TITLE:

Analysis of the product streams obtained on butanosolv pretreatment of draff

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HIGHLIGHTS:

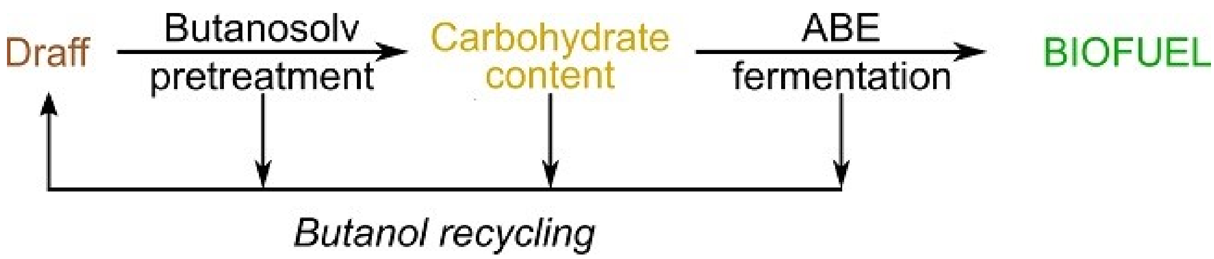
- A scalable pretreatment of the by-product draff using butanol gave three product streams
- A *pseudo*-lignin product stream was characterised in detail
- The hemicellulose-derived product stream contained butoxylated monosaccharides that were convert back to native sugars using chemical and enzymatic methods

- The cellulose-containing product stream was converted to glucose and fermented to give butanol completing a circular economy-type approach

KEYWORDS:

Sustainable Chemistry • Biomass • Draff • ABE fermentation • hemicellulose

GRAPHICAL ABSTRACT:



ABSTRACT:

The efficient use of biomass-derived waste streams from the food and drink industry is very important for achieving a circular economy. In this work, a pretreatment based on 1-butanol (butanosolv) was used to fractionate draff, a by-product from the brewing and distilling industries, leading to a solid pulp, a hemicellulose derived-fraction and a *pseudo* lignin. The pulp was enriched in glucans and showed a 4-fold improvement in enzymatic hydrolysis experiments relative to the starting biomass. The pulp could be fermented in an ABE process producing 32g/100g of solvents. The hemicellulose-derived fraction was analysed by 2D HSQC NMR and found to contain a mixture of predominantly butoxylated monosaccharides. The hydrolase enzymes present in Cellic® CTec3 were used to hydrolyse selectively the glucose and xylose derived butyl β-pyranose monomers. Alternatively, non-selective hydrolysis of both anomers was achieved using TFA/H_2O giving native sugars for fermentation and recovered 1-butanol. A detailed characterization of the *pseudo* lignin was also achieved.

155 words

1 INTRODUCTION

A decrease in our reliance on non-renewable fossil fuels and an increase in the use of renewable resources to produce clean and green energy is essential to attain a sustainable society.[1] In parallel, the steady rise in the level of waste being generated presents a challenge for today's society.[2] Attempts to overcome both of these challenges have led to the development of a range of technologies for the conversion of biowaste to bioenergy.[1] The major component of biowaste is biomass[1] and as a result biomass processing has become the focus of different bioenergy generating technologies, including in the context of the work discussed below, fermentation of agricultural waste to produce acetone, butanol and ethanol (ABE) as biofuels and commodity chemicals.[3] Ideally, the developed technologies are scalable enabling the establishment of biomass processing plants called biorefineries.[4] For the development of state-of-the-art biorefineries different approaches have been highlighted in the literature[5] including (i) the valorisation of the non-carbohydrate components of biomass (e.g. lignin, proteins), (ii) interdisciplinary development and research work and (iii) custom-designed and locally produced enzyme mixtures. Furthermore, the viability of the scale-up of technologies to commercial scale can be aided by conditional and economic optimisation of the system via extensive process modelling e.g. models for the enzymatic hydrolysis of lignocellulose such as the modified Holtzapple–Caram–Humphrey–1 kinetic model[6], a kinetic model considering heterogeneity[7] or mathematical modelling. [8]

One potential source of biomass for biorefineries is draff, also known as spent grain, which is produced as a by-product from the brewing and distilling industries. For example, in Scotland alone the Malt Whisky Industry produces 684 000 tonnes annually,[9] consisting mainly of barley grain residues.[10] Spent grains such as draff have been used traditionally as animal feed, although this is changing. One reason for this change is that this biowaste decomposes rapidly and so its use is time sensitive. Its short shelf life and seasonal demands have enforced a disposal requirement on distilleries and breweries which is a significant economic expense. As part of the strategy to avoid this, spent grains are increasingly being used in anaerobic digestion (AD)[11] and combined heat and power (CHP)[12] initiatives, providing some concern in the farming community over feed supplies.

<INSERT FIGURE 1 HERE>

The major chemical components of draff are cellulose and hemicellulose (mainly arabinoxylan[14] and β-1,3;1,4-glucan[15]), as well as a significant amount of protein (Figure 1).[11] Conversion of the high carbohydrate content into biochemicals and biofuels, *e.g.* via microbial fermentation, could provide a high value application.[11,12] More details on alternative applications of draff can be found in previously published reviews.[16–19] One possible approach for the valorisation of draff that is currently being explored involves a thermal pretreatment followed by enzymatic hydrolysis to generate a 'sugar platform' which can then undergo bacterial fermentation to produce biofuels (ABE).[20] Key challenges in this approach include controlling the generation of microbial inhibitors during the biomass pretreatment[21–23] and the cost of the required enzyme cocktail.[24,25]

Previously, we[26] and others[27–29] have shown that high-alcohol organosolv, particularly butanosolv, pretreatments can deliver cellulose pulps that are highly suitable for enzymatic hydrolysis. High yields of hemicellulose and lignin-derived product streams are also formed via this pretreatment and are amenable to further upgrading. Given the potential of draff as a biorefinery feedstock we were interested in investigating whether a butanosolv pretreatment could be used to facilitate ABE fermentation of the cellulose component of draff. From the five recovery stages discussed in the literature[30] three stages, the extraction (i.e. butanosolv pretreatment), the isolation and purification (i.e. chromatography) and the product formation (i.e. freeze drying) steps were explored. We also wanted to assess the co-product streams derived from the hemicellulose and lignin components. One advantage of the selected pretreatment is that it uses (bio)butanol, a biorenewable solvent, and so potentially facilitates a circular economy-type approach.[31]

2 MATERIALS AND METHODS

2.1 General Considerations

All chemicals used were obtained from commercial sources and used without further purification. The draff biomass was a kind donation from Celtic Renewables. Commercial Cellic® CTec2 enzyme mixture was kindly donated by Novozymes (Denmark) and used as received. Cellic® CTec3 enzyme mixture (Novozymes, Denmark) was kindly donated by Celtic Renewables and used as received. The elemental analysis was carried out by the Elemental Analysis Service at the London Metropolitan University.

