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Effects of operational parameters on dark fermentative hydrogen production from biodegradable complex waste biomass

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Abstract

This paper aimed to investigate the effect of the initial pH, combination of food to microorganism ratio (F/M) and initial pH, substrate pre-treatment and different inoculum sources on the dark fermentative biohydrogen (H_2) yields. Three model complex waste biomasses (food waste, olive mill wastewater (OMWW) and rice straw) were used to assess the effect of the aforementioned parameters. The effect of the initial pH between 4.5 - 7.0 was investigated in batch tests carried out with food waste. The highest H_2 yields were shown at initial pH 4.5 (60.6 ± 9.0 mL H_2/g VS) and pH 5 (50.7 ± 0.8 mL H_2/g VS). Furthermore, tests carried out with F/M ratios of 0.5, 1.0 and 1.5 at initial pH 5.0 and 6.5 revealed that a lower F/M ratio (0.5 and 1.0) favored the H_2 production at an initial pH 5.0 compared to pH 6.5. Alkaline pre-treatment of raw rice straw using 4% and 8% NaOH at 55 °C for 24 hours, increased the H_2 yield by 26 and 57 fold, respectively. In the dark fermentation of OMWW, the H_2 yield was doubled when heat-shock pre-treated activated sludge was used as inoculum in comparison to anaerobic sludge. Overall, this study shows that the application of different operating parameters to maximize the H_2 yields strongly depends on the biodegradability of the substrate.

Keywords: Biohydrogen; dark fermentation; waste biomass; biofuels; waste valorization

Highlights

- Combination of initial pH and F/M ratio affects H_2 yields from DF of food waste.
- Alkaline pre-treatment enhances the dark fermentative conversion of rice straw.
- Inoculum source and pre-treatment conditions influence H_2 yields in DF of OMWW.
- The selection of optimal operating parameters depends on substrate biodegradability.

1 Introduction

Dark fermentation (DF) of organic waste is one of the promising technologies for biohydrogen (H_2) production. The DF processes are usually preferred over other light dependent, photofermentation or biophotolysis processes because of the high bioreactor productivities and the potential to utilize a wide range of organic wastes as feedstock (Hallenbeck et al., 2009; Urbaniec and Bakker, 2015). In addition, the associated production of organic acids and alcohols, among others, can be either used in sidestream processes like anaerobic digestion for methane or photofermentative H_2 production for energy recovery, or can be used for the production of platform molecules (Bastidas-Oyanedel et al., 2015; Sarma et al., 2015).

Waste biomass is abundant and can sustain DF processes in scaled-up applications. An easily degradable food waste (the organic fraction of municipal solid waste (OFMSW)), more slowly degradable agricultural residues (i.e. rice straw) as well as agro-industrial waste such as olive mill wastewaters (OMWW) can serve as sustainable feedstock sources for dark fermentative H_2 production (Guo et al., 2010; Kapdan and Kargi, 2006; Ntaikou et al., 2010; Show et al., 2012). A major bottleneck in the utilization of these low cost waste biomasses is the rather low H_2 yields observed in the DF processes (Ghimire et al., 2015a; Urbaniec and Bakker, 2015). Nevertheless, H_2 yields and process kinetics can be enhanced by optimizing operating parameters, such as pre-treatment of inocula, food to microorganisms (F/M) ratio (also substrate to inoculum ratio), pre-treatment of substrates, culture temperature and pH (De Gioannis et al., 2013; Guo et al., 2010; Ntaikou et al., 2010; Wang and Wan, 2009). During recent years, extensive experimental research has been devoted to the establish the optimal operational conditions for maximizing H_2 production, with a special focus on operational pH, temperature and substrate utilization (De Gioannis et al., 2013; Ghimire et al., 2015a; Wong et al., 2014).

A wide range of optimal pH values have been reported for different substrates to enhance H_2 yields: an initial pH of 6.5 for food waste (Cappai et al., 2014), initial pH of 8.0 for food waste (Kim et al., 2011), a controlled pH of 7.0 for vegetable kitchen waste (Lee et al., 2008), an initial pH of 6.5 for rice straw (Chen et al., 2012), an initial pH of 6.0 for cheese whey (De Gioannis et al., 2014) and an initial pH of 4.5 for sucrose and starch (Khanal et al., 2004). This considerable variability in culture pH is mainly due to differences in temperature, substrate type and concentration (F/M ratio), inoculum types and their pre-treatment methods.

H_2 yields in DF of organic waste are strongly affected by the operational temperature as it can influence the rate of hydrolysis and the production of volatile fatty acids (VFAs) and thus the final pH of the fermentation (De Gioannis et al., 2013; Ghimire et al., 2015a). A thermophilic temperature has been reported to favor the dark fermentative H_2 production (Shin et al., 2004; Valdez-vazquez et al., 2005). Likewise, the physico-chemical characteristics of the substrates, and most importantly the biodegradability or bioavailability (can also be defined as the fraction of easily accessible carbohydrates for fermentative conversion) crucially affects the H_2 production (Monlau et al., 2013a). Therefore, several studies have established a strong correlation between H_2 yields and the initial carbohydrate fraction (soluble sugars in some cases) present in the substrates (Alibardi and Cossu, 2015; Guo et al., 2013; Monlau et al., 2012).

In this context, alkaline pre-treatment methods have been popularly adopted for the saccharification of lignocellulosic biomass (plant stalks, rice and wheat straw), which could enhance the production of H_2 in DF and CH_4 in DF coupled to anaerobic digestion, respectively and could thus give economic credentials (Monlau et al., 2015, 2013c; Sambusiti et al., 2013). Alkaline pre-treatment of lignocellulosic biomass has been reported to be carried out at different concentrations of alkaline agents (2 - 12% NaOH, weight basis), temperature (40 - 190 °C) and treatment period (30 minutes - 24 hours), with varying level of effectiveness in terms of increase in biogas yields (H_2 and CH_4) with consequent higher net energy recovery and economic return (Monlau et al., 2015, 2013b; Sambusiti et al., 2013). However, alkaline agents (i.e. Na^+ from NaOH) might exert inhibitory effects on dark fermentative microbial communities (Kim et al., 2009). Consequently, an investigation of selected alkaline pre-treatment conditions for a particular substrate type becomes vital to study the conditions that enhance the H_2 production.

H_2 production from organic waste is influenced by the presence of an effective hydrolyzing, H_2 producing microbial community, which depends on the inoculum source and inoculum pre-treatment method (Abreu et al., 2009; Bellucci et al., 2015; Chen et al., 2012; Pakarinen et al., 2008). Abreu et al. (2009) and Chen et al. (2012) showed that the H_2 yields mainly depend on the inoculum sources. However, the response of fermentative microorganisms towards the presence of inhibiting substances present in a substrate can influence the DF process. In a recent study, Bellucci et al. (2015) reported a varying response of fermentative microbial communities for H_2 production, when the inhibitor 5-hydroxymethylfurfural (HMF) was added. This was linked to the difference in inoculum pre-treatment methods applied. Likewise, the presence of polyphenolic compounds in substrates such as OMWW can exhibit inhibitory effects on fermentative microbial communities and H_2 yields (Hamdi, 1992; Ntaikou et al., 2009). Subsequently, investigating the effect of the inoculum source on

H_2 production performance from substrates like OMWW is fundamental to reach an optimum in H_2 production.

Despite some studies attempted to establish the optimal operational conditions of initial pH, F/M ratio, alkaline pre-treatment of substrate and inoculum selection, dissimilarities in H_2 production exist due to the differences between substrate types and experimental conditions. Therefore, it becomes essential to investigate the optimum initial pH for food waste under thermophilic DF conditions. So far, only few studies have considered the combined effects of F/M ratio and initial pH on thermophilic DF of food waste (Ginkel et al., 2001; Pan et al., 2008). Ginkel et al., (2001) revealed a profound impact of the concentration of substrate and pH on the H_2 yields in sucrose DF of, with an optimum pH and substrate concentration at pH of 5.5 and 7.5 g COD/L, respectively. In other study, Pan et al. (2008) established a F/M ratio of 6.0 as optimum for thermophilic DF of food waste, without the consideration of initial pH. Similarly, past studies on pre-treatment of substrates seemed more focused on maximizing the methane yields in anaerobic digestion by adopting higher concentrations of alkaline agents and treatment temperature (Monlau et al., 2013a). Therefore, optimum conditions of alkaline pre-treatment for dark fermentative H_2 production need to be investigated for lignocellulosic agricultural residues such as rice straw. Finally, different inoculum sources can be explored to study the effect on H_2 production from a typical poorly biodegradable feedstock such as OMWW, which contains polyphenolic compounds (Ntaikou et al., 2009).

