Hydrogen and Methane Production in Two-Stage Upflow Anaerobic Sludge Blanket Reactors from Crude Glycerol

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Abstract

This study aimed to investigate the impact of operational conditions, such as hydraulic retention time and substrate concentration, on the production of hydrogen (H_2) in an upflow anaerobic sludge blanket (UASB) reactor, which was followed by the production of methane (CH_4) in a sequential UASB fed with acidogenic effluent. The maximum yield of H_2 obtained was 0.23 ± 0.05 mol H_2/mol glycerol (∼8% of the maximum theoretical yield) by applying a maximum organic loading rate (OLR) of 50 kgCOD/m^3·d. The soluble metabolites detected in the UASB-H_2 effluent showed that 1,3-propanediol was the primary metabolite formed during the operation (1.8–3.7 g/L). UASB-CH_4 was operated stably with a maximum OLR of 19 kgCOD/m^3·d, removing 94% of organic matter and producing 0.092 m^3 of biogas daily (74% of CH_4). The ecological succession analysis of the UASB-H_2 showed the bacterial population parameters increase at average OLRs, promoting the dominance of generalist species because of a higher carrying capacity from a less specific substrate and increasing system stability because of niche diversification. An energy analysis was conducted in the condition with the highest daily CH_4 production, that is, 18.72 kgCOD/m^3·d. The two-stage system resulted in 171 MJ/m^3 reactor·d for a plant generating 34,350 kgCOD/d of glycerol. In contrast, a single CH_4 production system would be capable of generating only 94 MJ/m^3 reactor·d. Therefore, although hydrogen production was low in the hydrogenogenic reactor, acidogenesis in the first UASB reactor allowed the methanogenic reactor to achieve high OLR and, consequently, high energy yields.

Keywords: 1,3-propanediol, biodiesel by-product, UASB reactor, PCR-DGGE, biohydrogen

Introduction

Glycerol is an alcohol that is broadly used in the chemical industry. During the biodiesel production process, glycerol emerges as a by-product, with roughly 10 tons generated for every 100 tons of biodiesel. As biodiesel is increasingly incorporated into diesel, especially in Brazil, glycerol generation is also increasing. In addition, glycerol contains around 20% impurities,[1] which affect its market value. To increase the value of unpurified glycerol, researchers have focused on obtaining value-added products through dark fermentation and anaerobic digestion from this by-product.[2–4]

In dark fermentation, hydrogen (H_2) and carbon dioxide (CO_2) are produced as a gaseous phase, and the liquid effluent can be discharged with a high concentration of organic acids[5] and alcohols (1,3-propanediol).[6] One of the challenges related to glycerol fermentation is the use of high organic loading rates (OLRs), which may disfavor H_2 production.[7] Furthermore, the H_2 formation (generated by the oxidative route) can be harmed by a change to the reductive route with the production of 1, 3-propanediol (competitive route to H_2 production) as a strategy microorganisms use to balance Nicotinamide Adenine Dinucleotide oxidized/Nicotinamide Adenine Dinucleotide reduced (NAD+/NADH) ratio.[6] As expected in fermentative processes, the effluent has a high chemical oxygen demand (COD) due to producing organic metabolites (e.g., short-chain

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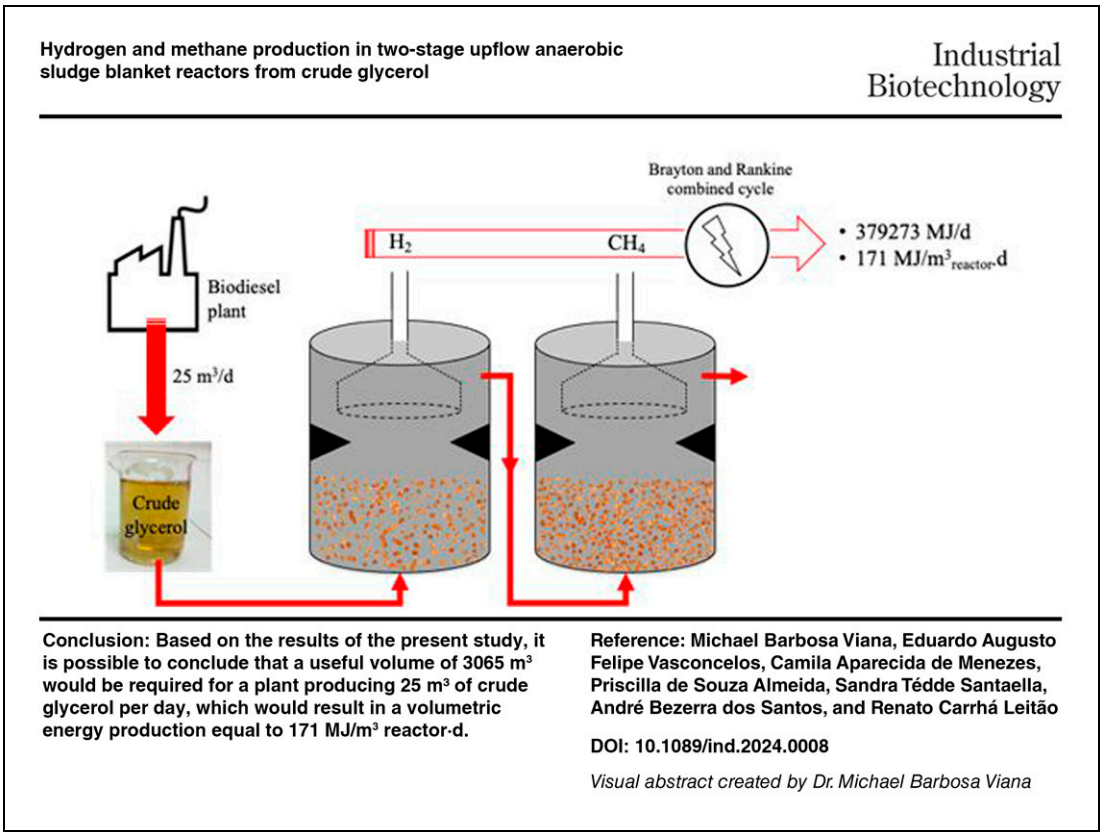

acids and alcohols). Therefore, the acidogenic effluent produced cannot be discarded without adequate treatment/use.

