## Sequential and Combinatorial Pre-Treatment Strategy of Lignocellulose for the Enhanced Enzymatic Hydrolysis of Spent Coffee Waste

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Abstract : Waste from the food-processing industry is produced in large amount and contains high levels of lignocellulose. Due to continuous accumulation throughout the year in large quantities, it creates a major environmental problem worldwide. The chemical composition of these wastes (up to 75% of its composition is contributed by polysaccharide) makes it inexpensive raw material for the production of value-added products such as biofuel, bio-solvents, nanocrystalline cellulose and enzymes. In order to use lignocellulose as the raw material for the microbial fermentation, the substrate is subjected to enzymatic treatment, which leads to the release of reducing sugars such as glucose and xylose. However, the inherent properties of lignocellulose such as presence of lignin, pectin, acetyl groups and the presence of crystalline cellulose contribute to recalcitrance. This leads to poor sugar yields upon enzymatic hydrolysis of lignocellulose. A pre-treatment method is generally applied before enzymatic treatment of lignocellulose that essentially removes recalcitrant components in biomass through structural breakdown. Present study is carried out to find out the best pre-treatment method for the maximum liberation of reducing sugars from spent coffee waste (SPW). SPW was subjected to a range of physical, chemical and physico-chemical pretreatment followed by a sequential, combinatorial pre-treatment strategy is also applied on to attain maximum sugar yield by combining two or more pre-treatments. All the pre-treated samples were analysed for total reducing sugar followed by identification and quantification of individual sugar by HPLC coupled with RI detector. Besides, generation of any inhibitory compounds such furfural, hydroxymethyl furfural (HMF) which can hinder microbial growth and enzyme activity is also monitored. Results showed that ultrasound treatment (31.06 mg/L) proved to be the best pre-treatment method based on total reducing content followed by dilute acid hydrolysis (10.03 mg/L) while galactose was found to be the major monosaccharide present in the pre-treated SPW. Finally, the results obtained from the study were used to design a sequential lignocellulose pre-treatment protocol to decrease the formation of enzyme inhibitors and increase sugar yield on enzymatic hydrolysis by employing cellulase-hemicellulase consortium. Sequential, combinatorial treatment was found better in terms of total reducing yield and low content of the inhibitory compounds formation, which could be due to the fact that this mode of pre-treatment combines several mild treatment methods rather than formulating a single one. It eliminates the need for a detoxification step and potential application in the valorisation of lignocellulosic food waste.

Keywords : lignocellulose, enzymatic hydrolysis, pre-treatment, ultrasound

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