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Short Communication

Modelling and optimization of xylose and glucose production from napier grass using hybrid pre-treatment techniques

Siphelele Mafuleka, E.B. Gueguim Kana*

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

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ABSTRACT

This work models and optimizes four hybrid techniques of Napier grass pre-treatment for xylose and glucose production, namely HCl and moist heat (HH), HCl and microwave (HM), NaOH and moist heat (NH) and NaOH and microwave (NM) using the Response Surface Methodology (RSM). The coefficients of determination (R^2) of 0.83 and 0.97 were obtained for xylose and glucose production respectively using HH hybrid pre-treatment, and 0.90 and 0.80 were obtained for xylose and glucose respectively using HM hybrid pre-treatment. The optimized pre-treatment conditions of HH gave 12.83 gL^{-1} xylose and 2.28 gL^{-1} glucose, and optimized HM pre-treatment gave 15.06 g L^{-1} and 2.44 gL^{-1} xylose and glucose respectively. A xylose to glucose concentration ratio of 5.6:1 was obtained for the optimized HH pre-treatment compared to 6.1:1 for the optimized HM pre-treatment. For NH and NM hybrid pre-treatments, low concentrations of fermentable sugars were observed (<0.5 gL^{-1}). The findings indicate that xylose and glucose production from Napier grass can be enhanced by an optimal combination of pre-treatments of HCl and moist heat at a volume fraction of 4.39% HCl, 93.07 °C for 180 min, or using a combination of microwave and HCl at a volume fraction of 5% HCl, 500 W for 30 min.

1. Introduction

Lignocellulosic biomass is an abundantly available and sustainable source of fermentable sugars for the production of biofuels and biomaterials [1,2]. It is composed of interlinked polymers of about 35–45% cellulose, 25–40% hemicellulose and 20–35% lignin dry mass fractions [3]. These bonds provide biomass structural support, impermeability and resistance to oxidative stress and microbial attack [4], and also limit the use of biomass as feedstock for microbial fermentations [5]. Hence efficient pre-treatment procedures are required to disrupt these interlinks and expose cellulose and hemicellulose to hydrolytic enzymes for fermentable sugar release.

Biological pre-treatment using fungal enzymes has been applied to hydrolyse lignocellulosic biomass, but with a slow degradation rate and a possible consumption of the substrate by the organisms [6]. Acid and alkaline pre-treatments effectively solubilize lignin and hydrolyse hemicellulose but acid

* Corresponding author. Tel.: +27 332605527.
E-mail address: kanag@ukzn.ac.za (E.B.G. Kana).
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causes corrosion to the bioreactor internal structures [6] while alkaline removes uronic acid substitutions on hemicellulose thus reducing the accessibility of hemicellulose to hydrolytic enzymes [7]. Microwave radiation and thermal pre-treatment strategies disrupt lignin, reduce the degree of polymerization of biomass and hydrolyse hemicellulose to xylose but with high energy requirement [8]. The use of steam explosion for biomass pre-treatment results in xylan fraction destruction, toxic and inhibitory phenolic compounds production, incomplete lignin-carbohydrate matrix disruption and has high-energy requirements [9]. Hybrid pre-treatment strategies of lignocellulosic biomass based on combination of physico-chemical inputs at optimized operational setpoints could reduce the pre-treatment duration, increase monomeric sugar yields at minimum cost.

The Response Surface Methodology (RSM) is a modelling and optimization technique that evaluates the interactive and synergistic effects of all input variables in the process to achieve a maximum output [10]. It has been reported in the optimization of various bioprocesses [10–12], but its application for the determination of optimal set points for biomass pre-treatment using hybrid techniques is scantily reported.

Napier grass (*Pennisetum purpureum*) is a C_4 perennial grass species widely distributed. It has high dry mass fraction of cellulose (36.46%) [13], shows rapid growth, has a high biomass yield and high adaptability [14–16] with a deep root system, low fertilizer requirements, high light, water and nitrogen utilizing efficiency [17,18]. Napier grass grows at optimum temperatures ranging from 25 to 40 °C and minimum temperature of 15 °C, it can be harvested 4 to 6 times in a year [18]. It is an attractive biofuel substrate because of its high potential of fermentative sugars [17,18].

