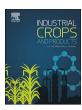
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Subcritical water hydrolysis of sugar beet pulp towards production of monosaccharide fraction



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ABSTRACT

Sugar beet pulp represents one of the most abundant by-product in food industry in terms of underexploited opportunities and amounts produced. In order to produce added-value product from this lignocellulosic material, subcritical water hydrolysis was applied in this study. Response surface methodology was used to evaluate the effects of hydrolysis parameters and optimize conditions for various responses. Hydrolysis temperature, hydrolysis time and HCl concentration were investigated as independent variables. The results obtained indicated highly promising application of corresponding hydrolysis technique towards valorization of proposed raw material. Relatively high yield of fermentable sugars with the addition of acid agent and the higher hydrolysis rates of lignocellulosic material compared to the previously published studies were obtained. Hydrolysis efficiency was higher than 50% and total monosaccharide yield reached up to 5.669 mg/mL which is significantly higher than previously reported results.

1. Introduction

As the oil markets entered a new era of insecurity in the market access, fluctuation in oil prices and environmental concerns are rising. Therefore, sugar factories are forced to consider finding new ways of energy supply in order to maintain sustainable development required to compete the growing sugar market. The sufficient knowledge regarding processes needed to produce valuable products from the polysaccharide-rich material, such as sugar beet pulp (SBP), is suggesting more comprehensive valorization of the proposed raw material (Sasaki et al., 2003; Mudhoo et al., 2011; Prado et al., 2014).

Sugar beet pulp, fibrous by-product left after the sucrose extraction from sugar beet cossettes, is normally pressed, dried, and mostly used as a cattle feed (Rombouts and Thibault, 1986, Asadi, 2006). However, this lignocellulosic material is rich in polysaccharides, particularly in cellulose, arabinan, galactan and pectin (Kelly, 1983). Therefore, as an agroindustrial residue, it represents especially favourable raw material for biomass fractionation, which can be performed in order to produce high-value products (Mussatto, 2016).

One of the possible solutions for valorisation of this kind of agroindustrial residue, could be the production of fermentation substrate adequate for generation of various combustible products, such

are liquid alkanes, bioethanol, etc. According to Serrano-Ruiz and Dumesic such substrate can be obtained by using lignocellulosic material, such as SBP (Serrano-Ruiz and Dumesic, 2011). According to Prado et al., production of fermentation substrate, in the form of fermentable sugars, can be performed through hydrolysis of cellulose to glucose or hemicellulose to pentoses and hexoses (Prado et al., 2014).

Various hydrolytic technologies and different biological and nonbiological treatment methods have been developed both for solubilisation or fractionation of lignocellulosic material (Gírio et al., 2010). Most of the proposed technologies were based on application of acid catalyst (Saeman, 1945; Malester et al., 1992) or enzyme (Gusakov et al., 2007) in order to conduct saccharification of cellulose and other lignocellulosic material to corresponding monomers. Recently, numerous researchers have suggested introduction of high pressure techniques, such as supercritical and subcritical techniques, in the area of agri-food by-products valorization (Toor et al., 2011; Prado et al., 2014; Pavlić et al., 2016). Their application in biomass and food waste streams processing has been particularly interesting due to recoveryextraction of valuable phytochemicals such as phenolics (phenolic acids, flavonoids), saccharides (simple sugars, starch, hemicellulose and cellulose) and bioactive compounds (alkaloids, essential oils, etc.) (Saldana and Valdivieso-Ramírez, 2015). Subcritical water (SW), as

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pressurized fluid, has gained special attention due to environmental aspect, since it is non-toxic to human health, safe to work with and has negligible environmental effect (Herrero et al., 2013). A considerable number of review articles have identified subcritical water as an effective solvent, catalyst and reactant for hydrolytic conversions and extractions (Brunner, 2014; Gbashi et al., 2017; Knez et al., 2018). Subcritical water attracted great attentions especially due to significant change of water properties, especially dielectric constant, caused by altered temperature. Furthermore, subcritical water, also has appropriate physical properties (lower viscosity but higher diffusivity) which favors the diffusion into the solid matrix and the release of compounds from solid to liquid phase (Teo et al., 2010). Therefore, application of subcritical water has significantly increased recently and this techniques has been applied for hydrolysis/extraction of various compounds such as polyphenols (Pavlić et al., 2016; Mayanga-Torres et al., 2017), sugars (Mayanga-Torres et al., 2017; Yedro et al., 2017), pectin (Wang et al., 2014), proteins (Powell et al., 2016) and others.

In this study subcritical water has been applied for hydrolysis of sugar beet pulp. The main scope of research was to investigate the operating conditions (process parameters effects) of sugar beet pulp hydrolysis using subcritical water with the addition of acid agent. The investigation was conducted using designed experiments and the results were analysed by application of response surface methodology (RSM).

2. Material and methods

2.1. Raw material characterization

SBP used in this study was obtained in the technological process of sugar production from the sugar factory "Šajkaška" (Žabalj, Serbia). Exhausted sugar beet cossettes with initial moisture content of 88–90% were pressed using industrial scale biconical press (Babbini, Italy) removing the certain amount of "sweet water" (containing 4–5% of sucrose). After removal of "sweet water", obtained pressed SBP contained 31.5% d.m. Corresponding SBP was used in the further experiments. The physicochemical properties of SBP regarding protein, insoluble and soluble fiber content were determined by AOAC Official Methods (AOAC, 2005; AOAC, 1991; AOAC, 1990). All experiments were performed in triplicates and results were expressed as mean ± standard deviation.

