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Continuous enzymatic hydrolysis of sugar beet pectin and L-arabinose recovery within an integrated biorefinery

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ABSTRACT

Sugar beet pulp (SBP) fractionated by steam explosion, released sugar beet pectin (SB-pectin) which was selectively hydrolysed using a novel α -L-arabinofuranosidase (AF), yielding monomeric L-arabinose (Ara) and a galacturonic acid rich backbone (GABB). AF was immobilised on an epoxy-functionalised resin with 70% overall immobilisation yield. Pretreatment of SB-pectin, to remove coloured compounds, improved the stability of the immobilised AF, allowing its reutilisation for up to 10 reaction cycles in a stirred tank reactor. Continuous hydrolysis of SB-pectin was subsequently performed using a packed bed reactor (PBR) with immobilised AF. Reactor performance was evaluated using a Design of Experiment approach. Pretreated SB-pectin hydrolysis was run for 7 consecutive days maintaining 73% of PBR performance. Continuous separation of Ara from GABB was achieved by tangential flow ultrafiltration with 92% Ara recovery. These results demonstrate the feasibility of establishing a continuous bioprocess to obtain Ara from the inexpensive SBP biomass.

1. Introduction

Sugar beet is one the most important crops worldwide mainly used for the production of sugar (sucrose) providing about 20% of the global demand (Finkenstadt, 2014). In the European Union alone, more than 112 million tonnes of sugar beet were grown in 2016 (Eurostat). Sugar beet pulp (SBP) is the main by-product following sucrose extraction, which is dried in an energy intensive process, pelleted and then sold as animal feed. After processing, 1 tonne of sugar beet provides approximately 160 kg of wet pulp which is then processed into 51 kg of dried pellets (88% dry matter) (Personal communication, British Sugar PLC, 2017).

SBP is a copolymer of cellulose (25% w/w) and pectin (50% w/w), and also contains low amounts of lignin (< 3% w/w), protein (< 11% w/w) and other compounds (Cardenas-Fernandez et al., 2017; Hamley-Bennett et al., 2016; Micard et al., 1996). Sugar beet pectin (SB-pectin) is formed of a linear methylated and acetylated D-galacturonic acid rich backbone (GABB) with intermittent blocks of alternating L-rhamnose (Rha) and D-galacturonic acid (GalAC) residues (rhamnogalacturonans) (Cardenas-Fernandez et al., 2017; Leijdekkers et al., 2013; Micard et al., 1996; Sakamoto and Sakai, 1995). SB-pectin also contains neutral sugar side chains, such as linear galactans formed of D-galactose (Gal) and highly branched arabinans formed of L-arabinose (Ara); both of these

can be feruloylated (Oosterveld et al., 2000). SBP thus represents an important renewable, carbohydrate-rich biomass feedstock with potential applications for the production of biofuels and value-added chemicals.

It has been described that Ara in SB-pectin arabinans is predominantly present as terminal (1 → 5)-linked, (1 → 3,5)-linked and (1 → 2,3,5)-linked residues. Small amounts of (1 → 2,4)-linked Rha and GalAc are also found indicating that arabinan chains originate from rhamnogalacturonan fractions in GABB (Oosterveld et al., 2000; Westphal et al., 2010). Unlike other sources of pectin (e.g. from citrus fruits or apple), SB-pectin has poor gelling properties due to its high degree of esterification and the presence of feruloylated arabinans and galactans (Chan et al., 2017; Chen et al., 2016). In spite of this, SB-pectin can be used as a thickener, emulsifier or as an agent to increase viscosity (Mesbahi et al., 2005). In addition, due to a high content of pentose and hexose sugars, SBP can be used for bioethanol production after enzymatic or chemical saccharification (Berowska et al., 2016; Rezić et al., 2013; Zheng et al., 2013).

This work focuses on obtaining Ara monomers from SB-pectin due to: the abundance of this carbohydrate in SB-pectin (almost 50% of the total monosaccharide content); the relative low price of SBP biomass; and the wide application of Ara in different industry sectors. For example, Ara is the starting material to produce arabinitol, one of the 12

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top value-added chemicals from biomass which is used as a building block for unsaturated polyester resins (Werpy and Petersen, 2004). Arabinitol can be synthesised from Ara either by chemical hydrogenation (Sifontes Herrera et al., 2012) or by yeast fermentation (Kordowska-Wiater, 2015). Chemical methods have been also developed for converting Ara into potentially useful starting materials for medicinal chemistry (Foster et al., 2015). Recently, we have described the enzymatic up-grading of Ara to L-gluco-heptulose, a rare heptose sugar, able to inhibit glucokinases and therefore with potential for utilisation in cancer treatment (Bawn et al., 2018; Subrizi et al., 2016). We have also demonstrated the feasibility of producing L-gluco-heptulose from Ara isolated from SBP in a batch process. This involved SBP fractionation by steam explosion, soluble SB-pectin treatment with α -L-arabinofuranosidase (AF) and enzymatic upgrading of the Ara monomers by a transketolase bioconversion (Cardenas-Fernandez et al., 2017).

In terms of recovery and separation of SBP monosaccharides; the recovery of Ara by counter current chromatography, after full acid hydrolysis of SB-pectin with sulfuric acid was previously described, achieving 96% (w/v) Ara recovery with 80% purity (Ward et al., 2017). The separation of pectin, cellulose and Ara after hydrolysis of SBP with a two enzyme system (AF and *endo*-arabinase) followed by dead-end ultrafiltration has also been reported (Spagnuolo et al., 1999). Furthermore, Kim et al. showed the production of Ara from commercially pure sugar beet arabinans using 40 g of co-immobilised *endo*- and *exo*-arabinases in a packed bed reactor, reaching a constant 83% conversion for up to 216 h (Kim et al., 2012).

The aim of this study was to establish a continuous process for the hydrolysis and recovery of Ara monomers from crude material containing SB-pectin (Fig. 1). The starting point is a crude SBP fraction following steam explosion within an integrated sugar beet biorefinery (Cardenas-Fernandez et al., 2017; Hamley-Bennett et al., 2016; Micard et al., 1996). The selective hydrolysis of arabinans into Ara monomers was achieved using an immobilised AF in a packed bed reactor and separation of Ara monomers from GABB was achieved by continuous tangential ultrafiltration. Pretreatment of crude material was also studied in order to improve immobilised AF performance.

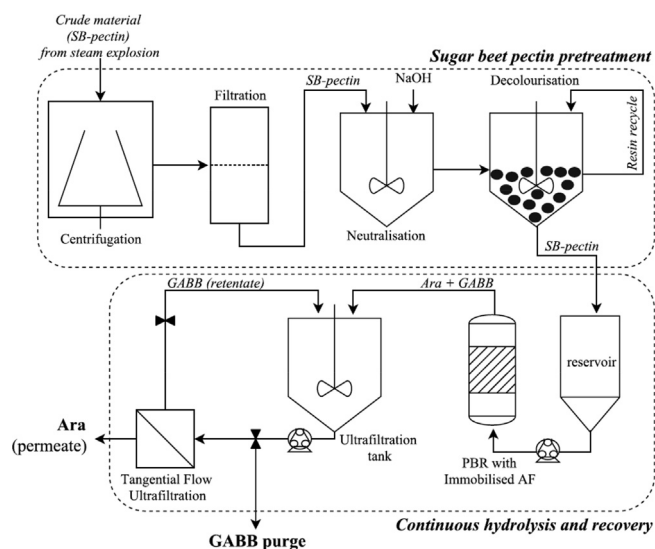


Fig. 1. Outline process flow sheet for the continuous recovery of L-arabinose from sugar beet pectin. Pretreatment section includes centrifugation, filtration, neutralisation and decolourisation. Recovery section includes continuous hydrolysis in a packed bed reactor (PBR), recovery of monomeric L-arabinose (Ara) and separation of galacturonic acid rich backbone (GABB) by tangential flow ultrafiltration (TFU).

