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Research paper

# Bioethanol and biobutanol production from sugarcorn juice

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## ARTICLE INFO

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*Abbreviations:*
GHG
greenhouse gases
AAFC
Agriculture and Agri-Food Canada
ABE
acetone butanol ethanol
SCJ
sugarcorn juice
SCJ A
sugarcorn juice A
SCJ B
sugarcorn juice B
SCJ-P2
sugarcorn juice-P2 medium
DNS
dinitrosalicylic acid
PS
phenol-sulfuric
PTS
phosphoenolpyruvate dependent phosphotransferase system
PEP
phosphoenolpyruvate
CCR
carbon catabolite repression
ATP
adenosine triphosphate

## ABSTRACT

Corn hybrids with high stalk sugar content or 'sugarcorn', are fast-growing energy crops recently developed by Agriculture and Agri-Food Canada. For the first time, this study uses juice extracted from sugarcorn plants for bioethanol and biobutanol production via microbial fermentation. Characterization results for sugarcorn juice from two different crop harvest times are presented. Juice characteristics such as, moisture content, total solids, total dissolved solids, pH, density, elemental analysis, protein, reducing sugars and total carbohydrates were determined for the two juice batches. Sugarcorn juice used in the study contained a maximum of 145 g $L^{-1}$ carbohydrates, with sucrose, glucose and fructose accounting for 80% of the sugars. *Saccharomyces cerevisiae* grown in sugarcorn juice supplemented with 3 g $L^{-1}$ yeast extract produced 45.6 g $L^{-1}$ ethanol in 72 h of fermentation (yield = 0.41 g ethanol per g carbohydrates). For biobutanol fermentation, a sporogenic strain of *Clostridium beijerinckii* was cultivated anaerobically in sugarcorn juice-P2 medium, achieving a butanol concentration of 8.3 g $L^{-1}$ in 257 h (yield = 0.31 g butanol per g total fermentable sugars). Sugarcorn is a new Canadian energy crop and a source of readily fermentable sugars, that has the potential to save on energy and enzyme costs, when compared to corn grain based biofuel production processes.

## 1. Introduction

The North American model of ethanol production from corn grain has been successful in the United States, which along with Brazil accounts for over 85% of global ethanol production. In comparison, the robust Brazilian ethanol industry driven by a year-round supply of sugarcane, achieves seven times the energy efficiency of ethanol from corn grain [1,2]. The difference may be attributed to the large amount

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of energy and enzymes expended in breaking down the starchy corn grain for fermentation, when compared to sugarcane juice which is a source of readily fermentable sugars. Also, sharp increases in the prices of corn over the past six years, underline the need for development of alternate energy crops suited to North America [3]. If we consider Canada's case in particular, the climate is not conducive for growth of tropical feedstocks like sugarcane, and the ethanol industry relies mainly on corn grain. Furthermore, Canada's short growth seasons require fast-growing crops, rendering the development of alternate energy crops even more challenging.

As an alternative to current feedstocks, hybrids of corn with high stalk sugar content, referred to as 'sugarcorn' were developed by Agriculture and Agri-Food Canada (AAFC, Ottawa, ON) [4,5]. Corn stalks are known to accumulate sucrose, glucose, and fructose, along with other soluble solids [6], until 2–3 weeks post silking. Over time, the concentration declines due to translocation of metabolites from stalk to grain, unless pollination is prevented [7,8]. Stalk sugar content is a genetically influenced trait and corn hybrids resistant to cold injury and stalk rot have been known to reach high sugar concentrations [4,9]. In an attempt to enhance the potential of corn stalks as an energy source, sugarcorn hybrids were developed by Reid et al., 2015 from various corn inbred lines as a potential Canadian energy crop [4].

Sugarcorn plants reach high concentrations of stalk sugars in the days following silking, facilitating an earlier harvest prior to corn grain maturity (Fig. 1). The germplasm is adapted to Canadian short growth seasons between May to September, and is particularly suited to the major corn growing regions in southwestern Ontario and southern Quebec [4,5].

Sugarcorn stalks contain readily fermentable sugars and can facilitate a direct bioconversion process which can circumvent the need for enzymes, unlike processes based on corn grain and lignocellulosic feedstocks. The juice extracted from the green stalks (Fig. 1) can supply sugars for production of biofuels, such as bioethanol and biobutanol [4].

Ethanol fermentation is one of the most mature and well-established bioprocesses [10]. With a global production of 97.2 billion liters in 2015, ethanol is expected to remain the most prominent and cost-effective biofuel for the foreseeable decades, with prices approaching that of gasoline [11].

