EMS-Based *In Vitro* Mutagenesis and Mutant Screening for Smut Resistance with Agronomic Traits in Sugarcane

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Abstract

Induced mutagenesis offers a useful method to improve desirable characters in sugarcane (*Saccharum* spp. hybrids). In this study, eleven Ethyl Methyl Sulfonate (EMS)-induced mutants of the Indian sugarcane cultivar CoC 671 were evaluated for smut disease resistance along with agronomic and quality traits viz. early maturity, high sucrose, high cane yield and for quality. PCR-based early detection assay for smut resistance was successfully adopted using the Internal Transcribed Spacer sequence of *Sporisorium scitamineum* and the same was correlated with field-grown mutants exhibiting resistance upon artificial smut pathogen inoculation. The smut resistant mutants were also found superior for juice and sugar quality parameters such as Brix %, Sucrose %, Purity % as well as Commercial Cane Sugar content at 10th and 12th month maturity. Mutants TC 2819 and TC 2826 exhibited superior for sucrose content (20–24%) than parent CoC 671 (18–21%), respectively, at 10th and 12th month of maturity. The study highlights potential of

Sections Figures References

Abstract

References

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ORIGINAL ARTICLE

Assessment of multiple pretreatment strategies for 2G L-lactic acid production from sugarcane bagasse

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Abstract
The bioprospecting of sugarcane bagasse (SCB) through alkali, acid, and hydrodynamic cavitation pretreatment methods and their combinations were evaluated based on bagasse composition, enzymatic hydrolysis, and lactic acid productivity using *Bacillus coagulans* NCIM 5648. From 100.0 g of SCB, L-lactic acid production of 26.16 g, 8.78 g, 14.15 g, 14.33 g, and 24.61 g in alkali, acid, sequential acid-alkali, sequential alkali-acid, and cavitation with alkali pretreatment was obtained, respectively. Considering the holistic approach from SCB to L-lactic acid, alkali pretreatment is found to be the best method with L-lactic acid titer of 68.7 g/L, the productivity of 2.86 g/L/h, and yield of 0.92 g/g which has resulted in 82.5% higher product yield from SCB as compared to alkali-acid pretreatment. Cavitation in presence of alkali evolved as the next better route with L-lactic acid titer of 62.5 g/L, the productivity of 2.60 g/L/h, and yield of 0.92 g/g. Though the highest glucose release of 89.3 g/L was achieved during enzymatic hydrolysis with sequential alkali-acid-pretreated SCB that resulted in the highest L-lactic acid titer of 71.8 g/L, the productivity of 2.99 g/L/h and fermentation yield of 0.90 g/g.

Keywords Sugarcane bagasse · Pretreatment · Enzymatic hydrolysis · L-lactic acid · *Bacillus coagulans*

1 Introduction

Lactic acid has numerous applications in food, chemical, textile, pharmaceutical, and other industries [1, 2]. It is estimated that the global demand for lactic acid will increase from 1220 kt in 2016 to 1960 kt by 2025 [3]. It can be produced commercially either chemically or by fermentation. Chemical synthesis results in a racemic mixture of two isomers. However, microbial fermentation can lead to an optically pure isomer depending on the strain, raw materials, and conditions used during fermentation [3, 4]. Besides, fermentative lactic acid production is a greener technique as compared to chemical synthesis due to limited use of harsh chemicals, mild operating conditions (lower reaction temperature and pressure), lesser byproducts, etc. [5].

Lignocellulosic biomass (LCB) is one of the most readily available renewable sources of energy. It can be utilized to generate various products ranging from biopower to biofuels, biobased bulk chemicals, and specialty products [6]. Sugarcane bagasse (SCB) is the by-product of the sugar industry with a well-established supply chain. It is the choice of the substrate because it was one of the surplus agro-industrial wastes in the year 2018–2019 in India. In sugar mills, SCB is commonly used for steam and electricity generation (cogeneration) using high-pressure boilers and turbo-alternators. There is a limitation on the use of SCB for cogeneration due to the diminishing market price of electricity. Hence, it is important to look for alternative products from SCB (biogas, ethanol, lactic acid, butanol, etc.) [7, 8].

