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Biohydrogen production in anaerobic fluidized bed reactors: Effect of support material and hydraulic retention time

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ABSTRACT

This study evaluated two different support materials (polystyrene and expanded clay) for biohydrogen production in an anaerobic fluidized bed reactor (AFBR) treating synthetic wastewater containing glucose (4000 mg L^{-1}). The AFBRs contained either polystyrene (R1) or expanded clay (R2) as support materials were inoculated with thermally pre-treated anaerobic sludge and operated at a temperature of 30 °C and a pH of approximately 5.5. The AFBRs were operated with a range of hydraulic retention times (HRTs) between 1 and 8 h. For R1 with an HRT of 2 h, the maximum hydrogen yield (HY) was 1.90 mol H_2 mol^{-1} glucose, with 0.805 mg of biomass (as total volatile solids, or TVS) attached to each g of polystyrene. For R2 operated at an HRT of 2 h, the maximum HY was 2.59 mol H_2 mol^{-1} glucose, with 1.100 mg of attached biomass (as TVS) g^{-1} expanded clay. The highest hydrogen production rates (HPR) were 0.95 and 1.21 L h^{-1} L^{-1} for R1 and R2, respectively, using an HRT of 1 h. The H_2 content increased from 16–47% for R1 and from 22–51% for R2. No methane was detected in the biogas produced throughout the period of AFBR operation. These results show that the values of HY, HPR, H_2 content, and g of attached biomass g^{-1} support material were all higher for AFBRs containing expanded clay than for reactors containing polystyrene.

1. Introduction

Today, global energy requirements are met primarily through the burning of fossil fuels (about 80% of the world's energy demand). Hydrogen is a promising alternative to fossil fuels, both from an economic and environmental standpoint. It is a clean and environmentally friendly fuel that produces water instead of green house gases when combusted [1,2]. Although most H_2 is generated from fossil fuels through thermochemical processes [1,3], it may also be produced by biological processes [4], which is potentially more attractive than thermochemical methods, especially if wastewater or other biomass can be used as the raw material [5–7]. Under anaerobic conditions, hydrogen is formed as a by-product during the conversion of organic wastes into organic acids, which are then used for methane generation. The acidogenic phase of anaerobic waste digestion can be manipulated to improve H_2 production [1,2].

Hydrogen fermentation has been carried out with a variety of substrates and different pure and mixed cultures [6–8].

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Notation

Symbols

COD	Chemical oxygen demand, mg L^{-1}
HRT	Hydraulic retention time, h
HPR	Hydrogen production rate, L h^{-1} L^{-1}
HY	Hydrogen yield, mol H_2 mol^{-1} glucose
HAc	Acetic acid concentration, mg L^{-1}
HBu	Butyric acid concentration, mg L^{-1}
HPr	Propionic acid concentration, mg L^{-1}
EtOH	Ethanol concentration, mg L^{-1}
Q	Total liquid flow rate, L h^{-1}
SMP	Soluble microbial products, mg L^{-1}
TVFA	Total volatile fatty acids, mg L^{-1}
TVS	Total volatile solids, mg L^{-1}
VFA	Volatile fatty acids, mg L^{-1}
VSS	Volatile suspended solids, mg L^{-1}

Abbreviations

AFBR	Anaerobic fluidized bed reactor
APBR	Anaerobic packed bed reactor
CSTR	Continuous stirred tank reactor
EPS	Extracellular polymeric substances
FID	Flame ionization detector
SEM	Scanning electron microscopy
TCD	Thermal conductivity detector
UASB	Upflow anaerobic sludge blanket

Many studies have examined the hydrogen production potential of various carbon sources, including glucose [9,10], sucrose [11–13], starch [14,15], and xylose [16,17]. Although biohydrogen production from simple sugars is well researched, only a handful of studies have explored the use of industrial/domestic wastewater as a potential feedstock. These sources include rice winery wastewater [18], food processing and domestic wastewater [19], glycerol-containing wastes [20], citric acid wastewater [21], dairy wastewater [22], and cheese processing wastewater [23].

Continuous stirred tank reactors (CSTRs) are the most frequently-used reactor type for continuous biohydrogen production. However, a range of other reactor types exist, mostly based on methanogenic anaerobic digestion [24]. Upflow anaerobic sludge blanket (UASB), anaerobic packed bed reactor (APBR) and the anaerobic fluidized bed reactors (AFBR) are considered superior to CSTRs because of their ability to retain large amounts of biomass in the reactor. In these systems, microorganisms are retained in an immobilized bed (UASB) or attached to solids (APBR and AFBR) in the form of biofilms or granules, and maintained in suspension by the drag force of upward wastewater flow [24,25].

The AFBR configuration with attached biofilms has been widely used as a biological treatment system for wastewater and is characterized by high efficiency and low hydraulic retention time (HRT) [26,27]. For high density support materials in fluidized bed reactors (sand, coal, clay and so forth), the attainment of adequate fluidization conditions requires the utilization of small diameter particles. Under these conditions, heavy biofilm accumulation and high fluidization velocity cause significant washout of bioparticles from the reactor. To overcome this problem, synthetic polymers previously subjected to chemical treatment to improve their surface characteristics (rugosity, porosity and electrical charge) have been successfully employed as support materials [28–30].

