Biohydrogen production from food waste by coupling semi-continuous dark-photofermentation and residue post-treatment to anaerobic digestion: A synergy for energy recovery

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Biohydrogen production from food waste by coupling semi-continuous dark-photofermentation and post treatment of residues by anaerobic digestion: a synergy for energy recovery

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Abstract

The study aimed at maximizing the energy yields from food waste in a three-step conversion scheme coupling dark fermentation (DF), photofermentation (PF) and anaerobic digestion (AD). Continuous H_2 production was investigated over a period of nearly 200 days in a thermophilic semi-continuous DF process with no pH control. The highest H_2 yield of 121.45 ± 44.55 N L H_2/kg VS was obtained at an organic loading rate of 2.5 kgVS/m^3·d and a hydraulic retention time of 4 days. The DF effluents mainly contained volatile fatty acids (VFAs) and alcohols as metabolites and un-hydrolyzed solid residues. The supernatant, after separation, was used to recover H_2 in a PF using *Rhodobacter sphaeroides*. The solid residual fraction along with PF effluent was converted into methane by anaerobic digestion. By combining DF and PF, the H_2 yield from the food waste increased 1.75 fold. Moreover, by adding AD as a post treatment, the total energy yield was substantially increased to reach 5.51 MJ/kg $VS_{food\ waste}$ added, versus 3.55 MJ/kg $VS_{food\ waste}$ when applying solely AD.

Keywords: Biohydrogen, food waste, dark fermentation, photofermentation, anaerobic digestion

1 Introduction

The inherent characteristics of hydrogen (H_2), such as higher energy content (142 MJ per kg), energy and water as the only by-products generated from its combustion, application in fuel cells for electricity generation and the ability to be produced biologically, makes H_2 a very interesting alternative future sustainable energy carrier [1]. Among several biological technologies proposed for H_2 production, dark fermentation (DF) is emerging as one of the prominent options, shown by the increasing research interests in this technology [2]. The advantages such as the flexibility to operate under different conditions of temperature and pressure, higher production rates, possibility to use renewable waste biomass as feedstock and its treatment capability makes the DF process attractive. Waste biomass such as agricultural residues, the organic fraction of municipal solid waste (OFMSW) and agro-industrial wastes are economically competitive when considering a supply of sustainable feedstock, aiming at the industrial development of DF systems for biological treatment of waste [3–5].

OFMSW which is mainly composed of food waste (FW) has been receiving a lot of attention because of its potential to be used for the production of biofuels and other value added products [6]. Especially, about 1.3 billion tonnes of food per year get wasted, which is approximately one-third of the food produced for human consumption [7]. FW is generated from agricultural production, industrial manufacturing processes and final consumption in households. In the European Union, the total annual generation of FW is estimated around 89.3 million tonnes, comprising 37.7 million tonnes generated from household consumption alone [8]. The volatile solids content in FW ranges from 21 to 27% which shows its high content of organic carbon that can be further valorized [9], and in particular for H_2 production by DF as demonstrated in the literature [10–15]. Some studies have reported the operational feasibility of continuous H_2 production using these food or kitchen wastes as a feed in DF processes [10,14,16].

With the advantage of a stable operation, continuous DF processes are usually preferred and scaling-up is more viable in comparison to batch processes which involve regular downtime periods of maintenance [17]. However, stable operation of continuous DF of FW is highly influenced by bioreactor operating parameters such as pH, temperature, organic loading rates (OLRs) and hydraulic retention times (HRTs) [4,5,18]. These factors also influence the microbial communities and thus the biochemical pathways that can affect the total H_2 yields

in mixed cultures [19]. In addition, there is growing interest in coupling DF either with photofermentation (PF) [20,21] or bioelectrochemical systems (BES) [22] to obtain higher overall H_2 yields or with anaerobic digestion (AD) for methane production [23–25], due to the post-treatment requirement of DF effluents (DFEs) and net positive energy gain from coupling these bioprocesses [26].

H_2 production rates and total H_2 yields are mainly a function of substrate types and OLRs applied [2]. A varying range of optimal OLR values has been reported for dark fermentative H_2 conversion from FW carried out in thermophilic DF processes [2]. Shin et al. [27] found an optimal H_2 yield of 126.25 L H_2/kg VS at an OLR of 8 kg VS/m^3/d while the H_2 production decreased when the OLR was increased to 10 kg VS/m^3/d. The authors reported 8 kg VS/m^3/d, 5 days and pH of 5.5, respectively, as optimal OLR, HRT and culture pH. In a study coupling DF and AD, Cavinato et al. [10] reported 66.7 L H_2/kg VS added at an optimum OLR of 16.3 VS/m^3/d, a HRT of 3.3 days and for a pH maintained in the range of 5-6 through the recirculation of AD effluent. Generally, HRTs in a range of 2-6 days have been reported as optimum for DF of organic FW in a CSTR process [2]. This range of HRTs is similar to the first stage of two-stage AD process [28]. Moreover, the HRT is also a function of the substrate types and bioreactor operational parameters.

It has been well documented that dark fermentative H_2 production is generally due to the conversion of the initial soluble fraction of carbohydrates present in the complex organic biomass, that will lead to accumulation of volatile fatty acids (VFAs) and alcohols in DFEs [29,30]. Some recent studies have shown the potential of these DFEs to be utilized in PF processes for H_2 production [20,21]. Combining DF with PF, Su et al. [31] achieved an increase in H_2 yield from 76.7 to 596.1 L H_2/kg VS from water hyacinth. Meanwhile, Rai et al. [20] achieved 43% higher volumetric H_2 yields from acid hydrolyzed sugarcane bagasse in two step DF-PF systems. However, during the conversion of complex organic biomass like FW, a part of the unhydrolyzed solid residues will remain that can be further valorized in AD systems producing methane (CH_4) in three steps conversion scheme (Fig. 1). Xia et al. [32,33] reported that a three-step conversion of algal biomass combining DF-PF-AD can achieve 1.7 and 1.3 times higher energy yields in comparison to a two-stage DF-AD and one stage AD process, respectively.

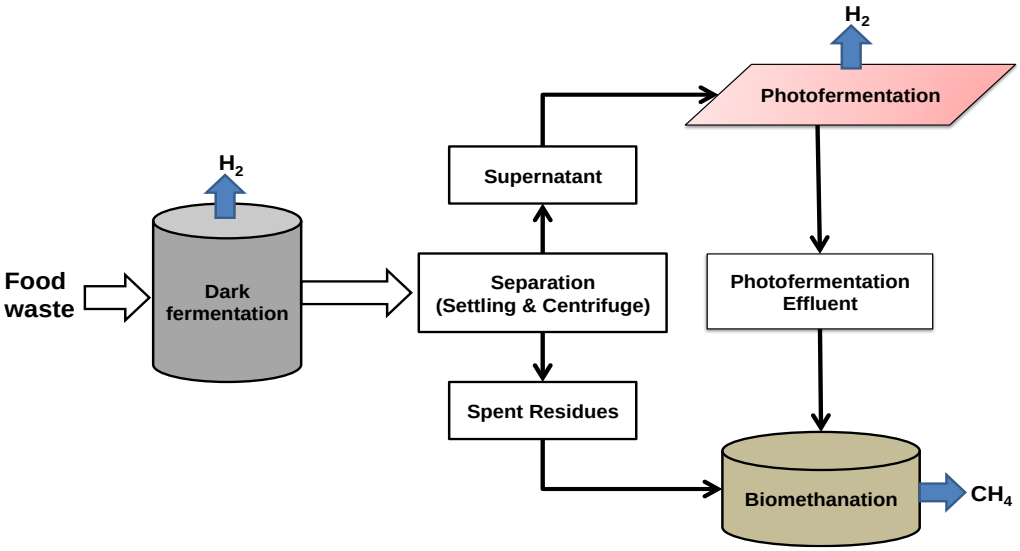

Fig. 1. Schematic of three-stage conversion of FW to hydrogen and methane

Furthermore, high OLRs are often responsible for a decrease in culture pH due to the accumulation of VFAs present in DFE. Thus, most of the continuous DF systems utilizing acidic substrates such as food waste require constant addition of external alkalinity sources such as alkaline chemicals (NaOH or KOH) or buffering agents (bicarbonate or phosphate buffers) [14,27,34]. A long-term study of continuous H_2 production at varying operating conditions of OLR and HRT to establish a long-term operability for continuous H_2 production in relation with the production of metabolites could provide further insights for the development of scaled-up DF systems. Similarly, a three step conversion process (DF, PF and AD) might contribute to an increase in overall energy yields and could provide the biological treatment to the by-products generated from DF systems.

