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A green process for simultaneous production of fructose and ethanol via selective fermentation

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ABSTRACT

Date *(Phoenix dactylifera* L.*)* fruits can provide a renewable source for the production of fructose and ethanol through selective fermentation processes. The effects of scale-up on ethanol yield and remaining fructose from date syrup in three 1 L, 7.5 L and 80 L fermentors were investigated. Fermentations were simultaneously conducted in the fermentors (working volume ratios of 1:10:100) using date syrups having an initial total sugars concentration of 142.5 g/L and *Saccharomyces cerevisiae* ATCC 36859 at 30 °C and 120 rpm. Experiments showed good and comparable results for the three fermentors. Glucose was completely consumed in the three fermentors. Fructose remained was 83.5%, 89.6% and 90.7% in the 1 L, 7.5 L and 80 L fermentors. The ethanol yields were 59.1%, 76.4% and 75.4%. The fructose fraction in the final syrup was >93% in the three fermentors. Statistically insignificant differences ($p > 0.05$) were obtained for the two larger fermentors. A kinetic model based on Monod equation was developed and successfully tested against the experimental data; also, the hydrodynamic was investigated using dimensionless correlations. A conceptual framework for the process was proposed. These results could provide a step forward towards commercialization of this clean process.

1. Introduction

Fructose, the sweetest natural sugar, is used commercially in foods, beverages, confectionery products, pharmaceuticals, and for diabetics (Haitao et al., 2011). Glucose and fructose can be produced from many raw materials including corn starch, sugar cane, sugar beet, rice and dates. On a commercial scale, fructose is produced from starch through enzymatic processes. The process involves enzymatic liquefaction and hydrolysis/saccharification of the starch followed by glucose isomerization to fructose. Due to reaction equilibrium limitations, the isomerization process produces syrups containing only 42% fructose and 51% glucose, commonly called HFS-42. Fructose-enriching techniques, such as multistage chromatographic separation, are often employed to produce concentrated fructose syrups (HFS-90) which are blended with HFS-42 to produce the commonly marketed HFS-55 (Gaily et al., 2010). Zhang et al. (2004) optimized a simulated moving bed reactor for the production of HFS-55. Moulik et al. (2015) used nanofiltration to produce fructose from its aqueous solutions. Lima et al. (2011) concluded that the high cost of the isomerization process and subsequent concentration prompted researchers to find alternative routes for fructose production. Among these routes is the technology that focused on the hydrolysis of inulin extracted from plants such as Jerusalem Artichoke and Dahlia (Anes and Fernandes, 2014). Abasaeed and Lee (1995) used acidic zeolite LZ-M-8 to produce a 75% fructose syrup from Jerusalem artichoke extract; a small quantity of hydroxymethyl furfural was produced in this process.

Ethanol is an important chemical that enjoys many uses, e.g. gasoline additive, solvent, and disinfectant. Ethanol can be produced through catalytic chemical processes that utilize the cracking products of crude oil, e.g., hydration of ethylene. Hui et al. (2013) argued that production by this route is limited due to availability, competition and cost of the feedstock as well as environmental

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concerns. The major route for ethanol production is the biochemical process that involves fermentation of carbohydrates (sugars and starchy crops) or lignocellulosic materials (Liu and Shen, 2008). Balat et al. (2008) argued that although lignocellulosic materials provide a low cost resource, their use for commercial production is hampered by the required costly pretreatments. Cardona et al. (2010) in their review of the use of sugarcane bagasse for production of ethanol showed that 48% ethanol yield can be obtained by using Candida Shehatae NCIM3501. Chng et al. (2016) used lipid-extracted biomass to obtain over 80% of the theoretical ethanol yield. It has been projected that in the next 30 years biomass will provide a means for producing more that 30% (by weight) of the chemicals; alleviating the burden on fossil resources (Sandersa et al., 2012).

The fruits of the date palm *(Phoenix dactylifera* L.*)* are rich in sugars. The fruit contains mainly equimolar amounts of glucose and fructose that constitute 71–80% of date's dry weight, a small amount of sucrose (2.9–4.0% of its dry weight), vitamins and other elements like iron, phosphorous, potassium, calcium, and fibers (Gaily et al., 2010). Dates are produced in many parts of the world; the total production in 2010 was 7.9 million tons (Jain, 2012). Large amounts of the production (20–30%) are unconsumed due to damaged fruits and/or low quality of some varieties (Al-Shreed et al., 2012); these wasted dates could cause environmental problems as well as global warming due to potential gas emissions (Lattieff, 2016).

