Cyprus University of Technology

Department of Chemical Engineering

Environmental Bioprocessing Laboratory

CHANIA 2023

Transcriptional Responses of *Saccharomyces cerevisiae* to Environmental Stresses During Bioethanol Production Using Biochar-Based Biocatalysts

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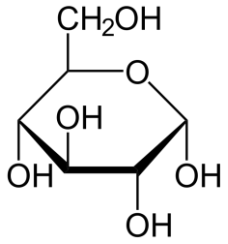
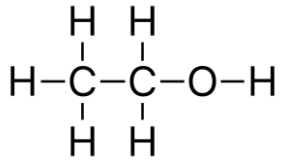
CHANIA2023 - 10th International Conference on Sustainable Solid Waste Management, Chania, Greece, 21-24 June 2023

Challenges in Bioethanol Production

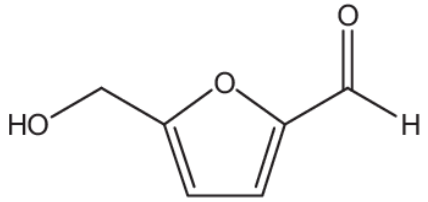
- **Substrate** inhibition



- **Product** inhibition

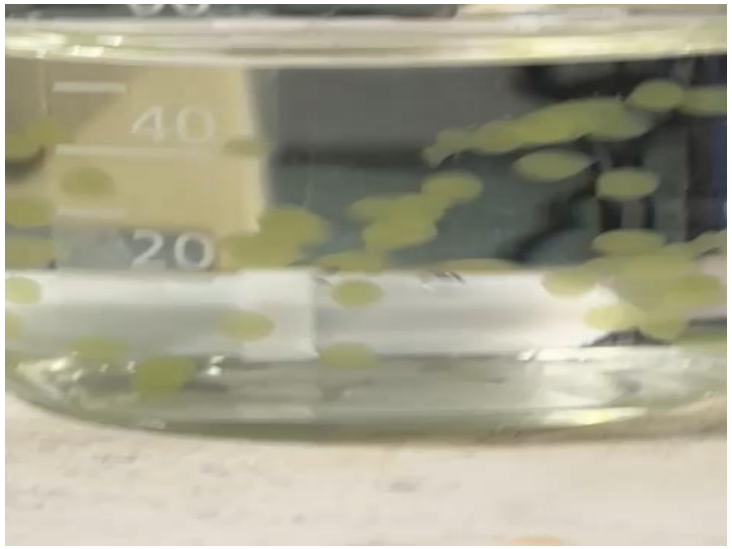


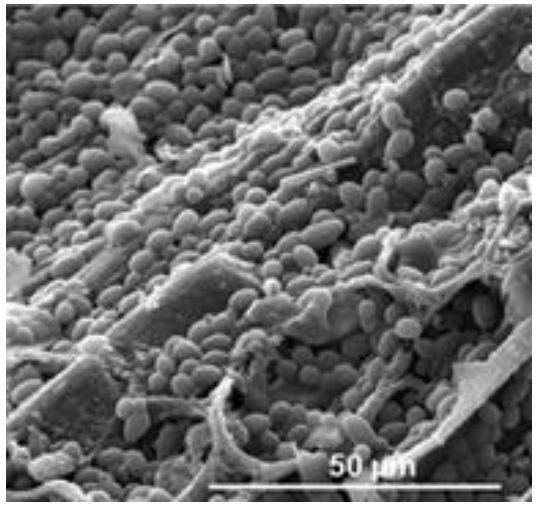
- **Inhibitors** released in pretreatment

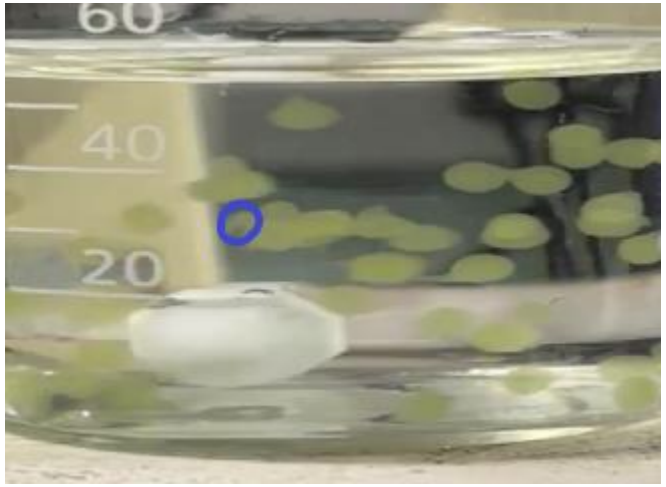


- High **temperatures** (> 37 °C *S. cerevisiae*)
- **Osmotic** stress
- **pH** variations

Immobilized Biocatalysts in Bioethanol Production







- Assist yeast tolerance
- Enhance ethanol production rate and yield
- Stable product formation
- Easy cell recirculation
- Entrapment in **alginate beads**
- **Poor mechanical properties**
- Novel eco-friendly and cost-effective **rigid carriers**

Biochar as a Carrier for Biocatalyst Development

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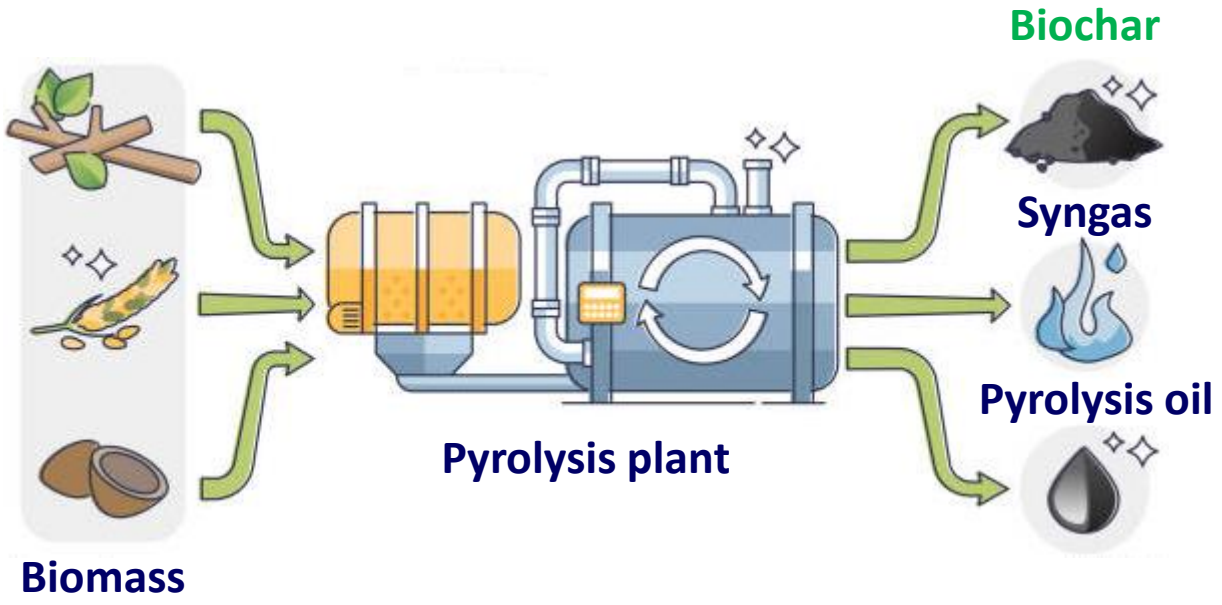
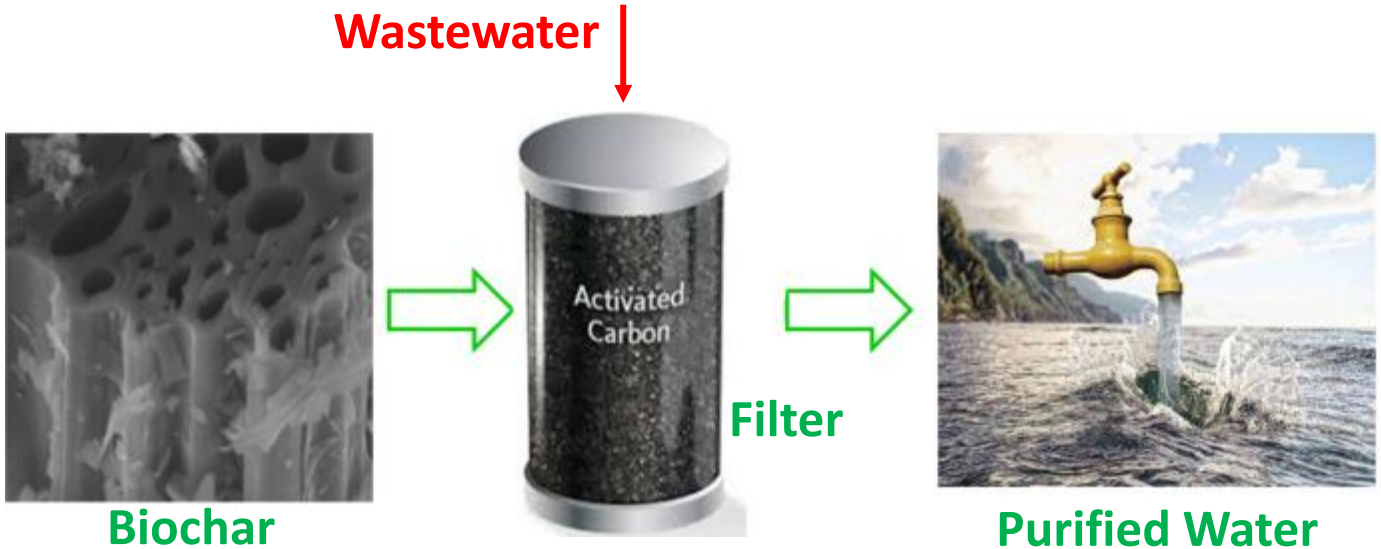
Biochar

- High-carbon, low cost and environmental friendly
- **Pyrolysis** of biomass
- **Multiple applications** (e.g. wastewater adsorbent, soil amendment)





