

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

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Introduction

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

Materials & Methods



- ✓ AUF CR described in Alexandropoulou et al. [3].
- ✓ Ceramic beads as support material for biomass attachment
- ✓ Reactor volume 0.5 L
- ✓ 35 ± 0.5°C
- ✓ Inoculum: indigenous microbial species contained in CW
- ✓ The feed was supplemented with NaOH, KH₂PO₄ and urea
- ✓ The reactor operated for two different operational periods, after two different start-ups

Table 1: CW used

Characteristic	Value
pH	6.4 ± 0.0
Total Suspended Solids-TSS (g/L)	4.3 ± 0.3
Volatile Suspended Solids-VSS (g/L)	4.1 ± 0.2
Total Carbohydrates (g/L)	45.9 ± 1.4
Soluble Carbohydrates (g/L)	41.4 ± 1.7
Total Chemical Oxygen Demand-COD (g/L)	51.9 ± 1.7
Soluble COD (g/L)	49.3 ± 1.7

Table 2: The conditions of two operational periods

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

1st operational period (effect of feed carbohydrates concentration, HRT=12 h)

Figure 1: The percentage of hydrogen in the gas phase and the hydrogen production rate.

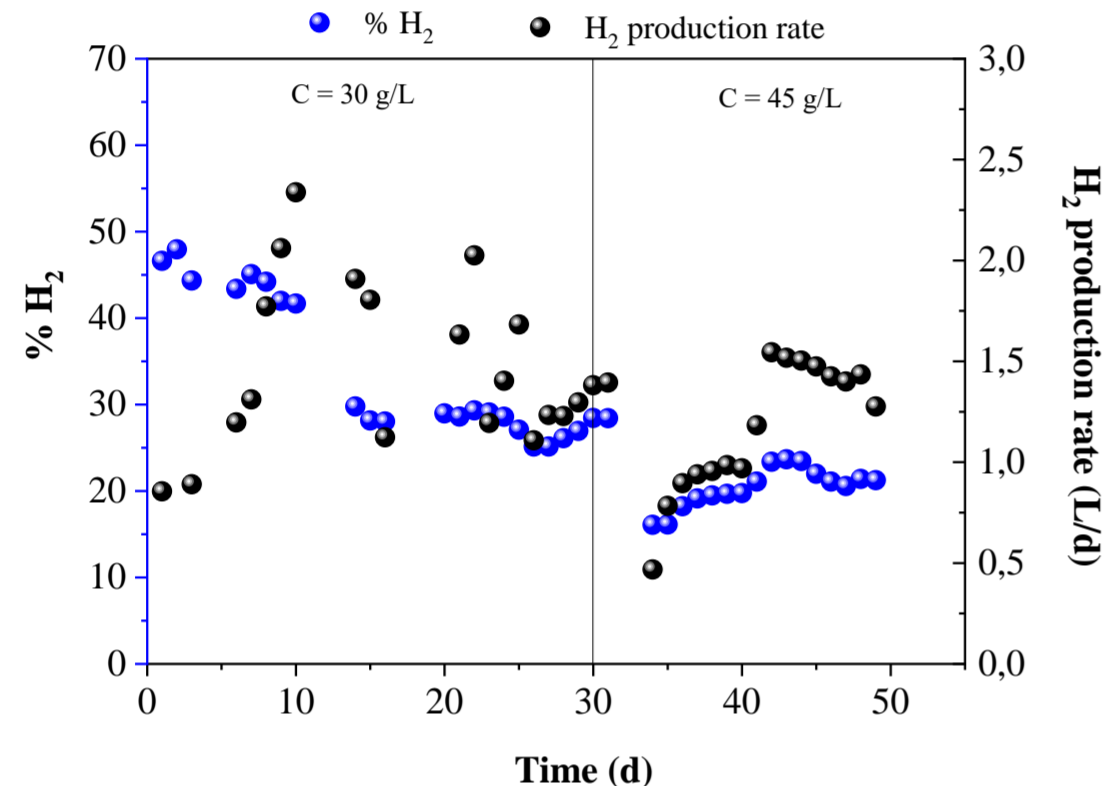


Figure 2: The distribution of the soluble metabolites during DF of CW in the AUF CR at the 1st operational period.

