The study of xylose fermenting yeasts isolated in the Limpopo province

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Introduction

- Energy and environmental challenges have become a huge problem
- These has led to implementation of biofuels
- Biofuels are renewable energy source made from lignocellulose
- Comprises of cellulose, hemicellulose and lignin polymers

Problem statement

- Lack of organisms that can efficiently ferment pentoses compared to hexoses
- Inhibitors present after hydrolysis hampers fermentation

Aim

- Select a promising xylose fermenting yeast from previously isolated yeasts in Limpopo and compare this yeast with *Pichia stipitis* in terms of carbohydrates and inhibitors during ethanol production

Objectives

- Screen yeasts, previously isolated in Limpopo, for their ability to produce ethanol from xylose
- Compare a selected yeast with *Pichia stipitis*
- Test a combination of glucose and xylose to improve ethanol production
- Ability of the selected yeast to tolerate inhibitors such as acetic acid and furfural during ethanol production

Screening of xylose fermenting yeast strains

- Ten yeast strains previously isolated were used
- Maintained on YPD media
- Fermentation media was composed of : 20 g/L xylose, 2 g/L yeast extract, 2g/L KH_2PO_4, 10 g/L $(NH_4)SO_4$ and 2 g/L $MgSO_4.7H_2O$
- Fermentation using serum bottles at 30 °C for 72 hours
- Analysis of ethanol using gas chromatography

Table 1: Maximum ethanol concentration produced by xylose fermenting yeasts from Limpopo

Yeast strain	source	Ethanol concentration (g/L)
Candida guilliermondii BP1	Soil	0.03
Candida guilliermondii MBI2	Outer part of sugar cane	0.87
Candida guilliermondii NCGRW5	Rotten wood	0.02
Candida guilliermondii TMB4	Timber waste	0.03
Candida intermedia TMB3	Timber waste	0.04
Candida membranifaciens TMB REC5	Wood	0.1
Candida silvae TMBC	Timber waste	0
Candida silvae TMBC1	Timber waste	0
Pichia stipitis GS115	UL BTEC unit	0.15
Trichosporon asahii ORT2	Corn cob	0.02
Trichosporon coremiiforme MBI1	Inner part of sugar cane	0

Ethanol production by selected yeast strain

- Xylose fermentation using 500ml Erlenmeyer flasks at 30 °C and 150rpm for 96 hours
- Sampling at 6 hour intervals
- Analysis using GC and HPLC
- Use *Pichia stipitis* as benchmark organism

