Applied Energy 189 (2017) 21–30

Contents lists available at ScienceDirect

Applied Energy

journal homepage: www.elsevier.com/locate/apenergy

Reduction in greenhouse gas emissions from vinasse through anaerobic digestion

Bruna S. Moraes [a,*], Søren O. Petersen [b], Marcelo Zaiat [c], Sven G. Sommer [d], Jin Mi Triolo [d]

[a] Interdisciplinary Center of Energy Planning (NIPE), University of Campinas (Unicamp), Rua Cora Coralina, 330, Cidade Universitária, P.O. Box 6166, Campinas, São Paulo 13083-896, Brazil
[b] Aarhus University, Department of Agroecology, Blichers Allé 20, DK-8830 Tjele, Denmark
[c] Laboratory of Biological Processes, University of São Paulo (USP), Av. João Dagnone, 1100, Santa Angelina, 13563-120 São Carlos, São Paulo, Brazil
[d] Institute of Chemical Engineering, Biotechnology and Environmental Technology, Faculty of Engineering, University of Southern Denmark, Campus vej 55, DK-5230 Odense M, Denmark

HIGHLIGHTS

- This study assessed the effect of AD treatment of vinasse on CH_4 and N_2O emissions.
- CH_4 emissions were not detected from digested vinasse during storage.
- Anaerobic digestion increased NH_3 emissions from vinasse during storage.
- CH_4 emissions of untreated vinasse were equivalent to 43.8 kg CO_2 eq kg^{-1} C-vinasse.
- AD of vinasse before soil application decreased N_2O emissions by up to 78%.

ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 2 December 2016
Accepted 3 December 2016

Keywords:
Nitrous oxide
Methane
Fertirrigation
Climate change
Bioethanol sector
Anaerobic digestion

ABSTRACT

Vinasse is a residue from bioethanol production that is produced in large quantities in Brazil and Europe and is applied to fields as a source of plant nutrients (fertirrigation). A side effect of this use is greenhouse gas (GHG) emissions during storage and transport in open channels to fields, and from fertirrigated soils. This study assessed GHG emissions in experiments simulating this vinasse management system, and the potential for reducing emissions of methane (CH_4) and nitrous oxide (N_2O) from vinasse *via* anaerobic digestion (AD) in biogas plants. During 21 days' storage of untreated vinasse, 29% of dry matter (DM) and 40% of volatile solids (VS) were lost, which resulted in cumulative CH_4 emissions of up to 43.8 kg CO_{2eq} kg^{-1} C-vinasse. In contrast, there were no CH_4 emissions from AD-treated vinasse (digestate) during storage. GHG emission was related to the biochemical characteristics of the untreated and digested vinasse. The accumulation of oxidised nitrogen (N) compounds was up to four-fold higher in soil amended with untreated vinasse than from digestate-amended soil. The N_2O emissions from soil amended with untreated vinasse were also higher than from soil amended with digestate, ranging from 0.173 to 0.193 kg CO_{2eq} m^{-2} in the former and from 0.045 to 0.100 kg CO_{2eq} m^{-2} in the latter. Extrapolation of the results to a Brazilian case indicated that AD treatment prior to storage/transport and field application could reduce GHG emissions from the vinasse management chain by at least 48%, with further reductions from the use of biogas in power production.

1. Introduction

Anaerobic digestion (AD) for biogas production is one of the most efficient technologies for providing clean and renewable energy from organic waste and also has the potential to reduce greenhouse gas (GHG) emissions from digestate [1,2]. The technology can therefore contribute towards meeting the mandatory national target for renewable energy set by the European Commission [3] of covering 20% of energy consumption by 2020 while also reducing GHG emissions [4]. AD technology can also increase the renewable contribution to the energy matrix of developing countries such as Brazil that have little experience in this particular field, but considerable potential. The Brazilian Decennial Expansion Energy Plan 2024 includes 4.5% annual growth in renewable energy production up to 2024, but the focus is on solar and wind

* Corresponding author.
E-mail address: bruna.moraes@nipe.unicamp.br (B.S. Moraes).

http://dx.doi.org/10.1016/j.apenergy.2016.12.009
0306-2619/

energy and biodiesel [5]. Biogas targets are being considered in the Brazilian National Energy Plan 2050 (under development), which includes the development of a biogas services chain in 2030 and the use of 13% of the theoretical biogas production potential from agricultural wastes for electricity generation in 2050 [6].

Vinasse is the main organic waste from bioethanol production and offers great potential for biogas production, although this alternative use is currently underexplored. In Brazil, as well as in Japan, Europe and the United States (US), vinasse has traditionally been managed [6] as an additive for animal feed [7,8], applied to fields as a nitrogen-phosphorous-potassium (NPK) fertiliser for crop production or been used to ameliorate soil organic matter [9]. Concerns about the potential environmental impacts on surface water and groundwater have resulted in a ban on the use of vinasse as a fertiliser in the US [10] and Uruguay [11]. In contrast, the application of vinasse is still recommended and legal in Brazil, the European Union and Canada. Between 1.0 and 1.7 million m^3 of vinasse are recycled annually to sugarcane fields by fertirrigation in Brazil – the largest sugarcane ethanol producer in the world [12]. Application rates are limited by the amount of K added per hectare set by a law aiming to protect soil, surface water and groundwater [13].

In addition to the risk of nutrient leaching from field-applied vinasse [14–16], its storage, transport and field application may be a source of methane (CH_4) and nitrous oxide (N_2O), which have respective Global Warming Potentials (GWP) of 34 and 298 times that of carbon dioxide (CO_2) [17]. The vinasse is stored in lagoons before transport or is transported directly in open channels from the bioethanol producer to fields for application to sugarcane crops. Methane is produced in vinasse during storage and transport, and may account for 98% of the total GHG emissions from vinasse management in this step, which has been assessed to be 1.43 kg CO_{2eq} m^{-3} [16]. After application of vinasse to fields with unburnt sugarcane, the accumulated N_2O emission corresponded to 142.3 kg CO_{2eq} ha^{-1} [18], and 361.5 kg CO_{2eq} ha^{-1} when applied to fields with sugarcane ratoon [19]. In the latter case, emissions were more than twice as high when N fertiliser was applied together with vinasse.

GHG emissions from the vinasse management chain may be reduced by AD treatment of vinasse to produce biogas. During AD, organic carbon is transformed into CH_4 and CO_2, and the potential for CH_4 emissions during subsequent storage is therefore reduced [20]. Similarly, N_2O emissions from vinasse after field application may be reduced, although this depends on soil conditions [21]. Potassium, N and P are not lost during AD, and this treatment technology therefore preserves the nutrient value of vinasse. The use of biogas for heat and power production may thus substitute fossil fuels and reduce GHG emissions. This conception is in accordance with the recent trends worldwide aiming to identify and exploit bioenergy technologies as mitigation measures [22].

