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Partially consolidated bioprocessing of mixed lignocellulosic feedstocks for ethanol production

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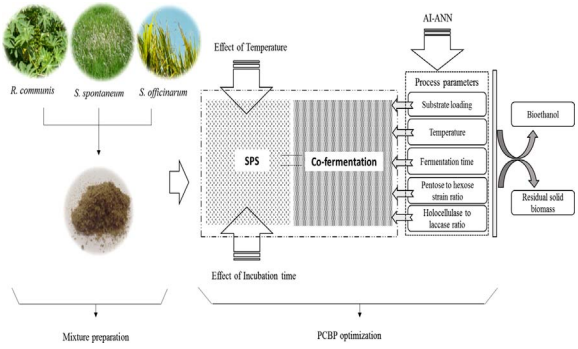
GRAPHICAL ABSTRACT



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ABSTRACT

Rapid urbanization and industrialization have accelerated the energy demand which cannot be met by decreasing fossil fuels thereby substantiate the need for lignocellulosic ethanol. The present study is one such attempt towards bioethanol production in an eco-friendly manner using enzymes in which a mixture of lignocellulosic biomass namely, *Ricinus communis*, *Saccharum officinarum* (tops) and *Saccharum spontaneum* were taken as a substrate. The mixed biomass was processed through partially consolidated bioprocessing (PCBP) approach which involves a non-isothermal simultaneous pretreatment and saccharification step where a concoction of laccase (*Pleurotus djamor*) and holocellulase (*Trichoderma reseei* RUT C30) was used followed by co-fermentation within the same reactor. The process parameters influencing PCBP were optimized using feed-forward ANN model which resulted in a maximum ethanol concentration of 7.86% (v/v) (62.01 g/L) at pentose to hexose strain ratio of 0.696 (v/v), substrate loading of 27.54% (w/v) and incubation time of 21.96 h.

1. Introduction

The carbohydrate polymers of lignocellulosic biomass are the renewable alternatives to crude oil based petro-fuels. The shift towards renewable carbons from fossil fuels is mainly driven by the alarming scenario of the world energy demand, which is 80% quenched by combusting fossil fuels, of which 58% of demand is from the transportation sector alone (Zabed et al., 2016). At this junction, lignocellulosics namely, energy crops, grasses, agricultural crop residues, forest remains and industrial wastes are foreseen as the prospective raw materials for commercial second generation (2G) ethanol production. These feedstocks are cost effective compared to starch and sugar rich first generation (1G) biomass and are fundamentally non-edible by humans due to highly unpalatable lignocellulose. Therefore,

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lignocellulosic biomass is strictly non-interfering as far as human food reserves are concerned thus avoiding food vs. fuel conflict. Besides, certain varieties of lignocellulosic biomass are toxin rich and thus cannot be used as fodder material. Such non-edible and non-grazable varieties overcome the food, fodder vs. fuel conflict and consequently can serve as vital biomass for 2G ethanol production both for domestic and international markets.

Lignocellulose entails the energy rich carbohydrate polymers which are an integral component of the plant cell wall that are intertwined to provide complexity to the cell structure. The polymer matrix is composed of a high energy density linear homopolymer, cellulose (40–50% w/w), and, a branched heteropolymer, hemicellulose (25–35% w/w), which can be harnessed for ethanol production. However, the hemicellulose moieties are covalently bound to the recalcitrant, cross linked phenyl propanoid polymer called lignin (15–20% w/w) which makes carbohydrate depolymerisation challenging (Rico et al., 2014). The 'n' I the empirical formula of lignin–$C_9H_{10}O_2(OCH_3)_n$ – varies significantly between hardwoods (n = 1.40), soft woods (n = 0.94) and grasses (n = 1.18) (Melero et al., 2012). Other minor components of the biomass include pectins, lipids, proteins, ash, minerals and fixed carbon. Till date several physical, chemical or physico-chemical technologies are being practised to overcome the natural recalcitrance of the polymer matrix. For this, the preliminary step of lignin removal from the carbon neutral lignocellulosics is inevitable since this step determines the success of bioethanol production process by influencing further access to the carbohydrate pool. However, the conventional energy intensive biomass pretreatment approaches are typically non-specific in their mechanism and cause significant loss of holocellulose (cellulose and hemicellulose) along with lignin. Moreover, the extreme reaction conditions viz. high pressure and temperature and harsh chemicals used for delignification induce the formation of undesirable by-products that intervene in the process efficacy. Besides, the other key limitations are the higher operating costs due to laborious and time consuming steps and higher energy input. Therefore, the challenge in 2G ethanol production lies in processing the precursor molecules of biorefineries through a simple and subtle pretreatment approach that is ideally substrate specific and rapid in action. In addition, it needs to be competent to process the biomass under mesophilic conditions with meagre inhibitor formation and require less water and net energy input. One such benign and industrially promising approach is the use of ligninolytic enzymes such as laccase, manganese peroxidase, lignin peroxidase and versatile peroxidase for biomass pretreatment. In the present study, laccase produced from a white rot fungi, *Pleurotus djamor* was used amongst other enzymes as it can catalyse oxidoreduction of phenolic and non-phenolic lignin in presence of oxygen and is independent of costly cofactors like biotin, pantothenic acid and thiamine.

