

An integrated biohydrogen refinery: Synergy of photofermentation, extractive fermentation and hydrothermal hydrolysis of food wastes.

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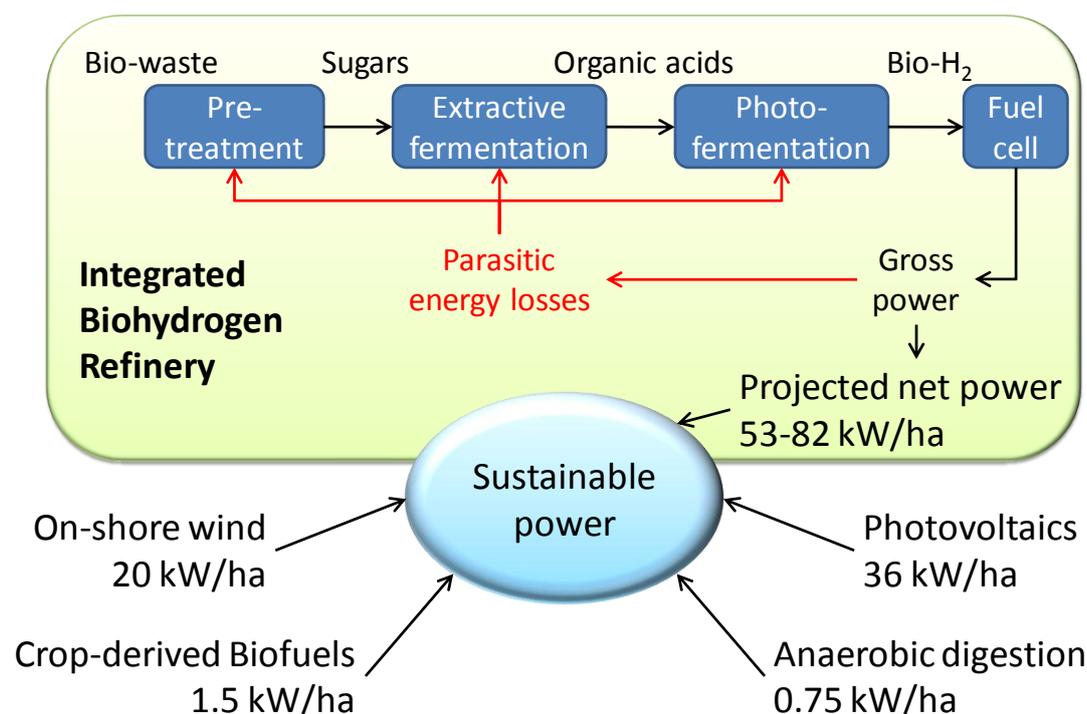
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Abstract

An *Integrated Biohydrogen Refinery* (IBHR) and experimental net energy analysis are reported. The IBHR converts biomass to electricity using hydrothermal hydrolysis, extractive biohydrogen fermentation and photobiological hydrogen fermentation for electricity generation in a fuel cell. An extractive fermentation, developed previously, is applied to waste-derived substrates following hydrothermal pre-treatment, achieving 83-99% biowaste destruction. The selective separation of organic acids from waste-fed fermentations provided suitable substrate for photofermentative hydrogen production, which enhanced the gross energy generation up to 11-fold. Therefore, electro dialysis provides the key link in an IBHR for 'waste to energy'. The IBHR compares favourably to 'renewables' (photovoltaics, on-shore wind, crop-derived biofuels) and also emerging biotechnological options (microbial electrolysis) and anaerobic digestion.

GRAPHICAL ABSTRACT



KEYWORDS: integrated biohydrogen refinery, waste to energy, electro-fermentation, photofermentation, hydrothermal hydrolysis

Abbreviations

IBHR	integrated biohydrogen refinery
EF	electro-fermentation
PF	photo-fermentation
DF	dark fermentation
OA	organic acids
PBR	photobioreactor
ASM	anion selective membrane
CSM	cation selective membrane
BPM	bipolar membrane
Ma	wholesaler's mango waste
Ap	wholesaler's asian pear waste
Av	wholesaler's avocado waste
CW1	catering waste 1
CW2	catering waste 2
CW3	catering waste 3
BG	brewers spent grain waste
HCW	hot compressed water
CE _{OAn}	current efficiency based on organic anion
CE _{Tan}	current efficiency based on total anion
HPP	hydrogen production potential
5-HMF	5-hydroxymethylfurfural
NER	net energy ratio
GBP	British pounds

4 1. INTRODUCTION

Biohydrogen provides opportunities for sustainable energy from biowastes using fermentative and photosynthetic microorganisms. We focus on the synergy of dark fermentation (DF) and photofermentation (PF), with a theoretical yield of 12 mol H₂/mol hexose equivalent. The concept has been advocated by many authors (Redwood et al., 2009 and references therein) and research continues to progress rapidly with at least 10 publications in 2011 (e.g. Abo-Hashesh et al., 2011; Afsar et al., 2011; 2011; Avcioglu et al., 2011; Chu et al., 2011; Lo et al., 2011; Özgür et al., 2011).

Organic acids (OAs) from DF can be valorised by re-use as substrates to produce methane (Martínez-Pérez et al., 2007), electricity (Kyazze et al., 2010) or H₂ via PF. Guwy et al. (2011) highlighted the challenge of recovering OA from DF for use in downstream processes including PF, which is inhibited by excess nitrogen sources (especially NH₄⁺) via the inhibition of nitrogenase (Redwood et al., 2009). Usually, OA are co-transferred with other solutes from DF to PF, hence the input feedstock must be low in N-sources to permit nitrogenase-mediated H₂ production. Biowaste feedstocks usually contain bioavailable N allowing microbial NH₄⁺ release. In several studies, DF extracts rich in OA contain excess N (Chen et al., 2008; Özgür et al., 2010; Redwood and Macaskie, 2006).

Extractive 'electro-fermentation' (EF) (Redwood et al., 2012) involves separating a fermenting culture from a permeate chamber with an anion selective membrane (ASM) to transfer anions specifically, rapidly and unidirectionally under DC. The ASM is impermeable to cations including NH₄⁺; hence electrodialysis renders the process robust and versatile, immune to the feedstock nitrogen/NH₄⁺ content.

Biomass is an abundant renewable source of fermentable sugars to support the future hydrogen economy (Claassen et al., 1999; Tseng et al., 2005). However, the application of

electrodialysis within a waste-fed bioprocess requires validation in three respects; (i) the energetic input for OA separation could exceed the potential energy output from bioH₂ production; (ii) inorganic anion present in real wastes could detract excessively from efficient target anion (OA) separation or upset the balance of retained anion with pH (Redwood et al., 2012); and (iii) liquefaction of feedstock could be restrictively complex or energetically costly. These factors would vary according to the waste stream. Therefore, a range of example wastes were processed to generate clarified solutions of soluble fermentable sugars.

