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Scientific Paper

Using fruit and vegetable waste to generate hydrogen through dark fermentation

Uso de resíduos de frutas e verduras para gerar hidrogênio por meio da fermentação escura

Camila Aparecida de Menezes[1], Maíra Saldanha Duarte[1], Isabelle Nascimento Teixeira[2], Willame de Araújo Cavalcante[3], Priscilla de Souza Almeida[1], Michael Barbosa Viana[3], Marcelo Zaiat[4], Renato Carrhá Leitão[1]*

ABSTRACT

Fruit and vegetable waste (FVW) are sugar-rich substrates that can result in hydrogen through dark fermentation. The success of dark fermentation depends on changing operation parameters, which vary based on the characteristics of the substrate. This study aimed to determine how FVW from a central horticultural wholesaler should be fermented to produce the maximum amount of hydrogen. The following goals were listed as determining the pre-treatment method (chemical, thermic, and acid) for brewery sludge and evaluating the fermentation of the liquid fraction of FVW in an anaerobic structured bed reactor (AnSTBR). The AnSTBR operation started with sucrose as substrate at a hydraulic retention time (HRT) of 6 h. Then, the sucrose was gradually replaced by FVW at the HRT of 6 h. The FVW was fed as the sole carbon source (5 g COD/L) at HRTs of 6, 12, and 3 h for at least 20 days on each condition. The heat treatment resulted in the highest cumulative hydrogen production and hydrogen production rate (HPR) (90 mL H_2 and 6.6 mL H_2/h). In the AnSTBR operation, the highest values of HPR were observed at 6 h (2094 L H_2/m^3 reactor.d). The attempt to decrease the HRT to 3 h caused a reduction in the HPR to 216 L H_2/m^3 reactor.d. It is highly encouraging the increase of the organic loading rate and the use of co-fermentation, aiming to reduce the size of the reactor and water expended on dilution by increasing substrate concentration in future studies.

Keywords: food waste; dark fermentation; structured bed reactor; green energy.

ABSTRACT

Resíduos de frutas e vegetais (RFV) são substratos ricos em açúcar que podem ser usados para produzir hidrogênio por meio da fermentação escura. O sucesso dessa fermentação depende dos parâmetros de operação, que variam com base nas características do substrato. Este estudo teve como objetivo determinar como os RFV de uma central de abastecimento de hortifrutigrangeiros devem ser fermentados para produzir a quantidade máxima de hidrogênio. Os seguintes objetivos foram listados: determinar o método de pré-tratamento (químico, térmico e ácido) para lodo de cervejaria e avaliar a fermentação da fração líquida dos RFV em reator anaeróbio de leito estruturado (AnSTBR). A operação do AnSTBR começou com sacarose como substrato operado com tempo de detenção hidráulica (TDH) de 6 horas. Em seguida, a sacarose foi gradualmente substituída por RFV usando TDH de 6 horas. O RFV foi usado como a única fonte de carbono (5 g DQO/L) com TDHs de 6, 12 e 3 horas por pelo menos 20 dias em cada condição. O tratamento térmico resultou na maior produção cumulativa de hidrogênio e taxa de produção de hidrogênio (TPH) (90 mL H_2 e 6,6 mL H_2/h). Na operação do AnSTBR, os maiores valores de TPH foram observados em 6 horas (2.094 L H_2/m^3 reator.d). A tentativa de reduzir o TDH para 3 horas causou uma redução no TPH para 216 L H_2/m^3 reator.d. É altamente recomendado o aumento da taxa de carga orgânica volumétrica e o uso de co-fermentação, visando reduzir o tamanho do reator e a água gasta na diluição, aumentando a concentração do substrato em estudos futuros.

Palavras-chave: resíduos de alimentos; fermentação escura; reator de leito estruturado; energia verde.

