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Research Paper

Ethanol production from mixtures of sweet sorghum juice and sorghum starch using very high gravity fermentation with urea supplementation

Nana Baah Appiah-Nkansah[a], Ke Zhang[a], William Rooney[b], Donghai Wang[a,*]

[a] Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS, 66506, United States
[b] Department of Soil and Crop Science, Texas A & M University, College Station, TX, 77843, United States

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ABSTRACT

The objective of this research was to enhance the production of ethanol from grain sorghum flour and sweet sorghum juice using very high gravity (VHG) fermentation. Various amounts of grain sorghum flour were combined with different concentrations of sweet sorghum juice to form (≈33 g/100 mL) mashes. Mashes were fermented to produce ethanol with and without urea supplementation via simultaneous saccharification and fermentation process. Based on one-way ANOVA results, all treatment mixtures had a significant effect on ethanol production and efficiency ($P < 0.05$). It is hypothesized that the combination of inoculation size (10^9 cells/ml), fermentation temperature (30 °C) and 16 mM urea supplementation had a synergistic effect on ethanol production. Free amino nitrogen consumption dynamics and distiller's dried grains with soluble results suggested that assimilable nitrogen was made available for yeast growth and metabolism. Results showed that 20.25% (v/v) of ethanol and up to 96% fermentation efficiency could be obtained from ≈ 33% (w/v) dissolved solids. Results also showed that the optimum sugar ratio of grain sorghum and sweet sorghum juice (18% sugar) for VHG fermentation is 1–1 (sugar to sugar).

1. Introduction

Biofuel processing technologies capable of increasing ethanol production, cost-effectiveness, energy saving, and water efficiency in the current dry-grind ethanol processes would significantly contribute to meeting the growing demand for fuel in commercial transportation. Very high gravity (VHG) fermentation, an evolving fermentation process, has shown to be environmentally friendly, high yield, and cost-effective in both ethanol and beer production (Udeh and Kgatla, 2013; Yu et al., 2012; Puligundla et al., 2011; Nuanpeng et al., 2010; Laopiaboon et al., 2009). VHG is described as "evolving" because it is progressively being explored in the dry-grind process around the world (Russell, 2003). VHG fermentation produces ethanol from mashes containing higher concentrations of dissolved solids. The following are some examples of VHG substrate concentrations reported by previous researchers: sugar cane molasses (47.6% (w/v)); wheat mash (35% (w/v)); rye (32–34% (w/v)); rice (31% (w/v)); and cornstarch (34% (w/v)) (Li et al., 2017; Chu-Ky et al., 2016; Puligundla et al., 2011). The advantages of VHG fermentation include a 58% potential reduction in process water requirement due to high solid mash preparation; low risks of bacterial contamination due to the inability for bacteria to thrive under higher osmotic conditions; higher enzymatic activities due to decreased starch-to-water ratios; and increased productivity and rate of fermentation (Nuanpeng et al., 2010; Puligundla et al., 2011). Industrial application of VHG fermentation technology is known to reduce the energy cost associated with downstream processes such as distillation and stillage evaporation by 4% as well as capital and labor costs (Nuanpeng et al., 2010; Bai et al., 2008).

A major challenge associated with VHG fermentation, however, is the long hours involved to complete the process. This is often referred to as stuck or sluggish fermentation (Russell, 2003; Peralta-Contreras et al., 2013). Sluggish fermentation may arise due to inadequate yeast metabolic activity to completely catalyze sugars into alcohol. The high initial sugar concentrations (without nutrients), deficient amino nitrogen, low water activity, and higher ethanol concentration result in high osmotic pressure, which causes high intracellular ethanol accumulation and creates stress to the yeast cells. This situation negatively impacts yeast dynamics and physiological fitness and slows the fermentation process (Kawa-Rygielska and Pietrzak, 2014; Udeh and Kgatla 2013Russell, 2003; Thomas et al., 1994). Long VHG fermentation duration (12 days) for wheat mash containing 36.5 g of dissolved solids per 100 mL at 17 °C using *Saccharomyces cerevisiae* has been reported (Jones and Ingledew, 1994). In another study, less than 50% of total sugars from wheat mash was fermented for 9 days (Thomas et al.,

* Corresponding author.
E-mail address: dwang@ksu.edu (D. Wang).

