Revista Brasileira de Ciência do Solo

Division – Soil Processes and Properties | Commission – Soil Chemistry

Methane Emission Induced by Short-Chain Organic Acids in Lowland Soil

Janielly Silva Costa Moscôso[(1)], Leandro Souza da Silva[(2)*], Stefen Barbosa Pujol[(1)], Sandro José Giacomini[(2)], Fabiane Figueiredo Severo[(1)], Laura Brondani Marzari[(3)] and Gustavo Dal Molin[(3)]

[(1)] Universidade Federal de Santa Maria, Departamento de Solos, Programa de Pós-Graduação em Ciência do Solo, Santa Maria, Rio Grande do Sul, Brasil.
[(2)] Universidade Federal de Santa Maria, Departamento de Solos, Santa Maria, Rio Grande do Sul, Brasil.
[(3)] Universidade Federal de Santa Maria, Curso de Agronomia, Santa Maria, Rio Grande do Sul, Brasil.

ABSTRACT: Methane (CH_4) is the second major greenhouse gas after CO_2, exerting a significant influence on the climate and the chemistry of the atmosphere. In lowland soil, acetate and H_2/CO_2 are the most important precursors of CH_4 and formed from organic matter fermentation in an anaerobic environment, giving rise to short-chain organic acids (ethanoic, propanoic, and butanoic), depending on the type of crop residue and the soil management system. Ethanoic acid can be directly converted to CH_4 by methanogenic microorganisms, but propanoic and butanoic acids must be converted to acetate before being converted to CH_4. This study aimed to quantify, in isolation, the dynamics and CH_4 emission potential of the three short-chain organic acids found in flooded lowland soils with rice crops. The study was carried out in a controlled environment using four standard carbon doses (0, 90, 180, and 270 mg kg^{-1}) of ethanoic, propanoic, and butanoic acids. The dynamics and the potential emission of CH_4 from soil were investigated when the acids were applied to flooded soil previously incubated for 20 days. The CH_4 emission dynamics were altered with the application of the three short-chain organic acids to the soil, even using an equal amount of carbon. The faster and more intense emission was achieved with the ethanoic acid application in relation to the other two acids application, while butanoic acid presents slower, delayed, and prolonged dynamics of CH_4 emission. Propanoic acid resulted in the lowest CH_4 emission due to its own stoichiometry and the temperature condition in which the experiment was conducted, which were unfavorable to the hydrogenotrophic bacteria. The addition of short-chain organic acids promoted a *priming effect* in the soil with conversion values of C to CH_4 above the calculated theoretical values.

Keywords: ethanoic acid, butanoic acid, propanoic acid, methanogenesis, irrigated rice.

*** Corresponding author:**
E-mail: leandrosolos@ufsm.br

Received: December 03, 2018
Approved: August 27, 2019

How to cite: Moscôso JSC, Silva LS, Pujol SB, Giacomini SJ, Severo FF, Marzari LB, Molin GD. Methane emission induced by short-chain organic acids in lowland soil. Rev Bras Cienc Solo. 2019;43:e0180252.
https://doi.org/10.1590/18069657rbcs20180252

INTRODUCTION

Methane (CH_4) is the second major greenhouse gas after CO_2, exerting a significant influence on the climate and the chemistry of the atmosphere. The addition of one mole of CH_4 to the atmosphere is approximately 28 times more effective in the infrared radiation absorption than one mole of CO_2 (IPCC, 2014). A complex microbial community, involving hydrolytic, fermenting, acetogenic, syntrophic, and methanogenic microorganisms, is responsible for the organic matter degradation in flooded soils, from the transformation of composite molecules into simpler forms until the CH_4 production (Liu et al., 2018; Zabranska and Pokorna, 2018). Acetate and H_2/CO_2 are the most important precursors of CH_4 in soils. Theoretically, acetate and H_2/CO_2 contribute about 70 and 30 % to the emission of CH_4, respectively. Acetate is used by the group of methanogenic acetoclastic bacteria, which reduce acetate to CH_4 and CO_2, and hydrogenotrophic bacteria, which reduce CO_2 by using H_2 as an electron donor (Leonzio, 2016).

These CH_4 precursors are formed through the organic matter fermentation, where simpler compounds are fermented intracellularly by fermenting bacteria giving rise to acetate and short-chain organic acids, such as propanoic and butanoic acid, in addition to the production of H_2 and CO_2 in a process called acidogenesis (Costa and Leigh, 2014). Temperature, redox potential, pH, pressure exerted by H_2, and availability of organic substrates are identified as the main factors influencing CH_4 production from these precursors (Cheng et al., 2013, 2014).

Research by Sousa (2001), Sousa et al. (2002), and Bohnen et al. (2005), related to lowland soils in Rio Grande do Sul cultivated with irrigated rice, describe ethanoic, propanoic, and butanoic acids as the three short-chain organic acids found in higher concentrations in lowland soil that accumulate after fermentation processes. These concentrations differ according to the type of crop residue and the soil management system used. These authors report a decrease in total acid concentration after two to four weeks of flooding. This decrease occurs due to the conversion of short-chain organic acids to CH_4 (Sousa et al., 2002). It is known that ethanoic acid can be used directly for conversion to CH_4 by methanogenic bacteria that use the methyl acid group for conversion. However, propanoic and butanoic acids need to be converted to acetate by the acetogenesis process before being converted to CH_4 (Madigan et al., 2016; Bedoya et al., 2019). When using legume residues (vetch) in the soil, Sousa (2001) demonstrated higher concentrations of ethanoic acid in soil solution, while grass residues (ryegrass) provided higher concentrations of propanoic and butanoic acids.

The means by which the carbon becomes available to soil microbiota for degradation influences the organic acids production, as well as the CH_4 emission, because the different vegetal residues will affect, in a differentiated way, the dynamics of elements and compounds release to the soil solution due to its chemical composition. The experimental hypotheses are: the main fatty acids (ethanoic, propanoic and butanoic acids) released during the organic matter decay under anaerobic soil conditions potentiate the CH_4 emissions by flooded soil; and the size of the organic chain, at equivalent C availability, determines the dynamic of CH_4 emission, being fastest for the shortest chain. This study aimed to quantify, in isolation, the dynamics and CH_4 emission potential of the three short-chain organic acids found in higher concentrations in flooded lowland soils for irrigated rice.