2.2 NMR analysis

Nuclear magnetic resonance (NMR) spectra were acquired on the following instruments: Bruker AVIII-HD 700 (^{1}H, 700 MHz; ^{13}C, 175 MHz), Bruker AVIII-HD 500 (^{1}H, 500 MHz; ^{13}C, 126 MHz), Bruker AVIII 500 (^{1}H, 500 MHz; ^{13}C, 126 MHz) and Bruker AVII 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Chemical shifts were expressed as δ in units of ppm. D_2O and d_6-DMSO solvents were used as lock for all NMR spectra. 0.03 V/V % dimethyl sulfoxide was added to D_2O and was used as an internal reference in the assignment of spectra. Data processing was carried out using MestReNova 12.0.3 (Windows) and TopSpin 4.0.5 (Windows). Volume integration of cross peaks in 2D HSQC spectra was performed using TopSpin 4.0.5 (Windows). Figures were generated using Inscape 0.92.4.

2.3 Butanosolv pretreatment of draff

The previously reported butanosolv pretreatment procedures[26,32] were followed where the lignocellulosic biomass, draff, was gently refluxed in a mixture of 95:5 V/V *n*-butanol and 4 M aqueous hydrochloric acid (10 mL g^{-1}) for 6 h. The mixture was allowed to cool, filtered and the residual pulp washed with a mixture of 9:1 V/V acetone and water (10 mL g^{-1}) and air dried for 48 h. The filtrate was concentrated *in vacuo* and the resultant gum-like residue was taken up in a mixture of 9:1 V/V acetone and water (5 mL g^{-1}). The dissolved residue was precipitated by drop-wise addition into rapidly stirred water (50 mL g^{-1}). Flowing complete $(NH_4)_2CO_3$ was added to aid flocculation. The precipitated *pseudo* lignin was separated from the aqueous hemicellulose-derived stream by filtration. When required the filtrate was extracted with ethyl acetate (3 x 2 mL mL^{-1}) and the resulting aqueous phase was freeze dried giving a caramel-like solid while the organic phase was dried over $MgSO_4$ and concentrated *in vacuo* resulting in a yellow viscous oil. Small scale extractions (15 g draff) were performed in triplicate, while the large scale extraction (800 g draff) was executed once.

2.4 Fermentation

Clostridium saccharoperbutylacetonicum NCIMB 12606 was purchased from National Collection of Industrial, Food and Marine Bacteria (Aberdeen, Scotland) and maintained cryogenically at -80°C. Cryogenic stocks consisted of cultures grown to exponential phase on TYA medium to which 5 % (w/v) glucose and glycerol was added to give a 15% (v/v) concentration per cryovial. One cryovial was defrosted slowly on ice and 1 ml of the thawed culture was used to inoculate 20 ml volumes of TYA supplemented with 1 % (w/v) xylose (for butoxylated xylose (**6α/β**) fermentation) or 1 % (w/v) glucose (for the cellulose pulp fermentations), these were grown overnight at 34°C under an atmosphere of N_2: CO_2: H_2 (80:10:10) in a Whitley A85 anaerobic workstation (Don Whitley Scientific). A 5 % (v/v) volume of this culture inoculum was then used to inoculate 50 ml volumes of

TYA supplemented with either 5 % (w/v) xylose or butoxylated xylose (**6α/β**) (for butoxylated xylose fermentation); 5 % (w/v) butanosolv cellulose pulp or Sigmacell cellulose type 50 (for the cellulose pulp fermentations). All media was pH adjusted to pH 6.2 with KOH 50% solution (Acros Organics) prior to bacterial inoculation. The fermentations were conducted for 72 hours with constant agitation provided by the use of a WTW OxiTop IS 12 inductive stirring system platform and addition of magnetic stirrers to culture flasks. Butoxylated xylose (**6α/β**) fermentations were carried out in replicates of five and fermentations of the cellulose pulp in triplicate.

3 RESULTS AND DISCUSSION

Consideration of the different composition of draff (Figure 1) compared to our previously used biomasses[26,32] led to an initial assessment of the use of a butanosolv pretreatment with draff Although this pretreatment provided three product streams (Table 1) similar to our previous studies[26,32], a significant difference in the nature of the obtained lignin stream was encountered. As part of the standard butanosolv process, sodium sulfate is added to aid the flocculation of lignins which are prone to form colloidal suspensions (common for herbaceous feedstocks in our experience).[32] In this study with draff, the precipitation of the (pseudo) lignin fraction also required the addition of a flocculant. However, it was found that ammonium carbonate, a volatile salt, could be used as a traceless flocculant instead of sodium sulfate, simplifying the overall work-up procedure (Figure 2).

<INSERT FIGURE 2 HERE>

Due to differences in the structure of the material obtained (vide infra), the term ‘pseudo lignin’ (rather than lignin) is used throughout to refer to the material obtained at the point in the procedure where lignin would normally be isolated (Figure 2). The overall positive mass balance

(Table 1, 110.3-116.5 wt%) was consistent with previous studies[26,32] and likely results from the incorporation of a significant quantity of the solvent, butanol, into some of the product streams (vide infra). Based on this initial assessment we decided to carry out an in-depth investigation into each of the product streams.

<INSERT TABLE 1 HERE>

3.1 Cellulose Pulp

The potential for using the butanosolv-derived draff cellulose pulp (CP) in a biorefinery context was tested by enzymatic hydrolysis of the CP with the commercial enzyme cocktail Cellic® CTec2 (Novozyme, Denmark) (Figure 3a). As a comparison, a draff pulp prepared using a mild acid hydrolysis pretreatment (0.5 wt% H_2SO_4, 140 °C, 1.5 hr)[33] and the untreated draff were included. This analysis showed that the butanosolv-derived CP performed better, releasing 55 wt% reducing sugars after 72 hours (*c.f.* mild acid hydrolysis pulp and untreated draff which released 35 wt% and 15 wt% respectively over the same time period, Figure 3a).

