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Effects of different nitrogen sources on methane production, free ammonium and hydrogen sulfide in anaerobic digestion of cheese whey with cow manure

Efecto de las diferentes fuentes de nitrógeno en la producción de metano, amonio libre y ácido sulfhídrico en la digestión anaeróbica del lactosuero con estiércol de vaca

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Abstract

In this present study, the effect of different nitrogen sources in the anaerobic digestion of cheese whey on methane production, free ammonia, and hydrogen sulfide. The results showed that supplementation with urea at a concentration of 1000 mg L^{-1} the maximum methane production values of 513.95 ± 2.12 mL CH_4 g VS^{-1} were obtained. On the other hand, supplementation with ammonium nitrate at a concentration of 1000 mg L^{-1} gave a value of methane of 415.93 ± 5.44 mL CH_4 g VS^{-1} and exhibited the lowest values hydrogen sulfide of 267.69 ± 0.37 ppm and free ammonium of 49.18 ± 9.66 mg L^{-1}. Supplementation with ammonium sulfate at a concentration of 2000 mg L^{-1}, methane values of 466.64 ± 9.93 mL CH_4 g VS^{-1} and hydrogen sulfide of 2768.43 ± 20.52 ppm were obtained. The findings from this research contributed to elucidate the role of supplementation with urea, ammonium sulfate, and ammonium nitrate in the anaerobic digestion process, which could help to solve some problems related to the reduction of methane production in cheese whey fed biodigesters.

Keywords: Anaerobic digestion, free ammonium, hydrogen sulfide, methane, nitrogen sources.

Resumen

En el presente estudio, se probó el efecto de diferentes fuentes de nitrógeno en la digestión anaeróbica del lactosuero sobre la producción de metano, amonio libre y ácido sulfhídrico. Los resultados mostraron que la suplementación con urea a una concentración de 1000 mg L^{-1} se obtuvieron los máximos valores de producción de metano de 513.95 ± 2.12 mL CH_4 g VS^{-1}. Por otro lado, la suplementación con nitrato de amonio a una concentración de 1000 mg L^{-1} dio un valor de metano de 415.93 ± 5.44 mL CH_4 g VS^{-1} y exhibió los valores más bajos de ácido sulfhídrico de 267.69 ± 0.37 ppm y amonio libre de 49.18 ± 9,66 mg L^{-1}. Con la suplementación con sulfato de amonio a una concentración de 2000 mg L^{-1} se obtuvieron valores de metano de 466.64 ± 9.93 mL CH_4 g VS^{-1} y de ácido sulfhídrico de 2768.43 ± 20.52 ppm. Los hallazgos de esta investigación contribuyen a dilucidar el rol que tiene la suplementación con urea, sulfato de amonio y nitrato de amonio en el proceso digestión anaerobia, lo que podría ayudar a resolver algunos problemas relacionados con la reducción de producción de metano en biodigestores alimentados con lactosuero.

Palabras clave: Digestión anaerobia, amonio libre, ácido sulfhídrico, metano, fuentes de nitrógeno.

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1 Introduction

The growing development of modern societies is closely linked to the use of energy. The high consumption of fossil fuels to meet the demand for energy and the growing scarcity of resources is causing civil and international conflicts, and negative effects on the environment such as global climate change (Ahmad and Zhang, 2020). The process of anaerobic digestion is a technology that has been given much attention since this process allows the biological conversion of organic wastes into biofuels such as biogas which is a mixture of mainly methane and carbon dioxide (Zhao *et al*., 2020; Monroy *et al*., 2020; Hernández *et al*., 2021).

many feedstocks used for biogas production have been previously studied Guiot and Frigon, (2012), and it has been reported that these resources are largely untapped, constituting an abundant source of bioenergy at the scale of a country (Guiot and Frigon, 2012). Cheese whey is considered a residue of the dairy industry, which corresponds to around 85-90% of the total volume of processed milk by-product. Utilization or, disposal has become a concern due to the need to comply with environmental regulations. The presence of biodegradable components in the cheese whey and the advantages of anaerobic digestion processes offer the potential production of biogas (methane), hydrogen, and other biofuels as ethanol (Chatzipaschali and Stamatis, 2012).

In 2016, the production of cow's milk in Mexico reached 11.61 million tons, of which approximately 23% is used for the production of cheeses. A Mexican cheese factory produces a large amount of cheese whey since, for each kilogram of cheese produced, between 8 and 9 L of whey are generated. This translates into a production of more than 2.4 million tons per year, of which only half is used and the other part must be managed as waste, which must be handled properly since mishandling of it can turn it into a highly polluting waste (Mazorra and Moreno, 2019). Therefore the anaerobic digestion of cheese whey for biogas production provides an excellent approach in its treatment (Muñoz *et al*., 2014). For some of the wastes, a nitrogen source supplement may be required since the anaerobic digestion process can be inhibited by a lack or excess of nutrients (Demirel and Scherer, 2008; El-Mashad and Zhang, 2010), but such supplements have not been reported widely (Wagner *et al*., 2012).

The production of biogas by anaerobic digestion also entails the formation of various metabolic products, such as free ammonia (FA) and hydrogen sulfide (H_2S), which have a negative effect on the anaerobic digestion process. Controlling the FA concentration in the anaerobic digestion process is very important as it can lead to failures and consequent economic loss (Bonk *et al*., 2018). The concentration of free ammonia depends on total ammonia nitrogen concentration, pH, and temperature (Krakat *et al*., 2017).

It has been reported that the inhibitory effect on anaerobic digestion varies from 55 to 150 mg NH_3 L^{-1}(Strik *et al*., 2006). Other studies have suggested that adapted inoculum are inhibited at FA concentrations of 70-1100 mg L^{-1}(Nielsen and Angelidaki, 2008). Different studies have elucidated the inhibitory effects of free ammonia on methanogen metabolism (Sterling *et al*., 2001). Different solutions have been suggested for ammonia inhibition, such as the direct removal of ammonia from the reactor, the prevention of high ammonia concentrations by dilution, and adaptation of the microbial community (Krakat *et al*., 2017). Hydrogen sulfide is formed during the anaerobic digestion of sulfate-containing feedstock by reduction of inorganic sulfate through sulfate-reducing bacteria (Nägele *et al*., 2017; Dannesboe *et al*., 2019). Hydrogen sulfide in biogas usually ranges from 50 up to 20000 ppm. It is corrosive (damages engines, reactor tanks, and pipelines) and produces sulfur oxides (SOx) due to combustion, and therefore, its removal is a prerequisite for energy production from biogas production systems (Dumont, 2015; Igarashi and Kuwabara, 2016).

The supplementation of inorganic nitrogen sources in the medium of anaerobic digestion may be an option to redirect the metabolism of anaerobic digestion to increase methane production and inhibit the formation of metabolic products that affect the anaerobic digestion process and equipment (Tanimu *et al*., 2014). The present study aims to evaluate the effect of supplementation of urea, ammonium sulfate, and ammonium nitrate at different concentrations to avoid ammonia's inhibitory effects, remove hydrogen sulfide, and increase methane production in the anaerobic digestion process fed with cheese whey.

Table 1. Physicochemical composition of the substrate and inoculum

Parameters	Substrate (Cheese whey)	Inoculum (cow manure)
Total carbohydrates (g L^{-1})	59.90±0.10	4.53±0.40
Total phosphorus (g L^{-1})	0.24±0.02	0.36±0.16
Total nitrogen (g L^{-1})	0.71±0.01	1.76±0.12
pH	6.52±0.09	7.12±0.00
Total protein (g L^{-1})	4.43±0.50	14.77±0.57
Volatile solids (g L^{-1})	59.73±0.50	11.42±0.19
Total solids (g L^{-1})	81.96±0.57	21.95±0.83

2 Materials and methods

2.1 Substrate and inoculum

The inoculum of this study was obtained from a biodigester located at Gómez Palacio, Durango, Mexico. The liquid manure was passed through a 3-mm mesh to remove solid materials. Cheese whey powder, the substrate for the study, was obtained from a dairy factory located at Torreon, Coahuila, Mexico. The physicochemical composition of the substrate and inoculum is given in Table 1 (Marchioro *et al*., 2018).

2.2 Batch anaerobic digestion

The experiments were carried out in 350-mL glass serum bottles with a working volume of 150 mL. The modified mineral medium used in each experiment was prepared according to the following composition (mg L^{-1}): 703, $NaH_2PO_4{\cdot}H_2O$; 600, K_2HPO_4; 111, $MgSO_4{\cdot}7H_2O$; 6, $CaCl_2$ and 8000, $CaCO_3$ (Cisneros *et al*., 2021).

Different concentrations of ammonium sulfate [$(NH_4)_2SO_4$], urea [$CO(NH_2)_2$], and ammonium nitrate (NH_4NO_3) were added, *viz*., 1000-5000 mg L^{-1} at an interval of 1000 mg L^{-1}. The amount of inoculum (I) and substrate (S) added for all tests was according to the I/S ratio of 1:2 based on the volatile solid content (Ma *et al*., 2019). A control treatment (cheese whey, inoculum, and mineral medium) was maintained to evaluate the effect of the added nitrogen sources. All the experiments were adjusted to a pH of 7, and to maintain anaerobic conditions, the bottles were sealed and purged with N_2/CO_2 (80/25% v/v). The temperature was maintained at 35 °C for 60 days. A factorial block design 1^5 was used and the treatments were maintained in duplicate.

2.3 Measurement of methane production

The biogas production was measured using a diaphragm manometer Dewit®. The measured pressure value is used to calculate the volume of biogas produced using equation (1) (Elasri and El Amin Afilal, 2016; Estevez *et al*., 2012):

$$V_{Biogas} = \frac{\Delta P \times V \times T_2}{T_1 \times P} \quad (1)$$

where ΔP = pressure difference between initial and final readings (kPa), V = volume of gas headspace of the reactor (mL), T_1 = temperature in the reactor, T_2 = standard condition of temperature (273.15 K), and P = atmospheric pressure at standard conditions (101.32 kPa).

The methane concentration was determined using the MQ-4 sensor Arduino® that was connected to a computer using a data acquisition card (Cadena *et al*., 2010). The methane production was normalized by being expressed in terms of specific methane production as mL CH_4 g VS^{-1} (Angelidaki *et al*., 2009).

2.4 Chemical and analytical analyses

Free ammonia (4500-NH_3-B) and pH (4500-H^+-B) were determined following the methods described in standard methods of water and wastewater (APHA, 2012).

Hydrogen sulfide was determined using a gas hydrogen sulfide Wintact® sensor, model WT8802, and the concentrations were expressed in ppm.

FOS/TAC is the ratio between volatile fatty acids concentration (FOS, expressed as mg/L of equivalents of CH_3COOH) and total alkalinity (TAC, expressed as mg/L of $CaCO_3$), and it was determined in the reactor according to Sun *et al*. (2017).

2.5 *Estimation of kinetic parameters*

Kinetic modeling of methane production, hydrogen sulfide production, and the relation of FOS/TAC was done by using the modified Gompertz equation (Equation (2)) (Wang and Wan, 2009; Hernández *et al*., 2021):

$$H = H_{\max} \exp\left\{-\exp\left[\frac{R_{\max} e}{H_{\max}}(\lambda - t) + 1\right]\right\} \qquad (2)$$

where $H_{\max}$ is the maximum cumulative value, $R_{\max}$ is a maximum rate, e is mathematical constant equivalent to 2.718282, λ is lag time, and t is cultivation time.

Excel version 2018 was used to analyze the data. To solve the equations this software utilizes the solver tool, which used the nonlinear-least-squares model estimation by the Levenberg-Marquardt method (Marquardt, 1963).

2.6 *Statistical analysis*

Statistical significance was assessed by analysis of variance (ANOVA) using Minitab software (Version 16, Minitab, Inc., State College, PA, USA).

3 Results and discussion

3.1 *Methane production and kinetic modeling*

The average cumulative methane production for treatments supplemented with urea, ammonium sulfate, and ammonium nitrate at different concentrations are presented in Fig. 1.

These profiles of curves resulted in a reverse L-shape cumulative curve characterized by high daily methane production in the initial phase of anaerobic digestion (approximately the first 15 days), followed by a decrease in daily methane production, until the end of the process at 60 days. At the end of the experimental period, the cumulative methane production from all treatments achieved average ranged from 7.1 up to 513 mL CH_4 g VS^{-1}.
This cumulative behavior curve is characteristic during the usage of organic waste that is composed of simple organic matter that is easily hydrolyzed into soluble compounds, enhancing the rate of the anaerobic digestion with subsequent methane production during the initial stages,

Table 2. Parameters obtained from the Gompertz model of the cumulative production of methane, hydrogen sulfide and FOS/TAC ratio for different sources of nitrogen. The values mean of parameters that do not share a letter are significantly different according to the one-way ANOVA. The first letter of the alphabet indicates higher mean values. Asterisks indicate the nitrogen source: * Urea, ** Ammonium Sulfate and *** Ammonium nitrate that were compared with the control

		Methane production				Hydrogen Sulfide				FOS/TAC ratio		
Source Nitrogen	**Concentration (mg L^{-1})**	**$H^{CH_4}_{max}$ (mL CH_4 g VS^{-1})**	**$R^{CH_4}_{max}$ (mL gVS^{-1} day^{-1})**	**λ_{CH_4} (day)**	**R^2**	**$H^{H_2SO_4}_{max}$ (ppm)**	**$R^{H_2SO_4}_{max}$ (ppm day^{-1})**	**$\lambda_{H_2SO_4}$ (day)**	**R^2**	**$R^{Fos/TAC}_{max}$ (mg L^{-1} $CaCO_3$/mgL^{-1} CH_3COOH) day^{-1}**	**$\lambda_{Fos/TAC}$ (day)**	**R^2**
Control	**0**	430.89±11.89^{B*,A**,A***}	87.56±1.89^{B*,BC**,A***}	1.86±1.3 1E$^{-03AB*,CD**,F***}$	0.99	1617.14±9.21^{D*,F**,A***}	253.35±4.37^{B*,F**,A***}	1.79±7.43E$^{-03A*,A**,D***}$	0.99	0.07±8.44E$^{-04C*,C**,D***}$	4.18±0.11^{D*,B**,B***}	0.95
	1000	513.95±2.12^{A*}	109.96±0.05^{A*}	1.63±5.08E^{-02B*}	0.98	1529.20±7.47^{D*}	212.24±5.58^{C*}	1.90±2.29E^{-02A*}	0.97	0.02±1.08E^{-03E*}	15.07±1.42E^{-02A*}	0.98
	2000	490.67±1.05^{A*}	102.66±0.03^{A*}	1.82±2.78E^{-02AB*}	0.98	1572.94±51.09^{D*}	219.19±4.90^{C*}	1.84±2.13E^{-02A*}	0.98	0.04±9.38E^{-04D*}	12.01±7.82E^{-02B*}	0.98
Urea	**3000**	346.49±12.54^{C*}	74.06±3.91^{C*}	2.03±1.63E^{-02AB*}	0.99	1735.10±15.17^{C*}	242.78±3.02^{A*}	1.52±5.52E^{-02B*}	0.97	0.05±1.86E^{-03D*}	5.76±0.42^{C*}	0.99
	4000	160.58±4.74^{D*}	33.39±0.51^{D*}	2.11±2.49E^{-01A*}	0.99	1996.67±9.93^{B*}	252.43±2.80^{A*}	1.53±2.98E^{-02B*}	0.99	0.07±3.61E^{-03B*}	3.35±4.23E^{-02E*}	0.96
	5000	89.57±0.25^{E*}	21.33±1.05^{E*}	2.18±4.76E^{-02A*}	0.98	2092.96±14.68^{A*}	257.25±1.35^{A*}	1.50±6.24E^{-02B*}	0.99	0.11±1.21E^{-03A*}	1.13±0.12^{F*}	0.97
	1000	445.37±1.71^{A**}	92.21±0.41^{AB**}	1.84±3.17E$^{-02CD**}$	0.98	2475.97±31.20^{E**}	343.82±4.64^{E**}	1.56±2.69E^{-02B**}	0.97	0.06±2.27E^{-03D**}	5.90±0.38^{A**}	0.97
	2000	461.76±10.11^{A*}	100.16±2.10^{A**}	1.77±5.60E^{-02D**}	0.97	2768.43±20.52^{D**}	391.81±1.00^{D**}	1.45±5.51E$^{-02BC**}$	0.98	0.06±1.26E^{-03D**}	5.83±.133^{A**}	0.91
Ammonium Sulfate	**3000**	325.63±16.50^{B**}	80.54±1.78^{C**}	1.99±4.18E$^{-02BC**}$	0.95	3231.88±24.82^{C**}	495.58±2.43^{C**}	1.34±4.59E$^{-02CD**}$	0.97	0.07±7.04E^{-04C**}	4.53±6.62E^{-02B**}	0.98
	4000	112.93±12.35^{C*}	29.64±4.24^{D**}	2.12±3.17E$^{-02AB**}$	0.95	3824.89±6.24^{B**}	554.64±2.16^{B**}	1.21±2.78E^{-02D**}	0.96	0.13±4.08E^{-04B**}	1.12±1.03E^{-02C**}	0.96
	5000	75.06±6.65^{C**}	21.44±1.88^{D**}	2.19±3.17E^{-02A**}	0.97	4044.90±41.87^{A**}	576.16±2.94^{A**}	0.94±3.17E^{-02E**}	0.95	0.35±3.24E^{-03A**}	0.69±1.32E^{-02D**}	0.97
	1000	415.93±5.44^{A***}	60.80±1.33^{B***}	2.05±2.22E$^{-02E***}$	0.98	267.69±0.37^{D***}	49.33±2.01^{D**}	2.65±2.40E$^{-02A***}$	0.94	0.04±5.49E$^{-05E***}$	13.64±1.91E$^{-03A***}$	0.98
	2000	71.08±1.49^{B***}	12.70±0.28^{C***}	2.53±9.47E$^{-03D***}$	0.99	280.39±3.16^{D***}	52.02±0.40^{D**}	2.64±5.40E$^{-02A***}$	0.97	0.16±5.39E$^{-03C***}$	1.11±1.58E$^{-02C***}$	0.99
Ammonium nitrate	**3000**	11.20±0.44^{C***}	6.36±0.18^{D*,**}	2.62±2.83E$^{-02C***}$	0.93	324.52±1.73^{C***}	76.62±0.04^{C**,*}	2.37±1.98E$^{-02B***}$	0.98	0.17±8.59E$^{-04BC***}$	0.96±2.34E$^{-02C***}$	0.97
	4000	7.88±0.24^{C***}	4.35±0.18^{D*,**}	2.73±3.10E$^{-02B***}$	0.90	342.25±3.49^{C***}	84.84±0.11^{B**,*}	2.19±6.33E$^{-02C***}$	0.93	0.17±1.96E$^{-03AB***}$	0.96±2.33E$^{-02C***}$	0.91
	5000	7.11±0.15^{C***}	4.24±0.04^{D*,**}	2.82±2.11E$^{-02A***}$	0.89	445.68±4.16^{B***}	87.65±0.63^{B**,*}	2.09±4.40E$^{-02C***}$	0.94	0.18±5.59E$^{-04A***}$	0.93±7.30E$^{-03C***}$	0.91

