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Comparison of the continuous fermentative hydrogen production efficiency from cheese whey in suspended and attached-biomass growth systems

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Abstract. In the present study, comparison of dark fermentation (DF) efficiency from cheese whey (CW) in an anaerobic UpFlow Column Reactor (AUFCR), filled with a support material for biomass attachment and a Continuous Stirred Tank reactor (CSTR), was assessed. The process efficiency and stability as well as the effect of the operational parameters, such as the initial carbohydrates concentration (dilution factor) and the hydraulic retention time (HRT) were assessed. The experiments showed that DF of CW in a CSTR led to higher hydrogen production yields compared to the AUFCR, which was 0.36 mol of H_2 per mol of consumed carbohydrates at the HRT of 12 h with initial carbohydrates concentration of 30 g/L, respectively.

Keywords: Cheese whey, Continuous stirred tank reactor, Upflow Column bioreactor, hydraulic retention time

1. 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]. The fact that the microbial cultures are suspended into the reactor liquor, is something that can effectively boost the mass transfer efficiency of the substrate in a CSTR.

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 [5].

The aim of this study was to assess the hydrogen production efficiency of CW using an attached biomass growth system, such as UFCR and a suspended-biomass system CSTR, operating at different HRTs and feed carbohydrate concentration.

2. Materials and Methods

CW

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.

AUFCR

The experiments were performed in the AUFCR described in Alexandropoulou et al. [6]. The reactor volume was 0.5 L and it was double-coated and temperature control (35 ± 0.5 ^oC) 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. 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. 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.

The start-up of both operational periods was performed using the indigenous microbial consortium of the CW, as described in Alexandropoulou et al. [6]. The feed was supplemented with NaOH, KH_2PO_4 and urea, as proposed by Alexandropoulou et al. [7], in order to keep the reactor pH at a suitable range for DF process.

CSTR

The experiments were performed in the CSTR described in Alexandropoulou et al. [7]. The reactor volume was 0.4 L and it was double-coated and temperature control (35 ± 0.5 ºC) was achieved via recirculation of water in the outer jacket. Diluted CW was fed periodically (8 times a day) while the treated effluent was removed from the reactor by overflow.

The CSTR like AUFCR operated for two different operational periods, after two different start-ups (Table 1). During the first one, the concentration of the total carbohydrates in the feed was initially 20 and increased to 30 g/L and 45 g/L, while the HRT was constant at 12 h. 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, to 6 and 4 h, respectively. The start-up of both operational periods was performed using the indigenous microbial consortium of the CW, as described in Alexandropoulou et al. [7], while NaOH, KH_2PO_4 and urea was also supplemented, as described above.

Table 1: The conditions of two operational periods of both reactors

Operational period	**Initial carbohydrates' concentration**	**HRT**
AUFCR		
1st	30, 45	12
2nd	30	12, 8, 6
CSTR		
1st	20, 30, 45	12
2nd	30	12, 8, 6, 4

Analytical methods

The reactor performance (biogas production rate and composition in H_2, pH, carbohydrates, COD, concentration) was monitored and characterized according to Alexandropoulou et al. [6,7].

3. Results and Discussion

AUFCR

In the first operational period where the effect of the carbohydrates' concentration was investigated (different dilution of CW), the reactor operated anaerobically for 30 days, with a total carbohydrates' concentration of 30 g/L and for 20 days, with a concentration of 45 g/L. Fig. 1a 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). 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. 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 2).

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
Hydrogen Content (%)	26.36 ± 1.39	21.92 ± 1.20
Hydrogen production rate ($L/L_{reactor}/d$)	2.29 ± 0.18	2.62 ± 0.15
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.30 ± 0.02	0.22 ± 0.02

The second experimental series of the AUFCR was conducted keeping the carbohydrates' concentration constant (equal to 30 g/L), and reducing the HRT from 12 h to 8 h and finally to 6 h. It has to be mentioned that in all cases the reactor reached a steady state. 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 (Fig.1b).

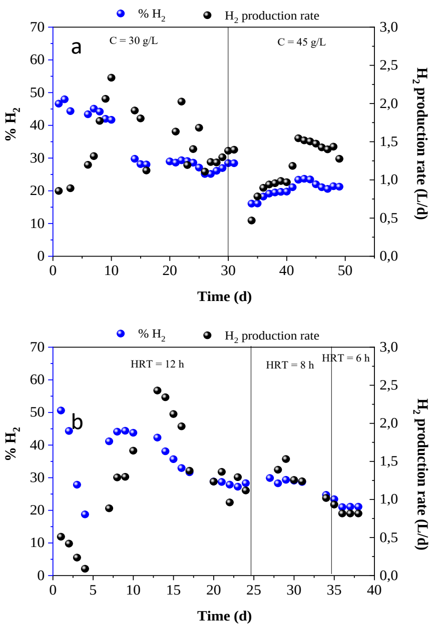

Figure 1: The percentage of hydrogen in the gas phase and the hydrogen production rate during DF of CW in the AUFCR a) at the 1st operational period and b) at the 2nd one

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.