This study aims to demonstrate the long-term operational feasibility of continuous H_2 production from FW using a semi-continuous thermophilic DF reactor at various low OLRs and HRTs without pH control. The experiment also aimed at reducing the dependency on chemical buffering agents that are used to maintain the culture pH at working conditions. H_2 production through different possible biochemical pathways was discussed in relation to major metabolites present in DFEs, obtained during the varying experimental conditions. The potential of coupling DF with photofermentative H_2 production was investigated in batch PF processes by using the liquid fraction of the DFE after physical separation. Further, the waste streams generated from the coupling of DF-PF were utilized in AD to maximize the energy yields and provide waste treatment solutions.

2 Materials and methods

2.1 Preparation of feedstock

An average composition of waste, as found in European countries, was prepared as cited elsewhere [9]. The waste mixture was prepared at the laboratory and was composed of (in % by weight): fruit and vegetables 72%, cooked pasta and rice 10%, bread and bakery 5%, dairy products (cheese) 2%, meat and fish 8% and snacks (biscuits) 3%. The FW ingredients were freshly bought at municipal markets in Naples (Italy), shredded with a blender and immediately stored at -20 ^{0}C to avoid acidification. The FW characteristics were (in g/kg FW): chemical oxygen demand (COD), 347.6 ± 47.4; carbohydrate content, 105.80 ± 0.7; total Kjeldahl nitrogen (TKN), 6.4 ± 0.18; lipids, 17.50 ± 1.19; total solids (TS), 23.79 ± 0.44%; volatile solids (VS), 22.8 ± 0.42% and the pH was 4.4 ± 0.1.

DFE were collected from the outlet of the fermenter and had a pH of 4.5 ± 0.1. After undergoing settling for 30 minutes and centrifugation at 4500 rpm for 20 minutes, the supernatant was collected. The DFE characteristics are presented in Table 1. The DFE was supplemented with KH_2PO_4, 3 g/L; $NaHCO_3$, 0.7 g/L; ferric citrate 24.5 mg/L and 10 mL of a trace metals solution (for composition, see below). pH was adjusted to 6.5 and then the DFE medium was autoclaved at 121 °C for 20 minutes.

Table 1. Characteristics of the DFE used in PF experiments

Parameters	**Values (mg/L)**
Chemical Oxygen Demand (COD)	3561.8±131.1
TOC	2447.7±7
TKN	208.0±7
NH_4^+	1.14±0.3
Phosphate	130.5±1
Total iron (Total-Fe)	≤ 0.7
Lactic Acid	33.0
Acetic Acid	465.9
Propionic Acid	449.6
Butyric Acid	1075.4
Ethanol	323.0

The solid residues left after settling and centrifugation of DFE along with the PF effluents mainly containing photofermentative biomass were used as feed for AD. The characteristics of the solid residues generated from solid-liquid separation was comprised of undigested FW which had a pH of 4.5 ± 0.1 and solid DF residue with content of: COD 2.64 ± 0.4 g/kg

residue; TS 2.42 ± 0.02% and VS 2.31 ± 0.02%. The PF effluent had a pH of 7.26 ± 0.01; and contained a soluble COD of 1407.69 ± 109 mg/L; with 0.71 ± 0.01 % TS and 0.28 ± 0.01 % VS contents.

2.2 Experimental setup and operational conditions

2.2.1 Dark fermentation bioreactor

Anaerobic digested sludge was collected from an anaerobic digestion plant of the farm "La Perla del Mediterraneo" (Campania, Italy). The sludge was used as start-up seed inoculum after thermal pretreatment at 105 ^{0}C for 4 hours to enrich the microbial consortia of H_2 producers, like spore forming *Clostridia*, and to inhibit the methanogens [35]. The inoculum had (in g/L): TS 29.54 ± 0.22; VS 18.36 ± 0.14; ammonium (NH^+_4), 0.28 ± 0.011; total alkalinity (as $CaCO_3$), 1.44 ± 0.014 and had a pH of 8.3 ± 0.1.

A continuously stirred serum bottle of 1500 ml working volume was used as DF bioreactor, which was maintained at a constant thermophilic temperature (55 ± 2 °C). The reactor was started with initial S/X ratio (substrate to inoculum ratio, as gVS substrate/gVS inoculum) of 0.5 and operated in semi-continuous mode with three different HRTs and four OLRs in six different operational conditions (Table 2).The pH of the initial feed (4.5 ± 0.1) was adjusted manually to an initial pH of 7.0 with 1 M NaOH. The culture pH in the reactor was not adjusted allowing the digesting mixture to reach indigenous chemical equilibrium.

Effluent and gas samples from the reactor were analyzed daily for determining the major metabolic intermediates, i.e. acetate, propionate, butyrate, lactate, ethanol and the gas composition (H_2 and CO_2). The total gas volume was measured by volumetric water displacement. The gas was passed through acidic water (1.5 % HCl) and the volume of water displaced corresponded to the volume of total gas. The volume of hydrogen produced was calculated by considering this volume and the gas composition and was then normalized for standard conditions.

Table 2. Experimental design used for the operation of semi-continuous reactor

Experimental periods	**I**	**II**	**III**	**IV**	**V**	**VI**
OLR (kg VS/**m^3**/d)	1	1	1.5	2	2	2.5
HRT (d)	12	6	6	6	4	4
Concentration (kg VS/**m^3**)	12	6	9	12	8	10

2.2.2 Photofermentation bioreactor

Rhodobacter sphaeroides AV1b (provided by professor Roberto De Philippis, University of Florence, Italy) was previously isolated from the Averno lake in Naples (Italy) cited elsewhere in Bianchi et al. [36] and was used as inoculum for PF. *R. sphaeroides* AV1b was first grown in a medium as previously described by Bianchi et al. [36], which was composed of (in g/L): DL-malic acid, 2; sodium glutamate, 1.7; K_2HPO_4 , 0.5; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.4; NaCl, 0.4; $CaCl_2.2H_2O$, 0.075; ferric citrate, 0.005; yeast extract, 0.4 and 10 mL of trace metals solution containing (in mg/L) $ZnSO_4.7H_2O$, 10; $MnCl_2.4H_2O$, 3; H_3BO_3, 30; $CoCl_2.6H_2O$, 20; $CuCl_2.2H_2O$, 1; $NiCl_2.6H_2O$, 2 and $Na_2MoO_4.2H_2O$, 30.

The *R. sphaeroides* AV1b pre-culture was grown again in a DFE supplemented with appropriated chemicals and autoclaved, as explained in section 2.1. It was mainly composed of (in mg/L): acetic acid, 848; propionic acid, 457; butyric acid, 1184; NH_4^+, 6; phosphate (as PO_4^{3-}), 35.8 and total Fe 0.045. Ten mL of the culture (1.52 g TSS/L) that represents 2.5 % V/V of the reactor working volume was used as inoculum in the PF experiments with DFE (Table 1).

Transparent 500 mL borosilicate serum glass bottles (Simax, Czech Republic) with 400 mL working volume were used as photofermentative batch reactor. The batch reactors were maintained at room temperature (24 ± 2 °C, April-May) under a luminance of about 4000 Lux and positioned on the top of the stirrers. Caps of the reactors presented two separate ports for biogas and culture medium sampling. The bottles were sealed with silica and flushed with argon to ensure anaerobic conditions and eliminate the nitrogen from the headspace since nitrogen can inhibit the activity of the nitrogenase enzyme responsible for photofermentative H_2 production [37]. The H_2 production was quantified as described in section 2.2.1.