This work models and optimizes four hybrid pre-treatment techniques for xylose and glucose production from Napier grass, namely HCl and heat (HH), HCl and microwave (HM), NaOH and heat (NH) and NaOH and microwave (NM). The interactive effects of pre-treatment inputs on the pattern of release of xylose and glucose are evaluated. Additionally, a preliminary assessment of fermentative hydrogen production using the optimally pre-treated Napier grass is carried out.

2. Materials and methods

2.1. Lignocellulosic biomass

The leaves of Napier grass (Bana cultivar, South Africa) were harvested with a hand knife (stem cut at 10–20 cm above the soil) at 6 months old (June 2013) from the Grassland Science tunnels of the University of KwaZulu-Natal, South Africa, located at altitude 721 m; 29° 37 ′ 40.8 ″ S, 30° 24′ 10.8″ E. This site is characterized by dry warm climate, mean annual rainfall 550 mm and mean annual air temperature for growing season is 18 °C. The soil is sandy loam. The harvested leaves were immediately dried at 60 °C for 72 h and reduced to particle size (<=1 mm) using a hammer mill (Model 915, Crust-Buster, Inc., USA) and stored in sealed paper bags at room temperature until use.

2.2. Experimental designs

Based on reported literature on efficient biomass pre-treatment techniques, the pre-treatments of HCl (volume fraction of 0.1–5%), NaOH (mass fraction of 0.1–5%), moist heat (60–100 °C) and microwave intensity (500–1000 W) were selected for this study. Four hybrid techniques were considered, namely HCl and heat (HH), HCl and microwave (HM), NaOH and heat (NH), and NaOH and microwave (NM). The Box-behnken design was used to generate seventeen experimental runs with varied input parameters for each of the hybrid procedures, thus a total of 68 experimental runs were carried out in duplicate.

2.3. Experimental set up

Moist heat based pre-treatments were conducted in Poly Science Analogue water bath with temperatures ranging from 5 to 100 °C. 10 g of Napier grass powder was immersed in 100 cm^3 dilute HCl or NaOH at concentrations specified in the design. The mixtures were placed in sealed 500 cm^3 Scotch bottles and exposed to moist heat at temperatures and durations specified in the design.

Microwave based pre-treatments were carried out in a Defy microwave oven (model DMO353) which provided radiation at variable power levels (100–1500 W) using dial settings. 10 g of Napier grass powder were immersed in 100 cm^3 dilute HCl or NaOH at concentrations stated in the design. The mixtures were placed in sealed 500 cm^3 Scotch bottles and exposed to microwave radiation (500, 750 and 1000 W). The pre-treatment duration was carried out as specified in the design.

2.4. Analytical methods

The xylose and glucose produced after each hydrolytic experiment were determined simultaneously using a glucose analyser (Model 2700 select-dual configuration, YSI, USA).

The experimental data obtained were used to fit four different polynomial model equations relating xylose and glucose to the process input treatment conditions in hybrid pre-treatments of HH and HM. The general form of the model is shown in Equation (1).

$$Y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 + \alpha_{33} x_3^2 + \alpha_{12} x_1 x_2 + \alpha_{13} x_1 x_3 + \alpha_{23} x_2 x_3 \quad (1)$$

where Y represents response output (glucose or xylose), α_0 is the intercept, $\alpha_1 x_1$ to $\alpha_3 x_3$ are the linear coefficients, $\alpha_{11} x_1^2$ to $\alpha_{33} x_3^2$ are quadratic coefficients and $\alpha_{12} x_1 x_2$ to $\alpha_{23} x_2 x_3$ represents the interaction of the coefficients. The significance of the model was assessed by Analysis of Variance (ANOVA) using Design Expert software, (Stat Ease, Inc.). The optimum experimental set points for maximum xylose and glucose production in the hybrid pre-treatments of HH and HM were obtained by solving the model equations and they were subsequently validated experimentally [19].