Anaerobic fluidized bed reactor with expanded clay as support for hydrogen production through dark fermentation of glucose

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Abstract

This study evaluated hydrogen production in an anaerobic fluidized bed reactor (AFBR) fed with glucose-based synthetic wastewater. Particles of expanded clay (2.8–3.35 mm) were used as a support material for biomass immobilization. The reactor was operated with hydraulic retention times (HRT) ranging from 8 to 1 h. The hydrogen yield production increased from 1.41 to 2.49 mol H₂ mol⁻¹ glucose as HRT decreased from 8 to 2 h. However, when HRT was 1 h, there was a slight decrease to 2.41 mol H₂ mol⁻¹ glucose. The biogas produced was composed of H₂ and CO₂, and the H₂ content increased from 8% to 35% as HRT decreased. The major soluble metabolites during H₂ fermentation were acetic acid (HAc) and butyric acid (HBu), accounting for 36.1–53.3% and 37.7–44.9% of total soluble metabolites, respectively. Overall, the results demonstrate the potential of using expanded clay as support material for hydrogen production in AFBRs.

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1. Introduction

One of the most important challenges of the new century is to develop new sources of renewable energy that might be able to replace fossil fuels. An ideal replacement would be a clean fuel that has a high efficiency of conversion. Hydrogen is a promising fuel because it is clean, renewable, and has a high energy density of 122 kJ g⁻¹. This is 2.75-fold greater than that of hydrocarbon fuels. Hydrogen can be produced by a number of processes, including electrolysis of water, thermocatalytic reforming of hydrogen-rich organic compounds, and biological processes. Currently, hydrogen is produced almost exclusively by electrolysis of water or by steam reforming of methane. Unfortunately, these processes are highly energy intensive, which makes hydrogen production costly and potentially environmentally unfriendly [1,2].

Biological H₂ production becomes more attractive when organic wastewater or other wastes are used as raw materials. Biological processes are particularly useful for this application because they are catalyzed by microorganisms at environmental temperature and pressure [3]. They require low energy investments, which makes them attractive as alternatives to...
conventional physical/chemical methods of H₂ production. In addition, these techniques are well suited for decentralized energy production at plants where small-scale biomass or waste are available, thus avoiding the expenses and energy costs of transport [4]. Biological H₂ production can be realized by photosynthetic and anaerobic microorganisms. Under anaerobic conditions, hydrogen is produced 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 digestion can be manipulated to improve H₂ production [1,2].

Most fermentative H₂ production utilizes cell suspensions in continuous stirred tank (CSTR) bioreactors [5–7]. However, these systems have difficulty in maintaining a sufficient bacterial population in bioreactors with a low hydraulic retention time (HRT). This causes operational instability and limits the H₂ production rate. Hence, the key to success is to make the system efficient and stable by using a high retention cell. As a result, many studies have used cell-entrapping and cell-attaching methods [8–10]. The anaerobic fluidized bed reactor (AFBR) with attached biofilm has been widely used as a biological treatment system for wastewater with high efficiency and low HRT. Although AFBRs possess favorable characteristics for the production on gaseous products like H₂, they have been less frequently utilized for H₂ dark fermentation [11]. The studies that have been done have used entrapping [9,11] and attaching methods [12,13] of biomass immobilization. According to Zhang et al. [13], the immobilized cells created by gel entrapping techniques are prone to mass transfer resistance. Additionally, the biogas produced inside the gel could lead to structural damage of the immobilized bioparticles.

One of the main parameters for the higher performance efficiency of AFBR with attached biofilm is the choice of support material. However, the selection of appropriate support material should take into account several important factors, in addition to those related to fluidization and biomass immobilization (shape, particle density, and surface area). The support material should be insensitive to abrasion, because depending on the turbulence of the system, the particle can easily be reduced in size, and it should have low cost [14,15]. Biomass retention support materials that have been tested for H₂ production in AFBR with attached biofilm include activated carbon [13] and celite [12]. Expanded clay, a cheap material that is resistant to abrasion and that has a high rigidity for biomass immobilization, has been successfully used as a support carrier for H₂ production in anaerobic packed bed reactors (APBRs) [8,16]. In addition, the apparent density of expanded clay is slightly higher than that of water (1.06 g cm⁻³), facilitating bed expansion and reducing operation costs.