MATERIALS AND METHODS

Conduction of the study and experimental design

The study was conducted in a controlled environment (BOD type incubator) in the Soil Chemistry and Soil Fertility Laboratory of the Federal University of Santa Maria (Universidade

Federal de Santa Maria-UFSM), Santa Maria, Rio Grande do Sul State. The soil used was a Planosol (IUSS Working Group WRB, 2015), which corresponds to *Planossolo Háplico Hidromórfico típico* (Santos et al., 2018), collected in the experimental field of the Soil Science Department of the UFSM (29° 43' 5" S; 53° 42' 20" W). This collection was carried out in the 0.00-0.20 m layer and the soil was then air-dried and sieved using a 4.0-mm mesh. Afterwards, a soil sample was sent for chemical analysis with the following results: pH (in water at a ratio of 1:1) = 4.9; table SMP= 5.5; M.O. = 2.8 dag kg^{-1}; clay= 400 g kg^{-1}; Ca^{2+}= 6.1 $cmol_c$ dm^{-3}; Mg^{2+}= 2.7 $cmol_c$ dm^{-3}; Al^{3+}= 1.8 $cmol_c$ dm^{-3}; H+Al= 7.7 $cmol_c$ dm^{-3}; Cation Exchange Capacity (CEC) effective = 10.8 $cmol_c$ dm^{-3}; CEC pH_7= 16.7 $cmol_c$ dm^{-3}; base saturation= 54.1 %; P-Mehlich= 8.5 mg dm^{-3}; K-Mehlich = 64 mg dm^{-3}. All the chemical analyses were performed according to the methodology of Tedesco et al. (1995).

The experimental units were composed of glass pots with a capacity of 995 mL with perforated plastic lids, containing a 0.20 m long silicone hose coupled to a three-way valve for sampling the gas in the "free atmosphere" of the bottles. In these units, 300 g of soil and 350 mL of distilled water were accommodated, forming a water layer sheet of 4.0 cm depth. Then, the soil was preincubated in a BOD-type incubator for 20 days keeping the water layer sheet and adopting an incubation temperature of 30±1 °C. The visual organic material previously present in the soil, such as root pieces or crop straw, was removed before weighing to avoid interference with CH_4 emissions. According to the methodology of Aulakh et al. (2001), containing some modifications (an increase in the size of the experimental units, vacuum performed with a syringe, and manual agitation).

After the preincubation period, all the experimental units were subjected to a vacuum using a 60 mL syringe, followed by the injection of N_2 in the "free atmosphere" of the bottles, potentiating the conditions favorable to the reduction processes in the soil. After exactly 24 h of this procedure, the treatments were applied. The treatments involved three different short-chain acids and four standardized doses of carbon that were used for each acid (Table 1), thus constituting a factorial arrangement (3 × 4) in a completely randomized design with three replicates. The three acids used were in the form of reagents for chemical analysis being the ethanoic acid with (purity ≥99.5 %), propanoic acid (purity ≥99.0 %), and butanoic acid (purity ≥99.7 %). A 30-mL aliquot corresponding to the treatments was applied to the soil with the aid of a calibrated

Table 1. Treatments with the addition of different doses of soil carbon via different short-chain organic acids

Treatment	Description	
	Amount of carbon	Amount of acid
	mg kg^{-1}	
T_1	0	0
T_2	90 via ethanoic acid	225.0
T_3	180 via ethanoic acid	450.0
T_4	270 via ethanoic acid	675.0
T_5	0	0
T_6	90 via propanoic acid	183.6
T_7	180 via propanoic acid	370.0
T_8	270 via propanoic acid	555.1
T_9	0	0
T_{10}	90 via butanoic acid	164.9
T_{11}	180 via butanoic acid	329.9
T_{12}	270 via butanoic acid	494.9

Dose values were based on preliminary tests and values found by Sousa et al. (2002).

syringe and the three-way valve coupled to the silicone hose attached to the lid of the experimental units.

Sampling and quantification of CH_4 gas emission

The CH_4 gas samples were always taken every 24 h after the treatments were applied for 15 consecutive days. The experimental period was defined by relevant literature results and pilot tests performed before this experiment. Samples were collected using a polypropylene syringe (BD®), previously evacuated and treated with N_2 gas, coupled to the three-way valve. Before each collection, the experimental units underwent vigorous manual agitation to homogenize the CH_4 gas which could be trapped in bubbles formed at aqueous layer. After the daily gas collection, a new vacuum and N_2 injection procedure were performed on the experimental units.

Samples contained in the syringes were transferred to pre-evacuated glass vials (Exetainer® vials, Labco Limited, UK) with a capacity of 12 mL for analysis by gas chromatography. The gas chromatograph (GC-2014, model Greenhouse, Shimadzu) was equipped with a flame ionization detector (FID) and analysis conducted at 250 °C with direct injection of 1 mL of sample. The production rate of CH_4 was calculated according to the methodology of Aulakh et al. (2001) using equation 1.

$$TP_{CH_4} = \frac{dc}{dt} \times \frac{V_{hs} \times MW}{W_S \times MV} \quad \text{Eq. 1}$$

In which: TP_{CH_4} is the rate of methane production (µg g^{-1} day^{-1}); *dc/dt* represents the change in CH_4 concentration in the "free atmosphere" of the flask measured in the apparatus (µmol day^{-1}) converted to µg; V_{hs} is the volume of the free atmosphere of the experimental unit (L); *MV* is the molecular volume of CH_4 at 30 °C (24.88 L mol^{-1}); W_s is the dry weight of the soil (g); and *MW* is the molecular weight of CH_4 (16 g mol^{-1}). The accumulated CH_4 production was calculated by adding the emission obtained during 24 h with the emission obtained the previous day, and so on.

The CH_4 produced from experimental units with addition of organic acids was subtracted from the CH_4 produced in the experimental units without addition of acids (dose of 0 mg kg^{-1}), using equation 2, to obtain the net production of CH_4 (P_{netCH4}).

$$P_{netCH_4} = PCH_4sa - PCH_4sc \quad \text{Eq. 2}$$

In which: P_{netCH_4} is the net CH_4 production from the added organic acids (μg g^{-1} day^{-1}); PCH_4sa is CH_4 produced in soil altered with organic acids (μg g^{-1} day^{-1}); and PCH_4sc is the CH_4 produced in the control soil (μg g^{-1} day^{-1}).

Statistical analysis

Means and standard deviations (SD) of the data from each treatment were presented. For total CH_4 emission, analysis of variance (ANOVA) and F test ($p<0.05$) were performed. Subsequently, according to ANOVA results, regression analysis was performed to evaluate the effect of acid dose and the Tukey test ($p<0.05$) was performed to verify the difference between the kind of acid. The graphs were made using SigmaPlot, version 12.0 (SigmaPlot, 2012).