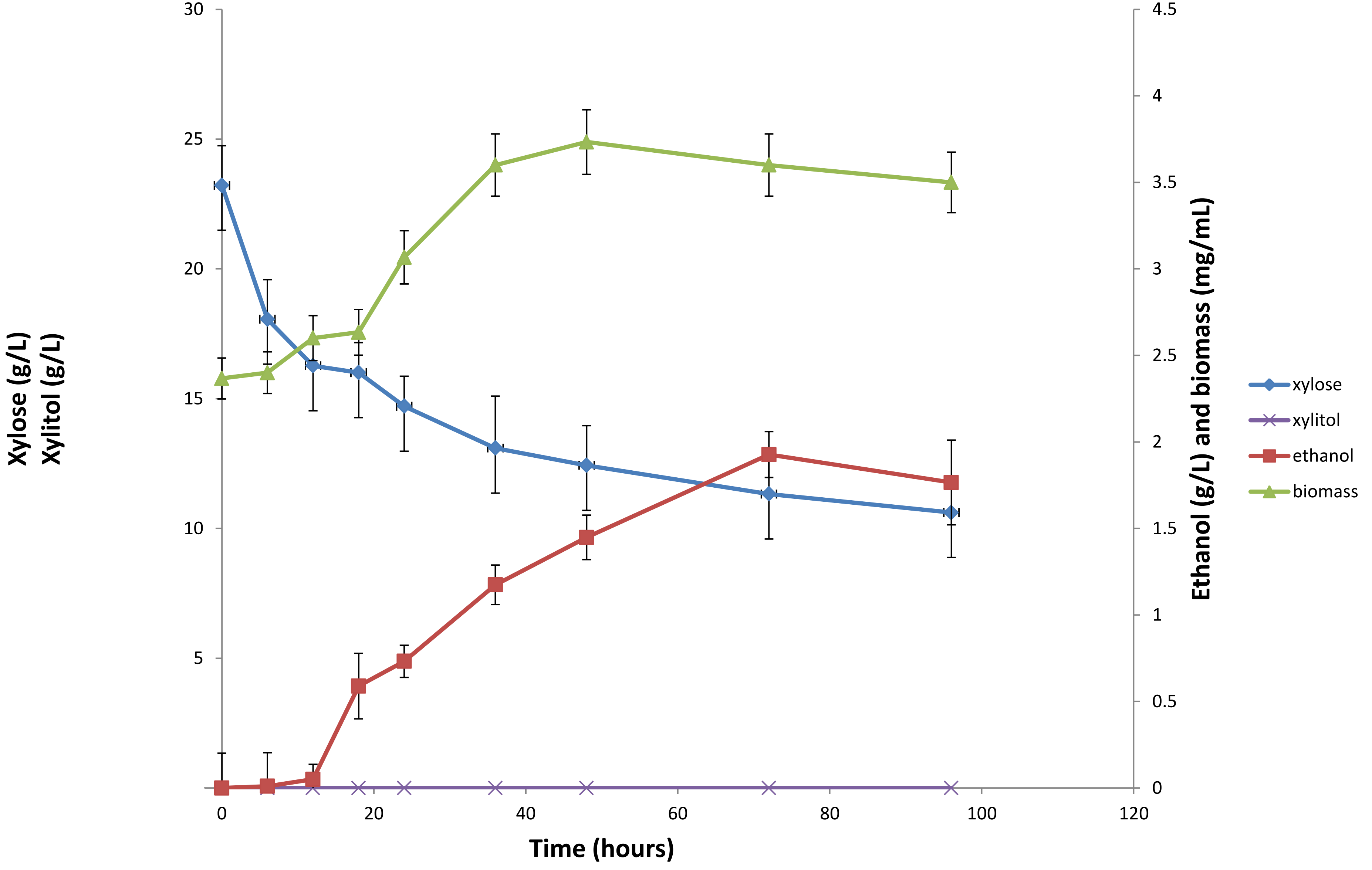

Figure 1: Xylose fermentation by *P. stipitis*

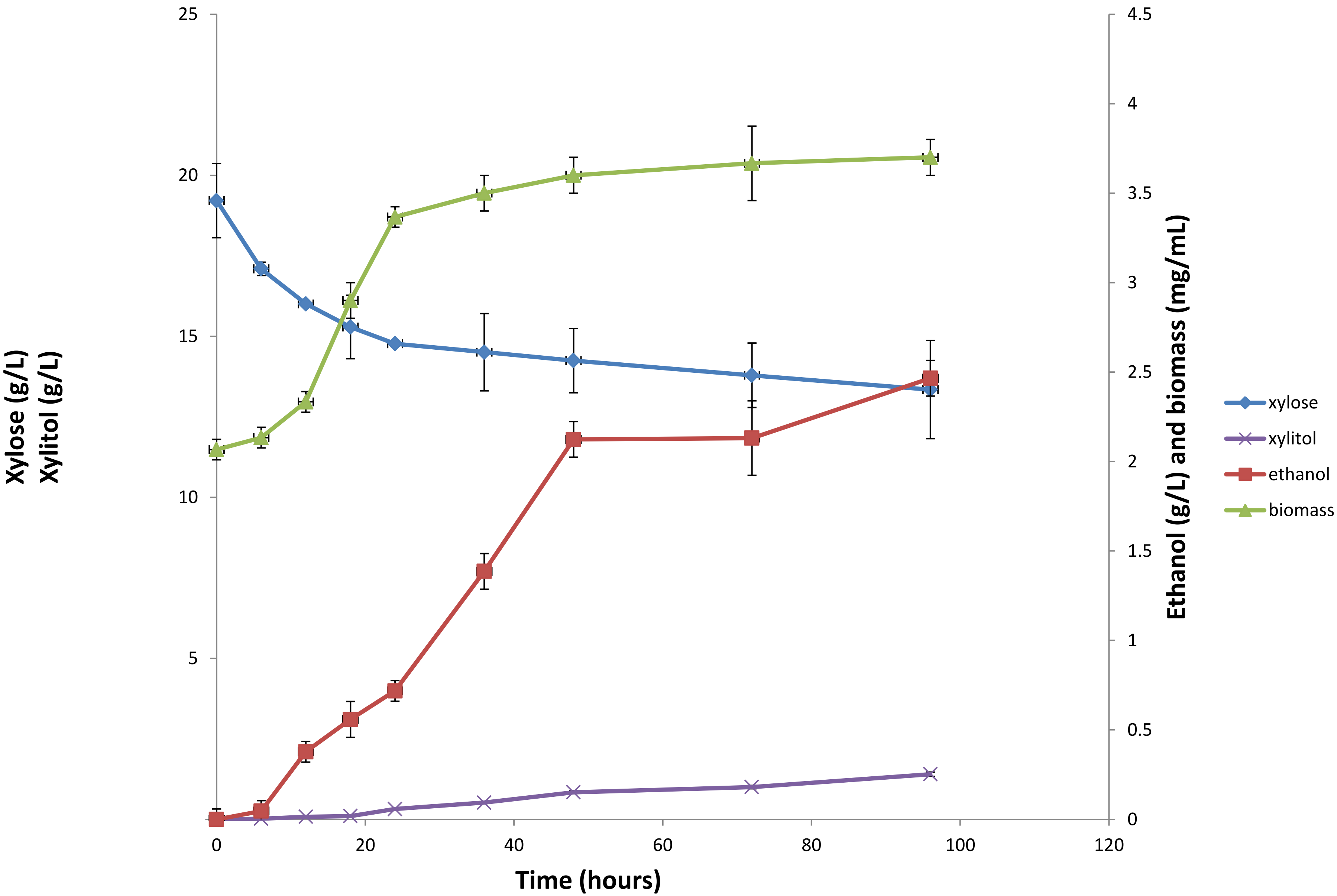

Figure 2: Xylose fermentation by *C. guilliermondii*

Influence of combination of glucose and xylose on ethanol production

- Co-fermentation using glucose and xylose
- 10 g/L of each carbohydrate
- Fermentation carried out for 96 hours with samples taken at 6 hour intervals
- Analysis by GC and HPLC

