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Using sweet sorghum bagasse for production of amylases required for its grain hydrolysis via a biorefinery platform

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ABSTRACT

Sweet sorghum plant, a widely grown energy crop, was utilized through a biorefinery process, by which its grains were hydrolyzed by the crude amylases produced from its bagasse. The hemicellulosic part of the bagasse was hydrolyzed with 0.5–1.0% sulfuric acid at 140–180 °C for 30–60 min and applied for amylase production using halotolerant bacterium *Nesterenkonia* sp. strain F. In the hydrolysate obtained at 140 °C for 60 min using 1% acid, *Nesterenkonia* showed 73.3 U/mL amylase activity by the consumption of 16.2 g/L xylose and 8.3 g/L other sugars. Supplementation of the hydrolysates with sorghum grain resulted in 38–67% higher amylase production. Furthermore, addition of biocompatible surfactants of Tween 20 and Tween 80 (0.1 g/L) increased the activity to 93 and 97 U/mL, respectively. The resulting crude enzyme was used in the process of ethanol production from both tannin-containing and tannin-free sorghum grains (6%), leading to 17.7 and 17.0 g/L bioethanol production, respectively. Through the cultivation of *Nesterenkonia* on the hemicellulosic hydrolysates, 5–10 g/L volatile fatty acids (VFA), 0.36–0.69 g/L acetone-butanol-ethanol (ABE), and 468–721 mg/L single cell protein (SCP) were also produced. The obtained SCP contained most of the essential amino acids and relatively high amounts of phenylalanine (8%), threonine (7%), methionine (6%), and lysine (6%).

1. Introduction

The large-scale production of fuels and chemicals from biomass is an important part in many climate change mitigation and energy supply scenarios. Considering food versus fuel conflict, lignocellulose is the only foreseeable renewable source of carbon required to supply the increasing demands of future fuels and chemicals (Bond et al., 2013). Lignocelluloses are complex composite of cellulose, hemicellulose, and lignin. To develop a real substitute for the current petroleum refinery, all parts of lignocellulose should be valorized through a biorefinery concept (Hörhammer et al., 2014; Menon and Rao, 2012; Moncada and Aristizábal, 2016).

Hemicellulose is a collective name for the polysaccharides of glucose, xylose, mannose, or galactose that are bound by a variety of glycoside linkages in lignocellulose structure. From near 180 billion tons of lignocellulosic material produced annually, more than 50 billion tons hemicellulose are obtainable (Mäki-Arvela et al., 2011). However, enzymatic hydrolysis typically used for the depolymerization of polysaccharide is not a practical process for obtaining hemicellulosic sugars, where hemicellulose polysaccharides with various types of branches requires different enzymes for complete depolymerization. Furthermore, the chemical structure of hemicellulose varies with species and cell types, making hemicellulose enzymatic hydrolysis more challenging (Wyman et al., 2005).

Hemicellulose polysaccharides can be depolymerized by using chemical catalysts, e.g., acids at elevated temperatures. Dilute acid pretreatment is the most recommended process for hemicellulose hydrolysis, where obtaining a hemicellulose free solid is the main objective of the pretreatment (Mosier et al., 2005). Although near complete solubilization of hemicellulose polysaccharides is obtainable by increasing the severity of the acid pretreatment, monomeric sugars are not the sole products of the process. Through this pretreatment, hemicellulose polysaccharides are converted to various products, including soluble oligomers, monomeric sugars, and their degradation products. Most of the individual forms of solubilized hemicellulose have the potential to be used as carbon source for the formation of microbial products. However, for efficient utilization of hemicellulosic hydrolysates, the microorganism should have the ability to utilize a wide

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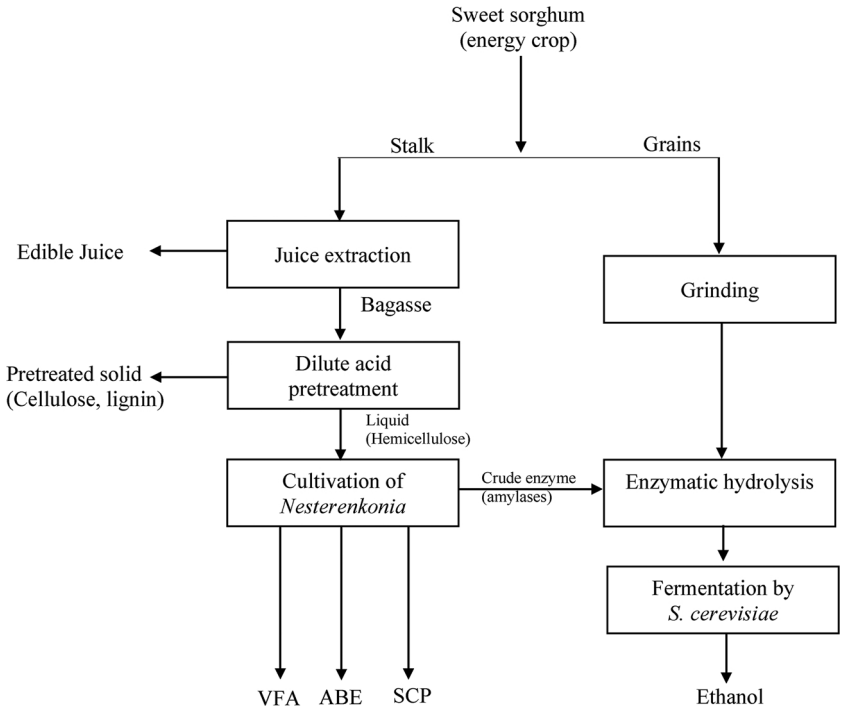

Fig. 1. Overview of processes for hemicellulose valorization using *Nesterenkonia* sp. stain F.

range of carbon sources and withstand high osmotic pressure of the neutralized hydrolysate. The salt formation through the neutralization of the hydrolysate intensifies the osmotic pressure and delimits the activity of typical microorganisms (Ezeji et al., 2007).