Although AFBRs possess characteristics favorable for the production of gaseous products such as H_2, they have been utilized less frequently for dark H_2 fermentation [31]. A literature survey revealed that the only support materials that have been tested for biohydrogen production in AFBRs were activated carbon [32], celite [33], and expanded clay [34,35]. Expanded clay is a cheap material and insensitive to abrasion. Meanwhile, activated carbon and celite are more expensive and require care with respect to abrasion because turbulence in the system can easily reduce these support materials in size. Moreover, there are no studies in the literature that have compared the startup, steady-state performance and biomass retention of AFBRs containing different types of attachment media under similar operating conditions in wastewater treatment for H_2 production.

However, it is critical that the media particles used are conducive to the rapid and extensive buildup of attached biomass to ensure that stable AFBR performance is attained shortly after startup. Although not all of the mechanisms and substances involved in biofilm adhesion and formation are known, most studies have emphasized that extracellular polymeric substances (EPSs) are primarily responsible for the structural and functional integrity of biofilms due to the cohesive forces they exert, which are responsible for keeping cells together in the form of biofilms, flocs and sludge [25,29,36]. In H_2-producing reactors, retention of more bacteria in the reactor should provide more driving power, allowing increased organic loading rates (OLRs) and volumetric H_2 production rates (HPR) [24]. It is obvious that substantial differences in hydrogen production rate still remain among these H_2-producing reactors, which could be attributed to the difference in the microbial population and the operating conditions.

Therefore, the present study focused on continuous biohydrogen production in AFBRs via mixed-culture biofilms grown on polystyrene and expanded clay support materials. Biofilms that formed in pores and on the surfaces of support material particles were evaluated to quantify biomass and characterize extracellular polymers. The effect of HRT on the performance of AFBRs was also investigated.

2. Materials and methods

2.1. *Heat-treatment of inoculum and synthetic wastewater*

The inoculum used in this study was obtained from the anaerobic sludge of a UASB reactor treating effluent from swine wastewaters [37]. The sludge was subjected to heat-treatment according to the methodology of Kim et al. [38]. This treatment consisted of preheating the sludge for 10 min at 90 °C.

Subsequently, the sludge was placed in an ice bath until the temperature reached 25 °C. This heat treatment eliminated nonsporulating vegetative methanogenic and acidogenic cells, while endospore-producing acidogenic cells (which are resistant to harsh environmental conditions) remained intact.

The synthetic wastewater contained glucose (4000 mg L^{-1}) as the main carbon source and was supplemented with nutrients as described by Leite et al. [39]. The wastewater pH was approximately 7.0; accordingly, 1000 mg L^{-1} of sodium bicarbonate and 1 mL L^{-1} of hydrochloric acid (10 M) were added to maintain the reactor pH at approximately 5.5.

2.2. Support materials

Particles of polystyrene and expanded clay were used in the AFBRs as support materials for biomass immobilization. The polystyrene particles were submitted to prior chemical treatment to enhance their surface roughness. This chemical treatment consisted of soaking the particles in sulphochromic solution for 50 min, rinsing in water, soaking in concentrated nitric acid for 20 min, rinsing in water again, and oven-drying at 40 °C [28,40]. The expanded clay pellets – commonly found at gardening stores – were ground, washed, oven-dried at 40 °C, and sifted to the desired grain size. The basic characteristics of the support materials are shown in Table 1.

2.3. Anaerobic fluidized bed reactor

Fig. 1 shows a schematic diagram of the two identical jacketed reactors used in this study. The reactors were constructed of transparent acrylic with the following dimensions: 190 cm tall, an internal diameter of 5.3 cm, and a total volume of 4192 cm^3. The effluent was recycled through a recycling pump connecting effluent outlet and feed inlet. The temperature in the AFBRs was maintained at 30 °C by recirculating heated water from a thermostatic bath through the column water jackets. A gas–liquid separator was used at the effluent outlet to collect gaseous and soluble products separately. A gas meter (TG1; Ritter Inc., Germany) was used to quantify the amount of hydrogen generated.

2.4. AFBR startup and operational conditions for biohydrogen production

The AFBRs were fed with synthetic wastewater containing glucose (4000 mg L^{-1}), nutrients, sodium bicarbonate (1000 mg L^{-1}), sufficient hydrochloric acid (1 mL L^{-1}; 10 M) to maintain a pH near 5.5, and 10% v/v of heat-treated inoculum (VSS ~ 20 g L^{-1}). In reactors R1 (polystyrene) and R2 (expanded clay), the total liquid flow (Q) was kept at 76 and 128 L h^{-1}, respectively (bed expansion = 30%). These flow rates generated a superficial velocity 1.3 times higher than the minimum fluidization velocity. The reactors were operated in batch mode for the first 48 h to activate H_2-producing microorganisms, and then switched to continuous mode with a designated HRT of 8 h.

