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Corn bran bioprocessing: Development of an integrated process for microbial lipids production

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HIGHLIGHTS

- Corn bran successfully used as feedstocks for microbial lipid production.
- RSM showed significant effect of pretreatment conditions on lipid accumulation.
- RSM demonstrated solid loading was a significant factor for lipid accumulation.
- Sugar yields of 0.53 g/g was achieved from de-starched corn bran.
- Lipid yields of 3.6 g/100 g was achieved from de-starched corn bran.

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ABSTRACT

This study investigated the potential of corn bran as a feedstock for microbial lipid production using oleaginous yeast, *Trichosporon oleaginosus* ATCC20509. Different conditions (solid loading of biomass, acid loading, and pretreatment duration) were applied to optimize pretreatment processes using the Box-Behnken design. The highest sugar yield of 0.53 g/g was obtained from corn bran hydrolysates at a pretreatment condition of 5% solid loading and 1% acid loading for 30 min. Compared with synthetic media, up to 50% higher lipid accumulations in *T. oleaginosus* were achieved using corn bran hydrolysates during fermentation. Also, the direct effect of pretreatment condition on the lipid accumulation of *T. oleaginosus* was investigated using response surface methodology (RSM). Solid loading of biomass during the pretreatment process significantly affected the fermentation process for lipid accumulation of *T. oleaginosus*. The RSM model can provide useful information to design an integrated bioconversion platform.

1. Introduction

Microbial lipids are preferable to alternate plant oils in the biodiesel and oleo-chemical industry due to their fatty acid composition, environmental impact, year-round production, and no requirement of broad lands (Sitepu et al., 2014a; Zhou et al., 2016). Oleaginous yeast efficiently accumulates lipids at least 20% (w/w of dry-cell mass), mainly as a form of triacylglycerides (TAG), using a broad array of agricultural wastes (Vieira et al., 2016; Matsakas et al., 2014; Lin et al., 2013; Kerkhoven et al., 2016). Many endeavors to improve an economically feasible production of microbial lipids have been attempted such as exploration of new sugar suppliers and development of bioconversion processes to reduce costs (Lopes, 2015; Sitepu et al., 2014b). Previous studies have successfully produced microbial lipids using renewable biomass — such as corn stover, wheat straw, and switchgrass — as substrates (Gong et al., 2014; Slininger et al., 2016; Yu et al., 2011).

Bran, the outer layer of cereals, is too often discarded during the milling process instead of being used as a food application, due to consumers' sensory expectations and technological drawbacks in the food industry (Coda et al., 2015). Frequent corn byproducts of dry milling are corn flour, corn bran, and hominy feed, and their economic disposal is the main concern of the food industry in fulfilling environmental regulations (ElMekawy et al., 2013). Corn bran is the most abundant, low-valued co-product of the industrial corn milling process in spite of high amounts of polysaccharide content with marginal amounts of lignin (Yadav et al., 2015). Corn bran is produced in yields of 60–70 g/kg, with a total production of 3×10^6 dry tons per year (Rose et al., 2010). Corn bran contains a large percentage of hemicellulose and has an arabinoxylan

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structure consisting of a β-1,4 linked d-xylopyranosyl backbone and α-l-arabinofuranosyl residues as side units linked (1 → 2) or (1 → 3) to the main chain (Rose et al., 2010; Yadav et al., 2015). Therefore, hemicellulose can be hydrolyzed into pentose (xylose and arabinose) and hexose (glucose, galactose, and mannose) (Peng et al., 2012), and is a promising substrate for microbial lipid production. Development of an effective pretreatment process is responsible for the recalcitrant of biomass structure toward hydrolysis of carbohydrate polymer (Guragain et al., 2014). Another bottleneck for integrated production of microbial lipids from renewable biomass is the generation of inhibitory compounds such as furfural and hydroxyfurfural (Keshav et al., 2016; Cavalaglio et al., 2016). High temperature and pressure with a strong acid or base should be applied to expose the structure of lignocellulosic biomass *via* pretreatment process (Lee et al., 2016). Therefore, generation of inhibitory compounds cannot be avoided in a biorefinery. To maximize utilization of corn bran hydrolysates, selection of an appropriate yeast strain, which has high tolerance to toxic compounds and enables the use of diverse monomer sugars as a carbon source — including xylose and arabinose, would be critical since the toxicity of carbon sources is dependent on the yeast strains (Lee et al., 2017; Fontanille et al., 2012; Fei et al., 2011).

Trichosporon oleaginosus ATCC 20509, which has been recently classified as basidomycetous, is known to accumulate up to 70% (w/w of dry mass) lipids using a variety of carbon sources such as pectin-derived sugar acids, *N*-acetylglucosamine, and whey permeate (Görner et al., 2016). Also, *T. oleaginosus* is able to consume carboxylic acids, which are known as inhibitory compounds during the fermentation process (Lee et al., 2017; Görner et al., 2016). *T. oleaginosus* efficiently produced 8 g/L of lipids using acetate-based nutrients, with a yield of 0.15 g/g and a productivity of 0.64 g/L/h (Gong et al., 2015). Lian et al. (2012) reported that acetate and formate were good energy sources for contribution to growth and lipid production of *T. oleaginosus*.