Normally, food and agricultural wastes contain complex polysaccharides requiring hydrolysis for their utilisation as fermentation substrates. Hydrolysis can be achieved by chemical, enzymatic and hydrothermal methods. Enzymatic hydrolysis requires optimisation to obtain the best combination of enzymes for each feedstock and cannot quickly adapt to variable feedstock composition, while chemical hydrolysis consumes chemicals and produces chemically aggressive effluents. Hydrothermal hydrolysis is, conversely, an environmentally benign method requiring only water, relatively moderate temperatures (200-260 °C) and pressure which also sterilises the feedstock, eliminating pathogens and competitor organisms.

An experimentally based model of a complete *integrated biohydrogen refinery* (IBHR) is described. Two hypotheses were tested; firstly that EF can function efficiently using real wastes and, secondly, that the IBHR can function as a net energy producing system, accounting for parasitic energy requirements (core scalable requirements for heat, power and mixing) and can, therefore, provide sustainable energy from biowaste.

5 2. MATERIALS AND METHODS

2.1 Extractive fermentation

Fermentations were connected to an electrodialysis cell to create an extractive 'electro-fermentation' as described previously (Redwood et al., 2012). Anions were actively transported out of the fermenter across an anion selective membrane (ASM) into the MA chamber (connected to a permeate vessel), in response to an externally applied current, regulated automatically in response to the fermentation pH.

In electro-fermentations (3 L), glucose (initially 28 mM) was completely consumed during an initial aerobic growth phase, before rendering anaerobic by nitrogen purge (30 min). At this point, waste-derived sugars (non-sterile) were added in pulses of 0.1 mol reducing sugars at intervals.

Current efficiency (CE), representing the fraction of passed charge attributed to target anion transfer, was calculated as described previously (Redwood et al., 2012).

2.2 Hot compressed water treatment

The HCW/CO₂ reactor contained 5 g (dry basis) homogenised waste in de-ionized water to a volume of 160 mL (±5 mL). Reactor operations (peak conditions: 200 °C, 50 bar, 15 min) and detoxification of hydrolysates (activated carbon, 5% w/v) were described previously (Orozco et al., 2012).

2.3 Fermentability tests

Escherichia coli strains HD701 and FTD67 (Redwood et al., 2008) were used in fermentability tests as described previously (Orozco et al., 2012) except using 10 mL of 'ED' medium (Redwood et al., 2012) (pH 6.5, sterile) and 5 mL of test solutions (non-sterile), diluted to ensure substrate limitation when >60 mM hexose equivalent was present.

2.4 Photofermentability tests

Rhodobacter sphaeroides ZX5 was selected for its substrate range (Tao et al., 2008), maintained and grown as described previously (Redwood and Macaskie, 2006). Carbon sources for PF were provided by permeates taken from the end of EFs without further purification. Cultures were grown photoheterotrophically using yeast extract (YE, 1 g/L) as the sole nitrogen source, harvested (4000 g, 15 min), washed and transferred into media without YE for H₂ production. To ensure cells were adapted to each OA mixture, the same batch of permeate provided the OA for growth and for H₂ production.

'Growth buffer' was as described previously (Redwood and Macaskie, 2006) omitting carbon sources and adding permeate samples (adjusted to pH 6.8 with 3 M NaOH) to provide 60 mM total OA carbon. For growth, preparations were incubated in fully-filled flat glass flasks (30 °C, static, 75 W/m²; tungsten-halogen lamp, 48 h). Light intensity was measured with a 400-1000 nm thermopile-type sensor (Skye, UK). After growth, cells were harvested and washed twice in 'HP buffer'; i.e. growth buffer omitting YE and including permeate samples (pH 6.8) diluted to 30-60 mM hydrogen production potential (HPP; Eroglu et al., 2004).

For H₂ production tests, washed cells were resuspended to 1 g dry wt./L (OD_{660 nm}: 3.3) and dispensed in 5 mL aliquots into 15 ml glass reactors. Controls used cells washed in HP buffer omitting carbon sources. Reactors were purged with Ar (30 min) before incubation (30 °C, static, 75 W/m² tungsten lamp, 48 h). H₂ was estimated as described previously (Orozco et al., 2012) using a value of 1 since PNS bacteria produce ~100% H₂ (v/v), due to the internal recycling of CO₂ (Ormerod and Gest, 1962).

2.5 Analysis

Inorganic anions and OAs were analysed by 'anion-HPLC' (Redwood and Macaskie, 2006) while sugars, 5-hydroxymethylfurfural (5-HMF) and ethanol were analysed by 'Refractive Index (RI) HPLC' and total reducing sugars were analysed using the dinitrosalicylic acid method (Orozco et al., 2010).

Total sugars and starch in solid wastes were measured after drying (60 °C) and grinding to pass through a 420 µm mesh. Starch was estimated by digestion in KOH (2 M, 4 °C, 2 h), then colorimetric analysis (A₅₇₀) of dextrans by the reaction with I₂/KI (0.0044%/2% w/v, respectively) with reference to an analytical grade starch standard (Birch and Priestley, 1973). Total sugars were estimated by digestion (Saeman et al., 1945) then reducing sugars analysis.

6 3. RESULTS AND DISCUSSION

3.1 Food wastes: Description and processing details

Commercial wastes were sourced from a fruit wholesaler, catering kitchen and brewery. The wastes were diverse (Table 1), containing 57-90 % water and 7-28% sugars by mass. Only catering waste 3 contained significant starch (21 % wet weight). Solids were processed to generate fermentable solutions (Figure 1).

3.2 Fermentability tests

Waste treatments yielded 13 clarified liquid preparations (3 juices, 3 infusions and 7 hydrolysates; Figure 1); all 13 were screened for fermentability (Table 1). Analysis of hydrolysates showed that treatment with activated carbon removed inhibitory 5-HMF (Orozco et al., 2012). Low H₂ yields (<1 L/kg raw waste) were obtained from Av waste and CW2, whereas Ap waste supported a high yield (5.7 L H₂/kg). CW3 gave the highest yield despite yielding no juice or infusion attributed to its low moisture content and high total sugars, primarily as starch (Table 1), which is highly

susceptible to HCW hydrolysis (Miyazawa and Funazukuri, 2005) to generate fermentable substrate (Orozco et al., 2012).

Additional fermentability tests used *E. coli* strain HD701/pUR400 (sucrose-capable; Penfold and Macaskie, 2004) but no additional H₂ was produced, in accordance with the absence of sucrose as shown by RI-HPLC. H₂ production in fermentability tests was limited by substrate availability (pH remained >5.5) i.e. higher concentrations of substrates (glucose/sucrose) enabled further H₂ production.

