EFFECT OF pH ON THE ANAEROBIC FERMENTATION OF FRUIT/VEGETABLES AND DISPOSABLE NAPPIES HYDROLYSATE FOR BIO-HYDROGEN PRODUCTION

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Abstract

- **Purpose:** The objective of this work was to optimize the anaerobic fermentation of a mixed waste stream, consisted of fruit and vegetables (FVW) that have lost their marketing value and a disposable nappies’ hydrolysate (DN). More specifically, the aim was to identify the optimal pH value for maximum hydrogen production and valuable metabolites such as volatile fatty acids and ethanol.

- **Methods:** A wide range of pH values was tested (from 4.5 to 7.5 with 0.5 increment) using an automatic controller system, in batch fermentations that took place in mesophilic temperature conditions (37 °C). The first set of experiments was carried out with the FVW mixture, diluted with water (2:3 v/v) and subsequent trials followed using a FVW mixture with DN hydrolysate at the same ratio (2:3 v/v).

- **Results:** The maximum hydrogen volume was produced at pH 6.0 (1.34 L H$_2$/L Reactor), for the fruit/vegetable stream whereas, the maximum concentration of ethanol and volatile fatty acids (15.60 g/L) was reached at pH 6.5 for the same substrate. Regarding the mixed waste stream, both hydrogen production and metabolites concentration reached a maximum at pH 7.5 with 4.09 L H$_2$/L Reactor and 17.16 g/L respectively.

- **Conclusions:** Different optimum pH value for bio-hydrogen production was observed between the anaerobic fermentation of the two substrates (FVW and FVW/DN hydrolysate mixture). Higher overall yields and concentrations of the metabolic products were obtained with the fermentation of the mixed substrate.

**Statement of novelty**

Disposable nappies represent a significant amount of municipal solid wastes that are currently sent to landfills, and their organic content remains thus unexploited. Up to date, there are limited research attempts and practical applications that use this waste stream as a source to produce added value products, besides compost. The present study provides quantitative data relating co-digestion of used disposable nappies and fruit/vegetables with pH effect on bio-hydrogen production. As such, it helps the better understanding of this waste stream regarding its valorization through anaerobic digestion for the production of gaseous bio-fuels and/or other metabolites. In addition, optimization of an important factor such as pH, is a useful tool for the successful design of scale-up processes, concerning the same substrates.

**Keywords:** biohydrogen, disposable nappies, food waste, anaerobic fermentation, pH effect
1- INTRODUCTION

Energy crisis as a result of fossil fuel stocks decline, rising fuel prices due to high demand and the dependence of all production processes on fuels, in combination with climate change, have incited researchers globally for alternative sustainable energy sources that could offer a solution to these concerns. Among the eco-friendly biofuels, hydrogen (H\textsubscript{2}) is the most promising due to its high energy yield (122 kJ/g) on mass basis, which is almost three times greater than that from hydrocarbon fuels, and high cleanness, since only water vapour is produced from its combustion [1–3]. H\textsubscript{2} is currently produced via conventional water electrolysis and thermo-catalytic reformation using natural gas and oil. These processes though, are characterized by a high greenhouse-gas footprint which is a contradiction from an environmental point view, considering that the scope is to produce a cleaner fuel [4, 5].

Dark or anaerobic fermentation (AF) is a practical method for bio-hydrogen production, consuming less energy than physicochemical ones. Metabolic pathways of facultative or obligate anaerobic bacteria, lead to bio-hydrogen production through the decomposition of organic carbon from various feedstock substrates. Besides bio-hydrogen, volatile fatty acids (VFAs) and ethanol can be also produced, as by-products of the process, which may be further utilized for methane, biodiesel and bio-plastics synthesis [2], among other uses. Anaerobic fermentation can be also considered as a waste management method, since organic wastes and wastewaters are treated and biologically processed this way, tackling the environmental burden of waste disposal [6].

Promising results of biological hydrogen production have been obtained using different substrates. Municipal solid wastes [7], cellulose containing wastes [8], agroindustrial wastewaters [9], cheese whey [10], food waste [11] as well as used nappies [12], have been recently reported as potential substrates for AF, taking into account that the type of substrate is of crucial importance for the effective H\textsubscript{2} production.