For a sustainable LCB based biorefinery, the researchers and engineers must emphasize on the production of platform and commodity chemicals at higher titers from hydrolyzed and fermentable sugars. However, it can only be accomplished when enzymatic liquefaction is performed at a high solid loading of pretreated lignocellulosic biomass with uncompromised and concentrated sugar yields [9]. Some of the factors that have a direct impact on enzyme hydrolysis are physico-chemical characteristics of pretreated feedstock, origin of the cellulases complex, and accessory enzymes associated with it, pH, water availability, and substrate feeding strategies [10, 11].

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Evaluation of alternative strategies for generating fermentable sugars from high-solids alkali pretreated sugarcane bagasse and successive valorization to L (+) lactic acid

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ABSTRACT

Economical bioprocessing of lignocellulosic biomass essentially demands production of fermentable sugars in the concentrated form before their valorization. The present study aimed towards hydrolysis of alkali pretreated sugarcane bagasse at high solid loading and its successive valorization to L (+) lactic acid (LA). Two strategies were evaluated, wherein pretreatment, 12.5% substrate loading, Cellic CTec2 enzyme complex and thermophilic *Bacillus coagulans* NCIM 5648 were common to the processes. In Process A, when Cellic CTec2 was dosed at 30 FPU g^{-1} dry biomass, it hydrolyzed 75.8 ± 1.7% cellulose and 88.6 ± 2.1% xylan in 24 h. However, when its loading was changed to 25 mg protein g^{-1} glucan in Process B, Cellic CTec2 hydrolyzed 72.3 ± 0.3% and 68 ± 0.8% cellulose and xylan respectively. Valorization of glucose-rich filtrates obtained from Process A and B using two different media resulted in 50.4 ± 1.2 g L^{-1} and 51.24 ± 1.31 g L^{-1} of LA production from 54.7 to 62.7 g L^{-1} of glucose respectively. Attaining 1.75–2.4 g L^{-1} h^{-1} LA productivity with two scenarios of separate hydrolysis and fermentation is highly encouraging. It opens newer avenues for bio-based LA production using a greener approach.

1. Introduction

Lignocellulosic biomass (LCB) is one of the most remunerative renewable sources of energy. Versatile products ranging from biopower to biofuels, bio-based bulk chemicals, and speciality products can be generated from LCB [1]. Two structural polysaccharides, namely cellulose and hemicellulose, together represent a significant fraction of LCB. Upon their successful depolymerization, fermentable sugars such as glucose and xylose are released predominantly. These sugars can be valorized to diverse and commercially important platform and commodity chemicals other than biofuels using thermal, chemical, biological route or their combination [1,2]. However, in a sustainable LCB based biorefinery, the high-titers of these products is inevitable, especially besides cellulosic ethanol for achieving an economically profitable and environmental trade-off [3,4]. Attaining high titres of desired products can only be accomplished, when enzymatic liquefaction is performed at high-solids of pretreated lignocellulosic biomass. Moreover, hydrolysis should be such that the sugar yields are not only concentrated but also uncompromised, thereby ascertaining the techno-commercial viability of the process.

However, several aspects govern the high solid loading enzymatic saccharification of any lignocellulosic biomass (Fig. 1). Feedstock selection and type of pretreatment have a direct impact on high solid loading hydrolysis. Besides this, biomass hydrolysis also depends on the choice of cellulase cocktail, its reaction towards water availability/constraint, effective mass transfer and feeding strategies for substrates [5–8]. However, a comprehensive review by Putro et al. highlights "pretreatment" as the primary decisive force that guides the successful commercial production of various bio-based fuels and chemicals [9].

