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Magnetically modified bacterial cellulose: A promising carrier for immobilization of affinity ligands, enzymes, and cells

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ABSTRACT

Bacterial cellulose (BC) produced by *Komagataeibacter sucrofermentans* was magnetically modified using perchloric acid stabilized magnetic fluid. Magnetic bacterial cellulose (MBC) was used as a carrier for the immobilization of affinity ligands, enzymes and cells. MBC with immobilized reactive copper phthalocyanine dye was an efficient adsorbent for crystal violet removal; the maximum adsorption capacity was 388 mg/g. Kinetic and thermodynamic parameters were also determined. Model biocatalysts, namely bovine pancreas trypsin and *Saccharomyces cerevisiae* cells were immobilized on MBC using several strategies including adsorption with subsequent cross-linking with glutaraldehyde and covalent binding on previously activated MBC using sodium periodate or 1,4-butanediol diglycidyl ether. Immobilized yeast cells retained approximately 90% of their initial activity after 6 repeated cycles of sucrose solution hydrolysis. Trypsin covalently bound after MBC periodate activation was very stable during operational stability testing; it could be repeatedly used for ten cycles of low molecular weight substrate hydrolysis without loss of its initial activity.

1. Introduction

Cellulose is the most abundant macromolecule in nature and conventionally found in plants as a structural component forming the lignocellulosic structure together with lignin and hemicellulose. Bacterial cellulose (BC), produced by bacterial strains of the genera *Acetobacter*, demonstrates high purity, high water holding capacity, high hydrophilicity, high crystallinity, high porosity and comparable mechanical behavior to other complex and synthetically produced polymers and fibers [1]. BC is nowadays considered a functional biomaterial with numerous applications in various fields including skin tissue repair [2], potential scaffold for tissue-engineering [3], wound healing applications [4], immobilization, paper manufacturing, cosmetics, dye decolorization [5] and as a thickening and stabilizing agent in the food industry [6]. One of the main disadvantages of industrial BC production is the high cost of manufacture mainly due to the commercial raw materials used in the formulation of fermentation media and the low productivity achieved during fermentation. To alleviate the former drawback, waste and by-product streams from various industrial sectors (e.g. the food industry, the biodiesel industry using oilseeds) could be used as low cost sources of nutrients [7]. For instance, the utilization of hydrolysates from confectionary industry waste streams or by-products from a sunflower-based biodiesel process (i.e. crude glycerol, sunflower meal hydrolysate) led to the production of around 13 g/L of BC [7].

Recently, diverse types of biological materials have been magnetically modified and used for various applications [8,9]. Also BC has already been converted into its magnetic derivatives using different approaches, e.g. by *in situ* thermal decomposition of iron(III) acetylacetonate under microwave irradiation [10] or by homogenizing the BC pellicle in the solution of ferrous and ferric salts, followed by the addition of sodium hydroxide [11]. Alternatively, magnetic bacterial cellulose (MBC) can be produced during the BC biosynthesis process in a culture medium containing dispersed magnetite nanoparticles [12].

The present work describes a new, extremely simple procedure to prepare magnetically responsive bacterial cellulose employing a simple entrapment of magnetic nanoparticles during the contact of BC with water-based magnetic fluid. MBC was used as an efficient carrier for the immobilization of affinity ligands, enzymes and cells.

Abbreviations: BC, bacterial cellulose; FF, ferrofluid; MBC, magnetic bacterial cellulose; PM-MBC, phthalocyanine-modified magnetic bacterial cellulose.

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2. Materials and methods

2.1. Materials

Reactive copper phthalocyanine dye (Ostazin turquoise V-G; C.I. Reactive Blue 21) was supplied by Spolek pro chemickou a hutni vyrobu, Usti nad Labem, Czech Republic. Crystal violet (C.I. 42555), N_{α}-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA), sodium (meta)periodate, 1,4-butanediol diglycidyl ether and glutaraldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). Crystalline trypsin from bovine pancreas and anhydrous Na_2CO_3 were from Lachema (Brno, Czech Republic). Reagent for the measurement of glucose concentration was obtained from Biosystems (Barcelona, Spain). *Saccharomyces cerevisiae* cells (compressed baker's yeast) were purchased in a local market. Other common chemicals were from Lachner (Neratovice, Czech Republic). Crude glycerol used in this study was kindly provided by the biodiesel producer P. N. Pettas S. A. industry (Patras, Greece). The crude glycerol (92.4% w/w) of slightly higher purity was obtained via decanting of the original crude glycerol using separation funnels [13].

2.2. Bacterial production of bacterial cellulose

Bacterial cellulose was produced by the bacterial strain *Komagataeibacter sucrofermentans* DSM 15973. Bacterial stock cultures were maintained at −85 °C in cryovials with a respective ratio of commercial glycerol to preculture of 1:1. Liquid media for the preparation of inoculum and fermentation media consisted of (g/L): 20 carbon source; 5 peptone; 5 yeast extract; 2.7 Na_2HPO_4; 1.15 citric acid. In the case of the inocula, the carbon source used was glucose, while in the case of fermentation medium, crude glycerol was employed. The pH of the medium was adjusted to 6 with 5 M NaOH.