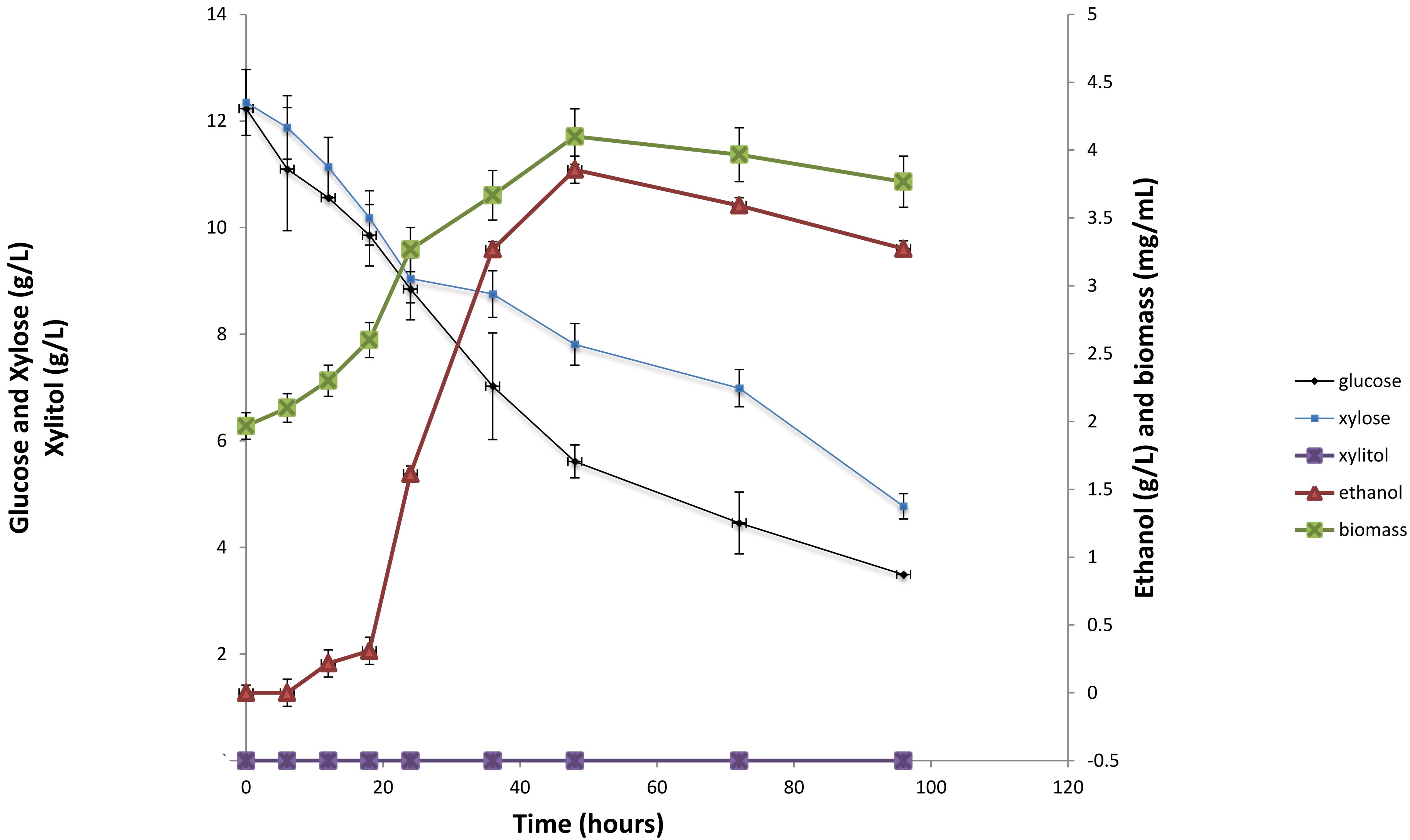

Figure 3: Influence of co-fermentation on *P. stipitis*

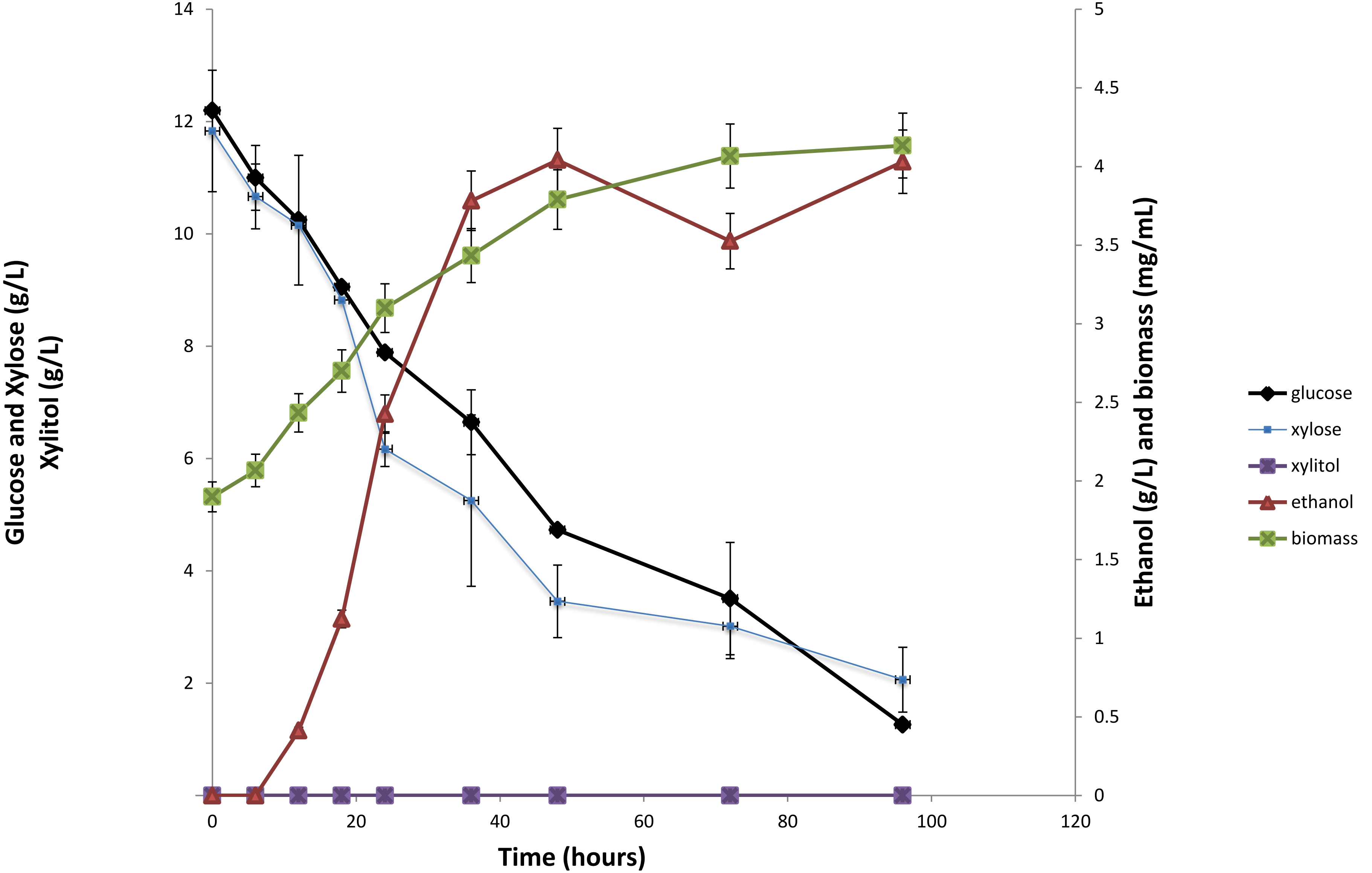

Figure 4: Influence of co-fermentation on *C. guilliermondii*

Effect of inhibitors on ethanol production

- Different concentrations (1-3 g/L) of acetic acid used on xylose fermentation media
- Fermentation lasted for 72 hours, and sample analysed every 24 hours using GC
- Different concentrations (1-3 g/L) of furfural used on xylose fermentation media
- Fermentation lasted 72 hours with samples taken every 24 hours and analysed using GC
- *Pichia stipitis* used as benchmark organism