This study aimed to collectively evaluate the use of expanded clay as a support material for H₂ production in an AFBR that is the site of dark fermentation of glucose without the addition of an alkalinity agent. The effect of HRT, organic loading rate and pre-heated treatment of inoculum in hydrogen production and soluble metabolites were also evaluated.

2. Experimental

2.1. Heat-treatment of H₂-producing sludge and fermentation medium

The inoculum was obtained from the sludge of an upflow anaerobic sludge blanket (UASB) reactor treating effluent from swine wastewaters. The sludge was subjected to heat-treatment at 90°C for 10 min in order to inactivate the H₂ consumers and harvest endospore-forming anaerobic bacteria such as Clostridium sp. and Bacillus sp. [17,18].

The medium used for H₂ fermentation contained glucose as the sole carbon source and sufficient amounts of inorganic supplements [16]. The composition of synthetic wastewater was according to Leite et al. [16].

2.2. Support material

Particles of expanded clay (2.8–3.35 mm) were used as support material for biomass immobilization. The material had an apparent density of close to 1.06 g cm⁻³ and a porosity of 23%.

2.3. Reactor

Fig. 1 shows a schematic of the AFBR system used in this study. The main body of the reactor was an acrylic tubular section with a 5.3 cm internal diameter and a height of 190 cm. The main body was equipped with a water jacket for temperature control. The reactor has a total volume of 4192 cm³, and the height of the static bed of support material was 74 cm.

2.4. Set-up and operation conditions of AFBR for H₂ production

The AFBR was fed with a synthetic wastewater containing glucose at a concentration of 2000 mg L⁻¹ and 10% v/v of heat-treated sludge. Nitrogen gas was used to sparge the fermentation medium in order to create an anaerobic environment. The operation temperature was kept constant at 30 ± 1°C. For
the AFBR system, the total liquid flow rate (Q) was controlled at 128 L h\(^{-1}\) (bed expansion = 30%). This flow rate produced a superficial velocity 1.30 times greater than the minimum fluidization velocity. The bioreactor was initially operated in batch mode for 48 h to activate the H\(_2\)-producing sludge. After this, it was switched to a continuous mode with a designated hydraulic retention time (HRT of 8 h).

To simplify the analysis of the results, the research was divided into five experimental phases with 15–20 days long each one, corresponding to different values of HRT. When a steady-state was reached (based on constant H\(_2\) production rate with a variation within 5–10% for 5–10 days), the HRT was decreased progressively from 8 to 1 h. The reactor was operated for 90 days, without the addition of an alkalinity agent. The compositions of gas products (H\(_2\) and CO\(_2\)) and soluble metabolites (volatile fatty acids) produced during H\(_2\) fermentation were monitored as a function of time. The pH and glucose concentrations were also recorded. The effluent of the reactor went to a gas–liquid separator, where the gaseous and liquid products were collected separately. A gas meter (Type TG1; Ritter Inc., Germany) was used to measure the amount of hydrogen generated.

2.5. Chemical and microbiological analysis

Hydrogen content in the biogas was determined by gas chromatography (GC-2010, Shimadzu, Tokyo, Japan) using a thermal conductivity detector (TCD) and argon as the carrier gas. The temperatures of the injector, detector, and column were kept at 30 °C, 200 °C, and 230 °C, respectively.

Volatile fatty acid concentrations (VFA) were assessed using Gas Chromatography (GC-2010, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID) and column of 30 m, internal diameter of 0.25 mm, and film thickness of 0.25 μm [19].

Alcohol concentrations were measured by gas chromatography (GC-2010, Shimadzu, Tokyo, Japan), equipped with FID and sample introduction system to COMBI-PAL headspace (AOC 5000 model and HP-INNOWAX column of 30 m × 0.25 mm × 0.25 μm of film thickness).

Glucose concentration was determined using the GOD-PAP enzymatic method. Chemical oxygen demand (COD), volatile suspended solids (VSS) and pH values were measured according to Standard Methods [20].

