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# Efficiency of pretreatments for optimal enzymatic saccharification of soybean fiber

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ABSTRACT

The effectiveness of several pretreatments [high-power ultrasound, sulfuric acid ($H_2SO_4$), sodium hydroxide (NaOH), and ammonium hydroxide ($NH_3OH$)] to enhance glucose production from insoluble fractions recovered from enzyme-assisted aqueous extraction processing of extruded full-fat soybean flakes (FFSF) was investigated. Sonication of the insoluble fraction at 144 $\mu m_{pp\ (peak\text{-}to\text{-}peak)}$ for 30 and 60 s did not improve the saccharification yield. The solid fractions recovered after pretreatment with $H_2SO_4$ [1% (w/w), 90 °C, 1.5 h], NaOH [15% (w/w), 65 °C, 17 h], and $NH_3OH$ [15% (w/w), 65 °C, 17 h] showed significant lignin degradation, i.e., 81.9%, 71.2%, and 75.4%, respectively, when compared to the control (7.4%). $NH_3OH$ pretreatment resulted in the highest saccharification yield (63%) after 48 h of enzymatic saccharification. A treatment combining the extraction and saccharification steps and applied directly to the extruded FFSF, where oil extraction yield and saccharification yield reached 98% and 43%, respectively, was identified.



## 1. Introduction

The continuous depletion of fossil fuel sources and increasing environmental concerns associated with the use of fossil-based fuel, such as carbon-dioxide ($CO_2$) gas emission, have triggered the interest in alternative sources of fuels and chemicals. In 2008, 19.5 million barrels per day (MBD) petroleum products were used in the United States with 12.9 MBD (66.2%) of total utilization being imported (EIA, 2009). In the future, the demand for transportation fuel and other fossil fuel is going to upsurge due to growing population. Therefore, a tremendous amount of work is underway to develop sustainable forms of alternative energy, such as wind, solar, geothermal, and biomass. Among several renewable resources, ethanol production from fermentation of 6- and/or 5-carbon sugars remains a promising alternative for transportation fuel. Currently, ethanol is primarily produced in the United States from corn, but only limited quantity (15–20 billion gallons) can be produced from this agricultural resource (NCGA, 2006) and therefore much attention is given to the lignocellulosic biomass considering its abundance, renewability, as well as the possibility of producing liquid fuels and other high-value chemicals. Moreover, the 2007 Energy Independence and Security Act also demands for 36 billion gallons biobased fuels by 2022. Of that, 16 billion gallons must come from lignocellulosic biomass (EIA, 2007).

The conversion of lignocellulosic biomass to fermentable monomers via enzymes has been technically achievable for decades. However, use of lignocellulosic biomass involves several technical and economical challenges (Mosier et al., 2005) and pretreatment of these biomasses remains one of the key hurdles and focus areas for industry and government agencies. Pretreatment is a prerequisite to make cellulosic biomass available to cellulosic enzymes. These pretreatments often involve drastic mechanical, chemical, or biological interventions and result in the production of toxic waste. Suitable pretreatment processes also depend on the physico-chemical properties of the biomass/agricultural residues. In this study, different strategies were explored to convert the fiber fraction recovered from enzyme-assisted aqueous extraction processing (EAEP) of extruded soybean flakes into fermentable sugars. EAEP is an environmentally friendly alternative to hexane (solvent) extraction of soybean oil and results in oil extraction yield of ~97% (de Moura et al., 2008). The process also produces a protein- and oil-lean fraction, and an insoluble carbohydrate-rich fraction. The soybean insoluble fraction recovered from the traditional hexane extraction is commonly used as a source of animal feed. Interestingly, this fraction has arisen limited interest for biofuel production, while some soybean materials such as soybean hulls or

*Abbreviations:* AC, accellerase 1000; AEP, aqueous extraction processing; AIL, acid insoluble lignin; ASL, acid soluble lignin; db, dry basis; EAEP, enzyme-assisted aqueous extraction processing; FFSF, full-fat soybean flakes; FPU, filter paper units; LSD, least significant difference; NA, not available; ND, not determined; NREL, national renewable energy laboratory; P6L, protex 6L; P50FP, protex 50FP; S.R., solid remaining; SLR, solid-to-liquid ratio.

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soybean molasses have been tested (Corredor et al., 2008; Qureshi et al., 2001). We previously demonstrated that the fiber fraction recovered from EAEP of extruded soybean flakes can be used as a source of fermentable sugars, saccharification yield up to 49.0% being obtained (Karki et al., 2011). The extrusion pretreatment applied to soybean flakes before aqueous extraction largely contributed to the increased accessibility of the saccharification enzyme to its substrate. Pretreatment methods such as steam-explosion (Corredor et al., 2008), acid treatment (Lloyd and Wyman, 2005), alkaline treatment (Ko et al., 2009; Zhao et al., 2008), ammonia fiber explosion (Teymouri et al., 2005), high-power ultrasound (Lomboy-Montalbo et al., 2010; Nitayavardhana et al., 2010), and extrusion (Dale et al., 1999) have been studied on a large variety of biomass. Our first objective was to determine if higher conversion of the soybean fiber recovered from EAEP can be achieved by adding a pretreatment before enzymatic saccharification. We compared the potential of a pretreatment having a physical effect, i.e., high power ultrasound, to chemical pretreatments, i.e., ammonium hydroxide, sodium hydroxide and sulfuric acid. Previous study has identified that water requirement plays a central role in liquid fuel production by affecting production costs and energy demands (Gerbens-Leenes et al., 2009). Therefore, in the perspectives of saving water usage and processing steps, the potential of combining the extraction and saccharification processes, with simultaneous or sequential addition of cellulase and protease enzymes, was investigated (Fig. 1). This combined step could also potentially add value to the skim fraction as a source of proteins and fermentable sugars that could be used as an enriched fermentation media.