3.3 Waste-fed extractive fermentations: Three case histories

After confirming fermentability (Table 1) the juices of mango waste (Ma), asian pear waste (Ap) and catering waste 1 (CW1) were selected (Figure 1) for sustained H₂ production and product separation in 3 L EFs.

The only significant substrates for H₂ production were waste-derived, since all initial glucose was consumed during the aerobic growth of *E. coli* before initiating anaerobic fermentation. Without additional feeding no H₂ production occurred. Residual formate from the growth medium (initially 23 mM) was depleted within 36 h and before the onset of detectable H₂ production and, therefore, this exogenous formate did not contribute to measured H₂ yields. Only permeates from the end of the EFs were tested in PF (below). Figure 2 shows the progress of three sustained dark EFs dosed 3 or 4 times each with waste-derived juices.

Figure 2A shows EF using Ma juice. The current efficiency (equation 1) on organic anion (CE_{OAn}) was 86% and based on total anion (CE_{TAn}) was 92%. The remainder is attributed to a combination of measurement error (e.g. fluid volumes) and current leakage, chemical reactions (e.g. water electrolysis) and the movement of non-measured anion. Anion analysis of extracts is shown in Table 2A. The OA concentration in the reactor (M chamber, not shown) was stable at ~80 mM total charge equivalents during H₂ production.

Conversely, using Ap juice (Figure 2B), the OA concentration (M chamber) fell progressively from ~100 mM to ~50 mM total charge equivalents during H₂ production. The H₂ production rate was initially slow, increasing after 96 h. The delay may be due to acclimatisation to unidentified inhibitors from Ap juice; nitrates (which could inhibit H₂ production) were not detected by anion-HPLC. CE_{OAn} was 86% and CE_{TAn} was 91%.

Using CW1 juice (Figure 2C), H₂ production commenced 20 h after feed 1 and progressed rapidly to an apparent yield of 2.5-3.0 mol H₂/mol hexose, which exceeds the maximum H₂ yield (2 mol/mol hexose; Redwood et al., 2008) predicted from the concentration of sugars in waste juice, measured by RI-HPLC. This is attributed to linear maltodextrins which are fermentable by *E. coli* (Boos and Shuman, 1998) and were detected by RI-HPLC, whereas galactose, sucrose, maltose, mannitol, mannose, xylose and citrate were absent. CE_{OAn} was 85% and CE_{TAn} was 95%.

It was concluded from three case histories that waste-fed EF is an efficient method for generating bioH₂ and purified OA (Table 2) from liquefied biowastes. Hence, the challenges posed to EF by solids and inorganic salts in wastes were addressed. Limited membrane biofouling was observed but this affected neither CE nor process efficiency (Redwood et al., 2012).

3.4 Photofermentability of permeates from waste-fed extractive fermentations

OA derived entirely from the EF of food wastes supported H₂ production in small-vial photofermentability tests. However, these simple reactors provided sub-optimal conditions and low substrate conversion efficiency (16-49%; Table 2B), whereas typical values in the range of 70-90% were obtained previously (Redwood, 2007) and are widely reported elsewhere (Akkerman et al.,

2002; Rocha et al., 2001; Sasikala et al., 1995). Therefore, the IBHR was modelled (Table 3) using an extrapolated PF efficiency of 80% (representing optimised full-scale PF). PF would increase the H₂ yield but also the 'parasitic energy'.

3.5 Net energy analysis of the IBHR

Our experimental data informs a model of an *Integrated Biohydrogen Refinery* (IBHR). Although only juices were available in sufficient volumes for EFs, fermentability was confirmed for all infusions and hydrolysates and we extrapolate on the basis of experimental OA yields from juice fractions (via EF) and experimentally measured energy requirements of OA separation. OAs in HCW hydrolysates were disregarded, being found at only ~0.01% of sugar levels.

In the UK the gross power generation of biogas plants is currently rewarded via the feed-in tariff (fIT) at a rate of GBP 0.14/kWh (Anon, 2011b) indicating fIT revenues of GBP 32/tonne plus revenues from the export of net power (variable rate: ~GBP 0.06/kWh) of ~GBP 13/tonne and 'gate fees' (paid to the waste processor) of GBP 50-90/tonne, and in contrast to landfill costs of GBP 76/tonne (inc tax) (Anon, 2010). The UK produces 16M tonnes of waste food per year, plus a further 90M tonnes of farm manures and slurries (Anon, 2011a). Assuming the food wastes are as productive as those assessed here (average gross output: 170 kWh/tonne; Table 2C) and that manures are ~half as productive (~100 kWh/tonne), applying the IBHR could generate potentially ~12 terawatt-hours pa with fIT revenues of GBP ~1.6 billion, plus net export revenues, gate fees, and avoided landfill costs.

However, to offer a solution for sustainable energy production the IBHR must perform independently of present subsidies. Therefore, for each case history was estimated the net energy ratio (NER) of a dark EF (Table 3A-B) and the complete IBHR (Table 3C-D). NER is defined as total process energy output over parasitic energy requirements (energy out / energy in). If a process generates a net energy output, then NER>1.

Parasitic energy was based on four factors (Table 3); HCW hydrolysis (0.022-0.032 kWh/kg raw waste), electro-separation of OA (0.021-0.091 kWh/kg), DF mixing energy (0.0008-0.0027 kWh/kg) and PF mixing energy (0.0008-0.0028 kWh/kg raw waste) as these are the core scalable elements, whereas other costs would be case-specific, e.g., an IBHR co-located with a dairy farm (manure and milk processing residues) would have near-zero transport and communitation costs, in contrast to an IBHR utilising organic fractions of municipal wastes located on a city outskirts. The energy demands of HCW treatment and mixing were estimated (Section 3.5), while that of OA separation was determined experimentally from the three waste-fed EFs (Figure 2). The presented parasitic energy demands would be applicable to production scale HCW treatment and electro-separation of OA.

The estimated energy demand of the experimental HCW reactor was ~100 kWh/kg dry matter, leading to parasitic costs of 3-11 kWh/kg raw waste, which would exceed the IBHR energy output. However, this misrepresents hydrolysis at production-scale because the experimental reactor and contents were heated and cooled in sequential batches without heat recovery, whereas a production scale system would operate continuously using an effluent-to-feed heat exchanger to provide ~95% of the heating and cooling (Chen and Yu, 2003; Jogwar et al., 2008) reducing the heat demand to 0.0102 kWh/kg HCW reactant (97% H₂O, approximated to 100%). The case-specific HCW energy demands (Table 3B) vary due to the different yields of washed pressings from raw wastes (Figure 1). The sensitivity of the NER to the fraction of heat recovered varied with the energy yield in each case. For example, CW1 yielded the lowest gross energy output (0.10 kWh/kg raw waste; Table 3A) requiring at least 83% heat recovery to break even (NER=1), whereas an Ap waste-fed IBHR (0.25 kWh/kg) would break even with only 64% heat recovery ([WEO3](#)).