Biochar as a Carrier for Biocatalyst Development

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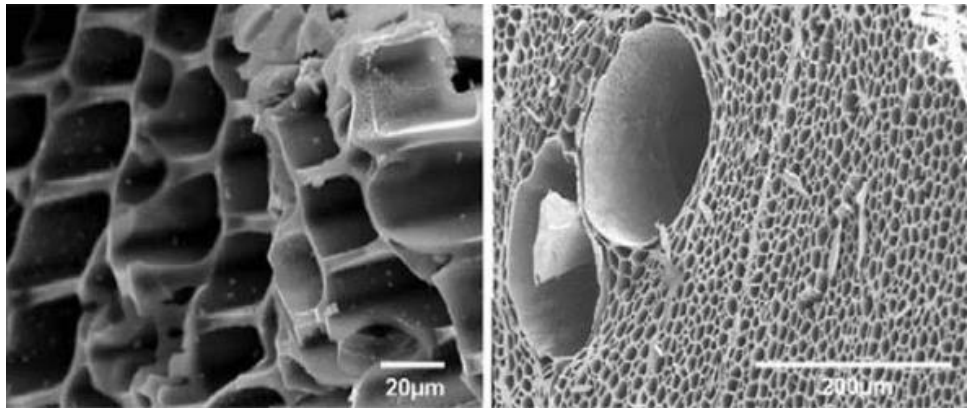
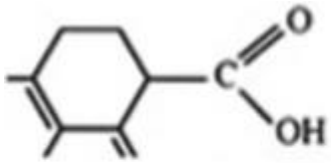
Potential for Cell Attachment

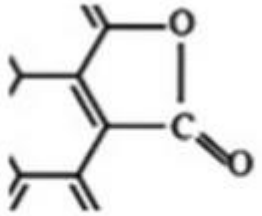
- ❑ **Porous** structure
- ❑ Increased **surface area**
- ❑ Surface **functional groups**
- ❑ Assists electro-active strains



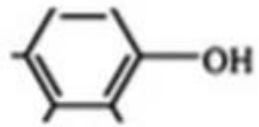
Carboxyl



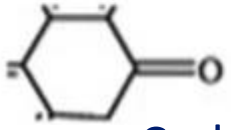
Lactone



Phenol

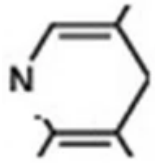


Acidic groups

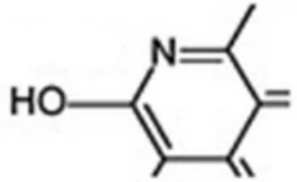


Carbonyl

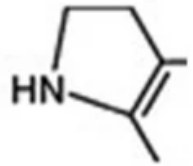
Ether



Pyridine



Pyridone



Pyrrole

Basic groups

Raw Materials, Pyrolysis Conditions & Biochar Properties

Seagrass

Corks

Sewage sludge

Peanut shells

Olive kernels

Pistachio shells

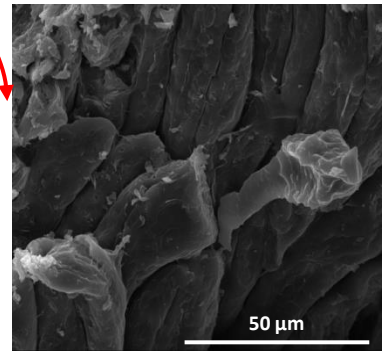
Vineyard prunings

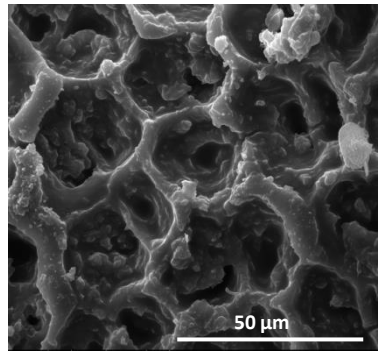
Car tyres

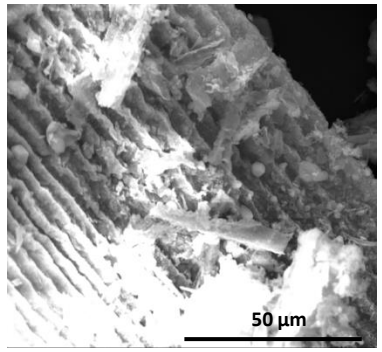
Microstructural details (SEM)

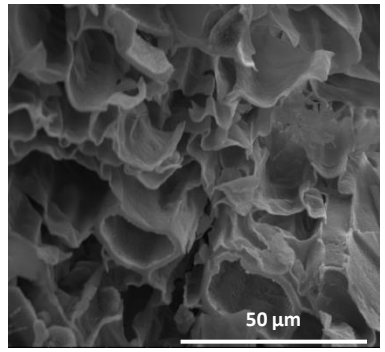
250 °C

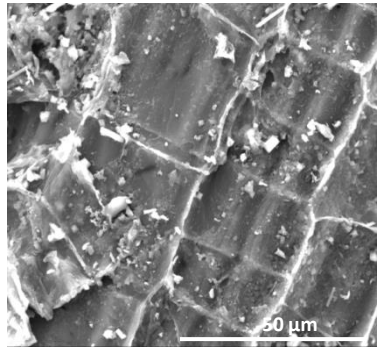
500 °C

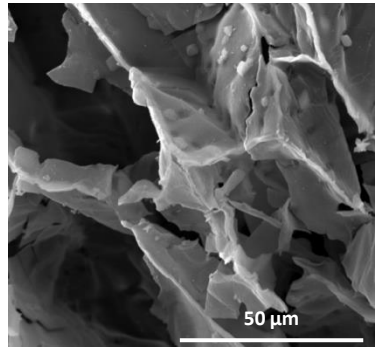








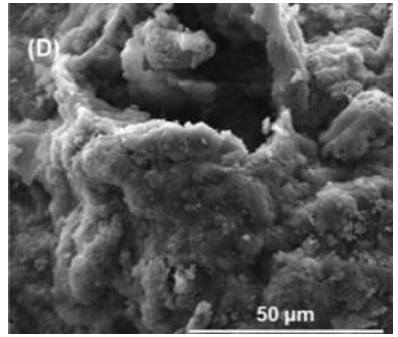


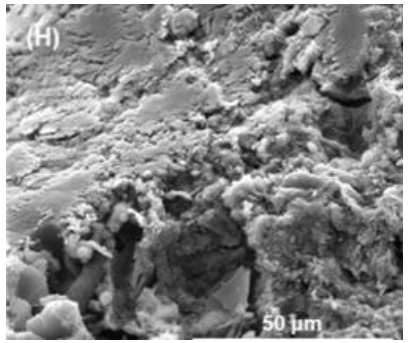


Activated sludge biochar (SEM)

250 °C

500 °C





Saccharomyces cerevisiae vs *Pichia kudriavzevii*

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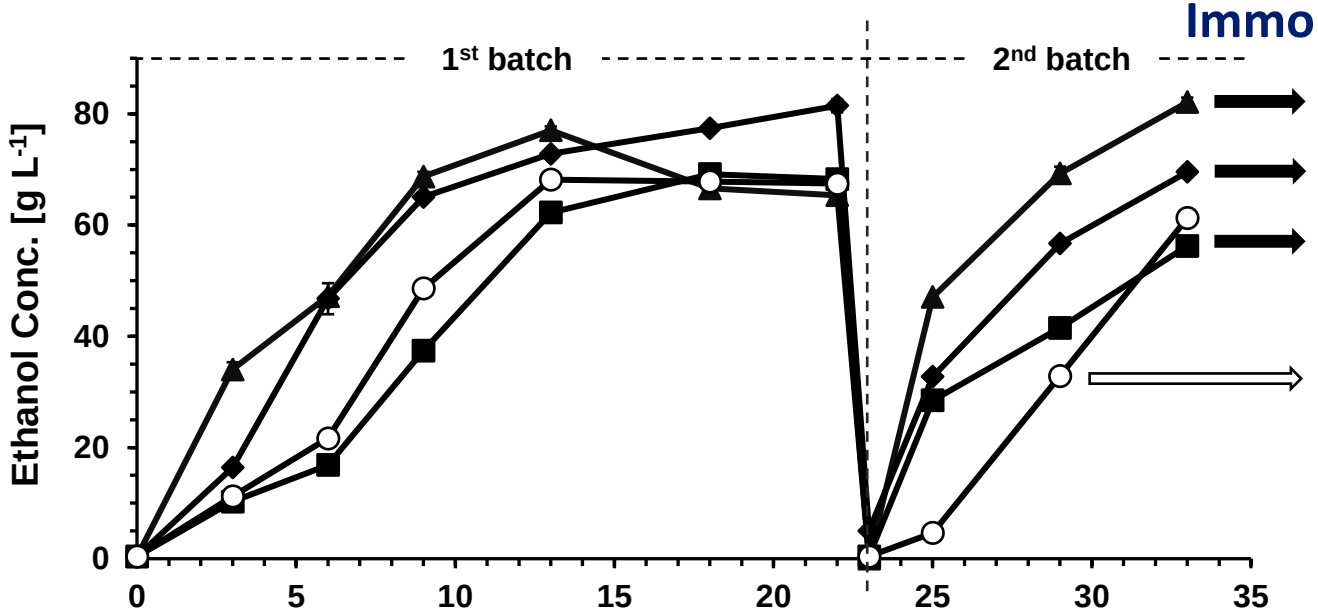
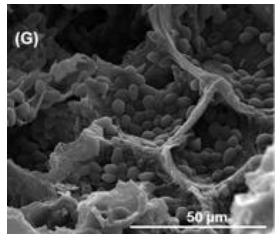
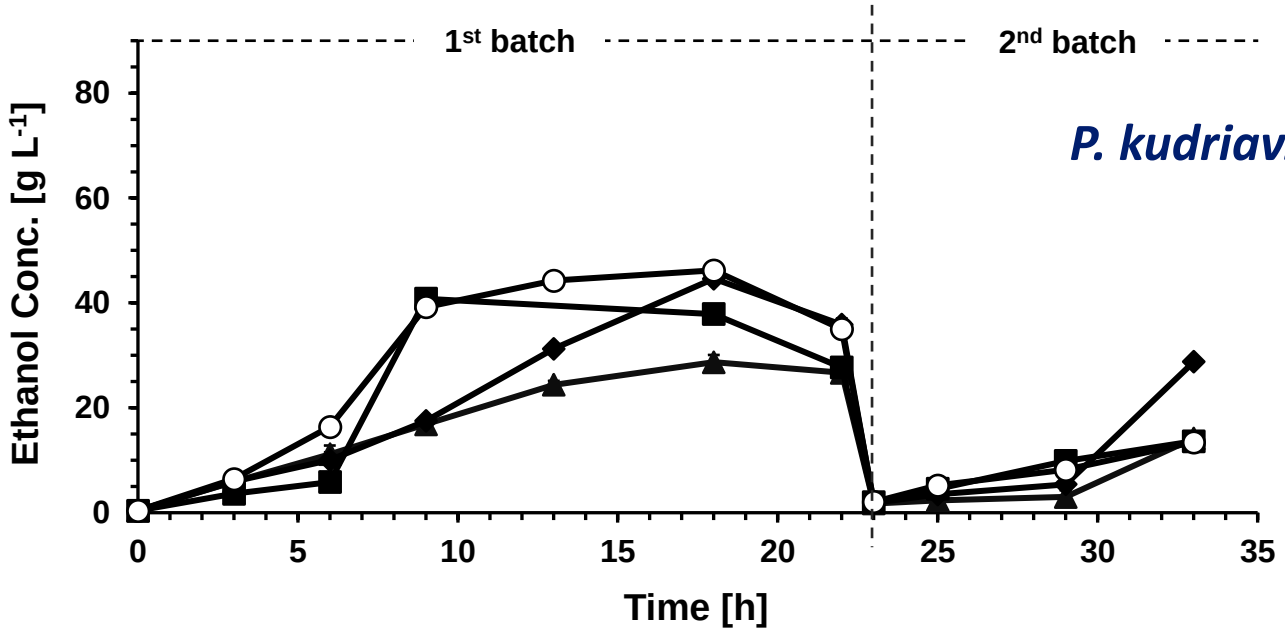
Immobilized *S. cerevisiae*





