

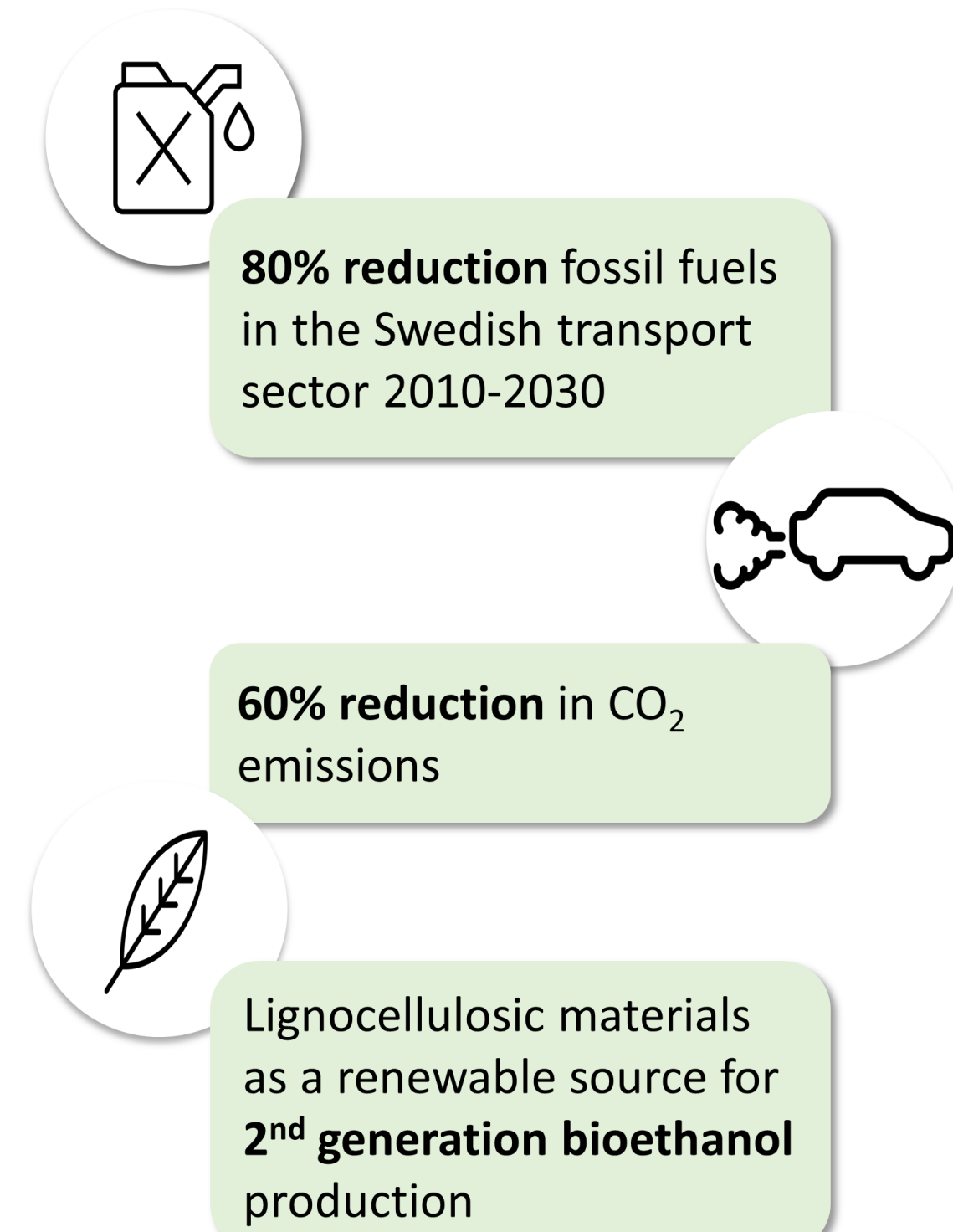
Novel methods for accelerating the development of more inhibitor tolerant yeast strains for cellulosic ethanol production

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Background

Towards a fossil-free transport sector



Saccharomyces cerevisiae as a bioethanol production host

Engineered *S. cerevisiae* is able to ferment glucose and xylose for ethanol production.

