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Biohydrogen and Methane Production from Cheese Whey in a Two-Stage Anaerobic Process

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The aim of the present study was to investigate the potential of hydrogen and subsequent methane production from raw cheese whey at 35 °C. The fermentative hydrogen production process from raw cheese whey was conducted in a continuous-type stirred tank bioreactor, operated at low hydraulic retention time (HRT; 24 h). In this stage, the carbohydrates contained in cheese whey are fermented to a mixture of acids and a gaseous mixture rich in hydrogen. The continuous fermentative hydrogen production was sustained by the indigenous microflora already contained in the raw cheese whey because the bioreactor was not seeded with any source of inoculum. At a HRT of 24 h, the hydrogen production rate was 7.53 L of H_2/day, while the yield of hydrogen produced was 0.041 $m^3$of H_2/kg of chemical oxygen demand (COD) added or 2.49 L of H_2/L of cheese whey. The mixed liquor from this stage was further digested to biogas in a periodic anaerobic baffled reactor (PABR), a baffled-type bioreactor. The PABR was operated at HRTs of 20, 10, and 4.4 days. The highest biogas and methane production rates were 105.9 L of biogas/day and 75.6 L of CH_4/day, respectively, and were obtained at an HRT of 4.4 days. During this stage, COD reduction reached 94%, obtained at an HRT of 4.4 days. Furthermore, the methane potential of the raw cheese whey was assessed by conducting a biochemical methane potential test. It was estimated to be 0.31 m^3 of CH_4/kg of COD added or 17.9 L of CH_4/L of cheese whey. This work demonstrated that biohydrogen production from cheese whey can be very efficiently coupled with methane production in a subsequent step, exploiting the gaseous biofuel potential of this wastewater type.

1. Introduction

Much attention has been paid to the hydrogen gas and its potential use as a fuel for transport purposes and electricity generation. Hydrogen is considered to be an alternative energy candidate because it is a clean and environmentally friendly fuel, oxidized to harmless water as a combustion product, instead of greenhouse gases.[1] It can be produced from renewable raw materials, such as organic wastes, and possesses a high-energy yield (122 kJ/g), which is about 2.75 times greater than that of hydrocarbon fuels.[2]

Certain hydrogen production processes, such as steam reforming or catalytic decomposition of natural gas, coal gasification, etc.,[3] are mature for commercial exploitation. However, these processes do not accomplish the environmental goal because nonrenewable fossil fuels are used to produce hydrogen gas. On the other hand, biological methods for hydrogen production are environmentally friendly and may be a viable alternative to the existing methods of hydrogen production.[4] Biohydrogen may be produced by cyanobacteria and algae through biophotolysis of water[5] or by photosynthetic and chemosynthetic− fermentative bacteria. The latter process seems to be the most promising one because it is carried out without photoenergy, while a variety of renewable feedstocks can be used as substrates. It is well founded that carbohydrates are the main source of hydrogen during fermentative processes and, therefore, wastes/wastewaters or agricultural residues rich in carbohydrates can be considered as potential sources of hydrogen.

Degradation of glucose (or its isomer hexoses or its polymers, starch and cellulose) during anaerobic conditions is accompanied by the production of hydrogen and various metabolic products, mainly volatile fatty acids (VFAs; acetic, propionic, and butyric acids), lactic acid, and alcohols (butanol and ethanol), depending on the microbial species present and the prevailing conditions. The hydrogen yield can be correlated stoichiometrically with the final metabolic products, through the reactions describing the individual processes of acidogenesis (eqs I and II). It is obvious that the production of acetic and butyric acids favors the simultaneous production of hydrogen,[6,7] with the fermentation of glucose to acetic acid giving the highest theoretical yield of 4 mol of H_2/mol of glucose (reaction I) and the conversion to butyric acid resulting in 2 mol of H_2/mol of glucose (reaction II):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \text{(I)}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \text{(II)}$$

In practice, the production of more metabolic products accompanied by a negative or zero yield in hydrogen results in lower total yields of hydrogen. Moreover, the metabolism toward acetate may occur via different, non-hydrogen-yielding pathways. In mixed fermentation processes, the microorganisms may select different pathways while converting sugars, as a response to changes in their environment (pH, sugar concentration, etc). The absence or presence of hydrogen-consuming microorganisms in the microbial consortium also affects the microbial metabolic balance and, consequently, the fermentation end products. In order to improve hydrogen production, all of these aspects should be taken into account and assessed.[8,9]

The waste material to be used for biological hydrogen production is based on its availability, cost, content in carbohydrates, and biodegradability.[4] Glucose, sucrose, and lactose

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are the fermentation substrates most studied in the laboratory.[10–14] Therefore, it is worthwhile to study hydrogen production from low-cost waste materials, residues, and biomass rich in carbohydrates and sugars. In this work, cheese whey, originating from the manufacturing of the Greek cheese type "feta", was used as a feedstock.

