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Ethanol fermentation of energy beets by self-flocculating and non-flocculating yeasts

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HIGHLIGHTS

- Energy beets grew at high yield and sucrose content in Arkansas' Delta region.
- Ground beet mash was an effective feedstock for ethanol production through SSF.
- Self-flocculating yeasts demonstrated enhanced fermentation performance.

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ABSTRACT

Specialized varieties of sugar beets (Energy Beets™) are being developed for producing industrial sugars in Arkansas' Mississippi River Delta. To evaluate their suitability for producing regional fermentation feedstocks, we report initial cultivation trials and ethanol fermentation of raw beet juice and combined juice with pulp mash (JPM) liquefied with enzymes, comparing ethanol yields under different regimes by self-flocculating and non-flocculating yeasts. Nine varieties produced root yields averaging 115 Mg/ha and 18.5% sucrose contents. Raw beet juice fermentation yielded ethanol up to 0.48 g/g (sugar). JPM was directly fermented through either a sequential (SeqSF) or simultaneous saccharification and fermentation (SSF) process. For both yeast types, SSF was a more efficient process than SeqSF, with ethanol yields up to 0.47 g/g (sugar) and volumetric productivity up to 7.81 g/L/h. These results indicate the self-flocculating yeast is suitable for developing efficient bioprocesses to ferment industrial sugar from energy beets.

1. Introduction

To address issues of U.S. energy independence and increasing greenhouse gas emissions from fossil fuels, considerable research is being conducted to develop alternative sources of biofuels and renewable chemicals. The U.S. Energy Independence and Security Act of 2007 proposed that at least 36 billion gallons of bio-based transportation fuels, mainly ethanol and biodiesel, be produced annually by the year 2022. Toward meeting this goal, the U.S. has expanded production to about 13.9 billion gallons (RFA, http://www.ethanolrfa.org). Ethanol is also the largest-scale biofuel produced worldwide with the U.S. (63%) and Brazil (24%) as the leading producers (Anonymous, 2013). The U.S. produces ethanol primarily from corn starch, while Brazil produces ethanol from cane sugar (sucrose). Starch-based ethanol production has a low net energy value and poor green-house gas reduction, compared to ethanol from sugar cane. U.S. fuel ethanol production consumes about the 45% of the total corn crop (USDA Economic Research Service, http://www.ers.usda.gov/media/866543/cornusetable.html). Second generation biofuels from lignocellulosic biomass are advancing to commercial-scale production in the U.S. and will provide energy and environmental benefits more comparable to sugar cane. Ethanol is produced commercially in Europe from sugar beets as a supplemental process in crystal sugar production. Traditional beet sugar processing is energy intensive and ethanol currently generated from conventional sugar beets is similar in net energy balance to starch ethanol, with somewhat lower greenhouse gas emission (Anonymous, 2013).

Alternative fermentation feedstocks that have high net energy output, reduced greenhouse gas emissions, and do not conflict with food supply are needed for sustainable production of biofuels and renewable chemicals in the U.S. Specialized varieties of sugar cane, sweet sorghum, and sugar beets are being developed as non-food industrial sugar crops to meet this need and to match regional production conditions (Panella and Kaffka, 2010; Tripp et al., 2009). New industrial sugar crops will support rural economic

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sustainability in regions outside the corn belt in U.S. Mid-West. Recent economic analyses indicate that sugar beets are feasible for producing bioethanol and biobased products (Shapouri and Salassi, 2006; Maung and Gustafson, 2011). Bioethanol produced from sugar crops qualify as advanced biofuels by potential for greenhouse gas reduction by at least 60% (Anonymous, 2013; Maung and Gustafson, 2011). There is ample opportunity to improve the energy balance for sugar beet ethanol through development of specialized higher yielding varieties (e.g., ‘‘Energy Beets™’’, Betaseed), alternative feedstock processing, and improved fermentation bioprocessing. Potentially larger and higher value markets are available for using industrial sugars in fermentative production of renewable platform chemicals and biopolymers (Bozell and Petersen, 2010; Eggleston et al., 2010; Powell et al., 2011).

Sugar beets grow in a wide range of temperate climates and soil types and yield high levels of sucrose (16–20%) (Asadi, 2007; Panella and Kaffka, 2010). The Mississippi River Delta region of Arkansas, located in the Mid-South of the U.S., has favorable agronomic and logistical features that may be advantageous for producing industrial sugar crops (Tripp et al., 2009). Agronomic benefits include potential for double cropping and ‘‘winter beet’’ production, require lower water and nitrogen inputs (compared to corn), and their high sugar contents enable at least double the ethanol production per acre as compared to corn (Panella and Kaffka, 2010; Maung and Gustafson, 2011). The cultivation potential of sugar beets in this area has not been reported, and performance of sugar beets in rotation with other row crops has not been reported.