The reactors were operated for 191 days. To facilitate data analysis, the study was divided into five experimental phases with different HRT values lasting 30–40 days each. After steady-state conditions were established (based on constant volumetric hydrogen production rates ranging from 5 to 10% over 5–10 days), HRT was progressively reduced from 8 h to 1 h. The composition of gaseous products (H_2 and CO_2) and soluble metabolites (volatile organic acids and alcohols) produced during fermentative H_2 production were monitored as a function of time. Glucose concentration, pH, chemical oxygen demand (COD) and solids were regularly measured. The results reported in this study were the average values of triplicate. Ten data obtained at steady-state were used for the determination of the mean values and standard deviations.

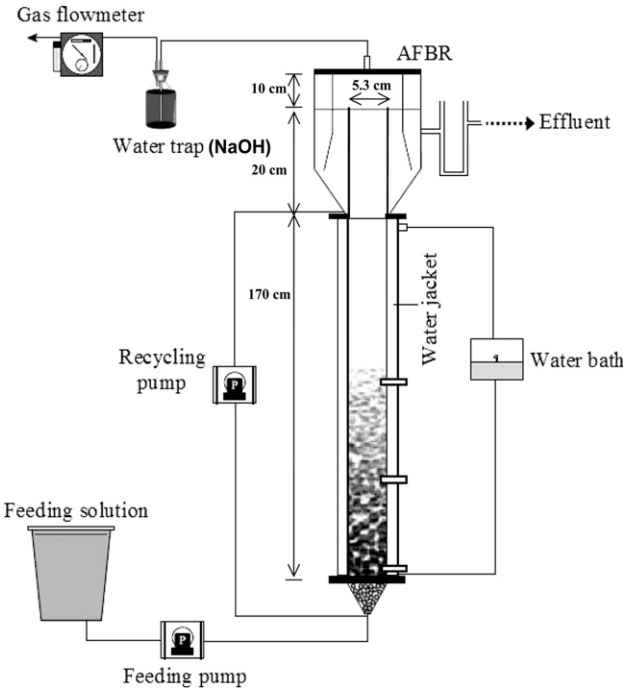

Fig. 1 – Schematic description of the anaerobic fluidized bed reactor (AFBR).

Table 1 – Support materials characteristics.

	Polystyrene	Expanded clay
Diameter (mm)	2.2 × 2.2	2.8–3.35
Density (g cm^{-3})	1.05	1.50
Shape	Cylinders	Granules
Roughness	14.6	18.1
Minimum fluidization velocity (cm s^{-1})	0.74	1.24

2.5. Chemical and microbiological analyses

The biogas hydrogen content was determined by gas chromatography (GC-2010, Shimadzu, Japan) using a thermal conductivity detector (TCD) with argon as the carrier gas. Injector, detector, and column temperatures were 30 °C, 200 °C, and 230 °C, respectively [37].

Concentrations of volatile fatty acids (VFA) and alcohols were also measured by gas chromatography (GC-2010, Shimadzu, Japan, equipped with FID and COMBI-PAL headspace injection; AOC 5000 model and 30 m HP-INNOWAX column × 0.25 mm × 0.25 μm film thickness) [37].

Glucose concentration was measured with an enzymatic GOD-PAP [34,35]. COD, pH, and solids (total solids, TS; volatile suspended solids, VSS; and total volatile solids, TVS) were measured in accordance with Standard Methods [41].

Structural analysis of biofilm samples was performed using a Zeiss DSM-960 digital scanning microscope. Samples were fixed for 12 h at 4 °C in 0.1 M phosphate buffer (pH 7.3) containing 2.5% glutaraldehyde. The samples were subsequently rinsed three times in 0.1 M phosphate buffer (pH 7.3) and gradually dehydrated via successive immersions in increasingly concentrated ethanol solutions (50, 70, 80, 90 and 95%). Each rinse/dehydration cycle took 10 min. The samples were then washed three times in 100% ethanol and immersed for 30 s in hexamethyldisilazane. Drying was completed at 60 °C for 2 h. The particles were then coated with gold powder, attached to supports with silver glue, and observed by digital scanning microscopy [42].

Biomass adhesion to the expanded clay and polystyrene particles was determined according to the methods of Chen and Chen [43]. Quantification of extracellular polymeric substances (EPSs) in protein form was performed in accordance with the method proposed by Lowry et al. [44] and modified by Peterson [45], using bovine serum albumin as a standard. Analysis of EPSs in carbohydrate form was carried out according to the methods of Dubois et al. [46] using lactose as a standard.

3. Results and discussion

3.1. H_2 content, H_2 yield production and H_2 production rate in AFBRs

Fig. 2 shows the variation in H_2 content as a function of HRT for the two AFBRs used in this study. H_2 content in the AFBR containing polystyrene (R1) increased from 16% to 47% when the HRT decreased from 8 h to 1 h. The AFRBR containing expanded clay (R2) performed similarly; when the HRT decreased from 8 h to 1 h, the H_2 content increased from 22% to 51%. Both AFBRs reached their highest H_2 concentrations when operated with an HRT of 1 h (Fig. 2). These values are in agreement with other studies using AFBRs with glucose concentrations of 2000 mg L^{-1} [34,35], 5000 mg L^{-1} [33], 10,000 and 30,000 mg L^{-1} [32], and sucrose concentrations ranging from 5000 to 40,000 mg COD L^{-1} [31]. The expanded clay reactor generated a higher H_2 concentration than the polystyrene reactor, reaching a maximum of 51% (Fig. 2). Methane was not detected during operation in either reactor.