2. Materials and methods

2.1. Reagents

All chemicals were of analytical grade and purchased, unless otherwise stated, from Sigma-Aldrich (Gillingham, UK). Epoxy methacrylate (ECR8209), amino C6 methacrylate (ECR8409), styrene tertiary amine (ECR1508) and polyacrylic quaternary ammonium (A860S) resins were supplied by Purolite® (South Wales, UK). Sugar beet pulp was provided by British Sugar PLC (Wissington, UK).

2.2. Analytical methods

2.2.1. α -L-arabinofuranosidase (AF) activity assay

Activity of the AF was measured using *p*-nitrophenyl- α -L-arabinofuranoside (*p*-NP-Ara) as substrate. 5 μ L of sample were added to 195 μ L of 2 mM *p*-NP-Ara in 0.1 M TRIS-HCl buffer pH 7 and incubated at 37 °C and agitated at 750 rpm in a Thermomixer™ C (Eppendorf, UK) for 5 min. 400 μ L of 0.2 M sodium borate pH 9.8 was then added and the absorbance measured at 405 nm (Aquarius spectrophotometer, Cecil Instruments, UK). A reaction blank was carried out as above using the same buffer but without *p*-NP-Ara. The extinction coefficient of the released *p*-NP was 18.5 mM⁻¹ cm⁻¹. One unit (U) of AF activity is defined as the amount of enzyme that hydrolyses 1 μ mol of *p*-NP-Ara to *p*-NP and Ara per minute at pH 7 and 37 °C.

2.2.2. Quantification of monosaccharides

Monosaccharides Rha, Ara, Gal, D-glucose (Glu), D-fructose (Fru) and GalAc were analysed using an Ion Chromatography System (ICS 5000+, Thermo Scientific, Hemel Hempstead, UK) and as described by Ward et al. (2017), using 15 mM of KOH for neutral sugars and 5% (v/v) of 1 M sodium acetate (electrochemical detection grade, Fisher Scientific, UK) for GalAc. Quantitative analyses were performed by measuring the peak area or height using the external standard method. Retention times for Rha, Ara, Gal, Glu, Fru and GalAc were 5.3, 6.3, 7.7, 8.2, 10.4 and 3.8 min respectively.

2.2.3. Full acid hydrolysis of SB-pectin

SB-pectin was fully hydrolysed into sugar monomers as previously described (Hamley-Bennett et al., 2016). 1 mL of pectin (containing ~10.5, ~3.9, ~4.5 g L⁻¹ polymeric Ara and GABB and monomeric Ara respectively), was mixed with 35 μ L of sulfuric acid 72% (v/v) and heated to 121 °C for 1 h in an autoclave. Then, the samples were centrifuged at 12,000 rpm at 4 °C for 10 min and the supernatant was recovered for ICS analysis.

2.3. Production of AF

2.3.1. Expression of AF

E. coli BL21 (DE3), expressing AF from a pET28a plasmid, was grown in 250 mL of Terrific Broth at pH 7 containing 0.05 g L⁻¹ kanamycin. Cultivation was carried out in 2 L baffled shake flasks at 37 °C and 250 rpm in a shaker incubator (Climo-shaker ISF1-X, Kuhner, Switzerland). AF expression was induced by adding 100 μ M IPTG after 6 h and continuing cultivation until 24 h. Cells were harvested by centrifugation (10,000 rpm at 4 °C for 15 min). Cell pellets were re-suspended in 100 mM TRIS-HCl buffer pH 7 (to reach a final cell concentration of 0.5 g L⁻¹). Then, the cells were disrupted using a Soniprep 150 sonicator (MSE, Sanyo, Japan) with 25 cycles of 10 s ON and 15 s OFF at 10 μ m amplitude. After disruption, the cell suspension was centrifuged (10,000 rpm at 4 °C for 30 min), the supernatant was then recovered and kept at 4 °C for enzymatic activity assay, protein quantification and purification.

2.3.2. Partial purification of AF

Ammonium sulphate powder was added to the clear cell lysate to

reach a saturation of 5% (w/v). This was then heated at 70 °C for 5 min and immediately cooled in an ice bath. Precipitated proteins were removed by centrifugation (10,000 rpm at 4 °C for 30 min). Then, ammonium sulphate powder was added to the supernatant to a final saturation of 50% (w/v) and kept under mild agitation at room temperature for 20 min. The precipitated AF was recovered by centrifugation and kept at 4 °C as a suspension in 50% (w/v) ammonium sulphate. Collected fractions were kept for activity assay, protein concentration and SDS-PAGE analyses.

2.4. Stability of free AF

Thermal and pH stability of free AF was evaluated by determining the activity over time from samples incubated under different conditions. The buffers used for pH stability studies were: pH 5 and 6 (100 mM sodium acetate), pH 7, 8 and 9 (100 mM TRIS-HCl) and pH 10 (100 mM sodium bicarbonate); experiments were carried out at 350 rpm in a Thermomixer™ C at room temperature for 6 days. Thermal stability was evaluated at 50, 60 and 70 °C in 100 mM TRIS-HCl buffer pH 7 and 350 rpm for 48 h in a Thermomixer™ C. Samples were taken periodically for enzyme activity analysis.

2.5. Immobilisation of AF

Immobilisation of partially pure AF (~30 U AF mg⁻¹ protein) was carried out by covalent attachment on epoxy and amino C6 resins (previously activated with 2% (v/v) glutaraldehyde) in 1 M sodium bicarbonate buffer pH 10. AF was also immobilised by weak ionic adsorption on tertiary amine resin in 10 mM sodium acetate buffer pH 6.2. Enzyme loading experiments were performed using 100, 500 and 1000 U of AF per g of wet carrier.

For all immobilisation experiments, the AF suspension was first centrifuged (10,000 rpm at 4 °C for 10 min) and the pellet (enzyme) was resuspended in the respective immobilisation buffer in a ratio of enzyme:carrier of 10:1 (v/w) and shaken at 7 rpm in a horizontal rotary agitator (Rotator LD-79, Labinco BV, The Netherlands) for 17 h at room temperature. Immobilised AF preparations were then recovered by vacuum filtration. For the desorption of non-covalently attached enzyme, the carriers were incubated with 10 volumes of 0.5 M NaCl in 10 mM TRIS-HCl buffer pH 7 for 15 min. Likewise, for ionic adsorption immobilisation, the carrier was incubated with MilliQ water for 15 min. Finally, the immobilised enzyme was recovered and then washed 3 times with 20 volumes of 10 mM TRIS-HCl buffer pH 7. All immobilised preparations were kept at 4 °C until utilisation.

