**Intensification of xylo-oligosaccharides production by hydrothermal treatment of brewer’s spent grains: Use of extremely low acid catalyst for reduction of degradation products associated with high solids loading**

**Lukas J. Swart1 · Oscar K.K. Bedzo1\* · Eugéne van Rensburg1 · Johann F. Görgens1**

1Dept. of Process Engineering, Stellenbosch University, Private Bag X1 Stellenbosch 7602 South Africa

\*Corresponding author - Email: 19123949@sun.ac.za

**Table S1** Comparing autohydrolysis with mineral acid catalysed XOS production for BSG with 15% solids loading using 12.5 mg acid/g dm at 150 °C for 10 min.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Acid Catalyst** | **Acid loading**  **(mol H+/g dm)** | **XOS yield (wt%)** | **XOS%a**  **(wt.%)** | **TDSyieldb (wt.%)** | **TRSc**  **(g/100g)** | **CSF** | **Cost Acid $/kg dm** |
| None | 0 | 41.7% | 96.9% | 26.8% | 1.77 | -1.71 | $0.00 |
| H2SO4d | 0.26 | 62.2% | 94.2% | 38.4% | 3.85 | -0.56 | $1.51 |
| HCl | 0.34 | 69.6% | 88.0% | 51.3% | 6.49 | -0.27 | $5.75 |
| H3PO4 | 0.38 | 56.8% | 96.6% | 35.8% | 2.73 | -1.15 | $2.17 |
| HNO3 | 0.20 | 71.7% | 94.7% | 42.0% | 4.03 | -0.87 | $4.96 |
| a XOS percentage of total xylan equivalent recovered  b Total dissolved solids hydrolysate  c Total reducing sugars per 100 gram dry feedstock | | | | | | | |
| d Mean of three replicates (This study) | | | | | | | |

ELA HTT results from full factorials with centre points, Table 0 2 for BSG-R (Run A1-A11) and Table 0 3 for BSG-SPD (Run B1-B11).

Table S2 Results for acid catalysed XOS production for BSG-R

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Run | Independent variablesa | | | XOS (g/l) | | XOS yield (wt%) | | ArOS yield (wt%) | XOS%b  (wt.%) | | | ArOS%c(wt.%) | Inhibitorsd (g/100g) | TDSe yield (wt.%) | | TDS NDf (g/100g) | | Mass balance closure | | CSF |
| AC | t | T |  |  | |  | | |  | |  |  |  |  | |  | |  | |
| A-1 | 5 | 5 | 130 | 11.39 | 52.4% | | | 66.5% | | | 98.2% | 90.8% | 0.10 | 39.8% | | 16.51 | | 95.6% | | -1.81 |
| A-2 | 5 | 15 | 130 | 10.89 | 47.2% | | | 45.9% | | | 97.1% | 74.4% | 0.15 | 32.6% | | 5.95 | | 91.2% | | -1.48 |
| A-3 | 5 | 5 | 170 | 14.54 | 67.5% | | | 42.6% | | | 94.4% | 56.1% | 0.71 | 41.2% | | 10.94 | | 89.9% | | -0.84 |
| A-4 | 5 | 15 | 170 | 16.57 | 76.4% | | | 29.7% | | | 91.7% | 45.9% | 1.18 | 42.9% | | 13.18 | | 83.0% | | -0.55 |
| A-5 | 20 | 5 | 130 | 11.92 | 54.5% | | | 41.9% | | | 96.9% | 51.9% | 0.16 | 31.6% | | 7.21 | | 92.8% | | -0.36 |
| A-6 | 20 | 15 | 130 | 10.60 | 48.0% | | | 30.3% | | | 95.2% | 41.6% | 0.19 | 35.9% | | 12.16 | | 89.4% | | -0.38 |
| A-7 | 20 | 5 | 170 | 15.89 | 72.6% | | | 22.8% | | | 78.5% | 28.7% | 1.31 | 47.4% | | 15.70 | | 87.4% | | 0.28 |
| A-8 | 20 | 15 | 170 | 10.87 | 50.2% | | | 13.2% | | | 62.9% | 23.5% | 1.77 | 41.0% | | 11.85 | | 82.9% | | 0.46 |
| A-9 | 12.5 | 10 | 150 | 13.25 | 60.9% | | | 38.8% | | | 94.3% | 47.1% | 0.39 | 38.0% | | 9.89 | | 91.8% | | -0.64 |
| A-10 | 12.5 | 10 | 150 | 13.96 | 63.0% | | | 40.1% | | | 94.0% | 46.4% | 0.41 | 39.8% | | 12.20 | | 95.7% | | -0.55 |
| A-11 | 12.5 | 10 | 150 | 13.25 | 60.6% | | | 36.6% | | | 94.2% | 44.1% | 0.29 | 37.4% | | 9.41 | | 86.8% | | -0.50 |
| a AC, Acid loading (mg/100g dm); t, Time (min); T, Temperature (°C). | | | | | | | | | | | | | | | | | | | | |
| b XOS percentage of total xylan equivalent recovered | | | | | | | | | | | | | | | | | | | | |
| c ArOS percentage of total arabinan equivalent recovered | | | | | | | | | | | | | | | | | | | | |
| d Combined acetic acid, formic acid, furfural and HMF | | | | | | | | | | | | | | | | | | | | |
| e Total dissolved solids hydrolysate | | | | | | | | | | | | | | | | | | | | |
| f Dissolved solids excluding quantified oligomers, sugars and inhibitors | | | | | | | | | | | | | | | | | | | | |

