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ORIGINAL PAPER

Food Industry Waste's Exploitation via Anaerobic Digestion and Fermentative Hydrogen Production in an Up-Flow Column Reactor

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Abstract The present study focuses on the exploitation of food industry waste for hydrogen and methane production, via biological processes. Fermentative hydrogen production in an anaerobic up-flow column reactor (AUFCR), using indigenous microbial species and fed with the soluble fraction of the waste coming after an extraction procedure, was investigated at different Hydraulic Retention Times (HRTs) in the range of 12–2 h. The highest hydrogen production rate (2.94 ± 0.37 L H_2/L/d) was obtained at the HRT of 2 h, while the highest yield of hydrogen produced per mol of consumed carbohydrates, expressed in glucose equivalents was achieved at the HRT of 12 h (0.14 ± 0.01 mol/mol). Methane production via anaerobic digestion (AD) in a CSTR—type reactor was also assessed, using the whole diluted food industry waste (without extraction). The HRTs tested were 20, 15 and 10 days, while when the reactor operated at the HRT of 10 days, the feeding medium was also thermally treated, in order to assess the effect of pretreatment in AD. The maximum methane production rate (0.24 ± 0.02 L CH_4/L/d) was observed for the HRT of 10 days and thermally treated substrate, while the maximum methane yield was obtained at the HRT of 15 days (247.9 ± 0.15 L CH_4/kg waste). The IWA ADM1 model was finally used to predict the anaerobic digesters' behavior through the operational period, resulting to a quite satisfactory simulation for a wide range of operational conditions.

Introduction

Owing to the rapid depletion of the non-renewable energy resources, such as fossil fuels, the climate changes as well as the global warming and environmental pollution, the use of renewable energy resources has become an imperative need for the future energy usage. Biomass including agricultural and forest residues, energy crops, livestock residues and municipal solid wastes, represents an attractive source for the production of chemicals and biofuels. The last category of biomass resources includes food industry wastes, which constitute ideal substrates for anaerobic fermentative processes. It was found that food industry wastes are desirable substrates due to their high content in carbohydrates and volatile solids, their high digestibility and well balanced carbon and nutrient contents [1]. The traditional approaches for food waste disposal are mainly landfilling, incineration and aerobic composting, but, alternative methods are needed in order to tackle the waste crisis [2]. This way, food production and processing could be achieved in conjunction with the production of energy in the form of methane and hydrogen, via anaerobic bioprocesses.

Biohydrogen is strategically important as it does not contain carbon, and therefore, combustion of hydrogen-based fuels do not lead to carbon-dioxide emissions [3]. Fermentative hydrogen production is a process which is carried out in the dark, under anaerobic conditions and it is directly related to the acidogenic stage of anaerobic digestion process [4]. The involved metabolic pathways of

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acidogenesis during hydrogen production, can either be promoted or prohibited, depending on the prevailing operating conditions, which govern the simultaneous production of specific volatile fatty acids (VFAs) including acetate, propionate and butyrate or reduced metabolic products such as ethanol or lactate [5]. The main challenge for efficient hydrogen production during fermentation, is the prevalence of the metabolic hydrogen generating pathways (towards acetate and butyrate), which can be secured through appropriate manipulation of operational parameters. Among them, the hydraulic retention time (HRT) is of crucial importance, since it influences the metabolic products distribution and the final hydrogen yields [5, 6]. In addition, the reactor configuration is quite important, influencing the reactor microenvironment, the prevailing microbial population, the established hydrodynamic behavior and the contact between substrate and consortia [6].

Up to now, numerous studies have been published dealing with fermentative hydrogen production of food waste using batch reactors [7, 8] or conventional continuous systems such as CSTR [9, 10]. An alternative for efficient fermentative hydrogen production could be the use of biofilm reactors such as column attached growth reactors, where biomass is immobilized on inert materials. In these systems the solid retention time (SRT) of the microbial biomass inside the reactor is independent of the HRT, due to physical retention, allowing high cell concentrations and thus high hydrogen volumetric production rates, with relatively small reactor volumes [4, 11]. Regarding food wastes, the potential of using such attached growth reactors with supported materials has not been extensively investigated. To our knowledge, only Júnior et al. [12] and Han et al. [13] investigated biohydrogen production from food waste in such types of bioreactors.

On the other side, anaerobic digestion (AD) through which the organic matter is biologically converted to methane, carbon dioxide, inorganic nutrients and a humus-like matter, is a mature technology, applied not only for food waste valorization, but also for waste treatment [14]. Microbial activities during AD involve various biochemical steps which are delicately balanced by interactions among different microbial consortia. Microbial communities in AD are strongly affected by changes of the operational conditions and substrate composition [15]. Applying AD on food wastes from restaurants, grocery stores, and produce markets throughout California and around the U.S., has a potential of producing 367 m^3 of biogas per dry ton with an energy content of 625 kWh/m^3 of biogas, yielding 849 TWh annually, which represents almost 5 % of the total global electricity energy consumption [16].

Modeling is an important tool for process design, determination of optimal operational conditions and controlling the AD process [17]. In order to describe the kinetics of the AD process, several anaerobic digestion models have been developed in the last years [18]. Among them, Anaerobic Digestion Model ADM No. 1 (ADM1) which was developed by an International Water Association (IWA) task group, is nowadays the most common platform tool for modeling the AD process and has also been extended for describing the fermentative hydrogen production process [19, 20]. ADM1 was used to describe the AD process of a wide range of wastes such as sewage sludge [17], microalgae [21] as well as the co-digestion of organic fraction of municipal solid waste (OFMSW) with waste activated sludge [22]. Recently, ADM1 was also used to simulate methane production of food waste [23–26].

This study focuses on the exploitation of food industry waste as a source of hydrogen and methane production. Specifically, fermentative hydrogen production of the diluted soluble fraction of the waste was investigated, using an anaerobic continuous up-flow column bioreactor (AUFCR), containing ceramic beads as support material for the attachment of bacterial biomass. The effect of the HRT on hydrogen productivities and yields, as well as on metabolites' distribution was also assessed. The solid fraction of the waste remained after extraction process was used for methane production in batch reactors, where the Biochemical Methane Potential (BMP), was assessed. In addition, the AD for methane production of the whole waste, was investigated in a CSTR- type reactor, at different HRT values. The ADM1 was also applied to the experimental data obtained from the methanogenic bioreactor. Some key parameters such as the specific maximum uptake rate constant for the volatile fatty acids consumption were estimated and used to simulate and predict the reactor performance during the operational period.

Materials and Methods

Food Industry Waste

The waste used consisted of expired solid baby foods, returned from the market to the company, in seven different flavours, which were provided from a Greek food industry, located in Athens, Greece. The average characteristics of the waste are presented in Table 1. The mixture of seven food products was homogenized and then diluted with deionized water at the desirable concentration for each experiment.

Hydrogen Production Process from the Soluble Fraction of the Wastes

The substrate for the fermentative hydrogen production process was the diluted soluble fraction of the food

Table 1 The main characteristics of the food waste used in this study

Characteristic	Value
TS (%)	94.88 ± 0.08
VS (% TS)	97.99 ± 0.00
Dissolved COD (g/g)	0.52 ± 0.07
Total carbohydrates (g/g)	0.78 ± 0.00
Dissolved carbohydrates (g/g)	0.41 ± 0.00
Proteins (g/g)	0.11 ± 0.01
Lipids (g/g)	0.06 ± 0.00

industry waste, coming after an extraction procedure, with an initial carbohydrates' concentration of 16.5 g/L. The extraction procedure was carried out due to the structural characteristics of the AUFCR and specifically to avoid blocking the support matrix. For the feed medium preparation, the procedure followed involved dilution with water at a concentration of 40 g waste/L, filtration with an 8 μm filter and, finally, centrifuging of the filtrate, at 4100 rpm for 10 min.

An anaerobic up-flow packed bed column reactor which was cylindrical in shape and made of Plexiglas (internal diameter 5.9 cm, height 30.5 cm and active volume of 0.5 L) operated at different HRT values. The reactor was double-coated and temperature control (35.0 ± 0.5 °C) was achieved via recirculation of water in the outer jacket. Cylindrical porous ceramic beads with specific surface of 600 m^2/L (height of 1.35 ± 0.05 cm, inner diameter of 0.84 ± 0.05 cm and outer diameter of 1.48 ± 0.03 cm) were used as support material for the attachment of bacterial cells. Fresh medium, maintained at a temperature below 4 °C, was fed continuously, via a peristaltic pump, at the bottom of the reactor, at a flow rate of 0.69, 1.04, 1.39, 2.08 and 4.17 mL/min, for HRTs of 12, 8, 6, 4 and 2 h, respectively. Simultaneous flow of the effluent occurred by overflow, at a height of 24.5 cm from the bottom, in order to maintain a constant reactor volume.

During start-up the reactor was filled with an aquatic solution of the mixture of the seven solid baby foods (the whole waste, without extraction process), and was operated anaerobically in batch mode for 48 h, in order to activate the indigenous microbial species contained into the waste, as proposed by Antonopoulou et al. [5]. The characteristics of the waste solution used as inoculum were: pH = 6.97, Total Suspended Solids (TSS) = 10.42 g/L, Volatile Suspended Solids (VSS) = 10.37 g/L, total carbohydrates = 13.5 g/L, dissolved carbohydrates = 8.5 g/L. Following start-up, the operation of the reactor was subsequently switched to continuous mode and the reactor was fed with the feed medium described above, also containing 5 g NaOH, 6.8 g KH$_2$PO$_4$ and 0.5 g yeast extract per liter

in order to maintain the pH of the acidogenic reactor, at the suitable levels for hydrogen production (almost 5–6). Furthermore, 2 g of urea (NH$_2$CONH$_2$) per liter of feed medium were added, in order to make up for N deficiency of the feed. A trace element solution was also used (10 mL/L feed) with concentration (in g/L): (NH$_4$)$_2$HPO$_4$ (7.21), FeSO$_4$.7H$_2$O (0.70), CaCl$_2$.2H$_2$O (22.50), NH$_4$Cl (35.90), MgCl$_2$.6H$_2$O (16.20), KCl (117.00), MnCl$_2$.4H$_2$O (1.80), CoCl$_2$.6H$_2$O (2.70), H$_3$BO$_3$ (0.51), CuCl$_2$.6H$_2$O (0.24), Na$_2$MoO$_4$.2H$_2$O (0.23), ZnCl$_2$ (0.19), NiCl$_2$.6H$_2$O (0.20), H$_2$WO$_4$ (0.01). The main characteristics of the feed medium used for the hydrogenogenic reactor, are presented in Table 2.

The reactor performance (biogas production rate and composition in H$_2$, pH, TSS, VSS, carbohydrates, dissolved Chemical Oxygen Demand (dCOD), VFAs, ethanol and lactate concentration) was monitored during the experimental period.

BMP Tests for Methane Production from the Solid Fraction of the Wastes

BMP assays were carried out in duplicate at 35 °C, based on Owen and Chynoweth [27] in order to determine the methane potential of the solid fraction obtained after the extraction procedure. Anaerobic sludge from the anaerobic digester of the Metamorphosis Athens wastewater treatment plant, treating municipal sewage sludge and operating at steady state at an HRT of 15 days, was used as inoculum. The main characteristics of the sludge were: pH: 6.87, dCOD: 0.11 g/L, TSS: 36.02 g/L and VSS: 19.40 g/L. Serum bottles of 160 mL were seeded with 20 mL mixed anaerobic culture, water and appropriate amounts of the solid fraction of the food waste were added, in order to acquire 2 g VS/L of the substrate, in a total volume of 100 mL. The microbial culture was supplemented with 10 mL/L of the trace metals solution described above. Control experiments using glucose as a substrate were carried out for checking the methanogenic biomass activity. Blank experiments were also carried out in order to determine the background gas productivity of the inoculum. The content of the vials was gassed with a mixture of

Table 2 The main characteristics of the feeding medium of the hydrogen producing AUFCR

Characteristic	Value
pH	12.0 ± 0.4
TSS (g/L)	2.93 ± 0.1
VSS (g/L)	1.18 ± 0.6
Dissolved COD (g/L)	21.7 ± 0.7
Dissolved carbohydrates (g/L)	16.5 ± 0.5

N₂/CO₂ (80/20), in order to secure anaerobic conditions. The vials were sealed with butyl rubber stoppers and aluminum crimps and methane production was monitored as a function of time according to Owen and Chynoweth [27].

Methane Production Process

A CSTR–type digester of 3 L active volume was operated anaerobically for 558 days. The reactor had a cylindrical shape, it was made of stainless steel and was stirred periodically, for 15 min, 2 times per hour. The reactor was equipped with a thermostatically controlled external water jacket, to maintain mesophilic temperature (35.0 ± 0.5 °C).

Anaerobic sludge obtained from the anaerobic digester of the Wastewater Treatment Plant of Patras was used as inoculum, during start-up. The characteristics of the sludge were: pH = 7.24, TSS = 25.3 g/L, VSS = 15.8 g/L and dCOD = 647 mg/L. The reactor was filled with 3 L of sludge supplemented with 10 mL/L of trace elements and was operated anaerobically in batch mode for 24 h and in the sequel, it was switched to continuous mode at the designated HRTs. The HRTs applied were equal to 20, 15, 10 and again 15 days. The feeding took place intermittently (every 8 h), using a peristaltic plump, while stirring. Simultaneous flow of the effluent occurred while feeding, by overflow.