Optimisation of immobilisation of AF on epoxy functionalised carrier was carried out with 500 U of AF per g of carrier (ratio 10:1) in 1 M sodium bicarbonate buffer pH 10 for 2, 4, 7 or 17 h, following a similar procedure to that explained above. Immobilisation yield and efficiency were calculated as shown in Eqs. (1) and (2), respectively:

$$\text{Immobilisation yield (\%)} = \frac{Sn Act_i - (Sn Act_f + Sn Act_w)}{Sn Act_i} \times 100 \quad (1)$$

$$\text{Immobilisation efficiency (\%)} = \frac{r_o \text{ immobilised enzyme}}{r_o \text{ free enzyme}} \times 100 \quad (2)$$

where *SnAct* represents the enzymatic activity (using *p*-NP-Ara as substrate, Section 2.2.1) of the supernatant at the beginning (*i*) and at the end (*f*) of the immobilisation process, and after the washing step (*w*). *r_o* represents the initial reaction rate of the hydrolysis reaction using crude material containing SB-pectin as substrate, this reaction was performed as follow: 5 U of free or immobilised AF (based on immobilisation yield calculation), 4.5 g L⁻¹ of polymeric Ara in SB-pectin in 50 mM TRIS-HCl buffer pH 7 at 50 °C and 900 rpm in a Thermomixer™ C for 24 h. Aliquots were withdrawn at different times for ICS analysis of Ara. For free enzyme reactions, the aliquots were mixed with similar volume of trifluoroacetic acid 0.5% (v/v) to stop the reaction.

2.6. Fractionation of SBP

SB-pectin was obtained by steam explosion (SE) at 5 bar for 24 min as described previously (Hamley-Bennett et al., 2016). The soluble fraction ('crude material' in this study) containing polymeric SB-pectin and monomeric sugars was recovered and kept at -20 °C until utilisation. Monosaccharides in the crude material were directly analysed by ICS (Section 2.2.2) whilst polymeric Ara and GalAc in SB-pectin were quantified by performing a full acid hydrolysis prior to ICS (Section 2.2.3). The concentration of polymeric Ara and monomeric Ara in the soluble fraction were ~10.5 and ~4.5 g L⁻¹ respectively; whilst the concentration of polymeric GalAc (as GABB in SB-pectin) was ~3.9 g L⁻¹, no monomeric GalAc was found in the soluble fraction.

2.7. Pretreatment of crude material containing SB-pectin

2.7.1. Early pretreatment of crude material

The crude material was first centrifuged at 10,000 rpm for 45 min at 4 °C, then the supernatant was vacuum filtered using a glass microfibre filter GF/F grade (Whatman™, 47 mm diameter and 0.7 μm pore size), then the pH was adjusted to 7 by adding 12.5 M NaOH (Fig. 1).

2.7.2. Decolourisation process of crude material

The decolourisation of crude material was carried out using a range of organic scavenger agents: granular activated charcoal (GAC), activated charcoal powder (ACP), amino C6 methacrylate resin (MC6), styrene tertiary amine resin (STA) and polyacrylic quaternary ammonium resin (PAQA). Initial decolourisation experiments were performed with 10% (w/v) solid loading at 900 rpm in a Thermomixer™ C for 15 min at room temperature and 1 mL final volume. Then, the supernatant was recovered immediately and diluted 100-fold for UV/Vis wavelength scanning (from 250 to 500 nm). The effect of the scavenger agents on monomeric sugars and SB-pectin was also studied; for that, the supernatant was both directly analysed by ICS and subjected of full acid hydrolysis and then analysed by ICS.

2.7.3. Pretreatment of crude material with PAQA resin

For optimisation of pretreatment of the crude material with PAQA, different solid loadings of 1, 5, 10 and 20% (w/v) were evaluated, with 30 min incubation. Then, incubation times of 5, 10, 15 or 30 min with 10% (w/v) solid loading were also tested. UV/Vis wavelength scanning and effect on monomeric and polymeric sugars were performed as in Section 2.7.2. Large scale PAQA pretreatment was performed in 2 L baffled shake flask with 10% (w/v) solid loading with 500 mL of crude material for 10 min at 120 rpm at room temperature.

2.8. Reutilisation of immobilised AF

The reutilisation of AF immobilised on epoxy-functionalised resin was carried out using non-pretreated and PAQA pretreated crude material under optimised conditions (Section 2.7.3) in a stirred tank reactor (STR) as follows: SB-pectin concentration (polymeric Ara) 8.0 g L⁻¹, 20 U of AF mL⁻¹ (~1.4 g of immobilised AF) in 50 mM TRIS-HCl buffer pH 7, 25 mL final working volume and agitated at 250 rpm with a Rushton turbine driven by a BDC 1850-220 motor (Caframo, Canada) and 50 °C for 3.5 h per cycle. In between reaction cycles, the immobilised enzyme was recovered by vacuum filtration and washed 3 times with 20 volumes of the same reaction buffer. Aliquots were withdrawn during the reaction time for analysis of monomeric Ara released; initial reaction rates and reaction yields were then calculated.

2.9. Continuous hydrolysis of SB-pectin in a packed bed reactor (PBR)

3 g of immobilised AF on epoxy-functionalised resin (~380 U AF per g of resin) were packed in a XK 16/20 column (200 mm height and 16 mm column i.d. GE Healthcare Bio-Science, Sweden). PAQA

Table 1

Experimental design to explore the continuous hydrolysis of arabinans in a steam exploded SB-pectin stream using a packed bed reactor (PBR) with 3 g of immobilised α -L-arabinofuranosidase (AF) on epoxy-functionalised resin. A 2-level, 3-factor full factorial design with 4 replicates at the centre point was used. Substrate concentration represents the concentration of polymeric Ara in SB-pectin.

Run	Factors			Responses	
	Dilution rate (h^{-1})	Substrate concentration (g L^{-1})	Temperature ($^{\circ}\text{C}$)	Reaction yield (%)	Process throughput ($\text{mg Ara h}^{-1} \text{g}_{\text{resin}}^{-1}$)
1	15	0.9	40	80.5	14.6
2	15	8.9	40	79.1	124.6
3	1.5	8.9	40	70.2	14.1
4	1.5	0.9	40	84.2	1.5
5	8.25	4.9	55	87.8	47.2
6	8.25	4.9	55	82.9	44.7
7	8.25	4.9	55	85.5	45.9
8	8.25	4.9	55	85.8	46.2
9	15	0.9	70	81.2	14.6
10	1.5	0.9	70	89.9	1.6
11	15	8.9	70	77.9	138.4
12	1.5	8.9	70	80.5	14.3

pretreated crude material (Section 2.7.3) was used in all PBR experiments. The height of the packed resin was approximately 2 cm equivalent to 4 mL column working volume. Column and crude material were kept at the set temperature in all cases using a TC-120 water bath (Grant Instruments, UK). Substrate was fed to PBR using a 120U peristaltic pump (Watson Marlow, Cornwall, UK). Samples were taken periodically and then analysed by ICS.

2.9.1. Statistical experimental design

A 2-level, 3-factor full factorial design with 4 replicates at the centre point was employed in this study with a total of 12 experiment runs (Table 1). The independent variables and levels used were as follows: SB-pectin concentration (as polymeric Ara): 0.9–8.9 g L^{-1} , temperature: 40–70 $^{\circ}\text{C}$ and dilution rate (calculated as the ratio of feed flow rate and column working volume): 1.5–15 h^{-1} . Reaction yield (%) and process throughput ($\text{mg Ara h}^{-1} \text{g}_{\text{resin}}^{-1}$) were the measured responses. Experiments were performed randomly and run for up to 2 h each. Design Expert® 9 software (Stat Ease, Minneapolis, USA) was used for experimental design and data analysis.

Validation of the statistical model for process throughput was performed using three different optimisation criteria: low (range: 1–10 $\text{mg Ara h}^{-1} \text{g}_{\text{resin}}^{-1}$), medium (range: 50–70 $\text{mg Ara h}^{-1} \text{g}_{\text{resin}}^{-1}$) and high (range: 120–140 $\text{mg Ara h}^{-1} \text{g}_{\text{resin}}^{-1}$). Substrate concentrations and dilution rates values were given by Design Expert® 9 software and all runs were carried out at 50 $^{\circ}\text{C}$ for 2 h.