When introducing a treatment technology for renewable energy production in a biomass management chain, it is important to assess how the technology affects all stages of the biowaste management chain [2,23,24], and a whole-farm approach is recommended for evaluating the overall effect of one or more GHG mitigation measures [23]. To the authors' knowledge, there are no published studies on the effect of AD treatment of vinasse on GHG emissions from the vinasse management chain. Therefore, this study aimed to quantify how AD treatment of vinasse affects CH_4 and N_2O emissions during storage or transport and after application to soil through bench-scale experiments. This study thus constitutes one of the first sources of experimental data on the theme and should contribute to stimulate the adoption of mitigation measures in a traditional and long-established management chain within the bioethanol sector in Brazil.

2. Material and methods

2.1. Sources of organic wastes and soil

The experiments were performed in the laboratory of the University of Southern Denmark. Vinasse from sugar beet ethanol production was provided by Nordic Sugar (Copenhagen, Denmark) and diluted 10 times prior to use to better represent the composition of sugarcane vinasse as used in Brazil. A digestate was produced in a stable codigestion process with the aforementioned vinasse, cow manure and straw (in proportions of 61:2:37% respectively in terms of total VS) and at a C/N ratio of 10:1 [25]. AD was performed in a mesophilic (37 °C) continuous stirred tank reactor (CSTR) at a hydraulic retention time (HRT) of 35 days [25]. The composition of the vinasse and the digestate used in the experiments are presented in Table 1. GHG emissions of this digestate were compared with emissions from untreated diluted vinasse during storage and after application to soil.

The soil was collected in November 2013 from 0 to 20 cm depth in a stubble field at the Foulum Research Centre in Denmark (55°52′ N, 9°34′ E). The soil is characterised as a sandy loam soil (Typic Hapludult) with 2.7% C, 0.18% N, pH (H_2O) 6.3, and a cation exchange capacity (CEC) of 8.7 cmol kg^{-1}. At the time of sampling the gravimetric soil moisture content was 15.2%, or around 80% of field capacity (FC). After sampling, the soil was passed through a 4-mm mesh sieve to remove roots and stones and stored at 4 °C. The soil was then stored at 22 °C for 24 h before the start of the experiment.

2.2. Gas emission from vinasse and digestate during storage

Storage conditions and emission measurements followed the methodology of Petersen et al. [26]. Aliquots of 15 L untreated and digested vinasse were stored in 25-L polyethylene containers in triplicate. For the experiments with untreated vinasse, 10% v/v of digestate was added in order to simulate the inoculation with a microbiota that is retained in vinasse stores or transport channels of sugarcane mills in Brazil [27]. The containers were immediately closed with a lid, to which a tube 1 cm in diameter and 15 cm long was connected to allow for the exchange of gases between the vinasse and the atmosphere.

Rates of CH_4, CO_2 and NH_3 emissions from stored untreated vinasse and digestate were measured during a period of 21 days at 37 °C, which is in accordance with the temperature of digestate

Table 1
Characterisation of sugar beet vinasse (diluted 10 times) and digestate used in the experiments.

Parameter	Unit	Sugar beet vinasse	Digestate from codigestion
Dry matter	g kg^{-1}	114.0	46.4
Volatile solids	g kg^{-1}	83.5	24.8
Total ammonia nitrogen	g L^{-1}	0.84	2.76
Total Kjeldahl nitrogen	g L^{-1}	4.90	3.56
Protein	% in VS	30.4	20.2
pH	n.a.	4.96	7.97
Chemical oxygen demand	g L^{-1}	77.41	33.16
C	g L^{-1}	29.03	12.44
C/N ratio	n.a.	5.92	3.5
Acetic acid	g L^{-1}	2.33	3.03
Propionic acid	g L^{-1}	0.02	1.64
Iso butyric acid	g L^{-1}	0.00	0.07
Butyric acid	g L^{-1}	0.03	0.08
Iso valeric acid	g L^{-1}	0.01	0.07
Valeric acid	g L^{-1}	0.00	0.00
Total volatile fatty acids	g L^{-1}	2.39	4.89

n.a.: not applied.

transferred from the biogas reactor but also of vinasse in open transport channels. Gas emissions were measured by transferring 600 mL of the liquid from the storage containers to 2-L Duran flasks. Two 5 cm-long Teflon tubes were connected to two holes in the lids of the flasks. A pump connected to one of the Teflon tubes provided a pressure that generated airflow of 2 L min^{-1} through the headspace. Prior to gas sampling this airflow was maintained for 30 min in order to establish steady-state concentration gradients above and below the liquid-air interface. A 5-L sample of exhaust air was then collected in a polyethylene terephthalate gas sampling bag fitted at the outlet with a Teflon tube. Air samples for analysis of CH_4 and CO_2 were collected from the gas-sampling bag with syringes and analysed by gas chromatography (see Section 2.4). The NH_3 concentration in each bag was determined using Kitagawa ammonia detection tubes (Duotec A/S, Glostrup, Denmark). The complete experimental set-up is presented in Fig. 1.

The pH, dry matter (DM), volatile solids (VS), volatile fatty acids (VFA), total ammoniacal N (TAN = NH_3-N + NH_4^+-N), total Kjeldahl N (TKN) and chemical oxygen demand (COD) of diluted untreated vinasse and digested vinasse were determined according to standard procedures (see Section 2.4) together with gas sampling at 7, 14 and 21 days after transfer of vinasse to the 25 L containers.

2.3. GHG emissions from vinasse and digested vinasse applied to soil

N_2O emissions from digestate and untreated vinasse applied to soil were studied by applying both materials at a rate corresponding to 100 m^3 ha^{-1} to soil packed (1.3 g cm^{-3}) in steel cylinders (6 cm diameter and 5 cm depth). The soil was amended with ammonium nitrate (NH_4NO_3) at a rate corresponding to 100 kg N ha^{-1}. The amounts of vinasse and digestate applied were selected according to the agricultural practice for use of vinasse to fertilise sugarcane in Brazil, and the fertiliser application rate corresponded to the average amount used in sugarcane fields at the ratoon stage (re-growth of cane stubbles) [12,15].

The treatments measured in triplicate and applied to the soil surface were as follows: (a) untreated vinasse or digestate without storage, (b) untreated vinasse or digestate stored for 7 days, (c) untreated vinasse or digestate stored for 14 days and (d) soil without amendments as a control (Fig. 1). The compositions of vinasse/digestate without storage and those stored are shown in Tables 1 and 2. After the application of fertiliser and vinasse/digestate, the cylinders were sealed at both ends with plastic lids that had been perforated to facilitate gas exchange. All the treatments were incubated at 22 °C. In total 95 cylinders were prepared, including five controls (soil without fertiliser and vinasse/digestate). The cylinders were weighed at regular intervals to determine water loss.