Cheese whey is the main byproduct of cheese manufacturing. It contains nutrients, such as lactose (4.5−5% w/v), soluble proteins (0.6−0.8% w/v), lipids (0.4−0.5% w/v), and mineral salts (8−10% of dried extract).[15] Because of its high organic content, cheese whey disposal constitutes a serious environmental problem, with lactose being mainly responsible for its high chemcial oxygen demand (COD) values. Anaerobic digestion of cheese whey offers an excellent approach from an energy conservation as well as pollution control point of view. However, raw whey is known to be quite problematic to be treated anaerobically because of its low bicarbonate alkalinity, its high COD concentration, and its tendency to get acidified very rapidly.[16]

The separation of acidogenesis from methanogenesis in a two-stage process may offer several advantages such as the production of a hydrogen-rich mixture in the acidogenesis stage and the higher performance of the process in terms of waste stabilization efficiency and net energy recovery.[17] To date, the feasibility of continuous fermentative hydrogen production from crude cheese whey has not been investigated extensively. Batch experiments were conducted by Ferchichi et al. (2005), who studied the influence of the initial pH on hydrogen production from cheese whey using a pure culture of *Clostridium saccharoperbutylacetonicum*.[18] In this study, continuous hydrogen production from cheese whey was studied using the indigenous mixed microbial culture already contained in the wastewater, a practice that has been scarcely applied and proposed by Antonopoulou et al. (2008).[19]

Moreover, an innovative high-rate bioreactor, the periodic anaerobic baffled reactor (PABR) was used in the second stage of the proposed process, for converting the acidified mixed liquor of the first stage to biogas. The PABR has been initially developed by Skiadas and Lyberatos[20] and, so far, it has been operated on synthetic feeding media of glucose and gelatin.[21,22] The PABR is a modified version of the anaerobic baffled reactor (ABR) and combines the advantages of common reactor designs, providing special flexibility.[23] Furthermore, the methane potential of the raw cheese whey was determined, and the overall potential of cheese whey for hydrogen and methane production was assessed.

2. Experimental Section

2.1. Analytical Methods. The measurements of dissolved and total COD, total (TSS) and volatile (VSS) suspended solids, alkalinity, pH, and Kjeldahl nitrogen (TKN) were carried out according to Standard Methods.[24] For the P determination, the persulfate digestion method and the ascorbic acid method[24] were applied. The oil and grease content of the wastewater was determined according to the Soxhlet extraction method,[24] while the total protein concentration was calculated based on the TKN content.[24] For determination of the total and soluble carbohydrates, a colored sugar derivative was produced through the addition of L-tryptophan and sulfuric and boric acids and subsequently measured colorimetrically at 520 nm.[25] D- and L-lactic acid were determined using the enzymatic reagent kit (Megazyme D-/L-lactic acid kit, K-DLATE 10/05).

For VFA and alcohol (ethanol and butanol) quantification, acidified samples with 20% H_2SO_4 were analyzed on 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 (held for 3 min) at a rate of 20 °C/min for VFA analysis and from 60 °C (held for 1 min) to 230 °C (held for 0.5 min) at a rate of 45 °C/min for alcohols analysis. Helium was used as the carrier gas at 15 mL/min, and the injector temperature was set at 175 °C and the detector at 225 °C. The hydrogen and methane contents of biogas were determined in a gas chromatograph (Varian STAR 3600) equipped with a thermal conductivity detector and a packed column (Poropak Q, 80−100 mesh) with nitrogen as the carrier gas. The injector, column, and detector temperatures were set at 70, 80, and 180 °C, respectively. The biogas production rate was measured using a water displacement technique.