[1]Embrapa Tropical Agroindustry, Biomass Technology Laboratory – Fortaleza (CE), Brazil.
[2]Universidade Federal do Ceará, Instituto de Ciências do Mar – Fortaleza (CE), Brazil.
[3]Instituto Federal de Educação, Ciência e Tecnologia do Ceará – Fortaleza (CE), Brazil.
[4]Universidade de São Paulo, Escola de Engenharia de São Carlos, Biological Processes Laboratory – São Carlos (SP), Brazil.
***Corresponding author:** renato.leitao@embrapa.br; renato.c.leitao@gmail.com
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INTRODUCTION

Population growth contributes to both the increase in waste generation and energy demand. According to waste generation statistics, human activity generates up to 62% of solid urban waste (Shanmugan *et al.*, 2023). Within the food waste category are fruit and vegetable waste (FVW) generated in large quantities in the harvesting process in food distribution centers (da Silva Júnior *et al.*, 2022). In Brazil, there are food supply centers (CEASAs) responsible for the distribution, in large part, of fruits and vegetables. For example, the CEASA located in Ceará is a significant generating source that sends 17 tons of FVW to the landfill daily (da Silva Júnior *et al.*, 2022). Concerning world energy demand, a counterpart is that FVW is rich in carbohydrates and, therefore, highly fermentable, making it a potential ideal substrate in fermentation processes for hydrogen (Martínez-Mendoza *et al.*, 2022) and methane production (da Silva Júnior *et al.*, 2022). Until now, studies on hydrogen production from this substrate with specific characteristics have not been identified.

The demand for hydrogen is relatively high due to its energy density and potential applications in producing other compounds (Saravanan *et al.*, 2021). One cost-effective way of obtaining hydrogen is through dark fermentation (Ji; Wang, 2021). However, its success depends on several operational conditions: the type (Martínez-Mendoza *et al.*, 2022) and pretreatment of inoculum (Viana *et al.*, 2019), substrate type, temperature, pH (Martínez-Mendoza *et al.*, 2022), organic loading rate (OLR) (Martínez-Mendoza; García-Depraect; Muñoz, 2023), and reactor type (Araujo *et al.*, 2024). A low-rate reactor was recently used for hydrogen production from FVW in different OLRs with promising results (11,800 L H_2/m^3 reactor.d) (Martínez-Mendoza; García-Depraect; Muñoz, 2023). Nevertheless, it is worth noting that high-rate reactors have the potential to generate a greater amount of hydrogen when compared to low-rate reactors (Saravanan *et al.*, 2021). However, it is important to highlight that, in the context of fermenting FVW, high-rate reactors have not been employed. Furthermore, when using high-rate fixed bed reactors for hydrogen production, the anaerobic structured bed reactor (AnSTBR) showed higher hydrogen productivity than the packed bed and bedless reactors (Araujo *et al.*, 2024). However, it is imperative to conduct further investigations to ascertain how various operational parameters may vary in response to the specific characteristics of the inoculum and substrate.

This study aimed to assess how the pretreatment method of the inoculum and the hydraulic retention time (HRT), consequently, the OLR, influence the process of dark fermentation of FVW for biohydrogen production in a high-rate AnSTBR. The HRT of 12, 6, and 3 h was applied, with substrate concentration fixed at 5 g chemical oxygen demand (COD)/L, reaching OLR of 10, 20, and 40 kg COD/m^3 d.

METHOD

The study proposed a three-step approach to evaluate the dark fermentation of FVW generated in CEASA-Ceará. The first step involved testing acid, chemical, and thermic methods of inoculum pretreatment. In the second step, an AnSTBR was tested as a reactor configuration with pretreated sludge and a sucrose-based substrate. Finally, in the third step, the AnSTBR was used for the dark fermentation of FVW with different HRT and OLR.

Substrate and Inoculum

CEASA-Maracanaú (Ceara state, Brazil) donated the raw FVW. Complete information on FVW composition and residue characterization is available elsewhere (da Silva Júnior *et al.*, 2022). The grounded residue showed the organic matter concentration (on a COD basis), total carbohydrates, total Kjeldahl nitrogen, phosphorus, and total solids equal to 163, 55, 2.8, 1.2, and 15 g/L, respectively. The residue used in the reactor feed was the liquid fraction after pressing the grounded residue, which showed a concentration equal to (in g/L) organic matter in a COD basis (116), total carbohydrates (45.7), total Kjeldahl nitrogen (1.5), total phosphorus (1.2), and total solids (7.8).