<INSERT FIGURE 3 HERE>

In addition, the butanosolv CP was shown to be suitable for ABE fermentation with a simultaneous saccharification and fermentation (SSF) experiment leading to the production of acetone, n-butanol and ethanol (ABE) as products (Figure 3b) with maximum solvent yields reaching 16 g/100 g for CP. No negative impacts of the CP on the growth of *C. saccharoperbutylacetonicum* or solvent production were observed, suggesting the absence of inhibitors. Whilst total solvent production levels using CP appeared lower than when an equal amount of Sigmacell cellulose control was used (Figure 3b), it should be noted that CP contained only 51 wt% fermentable sugars (Table 2) as compared to 100% in the Sigmacell cellulose control. This value (51%) is almost in agreement with the amount of reducable sugars in CP as determined by the enzymatic hydrolysis (55%, Figure 3a). At present, the identity of the material that constitutes the rest of the CP is unknown.

<INSERT TABLE 2 HERE>

Whatever the nature of this material, it does not impact on the performance of the fermentation, with CP delivering a higher level of solvent production based on the amount of sugars present, relative to the control (CP - 16g/100g solvent produced; *c.f.* Sigmacell 24g/100g solvent produced). This result seems promising compared to, for example the fermentation of glucose in an extractive fermentation process with free cells by Darmayanti *et al.* [35] which resulted in 37.8 g/100 g ABE produced. The same study[35] showed that using immobilised cells can significantly improve solvent yields (90-93.6 g/100g) highlighting an interesting avenue to explore in the future. The presence of a high amount of butyric acid at the CP fermentation endpoint (Table 3) suggested that there was insufficient sugar available for the culture to sustain the conversion of the organic acid into solvent. Indeed, a near complete sugar utilisation in the CP fermentation confirmed this (Table 3).

<INSERT TABLE 3 HERE>

3.2 A *pseudo* Lignin from Draff

Having demonstrated the potential of the butanosolv-derived draff CP for the production of butanol, attention turned to the characterisation of the (*pseudo*) lignin (PL) product stream. In general, butanosolv pretreatments[26] deliver high quality lignins with a high α-butoxylated β-O-4 content.[26] Such lignins are organic-solvent soluble and can be used directly in controlled lignin depolymerisations or as precursors to potential new materials.[26,36,37] Indeed, this is one of the advantages of the butanosolv pretreatment over other organosolv processes. However, in the case of draff, 2D HSQC NMR analysis of the PL revealed that this material was very different to a standard butanosolv lignin. In particular the main cross peaks observed in the aromatic region of the spectrum could be assigned to residual protein units containing phenylalanine and tyrosine[38] (Figure 4) rather than the expected lignin aromatic units (only visible at lower contour levels, Figure 4 inset box). This was consistent with the known relatively high protein content in draff,[13] which was further supported in the elemental analysis of PL which indicated that a substantial amount of nitrogen (Table 4; 2.87%) was retained in this product stream. Furthermore, the oxygenated alkyl region showed only weak cross peaks characteristic of lignin units (*e.g.* Figure 4, structure B), with the spectrum being dominated overall by signals corresponding to vinylic and aliphatic structures (Figures 4 and S2, ^{1}H: 5.0-5.5; ^{13}C: 127-129 ppm and ^{1}H: 0.5-2.5; ^{13}C: 15-42 ppm respectively). This probably results from the "high initial extractive content of draff",[39] of which a large proportion is a mixture of saturated and unsaturated lipids which partition with the hydrophobic fraction. HMBC NMR analysis of PL (Figure 4, lower panel) indicated that the majority of the lipids were present in the form of butyl esters, as evidenced by a correlation between the methylene protons of the ester chain ($RC(=O)OC\underline{H}_2CH_2CH_2CH_3$; ^{1}H: 3.88-4.00 ppm) and the ester carbonyl groups (^{13}C: 172-174 ppm). Additional correlations to these ester carbonyl carbons from signals in the aliphatic region were also observed. This analysis suggests that the naturally occurring triglycerides in draff are trans-esterified by butanol under the pretreatment conditions.

<INSERT FIGURE 4 HERE>

Fractionation of PL by partial dissolution in acetone:petroleum ether (5:95) allowed for the isolation and characterisation of a significant amount of lipid derived material (*ca.* 40 wt% of PL). 2D HSQC and HMBC NMR analysis of the lipid-enriched soluble fraction confirmed the assignment of the major components as fatty acid butyl esters (Figure S1a), which are potentially useful compounds for bioenergy applications, for example as a biodiesel.[41,42] Removal of triglycerides and trace amounts of maltose prior to butanosolv pretreatment using a room temperature ethanol wash led to smaller quantities of PL being isolated (Figure S2 and Table S1). In addition to the fatty acid butyl esters, butyl ferulate (**1**) and butyl coumarate (**2**) were isolated from PL by column chromatography of the acetone:petroleum ether (5:95) fraction (Figure S1b). To the best of our knowledge this is the first report of trans-esterification of triglycerides, ferulates and coumarates under butanosolv pretreatment conditions. ^{1}H NMR analysis of the major (*ca.* 60 wt% of PL) insoluble component of the PL from the partial dissolution protocol revealed very broad signals suggesting that this was a high molecular weight, heterogeneous material (Figure S1c) with only trace amounts of fatty acid butyl esters still present. It is important to note, that based on the above analysis, the *pseudo* lignin obtained during the butanosolv treatment of draff appears to be a distinct class, compared to those that originate from the condensation of carbohydrate and lignin fragments during other pretreatment processes.[43–45] Whilst the PL clearly contained small quantities of butanosolv lignin, it was not possible to obtain lignin that was of the same level of purity as that previously reported by us using dioxasolv processing of draff.[46]

<INSERT TABLE 4 HERE>

3.3 The Fate of the Hemicellulose Component

The majority of the carbohydrate content in draff is present as hemicelluloses (22-28% hemicelluloses *cf*. 17-25% cellulose[13]). A range of pretreatment methods[47], including lime pre-treatment[48], wet oxidation[49], steam[50] and dilute acid preatreatments[51], are known to partially depolymerise hemicellulose to form readily fermentable native monosaccharides such as xylose. In contrast, the butanosolv pretreatment of draff leads to complete depolymerisation of the hemicellulose component, as verified via DOSY NMR analysis and GPC analysis (Figure 5). However, during a butanosolv pretreatment the hemicellulose depolymerisation is linked, at least in part, to the incorporation of butanol at the anomeric position of many of the liberated monosaccharides,[26] giving a unique product stream. As the hemicellulose-derived fraction (HDF) was the largest isolated product stream from draff (53-64 wt%, Table 1), further investigations were carried out into its composition.