Table 1. Characteristics of the Cheese Whey Used in This Study

characteristic	value
pH	6.0 ± 0.1
TSS (g/L)	6.77 ± 0.5
VSS (g/L)	6.27 ± 0.4
total COD (g/L)	61.0 ± 1.5
soluble COD (g/L)	52 ± 3.0
total carbohydrates (g/L)	38.0 ± 2.1
soluble carbohydrates (g/L)	36.0 ± 1.7
lactic acid (g/L)	0.62 ± 0.05
total proteins (g/L)	4.675
oil and grease (g/L)	1.0 ± 0.1
TKN (g/L)	0.826
inorganic nitrogen (g/L)	0.078
total phosphorus (g/L)	0.24 ± 0.02
total alkalinity (mg of $CaCO_3$/L)	480 ± 90

2.2. Feedstock. The cheese whey used in this study was obtained from a cheese factory, producing mainly the white cheese "feta", located in the Achaia prefecture, nearby Patras, Western Greece. The wastewater was maintained in the refrigerator, replenishing it with fresh if signs of acidification were shown.

The average characteristics of the cheese whey used in this study are presented in Table 1. It is obvious that the wastewater mainly consists of carbohydrates (3.8% w/v). VFAs, ethanol, as well as butanol were not detected at all in raw cheese whey, while 0.62 g/L of lactic acid was found. The total bicarbonate alkalinity of cheese whey was extremely low (480 ± 90 mg of $CaCO_3$/L).

2.3. Hydrogen Production Stage. A 3 L working volume, double-wall, cylindrical, continuous stirred tank type reactor made of stainless steel was operated under mesophilic (35 °C) conditions at a hydraulic retention time (HRT) of 24 h. During start-up, the reactor was filled up with 3 L of undiluted cheese whey and operated anaerobically in a batch mode at 35 °C for 24 h.

After start-up, the operation of the hydrogen-producing reactor was subsequently switched to a continuous mode. The feed was provided in cycles; the undiluted cheese whey was fed intermittently for 7 min, every 3 h, maintaining the HRT at 24 h. The influent of the reactor was maintained at a temperature below 4 °C, while the mixed liquor inside the reactor was stirred periodically for 15 min, two times per hour. Feeding was programmed always with the stirring on. Simultaneous flow of the effluent occurred during feeding by liquid overflow, in order to maintain a constant reactor volume. As a result, a portion of the wastewater fed was removed with the effluent, and the concentration of the carbohydrates, after feeding was completed, had to be calculated, according to the mass balance equation (1) (assuming no conversion during feeding).

$$S = S_0 - (S_0 - S_{in})e^{(Q/V)t} \quad (1)$$

where S is the concentration immediately after feeding was completed, S_0 is the concentration in the feed, S_{in} is the concentration in the bioreactor prior to feeding, Q is the volumetric feeding rate, V is the reactor volume, and t is the duration of feeding.

Gas and liquid samples were taken 10−15 min before feeding started. The reactor performance (biogas production and composition in H_2, pH, TSS, VSS, carbohydrates, soluble COD, and VFA concentration) was monitored daily throughout the experimental period. Full characterization of the reactor performance (biogas production and composition in H_2, pH, TSS, VSS, carbohydrates, soluble COD, L- and D-lactic acid, ethanol, butanol, and VFA concentration) took place once the steady state was reached. By steady state, it is meant that the variation of the monitoring parameters in time was lower than 10%. Gas samples were analyzed for methane daily, in order to monitor whether methane production was taking place.

2.4. Methane Production Stage. A high-rate bioreactor, of the PABR type, was used for digesting the acidified effluent of the first stage to methane. Details of the PABR description can be found in Skiadas and Lyberatos (1998).[20] The reactor operating volume was 15 L, and the reactor consisted of four compartments. It was equipped with sample ports in every compartment placed 10 cm from the surface of the mixed liquor. There were also two biogas vents on the top of the reactor. The PABR was immersed in a tank full of water maintained at 35 °C through a temperature controller. The reactor was operated anaerobically under mesophilic (35 °C) conditions at HRTs of 20, 10, and 4.4 days.

Gas and liquid samples were taken at regular intervals, and biogas production, composition in CH_4, pH, soluble COD, and VFAs concentration were determined.