A brewery provided the methanogenic mesophilic flocculent sludge, with 40.65 g VS/L, collected from an upflow anaerobic sludge blanket reactor.

Batch Assays

The first step was to verify the best sludge treatment method (acid, thermal, or chloroform) to eliminate methanogenic archaea. In acid pretreatment, a hydrochloric acid solution (2 mol/L) was applied to the brewery sludge to lower the pH to 3.0. The pH was kept at 3.0 for 24 h and then increased to 8.2 (sodium hydroxide solution, 2 mol/L) before the assay assembly (Viana *et al.*, 2019). In thermal pretreatment, the sludge temperature was raised to 90°C for 10 min and then cooled to room temperature (25°C) with constant agitation before setting the essays (Kim; Han; Shin, 2006). In the treatment with chloroform, a solution containing 0.05% v/v of chloroform was added directly to the assays (Viana *et al.*, 2019).

The assays were carried out in triplicate in 330-mL flasks (Schott, Germany) with a working volume of 200 mL containing 20 mL of pretreated sludge, 5 g COD/L of sucrose, and nutrient solution (in g/L) (NH_4HCO_3 0.32, K_2HPO_4 0.125, $MgCl_2.6H_2O$ 0.015, $CuSO_4.5H_2O$ 0.005, and $CoCl_2.5H_2O$ 1.25 10^{-4}) (de Menezes; Silva, 2019). The flasks were sealed and maintained at 35°C with constant agitation (150 rpm) for 68 h. The amount of biogas produced was measured using the fluid displacement method (Walker *et al.*, 2019), and biogas composition was measured using gas chromatography, both at six intervals.

The examination of the total hydrogen output for each pre-treatment was made utilizing distinct models: the cone model equation (Eq. 1), the modified Gompertz equation (Eq. 2) (Wang; Guo, 2024), and the degradation model (Eq. 3) (Sganzerla *et al.*, 2023). The process of analysis relied on the Origin® software for accuracy and precision:

$$H = P\left[\frac{1}{\left(1+(kn*t)\right)^{n}}\right] \quad (1)$$

$$H = P\,e^{\left\{-e^{\left[\frac{R_h e}{P}(\lambda - t)+1\right]}\right\}} \quad (2)$$

$$H = M_0*(1 - e^{-k*t}) \quad (3)$$

where H is the cumulative hydrogen production (mL); P is the hydrogen production potential (mL); R_h is the maximum hydrogen production rate (HPR) (mL/h); *e* is the Euler's number (2.718); λ is the lag phase time (h); t is the fermentation time (h); $k_{hydrogen}$ is the hydrolysis rate constant (per h); M_0 is the maximum hydrogen productivity (mL); and n is the shape factor.

High-Rate Reactor

The AnSTBR (Figure 1) was built in glass, with a total volume of 4 L, a working volume of 3 L, and 10 cm of internal diameter. The reactor was inoculated

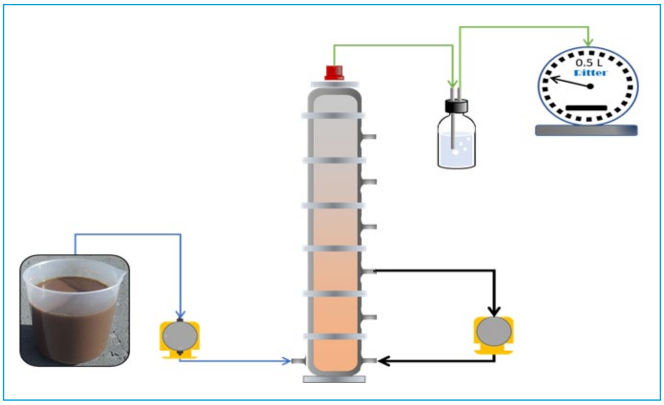

Figure 1 – Anaerobic structured bed reactor.

in batch mode without recirculation with 5 g COD/L sucrose, 10% v/v of thermally pretreated brewery sludge, and nutritional medium (de Menezes; Silva, 2019). The reactor was kept in batch mode for 4 days and then operated in continuous mode. Table 1 shows the operating parameters of the AnSTBR in continuous mode.