Almost 1.5 billion tons of food waste is being disposed annually around the globe, which accounts for the 33% of the food production for human consumption [6]. In Greece, large Super Market chains, return most of the expired food products or products of low quality (i.e. meat, fish, pasta, milk etc) to the production companies for waste management or lead it to animal food companies and social actions against poverty. Fruit and vegetables, due to their vulnerability, are being thrown away and led to landfiling. Interestingly, food waste is rich in carbohydrates and other nutrients and therefore could be utilized in fermentation-based bio-refining processes for the production of biofuels and/or added value products [5].

Disposable nappies are primarily composed of cellulose and synthetic fibres that can be treated by biological methods. According to EDANA [13] a typical composition of a disposable baby nappy is 36.6% cellulose pulp, 30.7% sodium polyacrylate (SAP), 16% polypropylene, 6.2% low-density polyethylene, and 10.5% of elastic and adhesive tapes. Cellulosic content is not the only biodegradable material that could be valorized from a used disposable nappy. The percentage of
organic content can reach almost 87% if urine and excreta are also added [14]. To date, the aforementioned recyclable materials and potential energy sources of this waste stream, are disposed of to landfills or led to incineration, due to collection of nappies with unsorted Municipal Solid Wastes (MSW) [15–17]. In the USA, 3.4 million tones of nappies are produced each year and more than 90% is sent to landfills [17].

Increasing living standards, consumerism and throw-away mentality, combined with unsustainable waste disposal practices have led to increased waste amounts in landfills. Landfilling is responsible for health hazards, fires and explosions, vegetation damage, unpleasant odors, landfill settlement, ground water pollution, air pollution, and global warming owing to the fact that biogas and leachate migrates away from the landfill boundaries and are released into the surrounding environment [18, 19]. Development of sustainable and effective waste valorization is mandatory to circumvent waste disposal and environmental problems.

This work aimed at assessing the effect of pH on the anaerobic fermentation of a mixed waste stream, consisted of fruit and vegetables (FVW), that cannot be consumed or are considered low quality from food suppliers, and a used disposable nappies’ hydrolysate (DN). More specifically, the purpose was to identify the optimal pH value for maximum bio-hydrogen production and valuable metabolites such as VFAs and ethanol. According to literature, pH regulation plays an important role on the final performance of AF. Even narrow changes of pH in the bioreactor, affect significantly the microbial balance, metabolic pathways and consequently the metabolic products. In general, acidic pH < 4 favors the accumulation of acidic metabolites and ethanol and inhibits H₂ production [20], while at pH > 7 propionate is predominant and H₂ synthesis is limited. Thus, it is crucial to maintain pH constant at an optimum value to maximize H₂ productivity [2, 9]. It should be noted that optimum pH for H₂ production, varies depending on the fermented substrate and therefore must be determined accordingly [21]. In the present study, constant pH, varying from 4.5 to 7.5, was studied in batch experiments at mesophilic conditions, with FVW and a mixture of FVW and DN hydrolysate as fermentation feedstocks.

2- MATERIALS AND METHODS

2.1 Anaerobic sludge

Anaerobic sludge, obtained from a municipal wastewater treatment plant in Metamorphosis (Attiki, Greece), was used as inoculum at 15% v/v for all batch experiments. The sludge was boiled at 100 °C for 20 min, prior to experiments, in order to enrich hydrogen-producing bacteria via the deactivation of methanogens and H₂ consumers [22]. The total solids (TS) content of the sludge was 15.6±3.6 g/L, with 70±2 % of it being volatile solids (VS).
2.2 Fermentation substrates

Fruit and vegetables, that did not meet quality standards for the supply chain, were collected from a Super Market in Patras (Achaia, Greece). Hard pieces like stalks, were removed and the rest were pulped, mixed and homogenized with an analytical mill (Sigma-Aldrich, IKA A11). Used disposable nappies were collected from a private nursery in Chalandri (Attica, Greece). The disposable nappies were cut manually with scissors and the process described by Conway et al. [23] was followed in order to obtain a hydrolysate with the containing cellulose and excreta. Both FVW and DN hydrolysate, were stored separately at -18 °C until further processing.