2.2.3 AD of residues from DF-PF process

A batch test was carried out in 1 liter transparent borosilicate serum glass bottles (Simax, Czech Republic) and was maintained at 34 ± 1°C in a water bath. The working volume of the reactor was 600 mL with an initial S/X ratio of 0.5 with a substrate concentration of 4.5 g VS/L. A low S/X ratio 0.5 was selected to assess the biomethane potential of the feed used. Based on the substrate type, a range of S/X ratio 0.5 - 2.3 gVS substrate/gVS inoculum is suggested to prevent the acidification of the AD reactor [38]. The source of inoculum used in

the tests was the same as the start up inoculum used in the semi-continuous DF reactor. The characteristics of the inoculum were (in g/L): TS, 23.71 ± 0.17; VS, 14.55 ± 0.11; ammonium (NH_4^+), 0.46 ± 0.02; and had a pH 8.2 ± 0.1. The tests were carried out in duplicates.

2.3 Analytical methods

Hydrogen was quantified with a Varian Star 3400 gas chromatograph equipped with a ShinCarbon ST 80/100 column and a thermal conductivity detector. Argon was used as the carrier gas with a front and rear end pressure of 20 psi. The duration of analysis was 14 minutes. The fermentation products (lactic, acetic, propionic and butyric acids) were quantified by High Pressure Liquid Chromatography (HPLC) (Dionex LC 25 Chromatography Oven) equipped with a Synergi 4u Hydro RP 80A (size 250×4.60mm) column and UV detector (Dionex AD25 Absorbance Detector). Gradient elution consisted of 20% methanol and 10% acetonitrile in 5 mM H_2SO_4 pumped at a rate of 0.9 mL/min, using a Dionex GP 50 Gradient pump. The elution time was 18.5 minutes. Ethanol was quantified by HPLC (Aminex HPX-87H column (300 mm on 7,8 mm, Bio-rad) using 5 mM H_2SO_4 as an eluent. The COD of the FW was measured described elsewhere [39]. The carbohydrate content was determined according to the Dubois method [40]. Total lipids were measured following a Bligh and Dyer chloroform/methanol total lipid extraction method [41]. The light intensity was measured with a light meter (Lutron-LX-107). The TS and VS of the seed sludge and TKN were determined according to the Standard Methods [42].

2.4 Data analysis

Hydrogen production rates (HPR) were expressed in L $H_2/m^3/d$ while the H_2 yields (HY) were determined considering the total daily organic load fed to the reactor and expressed as L H_2 /kg VS added. Average and deviations for daily production were determined during the steady state reached after 3-4 days of operation. The H_2 Production Stability Index (HPSI) was evaluated by considering the ratio of standard deviation and average HPR as reported by Tenca et al. [16]:

$$HPSI = 1 - \frac{S.D.(HPR)}{Avg.HPR} \quad (1)$$

A HPSI index closer to 1 represents a stable hydrogen production.

FactoMineR, an extension on R software, was used for multivariate analysis of the metabolites distribution from the different experimental periods in relation to the hydrogen yields and co-relation circles of the major metabolites were generated.

3 Results and discussion

3.1 Continuous dark fermentative biohydrogen production

3.1.1 Effect of operational parameters on H_2 production rate and yields

The results in terms of H_2 yields (HY), hydrogen production rates (HPR) and H_2 Production Stability Index (HPSI) during the different OLRs and HRTs investigated in the six operation periods (Table 2) are summarized in Table 3. Fig. 2 shows the HPR and pH trends over the operation period of 193 days. The results show an increase in HPR when OLRs were increased. During the operating periods II, III and IV at a constant HRT of 6 days, the HPR increased from 54.1 ± 41, to 109.5 ± 33 and 210.2 ± 30 N L/m^3/d, when the OLR was increased from 1 to 1.5 and 2 g VS/m^3/d, respectively (Tables 2 and 3). Meanwhile, the overall HY increased from 54.1 ± 41.3 N L/kg VS_{added} to 105.1 ± 14.9 N L H_2/kg VS_{added}. During the experimental period IV, the H_2 production had a comparatively better stability as shown by a HPSI of 0.86. However, no significant effect was observed on the total HY and HPR when the HRT changed to 4 days during operational period V (Table 3). When the OLR was changed from 2 to 2.5 g VS/L/m^3/d during period VI, both HY and HPR increased. However, the H_2 production was not stable, supported by a lower value of HPSI of 0.63. This instability could be explained by the accumulation of acids and a subsequent decrease in pH to 4.4 ± 0.1, which might have affected the microbial community.

During a short operation period (at the end of period IV), the culture pH inside the reactor was regulated manually to an initial culture pH 5.5 with 1 M NaOH, during feeding, with the objective to assess the influence of pH on the H_2 production performance (Fig. 2(b)). However, pH regulation did not show any effect on the HPR (Fig. 2(a)). Nevertheless, the increased HPSI (Table 3) showed that H_2 production was stable during that period in comparison to the experimental period when the culture pH was uncontrolled. The percentage of H_2 and CO_2 in the gas averaged 59 ± 6% and 39 ± 6%, respectively, when the H_2 production stabilized. However, the H_2 production performances in experimental period IV (HPR: 210.2 ± 29.8 N L/ m^3·d and HY: 105.1 ± 14.9 N L/kg VS_{added} at HRT of 6 days and OLR 2 g VS/L/m^3/d) were comparable to experimental period V (HPR: 208.0 ± 34.8 N L/

m^3/d and HY: 104.0 ± 17.4 N L/kg VS_{added} at a HRT of 4 days and OLR of 2 g VS/L/m^3/d). Thus, the operational conditions of period V were considered as ideal for the DF of FW in thermophilic semi-continuous reactors with lower HRT are generally more economically efficient in terms of bioreactor design and operation.

Table 3. H_2 production rate, yields and production stability from FW by mixed anaerobic cultures

Exp. Period	HPR (N L/m^3/d)	HY (N L/kg VS_{added})	H_2 in biogas (%)	HPSI
I	116.9±40.1	116.9±40.1	52.8%±1%	0.66
II	54.1±41.3	54.1±41.3	31.2%±1%	0.24
III	109.5±32.8	73.0±21.9	43.8%±20%	0.70
IV	210.2±29.8	105.1±14.9	59.4%±6%	0.86
V	208.0±34.8	104.0±17.4	57.2%±6%	0.83
VI	303.6±111.4	121.4±44.5	55.8%±10%	0.63