2.5. Biochemical Methane Potential (BMP) Experiments. BMP experiments were carried out in duplicates at 35 °C in 160 mL serum vials, according to Owen et al. (1979).[26] Serum bottles were seeded with 45 mL of a mixed anaerobic culture obtained from the anaerobic digester treating municipal sewage sludge, at Patras wastewater treatment plant, operated at steady state (HRT: 15 days) and pH = 7.1. The cheese whey (pH = 6), supplemented with 5 g/L of $NaHCO_3$, 10 mL/L of a solution of $(NH_4)_2HPO_4$ (0.721 g/L), and 10 mL/L of a solution with trace metals (Table 2), was added in the vials to a final volume of 50 mL. 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 gas mixture of N_2/CO_2 (80/20) in order to secure anaerobic conditions. The vials were sealed with butyl rubber stoppers and aluminum crimps, and methane production was monitored versus time according to Owen et al. (1979).[26] The pH values at the end of the experiments in the two serum bottles were 7.55 and 7.54, respectively.

Table 2. Stock Solution of the Trace Metals Used in the BMP Tests

compound	concentration (mg/L)
$CaCl_2 \cdot 2H_2O$	22 500
NH_4Cl	35 900
$MgCl_2 \cdot 6H_2O$	16 200
KCl	117 000
$MnCl_2 \cdot 4H_2O$	1800
$CoCl_2 \cdot 6H_2O$	2700
H_3BO_3	513
$CuCl_2 \cdot 2H_2O$	243
$Na_2MoO_4 \cdot 2H_2O$	230
$ZnCl_2$	189
$NiCl_2 \cdot 6H_2O$	200
H_2WO_4	10
$FeSO_4$	700

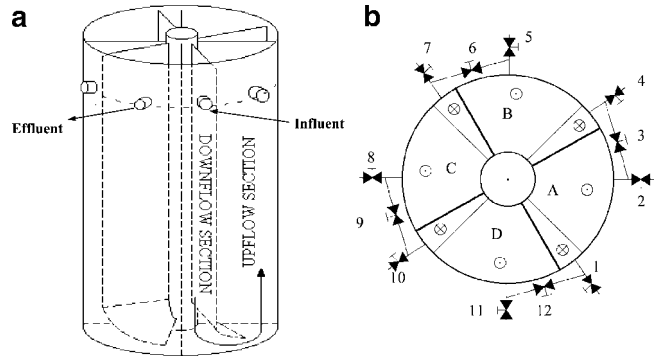

Figure 1. (a) Front view of a four-compartment PABR: the feed enters the compartment through a port attached to the downflow section, comes up in the upflow section passing below the baffle, and then enters the next compartment through external tubes. (b) Top view of a four-compartment PABR: 12 electronic valves positioned on tubes set outside the reactor. The valves vary the role of each compartment (influent, effluent, or intermediate) over time at a preselected switching period. Note that the external tubes connect the upflow section (⊙) of the compartment with the downflow section (⊗) of the subsequent compartment.

3. Results and Discussion

3.1. Continuous Hydrogen Production. The continuous stirred tank type bioreactor was operated in batch mode during start-up so that the indigeneous microorganisms would adapt to their new environmental conditions at 35 °C in the absence of air. The gas phase consisted of approximately 29% (v/v) 1 day later, indicating that the hydrogenogenic fermentation process had started. In the sequel, the bioreactor operation was switched to continuous mode, at an HRT of 24 h.

It was found that the pH in the reactor should be maintained at 5.2 ± 0.1 in order to detect H_2 in the gas phase at concentrations higher than 25%. This was achieved by adding $NaHCO_3$ in the cheese whey at a concentration of 20 g of $NaHCO_3$/L. The total bicarbonate alkalinity after the addition of $NaHCO_3$ increased to 11 390 ± 350 mg of $CaCO_3$/L. The pH value of the raw whey was increased from 6.0 ± 0.1 to 7.6 ± 0.1, after $NaHCO_3$ addition. It is well-known that anaerobic fermentative hydrogen production is suppressed by both low and high pH values[27] because the pH is a crucial parameter for bioprocesses. It has been reported that maximum hydrogen yields are obtained when the pH of the culture medium is between 5 and 6.[28,29]

The evolution of biogas and hydrogen versus time is presented in Figure 2. It should be noted that no methane was detected throughout the experimental period. The percentage of hydrogen in the gas phase at the steady state was 29.3 ± 1.6%, while the hydrogen production rate reached 7.53 L/day (or 2.51 ± 0.43 L/L of reactor/day). In terms of the organic load of cheese whey, the hydrogen production was calculated to be 0.041 m^3/kg of COD added.