The present study aims to investigate the effects of i) the initial pH and combined pH and F/M ratio on food waste, ii) alkaline substrate pre-treatment on dark fermentative H_2 production from rice straw and iii) the effect of inoculum source and pre-treatment on H_2 production from OMWW. Cumulative H_2 production, H_2 yields, H_2 production rates, lag phase and accumulation of DF metabolites (mainly organic acids and ethanol) were used to evaluate the efficiency of these various strategies to improve the H_2 production performance from these complex organic wastes.

2 Materials and methods

2.1 Inoculum

Two types of inoculum, i.e. anaerobic digested sludge (ADS) and waste activated sludge (WAS) were used in the experiments. ADS was collected from the effluent of an anaerobic digestion plant of a dairy farm located in Capaccio (Salerno, Italy). The plant features include a 100 m^3 CSTR operating at a hydraulic retention time of 24 days and operating within a pH and temperature range of 7.4 - 7.5 and 52 - 56 °C, respectively. The plant is continuously fed with buffalo manure, cheese whey of buffalo milk and sludge from an industrial wastewater treatment plant. WAS was collected from a secondary clarifier unit at the Nola Municipal Wastewater Treatment Plant located in Naples (Campania, Italy). The characteristics of the ADS and WAS before pre-treatment are presented in Table 1. The inocula were stored at 4 °C until used. The WAS and ADS underwent a heat shock treatment (HST) at 105 °C for 1.5 and 4 hours, respectively, in order to enrich spore forming *Clostridium* sp. and inhibit methanogens (Ghimire et al., 2015b). WAS had a shorter time for HST than ADS because it was obtained from an aerobic activated sludge process.

2.2 Preparation of feedstock

Three types of waste as reference models of complex waste biomass with different characteristic biodegradability, were used in this study: i) food waste, representative of moderately biodegradable organic waste was selected to study the effect of initial pH and substrate concentration on H_2 yields, ii) rice straw as a representative of slowly degrading lignocellulosic agricultural residues was used to study the technical feasibility of substrate pre-treatment on biohydrogen production and iii) OMWW was used to study the effect of the inoculum type and its adaptation to toxicants, as OMWW contains phenolic compounds and long chain fatty acid that can affect microbial growth (Hamdi, 1992; Ntaikou et al., 2009). Food waste was a mixed waste with a composition similar to the one reported by VALORGAS (2010) for European countries as (% 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%. To prepare the food waste, food was bought fresh from municipal markets in Naples (Italy), shredded with a blender (120 W Black and Decker, Kitchen Blender) for 5 minutes without adding water and immediately stored at frozen conditions (-20 ^{0}C) to avoid acidification. The rice straw was harvested from rice fields in Pavia (Italy) in 2012 and stored inside an airtight plastic bag at room temperature. Rice straw was reduced with the help of general paper scissors to a particle size of less than 2 mm (sieved with sieve size of 2mm by 2mm). OMWW was collected from a pressure olive mill of Frascati area (Lazio, Italy) in autumn 2013 and was stored at < 4 ^{0}C until use. The characteristics of the feedstocks are presented in Table 1.

2.3 Experimental set-up

Batch tests were carried out in one-liter borosilicate glass bottles (Simax, Czech Republic) maintained in thermophilic conditions (55 ± 2°C) with a thermostat in a water bath. The operating reactor volume in all experiments was 600 mL. The batch reactors were sealed with airtight caps having ports for sampling soluble metabolites and gas. The tests were carried out in duplicates with 30 reactors in total. The different sets of experiments were carried out to study the effect of the different operational parameters using the three selected model substrates (Table 2).

2.3.1 Effect of initial pH and F/M ratios on H_2 yield

The effect of initial pH and F/M ratio on biohydrogen production was studied with food waste and pretreated heat treated ADS as seed inoculum. The effect of the initial pH (4.5, 5, 5.5, 6.0, 6.5 and 7.0) was studied at a F/M ratio 0.5 and under thermophilic conditions (55 ± 2 °C). Another set of experiments was performed at F/M ratios 0.5, 1.0 and 1.5 with the two initial pH values of 5.0 and 6.5. The F/M ratios and two initial pH values were selected due to the fact that they are less affected by acidification at higher F/M ratios and the culture pH in the tests was not buffered with external alkalinity source. In addition, pH 6.5 was previously reported as optimal for food waste by Cappai et al. (2014), and thus considered for investigation in this study. The F/M ratios 0.5, 1.0 and 1.5 were obtained by adding 10 g, 18 g and 27 g food waste respectively, with a 190 g inoculum required to obtain the aimed F/M ratio. The final volume of the mixture was made up to 600 mL by adding distilled water. The initial pH was adjusted once, initially with 1 M HCl and 1 M NaOH prior to the start of the tests.

2.3.2 Effect of alkaline substrate pre-treatment on H_2 yield

Direct conversion of lignocellulosic biomass to biohydrogen is often limited due to their low biodegradability (Monlau et al., 2012; Pan et al., 2010). Biological hydrolysis is one of the limiting factors in DF. The evaluation of the effect of alkaline pre-treatment on H_2 yields was performed on rice straw. This study investigated an alkaline pre-treatment with 4 % NaOH (4 g/100g TS) and 8 % NaOH (8 g/100g TS) at a solid liquid ratio of 1:5 (w/v). This mixture was kept at 55 (± 2) °C for 24 hours in a one-liter borosilicate glass bottle (Simax, Czech Republic). The results were compared with untreated rice straw at thermophilic DF using 200 g of heat-treated WAS as inoculum. The concentration of rice straw was 45 gTS/L and the initial pH was adjusted to 6.5 during the batch tests that gave the optimal dark fermentative H_2 performance for rice straw as reported by Chen et al. (2012).

2.3.3 Effect of inoculum sources on H_2 yield

Heat shocked WAS and ADS was used as inoculum in a DF of OMWW carried out in batch tests and operated under thermophilic conditions (55 ± 2°C). The F/M ratio was fixed at approximately 1 gVS substrate/gVS inoculum in all sets of batch tests using 200 g of OMWW and a respective volume of ADS and WAS. The initial pH was adjusted to pH 6.0 in all experiments.

2.4 Analytical methods

Hydrogen was quantified with a gas chromatograph (VARIAN STAR 3400, USA) equipped with a ShinCarbon ST 80/100 column and a thermal conductivity detector. Argon was used as carrier gas with a front and rear end pressure of 20 psi. The duration of analysis was 14 minutes. The gas volume was measured with a volumetric displacement method. The biogas was passed through acidic water (1.5 % HCl) and the volume was quantified by water displacement (Ghimire et al., 2015c). The volume of hydrogen was calculated from the gas composition. Fermentation end products (lactic, acetic, propionic and butyric acids) were quantified by High Pressure Liquid Chromatography (HPLC) (Chromatography Oven LC 25 Model, Dionex, USA) equipped with a Synergi 4u Hydro RP 80A (size 250×4.60mm) column and an UV detector (AD25 Model, Dionex, USA). Gradient elution consisted of 20% methanol, 10% acetonitrile in 5 mM H_2SO_4 pumped at a rate of 0.9 mL/min by using a gradient pump (GP 50 Model, Dionex, USA). The elution time was 18.5 minutes. Ethanol and caproic acid were determined with an Aminex HPX-87H column (300 mm on 7,8 mm, Bio-rad), using 5 mM H_2SO_4 as an eluent at a flow rate of 0.4 mL/min. pH was measured with a pH meter (WTW, inolab, pH level 2). The COD of the food waste was measured as reported by Noguerol-Arias et al. (2012). The total lipid content was measured by the Bligh and Dyer chloroform/methanol total lipid extraction method (Bligh and Dyer, 1959). TS and VS concentrations were determined by the Method 2540 (Part 2000), alkalinity by titration (Method 2320, Part 2000) and TKN by macro-Kjeldahl (Method 4500-N_{org}, Part 4000) as described in the Standard Methods (APHA, 2005).

2.5 Measurements and data analysis

The biogas accumulated in the reactors was measured daily, except at the starting period of the experiments, i.e. 1-3 days, where it was measured twice a day, until the H_2 production completely ceased. The biogas volumes were normalized at 0 °C and 1 atm (NmL) and reported as a daily average. The average values were considered for the evaluations, while the data range based on the duplicate samples is provided and indicated by "±". H_2 yields were calculated by dividing the final cumulative recovery of H_2 by the amount of VS added at the start of the experiment.