The feed medium was not the same during the whole reactor operation. Thus, for the first 20 days at an HRT of 20 days (one HRT), the feed medium was an aquatic solution of the whole waste (without extraction) at an initial concentration of 2 g waste/L. The low organic load was used for inoculum acclimation with the new substrate. In the sequel (days 21–289 and 323–558), the feed was an aquatic solution of the whole waste, at an initial concentration of 11.2 g/L, supplemented with 7.5 g/L NaHCO₃ and the aforementioned solution containing trace elements. The same feed medium was used when the reactor was operated at an HRT of 10 days (days 290–322), but it was thermally treated at 90 °C for 15 min. The heat treatment was carried out as a pretreatment method, to enhance COD solubilization and assess its effectiveness on the reactor performance. The reactor operated twice at the HRT of 15 days, in order to perform kinetic experiments, as reported below. The reactor performance (biogas production rate and composition in CH₄, pH, TSS, VSS, dCOD and VFA concentration) was monitored and complete characterization of the effluent was carried out when steady state was reached.

Modeling of Methane Production Process

The IWA Anaerobic Digestion Model 1 (ADM1) was fitted to the data using non-linear parameter estimation. The software used was Aquasim 2.0 [28]. Monod kinetics were used for acetate and propionate uptake, with hydrogen inhibition of propionate uptake (noncompetitive), ammonia inhibition of acetate (noncompetitive), and pH inhibition of both groups.

In order to obtain appropriate kinetic data for parameter estimation, impulse disturbances were imposed to the bioreactor. Thus, when the anaerobic digester reached steady state at the HRT of 15 days (at the second time which operated at the HRT of 15 days), acetate, propionate and butyrate, one at a time, were spiked into the bioreactor and their concentration was monitored as it decreased towards the original steady state value. The impulses were imposed by injection of 50 mL of a concentrated solution of acetate (1200 mg COD/L), propionate (1000 mg COD/L) or butyrate (1200 mg COD/L) salts, respectively, shortly after feeding of the digester. The response of the bioreactor to these impulses was monitored through measurements of the biogas production rate, the percentage CH₄ content, the pH, and the VFAs concentration. The VFA impulses were simulated by a triangular pulse with 0.02 days duration. The maximum uptake rate (k_m) for each volatile fatty acid uptake process was estimated through fitting the model to the experimental data from the impulses.

A correction of the HRT was also included into the model structure, as it was observed that the SRT was higher than the HRT. This was due to the structural and operational characteristics of the bioreactor, resulting to a biomass and solids' accumulation. The secant method was applied for optimization. The values of the rest model parameters were set as suggested in the scientific and technical report of ADM1 [29], except from k_m for acetate, propionate and butyrate.

Analytical Methods

Determinations of dCOD, TSS, VSS, Total and Volatile Solids (TS, VS) were carried out according to Standard Methods [30]. The concentrations of the VFAs (acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, hexanoic) as well as that of ethanol were determined with a gas chromatograph (VARIAN CP-30), equipped with a flame ionization detector and a capillary column (Agilent technologies, INC. 30 m × 0.53 mm). The oven was programmed from 105 to 160 °C at a rate of 15 °C/min and subsequently to 235 °C (for 3 min) at a rate of 20 °C/min for VFA analysis and from 60 °C (for 1 min) to 230 °C (for 0.5 min) at a rate of 45 °C/min in case of ethanol analysis. Helium was used as the carrier gas at 15 mL/min, the injector temperature was set at 175 °C and the detector at 225 and 200 °C, for VFA and ethanol analysis respectively. Lactic acid concentration was measured with

Megazyme D-/L-Lactic acid assay kits. For the quantification of the carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan and sulphuric and boric acids and subsequently measured colorimetrically at 520 nm [31]. Total Kjeldahl Nitrogen (TKN) was carried out according to Standard Methods [30]. Crude protein content was determined by multiplying TKN by a factor of 6.25 [32]. The oil and grease content of the waste was determined according to the Soxhlet extraction method [30]. The produced gas composition in hydrogen and methane was quantified with a gas chromatograph (SRI 8610c MG#1), equipped with a thermal conductivity detector and a packed column. The carrier gas was nitrogen for hydrogen measurements and helium for methane. The injector, column and detector temperatures were set at 90, 35 and 100 °C, respectively. The volume of the produced gas was measured by the method of displacement of acidified water. The measurement of the pH was done using a HANNA (pH 211) pH-meter with a HANNA electrode (HI 1230).

Results and Discussion

Continuous Fermentative Hydrogen Production in the AUFCR

It is well known that the HRT is an important operational parameter that influences the hydrogen production rate and the stability of an acidogenic reactor [5, 33]. Thus, in the present study, its effect was explored, using an AUFCR which operated continuously for 54 days, at five different HRT values: 12, 8, 6, 4 and 2 h. The bioreactor was fed continuously with the feed medium, consisting of a carbohydrates concentration (in glucose equivalents), of 16.5 g/L. At each HRT value, a steady state was achieved, since the monitored parameters had a low variation (less than 10 %). In Table 3, the main characteristics of the reactor at each steady state, are presented. The biogas produced consisted mainly of hydrogen and carbon dioxide and was free of methane, indicating that there was no methanogenic activity in the bioreactor. The pH of the culture decreased with the HRT decrease and lied between 5.7 and 6.2. Although the optimum pH value for dark hydrogen production process is rather controversial, with suggestions varying from highly acidic to basic values, the range between 5 and 6 is most widely reported [11, 34]. TSS and VSS tended to accumulate in the bioreactor because of its constructional characteristics as well as of the fact that the feed medium contained solids, albeit at low concentration. The dissolved carbohydrates conversion efficiency was almost 100 % for all HRTs, indicating that the AUFCR was not kinetically limited at all HRT values.

These results are quite different than those presented in other studies on fermentative hydrogen production of sugar-based wastes [12] and glucose [35], using up-flow reactors with attached microbial cultures. In those studies an increase of the Organic Loading Rate (OLR), resulted to a significant reduction of substrate consumption.