Table S3 Results for acid catalysed XOS production for BSG-SPD

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Run | Independent variablesa | | | XOS (g/l) | | XOS yield (wt%) | | ArOS yield (wt%) | XOS%b  (wt.%) | | | ArOS%c(wt.%) | Inhibitorsd (g/100g) | TDSe yield (wt.%) | | TDS NDf (g/100g) | | Mass balance closure | | CSF |
| AC | t | T |  |  | |  | | |  | |  |  |  |  | |  | |  | |
| B-1 | 5 | 5 | 130 | 13.66 | 27.0% | | | 29.1% | | | 96.3% | 64.2% | 0.12 | 21.2% | | 6.94 | | 91.1% | | -1.31 |
| B-2 | 5 | 15 | 130 | 17.44 | 33.8% | | | 35.1% | | | 96.9% | 64.0% | 0.28 | 24.8% | | 7.08 | | 85.2% | | -1.19 |
| B-3 | 5 | 5 | 170 | 31.65 | 65.5% | | | 35.1% | | | 93.0% | 48.9% | 1.14 | 34.1% | | 8.97 | | 86.9% | | -0.13 |
| B-4 | 5 | 15 | 170 | 27.08 | 56.0% | | | 17.9% | | | 86.3% | 39.5% | 1.29 | 32.8% | | 9.66 | | 87.3% | | 0.14 |
| B-5 | 20 | 5 | 130 | 20.59 | 42.1% | | | 27.6% | | | 91.3% | 34.2% | 0.31 | 27.2% | | 8.05 | | 83.7% | | 0.08 |
| B-6 | 20 | 15 | 130 | 24.76 | 50.9% | | | 26.8% | | | 89.3% | 29.1% | 0.44 | 30.6% | | 8.12 | | 86.5% | | 0.27 |
| B-7 | 20 | 5 | 170 | 16.58 | 33.4% | | | 10.1% | | | 56.3% | 21.9% | 2.51 | 36.6% | | 14.94 | | 90.1% | | 0.99 |
| B-8 | 20 | 15 | 170 | 7.25 | 15.0% | | | 3.8% | | | 38.8% | 14.3% | 3.09 | 25.4% | | 8.49 | | 78.4% | | 1.07 |
| B-9 | 12.5 | 10 | 150 | 27.75 | 55.7% | | | 29.3% | | | 90.2% | 36.4% | 0.89 | 34.1% | | 10.55 | | 88.0% | | 0.04 |
| B-10 | 12.5 | 10 | 150 | 28.18 | 56.0% | | | 22.5% | | | 89.3% | 28.7% | 0.99 | 35.0% | | 10.76 | | 88.8% | | 0.03 |
| B-11 | 12.5 | 10 | 150 | 29.38 | 59.0% | | | 28.6% | | | 90.7% | 36.4% | 0.87 | 35.6% | | 11.05 | | 88.1% | | 0.07 |
| a AC, Acid loading (mg/100g dm); t, Time (min); T, Temperature (°C). | | | | | | | | | | | | | | | | | | | | |
| b XOS percentage of total xylan equivalent recovered | | | | | | | | | | | | | | | | | | | | |
| c ArOS percentage of total arabinan equivalent recovered | | | | | | | | | | | | | | | | | | | | |
| d Combined acetic acid, formic acid, furfural and HMF | | | | | | | | | | | | | | | | | | | | |
| e Total dissolved solids hydrolysate | | | | | | | | | | | | | | | | | | | | |
| f Dissolved solids excluding quantified oligomers, sugars and inhibitors | | | | | | | | | | | | | | | | | | | | |

