Bioresource Technology 209 (2016) 259–264

Contents lists available at ScienceDirect

Bioresource Technology

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

# Selective fractionation of Sugar Beet Pulp for release of fermentation and chemical feedstocks; optimisation of thermo-chemical pre-treatment

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## HIGHLIGHTS

- Valorisation of abundant waste stream.
- Optimisation of a steam pre-treatment for (SBP) using statistical design.
- Selective fractionation of pectin and cellulose in single step.
- No milling of SBP, addition of chemicals or dilution required.

## ARTICLE INFO

*Article history:*
Received 6 January 2016
Received in revised form 25 February 2016
Accepted 26 February 2016
Available online 3 March 2016

*Keywords:*
Sugar Beet Pulp
Biorefinery
Steam explosion
Design of experiments
Ethanol fermentation

## ABSTRACT

The effect of time and pressure on the selective extraction of sugar beet pectin using steam pre-treatment on unprocessed Sugar Beet Pulp was evaluated using a design of experiments approach. This process gave the highest solubilisation of pectin oligomers at a relatively low pressure and longer time (5 Bar, 24 min), whilst leaving the majority of the cellulose fraction intact. This method of steam pre-treatment fits into the concept of a sugar beet biorefinery as it valorises an existing waste stream without requiring any further physical processing such as milling or dilution with water. The residual cellulose fraction was enriched in cellulose and could be effectively fermented into ethanol by yeast after enzymatic digestion, producing 0.48 g ethanol per gram of glucose.



## 1. Introduction

Over 17 million tonnes of sugar beet were grown in Europe in the 2012/13 season (statistics from the European commission for agriculture and rural development, http://ec.europa.eu/index_en.htm). The factory processing wastes from sugar beet manufacture (Sugar Beet Pulp) have a dry-matter content of 18–23% w/w after sugar extraction (Kuhnel et al., 2011) and are mainly converted into a low value animal feed, incurring significant drying and transportation costs (Zheng et al., 2013). SBP has a very high carbohydrate content (~80% w/w) predominantly made up of glucose (26% of the total w/w) in the form of cellulose, together with arabinose (23%) and galacturonic acid (15%) in the form of sugar beet pectin. Unlike many waste lignocellulosic materials, it is very low in lignin (~1–2%), making it relatively easy to process and the mild conditions under which sucrose extraction is carried out (60 °C, with 75 wt% of water) make SBP a potential raw material for saccharification and subsequent conversion of sugars to value-added products (Olmos and Hansen, 2012). However, the % w/w of cellulose is not high enough to make SBP a cost effective feedstock in hexose based fermentations. Previous studies in this area have focused on either complete saccharification of SBP for bioethanol or biogas production (Kuhnel et al., 2011, Zheng et al., 2013) or the selective fractionation of pectin by enzymatic treatment (Leijdekkers et al., 2013). Extraction using acidic treatments often results in the loss of cellulose (Sun and Hughes, 1998), the generation of fermentation inhibitors (Larsson et al., 1999) and products which are enriched in homogalacturonans but low in neutral sugars (Rombouts and Thibault, 1986). Equally, scaling up enzymatic treatments is expensive and requires supplementation with cellulases to increase the yield of pectin, which reduces recovery of the cellulose fraction (Leijdekkers et al., 2013). Hot water treatment (HWT) has been shown to be an effective pre-treatment for SBP resulting in the solubilisation of 40–60% of the total biomass, depending on temperature and length of treatment (Hu et al., 2008). These treatments can solubilise all of the hemicellulose,

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http://dx.doi.org/10.1016/j.biortech.2016.02.131
0960-8524/

up to 22% of the cellulose and 60% of the lignin. HWT does not require any addition of other reagents and so has the advantage of being lower cost than chemical or enzymatic treatments and produces only small amounts of the degradation products that could inhibit subsequent fermentation when present at higher concentrations (Zieminski et al., 2014). Previous studies using HWT have been done at low dry matter concentrations between 1% and 8% (Zhang et al., 2011), with some requiring an additional milling step to reduce particle size before treatment (Kuhnel et al., 2011). Further size reduction or addition of water to SBP after sucrose extraction is not desirable, due to the additional costs incurred.

In this study we present the optimisation of a pressurised steam pre-treatment for the selective fractionation of SBP, which does not require the addition of water or a reduction in particle size. This selective release of arabinose and galacturonic acid allows cellulose to be recovered as an enriched by-product of the fractionation in a 'biorefinery' context, making the hydrolysis and utilisation of cellulose for ethanol production cost effective. A statistical design of experiments (DoE) approach has been used in order to: (i) obtain a detailed understanding of the factors influencing arabinose and galacturonic acid release and their interactions, and (ii) develop statistical models enabling accurate prediction of the optimum conditions for the solubilisation of arabinose and galacturonic acid while minimising hydrolysis of cellulose.

## 2. Methods

### 2.1. Pretreatment

#### 2.1.1. Pre-wash

SBP from the 2012/2013 UK harvest was supplied by AB sugar (Wissington, Norfolk, UK). The material was received frozen but had not undergone any further processing after sucrose extraction. One kilogram of SBP was defrosted and washed twice in ultrapure water (18.2 MΩ cm) at room temperature to remove residual sucrose, then pressed in a manual screw press to remove excess moisture. The percentage dry weight of washed and pressed SBP was calculated by drying samples at 105 °C for 48 h and calculating the percentage of the total starting weight. The dry weight of the washed and pressed SBP was 27.76% (±0.08). A small amount of this washed and pressed SBP was dried to ~98% dry solids for the exact carbohydrate composition to be determined.