The parameters monitored in the hydrogen-producing bioreactor reached levels of low variation from the 30th day and on. The mean values of the parameters and the standard deviations are given in Table 3. According to the table values, TSS and VSS concentrations were 10.0 ± 0.5 and 8.4 ± 0.4 g/L, respectively.

It is obvious that the main microbial products among metabolites measured were acetic acid (9.394 ± 0.544 g/L), lactic acid (7.543 ± 0.450 g/L), and butyric acid (7.199 ± 0.650 g/L). Propionic acid and ethanol were also detected, but to a

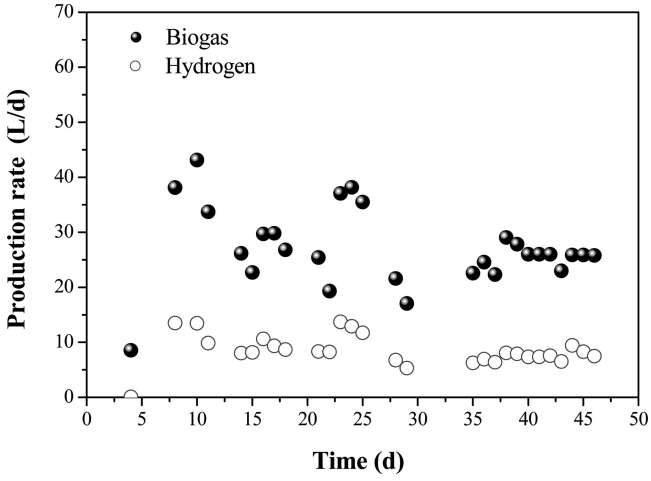

Figure 2. Evolution of biogas and hydrogen in the hydrogenogenic reactor throughout the experimental period.

Table 3. Characteristics of the Hydrogenogenic Reactor at Steady State

characteristic	value
flow rate (L/day)	3
pH	5.2 ± 0.1
TSS (g/L)	10.0 ± 0.5
VSS (g/L)	8.4 ± 0.4
H_2 in the gas phase (%)	29.3 ± 1.6
hydrogen production (L/L of reactor/day)	2.51 ± 0.43
carbohydrates (g/L)	11.30 ± 0.5
acetic acid (g/L)	9.394 ± 0.544
propionic acid (g/L)	undetected
butyric acid (g/L)	7.199 ± 0.650
lactic acid (g/L)	7.543 ± 0.450
ethanol (g/L)	0.308 ± 0.060
soluble COD (g/L)	47.4 ± 2.8
efficiency of soluble carbohydrate consumption (%)	68.6
hydrogen yield (mol of H_2/mol of consumed glucose)	0.9 ± 0.1
hydrogen yield (L of H_2/L of cheese whey)	2.49

lesser extent, while butanol, as well as isobutyric, valeric, and isovaleric acids, was not detected at all. On the basis of the experimental results obtained, a mixed acid fermentation occurs upon biological degradation of carbohydrates contained in raw cheese whey, and this could justify the lower obtained yields of hydrogen production, compared with the theoretical ones. At steady state, the calculated COD concentration, representing the sum of the different products (ethanol and lactic, butyric, propionic, and acetic acids) as well as the nonconsumed carbohydrates expressed in terms of COD, was equal to 45.54 ± 3 g/L. The calculated COD concentration accounted for 96% of the measured COD concentration (47.4 ± 2.8 g/L). Therefore, it can be assumed that the main metabolic products of cheese whey fermentation were already determined.

