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Red mud-based inorganic polymer spheres: innovative and environmentally friendly anaerobic digestion enhancers

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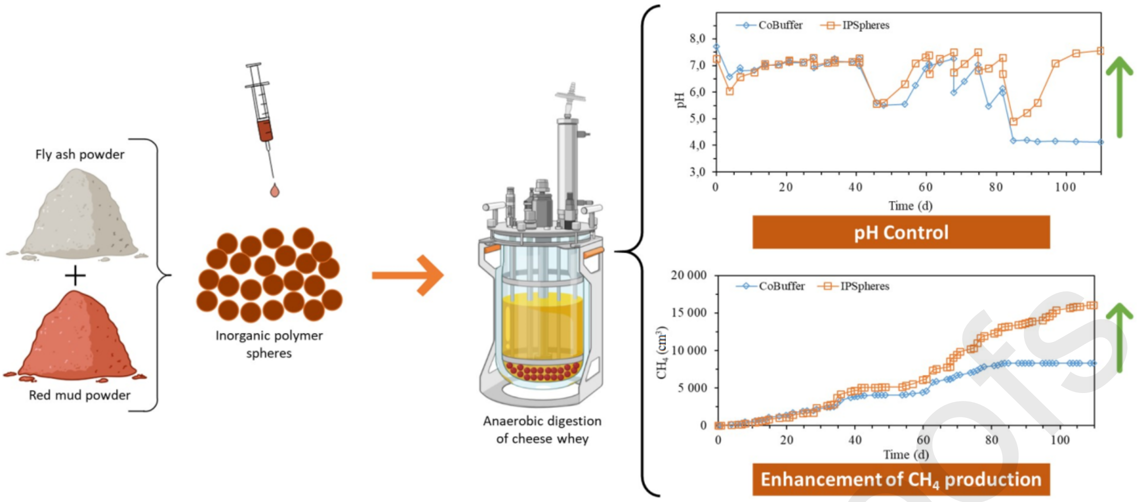
Conflicts of interest

There are no conflicts of interest to declare.

Graphical Abstract



Abstract

Red mud-based inorganic polymer spheres were used as alternative pH regulators and process enhancers in sequencing batch anaerobic reactors treating cheese whey. This byproduct tends to quickly acidify under anaerobic conditions, and the common route to control pH and ensure suitable conditions for methane production involves the use of commercial alkaline raw materials. The spheres were synthesized using significant amounts of hazardous and toxic waste, red mud (50wt.% of solid components), whose recycling is challenging. The inorganic polymeric spheres, when compared to virgin alkaline raw materials, improved organic matter removal by 44%, prevented VFA accumulation (acidification degree <20%), maintained pH values in a range (6.5–7.2) to ensure maximum methanogenic activity by archaea microorganisms, and boosted the methane volume by ~90%. These promising results demonstrate the feasibility and performance advantages of using these innovative spheres instead of virgin raw materials, which is an important tool towards sustainable development.

Keywords:

Anaerobic Digestion; Red Mud; Methane; Process improvement

1. Introduction

Anaerobic digestion (AD) is a powerful technology for converting organic waste into renewable energy and, as a result, has received increasing attention in recent years (Li et al., 2019), as it is expected to play a major role in the transition towards a circular economy. AD can be used as a substitute for fossil fuels regarding the generation of heat or electricity (Wu et al., 2019). Although is a well-known and widely applied process, some factors can hinder the efficiency of the AD process. According to Wu *et al.* (2019), the main causes of process instability include organic overload, ammonia inhibition, excessive acidification and long chain fatty acids inhibition. An increase in the reactor organic loading rate (OLR) significantly influences the structure and behavior of microbial consortia (Sun et al., 2017). Loading high amounts of organic material into the biological system at once can result in shock, due to the increased activity of hydrolyzing and acidogenic bacteria compared to methanogens (Neshat et al., 2017). Consequently, as the OLR increases, the risk of inhibition due to excessive production and accumulation of volatile fatty acids (VFA) also increases (Nghiem et al., 2017).

Another dominant factor affecting the VFA profile and, therefore, AD performance is pH (Lagoa-Costa et al., 2020). The pH level controls microbial activity and, as a result, organic biodegradation and biogas production (Mao et al., 2017). Strict pH control is crucial, especially when dealing with substrates susceptible to organic overload due to the rapid accumulation of VFA, such as cheese whey (Treu et al., 2019). The most common approach to buffering the pH of AD systems is the addition of commercial alkaline powders, such as sodium hydroxide or calcium carbonate. Nevertheless, this strategy can only ensure pH stabilization in the short-term and, in addition, chemical additives cannot be recovered after the AD process.

The possibility of using inorganic polymers as pH regulators was recently highlighted in a review paper by Novais et al. (2020), focusing on the most promising applications envisioned for inorganic polymeric foams. This review showed that most studies, regardless of the shape and size of the inorganic polymer, were tested in distilled water, while their performance under more realistic conditions was neglected. There are only two exceptions in which inorganic polymers have been evaluated in AD systems: i) Rugele *et al.* (2015) carried out short-term AD tests (25 days), using 2-8 mm granules; and ii) Novais et al. (2018b), using fly ash-based spheres (d = 3 mm) to control the pH inside the reactors for up to 70 days avoiding the need for continuous pH adjustment. These studies corroborated the potential of these materials to act as pH regulators under realistic conditions. However, the possibility of using 100% inorganic polymers based on waste remains unexplored and will be considered here.

Red mud, also known as bauxite residue, is a toxic waste produced from the alumina refining of the bauxite ore by the Bayer process (Khairul et al., 2019). One of the main challenges associated with recycling is the pH value, usually ranging between 9.2 and 12.8, due to the addition of sodium hydroxide during the refining process (Chen et al., 2019). This waste is disposed mainly in large lagoons in the form of slurry or in rejection dams as dry red mud, which can severely affect the environment (Khairul et al., 2019; Novais et al., 2018a). This is a critical concern, especially considering that 1 to 1.5 tonnes of red mud are generated for each ton of alumina produced and the world red mud reserve is greater than 2.7 billion tonnes (Khairul et al., 2019). Red mud is an ideal precursor to produce inorganic polymers with a low carbon footprint, since its intrinsic alkalinity makes it difficult to incorporation into Portland cement mixtures, thus making alkali-activation advantageous (Novais et al., 2019).

Recently, Novais *et al.* (2018a) reported the synthesis of red mud-based inorganic polymeric spheres and evaluated their ability to regulate pH by immersing the specimens for 28 days in an aqueous medium. So far, red mud-based inorganic polymers have never been used as pH regulators in anaerobic processes, and this possibility will be evaluated in this study. The use of red mud per se (not-activated) in AD is also uncommon, and only three investigations have evaluated its role in methanogenesis (Ye et al., 2018c, 2018b, 2018a). These studies showed that red mud enhanced methanogenesis due to the high content of alkaline compounds in the waste, inducing a high level of alkalinity, promoting the efficiency of hydrolysis-acidification and resulting in an increase in the activities of substrates and enzymes (Ye et al., 2018c, 2018b). Ye *et al.* (2018c) described that red mud increased methane production by 35.6 % compared to a control reactor when 2.0 g of red mud was added to anaerobic reactors with 10 mL of sludge inoculum and 90 mL of substrate, for 28 days. These are interesting findings, but the red mud cannot be recovered after AD. The strategy proposed in this study uses red mud as a precursor to synthesize mm-size spheres that can be used as AD enhancers, regulating the pH level and improving methane production. Then, after exhaustion, the spheres can be collected and reused as light aggregates in the production of new mortars, preventing the production of waste and closing the materials cycle.

