# Combining pyrolysis of biowaste to industrial bioprocesses: Enhancement of bioethanol production with the use of biochar-based biocatalysts

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## Abstract

***Purpose:*** This work explores the potential use of biochar as support material to enhance the efficiency of bioethanol production using different microorganisms and biochar types.

***Methods:*** Olive kernels (*Olea europaea*), vineyard prunings (*Vitis vinifera*), sewage sludge and sea grass (*Posidonia oceanica*) was used to produce biochar. The structural characteristics of biochar was studied via Brunauer-Emmett-Teller (BET), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) analyses. *S. cerevisiae*, *K. marxianus* and *P. kudriavzevii* were used for ethanol production using freely suspended cells while a non-biological biochar was also used for the immobilization of *S. cerevisiae*.

***Results:*** Biochar produced from vineyard prunings, sea grass and non-biological biochar achieved the highest specific surface area, which reached 41.73, 5.33 and 72.98 $m^2/g$ respectively. Commercial and sewage sludge biochar did not demonstrate significant porosity incorporating low values of specific surface area. Biochar produced from sewage sludge and sea grass consisted of Halite, while biochar produced from olive kernels and vineyard prunings as well as non-biological and commercial biochar consisted of Calcite ($CaCO_3$) and Silicone (Si).

*S. cerevisiae* produced 51.42 g/L of bioethanol at 37 °C, while *K. marxianus* and *P. kudriavzevii* yielded 45.50 g/L and 44.55 g/L of bioethanol respectively at 42 °C. *S. cerevisiae* immobilized cells using biochar of non-biological origin produced 68.43 g/L of bioethanol demonstrating the beneficial use of the material for the development of immobilized biocatalysts.

***Conclusions:*** These results are promising for the use of biochar as a novel support for enhancement of bioethanol production.

**Keywords**

Biochar, microbial immobilization, bioethanol, biowaste, *Saccharomyces cerevisiae.*

## 1. 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, decreasing the dependence on fossil fuel reserves and $CO_2$ emissions. Additional measures employed to mitigate global warming emissions are based on carbon sequestration. Thus, several strategies ranging from forestation and reforestation in terrestrial ecosystems to innovative technologies, such as underground geological and ocean $CO_2$ storage, have been evaluated. The potential use of materials with high carbon content as soil amendments and for long term carbon storage has been recently tested [1]. Biochar constitutes a carbon-rich material, which is formed as product of thermal degradation of organic materials in the absence of oxygen (pyrolysis process), and it is distinguished from charcoal by its use as a soil amendment [2]. Depending on the pyrolysis conditions (e.g. temperature) applied on different types of biomass, the resulting biochar may be characterized by several functional groups and an adequate porous structure useful for environmental and catalytic processes, where conventional support materials are commonly employed [3]. Therefore, pyrolysis can be applied to convert biomass into energy products, bio-oil and syngas, as well as biochar that could be used in multiple applications [4].

Biochar production has been proposed as a potential technology to mitigate climate change by sequestering carbon in soil, while the presence of biochar in the reduction of greenhouse gas emissions has been successfully studied in various countries worldwide [5]. Effects of fluxes of

nitrous oxide ($N_2O$), carbon dioxide ($CO_2$) and methane ($CH_4$) in the presence of biochar have been tested in southern Finland [6]. Moreover, Liu et al. [7] conducted a field experiment using biochar amendment for evaluation of $N_2O$ emissions from rice fields in southern China. Mukome et al. [8] investigated the effects of walnut shell and wood feedstock biochar produced at two different temperatures on $CO_2$ and $N_2O$ emissions from fertile agricultural soil amended with different types of organic synthetic fertilizer.

Although biochar incorporates a traditional focus on agronomic technologies, herein we evaluate its use in an area of high industrial interest where it could be employed as a renewable and low-cost support material. The high surface charge density of biochar enables the retention of cations by cation exchange [9], internal porosity, high surface area, and the presence of both polar and non-polar surface sites enabling biochar to adsorb organic molecules and associated nutrients. Moreover, the material is known to promote soil microbial activity [10] (particularly in mycorrhizal fungi) which are critically important for nutrient cycling [11]. Although activated carbon has been extensively applied as an immobilization carrier for different applications, the cost related to carbon activation elevates the overall investment. Therefore, similarly to the use of activated carbon in industrial biotechnology, biochar constitutes a carbonaceous material that can serve as a promising and cheaper alternative biomaterial for immobilization of microorganisms in different areas including biotechnology, food technology, biology and medicine. In the present study, the applicability of biochar to enhance the efficiency of bioethanol production through the immobilization of microbial producers has been explored.