#### 2.1.2. Steam pre-treatment

A stirred Parr pressure reactor (1 L capacity, Boston Instruments) controlled with a Parr 4843 control module was used for all treatments. Fifty grams of whole, washed SBP was loaded into the pre-heated reaction vessel and the heating jacket was fitted around the reaction vessel to help with heat retention. High-pressure steam (10 Bar, 184 °C) was allowed into the vessel until the required pressure was achieved (between 4 and 8 Bar(g), equivalent to 152–175.5 °C). Gauge pressure (Bar(g)) was monitored throughout each experiment and more steam allowed into the vessel as required in order to keep the pressure at a constant level. The reactor was fitted with a three-arm, self centering anchor stirrer with PTFE wiper blades, and set to 150 rpm. At the end of each experiment, the pressure was released instantly to achieve explosive decompression ('steam explosion'). The pressure release valve was connected to a collection bottle through ¼″ insulated tubing. Upon release of the pressure, some of the liquefied pectin fraction escaped through the connecting tubing and was collected in a 1 L Duran bottle cooled on ice. The release of steam and vapour ensured that no liquid was retained in the tubing during this process. The outside of the reaction vessel was quickly cooled with water until the gauge pressure inside the vessel had reached zero. The vessel contained the insoluble residue and remaining liquefied fraction. This was re-combined with the collected liquid fraction and the solid and liquid fractions separated by straining through a muslin cloth. The solid material was washed in 100 ml of ultrapure water and then pressed to remove excess liquid, which was added to the soluble fraction. The volume of the soluble fraction was measured and the insoluble fraction dried at 60 °C overnight.

#### 2.1.3. Statistical experimental design

Response surface methodology was used to determine the optimum conditions and effects of two independent variables A: time and B: pressure using Design Expert 9 software (Stat Ease, Minneapolis, USA) for experimental design and analysis. The yields of arabinose, galacturonic acid and glucose released in the soluble fraction were the measured responses. A central composite design consisting of 11 experimental runs was used, including three replicates at the centre point. The pressure ranged from 4 Bar to 8 Bar and the time from 1 min to 30 min with the centre point at 6 Bar for 15.5 min (see Table 1). The experiments were performed in a random order. The model was validated with triplicate experiments at the optimum conditions and analysing the resulting fractions.

### 2.2. Analysis of carbohydrates by Ion Chromatography

Ion Chromatography was performed using a Dionex 5000+ fitted with a 4 × 250 mm analytical CarboPac PA1 column. Flow rate was set to 1.0 ml/min running 0–15 min: 25 mM NaOH, 15–20 min: linear gradient of 25–75 mM NaOH, 20–30 min: 75 mM NaOH with linear gradient of 0–260 mM NaOAc, 32–34 min: 75 mM NaOH with 260 mM NaOAc, 34–42 min: 200 mM NaOH, 42–52 min: 25 mM NaOH (adapted from Kuhnel et al. (2012)). Calibration was performed with standard sugars obtained from Sigma and made up to the desired concentration in ultrapure $H_2O$. Monomeric sugar concentrations were calculated directly from the soluble fraction. Oligomeric sugars were first hydrolysed into their constituent monomers by the addition of 106 μL of 72% $H_2SO_4$ to 3 ml of each soluble fraction (in triplicate) and autoclaving for 1 h at 121 °C. Samples were neutralised with solid $CaCO_3$ and filtered before analysis. To calculate the carbohydrate composition of the insoluble residue from each pre-treatment; 100–300 mg of the washed and dried material was acid hydrolysed by the addition of 3 ml 72% $H_2SO_4$ for 1 h at 30 °C, 150 rpm. After 1 h, 84 ml of ultrapure $H_2O$ was added and the sample autoclaved for 1 h at 121 °C. Samples were neutralised with solid $CaCO_3$ and filtered before analysis. Hydrolysis and analysis of all samples was repeated in triplicate.

#### 2.2.1. Detection of degradation products

Fractions collected from the optimised pre-treatment condition were tested for the presence of furfural, hydroxymethylfurfural (HMF) and acetic acid by HPLC (Agilent 1200 Series HPLC) using a 300 × 7.8 mm Rezex ROA-Organic Acid H+ (8%) column (Phenomenex, Cheshire, UK), running at 65 °C in 5% $H_2SO_4$, 0.6 ml/min. Samples were not tested for the presence of aldehydes.

### 2.3. Fermentation of insoluble residue

#### 2.3.1. Enzymatic hydrolysis

The insoluble residue recovered from the optimised pre-treatment was combined with ultrapure $H_2O$ (equivalent to 5% dry solids) in a 250 ml Duran bottle and sterilised by autoclaving for 15 min at 121 °C. This sterilised mixture was incubated with 0.5 mg of cellulase 13 L–C013 L per gram of glucan (Biocatalysts Ltd, Cardiff, UK) containing a high proportion of cellulase activity