RESULTS

Dynamic and cumulative CH_4 emission

The addition of short-chain organic acids stimulated CH_4 production in the flooded Planosol, but with different emission dynamics between the three acids, at the same

carbon dose (Figure 1). With the ethanoic acid addition in the soil, there was a CH_4 emission peak on the third day after acid application, presenting values of 19.3, 34.5, and 49.4 μg g^{-1} day^{-1} at the carbon doses of 90, 180, and 270 mg kg^{-1} soil, respectively (Figures 1a, 1b, and 1c). A second peak of CH_4 emission was observed on the sixth day after ethanoic acid application, with values of 70.2, 82.4, and 94.0 μg g^{-1} day^{-1}, respectively, for the three carbon doses of 90, 180, and 270 mg kg^{-1} soil, respectively. It can be seen on the sixth day that, although the peaks are higher than at three days, the difference between the doses is lower; with 270 mg kg^{-1} there was 1.3-fold higher emission than with 90 mg kg^{-1}, while at three days this difference was 2.6 fold larger. Soon after this second peak, the emission rates of CH_4 with the ethanoic acid application decreased, presenting only more expressive emission at the tenth day. It is possible to observe that CH_4 emissions with ethanoic acid application were higher than the emissions obtained with another two acid application, except at the dose of 180 mg kg^{-1} where the accumulated CH_4 emission values of butanoic acid exceeded the values obtained by ethanoic acid. With respect to CH_4 emissions when propanoic acid is applied (Figures 1a, 1b, and 1c), the first emission peak occurred later in relation to ethanoic acid, namely on the forth day after application of propanoic acid, with emissions of 21.5, 24.4, and 26.6 μg g^{-1} day^{-1} for the three doses of 90, 180, and 270 mg kg^{-1}, respectively. The use of this acid also resulted in a second peak of emission that was more pronounced to the sixth day after application of the treatments representing three carbon doses, with values of 23.8, 35.5, and 55.8 μg g^{-1} day^{-1}, respectively.

Similar to ethanoic acid, this second CH_4 emission peak was followed by a decrease in emissions, but a new emission peak occurred on the nineth day after the acid application with the three doses used, with values similar to the second peak. For the dose of 270 mg kg^{-1}, a fourth emission peak could still be observed on the 11th day after the propanoic acid application, which did not occur with the other doses.

The accumulated emissions of CH_4 for this acid were less than the emissions when compared with the emissions obtained with the ethanoic acid application, at doses of 90 and 270 mg kg^{-1}, but without differing from the intermediate dose. Compared with butanoic acid, emissions were similar at doses of 90 and 270 mg kg^{-1} and lower at the dose of 180 mg kg^{-1} (Figures 1d, 1e, and 1f).

Butanoic acid, on the other hand, was the organic acid with the later and more prolonged emission peaks (Figure 1). At the dose of 90 mg kg^{-1}, the first CH_4 emission peak occurred only on the 11th day after the acid application, presenting an emission value of 37.9 μg g^{-1} day^{-1}. At doses of 180 and 270 mg kg^{-1}, the emissions were initially more similar to each other, with a first emission peak occurring on the 6th day after application of the acid. At the end of the evaluation period, the CH_4 production rate using the three organic acids decreased steadily with increasing carbon doses, reaching values similar to the rates observed with the control soil (Figures 1a, 1b, and 1c). From the results reported, it can be inferred that the greater stimulation of methanogenesis by addition of short-chain organic acids to the soil was restricted to a relatively short period lasting a maximun of 15 days.

CH_4 emission potential

The total CH_4 emissions after 15 days of short-chain organic acids application showed some statistically significant differences between the acids when the same dose of carbon was applied (Table 2). At 90 mg kg^{-1}, the value found for ethanoic acid differed from the value found for butritic acid, while propanoic acid was similar to ethanoic and butanoic acids. Only at the dose of 180 mg kg^{-1}, there was a distinction between the three acids, with the highest CH_4 emission value occurring with butanoic acid application and the lowest value with propanoic acid. At 270 mg kg^{-1}, ethanoic acid returned to have a higher total CH_4 emission than propanoic and butanoic acids, which had no statistical