Suspended cells



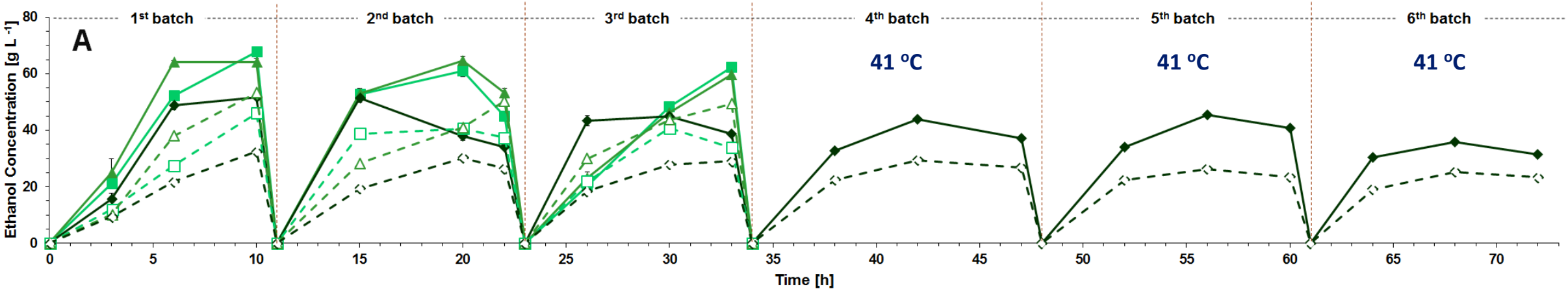
P. kudriavzevii

Vineyard prunings biochar: **8.21 g L^{-1} h^{-1}**

Different materials – different efficiencies

Different strains – different efficiencies

S. cerevisiae BBBs at Elevated Temperatures



BBBs vs suspended cultures

37, 39 & 41 °C to assess **thermotolerance**

Synthetic Citrus Peel Waste-based hydrolysate

Reduced bioethanol production at 41 °C

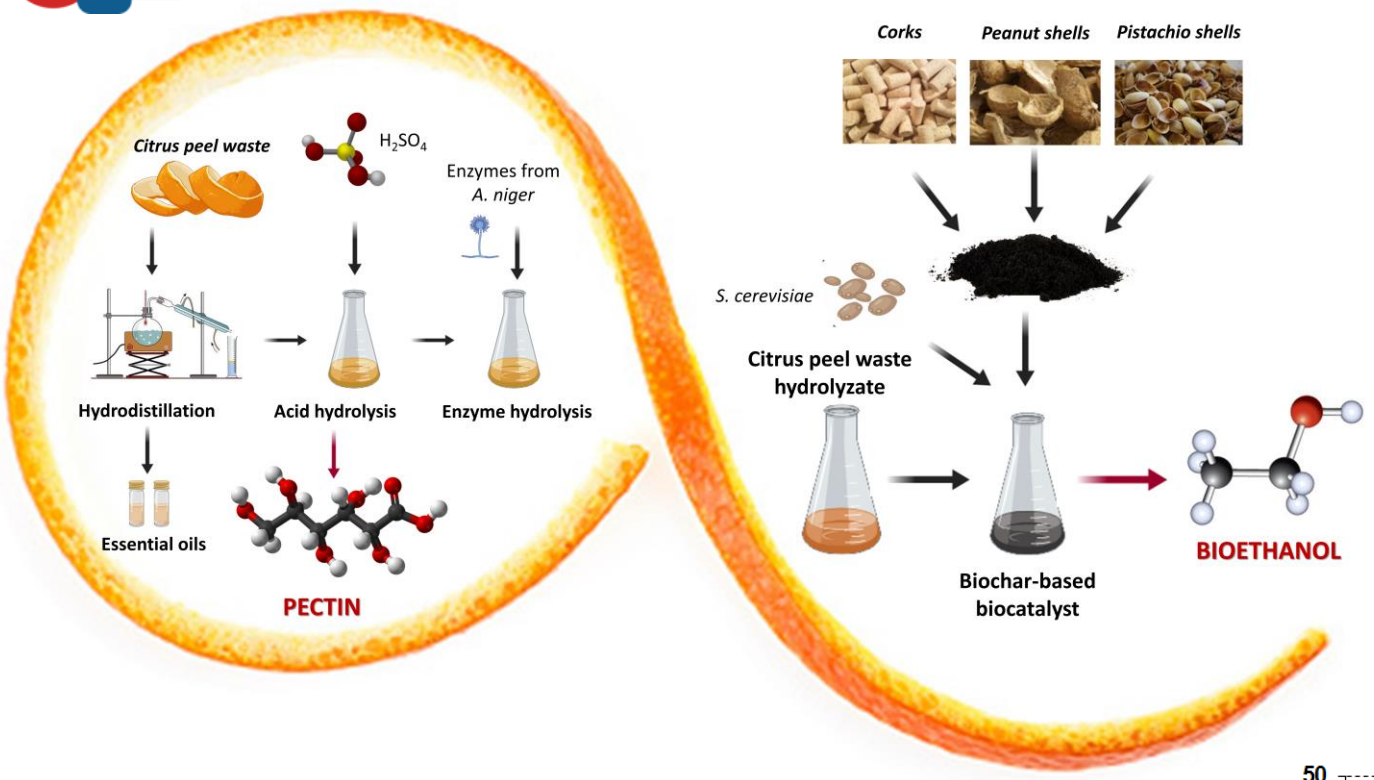
Productivity of last 3 batches (41 °C)

- **BBB:** 5.1-5.7 g L^{-1} h^{-1}
- **Free cells:** 3.3-3.7 g L^{-1} h^{-1}

Maximum **bioethanol concentration** at 41 °C

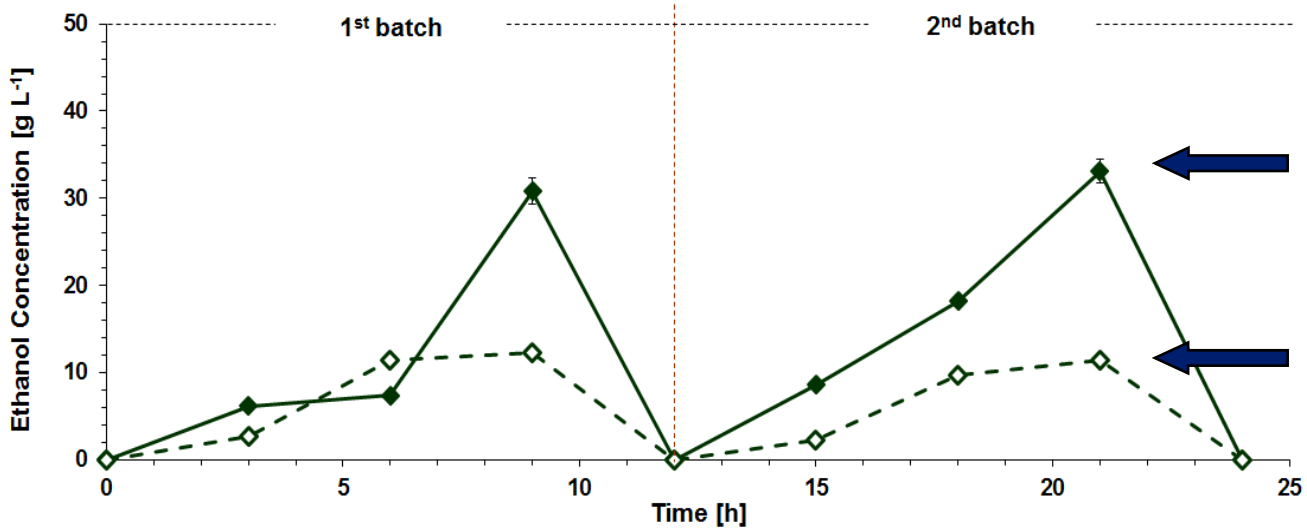
- **BBB:** 51.6 g L^{-1}
- **Free cells:** 32.4 g L^{-1}