De Gioannis et al. (2013) defined a parameter "t_{95}" as the time required to achieve 95% of the maximum H_2 yield. This parameter was used to compare the kinetics associated to the different batch tests, and to evaluate the effect of the experimental conditions.

$$t_{95} = \frac{H_o}{R.e}(1 - \ln(-\ln 0.95)) + \lambda \qquad (1)$$

$$H(t) = H_o \cdot \exp\left\{-\exp\left[\frac{R.e}{H_o}\right](\lambda - t) + 1\right\} \qquad (2)$$

Equation (1) corresponds to a rearranged form of the modified Gompertz equation (2), that has been widely used to model biohydrogen production kinetics (Gadhamshetty et al., 2010; Wang and Wan, 2009). This empirical formula gives biohydrogen production trends and includes five major parameters: i) cumulative biohydrogen production (or potential) (H_o, mL/g VS), ii) biohydrogen production rate (R, mL/h), iii) e is 2.71828, iv) lag time (λ, hours) and v) total cultivation time (t, hours). The cumulative biohydrogen production is a non-linear curve and in the present study, the parameters H_o, R and λ were estimated using the Curve Fitting Toolbox in MATLAB® (Version MATLAB R2012b, Curve Fitting Toolbox 3.3) with an associated 95% confidence limit. The total cumulative production, hydrogen production rates and lag phase time were used as parameters to compare the characteristics of the biohydrogen production systems. R software (OSX version 3.1.3) with the package Rcmdr (OSX version 2.1.7) was used for the statistical analysis of data obtained from the experiments. The *p* value was set at 0.05 and the significance of the results tested with *p* values: * < 0.05; ** < 0.01; *** < 0.001; while not significant results were with $p > 0.05$.

3. Results

3.1 Effect of the initial pH and combined effect of F/M ratio and pH on H_2 yields

The H_2 yields and the time required to achieve 95% of the maximum H_2 yield were plotted against the initial pH values (Fig. 1). The H_2 yields showed a decreasing trend to the increasing pH. Fig. 1 confirmed that H_2 production was favoured at the acidic pH range, i.e. at initial pH 4.5 and 5.0 with H_2 yields of 60.6 (± 9.0) and 50.7 (± 1.0) N mL H_2/g VS, respectively. This result is in agreement with the study reported by Khanal et al. (2004). The fermentative H_2 production patterns at the various pH values investigated are described by a modified Gompertz equation, as presented in Table 3 (Modeled plot is provided in Supplementary information S1). The different initial pH values in the tests were characterized by the differences shown in cumulative H_2 production, H_2 production rates and lag phase (Table 3). H_2 production rates (R, mL/h) were high at initial pH 7.0, however, higher rates were not co-related with higher H_2 yields (Fig. 1 and Table 3).

Unsurprisingly, the lag phase decreased when increasing the initial pH, which represents the time required for spore forming H_2 producers present in heat-treated ADS to germinate or adapt a sudden change of their environment (Ferchichi et al., 2005; Kim et al., 2011). Fig. 1 shows the time required to achieve 95% of the maximum H_2 yield decreased by increasing the initial pH, while the rate of H_2 production was higher at initial pH 7.0 (Table 3). H_2 production started faster at higher pH and lasted for a short time while it continued for longer time during the tests at lower pH. Thus, a decreasing lag phase did not correspond to an increase in H_2 yields. This can be explained by the methanogenic activities which started at higher initial pH, that was confirmed by the presence of methane in the biogas produced when H_2 production ceased completely. The final pH at the end of the tests was mainly lower than the initial pH (Table 3), which is mainly due to the production of VFAs (Table 3). As exception, the final pH in the batch tests with initial pH 4.5 was higher than the initial pH (Table 3), which could be due to the higher alkalinity of the inoculum (ADS) and the lower substrate concentration (F/M 0.5) used to avoid the use of chemical buffer. The final pH in all the tests were lower than 5.5, except for tests with initial pH 7.0 where the final pH was 6.6. This can be due to the higher alkalinity (buffering capacity) of the ADS inoculum (Table 1).

The concentrations of the main accumulated metabolites at the end of the tests are summarised in Table 3. Results confirm that different fermentation pathways occurred. The presence of propionate and ethanol generally does not indicate H_2 favorable pathways (Kim et al., 2011). The concentration of ethanol was comparatively higher in the tests with initial pH range 6.0 – 7.0, that could be linked to the low H_2 yields. In particular, the butyric to acetic acid ratio (B/A, mM:mM) co-related with the H_2 yields (Fig. 2). This observation is consistent with a study by Kim et al. (2006), which reported a higher corelation between B/A ratios (1.6 – 9.3) and H_2 yields. However, this ratio might not always give a good indication of high H_2 production. Guo et al. (2013) reported that the homoacetogenic activities can influence the concentration of end-metabolites due to acetate production from H_2 and CO_2. The presence of acetate in higher concentrations between pH 5.5 – 7.0 might indicate the prevailance of an homoacetogenic activity responsible of lower H_2 yields.

The results of the batch tests carried out at F/M ratios 0.5, 1.0 and 1.5 at two initial pH values (5.0 and 6.5) are presented in Table 4. Table 4 shows the major metabolites accumulated at the end of the tests. At the initial pH 5.0 and F/M ratios of 0.5, 1.0 and 1.5, H_2 yields were 50.7 (± 0.8), 60.3 (± 5.0) and 49.3 (± 12.2) mL H_2/g VS, respectively. Likewise, in tests carried out with an initial pH 6.5, respective H_2 yields of 28.2 (± 4.2), 43.2 (± 2.0) and 54.1 (± 4.4) mL H_2/g VS were obtained. An ANOVA analysis confirmed the significance of difference in H_2 yields at pH 5.0 and 6.5 for an F/M ratio of 0.5 (p value <0.05). However, it was not significant for F/M ratios 1.0 and 1.5 at both initial pH values tested. Likewise, at initial pH 5.0, the differences in H_2 yields were not significant for all the three tested F/M ratios. Interestingly, the differences in H_2 yields were significant (p value <0.05) at an initial pH of 6.5 for F/M ratios 0.5 and 1.5. This implies a combined influence of the F/M ratios and initial pH on dark fermentative H_2 production. The result also suggests that the comparable H_2 yields can be achieved through a combination of pH and F/M ratios by maximizing the utilization of substrates.

The different metabolites yields measured at the end of the batch tests explain the differences in H_2 yields (Table 4). The presence of different metabolites suggests a typical mixed type fermentation that can occur in complex substrates like food waste. Acetate yields were higher at initial pH 6.5 compared to pH 5.0, which was also confirmed in the tests carried out earlier at different initial pH (Table 3). Similarly, higher ethanol yields were obtained at increasing

F/M ratios and initial pH. High levels of butyrate yield at pH 6.5 and F/M ratios 1.0 and 1.5 can be associated to higher H_2 yields obtained in respective tests, as the production of butyrate is generally co-related to H_2 production (Kim et al., 2011).

3.2 Effect of alkaline substrate pre-treatment on H_2 yields

Fig. 3 shows the effects of alkaline substrate pre-treatment on biohydrogen production. The results illustrate that biohydrogen production can be significantly improved with alkaline pre-treatment of rice straw. As expected, the alkaline pre-treatment enhanced the saccharification of sugars from rice straw, which increased along with the concentration of NaOH. The COD values of hydrolysate after pre-treatment with 4% and 8% NaOH were 7.3 (± 0.8) and 8.3 (± 0.7) g/L respectively in comparison to the untreated rice straw with 3.8 (± 0.1) g/L soluble COD (determined with solid liquid ratio of 1:5). The results of end-product accumulation (Table 5) show that higher H_2 yields corresponded to higher B/A ratios (mM:mM), irrespective of the concentration of acids accumulated at the end of the tests.

3.3 Effect of inoculum sources on H_2 yields

The cumulative H_2 yields and accumulation of end metabolites during the application of two heat treated inoculum sources on biohydrogen production from OMWW is depicted in Fig. 4 and Table 6, respectively. The differences observed when using two inoculum types, i.e. ADS and WAS, at thermophilic temperature gave an indication of the level of inhibition of the polyphenols present in the OMWW on the microorganisms (Hamdi, 1992; Paraskeva and Diamadopoulos, 2006). The initial lag phase observed in Fig. 4 can give evidence for the adaptation of H_2 producing fermentative microbial communities to phenolic compounds present in OMWW. The maximum H_2 yield from OMWW with WAS was almost 2 fold higher than with ADS. In addition, WAS sludge required less heat-shock pre-treatment time to inhibit hydrogen consuming methanogens and showed a shorter lag phase (Fig. 4, Table 6). This shows that heat-shocked WAS is an appropriate inoculum for DF of OMWW for higher H_2 recovery.

The lower H_2 yield obtained from OMWW in tests inoculated with ADS is further supported by the analysis of the metabolic pathways (Table 6), which showed an accumulation of lactic acid. Metabolic pathways leading to lactic acid are not favorable to H_2 production (Hawkes et al., 2007), which explains the lower H_2 yields observed in the batch tests inoculated with ADS. Likewise, the higher levels of acetate in the tests carried out with WAS than ADS can explain the higher H_2 yields from OMWW, as acetate pathways generally yields to more H_2 per mole of glucose than the butyrate pathways (Hawkes et al., 2007).