2.3 Experimental set-up and procedure

All experiments were performed in 1-L double wall, cylindrical, stainless steel (INOX 316) bioreactors with a working volume of 750 mL at constant conditions. Temperature was controlled via a thermocouple and was maintained at 37 ± 0.2 °C using hot water circulation. A motor drive unit, on the top of the bioreactor, reserved continuous stirring. pH was automatically controlled (HACH controller, SC200) and kept constant with the addition of 0.5N NaOH solution through a peristaltic pump. The range of pH values tested was 4.5-7.5, using a step of 0.5 for the FVW substrate (diluted with tap water at 2:3 v/v ratio). Subsequently, batch experiments with FVW and DN hydrolysate at a 2:3 v/v ratio were conducted. The second set of experiments tested only the pH values that considered to produce adequate amounts of bio-hydrogen and VFAs. A total of four (4) pH values were tested in the mixture of FVW and DN hydrolysate, namely 6.0, 6.5, 7.0 and 7.5. A mixture of N2/CO2 gases at 80/20 % v/v ratio was used to purge the headspace of bioreactors, to ensure an initial anaerobic environment. In Fig. 1a the schematic diagram of experimental methodology followed for the batch tests using FVW and DN hydrolysate, is presented. Fig. 1b depicts the experimental apparatus used for the batch tests process. It should be noted that each experiment lasted until the end of the metabolites’ production (96-140h).

2.4 Analytical Methods

Physicochemical characterization of the used substrates, as well as chemical analysis of the effluents from the bioreactor, were performed. During the experiments, samples were taken in order to determine the composition of the produced biogas, other metabolites such as VFAS, lactic acid and ethanol as well as total and dissolved COD (t-COD and d-COD), total and dissolved carbohydrates, TSS and VSS. Samples were taken every 4-6 hours according to the evolution of the experiment. Off-line pH measurements were conducted using an electrode (Thermo Scientific, Orion ROSS Ultra Refillable pH/ATC Triode), while alkalinity, TS, VS, TSS, VSS, t-COD, d-COD, TKN, ammonium nitrogen, total and dissolved phosphorus
were determined according to *Standard Methods* [24]. Total and dissolved carbohydrates were measured according to Joseffson [25], phenolic compounds were determined according to Waterman and Mole [26], and VFAs as well as ethanol were analysed on a gas chromatograph (Agilent Technologies, 7890A) equipped with a flame ionization detector, as described by Dareioti et al. [9]. Lactic acid was measured with a DIONEX IC300 ion chromatography system using a thermostated (30 °C) Dionex IonPac analytical column (AS19 length 4 x 250 mm and 7.5 mm I.D), a guard column (4 x 50 mm length and 12 mm I.D) and an electron conductivity detector (Dionex). Fats and Oils were measured after extraction with hexane using a Soxhlet extractor (Velp Scientifica, SER 148). All experiments were carried out in triplicate. A custom-made equipment was used for the total biogas and bio-hydrogen volume quantification. The apparatus is composed of a U-tube filled with engine oil, an electron valve and a counter. The volume of gases is measured by counting the number of displacements of constant oil volume. Biogas composition analysis was performed by gas chromatography with a capillary column (HP-PLOT/Q, 30 m in length, 0.53 mm I.D. and 40 μm packing film), a thermal conductivity detector (TCD) and nitrogen as carrier gas. Biogas production was converted to standard conditions (i.e. STP = 0 °C and 1 atm).

### 3- RESULTS AND DISCUSSION

#### 3.1 Physicochemical characterization of fermentation substrates

Samples of the fermentation substrates were taken throughout the experimentation period, and measured in triplicate, in order to determine their characteristics and ensure that the batch tests are performed with constant feedstock. As shown in Table 1, DN hydrolysate is characterized by a neutral to alkaline pH (7.73 ± 0.04), which can be attributed to water and the presence of urine that has a high pH value when stored, due to the decomposition of urea and urate [27]. In general, pH of food waste is characterized as acidic with values close to 5.0 [28]; in this case pH of FVW is more acidic (3.48 ± 0.30) and therefore the resulting mixture of FVW/DN hydrolysate ends up with pH 4.67 ± 0.18. Furthermore, FVW presents high organic content, which is mainly attributed to the total carbohydrates concentration of this waste stream (64.53 ± 7.46 g/kg ww). Total solids (TS) of FVW is also high (111.55± 3.99 g/kg ww) with 90.5% of it being volatile solids (VS), and COD:N:P ratio is 303:4.5:1.