BBBs vs Suspended Cultures in CPW Biorefinery Hydrolysate

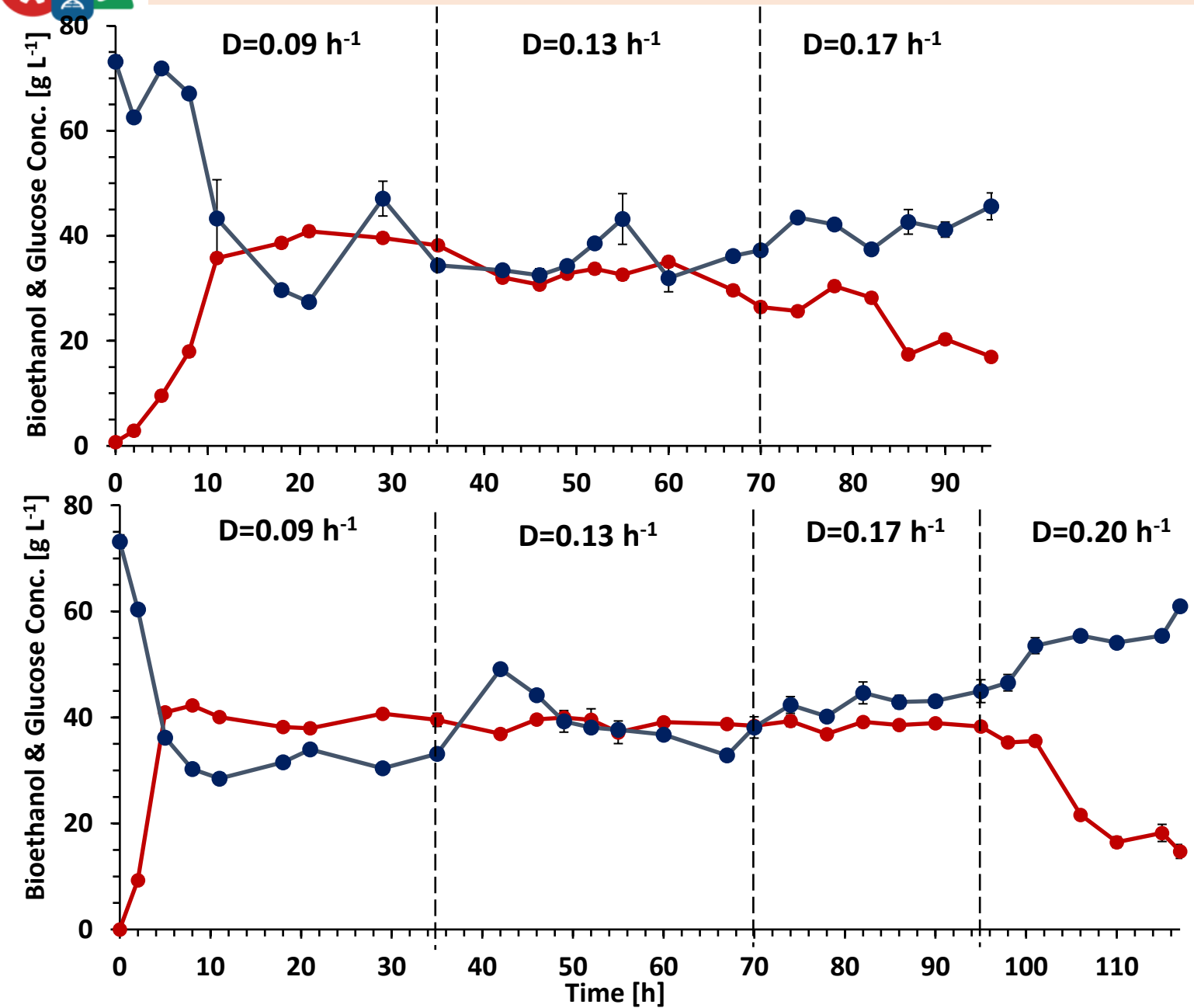


Maximum **bioethanol concentration** at **41 °C**

- **BBB: 33 g L^{-1}**
- **Free cells: 12 g L^{-1}**



Continuous Bioethanol Production by *S. cerevisiae*



37 °C, 75 g L^{-1} glucose feeding

Suspended cells

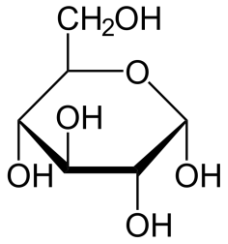
Immobilized cells

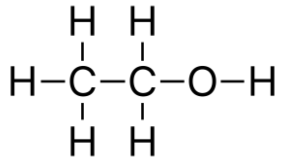
Bioethanol concentration

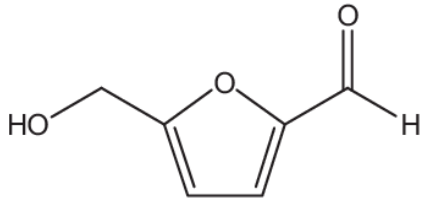
Glucose concentration

Challenges in Bioethanol Production

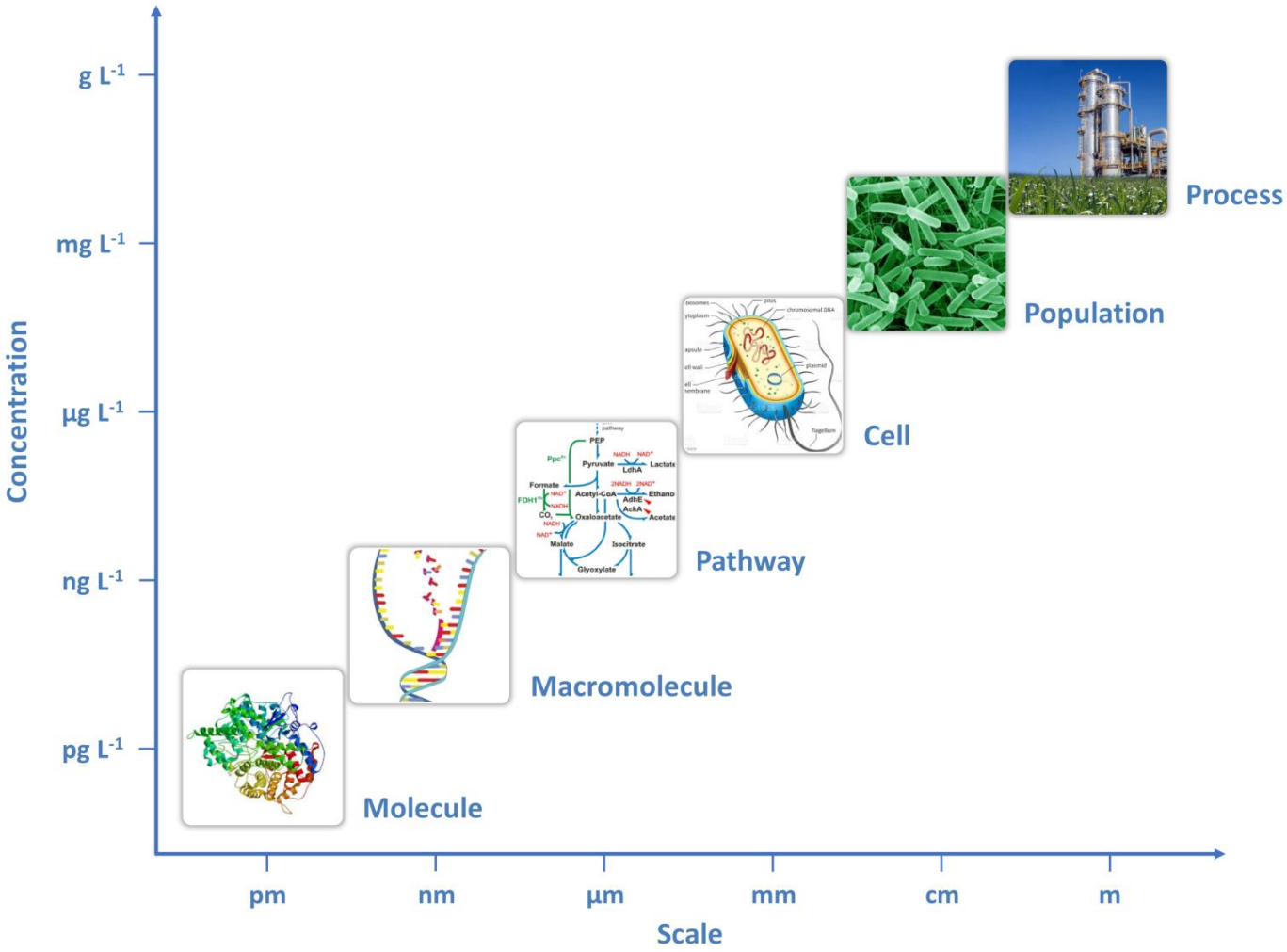
- **Substrate** inhibition
- **Product** inhibition
- **Inhibitors** released in pretreatment
- High **temperatures** (> 37 ºC *S. cerevisiae*)
- **Osmotic** stress
- **pH** variations



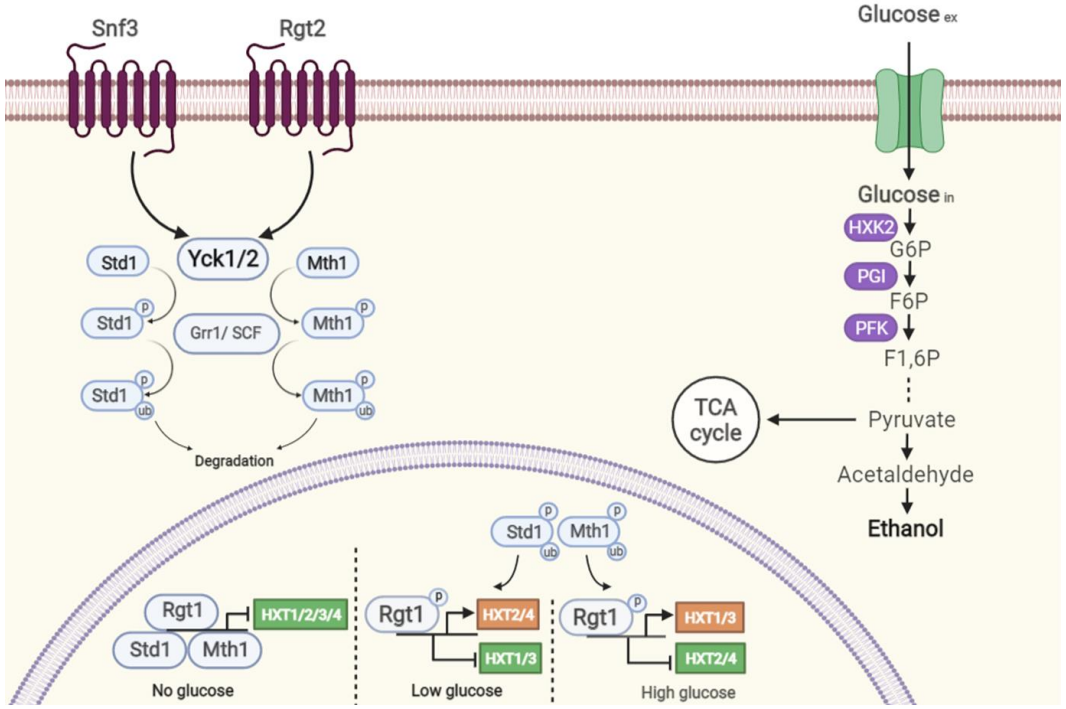




From the macro-scale to molecular mechanisms



Metabolic responses in *S. cerevisiae*



Sensing systems and complex signalling networks responding to variations in:

Temperature, osmolarity, inhibitors, etc.

Biochar confers stress multi-tolerance?