Structural analysis of the biofilms was evaluated in a scanning electron microscope (Digital Scanning Microscope DSM 960, ZEISS). The bioparticle samples were gently washed with phosphate buffer solution and allowed to settle naturally. The bioparticles were then fixed with 2.5% glutaraldehyde in phosphate buffer and left for 12 h. The fixed bioparticles were dehydrated by successive passages through 50%, 70%, 80%, 90%, 95%, and 100% ethanol, then dried in a bacteriological greenhouse at 35 °C and finally covered with gold and observed by scanning electron microscopy (SEM) [21].

3. Results and discussion

3.1. Composition of soluble and gaseous products

In this work, the onset of continuous operation of AFBR was at 8 h HRT. A glucose conversion of 87.5% was obtained on the 3rd day following AFBR start-up. This suggests the efficient utilization of carbon substrate by the H\(_2\)-producing culture. It was also found that the operation of the AFBR in batch mode for 48 h was suitable for efficient biomass adhesion on expanded clay particles.

The influent glucose concentration ranged from 2065 mg L\(^{-1}\) to 2279 mg L\(^{-1}\) in the V and I phases, respectively. Effluent glucose concentrations ranged from 139.59 mg L\(^{-1}\) to 240.83 mg L\(^{-1}\). The high values of substrate conversion achieved are consistent with comparable studies for fermentative H\(_2\) production in AFBR from glucose [12,13] and sucrose [9,11].

Fig. 2 shows that biogas produced by fermentative production in the AFBR consisted of H\(_2\) and CO\(_2\), whereas methane was undetectable. Glucose conversion was, in general, near or more than 90% for HRTs ranging from 8 to 1 h. The influent pH in AFBR was 6.40 and effluent pH ranged from 3.68 to 4.05 (Fig. 2). The results obtained from this work suggest that the absence of methanogenic activity in the reactor might be a consequence of lower HRT [13,22] and pH [13]. Also, the preservation of acidogenic conditions in AFBR was favored by a high recycle flow rate (R) value (ranging from 243 to 30 when HRT decreased from 8 to 1 h). Therefore, combining heat-treatment of inoculum with a lower pH value effectively prevented the activation of methane-forming populations [23,24].

The H\(_2\) content in the biogas increased significantly from 8% to 35% as the HRT decreased from 8 to 1 h, reaching the highest level (35%) when the reactor operated with an HRT of 1 h (Fig. 2). This highest value of 35% of H\(_2\) in biogas composition was lower than the range of 40–60% in other studies with AFBR with glucose concentrations of 10,000 and 30,000 mg COD L\(^{-1}\) [13] and sucrose concentrations ranging from 5000 to 40,000 mg COD L\(^{-1}\) [11].
Acidogenesis involves butyric acid, propionic acid, and ethanol-type fermentation patterns. Butyrate-type fermentation is characterized by the production of butyrate, acetate, H₂, and CO₂. Ethanol-type fermentation is associated with ethanol, acetate, and H₂ and CO₂ production. Propionate-type fermentation, associated with the production of acetate and some valerate, is associated with low production or consumption of H₂ [12,25,26]. Fig. 3 shows that butyric acid (HBu), acetic acid (HAc), and ethanol (EtOH) were major soluble microbial products (SMP) under different HRTs. Propionic acid was not detected in all experimental phases of AFBR. The butyric acid concentration (ranging from 410 to 98 mg L⁻¹) was greater than the acetic acid concentration (ranging from 225 to 532 mg L⁻¹) and ethanol concentration (ranging from 53 to 98 mg L⁻¹).

Table 1 shows that the formation of SMP tended to increase as HRT decreased from 8 to 2 h. The SMP value decreased slightly as the HRT was decreased for 1 h (Table 1). Also, the total volatile fatty acid (TVFA) contributed to most of the SMP, which indicates that fermentative hydrogen production occurring in AFBR was substantially the result of acidogenic metabolism. Similar results were obtained in AFBR [9,11–13].