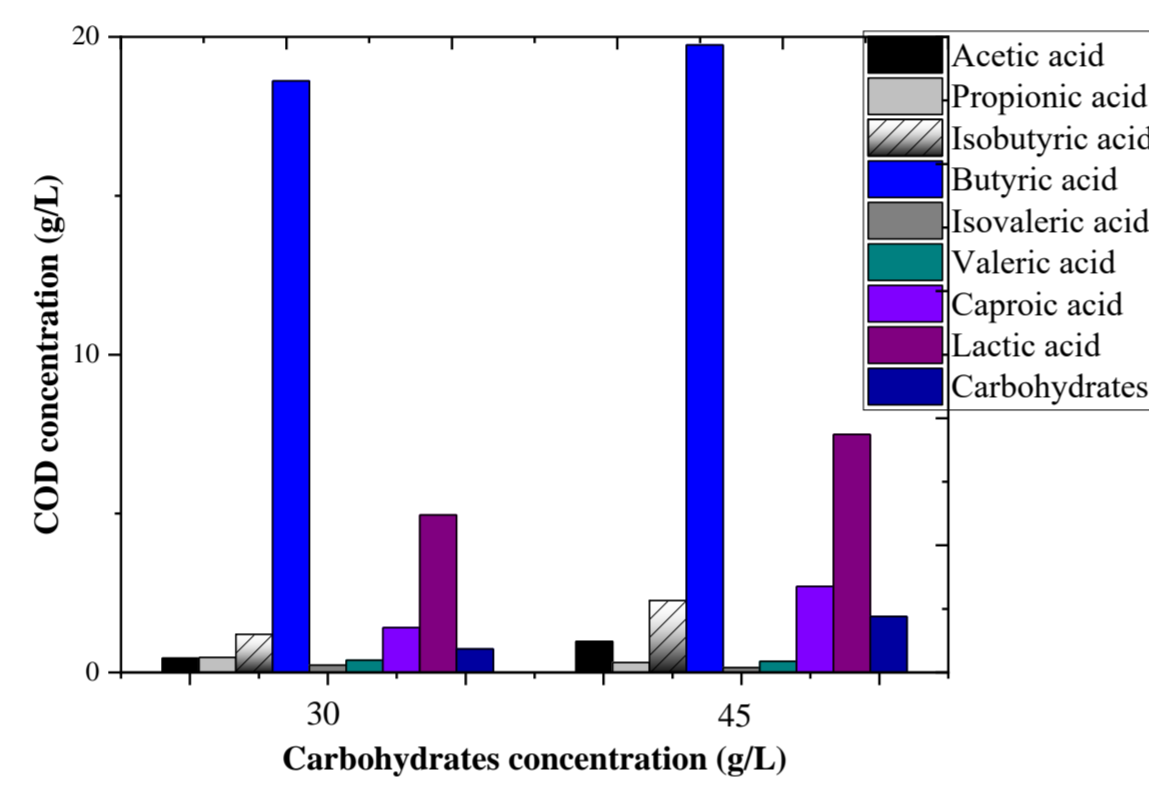


Figure 3: The concentration of the feed and non-consumed carbohydrates during DF of CW in the AUF CR at the 1st operational period.

