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Research article

A green and sustainable approach on statistical optimization of laccase mediated delignification of sugarcane tops for enhanced saccharification

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ABSTRACT

Bioethanol production from lignocellulosic biomass is a promising approach towards finding an alternative for transportation fuels that is driven by the prerequisite to lessen our dependency on fossil fuels, increase energy security and mitigate greenhouse gas emission. Recalcitrance of lignocellulosic biomass is a major hindrance in bioethanol production. Hence, an efficient pretreatment method is necessary for degradation of lignin and providing accessibility of holocellulose for hydrolysis. In an attempt to overcome this bottleneck, laccase mediated delignification of sugarcane tops was studied using central composite design (CCD) based on response surface methodology (RSM). The effect of different process parameters such as temperature, pH, solid loading, enzyme titre and incubation time were evaluated. It was observed that under optimum conditions of pH 7, solid loading of 21% (w/v), enzyme titre of 430.3 IU/mL, temperature of 40 °C and incubation of 6 h, maximum delignification of 79.1% was achieved. Compositional analysis, energy density measurement and water retention capacity of the biomass was also conducted along with GC-MS analysis for identification of low molecular compounds formed during delignification. Structural characterization of the biomass before and after pretreatment process were analysed by Scanning Electron Microscopy (SEM), Fourier-Transform Infra-Red Spectroscopy (FTIR) and X-Ray Diffraction Spectroscopy (XRD) that further substantiated the delignification of sugarcane tops.

1. Introduction

The oil crisis of 1973 is said to be the pivotal moment that facilitated the attention of the world towards biofuel production. Geo-political strife and wars in the twentieth century were critical drivers in losing assurance in the continuity of oil supplies for transportation fuels. Bioethanol production from lignocellulosic biomass has been recognized as a promising alternative to gasoline having high octane number and heat of vaporization that is well-suited with modern vehicles. Bioethanol is an oxygenated fuel containing 34.7% oxygen which is otherwise absent in gasoline. This in turn results in 15% higher combustion efficiency of bioethanol compared to gasoline thus lowering the emission of particulates and nitrogen oxides that poses threat to the health of living beings. There is negligible amount of sulphur in ethanol compared to gasoline consequently helping in lowering the sulphur content in the fuel when blended. In addition, blending can also help in lowering the emission of sulphur oxide, a carcinogenic agent that contributes in acid rain.

An extensive variety of lignocellulosic biomass are available that have the potential to be converted into high value bio-products like bioethanol. Lignocellulosic biomass consists of energy crops, forest residues, agricultural residues and organic solid municipal waste. Sugarcane tops is an agricultural residue that has the potential to be used as a source for bioethanol production. India is the second largest producer of sugarcane with an estimate of 330.36×10^9 kg sugarcane production for 2016/17 (USDA, 2016). Sugarcane generates huge amount of agricultural residues in the form of sugarcane tops which are basically top portion flanked by the upper end and the last stalk node attached with green leaves (Pereira et al., 2015). Survey conducted by NIIST–TIFAC, stated that for every 1000 kg of sugarcane, 250–300 kg (wet basis) of sugarcane tops is generated

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(Sindhu et al., 2012). Sugarcane tops can be used as fodder for animals but are low in nutritive value hence they are used as low quality roughage. Besides that it does not find any suitable use therefore it is usually left to rot in the field and burnt prior to the next cycle of cultivation.

Lignocellulosic biomass is the key building block of plant cell wall comprising of cellulose, hemicellulose, lignin, extractives, pectin and ash. Though relative fraction of the individual components may vary with the type of plant, species, age, climatic conditions and source of the biomass, lignin and holocellulose remain as the major fractions of the biomass. The relative abundance of these polymers among other things are significant factors in determining the ideal energy conversion pathway for different types of lignocellulosic biomass. The presence of lignin poses as a major recalcitrance of lignocellulosic biomass impeding the accessibility of holocellulose during saccharification. Several pretreatment methods consisting of physical, chemical, physico-chemical and biological approaches have been established to diminish the recalcitrance and enhance fermentable sugars of lignocellulosic biomass. Though there are various methods for pretreatment of biomass, every pre-existing technologies has its advantages and drawbacks concerning the cost of the process, consumption of energy and inventories. Generally chemical and physico-chemical pretreatment are associated with energy intensive process, production of inhibitors and solubilisation of hemicellulose.

