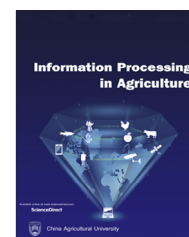


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Use of ultrasound to modify the pyrolyzed biomass of *Pinus* spp. and the implications for biological models

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ABSTRACT

Pine (*Pinus* spp.) needle biomass (PNB) was pyrolyzed at 400 °C for 3 h and then subjected to hydrothermal treatment at the same temperature for 10 min, with and without the addition of potassium chloride (KCl). The suspensions of the materials treated hydrothermally were submitted to ultrasound for 5, 10, 20, 30 and 60 min. Diffuse reflectance UV-Vis (DRUV) spectroscopy results for the materials with variations in sonication times were obtained and the band gap energy (*E*) was calculated. A culture medium containing *Saccharomyces cerevisiae* was monitored during 30 min of exposure to different materials for the calculation of the 10% (IC₁₀), 30% (IC₃₀) and 50% (IC₅₀) inhibitory concentrations. Of the samples that underwent ultrasonic treatment, the material pyrolyzed at 400 °C without the addition of potassium ions (PNB4003H60) presented the greatest inhibition of 10% of the *Saccharomyces cerevisiae* cultures. Of the materials without the addition of potassium, the material pyrolyzed and sonicated for 10 min (PNB4003H10) showed the best characteristics for use as a support for *Saccharomyces cerevisiae* organisms.

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1. Introduction

When carbon is subjected to ultrasonic vibration its pores break, its cracks propagate and its permeability is increased [1]. Ultrasonic treatment gathers residual oil from the pyrolysis process, breaks particles, forms cracks and modifies the surface of carbon [2]. Ultrasonic treatment can be used to promote the permeability of pyrolyzed material to alkaline metals [1,3,4], and this can be evaluated through UV spectroscopy, since the reflection of alkaline metals, such as Cs, K and Rb, generates band gaps between 0.6 and 3.0 eV [5] with reflectance bands at around 200 nm, which are characteristic of

the intercalated structure of alkaline metals in carbon structures [6].

Ultrasonic treatment reduces the viscosity of dispersions containing starch [7], improves the anaerobic digestion of plant tissues [8], reduces the size of sludge particles [9], modifies the structure of proteins, inducing tertiary structures [10], promotes the nanocomplexation of proteins and the reduction of the material hydrophobicity [11], contributes for the degradation of endocrine disrupting compounds, pharmaceuticals, and personal care products in water [12–14], reduces the contents of soluble oil and alcohol compounds, forming acid and ester compounds [15] and promotes the hydrolysis of methyl esters [16], besides selectively oxidizing alcohols to give aldehydes and ketones [17]. Ultrasonic treatment is also employed in the preparation of biological polysaccharides with the potential to act as immunoregula-

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tors and in the treatment of carcinomas [18]. Moreover, ultrasonic treatment promotes the acoustic cavitation (sonolysis) in plant tissues [19] and in metal structures [20,21], besides being used to prepare nanostructures [22–24] of starch, glycerol and ascorbic acid [25,26], solubilize organic matter [27] and disperse materials when preparing samples [28]. The evaluation of biological models contributes to an understanding of the molecular mechanism and it can be carried out through the study of the eukaryotic model *Saccharomyces cerevisiae*, which has a relatively short replication time [29].

The objective of this study was to evaluate carbons enriched with potassium and hydrothermally and ultrasonically treated as a support for the immobilization of *Saccharomyces cerevisiae*.

2. Method

2.1. Pyrolysis of biomass

The pine (*Pinus* spp.) needle biomass (PNB) was ground to a granulometry of ≤ 2.0 mm. The samples were pyrolyzed at 400 °C for 3 h and then subjected to hydrothermal treatment at the same temperature for 10 min [30,31] with the addition of 1 mL of water per gram of ground biomass (PNB4003H), without the addition of potassium and with the addition of 1 mL of KCl solution in a concentration of 0.25 mol L⁻¹ of potassium per gram of ground biomass (PNB4003H025K), in an adapted Mufla Quimis oven, model 318 D24.

