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A cleaner and eco-friendly bioprocess for enhancing reducing sugar production from pineapple leaf waste

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ABSTRACT

Conversion of agro-industrial wastes to energy is an innovative approach for waste valorization and management which also mitigates environmental pollution. In the present study, pineapple leaf waste rich in polymers such as cellulose (41.15% ± 0.25, w/w) and hemicellulose (21.02% ± 0.54, w/w) was utilised for the production of fermentable sugars, which are platform molecules for biofuel generation. The process involved in reducing sugar production from the pineapple leaf waste is entirely through enzymatic means where delignification and saccharification were carried out by laccase and cellulase-xylanase concoction respectively. The efficiency of enzymatic delignification was assessed in terms of reducing sugar production where saccharification without delignification yielded 91.9 mg/g and with delignification yielded 502 mg/g of reducing sugar accounting for a 5.5 fold increase. Delignification of 81.12% resulted in maximum reducing sugar production at high solid loading of 26.5% (w/v) in 6 h. The microscopic, structural and porosity studies of raw and delignified biomass further substantiated the efficiency of enzymatic delignification process. The competence of enzymatic delignification and saccharification process was assessed by mass and energy balance of the processes. Laccase mediated delignification of pineapple leaf waste offers an eco-friendly process for the conversion of wastes into biofuels.

1. Introduction

Increasing demand for energy and concerns regarding climate change due to greenhouse gas emissions have accelerated the search for alternative energy from renewable sources, of which lignocellulosic biomass derived from agriculture and energy crops is the most promising one. Agricultural residue is a carbon neutral source with advantages such as abundance, low cost, non interference with the food chain and environmental footprint. Presence of lignin moieties renders recalcitrance to the lignocellulosic biomass and hinders the access of carbohydratases towards holocelluloses. Delignification of biomass facilitates the hydrolysis of holocelluloses to fermentable sugars that are involved in the production of biofuels and other biochemicals. Until date, biofuel industries have their own well established physical, chemical and physico-chemical methods for delignification of biomass. The operating conditions for conventional delignification processes are very harsh that result in the production of harmful and inhibitory intermediates (Sannigrahi et al., 2011). Moreover, these are multi-targeted processes which not only degrade the lignin but also solubilise the cellulose and hemicelluloses moieties which are the precursors for biofuels and biorefinery (Karnchanawong et al., 2017; Bevilaqua et al., 2013). To the best of our knowledge no industry is associated with the enzyme mediated lignin removal or degradation which is an eco-friendly process. Implementation of an effective and eco-friendly pretreatment method that operates with low energy input, minimum inhibitor formation and short process time is beneficial from both industrial and environmental perspective.

Enzymatic delignification operates under mild conditions and owing to the specificity of the enzyme, it prevents the production of

Abbreviations: Mt, million tonnes; Mha, million hectares; w/w, weight per weight; w/v, weight per volume; rpm, revolutions per minute; h, hour; IU/g, International Units per gram; mg/g, milli grams per gram; 3D, three dimensional; min, minutes; nm, nano meter; Pa, Pascal; kJ/g, kilo joules per gram; KBr, potassium bromide; cm, centimeter; α, alpha; mA, milliampere; kV, kilovolts; θ, theta; m^2/g, square meter per gram; cm^3/g, cubic centimetre per gram; mL, milli litre; INR, Indian rupee; sp., species.

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harmful and inhibitory by-products (furfurals and hydroxymethylfurfurals). Laccase is an oxido-reductase enzyme that oxidizes phenolic and non-phenolic groups of lignin without affecting the structure of cellulose and hemicelluloses (Madhavi and Leela, 2009). The application of free and immobilised laccase in dye degradation (Zamora et al., 2003), wastewater (Osma et al., 2010) and soil treatment (Duran and Esposito, 2000) has already been reported but fewer studies are available in the scientific domain on laccase mediated delignification of biomass. Lignocellulosics such as *Bambusa bambos* (Mukhopadhyay and Banerjee, 2015), *Ricinus communis* (Mukhopadhyay et al., 2011) and *Lantana camara* (Kuila et al., 2011a) pretreated with laccase resulted in 73.90%, 85.69% and 88.79% delignification respectively whereas 50% delignification from *Eucalyptus globules* wood was reported using commercial recombinant laccase (Rico et al., 2014). Thus, the above cited literature shows the new technological developments that broaden the possibilities for application of tailor made oxido-reductive enzymes for biofuel generation.