2.9.2. PBR for the long term continuous hydrolysis of SB-pectin

Long term hydrolysis of arabinans in SB-pectin using a PBR packed with 3 g of immobilised AF was carried out at 3.9 g L^{-1} substrate concentration at 5 h^{-1} dilution rate and at 50 $^{\circ}\text{C}$ for 173 h. Samples were taken periodically for ICS analysis.

2.10. Tangential flow ultrafiltration (TFU) process for the recovery of Ara

TFU experiments were performed using a MidiKros hollow fibre membrane with a MWCO of 1 kDa and 115 cm^2 membrane area (SpectrumLab®, USA); a Millipore Filtration Tank (500 mL) fitted with pressure gauges in the feed and retentate streams and 1 L of feed mixture (from the long-term PBR run, Section 2.9.2) were used. Initially, 200 mL of feed mixture was placed in the tank. The flow rate from tank to membrane (feed flow rate) was set to 40 mL min^{-1} and pumped to the membrane with a 120 U peristaltic pump. The TFU process was performed working at 20 psi constant transmembrane pressure. The retentate was recirculated to the tank whilst the permeate (containing Ara) was collected in a bottle placed on a digital scale (Mettler BB2400, Mettler-Toledo, Switzerland); the permeate flow rate was calculated by

weighing the amount of permeate in a determined time (permeate density taken as 1 g mL^{-1}). Constant addition of fresh feed mixture to the tank was done with a 520S peristaltic pump (Watson Marlow, Cornwall, UK) and at a similar flow rate to the permeate flow rate. At the end of feeding (5 h), a discontinuous process (without addition of feed mixture) was carried out under similar operational conditions for 40 min. Samples of retentate and permeate were taken periodically. Concentration of Ara in samples was directly analysed by ICS, these samples were also subjected of full acid hydrolysis for the determination of GalAc concentration by ICS.

3. Results and discussion

3.1. Immobilisation of AF by covalent attachment and ionic adsorption

AF (EC 3.2.1.55) is an exo-type glycosidase that catalyses the successive removal of Ara residue from the non-reducing termini of α -1,2-, α -1,3-, α -1,5- and α -4,6-linked arabinofuranosyl residues (Saha, 2000). The recombinant thermostable AF from *Geobacillus thermoglucosidarius*, expressed in *E. coli* and previously characterised (60.2 kDa, pH and temperature optima of 7 and 80 $^{\circ}\text{C}$ respectively) (Espina Silva, 2015), is able to hydrolyse the natural substrate arabinan from different sources, such as arabinan branches from sugar beet pectin, yielding monomeric Ara and polymeric galacturonic acid rich backbone (GABB) (Cardenas-Fernandez et al., 2017). Immobilisation is the most common technique to improve the stability of biocatalysts and allows their reutilisation reducing process cost, as well as facilitating operation in different bioreactor configurations, such as a continuous PBR (Rao et al., 2009; Sheldon and van Pelt, 2013). In this work, immobilisation of AF via covalent attachment (on epoxy- and aldehyde- functionalised carriers) and ionic adsorption was attempted.

Prior to immobilisation, stability studies of partially purified AF were carried out at different pH (from 5 to 10) and temperature (50, 60 and 70 $^{\circ}\text{C}$). The AF was only partially purified as this would be appropriate for biorefinery applications. AF is very stable when incubated at different pH values for up to 140 h. The highest losses of activity were observed under acidic conditions (5 and 6), while over 80% of initial activity was retained between pH 7 and 10 (Supplementary Material). Temperature stability results showed that AF retained 80% of its activity at 50 $^{\circ}\text{C}$ after 50 h incubation, while losing 50 and 70% of activity at 60 and 70 $^{\circ}\text{C}$ respectively (Supplementary Material). As the optimum temperature for AF is 80 $^{\circ}\text{C}$, however our results demonstrate that working near the temperature optimum affects long-term enzyme stability. For an industrial process, it will be better to operate below the optimum temperature, compromising on enzyme activity rather than enzyme stability.

Table 2

Immobilisation of α -L-arabinofuranosidase (AF) on three different supports: tertiary amine resin (10 mM sodium acetate buffer pH 6.2), amino C6 resin (1 M sodium bicarbonate buffer pH 10) and epoxy resin (1 M sodium bicarbonate buffer pH 10) with various enzyme loadings. Immobilisation experiments were carried out at a ratio of enzyme solution to carrier of 10:1 (v/w) at room temperature. Values represent one standard deviation from the mean value (n = 2).

Immobilisation method Carrier	Ionic adsorption			Covalent attachment					
	Tertiary amine resin			Amino C6 resin (aldehyde)			Epoxy resin		
Enzyme loading (U g _{resin} ⁻¹)	Imm. Yield (%)	Imm. Efficiency (%)	Reaction yield (%)	Imm. Yield (%)	Imm. Efficiency (%)	Reaction yield (%)	Imm. Yield (%)	Imm. Efficiency (%)	Reaction yield (%)
100	92.7 ± 3.5	62.2 ± 2.9	81.6 ± 0.01	99.7 ± 3.2	73.6 ± 4.6	81.7 ± 3.1	99.9 ± 3.1	100.0 ± 2.0	82.3 ± 1.0
500	76.7 ± 1.9	18 ± 2.0	75.1 ± 1.1	93.4 ± 4.6	61.1 ± 2.0	74.5 ± 1.8	92.4 ± 2.9	73.1 ± 3.9	76.3 ± 1.1
1000	20.2 ± 2.0	2.77 ± 0.9	39.5 ± 5.3	81.0 ± 2.8	16.4 ± 7.5	66.2 ± 2.3	50.9 ± 4.8	61.7 ± 7.5	77.2 ± 1.6

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.biortech.2018.08.069>.

Immobilisation of enzymes by covalent attachment on epoxy- and aldehyde-functionalised carriers, involves nucleophilic attack between these groups and free γ -amino groups from lysine residues, found on the enzyme surface (Boller et al., 2002). This reaction tends to be faster when performed at high pH due to the increased deprotonation of the lysine amino groups. As already demonstrated, AF is stable at high pH and therefore its immobilisation on epoxy- and aldehyde-functionalised carriers was carried out at pH 10. Immobilisation by ionic adsorption using the weak tertiary amine resin was carried out at pH 6.2, close to the predicted AF isoelectric point (as calculated using the full enzyme amino acid sequence (Espina Silva, 2015) and the web.expasy.org/protparam/ bioinformatics software).

Table 2 shows the results of the three immobilisation methods performed with different enzyme loading of 100, 500 and 1000 U of AF per g of wet carrier. The metrics to compare immobilisation performance were: (i) immobilisation yield (ImmY), representing the relative amount of enzyme attached to the resin (Equation (1)); (ii) immobilisation efficiency (ImmE), which correlates the initial reaction rates of the immobilised with the free enzyme (Eq. (2)) and indicates the amount of attached enzyme still active after immobilisation; and (iii) reaction yield (RY), calculated as the maximum concentration of monomeric Ara released at the end of the AF-catalysed reaction using SB-pectin as substrate. When working at low enzyme loading (100 U g_{resin}⁻¹), ImmY was over 90% for all three cases, the highest ImmE was found for the epoxy carrier (100%), while RY values were around 82% after 24 h reaction for all the carriers, similar to the value observed for the free AF reaction. These initial results showed that all three carriers are suitable for the immobilisation of AF.

