Continuous fermentative hydrogen production from cheese whey in an attached growth biomass system: The influence of operational parameters

M. Alexandropoulou[1], E. Lenakaki[1], G. Lyberatos[1,2] and G. Antonopoulou[1,*]

[1]Institute of Chemical Engineering Sciences, Stadiou 1, Platani, Patras, GR 26504, Greece

[2]School of Chemical Engineering, National Technical University of Athens, GR 15780 Athens, Greece

*Presenting author email: geogant@chemeng.upatras.gr

ABSTRACT

Hydrogen production via dark fermentation (DF) of cheese whey (CW) was investigated using an anaerobic UpFlow Column Reactor (AUFCR), with attached biomass. The reactor operated for two different operational periods (after two different start-ups), where in the first, the effect of initial carbohydrates concentration i.e. 30 and 45 g/L was studied, while in the second one, the effect of the hydraulic retention time (HRT) i.e. 12, 8 and 6 h on DF performance, was investigated.

The optimal conditions were found to be at an HRT of 12 h and a feed carbohydrate concentration of 30 g/L. The hydrogen yield obtained under these conditions was 0.30 mol of H_2 per mol of consumed carbohydrates.

KEYWORDS

Hydrogen, up-flow column reactor, cheese whey, fermentation, substrate concentration, hydraulic retention time

INTRODUCTION

Biological hydrogen production from several types of wastes/wastewaters is considered as a promising process for sustainable energy production. Due to zero emissions when combusted and its high energy yield of 121 kJ/g (around 2.7 times higher than hydrocarbon fuels), hydrogen is one of the most promising sustainable energy resources [1]. Its production via dark fermentation (DF) is rather attractive as it can be conducted using mixed cultures at moderate temperatures, resulting to high production rates and yields. Among the different substrates that can be used as substrates, cheese whey (CW) is considered quite promising, as it is an agro-industrial by-product, rich in carbohydrates [2,3]. CW is the main by-product during cheese making process, which has strong pollution potential when disposed untreated to the environment [1]. Instead of its harmful disposal, as CW has a high lactose content (4.6 %), it is a very suitable substrate for biotechnological processes, especially for fermentations [4].

Up to now, DF from CW has been studied under different operational conditions, mainly in continuous stirred tank reactors (CSTR) and in upflow anaerobic sludge blanket (UASB) reactors [4]. A wide range of hydraulic retention times (HRTs) from 9-72 h has been tested in CSTRs, using mixed microbial cultures under mesophilic conditions [5 -7]. Apart from suspended-growth biomass systems, the attached-growth biosystems are also advantageous for DF, allowing operations with short HRTs, owing to the more significant biomass retention [3] and to the large surface areas for the formation of biofilm, provided from the supporting medium [8]. Lima et al. [9] studied DF of CW in an anaerobic sequencing biofilm batch reactor (AnSBBR), using polyethylene as an inert supporting matrix for biomass immobilization and the effects of feeding time, temperature, and influent concentration were assessed. The authors observed a hydrogen productivity of 660 mL /L/d and a yield of 0.80 mol H_2 /mol lactose, respectively, at an influent concentration of 5.4 g COD /L.

The objective of the present study was to investigate the possibility of using CW for the production of biohydrogen via DF in an anaerobic continuous up-flow column reactor (AUFCR), containing ceramic beads as support material for the attachment of bacterial biomass, at different HRTs and feed carbohydrate concentration.

MATERIALS AND METHODS

Cheese Whey

The CW used was obtained from a cheese factory located in Patras, Greece. The average characteristics of the wastewater were pH: 6.4 ± 0.0, total suspended solids (TSS): 4.3 ± 0.3 g/L, volatile suspended solids (VSS): 4.1 ± 0.2 g/L, total and soluble carbohydrates: 44.9 ±1.4 and 41.4 ± 1.7 g/L, respectively, total and soluble chemical oxygen demand (COD): 51.9 ± 1.7 and 49.3 ± 1.7 g/L, respectively.