Selection of right pretreatment technique remarkably alters the third vital component of LCB popularly known as lignin which acts as a barrier during depolymerization of cellulose. The unique

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; Page No.(1034-1037)

Back

BIOMETHANATION OF HIGH SOLID CONTAINING DISTILLERY SPENTWASH USING DEVELOPED ACCLIMATIZED MICROBIAL CONSORTIA

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Abstract

Biomethanation of spent wash (SW) is now a well established technology in distilleries. At present in distilleries average COD reduction could be achieved around 60-65% for SW having COD in the range of 100000-130000 mg/L. But SW obtained from integrated evaporation system has COD 230000-250000 mg/L. However, biomethanation of concentrated SW (20-30 Â°brix) is problematic due to relatively poorer digestion efficiency and intolerance of consortia of microbes to some inhibitors. The objective of the current study is to understand the acclimatization of methanogenic consortia to concentrated SW. The sludge containing methanogenic bacterial consortia from biogas plant was used for acclimatization. The developed acclimatized consortia of methanogenic bacteria were used for biomethanation of concentrated SW. The performance of developed bacterial consortia for the production of methane was evaluated on bench scale (10 l). This study successfully demonstrates the application of acclimatized consortia of methanogenic bacteria for operation on bench scale with enhanced COD reduction in the range of 64 to 74% using 25 to 30 Â°brix SW. The control biodigester using un-acclimatized inoculum at 30Â°brix SW showed maximum 43% COD reduction and less gas production compared to experiment.

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Sugarcane bagasse based biorefineries in India: potential and challenges

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Sugarcane bagasse (SCB) is one of the world's most abundant agricultural residues and in an Indian context, ~100 million tonnes per annum is produced. The current use of SCB is restricted to the cogeneration of steam and power, however considering its potential, cogeneration is not the best valorisation route. Furthermore, with falling electricity prices and reducing global sugar prices due to excess sugar stock, it is inevitable that the waste generated (SCB) by sugar mills are utilised for generating revenue sustainably. With this background, this review aims to put forth a biorefinery perspective based on SCB feedstock. Biogas and bioethanol are the Government of India's current focus with policies and subsidies clearly pointing towards a sizeable future market. Therefore, alongside these biofuels, high-value chemicals such as xylitol, succinic acid and lactic acid were identified as other desired products for biorefineries. This review firstly discusses SCB pre-treatment options based on end applications (saccharification or anaerobic digestion, AD). Next, state-of-the-art for each of these aspects was reviewed and our perspective on a profitable biorefinery is presented. We propose an AD based biorefinery where vortex-based hydrodynamic cavitation was found to be the best choice for pre-treatment. AD is considered not only a bioprocess for energy production here but also a 'pre-treatment', where partial conversion of holocellulose leads to a digestate rich in a loosened fibre matrix. This digestate rich in cellulose can be enzymatically hydrolysed and further valorised biochemically. This approach would be cost effective and provide a sustainable waste management route for sugar mills.

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Dr Sanjay Nagarajan is a Research fellow at the School of Chemistry and Chemical Engineering, Queen's University Belfast (QUB), UK investigating hydrodynamic cavitation as a biomass pre-treatment method for enhancing biogas yields. Earlier, he worked as a post-doctoral research associate at Birmingham City University, UK designing leach bed reactors for food waste digestion. He finished his PhD (QUB) on photocatalytic fermentable sugar production. He also worked as a research engineer on methane microbial electrosynthesis at the National University of Singapore, prior to his PhD. His research focus is bridging advanced oxidation processes and biomass pre-treatment, biofuel production and biorefinery applications.