Biological pretreatment based on lignin degrading microorganisms and their enzymes can play a major role in the efficient and eco-friendly use of lignocellulosic biomass for the sustainable production of bioethanol since they operate on mild conditions, low input of energy and no discharge of toxic effluents. Peroxidases and oxidases are classified under ligninolytic enzymes among which laccases (phenoloxidases, EC 1.10.3.2) is industrially the most sought after due to its high stability, ability to be produced on a large scale, broad substrate specificity and utilization of molecular oxygen instead of hydrogen peroxide in its catalytic reaction.

Laccases are extracellular multicopper oxidoreductases that act on phenolic/non-phenolic moieties using molecular oxygen as the final electron acceptor for oxidation. They are engaged in the oxidation of phenolic lignin and aromatic compounds that helps in the generation of phenoxy radicals that acts as natural mediators/enhancers. These low molecular weight mediators aids in the oxidation of bulky/non-phenolic units by overcoming steric hindrance and increases the redox potential (Heap et al., 2014).

In the present study, laccase produced from *Pleurotus djamor* was employed on sugarcane tops in pursuit of achieving effective delignification. Different process parameters, such as solid loading, temperature, pH, enzyme titre, and incubation time were optimized via response surface methodology to attain maximum lignin degradation. In addition, biochemical composition analyses, elemental analysis, energy density measurement, water retention capacity and structural changes of sugarcane tops were investigated. GC-MS analysis was also investigated for lignin degraded products. Saccharification of sugarcane tops before and after delignification further substantiated the effectiveness of laccase mediated delignification.

2. Materials and methods

2.1. Lignocellulosic biomass

Sugarcane (*Saccharum officinarum*) tops was accumulated from the local market of Indian Institute of Technology, Kharagpur, India. The biomass was chopped, sundried and milled to 0.2 mm particle size and consequently used for further investigation.

2.2. Enzymes

Laccase was extracted from *P. djamor* grown under solid state fermentation. The activity of laccase (1 IU) is defined as the ability of enzyme to oxidize 1 μmole of 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in 1 min per millilitre of solution (Bhattacharya et al., 2011). Cellulase-xylanase mixture was produced from *Trichoderma reesei* Rut C30 (Das et al., 2008). The activities of endo-glucanase, β-glucosidase and xylanase were determined according to the procedures of Zhang et al. (2007) and Jeffries et al. (1998). One unit of cellulase activity was described as the amount of enzyme necessary to release 1 μmol of glucose per minute in assay conditions.

2.3. Biochemical composition analysis

Lignin content of sugarcane tops was determined by the method followed by Rajak and Banerjee (2015), cellulose by semi micro determination method (Updegraff, 1969) and hemicellulose content was determined by anthrone method (Marlett and Lee, 2006).

2.4. Elemental analysis

The samples were quantitatively analysed by elemental/CHNS analyzer (M/s Elementar, VarioMicrocube, Germany) to observe changes in the carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) content before and after delignification (Singh et al., 2013). The weight % difference of C, H, N and S was measured for oxygen content of the sample.

2.5. Influence of various process parameters on laccase mediated delignification of sugarcane tops

Delignification was conducted by weighing 1 g of powdered sugarcane tops in an Erlenmeyer flasks (50 mL) to which required volume of laccase was added to maintain the substrate concentration in the range of 5–40% (w/v). The reaction was performed at pH (3–8), incubation time (2–12 h), and enzyme titre (100–1000 IU/mL) at temperature range of (30–60 °C). After completing the incubation time, the solid-liquid separation was conducted and the solid portion was oven dried for estimation of residual lignin. One variable at a time method was adopted for initial selection of the parameters range that were favourable for maximum delignification. CCD based RSM was used after selecting the boundary level in order to maximize delignification process under derived optimum conditions.

2.6. Statistical approach for optimization of laccase mediated delignification of sugarcane tops

CCD based RSM was used for optimization of process conditions for laccase mediated delignification of sugarcane tops. The boundary parameters chosen to establish individual and interactive influence on delignification were: pH (6–8), temperature (35–45 °C), solid loading (15–25%, w/v), enzyme titre (300–500 IU/mL) and incubation time (4–8 h). Based on CCD based RSM, design of experiments was attained and 32 experimental runs were conducted. All the experiments were conducted in triplicates. Table 1 depicts the sequence of experimental design. Response surface regression method was employed for analysis of the experimental data so as to fit the second-order polynomial equation as given below:

$$Y = \beta_{k0} + \sum_{i=1}^{5} \beta_{ki} x_i + \sum_{i=1}^{5} \beta_{kii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} \beta k_{ij} x_i x_j \quad (1)$$