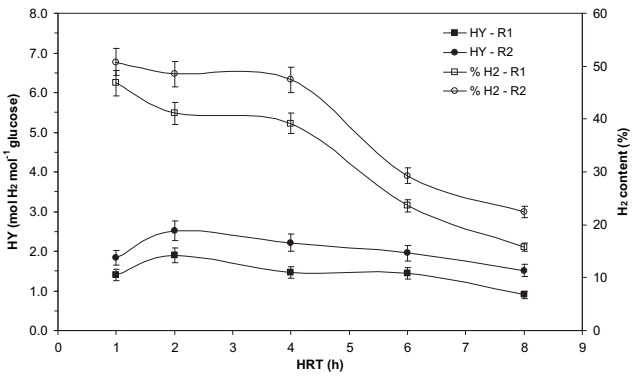

Fig. 2 – Effect of HRT on H_2 yield and H_2 content in AFBRs containing polystyrene (R1) and expanded clay (R2).

Fig. 2 also shows that hydrogen yield (HY) values for R1 (polystyrene) increased from 0.90 to 1.90 mol H_2 mol^{-1} glucose when HRT decreased from 8 h to 2 h, but HY declined to 1.40 mol H_2 mol^{-1} glucose when HRT decreased to 1 h. The same behavior was observed in R2 (expanded clay): HY values increased from 1.51 to 2.52 mol H_2 mol^{-1} glucose when HRT decreased from 8 to 2 h, but decreased to 1.84 mol H_2 mol^{-1} glucose when HRT decreased to 1 h. Therefore, the maximum HY values obtained for R1 (polystyrene) and R2 (expanded clay) were 1.90 and 2.52 mol H_2 mol^{-1} glucose, respectively, with an HRT of 2 h (Fig. 2). Considering that the maximum theoretical HY for glucose is 4 mol H_2 mol^{-1} glucose, the results obtained in the present study with glucose concentration of 4000 mg L^{-1}correspond to 47.5 and 63.0% yields for R1 (polystyrene) and R2 (expanded clay), respectively. Table 2 summarizes the maximum H_2 yields obtained in various types of H_2-producing systems for comparison. In these AFBRs, the maximum HY, 1.90 and 2.52 mol H_2 mol^{-1} glucose for R1 (polystyrene) and R2 (expanded clay), respectively, were comparable with those of other reactors. Furthermore, H_2 has been produced continuously and stably for over 6 months in these AFBRs. This might be attributed to the high biomass level retained in the systems.

Lin et al. [31] operated an AFBR with a draft tube using silicone gel for trapping anaerobic sludge, and used sucrose concentrations ranging from 5000 to 40,000 mg COD L^{-1}. The highest HY value (4.98 mol H_2 mol^{-1} sucrose, which corresponds to 62.3% yield considering that the maximum theoretical HY for sucrose is 8 mol H_2 mol^{-1} sucrose,) was obtained at 35,300 mg L^{-1} sucrose and the optimal HRT was 8.9 h. For an AFBR operated with a glucose concentration of 10,000 mg L^{-1} and activated carbon as a support material, Zhang et al. [32] obtained a maximum HY production value of 1.19 mol H_2 mol^{-1} glucose with the optimal HRT of 1 h. Amorim et al. [34] and Shida et al. [35] evaluated AFBRs containing expanded clay at glucose concentrations of 2000 mg L^{-1}, and obtained maximum HY values of 2.49 and 2.29 mol H_2 mol^{-1} glucose, respectively for HRTs of 2 h.

The HPR values improved in both R1 (polystyrene) and R2 (expanded clay) when HRT was decreased (Fig. 3). Maximum HPRs for R1 and R2 were 0.95 and 1.21 L h^{-1} L^{-1}, respectively, for an HRT of 1 h (Fig. 3). Similarly, HPR values increased with decreasing HRT in the AFBR studies of Lin et al. [31], Zhang et al. [32], Amorim et al. [34], and Shida et al. [35]. Lin et al. [31] obtained an HPR of 2.27 L h^{-1} L^{-1} with an HRT of 2.2 h and a sucrose concentration of 40,000 mg COD L^{-1}. Zhang et al. [32] obtained an HPR of 2.22 L h^{-1} L^{-1} with an HRT of 0.5 h and 10,000 mg L^{-1} glucose. Amorim et al. [34] and Shida et al. [35] obtained HPRs of 0.97 and 1.15 L h^{-1} L^{-1}, respectively, with an HRT of 1 h and 2000 mg L^{-1} glucose. These results show

Table 2 – Comparison of the maximum H_2 yield obtained in various types of H_2-producing reactor.