4. Discussion

4.1 Effect of the pH and F/M ratio on H_2 yield

This study showed that higher H_2 yields can be achieved from easily biodegradable organic waste like food waste, when compared to other complex substrates such as rice straw (Table 7). This is mainly a result of the high fraction of easily degradable carbohydrates contained in food waste, as already suggested by Guo et al. (2013). The combination of initial pH and substrate concentration is important to avoid inhibition of H_2 producers through elevated VFA accumulation and consequent pH depletion, and high hydrogen partial pressure (Ginkel et al., 2001). This is likely the case of substrates like food waste which generally show faster hydrolysis kinetics compared to lignocellulosic biomass such as rice straw (Table 7), that requires higher optimal substrate concentrations or F/M ratios compared to food waste.

Table 7 compares the results of the H_2 yields observed in this study with literature data reported under similar conditions. The highest H_2 yields observed at initial pH 4.5 and 5.0 (60.6 ± 9 and 50.7 ± 1 mL H_2/ g VS food waste, respectively) in this study were in contrast with Cappai et al. (2014), who obtained the highest H_2 yield (56.2 mL H_2/ g VS food waste) at pH 6.5. This difference in optimum initial pH might be due to the higher substrate concentrations used by Cappai et al. (2014) (Table 7). Furthermore, two possible explanations can be given for the relationship between initial pH (4.5 and 5.0) and the higher H_2 production: (i) a selection of hydrogen producers at pH range (4.5 – 5.0) and (ii) an inhibition of H_2 consuming methanogens. In addition, the differences in metabolic products accumulating at different initial pH ranges might support the growth of different microbial communinities which can influence the H_2 production as reported in the studies from Fang and Liu (2002) and Lee et al. (2008). Khanal et al. (2004) reported that a microbial shift to solventogenesis did not occur at a pH range 4.5 – 6.5, which provides further evidence of the importance of the initial microbial community and pH to reach higher H_2 yields. In addition, native microbial organisms present in the food waste might also influence the DF process in real conditions (waste type and storing conditions). In this study, the storage of food waste at freezing conditions might have impacted native microorganisms. Nevertheless, the comparison of the results between the tests operated at different initial pH remains unaffected as uniform substrates were used.

At lower F/M ratios (0.5 and 1.0), an initial of pH 5.0 favored the H_2 production whereas it was the inverse at a F/M ratio 1.5 and initial pH 6.5. At the initial of pH 5.0 and F/M 1.5, a lower H_2 yield was observed, which might be due to the shock load on the microbial systems. This was also confirmed in the study of Ginkel et al. (2001), who reported an inhibition of H_2 production at higher substrate loading rates due to shock loads. The conversion of substrates to metabolic products at pH 5.0 and F/M 1.5 is lower than at F/M ratios 0.5 and 1.0, which can be due to an inhibition of the substrate conversion. In addition, a low final pH (4.5 ± 0.1) at the end of the test at pH 5.0 and F/M 1.5 (Table 4) suggests that H_2 production might be inhibited due to a 'load shock'. This can be supported by the time required to achieve 95% of the maximum H_2 yield (t_{95} = 47 days) (Table 4). Pan et al. (2008) reported that a F/M ratio of 6.0 as appropriate for thermophilic (50 ± 2 °C) fermentation of food waste (Table 7). However, the initial pH in their study varied from 6.2 to 6.7. Therefore, in the DF systems where initial pH is not buffered, H_2 production is a combined function of suitable F/M ratio and initial pH. Likewise, an optimal operational pH range could be maitained through subsequent substrate feeding strategies which can garantee higher H_2 production and avoid the H_2 consuming activities i.e. methanogens and homoacetogens.

4.2 Effect of alkaline substrate pre-treatment on H_2 yield

The alkaline pre-treatment method applied in this study aimed at improving hydrolysis and solubilization of the organic matter that limit the dark fermentative substrate conversion (Monlau et al., 2015, 2013b). However, the level of effectiveness of the different pre-treatment methods depends on the nature of the substrate (Ariunbaatar et al., 2014; Carlsson et al., 2012). In the study of Monlau et al. (2013c), H_2 yields from sunflower stalks increased from 2.3 (± 0.9) to 4.4 (± 2.6) mL H_2/g VS, while in our study an increase from 0.3 (± 0.1) to 6.6 (± 0.1) from mL H_2/g VS from rice straw as the substrate was achieved under similar conditions of thermo-alkaline pre-treatment (Fig. 3 and Table 7). Meanwhile, H_2 yields further increased to 15.7 (± 1.0) mL H_2/g VS when 8 % w/w NaOH was applied (Fig. 3). This H_2 yield is lower than the value reported by Chen et al. (2012) with untreated rice straw, i.e. 24.8 mL/g TS at a substrate concentration of 90 g TS/L, whereas, it is 2.2 fold higher when the substrate concentration was 30 g TS/L (i.e. 7.1 mL H_2/g TS). This disagreement might be due to physico-chemical properties of the lignocellulosic substrates, such as particle sizes, soluble carbohydrates content and/or substrate concentration (Monlau et al., 2013a). Chen et al. (2012) reported an increasing trend of H_2 yields, when the particle size of rice straw decreased from 10 mm to < 0.297 mm. In their study, a maximum H_2 yield was obtained with a particle size of < 0.297 mm (6.4 mL H_2/g TS) at a substrate concentration of 30 g TS/L.

The effects of the chemical agents applied (NaOH) and or by-products formed (furfural, phenols) during the pre-treatment process and the response on the dark fermentative microbial community should be taken into consideration while selecting appropriate pre-treatment method. Kim et al. (2009) reported a decrease in H_2 yields when the Na^+ concentration in a continuous DF reactor gradually increased from 0.27 to 21.00 g Na^+/L while the acclimatized fermentative community maintained their activity up to 6.00 g Na^+/L. Nonetheless, in this study, the H_2 yields increased when 8 % w/w NaOH was applied compared to 4 % w/w NaOH (Fig. 3). Moreover, under similar pre-treatment conditions, 12 % w/w NaOH (i.e. 5.40 g Na^+/L) might either enhance the H_2 yields or exert effect on fermentative microbial community, depending on the inocula type and adaptation to Na^+ concentration. However, the application of pre-treatment methods should be based on the substrate type (biodegradability or bioavailability of easily fermentable carbohydrates), their practicability and economy viability.

4.3 Effect of inocula on H_2 yield

The application of two different inoculum types for the DF of OMWW showed differences in response of ADS and WAS in terms of dark fermentative conversion to H_2 and other metabolites (Fig. 4 and Table 6). Comparatively, WAS exhibited better performances in terms of H_2 production as shown by the H_2 production yields and kinetics in Table 6. The difference in H_2 yields might be a result of the effect of polyphenolic substances present in OMWW (total phenols in Table 1) on the fermentative communities present in ADS and WAS (Hamdi, 1992; Ntaikou et al., 2009). Ntaikou et al. (2009) used diluted OMWW to avoid growth inhibition, whereas, Hamdi (1992) observed an inhibition mainly on methanogens. Nonetheless, the difference in response of the two inocula could be also due to the difference in heat shock treatment time applied during the HST. ADS required a longer HST time to inhibit the activity of methanogens (Ghimire et al., 2015b) compared to WAS which has an aerobic origin. Therefore, the treatment time could have impacted the microbial communities that could contribute to fermentative H_2 production.

The use of WAS as better inoculum is supported by the studies of Chen et al. (2012) and Kim et al. (2011). Chen et al. (2012) achieved higher H_2 yields with a sludge originated from a municipal wastewater treatment plant when compared with other inoculum sources like cow dung, compost and paper mill sludge. The group attributed higher H_2 yields to the presence of a potential hydrolytic and fermentative bacterial microbial community. Kim et al. (2011) hypothesized that such increase in H_2 yields from sewage sludge addition was due to the presence of iron (Fe), calcium (Ca) and phosphorous (P) at much higher concentrations (no information on speciation was given). Further research on the nutrient and trace metal content in inocula and how these affect the DF rates is thus required.

The selection and application of various optimum operational parameters depends highly on the type of substrate, i.e. mainly its biodegradability. However, the improvement of dark fermentative H_2 production should bear the cost of application of different optimal operational parameters in terms of net energy and economy gain. It should be taken into consideration that DF of waste biomass is not a complete conversion of organic waste, i.e.

organic acids and alcohols accumulate in the effluent, for which a subsequent treatment needs to be provided. Valorization of these by-products can support the costs associated with the optimization of the DF process. Several studies have suggested the integration of DF with processes such as photofermentation (H_2), bioelectrochemical systems (H_2) and anaerobic digestion (CH_4) for further energy recovery and production of platform molecules of economic interest, such as biopolymers (Bastidas-Oyanedel et al., 2015; ElMekawy et al., 2014; Ghimire et al., 2015c; Xia et al., 2013).