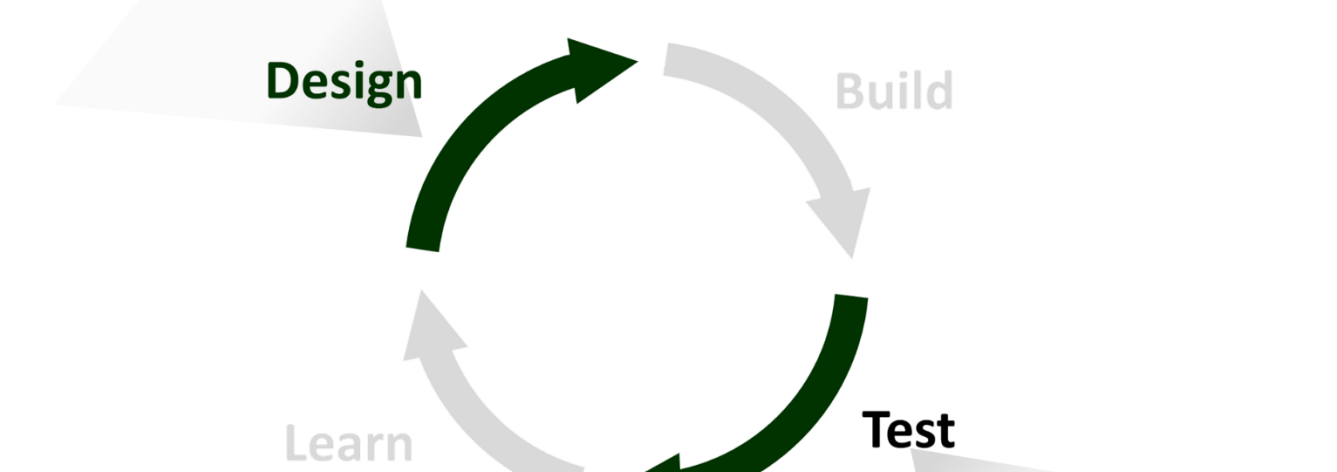
- Lignocellulosic hydrolysates contains a broad range of inhibitors for yeast
- Inhibitor composition depends on the type of raw material and pretreatment

An efficient genome editing strategy for engineering industrial yeast strains is needed

OBJECTIVE

To develop an efficient design-build-test-learn cycle for creating robust production strains

Use of CRISPR(i) based systems as a tool for studying the impact of genetic alterations



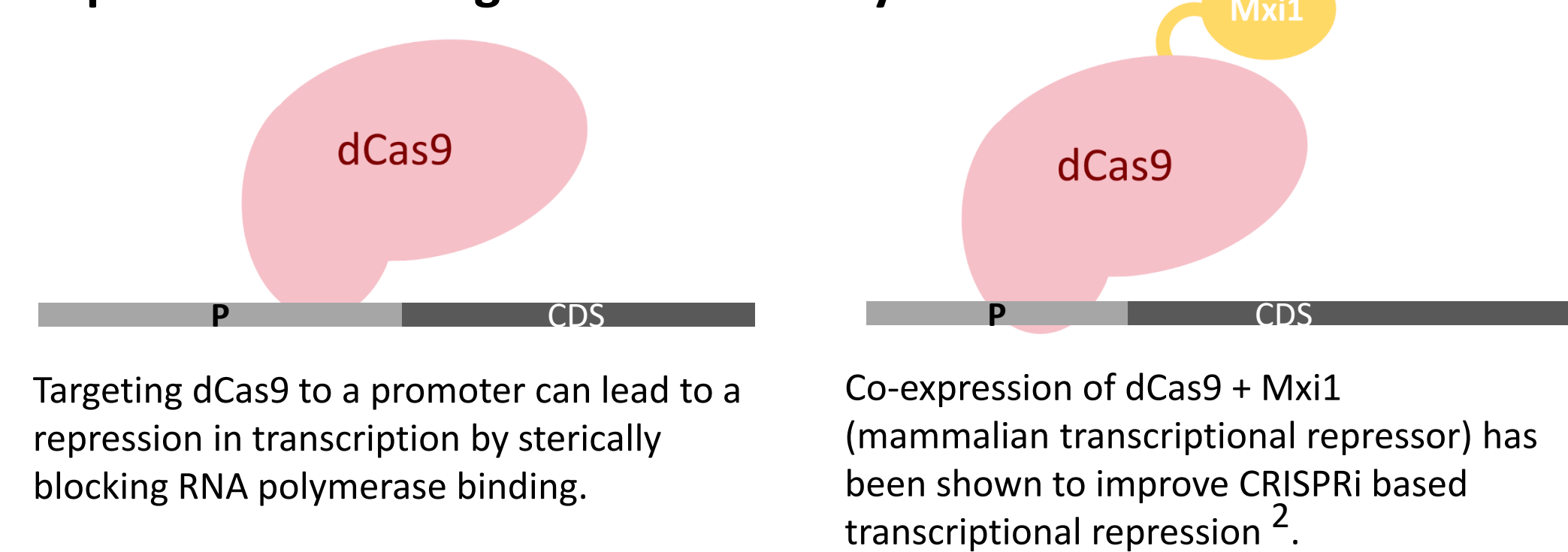
High-throughput analysis of yeast strains during fermentation in lignocellulosic hydrolysates