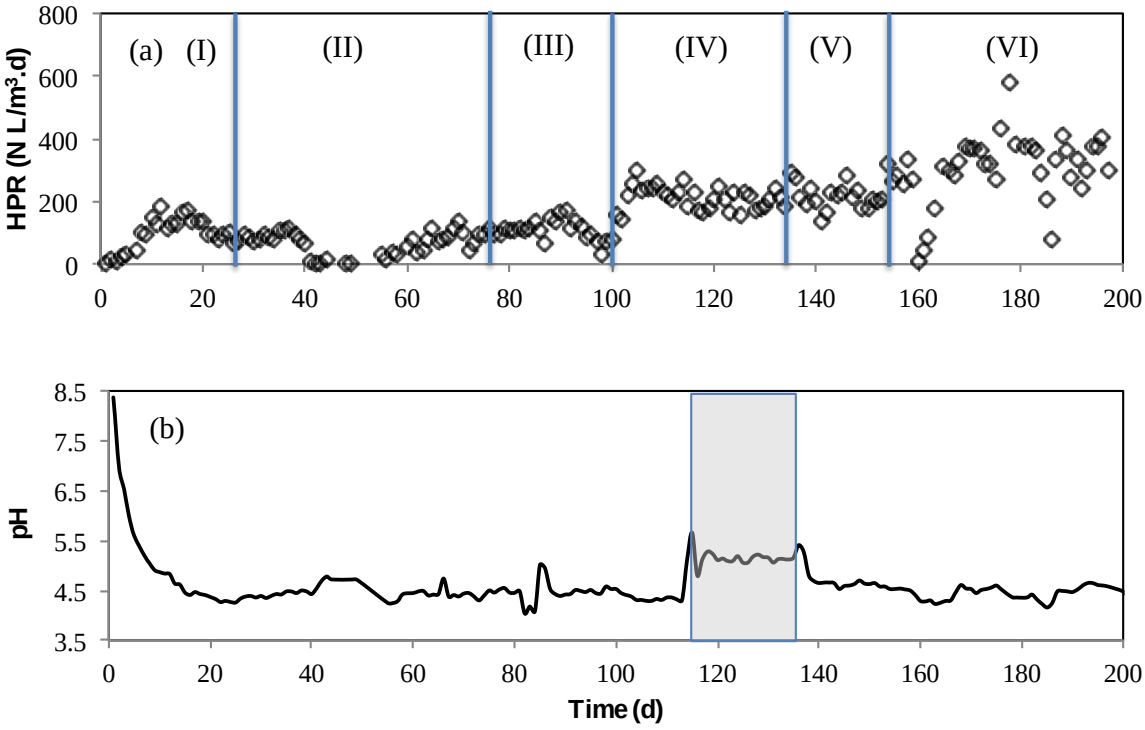

Fig. 2 (a) HPR (L H_2/m^3/d) and(b) pH trends in semi-continuous thermophilic reactor. Shaded region represents the experimental period when the culture pH inside the reactor was adjusted daily at pH 5.5 during the feeding operation.

A comparison of previous studies on dark fermentative H_2 production from FW with the results from this study (Table 4) suggests that comparable results in terms of H_2 production

can be achieved even at low OLRs and without pH control. Nonetheless, the characteristics of FW can also affect the overall HY as H_2 production is mainly function of the soluble fraction of carbohydrates present in the substrate [30]. The OLRs reported in the past studies were higher than in this study, and thus a source of alkalinity to balance the pH conditions at optimum was required. Valdez-Vazquez et al. [14] used $NaHCO_3$ and K_2HPO_4 to maintain the optimum pH at 6.4, while Lee et al. [43] used NaOH and H_3PO_4 to maintain the culture pH at 6. Thus, this pH decrease resulting from the production of acids can be minimized by the use of lower OLRs. Higher OLRs can exert detrimental effects on the microbial community, and thus H_2 production, by decreasing the pH due to the accumulation of metabolites [44].

Table 4. Comparison of dark fermentative H_2 production using FW by anaerobic mixed cultures

Substrate type	Reactor type	T (°C)	pH	OLR (kg VS/m^3·d)	Maximum assessed H_2 yield (N L H_2/kg VS_{added})	H_2 in biogas (%)	Reference
FW	Batch	55	4.5 (initial)	6	46.3	23	[45]
Vegetable kitchen waste	Intermittent-CSTR	55	6.0	28[a]	38.1[b]	40	[43]
FW and sewage sludge	Batch	35	5.0-6.0	-	122.9[c]	-	[46]
OFMSW (FW+paper)	Semi-continuous CSTR	55	6.4	11[d]	360	58	[14]
OFMSW	Packed bed reactor	38±2	5.6±0.2	16[e]	99	47	[47]
FW	Semi-continuous CSTR	55±2	4.7±0.2	2	104.0±17.4	57.2(±6)	This study

[a]gCOD/L·d, [b] mL H_2/g COD, [c] mL H_2/g carbohydrate COD, [d]g VS/kg wet mass reactor·d, [e]g VS/kg·d, FW=food waste, OFMSW= organic fraction of municipal solid waste

3.1.2 Metabolic intermediates

Lactate, acetate, propionate, butyrate and ethanol were the main metabolic intermediates observed during the different experimental periods. Such a mixture of intermediates is characteristic of mixed fermentation pathways occurring with complex substrates [30]. Average concentrations of the main metabolites during the six different experimental periods

295 are summarized in Table 5. There can be a number of possible H_2 production pathways
296 during mixed type fermentation, as represented by equations 2 – 5 (Table 6), whereas H_2
297 consuming or unfavorable pathways presented in equations 6 – 9 might exist at the same time
298 [17,19]. The presence of ethanol, acetate and butyrate are evidences for the presence of an
299 ethanol-acetate or butyrate-acetate pathway for H_2 production in the DF of the FW
300 investigated. On the other hand, the presence of lactate or propionate can be attributed to
301 fluctuations in H_2 production resulting in low H_2 yields.

302 **Table 5.** Characteristics of influent and effluents from DF of FW during different
303 experimental periods

Exp. Period	pH_IN	pH_OUT	Lactate (mM)	Ethanol (mM)	Acetate (mM)	Propionate (mM)	Butyrate (mM)
I	7.00	4.7±0.3	0.1±0.2	4.8±0.2	13.1±3.6	3.85±2.21	10.4±2.8
II	7.00	4.5±0.1	0.6±1.4	5.4±3.5	3.2±2.0	3.44±2.33	6.2±4.2
III	7.00	4.5±0.2	4.0±9.1	8.7±2.7	4.9±0.6	5.97±2.16	11.0±1.6
IV	7.00	4.9±0.4	0.0±0.0	17.2±8.6	8.5±1.8	9.65±2.91	12.0±2.9
V	7.00	4.7±0.2	0.0±0.0	17.1±6.6	6.7±1.9	5.70±2.15	9.9±3.2
VI	7.00	4.4±0.1	0.5±0.9	9.4±5.3	5.7±2.8	5.89±2.70	11.1±7.5

304

305 **Table 6.** Reaction stoichiometry in DF of glucose

Possible H_2 producing pathways	Metabolic pathway	$\Delta G'_0$[a] (kJ/mol)	Eqn
$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$	Acetate	-206.3	(2)
$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$	Butyrate	-254.8	(3)
$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2CO_2 + 2H_2$	Ethanol & acetate	-215.7	(4)
$4C_6H_{12}O_6 + 2H_2O \rightarrow 3CH_3CH_2CH_2COOH + 2CH_3COOH + 8CO_2 + 10H_2$	Butyrate & acetate	-254.0	(5)
Unfavorable and H_2 consuming pathways			
$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$	Propionate	-359.6	(6)
$1.5\ C_6H_{12}0_6 \rightarrow 2C_2H_5COOH + CH_3COOH + CO_2 + H_2O$	Propionate & acetate	-310	(7)
$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$	Ethanol	-235.0	(8)
$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$	Lactate	-198.1	(9)

306 [a] $\Delta G'_0$ values are adapted from [48,49]

307 Fig. 3 shows the plot of correlation circles of the five major metabolites and the HY. Fig. 3(a)
308 shows that the butyrate and acetate concentration is well correlated with the HY values. Not

surprisingly, propionate, lactate and ethanol are in the Dim 2 and are not correlated with the HY, which is supported by the Equations 6 - 9 (Table 6) in a DF with glucose as model substrate. However, the pathways leading to ethanol-acetate also yield H_2, as shown in Equation 4 [50,51]. Nonetheless, from Fig. 3, it can be seen that the ethanol is not correlated with acetate. Therefore, most of the H_2 yields can be attributed from the butyrate-acetate pathways, which showed a good correlation and is explained in Dim 1. The variable Dim 3 is mostly explained by lactate concentrations (Fig. 3 (b)), which correlated oppositely with HY and is an orthogonal and independent variable. The proximity of butyrate, ethanol and propionate suggests that these metabolites can be expected from DF by mixed microbial consortia. This is also supported in a study by Hwang et al. [50] who obtained butyrate, ethanol and propionate as the major metabolites during the DF at pH range 4-4.5, 4.5-5.0, 5.0-6, respectively.

