

Supplementary Materials

Use of ohmic heating to increase sugar yield from biowastes and potential for biohydrogen production from ohmically-generated hydrolysates.

This early example study evaluated the application of OH to increase fermentative bio-H₂ production from bio-waste. The sugar content was increased significantly but bio-H₂ production was inhibited; the toxic agent was not identified (Fig. S1A, B and C)

Fig. S1A: Increase in sugar content of the hydrolysate (%) by the application of ohmic heating. Wastes were as shown. Fig. S1B: Hydrogen production potential of the hydrolysate without and with ohmic heat treatment. Fig. S1C: HPLC chromatogram of OH-treated fruit waste shows higher content of organic acids (substrates for downstream photobiological hydrogen production). In addition, a new peak is visible (open arrow, unidentified) following ohmic heat treatment of the waste; this was a relatively minor component in comparison to the main peaks.

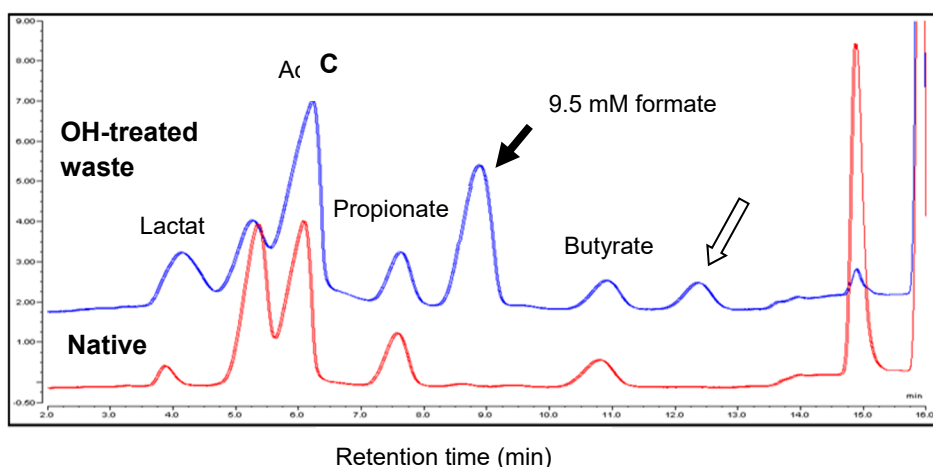
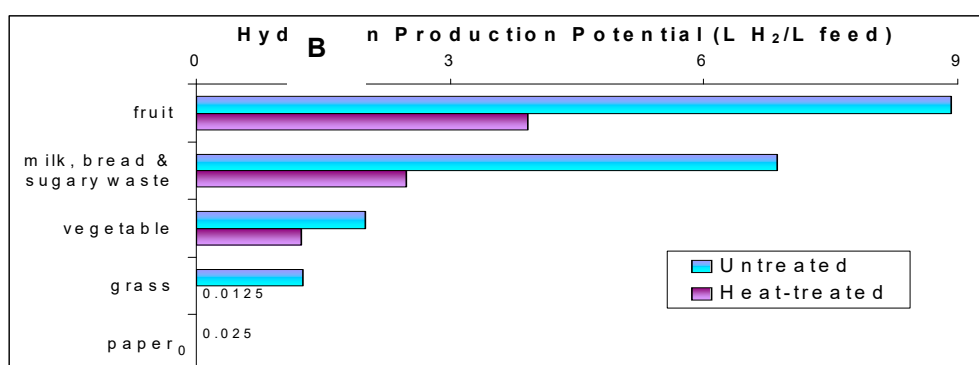
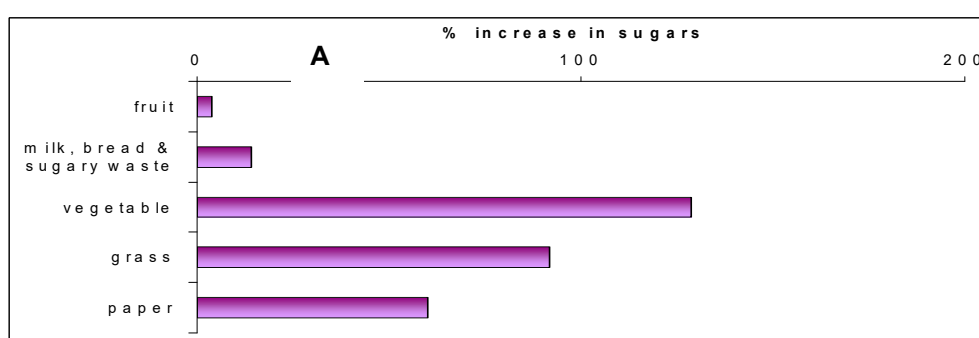


Figure S1. Increase in sugar content of hydrolysate (%) by the application of ohmic heating (A), Hydrogen production potential of the hydrolysate (B), HPLC chromatogram of OH treated fruit wastes show higher content of organic acids (substrates for downstream photobiological hydrogen production) (C). In addition, a new peak (unidentified) is apparent following ohmic treatment of the waste (open arrow) [67].

Biowastes: Spent grain, wheat straw and towards a biorefinery

Given a method to potentially mitigate against the parasitic energy demand upstream of the fermentation(s) (main text), attention must now turn to the high heat requirement of the downstream 5-HMF upconversion. Classically, chemical catalysts operate at high reaction rates for short residence times, with high value products justifying the energy investment. Notably, waste upvalorisation is not time-critical; in the current study dark fermentation is rate limiting, with a feed (using detoxified hydrolysate) residence time in excess of 24-48 hours for maximum hydrogen production. Thus, it would seem feasible to reduce the temperature of the 5-HMF catalytic upconversion, while accepting the slower reaction rate (the high temperature (260 °C) of the catalytic reaction in this work was adopted following early optimisation studies: I. Mikheenko, unpublished). Other work [59] showed little benefit from increasing the reaction temperature above 220 °C, however decreasing the temperature to 160 °C reduced the conversion by ~20% but ~ halved the yield for the same reaction time. Extending the reaction time restored the conversion but the DMF yield was reduced, indicating the formation of other co-products which were not examined in the earlier [47, 59] or in this current study.

Further to the work we report here, the hydrolysis of real wastes was examined (food wastes, spent brewery grains and wheat straw) together with an example dark fermentation of the food waste hydrolysate by *E. coli* following activated carbon treatment, prompted by the preliminary study shown in Fig. S2. The co-products were identified as sugars, 5-HMF and also furfural, in varying proportions depending on the waste source and hydrolysis temperature.

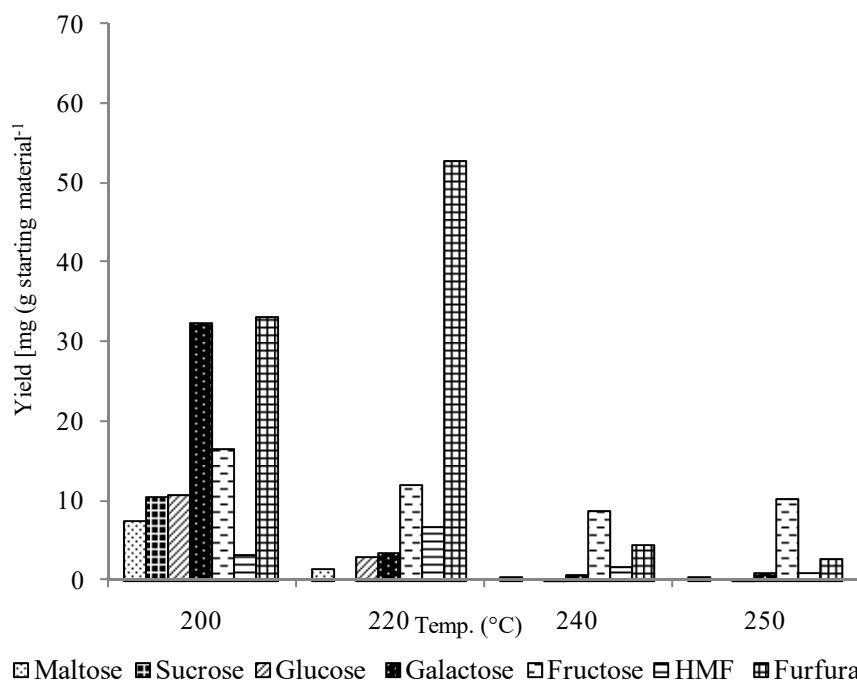


Figure S2. Yields of products in brewery grain hydrolysates before AC at different reaction temperatures.

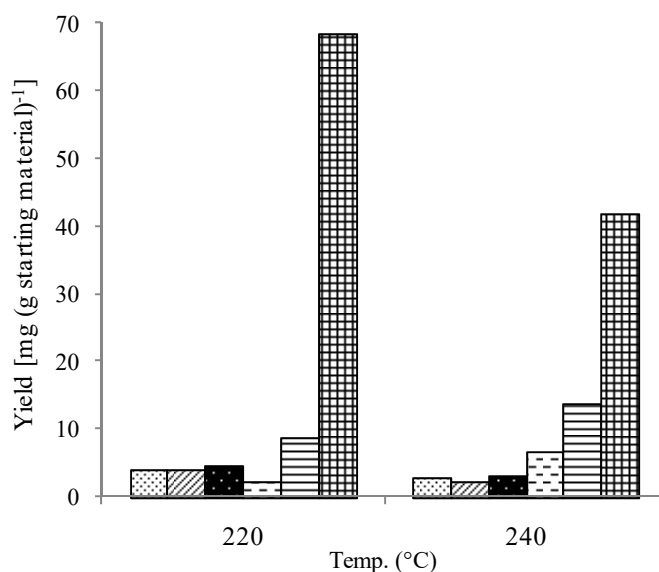


Figure S3. Yields of products of wheat straw hydrolysate before AC at 220 and 240 °C. Symbols are the same as in Fig. S2.