At 1, 4, 7, 14 and 21 days after initiation of the experiment, CO_2, CH_4 and N_2O emission rates were determined by randomly selecting three cylinders from each treatment and transferring these to 1-L glass jars closed with a lid equipped with a septum for gas sampling [28]. Gas was sampled using a 10 mL syringe immediately after closing the glass jars (t_0), and after 30, 60 and 90 min (t_1, t_2 and t_3). The headspace was mixed before gas sampling by exchanging 10 mL of air ten times with the syringe. The 10-mL gas samples were transferred to 6-mL evacuated Exetainers (Labco, High Wycombe, UK) for gas analyses.

For subsequent soil N analysis, about 10 g of soil from each replicate was transferred, after thorough mixing, to 50-mL extraction flasks and 40 mL of KCl (1 M) added. The flasks were closed and mixed end-over-end for 30 min and afterwards centrifuged for 1 h at 2000 rpm. The supernatant was passed through a 0.45 μm filter and stored at −18 °C until analysis (see Section 2.4) for TAN (NH_3-N + NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N). Soil sampling was performed simultaneously with gas sampling at 1, 4, 7, 14 and 21 days after initiation of the experiment.

2.4. Analytical methods

The concentrations of N_2O, CH_4 and CO_2 were analysed using an Agilent 7890 gas chromatograph with CTC Combipal autosampler. The configuration of the instrument is described in detail by Petersen et al. [26]. pH, TAN, TKN, NO_2-N, NO_3-N, DM, VS and VFA were all determined according to standard procedures [29]. The pH of biomasses and soil extracts were determined using a standard pH electrode (Radiometer, Copenhagen, Denmark). TKN was determined using a Kjeltec 2011 instrument (Foss, Höganäs, Sweden),

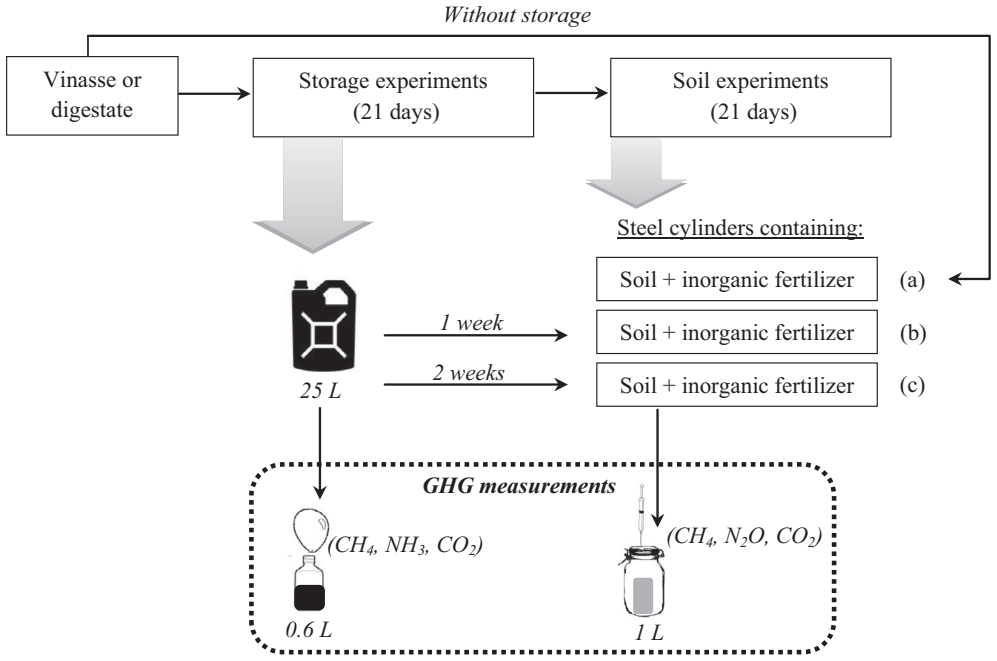

Fig. 1. Experimental set-up for evaluation of greenhouse gas (GHG) emissions from vinasse and digestate during storage and after soil application: (a) without storage; (b) after 1 week storage; (c) after 2 weeks storage.

Table 2
Characterisation of vinasse/digestate after 7, 14 and 21 days' storage used in the GHG emissions experiments from soil (average ± SE, n = 3).

Parameter	Unit	Vinasse			Digestate		
		7 days	14 days	21 days	7 days	14 days	21 days
Dry matter	g kg^{-1}	100.3 ± 0.8	91.7 ± 8.1	81.0 ± 12.8	39.9 ± 1.29	51.8 ± 2.87	45.3 ± 7.26
Volatile solids	g kg^{-1}	70.7 ± 1.1	60.2 ± 8.3	49.5 ± 12.5	19.3 ± 1.93	29.2 ± 2.85	23.9 ± 7.27
Total ammonia nitrogen	g L^{-1}	0.45 ± 0.10	1.57 ± 0.55	1.67 ± 0.36	2.54 ± 0.02	2.16 ± 0.05	2.22 ± 0.03
Total Kjeldahl nitrogen	g L^{-1}	4.48 ± 0.16	4.90 ± 0.92	4.84 ± 0.04	3.13 ± 0.11	3.74 ± 0.05	3.68 ± 0.09
Protein	% in VS	28.0 ± 1.0	30.6 ± 5.8	30.3 ± 0.3	19.6 ± 0.7	23.4 ± 0.3	23.0 ± 0.6
pH	n.a.	4.7 ± 0.1	6.18 ± 0.91	5.96 ± 0.83	7.84 ± 0.04	7.76 ± 0.02	7.90 ± 0.04
Chemical oxygen demand (COD)	g L^{-1}	79.2 ± 3.5	78.0 ± 0.4	77.4 ± 4.5	41.8 ± 4.6	35.8 ± 2.9	30.1 ± 2.2
C	g L^{-1}	29.7 ± 0.8	29.2 ± 1.0	29.0 ± 0.8	15.7 ± 1.7	14.2 ± 1.1	11.3 ± 0.8
C/N ratio	n.a.	6.6 ± 0.5	5.75 ± 0.30	5.99 ± 0.30	5.05 ± 0.37	3.85 ± 0.24	2.99 ± 0.17
Acetic acid	g L^{-1}	2.39 ± 0.09	2.41 ± 1.48	7.10 ± 3.00	4.65 ± 0.09	4.99 ± 0.12	4.96 ± 0.20
Propionic acid	g L^{-1}	0.32 ± 0.03	0.90 ± 0.52	2.21 ± 1.01	3.02 ± 0.10	3.09 ± 0.09	3.27 ± 0.08
Iso butyric acid	g L^{-1}	0.01 ± 0.00	0.01 ± 0.00	0.10 ± 0.10	0.21 ± 0.00	0.22 ± 0.13	0.24 ± 0.02
Butyric acid	g L^{-1}	0.05 ± 0.01	8.92 ± 5.19	10.4 ± 6.11	0.23 ± 0.01	0.25 ± 0.01	0.28 ± 0.02
Iso valeric acid	g L^{-1}	0.00 ± 0.00	0.01 ± 0.01	0.13 ± 0.08	0.00 ± 0.00	0.01 ± 0.01	0.25 ± 0.17
Valeric acid	g L^{-1}	0.00 ± 0.00	0.02 ± 0.01	0.09 ± 0.06	0.0 ± 0.00	0.01 ± 0.01	0.02 ± 0.06
Total volatile fatty acids	g L^{-1}	2.76 ± 0.95	12.3 ± 3.50	20.00 ± 4.39	8.11 ± 1.99	8.57 ± 2.11	9.03 ± 2.09