S. cerevisiae Fermentations Under Heat Shock

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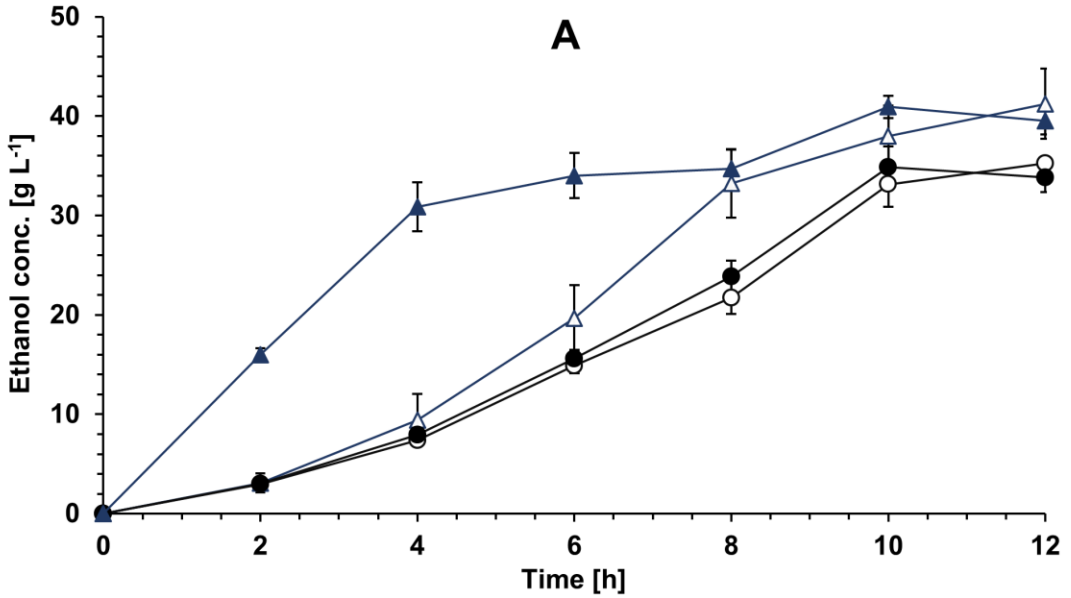
Metabolic Responses

- ❑ **Heat shock response**
- ❑ **Ethanol stress** response
- ❑ Osmotic stress response
- ❑ Oxidative stress response

***TPS*: controlling **trehalose** synthesis**

HSF1*: regulated by trehalose encoding for **heat shock transcription factor*

***HSP104*: **refolding** of misfolded proteins**



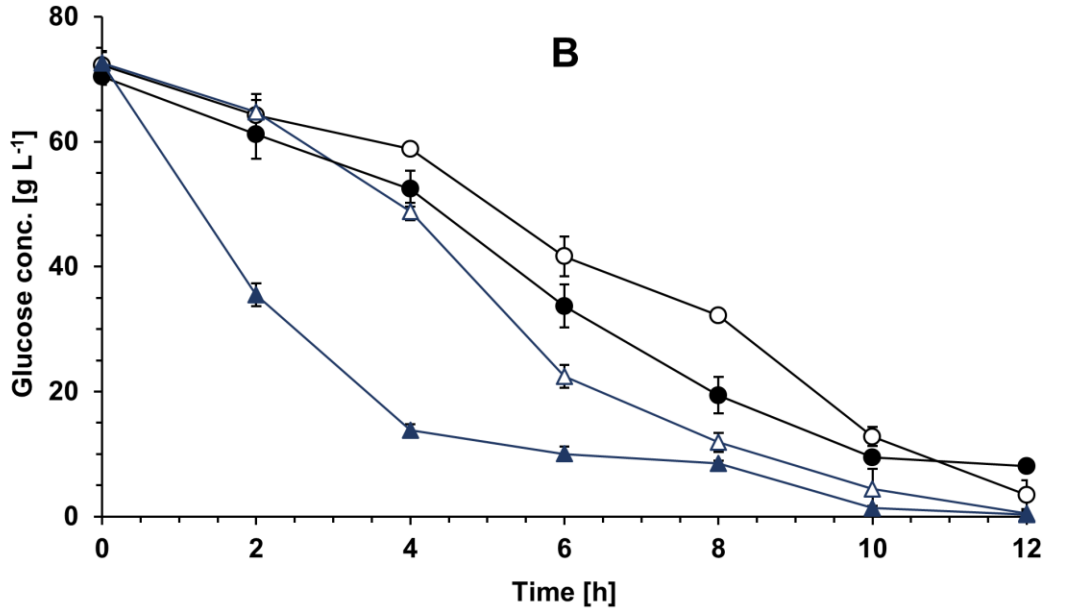
30 °C and **39 °C**

Similar results using free cells for 30 °C and 39 °C

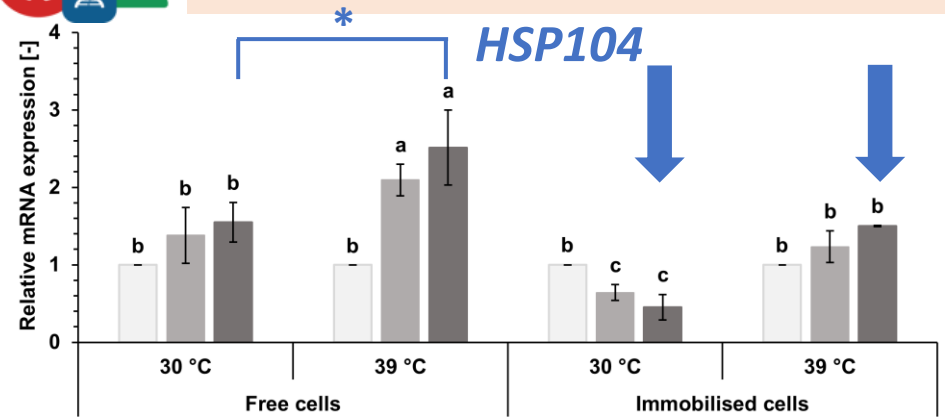
BBB vs free cells at 39 °C

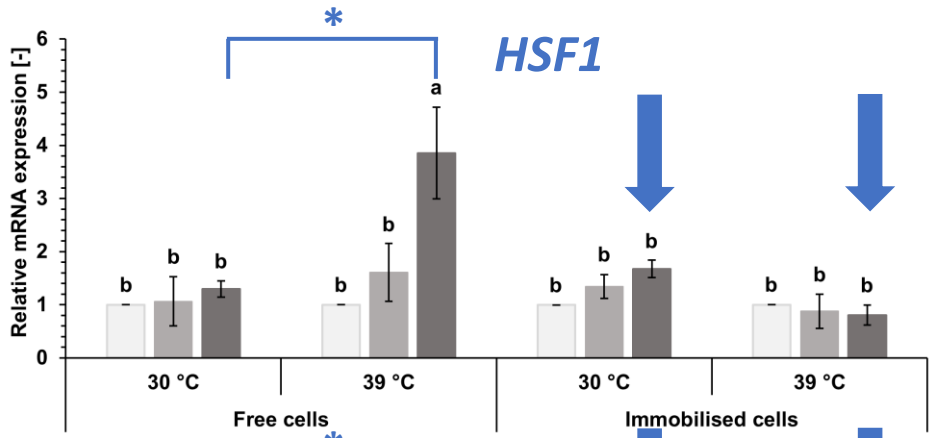
39 g L^{-1} vs 34 g L^{-1}

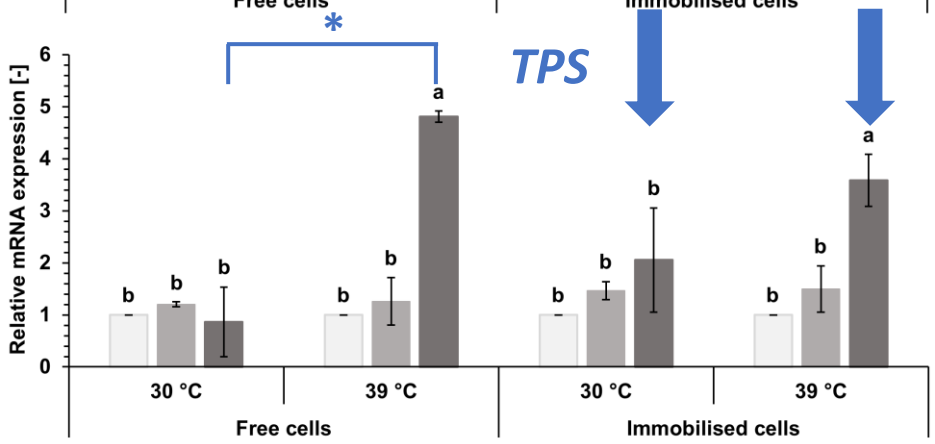
7.7 g L^{-1} h^{-1} vs 2.0 g L^{-1} h^{-1}



Expression from the Heat Shock Response Pathway

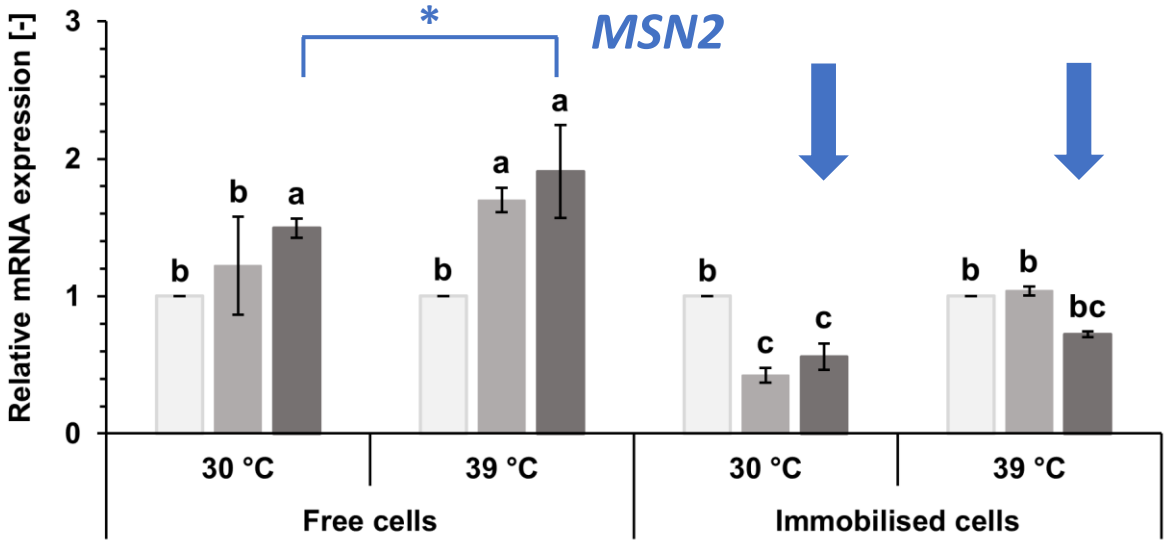


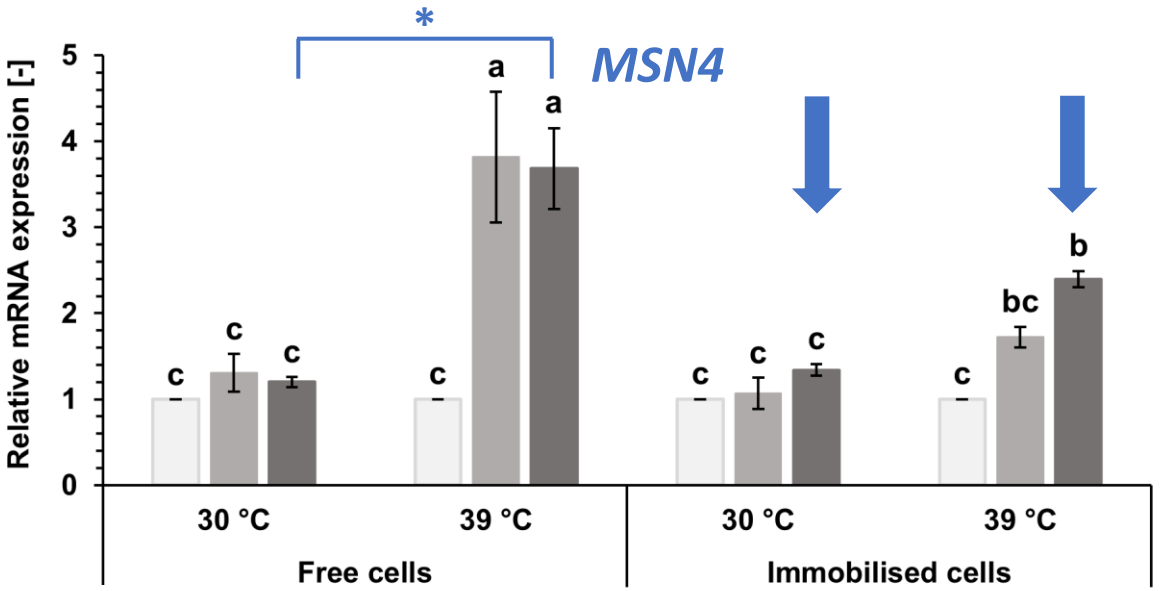




- Heat shock response route **not induced using BBB**
- ***HSF1***: **3-fold** increase in free cells and **0-fold** increase in BBB
- ***HSP104*: 2.2-fold** increase in free cells and **0.5-fold** increase in BBB
- Stress induction in free cells
- Biochar protects *S. cerevisiae* enhancing **heat tolerance**

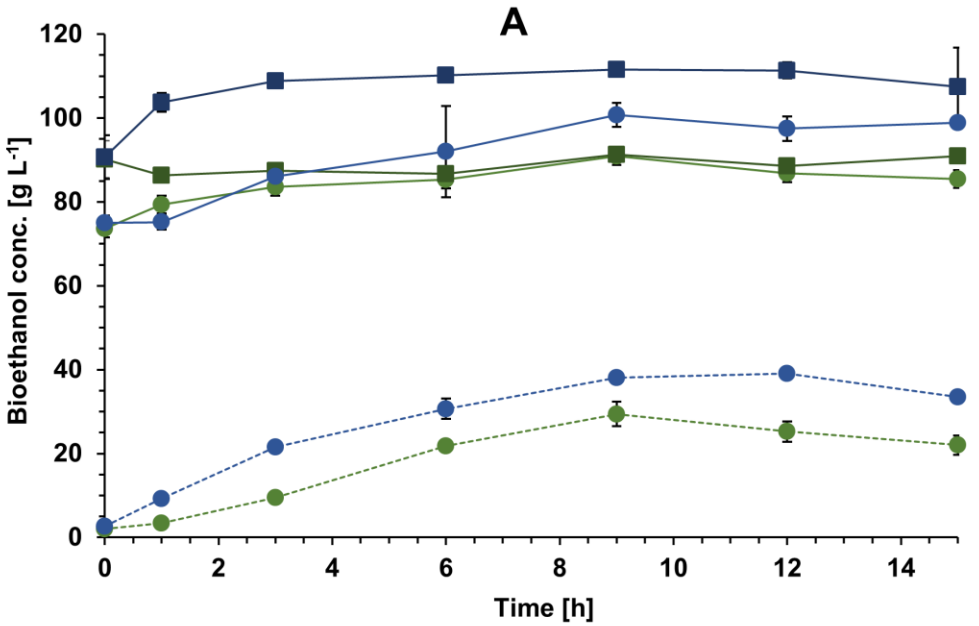
Expression from Genes Induced by Oxidative Stress

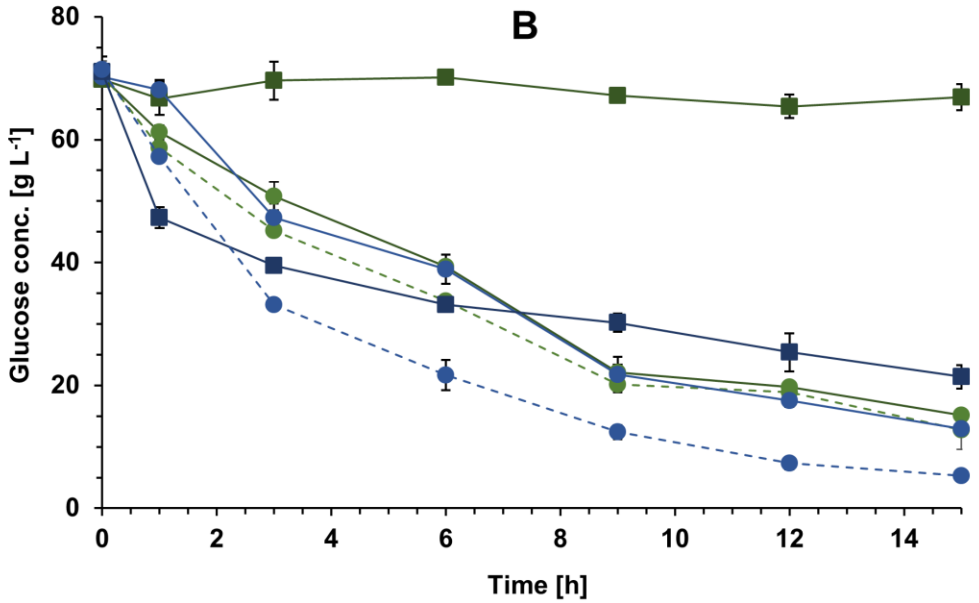




- Reactive oxygen species during heat shock
- **Oxidative stress** expressed by the ***MSN2/MSN4*** system
- ***MSN2***: **2.0-fold** increase in free cells and **0.3-fold <u>decrease</u>** in BBB
- ***MSN4*: 2.7-fold** increase in free cells and **1.4-fold** increase in BBB
- Stress induction in free cells
- Biochar protects *S. cerevisiae* **enhancing tolerance to oxidative stress**

S. cerevisiae Fermentations Under Ethanol Stress





70 g L^{-1} glucose, 30 ^{o}C

Similar results using 0 and 70 g L^{-1} initial ethanol conc.

<u>90 g L^{-1} initial ethanol conc.</u>

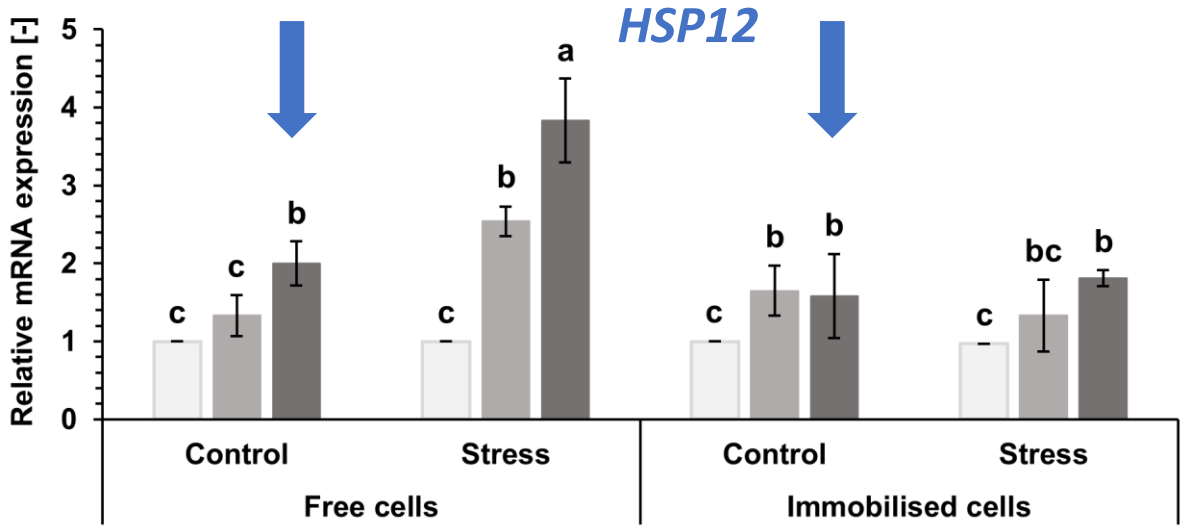
BBB: 21 g L^{-1} net ethanol, 7 g L^{-1} h^{-1} productivity, inhibition at 111 g L^{-1}

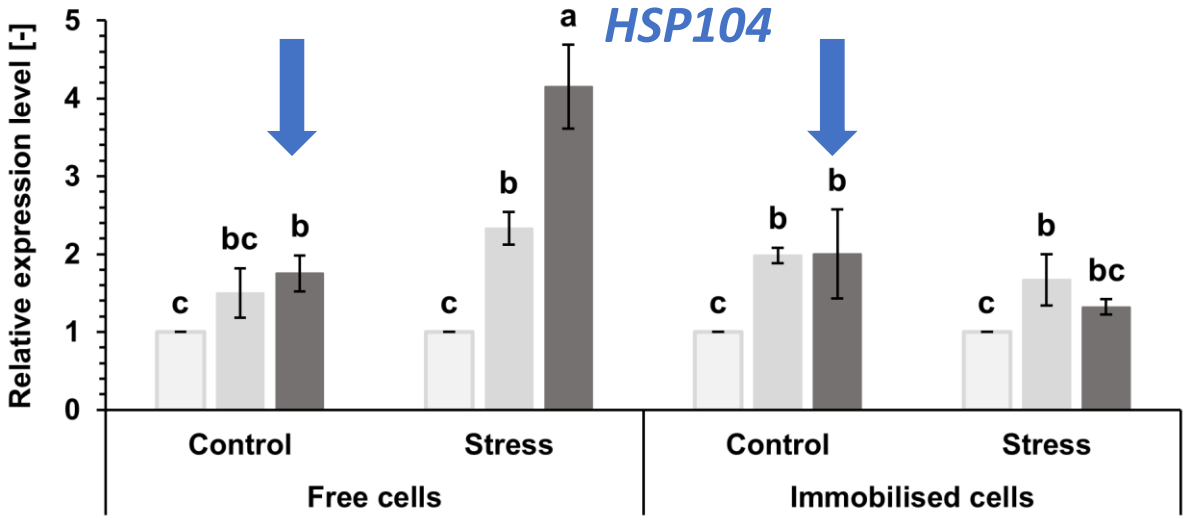
Free cells: complete inhibition (90 g L^{-1})

