Supporting Information for Research Article:

Brewers' spent grain liquor as a feedstock for lactate production with *Lactobacillus delbrueckii* subsp. *lactis*

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1. Material and Methods

1.1. Brewing conditions

Additional table 1: Brewing conditions for the brewing recipes of *Wheat bock*, *Wheat*, *Helles* and *May bock*; **kg:** Kilogram; **L:** Liter; **t:** Time; **T:** Temperature; °**Brix:** amount of sugar, dextrin and protein in weight percent (w%).

	Wheat bock	***Wheat***	***Helles***	***May bock***
Malt / kg	Wheat malt / 15 Pilsen malt / 8 Cara pils / 6	Wheat malt / 9.2 Pilsen malt / 9.2	Munich malt type II / 10.1 Vienna malt / 11.3	Munich malt type I / 10 Pilsen malt / 6.5 Vienna malt / 8.5 Cara malt dark / 0.2
Mashing water / L	72	70	67	67
Sparging water / L	25	50	50	45
Original gravity / °Brix	17.5	12.0	13.1	17
Protein rest / t,T	10 min, 55 °C	25 min, 45-55 °C	10 min, 57 °C	20 min, 54 °C
Maltose rest / t,T	30 min, 63 °C	40 min, 65 °C	35 min, 63 °C	55 min, 63 °C
Saccharification rest / t,T	25 min, 72 °C	30 min, 72 °C	20 min, 73 °C	20 min, 71 °C

1.2. Analysis of BSG liquor

Protein concentration was determined using Bradford assay, as originally described [1]. The pH value was measured using a pH meter (Microprocessor pH Meter 211, Hanna Instruments Deutschland GmbH, Vöhringen, Germany). Sugar and amino acid concentrations were determined with high-performance liquid chromatography (HPLC) (1.3). Samples for ion measurements were filtered through a 0.22 µm nylon filter (KX Syringe Filter Nylon, Cole-Parmer GmbH, Wertheim, Germany) previously to analysis. Sodium and potassium were measured by atomic emission spectroscopy (AES), according to DIN 38406. Atomic adsorption spectroscopy (AAS), according to DIN 348406 or DIN EN ISO 7980, was used for all other metal ions excluding aluminium. For this purpose, an atomic adsorption spectroscopy site (contrAA®, Analytik Jena, Jena, Germany) was used. A mixture of acetylene and air was applied as fuel gas, both for AAS as for AES. Aluminium analysis was carried out using a graphite furnace, according to DIN EN ISO 15586. Ammonium was determined photometrically (Spectroquant Pharo 100, Merck KGaA, Darmstadt, Germany), according to DIN 38406. Anions were analyzed by ion chromatography (883 Basic IC plus, Metrohm GmbH & Co. KG, Filderstadt, Germany). Phosphate was determined as described in DIN EN ISO 6878 using a spectrophotometer (DR 5000, Hach Lange GmbH, Düsseldorf, Germany).

1.3. HPLC analysis

Sugar was measured by HPLC. The modular HPLC system consisted of a two-channel degaser (Duratec GmbH, Hockenheim, Germany), a Merck Hitachi L-6200 pump (Merck KGaA, Darmstadt, Germany), a Midas cool autosampler (Spark Holland B.V., Emmen, Netherlands) and a Jetstream II plus column thermostat (Duratec GmbH, Hockenheim, Germany). The instrument control and data evaluation were carried out with a Clarity software system (Data Apex, Prague, Czech Republic). For separation purposes, a repro-gel Ca++ column, 300 mm x 8 mm (Dr. Maisch GmbH, Ammerbuch, Germany) with a precolumn Carbo Ca, 4 mm x 3.0 mm ID in the precolumn holder KJ0-4282 (both from Phenomenex Inc., Torrance, USA) at 80 °C and a flow of 0.5 $mL \cdot min^{-1}$ with ultrapure water as eluent, was used. Detection was performed with a RI 101 refractive index detector (Shodex, Kawasaki, Japan).