<INSERT FIGURE 5 HERE>

As expected,[32] 2D HSQC NMR analysis of HDF indicated the presence of both butoxylated and native glucose and xylose monosaccharides as the major components (Figure S3). Partitioning of the HDF between EtOAc and water allowed for the selective, but not exclusive, enrichment of the butoxylated monomeric sugars (Figure S4) into the EtOAc layer, [32] accounting for ca. 40 wt% of the original HDF. The enrichment in butoxylated monosaccharides in the EtOAc extract allowed for the identification of additional minor components, as well as those derived from glucose and xylose (Figure S4). For example, signals corresponding to α/β-L-arabinopyranose (**3α/β**), α/β-L-arabinofuranose (**4α/β**) and butyl α/β-L-arabinopyranoside (**5α/β**) were observed (Figure 6), consistent with the expected hemicellulose composition of draff which is known to contain D-xylose, D-glucose and L-arabinose.[52] The analysis of L-arabinose is significantly more challenging than for D-glucose and D-xylose due to the increased propensity of L-arabinose to exist in its native form as furanose isomers (Figure 6a). For example, the signals at ^{1}H: 4.9; ^{13}C: 102.3 ppm (Figure 6a) were assigned to the anomeric protons of the β-(**4β**) furanose anomer while the signals at ^{1}H: 5.0; ^{13}C: 96.2 ppm (Figure 6a) were assigned to the anomeric protons of the α-(**4α**) anomer of L-arabinofuranose. To date it has only been possible to confirm the presence of the pyranose β-(**5β**; ^{1}H: 4.0; ^{13}C: 103.7 ppm) and α-(**5α**; ^{1}H: 4.6; ^{13}C: 99.8 ppm) anomers of the butoxylated form of L-arabinose in the EtOAc extract by comparison with authentic samples (Figure 6b). In addition, further purification of the EtOAc extract of the HDF by column chromatography allowed for the identification of butoxylated monosaccharides derived from D-glucuronic acid (Figure S5), the formation and structure of which was verified by comparison with authentic samples (see ESI for details of synthesis and analysis). One possible source of the D-glucuronic acid-derived compounds could be the presence of glucuronoxylan and/or glucuronoarabinoxylan making up part of the hemicellulose component in draff although the presence of these polymers has not been discussed previously in the literature to the best of our knowledge.[52]

<INSERT FIGURE 6 HERE>

3.4 Scale up and a circular economy approach

As HDF was the major product stream from butanosolv processing of draff, it was decided to assess whether the butoxylated monosaccharides found in the ethyl acetate extract were substrates for ABE fermentation. Possible conversion of, for example, butyl α/β-D-xylopyranoside (**6α/β**) to D-xylose under the fermentation conditions would not only lead to the recovery of one equivalent of solvent-derived butanol per molecule of **6α/β** (hydrolysis step in Scheme 1a), but also enable the generation of additional butanol through ABE fermentation of the resulting D-xylose (0.3 eq. of butanol produced for every eq. of D-xylose formed,[53] fermentation step in Scheme 1a). Studies using an authentic sample of **6α/β** showed that under conditions where D-xylose could be fermented, no solvent was produced from **6α/β** (Tables S3 and Figure S6).

In further attempts to integrate the butoxylated monosaccharides present in the HDF into our proposed butanol production/recovery cycle (Figure 7), both chemical and enzymatic methods of converting butoxylated to native monosaccharides were investigated. Complete hydrolysis of both anomers of **6**α/β was achieved using aqueous TFA at 120 °C (Scheme 1b and Figure S7). Alternatively, enzymatic hydrolysis could potentially offer the advantage of a greener and milder approach as well as enabling selective processing of the anomeric mixture of butoxylated monosaccharides. As a proof of concept study, the potential “debutoxylating” ability of the commercially available enzyme cocktail, Cellic® CTec3, which is known to contain a range of cellulases and hemicellulases, was assessed.[54] Treatment of an authentic sample of **6**α/β with CTec3 resulted in the successful production of D-xylose by predominant conversion of the β anomer (**6**α, Scheme 1b, Figure S8 and Table S3). Analogous results were obtained when authentic samples of butoxylated D-glucose was used (Figure S9 and Table S4) and when the enzyme cocktail Cellic® CTec3 was replaced with the previously commercialized CTec2 version (data not shown).

<INSERT FIGURE 7 HERE>

<INSERT SCHEME 1 HERE>

4 CONCLUSIONS

The biowaste used in this work, draff, is depleted of a large proportion of its hexose sugars compared to the starting biomass (*e.g.* barley grain). However, draff still contains a significant portion of carbohydrates with as much as 50- 60 % of the dry matter consisting of carbohydrates[55] including glucans, starch, cellulose and arabinoxylans.[56] Here we reported a 63% carbohydrate content for the draff used, constituting of 35 % glucan and 28 % pentosan sugars.

We show that butanosolv pretreatment can be used to fractionate this draff into 3 streams: a cellulose enriched pulp; hemicellulose derived fraction; and *pseudo* lignin on both small (15 g) and large scale (800 g). We have identified ammonium carbonate as a traceless flocculant for the *pseudo* lignin. Enzymatic hydrolysis and ABE fermentation studies demonstrated that the pulp obtained from this process is highly suited to downstream bio-processing and, in terms of solvent production, outperformed commercial Sigmacell cellulose on a sugar basis during ABE fermentation. The judicious use of draff in ABE fermentation to derive extra value from the spent material is of significant industrial importance. For example, Celtic Renewables Limited has demonstrated that its technology[57] can take advantage of all of the sugars present in draff, including the pentoses that yeasts cannot traditionally use, demonstrating that draff can lead to high value commodity chemicals and biofuels as well as being used as an animal feed.

Attempted characterisation of the *pseudo* lignin fraction revealed an unusual material consisting mainly of fatty acid butyl esters, with small amounts of butyl ferulate, coumarate and lignin as well. In line with previous studies the hemicellulose derived stream contained a mixture of butoxylated and native monosaccharides, which were characterised by 2D HSQC, HBMC and DOSY NMR. In an effort to recover butanol from the process and to facilitate downstream microbial processing of butoxylated monosaccharides we demonstrate that the hydrolase enzymes in Cellic® CTec3 can selectively hydrolyse butyl β-pyranoses to give native sugars, whilst global hydrolysis can be achieved using aqueous TFA. The relevance of this work can be specifically appreciated in light of emerging technologies such as membrane processes that could be incorporated from the initial pretreatment step to the final solvent recovery.[58] This study highlights the potential of butanosolv pretreatments for the fractionation of draff prior to enzymatic and microbial processing, for example ABE fermentation, paving the way to a viable circular economy.

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Figure 1

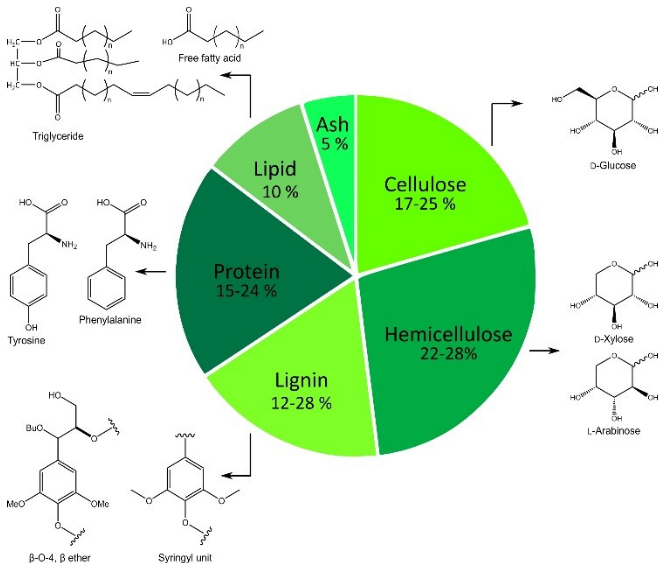


Figure 1. Apparent composition of brewer's spent grain or draff as reported by Mussatto *et al.*[13]

Structures of example monomeric units constituting the main components are shown.

Figure 2

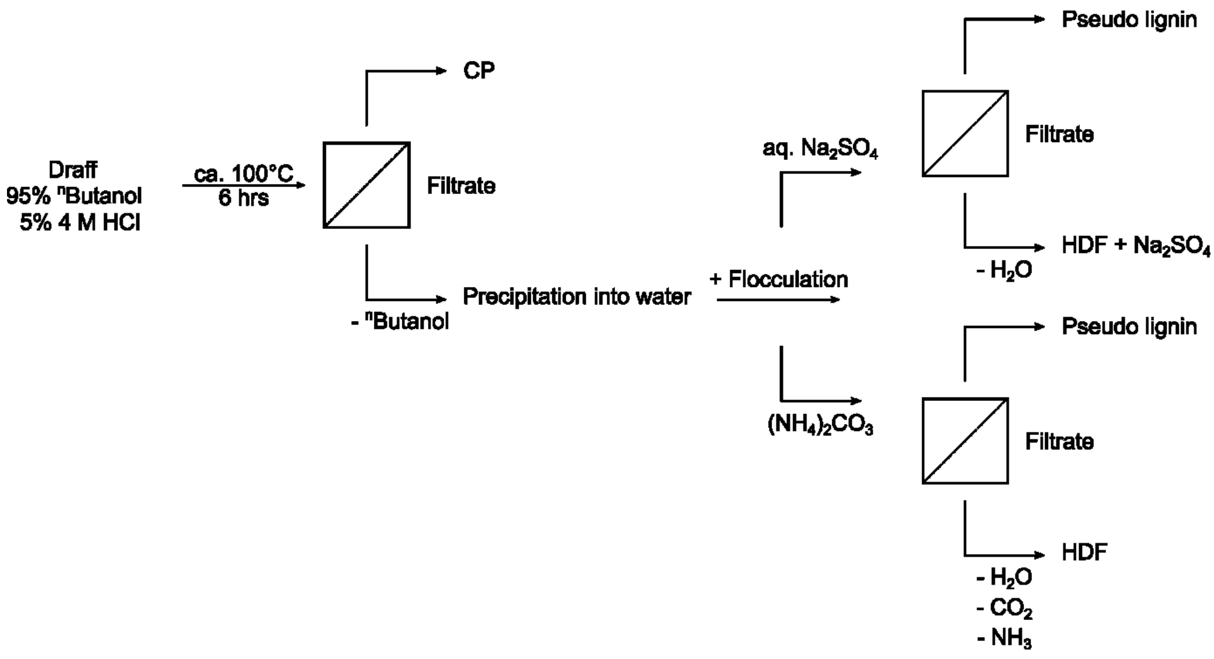


Figure 2. Schematic representation of the butanosolv pretreatment used in this study highlighting

the advantage of using a traceless flocculant (ammonium carbonate). CP = cellulose pulp; HDF = hemicellulose-derived fraction.

Figure 3

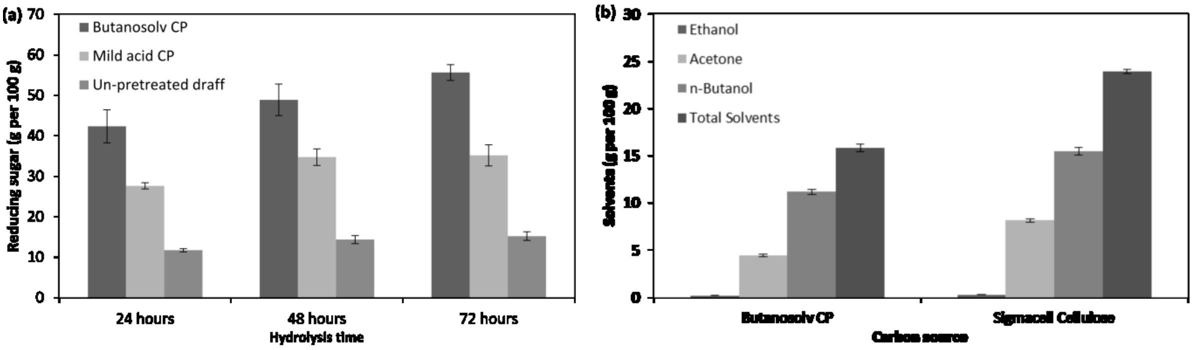


Figure 3. Assessment of the butanosolv-derived cellulose pulp (CP) for enzymatic hydrolysis and ABE fermentation. (a) Reducing sugars liberated via enzymatic hydrolysis of the starting draff and of the cellulose pulps (CPs) obtained from butanosolv or mild acid pretreatment of the draff, 5 wt % loading, 50°C, pH 5.5 and 10 FPU/g Cellic® CTec2; average of 3 replicates; (b) Solvent production during *C. saccharoperbutylacetonicum* fermentation of draff butanosolv CP and Sigmacell cellulose type 50, 5 wt % loading, at 52.5°C, pH 5.0 and 6 w/w % (g- Cellic® CTec3/100 g-cellulose); average of 3 replicates.