The worldwide pineapple production was estimated to be 24.80 Mt (million tonnes) from 1.02 Mha (million hectares) for the year 2013–14 (FAOSTAT, 2015), the production from India being 1.74 Mt from 0.11 Mha (Indian Horticulture Database, 2014). The leaf portion that remains in the field after fruit harvest amounts to around 2708.22 Mt, a part of which is generally used in making of rope fibres and paper industries. Utilisation of pineapple leaves, rich in holocellulose content (60–85%, w/w), for biofuel production is an endeavour towards valorization of waste (Daud et al., 2014). India being the fifth largest producer of pineapple in the world, the considered waste biomass is a feasible substrate for biofuel based industries (Agri Farming, 2015). The cost of cellulolytic enzyme is a major constraint in lignocellulosic bioethanol production. This calls for the development of an improved saccharification process with low dosage of cellulolytic enzyme at high solid loading. Laccase mediated delignification was reported to enhance saccharification efficiency of cellulase (Jegannathan and Nielsen, 2013).

In the present work, laccase mediated lignin degradation from pineapple leaf waste was attempted, followed by the production of reducing sugar. Optimum process conditions for enzymatic delignification and saccharification were obtained through response surface methodology (RSM) based on central composite design (CCD). Biochemical characterization and energy density of pineapple leaf waste was evaluated to assess its potential as a substrate for biofuel production. Porosity, microscopic and structural characterization studies of raw and delignified substrates corroborated the competence of enzymatic delignification process for improved production of reducing sugar and provide an eco-friendly route to biofuel and bioproduct industries.

2. Materials and methods

2.1. Substrate and its biochemical composition

Pineapple (*Ananas comosus*, Giant Kew variety) leaf waste was collected from the agricultural farm of Indian Institute of Technology Kharagpur, West Bengal, India. The substrate was chopped, sun-dried and pulverised to 0.2 mm particle size. Cellulose (Viles and Silverman, 1949), hemicellulose (Marlett and Lee, 2006), ash (TAPPI standard method), pectin (Sadasivam and Manickam, 1992) and lignin content of substrate were estimated by titrimetric (Hussain et al., 2002) and sulphuric acid methods (Theander and Westerlund, 1986). The reducing sugar was estimated by dinitrosalicyclic acid method (DNS) (Miller, 1959).

2.2. Enzyme

Enzymatic delignification and saccharification were carried out by laccase produced from *Lentinus squarrosulus* MR13 and cellulase-xylanase concoction produced from *Trichoderma reesei* Rut-C30 respectively. Laccase and cellulase-xylanase were produced in Microbial Biotechnology and Downstream Processing laboratory (MBDSP), IIT Kharagpur. Production of laccase was done according to Mukhopadhyay and Banerjee (2015) whereas saccharifying enzyme was produced by inoculating the spores (~3.6×10^6 spores per 1 mL) of *T. reesei* to wheat bran mixed with Czapek-Doc medium (pH 5) in 1:1 ratio (w/v) and incubated at 30 °C for 5 days. The enzyme was extracted in water and centrifuged at 10,000 rpm for activity measurement. The activity of laccase (Bhattacharya and Banerjee, 2008), cellulase (Mandels and Andreotii, 1976) and xylanase (Bailey, 1988) were measured following the standard assay protocol.

2.3. Methodology and optimization of enzymatic delignification and saccharification of substrate

The methodology for enzymatic delignification and saccharification of pineapple leaf waste was represented in Fig. 1.

Optimization of enzymatic delignification and saccharification of pineapple leaf waste was carried out by RSM based on three-level (−1, 0, +1), 2^5 factorial CCD using Minitab 16 software. The parameters studied for enzymatic delignification were: solid loading (15–25%, w/v), incubation time (4–6 h), temperature (35–45 °C), pH (5–7) and enzyme concentration (3500–4500 IU/g). For saccharification, the parameters considered were: solid loading (20–30%, w/v), incubation time (5–7 h), temperature (45–55 °C), pH (4–6) and enzyme (cellulase) concentration (60–100 IU/g) (corresponding xylanase concentration is 525–872 IU/g).

Based on the CCD, design of experiments (DOE) was obtained and experiments (32 runs) were performed. The results (Table S1 and S2, Supplementary Information (SI)) thus obtained were fed to the software and analysed based on RSM. A polynomial quadratic regression equation was obtained which represents the effect of independent factors and its interactions towards the output (% delignification and reducing sugar concentration (mg/g). The interactive effects of parameters were analysed based on 3D response surface plots. The optimizer predicts the optimized condition along with the predicted output. The validation experiment was carried out based upon the optimizer predicted output.

The enzymatic delignification of the substrate was carried out with required amount of substrate and laccase (according to solid loading) under defined reaction conditions. After specific

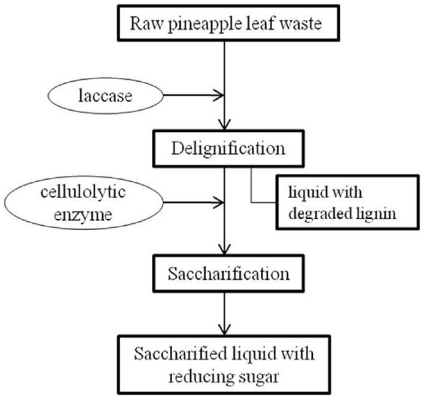

Fig. 1. Schematic representation of complete process for reducing sugar production.