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1994). Wang et al. (1998) also reported VHG fermentation duration of 120–114 h for rye and triticale mashes containing 28.5 g dissolved solids per 100 mL at 20 °C.

These long fermentation durations may be solved by mash supplementation. In VHG bioethanol production from cereal, the grain is ground and mixed with water to form an evenly suspended concentrated slurry, which is cooked in the presence of enzyme alpha-amylase, saccharified with glucoamylase, and fermented with *Saccharomyces cerevisiae* to produce ethanol. During the starch-to-sugar conversion process, also known as mashing or liquefaction, complex nitrogenous compounds (proteins) and nutrients are released into the media due to the digestion of protein and starch (Wang et al., 2008; Xu et al., 2007; Thomas and Ingledew, 1990). These nitrogenous materials are digested into amino acids, peptides, and free amino nitrogen (FAN) and then used by microorganisms (Peralta-Contreras et al., 2013; Thomas and Ingledew, 1990). FAN is a soluble protein that has been digested into free amino acids and small peptides, which are essential for yeast development and metabolism (Djameh et al., 2015; Goldammer, 2008). Therefore, supplementing mash with nitrogenous nutrients such as urea, yeast extract, peptone, spent brewer's yeast, corn steep liquor, and ammonium salts may stimulate ethanol production by promoting the growth of yeast cells (Li et al., 2017; Pradeep et al., 2012). Besides nitrogenous nutrients, mineral elements such as potassium, magnesium, zinc, calcium, and manganese, which are required in very little amounts, are known to be vital for yeast growth (Udeh and Kgatla, 2013). In particular, urea supplementation in VGH systems is known to be relatively cost-effective and result in favorable ethanol yields (Chang et al., 2011; Jones and Inglede, 1994).

In comparison to corn, sweet sorghum is considered a more efficient and cost-effective source of energy because of the lower nitrogen input and water requirement for its production (Pfeiffer et al., 2013). Sugar content in the juice may range from 13.7 to 15.89% (Wu et al., 2010) and can directly be fermented by yeast *Saccharomyces cerevisiae* into ethanol (Appiah-Nkansah et al., 2015). The juice is normally obtained by mechanically crushing harvested stalks using roller mills or screw presses (Harrison and O'Hara, 2013). Extraction of fermentable sugars and nonstructural carbohydrates from sweet sorghum biomass and grains by the diffusion method has recently been proposed (Appiah-Nkansah et al., 2016). Grain sorghum, a drought-resistant crop with good fermentation characteristics such as high starch content (62.9–73.3%, db) and fast fermentation, has also been recognized as a potential feedstock for the production of biofuels (Wu et al., 2010). Currently, grain sorghum is blended with corn in some commercial ethanol plants in the U.S. (Nghiem et al., 2016). While VHG ethanol fermentation studies have been conducted using sweet sorghum juice (Deesuth et al., 2015; Deesuth et al., 2016; Khongsay et al., 2012; Nuanpeng et al., 2010; Laopaiboon et al., 2009), sorghum grains (Chang et al., 2011), sweet potato mash (Zhang et al., 2010), cassava starch (Yingling et al., 2011), and finger millet mash (Pradeep et al., 2010), very little is known about VHG co-fermenting grain sorghum starch with sweet sorghum juice. Our previous work suggested that very high gravity fermentation using sweet sorghum juice and grain sorghum could be applied to boost ethanol yields (Appiah-Nkansah et al., 2015). To increase ethanol titers and productivity, it is therefore imperative that VHG fermentation from sweet sorghum juice co-fermented with grain sorghum starch is explored. Results obtained may be useful for sorghum growers and the ethanol industry.