Regarding DN hydrolysate, the absence of soluble carbohydrates leads to the conclusion that cellulosic fibers from the nappies are the main source of carbohydrates. Although the biodegradable content of a used nappy is considerable (cellulose fibers plus excreta), yet DN hydrolysate presents rather low concentrations of nutrients and organic carbon. This result is due to the high dilution ratio, that is needed for the pre-treatment. The latter has an impact on the TS as well, which is rather low (8.07 ± 0.97 g/kg ww).
Finally, the mixture FVW/DN hydrolysate has lower organic load than FVW, due to its dilution with the hydrolysate and its COD:N:P ratio is 317.5:6:1, which is almost ideal considering that for anaerobic processes the optimum operational ratio is 350:7:1 [29].

3.2 Effect of pH on FVW anaerobic fermentation

Batch experiments with FVW diluted with tap water at 2:3 v/v ratio were conducted. The range of pH values tested was 4.5-7.5, using a step of 0.5. The volume of produced biogas as well as its composition was analysed throughout the experiments. Other parameters such as, VFAs and ethanol, lactic acid, t-Carbohydrates, d-Carbohydrates, t-COD, d-COD, TSS and VSS were determined at regular basis, in order to monitor the process. Biogas production at STP conditions, is depicted in Fig. 2a. In all cases, biogas was composed of H₂ and CO₂, while CH₄ was not detected. At pH 4.5 and 5.0 there was practically no H₂, since acidic conditions inhibit metabolic activities related with H₂ production [2, 30, 31]. Maximum H₂ volume and yield (1008.1 ml, 1.345 L H₂/ L Reactor) was obtained at pH 6.0, in accordance with researchers that investigated the effect of pH on H₂ production with different substrates in batch experiments [9, 32]. At pH 7.0 and 7.5 though, H₂ volume prevailed CO₂, constituting 56.2% and 78.1% respectively of the total biogas volume obtained. VFAs and ethanol production (Fig. 2b) is a useful tool for the assessment of the process and information can be extracted regarding the H₂ yields, since acidogenesis and H₂ generation are closely related. At pH 4.5, acetic acid and ethanol were mainly produced, and their concentrations reached 1,683.77 and 1,380.94 mg/L, respectively, with the rest of the VFAs concentrations being less than 40 mg/L. Lactic acid appeared at pH 5.0, having a high concentration (4,681.94 mg/L), even though it was expected at lower pH values as well, according to literature [32, 33]. Lactate concentration was followed by acetic acid (1,468.06 mg/L) and ethanol (1,093.75 mg/L). Various intermediate metabolites were produced at pH 5.5. Acetic acid (2,135.90 mg/L) and ethanol (2,197.59 mg/L) were predominant at the end of the fermentation, while lactic acid was also produced but was not detected after 39.2h. Caproic acid appeared at 54.3h of fermentation and butyrate at 68h. Their concentrations reached 340.63 and 732.84 mg/L respectively. Isobutyrate and isovalerate were less than 85 mg/L, while propionate concentration was 684.40 mg/L. Total VFAs and ethanol production of 10,199.47 mg/L was obtained at pH 6.0, where the highest H₂ production was also observed, as stated previously. At the same pH value, butyrate had the highest concentration (6,262.82 mg/L), which is in agreement with literature [34] where it is mentioned that butyric and acetic acid type fermentations result in higher H₂ production. Zhang et al. [35] also reported butyric acid as the predominant VFA at pH 6.0, in kitchen waste anaerobic fermentation experiments. Acetic acid reached its maximum concentration (12,216.46 mg/L) at pH 6.5 followed by propionic and butyric acid with 1,210.98 and 1,500.01 mg/L respectively. Lower
concentrations of acetic acid and butyric acid are noticed at pH 7.0, whereas at pH 7.5 the same pattern as at pH 6.5 is observed. Acetic acid reached 11,856.98 mg/L and butyric acid 1,516.42 mg/L.

