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

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1. Introduction

Biorenewable energy is gaining increasing attention due to the negative effects imposed by global climate change, environmental pollution and exhaustion of fossil fuels (Ma *et al* 2017). Although *Saccharomyces cerevisiae* is the industrial workhorse of bioethanol production, the strain encounters a plethora of stress conditions during fermentation including high temperature, nitrogen limitation, osmotic stress from substrate sugars and ethanol inhibition (Elbakush and Güven, 2021). Different approaches have been used to tackle the inhibitory effects caused by the aforementioned stresses. However, 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 (BBB) 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 test the efficiency of BBB in ethanol production under inhibitory bioprocess conditions, including heat, osmotic and ethanol stress, as well as to assess the transcriptional patterns of genes involved in the molecular mechanisms controlling the metabolic responses of the strain in each case.

2. 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 *S. cerevisiae* as previously described (Kyriakou *et al* 2020). 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 and elevated bioethanol concentration.

3. Results and Discussion

Bioethanol fermentations of both freely suspended and supported cells of *S. cerevisiae* were conducted at 30 ^{o}C and 39 ^{o}C. Supported cells reached final concentration of 41 g L^{-1}, while the suspended culture yielded 34 g L^{-1}. Faster kinetics were obtained using BBB 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 confirmed the stress protective role of BBB against heat stress, given that relative expression of *HSF1* was significantly higher in suspended cells as opposed to BBB at 39 ^{o}C, demonstrating that the heat-shock response pathway was not triggered following attachment of the yeast on the biomaterial. Similarly, genes encoding *MSN2* and *MSN4* confirmed the absence of oxidative stress using the immobilized biocatalyst, while suspended cells exhibited increase of relative mRNA production over time. Moreover, the BBB system could efficiently sustain fermentations conducted under 90 g L^{-1} initial bioethanol content, which resulted in complete failure of conventional fermentations. Expression from *HSP12* and *HSP104* confirmed the protective role of biochar to cells attached on its surface, which included significantly lower activity of the aforementioned genes as opposed to the suspended culture.

4. Conclusions

The current work demonstrates that biochar-based biocatalysts can protect cells from heat shock stress and elevated bioethanol contents improving the performance of the fermentation process, while exemplifying the significance and novelty of the use of the proposed technology against a range of inhibitory conditions.

5. References

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