In general, hydrogen yields vary proportionally to the final metabolic products. It is well-known that production of acetic and butyric acids favors the production of hydrogen, while the production of propionic acid consumes hydrogen. Moreover, lactic acid and ethanol production is accompanied by no hydrogen generation.[19] On the basis of the reactions I and II, the anticipated hydrogen production according to the metabolic products measured could be calculated. This calculation corresponds to the production of 2 mmol of hydrogen/1 mmol of acetic acid produced and 2 mmol of hydrogen/1 mmol of butyric acid produced. In this study, the theoretically calculated hydrogen production based on the measured concentration of acetic acid at steady state was 939 mmol/day, while the respective value based on the measured concentration of butyric acid was 491 mmol/day. This rate was much higher compared to the experimentally measured hydrogen production rate (335 mmol/day). An explanation could be that hydrogen-consuming microorganisms have been established in the system, consuming an amount of the produced hydrogen. Such microorganisms could be either methanogenic bacteria converting hydrogen gas to methane or homoacetogenic bacteria producing acetic acid with hydrogen consumption (eq 1). Methanogens are reported to be limited under low pH values and operation of the reactor at short HRT.[30] Because no methane was detected in the reactor content and the pH and HRT values were extremely low for methanogenic bacteria to grow, the possibility of hydrogenotrophic methanogenesis is excluded. On the other hand, homoacetogenic bacteria could probably have been established in the reactor by consuming a considerable amount of the produced hydrogen, producing acetic acid, according to reaction III.

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad \text{(III)}$$

Another possibility for the discrepancy between the anticipated and measured hydrogen production rate could be that part of the acetate may be produced through reaction IV, where no hydrogen is produced via glucose degradation.

$$C_6H_{12}O_6 \rightarrow 3CH_3COOH \quad \text{(IV)}$$

The efficiency of total carbohydrates' consumption in glucose equivalents was 68.6% because the concentration of carbohydrates in the feed was 38.0 ± 2.1 g/L and the respective concentration in the mixed liquor was 11.30 ± 0.5 g/L. On the basis of the above observations, the yield of hydrogen produced was approximately 0.9 ± 0.1 mol of H_2/mol of glucose consumed and, consequently, 2.49 L of H_2/L of cheese whey. This corresponds to an energy yield of 24.85 kJ/L of cheese whey (assuming that the energy yield from hydrogen is 122 000 kJ/kg).

A common practice for seeding of a bioreactor with the aim of producing hydrogen is to use heat-treated sludge, in order to favor the spore-forming, hydrogen-producing clostridia.[30,31] In this work, the indigenous microorganisms of the cheese whey were used. This practice has also been applied in the fermentation of biomass (sweet sorghum) with success.[19] It should be noted that all common practices reported in the literature for starting up hydrogen-producing bioreactors failed, meaning that in these cases no spore-forming bacteria able to produce hydrogen prevailed. The fact that the raw cheese whey can be directly used for the production of hydrogen is very important for the operation of a full-scale plant, suggesting that no extra energy will be required either for the start-up of the reactor or for the pasteurization or sterilization of the influent.

From an environmental point of view, the fermentative hydrogen production process does not reduce the organic content of the feed. In this study, total COD removal was below 5% during the hydrogen production process and, therefore, another, subsequent stage is required for COD reduction.

3.2. Methane Production. A high-rate bioreactor was used for treating the acidified effluent of the first stage. PABR resembles a simple ABR. Its main concept lies on switching of the influent (and, consequently, the effluent) point within a period of time. The switching frequency (or equivalently the switching period, that is, the interval for the influent point to switch to all four compartments) allows flexibility in the operation of the PABR. The PABR can be operated as a simple ABR, if the switching frequency is set to zero and in the extreme case of a very high switching frequency, such as a single-

Table 4. Main Characteristics of the Influent of the PABR

characteristics	value
pH	4.8 ± 0.1
TSS (g/L)	8.43 ± 1.9
VSS (g/L)	6.78 ± 1.2
total COD (g/L)	58.0 ± 1.5
soluble COD (g/L)	46.2 ± 3.0
total alkalinity (mg of $CaCO_3$/L)	3417 ± 300
lactic acid (g/L)	9.6 ± 1.5
total VFAs (g of COD/L)	27.4 ± 3.1

compartment upflow bioreactor. It has been found that, under high organic loading rates, high switching frequencies (or equivalently low values of the switching period) lead to higher performance.[32] The switching period was selected to be 2 days in this study.

The average characteristics of the feed of the PABR after collection of the effluent of the hydrogen-producing reactor, homogenization and preservation at −20 °C, are presented in Table 4. The influent of the anaerobic digester was rich in VFAs, as anticipated, having a contribution of 59.3% in terms of COD, compared to the soluble COD concentration. Moreover, because of the addition of $NaHCO_3$ in raw cheese whey, the total alkalinity concentration of the effluent of the hydrogenogenic reactor was 3417 ± 300 mg/L.

