

Figure S1. Temperature change of brewer's spent grain (BSG) paste during ball milling with different milling-rest strategy (BSG paste prepared with DI water (20%), balls/wet biomass ratio was 3, other operating conditions are the same as "BSG pretreatment methods" section).

Table S1. Brewer's spent grain (BSG) composition before and after different pretreatments.

| | BSG | BM+EH ¹ * | BM-EH ² * | BM+EH | BM-EH |
|-------------------|--------------|----------------------|----------------------|--------------|--------------|
| BM setting | NA | 1 min-1 min | 1 min-1 min | 5 min-5 min | 5 min-5 min |
| Cellulose | 28.1% ± 0.5% | 16.6% ± 1.3% | 14.9% ± 0.4% | 16.2% ± 0.6% | 14.7% ± 1.1% |
| Glucose | - | 10.8% ± 0.4% | 11.7% ± 0.1% | 10.1% ± 0.3% | 11.2% ± 0.4% |
| Hemicellulose | 39.0% ± 0.5% | 24.2% ± 0.4% | 23.2% ± 0.6% | 23.3% ± 1.2% | 22.2% ± 0.9% |
| Xylose | - | 5.9% ± 0.1% | 5.9% ± 0.0% | 5.9% ± 0.3% | 5.9% ± 0.2% |
| ASL | 3.4% ± 0.0% | 3.2% ± 0.5% | 3.8% ± 1.1% | 2.9% ± 0.1% | 3.3% ± 0.1% |
| AIR | 29.5% ± 0.6% | 25.9% ± 0.7% | 22.1% ± 0.8% | 24.8% ± 0.5% | 22.0% ± 2.0% |
| Undetected | - | 13.2% ± 0.0% | 18.3% ± 0.0% | 16.6% ± 0.0% | 20.5% ± 0.0% |
| CNV cellulose | - | 36.9±6.0% | 41.5±1.9% | 35.9±3.8% | 40.8±5.4% |
| CNV hemicellulose | - | 17.5±1.9% | 18.2±2.3% | 18.1±6.5% | 18.9±4.3% |

¹ BM+EH: BSG sequentially went through ball milling (BM) (12 h) and enzymatic hydrolysis (EH) (21 h) pretreatments. ² BM-EH: BSG sequentially went through BM (6 h), synergistic BM-EH (6h) and EH (15 h) pretreatments.

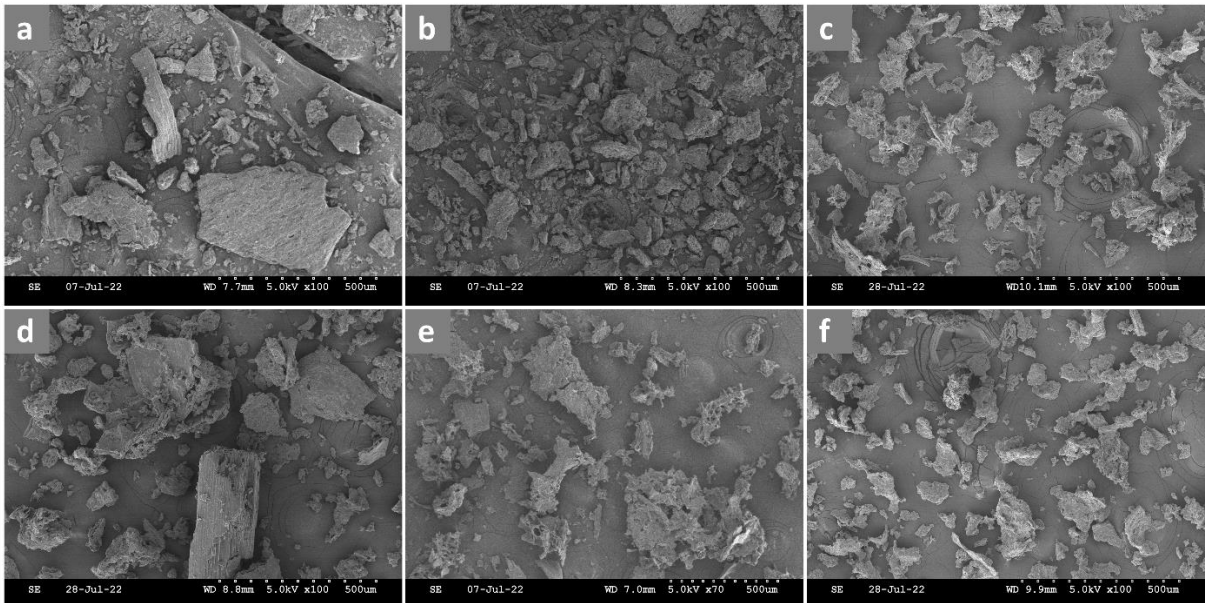


Figure S2. Scanning electron microscopic images of raw brewers' spent grain (BSG) (a) and BSG samples treated by ball milling for 1 cycle (BM-1) (b), ball milling for 2 cycles (BM-2) (c), enzymatic hydrolysis (EH) (d), synergistic BM-EH for 1 cycle (BM-EH-1) (e), and 2 cycles (BM-EH-2) (f), at lower magnification.