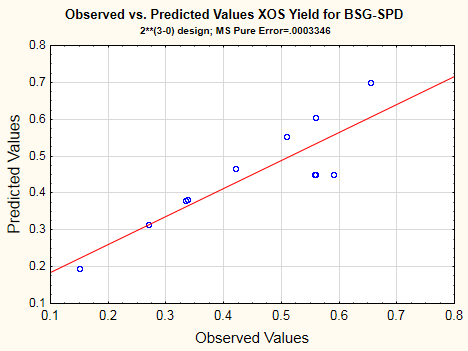
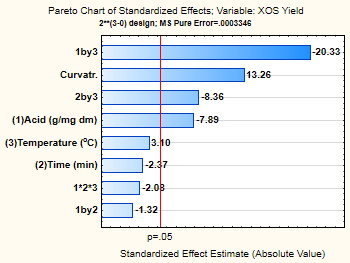


Fig. S1 Analysis of factorial results for BSG-SPD: standardised effects and regression model for XOS yield from BSG-SPD

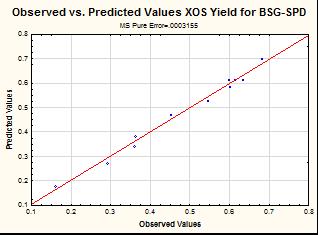
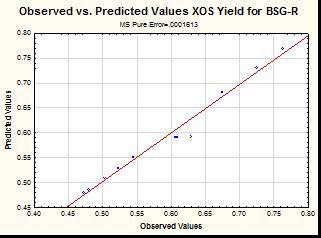


Fig. S2 Model response prediction for XOS yield vs. observed values (A) BSG-R and (B) BSG-SPD with quadratic term