In order to develop inhibitor tolerant strains, the expression of genes involved in tolerance will be systematically altered using **CRISPR interference (CRISPRi)**. This methodology uses a catalytically inactive, dead Cas9 (dCas9).

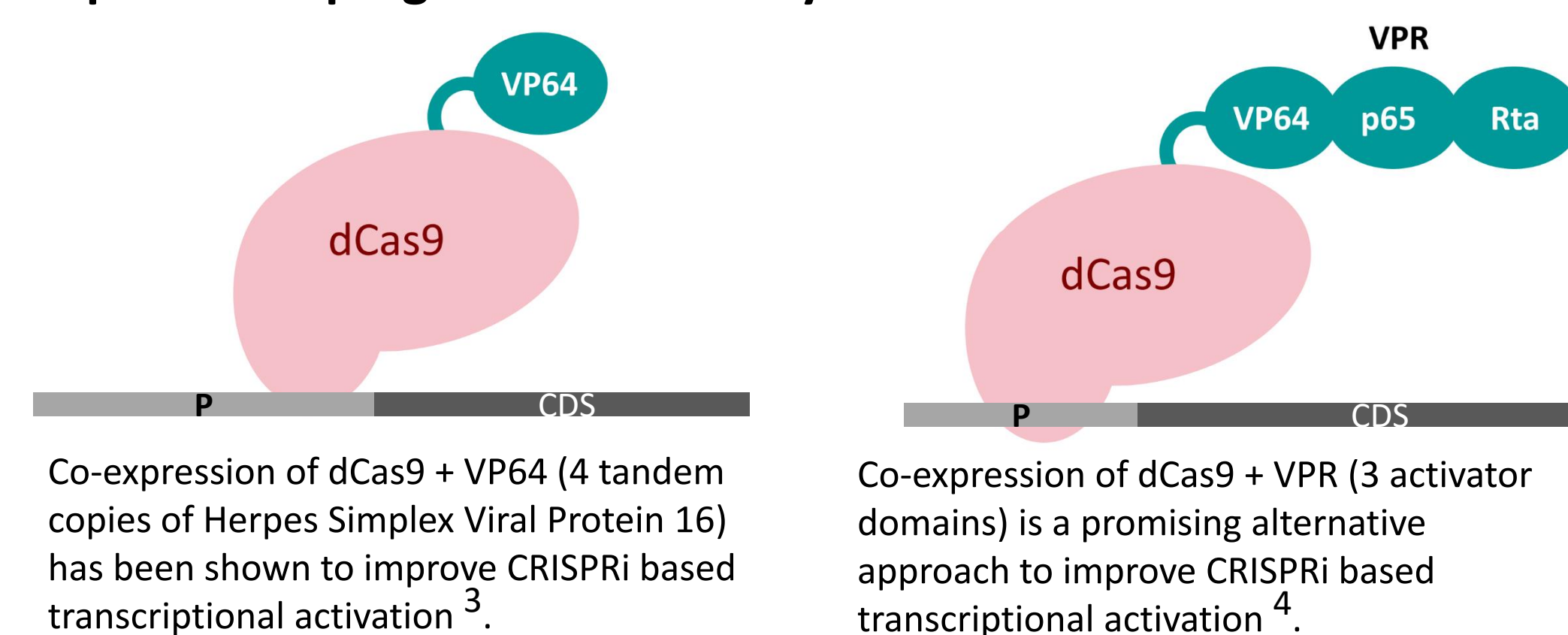
Vectors expressing dCas9+repressor/activator constructs have been constructed using the Moclo Yeast Toolkit ¹.