5. Conclusion

This study aimed to investigate the optimal operational parameters in the thermophilic DF of three types of complex wastes biomass with varying biodegradability, i.e. food waste, rice straw and OMWW. The DF applied to food waste was favored in the acidic pH range (4.5-5.0), though an appropriate substrate concentration that must be considered while selecting an acidic pH range. F/M ratios of 0.5 and 1.0 at an initial pH of 5.0 gave, respectively, 1.8 and 1.4 folds higher H_2 yields than at initial pH 6.5. Likewise, F/M ratios and pH can be optimized to achieve higher substrate utilization and H_2 yields. During the tests, higher B/A ratios (mM:mM) were associated with higher H_2 yields, a B/A ratio equivalent to 1.5 was related to the optimal H_2 yield. Similarly, pre-treatment of rice straw with 4% NaOH and 8% NaOH at 55 °C for 24 hours increased the H_2 yield by 26 and 57 fold, respectively. Furthermore, WAS showed adaptability to OMWW containing phenols and gave a nearly 2 fold higher H_2 yield when compared to ADS. In conclusion, the selection and application of optimal operational parameters for the optimization of H_2 production rely mainly on the substrate biodegradability. Therefore, these parameters should be optimized for each particular type of substrate prior to further application in scaled-up DF systems.

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Reference

Abreu, A.A.A., Danko, A.S.S., Costa, J.C.C., Ferreira, E.C.C., Alves, M.M.M., 2009. Inoculum type response to different pHs on biohydrogen production from l-arabinose, a component of hemicellulosic biopolymers. Int. J. Hydrogen Energy 34, 1744–1751. doi:10.1016/j.ijhydene.2008.12.020

Alibardi, L., Cossu, R., 2015. Effects of carbohydrate, protein and lipid content of organic waste on hydrogen production and fermentation products. Waste Manag. doi:10.1016/j.wasman.2015.07.049

American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association Water Works Association, American Water Environment Federation, Washington DC.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. doi:10.1016/j.apenergy.2014.02.035

Bastidas-Oyanedel, J.-R., Bonk, F., Thomsen, M.H., Schmidt, J.E., 2015. Dark fermentation biorefinery in the present and future (bio)chemical industry. Rev. Environ. Sci. Bio/Technology. doi:10.1007/s11157-015-9369-3

Bellucci, M., Botticella, G., Francavilla, M., Beneduce, L., 2015. Inoculum pre-treatment affects the fermentative activity of hydrogen-producing communities in the presence of 5-hydroxymethylfurfural. Appl. Microbiol. Biotechnol. 89–91. doi:10.1007/s00253-015-7002-6

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Cappai, G., De Gioannis, G., Friargiu, M., Massi, E., Muntoni, A., Polettini, A., Pomi, R., Spiga, D., 2014. An experimental study on fermentative H2 production from food waste as affected by pH. Waste Manag. 34, 1510–1519. doi:10.1016/j.wasman.2014.04.014

Carlsson, M., Lagerkvist, A., Morgan-Sagastume, F., 2012. The effects of substrate pre-treatment on anaerobic digestion systems: a review. Waste Manag. 32, 1634–50. doi:10.1016/j.wasman.2012.04.016

Chen, C.-C., Chuang, Y.-S., Lin, C.-Y., Lay, C.-H., Sen, B., 2012. Thermophilic dark fermentation of untreated rice straw using mixed cultures for hydrogen production. Int. J. Hydrogen Energy 37, 15540–15546. doi:10.1016/j.ijhydene.2012.01.036

De Gioannis, G., Friargiu, M., Massi, E., Muntoni, A., Polettini, A., Pomi, R., Spiga, D., 2014. Biohydrogen production from dark fermentation of cheese whey: Influence of pH. Int. J. Hydrogen Energy 39, 20930–20941. doi:10.1016/j.ijhydene.2014.10.046

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

ElMekawy, A., Srikanth, S., Vanbroekhoven, K., De Wever, H., Pant, D., 2014. Bioelectro-catalytic valorization of dark fermentation effluents by acetate oxidizing bacteria in bioelectrochemical system (BES). J. Power Sources 262, 183–191. doi:10.1016/j.jpowsour.2014.03.111

Fang, H.H.P., Liu, H., 2002. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour. Technol. 82, 87–93.

Ferchichi, M., Crabbe, E., Gil, G.-H., Hintz, W., Almadidy, A., 2005. Influence of initial pH on hydrogen production from cheese whey. J. Biotechnol. 120, 402–9. doi:10.1016/j.jbiotec.2005.05.017

Fernandes, T. V, Bos, G.J.K., Zeeman, G., Sanders, J.P.M., van Lier, J.B., 2009. Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. Bioresour. Technol. 100, 2575–9. doi:10.1016/j.biortech.2008.12.012

Gadhamshetty, V., Arudchelvam, Y., Nirmalakhandan, N., Johnson, D.C., 2010. Modeling dark fermentation for biohydrogen production: ADM1-based model vs. Gompertz model. Int. J. Hydrogen Energy 35, 479–490. doi:10.1016/j.ijhydene.2009.11.007

Ghimire, A., Frunzo, L., Pirozzi, F., Trably, E., Escudie, R., Lens, P.N.L., Esposito, G., 2015a. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Appl. Energy 144, 73–95. doi:10.1016/j.apenergy.2015.01.045

Ghimire, A., Frunzo, L., Salzano, E., Panico, A., Lens, P.N.L., Pirozzi, F., 2015b. Biomass Enrichment and Scale-up Implications for Dark Fermentation Hydrogen Production with Mixed Cultures. Chem. Eng. Trans. 43, 391–396. doi:10.3303/CET1543066

Ghimire, A., Valentino, S., Frunzo, L., Trably, E., Escudié, R., Pirozzi, F., Lens, P.N.L., Esposito, G., 2015c. Biohydrogen production from food waste by coupling semi-continuous dark-photofermentation and residue post-treatment to anaerobic digestion: A synergy for energy recovery. Int. J. Hydrogen Energy 40(46), 16045–16055. doi:10.1016/j.ijhydene.2015.09.117

Ginkel, S. V, Sung, S., Lay, J.J., 2001. Biohydrogen production as a function of pH and substrate concentration. Environ. Sci. Technol. 35, 4726–4730.

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

Guo, X.M., Trably, E., Latrille, E., Carrère, H., Steyer, J.-P., 2010. Hydrogen production from agricultural waste by dark fermentation: A review. Int. J. Hydrogen Energy 35, 10660–10673. doi:10.1016/j.ijhydene.2010.03.008

Hallenbeck, P.C., Ghosh, D., Skonieczny, M.T., Yargeau, V., 2009. Microbiological and engineering aspects of biohydrogen production. Indian J. Microbiol. 49, 48–59. doi:10.1007/s12088-009-0010-4

Hamdi, M., 1992. Toxicity and biodegradability of olive mill wastewaters in batch anaerobic digestion. Appl. Biochem. Biotechnol. 37, 155–163. doi:10.1007/BF02921667

Hawkes, F., Hussy, I., Kyazze, G., Dinsdale, R., Hawkes, D., 2007. Continuous dark fermentative hydrogen production by mesophilic microflora: Principles and progress. Int. J. Hydrogen Energy 32, 172–184. doi:10.1016/j.ijhydene.2006.08.014

Kapdan, I.K., Kargi, F., 2006. Bio-hydrogen production from waste materials. Enzyme Microb. Technol. 38, 569–582. doi:10.1016/j.enzmictec.2005.09.015

Khanal, S., Chen. WH., Li, L. and Sung, S., 2004. Biological hydrogen production: effects of pH and intermediate products. Int. J. Hydrogen Energy 29, 1123–1131. doi:10.1016/j.ijhydene.2003.11.002

Kim, D.-H., Kim, S.-H., Jung, K.-W., Kim, M.-S., Shin, H.-S., 2011. Effect of initial pH independent of operational pH on hydrogen fermentation of food waste. Bioresour. Technol. 102, 8646–52. doi:10.1016/j.biortech.2011.03.030

Kim, D.-H., Kim, S.-H., Shin, H.-S., 2009. Sodium inhibition of fermentative hydrogen production. Int. J. Hydrogen Energy 34, 3295–3304. doi:10.1016/j.ijhydene.2009.02.051

Kim, D.-H.H., Kim, S.-H.H., Kim, H.-W.W., Kim, M.-S.S., Shin, H.-S.S., 2011. Sewage sludge addition to food waste synergistically enhances hydrogen fermentation performance. Bioresour. Technol. 102, 8501–8506. doi:10.1016/j.biortech.2011.04.089

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

Lee, Z.-K., Li, S.-L., Lin, J.-S., Wang, Y.-H., Kuo, P.-C., Cheng, S.-S., 2008. Effect of pH in fermentation of vegetable kitchen wastes on hydrogen production under a thermophilic condition. Int. J. Hydrogen Energy 33, 5234–5241. doi:10.1016/j.ijhydene.2008.05.006

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J.-P., Carrère, H., 2013a. Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment. Crit. Rev. Environ. Sci. Technol. 43, 260–322.

Monlau, F., Kaparaju, P., Trably, E., Steyer, J.P., Carrere, H., 2015. Alkaline pretreatment to enhance one-stage CH4 and two-stage H2/CH4 production from sunflower stalks: Mass, energy and economical balances. Chem. Eng. J. 260, 377–385. doi:10.1016/j.cej.2014.08.108

Monlau, F., Sambusiti, C., Barakat, A., Guo, X.M., Latrille, E., Trably, E., Steyer, J.-P., Carrere, H., 2012. Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. Environ. Sci. Technol. 46, 12217–25. doi:10.1021/es303132t

Monlau, F., Trably, E., Barakat, A., Hamelin, J., Steyer, J.-P., Carrere, H., 2013b. Two-stage alkaline-enzymatic pretreatments to enhance biohydrogen production from sunflower stalks. Environ. Sci. Technol. 47, 12591–9. doi:10.1021/es402863v

Monlau, F., Trably, E., Barakat, A., Steyer, J., 2013c. Two-Stage Alkaline − Enzymatic Pretreatments To Enhance Biohydrogen Production from Sun flower Stalks.

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

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

Ntaikou, I., Kourmentza, C., Koutrouli, E.C., Stamatelatou, K., Zampraka, A, Kornaros, M., Lyberatos, G., 2009. Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. Bioresour. Technol. 100, 3724–30. doi:10.1016/j.biortech.2008.12.001

Pakarinen, O., Lehtomäki, A., Rintala, J., 2008. Batch dark fermentative hydrogen production from grass silage: The effect of inoculum, pH, temperature and VS ratio. Int. J. Hydrogen Energy 33, 594–601. doi:10.1016/j.ijhydene.2007.10.008

Pan, C., Zhang, S., Fan, Y., Hou, H., 2010. Bioconversion of corncob to hydrogen using anaerobic mixed microflora. Int. J. Hydrogen Energy 35, 2663–2669. doi:10.1016/j.ijhydene.2009.04.023

Pan, J., Zhang, R., Elmashad, H., Sun, H., Ying, Y., 2008. Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. Int. J. Hydrogen Energy 33, 6968–6975. doi:10.1016/j.ijhydene.2008.07.130

Paraskeva, P., Diamadopoulos, E., 2006. Technologies for olive mill wastewater (OMW) treatment : a review 1485, 1475–1485. doi:10.1002/jctb

Sambusiti, C., Monlau, F., Ficara, E., Carrère, H., Malpei, F., 2013. A comparison of different pre-treatments to increase methane production from two agricultural substrates. Appl. Energy 104, 62–70. doi:10.1016/j.apenergy.2012.10.060

Sarma, S.J., Pachapur, V., Brar, S.K., Le Bihan, Y., Buelna, G., 2015. Hydrogen biorefinery: Potential utilization of the liquid waste from fermentative hydrogen production. Renew. Sustain. Energy Rev. 50, 942–951. doi:10.1016/j.rser.2015.04.191

Shin, H.-S., Youn, J.-H., Kim, S.-H., 2004. Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis. Int. J. Hydrogen Energy 29, 1355–1363. doi:10.1016/j.ijhydene.2003.09.011

Show, K.Y., Lee, D.J., Tay, J.H., Lin, C.Y., Chang, J.S., 2012. Biohydrogen production: Current perspectives and the way forward. Int. J. Hydrogen Energy 37, 15616–15631. doi:10.1016/j.ijhydene.2012.04.109

Urbaniec, K., Bakker, R.R., 2015. Biomass residues as raw material for dark hydrogen fermentation – A review. Int. J. Hydrogen Energy 40, 3648–3658. doi:10.1016/j.ijhydene.2015.01.073

Valdez-vazquez, I., Riosleal, E., Esparzagarcia, F., Cecchi, F., Poggivaraldo, H., 2005. Semi-continuous solid substrate anaerobic reactors for H2 production from organic waste: Mesophilic versus thermophilic regime. Int. J. Hydrogen Energy 30, 1383–1391. doi:10.1016/j.ijhydene.2004.09.016

VALORGAS, 2010. Compositional analysis of food waste from study sites in geographically distinct regions of Europe-Valorisation of food waste to biogas. Available from:

http://www. valorgas.soton.ac.uk/Deliverables/VALORGAS_241334_D2-1_rev[1]_130106.pdf [accessed 12.01.2013]

Wang, J., Wan, W., 2009. Factors influencing fermentative hydrogen production: A review. Int. J. Hydrogen Energy 34, 799–811. doi:10.1016/j.ijhydene.2008.11.015

Wang, J., Wan, W., 2009. Kinetic models for fermentative hydrogen production: A review. Int. J. Hydrogen Energy 34, 3313–3323. doi:10.1016/j.ijhydene.2009.02.031

Wong, Y.M., Wu, T.Y., Juan, J.C., 2014. A review of sustainable hydrogen production using seed sludge via dark fermentation. Renew. Sustain. Energy Rev. 34, 471–482. doi:10.1016/j.rser.2014.03.008

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

Figure Captions

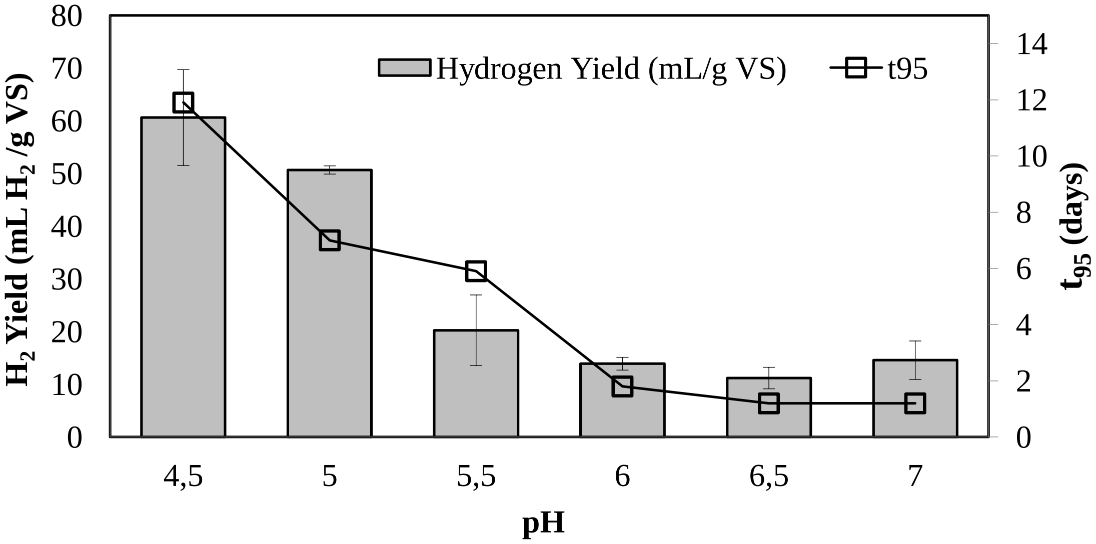
Fig. 1 Effect of initial pH on H_2 yield and time required for H_2 production to achieve 95% of the maximum yield during the DF of food waste at F/M ratio 0.5 and thermophilic temperature (55±1 °C) using ADS

Fig. 2 H_2 yields and B/A ratio as a function of pH in the thermophilic DF of food waste at F/M ratio 0.5

Fig. 3 Effect of alkaline pre-treatment of rice straw on H_2 yields

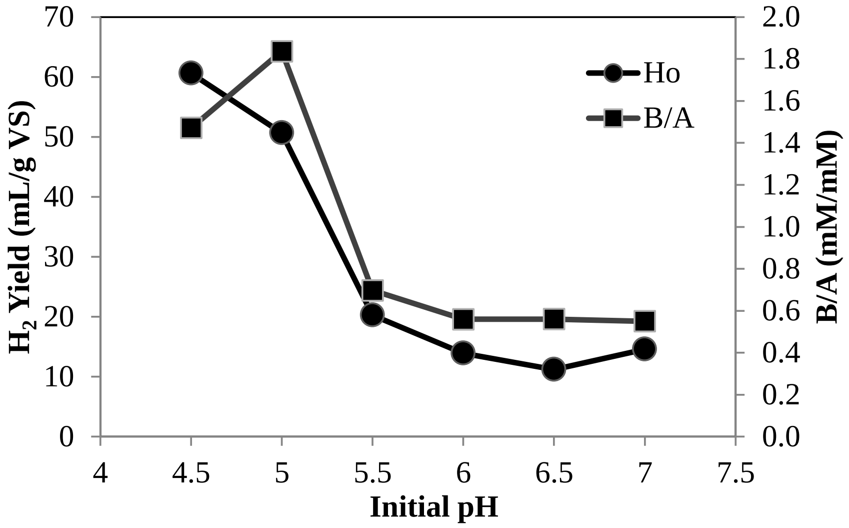
Fig. 4 Effect of inoculum source on cumulative H_2 production from the DF of OMWW using ADS (anaerobic digested sludge) and WAS (waste activated sludge) as inoculum

