Development of advanced biochar-based immobilized biocatalysts for bioethanol production by *Saccharomyces cerevisiae*

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ABSTRACT

Great effort has been devoted to produce ethanol as a second-generation biofuel derived from a variety of biomass-based feedstocks not competing with food products [1]. However, ethanol production under stress conditions (e.g. high temperature, substrate and product concentration) constitutes a major challenge inhibiting the yeast strains that usually perform high productivity and yield for the specific biofuel such as *S. cerevisiae* [2]. A potential method to achieve efficient ethanol production under stress conditions is the development of immobilized biocatalysts assisting yeast tolerance [3]. Biochar, a high-carbon, low-cost and environmental friendly material, holds great potential for application as an efficient support for cell attachment, which is known to enhance bioethanol production by *S. cerevisiae* as compared to the conventional technology [4]. This study aimed to test the efficiency of biochar-based biocatalysts (BBB) in ethanol production under conditions that usually inhibit the process. *S. cerevisiae* immobilized on pistachio nuts-biochar grown on a citrus peel waste (CPW) hydrolysate, exhibited 63 g L^{-1} ethanol concentration and 7.9 g L^{-1} h^{-1} productivity, substantially improving biosystem performance as compared to unsupported cultures. Alcoholic fermentations conducted at different elevated temperatures (37-41 ^{o}C) exhibited stable performance of the immobilized system for six repeated batch experiments. Fermentations conducted at 41 ^{o}C using BBB produced 30.8 g L^{-1} of ethanol, while free cells achieved significantly lower concentration (13.4 g L^{-1}). Monitoring of transcription from the heat-shock response and environmental stress response pathways of the strain revealed that under conditions that induced expression from the aforementioned pathways in free cells, biochar acted as a buffer alleviating the metabolic burden of the immobilized microorganism. Immobilization of *S. cerevisiae* on biochar enabled applying the workhorse of the current ethanol industry at high temperature alcoholic fermentations, which could potentially provide multiple technological merits such as increased ethanol production, reduction of operating costs, biocatalyst recyclability and low contamination risk.

KEYWORDS: Biochar, Bioethanol, *Saccharomyces cerevisiae*, Heat-shock response, Environmental stress response

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