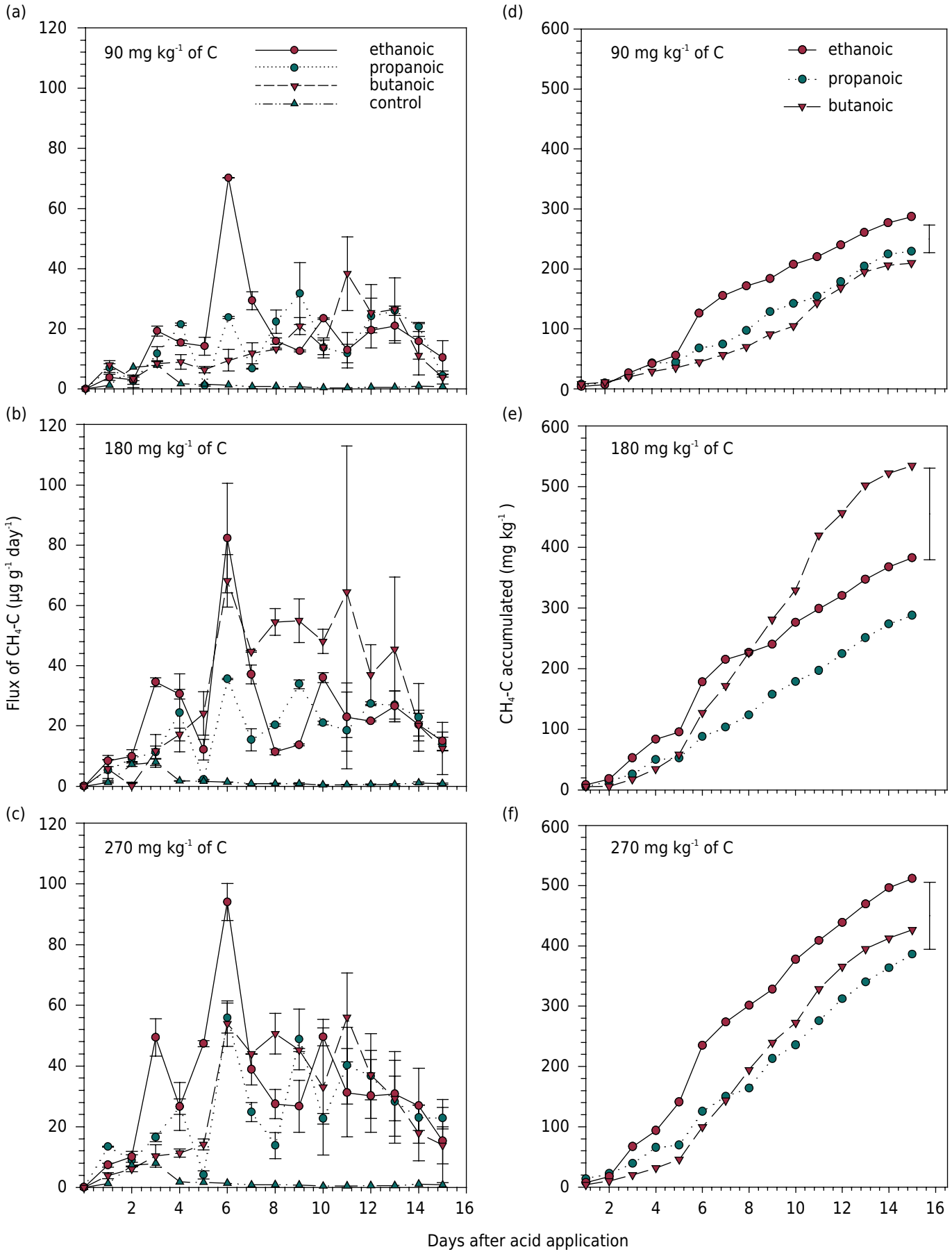

Figure 1. Flow of CH_4 after application of short-chain organic acids (a, b, and c). Vertical bars indicate the standard deviation. Accumulated net emission of CH_4 after application of short-chain organic acids (d, e, and f). The vertical bars indicate a significant difference between the means of the treatments using Tukey's test ($p<0.05$).

difference between themselves (Table 2). In this table, it is also possible to observe that the organic acids application raised the CH_4 emission above the values of carbon added in the soil. Propanoic acid reached the lowest values in the total CH_4 emission at the end of the experiment, at doses of 180 and 270 mg kg^{-1} (Table 2), as shown in the cumulative emission graphs (Figures 1e and 1f), although CH_4 emission exceeds the amount of carbon added to the soil.

The CH_4 emission data obtained with the application of ethanoic, propanoic, and butanoic acids were adjusted to a quadratic regression model as a function of the carbon doses (Figure 2). It can be seen from figure 2 and table 2 that the conversion rate of carbon applied as ethanoic acid is higher than the conversion rate of propanoic acid, resulting in lower emission with the last acid with increasing carbon doses. With respect to the behavior of butanoic acid, it can be inferred that there a saturation point was reached in the total emission of CH_4 with the dose of 180 mg kg^{-1}. Conversion of butanoic acid to CH_4 yielded almost the same ratio as for ethanoic acid, although the former was converted to more acetates. However, this conversion is slower, which delays the CH_4 emission when butanoic acid is applied to the soil.

Table 2. Average values (n = 36) obtained for the emission of total CH_4, after application of different short-chain organic acids under controlled laboratory conditions

Doses	Total CH_4–C emission			CV
	Ethanoic	Propanoic	Butanoic	
mg kg^{-1}	mg kg^{-1}			%
0	27.77 a	26.06 a	28.01 a	3.65
90	287.12 a	229.36 ab	209.94 b	7.60
180	382.41 b	287.72 c	508.85 a	15.28
270	511.65 a	386.06 b	426.60 b	10.08

Means followed by the same letter in the line do not differ by Tukey's test ($p<0.05$). CV: coefficient of variation.

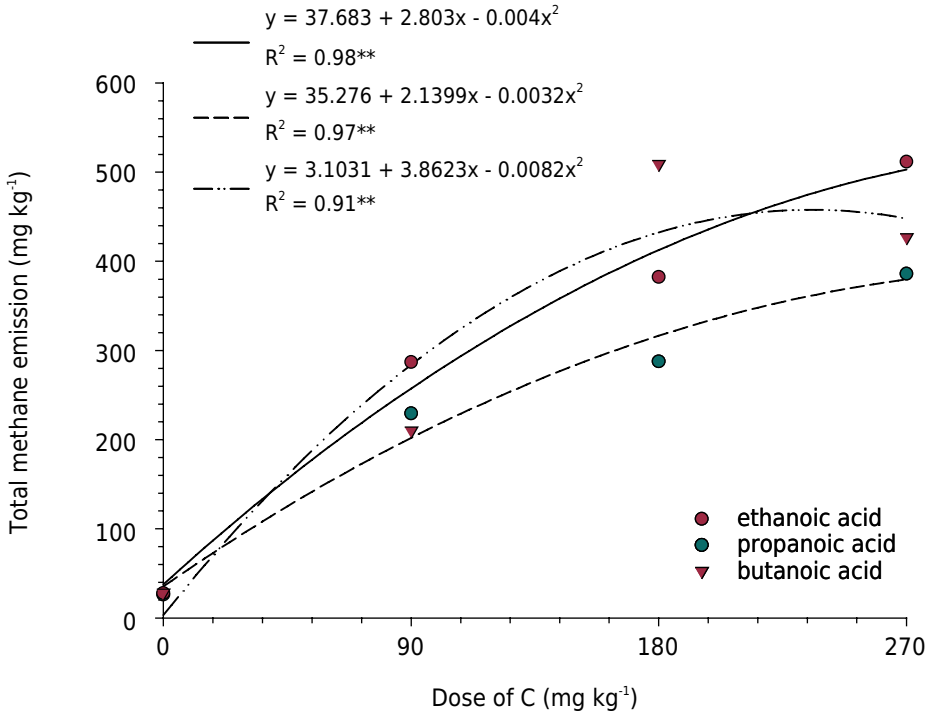

Figure 2. Regression of total CH_4 emission as a function of carbon doses from three short-chain organic acids. **: significant at 1 % using the F test.

DISCUSSION

Although occurring simultaneously, the sequence for the CH_4 generation follows the steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Dong et al., 2019). With ethanoic acid being readily available in the soil, the acetogenesis and methanogenesis stages were anticipated and, consequently, there was faster CH_4 emission compared to the other two acids and at all carbon doses, interfering in this way in the dynamics emissions of CH_4 (Figure 1). In previous studies which investigated the CH_4 emission in soils incubated under anaerobic conditions, Fey and Conrad (2000) and Kotsyurbenko et al. (2004) also reported a short time for conversion of acetate to CH_4, taking about three days. The microorganisms that participate in the methanogenesis process belong to the hydrolytic, fermentative, syntrophic, acetogenic, and methanogenic groups, which are stimulated by the introduction of substrates with different characteristics in the microbial system (Liu et al., 2018). This probably favored the group of methanogens that, consequently, allowed the highest CH_4 emission on the sixth day when ethanoic acid was applied.