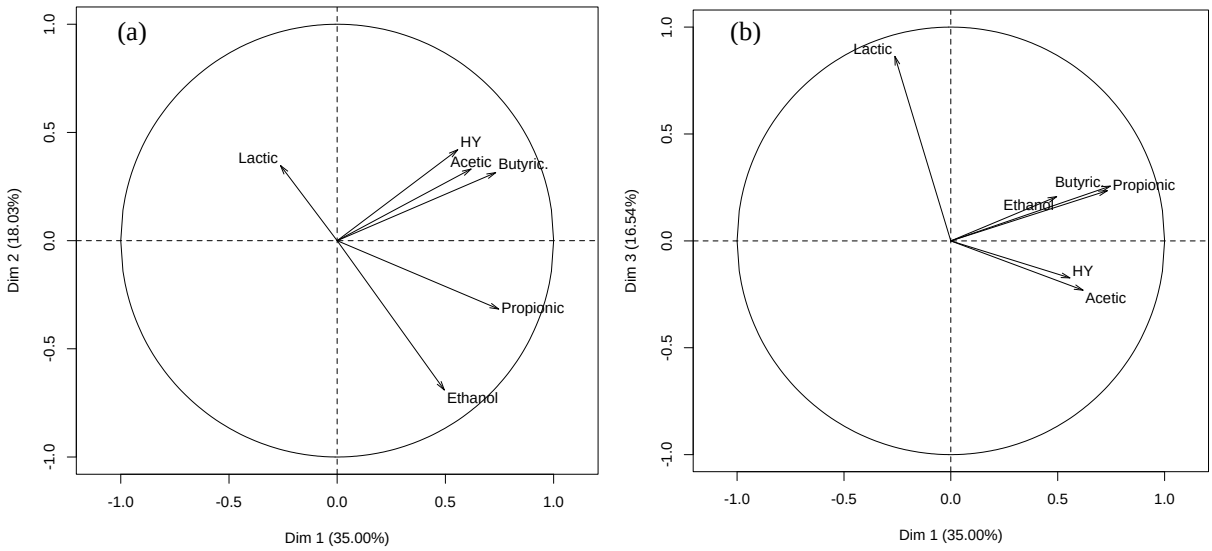

Fig. 3 Correlation circle of five metabolites and HY formed by the first three principle components Dim1, Dim 2 and Dim 3 representing 35.00, 18.03 and 16.54 % of the total variance, respectively. (a) Projections according to the first two factors (Dim 1 and Dim 2). (b) Projects according to the first and third factors (Dim 1 and Dim 3)

3.2 Photofermentative H_2 production from the liquid fraction of DF

The DFE from the semi-continuous DF reactor obtained during experimental period VI was further converted to H_2 by *R. sphaeroides* AV1b in a PF process. Cumulative H_2 production and VFA consumption trends during the PF experiments are shown in the Fig. 4 (a) and 4 (b), respectively. VFA and ammonium concentrations in the DFE medium (shown in Table 2)

were both in non-inhibiting levels for photofermentative H_2 production. Han et al. [52] reported that concentrations equal to 9.8 mM, 10.9 mM and 4.2 mM, respectively, for acetate, butyrate and propionate gave the optimum H_2 yield using *R. sphaeroides*. However, concentrations up to 30 mM of acetate have been reported in a study by Hustede et al. [53]. Similarly, the ammonium concentration was at non-inhibitory levels, as only a concentration higher than 2 - 5 mM of NH_4^+ has been reported to inhibit the photofermentative production of H_2 [54,55].

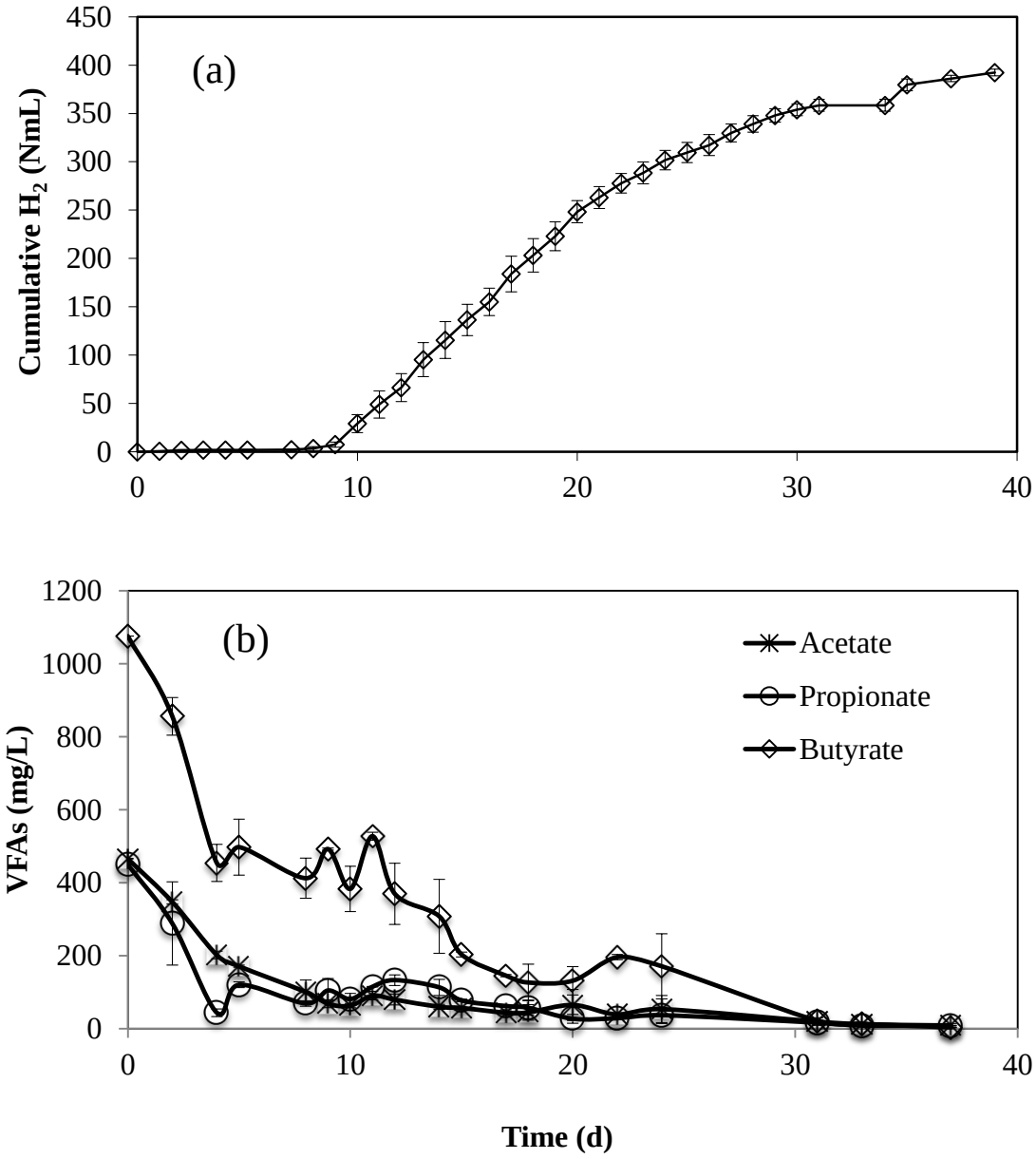

Fig. 4. (a) Cumulative hydrogen production and (b) depletion of major VFAs (acetate, propionate and butyrate) in a PF tests using DFE and *R. sphaeroides* AV1b

The PF of spent DFE yielded a cumulative production of 365.6 ± 3.2 NmL H_2, corresponding to a volumetric yield of 914 ± 8 N L H_2/m^3 and a substrate yield of 427 ± 6 N L H_2/kg COD consumed. The batch experiments were carried out for 40 days until the H_2 production completely ceased (Fig. 4 (a)). This is longer than any H_2 production time reported elsewhere [20,33]. The long lag phase (9 days) can partly explain this result. The final effluents were analyzed for COD, VFAs and biomass concentration which showed a COD reduction of 60.1%, while more than 98 ± 1% of VFAs were removed to reach a final biomass concentration of 1.6 g TSS/L. Theoretical COD removal calculated from the VFA concentration in final effluents showed a COD removal efficiency of 99.2%. However, the production of biomass and other bacterial carotenoids increased the final total COD of the PF effluent and thus reduced the total COD removal efficiency. This was evident by the reddish brown color of the effluent. The maximum percentage of H_2 in the biogas was 89% with 8.9% of CO_2.