Fermentations were carried out at 30 °C in a 32 L PVC container with working volume of 2 L. The container was sterilized under Ultra Violet light for 20 min. The volume of the inocula was 10% (v/v). The container was statically incubated for 8 days. After the completion of fermentation, BC was removed from the culture broth and treated with 2 M NaOH to remove bacterial cells and subsequently washed repeatedly until a neutral pH was achieved.

2.3. Characterization of materials

The morphology and structure of bacterial cellulose samples was studied by optical microscopy (OM) and scanning electron microscopy (SEM). Samples were analyzed using a Hitachi SU6600 scanning electron microscope (Hitachi, Tokyo, Japan) with accelerating voltage 1 or 3 kV. Energy dispersive X-ray spectra (EDS) were acquired in SEM using Thermo Noran System 7 (Thermo Scientific, Waltham, MA, USA) with Si(Li) detector; accelerating voltage was 5 or 15 kV and acquisition time was 300 s.

Fourier transform infrared (FTIR) absorption spectra were measured using Thermo Scientific Nicolet iS5 FTIR spectrometer (Thermo Nicolet Corp., Madison, WI, USA) with iD Foundation accessory (ZnSe crystal, range 4000–650 cm^{-1}, 32 scans, resolution 4 cm^{-1}). The IR absorption spectra are presented in transmittance after advanced attenuated total reflectance (ATR) and automatic baseline corrections.

2.4. Magnetic modification of bacterial cellulose

BC was magnetically modified using perchloric acid stabilized magnetic fluid (ferrofluid, FF) prepared according to the described procedure [14]. 25 mL of distilled water and 5 mL of FF (c = 30.24 mg/mL) were added to 10 g of BC particles (prepared using a mixer), followed by incubation on rotator mixer (Dynal, Oslo, Norway) for 24 h. Prepared magnetically responsive BC was thoroughly washed with water to remove excess magnetic fluid, sieved with a sieve with 0.7 × 0.7 mm opening, and stored at 4 °C. In the same way, cut pieces of BC pellicle were also magnetically modified.

2.5. Phthalocyanine modification of magnetic bacterial cellulose

MBC was allowed to sediment in a graduated cylinder for 18 h. Immobilization of reactive copper phthalocyanine dye was described previously by Safarik [15]. Briefly, 2 g of Ostazine turquoise and 6 g of NaCl were mixed with 100 mL of MBC suspension (1:1; sediment:water) and warmed to 70 °C. After 15 min, 5 g of anhydrous Na_2CO_3 was added and the suspension was stirred at 70 °C for next 3 h. Then the mixture was left overnight at ambient temperature without mixing. The phthalocyanine modified magnetic bacterial cellulose (PM-MBC) was thoroughly washed with water and methanol to dispose unbound dye and stored at 4 °C. The dry weight of 1 mL of settled MBC and PM-MBC was equal to 3 mg.

2.6. Adsorption of crystal violet

The adsorption of crystal violet by PM-MBC was tested in a batch system without any pH adjustment. Kinetic parameters were evaluated using 0.5 mL of settled PM-MBC mixed with 10 mL of 100 mg/L solution of crystal violet and incubated on rotator mixer (Dynal, Oslo, Norway) for 5–180 min at room temperature (22.0 °C). Thermodynamic parameters were determined at 11.0, 22.0 and 40.0 °C, using 0.3 mL of settled PM-MBC and dye concentrations ranging between 25 and 450 mg/L. Incubation time was set according to kinetic results to 2 h. To compare adsorption efficiency of MBC after phthalocyanine dye immobilization, the adsorption isotherm for MBC at room temperature was also prepared.

The absorbance of supernatant was measured after magnetic separation (separator DynaMag™-15, Dynal, Oslo, Norway) by spectrophotometer (Cintra 20, GBC Scientific Equipment, Braeside, Australia). The concentration of unbound dye in equilibrium (C_e, mg/L) or at time t (C_t, mg/L) was determined from calibration curve, while the amount of dye adsorbed on unit mass of adsorbent in equilibrium (q_e, mg/g) or at time t (q_t, mg/g) was calculated from these formulae:

$$q_e = \frac{V\,(C_0 - C_e)}{m} \tag{1}$$

$$q_t = \frac{V\,(C_0 - C_t)}{m} \tag{2}$$

where C_0 is the initial concentration of dye (mg/L) and m is the mass of adsorbent (g).

2.7. Adsorption kinetics

The data were analyzed using pseudo-first and pseudo-second-order kinetic models. The linear form of pseudo-first-order (P-1-O) kinetic model is given by the equation [16]:

$$\ln(q_e - q_t) = \ln(q_e) - k_1\,t \tag{3}$$

where the rate constant k_1 (1/min) can be obtained from the linear plot of ln ($q_e - q_t$) against time.

The linear form of pseudo-second-order (P-2-O) model can be expressed as described by Ho and McKay [17]:

$$\frac{t}{q_t} = \frac{1}{k_2\,q_e{}^2} + \frac{t}{q_e} \tag{4}$$

where the equilibrium adsorption capacity (q_e), and the second-order rate constant k_2 (g/mg min) can be determined from the slope and intercept of plot t/q_t versus t [18,19].