Total carbohydrates removal, expressed in glucose equivalents, ranged from 70.4 to 88.3%. The highest degradation was observed at pH 6.5, as shown in Fig. 2c. The increment of pH values was followed by an increment on the carbohydrates consumption as well. Dissolved carbohydrates consumption ranged from 88.6 to 96.7%. Maximum consumption was observed at pH 7.5. It is also worth mentioning that the H₂ production yield (mole of H₂ produced per mole of consumed carbohydrates) reached maximum efficiency at pH 5.5, which was equal to 0.55 mol H₂/mol equivalent glucose consumed, while the optimum theoretical yield is 4 mol H₂/mol equivalent glucose with acetic acid as the main end-product. Dareioti et al. and Thauer et al. [9, 36] observed similar low yields as well at pH 6.0 (0.642 mol H₂/mol equivalent glucose consumed) without acetic acid production at a study on the effect of pH regarding the optimization of H₂ production from agroindustrial wastewaters anaerobic acidogenesis. Likewise, Stavropoulos et al. [37] reported a maximum H₂ yield at pH 5.0, reaching 0.84 mol H₂/mol equivalent glucose consumed, with dairy products as an easily fermentable substrate.

**Fig. 3a** depicts the concentrations of main end-products as a function of time, regarding the batch experiment that took place at pH 6.0, which resulted in the maximum H₂ production. Fermentation lasted 118h and it is evident that acetic and ethanol type fermentations were predominant until the 70th h, with butyric type fermentation starting to evolve from that point on and then prevail by the end of experiment. Acetic acid reached a maximum of 4,140 mg/L at the 67th h of fermentation and then followed a downward trend. Ethanol concentration appears to follow the same pattern as well, while butyric acid concentration reached a maximum (6,016 mg/L) and appears to be stable until the end of the fermentation. Both acetic and butyric acid are usually produced during fermentation processes, but their dynamics vary due to alterations of environmental factors like pH, H₂ pressure and concentrations of metabolic products [38]. Hydrogen production (Fig. 3b) follows acetic acid production trend, which is in accordance with literature [36, 38]. The maximum H₂ production is observed at 30th h which remained stable during fermentation with a minor increase (1%) after the 90th h. Regarding total and dissolved carbohydrates (Fig. 3c), their consumption is rapid until the 30th h, whereas a lower further reduction is observed, which is not in line with the vast increase of the butyric acid concentration. This high concentration of butyric acid and the simultaneous reduction of acetic acid, enhance the assumption that the later may be enzymatically converted to butyric acid and AcCoA by butyric acid bacteria through simultaneous consumption of butyryl-CoA [39].

### 3.3 Effect of pH on FVW/DN hydrolysate anaerobic fermentation

Subsequent experiments, regarding the optimum pH value for maximum biohydrogen production from FVW and DN hydrolysate were conducted. The feedstock in these second series of experiments consisted of FVW and DN hydrolysate...
mixture at a 2:3 v/v ratio. A total of four (4) pH values were tested in the mixture, namely 6.0, 6.5, 7.0 and 7.5 due to previous experiment series’ findings. Hydrogen production at pH values below 5.5 was considered inadequate.

Results shown in Fig. 4a indicate that the highest volume of hydrogen was produced at pH 7.5 where it reached 3,021.91 mL and a yield of 4.02 L H₂/ Reactor. The change of the feedstock nature led to a threefold increment in H₂ volume, compared to FVW fermentation at a different pH value, probably due to microbial consortium enhancement from DN hydrolysate. This is evident also from the presence of 5% CH₄ (data not shown) at pH 7.5, which is an optimum value for methanogenesis, as methanogenic bacteria from the contained excreta of the used nappies could have been added in the mixture.