Figure 4

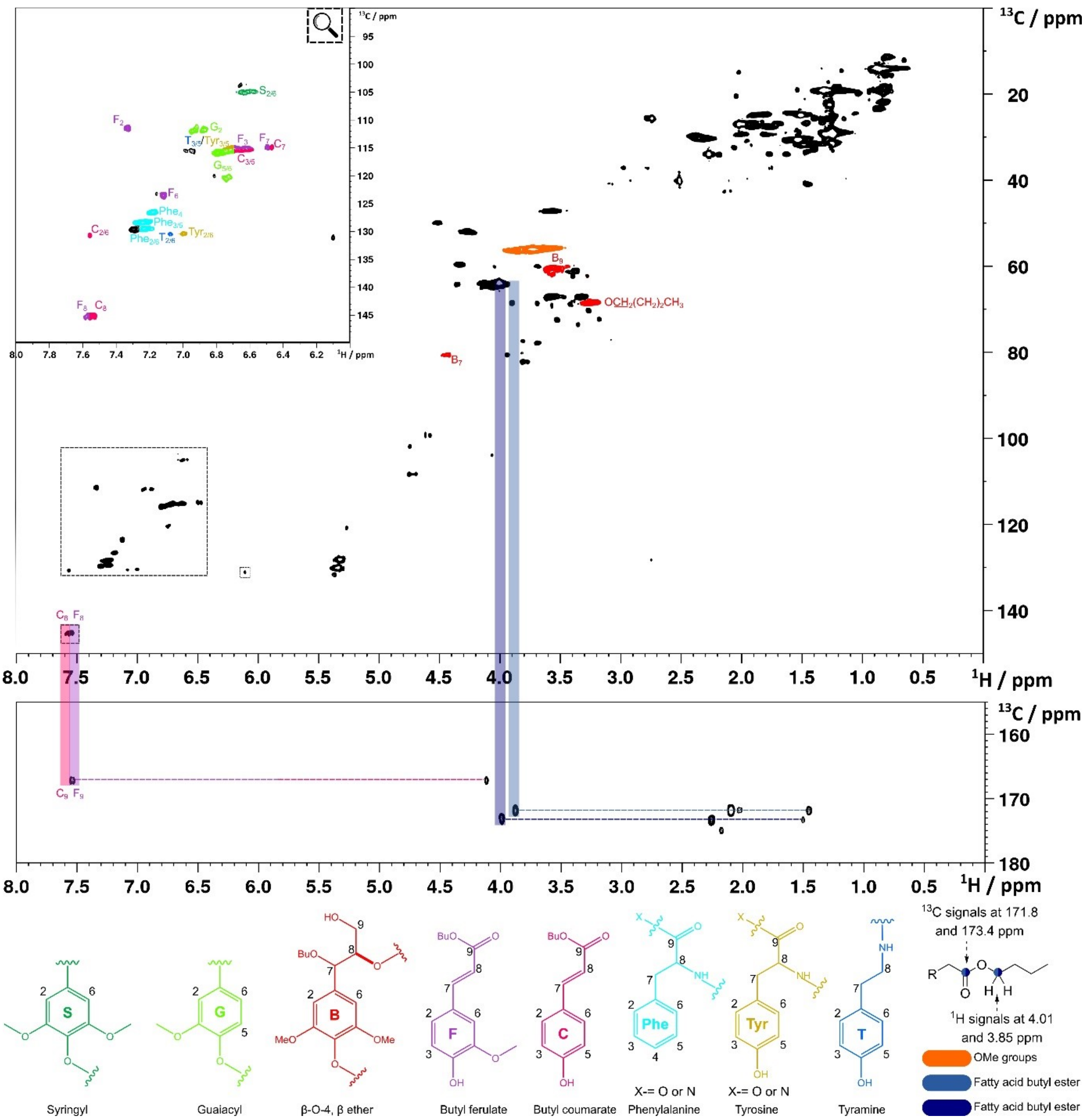


Figure 4. 2D HSQC and HMBC NMR spectra (700 MHz, d_6-DMSO) of draff butanosolv-derived *pseudo* lignin with assignments of the major characteristic peaks. The aromatic region is also displayed at a lower contour level in the inset box. Correlations of signals corresponding to butyl ester groups are highlighted between the HSQC and HMBC spectra. Assignments were based on literature data[26,38,40] and are colour coded corresponding to the assigned structural element.

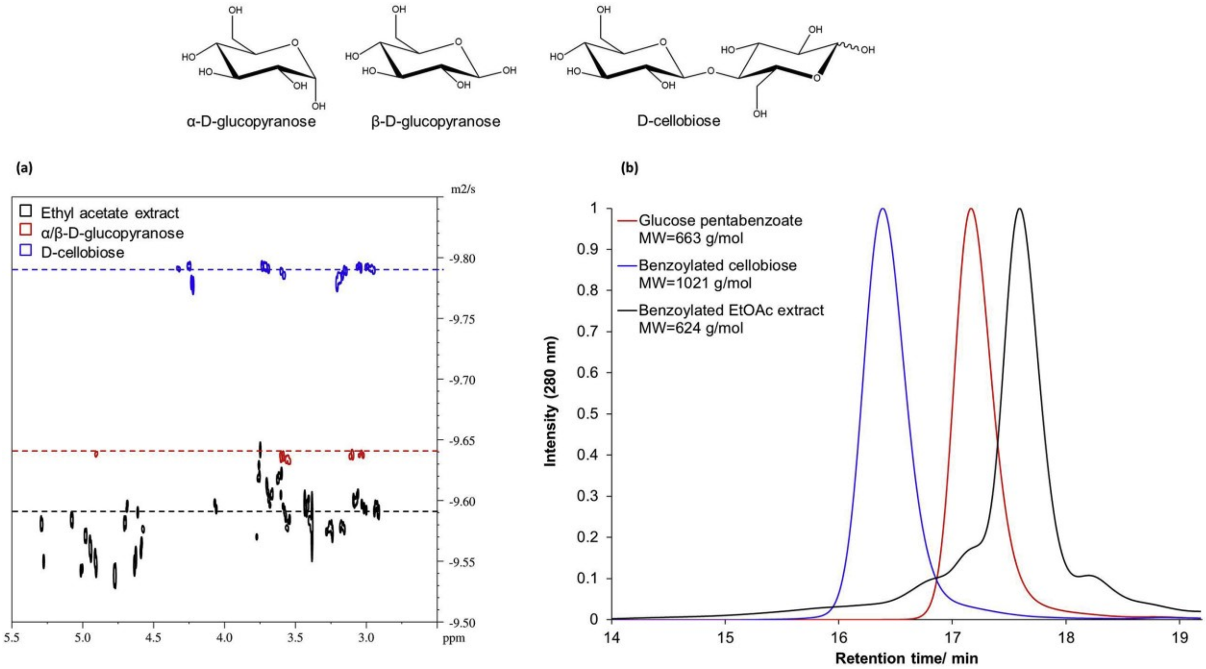


Figure 5. (a) DOSY NMR (700 MHz, d_6-DMSO) plot of the ethyl acetate extract, α/β-D-glucopyranose and D-cellobiose. The average diffusion coefficient of each examined species is indicated by the corresponding dashed line for ease of interpretation. The decrease of the diffusion coefficients correlates to an increase in the molecular masses (*e.g.* a more negative, hence smaller, value of the diffusion coefficient (-9.79 m^2/s for D-cellobiose) correlates to a higher molecular weight species). **(b)** GPC analysis of the ethyl acetate extract, α/β-D-glucopyranose and D-cellobiose after benzoylation.