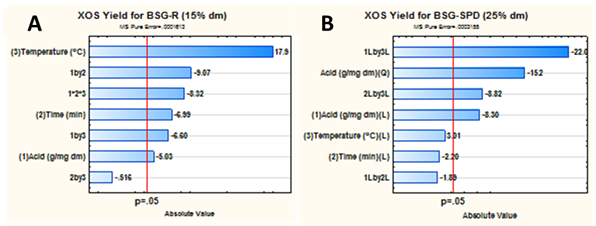


Fig. S4 Pareto chart of standardized effects, XOS yield (A) BSG-R (B) BSG-SPD

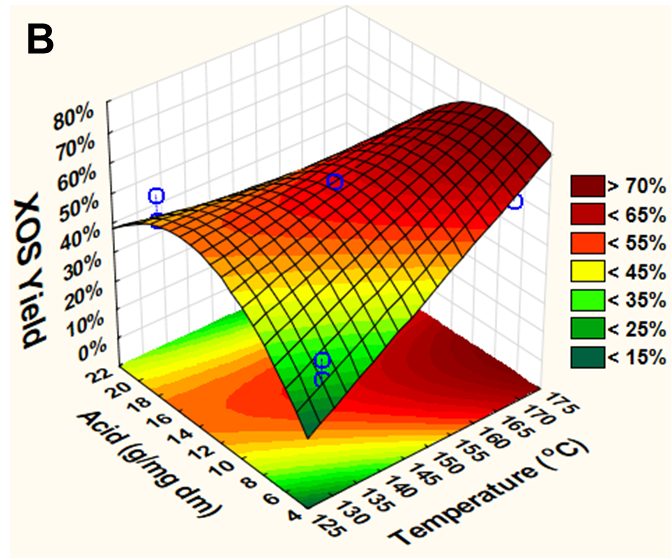
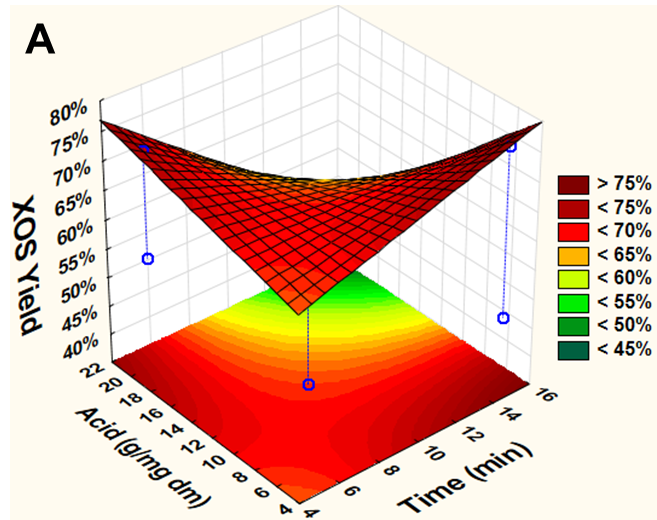


Fig. S5 Effect of acid loadings and treatment time in ELA HTT at 170 °C of BSG with XOS yields represented in surface plots for (A) BSG-R and (B) BSG-SPD with quadratic effect

Table S4 Regression coefficients and R2 assessing the correlation and significance of the fitted modelsa

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | BSG-R | |  | |  | |  | | BSG-SPD | |  | |
| Regression coefficients | XOS yield (wt.%) | | Inhibitors (g/100g) | | TDS yieldb (wt.%) | |  | | XOS yield (wt.%) | | Inhibitors (g/100g) | | TDS yieldb (wt.%) | | |
| a0 | 0.59267 | | -0.74538 | | 0.83956 | |  | | 0.56891 | | 0.91688 | | 0.34891 | | |
| a1 | -0.04524 | | -0.11249 | | -0.06177 | |  | | -0.10211 | | 0.88063 | | 0.01722 | | |
| a2 | -0.06283 | | -0.13141 | | -0.06098 | |  | | -0.03074 | | 0.25273 | | -0.01384 | | |
| a3 | 0.16135 | | 0.00537 | | -0.00277 | |  | | 0.04014 | | 1.71882 | | 0.06249 | | |
| a12 | -0.08149 | | -0.00016 | | 0.00500 | |  | | -0.01720 | | 0.10217 | | -0.02525 | | |
| a13 | -0.05932 | | 0.00090 | | 0.00040 | |  | | -0.26305 | | 0.70171 | | -0.04189 | | |
| a23 | -0.00464 | | 0.00106 | | 0.00038 | |  | | -0.10819 | | 0.11189 | | -0.04883 | | |
| a123 | -0.07478 | | 0.00000 | | -0.00003 | |  | | 0.00000 | | 0.00000 | | 0.00000 | | |
| a11 | 0.00000 | | 0.00000 | | 0.00000 | |  | | -0.32865 | | 0.46030 | | -0.11617 | | |
| R2 | 0.97784 | | 0.92095 | | 0.98125 | |  | | 0.99146 | | 0.99613 | | 0.95160 | | |
| a ANOVA for XOS concentration and yields models given | | | | | | | | | | | | | | |
| b Total dissolved solids yield in hydrolysate | | | | | | | | | | | | | | |
|  | | | | | | | | | | | | | | |