The predominant SMP was butyric acid (HBu) up to a HRT of 6 h, ranging from 37.7% to 44.9%. Starting from a HRT of 4 h acetate was produced in higher rates, ranging from 36.1% to 53.3% of the SMP (Table 1). Acetate contribution increased as HRT decreased, despite a slight deviation in butyric acid percentage during HRT of 8 and 6 h. In contrast, ethanol (EtOH) production, which is considered to be an unfavorable metabolite for H₂ production, was relatively insignificant (7.0–19.1% of SMP) and decreased at HRT of 1 and 2 h. Also, the absence of propionic acid (HPr) in the composition of soluble metabolites suggests that the activity of propionate formers was inhibited by the low pH [13]. Lin et al. [11] also reported that major soluble products were butyric acid and acetic acid, as they accounted for 62–73% and 17–23% of total SMP, respectively. They also showed that propionic acid and ethanol production were relatively insignificant (less than 19% of SMP).

However, Zhang et al. [13] observed the following products in descending order of SMP: acetate (43–46%), butyrate (20–31%), ethanol (14–21%), and hexanoate (7–10%), and an insignificant amount of propionate (0–3%). The authors observed that metabolites decreased slightly as the HRT was decreased, with the exception of butyrate, which initially increased (HRT from 4 to 1 h), but decreased with further decreases of HRT from 1 to 0.5 h.

This lack of consistency among the hydrogen studies suggests that other operating parameters, such as the dominant microbial population and the substrate, influenced the distribution of metabolites composition [13]. Grupe and Gottschalk [27] reported that decreasing the pH from 5.6 to 4.3 induced a shift from acetate and butyrate to butanol and acetone formation for cultures of Clostridium acetobutylicum fed with a glucose-based fermentation medium. In our work, which utilized AFBR with expanded clay performed in acidogenic conditions (pH near 4.0 in all experiment phases), the results showed that there was no shift in acidogenic to solventogenic metabolism.

The HAc/HBu ratio has been used as an indicator of H₂ production in acidogenesis systems [10–12,28–30]. In general, a higher HAc/HBu ratio gives a higher theoretical H₂ yield, according to the stoichiometric equations (Eqs. (1) and (2); [1]).

\[
\begin{align*}
C₆H₁₂O₆ + 2H₂O &\rightarrow 2CH₃COOH (acetic acid) + 2CO₂ + 4H₂ \\
C₈H₁₇O₆ &\rightarrow CH₃CH₂CH₂COOH (butyric acid) + 2CO₂ + 2H₂
\end{align*}
\] (1)

(2)

In this study, the HAc/HBu ratio increased from 0.80 to 1.34 when the HRT was reduced from 8 to 2 h. When the TDH was reduced to 1 h, the ratio was slightly reduced to 1.21 (Table 1). This behavior was also observed in other studies on dark fermentation [8,11,30].

3.2. Effect of HRT and OLR in the hydrogen production

Fig. 2 shows that hydrogen yield production (HY) increased from 1.41 to 2.23 mol H₂ mol⁻¹ glucose when HRT decreased from 8 to 4 h (OLR from 15.7 to 33.6 kg COD m⁻³ d⁻¹), and stabilized between 2.49–2.41 mol H₂ mol⁻¹ glucose for HRTs from 2 to 1 h (OLR from 66.5 to 116.6 kg COD m⁻³ d⁻¹). The
hydrogen production rate (HPR) significantly increased from 0.08 to 0.97 L h$^{-1}$ L$^{-1}$ when HRT decreased from 8 to 1 h (OLR from 15.7 to 116.6 kg COD m$^{-3}$ d$^{-1}$). Linear regression results show that the correlation between HPR ($y_1$) and OLR ($x_1$) can be expressed as $y_1 = 0.0084x_1 + 0.0074$ ($r^2 = 0.994$). van Ginkel et al. [31] emphasized that hydrogen yield production is essentially a function of the capacity of microorganisms to produce H$_2$ from organic material. These results suggested that changes in the metabolism of microorganisms occurred when HRT was decreased. Therefore, most of the glucose was used for producing SMP instead of bacterial growth or cell maintenance, which led to an increase in hydrogen production. This finding may be reinforced by the constant VSS value in the effluent, 100 ± 5 mg L$^{-1}$, when the TDH was reduced from 8 to 1 h. Most of the studies with AFBR observed similar behavior, with increases in HY and HPR as HRT decreased [9,11,13]. An AFBR containing alginate gel was investigated by Wu et al. [9] (the main characteristics are presented in Table 2). HRP and HY values increased when HRT was decreased from 6 to 1 h. However, when HRT changed from 2 to 1 h, the HRP and HY values decreased dramatically to 0.084 L h$^{-1}$ L$^{-1}$ and 0.126 mol H$_2$ mol$^{-1}$ sucrose, respectively. After thermal treatment, HRP and HY values reached 0.925 L h$^{-1}$ L$^{-1}$ and 2.67 mol H$_2$ mol$^{-1}$ sucrose, respectively, which indicates the prior domination of non-hydrogen producers in the culture. Lin et al. [11] operated an AFBR with silicon gel (the main characteristics are presented in Table 2) and showed that decreasing HRT or increasing sucrose concentration led to a marked increase in the HPR, but a gradual decrease in the HY. The best HPR (2.27 L h$^{-1}$ L$^{-1}$) occurred at 35,300 mg L$^{-1}$ and HRT = 2.2 h, whereas the highest HY (4.98 mol H$_2$ mol$^{-1}$ sucrose) was obtained at 35,300 mg L$^{-1}$ and HRT of 8.9 h. An AFBR containing granular activated carbon (the main characteristics are presented in Table 2) was investigated by Zhang et al. [13]. An HY of 0.94 mol H$_2$ mol$^{-1}$ glucose was found at 4 h HRT, but it stabilized at 1.12–1.19 mol H$_2$ mol$^{-1}$ glucose (average of 1.16 mol H$_2$ mol$^{-1}$ glucose) at an HRT from 0.5–2 h. The HPR increased significantly when HRT decreased and reached the maximum rate of 2.22 L h$^{-1}$ L$^{-1}$ at HRT of 0.5 h.