Fig. 1



Hydrogen Yield (mL/g VS)
t95
H2 Yield (mL H2 /g VS)
t95 (days)
80
70
60
50
40
30
20
10
0
14
12
10
8
6
4
2
0
4,5
5
5,5
6
6,5
7
pH

Fig. 2



70
60
50
40
30
20
10
0
H_2 Yield (mL/g VS)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B/A (mM/mM)
Ho
B/A
4
4.5
5
5.5
6
6.5
7
7.5
Initial pH

Fig. 3

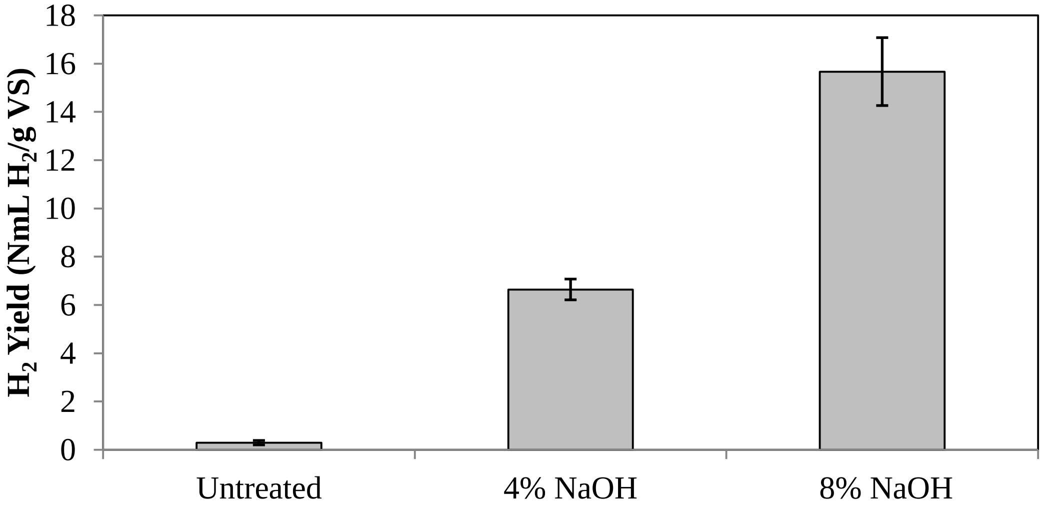


Fig. 4

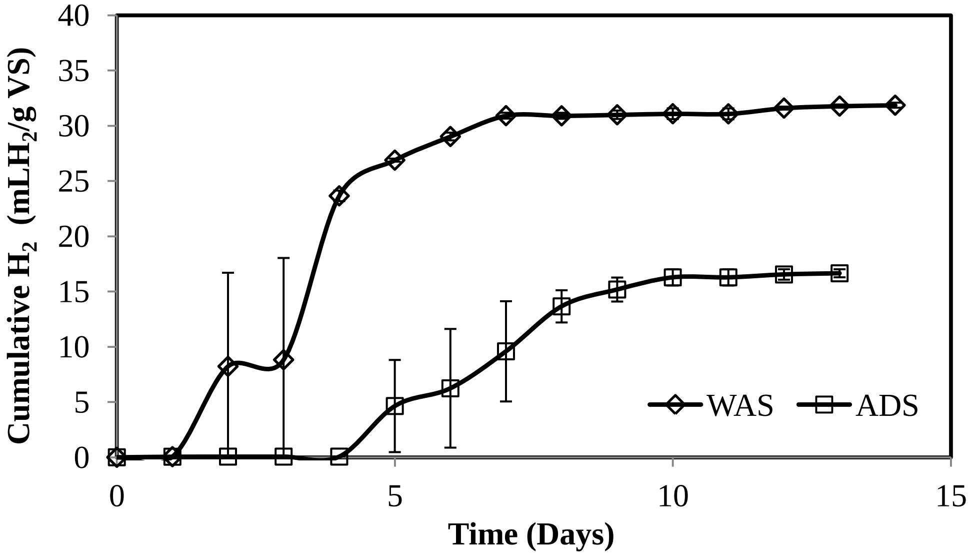

Cumulative H2 (mLH2/g VS)
40
35
30
25
20
15
10
5
0
0
5
10
15
Time (Days)
WAS
ADS

Table 1. Characteristics of the substrates and inocula used in this study

Characteristics	Food waste	OMWW	Rice Straw	ADS	WAS
pH	4.4 ± 0.1	4.6 ± 0.1	NA	8.3 ± 0.1	7.0 ± 0.1
Chemical Oxygen Demand (COD)	347.6 ± 47.0 $g/kg_{food\ waste}$	141.5 ± 13.0 g/L_{OMWW}	NA	NA	NA
Total solids	21.0 ± 0.1 %	4.7 ± 0.1 %	92.3 ± 0.2 %	2.33 ± 0.4 %	2.9 ± 0.2%
Volatile solids	20.2 ± 0.1 %	3.1 ± 0.3 %	80.9 ± 0.6 %	1.93 ± 0.1 %	1.8 ± 0.1%
Carbohydrate content	105.8 ± 0.7 $g/kg_{food\ waste}$	12.9 ± 0.2 g/L_{OMWW}	NA	NA	NA
Lipids	17.5 ± 1.0 $g/kg_{food\ waste}$	45.3 ± 4.0 g/L_{OMWW}	NA	NA	NA
TKN	6.4 ± 0.2 $g/kg_{food\ waste}$	0.5 g/L_{OMWW}	NA	NA	NA
NH_4-N	NA	NA	NA	283.5 ± 11.0 mg NH_4-N/L	203.1 ± 3.0 mg NH_4-N/L
Alkalinity	NA	NA	NA	1437.2 ± 14 mg $CaCo_3$/L	2605.7 ± 70.0 mg $CaCo_3$/L
Total phenols	NA	1.16 ± 0.03 g/L_{OMWW}	NA	NA	NA

NA-Not Analyzed

Table 2. Experimental conditions applied in the DF batch tests of the tested substrates

Investigation	Substrate	Inoculum	Initial pH	F/M
Effect of initial pH	Food waste	ADS	4.5, 5.0, 5.5, 6.0, 6.5 and 7.0	0.5
Combined effect of food waste and initial pH	Food waste	ADS	5.0 and 6.5	0.5, 1.0 and 1.5
Effect of pre-treatment of substrate	Rice straw	WAS	6.5	7.0
Effect of inoculum source and pre-treatment	OMWW	WAS and ADS	6.0	1.0

747 **Table 3.** Effects of initial pH on H_2 production performance and characteristics of accumulated end products in DF of food waste at F/M 0.5

Initial pH	Parameters derived from modified Gompertz model				Characteristics of digestate at the end of DF					
	H_o (mL/gVS)	L (h)	R (mL/h)	R^2	Average final pH	H_2 (mM/kg VS)	Acetate (mM/kg VS)	Propionate (mM/kg VS)	Butyrate (mM/kg VS)	Ethanol (mM/kg VS)
4.5	57.3	113.6	0.7	0.993	4.7 ± 0.1	1341.2 ± 201.3	1854.6 ± 114.0	964.5 ± 99.1	2728.7 ± 359.6	263.7 ± 16.1
5.0	50.9	68.1	1.0	0.999	4.9 ± 0.1	1121.3 ± 17.2	1611.8 ± 412	1686.7 ± 253.3	3018.7 ± 109.7	753.4 ± 290.6
5.5	20.3	41.2	0.4	0.995	5.2 ± 0.6	448.4 ± 148.2	2830.2 ± 381.0	1358.1 ± 392.1	1973.7 ± 374.9	623.7 ± 53.8
6.0	15.4	2.0	0.7	0.997	5.3 ± 0.1	308.0 ± 26.8	3558.9 ± 368.7	959.7 ± 6.4	1992.0 ± 238.1	2340.9 ± 263.7
6.5	11.2	3.3	0.8	0.995	5.5 ± 0.1	247.7 ± 45.3	3900.2 ± 838.5	260.0 ± 34.8	2185.5 ± 580.1	3056.7 ± 32.3
7.0	14.6	25.3	6.7	1.000	6.6 ± 0.1	322.6 ± 80.7	5922.4 ± 43.9	877.2 ± 41.4	3255.6 ± 308.1	1673.6 ± 48.4

