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

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Abstract

Introduction: Biorenewable energy is gaining increasing attention due to the global climate change, environmental pollution and exhaustion of fossil fuels (Ma et al., 2017). Although bioethanol has attracted growing attention as a green, renewable and non-polluting energy source, the use of agricultural land for growing energy crops compromises the food supply chain leading to an urgent need for alternative bioethanol feedstocks (Kassim et al., 2022; Liu et al., 2019;). Bioethanol can be produced via sugar fermentation of numerous biosources (Okolie et al., 2021), while *Saccharomyces cerevisiae* constitutes one of the most widely studied and applied microorganisms in both commercial and industrial levels (Khoshkho et al., 2022). Even though *Saccharomyces cerevisiae* is the industrial workhorse of bioethanol production, the strain encounters a plethora of stress conditions during the fermentation process including high temperature, nitrogen limitation, osmotic stress from substrate sugars and ethanol inhibition (Elbakush et al., 2021). Although different approaches have been used to tackle the inhibitory effects caused by the aforementioned stresses, the immobilization of yeast cells on biochar has exhibited substantial capacity to enhance bioethanol production (Kyriakou et al., 2019). Commonly used immobilizing agents comprise sodium and calcium alginate as well as agar-agar cubes, which are limited by unstable performance due to the poor mechanical properties of the carrier (Tesfaw and Assefa, 2014; Mongkolkajit et al., 2011). However, biochar-based biocatalysts have been proposed to enhance the production of renewable energy from biowaste, mitigating the environmental effects from food waste disposal while improving the sustainability of energy systems (Kyriakou et al., 2020). This study aimed to assess the stress-protective role of biochar-based biocatalysts against heat, osmotic and ethanol stress during fermentation.

Materials and methods: Biochar was obtained via conventional pyrolysis of pistachio shells (*Pistachia vera*) at 500 ^{o}C and it was used for the preparation of the biocatalyst via immobilization of *Saccharomyces cerevisiae* as previously described (Kyriakou et al., 2019). The biocatalyst prepared was employed in bioethanol production experiments at the elevated temperature of 39 ^{o}C while q-PCR analysis was conducted to determine mRNA expression from genes *HSF1* and *TPS1* known to impose instrumental effect in coping with heat shock stress. The expression levels of *HSP104* and *HSP12* were additionally investigated upon exposure of yeast cells to high bioethanol contents, while the intracellular proline level was determined to assess the protective effect of the biomolecule against various stresses, including heat-shock, oxidation and osmolarity.

Results and discussion: Bioethanol fermentations of both freely suspended and supported cells of *Saccharomyces cerevisiae* were conducted at 30 ^{o}C and 39 ^{o}C. Supported cells reached final concentration of 41 g L^{-1} following 10 h of incubation, while the suspended culture yielded 34 g L^{-1} over the same period (Figure 1). Faster kinetics were obtained using the biochar-based biocatalyst producing 30.9 g L^{-1} of bioethanol following 4 h of incubation as opposed to free cells that formed only 8 g L^{-1}. The mRNA expression levels monitored via q-PCR confirmed the stress protective role of the biochar-based biocatalyst against heat stress, given that the relative expression of *HSF1* was significantly higher in suspended cells as opposed to *Saccharomyces cerevisiae* immobilized on biochar at 39 ^{o}C (Figure 2), demonstrating that the heat-shock response pathway was not triggered following attachment of the yeast on the biomaterial.

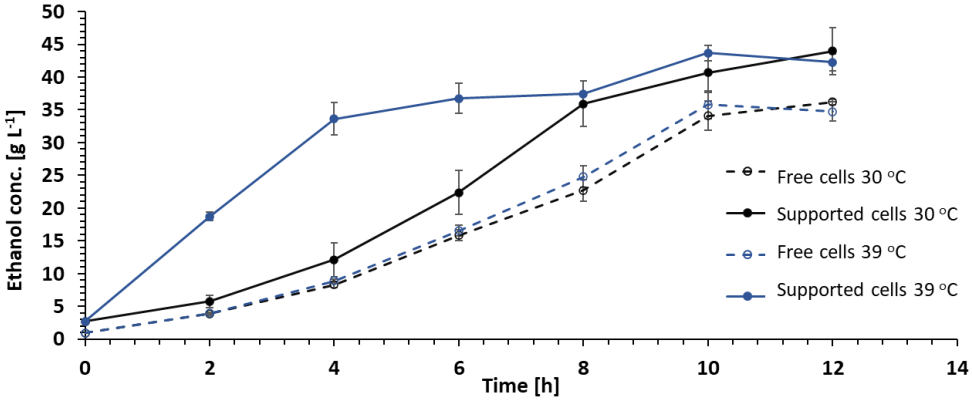

Figure 1. Evaluation of the biochar-based biocatalyst employing supported cells of *Saccharomyces cerevisiae* for bioethanol production at 30 °C and 39 °C.

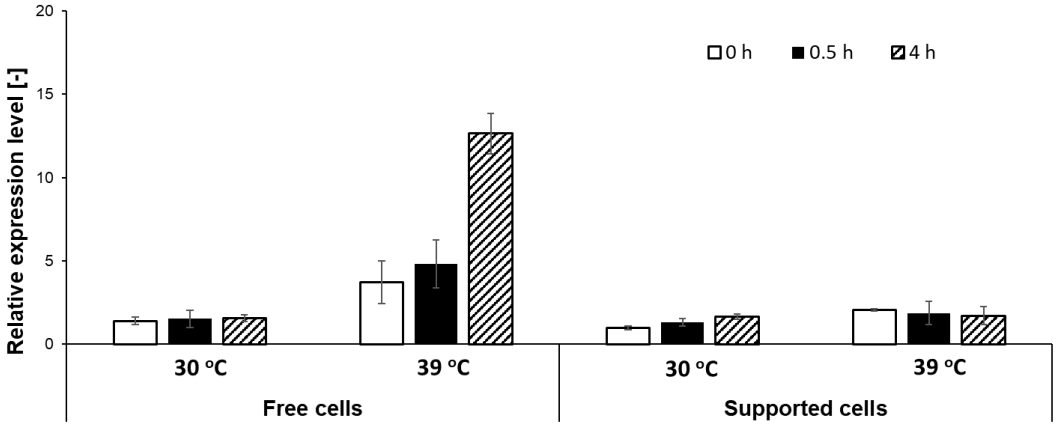

Figure 2. Relative expression of *HSF1* in bioethanol fermentations using freely suspended and supported cells of *Saccharomyces cerevisiae* at 30 °C and 39 °C.

Conclusions: The current work demonstrates that biochar-based biocatalysts can protect cells from heat shock stress and improve the performance of the fermentation process. Preliminary experiments exhibited that the heat-shock response pathway exhibited low expression using the immobilized biocatalyst, indicating that biochar serves as a promising support material enhancing bioethanol production using *Saccharomyces cerevisiae*. This work will also include fermentations of *Saccharomyces cerevisia*e exploring the effect of cell attachment on biochar during other environmental stresses such as ethanol and osmotic stress.

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