Hydrogen production process

The experiments were performed in the AUFCR described in Alexandropoulou et al. [10]. The reactor volume was 0.5 L and it was double-coated and temperature control (35 ± 0.5 ^{o}C) was achieved via recirculation of water in the outer jacket. Cylindrical porous ceramic beads were used as support material for the attachment of bacterial cells. Diluted CW (the dilution factor resulted in different feed carbohydrate concentration) was fed periodically (8 times a day) to the bottom of the up flow reactor, via a peristaltic pump. The treated effluent was removed from the reactor by overflow.

The reactor operated for two different operational periods, after two different start-ups, where new ceramic beads were used, and the main characteristics are presented in table 1:

Table 1: The conditions of two operational periods

Operational period	Initial carbohydrates' concentration	HRT
1st	30, 45	12
2nd	30	12, 8, 6

During the first one, the concentration of the total carbohydrates in the feed was initially 30 g/L and increased to 45 g/L, while the HRT was constant at 12 h (60 and 90 g carbohydrates/ Ld, respectively). During the second operational period, the concentration of the carbohydrates was constant at 30 g/L and the HRT was gradually reduced, from 12 to 8 and 6 h, respectively (60, 90 and 120 g carbohydrates/ Ld, respectively).

The start-up of both operational periods was performed using the indigenous microbial consortium of the CW, as described in Alexandropoulou et al. [10]. The feed was supplemented with NaOH, KH_2PO_4 and urea, as proposed by Alexandropoulou et al. [11], in order to keep the reactor pH at a suitable range for DF process.

Analytical methods

The reactor performance (biogas production rate and composition in H_2, pH, TSS, VSS, carbohydrates, COD, volatile fatty acids (VFA) and lactate concentration) was monitored and characterized according to Alexandropoulou et al. [10,11].

RESULTS AND DISCUSSION

1st operational period

In the first operational period where the effect of the carbohydrates' concentration was investigated (different dilution of CW), the reactor operated anaerobically for 49 days. Initially, it operated for 30 days, with a total carbohydrates' concentration of 30 g/L and then the concentration increased to 45 g/L. In each period, a steady state was reached, which means that the variation of every monitored parameter was less than 10%, for at least 5 successive measurements (on daily base). Fig. 1 illustrates both the hydrogen content of the produced biogas and the hydrogen production rate. As it can be seen, hydrogen was contained in the biogas since the beginning of the operation, as the hydrogen content of the gas phase was equal to 47 % (v/v) right after the inoculation. In the sequel, during the two steady states achieved, the hydrogen percentage was equal to 26 (30 g carbohydrates /L) and 22% (45 g carbohydrates /L). Also, no methane was detected during the reactor operation indicating that methanogenesis did not take place under the tested conditions.

The hydrogen production rate slightly increased with the carbohydrates' concentration increase. Specifically, when the concentration of the carbohydrates was 30 g/L, the hydrogen production rate was

1.25 ± 0.10 L H_2/d, which increased to 1.43 ± 0.08 L H_2/d, when the concentration of the carbohydrates increased to 45 g/L. As it was anticipated the hydrogen production rate in terms of L/$L_{reactor}$/d, also followed the same behavior and was 2.29 ± 0.18 and 2.62 ± 0.15 for 30 and 45 g/L respectively (Table 2).

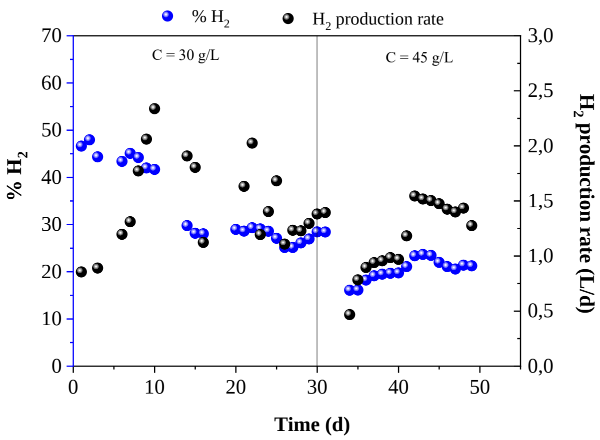

Figure 1: The percentage of hydrogen in the gas phase and the hydrogen production rate during DF of CW in the AUFCR at the 1st operational period.