The volumetric H_2 production obtained in this study (914±8 N L H_2/m^3) is higher than the study of Rai et al. [20] using *Rhodopseudomonas* BHU 01with a volumetric H_2 yield of 755 L H_2/ m^3. In another study by Uyar et al. [56] using *Rhodobacter capsulatus* (DSM 155) as biomass and DFE of *Miscanthus* hydrolysate as substrates, a volumetric yield of 1000 L H_2/ m^3 was obtained, which is slightly higher than in this study. The present study showed the potential of an integrated DF-PF system to achieve higher H_2 yields. Thus, the combined DF-PF processes can help in the industrial development of DF processes using FW. The residues generated from the downstream of these processes can nevertheless still be treated with anaerobic digestion in order to provide additional conversion of organic matter to further recover energy.

3.3 AD of DF-PF waste stream

The solid residues generated by the coupled DF-PF process can be ideal for AD as the undigested FW residues from the DF process and the PF effluent containing biomass generated from the PF can be converted to methane in a biorefinery model (Fig. 1). The result of the average cumulative methane production trends during the biomethane potential test using the waste stream generated from the DF-PF process is presented in Fig. 5. The cumulative CH_4 production stabilized after 50 days and the average cumulative CH_4 production was 871 ± 16 mL, corresponding to the total average yield of 324 ± 6 N L CH_4/g VS added (feed) and 0.9 kg COD/kg VS removed (calculated from CH_4 produced), evaluated

after subtracting the endogenous methane produced in the controls. The initial and final average pH in the BMP tests was 7.0 and 7.7, respectively, while the pH of the dark fermentation and photofermentation residues were respectively, 4.33 and 7.26. The pH was not adjusted with a buffering agent because the alkalinity of the inoculum was sufficient to maintain the pH, this further adds the practicability of the AD as a post-treatment option.

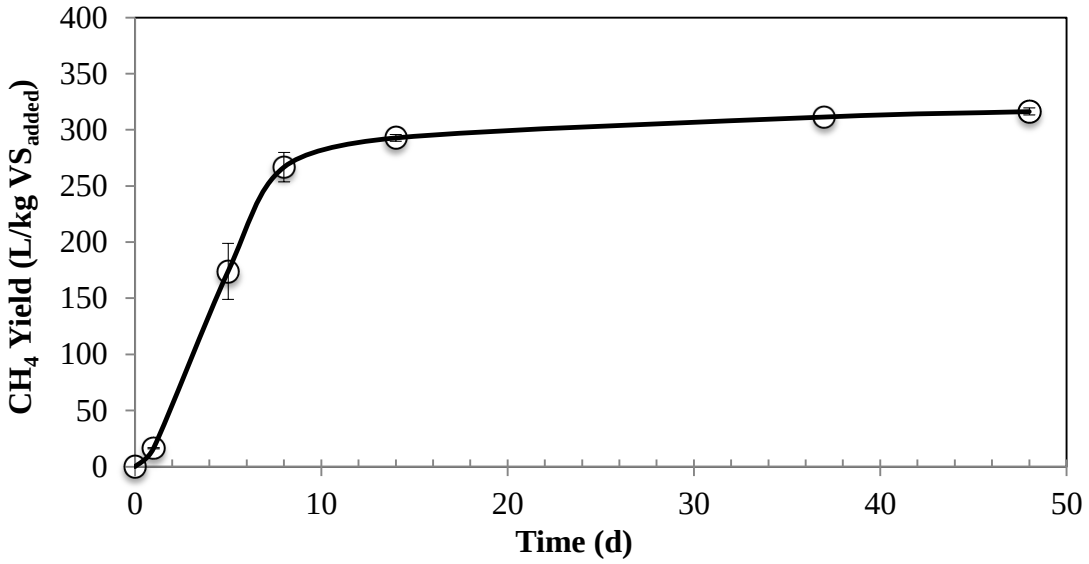

Fig 5. Methane yields from mesophilic AD of waste stream generated in the coupled DF-PF processes

3.4 Energy yields from gas biofuels produced from food waste

When considering the conversion of the initial VS added at the beginning of the DF process, the overall average H_2 yield from coupling of the DF-PF process was increased from 105.1 N L H_2/kg $VS_{initial}$ to 184.3 N L H_2/kg $VS_{initial}$, with an additional 79.2 N L H_2/kg $VS_{initial}$ from PF and 99.3 N L CH_4/kg $VS_{initial}$ from AD. The increase in energy yields obtained in his study was compared with energy yields from the coupled process previously reported in the literature (Table 7). The energy yields of hydrogen and methane from the stand alone DF as well as the two stage DF-PF and DF-AD was calculated based on the heating values of H_2 (242 kJ/mol) and methane (801 kJ/mol). These calculated energy yields represent the energy gain from the conversion of substrates by biological processes. However, the net energy gain can be estimated by considering the energy input in the processes, which is not representative in lab scale reactors and thus not calculated in this study.

Table 7. Comparison of energy yields from gaseous biofuels produced out of FW as feedstock using stand alone or coupling of different technologies

Feedstock	Process/ type	H_2 yield from DF / DF+PF (N L H_2/kg VS)	[a] Energy yield from H_2 (MJ/kg VS)	CH_4 yield from AD **(L CH_4/kg VS)**	[a] **Total energy yield (MJ/kg VS)**	**Reference**
FW+paper	Semi-continuous DF	360	3.89	-	3.89	[45]
FW	DF+PF (batch)	671[b]	7.25	-	7.25	[57]
Vinegar residue treated by HCl	DF+AD (batch)	53.2	0.57	192	7.4	[58]
FW	DF+AD (batch)	55	0.60	94	3.96	[25]
N. oceanica [c]	DF+PF+AD (batch)	183.9	1.98	161.3	7.74	[33]
C. pyrenoidosa [d]	DF+PF+AD (batch)	198.3	2.14	186.2	6.66	[32]
FW	Semi-continuous DF + PF (batch) +AD (batch)	184	1.99	99.3	5.55	This study

[a] The energy yield was calculated from the yield of biogas based on the heating values of hydrogen (242 kJ/mol and methane (801kJ/mol)

[b] L H_2/kg food waste

[c] Algal biomass pre-treatment by microwave heating with dilute H_2SO_4

[d] Algal biomass pre-treatment by steam heating with dilute H_2SO_4

By coupling DF with PF and AD processes, an additional 4.4 MJ/kg VS of energy yield can be achieved from food waste, which is higher than the coupled DF - AD process or stand alone DF processes (Table 7). Out of the overall energy recovered from the three-stage conversion (DF-PF-AD) of food waste, H_2 contributes only 35.8% out of 5.55 MJ/kg VS. However, this may be a positive add-on to the overall economic return compared to CH_4 productivity only. Therefore, the three-step process can definitely increase the recovered energy yield. Moreover, it is a very good solution for waste treatment as a higher FW conversion was accomplished. Table 7 shows that the energy yield of DF and PF from the study of Zong et al. [57] is higher than the energy yield reported in this study. This is likely because of the difference in H_2 yield achieved in these studies. In other studies by Xia et al. [32,33] and Wang et al. [58], although the overall energy yields obtained from the respective

three and two step conversion were high, the pre-treatment of the substrate required some energy input. Therefore, the overall energy yields obtained from the coupling of various processes depends on the H_2 and CH_4 yields and production rates in individual processes, which are mainly a function of process operational conditions such as pH, temperature, HRT and OLR as well as carbohydrate content and nature of the feedstock. However, the coupling of the PF and AD processes in the downstream process is not only advantageous from the energy point of view, but it also provides biological treatment of waste stream generated from DF processes by COD and pathogen removal [59].