Unlike the experimental HCW reactor, the experimental electro-separation cell was essentially representative of production scale. Nevertheless we overestimated the parasitic cost because the experimental cell contained a stack of 3 membranes (configured as BPM,ASM,CSM), whereas production systems employ manifold stacks configured as BPM,[ASM,BPM]_n,ASM,CSM (Redwood et al., 2012), thereby reducing the contribution of flanking membranes (non-separating) to the stack resistance. The observed variation in separation cost relates to differences in the separated OA profiles (Table 2A), which is important due to differences in the HPP/charge ratio. For example, succinate (divalent) provides only 3.5 mol HPP/mol ionic charge, whereas butyrate (monovalent) provides 10 mol HPP/mol. The dominant OAs were acetate and butyrate in all cases except CW1 juice where propionate was also produced. Note also that EF produces a third stream of H₂ via water electrolysis that, in operation, would be pooled with the bioH₂ streams (Figure 3) and is excluded in NER calculations.

The mixing requirement is lower in anaerobic culture, where mixing functions primarily to maintain homogeneity, than in aerobic culture, where it promotes oxygen transfer into the aqueous phase. Hence, adequate mechanical mixing requires only 1.7-3.8 W/m³ (Cumiskey et al., 2002). Mixing energy has been optimised in anaerobic digestion, where headspace re-circulation (or ‘gas mixing’) is a common method, requiring only 1-2 W/m³ (Cumiskey et al., 2002; Karim et al., 2005). For example, Ute Engineering (UK) manufacture digesters with a gas-based mixing system requiring 3 W/m³. Fully passive mixing uses the movement of biogas bubbles (e.g. BIMA system, Entec Biogas GmbH) or the accumulated pressure of formed gas (Lee et al., 1995). AgroEnergien (Germany) have applied this principle in a ‘Self Mixing Digester’. For EF, culture circulation by an external pump would make double use of the circulation to the electro dialysis cell, requiring 4 W/m³ for a turnover time of ~5 h (Mills, 1979), on which DF mixing energy was estimated (Table 3B).

PF mixing energy is reportedly 1.0 kWh/m²/year (Burgess and Fernandez-Velasco, 2007) for a tubular photobioreactor (PBR; *d*=90 mm). The space-time requirement was estimated using 5% light conversion efficiency and 80% substrate conversion efficiency with a horizontal irradiance of 2.12 MWh/m²/year (Šúri et al., 2007) ([WEO5](#)). Hence, the PBR would process 1667 mol HPP/m²/year, from which land usage was determined (Table 3D).

For the single-stage EF, NER < 1, so this system would consume energy. Conversely, the average NER for the IBHR was 2.4, with the PBR requiring only 2-3% of the total parasitic energy to produce 63-91% of the total bioH₂. Therefore, we conclude that (i) EF functions mainly to convert biowaste into purified OA and (ii) that IBHR is a viable route to energy from waste, independent of subsidies and credit systems.

A model of an IBHR utilising waste mango (Figure 3) shows a net output of 102 kWh/tonne raw waste with estimated revenues of ~GBP100/tonne from gate fees and electricity generation under current UK markets and incentives (Anon, 2010). A complete economic analysis would be beyond the remit of this study but would include capital and running costs, incentives for landfill and carbon avoidance, by-products (section 3.8) and electrolytic H₂ (Figure 3).

3.6 Hydrolysis and electro dialysis in the IBHR

Wastes were first pressed and infused with water to release soluble sugars (where possible; Figure 1). Finishing the process without treatment of the solid pressings by HCW would result in higher NERs of up to 6.6 (average: 4.7) but would also yield solid residues at 0.5-0.9 kg/kg waste ([WEO10](#)), requiring further disposal. HCW hydrolysis reduced the NERs to an average of 2.4 but eliminated 90% of the solid residua. HCW hydrolysis is, therefore, effective for the conversion of biowaste to energy.

The integration of DF and PF is challenging particularly with respect to the PBR's sensitivity to NH_4^+ ion (Section 1), which is addressed through the anion-selective property of EF, particularly against the cation NH_4^+ . In this study, fermentations contained initially 55 mM NH_4^+ from the starting ('ED') medium, whereas permeates contained $\ll 0.1$ mM NH_4^+ and supported H_2 production by *Rhodobacter sphaeroides* OU001 and ZX5, accordingly (Table 2). Other noteworthy features of EF were discussed previously (Redwood et al., 2012).

Electrodialysis is key to the integration of dark and light biohydrogen fermentations. Other approaches include co-culture, cell separation and immobilisation, all of which are sensitive to the nitrogen influx which may vary in a waste-fed IBHR. Due to the difficulty of balancing the growth rates of dark fermentative and photofermentative bacteria, co-culture requires precise control (Sun et al., 2010) and has not been applied to wastes. Cell separation from dark fermentation effluent is the most common laboratory approach but has limited scalability due to its reliance on slow and energy-intensive centrifugation or ultrafiltration, whereas EF does not rely on solvent flow through a membrane and hence requires no pressure gradient and is relatively immune to fouling. Immobilisation of the dark phase (e.g. granulation) has proven effective (Redwood et al., 2009) although immobilisation limits diffusion and mixing.

On average, 95% of parasitic energy in the IBHR was distributed equally between OA separation and HCW treatment. Therefore, eliminating these energy requirements would enhance the NER 20-fold. However, HCW treatment was highly effective in hydrolysing and liquefying the solid biomass residues. Without it, the process would achieve only 26% biowaste destruction. Electrodialysis is also key as it enables the IBHR to accept diverse feedstocks regardless of nitrogenous components which may inhibit PF. Therefore, HCW and electrodialysis offer 'good value' for their parasitic costs.

3.7 Comparison of IBHR and other sustainable energy technologies

Table 4 summarises the comparisons with other sustainable energy generation technologies, in terms of NER, energy yield and land requirements.

An alternative biowaste to H_2 method is the microbial electrolysis cell (MEC) in which occurs biocatalysed electrolysis. Using the data of Cheng et al. (2007), an MEC using a fuel cell to meet its power demand would have an NER of 2.0 (or 1.3 incorporating the same pre-treatment as the IBHR; [WEO11](#)). Therefore, the IBHR (average NER: 2.4) compares favourably with the MEC.