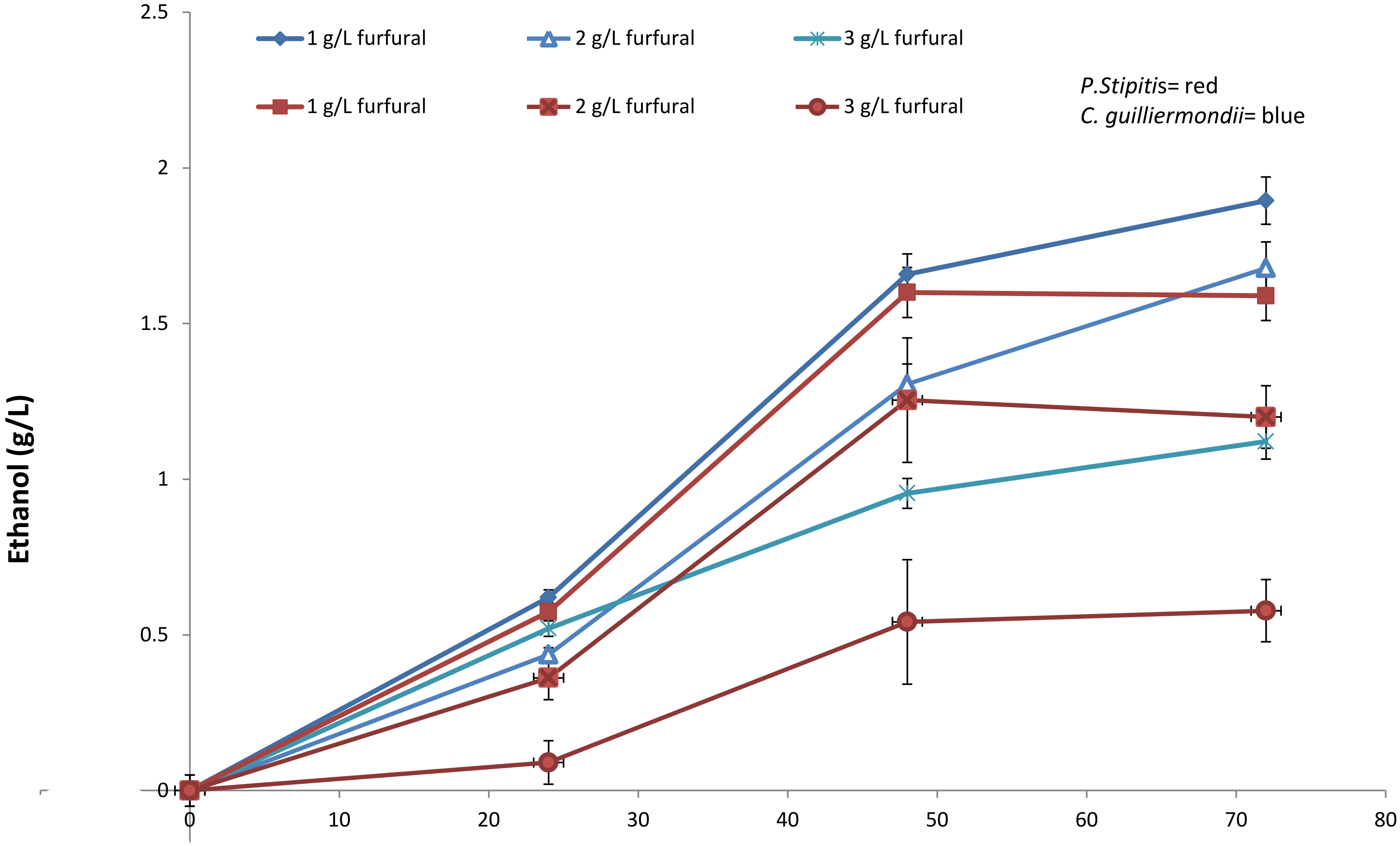

Figure 5: Effect of furfural on ethanol production of *C. guilliermondii* and *P. stipitis*