Fig. 4c presents the extent of carbohydrates degradation. Total carbohydrates consumption ranged from 88 to 90.5%, whereas dissolved carbohydrates followed the same pattern of removal and their percentage of consumption ranged from 97.4 to 98.2%. Besides the microbial enhancement from the DN hydrolysate, cellulose, which is part of the total carbohydrates, was also added in the mixture increasing thus the carbohydrates content of the feedstock. Concerning the main end-products formation (Fig. 4b), results denote that the main metabolic pathways followed by the anaerobic microbiota were butyric and acetic acid fermentation. Butyric acid reached a maximum (8,189.72 mg/L) at pH 6.0 and acetic (7,359.35 mg/L) at pH 7.5. Ethanol was again present in all batches but seemed to decrease with the increment of pH value.

Lactic acid was detected at pH 6.0 and 6.5 while caproic acid showed a maximum concentration at pH 7.0 (1,009.66 mg/L). Results were in accordance with other researchers as well [32, 35]. At pH 7.5, total VFAs and ethanol concentration show the highest value among the experiments (17,162.19 mg/L). According to literature [40], pH values close to neutral seem to enhance hydrogen consuming than hydrogen producing bacteria. However, low concentration of propionic acid, which is produced from hydrogen and glucose, indicates that hydrogen producers are predominant in the microbial consortium.

More details about the batch experiment (pH 7.5), where maximum H₂ yield was obtained, are given in Fig. 5. The alteration on the fermentation substrate due to DN hydrolysate addition on FVW, resulted not only at a different optimum pH value for H₂ maximization, but also at a difference on the metabolic pathways followed by the microbial consortia and the final concentrations of metabolic products. The fermentation of FVW/DN hydrolysate lasted 137.65 h and Fig. 5a presents the kinetics of main end-products formation. Acetic and butyric acid are produced from the beginning of the fermentation following the same trend and reaching both a concentration of 7000 mg/L after 89 h. Equal abundance of these two acids has been previously reported at a pH range of 6.5-7.0 in a glucose fermentation by a mixed culture [41]. This mixed-acid metabolic pathway is common, in similar waste streams fermentations i.e food waste [42]. In the present study, volatile fatty acids with high molecular weight, such as caproic, and ethanol were detected at low concentrations. Propionic acid reached 180 mg/L after the 64th h, probably due to the presence of acidogenic bacteria such as Corynebacteria, Propionibacterium and Bifidobacterium, which are responsible for this acid’s production via transcarboxylase cycle [43]. An alternative
metabolic pathway for propionic acid formation involves the presence of lactate [43], which in our case is undetectable, and
is thus most probably not followed.

Carbohydrates (Fig. 5c) present a reduction corresponding to the rate of main end-products formation, with 60.9% of them
being consumed until the 20th h of fermentation, while the rest followed a slow pace reduction. Hydrogen production reached
a maximum at the 32.7th h and remained stable from then onwards till the completion of fermentation (Fig. 5b).

4- CONCLUSIONS

The effect of pH on the anaerobic fermentation of FVW and DN hydrolysate for bio-hydrogen production was investigated
in this study. Bio-hydrogen was produced efficiently and maximized by properly adjusting the pH values according to the
utilized fermentation substrate. For FVW, as single feedstock, the maximum H2 yield was observed at pH 6.0 reaching 0.52
mol H2/mol equivalent glucose whereas, the maximum concentration of ethanol and organic acids (15,600 mg/L) was
reached at pH 6.5 for the same substrate. Regarding the mixed waste stream (FVW/DN hydrolysate), both hydrogen
production and metabolites concentration reached a maximum at pH 7.5 with 1.12 mol H2/mol equivalent glucose and 17,160
mg/L respectively. The use of FVW/DN hydrolysate instead of FVW alone, led to higher yields of H2 and main end-products
of the anaerobic fermentation, as well as greater carbohydrates consumption. Although the highest H2 production was
observed at pH 7.5, such pH value is not suggested for continuous operation of a fermentation system due to increased
possibility of converting the reactor to a methanogenic one. Moreover, the need to operate continuously at such high pH
value and sustain it constant, requires continuous addition of large quantities of alkaline solution, resulting thus to higher
operational cost than operating at suboptimal pH levels.