To obtain immobilised AF preparations with high enzyme loading for further applications in continuous processing, experiments with 500 and 1000 U g_{resin}⁻¹ were carried out. For ionic adsorption immobilisation; ImmY, ImmE and RY dropped drastically with increased enzyme load. The ImmY decreased slightly for both aldehyde and epoxy carriers; however, ImmE was reduced by 58% at the highest enzyme loading for the aldehyde carrier but only 38% for the epoxy carrier. Despite the decline in ImmY and ImmE for these two carriers, the RY were only slightly affected. In terms of overall immobilisation yield (calculated as the product of the ImmY and ImmE), the highest value of 67.5% was found when immobilising AF on epoxy-functionalised resin at 500 U g_{resin}⁻¹.

From the 17 h immobilisation procedures discussed above, shorter times (2, 4 and 7 h) were tested using 500 U per g of epoxy carrier. The maximum ImmY of around 92% was reached after 7 h; likewise, similar levels of ImmE were also reached after the same time, and RY was constant and around 80% in all cases (Supplementary Material). The overall immobilisation yield for 2, 4, 7 and 17 h of immobilisation were 61.8, 63.3, 70.1 and 67.5% respectively. Although similar overall immobilisation yields were obtained for 7 and 17 h, a shorter process time is economically important. Thus, the best conditions for the

immobilisation of AF were established, working with 500 U g_{resin}⁻¹ of epoxy-functionalised carrier at pH 10 with mild agitation conditions for 7 h and at room temperature, reaching ImmY, ImmE and RY of 92.3, 75.9 and 82.3% respectively. These results represent an important improvement compared to previous reported studies, where arabinofuranosidases from different sources where immobilised on magnetic nanoparticles and Duolite A568 with only 16 (Jia et al., 2016) and 10% (Kim et al., 2012) efficiency.

3.2. Pretreatment of crude material containing SB-pectin

The crude material (soluble fraction recovered after SBP fractionation by steam explosion, Section 2.6) contains mainly SB-pectin (containing ~10.5 g L⁻¹ of Ara as polymeric arabinans) and monomeric sugars such as Ara (4.5 g L⁻¹), Rha (0.23 g L⁻¹), Gal (0.22 g L⁻¹), Glu (0.18 g L⁻¹) and Fru (0.06 g L⁻¹); among other unknown soluble components. In fact, the crude material utilised in this work had a dark brown colour, which may be due to the presence of substances formed by Maillard or non-enzymatic browning reactions, which occur at the high temperatures required in SE (Bornik and Kroh, 2013; Wegener et al., 2015).

Decolourisation is a very common process used in the food industry, especially in sugar refining (e.g. decolourisation of sugar cane or sugar beet syrup before crystallisation) in order to remove coloured contaminants (Asadi, 2005). Organic scavenger agents such as activated carbon or ion exchange resins are the most common decolourisation agents used. In this work, five different agents were evaluated (Section 2.7.2). The degree of decolourisation was calculated by comparing the absorbance at 280 nm of pretreated and non-pretreated samples. The effect of the organic scavengers on monomeric Ara and SB-pectin (measure as initial Ara in arabinans) was also determined.

As shown in Fig. 2, the best decolourisation (> 95%) of the crude material was achieved using ACP with only 12% loss of monomeric Ara; however, 80% of SB-pectin was adsorbed onto ACP. Much lower decolourisation (22%) and low losses of Ara and SB-pectin were found for GAC. The difference in responses of both charcoal varieties could be due to the larger surface area of ACP, allowing higher adsorption of organic matter including the polymeric SB-pectin. Minimum losses of Ara (around ~10%) were observed for AC6, STA, PAQA; only around 15% of SB-pectin was adsorbed onto the AC6 and STA, while no SB-pectin adsorption was observed for PAQA resin. Decolourisation levels of 59 and 56% were reached for AC6 and PAQA respectively. Based on these results, the PAQA resin was selected as the best one for decolourisation pretreatment of the crude material.

All decolourisation experiments discussed above, were carried out with 10% (w/v) solid loading and 15 min contact time. In order to improve this process using the PAQA resin, different solid loading and contact times were evaluated. For solid loading experiments 1, 5, 10 and 20% (w/v) were tested with 30 min contact time (Supplementary Material). This showed that the higher the solid loading the higher the decolourisation level achieved. An enhancement of 9% decolourisation

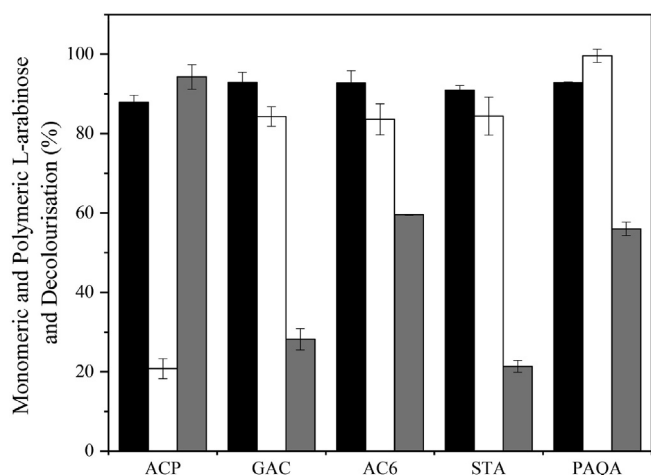


Fig. 2. Screening of various organic scavenger agents for the pretreatment of the crude SB-pectin material (from steam explosion of sugar beet pulp): activated carbon powder (ACP), granular activated carbon (GAC), amino C6 methacrylate resin (AC6), styrene tertiary amine resin (STA) and polyacrylic quaternary ammonium resin (PAQA). Monomeric Ara (black bars), polymeric Ara (white bars) in SB-pectin and decolourisation measured at 280 nm (grey bars). All experiments were carried out with 10% (w/v) solid loading, 900 rpm for 15 min at 22 °C. Error bars denote one standard deviation from the mean ($n = 3$).

was observed when increasing solid loading from 10 to 20% (w/v) without a major effect on Ara and SB-pectin recovery. Thereafter, contact times of 5, 10, 15 and 30 min with 10% (w/v) solid loading were evaluated (Supplementary Material), the decolourisation of the crude material happens very quickly reaching an average of 55% decolourisation after just 5 min contact time. The effect of contact time on Ara and SB-pectin recovery was not significant even after 30 min. In addition, the effect of this decolourisation step on the other monomeric sugars in the crude material (Rha, Gal, Glu and Fru) was similar to the one observed for Ara.

In summary, an optimised and reliable decolourisation method of the crude SB-pectin material using PAQA resin at 10% (w/v) solid loading for short contact time was established, which enables minimal losses of Ara and SB-pectin as well as allowing elimination of around 55% of coloured contaminating compounds.

3.3. Impact of SB-pectin pretreatment on immobilised AF reutilisation

One of the advantages of using immobilised biocatalysts is that they can be reutilised over several reaction cycles, reducing the process cost associated with biocatalyst production. Here, immobilised AF on epoxy carrier (Section 3.1) was reutilised in the hydrolysis of non-pretreated and PAQA-pretreated crude material (containing SB-pectin). These processes were carried out in a batch STR for 3.5 h per reaction cycle (Section 2.8). Initial reaction rate (r_0) and reaction yield were calculated for each reaction cycle.

r_0 is used to indicate enzyme activity and stability. The relative r_0 values for each reaction cycle (related to the r_0 in the first reaction cycle) are shown in Fig. 3. When performing the reaction using non-pretreated crude material, the immobilised AF lost 84% of its initial activity after 6 reaction cycles, whilst the reaction yield decreased by 30% at the end of the 7th cycle. This loss of activity could be associated to the presence of compounds in the crude material that would act as irreversible enzyme inhibitors or affect the enzyme activity and stability. Loss of activity due to thermal inactivation could be another explanation, although this is unlikely because AF was found to be very stable at 50 °C (Supplementary Material).

