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Characterizing *Yarrowia lipolytica* as a cell factory for sustainable bioprocesses

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Background

The utilization of an increasing amount of agro-waste and renewable low-cost substrates will be an ongoing challenge for the bio-based economy. Mobilization of these feedstocks is necessary for realization of biosustainability on an industrial scale. Cell factories traditionally applied In biotechnology are using a limited range of substrates (mainly glucose). However, a wider diversity in substrate range is highly desirable in developing biorefinery scenarios where seasonal variation in availability and composition of biomass feed-stocks typically appear.

Aim of the study

This study evaluates the applicability of the non-conventional yeast *Y. lipolytica* as a cell factory for the conversion of **glycerol** and the lignocellulose hydrolysate sugars **glucose, xylose and arabinose**. A quantitative physiological investigation was carried out in order to assess the cellular performance of three different *Y. lipolytica* wild type strains (**IBT 446, W29 and H222**) in single and mixed carbon source cultivations.

Benchmarking strains on single carbon substrate

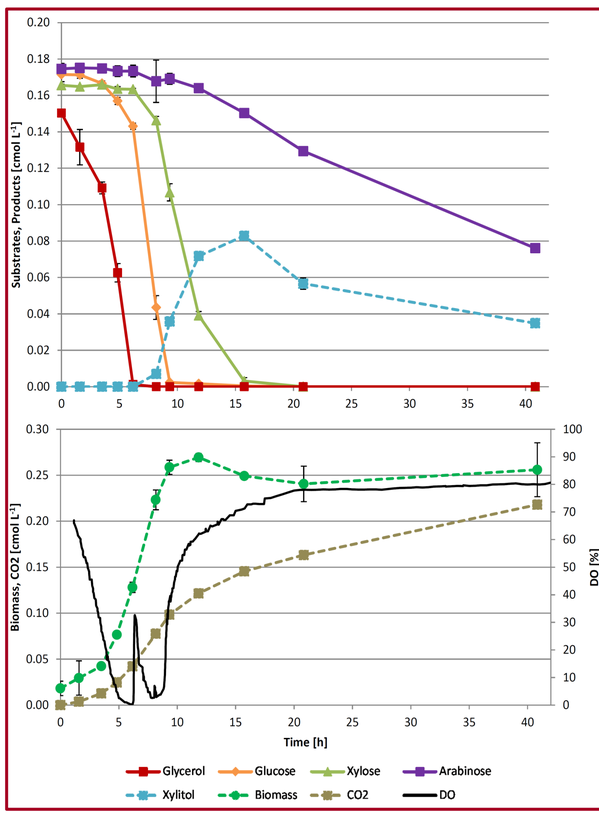
Glucose vs Glycerol

	IBT 446		W29		H222	
	Glucose[1]	Glycerol[1]	Glucose	Glycerol	Glucose	Glycerol
Growth rate						
µmax (h^{-1})	0,24 ± 0,01	0,30 ± 0,01	0.28 ± 0.02	0.31 ± 0.02	0.28 ± 0.01	0.35 ± 0.01
Yield coefficients						
Ysx (cmol $cmol^{-1}$)	0,69 ± 0,03	0,61 ± 0,01	0,67 ± 0,03	0,73 ± 0,01	0,64 ± 0,02	0.72 ± 0,01
Ysc (cmol $cmol^{-1}$)	0,30 ± 0,01	0,23 ± 0,00	0,34 ± 0,02	0,30 ± 0,01	0,35 ± 0,004	0,21 ± 0,01
Ysm (cmol $cmol^{-1}$)	N/A*	0,17 ± 0,00	N/A*	0,015 ± 0,01	N/A*	N/A**
Total	**0,99 ± 0,03**	**1,01 ± 0,01**	**1,02 ± 0,05**	**1,04 ± 0,01**	**0,98 ± 0,01**	**0,94 ± 0,01**

[1]Workman 2013, N/A: Not applicable: *below detection limit. **Carbon source not depleted at last sample point.

Xylose and Arabinose

Growth experiments in shake flasks and defined minimal media with 0.65 cmol L^{-1} (≈ 20 g L^{-1}) of the carbon source and cultivation time of 140 h: None of the strains showed growth on xylose or arabinose as the sole carbon source (verified by OD and HPLC measurements).



Set up: Bioreactor batch cultivation, minimal media with 0,163 cmol L^{-1} (≈ 5 g L^{-1}) of each carbon source.