On the contrary, anaerobic digestion uses organic acids to metabolize methane (CH_4). However, the methanogenesis step is limited by the OLR because the high biodegradability of glycerol can result in the accumulation of volatile acids,[8] which is not desirable in a methanogenic reactor. Therefore, the literature shows the frequent use of lower OLR for the CH_4 production from glycerol.[1] Thus, OLR proves to be one of the main parameters in the energy recovery of glycerol in the form of H_2 and CH_4 production. A little-explored alternative to achieve high OLRs is the digestion of glycerol in a high-rate sequential system, also called two-stage digestion. In addition to more significant energy generation when compared with single-stage anaerobic digestion, operating reactors in two stages enables the isolation of limiting steps (hydrolysis and acidogenesis in the first phase and methanogenesis in the second), reduces anaerobic toxicity to recalcitrant compounds, and controls the kinetic reaction because of the control of the ideal environmental conditions of each stage.[9] It should also be highlighted that the study of the sequential production of H_2 and CH_4 in a high-rate upflow anaerobic sludge blanket (UASB) reactor using crude glycerol as a substrate was not identified in the literature.

The aim of this study was to identify the operational factors that affect the production of H_2 and CH_4 from the anaerobic digestion of glycerol. These factors include hydraulic retention time (HRT), substrate concentration, cell immobilization, and inhibition of methanogenesis by chemical agents. The study also investigated the ecological changes that occurred throughout the operation of the H_2-producing reactor using Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and Sanger sequencing techniques.

Materials and Methods

SUBSTRATE AND INOCULUM

The residual glycerol was collected at the Usina de Biodiesel Quixadá (Quixadá, Ceará, Brazil). This glycerol presented an organic matter concentration equal to 1374 gCOD/L and physical–chemical characteristics as described in Viana et al.[10] Commercial glycerol with 99.5% purity had an organic matter concentration equal to 1418 gCOD/L.

The reactors were inoculated with sludge from a UASB reactor at the Ceará Water and Sewage Company (Cagece) to treat municipal sewage. Before inoculating the reactor, the following sludge characteristics were determined: total volatile solids (TVS) concentration, specific methanogenic activity,[11] and specific hydrogenogenic activity.[12] The sludge had a TVS concentration of 59 g/L and specific hydrogenogenic and methanogenic activities of 0.24 L H_2/kgTVS·d and 0.83 kgCOD/m^3·d, respectively.

UASB-H_2 OPERATION

The H_2-producing UASB reactor (UASB-H_2) was built in borosilicate glass with a diameter of 100 mm and a total height

Table 1. Operational Conditions in the UASB-H_2 Reactor Stages

STAGE	1	2	3		
STEP			I	II	III
Duration (d)	112	66	150	84	147
Glycerol type	Commercial	Commercial	Commercial	Crude	Crude
Chloroform	No	No	Yes	Yes	No
COD influent (g/L)	24–120	40.0	40.0		
Flow rate (L/d)	10	6.8–18.5	1.6–16.4		
Hydraulic retention time (d)	1.3	1.9-0.7	8.1–0.8		

COD, chemical oxygen demand; H_2, hydrogen; UASB, upflow anaerobic sludge blanket.

of 1.35 m, resulting in a working volume of 10.6 L (0.0106 m^3). The UASB-H_2 was initially filled with 9 L of inoculum for startup, resulting in a sludge mass of 531 gVTS. The operation of the UASB-H_2 was divided into three stages, all operated at room temperature (30°C), as detailed in *Table 1*. In Stage 1, the OLR increase was achieved by adding more commercial glycerol with a fixed HRT. In Stage 2, the OLR increase was achieved by decreasing the HRT with a fixed commercial glycerol concentration. Chloroform was not used to eliminate archaea in Stages 1 and 2. In Stage 3, kneaded pieces of corrugated polyvinyl chloride pipe with a specific area of 907 m^2/m^3 were added to the reactor.[13] Also in Stage 3, the UASB-H_2 was initially fed with commercial glycerol at a fixed concentration with the addition of chloroform (0.05% v/v).[10] However, on the 184th day of operation in Stage 3, all commercial glycerol was replaced by residual glycerol with the addition of chloroform. Chloroform was completely removed from the influent from the 234th day onward. In all stages of the H_2-producing reactor, a nutrient solution adapted from Lin and Lay[14] was added. An automatic controller (Hanna Instruments brand, model HI1006-3205) was used with a pH sensor connected to the reactor recirculation line to maintain a pH of up to 5.3.

UASB-CH_4 OPERATION

The reactor used to produce CH_4 (UASB-CH_4) was constructed using polyvinyl chloride and features a "Y" shape. It has a diameter of 100 mm, is 1.82 m tall, and has a working volume of 14.9 L (0.0149 m^3). The UASB-CH_4 was supplied with diluted UASB-H_2 effluent until the concentration of organic matter was consistent with the applied OLR. The OLR was increased from 2.5 to 20 kgCOD/m^3·d but only after the stabilization of CH_4 productivity and organic matter removal efficiency had been maintained for three times the hydraulic detention time. The pH of the influent was corrected to 7 with 50% (v/v) NaOH. No nutrient supplementation was added to the UASB-CH_4 influent. The scheme of the experimental apparatus of the two UASB reactors in series is presented in *Figure 1*.

ANALYTICAL METHODS

pH, alkalinity, concentration of TVS, and COD were determined following the procedures described in Standard Methods for the Examination of Water and Wastewater.[15] The concentration of total volatile fatty acids (VFAs) was determined following the Buchauer methodology.[16] A Ritter gasometer, model TG05/05, was used to measure the volume of biogas produced in the reactors. The concentrations of H_2, CH_4, CO_2, nitrogen (N_2), and hydrogen sulfide (H_2S) in the gas produced by the reactors were determined by a gas chromatograph (C2V-200 micro gas chromatograph (GC), Thermo Fisher Scientific, The Netherlands).