Table S5 Analysis of variance of the fitted models for XOS yield from BSG-R

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **SSa** | **d.f.b** | **MSc** | **F-value** | **p-value** | |
| **XOS yield (wt.%)** | |  |  |  |  | |
| AC | 0.0040928 | 1 | 0.004093 | 25.38037 | 0.037215 | |
| t | 0.0078949 | 1 | 0.007895 | 48.95843 | 0.019820 | |
| T | 0.0520682 | 1 | 0.052068 | 322.8886 | 0.003083 | |
| AC\*t | 0.0132805 | 1 | 0.013281 | 82.35597 | 0.011926 | |
| AC\*T | 0.0070387 | 1 | 0.007039 | 43.64898 | 0.022152 | |
| t\*T | 0.0000430 | 1 | 0.000043 | 0.266683 | 0.656993 | |
| AC\*t\*T | 0.0111830 | 1 | 0.011183 | 69.34848 | 0.014115 | |
| Lack of fit | 0.0018441 | 1 | 0.001844 | 11.43555 | 0.077427 | |
| Pure Error | 0.0003225 | 2 | 0.000161 |  |  | |
| Total SS | 0.0977677 | 10 |  |  |  | |
| a sum of squares; b degrees of freedom; c mean of squares. | | | | | |

Table S6 Analysis of variance of the fitted models for XOS yield from BSG-SPD

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **SSa** | **d.f.b** | **MSc** | **F-value** | **p-value** |
| **XOS yield (wt.%)** | |  |  |  |  |
| AC | 0.020853 | 1 | 0.020853 | 62.3138 | 0.015672 |
| t | 0.001890 | 1 | 0.001890 | 5.6487 | 0.140629 |
| T | 0.003223 | 1 | 0.003223 | 9.6307 | 0.090032 |
| AC\*t | 0.000592 | 1 | 0.000592 | 1.7676 | 0.315053 |
| AC\*T | 0.138388 | 1 | 0.138388 | 413.5346 | 0.002409 |
| t\*T | 0.023410 | 1 | 0.023410 | 69.9559 | 0.013995 |
| AC2 | 0.058916 | 1 | 0.058916 | 176.0547 | 0.005632 |
| Lack of fit | 0.001462 | 1 | 0.001462 | 4.3673 | 0.171812 |
| Pure Error | 0.000669 | 2 | 0.000335 |  |  |
| Total SS | 0.249403 | 10 |  |  |  |
| a sum of squares; b degrees of freedom; c mean of squares. | | | | | |

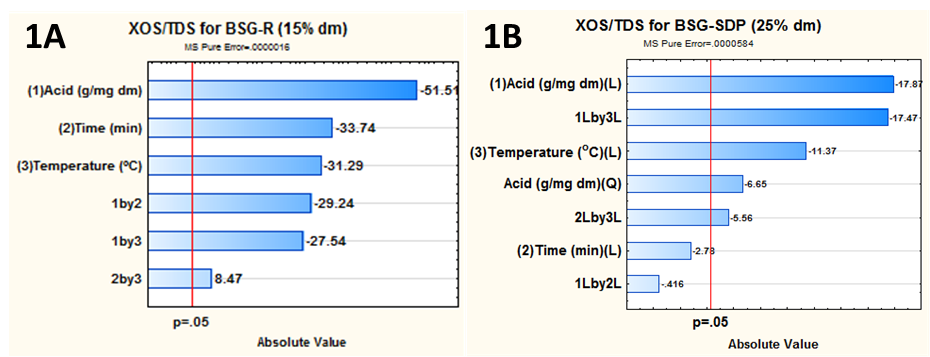
****

Fig. S6 Pareto standardised chart of effects for variable XOS/TDS (1A) BSG-R and (1B) BSG-SPD

