



## Temperature response of methane production in liquid manures and co-digestates



Lars Elsgaard\*, Anne B. Olsen, Søren O. Petersen

Department of Agroecology, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark

### HIGHLIGHTS

- Temperature dependence of methane emission in liquid slurry materials was quantified
- Arrhenius parameters were derived including 95% confidence limits
- Different slurry materials had similar temperature sensitivity of methane emission
- Temperature sensitivity of methane emission from slurry aligned with other ecosystems

### ARTICLE INFO

#### Article history:

Received 15 May 2015

Accepted 29 July 2015

Available online xxxx

Editor: D. Barcelo

#### Keywords:

Animal slurry

Arrhenius parameters

Methane emission

$Q_{10}$

Temperature sensitivity

### ABSTRACT

Intensification of livestock production makes correct estimation of methanogenesis in liquid manure increasingly important for inventories of  $\text{CH}_4$  emissions. Such inventories currently rely on fixed methane conversion factors as knowledge gaps remain with respect to detailed temperature responses of  $\text{CH}_4$  emissions from liquid manure. Here, we describe the temperature response of  $\text{CH}_4$  production in liquid cattle slurry, pig slurry, and fresh and stored co-digested slurry from a thermophilic biogas plant. Subsamples of slurry were anoxically incubated at 20 temperatures from 5–52 °C in a temperature gradient incubator and  $\text{CH}_4$  production was measured by gas chromatographic analysis of headspace gas after a 17-h incubation period. Methane production potentials at 5–37 °C were described by the Arrhenius equation (modelling efficiencies, 79.2–98.1%), and the four materials showed a consistent activation energy ( $E_a$ ) which averaged 81.0  $\text{kJ mol}^{-1}$  (95% confidence interval, 74.9–87.1  $\text{kJ mol}^{-1}$ ) corresponding to a temperature sensitivity ( $Q_{10}$ ) of 3.4. In contrast, the frequency factor ( $A$ ) differed among the slurry materials ( $30.1 < \ln A < 33.3$ ; mean, 31.3) reflecting that origin, age and composition of the manure affect this parameter. The  $E_a$  estimate, based on individual slurry materials, was intermediate when compared to published values of 63 and 112.7  $\text{kJ mol}^{-1}$  derived from composite data, but was similar to  $E_a$  estimated for  $\text{CH}_4$  production at microbial community level across aquatic ecosystems, wetlands and rice paddies (89.3  $\text{kJ mol}^{-1}$ ). This supports that the derived temperature sensitivity parameters may be applicable to dynamic modelling of  $\text{CH}_4$  emissions from livestock manure.

© 2015 Elsevier B.V. All rights reserved.

### 1. Introduction

Methane ( $\text{CH}_4$ ) is an atmospheric greenhouse gas (GHG) produced by methanogenic Archaea (methanogens) in diverse anaerobic environments, such as waterlogged soil and the digestive tract of animals (Le Mer and Roger, 2001). Large quantities of  $\text{CH}_4$  are also produced and released from man-made ecosystems such as landfills and rice paddies, and from confined animal feeding operations where both livestock and manure are sources of atmospheric  $\text{CH}_4$  (Knapp et al., 2014). Thus, manure management was recently estimated to account for about 10% of the total  $\text{CH}_4$  emissions from agriculture (Serrano-Silva et al., 2014). Intensification of livestock production can be observed in many regions

of the world (Bouwman et al., 2013) and, especially where manure is handled in liquid form (slurry), the emission of  $\text{CH}_4$  during storage can be significant (MacLeod et al., 2013; Opio et al., 2013). Accordingly, the correct estimation of methanogenesis in liquid manure becomes increasingly important for inventories of  $\text{CH}_4$  emissions.

Methane production in manure depends on storage temperature;  $\text{CH}_4$  emissions from storages have been observed at temperatures of <5 °C, but typically attains a maximum in the mesophilic temperature range, for example at 30–37 °C (Cullimore et al., 1985; Safley and Westerman, 1990). Other controlling factors include manure composition (e.g., organic matter degradability, ammonia concentration and pH) and size and composition of the methanogenic community as modified by storage conditions and pre-treatment (Zeeman, 1994; Chen et al., 2008; Witarso and Lansing, 2015). Most national inventories of  $\text{CH}_4$  emissions from manure management are based on guidelines

\* Corresponding author.

E-mail address: [lars.elsgaard@agro.au.dk](mailto:lars.elsgaard@agro.au.dk) (L. Elsgaard).

developed by the Intergovernmental Panel on Climate Change (IPCC, 1997, 2006) where the temperature dependency of CH<sub>4</sub> production is taken into account via fixed methane conversion factors (MCFs), defined for a range of average annual temperatures. However, local circumstances with respect to pre-treatment, storage conditions and residence time may significantly influence annual CH<sub>4</sub> emissions (Sommer et al., 2009), and proper accounting for management effects therefore may require a more dynamic approach.