This study developed the integrated process for microbial lipid production from de-starched corn bran. First, pretreatment conditions were optimized to obtain high sugar recovery and subsequent lipid yields. The pretreatment conditions were applied for de-starched bran, and their sugar recoveries were investigated. Also, corn bran hydrolysates, which were produced *via* different pretreatment conditions, were evaluated as nutrients for lipid production by *T. oleaginosus*. In addition, overall lipid yields from raw corn bran were calculated to investigate corn bran utilization as feedstocks for lipid production. Furthermore, the relationship between pretreatment conditions and lipid accumulation in *T. oleaginosus* was investigated using response surface methodology (RSM).

2. Materials and methods

2.1. Microorganisms and fermentation

Trichosporon oleaginosus ATCC20509 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), and grown in a yeast mold broth (YM broth, Difco, Detroit, MI, USA). Culture conditions were 25 °C at 200 rpm. Yeast cultures were preserved in a YM agar plate at 4 °C and re-cultivated to a fresh plate once a month.

2.2. Preparation of de-starched corn bran

Corn bran was obtained from LifeLine Foods, St. Joseph, Missouri, USA, and milled using a milling machine (Fitz-Mill, Fitzpatrick Company, Elmhurst, USA). After milling, corn brans were dried at 40 °C for 72 h.

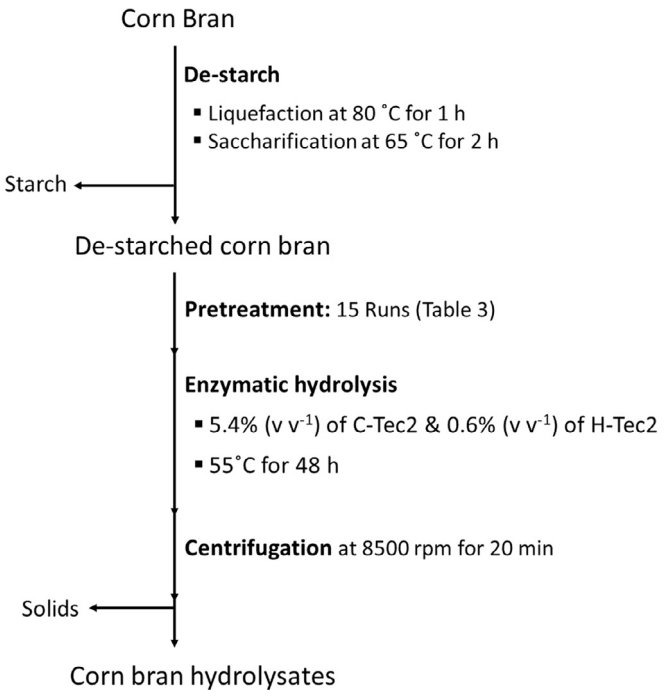

Fig. 1. Schematic of corn bran hydrolysates production.

Table 1
Composition of corn bran and de-starched corn bran (Probst and Vadlani, 2015).

Component (%, w/w)	Corn bran	De-starched corn bran
Cellulose	9.6 ± 0.2	14.2
Hemicellulose	25.8 ± 1.3	38.0
Starch	32.2 ± 3.3	–
Acid detergent lignin	1.4 ± 0.1	2.1

The overview of corn bran hydrolysate preparation is shown in Fig. 1. Alpha-amylase (Liquozyme, Novozymes Inc. Franklinton, NC, USA) and glucoamylase (GC480, Genencor International Inc., Palo Alto, USA) were provided by MGP Ingredients (Atchison, KS, USA) to remove starch *via* liquefaction and saccharification. Corn bran was mixed with distilled water at a rate of 15% (w/v) solid loading, and α-amylase was added with a concentration of 1 μL/g starch. Liquefaction was conducted at 80 °C for 1 h. Corn bran slurry was cooled to 65 °C and saccharification was performed by adding 5 μL/g starch of glucoamylase at 65 °C for 2 h. De-starched corn bran was washed with water to completely remove starch and dried at 40 °C until the moisture content was below 10% (w/w). The composition of corn bran, which was analyzed at an external laboratory (Agricultural Experimental Station Chemical Laboratories, University of Missouri, Columbia, USA), was shown in Table 1. A previous study regarding lipid production from bran showed that higher lipid yields were obtained from de-starched-bran hydrolysates compared with whole bran hydrolysates (Probst and Vadlani, 2015). Therefore, the starch fraction was removed *via* liquefaction and saccharification before the pretreatment process (Fig. 1).

2.3. Pretreatment and enzymatic hydrolysis of de-starched corn bran

Table 3 shows 15 runs of experimental conditions to optimize the pretreatment condition for de-starched bran. Different solid loading (5, 10, 15%), acid loading (0.5, 1, 1.5%), and pretreatment