## 2. Methods

### 2.1. Materials

Full-fat soybean flakes (FFSF) were prepared from variety IA 92M91 soybeans (Pioneer/DuPont, Johnston, IA, USA) harvested in 2008. The soybeans were cracked in a roller mill (Model 10X12SGL, Ferrel-Ross, OK, USA) and aspirated using a multi-aspirator (Kice, Wichita, KS, USA) to separate into meat and hull fractions. The meats were conditioned to 60 °C using Triple-deck seed conditioner (French Oil Mill Machinery Co., Piqua, OH, USA) and were flaked by using a smooth-surfaced roller mill (Rosskamp Mfg Inc., IA, USA) to 0.3 mm thickness and 3–5 mm width. Prior to extrusion, initial moisture content of the flakes was adjusted to 15% by spraying water in a Gilson mixer (Model # 59016A, MO, USA). The moisture adjusted FFSF were then stored at 4 °C in a double polyethylene bags until used. The enzymes, Protex 6L (P6L, alkaline serine endopeptidase), Protex 50FP (P50FP, endo/exo-peptidase), and Accellerase 1000 (AC, endo/exo-glucanase, hemi-cellulase, β-glucosidase) were provided by Genencor, a Division of Danisco (Rochester, NY, USA). AC had average activity of 66.6 filter paper units (FPU)/ml as determined by the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Standard Procedures NREL/TP-510-42628 (NREL, 2008a).

### 2.2. Conventional process

#### 2.2.1. Extrusion and aqueous extraction

The FFSF were extruded as described by Jung et al. (2009) using a Micro ZSE-27 twin-screw extruder (American Leistritz Extruders, NJ, USA). EAEP of extruded FFSF was carried out as described in Fig. 1 for the "conventional process". The extraction of the extruded FFSF took place in a 3 or 4 L jacketed reactor, with a bottom drain valve (Chemglass, NJ, USA). A 1:10 (w/w) flakes-to-water ratio (db), 50 °C extraction temperature (HAAKE Phoenix PI, Thermo HAAKE, NH, USA) and 9.0 slurry pH were used. Protease P6L was added to the slurry at concentration of 0.5% [g enzyme (as is)/g flakes (db)] and the slurry was stirred via an external stirrer at 600 rpm (LR 400C, Fisher Scientific, NJ, USA or BDC3030, Caframo, Ontario, Canada) for 1 h. The pH was kept nearly constant to 9.0 by adding 2 N NaOH during hydrolysis.

#### 2.2.2. Solid–liquid separation

Following EAEP of extruded flakes, the slurry was centrifuged at 3000*g* for 15 min at 20 °C (Avanti J-20 XPL, Beckman Coulter, CA, USA) to separate the insoluble fraction from the liquid fraction. The insoluble fraction was collected in plastic cups and stored at 4 °C for up to 3 weeks before use.

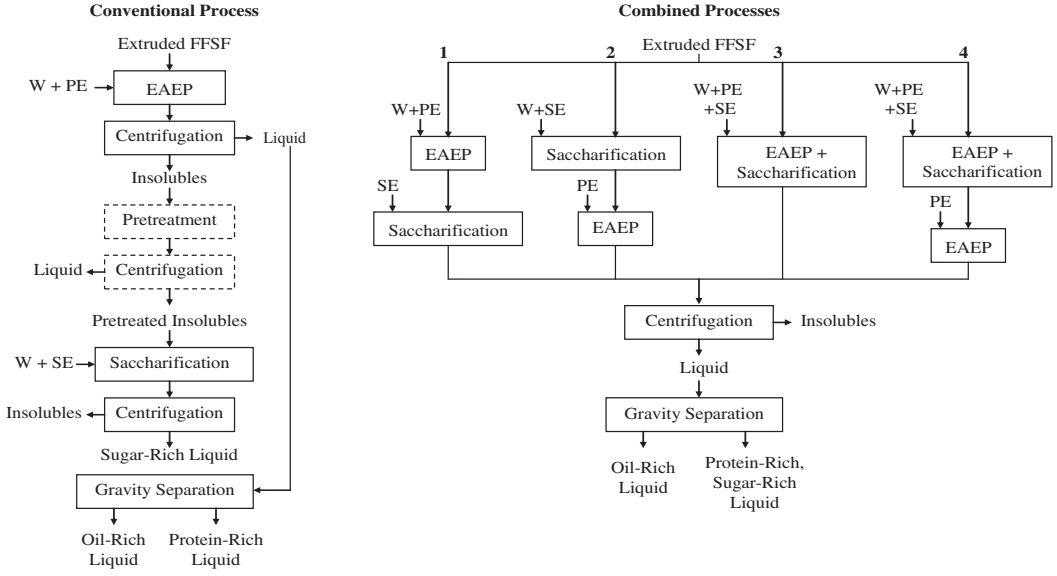


**Fig. 1.** Schematic of conventional and combined processes for recovery of oil-, protein- and sugar-rich fractions from full-fat extruded soybean flakes. The dashed boxes represent optional steps.