4. Conclusion

This study has shown the long-term feasibility of continuous H_2 production as well as the possibility to further recover energy through integration of PF and AD using FW as the substrate. In addition, the viability of H_2 production at low OLRs without the culture pH control can minimize the excessive use of chemical buffering agents for pH control. The integration of DF with PF can increase the overall H_2 yield 1.75 folds. On the other hand, coupling AD for the post treatment of waste streams generated by the coupling of the DF-PF processes can further increase the overall energy yield by 4.83 MJ/kg VS of food waste, adding a synergistic effect on the overall energy recovery during the conversion of food waste.

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6. References

[1] Kotay SM, Das D. Biohydrogen as a renewable energy resource -Prospects and potentials. Int J Hydrogen Energy 2008;33:258–63. doi:10.1016/j.ijhydene.2007.07.031.

[2] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl Energy 2015;144:73–95. doi:10.1016/j.apenergy.2015.01.045.

[3] Chong M, Sabaratnam V, Shirai Y, Ali M, Hassan MA. Biohydrogen production from biomass and industrial wastes by dark fermentation. Int J Hydrogen Energy 2009;34:3277–87. doi:10.1016/j.ijhydene.2009.02.010.

[4] Ntaikou I, Antonopoulou G, Lyberatos G. Biohydrogen Production from Biomass and Wastes via Dark Fermentation: A Review. Waste and Biomass Valorization 2010;1:21–39. doi:10.1007/s12649-009-9001-2.

[5] De Gioannis G, Muntoni a, Polettini a, Pomi R. A review of dark fermentative hydrogen production from biodegradable municipal waste fractions. Waste Manag 2013;33:1345–61. doi:10.1016/j.wasman.2013.02.019.

[6] Uçkun Kiran E, Trzcinski AP, Ng WJ, Liu Y. Bioconversion of food waste to energy: A review. Fuel 2014;134:389–99. doi:10.1016/j.fuel.2014.05.074.

[7] Gustavsson Jenny, Cederbery Christel, Sonesson Ulf, Van van Otterdijk Robert MA. Global food losses and food waste-Extent, causes and prevention. Rome: 2011.

[8] European Commission DE-DC. Prepatory study on food waste across EU 27-Final Report. European Commision; 2010. doi:10.2779/85947.

[9] VALORGAS. Compositional analysis of food waste from study sites in geographically distinct regions of Europe-Valorisation of food waste to biogas. 2010.

462 [10] Cavinato C, Giuliano A, Bolzonella D, Pavan P, Cecchi F. Bio-hythane production
463 from food waste by dark fermentation coupled with anaerobic digestion process: A
464 long-term pilot scale experience. Int J Hydrogen Energy 2012;37:11549–55.
465 doi:10.1016/j.ijhydene.2012.03.065.

466 [11] Xiao L, Deng Z, Fung KY, Ng KM. Biohydrogen generation from anaerobic digestion
467 of food waste. Int J Hydrogen Energy 2013;38:13907–13.
468 doi:10.1016/j.ijhydene.2013.08.072.

469 [12] Han, SK. and Shin H. Biohydrogen production by anaerobic fermentation of food
470 waste. Int J Hydrogen Energy 2004;29:569–77. doi:10.1016/j.ijhydene.2003.09.001.

471 [13] Elbeshbishy E, Hafez H, Nakhla G. Viability of ultrasonication of food waste for
472 hydrogen production. Int J Hydrogen Energy 2012;37:2960–4.
473 doi:10.1016/j.ijhydene.2011.01.008.

474 [14] Valdez-vazquez I, Riosleal E, Esparzagarcia F, Cecchi F, Poggivaraldo H. Semi-
475 continuous solid substrate anaerobic reactors for H2 production from organic waste:
476 Mesophilic versus thermophilic regime. Int J Hydrogen Energy 2005;30:1383–91.
477 doi:10.1016/j.ijhydene.2004.09.016.

478 [15] Sreela-or C, Imai T, Plangklang P, Reungsang A. Optimization of key factors affecting
479 hydrogen production from food waste by anaerobic mixed cultures. Int J Hydrogen
480 Energy 2011;36:14120–33. doi:10.1016/j.ijhydene.2011.04.136.

481 [16] Tenca A, Schievano A, Perazzolo F, Adani F, Oberti R. Biohydrogen from
482 thermophilic co-fermentation of swine manure with fruit and vegetable waste:
483 maximizing stable production without pH control. Bioresour Technol 2011;102:8582–
484 8. doi:10.1016/j.biortech.2011.03.102.

485 [17] Hawkes F, Hussy I, Kyazze G, Dinsdale R, Hawkes D. Continuous dark fermentative
486 hydrogen production by mesophilic microflora: Principles and progress. Int J
487 Hydrogen Energy 2007;32:172–84. doi:10.1016/j.ijhydene.2006.08.014.

[18] Guo XM, Trably E, Latrille E, Carrère H, Steyer J-P. Hydrogen production from agricultural waste by dark fermentation: A review. Int J Hydrogen Energy 2010;35:10660–73. doi:10.1016/j.ijhydene.2010.03.008.

[19] Li C, Fang HHP. Fermentative hydrogen production from wastewater and solid wastes by mixed Cultures. Crit Rev Environ Sci Technol 2007;37:1–39. doi:10.1080/10643380600729071.

[20] Rai PK, Singh SP, Asthana RK. Biohydrogen production from sugarcane bagasse by integrating dark- and photo-fermentation. Bioresour Technol 2014;152:140–6. doi:10.1016/j.biortech.2013.10.117.

[21] Chookaew T, O-thong S, Prasertsan P. Biohydrogen production from crude glycerol by two stage of dark and photo fermentation. Int J Hydrogen Energy 2015:2–7. doi:10.1016/j.ijhydene.2015.02.133.

[22] Chookaew T, Prasertsan P, Ren ZJ. Two-stage conversion of crude glycerol to energy using dark fermentation linked with microbial fuel cell or microbial electrolysis cell. N Biotechnol 2014;31:179–84. doi:10.1016/j.nbt.2013.12.004.

[23] Wieczorek N, Kucuker MA, Kuchta K. Fermentative hydrogen and methane production from microalgal biomass (Chlorella vulgaris) in a two-stage combined process. Appl Energy 2014;132:108–17. doi:10.1016/j.apenergy.2014.07.003.

[24] Pisutpaisal N, Nathao C, Sirisukpoka U. Biological hydrogen and methane production in from food waste in two-stage CSTR. Energy Procedia 2014;50:719–22. doi:10.1016/j.egypro.2014.06.088.

[25] Nathao C, Sirisukpoka U, Pisutpaisal N. Production of hydrogen and methane by one and two stage fermentation of food waste. Int J Hydrogen Energy 2013;38:15764–9. doi:10.1016/j.ijhydene.2013.05.047.

[26] Ruggeri B, Tommasi T, Sassi G. Energy balance of dark anaerobic fermentation as a tool for sustainability analysis. Int J Hydrogen Energy 2010;35:10202–11. doi:10.1016/j.ijhydene.2010.08.014.

[27] Shin H-S, Youn J-H. Conversion of food waste into hydrogen by thermophilic acidogenesis. Biodegradation 2005;16:33–44.

[28] Aslanzadeh S, Rajendran K, Taherzadeh MJ. A comparative study between single- and two-stage anaerobic digestion processes: Effects of organic loading rate and hydraulic retention time. Int Biodeterior Biodegradation 2014;95:1–8. doi:10.1016/j.ibiod.2014.06.008.