The concentration of the initial and non-consumed carbohydrates, expressed in glucose equivalents, is presented in Fig.2. As indicated, the concentration of non-consumed total and soluble carbohydrates slightly increased with the increase of the initial carbohydrates concentration. In particular, during the steady state of the first operational period, the concentrations of soluble and total carbohydrates were 0.69 ± 0.04 g/L and 1.45 ± 0.20 g/L, while for the second steady state the respective values were equal to 1.65 ± 0.13 g/L and 2.41 ± 0.19 g/L. The consumption of CW carbohydrates, was almost 95% in both cases.

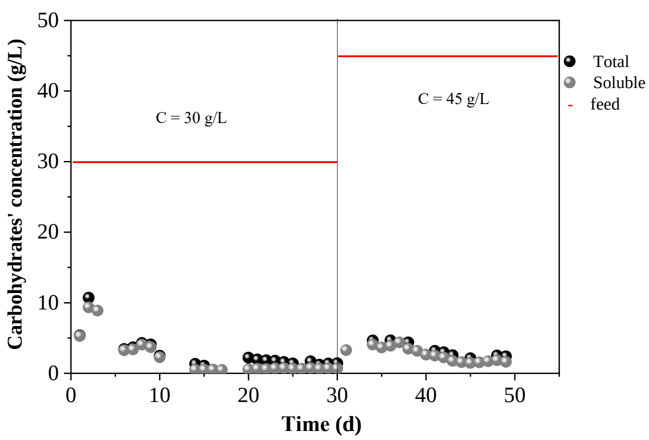

Figure 2: The concentration of the feed and non- consumed carbohydrates during DF of CW in the AUFCR at the 1st operational period.

The hydrogen yield expressed in terms of mol/mol of total carbohydrates consumed was higher for the lower feed concentration of carbohydrates (30 g/L) and it was equal to 0.30 ± 0.02. On the other hand, the hydrogen yield for initial carbohydrates' concentration of 45 g/L was 0.22 ± 0.02 (Table 1). The main metabolites produced during the 1st AUFCR operation were butyric, iso-butyric, acetic, caproic and lactic acid. Propionic acid was also detected, but at lower concentrations. As it can be seen from Fig.3, butyric and lactic acid were the two acids that prevailed during the whole experimental period. Specifically, butyric acid concentration was constant and almost equal to 10 g/L for the whole experimental period, while lactate concentration was equal to 4.65 ± 0.28 g/L and 7.02 ± 0.47 g/L for 30 and 45 g/L of carbohydrates', respectively. As shown in Table 1, the COD balance (theoretically calculated from the metabolic products compared to the experimentally measured one), was equal to 95.2% for 30 g carbohydrates/L and 78.8 % for 45 g/L, respectively. This could be attributed to un-identified metabolites, such as ethanol.

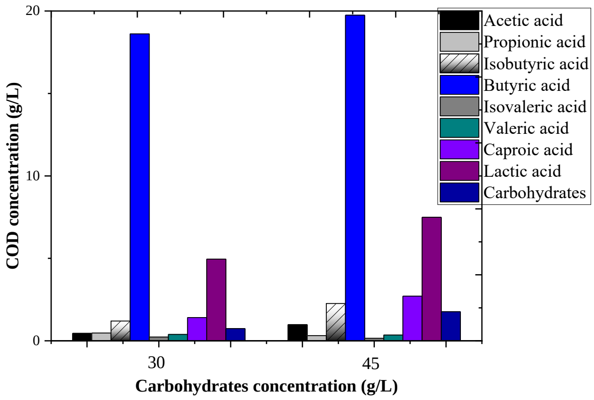

Figure 3: The distribution of the soluble metabolites during DF of CW in the AUFCR at the 1st operational period.

Table 2. The main characteristics of the two steady states during DF of CW in the AUFCR at the 1st operational period.