Reactor	Substrate	Optimal HRT (h)	Maximum HY	Reference
CSTR	Glucose	6	2.1 mol H_2 mol^{-1} glucose	[10]
CSTR	Glucose	13.3	1.63 mol H_2 mol^{-1} glucose	[47]
UASB	Sucrose	13	1.61 mol H_2 mol^{-1} sucrose	[13]
UASB	Rice winery wastewater	24	1.61 mol H_2 mol^{-1} hexose	[18]
APBR (horizontal flow)	Glucose	0.5	2.48 mol H_2 mol^{-1} glucose	[39]
APBR (vertical flow)	Sucrose	1	1.14 mol H_2 mol^{-1} sucrose[a]	[48]
AFBR (draft tube)	Sucrose	8.9	4.98 mol H_2 mol^{-1} sucrose	[31]
AFBR	Glucose	1	1.19 mol H_2 mol^{-1} glucose	[32]
AFBR	Glucose	2	2.49 mol H_2 mol^{-1} glucose	[34]
AFBR	Glucose	2	2.29 mol H_2 mol^{-1} glucose	[35]
AFBR (R1 – polystyrene)	Glucose	2	1.90 mol H_2 mol^{-1} glucose	This study
AFBR (R2 – expanded clay	Glucose	2	2.52 mol H_2 mol^{-1} glucose	This study

a Based on the article data.

that HPR values are influenced by both HRT and substrate concentration (or OLR).

Some studies employing 1–2 h HRTs have shown optimum HPR and HY values [32,34,35], which were attributed to the high flow effect washing away non-sporulated, methanogenic microorganisms [11]. The minimum HRT needed to maintain specific hydrogen production from glucose conversion is related to maintaining adequate concentrations of H_2-producing microorganisms in the system, and that contamination of the system by non-H_2-producing organisms, which compete for substrate, must be prevented. The H_2 production obtained with effluent pH of 5.5 in this study (Fig. 3) similar to that obtained by Zhang et al. [32], Amorim et al. [34] and Shida et al. [35] at pHs less than 4.0, and to results obtained by Lin et al. [31] under pH conditions between 5.8 and 6.8, which are regarded as favoring hydrogen production [10].

3.2. *Soluble microbial products and glucose conversion*

Table 3 shows the distribution of soluble microbial products (SMP) associated with HRT reduction in the AFBRs. The soluble metabolites for R1 (polystyrene) were acetate (HAc) (27.38–49.05%), butyrate (HBu) (30.40–38.22%), ethanol (EtOH) (9.97–38.41%), and propionate (HPr) (2.75–5.38%). For R2 (expanded clay), the soluble metabolites were HAc (32.99–46.81%), HBu (37.30–41.49%), EtOH (10.18–22.95%), and HPr (1.26–4.90%). Zhang et al. [32], who also employed glucose as a substrate, noted the predominance of HAc (43–46% of all soluble metabolites) over HBu (20–31%), and reported significant ethanol production in the effluent (14–21%). Only a small quantity of propionic acid was detected (less than 5.38%) during operation of the AFBRs. This observation corresponds to the increased H_2 production levels, considering that propionate production consumes 2 mol of hydrogen for every 2 mol of propionate produced (Eq. (1)). The absence of HPr may be attributed to low pH values [32].

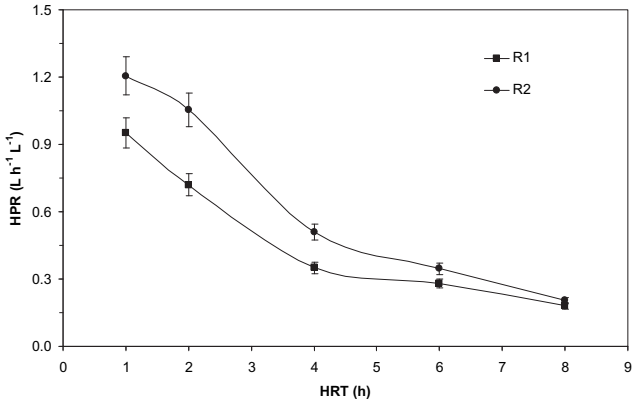

Fig. 3 – Effect of HRT on the H_2 production rate in AFBRs containing polystyrene (R1) and expanded clay (R2).

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (1)$$

The HAc/SMP and HBu/SMP values obtained from the R1 (polystyrene) and R2 (expanded clay) reactors were compared. The results indicated that acetate, followed by butyrate, predominated over other soluble metabolites in R2 (expanded clay), indicating superior H_2 production in this reactor. In this study, when the HRT of R1 (polystyrene) decreased from 8 to 2 h, the HAc/HBu ratio increased from 0.88 to 1.28. However, when the HRT decreased to 1 h, this ratio fell to 1.15. For R2 (expanded clay), similar behavior was observed; the HAc/HBu ratio increased from 0.81 to 1.21 when HRT decreased from 8 to 2 h. However, when HRT decreased to 1 h, this ratio diminished to 1.08. This phenomenon was also observed in other studies that used AFBRs for fermentative H_2 production [31,33]. HRT and OLR are important parameters that may affect the metabolic routes of hydrogen production [49]. This relationship is shown in Table 3, in which the metabolite proportions differed between the two AFBRs for every HRT tested.

Table 3 also shows that the optimum HRTs were 2 h for both R1 (polystyrene) and R2 (expanded clay). Favorable soluble metabolites were produced in greater proportions during operation at these HRTs, which maximized H_2 production.

Fig. 4 shows the glucose conversion behavior as a function of HRT. In reactor R1 (polystyrene), glucose conversion

Table 3 – Production of soluble metabolites in H_2 production under different operating conditions in AFBRs.