The biogas and methane production rates throughout the experiment are shown in Figure 3. The biogas and methane production rates increased when HRT was decreased from 20 to 4.4 days, as expected. The higher biogas and methane production rate was obtained (105.9 L of biogas/day and 75.6 L of CH_4/day, respectively) at a HRT of 4.4 days. This latter value corresponded to a yield of 22.23 L of methane/L of influent. In general, the biogas methane content was between 71.4 and 74.9%.

The mean values of the main parameters in the effluent of the PABR for the various HRTs are given in Table 5. The pH was approximately constant at all HRTs. Acetic acid was the only metabolic product detected at HRTs of 20 and 10 days, while at a HRT of 4.4 days, acetic and propionic acids were accumulated in the reactor. Butyric, isobutyric, valeric, and isovaleric acids were not detected at all. The influent and effluent COD concentrations in the PABR throughout the experiment are presented in Figure 4. The percentage of COD removal was approximately 99% at HRTs of 20 and 10 days and 94% at a HRT of 4.4 days. These observations imply that methanogenesis

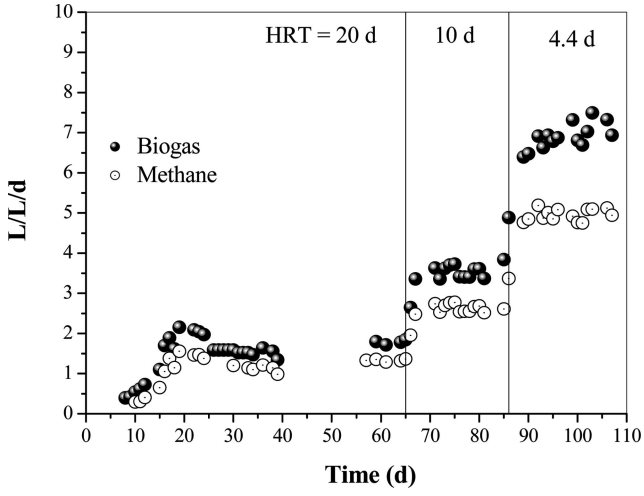

Figure 3. Biogas and methane production rates in the PABR throughout the experimental period.

Table 5. Characteristics of the PABR at Steady State at HRTs of 20, 10, and 4.4 days

	HRT (days)		
	20	10	4.4
pH	7.9 ± 0.1	7.9 ± 0.1	8.01 ± 0.1
% in CH_4	74.9 ± 1.0	73.6 ± 3.0	71.4 ± 2.2
biogas production (L/L of reactor/day)	1.73 ± 0.10	3.54 ± 0.16	7.06 ± 0.29
acetic acid (mg/L)	174 ± 50	96 ± 20	515 ± 150
propionic acid (mg/L)	NM	NM	918 ± 150
butyric acid (mg/L)	NM	NM	NM
isobutyric acid (mg/L)	NM	NM	NM
valeric acid (mg/L)	NM	NM	NM
isovaleric acid (mg/L)	NM	NM	NM
dissolved COD (mg/L)	786 ± 97	539 ± 102	3002 ± 258
COD removal (%)	98.3	98.9	94.2

started to get kinetically limited at a HRT of 4.4 days, while at HRTs of 20 and 10 days, there was no kinetic limitation. This indicated that further reduction of the HRT would lead to higher VFA accumulation and deterioration in the COD removal efficiency.

This study indicated that raw cheese whey could be treated anaerobically in a two-stage process at relatively short HRT values. To date, most of the studies on the anaerobic treatment of cheese whey dealt with diluted (or deproteinated) whey fed in high-rate bioreactors, operating at relatively short HRTs.[33] Otherwise, a HRT of 5 days is usually considered in the literature as a minimal admissible HRT to achieve stable operation of anaerobic bioreactors treating raw cheese whey.[34–36] Ergüder et al. (2001) stated that undiluted whey could be treated in a UASB reactor at short HRT values (2.06−4.95 days) with a COD removal efficiency of 95−97% at influent COD concentrations of 42 700 ± 141−55 100 ± 283 mg/L.[33]