The delay in the first CH_4 emission peak appearance, which occurred with the application of propanoic acid in relation to the application of ethanoic acid, can be explained by the degradation of propanoic acid starting only after the degradation of ethanoic acid present in the soil (Figure 1). In some studies using ethanoic and propanoic acids as the carbon source when evaluating the specific methanogenic activity in biological sewage sludge treatment reactors, Zhang et al. (2008) and Zhao et al. (2010) found that the propanoic acid degradation began only when the ethanoic acid, already contained in the sludge, was completely degraded. In figure 1, it can be observed that in the control treatment there is a small amount of CH_4 emission on the third day, similar to the first emission peak when ethanoic acid was used. This emission may be from endogenous ethanoic acid in the soil.

According to the stoichiometry of propanoic acid (Table 3), the amount of mol equivalent of CH_4 produced from this acid is 1.75, with a yield of 3 mols equivalent of H_2 and 1 mol equivalent of acetate in the acetogenesis process. The more H_2 there is in the medium, the more this favors the activity of methanogenic bacteria that use H_2 as an electron donor, reducing the CO_2 for the formation of CH_4; these are bacteria classified as hydrogenotrophic. However, the activity of these bacteria is most benefited by temperatures below 20 °C because they are classified as psychrophiles (Guneratnam et al., 2017).

However, in the present study, the temperature was maintained at 30 °C to benefit the acetoclastic bacteria that are classified as mesophilic (Junicke et al., 2016). Methanogenesis, from H_2/CO_2, occurs in small proportions under average ambient temperature of 25 to 30 °C for thermodynamic reasons. There are two possible explanations for this event. Firstly, the composition of the methanogenic microbial community changes with temperature and the same occurs with the relative activity of different physiological groups of microorganisms which depends on their classification, such as psychrophiles, mesophiles or thermophiles. Secondly, the fluidity of the microbial cytoplasmic membrane changes with temperature

Table 3. Molecular hydrogen (H_2), acetate, and methane (CH_4) equivalent from the anaerobic degradation of different compounds

Compound	H_2	Acetate	CH_4
	equivalent mol		
Ethanoic acid: $CH_3COO^- + H^+ = CH_4 + CO_2$	0	1	1.00
H_2: $H_2 + 0.25CO_2 = 0.25CH_4 + 0.5H_2O$	1	0	0.25
Propanoic acid: $CH_3CH_2COO^- + 3H_2O = CH_3COO^- + HCO_3^- + H^+ + 3H_2$	3	1	1.75
Butanoic acid: $CH_3(CH_2)_2COO^- + 2H_2O = 2CH_3COO^- + H^+ + 2H_2$	2	2	2.50

Adapted from Kotsyurbenko et al. (2004).

differently affecting the use of acetate and H_2 (Fey and Conrad, 2000). The decrease in temperature often results in a decrease in the efficiency of the transport of substances by the plasma membrane and decrease in the use of the substrate. In this way, the acetate must be transported through the membrane, while the H_2 is freely diffusible. When an inhibition occurs in the use of acetate in relation to H_2 with the decrease of temperature, as in high temperatures, there may be a limitation to the solubility of gaseous substrates, such as H_2 and CO_2 in the aqueous phase (Adams et al., 2010; Dong et al., 2018). This may explain the finding that when propanoic acid, which gives higher concentrations of H_2 to the system, was added, the CH_4 emissions were lower.

Before being transformed to CH_4, all short-chain organic acids are first degraded to H_2 and acetate (Wang et al., 2009). This sequence of processes clarifies the delay in the appearance of and the prolongation of the CH_4 emission peaks with the application of butanoic acid. In the processes of acetogenesis and methanogenesis, butanoic acid results in two mols equivalent of H_2 and two mols equivalent of acetate, resulting in 2.5 mols equivalent of CH_4 (Table 3). Thus, endogenous acetogenic microorganisms in the soil, besides converting the short-chain organic acids that were already present in the medium, must have converted a large amount of H_2 and acetate to CH_4 from the application of the butanoic acid, thus prolonging the peak CH_4 emissions.

The acids were applied using the same carbon doses but differed in the amounts of acetate, H_2 and CO_2 formed, which in turn determined the different amounts of CH_4 emitted in the experiment. In general terms, the conversion rates of butanoic acid to CH_4 were very similar to the conversion rates of ethanoic acid and higher than the conversion rates of propanoic acid to CH_4, especially at the two highest doses. This is in agreement with previous studies conducted by Ren et al. (2003) and Wang et al. (2009), in which reported conversion rates of short-chain organic acids to CH_4 varied in the following order: ethanoic acid > butanoic acid > propanoic acid.

When the mean values for the three evaluated acids are examined (Table 2), the average methane emission was greater than 300 or 400 mg kg^{-1} in the last two doses for ethanoic and butanoic acids, whereas for propanoic acid the emission was less than 300 or 400 mg kg^{-1}, indicating the closest methanogenic response between the first two and lower in the third. Results by Barredo and Evison (1991) and Demirel and Yenigün (2002) suggest that a large amount of propanoic acid results in a failure in the methanogenic process, leading to inhibition of methanogenic bacteria activity, whereas butanoic acid improves the conversion rates of short-chain organic acids to CH_4, increasing the yield of this acid by stimulating the activity of methanogenic bacteria.

Investigating CH_4 emission in lowland soils incubated anaerobically under various temperatures, (Fey and Conrad, 2000) reported that the concentrations of ethanoic, propanoic, and butanoic acids in the incubated soil were zero after one month of incubation at all temperatures, proving that the processes of acidogenesis, acetogenesis, and methanogenesis under these conditions are very rapid. The results of the present study are in agreement with the literature since the higher stimulation of the methanogenesis by addition of short-chain organic acids to the soil was restricted to a relatively short period of 15 days.