## 2. Materials and Methods

### *2.1 Biochar production*

According to the International Biochar Initiative (IBI), biochar should be produced through the use of waste-derived biomass. Suitable feedstocks may include agricultural and forestry wastes, as well as sludge from wastewater treatment plants and animal manure [3]. In this study, biochar was obtained via conventional pyrolysis at 250 $^{o}$C and 500 $^{o}$C from different agricultural waste. Specifically, biochar was derived from olive kernels (*Olea europaea*), vineyard prunings

(*Vitis vinifera*), sewage sludge and sea grass (*Posidonia oceanica*), while commercial and non-biological biochars, obtained from prunings and plastic tires respectively, were also analyzed and compared. All samples used in this work were initially dried (105 $^{o}$C) and subsequently stored in air tight plastic bags until application in pyrolysis. The samples were pyrolysed under controlled pyrolysis conditions through the supply of nitrogen gas.

### *2.2 Preliminary biochar characterization*

An overview of the structural, physical and chemical characteristics of the biochar formed is presented aiming to assess its potential use for the development of new products and support materials. Thus, the specific surface area of the studied materials was determined via the Brunauer-Emmett-Teller (BET) method [12]. X-ray Diffraction (XRD) was used to probe the presence of crystalline phases within the biochar samples. All measurements were performed in a theta-theta Rigaku Ultima IV diffractometer, equipped with a copper tube (Cu Kα radiation, $\lambda = 1.541$ Å), operated at 40 kV and 40 mA. The system is equipped with a multilayer mirror for parallel x-ray beam geometry and sample patterns were collected over the 10°–70° 2theta range, in a sample holder without rotation. The morphology of the samples was investigated through a Quanta 200 (FEI) Scanning Electron Microscope (SEM) in various accelerating voltages and all samples were sputter coated with a thin layer of gold (few nm) to prevent surface charging issues.

### 2.3 *Immobilization of microorganisms and bioethanol production*

*Saccharomyces cerevisiae, Kluyveromyces marxianus* and *Pichia kudriavzevii* were used for ethanol production using liquid media simulating an orange peel waste hydrolysate that consisted of (g/l): yeast extract 10, peptone 20, fructose 33.2, galactose 8.6, glucose 57.4, and sucrose 1.4 [13]. Both cultures of freely suspended and biochar-immobilized cells were compared. *S. cerevisiae* and *P. kudriavzevii* were pre-grown on liquid medium consisting of (g/l): yeast extract 10, peptone 20 and glucose 50, while *K. marxianus* was pre-grown on medium containing (g/l): yeast extract 3, malt extract 3, peptone 5 and glucose 50.

Each fermentation was conducted in 100 ml serum bottles sealed with screw caps containing 90 ml of the fermentation medium and 10 ml of yeast, while 50 mM citrate buffer was also used at pH 4.8. Serum bottles were incubated at 42 $^{o}$C and 37 $^{o}$C until complete consumption of sugars was achieved, while stirred at 100 rpm using a water bath shaker. Samples were withdrawn aseptically at several time points to measure ethanol production, sugars consumption and optical density (yeast growth).

The biocatalyst was prepared by the immobilization of *Saccharomyces cerevisiae* on a non-biological biochar material. For the support and immobilization process, 20 g of support material, 250 ml of fermentation medium and 5 g of *Saccharomyces cerevisiae* wet cells were placed in shake flasks. The flasks were incubated at 37 $^{o}$C and allowed to ferment overnight. The supernatant was decanted and the remaining biocatalyst was washed twice with 125 ml of fermentation medium and applied for bioethanol production.

The consumption of sugars was determined using the Phenol-Sulfuric Acid Method for Total Carbohydrates, which is based on the phenol-sulfuric acid reaction and it is useful for determination of simple sugars, oligosaccharides, polysaccharides and their derivatives [14].

Cell mass was determined through optical density at 600 nm using a UV/Vis spectrometer. Samples were diluted until the optical density reached the linear range of the calibration curve. A calibration curve was developed for each yeast correlating optical density to dry cell weight.