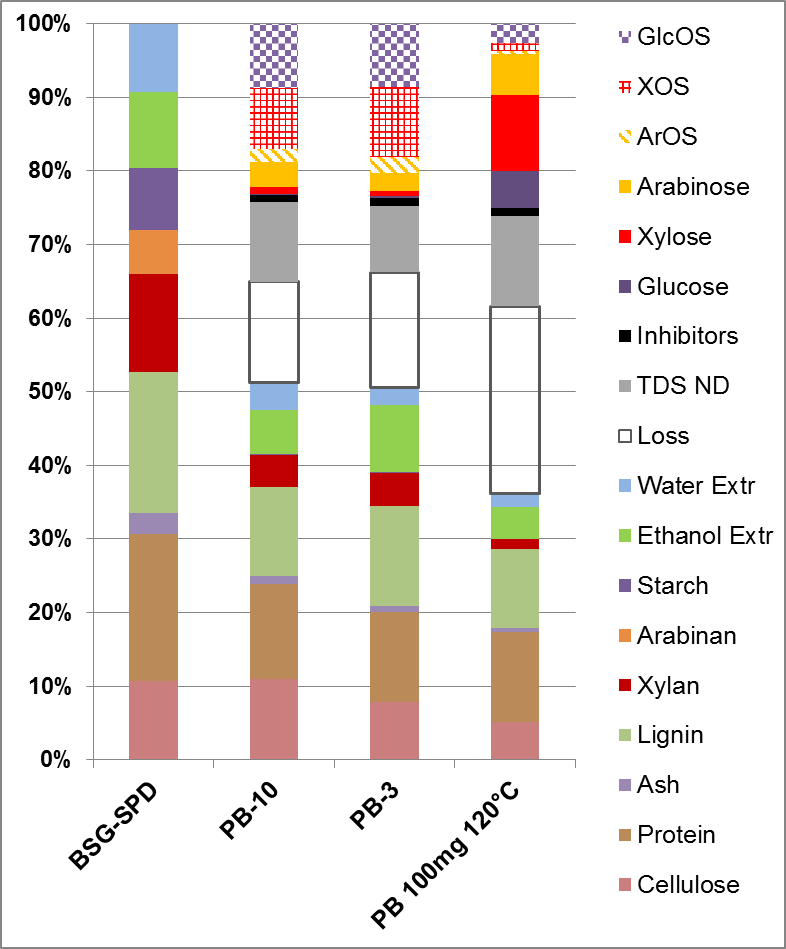
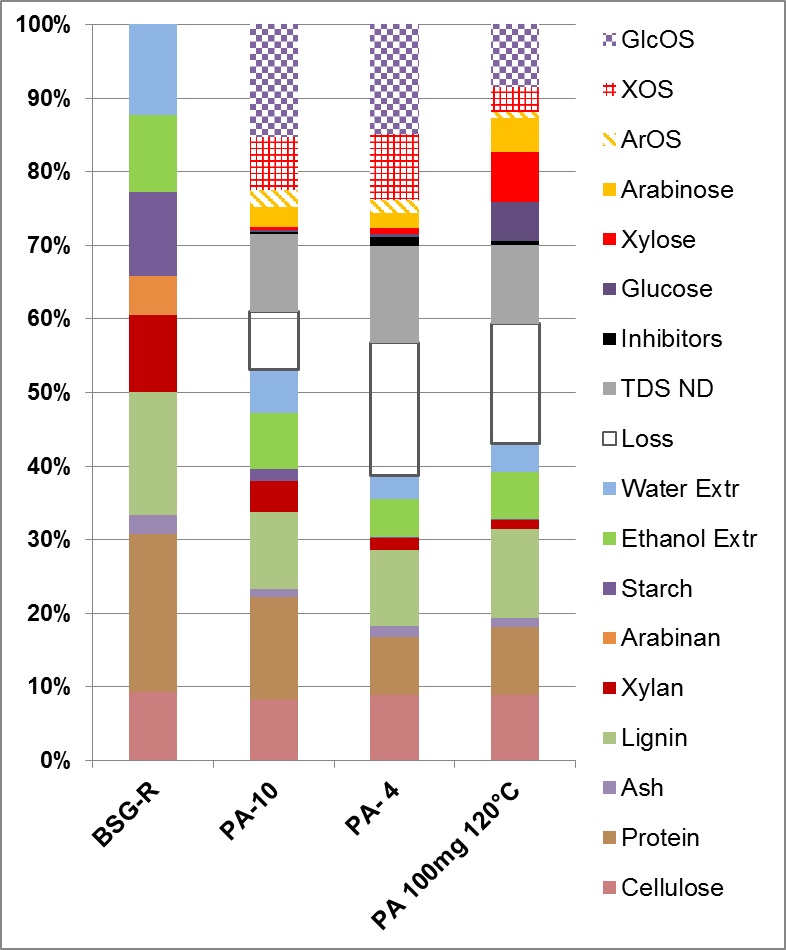