Efficient utilization of hemicellulose is considered as an essential part of the cost-effective production of biofuels from lignocelluloses. In this regards, a number of studies have been conducted to utilize hemicellulosic hydrolysates for the production of biofuels, e.g., ethanol (Castro et al., 2017) and butanol (Qureshi et al., 2010). The ethanologens that efficiently ferment glucose to ethanol, e.g., *Saccharomyces cerevisiae* and *Zymomonas mobilis*, cannot uptake xylose and arabinose (Saha, 2003). The yeasts with ability to ferment xylose to ethanol, e.g., *Pachysolen tannophilus*, *Pichia stipitis*, and *Candida shehate*, suffer from the low ethanol tolerance, slow fermentation rates, high sensitivity to inhibitors, and difficulties in controlling optimal rate of oxygen supply (Du Preez, 1994). Furthermore, natural yeast strains rarely ferment arabinose to ethanol (Dien et al., 1996). The toxic compounds generated during hemicellulose recovery is one of the major bottlenecks of hemicellulosic ethanol and xylitol production processes (Mateo et al., 2013). Solvent producing *Clostridia*, e.g., *Clostridium acetobutylicum* and *C. beijerinckii*, are able to uptake pentoses and hexoses for butanol production. However, the *Clostridia* are critically inhibited by the degradation products (particularly phenolic compounds), salts, and osmotic pressure of the hemicellulosic hydrolysates (Ezeji et al., 2007). Furthermore, despite extensive efforts on strain development, the performance of the engineered strains in fermenting hemicellulosic hydrolysates is not acceptable for industrial-scale biofuel production (Taherzadeh and Karimi, 2007).

Alternatively, hemicellulose polysaccharides can be utilized as a carbon source for the production of other important microbial products, either essential consumables or value-added byproducts, in the biofuel industry. In this regard, hemicellulosic hydrolysate from lignocelluloses like sugarcane bagasse has been utilized for xylitol production (Rodrigues et al., 2006). Among different required materials, enzymes have typically a significant share in the biofuel production cost. Considering the high price of pure enzymes, consolidated bioprocessing (CBP) has been suggested for the integration of enzyme production and usage, without necessity of enzyme purification (Favaro et al., 2015). Another advantageous approach is to utilize hemicellulose for the production of essential enzymes, leading to a biorefinery concept. However, the success of this approach is highly dependent on the abilities of the microbial candidate for enzyme production by utilizing dilute acid hemicellulosic hydrolysate. Recently, some halophile and halotolerant bacteria have been attracted interests for their capability in the production of stable amylases (Margesin and Schinner, 2001). Even though this class of bacteria can tolerate the osmotic pressure of dilute acid hydrolysates, to our knowledge, the halotolerant amylase producers have not been evaluated for amylase production from dilute acid hydrolysates.

Nesterenkonia sp. strain F, is a unique halotolerant bacterium that can produce amylases, which are stable in the presence of relatively high concentration of salts and solvents (Shafiei et al., 2011). Recently, this strain has attracted interests for its ability to produce acetone, butanol, and ethanol (ABE) as well as volatile fatty acids (VFA) such as acetic, propionic, and butyric acids under both aerobic and anaerobic conditions (Amiri et al., 2016). Furthermore, it has been shown that *Nesterenkonia* sp. strain F has the ability to utilize a wide range of carbon sources (Amoozegar et al., 2013). Therefore, this bacterium has unique characteristics for the bioconversion of hemicellulosic waste stream remained after dilute acid pretreatment.

Sorghum is an energy crop with several advantages over similar plants. High photosynthetic efficiency, drought resistance, little need to fertilizers, and easy sowing are among the advantages of this cereal crop (Almodares et al., 2011). Sweet sorghum or *Sorghum bicolor* (L.) Moench contains non-edible starchy grains along with about twenty-four-fold higher amounts of lignocellulosic biomass (Mirfakhar et al., 2017). Therefore, both starch and lignocellulosic parts of *S. bicolor* (L.) Moench plant have high potentials to be utilized for biofuel production.

In this study, *Nesterenkonia* sp. strain F was examined for the valorization of dilute acid hydrolysate of *S. bicolor* (L.) Moench bagasse. The hemicellulose content of this plant was used for the production of α-amylase as an essential enzyme for bioethanol production from the starchy grains of the sorghum plant. After assessing the effects of the hydrolysis conditions (time, temperature, and acid concentration), starch supplementation, and surfactant addition on the activity of α-amylase, this enzyme was evaluated for direct utilization in the process