Ethanol production was measured using gas chromatography (GC). A Shimadzu GC-2014 (Shimadzu, Milton Keynes, UK) using a flame ionization detector and a 30 m long Zebron ZB-5 capillary column (Phenomenex, Macclesfield, UK) with 0.25 mm internal diameter was employed. The mobile phase applied was nitrogen, while the stationary phase of the column was 5%-phenyl and 95% dimethylpolysiloxane. Samples were centrifuged for 10 min and the supernatant was filtered through 0.45 μm filters. Ethanol was extracted into hexane by vortexing 1 ml of the filtered sample with 2 ml of the solvent for 1 min. About 1 μl of the extract was injected and the temperature of the column was kept constant at 40 °C for 25 min followed by an increase of 30 °C min$^{-1}$ up to 160 °C, while it was maintained at 160 °C for an additional 5 min [15].

## 3. Results and Discussion

### *3.1 Properties of biochar produced*

Pyrolysis process conditions strongly affect the yield, the morphology and the physicochemical properties of biochar produced. An increase in temperature and heating rate led to reduction of biochar yield and surface functional groups, while the C content increased for all samples tested. The microstructures of biochar produced from olive kernels, vineyard prunings and sea grass at 500 ºC are shown in Figure 1, where smooth surface and porosity was observed. The materials produced from vineyard prunings, sea grass and non-biological biochar achieved the highest specific surface area values following processing at 500 ºC that reached 41.73, 5.33 and 72.98 $m^2$ $g^{-1}$ respectively. Nevertheless, commercial and sewage sludge biochar did not demonstrate any porosity (Figs. 1e,f) and incorporated low values of specific surface area. Thus, the aforementioned materials were not further considered as cell immobilization materials for ethanol production.

Based on the XRD analysis all types of biochar formed demonstrated a crystalline phase. Specifically, biochar produced from sewage sludge and sea grass consisted of Halite, while biochar produced from olive kernels and vineyard prunings as well as non-biological and commercial biochar consisted of Calcite ($CaCO_3$) and Silicone (Si). Previous studies have tested the crystalline phase in biochars produced from eucalyptus feedstocks, which resulted in the formation of Calcite ($CaCO_3$) following pyrolysis at 550 ºC [16].

### *3.2 Immobilization of microorganisms and bioethanol production*

Bioethanol fermentations of the three yeast strains were initially conducted using freely suspended cells in two different temperatures (37 and 42 $^oC$). *Saccharomyces cerevisiae* produced 51.42 g $L^{-1}$ of ethanol at 37 $^oC$ and 42.17 g $L^{-1}$ at 42 $^oC$ following 64 h of incubation. The temperature of 42 $^oC$ enhanced bioethanol production from *Kluyveromyces marxianus* and

*Pichia kudriavzevii* that yielded 45.50 g $L^{-1}$ and 44.55 g $L^{-1}$ of bioethanol respectively. However, the use of 37 $^{o}$C resulted in the production of 38.60 g $L^{-1}$ and 24.04 g $L^{-1}$ of ethanol using *Kluyveromyces marxianus* and *Pichia kudriavzevii* respectively (Fig. 2). Preliminary fermentations of immobilized *S. cerevisiae* using biochar of non-biological origin as support material produced 68.43 g $L^{-1}$ of ethanol while freely suspended cells produced 61.89 g $L^{-1}$ demonstrating the beneficial use of the material for the development of immobilized biocatalysts for a major bioprocess (Fig. 3). It has been previously reported that *Pichia kudriavzevii* KVMP10 is capable of producing 54 g $L^{-1}$ of ethanol at 42 °C using a hydrolysed Valencia orange peel model solution [15]. Moreover, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* produce 37.10 g $L^{-1}$ and 40.90 g $L^{-1}$ of ethanol following 72 h of fermentation using the feedstock of the current study [13]. Thus, given that under cell immobilization conditions the production of the biofuel was substantially enhanced highlights the importance of applying the specific material in the bioprocess.

## 4. Conclusions

The data presented demonstrate that both pyrolysis temperature and the type of feedstock strongly influence the physicochemical properties of biochar while the temperature of 500°C resulted in higher specific surface area and porosity as compared to 250°C. Preliminary fermentations indicate that biochar serves as a promising support material enhancing bioethanol production using *Saccharomyces cerevisiae*. The work will also include fermentations of the three yeast strains targeted using different biochar samples derived from vineyard prunings and sea grass while the adsorption capacity of each biochar will be examined.

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**Figure Captions**

**Fig 1.** SEM images of biochar. (a) Olive kernels biochar, (b) vineyard prunings biochar, (c) sea grass biochar, (d) non-biological biochar, (e) sewage sludge biochar and (f) commercial biochar, at 800× magnification.