RESEARCH PAPER

Transcriptional reprogramming and enhanced photosynthesis drive inducible salt tolerance in sugarcane mutant line M4209

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Abstract

Sugarcane (*Saccharum officinarum*) is a globally cultivated cash crop whose yield is negatively affected by soil salinity. In this study, we investigated the molecular basis of inducible salt tolerance in M4209, a sugarcane mutant line generated through radiation-induced mutagenesis. Under salt-contaminated field conditions, M4209 exhibited 32% higher cane yield as compared with its salt-sensitive parent, Co86032. In pot experiments, post-sprouting phenotyping indicated that M4209 had significantly greater leaf biomass compared with Co86032 under treatment with 50 mM and 200 mM NaCl. This was concomitant with M4209 having 1.9-fold and 1.6-fold higher K^+/Na^+ ratios, and 4-fold and 40-fold higher glutathione reductase activities in 50 mM and 200 mM NaCl, respectively, which suggested that it had better ionic and redox homeostasis than Co86032. Transcriptome profiling using RNA-seq indicated an extensive reprograming of stress-responsive modules associated with photosynthesis, transmembrane transport, and metabolic processes in M4209 under 50 mM NaCl stress. Using ranking analysis, we identified *Phenylalanine Ammonia Lyase (PAL)*, *Acyl-Transferase Like (ATL)*, and *Salt-Activated Transcriptional Activator (SATA)* as the genes most associated with salt tolerance in M4209. M4209 also exhibited photosynthetic rates that were 3–4-fold higher than those of Co86032 under NaCl stress conditions. Our results highlight the significance of transcriptional reprogramming coupled with improved photosynthetic efficiency in determining salt tolerance in sugarcane.

Keywords: Photosynthesis efficiency, redox homeostasis, salt tolerance, sugarcane, transcriptional reprogramming

Introduction

Soil salinity is one of the major environmental stresses that reduces crop yield and productivity worldwide (Qadir *et al.*, 2014; Zaib *et al.*, 2019), and it is estimated that 20% of total cultivated and 33% of irrigated agricultural land is currently affected by salinity (FAO and ITPS, 2015). Salinity triggers a distinct biphasic stress response in plants. At the onset, increased

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Research Article

Rapid Profiling for Sugar Estimation in Sugarcane by Using HPLC-RI and Genetic Evaluation by Using RAPD Molecular Markers

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ABSTRACT

The rapid HPLC-RI based sugars profiling method optimized and applied for the qualitative and quantitative estimation of different sugars present in sugarcane extract. The concentrations of sugars were found differentially modulated in high and low sucrose sugarcane cultivars during their active maturation phases of 300, 360 and 420 days after planting (DAP). The average decrease in fructose and glucose were estimated about 2.2 and 1.5 folds from 300- 420DAP respectively. However, the sucrose was enhanced to an average of 12.2 folds from initial to final time point. These saccharide analyses were found negative in correlation with the fructose and glucose while, positive with sucrose accumulation during maturation phase of sugarcane which was estimated by Pearson correlation. Further, the genetic evaluation was determined by using 10 RAPD primers, 40% of the primers (OPAB07, OPK07, OPK10 and OPK15) found polymorphic for the given locus. The polymorphic percentages were ranged from 17-40% obtained for OPK07 and OPK15 respectively. Total 61 alleles were amplified of which 8 alleles were found polymorphic with 13.11%. This HPLC-RI method was found significant towards the estimate of sugars in different samples with great precision and estimation in less than 12 minutes. Whereas, the sugar analysis and the RAPD markers provides major insights in complex polyploidy genome evaluation associated with the sucrose accumulation in commercial sugarcane hybrids.

KEY WORDS Brix, HPLC, sugarcane cultivars, genetic evaluation, molecular markers

Introduction

Pollution of the Sugarcane hybrid (Saccharum spp.) is an important cash crop which gives lucrative returns to growers in terms of sucrose, ethanol and allied products. It majorly cultivated in tropical and subtropical region of other countries [11].

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Present day, sugarcane hybrid cultivars are developed as an inter-specific hybrids (2n=100-300) focused to enhance sucrose and abiotic stresses resistance [11, 12]. The metabolite acting as osmo-protectent against the oxidative stresses and provides resistance to plants [32]. Despite of narrow gene pool, complex genome, poor flowers fertility and long breeding selection cycle, scientist perform physio-biochemical and genetic studies to