In this study, ethanol productivity is enhanced using simultaneous saccharification and fermentation from mixtures of sweet sorghum juice and ground sorghum grain under VHG conditions, both in the presence and absence of urea supplementation. The microorganism used is *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is generally used for ethanol fermentation and baking purposes (Russell, 2003). The ideal ratio of the starch and juice required for high ethanol yield and fermentation efficiency is established. FAN consumption dynamics by the yeast and their effect on ethanol levels and fermentation efficiency were also investigated.

2. Materials and methods

2.1. Materials

Regular grain sorghum provided by Kansas State University Agricultural Research Farm and sweet sorghum juice provided by Texas A & M AgriLife Research Sorghum Breeding Program were used in this study. The moisture content of the ground grain sorghum was determined using American Association of Cereal Chemists (AACC) and National Renewable Energy Laboratory (NREL) standard methods (AACC, 2000; Sluiter et al., 2008). The grain flour was stored in sealed plastic bags at room temperature. To obtain the juice, stalks were harvested and crushed using a three-roller mill (Ampro Sugar Cane Mill). Extracted juice was strained and immediately frozen at a temperature of −23 °C. Prior to use, it was thawed at room temperature. The juice was concentrated from about 11% sugars to 14%, 16%, and 18% sugar contents by a vacuum evaporation process at room temperature using a Rotavapor (Büchi Labortechnik AG, Flawil, Switzerland). Urea (Certified ACS) was obtained from Fisher Scientific (Fair Lawn, New Jersey, USA).

2.2. Starch and sugar content analysis

The total starch content of the materials was analyzed using a total starch kit (Megazyme International) in adherence to the AACC standard method (AACC, 2000). The sugar content of sweet sorghum juice was analyzed using HPLC with a Rezex RCM Monosaccharide (300 × 7.80 mm) column and a Refractive Index Detector RID—G1362A (Agilent Technologies, Santa Clara, CA) following the method described for ethanol in Section 2.9.

2.3. Protein content analysis

The crude protein analysis of the DDGS was completed using an LECO TruMac N (St. Joseph, MI, USA) analyzer. Initially, the instrument was prepared for operation as described in the TruMac operator's instruction manual. The system was then conditioned by analyzing 3–5 blanks. The calibration standard used was 0.5 g of EDTA. A crucible was used to weigh 0.5 g of the sample, and the sample mass and sample identification were entered into the software. The samples were run in duplicate and the percent crude protein was recorded.

2.4. Free amino nitrogen (FAN)

FAN in all the mashes was analyzed by the ninhydrin-based method as described in the modified International Ninhydrin Method (The Brewery Analysis software LZV936, 2014). FAN is an important chemical property in starch fermentation that significantly correlates to fermentation efficiency.

2.5. Mashing and liquefaction of sorghum with sweet sorghum juice

A detailed flowchart of the mashing, liquefaction, and saccharification process is shown in Fig. 1. For mashing, 18.14 g, 20.67 g, 23.2 g, and 41.32 g (db) grain sorghum flour were combined with 100 mL sweet sorghum juice of different sugar concentrations (14%, 16%, 18% and 0% (i.e., distilled water)) in 250 mL Erlenmeyer flasks to form ≈ 33% (w/v) mashes. The flour was gently dispersed in preheated juice (≈ 60–70 °C) in 100 mL Erlenmeyer flasks, and then 27.55 μL of Liquozyme®SC DS (α—amylase 267 KNU/g, 1.266 g/mL; Novozyme Inc., Franklinton, NC) and 0.14 g of KH_2PO_4 were added. The flasks were carefully transferred into a 70 °C water-bath shaker and set to operate at 180 rpm. The temperature of the water bath was gradually increased from 70 °C to 90 °C in a 30 min period, kept at 90 °C for a few minutes, and then lowered to 86 °C. Liquefaction continued for 60 min. Flasks were then removed from the water bath, and materials sticking