Ethanol stress induces heat shock proteins (HSP) similarly to heat shock

HSP12 and ***HSP104*** confirmed to influence yeast tolerance to ethanol

Expression from Genes Induced by Ethanol Stress





Control: 0 g L^{-1} ethanol, 70 g L^{-1} glucose, 30 °C

Stress: 90 g L^{-1} ethanol, 70 g L^{-1} glucose, 30 °C

Control: Minor differences in expression from *HSP12* and *HSP104* in suspended cells and BBB

Stress

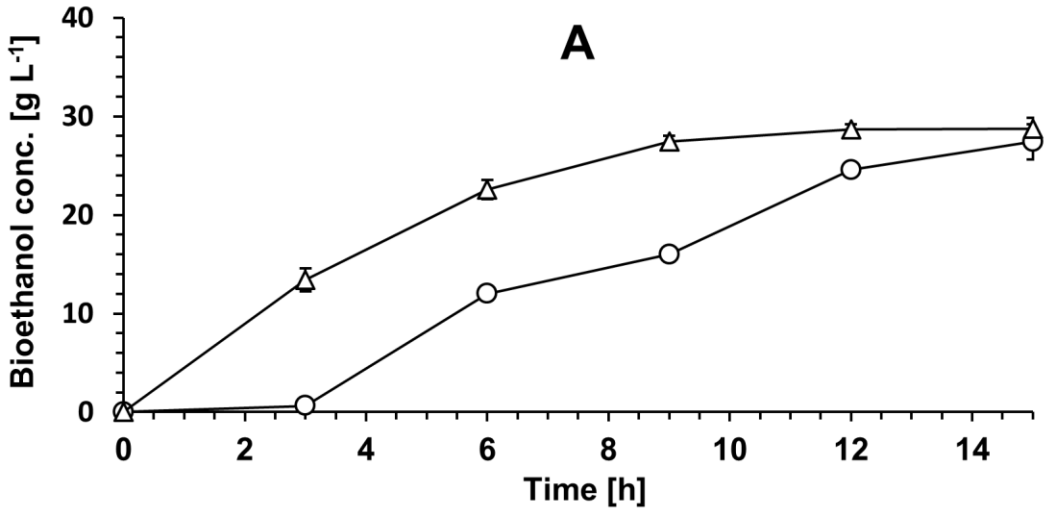
***HSP12*: 2.8-fold** increase in free cells and **0.8-fold** increase in BBB

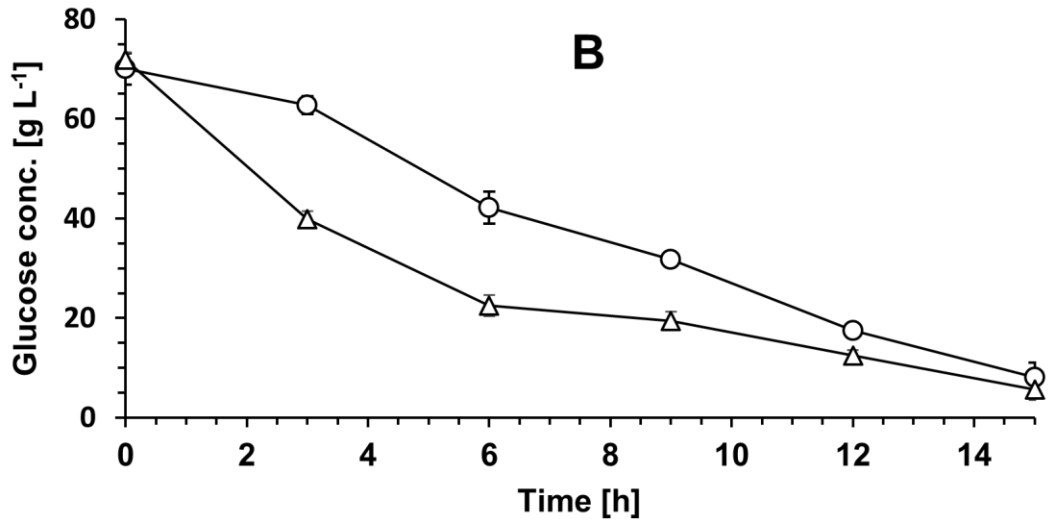
***HSP104*: 3.2-fold** increase in free cells and **0.3-fold** increase in BBB

Both genes upregulated in free cells **only at 90 g L^{-1} ethanol**

BBB enhanced **ethanol tolerance**

S. cerevisiae Fermentations Under Osmotic Stress





1 M NaCl, 70 g L^{-1} glucose, 30 ^{o}C

Similar final bioethanol concentration, but 74% higher productivity

Free cells: 1.8 g L^{-1} h^{-1}

BBB: 3 g L^{-1} h^{-1}

BBB induced osmoprotection

Osmotic stress response signalling pathway regulates osmoprotection

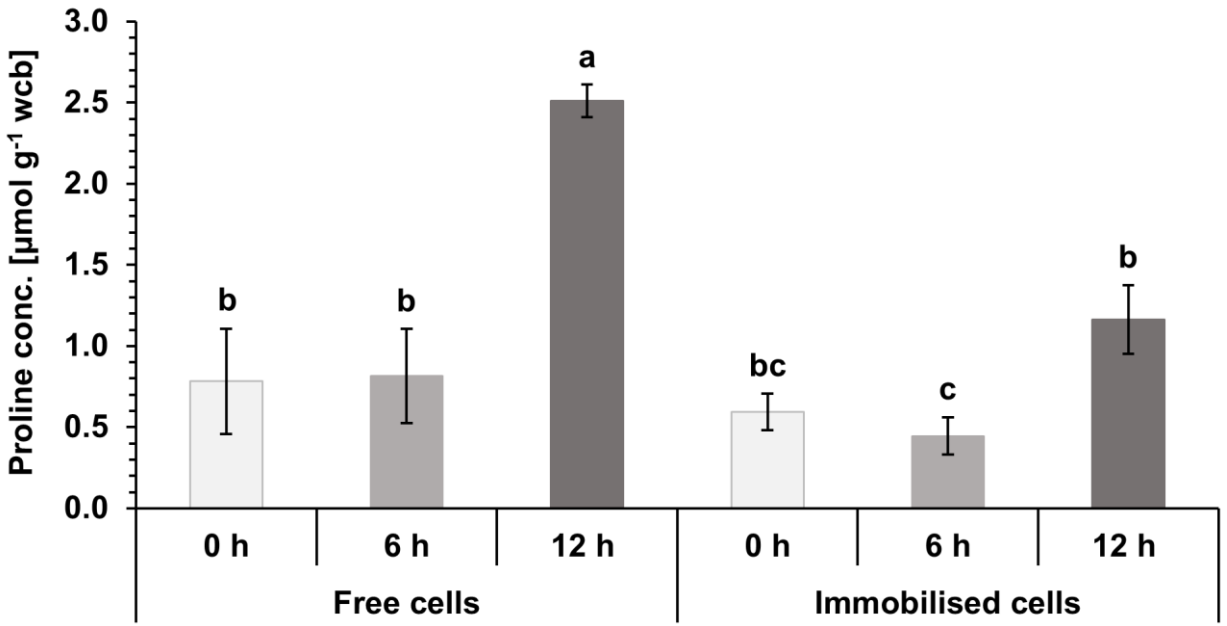
Proline accumulation:

Membrane stabilizer

Protein folding chaperone

Reactive oxygen species scavenger

Accumulation of Intracellular Proline by Osmotic Stress



1 M NaCl, 70 g L^{-1} glucose, 30 °C

Intracellular proline in free cells reached 2.5 µmol g^{-1} wet biomass

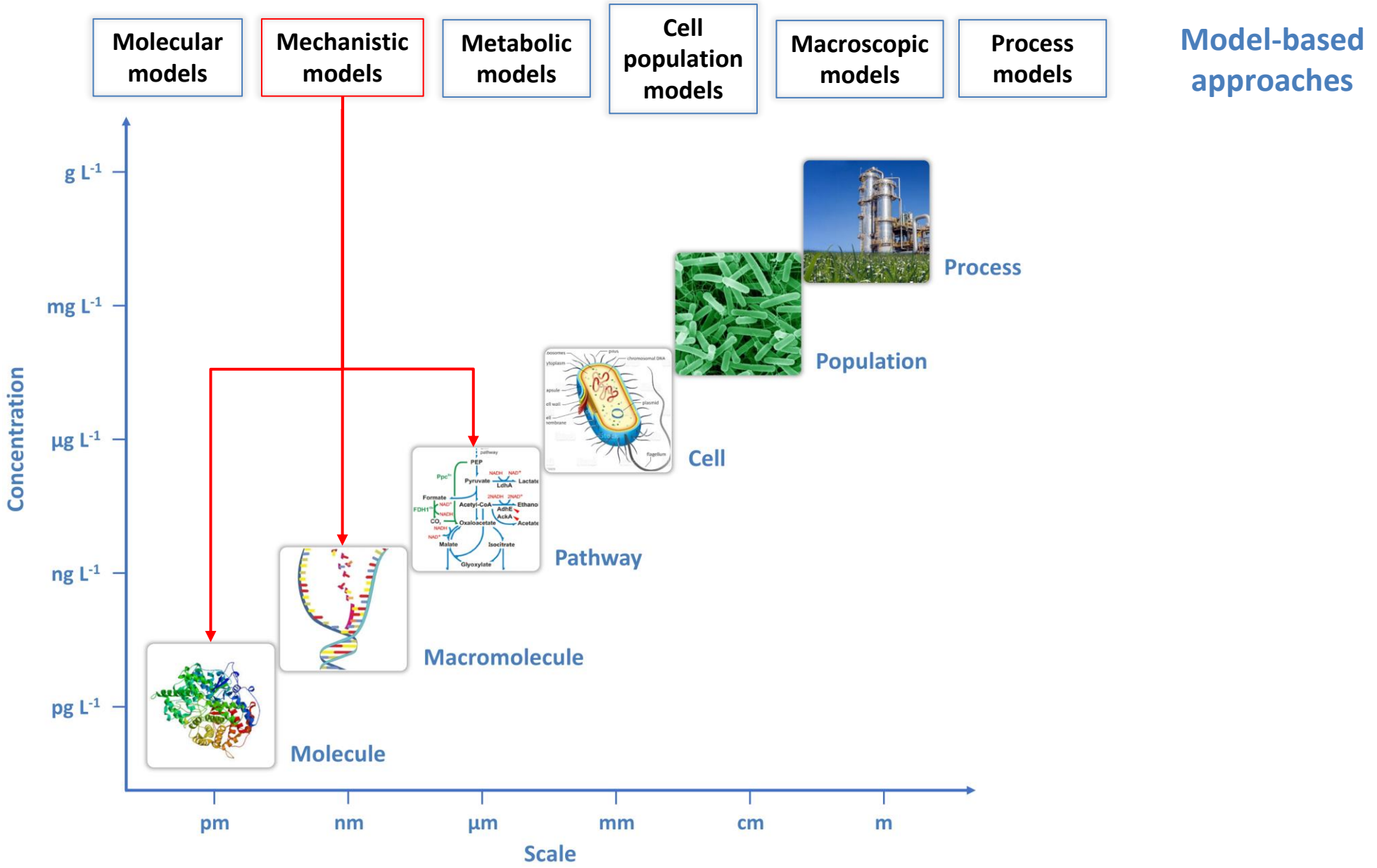
Increase of intracellular proline in BBB **was not significant**