Figure 6

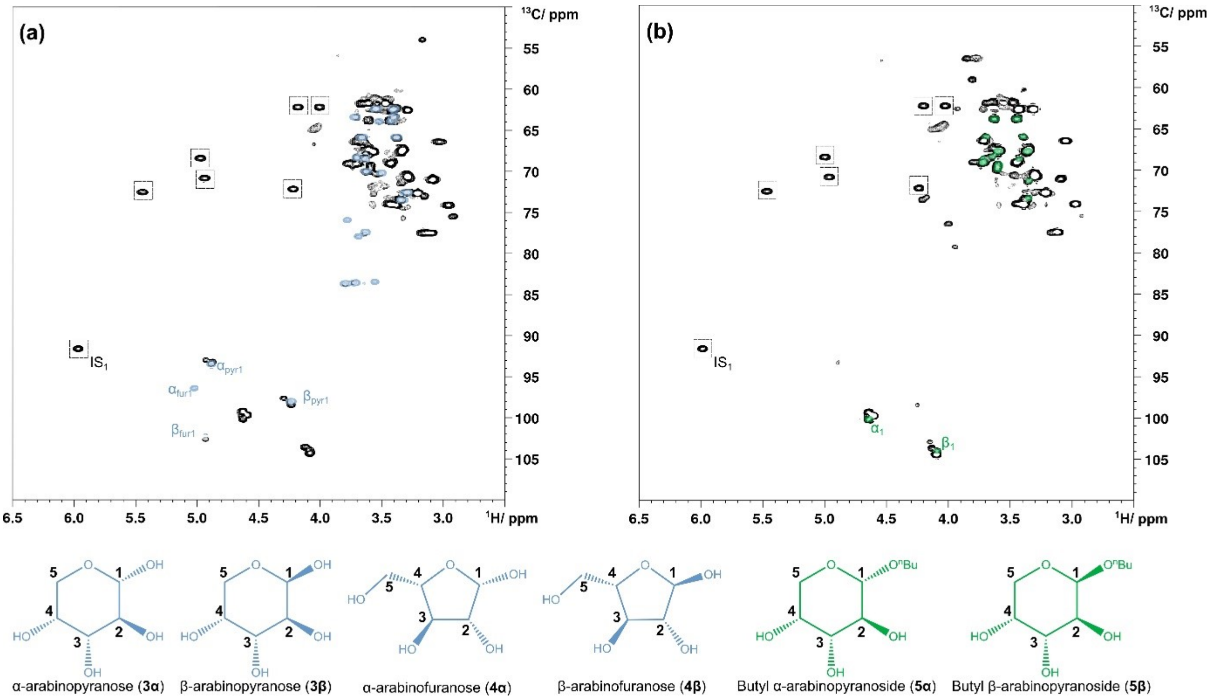


Figure 6. 2D HSQC NMR (700 MHz, d_6-DMSO) analysis of: **(a)** aqueous layer from extraction of HDF (black) overlaid with the spectrum of an authentic sample containing α/β-L-arabinopyranose (**3**α/β) and α/β-L-arabinofuranose (**4**α/β) (blue) and **(b)** EtOAc extract of HDF (black) overlaid with the spectrum of an authentic sample of butyl α/β-L-arabinopyranoside (**5**α/β) (green). α-D-glucose pentaacetate was used as an internal standard (IS), the signals for which are highlighted using boxes.

Figure 7

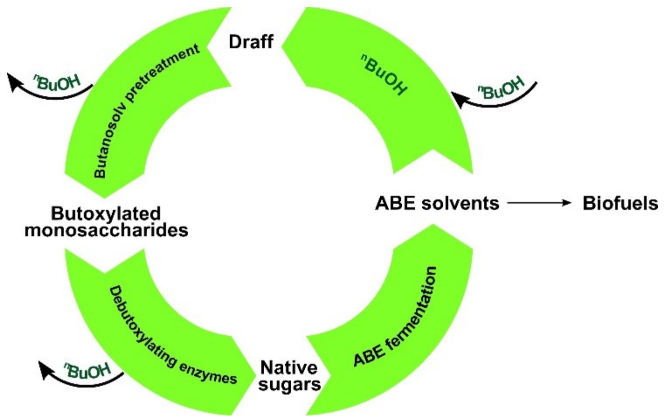


Figure 7. Our proposed circular economy approach based on "butanol production and formation" with draff used as the waste biomass.

Table 1

Product stream	**Small scale pretreatment**[a] (15 g)	**Large scale pretreatment** (800 g)
Cellulose pulp (CP)	28.5 ± 0.3 %	32.8 %
Hemicellulose-derived fraction (HDF)	64.2 ± 3.6 % [b]	53.4 %
Pseudo Lignin (PL) [c]	23.0 ± 0.6 % [b]	23.6 %
Mass balance	110.3 ± 1.5 % [d]	116.5 % [d]

Table 1. Product distribution resulting from the butanosolv pretreatment as weight % relative to the mass of the initial starting material on small scale (15 g draff) and on large scale (800 g draff).

[a] Average of 3 repeated extractions. [b] For practical reasons the total yield was back-calculated after work-up of exactly ½ of the extraction liquor. [c] The term *pseudo* lignin is used throughout for reasons that are described in the main text. [d] Increase of the overall mass balance can be attributed to the incorporation of *n*-butanol into the HDF and PL product streams.

Table 2

Monosaccharide	**Draff** (g per 100g)	**Butanosolv-derived cellulose pulp (CP)** (g per 100 g)
Glucose	34.47 ± 0.75	43.62 ± 0.45
Xylose	19.46 ± 0.94	6.14 ± 0.51
Arabinose	8.74 ± 0.34	1.27 ± 0.02
Total monosaccharide	62.67 ± 1.85	51.15 ± 0.30

Table 2. Carbohydrate content of starting draff and butanosolv-derived cellulose pulp (CP) determined using the method of Sluiter *et al*.[34] Average of 3 replicates.