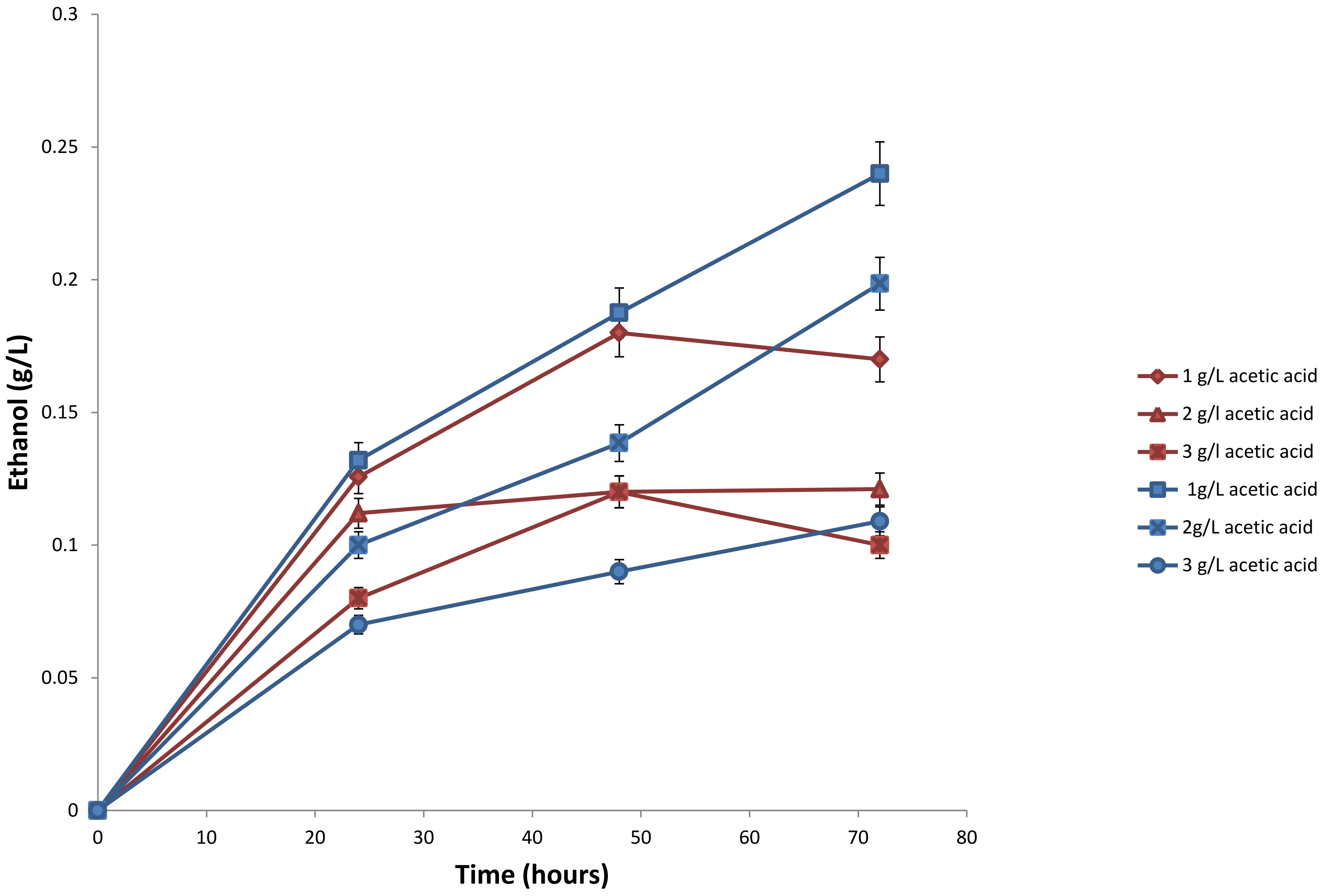

Figure 6: Effect of acetic acid on ethanol production of *C. guilliermondii* and *P. stipitis*

Conclusion

- *Candida guilliermondii* MBI2 produced the highest ethanol concentration
- *C. guilliermondii* MBI2 ferments xylose better than *P. stipitis*
- Lower ethanol production was observed in the presence of inhibitors
- *C. guilliermondii* MBI2 produces no xylitol when fermented using xylose and glucose

Future work

- Adapt strains on higher sugar concentrations, elevated temperatures and high acetic acid
- Repeat adaptation 50 times
- Ferment best selected strains in STR bioreactor at optimal conditions

Acknowledgements

- E. L Jansen van Rensburg
- D.C La Grange
- RSES