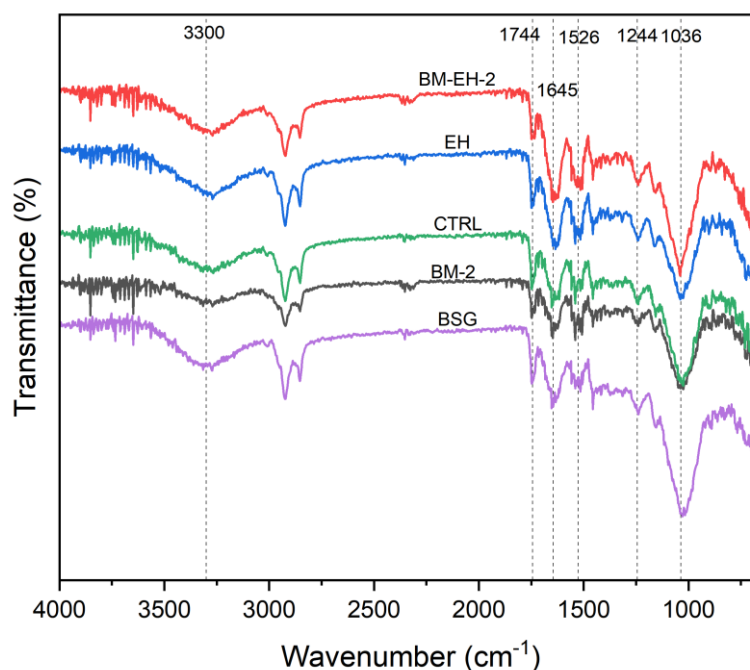


Figure S3. Fourier transform infrared spectroscopy (FTIR) spectra of raw and pretreated brewer's spent grain (BSG) samples (BM-EH-2 – BSG sample pretreated with synergistic ball milling and enzymatic hydrolysis for 2 cycles; EH – BSG sample pretreated by enzymatic hydrolysis; CTRL – controlled BSG sample subject with only autoclavation; BM-2 – BSG sample after ball milling for 2 cycles; BSG - raw BSG sample ground down to 0.5 mm).

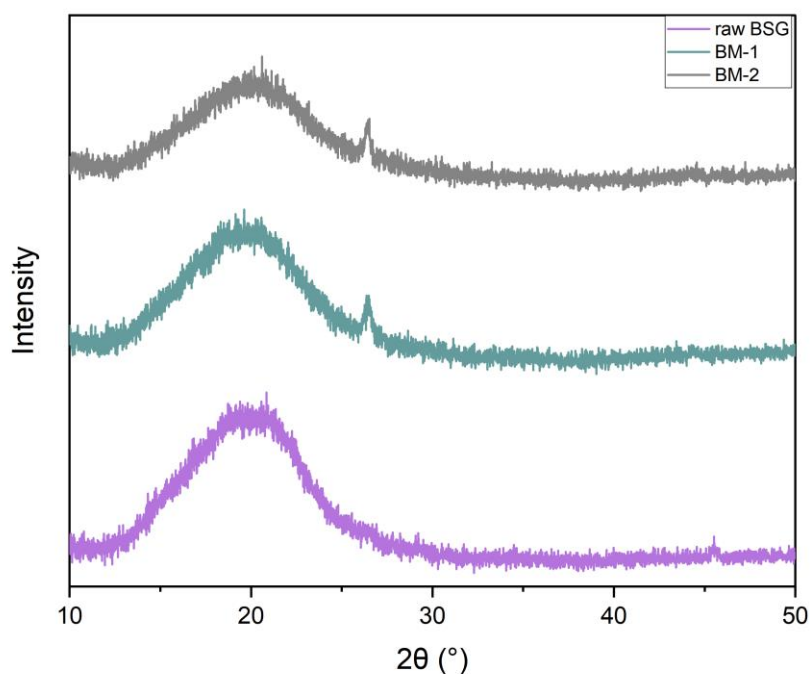


Figure S4. X-ray powder diffraction (XRD) spectra of raw and ball milled brewer's spent grain (BSG) samples (raw BSG - raw brewer's spent grain ground down to 0.5 mm; BM-1 – BSG sample after ball milling (BM) for 1 cycle; BM-2 – BSG sample after BM for 2 cycles).