The sequential reduction process and the different phases of CH_4 production have been characterized in previous anaerobic incubation studies using lowland soils cultivated with rice. However, published data on the dynamics of CH_4 involving the application of three different short-chain organic acids as the exogenous carbon source in soil are scarce, but only in the evaluation of the specific methanogenic activity in UASB reactors (Upflow Anaerobic Sludge Blanket) for biological treatment of sewage (Zhang et al., 2008; Wang et al., 2009; Schneiders et al., 2013; De Sá et al., 2014). Thus, the present study can elucidate some aspects of the dynamics of CH_4 emission from the degradation of short-chain organic acids that accumulate in the soil, resulting from the fermentation

of organic matter or organic residues at the beginning of the flooding period for the cultivation of irrigated rice.

In previous studies, Xu and Hosen (2010) and Zschornack et al. (2018) reported that the presence of ryegrass straw influenced soil CH_4 emissions in irrigated rice cultivation, with an increase of up to 25 %. The presence of residues in the soil from a crop used as winter cover can increase CH_4 emissions in flooded soil, increasing the supply of C to the methanogens present in the soil, as a result of a greater oxidation process (Kim et al., 2013). With regard to the soil solution, Sousa (2001) demonstrated higher ethanoic acid concentrations when vetch residues were present in rice cultivation, while ryegrass residues provided higher concentrations of propanoic and butanoic acids. Thus, the emission of CH_4 is not only related to the carbon content that is available to the microbiota, but also to the types of acids that are produced from the fermentation of different organic residues in anaerobic conditions.

The emission of CH_4 from the soil in quantities exceeding the carbon that was applied to the soil can be explained by the displacement of populations or the increase in the number of organisms active in the soil, resulting in a conversion of CH_4 from other soil carbon sources with values superior to what had been applied as a substrate for acetogenic and methanogenic bacteria. This could be called a priming effect, although the phenomenon is still unclear and more research is needed to increase understanding of its effect (Kuzyakov, 2010).

A factor that may favor this high emission was the constant use of a temperature suitable for the optimal activity of acetoclastic methanogenic bacteria, which may have exaggerated the emission of CH_4 because, in the field, such as in irrigated rice crops, daily variations in temperature occur which balance the activities of different groups of microorganisms involved in the whole hydrolysis process until the methanogenesis takes place. Applying glucose and acetate as an exogenous carbon source in anaerobically incubated soils, Lu et al. (2000) also found a conversion of these compounds that was greater than expected. Chidthaisong et al. (1999) observed that glucose and acetate supplementation of the soil increased the CH_4 emission by 1.6 to 500 times the expected level, depending on the type of fertilizer applied to the soil in the field before incubation. Lu et al. (2000), although they standardized glucose and acetate to the same carbon dose, also found that the conversion rate and conversion efficiency of the substrate to CH_4 largely exceeded the theoretical maximum values expected.

Even exceeding the expected theoretical maximum values were observed lower values in the total emission of CH_4 at the end of the experiment with the application of propanoic acid. This may have been a consequence of the high concentrations of H_2 present in the medium as a result of the degradation of this acid and according to its stoichiometry (Table 3). As the temperature at which the experiment was conducted did not benefit the activity of hydrogenotrophic bacteria, an accumulation of H_2 could have occurred in the system, thus inhibiting the activity of methanogenic acetoclastic bacteria. Temperature is a key factor that regulates CH_4 production in soils used for cultivation of irrigated rice (van Groenigen et al., 2013; Yvon-Durocher et al., 2014) as it affects, not only the production rate of CH_4, but also the methanogenesis-acetoclastic or hydrogenotrophic pathway (Lu et al., 2015). In the present study, the temperature may not have favored the hydrogenotrophic bacteria and their activities may have been compromised; this may have caused an accumulation of hydrogen in the system.

The concentration of hydrogen must be “controlled” during the methanogenic phase to maintain the equilibrium of the process at this stage; the ideal is a low hydrogen pressure level of around 5.82 Pa under standard conditions. A high concentration of hydrogen does not provide the necessary environmental conditions for the acetogenic bacteria to convert the organic acids generated to the acetate (Thauer, 2012). For this reason,

it is extremely important to reduce the amount of hydrogen in the mixture, where this fundamental task is carried out precisely by bacteria from the methanogenesis process, transforming hydrogen and carbon dioxide into methane (Baldacin and Pinto, 2015). In a previous study (Guiot et al., 2011; Bassani et al., 2015), a reduction in the activity of acetogenic bacteria in reactors used to treat effluents was observed when external H_2 was added, both in mesophilic and thermophilic conditions.

Based on thermodynamic considerations, specifically with regard to the Gibbs free energy (ΔG) resulting from fatty acid oxidation, it is predicted that acetoclastic methanogenic bacteria will be able to grow only in environments with low hydrogen pressures. This condition is achieved when hydrogen-consuming microorganisms are present in the system, such as hydrogenotrophic methanogenic arrays or sulfate-reducing bacteria (Karadagli and Rittmann, 2005). Most of the methanogenic environments maintain an H_2 concentration low enough to stimulate the growth of acetoclastic bacteria, avoiding the accumulation of acids. However, with the application of propanoic acid readily available in the soil, a high H_2 load was added to the system, which caused a decrease in the efficiency of the propanoic acid to be transformed to CH_4.

There is a great uncertainty in the values generated by the theoretical models of CH_4 emission since they do not take into consideration important factors that influence the process of anaerobic digestion, such as the physicochemical properties of the compounds, the biological inhibitors and the interactions and requirements among the different groups of bacteria involved in the process. Studies involving specific methanogenic activity have generally been carried out under sterile conditions in mini-reactors for effluent treatment, domestic or industrial, where standard organic acids are commonly used as the only source of carbon (Holmes et al., 2017; Li et al., 2018). However, the measurement of this activity in soil samples is somewhat more complex. Therefore, there is a need for more experiments and adjustments in methodologies to achieve more effective and more accurate results. Understanding the CH_4 emission dynamics, separately studying each short-chain organic acid that is produced by fermenting of different residues incorporated into the soil, can help in a future mitigation strategy for this greenhouse gas.

CONCLUSIONS

The dynamics of CH_4 emission are different by the application of three short-chain organic acids to the soil (ethanoic, propanoic, and butanoic) even the same amount of carbon was added. The CH_4 emission is faster and more intense when ethanoic acid is applied in relation to the other two acids application, while butanoic acid presents a slower, delayed, and prolonged CH_4 emission dynamics. Propanoic acid results in lower CH_4 emission values due to its own stoichiometric CH_4 convertion and the temperature condition under which the experiment was conducted, which was unfavorable for hydrogenotrophic bacteria. The addition of short-chain organic acids promotes a priming effect on soil with conversion values of C to CH_4 above the calculated theoretical values.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting scholarships and funding for research. This work was carried out with the support of the Coordination of Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

AUTHOR CONTRIBUTIONS

Conceptualization: Janielly Silva Costa Moscôso (lead), Leandro Souza da Silva (equal), and Sandro José Giacomini (supporting).