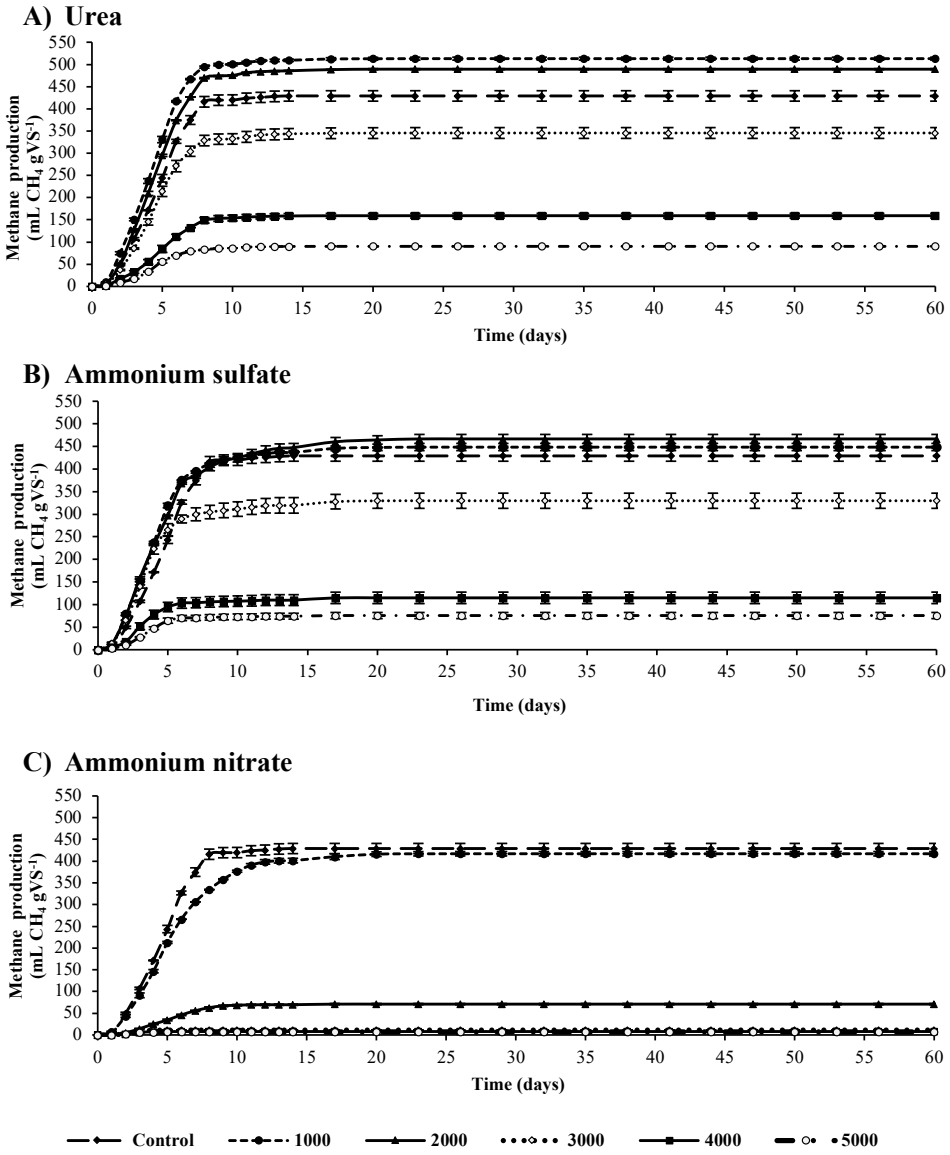

Fig. 1. Kinetic behavior of methane production of the different experimental treatments with A) Urea, B) Ammonium sulfate and C) Ammonium nitrate. Error bars represent standard error of the mean.

and after the majority of the organic material is consumed, methane production declines (Yao *et al*., 2018).

The methane production for different sources of nitrogen was modeled based on the modified Gompertz equation; kinetic parameter values are presented in Table 2. The high correlation coefficient (R^2) values that were at least 0.98 indicated a very strong linear relationship between experimental values and modeling data, which suggests that the modified Gompertz model fit the experimental data well. ANOVA complemented with the Tukey test was applied, to identify the statistical differences between nitrogen sources.

The experimental results of methane production presented in Fig. 1A showed that treatment supplemented with urea at a concentration of 1000 mg L^{-1} recorded 19.5% higher methane production than the control. The maximum production obtained was 513.0 ± 3.28 mL CH_4 g VS^{-1}, and 91.0% of this was produced within the first seven days of the digestion period. Likewise, the treatment at a concentration of 2000 mg L^{-1} exhibited the second-best effect on anaerobic digestion and recorded a

methane production level of 489.3 ± 1.16 mL CH_4 g VS^{-1}, which was 14.0% more than the control. About 87.2% of this methane production was reached in the first seven days. This could be attributed to the addition of urea which allowed achieving high values for the FOS/TAC ratio (Table 2), enhancing the production of methane. The treatments supplemented with urea at a concentration of 3000 mg L^{-1}, recorded a methane production of 345.5 ± 12.16 mL CH_4 g VS^{-1}, which was 19.4% less than the control. Concentrations of 4000 and 5000 mg L^{-1}, recorded values of 159.32 mL CH_4 g VS^{-1} and 90.22 mL CH_4 g VS^{-1}, respectively, which were 62.8% and 78.9% less than the control (Fig. 1A).

According to the results obtained relative to FA (Fig. 2) for the treatments at urea concentrations of 3000-5000 mg L^{-1}, a correlation between FA and decrease in methane production can be observed, since FA inhibits the activity of methanogens. The free ammonia produced in these concentrations could because of the direct hydrolyzed product of urea according to the following reaction (Equation (3)) (Tian *et al*., 2018):

$$CO(NH_2)_2 + 2H_2 \rightarrow 2NH_3 + H_2CO_3 \qquad (3)$$

Earlier, Jiang *et al*. (2012) also reported that an excess in urea concentration in anaerobic digestion led to inhibition of methanogens.

The kinetics values of the Gompertz equation for treatments supplemented with urea are presented in Table 2. The largest difference between experimental values and modeled methane production showed variability between 0.1% and 0.8%, indicating that the Gompertz equation adequately describes methane production.

The treatments at a urea concentration of 1000 mg L^{-1} showed a λ_{CH_4} value significantly lower than the control, indicating that the availability and biodegradability of the substrate favored hydrolysis, and reduced methane production. However, treatments supplemented with urea at concentrations beyond 1000 mg L^{-1}, but less than 3000 mg L^{-1} showed a positive effect on the rate of methane production, since $H_{\max}^{CH_4}$ and $R_{\max}^{CH_4}$ values were significantly higher than the control. However, for treatments with urea at concentrations of 4000 and 5000 mg L^{-1}, $H_{\max}^{CH_4}$ and $R_{\max}^{CH_4}$ values were significantly lower than the control (Table 2). In these concentrations, λ_{CH_4} value was significantly higher, which suggests that the microorganisms must have a longer adaptation period to overcome the stress caused by urea concentration. The results obtained can be due to the addition of urea after adjusting initial pH to 7 and 8 was a significantly stronger inhibitor with longer lag phases to methanogenesis than other nitrogen sources (Tian *et al*., 2018).

Results for the methane production using ammonium sulfate at different concentrations is shown in Fig. 1B. The highest methane production values were obtained at a concentration of 2000 mg L^{-1}, which was 466.64 ± 9.93 mL CH_4 g VS^{-1} and 8.78% higher than the control. At this concentration, 82.5% of the methane produced was within the first seven days of the digestion period. Supplementation at 1000 mg L^{-1} resulted in 448.41 ± 1.64 mL CH_4 g VS^{-1}, and this was 4.51% higher than the control. In the present study, the presence of photosynthetic bacteria (data not reported in this study) was detected in reactors supplemented with ammonium sulfate, which could have used the hydrogen sulfide produced as electron donor (Fig. 4B) and could have induced the displacement of NH_4^+ and helped to overcome ammonia (Fricke *et al*., 2007; Kitazaki, 2014).

At 3000 mg L^{-1}, 329.7 ± 16.78 mL CH_4 g VS^{-1} was recorded, which was 23.14% less than the control. A clear inhibitory effect on anaerobic digestion was observed at 4000 mg L^{-1} and 5000 mg L^{-1}, as the lowest methane production of 114.80 ± 12.73 mL CH_4 g VS^{-1} and 75.64 ± 6.80 mL CH_4 g VS^{-1} respectively was recorded. The inhibitory effect at higher concentrations could be the result of the breakdown of the potential membrane of microorganisms due to osmotic or ionic stress, as reported by Müller *et al*. (2006).

Previous studies have shown that methanogens are the most sensitive to high values of FA (Deublein and Steinhauser, 2008). Koster and Lettinga, (1988) reported that methanogens lost 56.5% of their activity at ammonia concentrations in the range of 4051-5734 mg L^{-1}, while the acidogenic population was hardly affected.

Results of kinetic parameters for treatments supplemented with sulfate ammonia are presented in Table 2. The values of $H_{\max}^{CH_4}$ and $R_{\max}^{CH_4}$ were significantly higher for 2000 mg L^{-1}, when compared to the control, while λ_{CH_4}) value was significantly lower (Table 2). For the treatment supplemented at 1000 mg L^{-1}, the values of $H_{\max}^{CH_4}$ and $R_{\max}^{CH_4}$ were slightly higher and the λ_{CH_4} value was slightly lower than the control. The results showed that supplementation with ammonia sulfate at concentrations 1000 and 2000 mg L^{-1} increased the rate of methane production and decreased the lag phase. This can be attributed to the fact that at

lower sulfate concentrations, sulfate-reducing bacteria (SRB) used propionate as substrate and reduced its toxicity, and the syntrophic activity with propionate oxidizing activity could have increased the methane production rate and decreased the lag phase (Reis *et al*., 1991; Zan and Hao, 2020). Li *et al*. (2015) also reported that the addition of sulfate (up to 200 mg L^{-1}) in the anaerobic digestion system could acclimate the sulfate-reducing bacteria to propionate utilization, causing those bacteria to oxidize propionate to acetate (Equation (4)), and having a positive effect on methane production.

$$C_2H_5COO^- + 0.75SO_4^{2-} \rightarrow CH_3COO^- + HCO_3^- + 0.75HS^- + 0.25H^+ \quad (4)$$

In the treatments supplemented with ammonium sulfate at concentrations of 3000 up to 5000 mg L^{-1}, the $H_{max}^{CH_4}$ and $R_{max}^{CH_4}$ values were significantly lower, while λ_{CH_4} values were significantly higher compared to the control (Table 2). These results indicated that at these concentrations, the methane production rate was affected. We attribute this behavior to the sulfate-reducing bacteria being able to successfully outcompete the methanogens for electrons, which affected the methane production (Schönheit *et al*., 1982; Bhattacharya *et al*., 1996).

The methane production values from the treatments supplemented with ammonium nitrate at different concentrations are shown in Fig. 1C. The methane production at concentration of 1000 mg L^{-1} was 417.01 ± 5.081 mL CH_4 gVS^{-1}, which was 2.80% lower compared to the control. At concentrations of 2000, 3000, 4000, and 5000 mg L^{-1}, methane production was significantly affected and was 83.4%, 97.4%, 98.16%, and 98.34% less than the control, respectively. This behavior can be due to the higher presence of nitrate rerouting the anaerobic digestion process towards denitrification (Ghyselbrecht *et al*., 2019a) and negatively affecting methane production. Similar behavior in the reduction of methane production concerning the increase in nitrate concentration was reported by Sheng *et al*. (2013). They reported that with the addition of sodium nitrate at a concentration of 0.5 g L^{-1}, a maximum methane production yield of 314.7 mL CH_4 g VS^{-1} was obtained, but the methane production yield was reduced by 50.1% when the added NO_3-N concentration exceeded 1.5 g L^{-1}.

The results of kinetic parameters $H_{max}^{CH_4}$ and $R_{max}^{CH_4}$ at a concentration of 1000 mg L^{-1} were significantly lower compared to the control, while λ_{CH_4} was significantly higher compared with control. For concentrations from 2000 to 5000 mg L^{-1}, $H_{max}^{CH_4}$ and $R_{max}^{CH_4}$ values were significantly lower, whereas λ_{CH_4} values were significantly higher than the control (Table 2). The presence of nitrate inhibits methanogenesis directly by changing the potential redox and as well as due to the formation of metabolic intermediates formed during denitrification, viz., nitrite, NO and N_2O (Roy and Conrad, 1999).

To determine the significant effects of supplemented treatments with urea, ammonium sulfate and ammonium nitrate on methane production, the Tukey test of a one-way ANOVA for media comparison of experimental methane production values was established as a statistical tool to help determine the best treatment (Table 2). For the treatment with urea at a concentration of 2000 mg L^{-1}, a methane production value of 513.0 ± 3.28 mL CH_4 g VS^{-1} was obtained, which was significantly higher compared to the control and other treatments. The maximum methane production results of this study were compared with other studies, which used cheese whey as a substrate (Table 3), and the methane production reported in this study is higher than the values reported in previous studies. Our results revealed that supplementation with urea at a concentration of 2000 mg L^{-1} improves the anaerobic digestion of cheese whey and increased methane production.

3.2 Free ammonia concentration

The result of FA concentration for different treatments is presented in Fig. 2. The significant difference between treatments is indicated with distinct letters based on ANOVA. Free ammonia concentrations for different treatments supplemented with urea are presented in Fig. 2A. The treatments supplemented with urea at concentrations between 3000, 4000, and 5000 mg L^{-1} at 60 days anaerobic digestion recorded free ammonium concentrations of 195.23 ± 1.12, 225.11 ± 4.28, and 239.40 ± 0.60, respectively, and were significantly higher than the control (150.26 ± 5.13). As mentioned earlier, the addition of higher concentrations of urea promoted the production of free ammonia and affected methane production (Lv *et al*., 2018). On the other hand, treatments supplemented with urea at concentrations of 1000 and 2000 mg L^{-1} recorded free ammonium concentrations of 158.92 ± 0.88 and 159.29 ± 1.10 mg L^{-1}, which were not significantly different when compared to the control. These results indicate that the supplementation of

urea in concentrations of 1000 and 2000 mg L^{-1} does not promote free ammonia production, but rather improves the nutritive value of the anaerobic digestion process, which induced a greater production of methane (Fig. 1A). Free ammonia concentration values at different concentrations of ammonia sulfate are shown in Fig. 2B. Free ammonia concentrations values for treatments supplemented with ammonia sulfate at concentrations of 1000 and 2000 mg L^{-1} at 60 days of anaerobic digestion were 157.88 ± 0.17 and 159.67 ± 1.33 mg L^{-1} respectively, which were not significantly different from the control. However, free ammonia concentrations were significantly higher than the control, with values of 218.84 ± 0.72, 256.70 ± 2.66, and 263.99 ± 1.22 mg L^{-1} at ammonia sulfate at concentrations between 3000 and 5000 mg L^{-1}. From the results obtained at concentrations of 1000 and 2000 mg L^{-1}, the free ammonium concentration did not increase, and hence did not show a negative effect on methane production (Fig. 1B). Previous studies have also shown that the increase in the addition of ammonia and sodium sulfate corresponded to an increase in free ammonium concentration, which caused a decrease in biogas production (Siles *et al*., 2010). Further, free ammonia could diffuse passively into the cell, causing proton imbalance and consequent changes in the intracellular pH, potassium deficiency, an increase in the maintenance energy requirement, and inhibit enzyme activity (Sprott and Patel, 1986). The treatments supplemented with ammonia nitrate at a concentration of 1000 mg L^{-1} at 60 days of anaerobic digestion recorded a free ammonia concentration of 49.18 ± 9.66 mg L^{-1}, which was significantly lower than the control (Fig. 2C). The results indicated that this concentration of ammonium nitrate did not show an inhibitory effect on the production of methane (Fig. 1C). Low concentrations of free ammonia may be due to the possibility that nitrate addition denitrification occurs for reduced to nitrite, which will stimulate the development of anammox bacteria that, under anaerobic conditions, converted ammonia and nitrite to nitrogen gas (González *et al*., 2019).

Table 3. Methane production values reported in anaerobic digestion studies that used cheese whey as substrate

Reactor	Inoculum	Temperature	Methane production	Reference
UASB**	SST	35 ºC	424 mL CH_4 /g COD	Ergüder *et al*. (2001)
UASB**	SAWW	21.6 ºC	238-308 mLCH_4 /g COD	Cárdenas *et al.* (2020)
AD***	SST	35 ºC	211 mL CH_4 / g VS	Comino *et al.* (2009)
AD****	CD	35 ºC	340 mL CH_4 / g VS	Comino *et al.* (2012)
AD*	PM	37 ºC	223 mL CH_4 / g VS	Carlini *et al.* (2015)
SBR*	SWW	32 ºC	270 mL CH_4/g VS	Antonelli *et al.* (2016)
ASBR*	SST	35 ºC	314 mL CH_4 / g COD	Fernández *et al* (2016)
AD*	PD	36.5 ºC	321 mL CH_4 / g VS	Kim *et al.* (2017)
AD*	BS	35 ºC	22-36 mL CH_4 / g COD	Mainardis *et al.* (2017)
AD*	CS	37 ºC	600 mL CH_4 / g VS	Escalante *et al.* (2017)
CSTR***	BS	37 ºC	381 mL CH_4 / g VS	Ramos *et al.* (2019)
AD*	CD	35 ºC	469 mL CH_4 / g VS	Cisneros *et al.* (2021)
AD*	CD	35 ºC	513 mL CH_4 / g VS	This Study

AD: Anaerobic digester; ASBR: Anaerobic sequencing batch reactors; BS: Biodigester sludge; CD: Cow dung; COD: Chemical oxygen demand; CS: Cattle slurry; CSTR: Continuous stirred-tank reactor; PD: Pig Droppings; PM: Poultry manure; SAWW: Sludge anaerobic of wastewater; SBR: Sequential batch reactors; SST: Sewage sludge treatment; SWW: Swine wastewater; UASB: Up-flow anaerobic sludge blanket; VS: Volatile solid; *One treatment phase; **Two treatment phases; *** Three treatment phases; ****Four treatment phases.

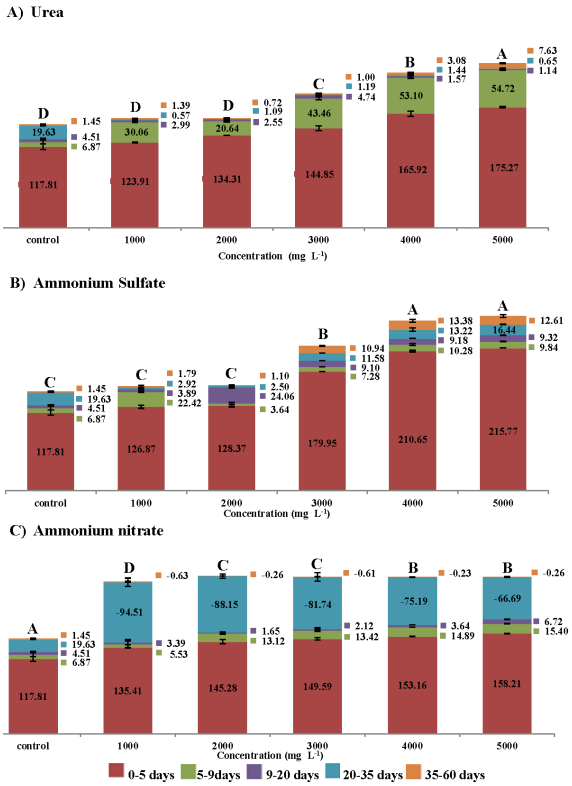

Fig. 2. Free ammonia (FA) in treatments with different concentrations of nitrogen source. The values in graphs show the FA concentration in mg L^{-1} according to the period of each treatment. The values mean of FA that do not share a letter are significantly different according to the one-way ANOVA. The first letter of the alphabet indicates higher mean values. Negative values indicate loss of FA concentration. Error bars represent standard error of the mean.

Therefore in such processes, the coordination of anammox and denitrifying bacteria for nitrogen removal the following reactions occurs (Equations (5) and (6)) (Waki *et al*., 2013; Li and Peng, 2020)

$$2NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$$

(Denitrification: nitrate reduction to nitrite) (5)

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

(*Anammox*) (6)

In the case of treatments supplemented with ammonium nitrate at concentrations from 2000 to 5000 mg L^{-1}, free ammonia concentrations of 71.64± 7.63, 82.77±9.14,96.25±0.49 and 113.37±0.15 mg L^{-1}, respectively, were recorded, which were significantly higher compared with treatment supplemented at a concentration of 1000 mg L^{-1}, but significantly lower than the control. Therefore in the supplemented with ammonium nitrate we attribute that the concentration of free ammonia was not the cause of the inhibitory effect of methane production (Fig. 1C). Percheron *et al*. (1999) reported that methane production stopped as soon as denitrification started. Concurrently, increases in the redox potential and transient nitrite production were observed.

3.3 FOS/TAC ratio

The FOS/TAC ratio is one of the most significant operative parameters in AD processes, since it allows us to know the digester's stability. The optimum FOS/TAC ratio for anaerobic digestion is between 0.3-0.4, in which the biogas production is maximized (Logan *et al*., 2019; Fiore *et al*., 2016).

The kinetic behaviors of FOS/TAC ratios for different sources of nitrogen are shown in figure 3. To better interpret and characterize the data obtained, the Gompertz model was used to determine the parameters of $R_{\max}^{Fos/TAC}$ and $\lambda_{Fos/TAC}$ (Table 2). The parameter of $\lambda_{Fos/TAC}$ indicated the time that the treatment remained in the optimal region of the FOS/TAC ratio for methane production. Meanwhile the $R_{\max}^{Fos/TAC}$ parameter indicated the maximum rate at which the anaerobic digestion process takes place as a function of the FOS/TAC ratio.

An ANOVA was performed on the data of the parameters obtained in the different treatments. Fig. 3A presents the graphs that show the kinetic behavior of the FOS/TAC ratio for the different treatments supplemented with urea. The graphs showed that the control and the treatments supplemented with urea at concentrations of 1000 up to 3000 mg L^{-1} show a decreasing trend in the FOS/TAC ratio values, obtained for 60 days values in a range between 0.3 and 0.2. On the other hand, for the treatments supplemented with 4000 and 5000 mg L^{-1}, the values obtained from the FOS/TAC ratio showed an increasing trend about the control, having values in a range between 0.8 and 1.2 for 60 days. These results indicated that at concentrations of 4000 and 5000 mg L^{-1} there is an accumulation of volatile fatty acids (VFA) which is attributable to an inhibition of methanogenesis. We attribute that the accumulation of VFA may be because, at concentrations 4000 and 5000 mg L^{-1}, a higher concentration of free ammonia was generated (Fig. 2A), which is potentially toxic to methanogenic microorganisms, which could have led to an inhibition of VFA consumption. Lv *et al*. (2018) reported that the accumulation of VFA could

affect methane production (Fig. 1A) since, in VFA, there are undissociated acids that have an inhibitory effect on the anaerobic digestion process. According to the ANOVA for the treatments supplemented with urea at concentrations of 1000 up to 3000 mg L^{-1}, the $\lambda_{Fos/TAC}$ were significantly higher than the control, while the treatments supplemented with the concentrations of 4000 and 5000 mg L^{-1} obtained significantly lower values. The $R_{\max}^{Fos/TAC}$ values for the concentrations of 1000 up to 3000 mg L^{-1} were significantly lower compared to the control, while the treatments supplemented with the concentrations of 4000 and 5000 mg L^{-1} obtained significantly higher values (Table 2).

This indicated that supplementation with urea at concentrations of 1000, 2000, and 3000 mg L^{-1} maintained the optimal FOS/TAC ratio for a longer time, which induced greater stability in the anaerobic digestion process. This could explain the highest values in methane production (Fig. 1A). This behavior could be due to the enzymatic hydrolysis of urea ($CO(NH_2)_2$) that produces CO2 and ammonia, resulting in the addition of 2 eq. of H^+ per mole of urea, thus increasing buffering capacity of the system and potentially improving the degradation of VFA to convert to methane (Boncz *et al*., 2012).

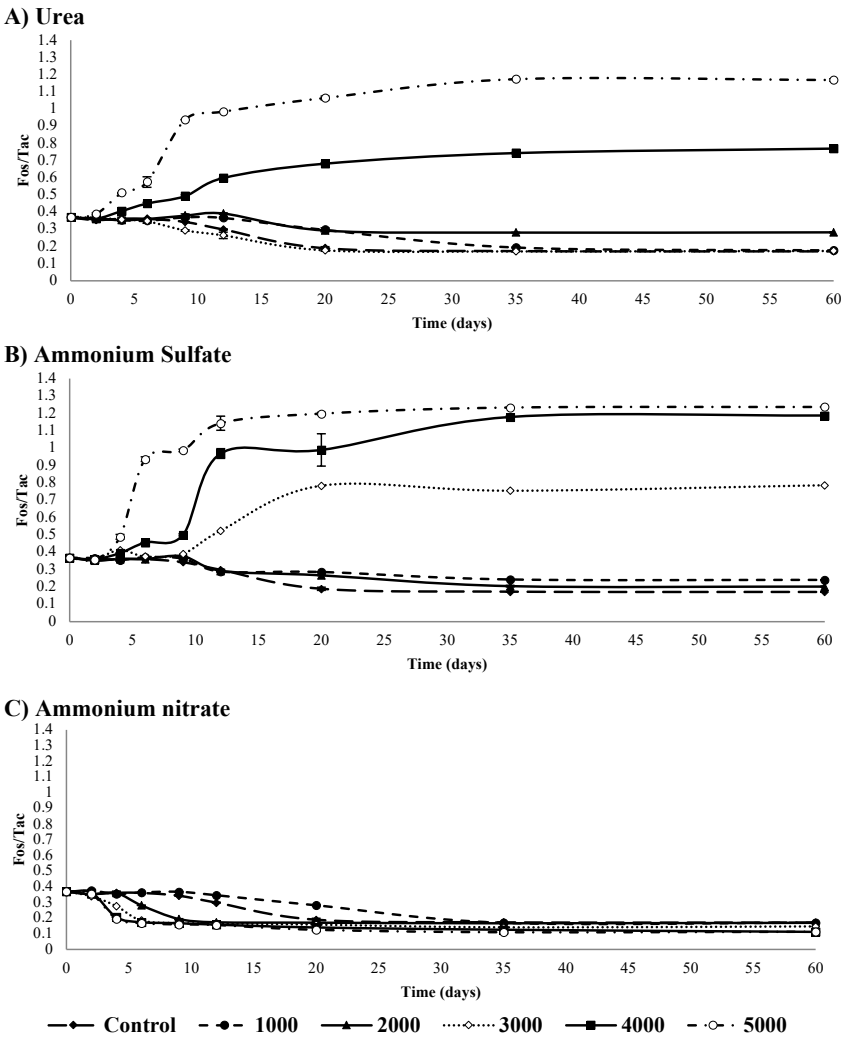

Figure 3. FOS/TAC trends measured during the lab scale tests performed of different treatments supplemented with different concentrations of A) Urea, B) Ammonium sulfate and C) Ammonium nitrate. Error bars represent standard error of the mean.

Fig. 3B shows the kinetic behavior of the FOS/TAC ratio values for the treatments supplemented with ammonium sulfate at concentrations of 3000, 4000, and 5000 mg L^{-1}, showing a trend of increase compared to the control, reaching values of 0.78 ± 1.31 E^{-02}, 1.18 ± 3.77 E^{-03} and 1.23 ± 1.05 E^{-03} respectively for 60 days of anaerobic digestion. Meanwhile for the concentrations of 1000 and 2000 mg L^{-1}, the kinetic behaviors showed a trend of decrease in the FOS/TAC ratio values, obtaining values for 60 days of anaerobic digestion of 0.24 ± 5.22 E^{-04} and 0.20 ± 3.16 E^{-03}, respectively.

According to the ANOVA, the treatments supplemented with ammonium sulfate at concentrations of 1000 and 2000 mg L^{-1}, the $\lambda_{Fos/TAC}$ values obtained were significantly higher compared to the control, while the $\lambda_{Fos/TAC}$ at a concentration of 3000 mg L^{-1} was without significant differences compared to the control. On the other hand, for the concentrations of 4000 and 5000 mg L^{-1}, the $\lambda_{Fos/TAC}$ obtained were significantly lower compared to the control (Table 2). The $R_{\max}^{Fos/TAC}$ for the concentrations of 1000 and 2000 mg L^{-1} were significantly lower than the control, while for the concentration of 3000 mg L^{-1}, there was no significant difference compared to the control. For the concentrations of 4000 and 5000 mg L^{-1}, the $R_{\max}^{Fos/TAC}$ values obtained were significantly higher compared to the control (Table 2). These results indicated that with supplementation with ammonium sulfate at concentrations of 1000 and 2000 mg L^{-1}, there is greater stability of the anaerobic digestion process, and this may have influenced the increase in methane production (Fig. 1B). The gradual increase in the concentration of ammonium sulfate from 3000 to 5000 mg L^{-1} causes instability in the anaerobic digestion process, suggesting an inadequate conversion of the substrate to methane. This is because, at concentrations of 3000 to 5000 mg L^{-1}, the concentration of H_2S was higher (Fig. 4A), which is an indicator of the proliferation of reducing sulfate bacteria, which presumably in the presence of sulfate tend to consume hydrogen and acetate (Equations (7) and (8)). This causes causing instability in the anaerobic digestion process that leads to an inhibitory effect during methanogenesis (Karhadkar *et al*., 1987; McCartney and Oleszkiewicz, 1991).

$$SO_4^{2-} + 4H_2^2 \rightarrow H_2S + 2H_2O + 2OH^- \quad (7)$$

$$SO_4^{2-} + CH_3COOH \rightarrow H_2S + 2H_2CO_3^- + 2OH^- \quad (8)$$

Another effect that may have caused the destabilization of the anaerobic digestion process was reported by Yuan and Zhu (2016) and Sürmeli *et al*. (2019), where hydrogen sulfide can easily diffuse through the cell membrane and suppress the activity of methanogens.

Fig. 3C shows the kinetic behaviors for the different treatments supplemented with ammonium nitrate from 1000 up to 5000 mg L^{-1}, showing a decreasing trend in the FOS/TAC ratio, obtaining values for 60 days of anaerobic digestion in a range from 0.1 to 0.17. These results indicate that the decrease in the FOS/TAC ratio in the anaerobic digestion process of the different treatments was because of the lack of VFA (Logan *et al*., 2019). From the ANOVA, it was obtained that the $\lambda_{Fos/TAC}$ values for the treatments supplemented with ammonium nitrate at a concentration of 1000 mg L^{-1} were significantly higher compared with the control, while the values from the concentration 2000 to 5000 mg L^{-1} were significantly lower (Table 2). The $R_{\max}^{Fos/TAC}$ values obtained in the treatments supplemented with ammonium nitrate at a concentration of 1000 mg L^{-1} were significantly lower than the control, while for the treatments supplemented at concentrations of 2000 to 5000 mg L^{-1}, significantly higher values were obtained (Table 2).

We correlated these results with the data obtained in the production of methane (Fig. 1C and 2C) since only for the concentration of 1000 mg L^{-1} the highest production of methane values were obtained, suggesting that the metabolic transformation of the VFA was directed to methane and reduction of ammonia by denitrification.

However, for concentrations from 2000 to 5000 mg L^{-1}, there was a low production of methane, which indicates that most likely, as there was a greater quantity of nitrates, the denitrification process was increased, consuming more VFA and part of the methane produced, and therefore there was a greater destabilization of the anaerobic digestion process.

Earlier, Lin and Gu. (2020) and Soto *et al*. (2007) reported that denitrification is carried out by anoxic microorganisms that can use nitrate as a terminal electron acceptor and organic compounds as electron donors for microbial respiration.

3.4 Hydrogen sulfide concentration

The cumulative H_2S concentration over the digestion period of different sources of nitrogen is illustrated in Fig. 4. In general, the H_2S concentrations of all treatments were in the range of 266.5 to 4024.1 ppm.

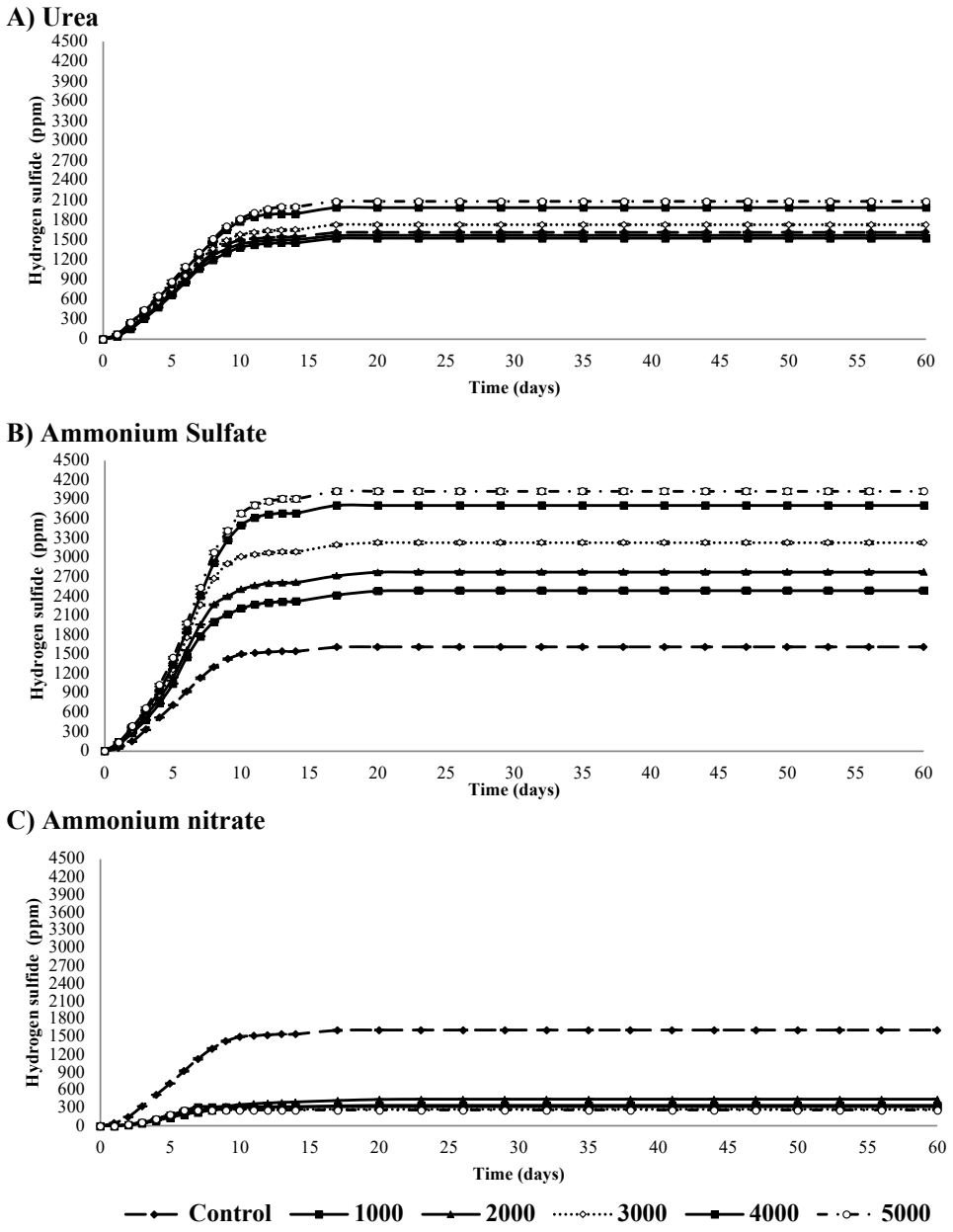

Figure 4. Hydrogen sulfide concentration accumulation in treatments with different concentrations of A) Urea, B) Ammonium sulfate and C) Ammonium nitrate. Error bars represent standard error of the mean.

Table 2 shows the parameters of the maximum H_2S production rate ($R_{\max}^{H_2SO_4}$), maximum production ($H_{\max}^{H_2SO_4}$), and period of duration of the lag phase ($\lambda_{H_2SO_4}$) determined from the Gompertz model, which allowed us to learn more about the effect of different treatments on the anaerobic digestion process. According to the ANOVA, the $H_{\max}^{H_2SO_4}$ and $R_{\max}^{H_2SO_4}$ values obtained for treatments supplemented with urea at concentrations of 3000 to 5000 mg L^{-1} were significantly higher compared to the control, while the $\lambda_{H_2SO_4}$ values were significantly lower compared to the control, which indicated that at these concentrations, the metabolism of H_2S production is favored during the anaerobic digestion process. On the other hand, treatments supplemented with urea at concentrations of 1000 and 2000 mg L^{-1} had $H_{\max}^{H_2SO_4}$, $R_{\max}^{H_2SO_4}$, and $\lambda_{H_2SO_4}$ values without significant differences compared to the control (Table

2), suggesting that at these concentrations, the metabolic synthesis of H_2S is not favored. The treatments supplemented with ammonium sulfate at concentrations of 1000 up to 5000 mg L^{-1} had $H_{max}^{H_2SO_4}$ and $R_{max}^{H_2SO_4}$ values significantly higher compared to the control, while the $\lambda_{H_2SO_4}$ values were significantly lower compared to the control (Table 2). This behavior can be attributed to the sulfate supplement serving as an electron source for sulfate-reducing bacteria since these bacteria use inorganic sulfate as an external electron acceptor in the oxidation of energy substrates, resulting in the production of H_2S (Barton and Fauque, 2009; Hedderich *et al*., 1998). In treatments supplemented with ammonium nitrate at concentrations from 1000 to 5000 mg L^{-1}, significantly lower $H_{max}^{H_2SO_4}$ and $R_{max}^{H_2SO_4}$ values were obtained compared to the control, while the $\lambda_{H_2SO_4}$ values obtained were significantly higher than the control. Comparing the different treatments supplemented with urea, ammonium sulfate and ammonium nitrate shown in Fig. 4, the supplementation of ammonium sulfate had the greatest positive effect on the metabolic production of H_2S. While on the other hand, with the supplementation with ammonium nitrate, the production of H_2S decreased notably, since the lowest concentration values were obtained.

One possible explanation is that nitrate-reducing bacteria used nitrate as an alternative terminal acceptor leaving sulfate aside, which led to a decrease in H_2S (Marietou, 2016). Another explanation is that nitrate produces inhibitory osmotic stress in sulfate-reducing bacteria, affecting their metabolism and growth (He *et al*., 2010).

Conclusions

The highest methane production was recorded in the treatment supplemented with urea at 1000 mg L^{-1}, which showed a 19% increase from the control. Furthermore, the addition of 1000 mg L^{-1} of urea led to less variation in the FOS/TAC ratio, which is one of the crucial parameters related to the anaerobic digestion stability. The addition of ammonium nitrate at a concentration of 1000 mg L^{-1} resulted in a 63.2% reduction of free ammonium concentration when compared to the control. However, with this treatment, it was possible to reduce the concentration of H_2S, obtaining lower values of 267.69 ± 0.37 ppm, which is very important and interesting in terms of the quality of the biogas and production costs.

The addition of ammonium sulfate at higher concentrations increased the hydrogen sulfide production, and the highest values were recorded at 5000 mg L^{-1}, which negatively affected the anaerobic digestion process. These results revealed that the effect of supplementation of nitrogen varied with type and its concentrations in the anaerobic digestion process of cheese whey.

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References

Ahmad, T., and Zhang, D. (2020). A critical review of comparative global historical energy consumption and future demand: The story told so far. *Energy Reports 6*, 1973-1991. https://doi.org/10.1016/j.egyr.2020.07.020

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., Kalyuzhnyi, S., Jenicek, P., and Van Lier, J. B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science and Technology 59*, 927-934. https://doi.org/10.2166/wst.2009.040

Antonelli, J., Lindino, C.A., de Azevedo, J.C.R., de Souza, S.N.M., Cremonez, P.A., and Rossi, E. (2016). Biogas production by the anaerobic digestion of whey. *Revista de Ciências Agrárias 39*, 463-468. https://doi.org/10.19084/RCA15087

APHA. (2012). *Standard Methods for the Examination of Water and Wastewater*, 22th. American Public Health Association.New York

Barton, L.L., and Fauque, G.D. (2009). Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Advances in Applied Microbiology 68*, 41-98. https://doi.org/10.1016/S0065-2164(09)01202-7

Bhattacharya, S.K., Uberoi, V., and Dronamraju, M.M. (1996). Interaction between acetate

fed sulfate reducers and methanogens. *Water Research 30*, 2239-2246. https://doi.org/10.1016/0043-1354(95)00238-3

Boncz, M.A., Formagini, E.L., Santos, L.D.S., Marques, R.D., and Paulo, P.L. (2012). Application of urea dosing for alkalinity supply during anaerobic digestion of vinasse. *Water Science and Technology 66*, 2453-2460. https://doi.org/10.2166/wst.2012.476

Bonk, F., Popp, D., Weinrich, S., Sträuber, H., Kleinsteuber, S., Harms, H., and Centler, F. (2018). Ammonia inhibition of anaerobic volatile fatty acid degrading microbial communities. *Frontiers in Microbiology 9*, 1-13. https://doi.org/10.3389/fmicb.2018.02921

Cadena, R.O., Rivera, E.M., and Herrera, G. (2010). Automatic volumetric gas flow meter for monitoring biogas production from laboratory-scale anaerobic digester. *Sensors and Actuators, B: Chemical 147*, 10-14. https://doi.org/10.1016/j.snb.2010.03.053

Cárdenas, K.N., Fajardo, M.C., Schettino, B.S., Meraz, M.A., and Castilla, P. (2020). Acidogenesis/methanogenesis from acid cheese whey in hybrid-UASB reactors. *Revista Mexicana de Ingeniería Química 19*, 17-27. https://doi.org/10.24275/rmiq/IA1420.

Carlini, M., Castellucci, S., and Moneti, M. (2015). Biogas production from poultry manure and cheese whey wastewater under mesophilic conditions in batch reactor. *Energy Procedia 82*, 811-818. https://doi.org/10.1016/j.egypro.2015.11.817

Chatzipaschali, A.A., and Stamatis, A.G. (2012). Biotechnological utilization with a focus on anaerobic treatment of cheese whey: Current status and prospects. *Energies 5*, 3492-3525. https://doi.org/10.3390/en5093492

Cisneros, S., Veana, F., Arjona, M.A., Álvarez, C.L., and Pérez, S.B. (2021). Optimization of variables from the anaerobic digestion process of cheese whey in biogas production. *Revista Internacional de Contaminación Ambiental 37*, 307-18. https://doi.org/10.20937/RICA.53879.

Comino, E., Rosso, M., and Riggio, V. (2009). Development of a pilot scale anaerobic digester for biogas production from cow manure and whey mix. *Bioresource Technology 100*, 5072-5078. https://doi.org/10.1016/j.biortech.2009.05.059

Comino, E., Riggio, V.A., and Rosso, M. (2012). Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. *Bioresource Technology 114*, 46-53. https://doi.org/10.1016/j.biortech.2012.02.090

Dannesboe, C., Hansen, J.B., and Johannsen, I. (2019). Removal of sulfur contaminants from biogas to enable direct catalytic methanation. *Biomass Conversion and Biorefinery 9*, 1-12. https://doi.org/10.1007/s13399-019-00570-7

Demirel, B., and Scherer, P. (2008). Production of methane from sugar beet silage without manure addition by a single-stage anaerobic digestion process. *Biomass and Bioenergy 32*, 203-209. https://doi.org/10.1016/j.biombioe.2007.09.011

Deublein, D., and Steinhauser, A. (2008). Process parameters. *Biogas from Waste and Renewable Resources* (Deublein, D., and Steinhauser, A. eds.), 125-127. Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany https://doi.org/10.1002/9783527621705

Dumont, E. (2015). H_2S removal from biogas using bioreactors: a review. *International Journal of Energy and Environment 6*, 479-498.

El-Mashad, H.M., and Zhang, R. (2010). Biogas production from co-digestion of dairy manure and food waste. *Bioresource Technology 101*, 4021-4028. https://doi.org/10.1016/j.biortech.2010.01.027

Elasri, O., and El amin Afilal, M. (2016). Potential for biogas production from the anaerobic digestion of chicken droppings in Morocco. *International Journal of Recycling of Organic Waste in Agriculture 5*, 195-204. https://doi.org/10.1007s40093-016-0128-4

Ergüder, T., Tezel, U., Güven, E., and Demirer, G. (2001). Anaerobic biotransformation and methane generation potential of cheese whey in batch and UASB reactors. *Waste Management*

21, 643-650. https://doi.org/10.1016/S0956-053X(00)00114-8

Escalante, H., Castro, L., Amaya, M.P., Jaimes, L., and Jaimes, J. (2018). Anaerobic digestion of cheese whey: Energetic and nutritional potential for the dairy sector in developing countries. *Waste Management 71*, 711-718. https://doi.org/10.1016/j.wasman.2017.09.026

Estevez, M.M., Linjordet, R., and Morken, J. (2012). Effects of steam explosion and co-digestion in the methane production from Salix by mesophilic batch assays. *Bioresource Technology 104*, 749-756. https://doi.org/10.1016/j.biortech.2011.11.017

Fernández, C., Martínez, E.J., Morán, A., and Gómez, X. (2016). Biological treatments of cheese whey for biogas and hydrogen production. Review. *Revista Investigación, Optimización y Nuevos Procesos en Ingeniería 29*, 47-62. https://doi.org/10.18273/revion.v29n1-2016004

Fiore, S., Ruffino, B., Campo, G., Roati, C., and Zanetti, M.C. (2016). Scale-up evaluation of the anaerobic digestion of food-processing industrial wastes. *Renewable Energy 96*, 949-959. https://doi.org/10.1016/j.renene.2016.05.049

Fricke, K., Santen, H., Wallmann, R., Hüttner, A., and Dichtl, N. (2007). Operating problems in anaerobic digestion plants resulting from nitrogen in MSW. *Waste Management 27*, 30-43. https://doi.org/10.1016/j.wasman.2006.03.003

Ghyselbrecht, K., Monballiu, A., Somers, M.H., Sigurnjak, I., Meers, E., Appels, L., and Meesschaert, B. (2019b). The fate of nitrite and nitrate during anaerobic digestion. *Environmental Technology 40*, 1013-1026. https://doi.org/10.1080/09593330.2017.1415380

González, G., Pérez, V. Orozco, J. Aguirre, J.F. Beristain, R., and Buendía, L. (2019). Kinetics and microbial structure of nitrogen cycle bacteria contained in the rhizosphere of natural wetland polluted with chromium. *Revista Mexicana de Ingeniería Química 19*, 543-53. https://doi.org/10.24275/rmiq/IA660.

Guiot, S.R., and Frigon, J.C. (2012). Microbial technologies in advanced biofuels production. *Microbial Technologies in Advanced Biofuels Production* (P.C. Hallenbeck, eds.), 143-161. Springer, US. https://doi.org/10.1007/978-1-4614-1208-3

He, Q., He, Z., Joyner, D.C., Joachimiak, M., Price, M.N., Yang, Z.K., Yen, H.C.B., Hemme, C.L., Chen, W., Fields, M.M., Stahl, D.A., Keasling, J.D., Keller, M., Arkin, A.P., Hazen, T.C., Wall, J.D., and Zhou, J. (2010). Impact of elevated nitrate on sulfate-reducing bacteria: a comparative study of *Desulfovibrio vulgaris*. *ISME Journal 4*, 1386-1397. https://doi.org/10.1038/ismej.2010.59

Hedderich, R., Klimmek, O., Kröger, A., Dirmeier, R., Keller, M., and Stetter, K.O. (1998). Anaerobic respiration with elemental sulfur and with disulfides. *FEMS Microbiology Reviews 22*, 353-381. https://doi.org/10.1016/S0168-6445(98)00035-7

Hernández, V. C., Benítez, G., Fajardo, M. C., Rojas, U., and Salazar, M. L. (2021). Analysis of the transient inhibited steady-state in anaerobic digestion of a semisolid from pretreated bovine slaughterhouse wastewater. *Revista Mexicana de Ingeniera Quimica 20*, 541-553. https://doi.org/10.24275/rmiq/IA2012

Igarashi, K., and Kuwabara, T. (2016). Hydrogen-sulfide-free methane production by fermenter-methanogen syntrophy using dacite pumice under aerobic gas phase. *Energy and Fuels 30*, 4945-4950. https://doi.org/10.1021/acs.energyfuels.6b00494

Jiang, Y., Heaven, S., and Banks, C.J. (2012). Strategies for stable anaerobic digestion of vegetable waste. *Renewable Energy 44*, 206-214. https://doi.org/10.1016/j.renene.2012.01.012

Karhadkar, P.P., Audic, J.M., Faup, G.M., and Khanna, P. (1987). Sulfide and sulfate inhibition of methanogenesis. *Water Research 21*, 1061-1066. https://doi.org/10.1016/0043-1354(87)90027-3

Kim, M.J., and Kim, S.H. (2017). Minimization of diauxic growth lag-phase for high-efficiency biogas production. *Journal of Environmental*

Management 187, 456-463. https://doi.org/10.1016/j.jenvman.2016.11.002

Kitazaki, S. (2014). Hydrogen sulfide generation suppression by nitrate addition application to solid waste landfill site. *American Journal of Environmental Protection 3*, 267-274. https://doi.org/10.11648/j.ajep.20140305.20

Koster, I.W., and Lettinga, G. (1988). Anaerobic digestion at extreme ammonia concentrations. *Biological Wastes 25*, 51-59. https://doi.org/10.1016/0269-7483(88)90127-9

Krakat, N., Anjum, R., Dietz, D., and Demirel, B. (2017). Methods of ammonia removal in anaerobic digestion: A review. *Water Science and Technology 76*, 1925-1938. https://doi.org/10.2166/wst.2017.406

Li, Z., and Peng, Y. (2020). Biphasic effect of nitrate on anaerobic ammonium oxidation (anammox) and related kinetic modeling. *Chemosphere 238*, 1-36. https://doi.org/10.1016/j.chemosphere.2019.124654