The main objective of this work was to evaluate the use of inorganic polymer spheres based on red mud of the size of mm as pH regulators and AD enhancers during the operation of anaerobic sequential batch reactors (AnSBR), and this has never been studied. This study was performed using much more demanding and unfavorable conditions (e.g. eleven consecutive substrate additions of cheese whey at high organic loads and 110 days of incubation) in comparison with those used in previous studies (e.g. single substrate addition and batch operation for 70 days) (Novais et al., 2018b). In addition, in the present study, the volume of the reactor is five times larger (5 L instead of 1 L), which is expected to provide valuable insights regarding the scale-up of the process and the perspectives for implementation on a pilot or full-scale. Moreover, and for the first time, the solid precursors used for producing the spheres were 100 % waste-based and a much smaller amount of activating solution was used in their synthesis (38.3 wt.% instead of 56.0 wt.%), being a much more eco-friendly strategy.

This research promotes the valorization of waste, both by biological processes and by the incorporation of complex wastes in new products, contributing towards a circular economy, sustainable use of resources and reduction of the use of virgin raw materials in well-established processes, such as AD.

2. Materials and Methods

2.1. Materials

Due to its high acidogenic potential, cheese whey powder, an industrial by-product of a medium-scale cheese factory, was selected as a carbon source for AD experiments. A concentrated solution was prepared, with sCOD = 106.8 ± 0.62 gO2/L and pH = 5.7, and the volume of substrate to be added to the reactors estimated in accordance with the organic loading rate defined for each cycle. The mixed anaerobic microbial culture used as an inoculum was collected from the mesophilic digester at a local wastewater treatment plant. Prior to inoculation, biomass was acclimatized at 37 ± 1 °C for approximately 48 h. The volume of inoculum added at the beginning of the experiments was calculated to reach the initial concentration of volatile suspended solids (VSS) of 4

± 0.2 gVSS/L in the reactors. These initial conditions were selected to ensure that the assays started with a lower ratio of food to microbes (F/M), and then the organic load during the experiment was increased progressively to promote an organic load shock and, thus, evaluate better the pH buffering capacity of the red mud-based spheres under these adverse conditions.

The red mud was supplied by a bauxite mining company and used as a source of alumina, while biomass fly ash waste from a Portuguese pulp and paper industry was used as a source of silica. Prior to their alkaline activation, the red mud was dried, milled and sieved to reach a particle size below 75 μm, while fly ash was simply sieved to attain sizes below 63 μm. These solid precursors were activated using commercial sodium silicate (silica modulus = 3.09; Chem-Lab, Belgium) and sodium hydroxide (ACS reagent, 97%; Sigma Aldrich). The foaming agent (sodium dodecyl sulfate) and the consolidation medium (polyethylene glycol 600) were supplied by Sigma Aldrich.

2.2. Synthesis of the red mud-based inorganic polymer spheres

The spheres were synthesized following a previously reported method, in which the inorganic polymer slurry is injected in a hot bath to ensure fast consolidation (Novais et al., 2017). The mixture composition used to produce the red mud spheres was selected from previous work by Novais *et al.* (2018a), considering the spheres performance in aqueous medium. Briefly, a mixture of red mud and biomass fly ash (10 g each) was alkali-activated using 15 g of activator, and 4.15 g of water. The mixture between the solid components and the activator was performed in a planetary mixer for 2 min. Then, a foaming agent (0.30 g) was added to the slurry and mixed for 5 min. The foamed paste was then injected in a polyethylene glycol medium to produce the porous spheres. The spheres were collected and cured for 24 h at 40 ºC (inside a plastic container to prevent fast dehydration), and then cured in open conditions at ambient temperature until the 14th day.

2.3. Anaerobic digestion experiments

2.3.1. Reactor set-up

The assays were performed in 5 L AnSBR with periodic stirring, incubated in a thermostatic bath to maintain the temperature in mesophilic conditions (37 ± 1 °C), and the volume of biogas produced was measured using a water displacement system. Inorganic nutrients were added, as described by Van Lier *et al.* (1997), to suppress microorganisms needs, since trace elements are of vital importance as a support of enzymatic activities and chemical reactions (Mao et al., 2015), and promote their growth (Tabatabaei et al., 2018). Stock solutions with macronutrients (g/L): NH_4Cl (165), KH_2PO_4 (36.2), $CaCl_2.2H_2O$ (7.79), $MgSO_4.7H_2O$ (8.79), with micronutrients (g/L): $FeCl_2.6H_2O$ (0.98), $CoCl_2.6H_2O$ (0.98), $MnCl_2.4H_2O$ (0.24), $CuCl_2.2H_2O$ (0.02), $ZnCl_2$ (0.02), H_3BO_3 (0.03), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.04), $Na_2SeO_3.5H_2O$ (0.05), $NiCl_2.6H_2O$ (0.02) and EDTA (0.49) were prepared. For each reactor, 5 mL of each solution were added to provide mineral media (Silva et al., 2013). Prior to sealing, the reactors were purged with nitrogen gas to remove any residual O_2.

2.3.2. Experimental conditions

To evaluate the potential of red mud-based inorganic polymeric spheres as a pH buffering material in AD systems, preliminary tests (not shown for the sake of brevity) addressing the influence of the amount of spheres on the pH were carried out, using 200 mL glass vessels for 35 days. These tests showed that the use of dosages higher than 15 g/L of the spheres resulted in pH values above the range that favors methanogenesis

(6.5 – 7.2) (Zhang et al., 2014). For this reason, the spheres amount was limited to 15 g/L. The amount of commercial alkalinity was selected considering also preliminary tests, which show near neutral pH after 7 days of incubation, with soluble chemical oxygen demand (sCOD) removals higher than 85 % and methane content in biogas around 60 % after 30 days of incubation.

Three different assays (5 L working capacity) were studied during 110 days: i) a control reactor (*blank*) – without the addition of commercial alkaline reagents or inorganic polymer spheres; ii) a reactor containing a commercial buffer, hereinafter coded as *CoBuffer*, prepared using $NaHCO_3$ and $KHCO_3$ to achieve 2 g/L of initial alkalinity measured as $CaCO_3$; iii) a reactor containing the red mud-based inorganic polymer spheres (15 g/L), coded as *IPSpheres*.

During the experiments, biological systems were intentionally disturbed by the multiple additions (11) of the substrate, with increasing organic loads imposed. Substrate additions were made every week of incubation, except for the 7th addition made after 20 days, due to the longer time needed to consume the substrate. In the *IPSpheres* reactor, the first eight substrate additions were performed when the organic matter content of the reactor, expressed here by sCOD, was close to 2 gO_2/L, and in the last three substrate additions, the remaining sCOD from previous AD cycles presented values around 3 – 4 gO_2/L.

In the first four AD cycles, 4 gO_2/L were added to the reactors. In the following three cycles (5th, 6th and 7th), the OLR increased to 8 gO_2/L, but in this last cycle (7th) the consumption of organic matter in the reactor took longer and, therefore, the organic load added in the subsequent cycle (8th) was again 4 gO_2/L. In the 9th, 10th and 11th AD cycles, the OLR was successively increased to 10, 14 and 20 gO_2/L, respectively. This escalation in the OLR was intended to assess the performance of inorganic polymer spheres under stress conditions and compare it with the reactor with commercial reference buffer solutions.

2.4. Characterization of materials and physical-chemical analysis

The morphology of the inorganic polymer spheres was evaluated by optical microscopy (Leica EZ4HD microscope), while their microstructure, before and after AD, assessed by scanning electron microscopy (SEM; Hitachi S4100 coupled with energy dispersion spectroscopy (EDS; Rontec). After AD, the spheres (110 incubation days) were collected by settling and washed with water to remove residual biomass, prior to their microstructural characterization.

During the incubation of the AnSBR reactors, samples with a maximum of 10 mL of volume were collected every 2 to 3 days to analyze the reactor's performance. pH, organic matter (total and soluble COD), total and volatile suspended solids were performed in accordance with Standard Methods (APHA, 2012) and the equipment used was described by Gameiro *et al.* (2016). The biogas composition and the quantification of VFA were carried out by gas chromatography, as detailed by Gameiro *et al.* (2016). As described by Gameiro et al. (2016), oxidation stoichiometry values for each VFA specie analyzed (1.067 $mgO2/mg_{\text{acetic acid}}$, 1.514 $mgO2/mg_{\text{propionic acid}}$, 1.818 $mgO2/mg_{\text{butyric acid}}$, 2.039 $mgO2/mg_{\text{valeric acid}}$, and 2.207 $mgO2/mg_{\text{caproic acid}}$) were used to express the individual VFA concentration as COD equivalent.

3. Results and discussion

3.1. Red mud-based inorganic polymer spheres characterization before and after AD

Using optical micrographs of the red mud-based inorganic polymer spheres after synthesis the particles' size distribution was measured, being d = 2.36 ± 0.10 mm. SEM micrographs reveal the presence of a significant amount of pores on the sphere's surface, which should allow the diffusion of alkali from inside and, as a result, regulates the pH in the AD reactor. The EDS spectrum shows that iron, silicon and calcium are the most abundant elements in the spheres, while other elements such as aluminum, titanium, sodium, and potassium are presents in smaller quantities. These elements are aligned with the chemical composition of the precursors (red mud and fly ash) deeply discussed in the work of Novais et al. (2018a). After AD assays, the spheres keep their integrity, despite the long immersion period, and this suggests that strong geopolymerisation has occurred. This feature is very relevant, as it can allow the reuse of the spheres after the AD in another application, such as heavy metal adsorbent. It is not surprising that the sodium content in the samples sharply decreases during AD experiments, which explains their high pH regulation capacity (discussed in detail in the following sections). Interestingly, after AD, the samples show the presence of significant amounts of phosphorus on their surface and smaller amounts in their internal structure (results not shown for the sake of brevity), absent in the spheres chemical composition before being used in AD, and this suggests that the spheres may be acting as a support medium for bacteria. This topic will be addressed in future work.

3.2. Influence of the alkaline source on the performance of anaerobic digestion

3.2.1 pH evolution

Figure 1 shows the evolution of pH over time during 110 days incubation period for all reactors: *Blank* (without the addition of alkalinity or spheres); *CoBuffer* (containing 2 g/L of alkalinity measured as $CaCO_3$); and *IPSpheres* (containing 15 g/L of red mud inorganic polymer spheres). The vertical dashed lines in Figure 1 identify the various substrate additions made during the AnSBR assays. The concentration of substrate (represented as S_0 and expressed in gO_2/L) added in each of the AD cycles, was also included in the figure. In addition, the most favorable methanogenic pH range, between 6.5 and 7.2, suggested by Zhang *et al.* (2014), was highlighted in the figure by a grey bar.

In the blank assay, after the 1st substrate addition (4 gO_2/L), the pH decreased to 4.66 after 3 days, and then remained below this value until the end of the experiment. At the end of the experiment, after eleven substrate additions, the pH was 3.49, indicating system failure due to low pH values, with inhibition of the entire microbial community (Kim et al., 2002). Similar behavior was observed by Novais *et al.* (2018) in the AD of cheese whey (8 gO_2/L). The authors reported that, without pH regulation, the pH inside the reactor was less than 4.38 during the 70 days of incubation (Novais et al., 2018b).

In the other reactors (*CoBuffer* and *IPSpheres*), the pH also dropped after the first day of incubation due to the rapid degradation of the carbon source, reaching pH values of 6.57 and 6.05, respectively, on the 3rd day. Figure 1 also shows that in these reactors, following a new substrate addition, the pH immediately decreased, but a rapid recovery was observed. This is particularly evident when the organic load increased from 4 to 8 gO_2/L, in the 5th AD cycle and in the following cycles. Interestingly, the pH fluctuation within these reactors remains quite similar for the first 41 days (6 AD cycles), showing for the first time that red mud spheres can be effective pH regulators in AD systems. A much more pronounced decline in pH was observed after the 7th substrate addition, with the pH in the reactors decreasing to values close to 5.5. Contrary to what was observed in the first six AD cycles, in the 7th AD cycle, the reactors took more than a week in this situation, the duration of which depends on the reactor content to recover from the low pH values (below 6.0) caused by the accumulation of intermediate compounds (VFA). This feature can be explained by the fact that the production of intermediates by acidogenic and acetogenic bacteria is faster than their consumption by the methanogenic archaea community (Franke-Whittle et al., 2014). The reactor containing the red mud spheres recovered more quickly than the one containing the commercial buffer solution. In the first, the pH level inside the reactor started to increase 7 days after the beginning of the cycle (day 48), reaching neutral values after 16 days (day 57); while in the second, a delay was observed, in which the pH increase started after the 13th day and the neutral pH was reached after 20 days. The different behavior of these two reactors in the 7th cycle was more noticeable and intensified in the subsequent AD cycles.

After the 7th AD cycle, the reactor containing the commercial buffer solution (*CoBuffer*) showed a more unstable operation in terms of pH fluctuations, with a tendency to reach lower pH values in subsequent incubation periods and substrate additions. This behavior is characteristic of a system with a lack or insufficient buffer capacity, reacting with a sharp drop in pH when the organic load is added to the anaerobic system (Park et al., 2018). During the 11th AD cycle, this reactor was unable to recover from the low pH values induced by the addition of a high substrate load (20 gO_2/L) on day 82 of incubation. In this last AD cycle, the pH dropped to 4.18 and the reactor did not recover, remaining at acidic pH values until the end of the experiment. This shows that the chemical alkalinity added at the beginning of the experiment was not sufficient to provide adequate and prolonged buffering capacity for the high loads applied to the anaerobic system treating cheese whey. Contrary to what was observed in this work for high organic loads, some studies have suggested that an alkalinity of approximately 2 g/L as $CaCO_3$ is adequate to neutralize the VFA formed at low organic loading rates (Fernández et al., 2015), comprehended between 0.4 and 2.0 $gO_2/L/d$.

It is notable that, in the reactor containing the waste-based spheres, the system quickly recovered from the pH decline at the beginning of the 8th, 9th, and 10th AD cycles, remaining slightly above the optimum pH range for methanogenic community at the end of each cycle. Despite the sharp drop in pH to 4.9 observed after the 11th substrate addition (the highest load), the reactor still managed to recover from the organic loading shock after ~ 7 days, reaching values close to neutrality after 15 days (97th day), differing from the behavior observed in the reactor containing the commercial buffer solution. Interestingly, in the 11th cycle, the system took exactly the same time (7 days) to recover from the instability caused by the organic loading shock, as seen in the 7th AD cycle. This result suggests that the slow but prolonged OH^- leaching from the porous red mud-based inorganic polymer spheres is a very efficient approach to regulate the pH in AD systems, even under demanding conditions of high organic loads in the medium. This contrasts with what was observed when using commercial chemical compounds, which were unable to control the pH under high, consecutive OLRs.