In this study, a high Q value (128 L h$^{-1}$) in AFB, which was needed for the fluidization of expanded clay particles, guaranteed good bed motion and complete mixing of the liquid, and avoided particle agglutination in the reactor. It was also observed that biogas easily detached immobilized bioparticles and there was a low coalescence of rising biogas bubbles. Although the high Q value may have caused abrasion due to intraparticle collisions and shear stress, particle disintegration was not remarked upon in this work.

Wu et al. [9] investigated the effect of OLRs ranging from 80 to 480 kg COD m$^{-3}$ d$^{-1}$ on HPR and HY for AFBR (the main characteristics are presented in Table 2). The highest HY value reached 2.77 mol H$_2$ mol$^{-1}$ sucrose for HRT of 2 h. At the same HRT and glucose concentration as in this study, HPR reached a highest value of 0.925 L h$^{-1}$ L$^{-1}$.

The effects of an OLR ranging from 13.5 to 436.4 kg COD m$^{-3}$ d$^{-1}$ on HPR and HY was evaluated by Lin et al. [11] (the main characteristics are presented in Table 2). Unlike HPR, the HY values did not have a universal trend against the OLR. The HY tended to increase with OLR for higher HRT (HRT of 6 and 8.9 h), but was nearly independent of OLR for lower HRT (HRT of 2.2 and 3 h). According to the authors, sucrose concentration and HRT affected the production of H$_2$.

H$_2$ production by dark fermentation in AFBR is highly dependent on the process conditions such as temperature, method of biomass immobilization, support material, type and concentration of substrate, mineral medium formulation, HRT, pH, and OLR (Table 2). The results presented were obtained under mesophilic conditions (25–40°C). Acid and heat-treatment were used as methods for increasing hydrogen production by altering the microbial communities present in the starting mixed population (sewage and swine

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**Table 1 – Production of soluble metabolites for the H$_2$ production under different operating conditions in the AFBR.**