When the reactor was inoculated with the indigenous microbial consortium and operated for 2 days in batch mode, the hydrogen content in the gas phase was very high, almost 57 % (Fig. 1a). However, with the operation of the AUFCR in continuous mode, the hydrogen content decreased gradually to 16 % at day 12 (HRT = 12 h) and thereafter levelled off for this HRT value. In the sequel, at the HRTs of 8, 6 and 4 h the percentage of hydrogen of the produced biogas, ranged between 10 and 11 %. Finally, at the HRT of 2 h, the hydrogen content stabilized at 17 %. These values are lower than the respective obtained by other studies dealing with mesophilic fermentative hydrogen production of food wastes, with different bioreactor configurations. Specifically, Castillo-Hernandez et al. [10] who studied biohydrogen production from food waste in a CSTR at a range of HRTs of 24, 12 and 8 h, reported a hydrogen content in the gas phase of 39 % for the first two HRTs (24 and 12 h) and 35 % for the last one (8 h). Furthermore, Nam et al. [36] reported 50 % hydrogen, when studying biohydrogen production from the co-digestion of food waste and sewage sludge, in a sequencing batch reactor (SBR) at an HRT of 72 h.

The biogas and hydrogen production rates were significantly affected by the HRT decrease. Thus, the maximum hydrogen production rate was obtained at the HRT of 2 h and corresponded to 1.47 ± 0.19 L H_2/d (Fig. 1b), as was anticipated, since at the HRT of 2 h the reactor OLR was the highest (198 g carbohydrates/L/d or 260.4 g COD/L/d).

On the other side, the yield of hydrogen produced (expressed as mol per mol of consumed carbohydrates, measured in glucose equivalents and as L per kg of waste), was maximized at the HRT of 12 h (Table 3). The yield was 0.14 ± 0.01 mol H_2/mol of consumed carbohydrates. This value is much higher than the respective obtained at the HRTs of 8, 6 and 4 h. These lower yield values could be attributed to the fact that while the OLR increased at the lower HRTs, the hydrogen production rate did not increase in proportion to the HRT decrease, although the consumption of carbohydrates was complete. Only at the HRT of 2 h, when the hydrogen production rate was high enough (2.94 ± 0.37 L/L/d) the hydrogen yield increased to 0.11 ± 0.01 mol H_2/mol of consumed carbohydrates.

Anaerobic hydrogen production from organics produces, along with hydrogen, VFAs and alcohols as fermentation end products [37, 38]. Lactic acid production has also been reported in several studies. The distribution of the soluble

Table 3 The characteristics of the up-flow hydrogen producing reactor at each steady state

HRT (h)	12	8	6	4	2
pH	6.23 ± 0.03	6.04 ± 0.10	5.85 ± 0.02	5.76 ± 0.02	5.73 ± 0.02
TSS (g/L)	2.55 ± 0.06	12.45 ± 1.27	13.25 ± 1.25	15.40 ± 1.82	14.94 ± 0.69
VSS (g/L)	1.76 ± 0.05	9.80 ± 1.16	10.67 ± 1.18	12.10 ± 0.99	11.66 ± 0.89
Efficiency of carbohydrates consumption	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Biogas production rate (L/L/d)	3.96 ± 0.26	4.72 ± 0.29	6.44 ± 0.15	10.34 ± 0.25	17.35 ± 1.60
H_2 production rate (L/L/d)	0.64 ± 0.06	0.43 ± 0.04	0.69 ± 0.05	1.18 ± 0.04	2.94 ± 0.37
H_2 yield (mol H_2/mol consumed carbohydrates)	0.14 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.01
H_2 yield (L H_2/kg waste)	8.01 ± 0.76	4.03 ± 0.34	4.33 ± 0.32	4.90 ± 0.17	6.12 ± 0.77
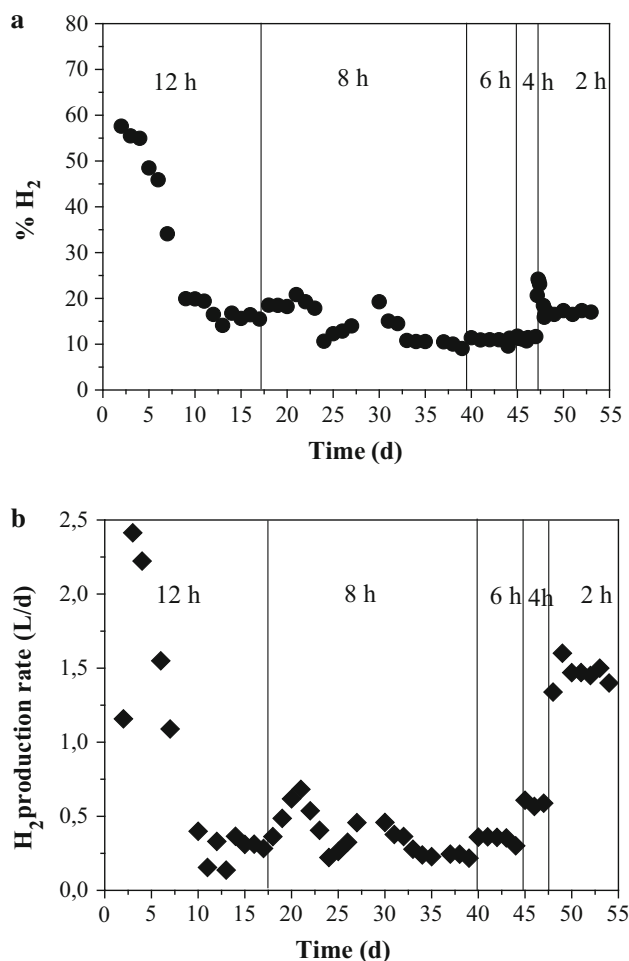
Fig. 1 The percentage of the hydrogen in the gaseous phase (**a**) and daily hydrogen production rate (**b**) during the operational period of the acidogenic bioreactor

metabolites at the steady states of the different HRT values, is presented in Table 4, where it is obvious that the main metabolic products during the experimental period were acetic, propionic, butyric, caproic and lactic acids. Valeric,