SE: standard error.

and TAN either by the distillation and titration method or by the phenate method based on absorbance at 640 nm. NO_2^--N and NO_3^--N were measured colorimetrically at 543 nm and 220 nm respectively [29]. DM was measured gravimetrically after a 24-h drying period at 103 °C. VS was determined following combustion at 550 °C for 2 h. VFA (C2–C5) concentrations were determined using a gas chromatograph (Hewlett Packard 6890, USA) with a flame ionisation detector and 30 m X 0.25 mm X 0.25 μm column (HP-INNOWax, Germany). The carrier gas was He. The temperature of the column was increased gradually from 110 °C to 220 °C at a rate of 10 °C min^{-1}.

2.5. Calculations and data analysis

The emission rates (E_i, g m^{-2} h^{-1}) of NH_3 and CH_4 from the stored digestate and vinasse were calculated per surface area according to Eq. (1).

$$E_i = (C_i \cdot F \cdot M_i)(V \cdot A)^{-1} \quad (1)$$

where C_i is the concentration of NH_3 or CH_4 in the sampled air (L L^{-1}), F is the air flow (L h^{-1}), M_i is the molar mass of NH_3 or CH_4 (g mol^{-1}), V (L mol^{-1}) is the molar volume of NH_3 or CH_4 in the headspace gas at 37 °C and ambient pressure, and A is the surface area of the storage unit (m^2). There were no emissions of N_2O during storage.

The fluxes of CO_2, CH_4 and N_2O from soil were calculated using linear regression of the gas concentration *versus* the time after closing the jars with lids (t_0, t_1, t_2 and t_3). Gas flux (F, g gas m^{-2} h^{-1}) was calculated according to Eq. (2).

$$F = \frac{dC}{dt} \cdot \frac{V}{A} \quad (2)$$

where dC/dt is the rate of change in gas concentration (CO_2, CH_4 or N_2O) in the closed glass jar (g L^{-1} h^{-1}), V is the glass jar volume (L) and A is the surface area circumscribed by the glass jar (m^2).

Cumulative emissions over the experimental period were obtained through numerical integration of the area under the curve of gas fluxes over time by applying the trapezoid rule using the software Origin 8.1. Global warming potential was calculated in tonnes of equivalent carbon (t CO_{2eq}), by using the factors of 298 kg CO_{2eq} kg^{-1} N_2O and 34 kg CO_{2eq} kg^{-1} CH_4 [17] for a 100-year horizon.

Data of GHG fluxes and emission rates were compared through analysis of variance (one-way ANOVA, n = 3) to evaluate whether there were treatment effects at 95% confidence interval. Treatment differences were identified by Tukey's honest significant difference test.

2.6. Emission scenarios for the Brazilian case

The overall GHG emissions from vinasse in scenarios with and without biogas production were compared using experimental data from this study as the basis for discussing the effect of biogas production of vinasse within the context of sugarcane ethanol production. The emission scenarios were defined using common practices of vinasse management from Brazilian sugarcane biorefineries, which include storage lagoons or transportation in open distribution channels [16] prior to vinasse application to sugarcane fields. The application rate to sugarcane fields was set as 100 m^3 ha^{-1}, a rate normally used for fertirrigation in Brazil [30]. In the scenarios, the retention time in the open channels or storage lagoons was set at 7 and 14 days, and application of vinasse/digestate without storage before fertirrigation was included in the calculations. The vinasse retention time in sugarcane mills in Brazil is variable, but values from 2 to7 days can be normally found, as well as the application without storage or not using open channels [16,31]. The reduction in GHG emissions due to substitution of fossil fuels for power generation with biogas from vinasse was calculated from the measured methane yield of 0.69 L L^{-1} d^{-1} during codigestion of vinasse reported by Moraes et al. [25] and assuming that 100% of CH_4 in biogas is combusted. Leakage losses of CH_4 from the biogas plant were estimated as 1.5% of total CH_4 production [32]. A scheme of the assessed scenarios based on typical Brazilian vinasse management chain is presented in Fig. 2. The overall GHG emissions balance results were expressed in terms of CO_{2eq} per volume of vinasse or digestate, so that they can be applied to different production scales of sugarcane biorefineries.

3. Results and discussion

3.1. Changes in C and N species during storage

In untreated vinasse, pH increased (Table 1) from 4.7 to 6.2 ($p < 0.05$), probably due to the production of total inorganic carbon (TIC) which buffers the solution to a pH between 6 and 8 [33]. The optimal pH range for CH_4 production is between 6 and 8.5, so the pH of the untreated vinasse during storage reached a level that would support CH_4 production. In the digestate, the pH was at the upper end of this range throughout the storage period.