Analytical Methods

COD (5220 D. Closed Reflux, Colorimetric Method), total solids (TS) (2540 B. Total Solids Dried at 103–105°C), volatile solids (VS) (2540 E. Fixed and Volatile Solids Ignited at 550°C), and nitrogen total Kjeldahl (4500-N_{org} B. Macro-Kjeldahl Method) were analyzed according to the Standard Methods for Examination of Water and Wastewater (APHA; AWWA; WEF, 2005). The total carbohydrate content was determined using Dubois's colorimetric method with sucrose as the reference sugar (Dubois *et al.*, 1956). Volatile fatty acids (VFA) were determined in high-performance liquid chromatography using a Shimadzu chromatograph with refractive index detector (RID – M20A) and Aminex HPX-87 column (Bio-Rad, 300 × 7.9 mm). The mobile phase used was a 5 mM sulfuric acid solution, in isocratic mode, with a flow rate of 0.6 mL/min, an injection volume of 20 µL, and an oven temperature of 65°C for 35 min. The volumetric biogas production was determined using a Ritter automatic meter connected to the reactor headspace. The biogas composition was evaluated using gas chromatography (GC-2010, Shimadzu) with hydrogen, methane, carbon dioxide, and hydrogen sulfide as reference gases in the calibration curve (Paranhos; Silva, 2018). The equipment used a thermal conductivity detector and temperatures of the injector, detector, and column at 30, 200, and 230°C, respectively. Argon was applied as carrier gas.

RESULTS AND DISCUSSION

Batch Assays

Table 2 shows the produced hydrogen and the parameters of the curves obtained by fitting the experimental data with the modified Gompertz, cone, and degradation kinetic models. The Gompertz, cone, and degradation models provided valuable information on the sucrose conversion patterns and hydrogen production results. This study applied these kinetic models to predict hydrogen production from acidic, thermic, and chemically pretreated sludge. According to the analysis of the three models, the predicted maximum hydrogen production was observed in thermic-pretreated sludge, followed by chemically pretreated sludge. In the Gompertz model, the maximum HPR (R_h) was 6.6 mL/h, and the lag phase (λ) was 0.39 h for the reactor in thermic-pretreated sludge, indicating a fast hydrogen production after the startup.

The findings of this study contrast with those of Viana *et al.* (2019), who reported a higher hydrogen production in the reactor with chloroform-treated brewery sludge as compared to thermally pretreated sludge. The authors justified that chloroform acts exclusively on methyl-coenzyme M reductase, an enzyme present only in archaea, not interfering with the bacterial community. In contrast, Vilela *et al.* (2019) justified that the heat treatment, in addition to eliminating archaea, selected microorganisms belonging to the genus *Clostridium*. In this study, the thermic pretreated sludge returned the highest hydrogen production and sucrose degradation. Acid treatment returned the worst results of substrate degradation and hydrogen production. This may be attributed to cell denaturation by adding the acid, which is a positive welcome because this method involves the highest cost among those tested.

Table 1 – Operational conditions in the AnSTBR fed with a fixed concentration of 5 g COD L^{-1}.

Condition	Day	Substrate	HRT (h)	OLR (kg COD/m³ d)	Recycling
I	1-17	Sucrose	6	20	No
II	18-41	Sucrose	6	20	Yes
III	42-58	50% sucrose + 50% FVW	6	20	Yes
IV	59-82	FVW	6	20	Yes
V	83-112	FVW	12	10	Yes
VI	113-124	FVW	3	40	Yes

Table 2 – Kinetic models applied to the hydrogen production from acidic, thermic, and chemically pretreated sludge.