	Polystyrene (R1)					Expanded clay (R2)				
	HRT (h)					HRT (h)				
	8	6	4	2	1	8	6	4	2	1
pH	5.87	5.36	5.68	5.67	5.72	5.42	5.00	4.92	5.59	5.69
TVFA (mM)	13.49 ± 0.34	18.20 ± 0.53	17.78 ± 0.83	17.67 ± 0.39	5.80 ± 0.94	14.60 ± 0.23	21.42 ± 0.47	23.98 ± 0.91	24.13 ± 0.44	18.42 ± 0.88
SMP (mM)	21.91 ± 0.45	26.33 ± 0.62	24.53 ± 0.91	19.62 ± 0.43	7.54 ± 0.98	18.94 ± 0.31	26.85 ± 0.52	26.70 ± 0.93	28.48 ± 0.55	20.70 ± 0.91
Eth/SMP (%)	38.41	30.89	27.53	9.97	23.07	22.95	20.24	10.18	15.27	11.02
HAc/SMP (%)	27.38	34.18	38.22	49.05	38.25	32.99	37.24	46.81	45.06	43.74
HBu/SMP (%)	31.12	31.08	30.40	38.22	33.30	40.49	41.26	41.49	37.30	40.34
HPr/SMP (%)	3.08	3.85	3.86	2.75	5.38	3.57	1.26	1.52	2.37	4.90
HAc/HBu	0.88	1.10	1.26	1.28	1.15	0.81	0.90	1.13	1.21	1.08

HAc: acetate, HBu: butyrate, EtOH: ethanol, TVFA = HAc + HBu + HPr, SMP = TVFA + EtOH, EtOH/SMP: molar ethanol to SMP ratio, HAc/SMP: molar acetate to SMP ratio, HBu/SMP: molar butyrate to SMP ratio, HAc/HBu: molar acetate to butyrate ratio.

remained virtually constant as HRT decreased from 8 to 4 h, with values ranging between 80.66 and 83.70%. When the HRT decreased to 2 h and 1 h, glucose conversion rates in R1 dropped to 59.54 and 49.11%. Reactor R2 (expanded clay) showed similar behavior, with glucose conversion values ranging between 88.20 and 96.30% for HRTs from 8 to 4 h. These values decreased to 76.30 and 70.50% when HRT was decreased to 2 h and 1 h, respectively. The higher glucose conversion rate obtained in R2 reinforces the superior performance of this reactor for fermentative H_2 production. The high values of substrate conversion achieved are consistent with comparable studies in AFBRs using glucose [32–35] and sucrose [31]. These high rates may be attributed to the high accumulation of biomass in the system, which is an advantageous feature of adhered-growth systems [50]. Sucrose conversions ranging from 92–99% were obtained by Lin et al. [31] in AFBRs using HRTs decreasing from 8.9 to 2.2 h. Similar results (glucose conversions of 89–98%) were verified by Amorim et al. [34] and Shida et al. [35] in AFBRs using HRTs ranging from 8 to 1 h. However, Zhang et al. [32] verified glucose conversions ranging from 99.47% to 71.44% when HRT was decreased from 4 to 0.5 h. Although the present study has also achieved lower glucose conversions, most of the substrate was channeled to final product reactions instead of bacterial growth and maintenance [32,35]

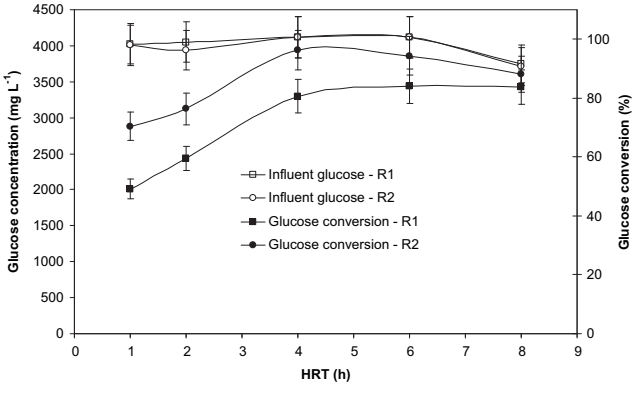

Fig. 4 – Effect of HRT on the glucose concentration and glucose conversion in the AFBRs containing polystyrene (R1) and expanded clay (R2).

3.3. *Attached biomass and extracellular polymeric substances*

It is generally believed that hydrogen production rates are closely related to the dominant microorganisms and environmental conditions present during anaerobic fermentation but are independent of reactor configuration. For example, a hydrogen yield production equivalent to 1.6–2.1 mol H_2 mol^{-1} glucose was achieved in a CSTR reactor [47], a UASB reactor [18] and a fixed bed reactor [48] using mixed cultures rich in *Clostridium* sp. Fig. 5 shows that micro-shaped bacilli resembling *Clostridium* sp. are dominant in the biofilm. Also, the maximum HY (2.52 mol H_2 mol^{-1} glucose) obtained in this study was similar the maximum obtained (2.45 mol H_2 mol^{-1} glucose) using a mixed culture rich in *Clostridium* sp. [9]. It seems likely that the low pH conditions influenced the efficiency of bacterial hydrogen formation in these experiments. In other studies, maximum HY occurred at an optimum pH range of 5.2–5.7, but decreased significantly when the pH dropped to 4.7 [10,51]. These results can be explained by the activity of hydrogenase, an enzyme in *Clostridium* sp. that is inhibited by low pH [49], although *Clostridium butyricum* activity was noticeable even at pH 4.0 [52]. Furthermore, additional substrates may be required to maintain bacterial growth in stressed environments, resulting in lower than optimal hydrogen production.

