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Sesbania aculeate biomass hydrolysis using magnetic nanobiocomposite of cellulase for bioethanol production

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ABSTRACT

Cellulase bound magnetic nanoparticles was used as nanobiocatalyst for the hydrolysis of *Sesbania aculeate* biomass in the present study. The characteristic peak at 1624 cm^{-1} in FT-IR spectrum confirmed the presence of cellulase on MNP. The particle size of the nanobiocatalyst was found in the range of 90–100 nm and it was structurally found to be cubic in nature. The paramagnetic behavior of nanobiocatalyst was confirmed by VSM analysis. The optimal parameters are 1.5% (w/v) of nanobiocatalyst concentration, biomass concentration of 4% (w/v) and temperature at 30 °C. The maximum bioethanol yield of 5.31 g/l was obtained using *Sesbania aculeate* biomass hydrolysate under optimal conditions. The produced bioethanol was confirmed by GC-MS analysis. Reusability study was analyzed and proved that the nanobiocatalyst was efficient for the feasible production of bioethanol.

1. Introduction

Energy consumption has increased drastically due to increase in population and exploration by various industries with certain environmental issues such as pollution and global warming. In recent times, depletion of fossil fuels is the major concern in the production of conventional sources. This has led to the shift in global energy towards alternative fuels especially from bio-based resources [1]. Bioethanol produced from renewable source is considered as clean fuel and substitute for gasoline. The first and second generation bioethanol are mainly produced from food crops and lignocellulosic materials. The availability and low cost of substrate gives an additional advantage on substrate selection [2]. The lignocellulosic biomass is widely used for the production as there is existence of food competence in the society. The economy of the production of bioethanol mainly depends on the substrate and feedstock used for fermentation [3]. The lignocellulosic biomass is composed of cellulose (40–50%), hemicelluloses (25–30%) and lignin (15–20%) in nature [4]. The cellulose and hemicelluloses are hydrolyzed to soluble sugars and further converted to liquid fuel by a fermentation process [5,6]. Lignocellulosic biomass, the main source for bio-based chemicals are enzymatically hydrolyzed by cellulase consisting of endoglucanases, cellobiohydrolases and β-glycosidase [7,8]. Lignin is a hydrophobic aromatic polymer tightly bound with hemicelluloses. It is necessary that lignin should be removed for the liberation of cellulosic polymer [9,10]. Due to the complex cross linking in substrate, the conversion of biomass to biofuels is a great challenge. This issue is minimized by subjecting substrate to pretreatment and fractionation for the better conversion of substrate into biofuels [11]. The biomass of *Sesbania aculeate* used in this study was found to be abundant in agricultural lands that has low moisture and decomposes at easy bases. *Sesbania aculeate* plant grows in dry land, requires less water, and produces large amount of biomass. It was reported that the starch and sugar content were found to be more in terms of dry weight. The biomass was mainly utilized as the availability of dry biomass was more than the other sources.

The efficient removal of lignin and hemicelluloses is achieved by pretreatment of substrate either by physical, chemical or by thermal hydrolysis [12]. The enzymes used for the production are immobilized in nanomaterials by covalent binding as they provide stable bonding between amine and carbonyl groups of glutaraldehyde [13,14]. The enzyme immobilization reduces the cost of the enzyme by enabling the process of reusability with increased stability. This process is considered as superior because of the added advantages such as thermostability and storage. The advantage of high surface to volume ratio of nanomaterials has a direct impact on the behavior of the enzyme. The other added advantage of using

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immobilized enzyme is that they can be used in both aqueous and low water media [15,16]. Covalent binding and affinity interaction are widely used because of less enzyme leakage from the support. In order to improve the viability of the process, immobilization of nanomaterials on enzyme has been extensively used for the production of biofuels [17,18]. The major issues on using magnetic particles are the presence of insoluble in the suspension making it difficult for the separation process [19–21]. Researchers have reported the use of cellulase enzyme immobilized on magnetic nanoparticle for the effective hydrolysis of wheat straw. In the present work, the *Sesbania aculeate* biomass was hydrolyzed using cellulose immobilized magnetic nanoparticles as nanobiocatalyst for bioethanol production.

2. Materials and methods

2.1. Materials used

The *Sesbania aculeate* biomass used for the bioethanol production was collected from agriculture land in Perambalur district, Tamilnadu. The laboratory grade chemicals used for synthesis of magnetic nanoparticles such as ferrous sulphate, ferric chloride and sodium hydroxide were purchased from Reachem Laboratory, Chennai. Aminoethyl aminopropyl (APTS), Tetra ethyl orthosilicate (TEOS), Sodium fluoride of analytical grade were purchased from Sigma Aldrich and Fisher Scientifics, India. Glutaraldehyde was obtained from LOBA Chemical Laboratory, India. Cellulase (CAS No. 9012-54-8) enzyme from *Trichoderma longibrachiatum* (≥1.0 unit/mg solid) used for hydrolysis was purchased as commercial enzyme from Sigma Aldrich, India. All these chemicals were used without any further purification.