When performing the same reaction using PAQA pretreated crude material, a significant improvement in stability was observed. The r_0

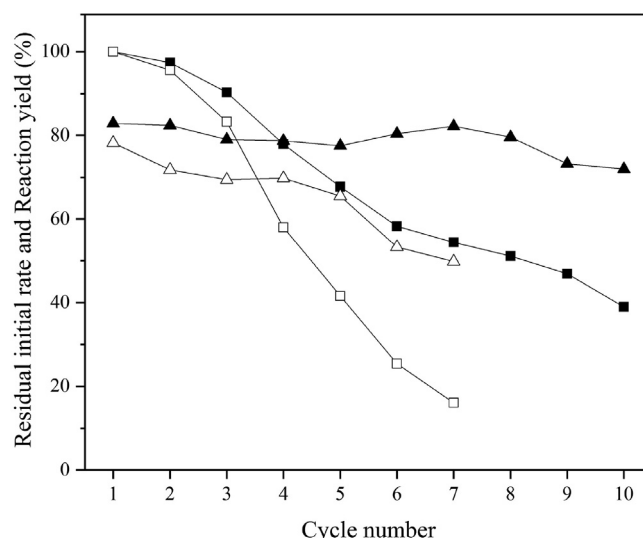


Fig. 3. Reutilisation of immobilised α -L-arabinofuranosidase (AF) for the hydrolysis of arabinans from SB-pectin. Reactions were performed in a 25 mL working volume stirred tank reactor with 8.0 g L⁻¹ SB-pectin, 20 U AF mL⁻¹ (~1.4 g of immobilised AF) in 50 mM TRIS-HCl buffer pH 7 at 250 rpm and 50 °C for 3.5 h per reaction cycle. (■) Initial reaction rate using PAQA pretreated crude material, (□) initial reaction rate using non-pretreated crude material, (▲) reaction yield using PAQA pretreated crude material and (△) reaction yields using non-pretreated crude material.

was 3.4-fold higher than the reaction with non-pretreated crude material after 6 reaction cycles. The immobilised AF kept 40% of its initial activity after 9 reaction cycles. Moreover, the reaction yields were similar (~80%) after 8 cycles and a slight decrease of 5% was observed at the end of the experiment (Fig. 3). Partial decolourisation of the crude material helped to eliminate 55% of coloured contaminants (Section 3.2) which could explain the high loss of enzyme activity when using the non-pretreated crude material. Pretreatment by decolourisation of the crude material is thus a crucial step within an integrated biorefinery context, which improves the lifetime of the immobilised AF. The stability of the immobilised enzyme could potentially be improved further by increasing the decolourisation levels; however, this should be achieved without affecting the concentration of SB-pectin in the crude material.

3.4. Continuous hydrolysis of SB-pectin using a PBR

A PBR represents a good option when working with immobilised biocatalysts as mechanical damage to the particles due to mixing is avoided, it can be easily scaled-up as well as allowing continuous operation avoiding unnecessary separation steps. Here, the continuous hydrolysis of SB-pectin of the PAQA pretreated crude material using immobilised AF in a PBR was studied.

Continuous process performance was evaluated using a statistical DoE approach using a 2-level, 3-factor full factorial design (Table 1). The following factors were studied: reactor dilution rate, substrate concentration (polymeric Ara concentration in SB-pectin) and temperature. Reaction yield (%) and process throughput (mg Ara h⁻¹ g_{resin}⁻¹) were the measured responses. Each experimental run was carried out for up to 2 h and the concentration of monomeric Ara released was monitored periodically. Average values of Ara concentration during steady state period ranged from 0.7 to 7.2 g L⁻¹. These were then used for yield and throughput calculations.

The measured Ara concentrations varied with dilution rate (reduced mass transfer resistance to bead surface), substrate concentration (increased solute driving force) and temperature. A separate model for Ara concentration was also developed for which substrate concentration

was the primary factor. The calculated reaction yields showed a variation ranging between 70 and 90% (Table 1). The Analysis of Variance (ANOVA) of the reaction yield model was not significant (P -value = 0.35). The P -values for all individual factors evaluated were higher than 0.1 confirming that none of the factors studied, nor their interactions, have an effect on the reaction yield over the ranges studied. A separate model for Ara concentration was also developed (data not shown) for which model and substrate concentration were significant; however, ‘Lack of fit’ was found not significant and therefore the model cannot be used for prediction.

For process throughput, the calculated values were between 1.6 and 138.4 mg Ara h⁻¹ g_{resin}⁻¹ with the highest value being obtained when dilution rate, substrate concentration and temperature were all at their ‘high’ factor settings (run 11). For model development, the response values were transformed by taking the natural logarithm as recommended by Box-Cox analysis. ANOVA for a throughput model adjusted for curvature, demonstrated that the model was highly significant (P -value < 0.0001). Both dilution rate and substrate concentration were significant factors (P -value < 0.0001) having a positive effect on process throughput. Temperature was found not significant and no factor interactions were identified. In addition, the ‘Lack of fit’ F -value of 2.99 is not significant relative to pure error indicating that the model, represented by Eq. (3) (where DR and SC are reactor dilution rate and substrate concentration respectively), can be used for prediction. Furthermore, the coefficient of determination (R^2) is 0.8441, indicating that only 15.6% of the total variation is not explained by the model.

$$\ln(\text{Throughput}) = 0.34 + (0.17 \times \text{DR}) + (0.28 \times \text{SC}) \quad (3)$$

Fig. 4 shows a surface response plot of the model indicating how process throughput varies as a function of substrate concentration and dilution factor. The process throughput increases almost exponentially with increasing substrate concentration and dilution factor (independent of temperature) up to a predicted value of nearly 250 mg Ara h⁻¹ g_{resin}⁻¹. The fact that no clear optimum is reached suggests that these preliminary PBR experiments occurred in a kinetically controlled operating region. Further advances in throughput are clearly possible, which is limited by the initial substrate concentration (SB-pectin in crude material) after SE process.

In order to validate the model predictions, three additional experiments with different ranges of process throughput were identified (Supplementary Material). PBR operating conditions (dilution rate and

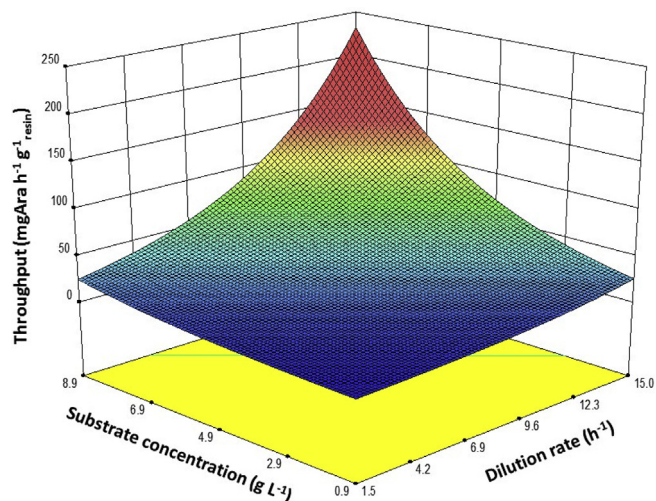


Fig. 4. Variation of process throughput in the packed bed reactor as a function of feed concentration (polymeric Ara in SB-pectin (g L⁻¹)) and dilution factor (h⁻¹). Response surface generated from a 2-level, 3-factor full factorial experimental design as described in Table 1.

substrate concentration) for each criterion were predicted by the software (based on Eq. (3)) aiming to maximise process throughput within each range. Temperature was fixed at 50 °C in order to minimise thermal inactivation of the immobilised AF and prevent microbial contamination. The experimental process throughput values for all three criteria were close to those predicted as were the reaction yields (even though the yield model was not found to be statistically significant).