Lin, Y.H., and Gu, Y.J. (2020). Denitrification kinetics of nitrate by a heterotrophic culture in batch and fixed-biofilm reactors. *Processes 8*, 2-20 https://doi.org/10.3390/PR8050547

Logan, M., Safi, M., Lens, P., and Visvanathan, C. (2019). Investigating the performance of internet of things based anaerobic digestion of food waste. *Process Safety and Environmental Protection 127*, 277-287. https://doi.org/10.1016/j.psep.2019.05.025

Lv, Z., Jiang, J., Liebetrau, J., Richnow, H.H., Fischer, A., Ács, N., and Nikolausz, M. (2018). Ammonium chloride vs urea-induced ammonia inhibition of the biogas process assessed by stable isotope analysis. *Chemical Engineering and Technology 41*, 671-679. https://doi.org/10.1002/ceat.201700482

Ma, X., Jiang, T., Chang, J., Tang, Q., Luo, T., and Cui, Z. (2019). Effect of substrate to inoculum ratio on biogas production and microbial community during hemi-solid-state Batch anaerobic co-digestion of rape straw and dairy manure. *Applied Biochemistry and Biotechnology 189*, 884-902. https://doi.org/10.1007/s12010-019-03035-9

Mainardis, M. (2017). Characterization and BMP tests of liquid substrates for high-rate anaerobic digestion. *Chemical and Biochemical Engineering Quarterly 31*, 509-518. https://doi.org/10.15255/CABEQ.2017.1083

Marchioro, V., Steinmetz, R.L.R., do Amaral, A.C., Gaspareto, T.C., Treichel, H., and Kunz, A. (2018). Poultry litter solid state anaerobic digestion: effect of digestate recirculation intervals and substrate/inoculum ratios on process efficiency. *Frontiers in Sustainable Food Systems 2*, 1-10. https://doi.org/10.3389/fsufs.2018.00046

Marietou, A. (2016). Nitrate reduction in sulfate-reducing bacteria. *FEMS Microbiology Letters* 363, 1-4. https://doi.org/10.1093/femsle/fnw155

Marquardt, D.W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *Journal of the Society for Industrial and Applied Mathematics 11*, 431-441. http://www.jstor.org/stable/2098941

Mazorra, M.Á., and Moreno, J.M. (2019). Properties and options for the valorization of whey from the artisanal cheese industry. *CienciaUAT 14*, 133-144. https://doi.org/10.29059/cienciauat.v14i1.1134

McCartney, D.M., and Oleszkiewicz, J.A. (1991). Sulfide inhibition of anaerobic degradation of lactate and acetate. *Water Research 25*, 203-209. https://doi.org/10.1016/0043-1354(91)90030-T

Monroy, S.G., Jiménez A., Gutiérrez M., and Medina S.A. (2020). Biodigester with Mixing by hydraulic recirculation of the wastewater on biogas production: fundamentals in the design and scaling by a dimensional analysis. *Revista Mexicana de Ingeniería Química 19*, 81-99. https://doi.org/10.24275/rmiq/Bio1545.

Müller, T., Walter, B., Wirtz, A., and Burkovski, A. (2006). Ammonium toxicity in bacteria. *Current Microbiology 52*, 400-406. https://doi.org/10.1007/s00284-005-0370-x

Muñoz, K., Poggi, H., García, J., Ponce, M., Ramos, A., Barrera, J., Robles, I., Ruiz, N., Villa, L., and Rinderknecht, N. (2014). Cheese whey as substrate of batch hydrogen production: Effect

of temperature and addition of buffer. *Waste Management & Research 32*, 434-440. https://doi.org/10.1177/0734242X14527333

Nägele, H.J., Steinbrenner, J., Hermanns, G., Holstein, V., Haag, N.L., and Oechsner, H. (2017). Innovative additives for chemical desulphurisation in biogas processes: A comparative study on iron compound products. *Biochemical Engineering Journal 121*, 181-187. https://doi.org/10.1016/j.bej.2017.01.006

Nielsen, H.B., and Angelidaki, I. (2008). Strategies for optimizing recovery of the biogas process following ammonia inhibition. *Bioresource Technology 99*, 7995-8001. https://doi.org/10.1016/j.biortech.2008.03.049

Percheron, G., Bernet, N., and Moletta, R. (1999). Interactions between methanogenic and nitrate reducing bacteria during the anaerobic digestion of an industrial sulfate rich wastewater. *FEMS Microbiology Ecology 29*, 341-350. https://doi.org/10.1016/S0168-6496(99)00028-8

Ramos, J.L., Vargas, C.L., Mata, J., and Camacho, Á. (2019). Evaluation of poultry manure and goat cheese whey anaerobic co-digestion. *Spanish Journal of Agricultural Research 17*, 1-14. https://doi.org/10.5424/sjar/2019172-14577

Reis, M.A.M., Lemos, P.C., Martins, M.J., Costa, P.C., Gonçalves, L.M.D., and Carrondo, M. J. T. (1991). Influence of sulfates and operational parameters on volatile fatty acids concentration profile in acidogenic phase. *Bioprocess Engineering 6*, 145-151. https://doi.org/10.1007/BF00369251

Roy, R., and Conrad, R. (1999). Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiology Ecology 28*, 49-61. https://doi.org/10.1111/j.1574-6941.1999.tb00560.x

Schönheit, P., Kristjansson, J.K., and Thauer, R.K. (1982). Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate. *Archives of Microbiology 132*, 285-288. https://doi.org/10.1007/BF00407967

Sheng, K., Chen, X., Pan, J., Kloss, R., Wei, Y., and Ying, Y. (2013). Effect of ammonia and nitrate on biogas production from food waste via anaerobic digestion. *Biosystems Engineering 116*, 205-212. https://doi.org/10.1016/j.biosystemseng.2013.08.005

Siles, J.A., Brekelmans, J., Martín, M.A., Chica, A.F., and Martín, A. (2010). Impact of ammonia and sulphate concentration on thermophilic anaerobic digestion. *Bioresource Technology 101*, 9040-9048. https://doi.org/10.1016/j.biortech.2010.06.163

Soto, O., Aspé, E., and Roeckel, M. (2007). Kinetics of cross-inhibited denitrification of a high load wastewater. *Enzyme and Microbial Technology 40*, 1627-1634. https://doi.org/10.1016/j.enzmictec.2006.11.014

Sprott, G.D., and Patel, G.B. (1986). Ammonia toxicity in pure cultures of methanogenic bacteria. *Systematic and Applied Microbiology 7*, 358-363. https://doi.org/10.1016/S0723-2020(86)80034-0

Sterling, M., Lacey, R., Engler, C., and Ricke, S. (2001). Effects of ammonia nitrogen on H_2 and CH_4 production during anaerobic digestion of dairy cattle manure. *Bioresource Technology 77*, 9-18. https://doi.org/10.1016/S0960-8524(00)00138-3

Strik, D.P.B.T.B., Domnanovich, A.M., and Holubar, P. (2006). A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. *Process Biochemistry 41*, 1235-1238. https://doi.org/10.1016/j.procbio.2005.12.008

Sun, H., Guo, J., Wu, S., Liu, F., and Dong, R. (2017). Development and validation of a simplified titration method for monitoring volatile fatty acids in anaerobic digestion. *Waste Management 67*, 43-50. https://doi.org/10.1016/j.wasman.2017.05.015

Sürmeli, R.Ö., Bayrakdar, A., Molaey, R., and Çalli, B. (2019). Synergistic effect of sulfide and ammonia on anaerobic digestion of chicken manure. *Waste and Biomass Valorization 10*, 609-615. https://doi.org/10.1007/s12649-017-0090-z

Tanimu, M.I., Mohd Ghazi, T.I., Harun, R.M., and Idris, A. (2014). Effect of carbon to nitrogen ratio of food waste on biogas methane production in a batch mesophilic anaerobic digester. *International Journal of Innovation, Management and Technology 5*, 116-119. https://doi.org/10.7763/IJIMT.2014.V5.497

Tian, H., Fotidis, I.A., Kissas, K., and Angelidaki, I. (2018). Effect of different ammonia sources on aceticlastic and hydrogenotrophic methanogens. *Bioresource Technology 250*, 390-397. https://doi.org/10.1016/j.biortech.2017.11.081

Wagner, A.O., Hohlbrugger, P., Lins, P., and Illmer, P. (2012). Effects of different nitrogen sources on the biogas production-a lab-scale investigation. *Microbiological Research 167*, 630-636. https://doi.org/10.1016/j.micres.2011.11.007

Waki, M., Yasuda, T., Fukumoto, Y., Kuroda, K., and Suzuki, K. (2013). Effect of electron donors on anammox coupling with nitrate reduction for removing nitrogen from nitrate and ammonium. *Bioresource Technology 130*, 592-598. https://doi.org/10.1016/j.biortech.2012.12.101 Wang, J., and Wan, W. (2009). Kinetic models for fermentative hydrogen production: A review. *International Journal of Hydrogen Energy 34*, 3313-3323. https://doi.org/10.1016/j.ijhydene.2009.02.031

Yao, Y., Bergeron, A.D., and Davaritouchaee, M. (2018). Methane recovery from anaerobic digestion of urea-pretreated wheat straw. *Renewable Energy 115*, 139-148. https://doi.org/10.1016/j.renene.2017.08.038

Yuan, H., and Zhu, N. (2016). Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. *Renewable and Sustainable Energy Reviews 58*, 429-438. https://doi.org/10.1016/j.rser.2015.12.261

Zan, F., and Hao, T. (2020). Sulfate in anaerobic co-digester accelerates methane production from food waste and waste activated sludge. *Bioresource Technology 298*, 122536. https://doi.org/10.1016/j.biortech.2019.122536

Zhao, J., Hou, T., Lei, Z., Shimizu, K., and Zhang, Z. (2020). Effect of biogas recirculation strategy on biogas upgrading and process stability of anaerobic digestion of sewage sludge under slightly alkaline condition. *Bioresource Technology 308*, 123293. https://doi.org/10.1016/j.biortech.2020.123293