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FIGURE AND TABLE CAPTIONS

Fig. 1  a) Schematic diagram of batch experimental methodology for FVW and DN hydrolysate, b) Experimental apparatus for the batch tests process

Fig. 2  a) H₂, CO₂ and specific volumetric hydrogen evolution at STP conditions, b) Main end-products, c) removal of total (t-CH) and dissolved (d-CH) carbohydrates and H₂ production yield per mole of consumed carbohydrates expressed in glucose equivalents, at the pH values tested for FVW anaerobic fermentation

Fig. 3  a) Main end-products, b) biogas, H₂ and CO₂ volume at STP conditions, c) degradation of total (t-CH) and dissolved (d-CH) carbohydrates expressed in glucose equivalents, as a function of experimental time at the optimum pH value (6.0) for FVW anaerobic fermentation

Fig. 4  a) H₂, CO₂ and specific volumetric hydrogen evolution at STP conditions, b) Main end-products, c) removal of total (t-CH) and dissolved (d-CH) carbohydrates and H₂ production yield per mole of consumed carbohydrates expressed in glucose equivalents, at the pH values tested for FVW/DN hydrolysate anaerobic fermentation

Fig. 5  a) Main end-products, b) biogas, H₂ and CO₂ volume at STP conditions, c) degradation of total (t-CH) and dissolved (d-CH) carbohydrates expressed in glucose equivalents, as a function of experimental time at the optimum pH value (7.5) for FVW/DN hydrolysate anaerobic fermentation

Table 1: Chemical composition of fermentation substrates used in this study. Data are mean values (n=3, ± SD)
Figure 2

(a) Total H\textsubscript{2}, CO\textsubscript{2} at STP (mL)

(b) Main end-products (mg/L)

(c) Carbohydrates Removal (%) (eq Glucose)
Fig. 3
(a) 

(b) 

(c) 

Fig. 4
(a) Main end-products (mg/L) vs. Experimental time (h)

(b) Biogas, H₂, CO₂ Volume at STP (mL) vs. Experimental time (h)

(c) Carbohydrates (g/L) vs. Experimental time (h)

Fig. 5
| Physicochemical parameter | FVW (g/kg ww) | DN hydrolysate (g/L) | Mixture (2:3 v/v) (g/L) |
|---------------------------|---------------|----------------------|------------------------|
| pH                        | 3.48 (0.30)   | 7.73 (0.04)          | 4.67 (0.18)            |
| Moisture (%)              | 88.85 (0.40)  | 99.19 (0.10)         | 96.72 (0.31)           |
| t-Carbohydrates*          | 64.53 (7.46)  | 1.37 (0.13)          | 28.40 (2.15)           |
| d-Carbohydrates*          | 46.93 (1.41)  | 0.24 (0.01)          | 23.04 (3.39)           |
| Phenols**                 | 0.99 (0.02)   | 0.04 (0.00)          | 0.35 (0.01)            |
| t-COD                     | 103.77 (22.08)| 5.87 (1.26)          | 42.11 (1.67)           |
| d-COD                     | 72.39 (4.71)  | 1.51 (0.22)          | 23.97 (0.49)           |
| Fats and Oils             | 0.63 (0.07)   | 0.06 (0.01)          | 0.49 (0.01)            |
| t-Phosphorus              | 0.34 (0.03)   | 0.02 (0.00)          | 0.12 (0.00)            |
| d-Phosphorus              | 0.28 (0.02)   | 0.02 (0.00)          | 0.10 (0.00)            |
| TKN                       | 1.54 (0.13)   | 0.26 (0.02)          | 0.75 (0.06)            |
| NH₃-N                     | 0.36 (0.07)   | 0.26 (0.02)          | 0.12 (0.01)            |
| TSS                       | 39.83 (5.97)  | 4.45 (0.18)          | 14.20 (0.71)           |
| VSS                       | 39.00 (4.58)  | 4.30 (0.32)          | 13.93 (0.63)           |
| TS                        | 111.55 (3.99) | 8.07 (0.97)          | 32.83 (3.07)           |
| VS                        | 101.03 (3.72) | 4.99 (0.98)          | 29.30 (2.80)           |

*In glucose equivalent

**In syringic acid equivalent