MICROBIAL COMMUNITY ANALYSIS

Bacterial and archaeal samples were analyzed using DNA extraction and PCR amplification techniques. The FastDNA™ Spin Kit for Soil from MP Biomedicals (Santa Ana, CA) was used to extract DNA from the samples with the modifications observed in Vasconcelos et al.[17] The amount of DNA using a NanoDrop 2000c spectrophotometer from Thermo Fisher Scientific was measured, and the samples were diluted before performing PCR amplification. Specific primers containing GC-clamps were used for analysis by DGGE, and the 16S rRNA gene hypervariable regions V2, V3, V6, and V8 were targeted. The DGGE patterns were analyzed using Bionumerics software v. 6.1 to study the community functional distribution and environmental distribution of the organisms.

Results and Discussions

UASB-H_2 REACTOR

Table 2 presents the average values of the monitored variables during the 559 days of UASB-H_2 operation. In Stage 1, when the substrate concentration was increased to 74 gCOD/L, the hydrogen production rate (HPR) increased to 0.9 L H_2/L·d. This increase may have resulted from the inactivation of the activity of methanogenic archaea because of the toxicity caused by the excess substrate,[16] as CH_4 was not detected in the biogas under this operational condition. However, this increase in the HPR values was not observed for concentrations lower than 66.1 gCOD/L. Conversely, when the substrate concentration was increased to 121.5 gCOD/L, the HPR was reduced to 0.7 L H_2/L·d. The reduction can be attributed both to the toxicity caused by the high concentration of undissociated VFAs formed[17] and to the biomass drag because of the increased biogas production at the beginning of this operational condition.[18]

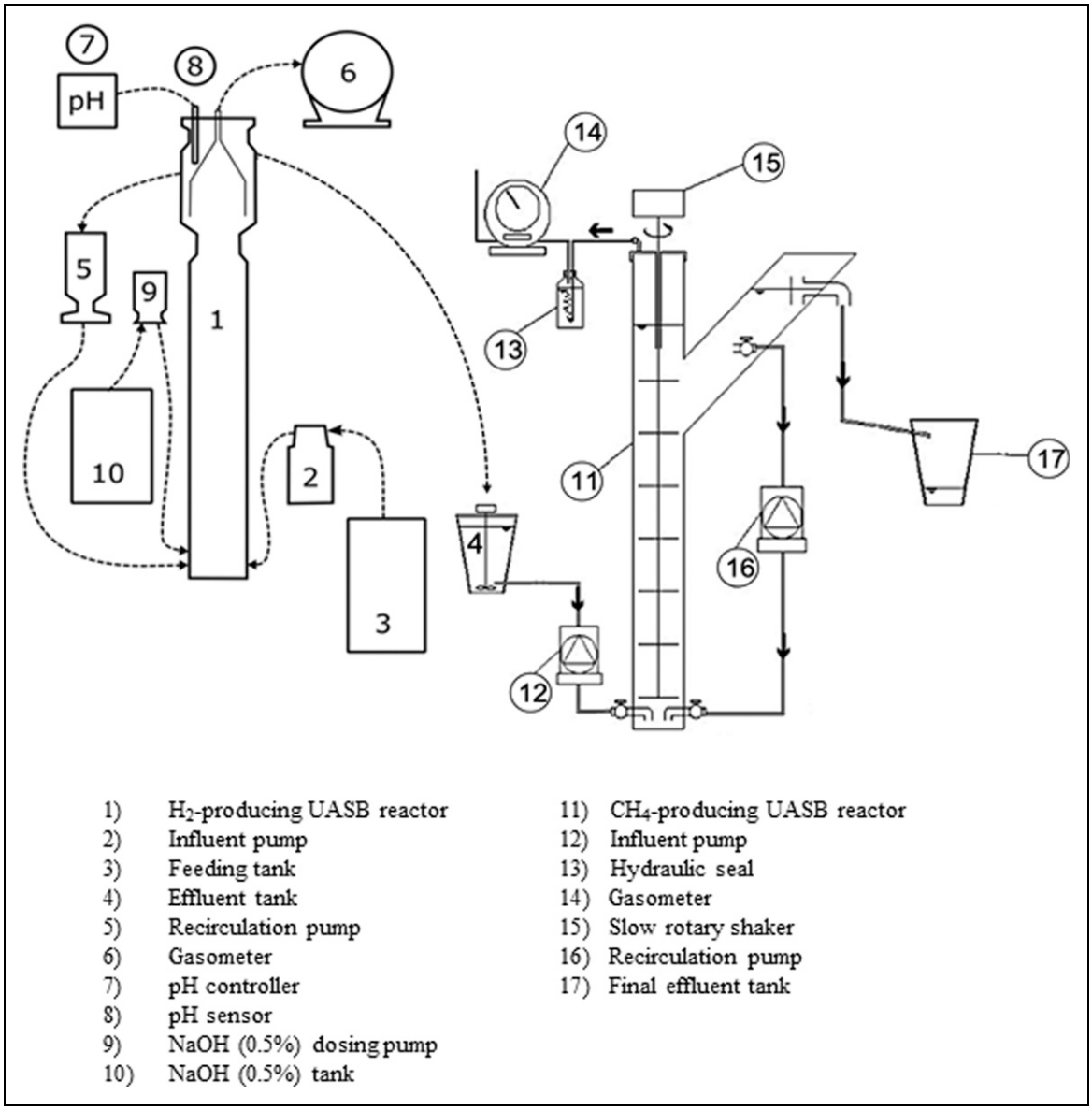

Fig. 1. Scheme of the experimental apparatus of the two UASB reactors in series used to produce H_2 and CH_4 from crude glycerol. CH_4, methane; H_2, hydrogen; UASB, upflow anaerobic sludge blanket.