Anaerobic digestion (AD) is a ubiquitous process, applied worldwide domestically to produce biogas for cooking (Limmechokchai and Chawana, 2007) and at scale for biowaste treatment. AD generates, 40-80 kWh (power)/tonne feedstock (Reith et al., 2003) and requires ~ 6 kWh/tonne for mass transfer (Redman, 2011) and ~ 10 kWh/tonne if mixed (Section 3.5) giving an NER of 2.5-5 but this excludes additional costs such as feedstock and digestate transport, dewatering and communitation. A renewed interest in AD bioenergy has prompted incentives and regulatory frameworks to valorise digestate sustainably as fertilizer (Anon, 2011a) which limit the use of digestate in accordance with local soil characteristics to prevent nutrient overloading.

As a solar process, the IBHR has a significant land requirement. The calculated land usage for PF was 0.8-2.8 m^2 ·years/tonne, varying with the feedstock (Table 3D). For example, to match the capacity of a typical AD plant (5000 tonnes/year), an equatorial IBHR would require 1.4 hectares of any land-type processing Ap waste or 0.4 ha for CW1. An equivalent AD process would require ≥ 120 ha of agricultural land for spreading digestate within nitrate release regulations (Redman, 2011). Therefore, the IBHR has 0.3-1.2% of the land "requirement" of AD. Furthermore, the land taken for the IBHR need not be arable, including industrial areas, contaminated or infertile

land, rooftops and slopes. Due to a ~2-fold lower solar irradiance ([WEO5](#)) the requirement for the IBHR would be ~2-fold higher in locations 50° from the equator (e.g. Northern Europe, Northern USA or Australia) but due to the minor contribution of PF energy requirements (~2.5%; [WEO6](#)), the effect on NER would be negligible.

To compare the IBHR with other solar processes, the power/land ratios in the range 53-82 kW/ha (equator) or 27-41 kW/ha (~50° N or S) were calculated ([WEO7](#)). For comparison, the Sarnia photovoltaic (PV) power plant, currently the world's largest (380 ha), outputs 36 kW/ha while onshore wind (UK) produces ~20 kW/ha (MacKay, 2008). The best crop-derived biofuels may capture up to 5 kW/ha as chemical energy in the biofuel, which leads to a maximum of ~1.5 kW/ha energy at point of use, using an efficiency of 30% in a generator or vehicle engine. Therefore, in locations ~50° from the equator (e.g. UK, Germany) the IBHR could easily out-produce wind and crop-derived biofuels, with a similar output to PV, while also providing sustainable biowaste treatment.

3.8 Integrated biorefineries

An integrated biorefinery combines multiple integrated technologies to convert a biomass feedstock into a spectrum of products in order to maximize its value and overall effects. The IBHR represents a potentially valuable module within a fully integrated biorefinery because, in addition to the efficient conversion of biomass to biohydrogen and net recoverable energy, there are several potentially valuable co-products where routes for further processing and value recovery remain to be determined. The unused feedstock mass (average 8%; [WEO10](#)) could be used in wet combustion, further hydrolysis, gasification, anaerobic digestion or to produce building materials. 5-hydroxymethylfurfural (5-HMF), a byproduct of HCW hydrolysis is a potentially valuable platform chemical for sustainable plastics and biofuels (Orozco, 2011). Photofermentative bacterial cells can be used as animal feeds or sources of other plastics precursors, carotenoids and single cell protein (Sasikala et al., 1995). The authors have shown previously (e.g. Orozco et al., 2010) that bionanocatalyst (for clean chemistry and fuel cells) can be made using excess bacterial cells from biohydrogen dark fermentation and photofermentation, while CO₂ from dark fermentation can provide the carbon source for photosynthetic algae (X. Zhang, R. L. Orozco and L. E. Macaskie, unpublished) avoiding any requirement for additional sunlight-capture space through the use of dichroic 'beam-sharing' (Redwood et al., 2012).

4. CONCLUSIONS

The results of this study support the hypotheses; biowastes are suitable for electro-fermentation and the IBHR is a practical approach for biowaste-to-energy. Extracts from waste-fed electro-fermentation were suitable for photofermentation, which is required for the IBHR to achieve a positive energy balance. Hydrothermal hydrolysis and electrodialysis represent 95% of the parasitic energy but enable ≤99% destruction of biowaste and NH₄⁺-immune solar bioenergy production at 67 kW/ha, with a net energy ratio of 2.4. The IBHR compares favourably with leading biological waste-to-energy processes and could out-produce some core renewables (wind, crop-biofuels and photovoltaics) while also disposing biowastes.

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SUPPLEMENTARY DATA CAPTION

Mathematical derivations for Table 3 and values given in text. The spreadsheet may be downloaded from <http://epapers.bham.ac.uk/1554/>.

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TABLES**Table 1.** Food wastes characterisation and fermentability

Waste ^a	Description	Moisture content (% w/w)	Total sugars (% w/w wet matter)	Starch content (% w/w wet matter)	Total monosaccharide (mM) ^g			Fermentative H ₂ yield (L/kg raw waste)			
					Juice	Infusion	Hydrolysate ^f	Juice	Infusion	Hydrolysate ^f	Total
Ma ^d	Variety: 'keit' Stones removed.	84.9	10.6	0.23	409	57.4	16.8	0.85	0.47	1.33	2.65
Ap ^d	Variety: <i>Purus x bretschnideri</i> Whole fruit used.	87.2	7.25	0.18	338	82.0	13.1	1.08	1.60	0.64	3.32
Av ^e	Variety: 'Avo Hass' Stones removed.	70.6	8.83 ^b	ND ^c	e	e	0.65	e	e	0.52	0.52
CW1 ^d	Red onion, tomato, lettuce, spring onion, pepper, pasta, lemon peel.	89.7	7.02	1.35	112	21.2	10.2	0.77	0.44	0.66	1.87
CW2 ^e	Onion, pea, potato, carrot, courgette.	87.3	4.08	1.51	e	e	3.3	e	e	0.93	0.93
CW3 ^e	Rice, pasta (cooked).	57.4	28.5	21.1	e	e	12.4	e	e	5.99	5.99
BG ^e	Malted barley from beer process.	75.5	16.4	1.22	e	e	7.6	e	e	1.07	1.07

^aFor waste abbreviations see Figure 1; ^bThe oil fraction was omitted from acid digestion; ^cAvocado was too oily for starch analysis; ^dpressed to extract juice and solids were infused, see Figure 1; ^eNo juice or infusion could be produced due to the physical nature of the waste; ^fHCW hydrolysis took place at 200 °C except for grain which was at 220 °C and hydrolysates were detoxified with activated carbon as reported previously (Orozco et al., 2012); ^gpredominantly glucose and fructose with 4.1 mM and 3.2 mM xylose in Ap and Ma, respectively. For CW1-3, the description is not exhaustive. H₂ yield was determined in dark fermentability tests (Section 3.2).