Based on the process insights gained from the PBR model, the long-term hydrolysis of polymeric Ara in SB-pectin was subsequently performed using a PBR packed with 3 g of immobilised AF with 3.9 g L⁻¹ initial substrate concentration, 5 h⁻¹ dilution rate at 50 °C. This process was carried out for 173 h and the concentration of monomeric Ara released from SB-pectin was measured periodically (Supplementary Material). The average reaction yield and process throughput for the first 72 h were 87% and 22.2 mg Ara h⁻¹ g_{resin}⁻¹ respectively; these values were also close to those predicted by the model (82.8% and 23.1 mg Ara h⁻¹ g_{resin}⁻¹). At the end of the process the reaction yield decreased by 30%, however, the overall reaction yield and process throughput were maintained at 79% and 20 mg Ara h⁻¹ g_{resin}⁻¹ respectively. Kim et al. (2012) reported slightly higher conversion of 83% for up to 216 h; however, commercially pure sugar beet arabinans and co-immobilised *endo*- and *exo*-arabinases in a PBR were used in this study, as well as 13 times more carrier than reported in our current work.

3.5. Tangential flow ultrafiltration (TFU) for the recovery of monomeric Ara

After having selectively hydrolysed the arabinans in SB-pectin into monomeric Ara using a PBR packed with immobilised AF (Section 3.4), we aimed to establish a TFU step in order to continuously separate Ara from GABB (residual polymer after selective enzymatic process). Previously, we demonstrated that this separation can be achieved by TFU using a hollow fibre membrane (1 kDa MWCO) in a batch operation mode (Cardenas-Fernandez et al., 2017). In this work, the continuous TFU process was performed with 1 L of the stream coming from the PBR process, which contained 5.1 g L⁻¹ of monomeric Ara (3.2 g L⁻¹ from PBR hydrolysis and 1.9 g L⁻¹ from SE process) and 1.7 g L⁻¹ of GalAc (from the GABB after acid hydrolysis).

The permeate flux reached steady state after 3.5 h decreasing only by 31% compared to its initial value (Fig. 5). The transmission of Ara into the permeate was constant during the whole process, recovering 91.5% of the total Ara, while 92.7% of GABB was rejected and recovered in the retentate stream. A small fraction of the GABB was found in the permeate stream, which suggests that there was some oligomeric GABB with a molecular weight below 1 kDa. Other monosaccharides in the crude material, arising from the initial SE pretreatment (Rha, Gal, Glu and Fru), were also found in the permeate but in very low concentrations (0.07, 0.09, 0.05 and 0.02 g L⁻¹ respectively). Ara represented 95% of the total sugar content in the permeate stream. The retained GABB was concentrated 4.6-fold after 5 h of continuous operation.

In order to increase Ara recovery and concentrate GABB further, an additional 40 min of discontinuous process operation (without feeding of fresh mixture) was carried out (data not shown). During this time, GABB was concentrated 12.1-fold and the permeate flux maintained 60% of its original value at the end of the process. The overall throughput of the TFU process was 0.93 g Ara h⁻¹ suggesting that the hollow fibre membrane could efficiently process the eluent from the PBR recovering the released monomeric Ara in a relatively pure (95%) product stream.

Regarding the membrane performance, normalised water permeability (NWP) was measured before and after the TFU process as well as after membrane cleaning (250 mL of NaOH 0.5 M followed by 250 mL of MilliQ water). NWP is a critical metric in membrane processes as it characterises the degree of membrane irreversible fouling. Initial and

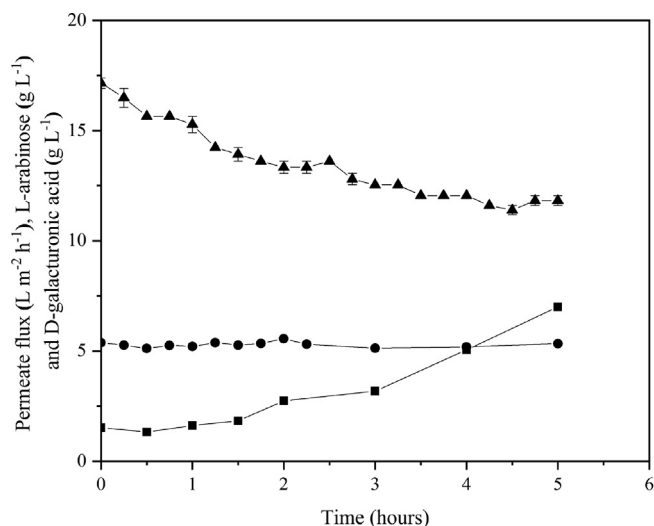


Fig. 5. Continuous tangential flow ultrafiltration for the recovery of monomeric Ara from the packed bed reactor product stream. Filtration performed using a MidiKros hollow fibre membrane (1 kDa MWCO). The feed flow rate (from tank to membrane) was set to 40 mL min⁻¹ and filtration was performed at a constant transmembrane pressure of 20 psi. Fresh mixture was fed at the same flow rate as permeate flow rate for 5 h: (●) monomeric Ara in the permeate, (■) D-galacturonic acid (from galacturonic rich acid backbone) in the retentate and (▲) permeate flux.

final NWP were 22.5 and 10.5 L h⁻¹ m⁻² bar⁻¹ respectively. After membrane cleaning, the NWP was restored to a level comparable to the initial one (21.5 L h⁻¹ m⁻² bar⁻¹), indicating that little irreversible fouling occurred during the process and that the membrane could be easily reused.

4. Conclusions

This work demonstrates the feasibility of establishing a continuous bioprocess sequence to obtain Ara from low-value SBP biomass within the context of an integrated sugar beet biorefinery. The process consists of SBP fractionation by steam explosion, pretreatment of crude material containing SB-pectin, continuous enzymatic hydrolysis of SB-pectin using immobilised AF in a PBR, and finally, recovery of Ara using continuous TFU. Processes such as PBR and TFU are widely used in industry and straightforward to scale-up. They can therefore be easily implemented in a sugar beet biorefinery to produce an Ara stream suitable for further enzymatic or chemical upgrading.

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References

Asadi, M., 2005. Sugarbeet rocessing. In: *Beet-sugar Handbook*. John Wiley & Sons, Inc., pp. 99–465.

Bawn, M., Subrizi, F., Lye, G.J., Sheppard, T.D., Hailes, H.C., Ward, J.M., 2018. One-pot, two-step transaminase and transketolase synthesis of l-gluco-heptulose from l-arabinose. *Enzyme Microb. Technol.* 116, 16–22.

Berowska, J., Pielech-Przybylska, K., Balcerak, M., Dziekoska-Kubczak, U., Patelski, P., Dziugan, P., Krgiel, D., 2016. Simultaneous saccharification and fermentation of sugar beet pulp for efficient bioethanol production. *BioMed Res. Int.* 2016, 1–10.