Biobutanol is a relatively new bio-based chemical, which has some advantages over ethanol for use as transportation fuel. Firstly, butanol has an energy density value (29.2 MJ $L^{-1}$) that is closer to that of gasoline (32 MJ $L^{-1}$) than ethanol (19.6 MJ $L^{-1}$) [10,12]. Furthermore, butanol can be blended with gasoline to any proportion. Ethanol on the other hand, can be blended only up to 10–15% without necessitating the modification of automobile engines [13].

*Saccharomyces cerevisiae* is the most commonly used yeast in small and Industrial scale bioethanol production. The robust yeast can operate in a wide pH range, has the ability to tolerate high levels of ethanol and other inhibitory compounds when compared to other fermentative microbes [14–17].

Distinct characteristics of some species of *Clostridium*, such as, their ability to utilize a variety of carbon sources, have made them interesting candidates for industrial biobutanol production. Primary solvent producers of the genus include, *C. acetobutylicum, C. beijerinckii, C. saccharobutylicum*, and *C. saccharoperbutylacetonicum,* all of which are spore-forming and anaerobic bacteria [18,19].

This study aims to characterize the juice extracted from the stalks of sugarcorn hybrids. The paper also evaluates the potential of sugarcorn juice (SCJ) to serve as a medium for production of bioethanol and biobutanol via microbial fermentation.

## 2. Material and methods

### 2.1. Characterization of sugarcorn juice medium for biofuel production

Sugarcorn hybrids were grown at the University of Guelph, Ridgetown campus, Ontario, Canada (42°26′N, 81°53′W). The plants were harvested late August to early September, about 5–10 days post-silking. The stalks of the sugarcorn plants were cut 12–13 cm above the soil and whole plants were fed through a three-roller press to extract sugarcorn juice (SCJ). The juice was brought to University of Western Ontario, and was frozen at −20 °C. Two juice batches were used in this research, namely, sugarcorn juice A (SCJ A) and sugarcorn juice B (SCJ B), both harvested in September 2014 with an interval of two weeks.

All analyses involving characterization of the sugarcorn juice were performed in triplicate. The total solids, total dissolved solids, moisture content and ash content in the sugarcorn juice samples were measured as per National Renewable Energy Laboratory protocols [20,21]. The pH was determined using a pH meter (VWR symphony SB70P, Beverley, USA). Density of the sugarcorn juice was estimated gravimetrically at 20 °C, using an analytical balance and a standard 50 mL pycnometer. Density calibration for the method was carried out using distilled water at 20 °C.

A Flash EA 1112 Series elemental Analyzer (Thermoscientific, Waltham, USA) was used to measure nitrogen, oxygen, hydrogen and

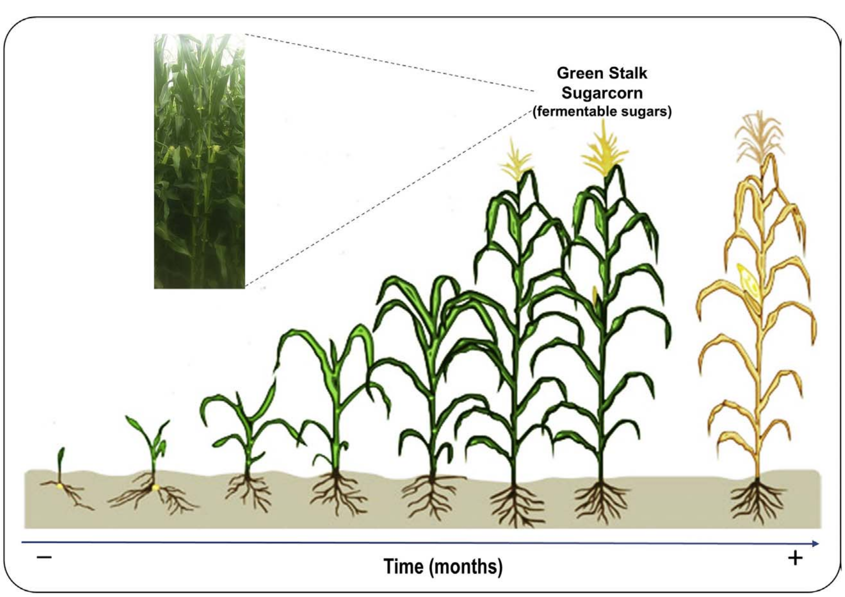


**Fig. 1.** Harvest stages of biofuel feedstocks from corn: sugarcorn, corn kernel and corn stover.