2.2. Ultrasound treatment

The suspensions of the materials treated hydrothermally were submitted to the ultrasound treatment (UT) using a Cristófoli ultrasonic vessel, with constant power of 170 W and ultrasonic frequency of 42 kHz, in the absence and presence of KCl, respectively, for 5 min (PNB4003H5 and PNB4003H025K5), 10 min (PNB4003H10 and PNB4003H025K10), 20 min (PNB4003H20 and PNB4003H025K20), 30 min (PNB4003H30 and PNB4003H025K30) and 60 min (PNB4003H60 and PNB4003H025K60).

2.3. Determination of cytotoxic activity

The culture medium containing *Saccharomyces cerevisiae* [32,33] was transferred to test tubes at 40 °C, in a bain-Marie, containing 0, 1 · 10³, 5 · 10³, 1 · 10⁴ and 5 · 10⁴ ppm of the PNB materials, PNB4003H, PNB4003H5, PNB4003H10, PNB4003H20, PNB4003H30, PNB4003H60, PNB4003H025K, PNB4003H025K5, PNB4003H025K10, PNB4003H025K20, PNB4003H025K30 and PNB4003H025K60, and KCl. The growth curve for *S. cerevisiae* was monitored for 30 min during the exposure of the culture medium to the different hydrothermally treated materials [34,35]. For the evaluation of where foam production were used glass test tubes of capacity for 20 mL with internal diameter of 1 cm. At times of 5, 10, 15, 20, 25 and 30 min where foam expansion was determined with the aid of a millimeter ruler from the culture medium. Based on the *S. cerevisiae* growth curves, the 10% (IC₁₀), 30% (IC₃₀) and 50% (IC₅₀) inhibitory concentrations were calculated.

2.4. DRUV characterization

The spectra for the diffuse reflectance UV-Vis (DRUV) spectroscopy were obtained with the aid of a PerkinElmer LAMBDA 365 spectrophotometer, at between 200 and 400 nm. The band gap energy (E) was calculated using Eq. (1), where *h* is the Planck constant (6.63 · 10⁻³⁴ J s), *c* is the speed of light (3.00 · 10⁸ m s⁻¹) and λ is the wavelength where the band gap occurs (nm) [36].

$$E = \frac{h \cdot c}{\lambda} \quad (1)$$

3. Results and discussion

3.1. Spectroscopic results

The spectroscopic results (Fig. 1a, Table 1) show that the PNB material submitted to hydrothermal treatment at 400 °C for 3 h (PNB40003H), without the addition of K⁺ ions, had the highest ratios for the intensities of reflectance bands in the regions of 200/250 and 200/280 nm. This reflects in the

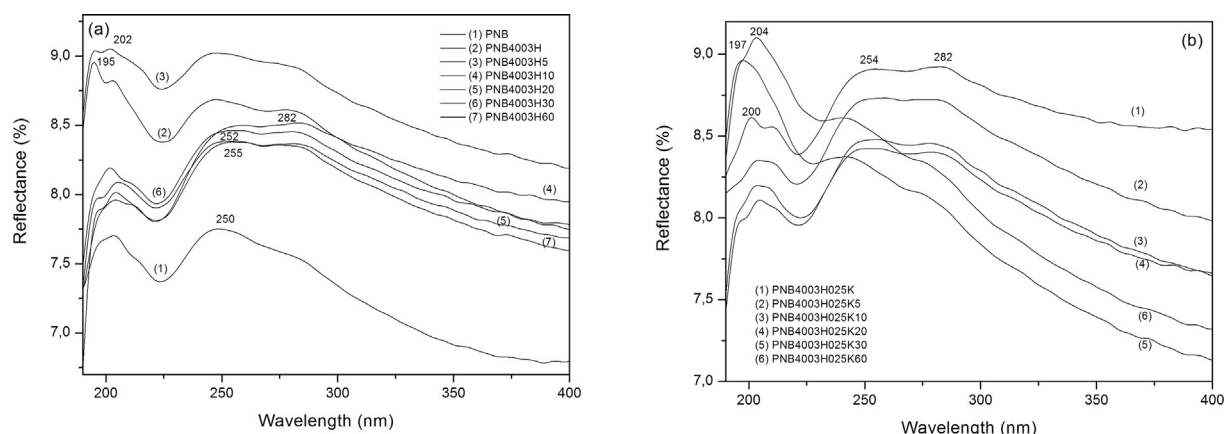


Fig. 1 – Diffuse reflectance UV-Vis (DRUV) spectroscopy results for the materials with variations in sonication times: (a) pyrolyzed biomass without the addition of K⁺ ions; (b) pyrolyzed biomass with the addition of K⁺ ions.

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