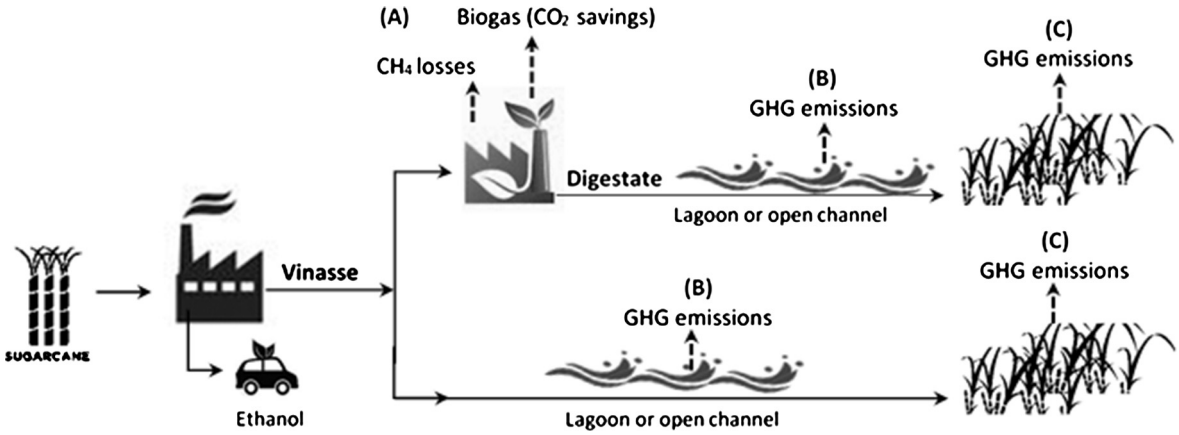

Fig. 2. Schematic illustration of the assessed scenarios with and without biogas production accounting for overall GHG emissions from vinasse management chain within a typical Brazilian case. (A) biogas production data from a codigestion reactor treating vinasse [25] and CH_4 losses estimation from Sommer et al. [32]; (B) GHG emission data from storage experiments of the present study; (C) GHG emission data from soil experiment of the present study.

In untreated vinasse, acetic acid was initially predominant, but during storage the butyric acid concentration increased from 0.05 to 10.4 g L^{-1} and propionic acid from 0.3 to 2.2 g L^{-1} although there was a wide variation between replicates (Table 2). In contrast to the digestate, propionic and butyric acid concentrations in untreated vinasse increased significantly ($p < 0.05$) during storage. Accumulation of propionic acid, butyric acids and total VFA in untreated vinasse indicated that the complete degradation of organic matter to CH_4 during storage was inhibited or delayed, either because the concentration of methanogens was initially low or the activity of methanogens was inhibited due to a stressful and changing environment [34–38]. Accumulation of these acids may occur if further degradation becomes thermodynamically unfavourable due to the accumulation of H_2 and/or acetic acid in the absence of methanogenesis, *i.e.* VFA accumulation due to hydrolysis and acid formation is faster than CH_4 formation [39].

The total concentration of VFA in the codigestate with vinasse, manure and straw did not change significantly during storage, and there was no significant change in DM or VS ($p > 0.05$), presumably because the digestible organic carbon had been degraded during biogas production and was therefore absent from the digestate from the start of storage (Table 2). In contrast, the total VFA of the untreated vinasse increased from 2.8 to 20 g L^{-1}, indicating that hydrolysis and acidogenesis were taking place. Transformations of organic matter during storage of untreated vinasse resulted in significant ($p < 0.01$) reductions in DM and VS of 29% and 40% respectively. The corresponding concentrations in digestate were considerably lower than in untreated vinasse, with no clear trends.

In untreated vinasse the TAN concentration increased almost three-fold during storage, while no increase was measured in the digested vinasse. The increase of TAN in stored untreated vinasse was probably related to mineralisation of organic N, as reflected in the reduction of VS. The absence of changes in TAN during storage of digestate was a result of N mineralisation during AD prior to storage. In both waste materials the TAN concentration was always below the level of 4–6 g L^{-1}, which may inhibit methanogenesis [40].

3.2. Soil N dynamics after soil application

In soil amended with digestate, the initial NH_4^+-N concentration was significantly ($p < 0.05$) higher than that in soil with untreated vinasse (Fig. 3). This was probably because most N in the digestate was in an inorganic form following digestion, while in untreated vinasse more N was in organic forms. After day 1, NH_4^+-N in the soil amended with fresh or stored untreated vinasse (Fig. 3, left) increased until day 7 and then declined over time, a pattern also seen to a lesser extent in soil amended with non-stored digestate (Fig. 3a, right). There was a delay in net N mineralisation in soil amended with untreated stored vinasse (Fig. 3b and c, left), which may have been due to microbial immobilisation during the metabolism of volatile fatty acids that had accumulated during storage [41].

There was a significant decline in soil NO_3^--N concentrations during the first 7 days after application of non-stored vinasse, and of vinasse stored for 7 days before application to soil (Fig. 3a, b left), which is indicative of denitrification. Between 7 and 14 days of incubation, the NO_3^--N concentration did not change much in soil amended with unstored vinasse (Fig. 3a, left) or vinasse stored for 7 days (Fig. 3b, left), after which the concentration increased four-fold ($p < 0.05$). Addition of liquid vinasse may initially have caused a reduction in oxygen concentration due to microbial degradation of labile organic C [15], causing anaerobiosis in microsites, and this may have delayed oxidation of NH_4^+ and enhanced denitrification of NO_3^- [42]. Later the declining NH_4^+ concentration and concomitant increase in NO_3^--N and NO_2^--N concentrations in all treatments showed that aerobic conditions probably dominated. This was in accordance with the decrease in N_2O emissions towards the end of measuring period (see Section 3.4).

The accumulation of oxidised N was always lower in the treatments with digestate, which may be related to the lower potential for N mineralisation. In soil to which non-stored digestate was added, no increase in NO_3^--N was observed (Fig 3a, right). In soil amended with vinasse stored for 14 days, the NO_3^--N concentration increased significantly ($p < 0.05$) after day 7 (Fig. 3c, left), while the increase in NO_3^--N measured in the soil with stored digestate (Fig. 3b and c, right) was not significant. Stabilisation of organic wastes through AD before their application to soil will reduce short-term microbial activity, as shown by Möller [43], which may have contributed to the more moderate N dynamics in the soil amended with digestate compared with undigested vinasse.

Nitrite (NO_2^--N) accumulated in all treatments and remained high except in the soil to which digestate stored for 7 days was added (Fig 3b, right). The accumulation of NO_2^--N in the soil may be due to an imbalance between ammonia-oxidising and nitrite-oxidising bacteria or incomplete activity of heterotrophic denitrifiers, or both. The apparent coexistence of aerobic and anaerobic microsites in vinasse and digestate-amended soils was in accordance with previous research on soils receiving liquid amendments, *e.g.* liquid manure [44] or liquid fertiliser [15].