At least **2-fold higher proline accumulation** in free cells

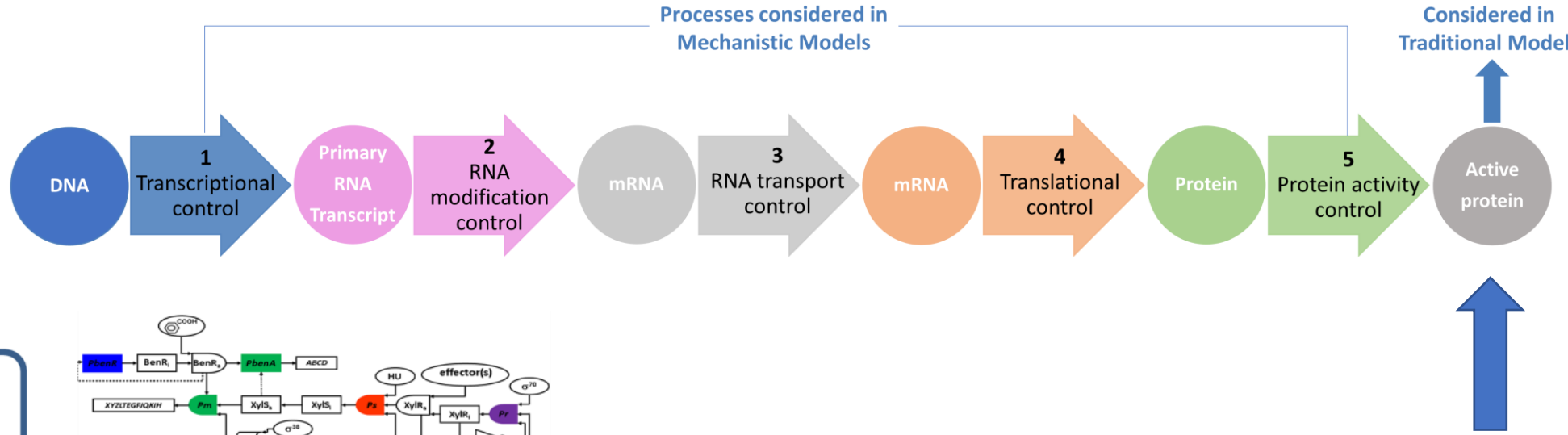
Cells tackle the inhibition resulting in reduced bioprocess performance

From a "*Trial-and-Error*" to a Model-Based Approach

Cyprus University of Technology

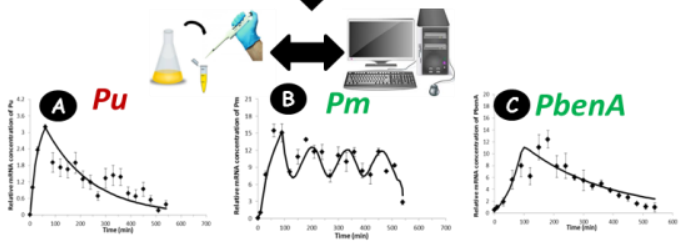


Mechanistic Models of Gene Regulation

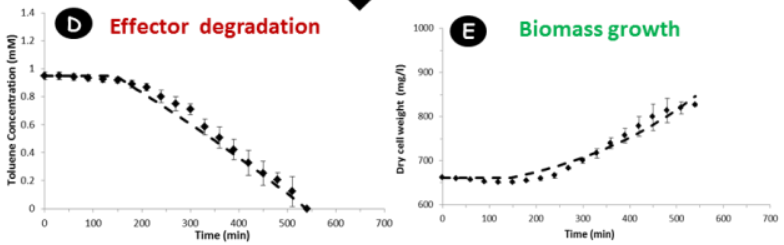


Genetic circuit design

Gene expression kinetics



Prediction of bioprocess performance



Alternative to **Monod-type** traditional models

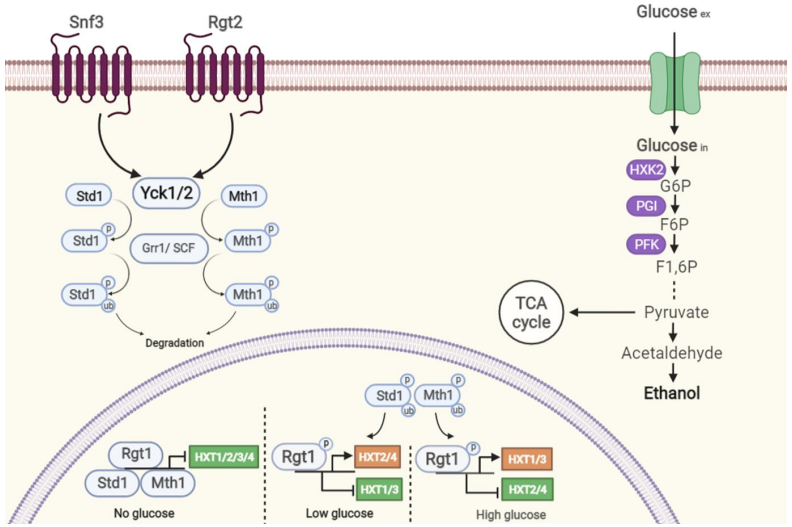
- Kinetics of a **simple enzyme catalysed reaction**
- **Microbial growth** based on the consumption of a single substrate

Linking Gene Regulation to Ethanol Bioprocess Kinetics

Cyprus University of Technology

Glucose sensing, signalling and bioconversion

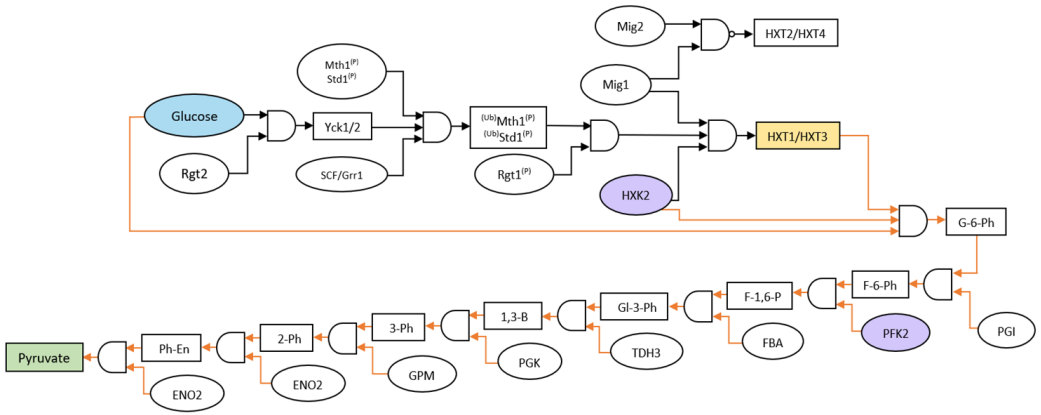
Biochemical Description



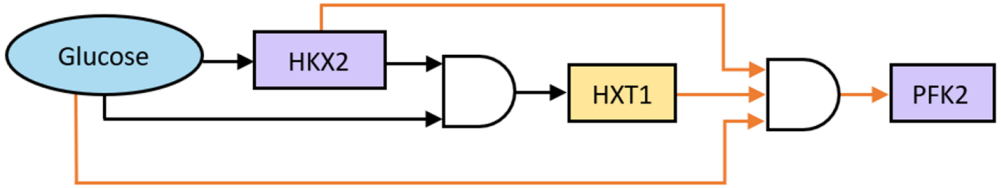
Genetic Circuit Model

C_{mHXK2}	$\frac{d[C_{mHXK2}]}{dt} = \beta_{HXK2} \cdot \frac{[C_{Glu}]^{n_{Glu,HXK2}}}{[K_{Glu,HXK2}]^{n_{Glu,HXK2}} + [C_{Glu}]^{n_{Glu,HXK2}}} - \alpha_{HXK2}\, C_{mHXK2}$
C_{mHXT1}	$\frac{d[C_{mHXT1}]}{dt} = \beta_{HXT1} \cdot \left[\frac{[C_{Glu}]^{n_{Glu,HXT1}}}{[K_{Glu,HXT1}]^{n_{Glu,HXT1}} + [C_{Glu}]^{n_{Glu,HXT1}}} \cdot \frac{[C_{mHXK2}]^{n_{HXK2,HXT1}}}{[K_{HXK2,HXT1}]^{n_{HXK2,HXT1}} + [C_{mHXK2}]^{n_{HXK2,HXT1}}} \right] - \alpha_{HXT1}\, C_{mHXT1}$
C_{mPFK2}	$\frac{d[C_{mPFK2}]}{dt} = \beta_{PFK2} \cdot \left[\frac{[C_{Glu}]^{n_{Glu,PFK2}}}{[K_{Glu,PFK2}]^{n_{Glu,PFK2}} + [C_{Glu}]^{n_{Glu,PFK2}}} \cdot \frac{[C_{mHXK2}]^{n_{HXK2,PFK2}}}{[K_{HXK2,PFK2}]^{n_{HXK2,PFK2}} + [C_{mHXK2}]^{n_{HXK2,PFK2}}} \cdot \frac{[C_{mHXT1}]^{n_{HXT1,PFK2}}}{[K_{HXT1,PFK2}]^{n_{HXT1,PFK2}} + [C_{mHXT1}]^{n_{HXT1,PFK2}}} \right] - \alpha_{PFK2}\, C_{mPFK2}$

Logic Description



Reduced Logic Description



Conclusions & Future Plans

- Biochar includes advanced characteristics for immobilization
- Different raw materials – **different properties**
- Different strains – **different efficiencies**
- **Enhanced ethanol production**
- High ethanol productivity via *S. cerevisiae* at **elevated temperatures**
- Immobilization conferred cells with **multi-stress tolerance** (heat tolerance, ethanol tolerance, osmotolerance)
- **Tailor-made (nano)biochar** for cells and hydrolytic enzymes immobilization

Thank You!

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