Table 3

	Butanosolv cellulose pulp	Sigmacell cellulose
Ethanol (g/L)	0.10 ±0.00	0.15 ±0.00
Acetone (g/L)	2.24 ±0.06	4.08 ±0.09
Butanol (g/L)	5.59 ±0.14	7.76 ±0.20
Total Solvents (g/L)	7.93 ±0.21	11.99 ±0.11
Acetic acid (g/L)	0.52 ±0.02	0.52 ±0.12
Butyric acid (g/L)	5.12 ± 0.17	5.22 ±0.31
Total acids (g/L)	5.64 ± 0.15	5.74 ±0.19
Sugar used (%)	88.01 ± 0.41	92.37 ± 0.23
Yield Butanol (g/g)	0.25 ±0.01	0.17 ±0.00
Yield Total Solvents (g/g)	0.35 ±0.01	0.26 ±0.00

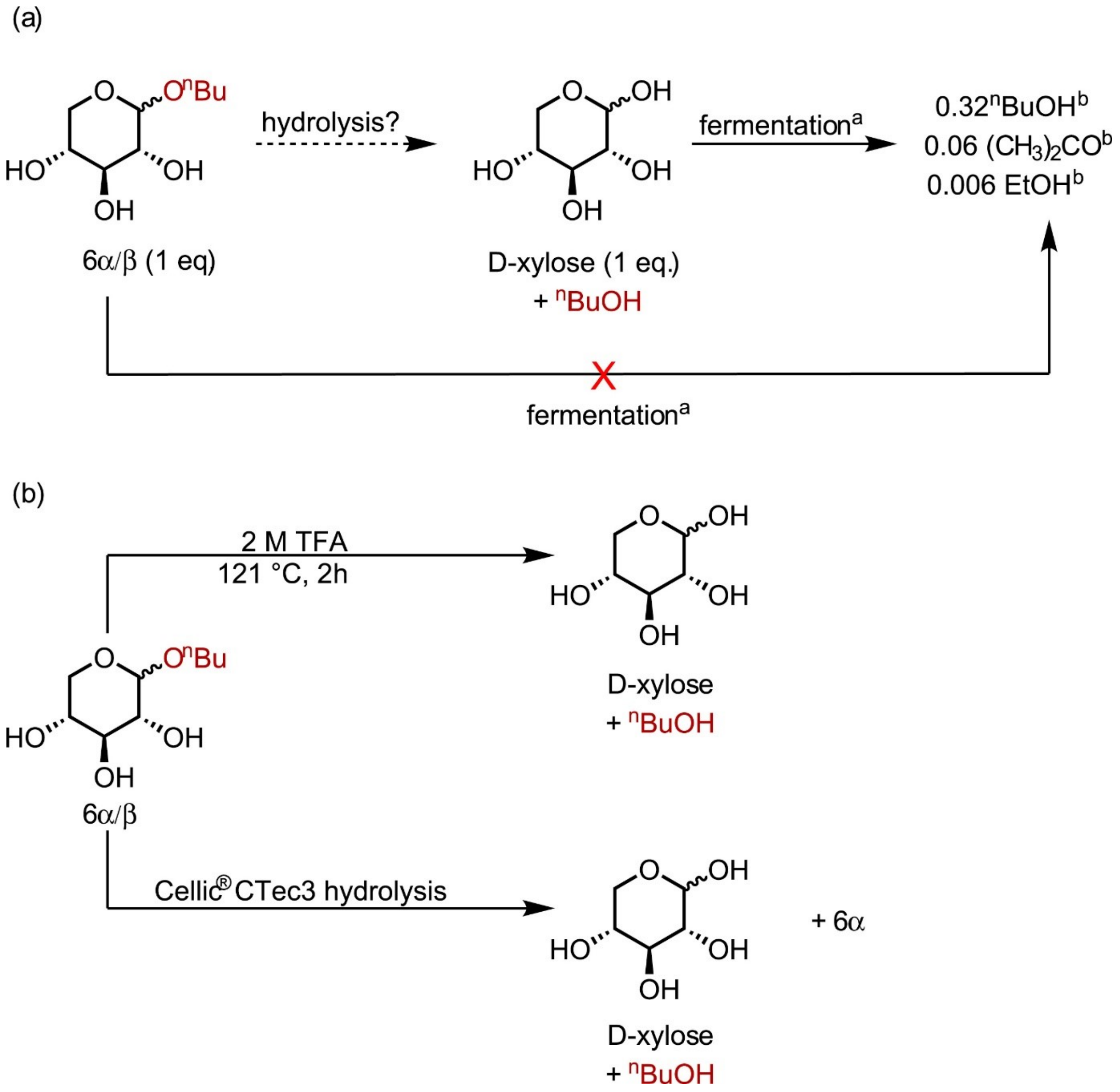
Table 3. End products and solvent yields from the *C. saccharoperbutylacetonicum* fermentation of the butanosolv cellulose pulp and the Sigmacell cellulose type 50 control, 5 wt % loading, at 52.5°C, pH 5.0 and 6 w/w % (g- Cellic® CTec3/100 g-cellulose); average of 3 replicates.

Table 4

Sample	C(wt %)[a]	H(wt %)[a]	N(wt %)[a]	O(wt %)[a,b]	C/O ratio
Draff	49.87	7.19	4.44	38.51	1.29:1
CP	40.18	5.83	3.86	50.13	0.8:1
PL	62.46	8.30	2.87	26.38	2.37:1

Table 4. Elemental composition of the starting biomass, draff, the cellulose pulp (CP) and the draff-derived *pseudo* lignin (PL) resulting from butanosolv extraction.

[a] Average of 2 repeats. [b] The oxygen content was determined by the subtraction method.



Scheme 1. Processing of butoxylated xylose (**6α/β**). (a) Theoretical conversion of **6α/β** via ABE fermentation[53] resulting in the theoretical production of 1.3 eq of *n*-butanol in total (top row). Results of fermentation experiments showed that fermentation **6α/β** does not lead to solvent production under the same conditions as fermentation of xylose (bottom row; Figure S6 and Table S2). [a]ABE fermentation using *C. saccharoperbutylacetonicum.* [b]Calculated based on results shown in Table S2. (b) Chemical conversion of **6α/β** led to the complete regeneration of xylose, while enzymatic processing of **6α/β** led to the selective regeneration of xylose.