748 R^2 represents the regression coefficient

Table 4. Effects of initial pH and F/M ratio on H_2 production performance and characteristics of accumulated end products in DF of food waste

pH	F/M	Parameters derived from modified Gompertz model					Characteristics of digestate at the end of DF							
		H_o (mL/g VS)	L (h)	R (mL/h)	t_{95} (day)	R^2	Average final pH	H_2 (mM/kg VS)	Lactate (mM/kg VS)	Acetate (mM/kg VS)	Propionate (mM/kg VS)	Butyrate (mM/kg VS)	Ethanol (mM/kg VS)	Caproate (mM/kg VS)
	0.5	50.9	68.1	1.0	7.0	0.949	4.9 ± 0.1	2264.9 ± 34.8	17.5 ± 8.1	1610.7 ± 411.8	1687.0 ± 253.3	3018.7 ± 109.7	753.4 ± 290.6	0.0 ± 0.0
5.0	1.0	58.5	81.9	1.4	9.7	0.997	4.7 ± 0.1	2690.9 ± 206.5	18.1 ± 2.2	1264.0 ± 27.1	3135.4 ± 245.7	2959.9 ± 35.2	1876.5 ± 5.9	0.0 ± 0.0
	1.5	54.2	87.9	0.3	46.5	0.991	4.5 ± 0.1	2202.1 ± 545.2	98 ± 10.3	420.3 ± 119.7	842.8 ± 59.2	2638.1 ± 202.9	1402.9 ± 325.6	0.0 ± 0.0
	0.5	11.2	3.4	0.8	1.2	0.995	5.5 ± 0.1	1259.7 ± 188.4	0.0 ± 0.0	6043.0 ± 357.2	830.3 ± 38.9	2344.0 ± 73.3	3056.7 ± 32.3	0.0 ± 0.0
6.5	1.0	42.6	17.0	1.6	4.6	0.938	5.7 ± 0.1	1928.7 ± 89.3	126.3 ± 124.2	1700.0 ± 305.8	775.8 ± 91.1	2062.9 ± 169.1	3602.1 ± 20.7	70.3 ± 9.4
	1.5	56.9	2.3	1.8	7.0	0.944	5.3 ± 0.1	2413.4 ± 197.0	0.0 ± 0.0	2364.5 ± 216.1	655.5 ± 166.3	2410.5 ± 47.5	2206.0 ± 63.1	263.3 ± 23.1

Table 5. Effect of substrate pre-treatment on H_2 production performance measured by the modified Gompertz model and characteristics of accumulated end products in DF of rice straw

Pre-treatment method	Parameters derived from modified Gompertz model				Characteristics of digestate at the end of DF						
	H_o (mL/g VS)	L (h)	R (mL/h)	R^2	Average final pH	H_2 (mM/kg VS)	Acetate (mM/kg VS)	Propionate (mM/kg VS)	Butyrate (mM/kg VS)	Ethanol (mM/kg VS)	B/A (mM:mM)
Without treatment	0.3	37.3	0.1	0.958	4.7 ± 0.1	12.8 ± 4.1	462.6 ± 42.7	50.8 ±15.8	46.4 ±13.7	41.0 ± 7.2	0.10
4% NaOH	6.7	23.9	2.9	0.999	4.9 ± 00	296.3 ± 19.2	775.0 ± 13.5	189.4 ±18.5	227.7 ± 38.5	129.4 ± 44.8	0.29
8% NaOH	15.4	11.3	3.6	0.965	5.2 ± 0.6	699.4 ± 62.8	468.6 ± 84.4	55.6 ± 15.4	614.1 ±105.8	148.9 ± 11.8	1.31

Table 6. Effects of inoculum source on H_2 production performance measured by the modified Gompertz model and characteristics of accumulated end products in DF of OMWW

Inoculum type	Parameters derived from modified Gompertz model				Characteristics of digestate at the end of DF							
	H_o (mL/g VS)	L (h)	R (mL/h)	R^2	Average final pH	H_2 (mM/kg VS)	Lactate (mM/kg VS)	Acetate (mM/kg VS)	Propionate (mM/kg VS)	Butyrate (mM/kg VS)	Ethanol (mM/kg VS)	B/A (mM:mM)
ADS	106.1	101.0	1.0	0.996	5.6 ± 0.1	751.2 ± 15.2	1651.8 ± 573.4	1752.2 ± 510.9	269.5 ± 183.3	4293.5 ± 93.1	3423.2 ± 1104.2	1.95
WAS	204.1	34.4	2.2	0.984	5.5 ± 0.2	1479.7 ± 46.3	0.0 ± 0.0	6823.0 ± 904.1	282.0 ± 217.1	5062.5 ± 131.0	3022.6 ± 0.8	0.44

Table 7. Summary of various strategies to improve the H_2 yields from substrates with different biodegradability

Substrates	Optimization parameters	Optimal conditions	Substrate concentrati on (g VS/L)	Culture system	H_2 Yield (NmL/g VS_{added})	Reference
Food waste	Initial pH (4.5-8.5)	pH 6.5	53.1 ± 0.9	Activated sludge, 39 °C, batch	56.2	(Cappai et al., 2014)
Food waste	Initial pH (4.5-7)	pH 4.5 – 5.0	3.4	Anaerobic sludge, 55 ± 2 °C, batch	61.0 ± 9.0 at pH 4.5 51.0 ± 1.0 at pH 5.0	This study
Food waste	F/M ratio (1-10)	F/M ratio of 6.0	18.5	Anaerobic sludge, thermophilic (50 °C), batch	39.0	(Pan et al., 2008)
Food waste	F/M ratio (0.5, 1, 1.5) at pH 5 & 6.5	F/M ratio of 1 at pH 5.0	6.1	Anaerobic sludge, 55 ± 2 °C, batch	60.3 ± 5.0	This study
Sun flower stalks	Substrate pre-treatment (thermo-alkaline)	4% NaOH at 55 °C, 24 hour	5.0	Anaerobic sludge, 35 °C, pH 5.5	4.4 ± 2.6	(Monlau et al., 2013b)
Rice straw	Thermal alkaline pre-treatment	8% NaOH at 55 °C, 24 hour	43.0	Activated sludge, thermophilic (55 °C), initial pH 6.0, batch	15.7 ± 1.0	This study
Rice straw	Inoculum source (MWWS[b], PMS[c] & CDC[d])	MWWS	30.0 g TS/L	55 ^{0} C, initial pH 6.5, batch	7.1[e]	(Chen et al., 2012)
OMWW	Inoculum source (activated sludge & anaerobic digestate)	Activated sludge	10.5	55 °C, initial pH 6.0, batch	33.1 ± 1.0	This study

[a]N L H_2/kg total organic carbon
[b]MWWS: Municipal wastewater plant sludge
[c]PMS: Paper Mill Sludge
[d]CDS: Cow Dung Compost
[e]mL H_2/g TS

Supplementary Information on

Effects of operational parameters on dark fermentative hydrogen production from biodegradable complex waste biomass

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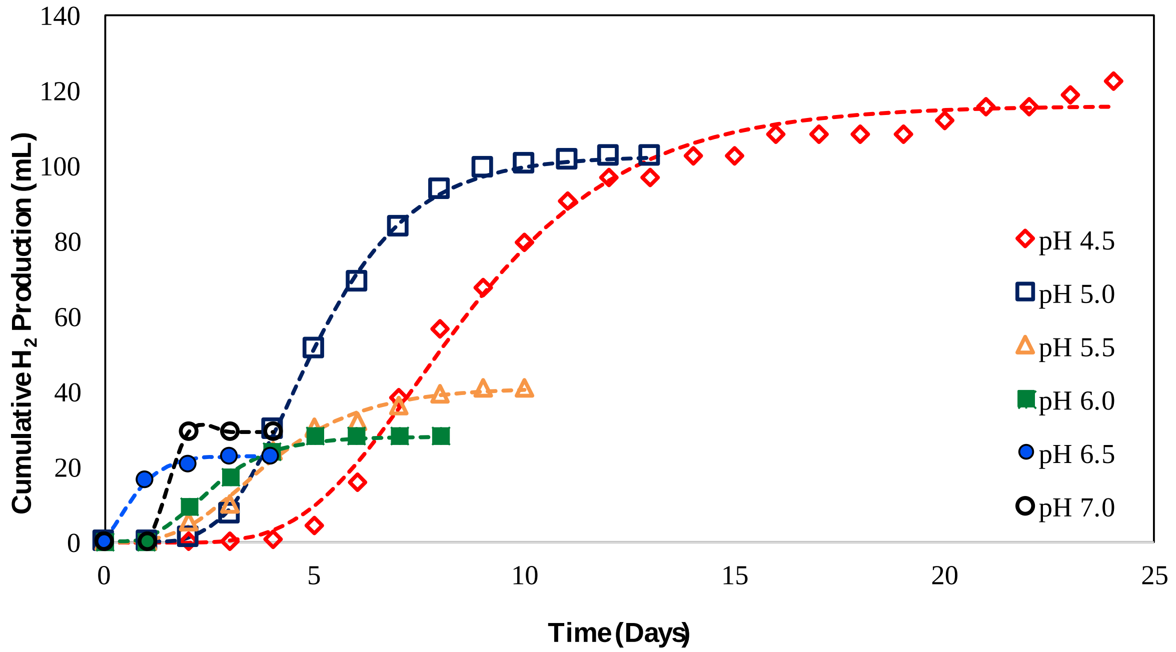

Fig. S1. Cumulative H_2 production at different initial pH values using food waste at a F/M ratio 0.5 and ADS as inoculum (dotted lines represents the results from a modified Gompertz model)