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Optimization of dilute acid-based pretreatment and application of laccase on apple pomace

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HIGHLIGHTS

- ▶ Apple pomace is a polysaccharide-rich under-utilized substrate for bio-processes.
- ▶ Optimization of the key pretreatment conditions using surface response methodology.
- ▶ Laccase treatment degraded 85% of inhibitory phenolic compounds of the hydrolyzate.
- ▶ The mild acid pretreatment produced low amounts of inhibitory furans and acetate.
- ▶ Enhancements of digestibility of apple lignocellulose allow efficient bioconversion.

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ABSTRACT

The present study was aimed to optimize acid-based pretreatment of apple pomace in relation to acid concentration, temperature and reaction time using response surface method with glucose as response variable. In addition, laccase (EC. 1.10.3.2) from *Trametes versicolor* was applied for degradation of polyphenols in apple pomace that could inhibit the further bioconversion steps involving enzymes and fermenting micro-organisms. The optimized conditions were: 1.5 g/100 mL acid concentration, 16 min reaction time and 91 °C reaction temperature, producing 13.9 g glucose/100 g on a dry matter basis. Subsequent application of laccase to hydrolyzates degraded most of the phenolic compounds in apple pomace by more than 85%. The optimized pretreatment conditions resulted in lower concentrations of other inhibitors such as furan compounds and acetic acid. Therefore, dilute acid pretreatment in combination with laccase application can be used for enhancing subsequent hydrolysis of polysaccharides and fermentation of apple pomace.

1. Introduction

The world's total apple production in the year 2008–2009 has been reported to be more than 69.5 million t (FAO, 2008), of which Canada contributed 455,361 t (Statistics Canada, 2010). Every year apple processing industries produce more than 5500 t of apple processing by-products, mainly pomace (residue left after juice extraction) in Nova Scotia, Canada (Rupasinghe, 2003). Apple pomace presents a serious disposal challenge for the industry and is considered as an under-utilized biomass. Nevertheless, it is rich in both soluble (fructose, glucose, sucrose) and insoluble (cellulose, hemicellulose, pectin) carbohydrates; therefore, is suggested to be one of the excellent substrates for bio-processes (Vendruscolo et al., 2008). Apart from carbohydrates, apple pomace also contains lignin, which along with other factors such as cellulose crystallinity affects the use of this biomass for bio-conversion (Hendriks and Zeeman, 2009). The complex physico-chemical associations between lignin, cellulose and hemicelluloses hinder the accessibility of cellulose for hydrolytic enzymes. High lignin to cellulose ratio (Villas-Bôas et al., 2003) and presence of pectin-cellulose, hemicellulose-cellulose, hemicellulose–lignin interactions in apple pomace also limit enzymatic hydrolysis of polysaccharides of apple pomace.

Cellulose could be made accessible to cellulolytic enzymes by pre-treating the plant-based substrate with milling, steam (heat), acid, alkaline, sulfur dioxide, ionic liquids etc. (for review, see Hendriks and Zeeman, 2009). An effective pretreatment is expected to decrease the cellulose crystallinity and degrade the lignin–cellulose network and increase the surface area, thereby allowing the cellulose conversion into glucose by cellulases. Furthermore, pretreatment of fruit-based biomass is also known to release phenolic compounds, essential oils, pectin, and carotenoids (Galanakis, 2012). Pretreatment is considered to be one of the most crucial and expensive steps due to high input involved in terms of heat, acid/base and reaction time. Dilute acid pretreatment is an

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