Laccase extracted from fungal source is considered to be a potent candidate for delignification considering its specificity towards lignin. A crucial measure to gauge the efficacy of laccase is the redox potential of its T1 copper site which in case of fungal laccases lies in the range of 400–800 mV with reference to standard hydrogen electrode (Xu et. al., 1996). Although the range of redox potential is not sufficient to oxidize the major fraction of lignin within a lignocellulosic biomass i.e., non-phenolic subunits (Li et al., 1999) still there exists a scope considering the role of lignin degradation intermediates which act as mediators for laccase to attain redox potential sufficient to oxidize the non-phenolic units of lignin (Bourbonnais and Paice, 1990) thereby leading to enhanced access of carbohydratases towards the holocelluloses.

Laccases act on both lignin and its related substrate analogues. Due to which these enzymes are applied industrially for several purposes namely degradation of dye from textile effluents, bioremediation of water bodies polluted with phenolic compounds and treatment of waste liquors emanating from softening and bleaching of paper and pulp (Gonçalves et al., 2015). But the industrial scale application of laccase towards biomass pretreatment for biofuels has not been attempted yet by any major player in the Indian biofuel sector.

The rate determining step in lignocellulosic ethanol production is saccharification where the lignin free carbohydrates are depolymerized to hexoses and pentoses using cellulases and xylanases from microbial origin. *Trichoderma reesei* RUT C30 is an efficient industrial strain known for secreting the complete holocellulolytic system. Cost effective saccharification of biomass is still a major challenge in 2G ethanol scheme which needs particular attention as it directly influences the minimum ethanol selling price (MESP) (Cannella et al., 2014). The hydrolysis approach needs to be modified such that higher biomass loading is efficiently processed at low enzyme dose to mark the overall process economical. The reducing sugars released upon saccharification are then co-fermented to ethanol using hexose and pentose fermenting strains since utilization of pentose sugars along with hexoses is prerequisite for commercial viability of 2G ethanol, which is the major setback of glucose fermenting industrial strain, *Saccharomyces cerevisiae*. These steps are generally performed separately similar to the study of Naseeruddin and co-workers who conducted pretreatment of *Prosopis juliflora* biomass (10 g) using 2% (w/v) $Na_2S_2O_4$ for 18 h followed by enzymatic saccharification using cellulase for 36 h leading to the release of reducing sugars (39.37 g/L) which were separately fermented to ethanol (10.85 g/L) using *S. cerevisiae* VS3 and *Pichia stipitis* NCIM 3498 within 36 h in a sequential manner (Naseeruddin et al., 2017). In contradiction to the conventional sequential approach, in the present study, a simplified version of fermentation, referred to as partially consolidated bioprocessing (PCBP) was attempted in the present study to convert the mixed lignocellulosic biomass to ethanol in a shorter incubation time and at higher substrate loading. PCBP involves a prior non-isothermal simultaneous pretreatment and saccharification (SPS) of biomass followed by co-fermentation. This approach is a novel contribution to the pool of existing knowledge where the entire biotechnology process is conducted in a single reactor without compromising the optimum temperatures for maximum activity of lignocellulytic enzymes (laccase, cellulase and xylanase) and yeasts involved in the process. The main advantage of this process is that no intermittent solid–liquid separation is needed, thereby reducing the overall labour, time and cost. In addition, both pretreatment and saccharification are conducted together in PCBP, therefore the enzyme loading is also significantly reduced.

Thus the objective of the present study is to address the knowledge gap and the challenges involved in lignocellulosic ethanol through an integrated PCBP approach for maximum bioethanol production in a shorter time and with minimum allied waste streams. Mixed non-edible lignocellulosic feedstocks comprising whole plant material of *Ricinus communis* (castor) and *Saccharum spontaneum* (kans grass or wild sugarcane) as well as top portions of *Saccharum officinarum* (sugarcane) were used as the substrate to explore the scope of the present ethanol production strategy. A combination of these substrates has not been evaluated for ethanol production till now and this appears to be the maiden attempt not so far reported in literature where in-house laccase from *P. djamor* and cellulases and xylanases from *T. reesei* RUT C30 were used to depolymerize mixed non-edible lignocellulosic biomass for reducing sugars production and co-fermented to ethanol using yeasts through integrated approach of PCBP. The effect of SPS temperature, SPS time, substrate loading, co-fermentation temperature, holocellulase to laccase ratio, fermentation time and pentose to hexose fermenting strain ratio on ethanol yield was investigated. The influential process parameters were further studied through artificial intelligence (AI) based artificial neural network (ANN) model to optimize and validate ethanol production from mixed lignocellulosics. Based on the experimental data obtained from this study and the advantage of the using non-edible mixed lignocellulosics, the strategic approach of PCBP is revealed as an attractive way out to address feedstock needs and allow energy production in an eco-friendly and economical manner.