Note that furfural is a major component of the hydrolysis products from these real wastes. Furfural is a platform chemical which has received increased attention due to its production in hydrolysis of hemicelluloses (reviewed by Delbecq et al. [69] and Shen et al. [70]). The market for furfural *per se* is not large but, key, to the work we report here, MTHF (the extraction solvent used in this work) can be produced from it readily and potentially economically via hydrogenation [71].

Bio-Pd made on post-fermentation *E. coli* was an effective catalyst in the hydrogenation of 2-pentyne and soybean oil [32] (including catalyst made by *E. coli* from precious metal metallic scrap [72]) and the future scope is clear for ‘in process’ replenishment of MTHF (neo-solvent) within the upconversion process (following removal of 2,5-DMF) by a sequential use of bio-Pd/Ru (for upconversion of 5-HMF to 2,5 DMF) and bio-Pd (for hydrogenation of furfural to MTHF). Both catalysts are produced by *E. coli*; bio-Pd/Ru is made by simply using bio-Pd as a catalyst for sequential galvanic reductive deposition of a second metal that can be from Ru(III) (this study) or, indeed, from Au(III) to make catalytically active bio Pd/Au core-shell nanostructures [73]. In addition, it is noteworthy that supported Pd/Au bimetallic (chemical) catalyst has been reported for the selective hydrogenation of biomass-derived 5-HMF to DMF, with suggested charge transfer from Pd to Au atoms [74] and future studies would aim to compare the bio-Pd/Ru and bio-Pd/Au bio-bimetallics in this reaction. The two reactions can be easily separated spatio-temporally via the use of self-immobilised bio-metallic catalyst as described elsewhere in the case of bio-Pd-catalysis [75, 76].

Clearly more work is needed to optimise the proportions in the bionanocatalyst of the various metals (and nano structures, e.g., core-shells or alloys) according to the preferred reaction outcome, i.e the best combination of yield versus energy input, informed by an energy mass balance from an integrated process.

A full cost-benefit analysis also requires factoring-in of additional features, which are becoming appreciated within a ‘biorefinery concept’ but are beyond this scoping study. The use of metallic wastes as a source for biomanufacturing of metal catalysts by *E. coli* was shown to be economically attractive in upgrading of hydrocarbons and such ‘bio-Pd’ was shown as an effective hydrogenation catalyst (see above). In addition, bio-metallic catalyst made from waste can also be used as an effective electrocatalyst in a downstream fuel cell for direct conversion of biohydrogen into power (see Introduction). This integrated approach

needs to be developed within the context of a Life Cycle Analysis of an integrated biorefinery and coupled fuel cell system. Such a LCA would also factor-in the environmental benefit by widening the system boundaries to include the value of avoided carbon equivalents (\$50/t). It is also necessary to factor-in other considerations such as re-use of the extraction solvent, volume to be used and scope for in-process recycle (see above).

While waste biomass of *Rhodobacter sphaeroides* is considered to be potentially valuable with respect to the useful materials that can be extracted (e.g. poly β -hydroxybutyrate and pigments [77]), *E. coli* biomass has little recycle potential with respect to valuable chemicals and, if not recycled 'in process' would require disposal as biowaste. Upconversion into supported nanocatalyst for use in side stream valorisation is a major advance towards circularity, but the tonnage of waste *E. coli* generated versus that actually consumed to make catalyst requires balancing, noting that the dark fermentation uses resting (non-growing) cells. Manufacturing and proof of active catalysts by combining waste metals and post-fermentation *E. coli* was economically validated with respect to generating a high value upgraded oil product from heavy fossil oils and the same approach was applied to biomass-pyrolysis oil upgrading [78]. While the current study shows little potential for stand-alone fermentation processes to realise surplus energy from biomass wastes it points clearly to the potential of an integrated biorefinery, based on initial benchmark data.

Integrating process biotechnology with applied catalysis is a relatively recent development. Lee et al. in 2019 [79] stated that biomass to energy conversion will not be cost-effective unless combined with catalytic upgrading of biomass products, specifically citing (amongst others) the use of Pd/C catalysts for pyrolysis oil upgrading. The current study proves this integration concept by avoiding the need to source 'from new' a catalyst for upgrading of product obtained from a mild reaction, using active catalysts fabricated from wastes in a cost and energy efficient way, for one pot production of a biofuel along with directly-usable clean hydrogen. More recently, it is appreciated that for full process viability the biorefinery concept must embrace catalytic upgrading of biomass-derived platform chemicals more generally [80] in addition to 5-HMF, the worked example that we platform here.