Table 2.**A:** Anion analysis of extracts from waste-fed extractive fermentations

Fermentation substrate ^a	Inorganic anion (mM)					Organic anion (mM)							
	Chloride	Nitrite	Nitrate	Sulphate	Phosphate	Lactate	Acetate	Propionate	Formate	Butyrate	Succinate	Citrate	Total HPP ^b
Ma juice	2.7	0.0	0.0	5.6	2.4	54.6	90.4	0.0	34.5	98.8	8.1	0.6	1777
Ap juice	2.7	0.3	0.0	5.5	2.6	8.0	75.5	0.0	47.0	84.0	25.9	0.3	1422
CW1 juice	6.9	0.0	0.04	12.7	4.5	10.2	98.1	58.4	17.4	39.5	3.2	0.0	1297

B: Experimental and potential H₂ yields by photofermentation of waste juices

Original waste	Initial substrate concentration	Substrate conversion efficiency	Yield of OA from EF	Extrapolated PF H ₂ yield from waste juices.
(source of OA via EF)	mM HPP ^c	% ^d	mol HPP/kg juice	L H ₂ /kg juice ^f
Ma juice	34.64	17.7%	5.748 ^e	110.6
Ap juice	39.60	48.9%	5.257 ^e	101.2
CW1 juice	61.46	15.6%	0.956 ^e	18.4

^asource of organic acids (OA) via extractive fermentation; ^bHPP values (mol H₂/mol OA) are: Lactate 6; Acetate 4; Propionate 7; Butyrate 10; Succinate 7; Citrate 14; (Sasikala et al., 1995) ^cHPP, hydrogen production potential as proposed previously (Eroglu et al., 2004) is a convenient unit for the potential H₂ production from any mixture of substrates, e.g. a solution of 1 mM acetate and 1 mM butyrate contains 14 mM HPP, because acetate=4 H₂/mol and butyrate=10 H₂/mol (Sasikala et al., 1995); ^dSubstrate conversion efficiency (Sasikala et al., 1995); ^eExtractive fermentations fed with the juices of wastes Ma, Ap and CW1 (Figure 1), yielded respectively 4.02 mol HPP from 0.70 kg juice, 4.02 mol HPP from 0.77 kg juice and 0.80 mol HPP from 0.84 kg juice; ^fTo extrapolate the productivity at full scale, a substrate conversion efficiency of 80% was used, typical for optimised photobioreactors (as opposed to static vial tests, used here as a high throughput method to confirm the suitability of electro-separated OA; Section 3.4); EF: extractive fermentation; PF: photofermentation.

Table 3 Energy balances for 3 IBHR case histories**A: Gross energy production by a single-stage extractive fermentation.**

Waste	H ₂ yield in juice-fed EF	H ₂ yield (Juice fraction)	Fraction of total H ₂ from juice ^b	H ₂ yield from juice, infusion & hydrolysate	Gross electricity production potential ^c
Unit	mol H ₂ / kg juice	mol H ₂ from the juice of 1 kg raw waste ^a	%	mol H ₂ / kg raw waste	kWh/kg raw waste
Ma	0.631	0.102	31.9%	0.319	0.019
Ap	0.433	0.127	32.6%	0.391	0.023
CW1	0.457	0.262	41.1%	0.638	0.038

B: Parasitic costs and net energy production of a single-stage extractive fermentation.

Waste	Hot Compressed Water (HCW) treatment			Electro-separation of organic acids			Mixing energy for dark fermentation ^f			Total parasitic energy	Net Energy Ratio ^h
Unit	Moisture of washed pressings	kg HCW reactant / kg raw waste ^a	kWh / kg raw waste ^d	kWh / mol HPP ^e	kWh / kg juice	kWh / kg raw waste ^f	Days of mixing	kWh / kg juice ^g	kWh / kg raw waste ^f	kWh / kg raw waste	Out / In
Ma	84.9%	3.126	0.0318	0.0073	0.0422	0.021	3.863	0.0016	0.00080	0.0539	0.35
Ap	87.2%	2.116	0.0216	0.0192	0.1011	0.091	4.042	0.0015	0.00137	0.1142	0.20
CW1	84.7%	2.349	0.0239	0.0128	0.0123	0.017	5.617	0.0019	0.00269	0.0438	0.87

C: Gross energy production by an integrated biohydrogen refinery (IBHR).

Waste	H ₂ yield in juice-fed fermentations			Total H ₂ yield from juice, infusion and hydrolysate ^b	Gross electricity production potential ^c
Unit	Dark fermentation	Photo-fermentation	Total	mol H ₂ / kg raw waste	kWh / kg raw waste
Unit	mol H ₂ / kg juice ^a	mol H ₂ / kg juice	mol H ₂ from the juice of 1 kg raw waste ^b	mol H ₂ / kg raw waste	kWh / kg raw waste
Ma	0.631	4.598	0.842	2.642	0.157
Ap	0.433	4.206	1.364	4.187	0.249
CW1	0.457	0.769	0.703	1.711	0.102

D: Parasitic costs and net energy production of an integrated biohydrogen refinery (IBHR).

Waste	Yield of organic acids (HPP) via extractive fermentation of wastes		Land usage	Mixing energy for photofermentation	Total parasitic energy ^k	Net Energy Ratio ^h
Unit	mol HPP from the juice of 1 kg raw waste ⁱ	mol HPP / kg raw waste ^j	m ² -years / kg raw waste	kWh / kg raw waste	kWh / kg raw waste	Out / In
Ma	0.925	2.905	0.00174	0.0017	0.056	2.83
Ap	1.546	4.746	0.00285	0.0028	0.117	2.13
CW1	0.549	1.334	0.00080	0.0008	0.045	2.29

^aCalculated using the mass yields shown in Figure 1; ^bCalculated in Table 1; ^cwith a power conversion efficiency of 75% via a fuel cell, 285.9 kJ/mol H₂; ^dAuthor's estimation for the power demand for heating in a continuous flow HCW system (Section 3.5); ^eExtractive fermentations fed with the juices of waste mango, asian pear and catering waste 1 required for electro-separation 29.5 Wh, 77.3 Wh and 10.3 Wh, respectively; ^ffor juice, infusion and hydrolysate; ^gEstimated using 4 W/m³ mixing power (Mills, 1979) using the fed juice volumes (Table 2B, legend); ^hNet Energy Ratio (NER) includes the necessary and scalable process energy requirements and excludes variable requirements such as feedstock transport, and communitation; ⁱCalculated using the yields of juice from raw waste (Figure 1) and yields of HPP from juice (Table 2B); ^jEstimated HPP yield via extractive fermentation of all waste fractions (juice, infusion and hydrolysate), assuming that HPP yield is proportional to H₂ yield (Table 2); ^kSum of PF mixing energy and single-stage parasitic energy (B); For further detail see [WEO2-WEO5](#). Data represent the average measurements in repeatedly-fed sustained EFs.