CRISPR will be used to stablish permanently gene modifications successfully tested with CRISPRi.

Expression downregulation driven by:

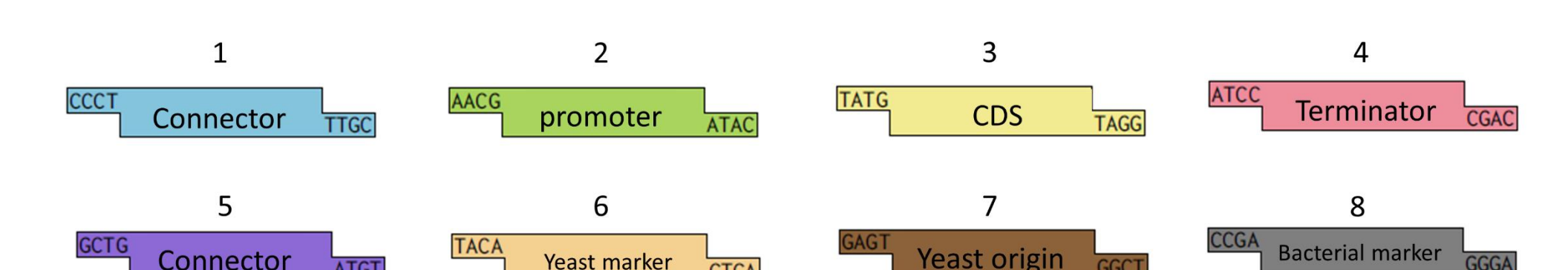


Expression upregulation driven by:

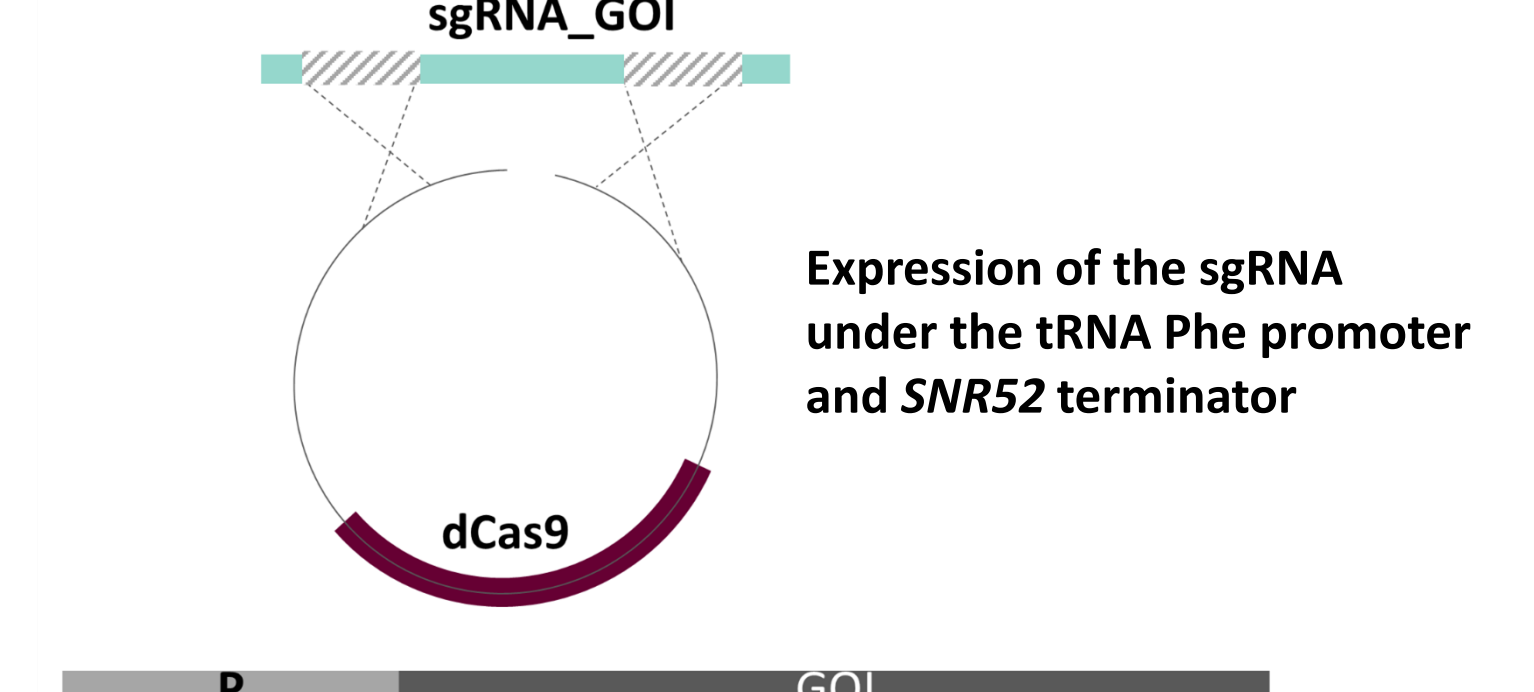


Vector construction

1. Assembly of parts to obtain vectors expressing a CDS or a domain of interest (dCas9 or activator/repressor, respectively).



2. Assembly of a vector containing multiple transcription units, expressing dCas9 + activator/repressor and a cassette for sgRNA expression. The sgRNA is assembly into the vector through *in vivo* homologous recombination.