Model	Parameter	Pretreatment		
		Acid	Thermic	Chemical
Cone	H (mL)	45.74	89.71	70.16
	K_h (1/h)	2.32	3.05	-
	n	0.04659	0.05418	0.44362
	R^2	0.997	0.996	0.992
Gompertz	R_h (mL/h)	1.97	6.60	5.36
	λ (h)	0.43	0.39	0.56
	R^2	0.999	0.999	0.966
Degradation	M_0 (mL)	45.84	89.97	78.18
	K (1/h)	3.48	3.07	0.88

High-Rate Reactor Operation

Figure 2 presents the results of carbohydrate conversion efficiency (a) and HPR (b) of the AnSTBR. Interestingly, carbohydrate removal efficiency was consistently high, above 90%, in all operational conditions. This was likely due to sucrose being a substrate known to be readily fermentable. However, a surprising finding in the study by de Araujo *et al.* (2024) was that an AnSTBR provided with sucrose at an HRT of 6 h had a carbohydrate removal efficiency of only 52.6%.

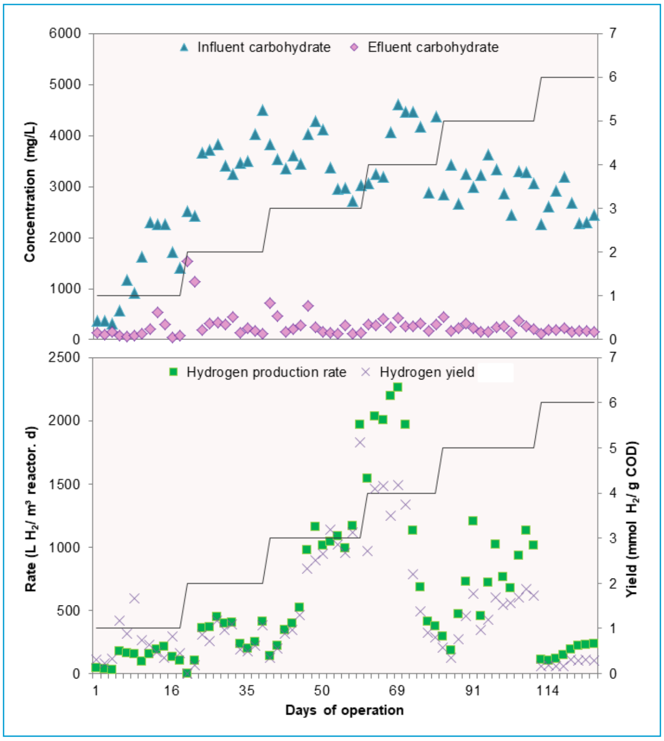

Figure 2 – Results of influent and effluent carbohydrate concentration (a) and hydrogen production rate (b) of hydrogen production from FVW in AnSTBR.

This may be because this study used brewery sludge as an inoculum, leading to a higher amount of active biomass and higher carbohydrate conversion efficiency than in the study by de Araujo *et al.* (2024) using sugarcane vinasse as an inoculum and presenting lower carbohydrate conversion efficiency.

The high concentration of active biomass within the acidogenic reactor might be beneficial to substrate conversion, i.e., the higher the concentration of active biomass, the greater the degree of acidification (VFA produced per added COD) (Bernal; de Menezes; Silva, 2021). In the AnSTBR, the VFA generation was equal to 2826 ± 234 mg acetate/L in the operation with sucrose. Therefore, approximately 60% of the added COD was converted to VFA. In operating an expanded granular sludge bed reactor fed with sugarcane juice, known for having high retention of active biomass, the acidification degree remained between 55% and 75%, comprising the range observed in the present study (de Menezes; Silva, 2019). Acidification remained higher than 50% when the FVW was added to feed with sucrose (2145 mg acetate/L) and remained unchanged when adding only FVW at the HRT of 12 h (2123 mg acetate/L) and 6 h (2331 mg acetate/L). The reduction of HRT for 3 h caused a drop in VFA production to 1648 mg acetate/L, which may be associated with pathways change and the production of other metabolites not identified (alcohols, ketones).