Table 4 Comparison of IBHR and other sustainable energy technologies

Technology	Net energy productivity metrics			Source and details	
	NER	Yield (kWh/tonne)	Power (kW/ha)		
IBHR	2.4 (average)	97 (average)	67 (average)	This study	
Waste	Anaerobic Digestion (AD)	2.5-5 ^a	40-80	<1 ^b	Redman (2011)
	Microbial Electrolysis Cell (MEC)	1.3/2.0 ^c	30/58 ^c	N/A	Cheng et al. (2007)
Non-waste	Photovoltaic cells (PV)	N/A	N/A	36	Sarnia, Canada ^d
	On-shore wind turbines	N/A	N/A	20	UK, Mackay (2008)
	Crop-derived biofuels	N/A	N/A	1.5 ^e	

^aexcluding the parasitic energy costs of feedstock and digestate transport, dewatering and comminution. ^bincluding the area of agricultural land required for digestate disposal within nitrate release regulations (see text). ^cFirst value assumes the same pre-treatment as the IBHR (See [WEO11](#)); second value assumes no pretreatment required. ^dSarnia photovoltaic power plant, currently the world's largest (380 ha; 120 GWh pa). NER: net energy ratio. ^eSource value (5 kW/ha) was reduced to account for energy conversion efficiency (~30%) in a typical combustion engine (i.e. generator or vehicle).

Figures

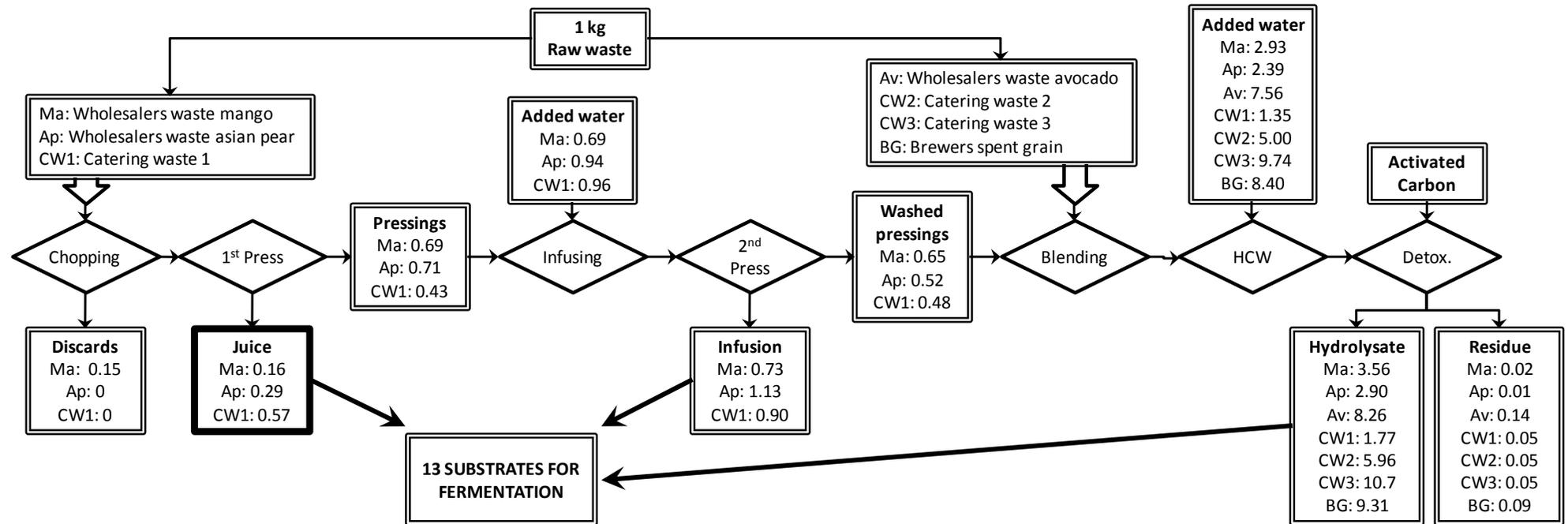


Figure 1. Food waste processing scheme and mass flows.

Values represent masses (kg/kg raw waste). Wastes comprised 3 samples of spoiled fruits, 3 samples of catering wastes and brewers malted grain. Solids were pressed using a Ferrari 5 L hand-cranked fruit press. Solid residues were homogenised (except for grain) using a standard kitchen blender and the moisture content was determined (by drying at 60 °C) before wet samples were treated with hot compressed water (HCW). Av, CW2, CW3 and BG were unsuitable for pressing and were treated only by blending and hot compressed water (HCW), which took place at 200 °C except for grain which was at 220 °C. Raw wholesalers waste avocado contained 0.16 g stones which were removed before blending (not shown). Detoxification (Detox.) used activated carbon (5% w/v; Orozco et al., 2012). Bold outline: Three juices tested in 3 EF tests.