[29] Monlau F, Sambusiti C, Barakat A, Guo XM, Latrille E, Trably E, et al. Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. Environ Sci Technol 2012;46:12217–25. doi:10.1021/es303132t.

[30] Guo XM, Trably E, Latrille E, Carrere H, Steyer J. Predictive and explicative models of fermentative hydrogen production from solid organic waste: Role of butyrate and lactate pathways. Int J Hydrogen Energy 2013;39:1–10. doi:10.1016/j.ijhydene.2013.08.079.

[31] Su H, Cheng J, Zhou J, Song W, Cen K. Hydrogen production from water hyacinth through dark- and photo- fermentation. Int J Hydrogen Energy 2010;35:8929–37. doi:10.1016/j.ijhydene.2010.06.035.

[32] Xia A, Cheng J, Ding L, Lin R, Huang R, Zhou J, et al. Improvement of the energy conversion efficiency of Chlorella pyrenoidosa biomass by a three-stage process comprising dark fermentation, photofermentation, and methanogenesis. Bioresour Technol 2013;146:436–43. doi:10.1016/j.biortech.2013.07.077.

[33] Xia A, Cheng J, Lin R, Lu H, Zhou J, Cen K. Comparison in dark hydrogen fermentation followed by photo hydrogen fermentation and methanogenesis between protein and carbohydrate compositions in Nannochloropsis oceanica biomass. Bioresour Technol 2013;138:204–13. doi:10.1016/j.biortech.2013.03.171.

[34] Elsamadony M, Tawfik a. Potential of biohydrogen production from organic fraction of municipal solid waste (OFMSW) using pilot-scale dry anaerobic reactor. Bioresour Technol 2015;196:9–16. doi:10.1016/j.biortech.2015.07.048.

543 [35] Ghimire A, Frunzo L, Salzano E, Panico A, Lens PNL, Pirozzi F. Biomass Enrichment
544 and Scale-up Implications for Dark Fermentation Hydrogen Production with Mixed
545 Cultures. Chem Eng Trans 2015;43:391–6. doi:10.3303/CET1543066.

546 [36] Bianchi L, Mannelli F, Viti C, Adessi A, De Philippis R. Hydrogen-producing purple
547 non-sulfur bacteria isolated from the trophic lake Averno (Naples, Italy). Int J
548 Hydrogen Energy 2010;35:12216–23. doi:10.1016/j.ijhydene.2010.08.038.

549 [37] Koku H, Eroglu I, Gunduz U, Yucel M, Turker L. Aspects of the metabolism of
550 hydrogen production by Rhodobacter sphaeroides. Int J Hydrogen Energy
551 2002;27:1315–29. doi:10.1016/S0360-3199(02)00127-1.

552 [38] Esposito G, Frunzo L, Liotta F, Panico A, Pirozzi F. Enhanced bio-methane production
553 from co-digestion of different organic wastes. Open Environ Eng J 2012;5:1–8.

554 [39] Noguerol-Arias J, Rodríguez-Abalde A, Romero-Merino E, Flotats X. Determination
555 of chemical oxygen demand in heterogeneous solid or semisolid samples using a novel
556 method combining solid dilutions as a preparation step followed by optimized closed
557 reflux and colorimetric measurement. Anal Chem 2012;84:5548–55.
558 doi:10.1021/ac3003566.

559 [40] DuBois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith F. Colorimetric method for
560 determination of sugars and related substances. Anal Chem 1956;28:350–6.

561 [41] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J
562 Biochem Physiol 1959;37:911–7.

563 [42] APHA. Standard Methods for the Examination of Water and Wastewater. vol.
564 Standard M. 20th ed. Washington DC: American Public Health Association Water
565 Works Association, American Water Environment Federation; 2005.

566 [43] Lee Z-K, Li S-L, Kuo P-C, Chen I-C, Tien Y-M, Huang Y-J, et al. Thermophilic bio-
567 energy process study on hydrogen fermentation with vegetable kitchen waste. Int J
568 Hydrogen Energy 2010;35:13458–66. doi:10.1016/j.ijhydene.2009.11.126.

[44] Van Ginkel S, Logan BE. Inhibition of biohydrogen production by undissociated acetic and butyric acids. Environ Sci Technol 2005;39:9351–6.

[45] Shin H, JH. Y, SH. and K. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. Int J Hydrogen Energy 2004;29:1355–63. doi:10.1016/j.ijhydene.2003.09.011.

[46] Kim S-H, Sun-Kee H, Hang-Sik S. Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. Int J Hydrogen Energy 2004;29:1607–16. doi:10.1016/j.ijhydene.2004.02.018.

[47] Alzate-Gaviria LM, Sebastian PJ, Pérez-Hernández A, Eapen D. Comparison of two anaerobic systems for hydrogen production from the organic fraction of municipal solid waste and synthetic wastewater. Int J Hydrogen Energy 2007;32:3141–6. doi:10.1016/j.ijhydene.2006.02.034.

[48] Thauer RK, Jungermann K, Decker K, Pi PPH--. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 1977;41:809.

[49] Kim S-H, Han S-K, Shin H-S. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. Process Biochem 2006;41:199–207. doi:10.1016/j.procbio.2005.06.013.

[50] Hwang MH, Jang NJ, Hyun SH, Kim IS. Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH. J Biotechnol 2004;111:297–309. doi:10.1016/j.jbiotec.2004.04.024.

[51] Lin C, Hung W. Enhancement of fermentative hydrogen/ethanol production from cellulose using mixed anaerobic cultures. Int J Hydrogen Energy 2008;33:3660–7. doi:10.1016/j.ijhydene.2008.04.036.

[52] Han H, Liu B, Yang H, Shen J. Effect of carbon sources on the photobiological production of hydrogen using Rhodobacter sphaeroides RV. Int J Hydrogen Energy 2012;37:12167–74. doi:10.1016/j.ijhydene.2012.03.134.

595 [53] Hustede E, Steinbiichel A, Schlegel HG. Relationship between the photoproduction of
596 hydrogen and the accumulation of PHB in non-sulphur purple bacteria. Appl Microbiol
597 Biotechnol 1993;39:87–93.

598 [54] Lee C-M, Hung G-J, Yang C-F. Hydrogen production by Rhodopseudomonas palustris
599 WP 3-5 in a serial photobioreactor fed with hydrogen fermentation effluent. Bioresour
600 Technol 2011;102:8350–6. doi:10.1016/j.biortech.2011.04.072.

601 [55] Argun H, Kargi F, Kapdan I. Light fermentation of dark fermentation effluent for bio-
602 hydrogen production by different Rhodobacter species at different initial volatile fatty
603 acid (VFA) concentrations. Int J Hydrogen Energy 2008;33:7405–12.
604 doi:10.1016/j.ijhydene.2008.09.059.

605 [56] Uyar B, Schumacher M, Gebicki J, Modigell M. Photoproduction of hydrogen by
606 Rhodobacter capsulatus from thermophilic fermentation effluent. Bioprocess Biosyst
607 Eng 2009;32:603–6. doi:10.1007/s00449-008-0282-9.

608 [57] Zong W, Yu R, Zhang P, Fan M, Zhou Z. Efficient hydrogen gas production from
609 cassava and food waste by a two-step process of dark fermentation and photo-
610 fermentation. Biomass and Bioenergy 2009;33:1458–63.
611 doi:10.1016/j.biombioe.2009.06.008.

612 [58] Wang Z, Shao S, Zhang C, Lu D, Ma H, Ren X. Pretreatment of vinegar residue and
613 anaerobic sludge for enhanced hydrogen and methane production in the two-stage
614 anaerobic system. Int J Hydrogen Energy 2015;40:4494–501.
615 doi:10.1016/j.ijhydene.2015.02.029.

616 [59] Ward AJ, Hobbs PJ, Holliman PJ, Jones DL. Optimisation of the anaerobic digestion
617 of agricultural resources. Bioresour Technol 2008;99:7928–40.
618 doi:10.1016/j.biortech.2008.02.044.

619