Models with different levels of complexity have been presented to describe CH<sub>4</sub> emission from liquid manure storage with a daily to monthly time resolution (Mangino et al., 2001; Sommer et al., 2004; Chianese et al., 2009). Yet, significant knowledge gaps remain with respect to CH<sub>4</sub> production potentials for specific storage conditions, including the detailed effect of slurry temperature. Generally, the temperature response of microbial activity below the optimum temperature can be described by the Arrhenius equation (Elsgaard and Jørgensen, 2002; Davidson and Janssens, 2006), i.e.,  $\text{rate} = A \exp(-E_a/RT)$ , where  $A$  is the frequency factor,  $E_a$  is the activation energy (J mol<sup>-1</sup>),  $R$  is the gas constant (8.314 J mol<sup>-1</sup> K<sup>-1</sup>) and  $T$  is temperature (K). Previous attempts to estimate methanogenesis in livestock manure have relied on compilations of data from dissimilar studies to derive an exploratory temperature relationship, with no possibility to estimate slurry-specific variation or parameter uncertainties (Safley and Westerman, 1990; Sommer et al., 2004).

The objective of this study was to determine the temperature dependency of CH<sub>4</sub> production in separate liquid manure materials, including cattle and pig slurry, and co-digested slurry from a thermophilic biogas plant. To ensure a superior data coverage we used a temperature-gradient incubator (TGI) to allow for simultaneous slurry incubation at 20 different temperatures ranging from 5 to 52 °C (Elsgaard and Jørgensen, 2002). We expected this methodology to allow for invention of robust temperature relationships for methane production (i.e., with low parameter uncertainties) and to allow for tests of potential differences among the slurry types.

## 2. Materials and methods

### 2.1. Slurry materials

Cattle slurry was collected from the storage tank of a beef cattle farm (Nedergaard, Tjele) in April 2013. The animals were fed grass-clover and whole-crop silage, with only a minor group of calves receiving concentrates. The slurry had been collected during six months, and was mixed on the day of sampling. Pig slurry originated from different production facilities at the Research Centre AU-Foulum and represented both finishing pigs and farrowing sows. Pig slurry was collected from a mixed storage tank in January 2014; age of the slurry at the time of sampling was at least six months. Livestock slurry co-digested with other organic substrates was collected in May 2014 from a biogas plant at Research Centre AU-Foulum with an 1100-m<sup>3</sup> reactor operated at 52 °C (hydraulic retention time, 13–14 d). Various organic materials, including maize silage and glycerol/fish silage that together constituted c. 20% by volume, were co-digested with cattle and pig slurry (Dr. Alastair Ward, pers. comm.). At the biogas plant, digestate is first stored in a post-digestion storage tank (from where gas is collected during the cooling phase), and then transferred at monthly intervals to a secondary tank for final storage. For this study, fresh digestate was collected directly from the outlet of the reactor, while stored digestate (>1 month) was collected from the secondary tank.

Before use, the collected slurry materials were sieved (<2 mm) to enable reproducible incubation in test tubes (see below) – it was thus assumed that the methanogenic community of the sieved fraction had the same temperature response as that of bulk slurry. The sieved slurry materials were stored in (almost filled) stoppered 300-mL infusion bottles at 2 °C for a maximum of 14 d before determination of temperature responses.

### 2.2. Temperature gradient incubator

The TGI used was described in detail by Elsgaard and Jørgensen (2002). Briefly, the TGI consists of an insulated aluminum bar (240 × 79 × 65 cm) with 30 rows of six replicate sample wells for incubation of 28-mL test tubes. The incubator is heated at one end by an electric plate and cooled at the other end by thermoelectric Peltier elements; this produces a linear thermal gradient over the 30 sample rows. Temperatures are monitored continuously and controlled by three automated PC-operated control loops. In the present study temperature gradients ranging from 5 to 52 °C were produced, corresponding to increments of ~1.6 °C between adjacent incubation temperatures (20 of the 30 incubation temperatures were used for slurry incubations). During operation the standard deviation around mean temperatures was 0.2–0.4 °C, as calculated from temperatures logged at 5-min intervals at 15 sample rows along the thermal gradient.

### 2.3. Incubation procedure

For determination of the temperature response of a slurry material, a stoppered 300-mL infusion bottle with the slurry was first pre-incubated at 20–22 °C for 4 h to activate methanogenesis. During this time, a flow of N<sub>2</sub> was passed through the headspace of the infusion bottle to ensure that oxygen was excluded. