Cyprus University of Technology

Environmental Bioprocessing Laboratory

Metabolic responses of *Saccharomyces cerevisiae* to environmental stresses during bioethanol production using biochar-based biocatalysts

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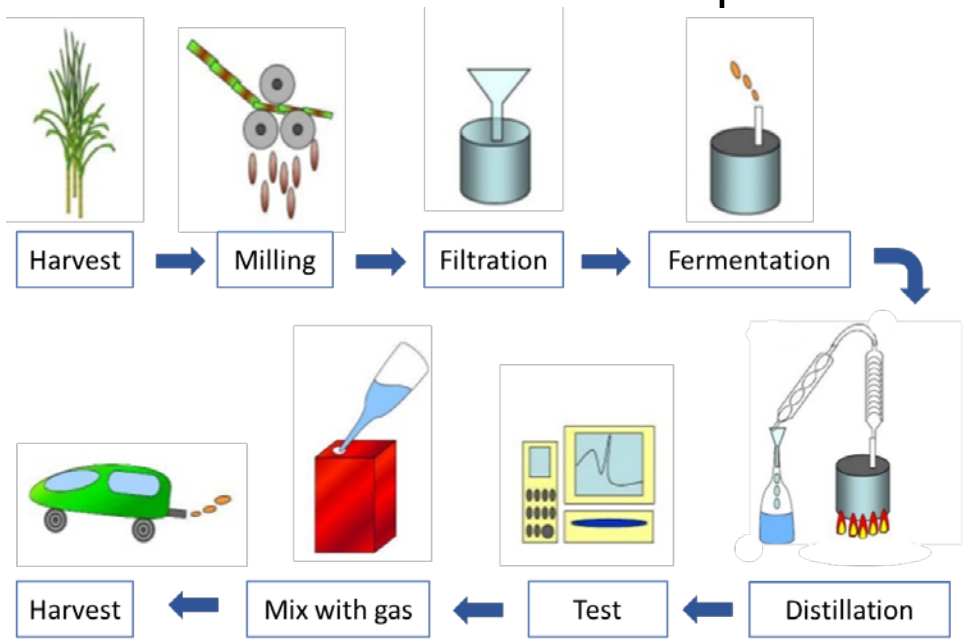
Technology of bioethanol

- Biodiesel and bioalcohols constitute the most important biofuels



- Highlights the need to identify alternative feedstocks for sustainable manufacturing
- Current limitations of bioethanol technology:
  - **Cooling and distillation cost**
  - **Lack of rigid carriers for recirculation of yeasts**

Biochar as a yeast immobilization carrier

- Substrate and product inhibition
- **Immobilized biocatalysts**
- Alginate gel beads have poor mechanical properties
- **Biochar**
- Environmental management and soil amendment
- Immobilization of heavy metals
- Enhance methane production in anaerobic digestion
- Promotes interspecies electron transfer
- Improves cell activity and growth
- Assists buffering capacity and nutrient adsorption into their surface
- **Biochar-based biocatalyst technology**

Environmental stresses of *S. cerevisiae*

- Faces two important environmental challenges
- **Heat shock**
- **Oxidative stress**

- *Saccharomyces cerevisiae* exhibits optimal growth at temperatures between **25 – 30 ºC**
- At temperatures > **36 – 37 ºC** → **Heat Shock Response**

- **Ethanol stress**
- Ethanol in excess of **9%** can affect the growth of *Saccharomyces cerevisiae* cells.

Aim of the study

- Evaluate the use of **biochar-based biocatalysts** for bioethanol production at **elevated temperatures.**

- Assess the **stress-protective role** of the technology proposed against **heat**, **oxidative** and **ethanol** stress during fermentation.