Although the high concentration of active biomass is associated with high acidification, on the contrary, this can lead to low hydrogen productivity (Bernal; de Menezes; Silva, 2021). This occurs because substrate fermentation via the acetate production pathway, which results in greater hydrogen production, can increase the partial pressure of hydrogen in the reactor. In turn, the increase in hydrogen partial pressure leads microorganisms to change their metabolism from pathways with less hydrogen production to pathways with hydrogen consumption (de Menezes *et al.*, 2023). In operation with sucrose in the AnSTBR of the present study, probably inoculated with a higher concentration of active biomass than the AnSTBR of the study by de Araujo *et al.* (2024), the HPR was equal to 340 L H_2/m^3 reactor d, against 743 L H_2/m^3 d reactor in the study by de Araujo *et al.* (2024).

The insertion of the recirculation caused an increase in the HPR by 50%, which is an expected value since the turbulence caused by the increase in the upflow velocity generates the release of hydrogen from the medium. Besides, the recycling of effluent can increase the mass transfer between the phases, leading to higher hydrogen production (Lima; Zaiat, 2012). Adding FVW to sucrose (50%/50% on a COD basis) and feeding FVW without sucrose increased HPR to 1174 and 2094 L H_2/m^3 reactor. d, respectively. The trend observed in the AnSTBR of the present study differs from that observed in the AnSTBR of Ribeiro *et al.* (2022). The HPR values decreased from 2096 to 500 L H_2/m^3 reactor.d by progressively increasing the cheese whey to sucrose ratio in the authors' study (Ribeiro *et al.*, 2022). While the decrease in hydrogen production in the authors' study can be associated with the already expected low generation of hydrogen from cheese whey, in the present study, the increase in hydrogen production can be associated with the stabilization of the reactor due to more than 50 days of operation (Vilela *et al.*, 2021). This may be true because the concentration of carbohydrates flowing to the reactor remained unchanged when replacing sucrose with FVW. Additionally, FVW still has more significant nutritional supplementation than cheese whey (Ribeiro *et al.*, 2022), for example.

The reduction of the HRT to 3 h reduced the HPR to 216 L H_2/m^3. d. The reduction of hydrogen production and acidification at this HRT leads us to believe that the active biomass washed from the reactor in this operational condition. However, the percentage of VS concerning total solids remained stable under all operating conditions (~60%), and the concentration of total solids in the effluent remained unchanged. Therefore, the metabolites generated in all operating conditions can explain the lowest values of HPR in the sucrose operation and the lowest values of HPR at 3 h HRT (Table 3). However, only organic acids were determined in the analysis; that way, the lower hydrogen production and VFA concentration at the HRT of 3 h can be associated with the formation of other metabolites not identified, such as alcohols, for example.

Among the operational conditions, the highest average values of acetate and butanoate concentration, routes with the highest generation of hydrogen, respectively, were observed in the operation with sucrose. However, the lowest hydrogen production values were observed in the operation with sucrose. de Araujo *et al.* (2024) attributed the hydrogen deficit relationship to the theoretical occurrence of homoacetogenesis in the AnSTBR operation. The authors justified that 39.3% of the acetate detected in the AnSTBR was produced autotrophically through hydrogen consumption (4 mol H_2 per mol acetate) (Saady, 2013). In the AnSTBR of the present study, when applying the formulation presented by de Menezes and Silva (2019) and used by de Araujo *et al.* (2024) to measure homoacetogenesis, it was possible to verify that 54% of the acetate identified in the reactor effluent was synthesized autotrophically. These data corroborate the previous discussion about the influence of the concentration of active biomass in the reactor since, at large concentrations of active biomass, homoacetogenesis may occur (Carosia *et al.*, 2021). The hypothesis is that, at a higher concentration of active biomass, there is a proportionally greater occurrence of homoacetogenesis, consequently explaining why the HPR in operation with

Table 3 – VFA generated at each operational condition applied to the AnSTBR.