Fig. S7 Insoluble residue and hydrolysates obtained from hydrothermal treatments for (A) BSG-R and (B) BSG-SPD

Table S7 Protein analysis from BSG, press liquid and HTT solid residue

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino acid**  **(wt.%)** |  | **Feedstock** | | |  | **Solid Residue** | | |
|  | **BSG-R** |  | **Screw press liquid-solid filtrate** |  | **A-4**  **170°C**  **15min 5mg** |  | **B-3**  **170°C**  **5min 5mg** |
|  |  |  |  |
| **Basic amino acids** |  |  |  |  |  |  |  |  |
| Arginine |  | 6.5% |  | 5.6% |  | 3.1% |  | 3.3% |
| Histidine\* |  | 3.2% |  | 1.5% |  | 2.8% |  | 2.7% |
| Lysine\* |  | 2.3% |  | 3.5% |  | 0.9% |  | 1.0% |
| **Subtotal** |  | 12.0% |  | 10.5% |  | 6.9% |  | 7.0% |
| **Hydrophobic amino acids** |  |  |  |  |  |  |  |  |
| Alanine |  | 4.5% |  | 4.8% |  | 5.2% |  | 5.4% |
| Glycine |  | 4.9% |  | 4.4% |  | 4.8% |  | 5.0% |
| Isoleucine\* |  | 3.7% |  | 4.1% |  | 4.2% |  | 4.7% |
| Leucine\* |  | 7.8% |  | 7.8% |  | 9.1% |  | 9.4% |
| Methionine\* |  | 2.4% |  | 2.2% |  | 2.8% |  | 2.4% |
| Phenylalanine\* |  | 7.5% |  | 5.1% |  | 8.7% |  | 8.0% |
| Proline |  | 7.2% |  | 12.2% |  | 7.3% |  | 7.3% |
| Valine\* |  | 5.8% |  | 5.4% |  | 6.3% |  | 6.5% |
| **Subtotal** |  | 43.8% |  | 46.0% |  | 48.4% |  | 48.7% |
| **Hydrophilic amino acids** |  |  |  |  |  |  |  |  |
| Asparagine |  | 6.3% |  | 7.3% |  | 5.2% |  | 5.9% |
| Glutamine |  | 23.2% |  | 24.4% |  | 24.5% |  | 23.8% |
| Serine |  | 5.8% |  | 4.6% |  | 5.6% |  | 5.5% |
| Threonine\* |  | 4.6% |  | 3.8% |  | 4.6% |  | 4.7% |
| Tyrosine |  | 4.1% |  | 3.4% |  | 4.9% |  | 4.4% |
| **Subtotal** |  | 44.2% |  | 43.4% |  | 44.8% |  | 44.3% |
| **Essential amino acids\*** |  |  |  |  |  |  |  |  |
| **Subtotal included** |  | 37.3% |  | 33.4% |  | 39.5% |  | 39.3% |
| **Total amino acidsa** |  | 23.3% |  | 43.0% |  | 25.9% |  | 22.2% |
| **Crude Protein** |  | 23.4% |  | 39.5% |  | 26.5% |  | 23.3% |
| **a Tryptophan and Cysteine not determined** | | | | | | | | |