Bioresource Technology 262 (2018) 32–41

Contents lists available at ScienceDirect

Bioresource Technology

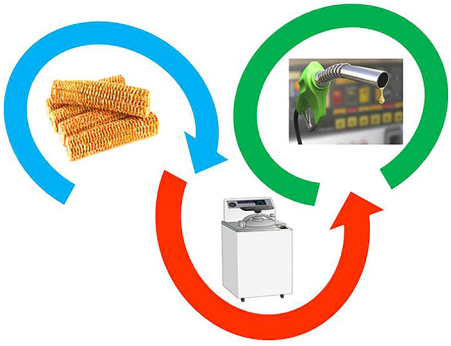
journal homepage: www.elsevier.com/locate/biortech

Simultaneous saccharification and bioethanol production from corn cobs: Process optimization and kinetic studies

Yeshona Sewsynker-Sukai, E.B. Gueguim Kana*

University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg, South Africa

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bioethanol
Prehydrolysis
Simultaneous saccharification and fermentation
Corn cobs

ABSTRACT

This study investigates the simultaneous saccharification and fermentation (SSF) process for bioethanol production from corn cobs with prehydrolysis (PSSF) and without prehydrolysis (OSSF). Two response surface models were developed with high coefficients of determination (> 0.90). Process optimization gave high bioethanol concentrations and bioethanol conversions for the PSSF (36.92 ± 1.34 g/L and 62.36 ± 2.27%) and OSSF (35.04 ± 0.170 g/L and 58.13 ± 0.283%) models respectively. Additionally, the logistic and modified Gompertz models were used to study the kinetics of microbial cell growth and ethanol formation under microaerophilic and anaerobic conditions. Cell growth in the $OSSF_{microaerophilic}$ process gave the highest maximum specific growth rate (μ_{max}) of 0.274 h^{-1}. The $PSSF_{microaerophilic}$ bioprocess gave the highest potential maximum bioethanol concentration (P_m) (42.24 g/L). This study demonstrated that microaerophilic rather than anaerobic culture conditions enhanced cell growth and bioethanol production, and that additional prehydrolysis steps do not significantly impact on the bioethanol concentration and conversion in SSF process.

1. Introduction

Second generation biofuels such as lignocellulosic bioethanol production has gained significant interest as a potential replacement for fossil fuel-derived sources (Aguilar-Reynosa et al., 2017). Bioethanol exhibits several advantages over conventional fossil fuels which include its renewable and sustainable nature, ease of storage, higher oxygen content and higher octane number, among others (Putra et al., 2015). Lignocellulosic biomass sources such as corn cobs have emerged as suitable feedstocks for bioethanol production processes. However, economical cellulosic bioethanol production is associated with several key technological issues. Identification of the bottlenecks that limit

* Corresponding author.
E-mail address: kanag@ukzn.ac.za (E.B. Gueguim Kana).

https://doi.org/10.1016/j.biortech.2018.04.056
Received 27 February 2018; Received in revised form 11 April 2018; Accepted 13 April 2018
Available online 17 April 2018

industrial ethanol fermentation with subsequent development of attractive high-ethanol-performance processes is considered fundamental for scale up. These include the need for cost-effective lignocellulosic pretreatment regimes that result in high sugar yields and low fermentation inhibitor compounds, efficient utilization of feedstock, fermentation processes that result in high ethanol yields and shorter fermentation times (Aguilar-Reynosa et al., 2017; Zhao et al., 2015).

Simultaneous saccharification and fermentation (SSF) processes are being considered as effective operational strategies to reduce production costs, increase ethanol concentration and ethanol conversion with shorter times due to the elimination of separate, long saccharification steps. SSF processes are performed in a single reactor with the same working temperature and the glucose that is produced is simultaneously metabolized by the bioethanol producing microorganism. Moreover, the inhibitory effects caused by high glucose yields during the enzymatic hydrolysis stage are significantly reduced. Nevertheless, differences in the optimal temperature for enzymatic activity (50 °C) and yeast growth (30 to 37 °C) have limited the implementation of SSF processes (Gonçalves et al., 2016; Ruiz et al., 2012).

Several studies have focussed on the enhancement of SSF processes (Zhao et al., 2015; Aguilar-Reynosa et al., 2017; Zhu et al., 2015). Zhu et al. (2015) observed a 1.4-fold higher bioethanol concentration when the solid loading was increased from 15 to 25%. Likewise, Aguilar-Reynosa et al. (2017) recorded a 9% improvement in the bioethanol conversion using a 10% solid loading compared to 12.5%. Similarly, Zhao et al. (2015) observed an 18% increase in the ethanol yield when the yeast titre was increased up to four times its base level. SSF processes are affected by several process parameters that include solid loading, enzyme loading and yeast titre. An optimum combination of these process inputs may overcome challenges associated with mass and heat transfer in addition to improving the overall ethanol concentration and conversion. Therefore, optimization of key parameters that influence the SSF process is crucial for achieving maximum bioethanol concentration and conversion. Bioprocess optimization is a complex stage that is necessary to improve product yield and maintain a level of consistency during scale up (Cheng et al., 2017). Statistical models such as the response surface methodology (RSM) can be used to identify the individual and interactive effects of process variables on the responses and to determine the optimum conditions during SSF processes.

