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Bioreactor Feeding Strategies to Improve Biogas Production and Pig Slurry Management Flexibility

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ABSTRACT

Pig slurry (PS) management is a challenge that needs appropriate strategies. Anaerobic digestion (AD) has proved to be an interesting option to follow when it comes to livestock effluent management. Although this technology is well established, it is crucial to investigate different scenarios that demonstrate its suitability to motivate farmers to adopt new strategies for PS management. Previous research, based on a daily feeding regime and a 2-day starvation-induced period, investigated the impact of feast/famine cycles on the AD process. There was evidence of a positive link between AD performance and the starvation regime. From this assumption, new scenarios were designed combining different feeding frequencies: a) one daily feeding (F1) and six feedings per day (F6), b) two days of starvation with one feeding (S1) and with six feedings (S6). The operational parameters were settled in advance: organic loading rate (1.5±0.2 g $VS/L_{reactor}$.d), hydraulic retention time (15 days), and mesophilic conditions (37±1 ºC). The results obtained in this work indicate a significant improvement ($P < 0.05$) of 92% in specific methane production when comparing the trial F1 with F6. VS reduction remained constant in F1 and F6, but the starvation period (S1) led to an increase in VS reduction compared to both F1 (27%) and F6 (33%). The results obtained are in agreement with the previous work conducted by the authors. This study highlights how feeding frequency and starvation affect biogas production, assessing their effectiveness on biogas yield. This tool will give farmers a key decision factor to make an evidence-based decision and can work as a contingency planning strategy, aligning their PS management needs with AD versatility.

Keywords: anaerobic digestion, biogas production, biowaste management, feeding frequency, feast/famine.

INTRODUCTION

The world's population is growing at a rapid rate, which has led to an increase in food consumption and an intensification of livestock production (Samoraj *et al.*, 2022). Particularly, pork meat has increased over the years, being the most consumed meat worldwide and its production is expected to continue increasing. This pork meat rise implies an increase in biowaste production needing attention and proper management, namely concerning pig slurry (PS). If not treated properly, it has the potential to be dangerous to the environment (Xie *et al.*, 2017). For this reason, is of the utmost importance to search for effective management alternatives to serve as guidelines for PS control and valorisation.

AD emerges as one of the viable options for livestock management since animal slurry is a valuable resource due to its high organic content. For years, AD has proved to be an interesting option that provides a clean energy renewable source. Giving rise to biogas and a digestate with potential for agricultural use (Syahri *et al.*, 2022), it is undeniable that AD has become increasingly attractive in the search for economic circularity. The flexibility of the best available techniques, manure storage time minimization, GHG emission reduction, bioenergy recovery, and digestate valorisation are key factors to circular bioeconomy goals (Kowalczyk-Juśko *et al.*, 2023; Gontaruk *et al.*, 2024). More recently, studies have focused on exploring ways to improve this technology, enhancing biogas production and quality (Prabhu *et al.*, 2021; Cândido *et al.*, 2022). However, it is important to focus on solutions that can be scaled up, considering the reality of farms.

Several articles about bioreactor feeding management appeared around 2017 to investigate the flexibility of biogas production (Zealand *et al.*, 2017; Piao *et al.*, 2018; Svensson *et al.*, 2018). The results obtained with the substrates used in those articles were promising (rice straw, food waste, and glucose), but a lack of information needed to be fulfilled regarding the feeding management with PS, since substrate type has a great influence on the process performance and can compromise the results (Syahri *et al.*, 2022). The wide variety of conclusions confirms that the results cannot be generalized to bioreactors running with different substrates.

Silva *et al.* (2021) studied the effects of starvation on AD with a single feeding and concluded that the starvation period improves biogas production and quality. The present study builds on the conclusions drawn by Silva *et al.*, bringing forward new scenarios to assess the impact on the AD process of varying the feeding frequency, namely the number of feedings.

In this sense, this work intended to investigate how the feeding frequency range (one per day and every four hours) would affect the AD process and subsequently, the biogas production and/or efficiency, following a feeding management strategy. The conclusions of this work demonstrate, once again, the versatility of AD and how it is possible to adjust the process to the reality of each farm, under certain conditions. The authors expect that this study will provide guidelines to help farmers plan manure management and be able to make better decisions towards bioenergy production.

MATERIAL AND METHODS

Experimental design

After the previous study conducted by Silva *et al.* (2021) that compared the regime of daily feedings with the application of a starvation period of 2 days with a single load, the present work intended to evaluate the effect of increasing the frequency of feeding on AD performance, keeping the operational parameters selected in advance: organic loading rate (1.5±0.2 g $VS/L_{reactor}$.d), hydraulic retention time (15 days), and mesophilic conditions (37±1 °C). Hence, four scenarios were analysed in this study, which are summarised in Table 1: one daily feeding (F1), daily feeding with six feedings (F6), a 2-day starvation period with a single feeding (S1), and lastly a 2-day starvation period with six feedings (S6).

Physicochemical characterization

pH and electrical conductivity (EC) were measured using VioLab benchtop multiparameter probe. The biogas quality (expressed in % v/v CH_4) was quantified every week, using a portable analyser (LMSxi Multifunction Landfill Gas Analyser, Gas Data, United Kingdom). At the beginning and end of each cycle, both the PS and digestate were characterized for physicochemical parameters. The average values for pig slurry characterisation after pre-treatment are presented in Table 1. The characterization comprised the determination of total and volatile solids (TS and VS, respectively), total chemical oxygen demand (TCOD), VS reduction, ammoniacal nitrogen ($N\text{-}NH_4^+$), and Kjeldahl nitrogen (N_K). The methodologies used followed the methods of the American Public Health Association (APHA, 2012).

Table 1. Feeding regimes description and data origin

Code	Feeding regime	Origin of data
F1	Daily in a single load	Silva *et al.* (2021)
F6	Daily in six feedings	This study
S1	A single load and a 2-day starvation period	Silva *et al.* (2021)
S6	Six feedings and a 2-day starvation period	This study

Also, soluble chemical oxygen demand (SCOD) was determined by using COD kits (Nanocolor CSB 15000, Macherey-Nagel, Germany).

Substrate collection and preparation

PS samples used during the AD trials were collected directly from the fattening/finishing phase from a pig farming located in Torres Vedras, Portugal (39º11'37.43"N; 9º15'01.72"W) with a capacity for 1862 fattening/finishing places. The samples were collected in appropriate containers and transported to the laboratory for storage at 4 °C to preserve their characteristics until further use (Silva *et al*., 2023). Before each cycle, to promote the correct homogenization of the samples and to enhance the availability and degradation of the substrate (Lopes *et al.*, 2019), PS was subjected to agitation for 1 minute with an industrial stirrer (250 W, 2500 rpm, Bertrand – Groupe Dito, France). Then, due to equipment constraints regarding the feeding pipes of the reactor where the experiments run, the samples had to be sieved, with a mesh size of 2 mm to remove grains and coarse material to prevent clogging. The PS used in this study is similar to the one used in the study with which we intend to compare feeding frequencies, detailed information and the characteristics of the PS collected for the F1 and S1 trials can be found in Silva *et al.* (2021). Table 2 shows the characterization of PS for feeding frequency of six feedings per day under study, after the pre-treatment.

Table 2. Characterization of pig slurry after mechanical pre-treatment

Parameters	F6	S6
pH	6.9 ± 0.2	7.0 ± 0.1
EC (mS/cm)	7.9 ± 2.5	9.2 ± 1.1
TS (g/kg)	37.3 ± 1.4	37.5 ± 1.6
VS (g/kg)	24.5 ± 1.6	25.0 ± 1.3
VS/TS (%)	66	67
TCOD (g/L)	69.5 ± 4.0	58.6 ± 4.3
SCOD (g/L)	36.0 ± 1.7	36.0 ± 0.85
SCOD/TCOD (%)	52	67
$N\text{-}NH_4^+$ (g/L)	3.6 ± 0.1	3.3 ± 0.1
N_K (g/L)	4.7 ± 0.1	4.4 ± 0.1
TOC	14	15
C/N ratio	3.0	3.4

Note: EC – electrical conductivity; TS – total solids; VS – volatile solids; TCOD – total chemical oxygen demand; SCOD – soluble chemical oxygen demand; $N\text{-}NH_4^+$ – ammoniacal nitrogen; N_K – Kejdahl nitrogen; TOC – total organic carbon.

Anaerobic digestion experiments

To investigate the effect of increasing feeding frequency, a methodology was designed based on raising the number of feedings to six. Experiments S6 and F6 were carried out in the same continuous stirred-tank reactor (CSTR) as F1 and S1 (Fig. 1), with a working volume of 4.8 L, under mesophilic conditions. To maintain the temperature under mesophilic conditions (37±0.2 °C) there is a heating system coupled with the CSTR which is controlled by computer software.

Before each feeding, the reactor was under agitation for one minute and the PS under 30 seconds to promote total homogenization. During the feeding, both reactor and PS continue under agitation until the end of the feeding period (feed pump from Watson Marlow, 120 rpm, 24 W). Both agitations were assured by two mechanical stirrers (VELP Scientifica, 50 rpm, 60 W). To promote homogenization inside the reactor, the reactor agitation remained unchanged even on days when there was no feeding, being activated every 4 hours with a duration of 2 minutes. To monitor the AD process over time, the biogas production was recorded daily through a flow meter (MilliGascounter, Ritter, Germany). The temperature inside the reactor was also permanently controlled and recorded daily. To evaluate the efficiency of the AD process, the following parameters were calculated: methane production (MP), biogas production rate (GPR), specific biogas production (SGP), and specific methane production (SMP).

Bioenergy recovery

To determine the specific electric energy generated in each feeding frequency and allow a comparison between them, energy performance (EP) was calculated based on a simplified approach presented by Silva *et al.* (2024) and adapted for this experimental study. This calculation is performed by multiplying the methane-specific weight (0.72 kg/m^3), the methane low calorific value (50 MJ/kg), and the SMP (m^3/kg VS) of each trial. Then, using the conversion factor of MJ to kWh (0.28), the predicted value of bioenergy produced

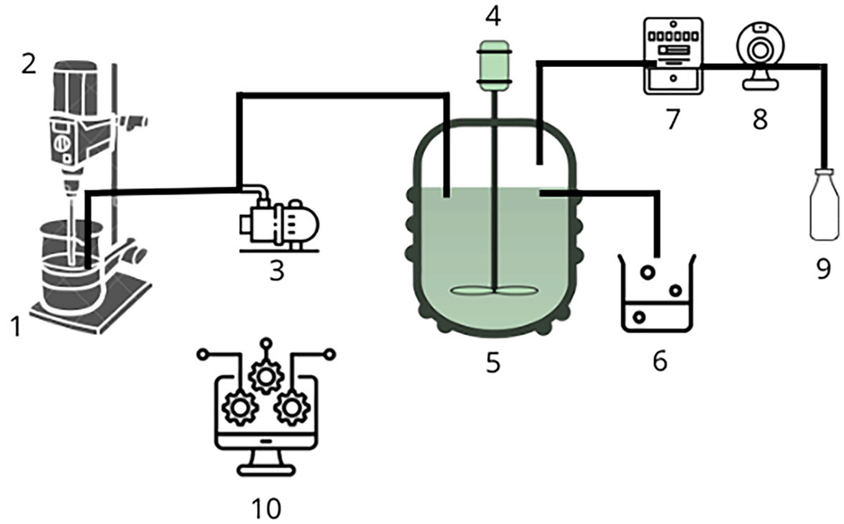

Figure 1. Schematic representation of the AD unit. Legend: 1 – feeding tank; 2 – feeding stirrer; 3 – feeding pump; 4 – Stirrer of the continuous stirred-tank reactor (CSTR); 5 – CSTR; 6 – digestate tank; 7 – biogas flow meter; 8 – video monitoring; 9 – gas holder; 10 – control software

obtained is expressed in kWh/kg VS. From the base scenario of a single feeding for different regimes (F1 and S1), the study of the increment of feeding frequencies was performed, increasing to six feedings for the same regimes (F6 and S6). In this context, an energy performance indicator (EPI) is proposed to facilitate the comparison between different feeding frequencies to compare different regimes, namely daily feeding (Equation 1) and 2-day starvation trials (Equation 2):

$$EPI_{daily\ feeding} = \frac{EP_{F6} - EP_{F1}}{EP_{F1}} \times 100 \quad (1)$$

$$EPI_{2-day\ starvation} = \frac{EP_{S6} - EP_{S1}}{EP_{S1}} \times 100 \quad (2)$$

where: EP_{F6}, EP_{F1}, EP_{S6} and EP_{S1} correspond to the energy performance for F6, F1, S6 and S1 trials, respectively (expressed in %).

Afterwards, a comparison between the indicators is possible, allowing a better understanding of the combined effect of altering feeding regimes and frequencies.

Statistical analysis

Data are presented as the mean and standard deviation of three replicates. A statistical analysis was performed using GraphPad Prism software (version 5.0). The Student's t-test was performed with a 95% degree of confidence (P = 0.05) to compare two groups or samples. Differences were deemed statistically significant when P-values were less than 0.05.

RESULTS AND DISCUSSION

Impact of six feedings in the two regimes

Monitoring a bioreactor involves following several parameters to assess the evolution of the process performance. Analyzing the values shown in Table 3 it is clear the positive influence of the starvation period (S6) in comparison with daily feeding (F6), except for biogas quality (expressed in % v/v CH_4), where starvation trials had a decrease of 3% compared to daily feeding. This pattern is not aligned with the single feeding trials (F1 and S1), where the starvation period (S1) had better biogas quality (75% v/v CH_4) than daily feeding (F1), which presented 73% v/v CH_4. Although this is the only parameter that differs from the single feeding trials (Silva *et al.*, 2021), the results obtained are of the same order of magnitude and do not vary greatly.

The improvement of VS reduction, around 20%, in S6 indicates the better degradation of the organic matter in the 2-day starvation period, which is aligned with the higher MP (15%

Table 3. Operational parameters

Parameters	F6	S6
MP (L CH_4/d)	5.5 ± 1.5	6.3 ± 1.6
% v/v CH_4	75	73
SGP (L/g VS)	0.97 ± 0.26	1.08 ± 0.23
VS reduction (%)	41.4	49.8

Note: MP – methane production; SGP – specific biogas production; VS – volatile solids

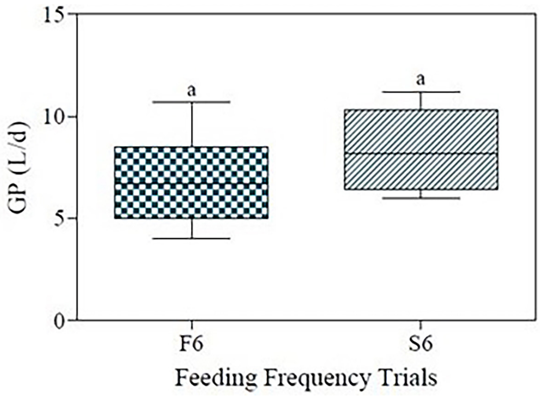

Figure 2. Gas production (GP) of six feeding trials, without (F6) and with the starvation period (S6) (different letters indicate significantly different results, P-value < 0.05, while equal letters indicate not significantly different results, according to the Student's t-test)

increase) and SGP (11% increase). This can also be related to the higher SCOD/TCOD ratio of PS used for S6 trials (29% higher than F6) (Table 1), which suggests a higher bioavailability and, thus, a higher biogas production.

Regarding biogas production, it was observed in the study conducted by Silva *et al.* (2021) that the starvation period (S1) led to an increase of 34%, compared to F1. In the present study, this behavior was also noticed by an increase of 18% from F6 to S6 (Fig. 2). However, statistical analysis from both studies proved that the trials were not statistically significantly different, suggesting that the famine period did not significantly impact biogas production (P-value > 0.05).

The effect of increasing the feeding frequency

The increment of the number of feedings per day increased the SMP by almost double: F1 registered 0.38±0.08 L CH_4/g VS and F6 0.73±0.20 L CH_4/g VS. The Student's t-test compared the means of SMP for each feeding frequency (F1 and F6). The P-values obtained were less than 0.05, confirming that the differences between SMP values were statistically significant. However, with more feedings, the standard deviation increased, as can be seen in Figure 3i; this behavior has also been reported by other authors (De Vrieze *et al*., 2013; Mulat *et al.*, 2016).

Similar behavior and P-value were observed for starvation trials (Figure 3ii). In these trials, there is also an increase in SMP of 46% regarding the increase in feeding frequency, keeping the same average OLR and HRT. Once again, the P-value confirmed the significant impact of feeding frequency in SMP even when a starvation period is induced.

Regarding AD trials on continuously stirred tank reactors (CSTRs), different results are reported in the literature. For example, Mulat *et al*. (2016) performed an experimental study on a laboratory scale CSTR under an OLR of 4 g VS/L.d (2.6 times higher than the OLR in this study) using a by-product of bioethanol production plants and reported lower specific methane production with more frequent feeding. On the other hand, with a lower OLR than the one used in this study, Piao, Lee and Kim (2018) stated that working with an OLR of 0.5 g glucose/L.d showed a similar pattern for different feeding frequencies: twice a day, once a day, and every two days. In contrast, Svensson *et al*. (2018) demonstrated that frequent feeding led to an increase in methane production of 20% when working with food waste at a very high OLR (21 g COD/L.d).

However, over time, an interesting pattern was detected as shown in Figure 4: the trials without famine period (F6) tend to increase

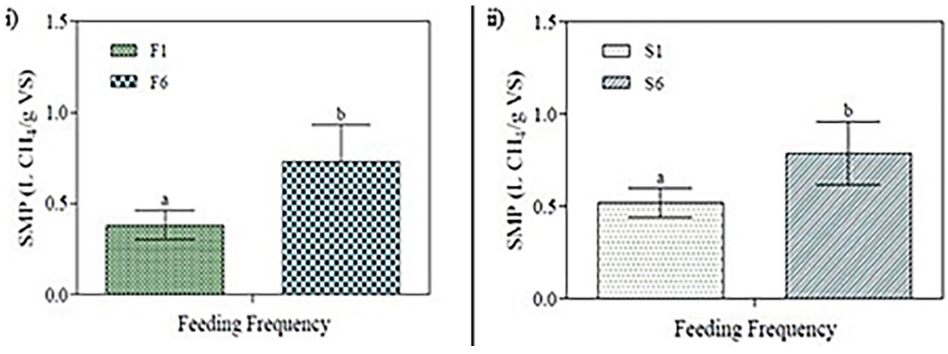

Figure 3. Comparison of SMP values for different feeding frequencies (one and six feedings): i) daily feedings, ii) 2- day starvation period. Error bars represent standard deviation (SD). Different letters in each graph indicate significantly different results (P-value < 0.05) according to the Student's t-test

their specific methane production over time, while when inducing a starvation period (S6) leads to a decrease in this parameter. However, the statistical analysis of both SMPs presented a P-value higher than 0.05, meaning that there are no statistically significant differences between trials, suggesting that the production of biogas is not substantially affected during the two studied cycles. This indicates that the increase in feeding frequency (six feedings/day) combined with a starvation period may not be a strategy to adopt in the long term, since after one HRT the SMP of S6 tends to decline (Fig. 4). However, during the first cycle, it seems that the AD performance is not compromised and can be considered as a management feeding strategy to be applied by large-scale pig farm units when some unexpected problems occur, namely energy failure, equipment replacement, and lack of human resources.

Bioenergy production forecast

Figure 5 shows that for the assays with only one feeding regime (F1, S1), the average EP calculated is lower than for six feedings (F6, S6). For the daily feeding regime, the increase in feedings (from F1 to F6) resulted in an EPI of 92%. Also, in starvation trials (S1 and S6), it is possible to see an improvement of nearly 50% in EPI with the adoption of more feedings (S6).

According to the EPIs obtained, it is possible to state that a higher feeding frequency corresponds to an increase in energy production. The increase of EP with the feeding frequency may be linked to a higher degradation of the substrates introduced into the bioreactor. Dividing the load into several smaller feedings throughout the day leads to greater degradation of the organic matter supplied. The data reinforces the view that producers have flexibility in deciding how often to feed the digester, according to the reality and needs of their farms.

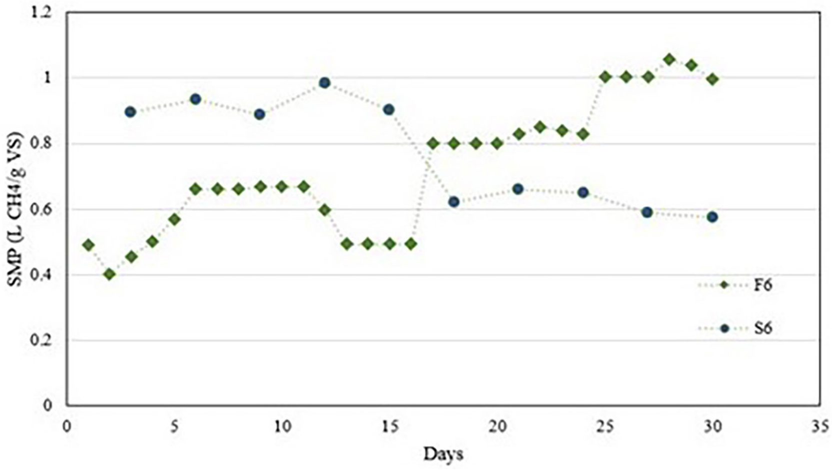

Figure 4. SMP evolution of F6 and S6 throughout the trials

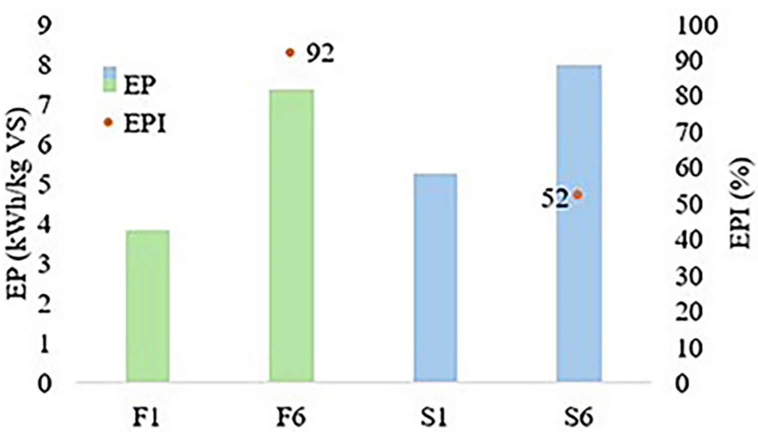

Figure 5. Improvement of EP with the increase in feeding frequency

CONCLUSIONS

This study has shed new light on feeding management strategies, combining the effects of a starvation-induced period with a change in feeding frequency. The results obtained show not only the flexibility and versatility of both the anaerobic digestion process and pig slurry management, allowing a more in-depth understanding of feeding management strategies.

Overall, the AD performance was improved. The increase in feeding frequencies improved the specific methane production for both regimes: a two-fold increase in daily feedings (from F1 to F6) and 46% in 2-day starvation period trials (from S1 to S6). In addition, the estimated energy performance increased with more feedings per day (92% and 52% in daily and starvation trials, respectively).

It is possible to conclude that the alteration of feeding regimes can benefit the flexibility of bioenergy production, which gives farmers more freedom to choose the best route to follow. This study highlights that the possibility of changing feeding regimes can be considered as a contingency plan to deal with unforeseen events such as equipment replacement and energy failure.

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