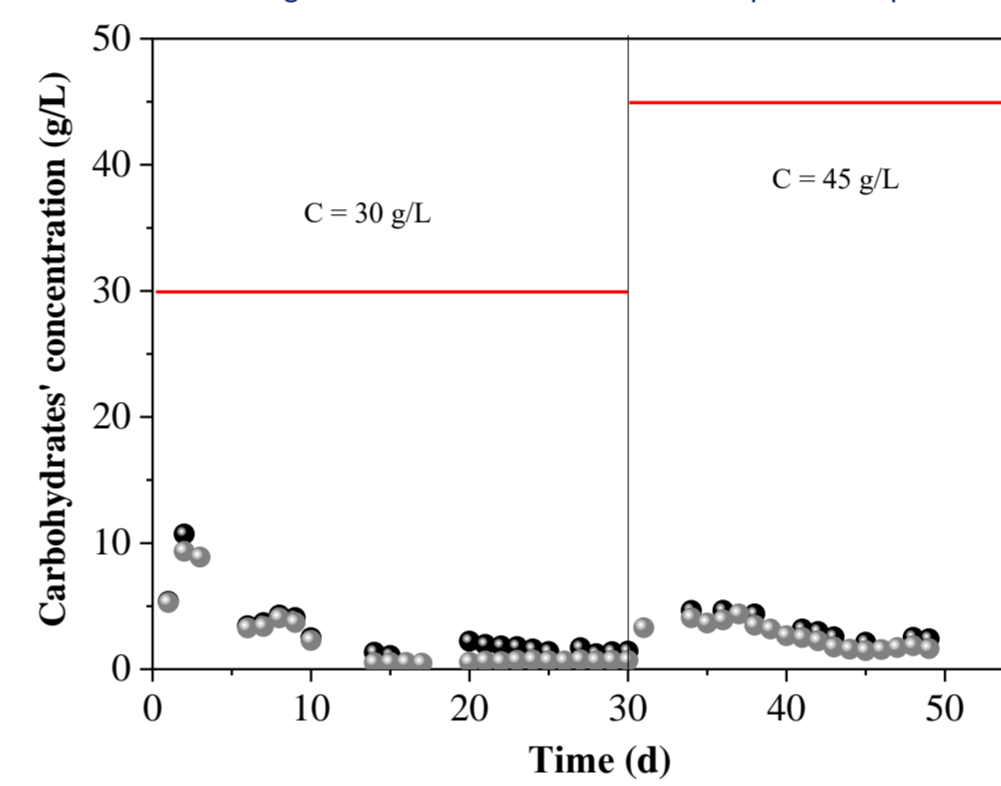


Table 3: The main characteristics of the two steady states during DF of CW in the AUF CR at the 1st operational period.

	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

- ✓ The hydrogen percentage was equal to 26 (30 g carbohydrates /L) and 22% (45 g carbohydrates /L).
- ✓ The hydrogen production rate was 1.25 ± 0.10 L H₂/d and increased to 1.43 ± 0.08 L H₂/d, with the increase of carbohydrates concentration.
- ✓ The consumption of CW carbohydrates, was almost 95% in both experimental phases.
- ✓ The hydrogen yield (mol/mol of total carbohydrates consumed) was 0.30 ± 0.02 for the lower feed concentration of carbohydrates and 0.22 ± 0.02 for the higher one.
- ✓ The main metabolites produced during the 1st AUF CR operation were butyric, iso-butyric, acetic, caproic and lactic acid. Butyric and lactic acid were the two acids that prevailed during the whole experimental period.

Results & Discussion

2nd operational period (effect of HRT, feed carbohydrates concentration =30 g/L)

Figure 4: The percentage of hydrogen in the gas phase and the hydrogen production rate.

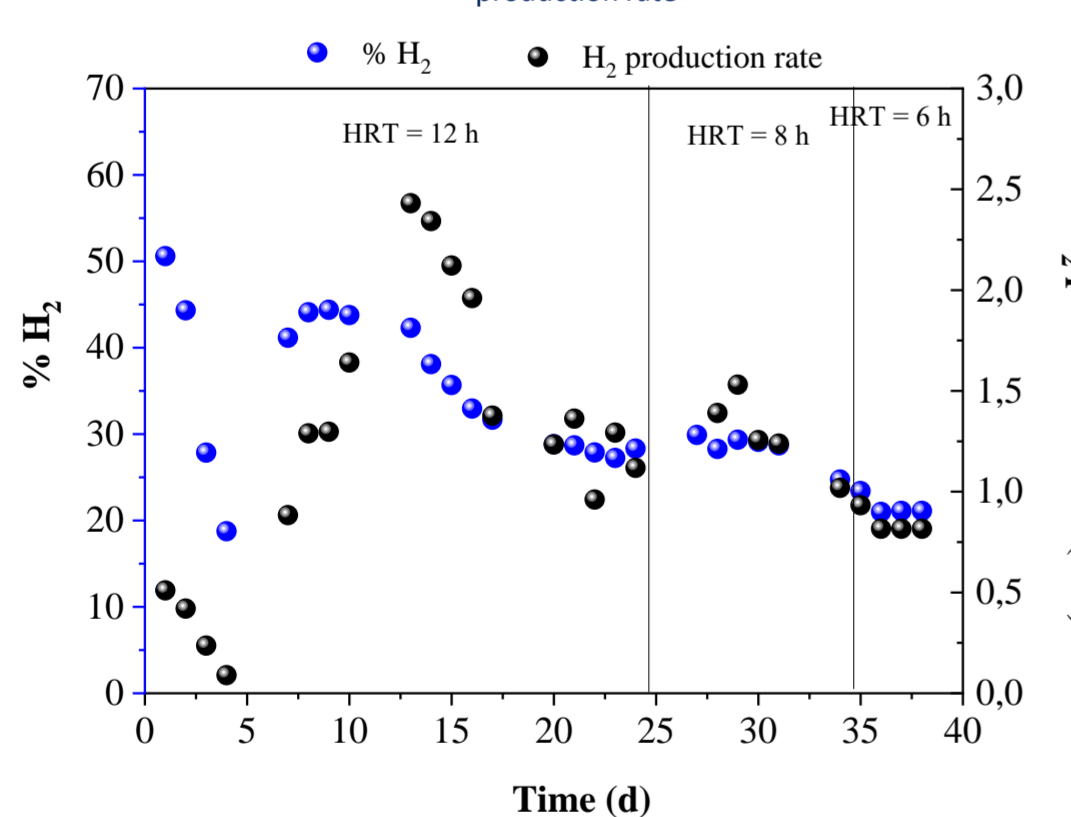


Figure 5: The distribution of the soluble metabolites during DF of CW in the AUF CR at the 2nd operational period.