isovaleric and isobutyric acids as well as ethanol, were not detected during the experimental period. The distribution of the metabolites varied as the HRT decreased from 12 to 2 h. At the HRT of 12 h, the dominant metabolic products were acetic and butyric acids, while at the other HRTs, butyric acid prevailed. Thus, the maximum butyric acid concentrations were determined for the HRTs of 4 and 2 h (5.60 ± 0.23 and 5.47 ± 0.27 g/L, respectively). On the other side, the concentration of acetic acid, decreased gradually as the HRT decreased (from 4.46 ± 0.36 g/L at the HRT of 12 h to 0.96 ± 0.04 g/L at the HRT of 2 h). Reungsang et al. [9] who studied biohydrogen production from food waste in a CSTR bioreactor at HRTs of 84, 72, 60 and 48 h reported butyric acid as the dominant metabolic product throughout the experimental period, while acetic acid was detected in lower concentrations. It is well known that hydrogen is correlated to acetate and butyrate concentration [5]. It was observed that the measured hydrogen production yield was much lower than the predicted hydrogen production based on the metabolic products measured. An explanation could be that a considerable amount of the produced hydrogen is probably consumed by hydrogen consuming microorganisms such as homoacetogenic bacteria which may be establish in the reactor, producing acetic acid (Reaction 1) [5, 19]:
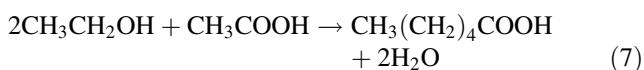
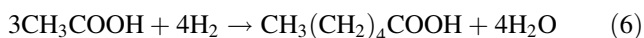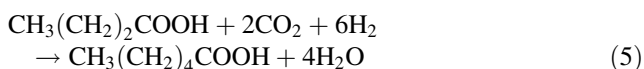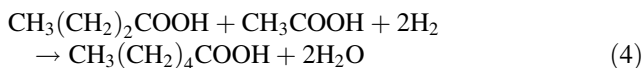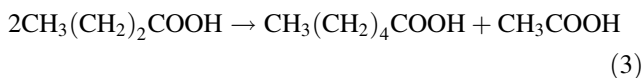$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \qquad (1)$$
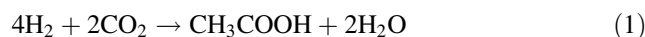
As presented in Table 4, at the HRT of 6 h, production of lactic acid was detected, the concentration of which increased with the HRT reduction to 4 and 2 h and maximized at the lower HRT value (0.94 ± 0.02 g/L, at the HRT of 2 h). This is a strong indication that lactate is an intermediate metabolic product, which is converted to other products, such as acetate and propionate (Reaction 2) [5]. At the higher HRT values (12 and 8 h), lactate is totally converted to products, while at the lower HRT values, lactate is accumulated, due to kinetic limitation of its consumption to acetic and propionic acids.

Table 4 The distribution of the main metabolic products at each steady state of the AUFCR

HRT (h)	Butyric acid (g/L)	Acetic acid (g/L)	Propionic acid (g/L)	Caproic acid (g/L)
12	4.00 ± 0.21	4.46 ± 0.36	0.55 ± 0.08	1.14 ± 0.14
8	4.77 ± 0.24	2.05 ± 0.11	1.25 ± 0.07	0.94 ± 0.05
6	4.98 ± 0.14	1.73 ± 0.12	1.00 ± 0.04	0.96 ± 0.05
4	5.60 ± 0.23	1.61 ± 0.13	0.89 ± 0.02	0.89 ± 0.06
2	5.47 ± 0.27	0.96 ± 0.04	0.65 ± 0.02	0.39 ± 0.02

$$3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH$$
$$+ CO_2 + H_2O$$
$$(2)$$
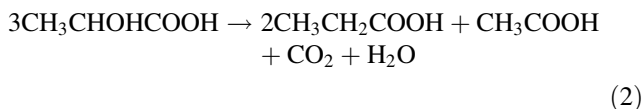
The experimental results obtained are in agreement with the results of Sivagurunathan et al. [33] who reported that the maximum concentration of lactic acid was obtained for the shortest HRT value (1.5 h), when using a continuous reactor with immobilized cells, treating beverage wastewater at the HRTs of 8–1.5 h. It is also remarkable, that in the present study, the production of caproic acid or hexanoete was detected among the metabolites. Its production was enhanced at the higher HRT values (1.14 ± 0.14 g/L at the HRT of 12 h and just 0.39 ± 0.02 g/L at the HRT of 2 h). This could be attributed to the assumption that caproic acid is not produced directly from pyruvate, like the other VFAs, but secondarily via the consumption of butyrate, acetate and/or ethanol (Reactions 3–7) [39, 40]. Thus, at the higher HRT values, probably there is no kinetic limitation for the conversion of butyrate, acetate and/or ethanol to caproic, and its production is limited at the lower HRTs. Ding et al. [40] reported that caproic acid is produced by some special species like *Clostridium kluyveri*. The production of caproic acid in this study, could possibly be related to the specific reactor configuration, as its production has also been reported in other studies dealing with fermentative hydrogen production using attached mixed acidogenic consortia [11, 40].

$$2CH_3(CH_2)_2COOH \rightarrow CH_3(CH_2)_4COOH + CH_3COOH$$
$$(3)$$

$$CH_3(CH_2)_2COOH + CH_3COOH + 2H_2$$
$$\rightarrow CH_3(CH_2)_4COOH + 2H_2O \qquad (4)$$

$$CH_3(CH_2)_2COOH + 2CO_2 + 6H_2$$
$$\rightarrow CH_3(CH_2)_4COOH + 4H_2O \qquad (5)$$

$$3CH_3COOH + 4H_2 \rightarrow CH_3(CH_2)_4COOH + 4H_2O \qquad (6)$$

$$2CH_3CH_2OH + CH_3COOH \rightarrow CH_3(CH_2)_4COOH$$
$$+ 2H_2O \qquad (7)$$

In Table 5, the ratio of the measured to the theoretically calculated dissolved COD concentration, is presented. Theoretical calculated COD concentration represents the

Table 5 The percentage of the measured in relation with the theoretical calculated -dissolved COD, at the steady states of the AUFCR

HRT (h)	COD theoretical calculated/ measured (%)
12	78
8	80
6	79
4	85
2	80

sum of COD of different products (lactic, butyric, propionic, caproic and acetic acid) as well as the COD of non-consumed carbohydrates measured as glucose equivalents. Specifically, the measured COD values were 18.5 ± 1.3, 18.1 ± 0.7, 17.9 ± 0.8, 17.5 ± 1.0 and 17.6 ± 0.9 g/L, at the HRT values of 12, 8, 6, 4 and 2 h, while the respective theoretically calculated were 14.5, 14.5, 14.1, 14.9 and 14.0 g/L. Taking into account that the dCOD concentration of the feed medium is approximately 21.7 ± 0.7 g/L it is obvious, that there is a dCOD removal, which could be attributed partly to the production of hydrogen in the gas phase and partly to the production of biomass, in the particulate phase. Given that 17.6 ± 0.8 g/L (16.5 ± 0.5 g/L carbohydrates in the feed medium and 100 % efficiency of consumption, at all HRT values) of dCOD is converted during fermentation it is obvious that hydrolysis took place to some degree, and this was enhanced at the higher HRT values (at the HRT of 12 h the measured dCOD concentration was higher). Finally, the fact that the ratio presented in Table 5 is lower than 100 %, indicates that some additional unidentified metabolic products were produced during the fermentation.