3.2.2 VFA accumulation

The AD process is very sensitive to VFA accumulation in the liquid medium, since the degradation rate of VFA generated by methanogenic archaea is lower than its production by acidogenic bacteria (Prazeres et al., 2012). The values for sCOD and VFA concentrations, expressed in COD equivalents, measured in the different reactors throughout the experiments, are depicted in Figure 2. It should be highlighted that only the most abundant species in the VFA mixture were included, namely acetic, propionic, and n-butyric acids. Other VFA species (iso-butyric, iso-valeric, n-valeric and n-caproic acids) were also determined individually but the concentrations are (mainly) lower than 1 gO_2/L and, for this reason, were indicated in the Figure 2 as “others”. The low pH values observed after each substrate addition (see Figure 1) are closely related to the conversion of organic matter into intermediates in the anaerobic process, such as VFA. These intermediates, in well-buffered systems, act as substrates for methanogenic microorganisms, to convert them into methane-rich biogas (Franke-Whittle et al., 2014). In non-buffered systems, such as the blank assay, microorganisms are unable to convert VFA present in the medium into methane, inhibiting the activity of acidogenic and methanogenic microorganisms (Wang et al., 2012) and, as a result, the pH remains at acidic range. In fact, acidogenic microorganisms are more tolerant to low pH values than methanogenic microorganisms, and can tolerate mildly acidic conditions, being the optimal pH range for their growth and activity, generally considered between 4.0 and 6.5 (Speece, 1996).

In the 1st AD cycle, the total VFA concentration was similar in all studied reactors. However, in subsequent cycles, VFA accumulates continuously in the blank reactor, increasing with the addition of substrate, until the 6th cycle. After the 7th AD cycle, the pH dropped below 4.0 and the VFA concentration remained almost constant (≈ 7 gO_2/L), suggesting complete inhibition of acidogenic microorganisms by product accumulation. With the following substrate additions, the sCOD in the liquid fraction increased, reaching near 60 gO_2/L at the end of the experiment.

Different behavior was observed in the reactors containing a commercial buffer solution or spheres. In these systems, the addition of substrate until the 7th AD cycle had a positive effect on the microorganism's activity. The high sCOD concentration observed immediately after cheese whey addition, subsequently decreased during each AD cycle, reaching values below 1.5 gO_2/L, and the total VFA concentration was less than 1 gO_2/L at the end of all AD cycles. These features demonstrate that within these reactors, stable conditions were achieved in each AD cycle, enabling the success of the anaerobic treatment of cheese whey, with low concentration of VFA (intermediates) and organic matter, which were converted into methane (final product). Treu *et al.* (2019) explained that when a high amount of organic carbon is fed into a reactor, the VFA concentration increases rapidly. As a result, fermentative microorganisms can take over the AD microbiome and methanogenic archaea become severely stressed by the accumulation of increasing amounts of process intermediates. This was particularly observed in the 7th AD cycle, when an accumulation of organic matter (more than 7 gO_2/L during 13 days) was observed in both reactors (*CoBuffer* and *IPSpheres*), with a remarkable increase in total VFA concentrations to values close to 6 gO_2/L. The acidic conditions in the reactors led to the accumulation of n-butyric acid, the most abundant VFA specie in this 7th cycle (≈ 3 gO_2/L). However, n-butyric acid was consumed mainly as soon as the organic matter started to be removed. Propionic acid was also formed in lower amounts (≈ 0.6 gO_2/L) during the 7th AD cycle, but, in contrast to n-butyric acid, methanogenic microorganisms did not consume this specie at the same rate to form methane.

After the 7th cycle, the reactors containing the commercial buffer or the spheres began to behave differently regarding the VFA fluctuation, both in total and individual quantities, during the remaining incubation period. In the reactor containing the commercial buffer after the 75th day, the VFA concentration in the medium increases continually, suggesting a decrease in the buffer capacity of the anaerobic system. After the additions of high organic loads in cycles 10 (14 gO_2/L) and 11 (20 gO_2/L), the microorganisms in the reactor with the commercial buffer stopped to consume n-butyric acid, which accumulated in the liquid fraction (4 – 5 gO_2/L). In addition, a very high propionic concentration (3 – 4 gO_2/L) was also achieved, resulting in the acidification of the reactor. With the increase in VFA concentration, microorganisms were unable to remove the dissolved organic matter, leading to its accumulation in the medium, reaching ~ 28 gO_2/L at the end of the experiment, with VFA representing 50 % (13.9 gO_2/L).

Unlike the reactor with a commercial buffer solution, in the reactor with red mud spheres, n-butyric acid continued to be consumed, even with additions of high substrate concentration (10th and 11th cycles). However, in the 11th AD cycle, the concentration of propionic acid increased in the system, reaching 3.5 gO_2/L at the end of the experiment, accounting for more than 50 % of the sCOD and more than 80 % of the total VFA mixture. The reactor containing the red mud spheres showed superior buffer capacity in comparison with the use of the commercial buffer solution. In fact, even in the most stressful OLR (11th AD cycle), the system was able to recover from the pH drop after 15 days. As a result, organic matter was mostly consumed and the VFA accumulation in the medium decreased significantly from 14.6 gO_2/L at day 92 to 4.2 gO_2/L at the end of the experiment (day 110). This behavior can be explained by the controlled release of alkalis from the porous red mud-based inorganic polymer spheres that effectively buffer the pH in anaerobic systems during long operation cycles and under organic loading shocks and substrate overload.

3.2.3 Methane production

Figure 3 shows the methane production, that is, the biogas composition (expressed as the ratio between CH_4 and CO_2) and the amount of methane volume accumulated over time observed in all reactors. In this figure, a zero CH_4/CO_2 ratio means that no methane has been formed, while higher ratios correspond to a higher methane content in biogas. As expected from the pH level observed in the blank assay (see the discussion in *section 3.2.1*), methane was detected only in small amounts in the first AD cycle, with a maximum methane content in the biogas of 25 %. This very low amount of methane may be associated with the intrinsic activity of the anaerobic biomass prior to its use to inoculate the AnSBR, not resulting from the degradation of the organic matter present in the cheese whey, as the acidic conditions in the medium do not favor methanogenesis. As a result, during the entire experiment, the blank reactor accumulated only 66 cm^3 of methane.

The results observed for the other two reactors suggest that successive additions of substrate led to instability in the biogas composition, reducing the contribution of methane in the gas phase at the higher organic loads applied. However, an increasing trend in the contribution of methane is observed after each cycle in the reactors containing the commercial buffer or the waste-based spheres, especially at the first six cycles. Immediately after the 1st day of incubation, methane was produced in both reactors, which showed a similar behavior and methane accumulation volume (≈ 2700 cm^3) until the end of the 5th AD cycle. After that, methane production started to differ, with higher values observed in the reactor containing the red mud spheres. In fact, after the 6th substrate addition, the methane volume was already 21 % higher than that measured in the reactor containing the commercial pH buffer. In the 7th AD cycle, methane production in both reactors dropped in comparison with that produced in previous AD cycles, and this may be related to the organic matter accumulation (see discussion in *section 3.2.2*). According to Lerm *et al.* (2012), an organic overload results in a subsequent collapse of gas production that requires long recovery periods, as observed in the 7th AD cycle (20 days instead of 7), and also in the last cycle (29 days). This behavior was also reported by Sun *et al.* (2017), who stated that when an increase in the OLR is made beyond a specific range supported by microorganisms, the biogas yield decreases and this can inhibit the anaerobic system.

The difference between methane yields was further intensified from the 7th AD cycle until the end of the experiment, reaching about 16 000 cm^3 of methane on the 110th day, which corresponds to 40.9 gO_2 (expressed as COD equivalent) in the reactor containing the red-mud spheres. This value almost doubles that produced in the reactor containing the commercial buffer, which reached 8 300 cm^3 of methane (21.1 gO_2, as COD equivalent). Future work should be carried out using multivariate statistical techniques to validate these findings and provide additional information on the influence of inorganic polymer spheres on methane yield.

An increase in methane yield when adding red mud to anaerobic sludge digesters has been previously reported by Ye *et al.* (2018c). In their study, powdered red mud enhanced methane accumulation by 35.5 % in comparison with the blank reactor (non-containing red mud). However, in the present study, a much higher methane yield (93.6 %) was observed in comparison with the reactor containing the commercial buffer. These very promising results demonstrate the potential of these innovative and waste-based materials to act as pH regulators and AD enhancers.

3.2.4 Anaerobic digestion balances

Figure 4 shows the COD balances at the end of each AD cycle for the reactors studied, considering the acidified (expressed as gO_2) and non-acidified sCOD fractions, the gas (methane) formed (expressed as gO_2) and the removed sCOD, channeled for microbial growth and maintenance.

It can be observed that the blank reactor did not show methanogenic or acidogenic behavior, since no methane was formed and the acidified sCOD fraction did not exceed 40 % until the 6th AD cycle, decreasing even further to values below 20 % after the 7th AD cycle and until the end of the experiment. This COD profile demonstrates that the blank reactor, without pH regulation, was inhibited by the accumulation of substrate. Thus, under these conditions, it was not possible to recover energy in the form of methane, but only materials (VFA), in about 20 to 50%.

The reactors containing the commercial buffer or the spheres presented a similar COD balance until the end of the 5th AD cycle. In the 6th AD cycle, with the increase of organic matter added to the reactors, the performance of the reactor with commercial buffer decreased, with an increase in VFA concentration and a decrease in methane production. On the other hand, in the reactor with red mud spheres, the amount of methane formed increased while the acidified and non-acidified fractions of soluble COD were maintained with the increase of the organic load applied.

As previously discussed, from the 7th AD cycle onwards, the profile of both reactors with alkaline materials addition was different. The reactor with commercial buffer addition increased its share in the acidified organic matter (VFA), with a substantial reduction of the methane formed with the increase of the organic load. The reactor with red mud spheres maintained its VFA and methane shares in each AD cycle, despite the instability in the pH values observed in the 7th and 11th AD cycles. These results demonstrate that the use of mm-size red mud-based inorganic polymer spheres instead of commercial buffer solutions has performance advantages. These advantages are further illustrated in Figure 5, where the global parameters as sCOD removal (determined considering the sCOD added and removed during the entire incubation period), the methanization degree (i.e., the amount of sCOD that was added and converted to methane), and the degree of acidification (i.e., the amount of sCOD converted to VFA) are shown. Figure 5 shows that the sCOD removal and the methanization degree increased significantly by 44 and 80 %, respectively, while the acidification degree decreased by 62 %, when red mud spheres were used instead of the commercial buffer. In addition, the methane yield in the reactor with the spheres, considering the eleven AD cycles, was 210 cm^3_{CH4}/gO_2 removed, which is equivalent to a methane production rate of 1.9 cm^3_{CH4}/gO_2 removed per day, and is 25 % higher than the yield obtained when the commercial buffer solution was used (169 cm^3_{CH4}/gO_2 removed, which correspond to 1.5 cm^3_{CH4}/gO_2 removed per day). Similarly, the biogas yield for the reactor with the spheres was 25 % higher than the yield for the reactor with commercial buffer solution addition (3.6 and 2.9 cm^3_{biogas}/gO_2 removed per day, respectively).

4. Conclusions

This study evaluated the use of red mud-based inorganic polymer spheres as pH regulators and AD intensifiers in a demanding environment, and the performance of this novel material was compared with the performance of commercial buffer solution. Results show that the use of inorganic spheres can not only buffer the pH of anaerobic reactors for long periods without additional alkalis sources, but also strongly increase the accumulated methane volume (by 90%) and methane yield (by 25%), compared to the commercial buffer solution. These promising results demonstrate the viability of inorganic spheres to improve the stability and performance of anaerobic reactors.

E-supplementary data of this work can be found in online version of the paper

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References

1. APHA, 2012. Standard methods for the examination of water and wastewater, 22nd ed. American Water Works Association, Water Environment Federation.
2. Chen, X., Guo, Y., Ding, S., Zhang, H., Xia, F., Wang, J., Zhou, M., 2019. Utilization of red mud in geopolymer-based pervious concrete with function of adsorption of heavy metal ions. J. Clean. Prod. 207, 789–800. https://doi.org/10.1016/j.jclepro.2018.09.263
3. Fernández, C., Cuetos, M.J., Martínez, E.J., Gómez, X., 2015. Thermophilic anaerobic digestion of cheese whey: Coupling H2 and CH4 production. Biomass and Bioenergy 81, 55–62. https://doi.org/10.1016/j.biombioe.2015.05.024
4. Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H., 2014. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. Waste Manag. 34, 2080–2089. https://doi.org/10.1016/j.wasman.2014.07.020
5. Gameiro, T., Lopes, M., Marinho, R., Vergine, P., Nadais, H., Capela, I., 2016. Hydrolytic-Acidogenic Fermentation of Organic Solid Waste for Volatile Fatty Acids Production at Different Solids Concentrations and Alkalinity Addition. Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-3086-6
6. Khairul, M.A., Zanganeh, J., Moghtaderi, B., 2019. The composition, recycling and utilisation of Bayer red mud. Resour. Conserv. Recycl. 141, 483–498. https://doi.org/https://doi.org/10.1016/j.resconrec.2018.11.006
7. Kim, M., Ahn, Y.H., Speece, R.E., 2002. Comparative process stability and

efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Res. 36, 4369–4385. https://doi.org/10.1016/S0043-1354(02)00147-1

8. Lagoa-Costa, B., Kennes, C., Veiga, M.C., 2020. Cheese whey fermentation into volatile fatty acids in an anaerobic sequencing batch reactor. Bioresour. Technol. 308, 123226. https://doi.org/10.1016/j.biortech.2020.123226
9. Lerm, S., Kleyböcker, A., Miethling-Graff, R., Alawi, M., Kasina, M., Liebrich, M., Würdemann, H., 2012. Archaeal community composition affects the function of anaerobic co-digesters in response to organic overload. Waste Manag. 32, 389–399. https://doi.org/10.1016/J.WASMAN.2011.11.013
10. Li, Y., Chen, Y., Wu, J., 2019. Enhancement of methane production in anaerobic digestion process: A review. Appl. Energy 240, 120–137. https://doi.org/10.1016/J.APENERGY.2019.01.243
11. Mao, C., Feng, Y., Wang, X., Ren, G., 2015. Review on research achievements of biogas from anaerobic digestion. Renew. Sustain. Energy Rev. 45, 540–555. https://doi.org/10.1016/j.rser.2015.02.032
12. Mao, C., Wang, X., Xi, J., Feng, Y., Ren, G., 2017. Linkage of kinetic parameters with process parameters and operational conditions during anaerobic digestion. Energy 135, 352–360. https://doi.org/10.1016/j.energy.2017.06.050
13. Neshat, S.A., Mohammadi, M., Najafpour, G.D., Lahijani, P., 2017. Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. Renew. Sustain. Energy Rev. 79, 308–322. https://doi.org/10.1016/j.rser.2017.05.137
14. Nghiem, L.D., Wickham, R., Ohandja, D.G., 2017. Enhanced biogas production and performance assessment of a full-scale anaerobic digester with acid phase digestion. Int. Biodeterior. Biodegrad. 124, 162–168.

https://doi.org/10.1016/j.ibiod.2017.04.001

15. Novais, R.M., Carvalheiras, J., Seabra, M.P., Pullar, R.C., Labrincha, J.A., 2019. Red mud-based inorganic polymer spheres bulk-type adsorbents and pH regulators. Mater. Today 23, 105–106. https://doi.org/10.1016/j.mattod.2019.01.014
16. Novais, R.M., Carvalheiras, J., Seabra, M.P., Pullar, R.C., Labrincha, J.A., 2018a. Innovative application for bauxite residue: Red mud-based inorganic polymer spheres as pH regulators. J. Hazard. Mater. 358, 69–81. https://doi.org/10.1016/j.jhazmat.2018.06.047
17. Novais, R.M., Gameiro, T., Carvalheiras, J., Seabra, M.P., Tarelho, L.A.C., Labrincha, J.A., Capela, I., 2018b. High pH buffer capacity biomass fly ash-based geopolymer spheres to boost methane yield in anaerobic digestion. J. Clean. Prod. 178, 258–267. https://doi.org/10.1016/j.jclepro.2018.01.033
18. Novais, R.M., Pullar, R.C., Labrincha, J.A., 2020. Geopolymer foams: An overview of recent advancements. Prog. Mater. Sci. 109, 100621. https://doi.org/10.1016/j.pmatsci.2019.100621
19. Novais, R.M., Seabra, M.P., Labrincha, J.A., 2017. Porous geopolymer spheres as novel pH buffering materials. J. Clean. Prod. 143, 1114–1122. https://doi.org/10.1016/j.jclepro.2016.12.008
20. Park, J.G., Lee, B., Jo, S.Y., Lee, J.S., Jun, H.B., 2018. Control of accumulated volatile fatty acids by recycling nitrified effluent. J. Environ. Heal. Sci. Eng. 16, 19–25. https://doi.org/10.1007/s40201-018-0291-9
21. Prazeres, A.R., Carvalho, F., Rivas, J., 2012. Cheese whey management: A review. J. Environ. Manage. 110, 48–68. https://doi.org/10.1016/j.jenvman.2012.05.018
22. Rugele, K., Bumanis, G., Mezule, L., Juhna, T., Bajare, D., 2015. Application of industrial wastes in renewable energy production. Agron. Res. 13, 526–532.

23. Silva, F.C., Serafim, L.S., Nadais, H., Arroja, L., Capela, I., 2013. Acidogenic fermentation towards valorisation of organic waste streams into volatile fatty acids. Chem. Biochem. Eng. Q. 27, 467–476.

24. Speece, R.E., 1996. Anaerobic biotechnology for industrial wastewaters. Archae Press, Nashville, USA.

25. Sun, M.-T., Fan, X.-L., Zhao, X.-X., Fu, S.-F., He, S., Manasa, M.R.K., Guo, R.-B., 2017. Effects of organic loading rate on biogas production from macroalgae: Performance and microbial community structure. Bioresour. Technol. 235, 292–300. https://doi.org/10.1016/j.biortech.2017.03.075

26. Tabatabaei, M., Valijanian, E., Aghbashlo, M., Ghanavati, H., Sulaiman, A., Wakisaka, M., 2018. Prominent parameters in biogas production systems, in: Tabatabaei, M., Ghanavati, H. (Eds.), Biogas: Fundamentals, Process and Operation, Biofuel and Biorefinery Technologies. Springer International Publishing, Cham, pp. 135–163. https://doi.org/10.1007/978-3-319-77335-3

27. Treu, L., Tsapekos, P., Peprah, M., Campanaro, S., Giacomini, A., Corich, V., Kougias, P.G., Angelidaki, I., 2019. Microbial profiling during anaerobic digestion of cheese whey in reactors operated at different conditions. Bioresour. Technol. 275, 375–385. https://doi.org/10.1016/j.biortech.2018.12.084

28. Van Lier, J.B., Rebac, S., Lens, P., van Bijnen, F., Oude Elferink, S.J.W.H., Stams, A.J.M., Lettinga, G., 1997. Anaerobic treatment of partly acidified wastewater in a two-stage expanded granular sludge bed (EGSB) system at 8°C. Water Sci. Technol. 36, 317–324. https://doi.org/10.1016/S0273-1223(97)00538-6

29. Wang, L.H., Wang, Q., Cai, W., Sun, X., 2012. Influence of mixing proportion on the solid-state anaerobic co-digestion of distiller's grains and food waste. Biosyst. Eng. 112, 130–137. https://doi.org/10.1016/j.biosystemseng.2012.03.006

30. Wu, D., Li, L., Zhao, X., Peng, Y., Yang, P., Peng, X., 2019. Anaerobic digestion: A review on process monitoring. Renew. Sustain. Energy Rev. 103, 1–12. https://doi.org/10.1016/J.RSER.2018.12.039

31. Ye, J., Hu, A., Cheng, X., Lin, W., Liu, X., Zhou, S., He, Z., 2018a. Response of enhanced sludge methanogenesis by red mud to temperature: Spectroscopic and electrochemical elucidation of endogenous redox mediators. Water Res. 143, 240–249. https://doi.org/10.1016/j.watres.2018.06.061

32. Ye, J., Hu, A., Ren, G., Chen, M., Tang, J., Zhang, P., Zhou, S., He, Z., 2018b. Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud. Water Res. 134, 54–62. https://doi.org/10.1016/j.watres.2018.01.062

33. Ye, J., Hu, A., Ren, G., Zhou, T., Zhang, G., Zhou, S., 2018c. Red mud enhances methanogenesis with the simultaneous improvement of hydrolysis-acidification and electrical conductivity. Bioresour. Technol. 247, 131–137. https://doi.org/10.1016/j.biortech.2017.08.063

34. Zhang, C., Su, H., Baeyens, J., Tan, T., 2014. Reviewing the anaerobic digestion of food waste for biogas production. Renew. Sustain. Energy Rev. 38, 383–392. https://doi.org/10.1016/j.rser.2014.05.038

Figure captions

Figure 1 pH evolution during time for fed-batch assays testing no alkaline addition (blank) (○); with commercial buffer solution (◊), and with red mud-based inorganic polymer spheres (□□).

Figure 2 sCOD and VFA species (n-butyric, propionic and acetic acids), expressed as COD equivalents, during fed batch assays: a) no alkaline addition, b) assay with commercial buffer solution, and c) assay with red mud-based inorganic polymer spheres. Note: “Others” refers to VFA species as iso-butyric, iso-valeric, n-valeric and n-caproic acids, which were determined individually and presented low concentrations.

Figure 3 Biogas composition expressed by the CH4/CO2 ratio (a) and methane volume accumulated (b) during time, for fed-batch assays testing no alkaline addition (blank) (○), with commercial buffer solution (◊), and with red mud-based inorganic polymer spheres (□□).

Figure 4 COD balance including the acidified and non-acidified fraction of the liquid phase, the sCOD converted into methane, represented in COD equivalents of gas, and the sCOD removed from the mixture, for biomass growth and metabolism. a) Blank assay; b) assay with commercial buffer solution; c) assay with red mud-based inorganic polymer spheres.

Figure 5 Anaerobic performance during the overall experience (eleven consecutive substrate additions), regarding sCOD removal, degree of acidification and methanization degree, for reactors performed without alkaline addition (Blank), with commercial buffer solution (CoBuffer) and with red mud-based inorganic polymer spheres (IPSpheres).

Figures:

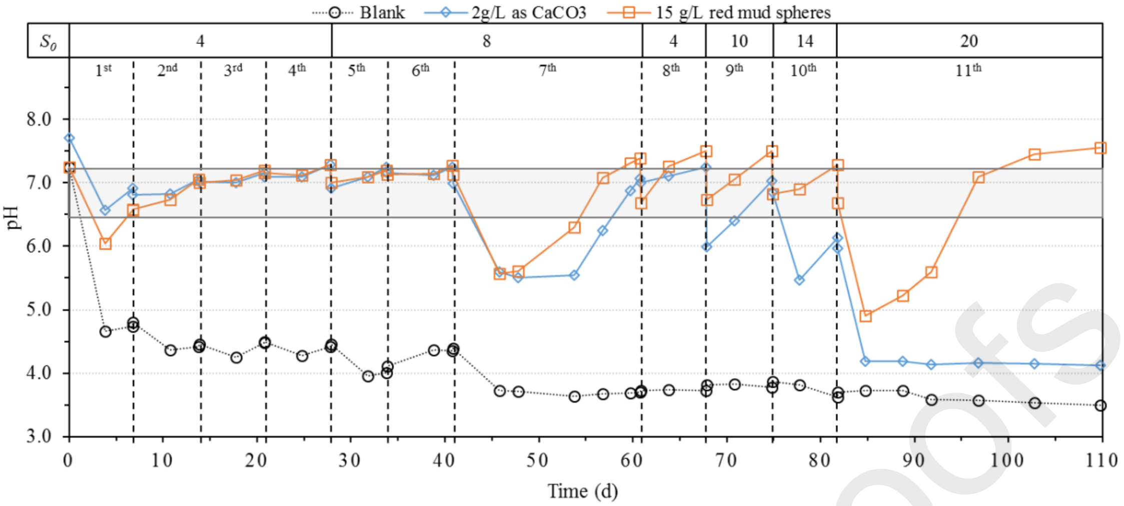
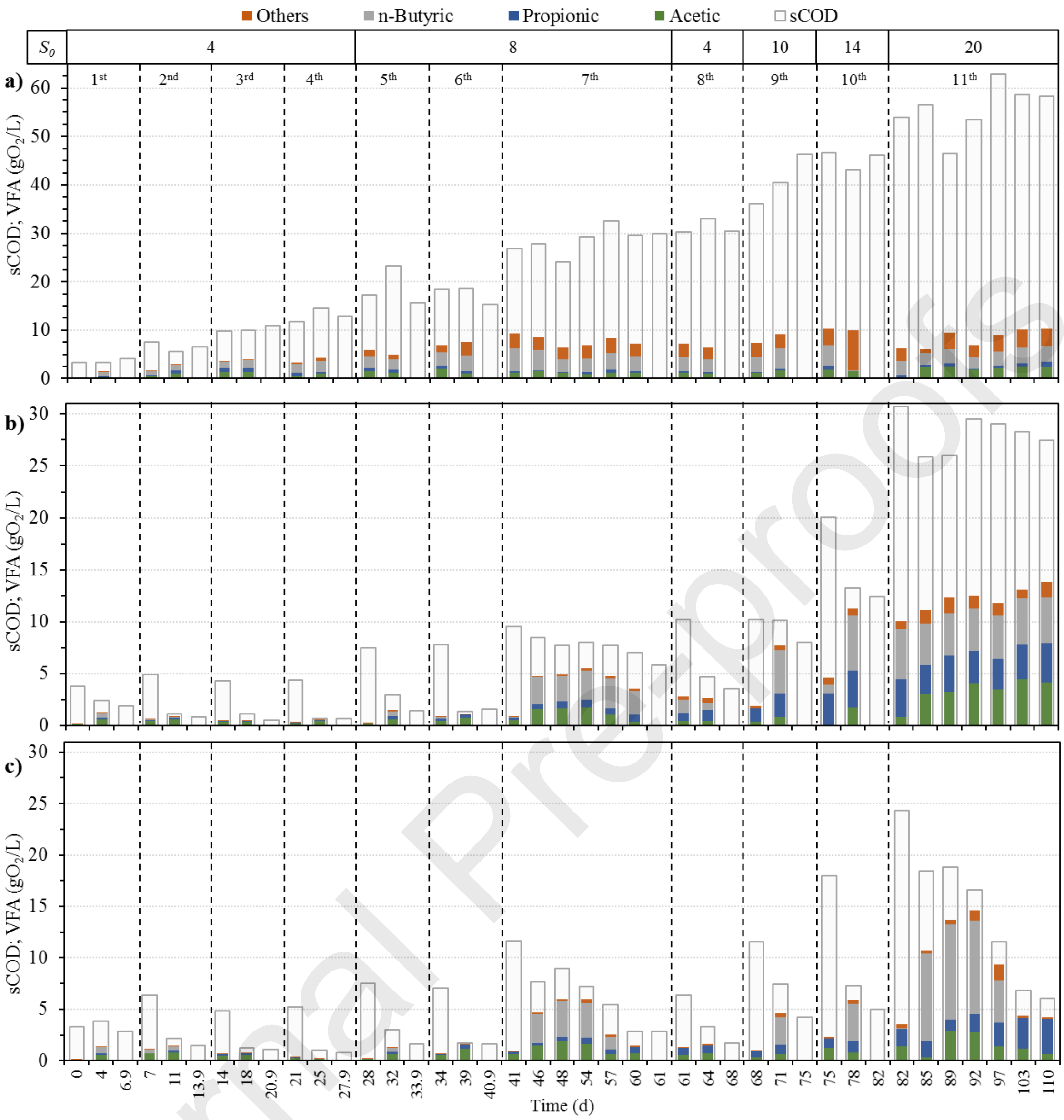


Figure 1 pH evolution during time for fed-batch assays testing no alkaline addition (blank) (○); with commercial buffer solution (◊), and with red mud-based inorganic polymer spheres (□☐).



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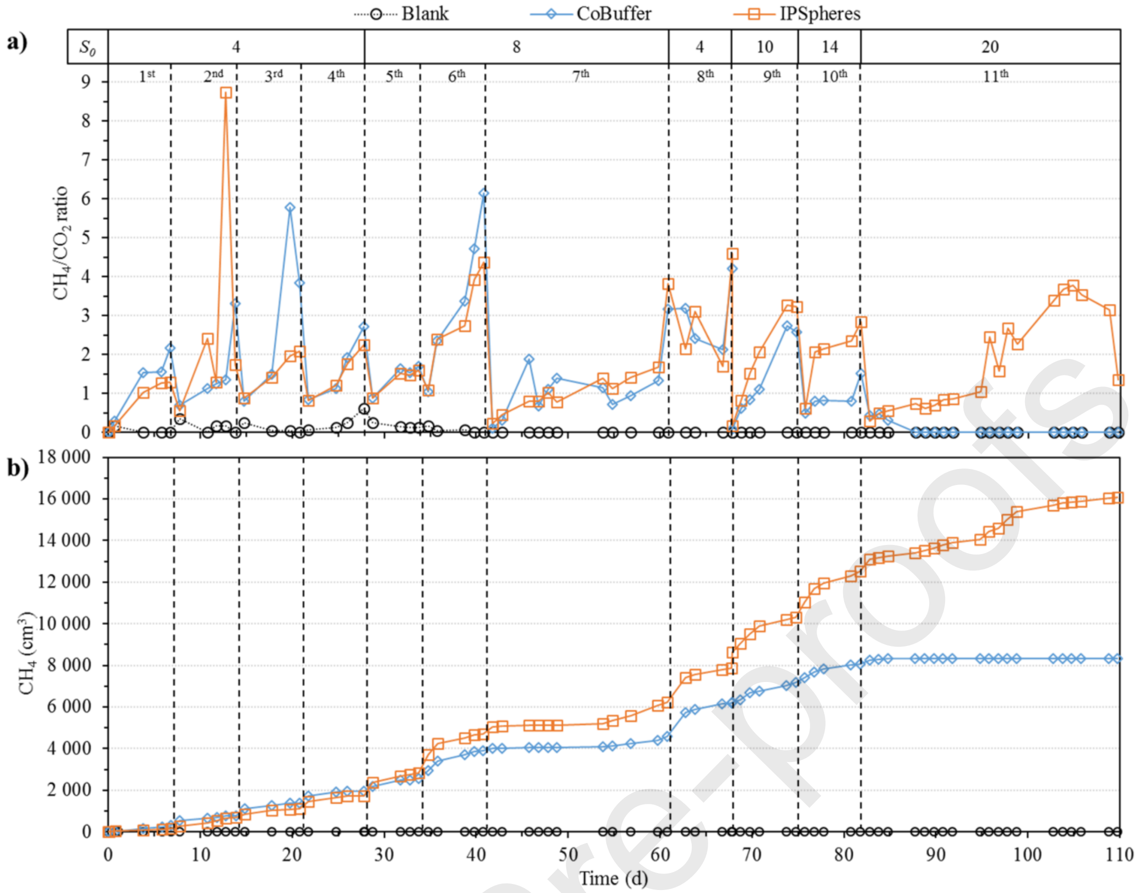
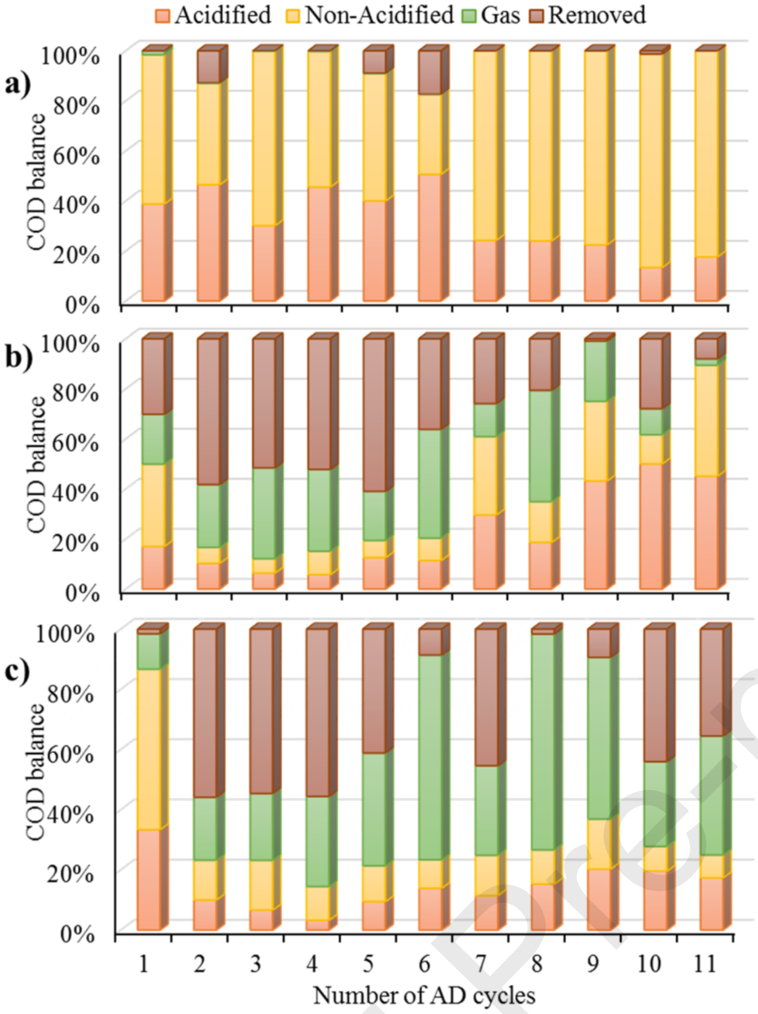


Figure 3 Biogas composition expressed by the CH_4/CO_2 ratio (a) and methane volume accumulated (b) during time, for fed-batch assays testing no alkaline addition (blank) (○), with commercial buffer solution (◊), and with red mud-based inorganic polymer spheres (□☐).



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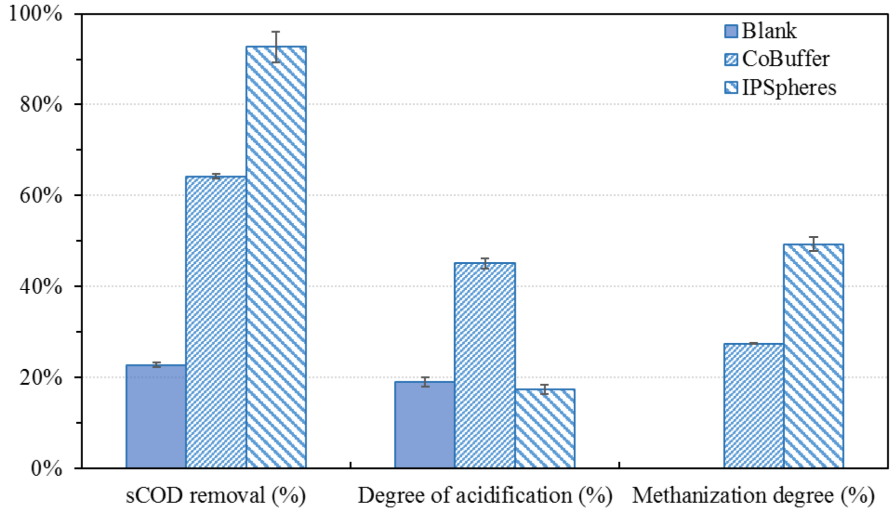

Figure 5 Anaerobic performance during the overall experience (eleven consecutive substrate additions), regarding sCOD removal, degree of acidification and methanization degree, for reactors performed without alkaline addition (Blank), with commercial buffer solution (*CoBuffer*) and with red mud-based inorganic polymer spheres (*IPSpheres*).

CRediT authorship contribution statement

Tânia Gameiro: Conceptualization, Investigation, Formal analysis, Roles/Writing - original draft; Visualization

Rui M. Novais: Conceptualization, Methodology, Roles/Writing - original draft, Funding acquisition

Catarina L. Correia: Investigation, Formal analysis, Visualization

João Carvalheiras: Investigation, Visualization

Maria P. Seabra: Conceptualization, Validation, Writing - review & editing

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Armando C. Duarte: Writing - review & editing, Supervision

Isabel Capela: Conceptualization, Validation, Resources, Writing - review & editing, Funding acquisition

Highlights:

- An eco-friendly approach to control pH in Anaerobic Digestion (AD) is presented
- Red mud-based inorganic polymer spheres were used as buffer material in AD
- The use of inorganic spheres boosted methane volume by 90%
- Inorganic spheres allowed low acid content and high organic removal inside digester
- Inorganic spheres were used repeatedly without losing buffer capacity and integrity