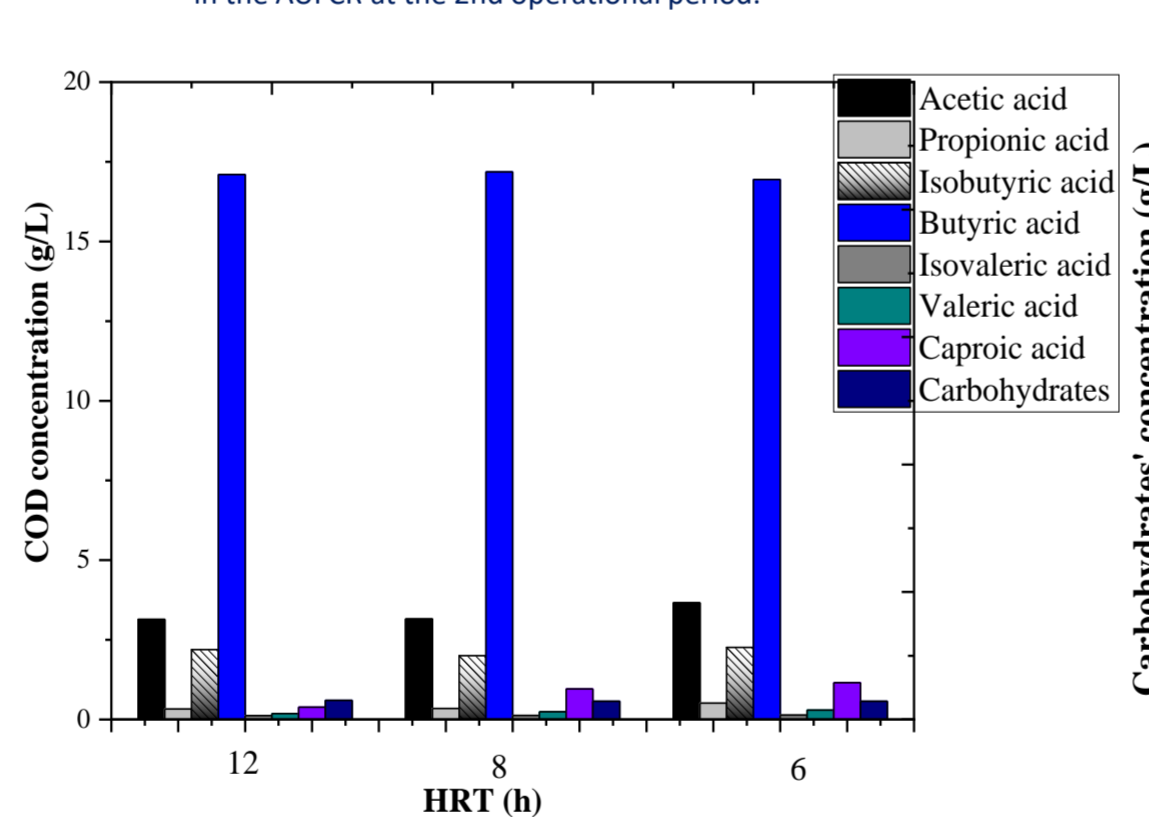


Figure 6: The concentration of the feed and non-consumed carbohydrates during DF of CW in the AUF CR at the 2nd operational period.