IBT 446 - mixed substrates

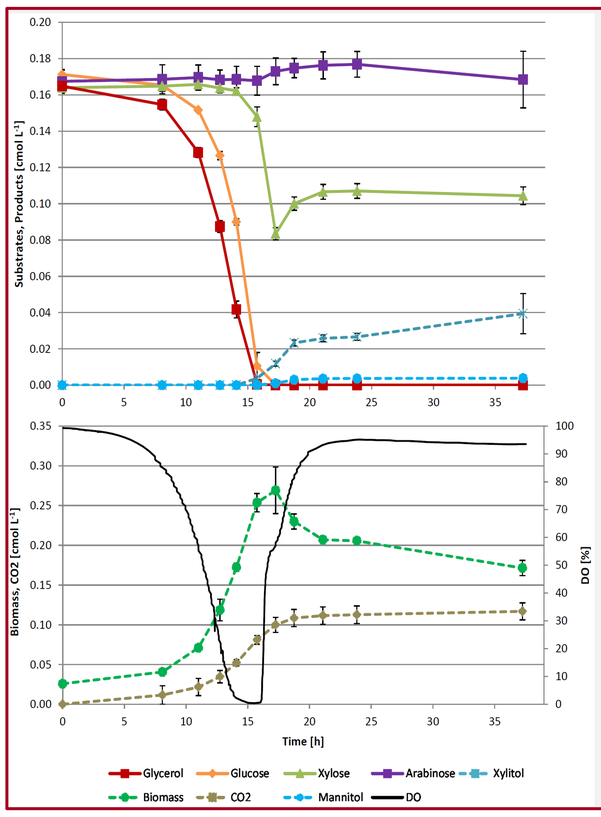
Results:

Uptake cascade: 1. glycerol, 2. glucose, 3. xylose, 4. arabinose

Xylitol production and re-utilization

Growth on glycerol and glucose (primary carbon sources)

Maintenance of biomass after depletion of primary carbon sources



W29 - mixed substrates

Results:

Uptake cascade: 1. glycerol, 2. glucose, 3. xylose (half consumed)

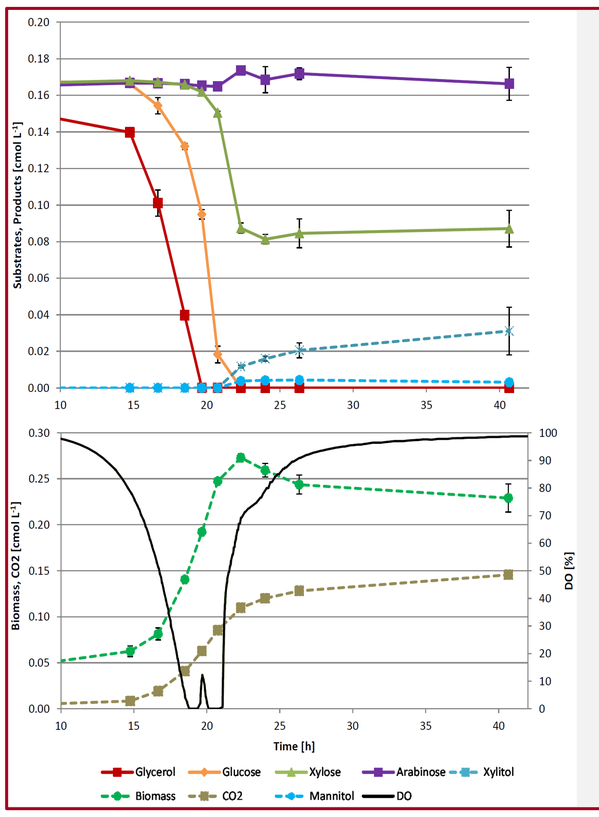
Co-consumption glycerol/glucose

Arabinose not consumed

Xylitol production but no re-utilization

Growth on glycerol and glucose (primary carbon sources)

Drop of biomass after depletion of primary carbon sources



W29 - mixed substrates

Results:

Uptake cascade: 1. glycerol, 2. glucose, 3. xylose (half consumed)

Arabinose not consumed

Xylitol production but no re-utilization

Growth on glycerol and glucose (primary carbon sources)

Drop of biomass after depletion of primary carbon sources

Summary and Conclusions

With current global focus on renewable and sustainable technologies, there is a strong need to develop innovative solutions in industrial biotechnology. The non-conventional yeast *Yarrowia lipolytica* has considerable potential as a versatile cell factory, especially when compared to the typically applied organisms such as *Saccharomyces cerevisiae* which utilizes glycerol very slowly and requires genetic engineering for conversion of lignocellulosic sugar monomers xylose and arabinose. In this study we could show that *Y. lipolytica* IBT 446 is versatile and interesting as a biorefinery cell factory, as it is natural capable to utilize sugars typically found in plant hydrolysates (glucose, xylose, arabinose) together with glycerol in mixed carbon cultivations. Furthermore the strains W29 and H222 are naturally capable of conversion of glycerol, glucose and xylose.

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