Use of vineyard prunings biochar as a carrier for cell immobilization and ethanol overproduction by *Saccharomyces cerevisiae*

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Introduction

Substantial research interest has focused over the past decades on the reduction of greenhouse gas emissions through exploitation of alternative energy sources and renewable fuels. Although, biodiesel and bioalcohols constitute the most important biofuels, ethanol is more preferable incorporating a series of environmental benefits (*Ma et al., 2017*). Ethanol is often produced from energy crops using agricultural land, a practice that could impact food supply, highlighting the need to identify alternative feedstocks for sustainable manufacturing (*Escobar et al., 2009)*. Immobilized biocatalysts could assist yeasts in the presence of inhibitors performing stable and elevated biofuel production (*Kirdponpattara et al., 2013*). Thus, there is a need to identify novel eco-friendly and cost-effective carriers with rigid structure improving the stability of ethanol bioprocesses.

Biochar constitutes a carbon-rich material produced from thermal decomposition of biomass in the absence of oxygen, which is distinguished from charcoal through use as soil amendment (*Lehmann et al., 2009*). A wide range of biowaste can be converted to biochar under varying pyrolysis conditions yielding products characterized by different surface functional moieties and a porous structure useful for environmental and catalytic processes (*Niazi et al., 2018*). Biochar has been extensively used in environmental management applications and as soil amendment enhancing the availability of nutrients, improving soil fertility and crop yields, as well as remediating heavy metal contaminated soil, more efficiently compared to other organic materials (*Huang et al., 2019*). Moreover, biochar demonstrated effective immobilization of heavy metals, minimizing in situ the bioavailability of inorganic and organic contaminants to earthworms, microbes, and plants (*Uchimiya et al., 2011*) whereas its use in anaerobic digestion is known to enhance methane production (*Mohan et al., 2006*).

Recent advances exhibit novel biochar applications due to a number of properties that include high surface charge density enabling retention of cations through cation exchange (*Liu et al., 2012*), high internal porosity and surface area, as well as presence of both polar and non-polar surface sites allowing adsorption of organic molecules and other nutrients. Herein, we evaluate the utilization of biowaste for biochar production and its subsequent use as carrier for efficient manufacturing of liquid transportation fuels from an abundant food waste residue, such as citrus peel waste. Different biowaste were applied for biochar production using varying pyrolysis temperatures and the properties of the materials generated were assessed using scanning electron microscopy observations as well as surface area and elemental composition measurements. The most promising materials were used for immobilization of *S. cerevisiae*, which was subsequently applied for enhancing the production of ethanol from citrus peel hydrolyzates in repeated batch fermentations. In the present study, pyrolysis temperature, biochar particle size and the adsorption capacity of the material will be explored aiming to enhance the efficiency of bioethanol production through the immobilization of *S. cerevisiae* on vineyard prunings biochar.

Materials and Methods

Biochar production: Biochar was obtained via conventional pyrolysis at 250 ^{o}C and 500 ^{o}C from different agricultural waste. Specifically, the biochar used in this work was derived from VP (*Vitis vinifera*) prunings and sea grass (*Posidonia oceanica*) residues (SGR), while a non-biological char (NBC) was also analyzed and compared.

Characterization of biochar properties: An overview of the structural, physical and chemical characteristics of the biochars prepared, such as specific surface area and porosity, aiming to assess their potential applications as support materials. Thus the specific surface area of the materials generated was determined via the Brunauer-Emmett-Teller (BET) method, while the porosity was measured using Scanning electron microscopy (SEM) imaging analysis. X-ray diffraction analyses (XRD) was also performed to identify any crystallographic structure in the biochar produced.

Immobilization of microorganisms and bioethanol production: Biocatalysts were prepared through immobilization of *S. cerevisiae* on biochars derived from VP and SGR, as well as NBC. Both cultures of freely suspended and biochar-immobilized cells were compared. The consumption of sugars was measured using the Phenol-Sulfuric Acid Method for Total Carbohydrates, while ethanol production was monitored using gas chromatography (GC).

Results and Discussion

Characterization of biochar properties: Apart from VP pyrolyzed at 250 ºC, all other carbonaceous materials presented crystalline phases including halite, calcite, sylvite and/or silicon. Moreover, increase in pyrolysis temperature enhanced biochar's porosity and BET-specific surface area, which reached 41.7 m^2 g^{-1} for VP-based biochar remaining at lower levels (0.15-5.3 m^2 g^{-1}) in other specimens tested.

Microbial immobilization and bioethanol production: The biocatalyst developed using *S. cerevisiae* immobilized on VP-based biochar exhibited exceptional ethanol productivity as compared to the relevant literature, which reached 7.2 g L^{-1} h^{-1} in repeated batch fermentations using a citrus peel waste hydrolysate as feedstock. The aforementioned strain improved biofuel production by 36% compared to the conventional process (Figure 1), demonstrating a novel application of biochar in industrial biotechnology incorporating important technological advances such as enhanced biofuel production and biomass recycling.

Conclusions

The data presented demonstrate that both pyrolysis temperature and the type of feedstock strongly influence the physicochemical properties of biochar. Preliminary fermentations indicate that biochar serves as a promising support material enhancing bioethanol production using *S. cerevisiae*. The work will also include fermentations of *S. cerevisiae* using vineyard prunings biochar optimized under different bioprocess conditions.

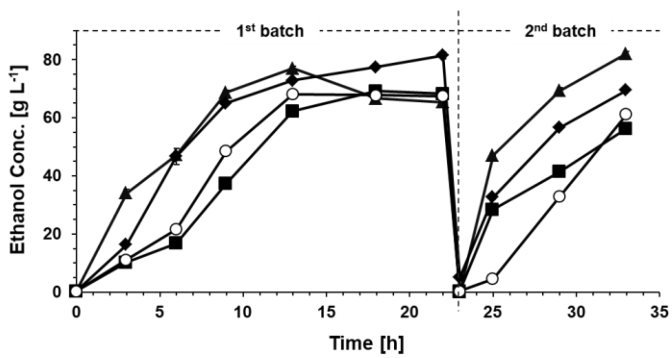

Figure 1. Evaluation of *S. cerevisiae* immobilized biocatalysts for ethanol production. Symbols correspond to: i) ▲ : cells immobilized on VPB; ii) ■ : cells immobilized on SGRB; iii) ◆ : cells immobilized on NBC; iv) ○ : freely suspended cells.

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