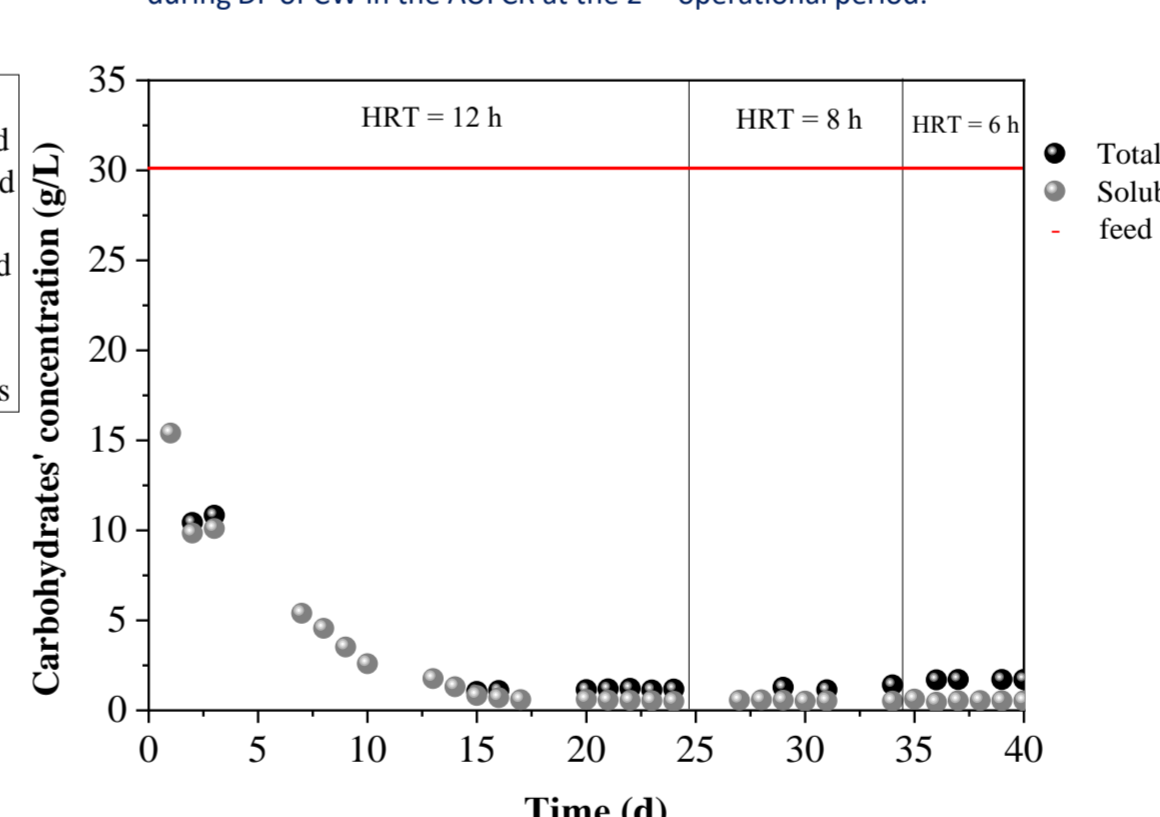


Table 4: The main characteristics of the two steady states during DF of CW in the AUF CR at the 2nd operational period.

	HRT = 12 h	HRT = 8 h	HRT = 6 h
pH	5.67 ± 0.04	5.69 ± 0.07	5.58 ± 0.03
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

- ✓ The hydrogen production rate at the steady states was equal to 1.27 ± 0.10 L H₂/d for the first phase (HRT=12 h), which slightly increased to 1.29 ± 0.19 L H₂/d when the HRT decreased to 8 h and finally decreased to 0.84 ± 0.06 L H₂/d with a further reduction of the HRT to 6 h.
- ✓ The carbohydrate consumption was as high as 95 % during the whole experimental period.
- ✓ The dominant metabolic products for the whole working period were butyric, iso-butyric and acetic acid.
- ✓ 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.

Conclusions

- ✓ Fermentative hydrogen production of CW was investigated in an anaerobic AUF CR filled with a ceramic support material, at different feed carbohydrate concentrations (dilutions) and HRT values
- ✓ 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 AUF CR 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 H₂ per mol of consumed carbohydrates.

[1] Lovato G, Lazaro CZ, Zaiet M, Ratusznei SM, Rodrigues JAD. Biohydrogen production by co-digesting whey and glycerin in an AnSBBR: Performance optimization, metabolic pathway kinetic modeling and phylogenetic characterization. *Biochem Eng J* 2017;128:93–105. <https://doi.org/10.1016/j.bej.2017.09.011>.

[2] Rao R and Basak N. Fermentative molecular biohydrogen production from cheese whey: present prospects and future strategy. *Applied Biochemistry and Biotechnology* <https://doi.org/10.1007/s12010-021-03528-6>

[3] Alexandropoulou M, Antonopoulou G, Lyberatos G. Food industry waste's exploitation via anaerobic digestion and fermentative hydrogen production in an Up-Flow Column Reactor. *Waste and Biomass Valorization* 2016;7:711–23. <https://doi.org/10.1007/s12649-016-9544-y>.