Methodology: Janielly Silva Costa Moscôso (lead), Leandro Souza da Silva (equal), Stefen Barbosa Pujol (supporting), Sandro José Giacomini (supporting), Fabiane Figueiredo Severo (supporting), Laura Brondani Marzari (supporting), and Gustavo Dal Molin (supporting).

Formal Analysis: Janielly Silva Costa Moscôso (lead) and Stefen Barbosa Pujol (supporting).

Investigation: Janielly Silva Costa Moscôso (equal), Leandro Souza da Silva (equal), Fabiane Figueiredo Severo (supporting), Laura Brondani Marzari (supporting), and Gustavo Dal Molin (supporting).

Writing – Original Draft: Janielly Silva Costa Moscôso (lead).

Writing – review & editing: Leandro Souza da Silva (lead), Stefen Barbosa Pujol (supporting), and Sandro José Giacomini (equal).

REFERENCES

Adams HE, Crump BC, Kling GW. Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams. Environ Microbiol. 2010;12:1319-33. https://doi.org/10.1111/j.1462-2920.2010.02176.x

Aulakh MS, Wassmann R, Bueno C, Rennenberg H. Impact of root exudates of different cultivars and plant development stages of rice (*Oryza sativa* L.) on methane production in a paddy soil. Plant Soil. 2001;230:77-86. https://doi.org/10.1023/A:1004817212321

Baldacin ACS, Pinto GMF. Biodigestão anaeróbia da vinhaça: aproveitamento energético do biogás. Revista Eletrônica FACP. 2015;7:1-47

Barredo MS, Evison LM. Effect of propionate toxicity on methanogen-enriched sludge, *Methanobrevibacter smithii*, and *Methanospirillum-hungatii* at different pH values. Appl Environ Microbiol. 1991;57:1764-9.

Bassani I, Kougias PG, Treu L, Angelidaki I. Biogas upgrading via hydrogenotrophic methanogenesis in two stage continuous stirred tank reactors at mesophilic and thermophilic conditions. Environ Sci Technol. 2015;49:12585-93. https://doi.org/10.1021/acs.est.5b03451

Bedoya K, Coltell O, Cabarcas F, Alzate JF. Metagenomic assessment of the microbial community andmethanogenic pathways in biosolids from a municipal wastewatertreatment plant in Medellín, Colombia. Sci Total Environ. 2019;648:572-81. https://doi.org/10.1016/j.scitotenv.2018.08.119

Bohnen H, Silva SL, Macedo VRM, Marcolin E. Ácidos orgânicos na solução de um Gleissolo sob diferentes sistemas de cultivo com arroz irrigado. Rev Bras Cienc Solo. 2005;29:475-80. https://doi.org/10.1590/S0100-06832005000300018

Cheng K, Ogle SM, Parton WJ, Pan G. Simulating greenhouse gas mitigation potentials for Chinese Croplands using the DAYCENT ecosystem model. Glob Change Biol. 2014;20:948-62. https://doi.org/10.1111/gcb.12368

Cheng K, Ogle SM, Parton WJ, Pan G. Predicting methanogenesis from rice paddies using the DAYCENT ecosystem model. Ecol Model. 2013;261-262:19-31. https://doi.org/10.1016/j.ecolmodel.2013.04.003

Chidthaisong A, Obata H, Watanabe I. Methane formation and substrate utilisation in anaerobic rice soils as affected by fertilisation. Soil Biol Biochem. 1999;31:135-43. https://doi.org/10.1016/S0038-0717(98)00114-X

Costa KC, Leigh JA. Metabolic versatility in methanogens. Curr Opin Biotech. 2014;29:70-5. https://doi.org/10.1016/j.copbio.2014.02.012

De Sá LRV, Cammarota MC, Ferreira-Leitão VS. Produção de hidrogênio via fermentação anaeróbia - aspectos gerais e possibilidade de utilização de resíduos agroindustriais brasileiros. Quim Nova. 2014;37:857-67. https://doi.org/10.5935/0100-4042.20140138

Demirel B, Yenigün O. The effects of change in volatile fatty acid (VFA) composition on methanogenic upflow filter reactor (UFAF) performance. Environ Technol. 2002;23:1179-87. https://doi.org/10.1080/09593332308618336

Dong N, Bu F, Zhou Q, Khanal SK, Xie L. Performance and microbial community of hydrogenotrophic methanogenesis under thermophilic and extreme-thermophilic conditions. Bioresour Technol. 2018;266:454-62. https://doi.org/10.1016/j.biortech.2018.05.105

Dong B, Xia Z, Sun J, Dai X, Chen X, Ni B-J. The inhibitory impacts of nano-graphene oxide on methane production from waste activated sludge in anaerobic digestion. Sci Total Environ. 2019;646:1376-84. https://doi.org/10.1016/j.scitotenv.2018.07.424

Fey A, Conrad R. Effect of temperature on carbon and electron flow and on the archaeal community in methanogenic rice field soil. Appl Environ Microbiol. 2000;66:4790-7. https://doi.org/10.1128/AEM.66.11.4790-4797.2000

Guiot SR, Cimpoia R, Carayon G. Potential of wastewater-treating anaerobic granules for biomethanation of synthesis gas. Environ Sci Technol. 2011;45:2006-12. https://doi.org/10.1021/es102728m

Guneratnam AJ, Ahern E, FitzGerald JA, Jackson SA, Xia A, Dobson ADW, Murphy JD. Study of the performance of a thermophilic biological methanation system. Bioresour Technol. 2017;225:308-15. https://doi.org/10.1016/j.biortech.2016.11.066

Holmes DE, Shrestha PM, Walker DJF, Dang Y, Nevin KP, Woodard TL, Lovley DR. Metatranscriptomic evidence for direct interspecies electron transfer between *Geobacter* and *Methanothrix* species in methanogenic rice paddy soils. Appl Environ Microbiol. 2017;83:e00223-17. https://doi.org/10.1128/AEM.00223-17

Intergovernmental Panel on Climate Change - IPCC. Climate change 2014: mitigation of climate change. Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2014.

IUSS Working Group WRB. World reference base for soil resources 2014, update 2015: International soil classification system for naming soils and creating legends for soil maps. Rome: Food and Agriculture Organization of the United Nations; 2015. (World Soil Resources Reports, 106).