The biomass drag can be attested by the increase in the volatile suspended solid concentration from 1.1 to 17 g/L, which reduced the sludge retention time from 69 days to 1 day.

In Stage 2, the expectation was that the decrease at the HRT would cause the washing of the archaea from the medium, which, within a relative acidic pH (between 5.3 and 6.0), provided an exclusively acidogenic system.[19] However, it was possible to observe that for similar conditions, that is, 45 gCOD/L and influent flow of 10 L/d, the percentage of CH_4 in biogas was 25% for the strategy of increasing substrate concentration (Stage 1) against 89% for the strategy of decreasing HRT (Stage 2). Owing to CH_4 at all OLR applied, the maximum HPR observed in Stage 2 was 0.1 L H_2/L·d.

The comparison of the outcomes obtained in Stages 2 and 3 demonstrates how the presence of archaea has a negative impact on CH_4 productivity. In Stage 2, it was observed that the biogas produced had a CH_4 composition of 56%, and no H_2 was detected. However, in Stage 3 (Step 1), no CH_4 was detected in the biogas despite a similar inflow rate of 12.9 L/d and a COD concentration of 44.9 g/L. Because chloroform selectively inhibits the activity of methyl-coenzyme M reductase, which is only present in methanogenic archaea,[20] the HPR value was 1.2 L H_2/L·d in Stage 3. Moreover, the results from Stage 3 also support the low HPR values observed in Stages 1 and 2, which can be associated with washing the reactor biomass. In environments characterized by high liquid or gas flow, the inclusion of support media can enhance biomass retention within the reactor. In addition, using support media can lead to higher activity of the H_2-producing biomass and reduced metabolic stress, including pH,

Table 2. Average Values of the Variables Monitored During the 559 Days of UASB-H_2 Operation

STAGE		DAYS OF OPERATION	MEASURED OLR (KGCOD/M^3·D)	INFLUENT FLOW (L/D)	COD INFLUENT (G/L)	% H_2	% CH_4	HPR (L H_2/L·D)	HY (MOL H_2/MOL GLYCEROL ADD)
1		1–24	14.4 ± 3.9	8.3 ± 2.3	26.6 ± 1.3	18 ± 10	12.0 ± 2.8	–	–
		25–45	26.5 ± 5.3	9.7 ± 1.7	43.4 ± 9.0	20 ± 1	25.0 ± 2.4	0.1 ± 0	0.02 ± 0.01
		46–59	39.2 ± 5.5	10.2 ± 0.7	58.2 ± 8.3	19 ± 0	30.0 ± 0	0.1 ± 0	0.01 ± 0
		60–73	48.6 ± 1.6	10.1 ± 1.1	66.1 ± 3.2	68 ± 0	13.0 ± 0	0.5 ± 0.2	0.04 ± 0.03
		74–84	54.5 ± 14.7	10.4 ± 0.7	74.0 ± 21.0	85 ± 0	–	0.9 ± 0.2	0.07 ± 0.02
		85–112	79.1 ± 11.4	9.0 ± 2.2	121.5 ± 27.4	71 ± 8	–	0.7 ± 0.2	0.04 ± 0.02
2		1–9	18.8 ± 2.7	6.8 ± 0.5	37.4 ± 5.5	–	–	–	–
		10–23	28.9 ± 4.9	8.3 ± 1.2	45.4 ± 5.1	4 ± 0	89.0 ± 0	–	–
		24–37	37.4 ± 3.8	11.8 ± 1.1	34.6 ± 19.5	5 ± 2	56.0 ± 0	–	–
		38–50	54.0 ± 4.6	17.6 ± 1.6	41.9 ± 0.8	39 ± 0	9.0 ± 0	0.1 ± 0.1	0.01 ± 0.01
		51–66	61.5 ± 5.8	18.5 ± 1.3	45.6 ± 2.9	39 ± 0	9.0 ± 0	0.1 ± 0.1	0.01 ± 0.01
3	I	1–6	4.2 ± 1.0	1.4 ± 0.3	31.1 ± 0.9	4 ± 0	87.0 ± 2.0	–	–
		7–10	9.0 ± 2.4	3.8 ± 1.0	31.0 ± 1.7	4 ± 0	85.0 ± 1.0	–	–
		11–26	12.8 ± 1.2	4.8 ± 0.3	35.7 ± 3.1	44 ± 9	–	0.1 ± 0.1	0.03 ± 0.02
		27–45	20.0 ± 3.4	5.7 ± 0.8	44.3 ± 2.5	39 ± 1	–	0.2 ± 0.1	0.04 ± 0.02
		46–103	28.9 ± 4.6	8.4 ± 2.3	47.7 ± 9.1	37 ± 3	–	0.3 ± 0.1	0.04 ± 0.02
		104–150	42.9 ± 10.9	12.9 ± 3.4	44.9 ± 11.7	49 ± 4	–	1.2 ± 0.8	0.11 ± 0.05
	II	151–184	41.2 ± 4.0	12.3 ± 2.0	43.4 ± 7.9	52 ± 8	–	0.8 ± 0.4	0.08 ± 0.04
		185–234	50.1 ± 3.1	18.2 ± 2.3	36.4 ± 5.4	45 ± 2	–	1.6 ± 0.3	0.13 ± 0.02
	III	235–381	50.0 ± 3.4	15.6 ± 3.6	44.6 ± 11.0	51 ± 10	–	1.3 ± 0.6	0.11 ± 0.05

The parameter was either not measured or was found to be below the detection limits.
CH_4, methane; HPR, hydrogen production rate; HY, hydrogen yield.

temperature, OLR, and toxic elements from the residual glycerol, such as methanol and salts.[21]