Boller, T., Meier, C., Menzler, S., 2002. EUPERGIT oxirane acrylic beads: how to make

enzymes fit for biocatalysis. *Org. Process Res. Dev.* 6 (4), 509–519.

Bornik, M.-A., Kroh, L.W., 2013. D-Galacturonic acid as a highly reactive compound in nonenzymatic browning. 1. Formation of browning active degradation products. *J. Agric. Food Chem.* 61 (14), 3494–3500.

Cardenas-Fernandez, M., Bawn, M., Bennett, C., Penumathsa, B., Subrizi, F., Suhaili, N., Ward, D., Bourdin, S., Dalby, P.A., Hailes, H., Hewitson, P., Ignatova, S., Kontoravdi, C., Leak, D.J., Shah, N., Sheppard, T., Ward, J.M., Lye, G.J., 2017. An integrated biorefinery concept for conversion of sugar beet pulp into value-added chemicals and pharmaceutical intermediates. *Faraday Discuss.* 202, 415–431.

Chan, S.Y., Choo, W.S., Young, D.J., Loh, X.J., 2017. Pectin as a rheology modifier: origin, structure, commercial production and rheology. *Carbohydr. Polym.* 161, 118–139.

Chen, H.-M., Fu, X., Luo, Z.-G., 2016. Effect of molecular structure on emulsifying properties of sugar beet pulp pectin. *Food Hydrocolloids* 54 (Part A), 99–106.

Espina Silva, G.A., 2015. Carbohydrate-degrading enzymes from the thermophilic ethanologen *Geobacillus thermoglucosidarius*. Department of Biology and Biochemistry, vol. Doctor.

Eurostat. < http://ec.europa.eu/eurostat/statistics-explained/index.php/Agricultural_production_-_crops#Potatoes_and_sugar_beet > .

Finkenstadt, V.L., 2014. A review on the complete utilization of the sugarbeet. *Sugar Technol.* 16 (4), 339–346.

Foster, R.W., Tame, C.J., Bučar, D.-K., Hailes, H.C., Sheppard, T.D., 2015. Sustainable synthesis of chiral tetrahydrofurans through the selective dehydration of pentoses. *Chem. Eur. J.* 21 (45), 15947–15950.

Hamley-Bennett, C., Lye, G.J., Leak, D.J., 2016. Selective fractionation of sugar beet pulp for release of fermentation and chemical feedstocks; optimisation of thermo-chemical pre-treatment. *Bioresour. Technol.* 209, 259–264.

Jia, L., Budinova, G.A.L.G., Takasugi, Y., Noda, S., Tanaka, T., Ichinose, H., Goto, M., Kamiya, N., 2016. Synergistic degradation of arabinoxylan by free and immobilized xylanases and arabinofuranosidase. *Biochem. Eng. J.* 114, 268–275.

Kim, Y.-S., Lim, Y.-R., Oh, D.-K., 2012. L-arabinose production from sugar beet arabinan by immobilized endo- and exo-arabinanases from *Caldicellulosiruptor saccharolyticus* in a packed-bed reactor. *J. Biosci. Bioeng.* 113 (2), 239–241.

Kordowska-Wiater, M., 2015. Production of arabinol by yeasts: current status and future prospects. *J. Appl. Microbiol.* 119 (2), 303–314.

Leijdekkers, A.G.M., Bink, J.P.M., Geutjes, S., Schols, H.A., Gruppen, H., 2013. Enzymatic saccharification of sugar beet pulp for the production of galacturonic acid and arabinose; a study on the impact of the formation of recalcitrant oligosaccharides. *Bioresour. Technol.* 128, 518–525.

Mesbahi, G., Jamalain, J., Farahnaky, A., 2005. A comparative study on functional properties of beet and citrus pectins in food systems. *Food Hydrocolloids* 19 (4), 731–738.

Micard, V., Renard, C.M.G.C., Thibault, J.F., 1996. Enzymatic saccharification of sugar-beet pulp. *Enzyme Microb. Technol.* 19 (3), 162–170.

Oosterveld, A., Beldman, G., Schols, H.A., Voragen, A.G.J., 2000. Characterization of arabinose and ferulic acid rich pectic polysaccharides and hemicelluloses from sugar beet pulp. *Carbohydr. Res.* 328 (2), 185–197.

Rao, N.N., Lütz, S., Würges, K., Minör, D., 2009. Continuous biocatalytic processes. *Org. Process Res. Dev.* 13 (3), 607–616.

Rezić, T., Oros, D., Marković, I., Kracher, D., Ludwig, R., Šantek, B., 2013. Integrated hydrolyzation and fermentation of sugar beet pulp to bioethanol. *J. Microbiol. Biotechnol.* 23 (9), 1244–1252.

Saha, B.C., 2000. α -l-Arabinofuranosidases: biochemistry, molecular biology and application in biotechnology. *Biotechnol. Adv.* 18 (5), 403–423.

Sakamoto, T., Sakai, T., 1995. Analysis of structure of sugar-beet pectin by enzymatic methods. *Phytochemistry* 39 (4), 821–823.

Sheldon, R.A., van Pelt, S., 2013. Enzyme immobilisation in biocatalysis: why, what and how. *Chem. Soc. Rev.* 42 (15), 6223–6235.

Sifontes Herrera, V.A., Saleem, F., Kusema, B., Eränen, K., Salmi, T., 2012. Hydrogenation of l-arabinose and d-galactose mixtures over a heterogeneous Ru/C catalyst. *Topics Catal.* 55 (7), 550–555.

Spagnuolo, M., Crecchio, C., Pizzigallo, M.D.R., Ruggiero, P., 1999. Fractionation of sugar beet pulp into pectin, cellulose, and arabinose by arabinases combined with ultrafiltration. *Biotechnol. Bioeng.* 64 (6), 685–691.

Subrizi, F., Cardenas-Fernandez, M., Lye, G.J., Ward, J.M., Dalby, P.A., Sheppard, T.D., Hailes, H.C., 2016. Transketolase catalysed upgrading of l-arabinose: the one-step stereoselective synthesis of l-gluco-heptulose. *Green Chem.* 18 (10), 3158–3165.

Ward, D.P., Hewitson, P., Cárdenas-Fernández, M., Hamley-Bennett, C., Díaz-Rodríguez, A., Douillet, N., Adams, J.P., Leak, D.J., Ignatova, S., Lye, G.J., 2017. Centrifugal partition chromatography in a biorefinery context: optimisation and scale-up of monosaccharide fractionation from hydrolysed sugar beet pulp. *J. Chromatogr. A* 1497, 56–63.

Wegener, S., Bornik, M.-A., Kroh, L.W., 2015. D-Galacturonic acid: a highly reactive compound in nonenzymatic browning. 2. Formation of amino-specific degradation products. *J. Agric. Food Chem.* 63 (28), 6457–6465.

Werpy, T., Petersen, G., 2004. Top value added chemicals from biomass. Results of screening for potential candidates from sugars and synthesis gas, office of scientific and technical information (OSTI), 1.

Westphal, Y., Kühnel, S., de Waard, P., Hinz, S.W.A., Schols, H.A., Voragen, A.G.J., Gruppen, H., 2010. Branched arabino-oligosaccharides isolated from sugar beet arabinan. *Carbohydr. Res.* 345 (9), 1180–1189.

Zheng, Y., Lee, C., Yu, C., Cheng, Y.-S., Zhang, R., Jenkins, B.M., VanderGheynst, J.S., 2013. Dilute acid pretreatment and fermentation of sugar beet pulp to ethanol. *Appl. Energy* 105, 1–7.