2.2. Subcritical water hydrolysis (SWH)

SWH was performed in batch-type high-pressure extractor (Parr Instrument Company, USA) with internal volume 450 mL and maximum operating pressure of 200 bar and temperature 350 °C, connected with temperature controller (4838, Parr Instrument Company, USA). Auto tuning for each temperature was performed by temperature controller prior extraction. Detailed procedure was previously described elsewhere (Zeković et al., 2014). For each experimental run, 10.0 g of SBP was mixed with 200 mL of solvent and all extractions were performed at regulated isobaric conditions (50 bar). Stirring was employed by magnetic stirrer (1000 rpm) in order to increase mass and heat transfer, and prevent local overheat on the inner walls of reactor. Temperature (150, 200 and 250 °C), hydrolysis time (25, 35 and 45 min) and HCl concentration in solvent (0.5, 1 and 1.5%) were independent variables. Total time of the process could be separated to heating, stationary (hydrolysis) and cooling phase. Moment when temperature reaches stationary phase (150 ± 2 °C; 200 ± 2 °C; 250 ± 2 °C) was chosen as start of hydrolysis and from that point, until cooling, hydrolysis time was measured. Therefore, time in heating and cooling phase was excluded from the total hydrolysis time (Alaejos et al., 2008). Hydrolysis time was measured during stationary phase since it was the only constant period for each experimental run, therefore, only this period could be appropriately used as independent variable in applied experimental design. Hence, heating phase lasted for

16, 23 and 32 min for 150, 200 and 250 °C extractions, respectively, while cooling phase in ice bath was approximately 10 min for all experimental runs. After hydrolysis, liquid and solid phase were immediately filtered through filter paper under vacuum (V-700, Büchi, Switzerland). Liquid phase was collected into glass flasks and stored at 4 °C until the analysis. Total hydrolysis yield (Y) was determined by vacuum evaporation and further drying of certain volume (10 mL) of crude liquid extract. Results were expressed as percentage of total extractable solids per 100 g of SBP.

2.3. Determination of pH

The pH of the obtained SBP hydrolysates was determined using a digital pH meter (WTW, Germany).

2.4. Determination of total reducing sugar content

Total reducing sugars (open-chain form sugars with an aldehyde group or a free hemiacetal group) were determined by Luff-Schoorl method. This redox titration is based on iodometric determination of the unreduced Cu^{2+} ions (oxidizing agent) remaining after the reaction with reducing sugars (Egan et al., 1981).

2.5. Determination of saccharides and hydroxymethylfurfural (HMF)

Samples were adjusted to pH ~ 4 by adding 10 M NaOH, diluted 10 times with methanol and filtered through 0.45 µm pore size regenerated cellulose syringe filters (Agilent Technologies) before injection into the HPLC system. HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD) and an evaporative light-scattering detector - ELSD (Agilent G4218A LT-ELSD) on an Agilent, ZORBAX Carbohydrate, 5 µm, $4.6 \times 250 \,\mathrm{mm}$ column, at a flow-rate of $1.400 \,\mathrm{mL}$ min⁻¹. Solvent gradient was performed by varying the proportion of solvent A (water) to solvent B (acetonitrile) as follows: initial 85% B; 0-14 min, 85-80% B; 14-30 min, 80-60% B. The total running time and post-running time were 30 and 5 min, respectively. The column temperature was 30 °C. $(\ge 98.0\%)$, D-(+)-galactose $(\ge 99.0\%)$, D-(-)-fructose $(\geq 99.5\%)$, sucrose $(\geq 99.5\%)$, D-(+)-glucose monohydrate $(\geq 99.5\%)$ and L-(+)-arabinose(≥99.0%) analytical standards were obtained from Sigma-Aldrich. Stock solutions were prepared by dissolving 10 mg of standards in 10 mL water. Working standards were prepared by appropriate dilution of these solutions with deionized water to obtain concentration range of 0.001-0.300 mg/mL for HMF and 0.01-3.00 mg/ mL for other investigated analytes. The injected volume of samples and standards was $50\,\mu\text{L}$ and it was done automatically using autosampler. HMF was detected using DAD at 285/10 nm with reference wavelength set at 500/100 nm and the spectra were acquired in the range 210-400 nm. Arabinose, fructose and glucose were detected using ELSD detector set up as follows: nitrogen carrier gas pressure 3.5 bar, temperature 40 °C, gain 2.

2.6. Experimental design

Response surface methodology (RSM) was applied to evaluate the effects of hydrolysis parameters and optimize conditions for various responses. Box-Behnken experimental design (BBD) with three numeric factors on three levels was used. Design consisted of fifteen randomized runs with three replicates at the central point. Temperature (X_1 ; 150, 200 and 250 °C), hydrolysis time (X_2 ; 25, 35 and 45 min) and HCl concentration (X_3 ; 0.5, 1 and 1.5%) were investigated as independent variables. Independent variables were coded in range from -1 to 1, so the units of variables were irrelevant. The response variables were fitted to the quadratic polynomial model (Eq. (1)) which is generally able to describe relationship between the responses and the independent variables (Bezerra et al., 2008):

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