The SEM micrographs (Fig. 5) show that particles of polystyrene and expanded clay were appropriate support materials for biomass immobilization. However, the biofilms were not uniformly distributed on the particle surfaces; some areas were not covered (Fig. 5a, c). The production of acetic and butyric acids in the study conditions shows that the microorganisms in the inoculum were metabolically similar to *Clostridium* sp. and *Bacillus* sp. [9,53,54]. The presence of these groups is probably related to their morphological use of organic acids (such as acetate and butyrate) and to hydrogenotrophic metabolisms (H_2/CO_2). These findings show that the heat treatment of the inoculum was important to increase the conversion efficiency of glucose and H_2 production.

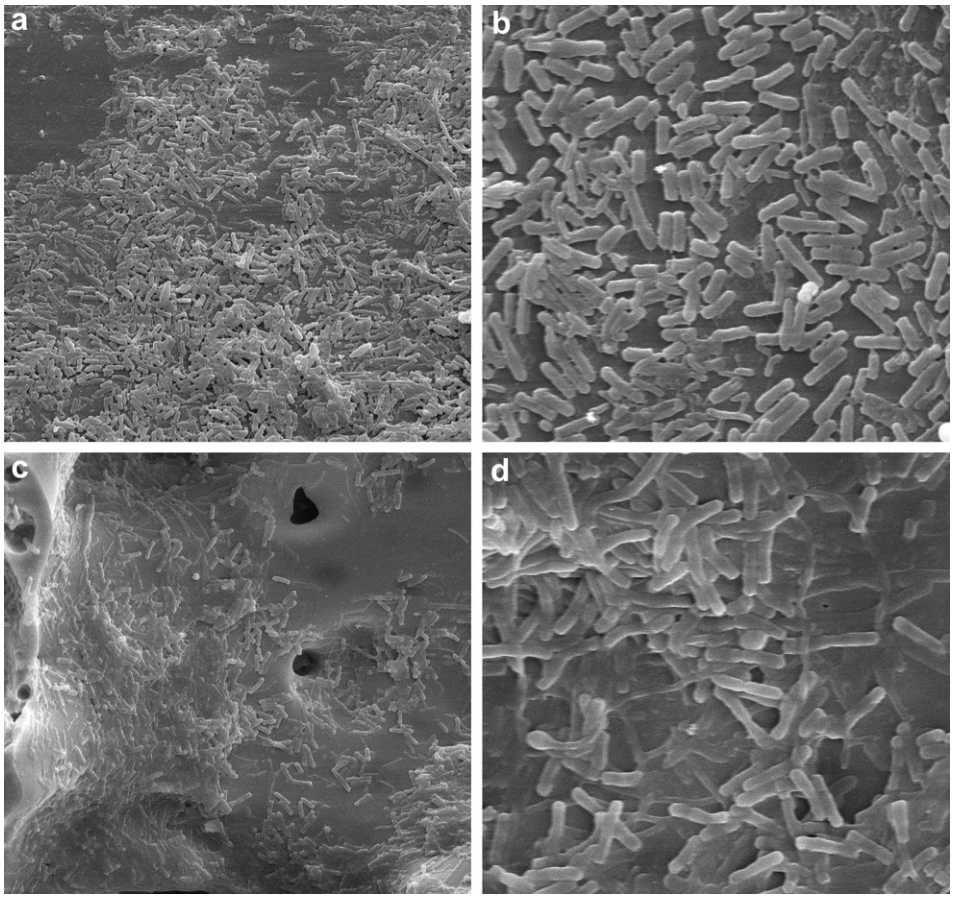

Fig. 5 – SEM micrograph of biofilm attached to support materials: (a, b) polystyrene and (c, d) expanded clay (HRT = 2 h; a, c, magnification 1000×; b, d, magnification 3000×).

In general, the proportion of EPS in biofilms can vary between roughly 50 and 90% of the total organic matter. The best-investigated component of EPS is the polysaccharide moiety. EPSs such as polysaccharides are key compounds for microbial adhesion. However, the matrix is also composed of other components such as proteins, nucleic acids and lipids [36]. Biofilm accumulation on a support is a dynamic process that is the net result of growth and detachment. Biofilm formation is affected by several external factors, including the composition and the concentration of the feed, the velocity of the liquid phase (shear stress), the concentration of particles, particle-particle collisions, and particle-wall collisions [55,56]. In addition, the nature and the concentrations of substrates may affect biofilm growth and composition [55,57].

Under a high substrate loading rate (low HRT), biofilm accumulation is higher, which can affect the structures formed. The amount of EPS synthesis within the biofilm depends greatly on the availability of carbon substrates (both inside and outside the cell) and on the balance between carbon and other limiting nutrients. The presence of excess available carbon and limitations in other nutrients (such as nitrogen, potassium, or phosphate) may favor EPS production over cell formation and consequently the concentration of active biomass [55,58].