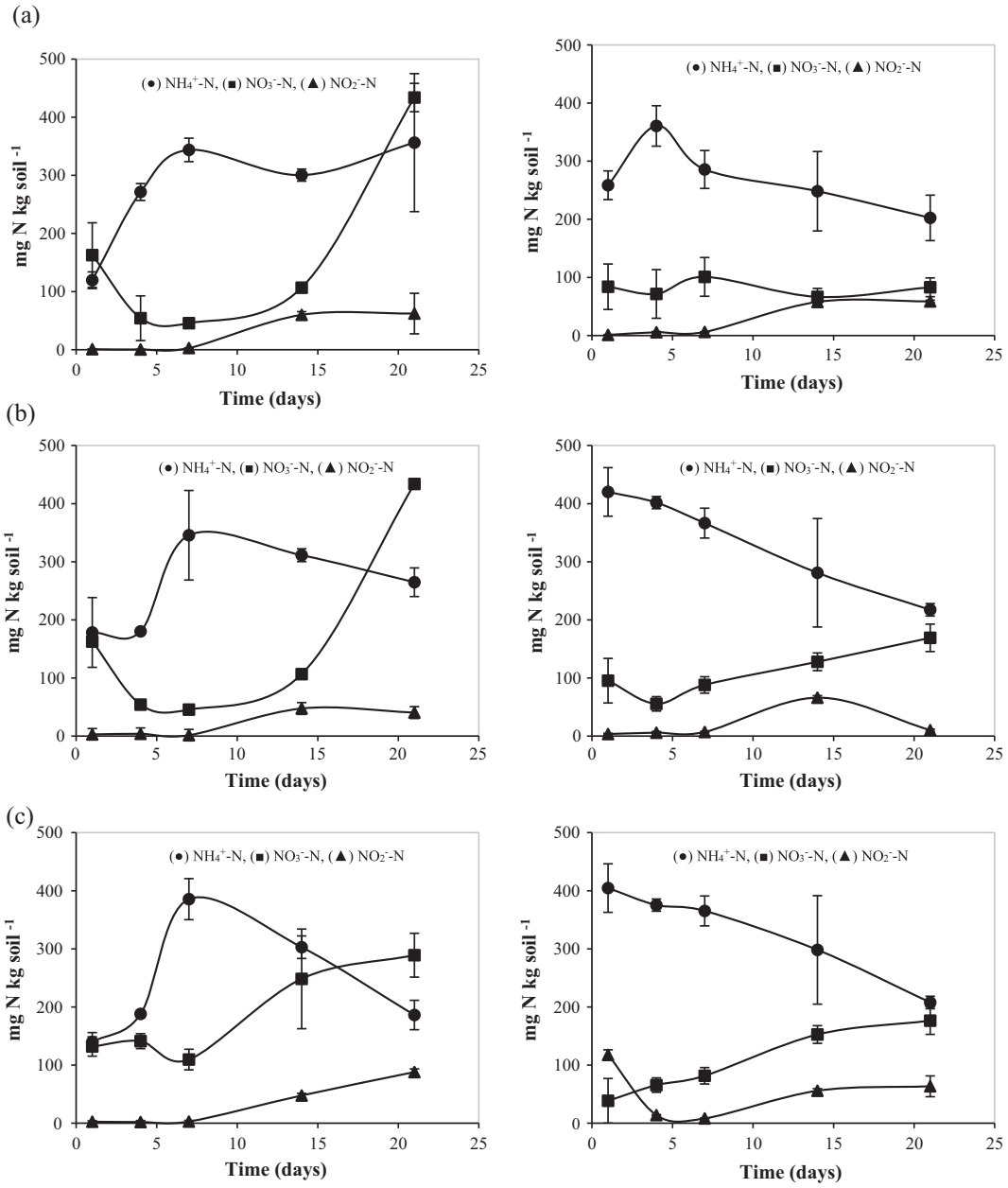

Fig. 3. Concentration of (●) NH_4^+-N, (■) NO_3^--N, (▲) NO_2^--N in the soil after application of vinasse (left) and digestate (right): (a) without storage; (b) after 7 days' storage; (c) after 14 days' storage (error bars = SEM, n = 3).

3.3. Gas emissions from untreated vinasse and digestate during storage

No CH_4 was emitted from the digestate during storage, indicating that degradable organic matter had been effectively transformed to CO_2 and CH_4 during the preceding biogas production process [32], i.e., the remaining organic matter in the digestate was not susceptible to anaerobic degradation to methane, and thus, no carbon emissions were detected. In contrast, CH_4 emission from untreated vinasse was measured from the day 7 of storage (Fig. 4), reflecting the observed decline in organic carbon. The lag phase in methanogenesis may be due to a suboptimal environment with low pH (Table 2) to which methanogens in untreated vinasse were not adapted. The standard error of observed mean CH_4 emissions was high, so differences were generally not significant. However, one of the three replicates was considered an outlier since the cumulative CH_4 production per volume of vinasse was an order of magnitude higher (333 g CH_4 L^{-1} vinasse) than from the two other replicates, and higher than the biochemical methane potential of sugar beet vinasse [25]. Excluding this outlier from data analysis, AD was effective in mitigating GHG emissions during storage compared to untreated vinasse. This supports the findings of Oliveira et al. [16] who showed that emissions of CH_4 from vinasse in open distribution channels are accompanied by a reduction in organic matter content. The emission in their study was attributed to the anaerobic conditions in the vinasse and to the huge volume of organic material sedimented in the channel. It therefore appears that AD would also be able to reduce emissions during transport.

Ammonia emissions in this study were low due to the absence of air exchange above the vinasse and digestate during storage. Ammonia was emitted from the digestate (Fig. 5), since inorganic-N was available in the digestate due to previous organic-N conversion in the anaerobic digestion process; however

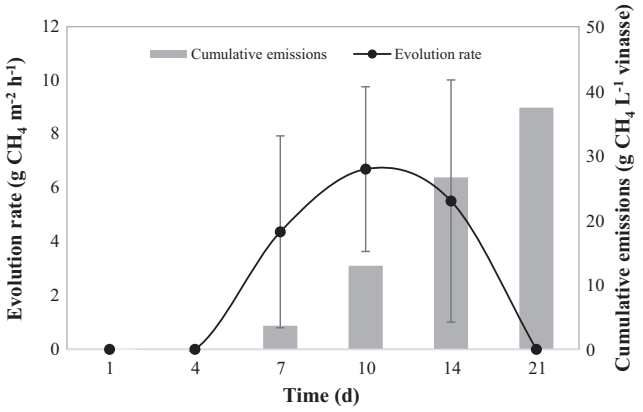

Fig. 4. Emission rates (●) and cumulative emissions (bars) of methane from stored untreated vinasse at 37 °C (error bars = SEM, n = 2). One of the triplicates was considered as an outlier due to inconsistent experimental results.

no ammonia was emitted from untreated vinasse (data not shown), which was not surprising in view of the contrasting pH of digestate and untreated vinasse [45]. This result is in line with earlier studies showing that the NH_3 emission potential of a biomass is increased by AD due to an increase in pH and TAN concentrations [46].