Biochar-based biocatalyst production

Pistachios shells

Pyrolysis 500°C

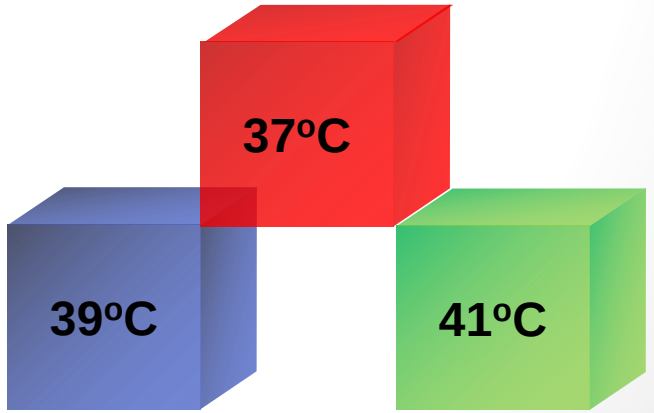
Biochar

Immobilization

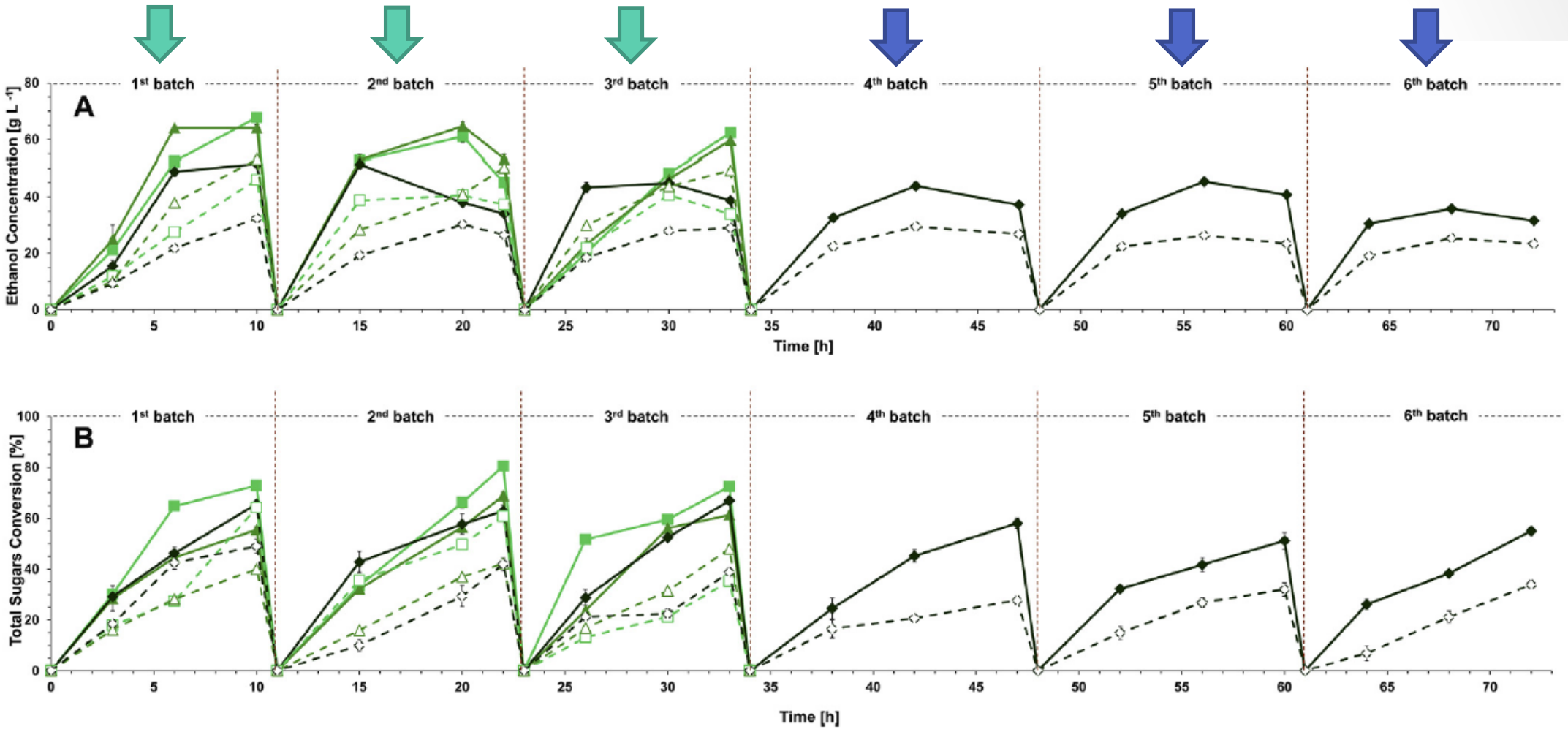
Bioethanol fermentations

Liquid media simulating an orange peel waste hydrolysate that consisted of :

- yeast extract
- peptone
- fructose
- galactose
- glucose
- sucrose



Application of BBB in repeated batch fermentations



Immobilized cells 37 ºC

Immobilized cells 39 ºC

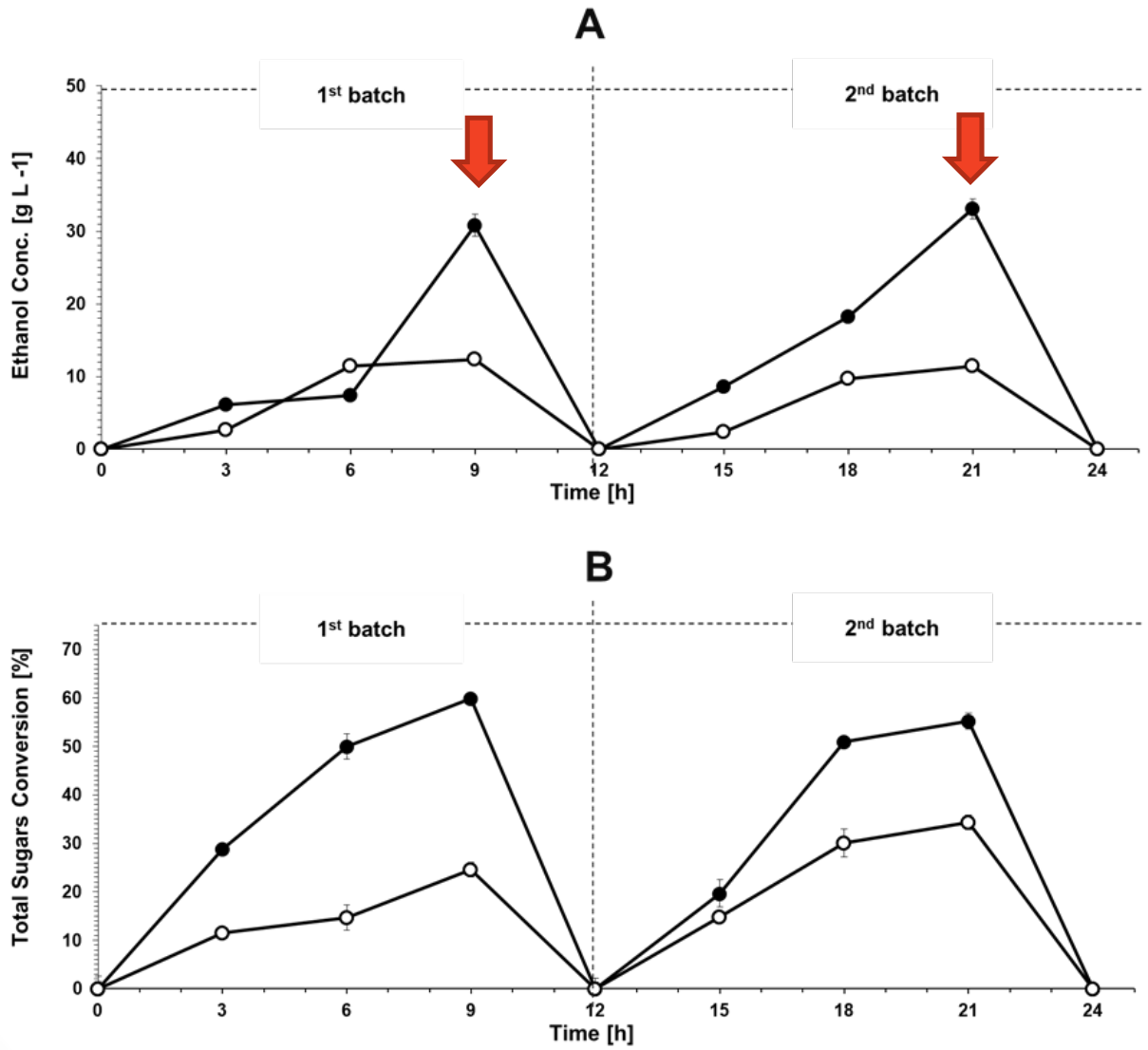
Immobilized cells 41 ºC

Free cells 37 ºC

Free cells 39 ºC

Free cells 41 ºC

Application of BBB in CPW hydrolysate



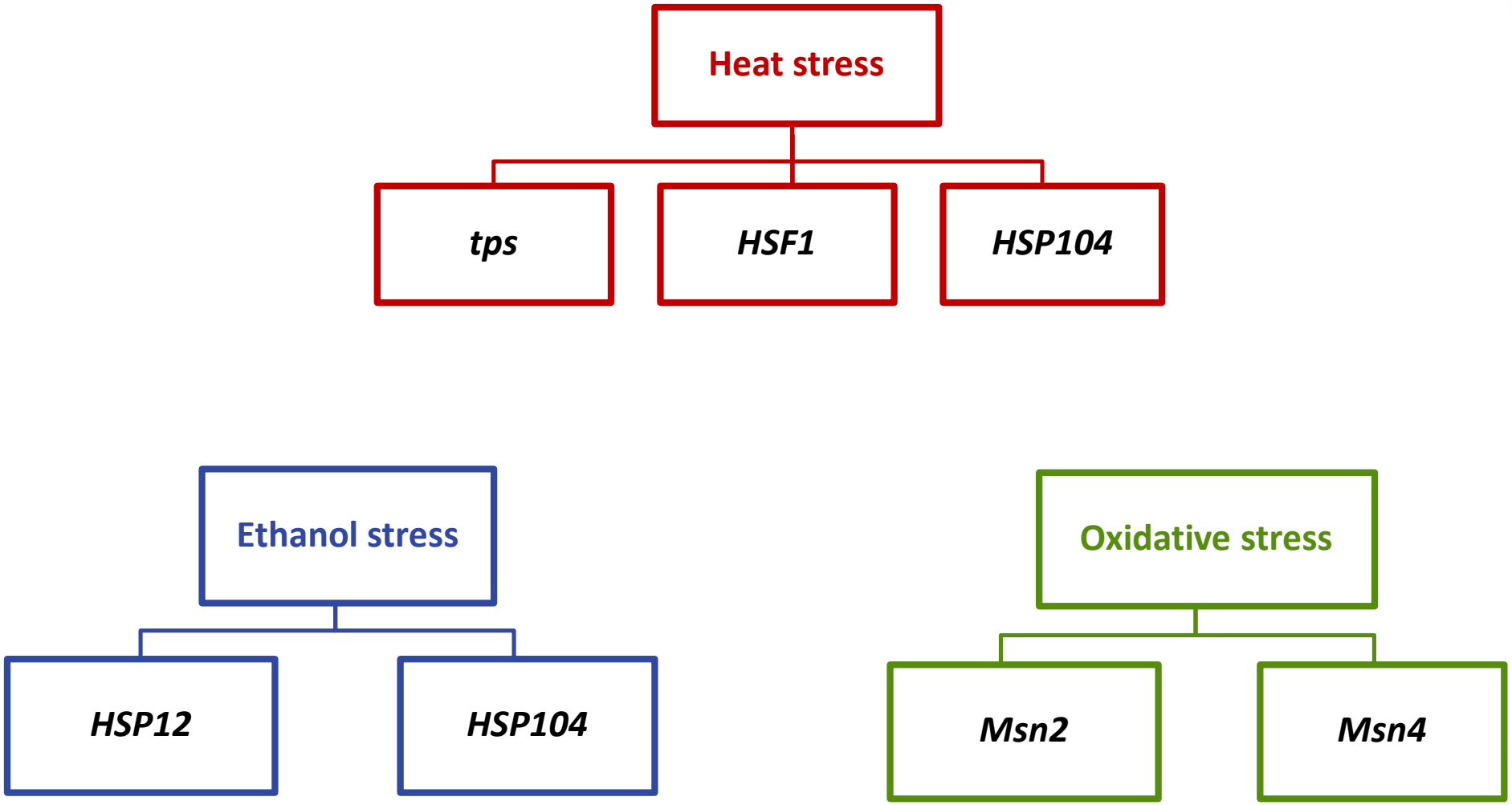
Temperature

41 °C

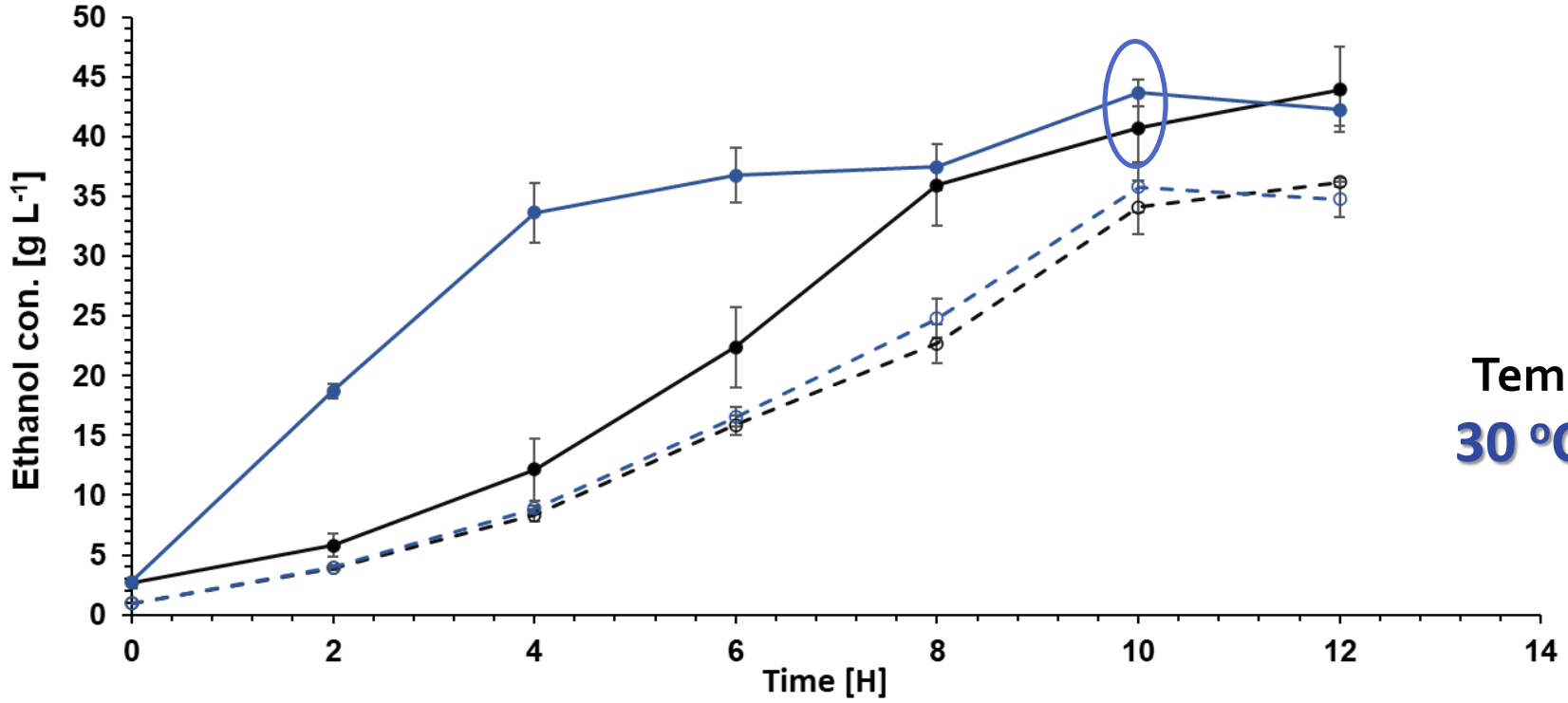
Immobilized cells 33 g L^{-1}

Free cells 12 g L^{-1}

Environmental stresses of *S. cerevisiae*



Heat shock response of *S. cerevisiae*



Temperature

30 °C & 39 °C

Liquid media consisted of :

- yeast extract
- peptone
- glucose

Immobilized cells 39 °C

Free cells 39 °C

Immobilized cells 30 °C

Free cells 30 °C

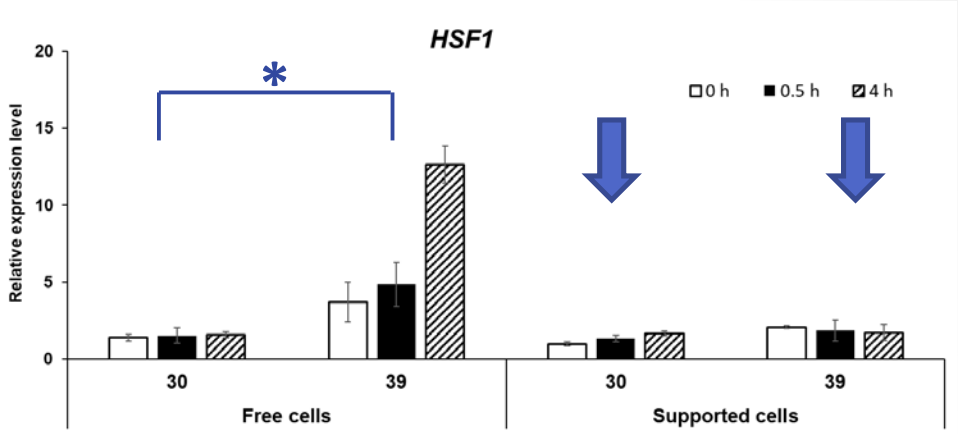
Heat Shock Stress Response

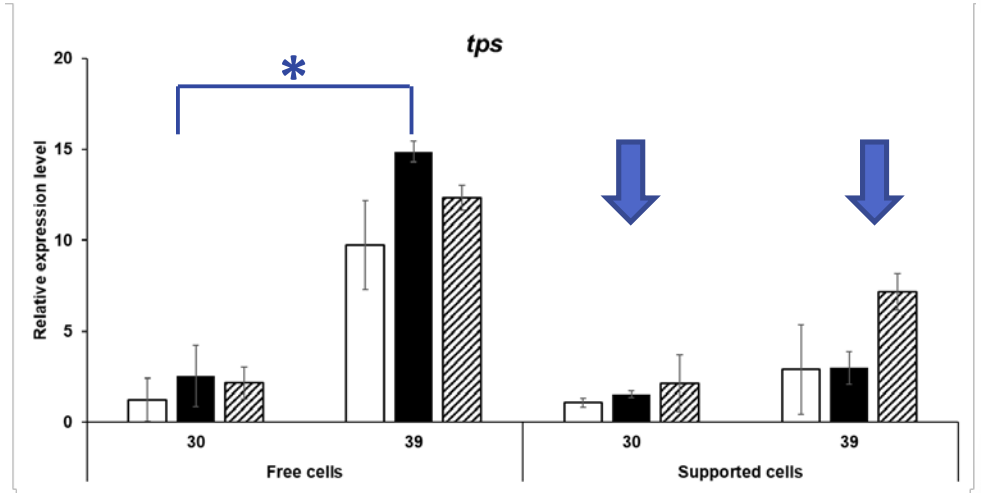
- Freely suspended cells

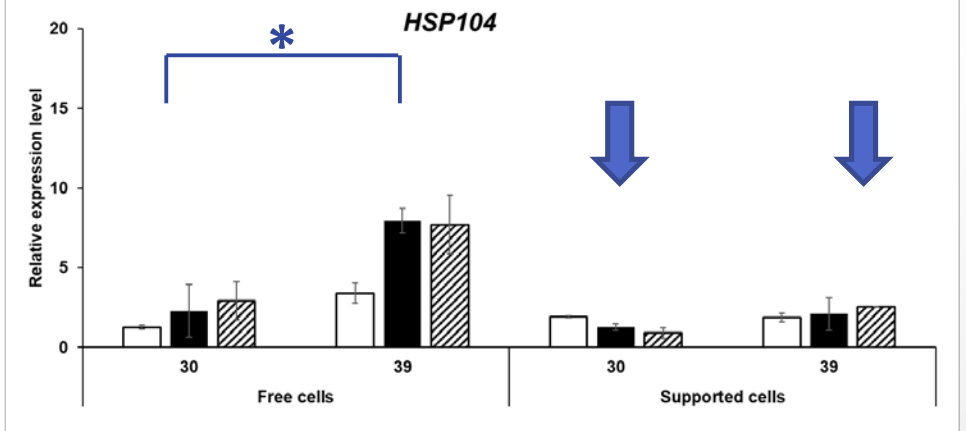
HSF1 | HSP104 | tps

- **Higher** Relative expression

The heat-shock response pathway was not triggered using biochar-based biocatalyst