In regions where sugar beets are currently grown and processed to crystal sugar and related food products, ethanol fermentation facilities may be economically added to existing beet sugar production factories and use conventional raw diffuser juice, thick juice, or molasses. New industrial sugar production in non-traditional growing regions will need highly efficient technologies to reduce energy and water inputs during processing of beet roots. Because beet roots are herbaceous materials (lacking lignified cell walls; Micard et al., 1996; Asadi, 2007), the tissue can be readily fractured by combined grinding and pressing to express sucrose-rich intracellular contents. Enzymes, such as Pectinases and Cellulase, can improve juice yields in the tissue mash and can potentially contribute additional fermentable glucose from cellulose (Nahar and Pryor, 2013; Srichuwong et al., 2010). Yeasts readily ferment beet juice and enzyme-liquefied mash, and can provide ethanol yields reaching 0.46 g g^{-1} (sugar) (Nahar and Pryor, 2013; Ogbonna et al., 2001; Wu et al., 1989).

To reduce ethanol production costs, it is critical to optimize the fermentation bioprocess to obtain rapid and complete sugar utilization. This can be achieved for pulpy beet mashes through a simultaneous saccharification and fermentation (SSF) process (Nahar and Pryor, 2013; Rezić et al., 2013). Fermentation using a self-flocculating yeast, where the cells reversibly aggregate to form flocs, has been demonstrated to have the dual advantages of enhanced ethanol tolerance and easy cell from fermentation broth by cost-effective sedimentation instead of high-speed centrifugation, which greatly reduces capital investment and energy consumption (Bai et al., 2004; Zhao and Bai, 2009). Continuous ethanol fermentation with self-flocculating yeast has been achieved at commercial scale (Zhao and Bai, 2009). In addition, self-flocculating yeasts showed a higher tolerance than non-flocculating yeasts to fermentation inhibitors, such as acetic acid, furfural and hydroxymethyl furfural released from thermochemically-pretreated lignocellulosic biomass (Landaeta et al., 2013; Purwadi et al., 2007; Westman et al., 2012).

The objectives of this research were to demonstrate capacity for cultivating energy beets in the Arkansas Delta region, evaluate minimal processing of beet roots for fermentation feedstock, and determine effectiveness for a self-flocculating yeast to ferment minimally processed beet root feedstocks to ethanol.

2. Methods

2.1. Sugar beet cultivation conditions

Nine varieties of conventional (non-glyphosate resistant) sugar beet (*Beta vulgaris* L.) provided by Betaseed, LLC (Shakopee, MN) were planted at the Arkansas State University Research Farm Complex in Jonesboro, Arkansas in the spring of 2012 and harvested in late summer and fall of 2012 and in early spring of 2013. The sugar beets were planted in a 0.16 ha field consisting of a collins silt loam soil. Beets were planted on April 05, 2012 at a target rate of 22,250 seed/ha and at a two centimeter depth. Herbicides and fungicides were applied regularly according to BetaSeed recommendations, and beets were irrigated on a weekly basis beginning the last week of May and continuing through August. On August 15 and November 02, 2012, a 3.05 m section of row from each plot was harvested, topped, and the mass determined.

2.2. Processing beet roots

Beet roots from one variety harvested in fall of 2012 and early spring of 2013 were immediately washed, peeled, cut into cubes of approximately 2 × 2 cm, and ground to a fine pulp using a commercial juicer (Omega, Harrisburg, PA). The masses recovered for the separated juice and wet pulp (fresh weight; FW) were determined gravimetrically to determine yields. The raw juice and wet pulp were used either fresh or stored at −20 °C until use. The raw juice was directly fermented with yeast for ethanol production. To prepare whole beet root lysates as fermentation substrate, the raw juice and pulp were recombined quantitatively and this juice and pulp mash (JPM) was subjected to either sequential saccharification and fermentation (SeqSF) or a simultaneous saccharification and fermentation (SSF) processes (Fig. 1).

2.3. Composition analyses

The specific content of free glucose in juice and JPM was determined by using a glucose assay kit following the manufacturer’s

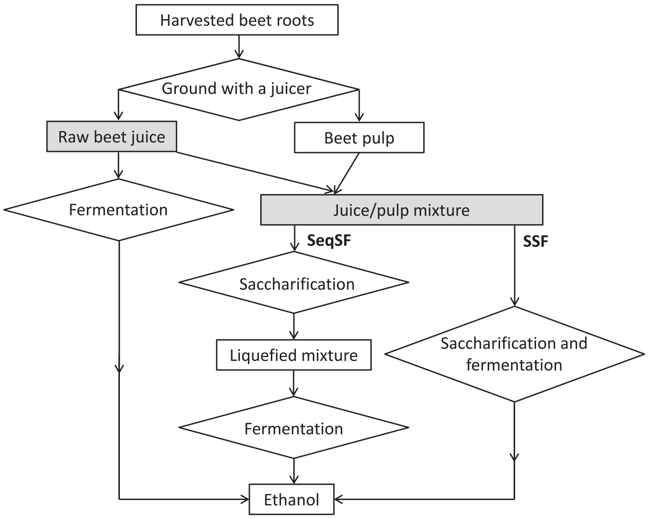

Fig. 1. Flow chart of processing the harvested energy beet roots to convert to ethanol. The raw beet juice was either used directly as feedstock for fermentation or re-combined with the beet pulp as feedstocks for the SeqSF and SSF processes.