The term "selective fermentation" is used when a microorganism ferments, preferentially, one substrate in a multi-substrate medium leaving all other substrates almost unfermented. Date syrups contain, mainly, a mixture of glucose and fructose. In order to simultaneously produce ethanol and fructose from date syrups, the microorganism is expected to selectively ferment the glucose component to ethanol while leaving the fructose component almost untouched. Microorganisms such as *Zymomonas mobilis* and some strains of *Saccharomyces cerevisiae* have been used in selective fermentation. Significant losses of fructose and high production of sorbitol as a byproduct were observed with *Z. mobilis* (Carvalho et al., 2008). When *Z. mobilis CP4* was used in a fed-batch fermentation of glucose-fructose mixtures, the main products were ethanol and sorbitol (Shene and Bravo, 2001). *Saccharomyces cerevisiae* ATCC 36858 and ATCC 36859 have been shown to produce fructose and ethanol from glucose/fructose mixtures, sucrose, molasses, and date syrups media (Putra and Abasaeed, 2015). Atiyeh and Duvnjak (2001) used ATCC 36858 to obtain 92% fructose yield from a medium containing high sucrose concentration. Upon using the sugarcane molasses and ATCC 36858, Atiyeh and Duvnjak (2003) obtained ethanol yields of 66–77% and fructose yield above 90%. These strains were able to selectively ferment the glucose from high initial sugar concentrations with minimal formation of sorbitol. Putra et al. (2013) showed that using higher initial sugar concentrations produces higher ethanol concentrations that reduce the cost of downstream processing. The concentrations increase is limited by substrate and/or product inhibitions. Elleuch et al. (2008) claimed that via selective fermentation, the wasted dates can be used to produce value-added products, e.g., fructose and ethanol. Preliminary economic analysis of this selective fermentation process for commercial scale production has shown its feasibility (Carvalho et al., 2008). The potential of this fermentation in a large scale has not been investigated yet.

In this contribution, we attempted to advance our previous results on selective fermentation of date syrups a step further towards commercialization of the process that will eventually produce concentrated fructose syrups and ethanol. The yeast, *S. cerevisiae* ATCC 36859 was used in this investigation. Results from three fermentors (1 L, 7.5 L and 80 L) operated under identical conditions will be presented. A kinetic model that combines mass transfer process with modified Monod equations to predict the sugars and products profiles will be presented. Dimensionless analysis leading to Sherwood correlation that elucidates the hydrodynamics of the scaled up process will be performed. The findings of this investigation will help in understanding and alleviating potential scale-up problems. A conceptual layout of the proposed process will also be presented.

2. Materials and methods

2.1. Substrate preparation

In a typical experiment, the date fruit was cut into two halves and the inner seed was removed. Four hundred grams of the pitted date were added to one liter of deionized water. The sugar extraction process was performed at 40 °C for two hours to avoid sugar caramelization. To remove suspended solids and fibers, the extract was centrifuged at 6500 rpm for 6 min. The final sugar concentration of the syrup was adjusted to 158 g/L by diluting the extracted syrup with distilled water. The concentrations of the various sugars were determined as detailed in section 2.3 below. The sugars were then sterilized (Astell AMB230N, Sidcup, Kent, UK) at 121 °C for 15 min prior to fermentation experiments.

2.2. Inoculum preparation

S. cerevisiae ATCC 36859 was grown on a solid medium (Agar) and propagated in a liquid medium (Broth). The Agar was prepared using 41 g Difco-Agar in 1 L deionized water near its boiling point and then transferred quickly to test tubes and glass Petri dishes. The test tubes and Petri dishes were sterilized for 15 min at 121 °C, and then cooled overnight to solidify the Agar. The broth was prepared using 10 g glucose, 30 g yeast extract, 3.5 g peptone, 2 g KH_2PO_4, 1 g $MgSO_4.7H_2O$ and 1 g $(NH_4)_2SO_4$ all in 1 L deionized water followed by sterilization at 121 °C for 15 min. The yeast was initially grown in 500 ml conical flasks at 30 °C and 120 rpm in a shaker bath. Detailed description of the procedure is given in Putra et al. (2015).

For the scale-up experiments, strain ATCC 36859 was propagated in an aerated 7.5 L fermentor (New Brunswick- BioFlo/GelliGen115, New Jersey, USA). Two liters of a solution having a composition similar to the broth without glucose was prepared and added to the 7.5 L fermentor. The fermentor and its contents were autoclaved at 121 °C for 15 min. Five hundred ml of the broth containing the initially grown yeast were aseptically transferred from the conical flasks and placed in the sterilized, aerated fermentor. One hundred ml of a 10 g glucose/L was intermittently fed when the concentration of glucose drops to <0.05 g/L in the fermentor. Glucose and ethanol concentration were periodically measured by HPLC. Addition of the 10 g glucose/L continued until the final volume in the fermentor was 4.6 L. The yeast concentration was about 1.87 g/L. During the whole operation, air was initially introduced at a rate of 1 vvm and increased to maintain 10% oxygen saturation, the temperature was set at 30 °C, and the agitation was maintained at 200 rpm.

2.3. Scale-up fermentation experiments

In order to investigate the scaling of the fermentation, experiments were performed simultaneously in three different fermentors with total volumes of 1 L (Minifore Lambda, Sihlbruggstrasse, Switzerland), 7.5 L (New Brunswick- BioFlo/GelliGen115, New Jersey, USA) and 80 L (Elettronica Veneta-FERA/EV, Motta di Livenza-Treviso, Italy). Table 1 shows the dimensions and major characteristics of each fermentor. The working volumes used in the 1 L,