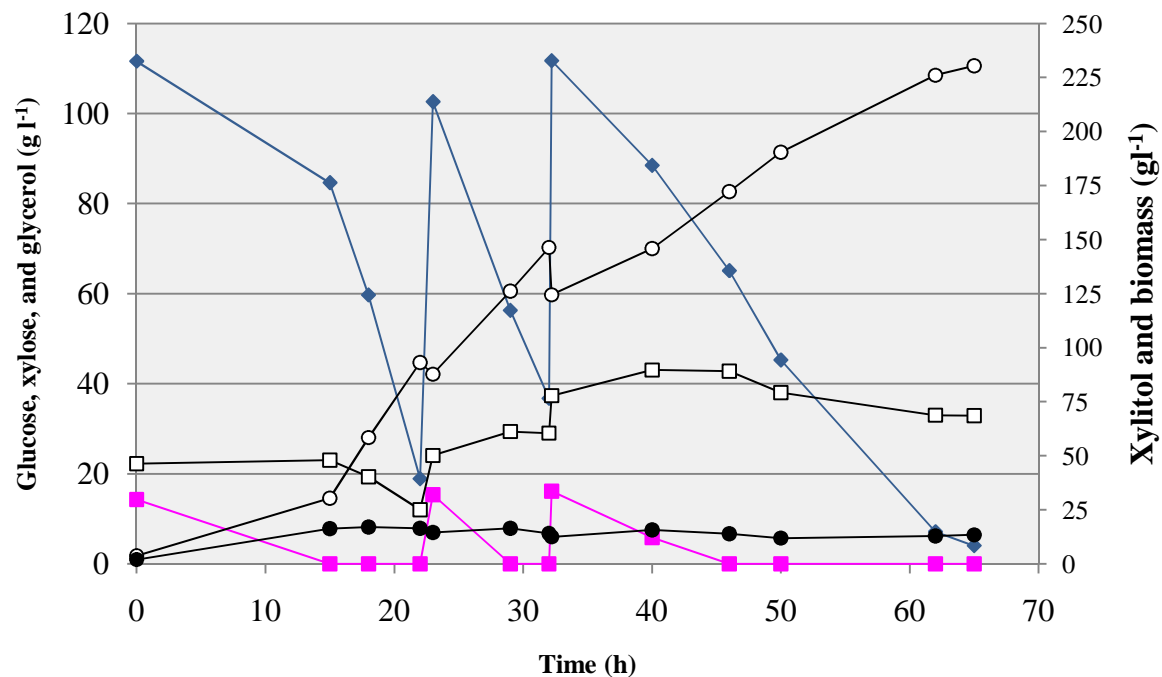
Symbols: ■, glucose; ◆, xylose; , glycerol; ▲, ethanol; , xylitol; ●, biomass

Schematic Process for concentrated xylose stream from Sugar cane bagasse



Fed batch fermentation profile of SS2 using sugarcane bagasse hydrolysate and glycerol

$$Y_{p/s} = 0.97, \text{ Productivity} = 3.5 \text{ g l}^{-1} \text{ h}^{-1}$$



Fed-batch fermentation profile using sugarcane bagasse hydrolysate and glycerol under the environmental conditions at pH=4.5, 30° C, aeration rate=1vvm, and 500rpm Symbols: ■, glucose; ◆, xylose; , glycerol ; , xylitol; ●, biomass.

Summary

- Efficient microbial xylitol production process developed via chemical mutagenesis
- Evaluation of glycerol as primary substrate for cell growth
- Evaluation of xylitol production process as a higher value product in bio-refinery using acid sugarcane bagasse hydrolysate
- Achieved xylitol product yield, 0.97 g g^{-1}
- Productivity $3.4 \text{ g l}^{-1} \text{ h}^{-1}$

Acknowledgment

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- Dr Simon Shin from LP Bio Ltd., Korea
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- Lab colleagues from UNSW Biofuel Laboratory