3.3. BMP Experiments. The methane production during the batch experiment for the determination of the methane potential of the cheese whey is presented in Figure 5. The calculated methane production of the wastewater, after subtraction of the methane produced from the blank experiment, was 89.5 mL of CH_4. It is obvious that a significant portion of the methane produced totally (80%) was evolved within in the first 13 days, while the experiment lasted more than 3 months. These values indicated that the biological methane potential of the cheese whey was 0.31 m^3 of CH_4/kg of COD added or 17.9 L of CH_4/L of cheese whey. The corresponding energy yield is 897 kJ/L of cheese whey (assuming that the energy yield from methane is 50 120 kJ/kg). The obtained methane potential is lower than

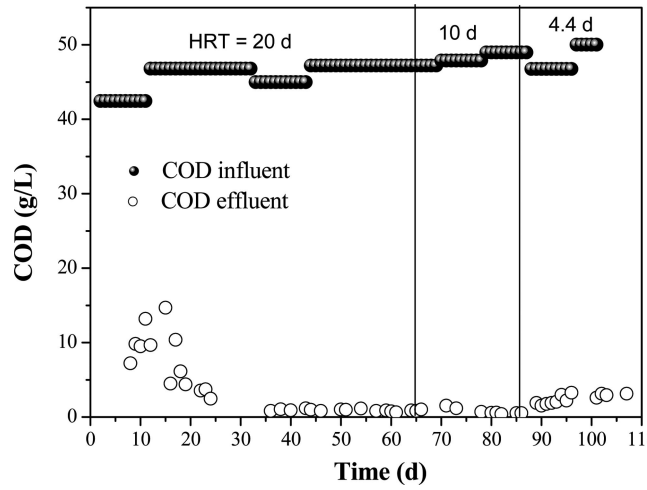

Figure 4. Influent and effluent CODs in the PABR.

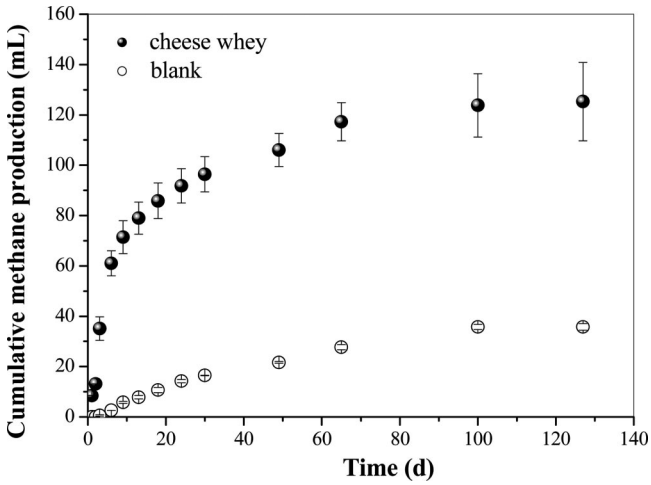

Figure 5. Methane production during the batch experiment for the determination of the methane potential of cheese whey.

the respective value obtained from Ergüder et al. (2001), who found the methane potential to be 23.4 L of CH_4/L of cheese whey.[33] The higher yields could probably be due to the different organic load of the wastewater.

4. Conclusions

The present study focused on the combined hydrogen and methane production from raw cheese whey in a two-stage process. It has been shown that continuous fermentative hydrogen production from cheese whey is possible and stable at a HRT of 24 h in mesophilic conditions. The biogas and hydrogen production rates were 25.7 and 7.53 L/day, respectively, while the yield of hydrogen produced was approximately 0.9 ± 0.1 mol of H_2/mol of glucose consumed. This yield corresponds to the production of 2.49 L of H_2/L of cheese whey or to an energy yield of 24.85 kJ/L of cheese whey. The indigenous microorganisms of the whey wastewater were capable of hydrogenogenic fermentation, and there was no need for using other sources of inoculum during the bioreactor start-up.

Further conversion of the acidified effluent of the first stage to methane was conducted in a high-rate bioreactor of the PABR type. The higher biogas and methane production rates were 105.9 and 75.6 L/day, respectively, and obtained at a HRT of 4.4 days. At this HRT, signs of VFA accumulation indicated that further reduction of the HRT would decrease the performance. Moreover, the BMP of cheese whey was found to be 0.31 m^3 of CH_4/kg of COD added or 17.9 L of CH_4/L of cheese whey.

Overall, this work demonstrates that biohydrogen production can be very efficiently coupled with a subsequent step of methane production, and cheese whey can be an ideal feedstock for the proposed gaseous biofuel production process.

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