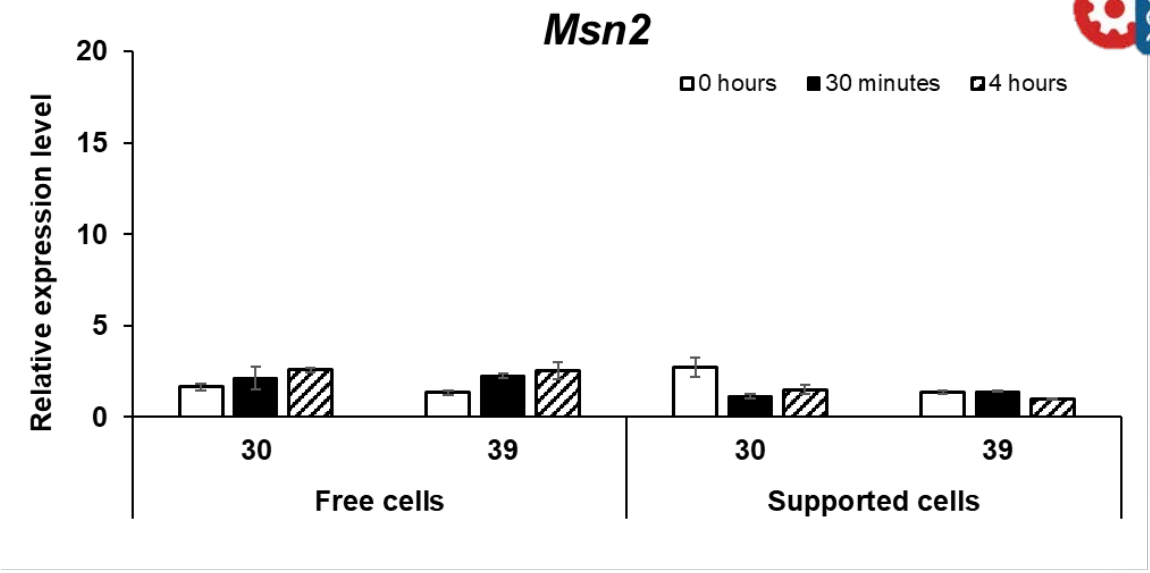


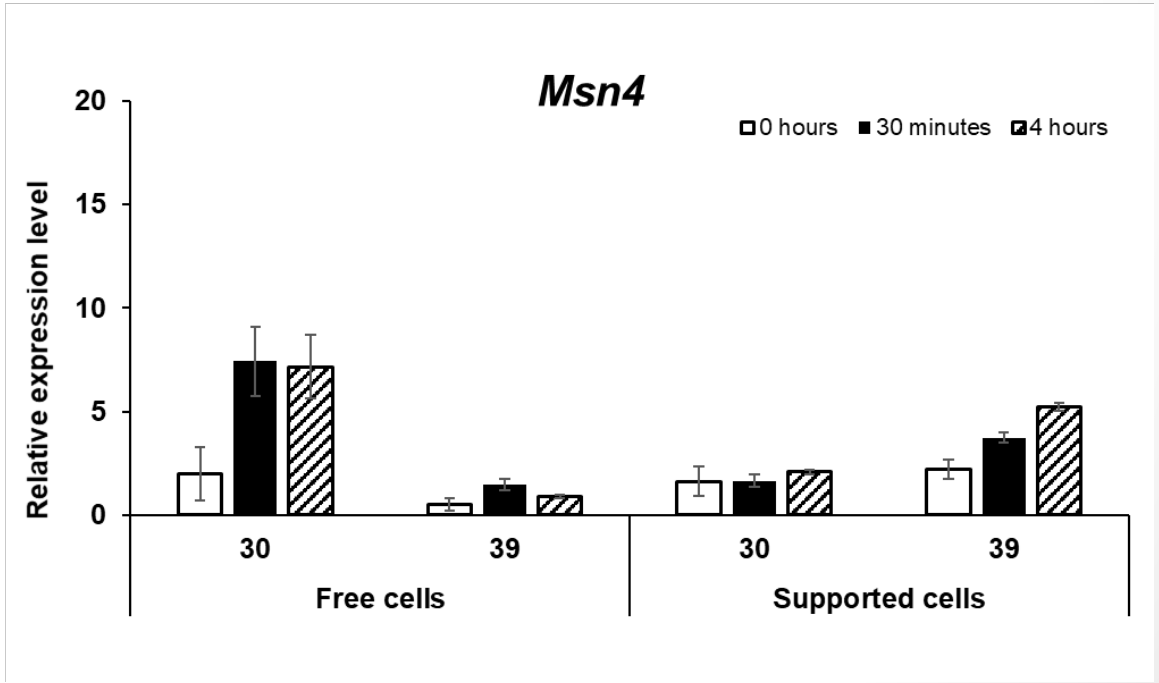




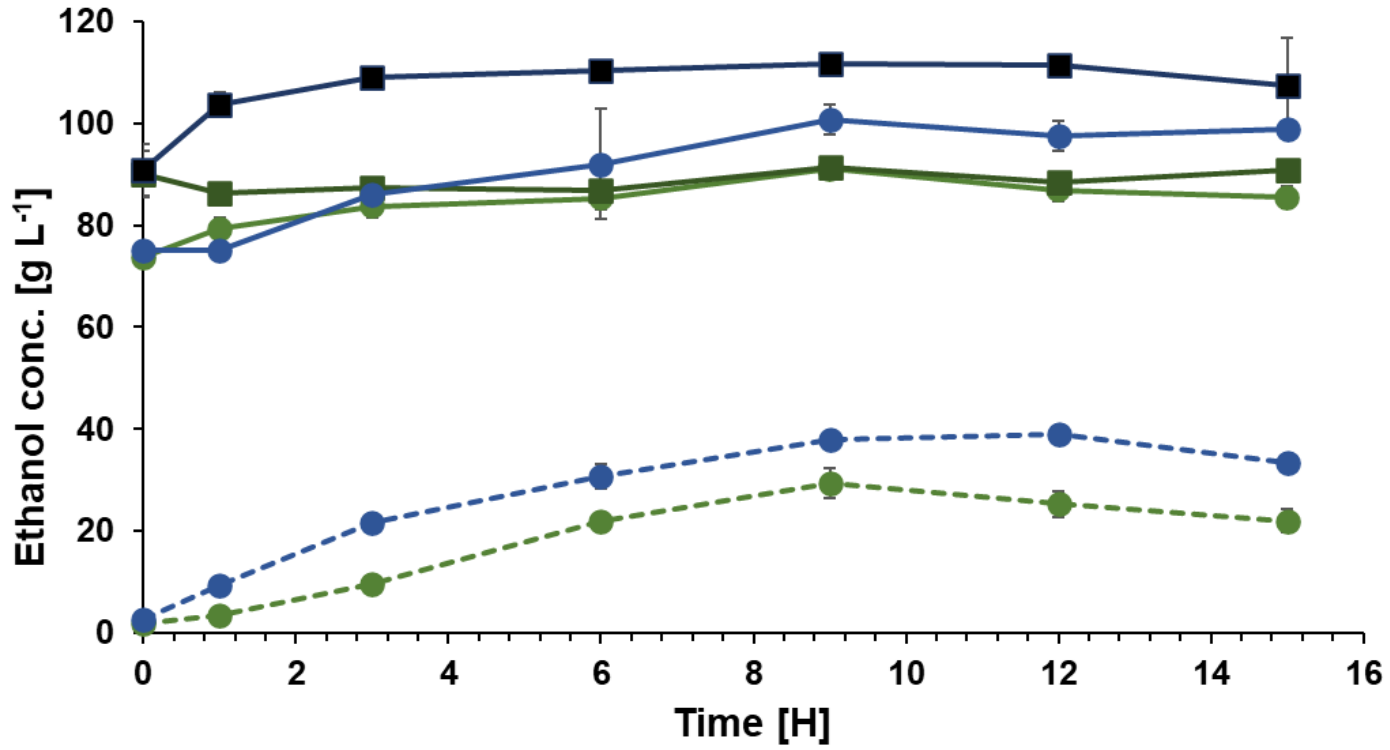
Oxidative Stress Response

- No difference between freely suspended and immobilized cells
  - MDA & H_2O_2





Ethanol stress response of *S. cerevisiae*



Ethanol concentrations

70 g L^{-1} & 90 g L^{-1}

Liquid media consisted of :

- yeast extract
- peptone
- glucose

Immobilized cells 90 g L^{-1}

Free cells 90 g L^{-1}

Immobilized cells 70 g L^{-1}

Free cells 70 g L^{-1}

Immobilized cells 0 g L^{-1}

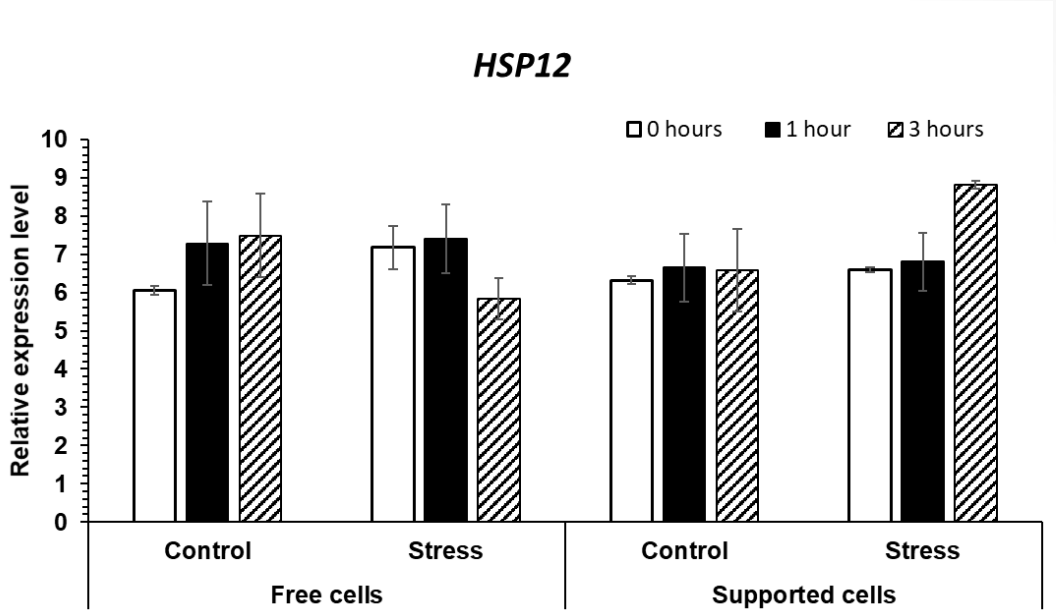
Free cells 0 g L^{-1}

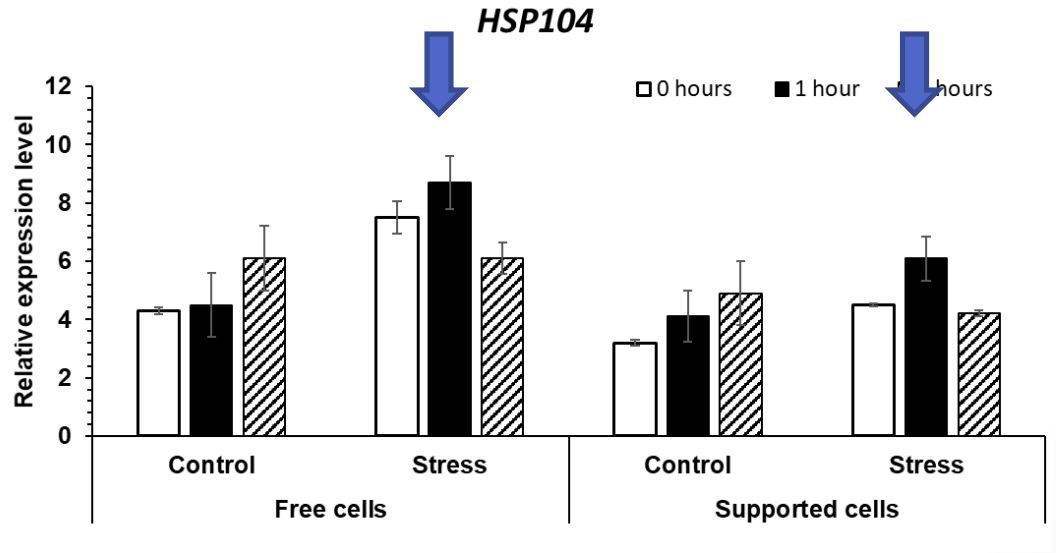
Ethanol Stress Response

- Freely suspended cells

HSP104

- **Higher** Relative expression

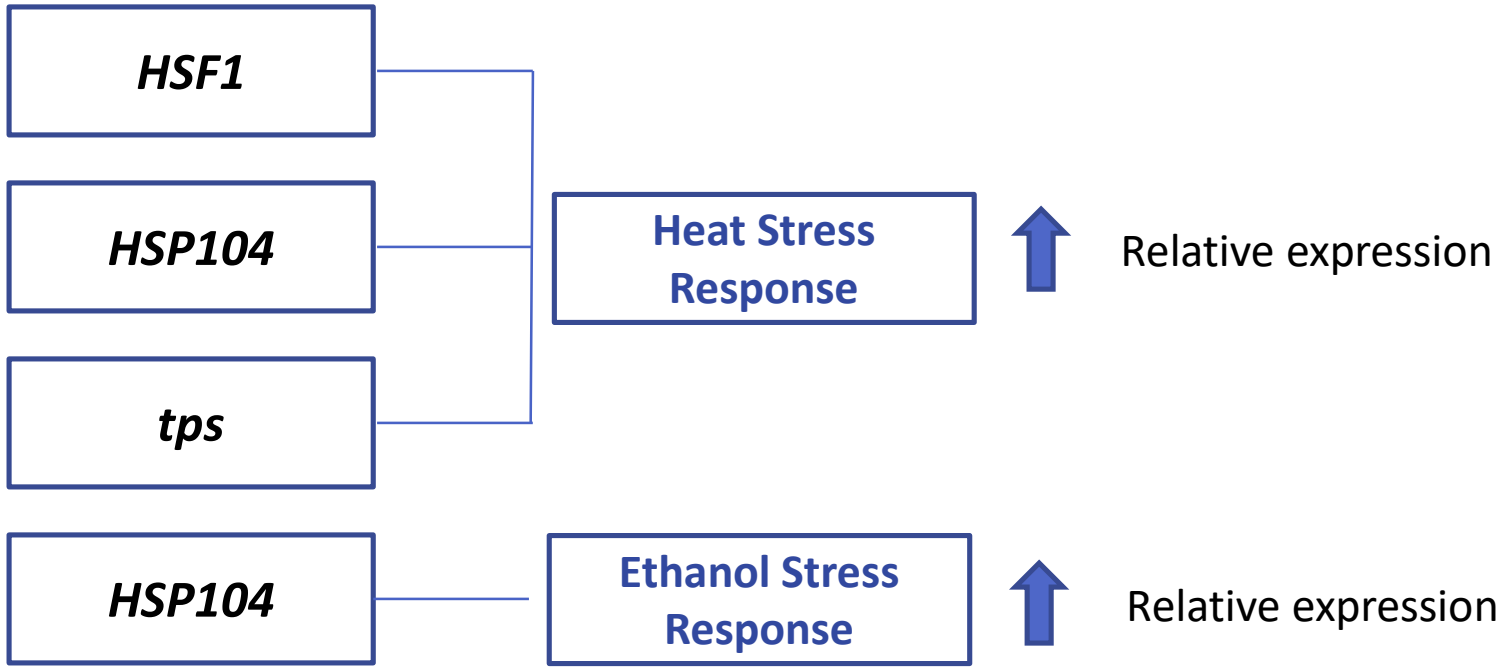




Conclusions

- BBB enhanced ethanol production using both synthetic media and CPW hydrolysate at elevated temperatures.

- Reduction of the operation cost
- Biocatalyst recyclability



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Thank you