BMP Tests for Methane Production from the Solid Fraction of the Wastes

Methane production during the BMP test of the solid fraction obtained after pretreatment is presented in Fig. 2. The biogas production rate was initially high (0–15 days), while the rate decreased later. The calculated yield in
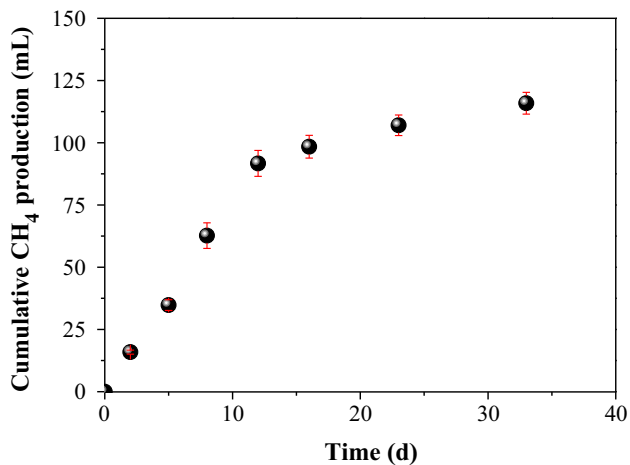
Fig. 2 Cumulative methane production of the solid fraction obtained after extraction process

methane after subtracting the methane produced from blank experiments, was almost 461.96 ± 20.92 L CH₄/kg VS, indicating its high biodegradability. In this respect, food wastes could be fully exploited after a separation/extraction process, where the liquid-diluted fraction could be used for hydrogen production using an AUFCR, while the solid fraction could be used for methane production.

Continuous Methane Production in a CSTR-Type Reactor

The digester was operated for 558 days, during which the effect of HRT and feed pretreatment were studied. Initially,

the CSTR was operated at an HRT of 20 days with an organic load of 2 g/L for the first 20 days (for inoculum acclimatization), and in the sequel the organic load was increased to 11.2 g/L until a steady state was reached. Thereafter, the HRT was reduced to 15 and 10 days, sequentially. At the HRT of 10 days, the reactor was fed with thermally pretreated feed medium (90 °C for 15 min) so as to assess the influence of pretreatment on its performance. Finally, the HRT of the digester increased again to 15 days, where a new steady state was reached and impulses with acetic, propionic and butyric acids, were imposed for modelling purposes. The main characteristics of the feed medium of the bioreactor with and without thermal treatment are given in Table 6. As shown in the table, the application of thermal treatment caused an increase of the dissolved carbohydrates' concentration by around 19 %.

The characteristics of the reactor performance at each steady state, are presented in Table 7. The percentage of CH₄ in the gas phase was not affected during the operational period and was in the range of 55.5 ± 2.3 to 60.9 ± 2.7 %. These values for the methane content are in agreement with several studies dealing with AD of food wastes [41, 42]. The biogas and CH₄ production rate varied at different operational periods as shown in Fig. 3a and were maximized at the HRT of 10 days and specifically when the reactor was fed with the thermally treated feed (1.3 ± 0.15 L biogas/d and 0.73 ± 0.07 L CH₄/d). The latter methane production rates are almost 9 % higher than the respective ones obtained at the HRT of 10 days when the reactor was fed with the medium of food wastes,

Table 6 The main characteristics of the feeding medium of the methanogenic bioreactor with and without thermal treatment

Characteristic	Feed	Thermally treated feed
pH	7.99 ± 0.13	8.59 ± 0.07
TSS (g/L)	5.34 ± 0.63	6.08 ± 0.39
VSS (g/L)	4.87 ± 0.68	5.54 ± 0.37
Dissolved COD (g/L)	5.16 ± 0.60	6.86 ± 0.95
Total carbohydrates (g/L)	8.71 ± 0.05	8.71 ± 0.05
Dissolved carbohydrates (g/L)	4.56 ± 0.04	5.43 ± 0.07

Table 7 The main characteristics of the anaerobic digester at all steady states

HRT (days)	Days	CH₄ (%)	CH₄ production rate (L/d)	CH₄ production rate (L/L/d)	Methane yield (L/kg waste)	Dissolved COD (g/L)	pH
20	1–141	60.2 ± 2.1	0.26 ± 0.02	0.09 ± 0.00	156.2 ± 0.11	0.27 ± 0.06	7.3 ± 0.1
15	142–220	60.9 ± 2.7	0.56 ± 0.04	0.19 ± 0.01	247.9 ± 0.15	0.23 ± 0.06	7.2 ± 0.1
10	221–289	55.5 ± 2.3	0.67 ± 0.10	0.22 ± 0.03	199.6 ± 0.50	1.05 ± 0.35	7.1 ± 0.1
10 (thermally treated feed)	290–322	57.4 ± 5.3	0.73 ± 0.07	0.24 ± 0.02	216.2 ± 0.32	0.89 ± 0.25	7.3 ± 0.1
15	323–558	59.5 ± 2.6	0.37 ± 0.00	0.12 ± 0.00	165.4 ± 0.04	0.34 ± 0.21	7.2 ± 0.2
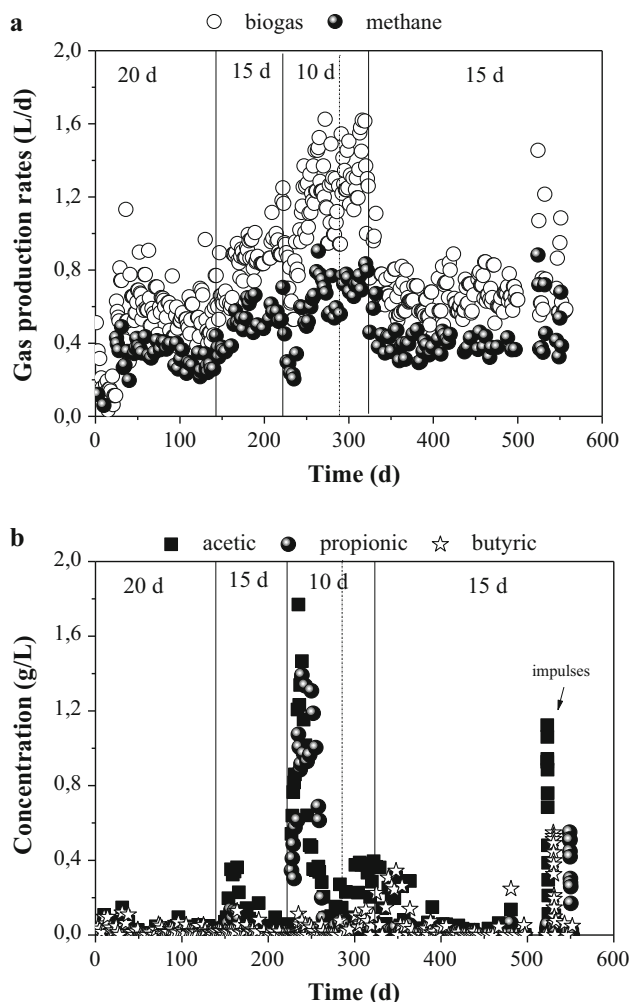
Fig. 3 The daily biogas and methane production rates (a) and the distribution of the main metabolic products (b) of the digester during the experimental period

without thermal treatment. Although the biogas production rate was maximized at the HRT of 10 days and pretreated feed medium, the methane yield was maximum (247.9 ± 0.15 L CH₄/kg waste) when the digester operated at the HRT of 15 days for the first time. In order to assess the efficiency of pretreatment e.g. for a full-scale plant, it is necessary to take into account both economic (based on heating costs) and technical (based on methane production rate) aspects.