Junicke H, van Loosdrecht MCM, Kleerebezem R. Kinetic and thermodynamic control of butyrate conversion in non-defined methanogenic communities. Appl Microbiol Biotechnol. 2016;100:915-25. https://doi.org/10.1007/s00253-015-6971-9

Karadagli F, Rittmann BE. Kinetic characterization of *Methanobacterium bryantii* M.o.H. Environ Sci Technol. 2005;39:4900-5. https://doi.org/10.1021/es047993b

Kim SY, Lee CH, Gutierrez J, Kim PJ. Contribution of winter cover crop amendments on global warming potential in rice paddy soil during cultivation. Plant Soil. 2013;366:273-86. https://doi.org/10.1007/s11104-012-1403-4

Kotsyurbenko OR, Chin K-J, Glagolev MV, Stubner S, Simankova MV, Nozhevnikova AN, Conrad R. Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West Siberian peat bog. Environ Microbiol. 2004;6:1159-73. https://doi.org/10.1111/j.1462-2920.2004.00634.x

Kuzyakov Y. Priming effects: interactions between living and dead organic matter. Soil Biol Biochem. 2010;42:1363-71. https://doi.org/10.1016/j.soilbio.2010.04.003

Leonzio G. Process analysis of biological Sabatier reaction for bio-methane production. Chem Eng J. 2016;290:490-8. https://doi.org/10.1016/j.cej.2016.01.068

Li J, Xiao L, Zheng S, Zhang Y, Luo M, Tong C, Xu H, Tan Y, Liu J, Wang O, Liu F. A new insight into the strategy for methane production affected by conductive carbon cloth in wetland soil: beneficial to acetoclastic methanogenesis instead of CO_2 reduction. Sci Total Environ. 2018;643:1024-30. https://doi.org/10.1016/j.scitotenv.2018.06.271

Liu P, Klose M, Conrad R. Temperature effects on structure and function of the methanogenic microbial communities in two paddy soils and one desert soil. Soil Biol Biochem. 2018;124:236-44. https://doi.org/10.1016/j.soilbio.2018.06.024

Lu Y, Fu L, Lu Y, Hugenholtz F, Ma K. Effect of temperature on the structure and activity of a methanogenic archaeal community during rice straw decomposition. Soil Biol Biochem. 2015;81:17-27. https://doi.org/10.1016/j.soilbio.2014.10.031

Lu Y, Wassmann R, Neue HU, Huang C, Bueno CS. Methanogic responses to exogenous substrates in anaerobic rice soils. Soil Biol Biochem. 2000;32:1683-90. https://doi.org/10.1016/S0038-0717(00)00085-7

Madigan MT, Martinko JM, Bender KS, Buckley DH, Stahl DA. Microbiologia de Brock. 14. ed. Porto Alegre: Artmed Editora; 2016.

Ren NQ, Liu M, Wang AJ, Ding J, Li H. Organic acids conversion in methanogenic - phase reactor of the two phase anaerobic process. Environ Sci. 2003;24:89-93.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araújo Filho JC, Oliveira JB, Cunha TJF. Sistema brasileiro de classificação de solos. 5. ed. rev. ampl. [e-book]. Brasília, DF: Embrapa; 2018.

Schneiders D, Silva JD, Till A, Lapa KR, Pinheiro A. Atividade metanogênica específica (AME) de lodos industriais provenientes do tratamento biológico aeróbio e anaeróbio. Ambi-Agua. 2013;8:135-45. https://doi.org/10.4136/ambi-agua.1098

SigmaPlot. SigmaPlot for windows, version 12.0. San Jose: Systat Software, 2012.

Sousa RO. Oxirredução em solos alagados afetada por resíduos vegetais [thesis]. Porto Alegre: Universidade Federal do Rio Grande do Sul; 2001.

Sousa RO, Peralba MCR, Meurer EJ. Short chain organic acid dynamics in solution of flooded soil treated with ryegrass residues. Commun Soil Sci Plant Anal. 2002;33:779-87. https://doi.org/10.1081/CSS-120003065

Tedesco MJ, Gianello C, Bissani CA, Bohnen H, Volkweiss SJ. Análise de solo, plantas e outros materiais. 2. ed. Porto Alegre: Universidade Federal do Rio Grande do Sul; 1995. (Boletim técnico, 5).

Thauer RK. The Wolfe cycle comes full circle. PNAS. 2012;109:15084-5. https://doi.org/10.1073/pnas.1213193109

van Groenigen KJ, van Kessel C, Hungate BA. Increased greenhouse-gas intensity of rice production under future atmospheric conditions. Nat Clim Change. 2013;3:288-91. https://doi.org/10.1038/nclimate1712

Wang Y, Zhang Y, Wang J, Meng L. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass Bioenerg. 2009;33:848-53. https://doi.org/10.1016/j.biombioe.2009.01.007

Xu H, Hosen Y. Effects of soil water content and rice straw incorporation in the fallow season on CH_4 emissions during fallow and the following rice-cropping seasons. Plant Soil. 2010;335:373-83. https://doi.org/10.1007/s11104-010-0426-y

Yvon-Durocher G, Allen AP, Bastviken D, Conrad R, Gudasz C, St-Pierre A, Thanh-Duc N, del Giorgio PA. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. Nature. 2014;507:488-91. https://doi.org/10.1038/nature13164

Zabranska J, Pokorna D. Bioconversion of carbon dioxide to methane using hydrogen and hydrogenotrophic methanogens. Biotechnol Adv. 2018;36:707-20. https://doi.org/10.1016/j.biotechadv.2017.12.003

Zhang ZY, Yang YN, Lei ZF, Chen HJ, Sugiura N. Kinetic difference between acetate and propionate pregrown reactors through batch methanogenesis experiments. Japan Wat Treat Biol. 2008;44:95-107. https://doi.org/10.2521/jswtb.44.95

Zhao R, Zhang Z, Zhang R, Li M, Lei Z, UtsumI M, Sugiura N. Methane production from rice straw pretreated by a mixture of acetic–propionic acid. Bioresour Technol. 2010;101:990-4. https://doi.org/10.1016/j.biortech.2009.09.020

Zschornack T, Rosa CM, Reis CES, Pedroso GM, Camargo ES, Santos DC, Boeni M, Bayer C. Soil CH_4 and N_2O emissions from rice paddy fields in Southern Brazil as affected by crop management levels: a threeyear field study. Rev Bras Cienc Solo. 2018;42:e0170306. https://doi.org/10.1590/18069657rbcs20170306