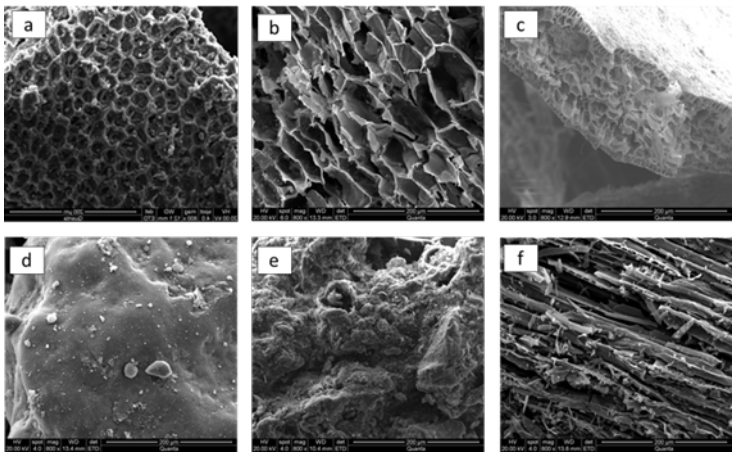

**Fig 2.** Ethanol production at 42 ºC (a) and 37 ºC (b) using freely suspended cultures of *S. cerevisiae, K. marxianus* and *P. kudriavzevii*.

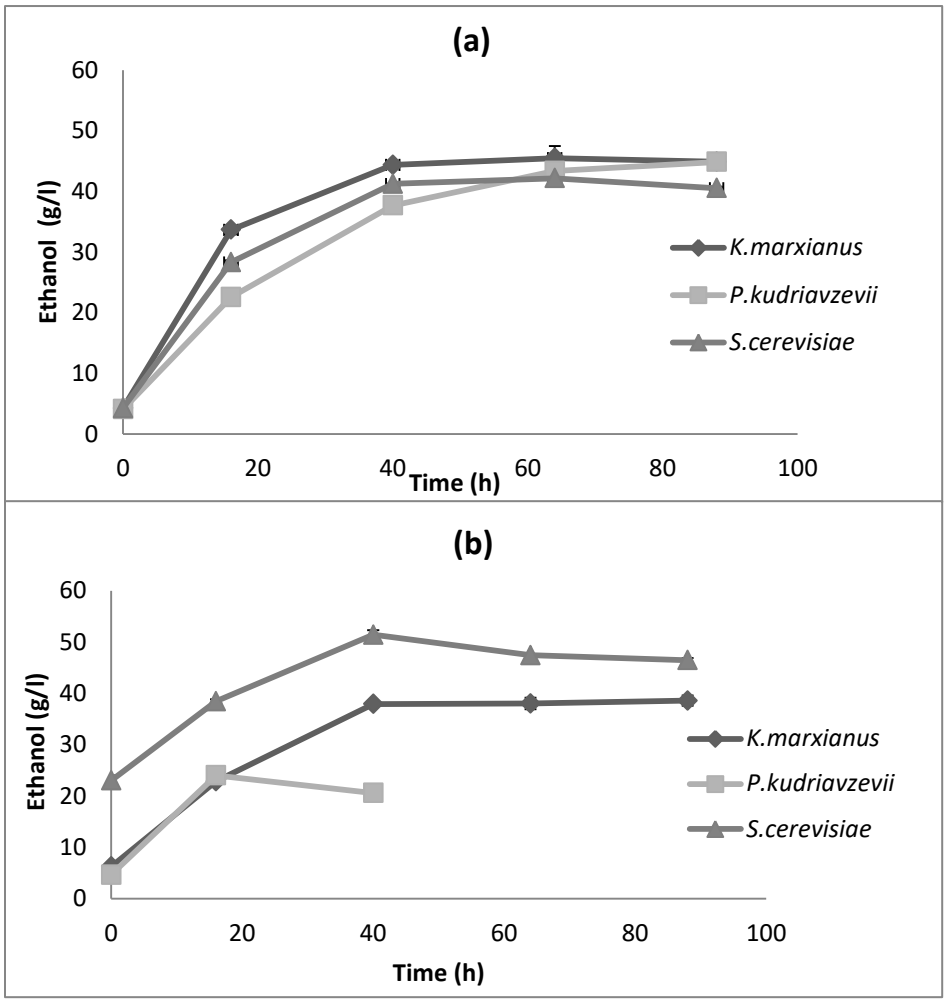

**Fig 3.** Ethanol production of *S. cerevisiae* immobilized cells using non-biological biochar as support material.