At the HRT of 20 days the reactor performance was stable with VFAs (mainly acetic and butyric acids) at low levels (almost zero), while ethanol and propionic acid were not detected at all. When the HRT decreased from 20 to 15 days, a negligible VFAs accumulation occurred, in contrast with the next HRT change (from 15 to 10 days), where a significant accumulation was detected (Fig. 3b).

However, the digester recovered and a new steady state was reached at the HRT of 10 days. The concentration of dissolved COD was 1.05 ± 0.35 g/L, which corresponded to a 79 % dCOD removal efficiency. At the higher HRTs, the dCOD removal efficiency was higher: more than 98 % both at the HRTs of 20 and 15 days. From Table 7, it is obvious that the methane production rate at the HRT of 15 days applied for the second time (day 323–558) was lower than the respective one at the same HRT value, reached from day 142 to 220, while the percentage of methane in the gas phase and the pH culture were similar. This discrepancy could be attributed to the fact that the reactor did not fully recover after the decrease of the HRT from 15 to 10 days where a VFAs accumulation occurred which possibly resulted to a loss of a portion of the active biomass, leading to lower methane production rates and yields (if someone compares both steady states at the HRT of 15 days). In other word, most likely, some species were washed out while operating at the low HRT.
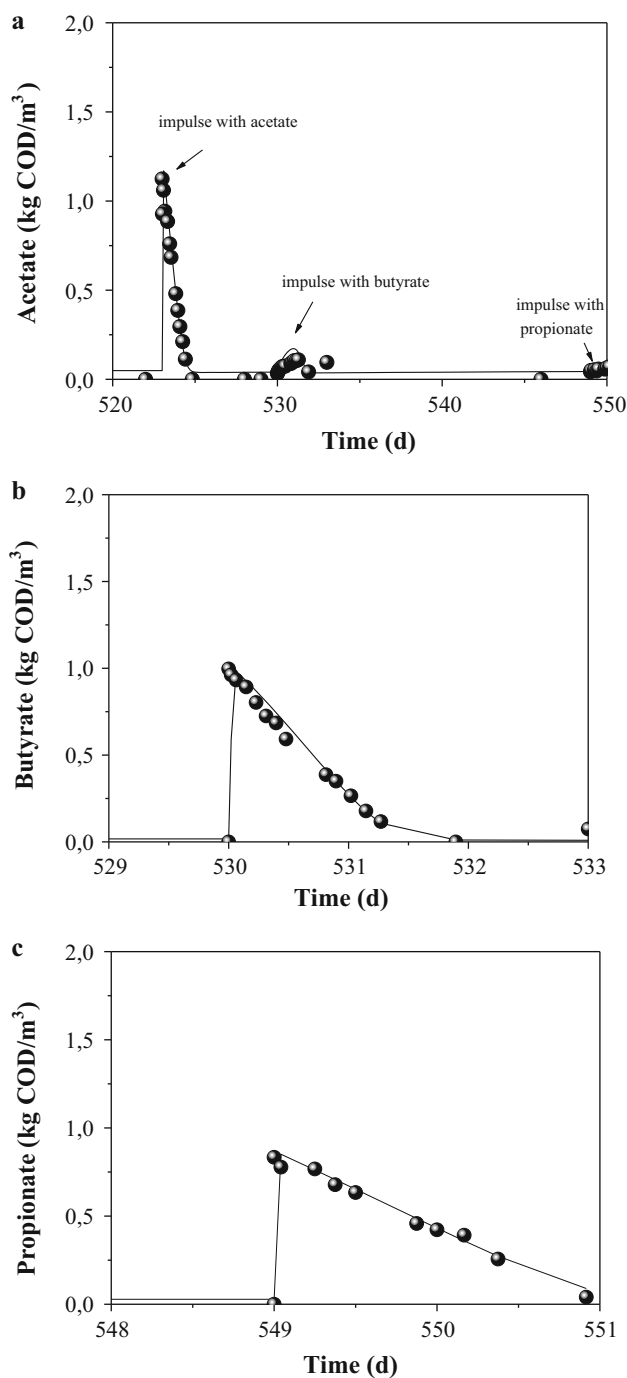
Modeling of the Methane Production in a CSTR-Type Reactor

Feed Composition

The feed medium was assumed to consist of carbohydrates, proteins, lipids (which comprise the solid phase), sugars, amino acids and long chain fatty acids (which constitute the liquid phase) and inerts (particulate and soluble). The composition of the feed as introduced in the model as kg COD/m³ was: carbohydrates 4.42, proteins 1.77, lipids 1.79, sugars 4.86, while aminoacids and long chain fatty acids were set to zero. In order to introduce the realistic inorganic inputs of the process, the concentrations of trace metals which were added in the reactor, were taken into account, as cations and anions concentration. In addition, the $NaHCO_3$ addition of the feed was considered as input to the methanogenic reactor. Specifically, the concentrations of carbon dioxide coming from $NaHCO_3$ hydrolysis were used for calculating the input concentrations of inorganic carbon, while the Na^+ contained in $NaHCO_3$ were accounted for, as cations.