Table 3: The main characteristics of the three steady states during DF of CW in the AUFCR at the 2nd operational period

	HRT=12 h	HRT=8 h	HRT=6 h
pH	5.67 ± 0.04	5.69 ± 0.07	5.58 ± 0.03
Hydrogen Content (%)	28.95 ± 1.64	28.03 ± 1.88	21.56 ± 1.16
Hydrogen production rate(L/$L_{reactor}$/d)	2.43 ± 0.20	2.45 ± 0.36	1.61 ± 0.11
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.30 ± 0.02	0.20 ± 0.04	0.10 ±0.00

CSTR

In the first operational period of the CSTR, the effect of the carbohydrates' concentration (20, 30 and 45 g/L) was investigated and the reactor operated anaerobically at the HRT of 12 h. Fig. 2a illustrates both the hydrogen content of the produced biogas and the hydrogen production rate. As it can be seen, the hydrogen content of the gas phase was equal to 45 % (v/v) right after the inoculation. In the sequel, during the three steady states achieved, it was equal to 33.4, 34.5 and 32.9 %, at the concentration of carbohydrates of 20, 30 and 45 g /L. The hydrogen production rate was 0.92 ± 0.07 L H_2/d and increased to 1.09 ± 0.09 and 1.65 ± 0.12 L H_2/d, respectively.

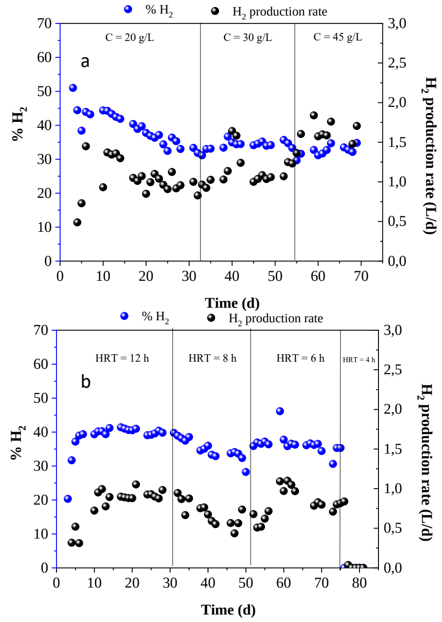

Figure 2: The percentage of hydrogen in the gas phase and the hydrogen production rate during DF of CW in the CSTR a) at the 1st operational period and b) at the 2nd one.

The hydrogen yield was higher for the lower feed concentration of carbohydrates (20 g/L) and it was equal to 0.32 ± 0.01 mol/mol of total carbohydrates consumed. On the other hand, the hydrogen yield for initial carbohydrates' concentration of 30 and 45 g/L was 0.20 ± 0.01 and 0.20 ± 0.02, respectively (Table 4).

Table 4: The main characteristics of the three steady states during DF of CW in the CSTR at the 1st operational period

	C= 20 g/L	C= 30 g/L	C= 45 g/L
pH	5.37 ± 0.10	5.17 ± 0.05	5.40 ±0.13
Hydrogen Content (%)	33.38 ±1.43	34.46 ± 0.75	32.91 ± 1.24
Hydrogen production rate(L/$L_{reactor}$/d)	2.06 ± 0.16	2.44 ± 0.21	3.66 ± 0.28
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.32 ± 0.01	0.20 ±0.01	0.20 ±0.02

Like AUFCR case, the second operational period of the CSTR was conducted keeping the carbohydrates' concentration constant to 30 g/L and reducing the HRT from 12 h to 8 h, 6 h and finally to 4 h. It has to be mentioned that in three initial cases the reactor reached a steady state, except from the case of 4 h, where the reactor operation failed. Fig. 2b illustrates both the hydrogen content of the produced biogas and the hydrogen production rate. The hydrogen content of the gas phase was 39.6, 34.8 and 35.4 30 at the HRT of 12, 8 and 6 h. The hydrogen production rate was 0.93 ± 0.04 L H_2/d and decreased to 0.59 ± 0.07 and to 0.82 ± 0.07 L H_2/d, respectively.

The hydrogen yield expressed in terms of mol/mol of total carbohydrates consumed was maximized for the higher HRT (12 h) and was 0.36 ± 0.01 and decreased with the HRT reduction (Table 5).

It is worth to mention that comparing the CSTR with the AUFCR, the yield of hydrogen was higher for CSTR operating at the HRT of 12 h with initial carbohydrates concentration of 30 g/L.

Table 5: The main characteristics of the three steady states during DF of CW in the CSTR at the 2nd operational period

	HRT=12 h	**HRT=8 h**	**HRT=6 h**
pH	5.12 ± 0.03	5.02 ± 0.05	5.17 ± 0.11
Hydrogen Content (%)	39.60 ± 0.53	34.77 ± 1.69	35.38 ± 2.15
Hydrogen production rate($L/L_{reactor}$/d)	2.22 ± 0.10	1.43 ± 0.17	1.98 ± 0.19
Hydrogen yield (mol H_2/ mol consumed carbohydrates)	0.36 ± 0.10	0.16 ± 0.02	0.17 ± 0.01

4. Conclusions

Fermentative hydrogen production of Cheese Whey (CW) was investigated in an anaerobic UpFlow Column Reactor (AUFCR) filled with a ceramic support material and in a continuous stirred tank reactor (CSTR), 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, for both reactor configurations. Only DF in CSTR at the HRT of 4 h, the hydrogen production ceased and the process failed. The experiments showed that DF of CW in a CSTR led to higher hydrogen production yields compared to the AUFCR, which was 0.36 mol of H_2 per mol of consumed carbohydrates at the HRT of 12 h with initial carbohydrates concentration of 30 g/L.

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