	C= 30 g/L	**C= 45 g/L**
pH	5.65 ± 0.03	5.50 ± 0.07
TSS (g/L)	7.65 ± 1.34	10.49 ± 0.39
VSS (g/L)	5.61 ± 1.11	7.54 ± 0.26
Hydrogen Content (%)	26.36 ± 1.39	21.92 ± 1.20
d COD (g/L)	29.89 ± 0.69	45.42 ± 4.11
% COD (theoretical/measured)	95.20	78.75
Hydrogen production rate ($L/L_{reactor}/d$)	2.29 ± 0.18	2.62 ± 0.15
Hydrogen yield ($L\ H_2/L_{feed}$)	1.14 ± 0.09	1.31 ± 0.07
Hydrogen yield ($L\ H_2/L_{cheese\ whey}$)	2.08 ± 0.17	1.54 ± 0.09
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.30 ± 0.02	0.22 ± 0.02

2nd operational period

The second experimental series of the AUFCR was conducted keeping the carbohydrates' concentration constant (equal to 30 g/L), and reducing the HRT. In particular, the reactor operated for 40 days and its operation could be divided into three phases. During the first one the HRT was 12 h (days 1-24) and in the sequel the HRT decreased to 8 h (days 25-34) and finally to 6 h (days 35-40). It has to be mentioned that in all cases the reactor reached a steady state. As becomes obvious from Fig. 4, a long and stable reactor operation was achieved, despite the fact that right after inoculation the hydrogen production almost ceased. This is generally attributed to a gradual change in microbial community composition [3]. The hydrogen production rate at the steady states was equal to 1.27±0.10 L H_2/d for the first phase (HRT=12 h), which slightly increased to 1.29±0.19 L H_2/d when the HRT decreased to 8 h and finally decreased to 0.84±0.06 L H_2/d with a further reduction of the HRT to 6 h. Similarly, the hydrogen production rates achieved in terms of $L/L_{reactor}/d$ were equal to 2.43 ± 0.20, 2.45 ± 0.36 and 1.61 ± 0.11 for the three steady states, respectively.

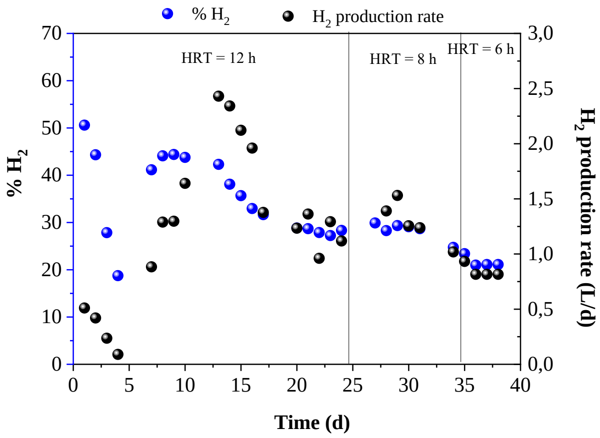

Figure 4: The percentage of hydrogen in the gas phase and the hydrogen production rate during DF of CW in the UFCR at the 2nd operational period.

In Fig. 5, the concentrations of the influent and effluent (non-consumed) reactor carbohydrates, are presented. It can be seen that although carbohydrates' concentrations were initially quite high, after a period of almost 20 days, the reactor operation reached a steady state and the carbohydrates were almost consumed. In particular, the concentration of the soluble carbohydrates was almost 0.5 g/L during the whole experimental period. The concentration of the non-consumed total carbohydrates was 1.16 ± 0.03 g/L at the HRT of 12 h, 1.27 ± 0.19 g/L at the HRT of 8 h and 1.70± 0.01 g/L at the shorter HRT (6 h).Therefore, it can be concluded that the carbohydrate consumption was as high as 95 % during the whole experimental period. The hydrogen yield expressed in terms of mol/mol of total carbohydrates consumed was maximized for the higher HRT (12 h) and was 0.30 ± 0.02 and decreased to 0.22 ± 0.04 and to 0.10± 0.00 for the HRTs of 8 and 6h (Table 3). It is worth to mention that when the two reactors operated under the same conditions (HRT = 12 h, carbohydrates' concentration 30 g/L) the hydrogen yields obtained were the same (i.e 0.30 mol/mol). This fact confirms the repeatability of the two experiments.