Additionally, reports on SSF processes have indicated that a prehydrolysis step could significantly enhance the fermentation process and ethanol concentration and conversion (He et al., 2016; Zhu et al., 2015; Liu et al., 2014). This is due to the higher saccharification efficiency at elevated temperatures which are usually required for optimal enzymatic activity and reduced initial viscosity at the beginning of fermentation (He et al., 2016; Zhu et al., 2015). Even with these advantages, prehydrolysis stages require a longer process time and a higher energy input, thus reducing its economic feasibility. Combining the enzymatic hydrolysis and fermentation steps reduces the number of unit operations. A decrease in capital investment has been estimated to be more than 20% when SSF processes without prehydrolysis have been used (Wingren et al., 2003). This reduction is substantial since lignocellulosic bioethanol production is already limited by its high cost. There has been a lack of consensus on the effect of prehydrolysis stages in SSF processes (Zhu et al., 2015; He et al., 2016). Thus, modelling and optimization of SSF processes with and without prehydrolysis is imperative to determine its effect on the bioethanol concentration and conversion.

Kinetic modelling is considered as one of the most crucial steps in developing fermentation processes for large scale application. These process models define the production process under different input conditions which can help improve the product yield, productivity and reduce undesirable by-products. This will reduce costs and increase the product quality. Logistic models are employed to describe the changes in microbial cell growth as a function of growth rate, initial and maximum biomass concentration, and time (Phukoetphim et al., 2017). The modified Gompertz model has been used to determine production lag time, maximum production rate, and maximum product concentration on a given substrate (Dodic et al., 2012).

There is a dearth of knowledge on the kinetics of *Saccharomyces cerevisiae* growth and ethanol formation from corn cob wastes under microaerophilic and anaerobic conditions in SSF processes. A high cost is associated with maintaining anaerobic conditions at large scale thus reducing its economic viability (Podkaminer et al., 2012; Azhar et al., 2017). *S. cerevisiae* is one of the few yeasts that are able to grow under aerobic, microaerophilic and anaerobic environments however, the former two favours microbial cell growth and replication while the latter proceeds directly towards bioethanol fermentation (Lin et al., 2012). *S. cerevisiae* shifts to a mixed respiro-fermentative metabolism which produces ethanol when sufficient glucose concentration is available (0.8 mM) (Verduyn et al., 1984). Generally, aerobic ethanol production by *S. cerevisiae* depends on the relative capacities of the fermentative and respiratory pathways. This microbe does not generate ethanol under aerobic conditions when low glucose concentrations are present (Kappeli, 1986). Glucose uptake in *S. cerevisiae* is controlled by multiple hexose transporters (Ozcan and Johnston, 1999) which have demonstrated different substrate specificity and affinity when expressed under different, overlapping conditions (Reifenberger et al., 1997). Some studies have indicated that the presence of oxygen stimulates high levels of pyruvate decarboxylase in *S. cerevisiae* whereas yeasts such as *Candida utilis* and *Kluyveromyces lactis* display high levels of this enzyme under oxygen-limited conditions (Snoek and Steensma, 2007; Kiers et al., 1998; Weusthuis et al., 1994). Oxygen is required for lipid biosynthesis in *S. cerevisiae* and is essential for cell growth, plasma membrane integrity, and the maintenance of high glycolytic and ethanol production rates (Rosenfeld et al., 2003). Therefore, knowledge on the kinetics of microbial cell growth and bioethanol production under microaerophilic and anaerobic process conditions will significantly impact on bioethanol process design for large scale application.

The specific objectives of this work was to: (1) optimize the simultaneous saccharification and fermentation (SSF) process of bioethanol production from corn cobs with prehydrolysis (PSSF) and without prehydrolysis (OSSF), (2) determine the individual and interactive effects of yeast titre, solid loading and enzyme loading on the bioethanol concentration and bioethanol conversion, and (3) study the kinetics of microbial cell growth and bioethanol formation under microaerophilic and anaerobic process conditions using the logistic and modified Gompertz models.

2. Materials and methods

2.1. Materials

The corn cobs used in this study were obtained from the Ukulinga research farm (Pietermaritzburg, South Africa) (29° 67′ E, 30° 40′ S). These were then oven dried at 60 °C for 24 h and thereafter milled to a particle size of less than 1–2 mm by a centrifugal miller (Retsch ZM-1, South Africa). The powdered corn cobs were stored at room temperature. All chemicals used in this study were purchased from Merck, South Africa.

2.2. Pretreatment of corn cobs

Milled corn cobs were pretreated using an optimized sequential alkalic salt and dilute acid pretreatment as described in our previous study (Sewsynker-Sukai et al., 2018). During the first stage, the milled corn cobs with a solid loading of 14.49% (w/v) was treated with 12.70% (w/v) $Na_3PO_4{\cdot}12H_2O$ at 121 °C for 15 min then washed and dried at 60 °C and was thereafter treated with 1.04% (v/v) H_2SO_4 at 121 °C for 15 min. The treated biomass was filtered, washed and dried as previously described and the solid residue obtained was used for the