In this study, the formation of EPS was related to the HY in AFBRs. Fig. 6 shows the variations in total volatile solids (TVS) content, EPS content in the form of proteins, and EPS content in the form of carbohydrates in the biomass attached to polystyrene and expanded clay particles as a function of

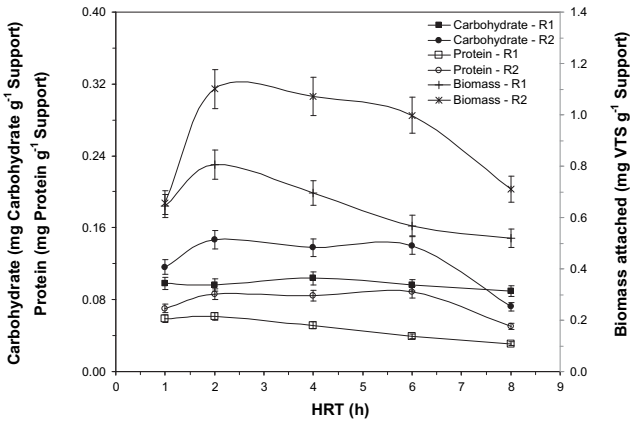

Fig. 6 – Effect of HRT on the biomass attached and EPS content (carbohydrate and protein forms) in the AFBRs containing polystyrene (R1) and expanded clay (R2).

operating HRT in AFBRs. Attached biomass, EPS proteins, and EPS carbohydrates were higher for expanded clay particles. When HRT decreased from 8 h to 2 h, the TVS/expanded clay, protein/expanded clay, and carbohydrate/expanded clay ratios increased from 0.711 to 1.100 mg TVS g^{-1} expanded clay, from 0.050 to 0.086 mg protein g^{-1} expanded clay, and from 0.071 to 0.147 mg carbohydrate g^{-1} expanded clay, respectively. However, these values dropped to 0.658 mg TVS g^{-1} expanded clay, 0.070 mg protein g^{-1} expanded clay, and 0.116 mg carbohydrate g^{-1} expanded clay, respectively, for an HRT of 1 h.

The same behavior was observed for polystyrene particles. The TVS/polystyrene, protein/polystyrene, and carbohydrate/polystyrene ratios increased from 0.519 to 0.805 mg TVS g^{-1} polystyrene, from 0.031 to 0.061 mg protein g^{-1} polystyrene, and from 0.090 to 0.104 mg carbohydrate g^{-1} polystyrene, respectively, when HRT decreased from 8 h to 2 h. However, these values dropped to 0.645 mg TVS g^{-1} polystyrene, 0.059 mg protein g^{-1} polystyrene, and 0.098 mg carbohydrate g^{-1} polystyrene, respectively, for an HRT of 1 h.

The decreasing TVS/support ratio for polystyrene and expanded clay particles may have contributed to the reduction in HY values in each reactor at the HRT of 1 h (Fig. 2). The increasing OLR (with decreasing HRT) may have increased the thickness of the biofilm, and therefore attachment to the support material might have become weaker. As a result, some biofilm may have separated from support materials due to particle–particle collisions, causing a decrease in the observed values of TVS/support, protein/support, and carbohydrate/support ratios when the highest OLR value was reached. These effects would subsequently result in reduced HY. Another hypothesis is that once the AFBRs became overloaded, the systems were limited with respect to glucose conversion, while the HPR continued to increase as the HRT decreased (OLR increased).

Moreover, the superior performance of AFBRs containing expanded clay (R2) could be credited to the surface characteristics of this support material. The surface roughness of expanded clay (18.1%) particles is higher than that of polystyrene (14.6%) particles. Although the polystyrene particles were previously submitted to chemical treatment to enhance their surface roughness, the majority of their surface area remained smooth. Moreover, expanded clay particles have more creviced surfaces than polystyrene particles, and these crevices protect developing biofilms from shear forces, allowing more uniform biomass colonization.

4. Conclusion

Based on the experimental results, we conclude that glucose fermentation in the AFBRs containing polystyrene (R1) and expanded clay (R2) was adequate for hydrogen production. HY increased in R1 and R2 when HRT decreased from 8 h to 2 h. When HRT decreased to 1 h, the performance of the AFBRs declined considerably. R2 had HY values ranging from 1.51–2.52 mol H_2 mol^{-1} glucose, while the HY values for R1 ranged between 0.90 and 1.90 mol H_2 mol^{-1} glucose. The highest hydrogen production rate (HPR) values were 0.95 and 1.21 L h^{-1} L^{-1} for R1 and R2 with an HRT of 1 h. The H_2 content increased from 16–47% for R1 and from 22–51% for R2 with decreasing HRT. No methane was detected in the biogas throughout the period of operation of the AFBRs. It was possible to verify that larger levels of attached biomass and EPS content on support materials resulted in higher HY and HPR values in AFBRs. R2 displayed a more favorable distribution of SMP for hydrogen production, with acetic and butyric acids as the dominant species. Glucose conversion in R2 was greater than in R1, which may be attributed to better surface characteristics of the expanded clay material for biomass attachment.

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