In Stage 3, the production of H_2 was higher than in Stages 1 and 2, but the yield of hydrogen production (HY) in the three steps of Stage 3 remained below 0.23 mol H_2/mol glycerol in Step 2. This could be because of the generation of 1,3-propanediol in this operating condition (0.27 mol 1,3-propanediol/mol glycerol). In Step 3, there was a reduction in H_2 yield (0.23 mol H_2/mol glycerol) and 1,3-propanediol yield (0.27 mol 1,3-propanediol/mol glycerol). The formation of H_2 occurs during the oxidative branch of glycerol fermentation, which also generates acetic and butyric acids as liquid metabolites.[5] In some cases, the resulting increase in H_2 partial pressure triggers the production of 1,3-propanediol through the reductive branch as a means of H_2 consumption.[6] However, the reduction in 1,3-propanediol yield without a corresponding increase in H_2 production in Step 3 leads to propionic acid as the preferable metabolite via the oxidative route because propionic acid does not result in the production or consumption of H_2 from glycerol.[6] In fact, applying crude glycerol in Step 3 caused an increase in propionic acid concentration from 1.2 to 2.4 g/L. Glycerol and propionic acid, which both have the same degree of reduction, facilitate this strategy.[17]

Table 3 shows the ecological parameters of the reactor for Stages 1, 2, and 3 for the domains Bacteria and Archaea. The study found that although H_2 production and yields would increase whenever H_2-producing species became dominant, there was a decrease in bacterial richness and diversity in Stage 3 because of increasing selective pressures for H_2-producing bacteria. CH_4-producing archaea are sensitive to OLR, as observed from the absence of CH_4 in Stage 1, for OLRs higher than 54.5 kgCOD/m^3·d, and by reducing population in earlier stages. Results indicate that higher OLR could reduce H_2 production because of the resporulation of H_2-producing bacteria.[22] These results and explanations are corroborated by the functional organization (Fo) calculated for each stage because a higher Fo indicates a more specialized community, with fewer species but more productivity because of specialization. However, the decrease in niche diversification reduces the general resistance and resilience of the system to external changes because of a lower gene pool and functional redundancy.[17,23]

Table 3. Ecological Parameters of the Reactor for Stages 1 (a), 2 (b), and 3 (c) for Domains Bacteria and Archaea

STAGE 1						STAGE 2						STAGE 3					
SAMPLE	TIME	OLR	RR	H	FO	SAMPLE	TIME	OLR	RR	H	FO	SAMPLE	TIME	OLR	RR	H	FO
BACTERIA																	
Seed	N/A	N/A	38.72	2.32	48.35	Seed	N/A	N/A	43.3	2.41	44.7	Seed	N/A	N/A	40.0	2.42	4.1
I	1–24	14.4 ± 3.9	136.23	2.98	53.69	I	1–9	18.8 ± 2.7	85.5	2.64	51.4	I	1–45	20 ± 3.4	67.7	2.61	59.8
II	25–45	26.5 ± 5.3	194.06	3.32	54.73	II	10–23	28.9 ± 4.9	122.9	2.88	60.9	II	46–103	28.9 ± 4.6	117.0	2.79	67.6
III	46–59	39.2 ± 5.5	59.63	2.52	59.60	III	24–37	37.4 ± 3.8	41.0	2.21	63.0	III	104–150	42.9 ± 10.9	27.8	2.20	79.1
IV	60–73	48.6 ± 1.6	25.87	2.45	61.24	IV	38–50	54.0 ± 4.6	30.7	1.93	72.4	IV	151–184	41.2 ± 4.0	58.9	2.29	51.5
V	74–84	54.5 ± 14.7	11.53	2.04	59.56	V	51–66	61.5 ± 5.8	19.4	1.84	62.3	V	185–234	50.1 ± 3.1	34.0	2.08	54.1
ARCHAEA																	
Seed	N/A	N/A	119.24	3.35	31.93	Seed	N/A	N/A	134.3	2.47	37.2	Seed	N/A	N/A	126.6	2.48	31.9
I	1–24	14.4 ± 3.9	169.44	3.65	31.90	I	1–9	18.8 ± 2.7	75.1	2.24	33.7	I	1–45	20 ± 3.4	5.4	1.66	14.9
II	25–45	26.5 ± 5.3	135.36	3.53	30.35	II	10–23	28.9 ± 4.9	34.5	2.05	31.2	II	46–103	28.9 ± 4.6	3.7	1.42	11.0
III	46–59	39.2 ± 5.5	92.40	3.43	26.47	III	24–37	37.4 ± 3.8	25.8	1.63	28.2	III	104–150	42.9 ± 10.9	3.0	1.23	7.5
IV	60–73	48.6 ± 1.6	59.33	3.15	24.67	IV	38–50	54.0 ± 4.6	16.7	1.41	21.5	IV	151–234	41.2 ± 4.0	4.3	1.21	8.8
V	74–84	54.5 ± 14.7	29.01	2.83	24.25	V	51–66	61.5 ± 5.8	8.7	1.28	18.6	V	235–381	50.1 ± 3.1	10.9	1.32	14.6

Time in days; OLR in kgCOD/m^3·d.
FO, functional organization; OLR, organic loading rate; RR, Range-weighted richness.

Some of the species that are inhibited in these ecological interactions were also probably sensitive to substrate concentration. Therefore, higher OLR stages resulted in the inhibition by excess of substrate. The lower bacterial Fo also corroborates this explanation in the initial stages, which gradually increased from the inoculum to seeds I, II, III, IV, and V. Decreases in population parameters followed by increases in Fo indicate a higher degree of specialization of the community toward the use of substrates over time, which can be seen in the increase in H_2 production, yield, and concentration.[17,23]

Among the three stages, the third one presented a different pattern than the other two because chloroform was added to the influent and crude glycerol was added in Stage 3, Steps 2 and 3. Both population and functional parameters for archaea decreased from inoculum to Stage 3, because of the presence of chloroform, increasing slightly in seed IV and significantly in seed V. The increase in population parameters could be explained by the introduction of crude glycerol, which contains impurities such as long-chain fatty acids and others, which could potentially increase the number of generalist species taking advantage of a general substrate rather than the previous.[24] Furthermore, the highest bacterial Fo observed occurred at Stage 3, where biogas production, H_2 concentration, and yield increased significantly, implying an increase in specialization.