Parameter Estimation of the Specific Maximum Uptake Rates

The parameters estimated were the specific maximum uptake rate (k_m) for acetate, propionate and butyrate through fitting the model to volatile fatty acid experimental data during the impulses with the three acids. The impulses were carried out, when the reactor was operated at the HRT of 15 days, for the second time. Initially, the k_m value for acetate was determined through fitting of the acetate
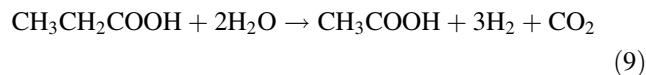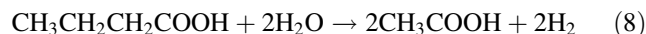
Fig. 4 Experimental (*cycles*) and model predicted volatile fatty acids (acetate (**a**), butyrate (**b**) and propionate (**c**)) concentrations (*line*) of CSTR-type digester fed with industrial food wastes during the operational period when impulses with VFAs occurred
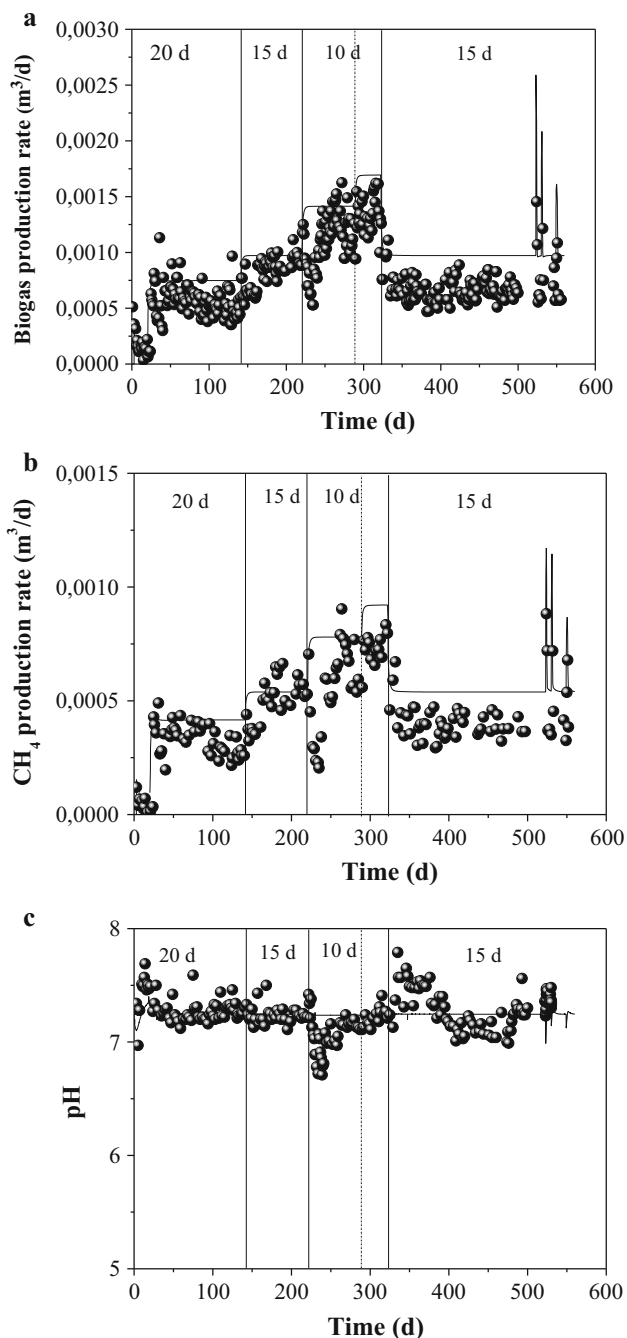
impulse to the experimental data and this value was used for simulation of acetate uptake during the impulse with butyrate and propionate, since acetate is also produced by butyrate and propionate degradation according to the reactions:

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (8)$$

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 \quad (9)$$

Afterwards, the k_m values for butyrate and propionate were determined through model fitting to the experimental data of the impulses with butyric and propionic salts, respectively. In Fig. 4, the simulated and experimental concentrations for VFAs during the three impulses, are presented. It can be seen that the model predicted very well the reactors' response to the VFA spikes. The values of the uptake rates (k_m) of the volatile fatty acids, estimated after the model fitting, are listed in Table 8 and are compared with the respective values estimated by Kalfas et al. [43] and Koutrouli et al. [44] who both modeled the AD of olive pulp in a CSTR-type bioreactor, and also implemented impulse disturbances of VFAs to their reactors. The comparison was also made with the parameter values suggested by Batstone et al. [29]. It can be seen that the values estimated in this study, for the volatile fatty acid degradation parameters, are in the same order of magnitude with those reported by the other studies. The fact that there are some differences could be attributed to the different wastes used (olive pulp, sludge) in different anaerobic digesters systems. It can also be observed that there is a wide range for k_m values for propionic acid, in the four studies presented in Table 8, since propionic acid degraders are considered to be a sensitive group of acetogenic bacteria [44].

Model Prediction for the Performance of the CSTR-Type Reactor

Using the k_m values estimated as reported above, the ADM1 was used to predict the performance of the methanogenic bioreactor under all the operating conditions, without any other modification or parameter estimation. The ADM1 simulation of the biogas and methane production rates and pH, against the respective experimental data at HRTs of 20, 15, 10 and 15 days (the second time) are shown in Fig. 5. It is obvious that the model was able to

Table 8 k_m values for acetate, butyrate and propionate estimated in this study and compared to these reported by other studies	k_m (kg COD kg/COD/d)	This study	Koutrouli et al. [44]	Kalfas et al. [42]	Batstone et al. [29]
	Acetate	5.98	8.34	9.99	8.00
	Butyrate	14.43	15.55	20.61	20.00
	Propionate	8.22	2.02	3.50	13.00

Fig. 5 Experimental (*cycles*) and model predicted biogas (**a**) and methane (**b**) production rates as well as pH (**c**) of CSTR-type digester fed with food wastes during all the experimental period

satisfactorily predict the experimental data over a wide range of operating conditions. However, for the HRT of 15 days (when the rector operated for second time from days 323 to 558), the predicted methane and biogas production rates were higher than the experimentally obtained rates, and were similar to the experimental values for the HRT of 15 days (days 142–220 when the reactor operated at the HRT of 15 days for first time). As mentioned earlier,

this was probably due to a washout of some microbial species when the reactor retention time was reduced to 10 days and the reactor seemed to be kinetically limited due to VFAs accumulation.

Conclusions

In the present study, the possibility of gaseous biofuels production using food industry wastes was investigated. Specifically, fermentative hydrogen production was studied in an anaerobic AUFCR at the HRTs of 12–2 h. The highest hydrogen production rate (1.5 ± 0.19 L H_2/d) was obtained at the HRT of 2 h, while the highest yield of hydrogen per mol of consumed carbohydrates (0.14 ± 0.01 mol/mol) was achieved at the HRT of 12 h. The distribution of the metabolic products was different at the various HRTs, with butyric acid being the main metabolic product during the whole operational period. Lactate was observed for the lower HRT values, while higher concentrations of caproic acid were detected at higher HRT values. Methane production of food wastes via AD was conducted in a CSTR type digester at the HRTs of 20, 15 and 10 days. It was shown that AD is a viable bioprocess, leading to a maximum methane production rate (0.73 ± 0.07 L/d) at the HRT of 10 days and thermally treated feed medium and a maximum methane yield at the HRT of 15 days (247.9 ± 0.15 L CH_4/kg waste). Finally, the ADM1 model was able to satisfactorily predict the digesters' behavior over a wide range of operating conditions. To sum up, it was demonstrated that food industry wastes and especially returned solid baby food is an appropriate substrate for prolonged and stable biofuels' production through biological processes.

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