	Condition	Substrate	HRT (h)	Lactate (mg/L)	Acetate (mg/L)	Propanoate (mg/L)	Isobutanoate (mg/L)	Butanoate (mg/L)
Average	I	Sucrose	6	N.M.	N.M.	N.M.	N.M.	N.M.
σ								
Average	II	Sucrose	6	89	1331	124	112	1617
σ				4	7	3	23	32
Average	III	50% sucrose + 50% FVW	6	0	695	147	142	246
σ				0	17	8	20	3
Average	IV	FVW	6	891	403	93	88	324
σ				137	11	3	10	69
Average	V	FVW	12	N.M.	N.M.	N.M.	N.M.	N.M.
σ								
Average	VI	FVW	3	305	217	179	37	555
σ				3	1	4	11	12

N.M.: not measured.

sucrose in the study by de Araujo *et al.* (2024) was higher than that observed in the present study.

Homoacetogenesis was reduced by inserting FVW as a co-substrate and as a substrate to AnSTBR at 6 h HRT, i.e., the percentage of autotrophically produced acetate was reduced to zero. One explanation for this occurrence is the substitution of the reactor's biomass for solids present in the residue because it may have caused an increase in the rate of hydrolysis instead of acidification, reducing the partial pressure of hydrogen in the reactor. In turn, reducing hydrogen partial pressure in the reactor favored hydrogen production routes instead of consumption. In fact, changing the diet from 50% sucrose + 50% FVW to FVW caused a reduction in the molar fractions of acetate and propanoate from 64% to 30% and from 11% to 6%, respectively. The molar fraction of lactate increased from 0 to 40%, and butanoate remained stable at 16%. The residue insertion had a significant impact on the metabolic profile in the reactor despite the constant concentration of carbohydrates. Instead of producing acetate and butanoate through pyruvate, the reactor shifted towards generating lactate and utilizing lactate and acetate for the co-synthesis of butanoate and hydrogen. This change suggests that optimizing the reactor's performance may be possible by adjusting the influent's composition.

Based on the operational conditions applied, it was found that the most favorable condition for hydrogen production from 5 g COD/L of FVW was the HRT of 6 h, resulting in an OLR of 20 kg COD/m^3 d. Interestingly, the optimal OLR applied to AnSTBR was lower than that for cheese whey fermentation (90 kg COD/m^3 d) (Ribeiro *et al.*, 2022) and molasses (120 kg COD/m^3 d) (Vilela *et al.*, 2021) in the same reactor configuration. However, it was observed that when an OLR of 40 kg COD/m^3 d was used, which translates to 5 g COD/L and 3 h HRT, there was a reduction in hydrogen production due to probable pathway change, which reinforces the fact that the process presents an optimal HRT and OLR to operate. This finding challenges the main self-criticism about the study: using water for dilution. An alternative to the use of clean water in FVW digestion would be the use of co-fermentation with municipal sewage or industrial wastewater. To improve future studies, increasing the concentration of substrate influent in the reactor is recommended for a higher OLR, as is the employment of co-fermentation for water saving and improvement of the process.

CONCLUSIONS

The dark fermentation of FVW in a high-rate reactor (AnSTBR) yielded the most favorable results for biohydrogen production, an HPR 2094 L H_2/m^3 reactor. d, with an OLR of 20 kg COD/m^3 d (HRT of 6 h) and thermally treated sludge as the selected and more effective pretreatment method of the inoculum. A low HRT of 3 h was demonstrated to be inadequate for the process, resulting in lower carbohydrate conversion to VFA, i.e., a reduction of VFA production from 2331 to 1648 mg acetate/L. Because of lower VFA production at the HRT of 3 h, lower HPRs were observed than at the other HRT (216 L H2/m^3. d). The thermally pretreated sludge returned the best sucrose conversion and hydrogen production results among the tested methods. The results of hydrogen productivity of AnSTBR operated with sucrose were lower than those observed in the application of the residue, probably due to autotrophic acetate synthesis. Future aspects of this research involve increasing OLR by increasing substrate concentration.

AUTHORS' CONTRIBUTIONS

de Menezes, C.A.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. Duarte, M.S.: Investigation, Methodology, Writing – review & editing. Teixeira, I.N.: Investigation, Methodology. Cavalcante, W.A.: Software, Writing – original draft. Almeida, P.S.: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. Viana, M.B.: Zaiat, M.: Conceptualization, Formal analysis, Funding acquisition, Resources, Supervision, Validation. Leitão, R.C.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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