2.2. Collection and pretreatment of substrate

The *Sesbania aculeate* biomass was collected from agriculture fields in Perambalur District, Tamilnadu. It was dried in sunlight for 2 days and was further dried in oven for at 60 °C for overnight. The dried biomass was ground to fine particles and stored at room temperature for further use. The ground biomass particles (substrate) were hydrolyzed in autoclave at 121 °C for 30 min using 0.5% (v/v) of sulphuric acid, nitric acid or hydrochloric acid. The pretreated mixture was filtered and dried overnight at 60 °C in hot air oven to remove the excess moisture content. The chemically pretreated biomass was subjected to cellulose estimation by anthrone method [22].

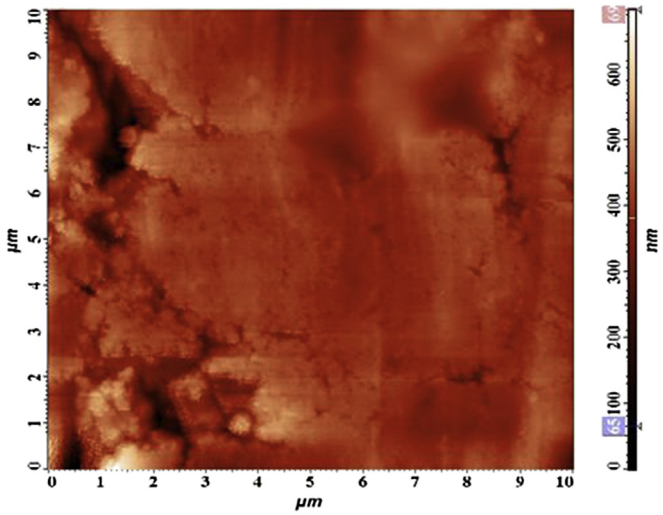

Fig. 1. Surface characterization of cellulase bound magnetic nanoparticle by AFM.

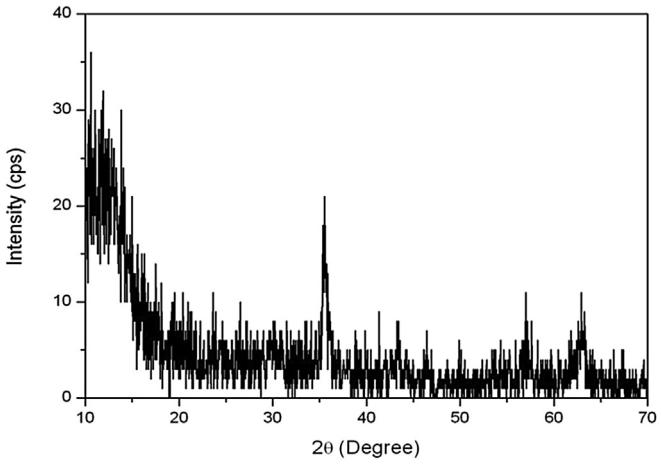

Fig. 2. Structural information of enzyme bound magnetic particle.

2.3. Synthesis of magnetic nanoparticles

Magnetic nanoparticle (MNP) was synthesized by modified co-precipitation method. In this method, 0.2 M of $FeCl_3$ and 0.1 M of $FeSO_4$ was prepared for 50 ml by keeping the solution under constant stirring condition. 1% NaOH of was added drop wise under stirring until a black precipitate is obtained. The solution was left undisturbed until the particle settles at the bottom. The obtained precipitate was separated by magnetic decantation and washed twice with distilled water. The final product was dried at 80 °C in hot air oven and made into fine powder. The synthesized magnetic particle was modified functionally by suspending the magnetic particles in 20 ml of distilled water with the addition of APTS, sodium fluoride, methanol and TEOS respectively. The mixture was stirred vigorously for 24 h. The obtained precipitate was washed with ethanol, water and dried [23].

2.4. Immobilization of cellulase with magnetic nanoparticles

MNP (500 mg) was suspended in 100 ml of deionized water and subjected to ultrasonication to reduce the size of the particle. To this solution, glutaraldehyde (1 M) was added and incubated for 1 h at 25 °C in a shaker. The mixture was washed twice with sodium acetate buffer and deionized water. The cellulase was added with respect to the amount of magnetic particle and incubated for 2 h at 25 °C. The nanobiocomposite was washed with deionized water and buffer to remove any loosely bound particles [24].

2.5. Characterization of magnetic nanobiocomposite of cellulase

The surface morphology of the nanobiocomposite was determined by Atomic Force Microscopy (AFM) (NTMDT, Ireland). The phase structure and functional bonding of the nanobiocomposite was determined by X-Ray Diffraction (XRD) (RIKAGU, Japan) and Fourier Transform Infra Red spectroscopy (FTIR) FT-IR 6300, Jasco, International Co., Japan respectively. The property of magnetic nanoparticles after cellulase binding was confirmed by Vibrating Sample Magnetometer (VSM) (VSM-Lakeshore-7410).

2.6. Enzymatic hydrolysis of biomass

The prepared nanobiocomposite of cellulase was used as