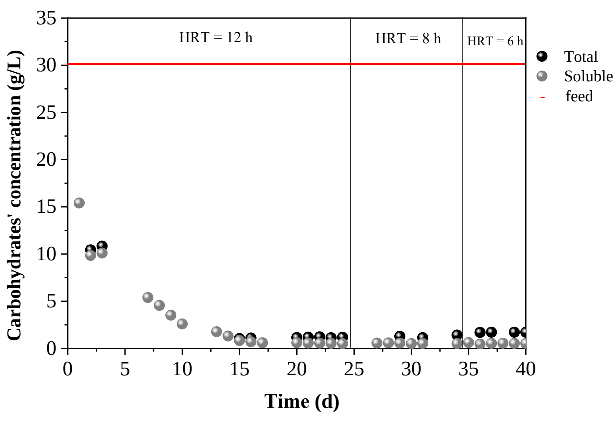

Figure 5: The concentration of the feed and non- consumed carbohydrates during DF of CW in the AUFCR at the 2nd operational period.

In Fig. 6, the distribution of the soluble metabolites during the 2nd experimental period of the AUFCR under different HRT values is presented. As it can be seen, the dominant metabolic products for the whole working period were butyric, iso-butyric and acetic acid.

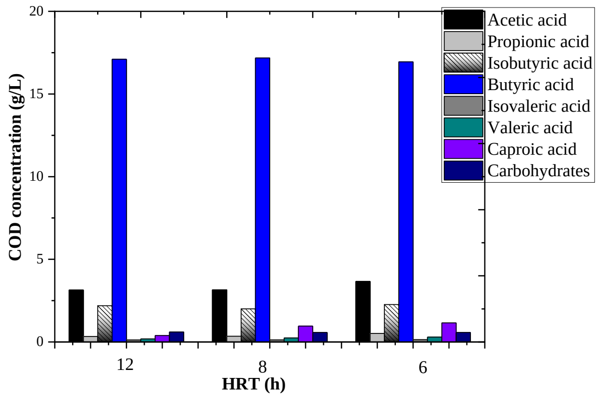

Figure 6: The distribution of the soluble metabolites (in COD) during the steady states of DF of CW in the AUFCR at the 2nd operational period.

Table 3: The effect of HRT on the main characteristics of the AUFCR.

	HRT = 12 h	**HRT = 8h**	**HRT = 6h**
pH	5.67 ± 0.04	5.69 ± 0.07	5.58 ± 0.03
TSS (g/L)	8.13 ± 0.05	8.31 ± 1.12	7.69 ± 0.00
VSS (g/L)	5.78 ± 0.05	5.97 ± 0.86	5.62 ±0.00
Hydrogen Content (%)	28.95 ± 1.64	28.03 ± 1.88	21.56 ± 1.16
d COD (mg/L)	34.11 ±0.45	34.72 ± 1.81	32.89 ± 1.82
Hydrogen production rate ($L/L_{reactor}/d$)	2.43 ± 0.20	2.45 ± 0.36	1.61 ± 0.11
Hydrogen yield ($L\ H_2/L_{feed}$)	1.21 ± 0.10	0.81 ± 0.00	0.40 ±0.00
Hydrogen yield ($L\ H_2/L_{cheese\ whey}$)	2.02 ± 0.17	1.35 ±0.00	0.67 ± 0.00
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.30 ± 0.02	0.20 ± 0.04	0.10 ±0.00

CONCLUSIONS

Fermentative hydrogen production of Cheese Whey (CW) was investigated in an anaerobic UpFlow Column Reactor (AUFCR) filled with a ceramic support material, at different feed carbohydrate concentrations (dilutions) and HRT values. The results showed a long and stable reactor operation with satisfactory rates and yields, even at high organic loadings. Both experiments showed that an immobilized system such as the AUFCR could be a potential candidate for fermentative hydrogen production from CW and that the optimal conditions could be an HRT of 12 h and carbohydrates' concentration of 30 g/L. The hydrogen yield obtained under these conditions was 0.30 mol of H_2 per mol of consumed carbohydrates.

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