<table>
<thead>
<tr>
<th>HRT (h)</th>
<th>EIOH/SMP (%)</th>
<th>HAc/SMP (%)</th>
<th>HBu/SMP (%)</th>
<th>HAc/HBu</th>
<th>TVFA (mg COD L$^{-1}$)</th>
<th>SMP (mg COD L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>19.1</td>
<td>36.3</td>
<td>44.6</td>
<td>0.81</td>
<td>987.9 ± 45.0</td>
<td>1115.1 ± 85.7</td>
</tr>
<tr>
<td>6</td>
<td>19.0</td>
<td>36.1</td>
<td>44.9</td>
<td>0.80</td>
<td>986.7 ± 28.1</td>
<td>1113.2 ± 43.1</td>
</tr>
<tr>
<td>4</td>
<td>13.4</td>
<td>48.9</td>
<td>37.7</td>
<td>1.30</td>
<td>1459.7 ± 98.1</td>
<td>1596.6 ± 96.3</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>53.3</td>
<td>39.7</td>
<td>1.34</td>
<td>1622.9 ± 26.4</td>
<td>1697.7 ± 56.9</td>
</tr>
<tr>
<td>1</td>
<td>7.9</td>
<td>50.5</td>
<td>41.6</td>
<td>1.21</td>
<td>1463.0 ± 99.8</td>
<td>1537.4 ± 61.6</td>
</tr>
</tbody>
</table>

HAc: acetate; HBu: butyrate; EIOH: ethanol; TVFA = HAc + HBu; SMP = TVFA + EIOH; TVFA and SMP were based in COD; EIOH/SMP, molar ethanol to SMP ratio; HAc/SMP ratio, molar acetate to SMP ratio; HBu/SMP ratio, molar butyrate to SMP ratio; HAc/HBu ratio, molar acetate to butyrate ratio.
slaughterhouse sludge). Entrapping (alginate gel and silicon gel) and attaching methods (celite and activated carbon) were used to immobilize biomass. Sucrose (5–40 g COD L\(^{-1}\)) and glucose (2–120 g L\(^{-1}\)) were studied as carbon substrates for H\(_2\) production. The AFBR operated with HRTs ranging from 8.9 to 30 h and a pH between 3.7 and 6.8 with highest HY values of 1.19–2.49 mol H\(_2\) mol\(^{-1}\) glucose. The AFBR operated with HRTs ranging from 0.5 to 8.9 h. These results indicated that expanded clay seems to be successful and feasible for continuous fermentation of H\(_2\) production in AFBR.

### Table 2 – Comparative study of operating conditions and efficiency of hydrogen fermentative production in anaerobic packed bed and anaerobic fluidized bed reactors.

<table>
<thead>
<tr>
<th>Reactor, temperature</th>
<th>Microorganism, pre-treatment</th>
<th>Biomass immobilization, support material</th>
<th>Substrate, Concentration</th>
<th>HRT, pH</th>
<th>HY, ORL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APBR (vertical flow)</td>
<td>Sewage sludge</td>
<td>Attachment</td>
<td>Sucrose 20 g COD L(^{-1})</td>
<td>5.0–8.5 h</td>
<td>0.08–1.14 mol H(_2) mol(^{-1}) sucrose(^a)</td>
<td>Chang et al. [8]</td>
</tr>
<tr>
<td>35 °C APBR (horizontal flow)</td>
<td>Acid</td>
<td>Natural fermentation</td>
<td>Glucose 0.5 mol L(^{-1})</td>
<td>6.7</td>
<td>96–960 kg COD m(^{-3}) d(^{-1})</td>
<td>Leite et al. [16]</td>
</tr>
<tr>
<td>30 °C AFBR</td>
<td>None</td>
<td>Sewage sludge</td>
<td>Glucose 2 g L(^{-1})</td>
<td>3.9–7.3 h</td>
<td>96 kg COD m(^{-3}) d(^{-1})</td>
<td>Wu et al. [9]</td>
</tr>
<tr>
<td>35 °C AFBR (draft tube)</td>
<td>Acid and heated 70 °C – 30 min</td>
<td>Alginate gel</td>
<td>Sucrose 20 g COD L(^{-1})</td>
<td>5.8–6.8</td>
<td>80–480 kg COD m(^{-3}) d(^{-1})</td>
<td></td>
</tr>
<tr>
<td>40 °C AFBR</td>
<td>Heated 100 °C – 1 h</td>
<td>Silicon gel</td>
<td>Glucose 5–40 g COD L(^{-1})</td>
<td>2.2–8.9 h</td>
<td>1.83–4.98 mol H(_2) mol(^{-1}) sucrose</td>
<td>Lin et al. [11]</td>
</tr>
<tr>
<td>35 °C AFBR</td>
<td>Acid</td>
<td>Sewage sludge</td>
<td>Celite R-633 5 or 10 g L(^{-1})</td>
<td>1.4–5.6 h</td>
<td>13.5–436.4 kg COD m(^{-3}) d(^{-1})</td>
<td>Koskinen et al. [12]</td>
</tr>
<tr>
<td>37 °C AFBR</td>
<td>Heated 105 °C – 45 min</td>
<td>Activated carbon</td>
<td>Sucrose 10 g L(^{-1})</td>
<td>4.0</td>
<td>21.4–85.7 kg COD m(^{-3}) d(^{-1})</td>
<td>Zhang et al. [13]</td>
</tr>
<tr>
<td>30 °C AFBR</td>
<td>Heated 90 °C – 10 min</td>
<td>Expanded clay</td>
<td>Sucrose 2 g L(^{-1})</td>
<td>3.7–4.1</td>
<td>19.7–116.6 kg COD m(^{-3}) d(^{-1})</td>
<td>This study</td>
</tr>
</tbody>
</table>