UASB-CH_4 REACTOR

Figure 2 shows the variation in OLR, influent and effluent COD, organic matter removal efficiency, and daily biogas production as a function of the operation time of the UASB-CH_4 reactor fed with the UASB-H_2 reactor effluent on Step 3 and Stage 3. Average values of the variables monitored during the 177 days of UASB-CH_4 operation are presented in *Table 4*.

The OLR of the UASB-CH_4 reactor was gradually increased up to 6.5 kgCOD/m^3·d, which resulted in a high average removal efficiency of organic matter (between 81% and 94%) and an average CH_4 yield of up to 0.34 m^3·CH_4/kgCOD. However, when the OLR of 11 kgCOD/m^3·d was reached, the reactor experienced an accumulation of VFAs, which reduced the pH from 7.0 to 5.8 and lowered the average removal efficiency of organic matter to 56%. During this period, biogas production declined from 50 to 2 L/d. After temporarily reducing the OLR to 6.0 kgCOD/m^3·d, it was

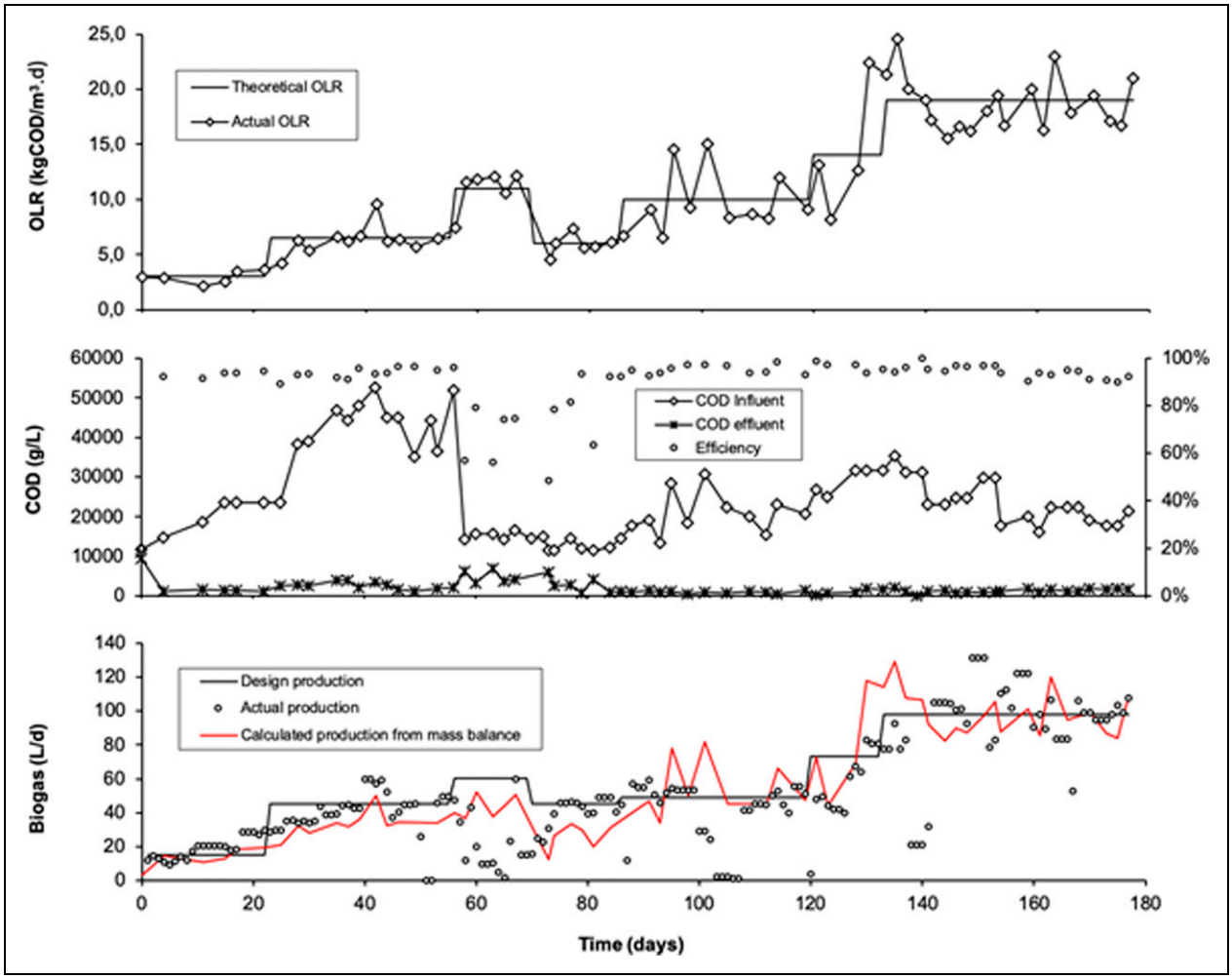

Fig. 2. Variations in influent and effluent COD, organic matter removal efficiency, and daily biogas production as a function of the OLR applied on each operation stage of the UASB-CH_4 operation. COD, chemical oxygen demand; OLR, organic loading rate.

Table 4. Average Values of the Variables Monitored During the 177 Days of UASB-CH_4 Operation

ACCUMULATED TIME (DAYS)	DURATION (DAYS)	MEASURED OLR (KGCOD/M^3·D)	INFLUENT FLOW (L/D)	COD INFLUENT (G/L)	COD EFFLUENT (G/L)	COD REMOVAL EFFICIENCY (%)	% CH_4	DAILY CH_4 VOLUME (L/D)	SPECIFIC PRODUCTION (M^3 CH_4/KGCOD)
6	6	2.91	3.01	13.3	5.3	56	73.5	8.80	0.09
22	16	2.95	2.23	22.3	1.4	93	73.5	15.97	0.39
38	16	5.72	2.24	38.4	3.1	92	73.8	27.37	0.32
55	17	6.82	2.40	43.8	2.2	95	74.4	31.24	0.35
69	14	10.92	10.09	21.4	4.4	73	74.0	16.30	0.13
85	16	5.87	7.27	12.9	2.9	76	74.4	29.13	0.35
111	26	9.74	6.95	20.5	1.0	95	74.2	27.57	0.23
119	8	9.76	6.60	19.7	0.9	95	74.2	36.66	0.26
125	6	10.65	4.82	25.8	0.5	98	74.2	28.46	0.23
132	7	17.50	7.78	31.6	1.4	96	74.4	50.64	0.23
177	45	18.72	12.40	24.1	1.3	94	74.4	68.55	0.25

further increased until an average OLR of 19 kgCOD/m^3·d was reached. At the end of the operation (19 kgCOD/m^3·d), the UASB-CH_4 was able to remove 94% of organic matter and produce 92 L of biogas per day, 74% of which was composed of CH_4. The reactor's CH_4 production rate was 4.6 L CH_4/L·d, which was higher than the maximum CH_4 production rate reported in the literature from glycerol in an anaerobic fluidized bed reactor (2.04 L CH_4/L·d).[25] It is worth noting that the OLR achieved in this study is twice as high as the maximum reported in the literature for a reactor treating effluent from an acidogenic reactor treating glycerol.[26]

DATA AND ENERGY ANALYSIS

A summary of the answers obtained through the data is that increasing OLR by increasing concentration is more suitable than decreasing the HRT for eliminating archaea, which goes against common sense.[6] Furthermore, the increase in the OLR caused a reduction in active biomass and reduced the H_2 productivity more than the toxicity caused by acids. The reduction in active biomass was a mitigating factor for inhibition because of the increase in OLR, as demonstrated in the analysis of ecological parameters. Therefore, adding support material can be an alternative for maintaining a high concentration of active biomass within the reactor, which can guarantee conditions for high acidification with reduced inhibition by the concentration of acids within the acidogenic reactor.

The high acidification of the acidogenic reactor was adequate so that the CH_4 production results were twice the highest observed in the literature for glycerol,[25] and the reactor could reach OLR twice the maximum reported for a two-stage system treating glycerol.[26]

The data used in the energy analysis were selected based on the average values obtained during the OLR that provided the highest HPR (1.6 L H_2/L·d at 50.1 kgCOD/m^3·d) and CH_4 production rate (4.6 L CH_4/d at 18.7 kgCOD/m^3·d). Based on laboratory data, this scenario estimates the energy potential of a biodiesel plant capable of producing 25 m^3 of glycerol per day. The lower calorific values of H_2 and CH_4 at 25°C and 1 atm are assumed to equal 10.71 and 36.03 MJ/m^3 of gas, respectively. An electrical energy cogeneration system is used with a Brayton and Rankine combined cycle to generate both electrical and thermal energy. This system has a relatively high efficiency of 40%.[27]

Owing to the low yield of H_2 from residual glycerol, the total energy generated would be practically all from CH_4. Despite this, when comparing the energy estimation results between the use of a two-phase system (present work) and a single reactor producing CH_4, it is possible to observe the superiority of the two-phase system, both in terms of energy produced and in terms of energy per unit reactor volume. In the present simulation, a useful volume of 3065 m^3 would be required, resulting in a volumetric energy production equal to 171 MJ/m^3 reactor·d. A single CH_4 production system with a required useful volume of 3065 m^3 would generate only 94 MJ/m^3 reactor·d.

Conclusions

Increasing the substrate concentration while keeping the hydraulic retention time (HRT) fixed was found to be more effective at removing methanogenic archaea from the UASB-H_2 reactor than maintaining a fixed substrate concentration and decreasing the HRT. The presence of archaea in the reactor was believed to be one of the reasons for the low H_2 productivity. In addition, it was observed that at OLRs higher than around 50 kgCOD/m^3·d, the biomass in the reactor may have been washed out. However, after adding a support medium and inhibiting methanogenesis, there was a significant increase in HPR, which peaked at a value of 1.6 L H_2/L·d, confirming the previous hypotheses.

As for CH_4 production, the greatest volumetric methane production (4.6 L CH_4/L·d) was noted at an OLR of ~19 kg COD/m^3·d. It is important to note that the OLR achieved in the two-stage methanogenic reactor was twice the maximum previously observed in the literature.

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Authors' Contributions

The authors confirm contribution to the article as follows: Study conception and design: M.B.V., E.A.F.V., S.T.S., A.B.S., and R.C.L. Data collection: M.B.V. and E.A.F.V. Interpretation of results: M.B.V., E.A.F.V., C.A.M., P.S.A., and R.C.L. Draft article preparation: M.B.V., E.A.F.V., C.A.M., P.S.A., and R.C.L. All authors reviewed the results and approved the final version of the article.

Author Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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REFERENCES