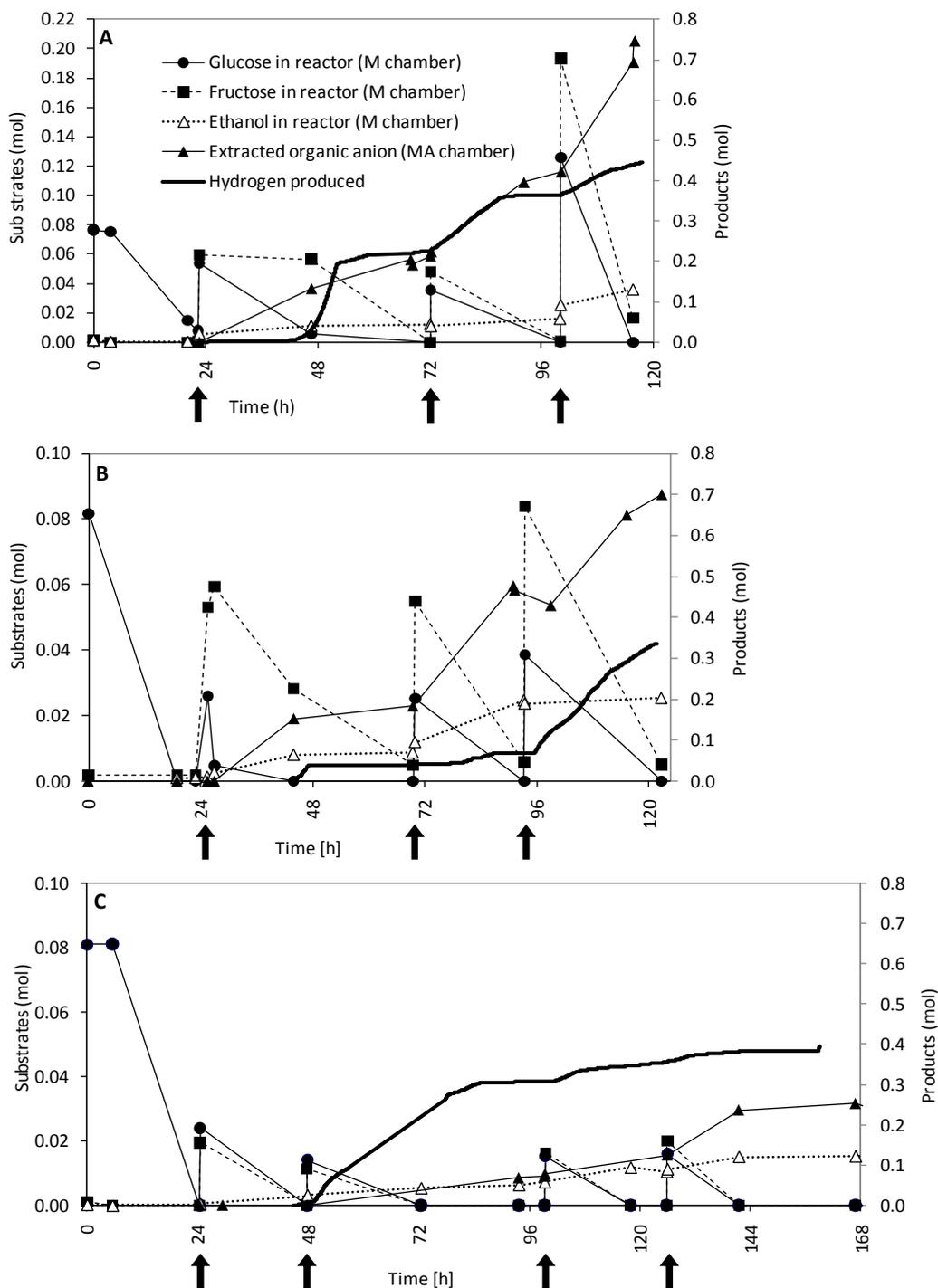


Figure 2. Waste-fed extractive dark fermentations.

Fermentations were fed with the juices of A, Ma; B, Ap; and C, CW1 (Figure 1). Arrows indicate repeated substrate dosings into the fermenter. Volumes added were A, 238mL; B, 255mL; C, 245mL, designed to provide ~0.1 mol of reducing sugars content per dose. However, due to the variable nature of waste feedstocks variation in substrate dosing was unavoidable in practice. Major sugars (glucose, fructose) are shown; maltose, mannose, xylose and mannitol were detected in low concentrations (not shown); sucrose was not detected. Total organic anion represents the summed charges of lactate, acetate, propionate, formate, butyrate and succinate. All except succinate have single pK_a values in the range 3.8-4.9 and were treated as fully dissociated at pH 6.0. Succinate (pK_a 4.2, 5.6) has theoretically 1.59 charge equivalents/mol at pH 6.0.

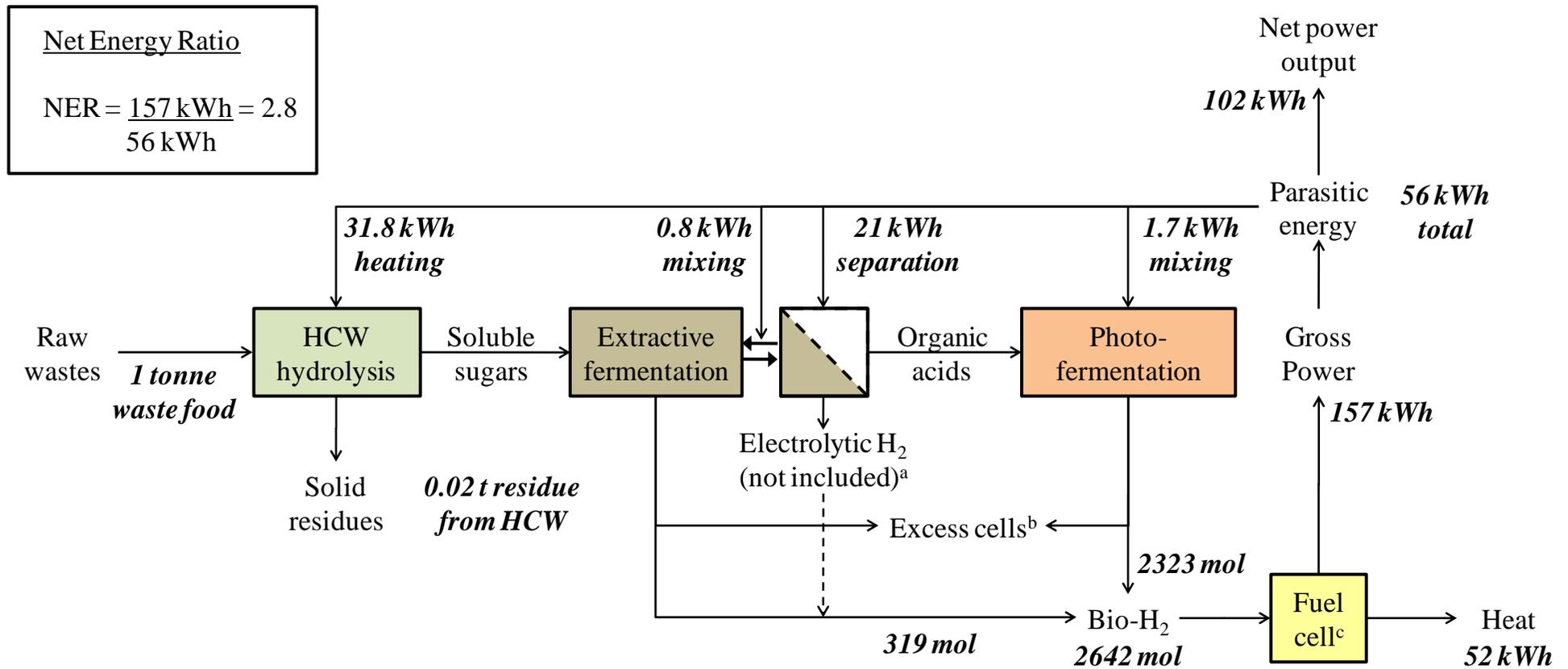


Figure 3. Net energy analysis of an integrated biohydrogen refinery (IBHR) using pre-treatment by HCW and extractive fermentation. Annotations in bold italics indicate the features of a process using wholesalers mango waste, where values are derived from the experimental data of this study; ^aIn addition to bioH₂, the IBHR also produces H₂ via electrolysis however the yield of electrolytic H₂ in a full scale IBHR is unclear hence only bioH₂ was included in this analysis; ^bExcess *E. coli* and *R. sphaeroides* cells can be valorised via metal recovery for fuel cell manufacture (Orozco et al., 2010); ^cFuel cell efficiency: 75%; HCW: hot compressed water hydrolysis. See also [WEO8](#).