\(a\) Based on the article data.

Table 2 also includes the operation conditions for H\(_2\) production in expanded clay APBR. Leite et al. [16] obtained an HY value of 2.48 mol H\(_2\) mol\(^{-1}\) glucose with an HRT = 0.5 h and pH 3.9–4.0. In this work, the AFBR was fed with the same glucose-based synthetic wastewater used by Leite et al. [16] and HY values around 2.41–2.49 mol H\(_2\) mol\(^{-1}\) glucose with an HRT from 1 to 2 h. These results indicated that expanded clay seems to be successful and feasible for continuous fermentative H\(_2\) production in AFBR.

### 3.3. Microbiological analysis

In mesophilic processes using microbial communities, optimal H\(_2\) fermentation performance has often been achieved with a high HAc/HBu ratio [12,17,26,30,32]. This was also the case in this work, since the highest H\(_2\) production was observed with a high HAc/HBu ratio (HRT = 2 h). The high hydrogen yield production may be attributed to a change in the metabolite flow deriving from the fermentation of butyric acid into acetic acid. This finding may be attested by the HAc/ HBu ratio, which ranged from 0.80 to 1.34 when the TDH was reduced from 8 to 1 h. During this TDH transition more electrons were diverged to produce HAc and hydrogen than to produce HBu and hydrogen. SEM micrographs of bioparticles show that cells, mainly in rod-like and spherical shapes, and some endospores, covered the surface and cavities of expanded clay, which indicates high biomass retention in AFBR (Fig. 5). During all operation phases, the butyrate (major metabolite) and acetate production indicate that heat-treatment was effective in enriching the inoculum with acidogenic bacteria that produce H\(_2\), since they had metabolism similar to the species *Clostridium* sp. and *Bacillus* sp. [24,33].

![Fig. 5 – SEM micrography of biofilm attached on expanded clay (increase: 3000×).](image)
4. Conclusions

The anaerobic fluidized bed reactor using expanded clay proved to be efficient for hydrogen production. The expanded clay has a good grip on the biomass particles, especially when operating with a low hydraulic retention time. The density of expanded clay next to the water facilitates the movement of the bed, and is a low cost material.

The hydrogen content ranged between 8 and 35% with the remainder of the biogas being comprised of CO₂. The presence of methane gas was not detected in any phase of the study. The hydrogen content increased significantly as HRT decreased. The acetate and butyrate were dominant as the soluble metabolite, which indicates that the microbial community in this AFBR system was efficient in H₂ production. The hydrogen yield production and hydrogen production rate were linearly correlated with the effect of HRT, with a hydrogen yield production of 0.97 L h⁻¹ L⁻¹, when the HRT was 1 h and 2.49 mol H₂ mol⁻¹ glucose when the HRT was 2 h. The microscopic analyses showed that the cells were mainly rod-like, spherical shapes, with some endospores. This shows that the species producing the hydrogen gas are similar to Clostridium sp and Bacillus sp.

The development of anaerobic biofilm is a complex process that depends on several factors. Many complex interactions occur between the surface of the medium, the microorganisms, and the substrate, and all of them influence the microbiological retention.

These results indicate that the expanded clay used in AFBR is a promising support material. It outperformed the majority of systems in the production of hydrogen that have been reported so far.

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