PHASE 2 – Establishment of a high-throughput screening platform

In order to analyse a large number of strains a rapid, high-throughput system to characterise the performance of new strains in lignocellulosic hydrolysates is essential.

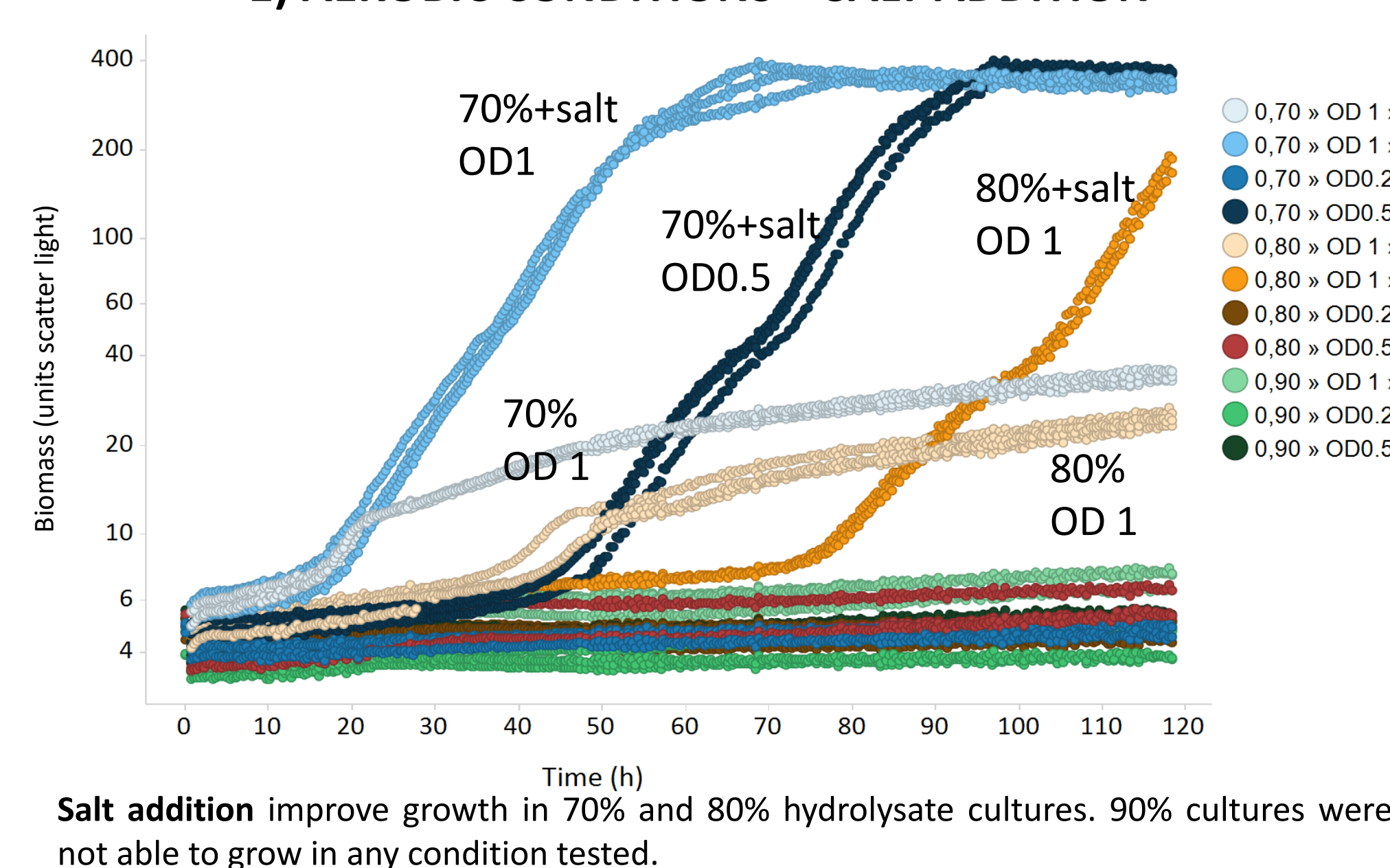
Various culture conditions and parameters have been tested in the Biolector® microbioreactor platform:

- Lignocellulose hydrolysate concentration (% v/v)
- Size of inoculum
- Addition of salt to the medium
- Preculture adaptation
- Aerobic / anaerobic conditions

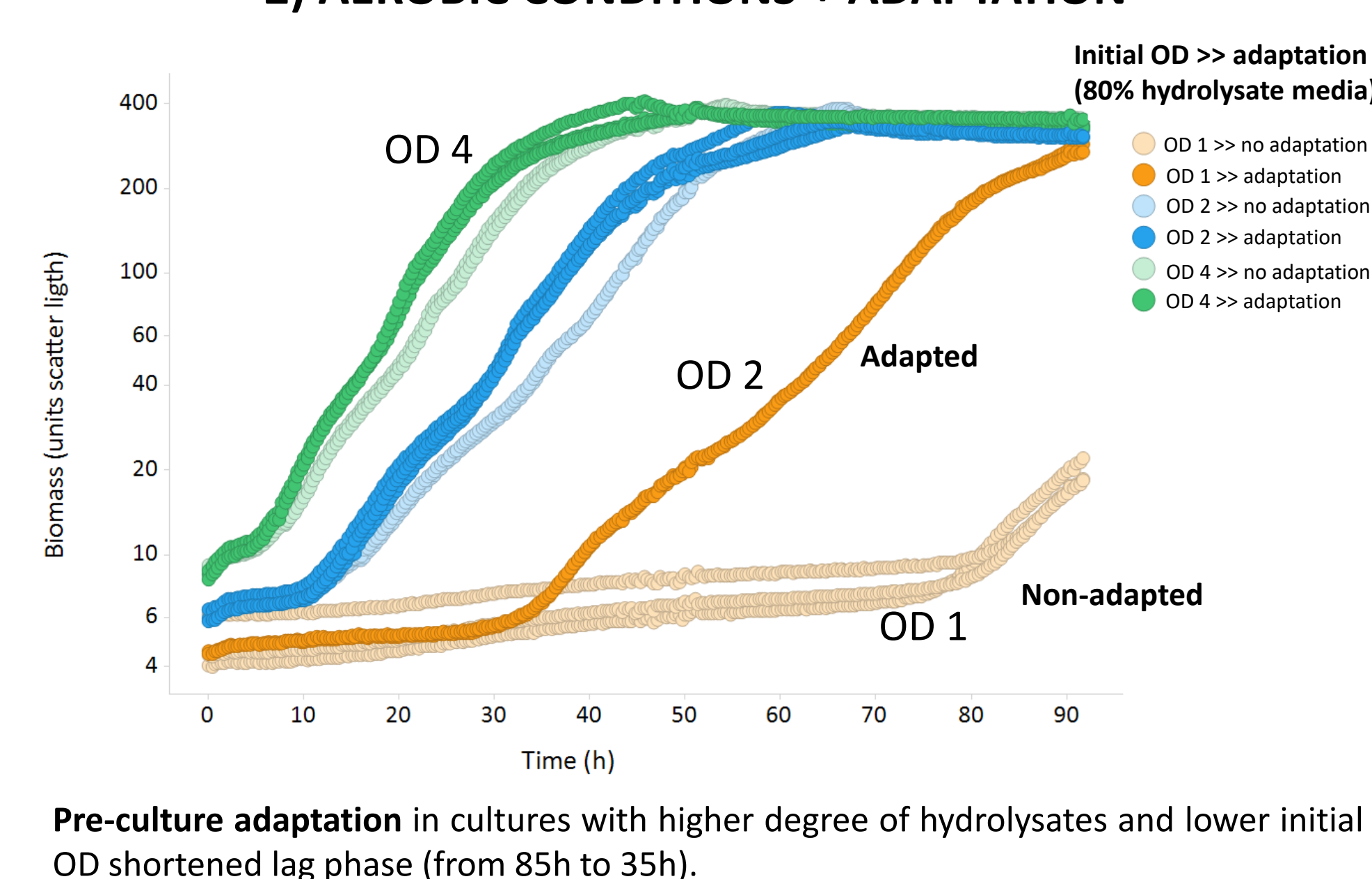
MATERIALS AND METHODS

- Culture volume: 1 mL
- Polyloid industrial strain
- Media: Wheat straw hydrolysate with YPD supplementation (final sugar concentration: 60 g/L glucose + 30 g/L xylose), pH 5.5
- Adaptation in anaerobic conditions was performed by addition of different lignocellulosic hydrolysate concentration (0%, 14% and 40%) in the YPD preculture media, with an overnight incubation

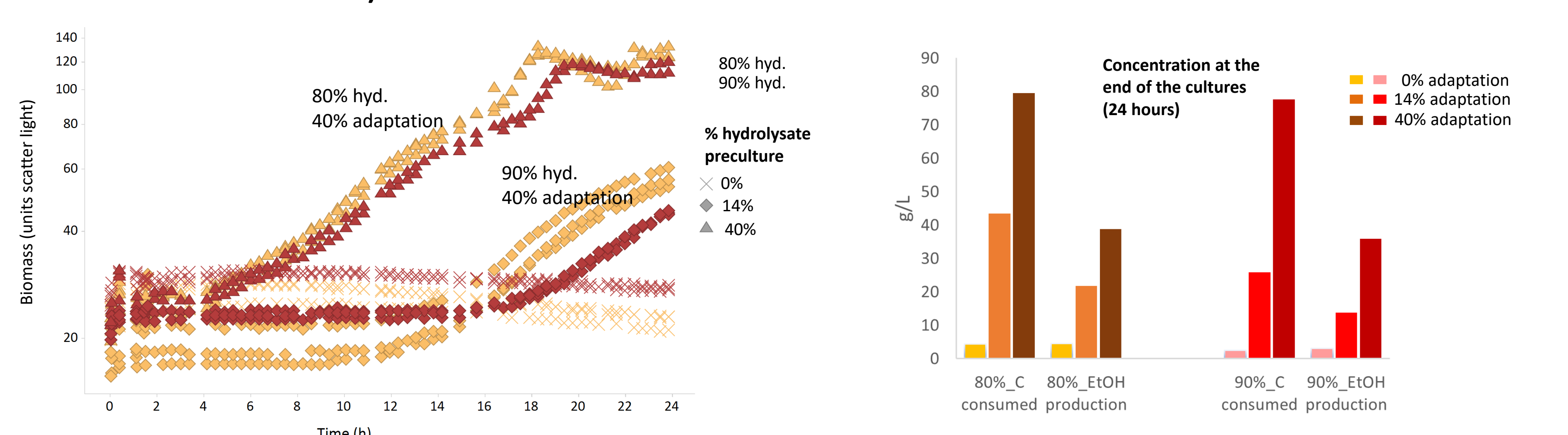
1) AEROBIC CONDITIONS + SALT ADDITION



2) AEROBIC CONDITIONS + ADAPTATION



3) ANAEROBIC CONDITIONS + ADAPTATION LEVELS



Under **anaerobic conditions**, preculture adaptation at high hydrolysate concentration had a high impact in growth performance. Furthermore, HPLC measurements showed a drastic increase in ethanol production. Concretely, about a 10-fold increase in ethanol production was measured for the strains adapted in 40% hydrolysates compared with non-adapted strains.

Conclusions

- An efficient platform for high-throughput screening of yeast directly in lignocellulosic hydrolysates was established
- Preliminary results showed a reliable correlation in terms of growth with shake flask cultures

References

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