Lactate and amino acids were also measured by using HPLC with an Alliance 2695 with integrated degaser, column thermostats and Empower 3 software (all from Waters Corporation, Milford, USA). The separation of lactate was carried out with a repro-gel H+ column, 300 mm x 4.6 mm with integrated precolumn (Dr. Maisch GmbH, Ammerbuch, Germany) at 55 °C. The eluent was 9 mM H_2SO_4 with an isocratic flow profile of 0.3 $mL \cdot min^{-1}$. Detection was performed with a 2996 photodiode array detector (Waters Corporation, Milford, USA). The quantification was performed at a wavelength of 210 nm. Amino acids were separated with a resolve C18 column, 150 mm x 3.9 mm (Waters Corporation, Milford, United States) at 30 °C and a flow rate of 1 $mL \cdot min^{-1}$. The detection occurred with an alliance 2475 multi λ fluorescence detector (Waters Corporation, Milford, United States). Previously to injection, all samples were filtered through a 0.22 µm nylon filter (KX Syringe Filter Nylon, Cole-Parmer GmbH, Wertheim, Germany) and diluted to a concentration inside the external calibration range.

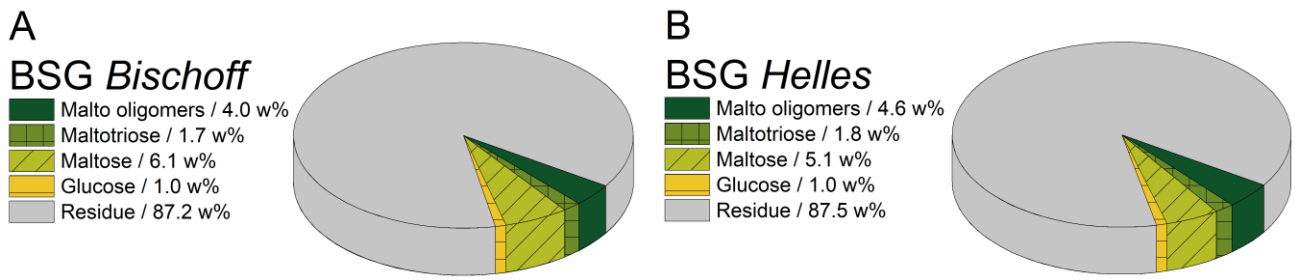
1.4. Chemicals and enzyme specification

The following chemicals were obtained from Carl Roth GmbH & Co. KG [CAS No.]: D(+)-Glucose >99.5 % [50-99-7], Meat extract [68990-09-0], Yeast extract [8013-01-2], Tween 80 [9005-65-6], $(NH_4)_3$ citrate >97 % [3458-72-8], L-Cysteine-hydrochloride monohydrate >98.5 % [7048-04-6], NaOH >98 % [1310-73-2], Glycerol >99,5 % [56-81-5]. Sigma-Aldrich supplied: Peptone from casein, tryptic digest [91079-40-2], K_2HPO_4 >98 % anhydrous [231-834-5], Sodium acetate >99 % [127-09-3], $MnSO_4 \cdot H_2O$ >99 % [10034-96-5], $MgSO_4 \cdot 7\ H_2O$>98 % [10034-99-8] and Resazurin sodium salt [62758-13-8]. D(+)-Maltose monohydrate >97 % [6363-53-7] and Maltotriose hydrate >95 % [1109-28-0] were purchased from Santa Cruz Biotechnology. Coomassie Bradford reagent [Art. No. 23236] was bought from ThermoFisher Scientific.

The applied 1,4-α-glucoamylase Attenuzyme® Core was produced by Novozymes A/S with the strain *Aspergillus niger* and had an activity of 1600 $AGU \cdot g^{-1}$ [2].

2. Results

2.1. Comparison of industrial and self-made BSG



Additional figure 1: Sugar content of BSG based on dry weight. **A)** Commercial BSG *Bischoff* **B)** Self-made BSG *Helles*; **BSG**, Brewers' spent grain, **w%**, weight percentage.

Nomenclature

AAS	[-]	Atomic adsorption spectroscopy
AES	[-]	Atomic emission spectroscopy
AGU	[-]	Amyloglucosidase unit
BSG	[-]	Brewers' spent grain
HPLC	[-]	High-performance liquid chromatography

3. References

[1] Bradford, M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. 1976, *254*, 248–254.

[2] Attenuzyme ® Core Product Data Sheet, Novozymes A/S, 2014.