3.4. Gas emission from digestate and vinasse applied to soil

The cumulative N_2O emissions of 115–130 g CO_2 eq m^{-2} from the sugar beet vinasse applied to soil (Table 3) were higher than emissions measured from soil amended with sugarcane vinasse under field conditions, which have been found to range from 12.4 to 43.9 g CO_2 eq m^{-2} [18,19]. The main reason was probably the high N content of sugar beet vinasse of 4–5 g L^{-1} (Table 2) compared to sugarcane vinasse with <1 g N L^{-1} [47]. Nitrous oxide emissions were significantly ($p = 0.003$) lower (73%) from soils amended with digestate compared to untreated vinasse (Fig. 6a and b), and no emissions were detected from unamended soil. After 14 days of incubation, the emission of N_2O peaked and thereafter declined. These trends were similar to those seen in studies from Brazil with the application of sugarcane vinasse [15,18]. During the first 7 days, the declining NO_3^--N concentrations suggested that N_2O emission was mainly due to incomplete heterotrophic denitrification in the soil (also reported in the literature as the main source of N_2O emissions in soil, [48]), although other biological processes cannot be excluded, *e.g.* heterotrophic nitrification [49] and nitrifier denitrification [50]. It is likely that soil NO_3^--N availability limited N_2O emissions during this period [42].

Degradable organic matter enhances microbial activity and oxygen consumption, as reflected in higher CO_2 emissions from soils amended with vinasse (Fig. 7), and consequently N_2O emissions probably depended on a partial depletion of O_2 in the soil during the first 14 days. The delay between application of vinasse and time of maximum N_2O emissions indicates that there was a coupling between nitrification and denitrification activity, as previously shown for manure application by Nielsen and Revsbech [51] who also saw a gradual increase in N_2O emissions towards a maximum after 12–14 days. In the present study the increase in N_2O emissions measured from day 7 occurred in parallel with NO_2^--N accumulation. Nitrous oxide emissions declined after 14 days whereas NO_2^- concentrations remained high, indicating that NO_2^- was probably not the direct source of N_2O and reflected a soil environment in which stress factors caused an imbalance between ammonia oxidation and nitrite oxidation [52]. The behaviour of N compounds in soil observed through these results is in accordance with the main N_2O generating pathways (Fig. 8).

Soil N dynamics were stronger, and N_2O emissions higher, in soil amended with vinasse compared with digestate. This is in line with previous results on N_2O emission from this soil after application of untreated and digested liquid manure [53]. It should be stressed that the balance between N_2O and N_2 depends on the balance between oxygen supply and demand, and therefore mitigation of N_2O emissions *via* AD of vinasse is most likely to occur when the soil oxygen supply is high, *i.e.* in light-textured and/or well-drained soil.

There was no significant CH_4 emission from digestate or vinasse applied to soil (data not shown), as also observed by Carmo et al. [15].

3.5. GHG emission from the two systems: scenario assessment within the Brazilian case

Table 4 shows the overall balance of CH_4 and N_2O emissions during management of vinasse with or without AD. Cumulative CO_2-C emissions during storage and from soils were not included in the GHG balance since they are considered biogenic carbon, *i.e.* the CO_2 emitted during the biomass-based fuel (ethanol in this case) lifecycle does not increase atmospheric CO_2 concentrations, assuming that the biogenic carbon emitted is offset by the uptake of CO_2 resulting from the growth of new biomass [10]. Carbon dioxide savings from the power generated by CH_4 production in

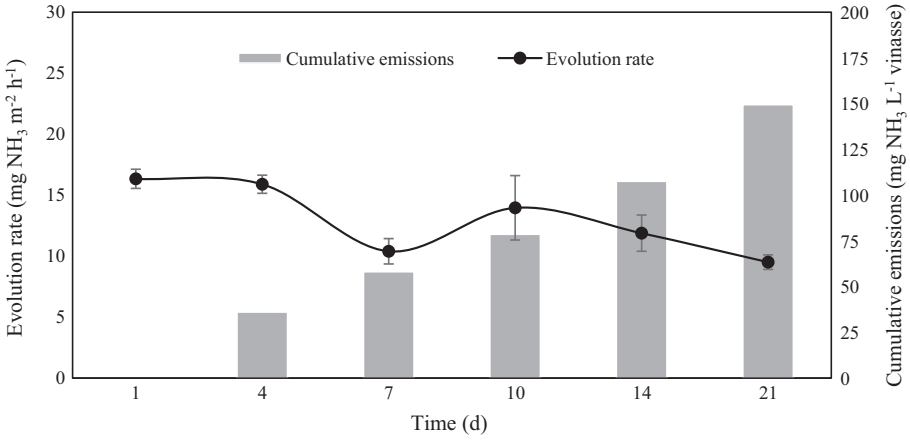

Fig. 5. Emission rates (●) and cumulative emissions (bars) of ammonia from stored AD treated vinasse at 37 °C (error bars = SEM, n = 3).

Table 3
Cumulative N_2O emissions from soils treated with vinasse and digestate as affected by storage prior to application to soil. Letters (a, b, c, d) indicate significant statistical ($P < 0.05$) differences within each column (n = 3).

Management	Treatment[a]			
	Vinasse		Digestate	
	N_2O-N (mg m^{-2})	g CO_{2eq} m^{-2}	N_2O-N (mg m^{-2})	g CO_{2eq} m^{-2}
Without storage	418.22 a	130	249.98 bc	77
7 days' storage	405.21 a	126	202.12 cd	63
14 days' storage	370.32 a	115	116.85 d	36

[a] No emissions were detected from soils without treatment (control).

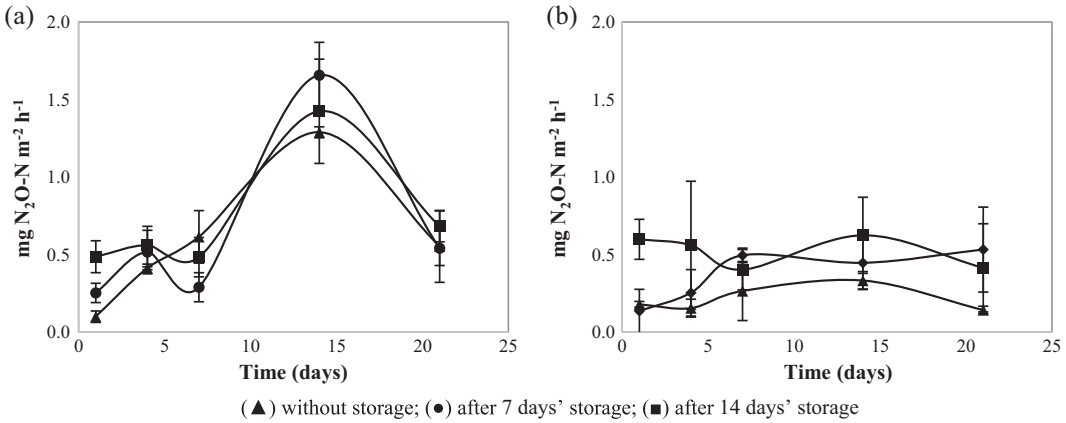

Fig. 6. Nitrous oxide emission rates of untreated vinasse (a) and digestate (b), related to the management before application: (▲) without storage; (●) after 7 days' storage; (■) after 14 days' storage (error bars = SEM, $n = 3$).