1. Viana MB, Freitas AV, Leitão RC, et al. Anaerobic digestion of crude glycerol: A review. Environ Technol Rev 2012;1(1):81–92; doi: 10.1080/09593330.2012.692723
2. Viana QM, Viana MB, Vasconcelos EAF, et al. Fermentative H_2 production from residual glycerol: A review. Biotechnol Lett 2014;36(7):1381–1390; doi: 10.1007/s10529-014-1507-4
3. Sarma SJ, Brar SK, Sydney EB, et al. Microbial hydrogen production by bioconversion of crude glycerol: A review. Int J Hydrogen Energy 2012;37(8):6473–6490; doi: 10.1016/j.ijhydene.2012.01.050
4. Wang J, Yin Y. Biological production of medium-chain carboxylates through chain elongation: An overview. Biotechnol Adv 2022;55:107882; doi: 10.1016/j.biotechadv.2021.107882
5. Chen Y, Yin Y, Wang J. Comparison of fermentative hydrogen production from glycerol using immobilized and suspended mixed cultures. International J Hydrogen Energy 2021;46(13):8986–8994; doi: 10.1016/j.ijhydene.2021.01.003
6. Simões AN, da Costa TB, de Menezes CA, et al. One waste and two products: Choosing the best operational temperature and hydraulic retention time to recover hydrogen or 1,3-propanediol from glycerol. Bioprocess Biosyst Eng 2021;44(12):2491–2502; doi: 10.1007/s00449-021-02620-9
7. Sittijunda S, Reungsang A. Valorization of crude glycerol into hydrogen, 1,3-propanediol, and ethanol in an Up-Flow Anaerobic Sludge Blanket (UASB) reactor under thermophilic conditions. Renew Energy 2020;161:361–372; doi: 10.1016/j.renene.2020.07.053
8. de Mello BS, Pozzi A, Rodrigues BCG, et al. Anaerobic digestion of crude glycerol from biodiesel production for biogas generation: Process optimization and pilot scale operation. Environ Res 2024;244:117938; doi: 10.1016/j.envres.2023.117938
9. de Menezes CA, Almeida PS, Camargo FP, et al. Two problems in one shot: Vinasse and glycerol co-digestion in a thermophilic high-rate reactor to improve process stability even at high sulfate concentrations. Sci Total Environ 2023;862:160823; doi: 10.1016/j.scitotenv.2022.160823
10. Viana MB, Dams RI, Pinheiro BM, et al. The source of inoculum and the method of methanogenesis inhibition can affect biological hydrogen production from crude glycerol. Bioenerg Res 2019;12(3):733–742; doi: 10.1007/s12155-019-09994-5
11. Soto M, Méndez R, Lema JM. Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up. Water Res 1993;27(8):1361–1376; doi: 10.1016/0043-1354(93)90224-6
12. Davila-Vazquez G, Alatriste-Mondragón F, de León-Rodríguez A, et al. Fermentative hydrogen production in batch experiments using lactose, cheese whey and glucose: Influence of initial substrate concentration and pH. Int J Hydrogen Energy 2008;33(19):4989–4997; doi: 10.1016/j.ijhydene.2008.06.065
13. Andrade Neto CO, Pereira MG, Melo HN. Materiais alternativos para enchimento de filtros anaeróbios: Conduíte cortado e tijolo cerâmico vazado. Oficina e Seminário Latinoamericano De Digestão Anaeróbia 2000;6:28–35. (In portuguese)
14. Lin C, Lay C. A nutrient formulation for fermentative hydrogen production using anaerobic sewage sludge microflora. Int J Hydrogen Energy 2005;30(3):285–292; doi: 10.1016/j.ijhydene.2004.03.002
15. Eaton AD, Clesceri LS, Rice EW, et al. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WEF: Washington; 2005.
16. Bekins BA, Warren E, Godsy M. A comparison of zero-order, first-order, and Monod biotransformation models. Ground Water 1998;36:261–268.
17. de Menezes CA, Almeida PS, Delforno TP, et al. Relating biomass composition and the distribution of metabolic functions in the co-fermentation of sugarcane vinasse and glycerol. Int J Hydrogen Energy 2023;48(24):8837–8853; doi: 10.1016/j.ijhydene.2022.11.271
18. Wang X, Ren NQ, Liu BF, et al. CFD simulation of an Expanded Granular Sludge Bed (EGSB) reactor for biohydrogen production. Int J Hydrogen Energy 2009;34(24):9686–9695; doi: 10.1016/j.ijhydene.2009.10.027
19. Nirmala N, Praveen G, Amitkumar SA, et al. A review on biological biohydrogen production: Outlook on genetic strain enhancements, reactor model and techno-economics analysis. Sci Total Environ 2023;896:165143; doi: 10.1016/j.scitotenv.2023.165143
20. Hu B, Chen S. Pretreatment of methanogenic granules for immobilized hydrogen fermentation. Int J Hydrogen Energy 2007;32(15):3266–3273; doi: 10.1016/j.ijhydene.2007.03.005
21. Kumar G, Mudhoo A, Sivagurunathan P, et al. Recent insights into the cell immobilization technology applied for dark fermentative hydrogen production. Bioresour Technol 2016;219:725–737; doi: 10.1016/j.biortech.2016.08.065
22. Selembo PA, Perez JM, Lloyd WA, et al. Enhanced hydrogen and 1,3-propanediol production from glycerol by fermentation using mixed cultures. Biotechnol Bioeng 2009;104(6):1098–1106; doi: 10.1002/bit.22487
23. Marzorati M, Wittebolle L, Boon N, et al. How to get more out of molecular fingerprints: Practical tools for microbial ecology. Environ Microbiol 2008;10(6):1571–1581; doi: 10.1111/j.1462-2920.2008.01572.x
24. Lebrero R, Rodríguez E, Pérez R, et al. Abatement of odorant compounds in one- and two-phase biotrickling filters under steady and transient conditions. Appl Microbiol Biotechnol 2013;97(10):4627–4638; doi: 10.1007/s00253-012-4247-1
25. Costa TB, Simões AN, de Menezes CA, et al. Anaerobic biodegradation of biodiesel industry wastewater in mesophilic and thermophilic fluidized bed reactors: Enhancing treatment and methane recovery. Appl Biochem Biotechnol 2021;193(10):3336–3350; doi: 10.1007/s12010-021-03606-9
26. Tangkathitipong P, Intanoo P, Butpan J, et al. Separate production of hydrogen and methane from biodiesel wastewater with added glycerin by two-stage anaerobic sequencing batch reactors (ASBR). Renew Energy 2017;113:1077–1085; doi: 10.1016/j.renene.2017.06.056
27. MesserlieRL, Strother JR. Integration of the Brayton and Rankine cycle to maximize gas turbine performance: A cogeneration option. In: ASME 1984 International Gas Turbine Conference and Exhibit. American Society of Mechanical Engineers: New York; 1984;4:1–6.

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