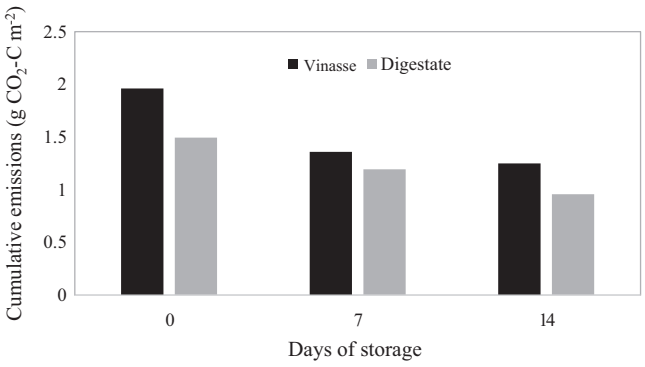

Fig. 7. Carbon dioxide cumulative emissions of untreated vinasse (black bars) and digestate (grey bars), related to the management before application t: 0 days' storage (*i.e.* without storage); after 7 days' storage; after 14 days' storage.

the biogas reactor were 47.0 kg CO_{2eq} m^{-3}, applying the results of biogas production of vinasse from Moraes et al. [25]. With a retention time of vinasse in open channels or lagoons of 7 and 14 days, GHG emissions from this stage may be important for the total emission during management of the vinasse. Thus Oliveira et al. [16] showed that CH_4 emissions from vinasse transported with a retention time of 3 d in open distribution channels account for 98% of the total emissions. According to the bench-scale study presented here, AD before transport or storage could reduce CH_4 emissions during this stage dramatically. After field application, the digestate showed N_2O emissions 48–78% lower than those from untreated vinasse, depending on the retention time before application to soil.

The introduction of AD in the vinasse management chain will probably reduce GHG emissions, regardless of the length of storage, but the relative effect on GHG mitigation will increase with increasing storage time of the vinasse in lagoons or retention in channels before application to fields, *i.e.* from 56.3 to 966.1 kg CO_{2eq} m^{-3} reduction when including substitution of fossil fuel used for power production. AD of vinasse increases the risk of losing NH_3 during transportation or storage due to the increase in pH. Future studies should be undertaken in conditions that are applicable to Brazil (*e.g.* use of sugarcane vinasse, soil from sugarcane crops) to confirm these results.

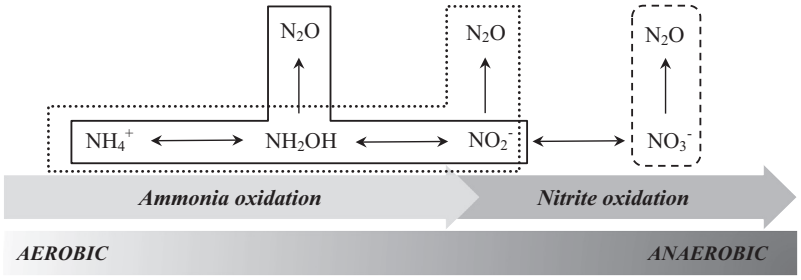

Fig. 8. Main nitrous oxide generating pathways in soil: (–) nitrification, (. . .) nitrifier denitrification, (**- -**) denitrification.

Table 4
Global GHG balance for scenarios of vinasse management as affected by the biodigestion process considering the cumulative emissions from storage lagoons of vinasse or digestate or its transportation in open distribution channels (CH_4 emissions) and posterior application to the soil (N_2O emissions), as affected by storage time.

GHG balance	[a]Storage time (d)					
	Vinasse			Digestate		
	0[b]	7	14	0[b]	7	14
[c]Biogas (kg CO_{2eq} m^{-3})	0.0	0.0	0.0	−47.0	−47.0	−47.0
[d]Storage (kg CO_{2eq} m^{-3})	0.0	124.8	906.3	0.0	0.0	0.0
[e]Field (kg CO_{2eq} m^{-3})	19.3	19.0	17.3	10.0	7.9	4.5
Total CO_{2eq} (kg CO_{2eq} m^{-3})	19.3	143.8	923.6	−37.0	−39.1	−42.5

[a] Equivalent to days of storage in the current study prior to the field application management.
[b] Means vinasse/digestate was directly applied to the soil without storage or without being transported in open distribution channels.
[c] Equivalent to the CO_{2eq} savings from the power generated by CH_4 production in the biogas reactor treating sugar beet vinasse, considering the codigestion process with straw and 3% of manure (data from Moraes et al. [25]) during the steady state operation at an organic loading rate of 2.0 g VS L^{-1} d^{-1}) and deducting the CH_4 emission at a biogas plant, estimated as 1.5% of total CH_4 production (Sommer et al. [32]); assumption that 100% of methane in biogas is combusted.
[d] GHG emission from storage lagoons of vinasse or digestate or its transportation in open distribution channels (CH_4 emissions).
[e] GHG emission from application of vinasse or digestate to the soil (N_2O emissions), considering the application rate of 100 m^3 ha^{-1}.

4. Conclusions

This study indicated that untreated vinasse may have a high GHG emission potential. A seven-day lag phase was observed before CH_4 was emitted from stored untreated vinasse, possibly due to a low initial pH. Cumulative CH_4 emissions during 21 days of storage of untreated vinasse were equivalent to 43.8 kg CO_2 eq kg^{-1} C-vinasse, while no CH_4 emissions were detected from digestate during the storage period. Hence, the selective removal of degradable organic matter during AD is likely to reduce CH_4 emissions during storage and transport of vinasse, depending on the retention time. Nitrous oxide emissions from anaerobically digested vinasse were up to 78% lower than from untreated vinasse when applied to soil. Calculations of scenarios with and without AD, and considering the vinasse management chain in Brazil, showed increasing GHG mitigation with storage or transportation time, although mitigation was always achieved for any scenario including AD.

Acknowledgements

The authors wish to acknowledge the support given by the Danish Council for Strategic Research (DSF) under the 'Strategic Research in Sustainable Energy and Environment' research programme to the 'Optimisation of value chains for biogas production in Denmark (Biochain)' project and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) processes no. 2012/00414-7 and no. 2013/03578-3.

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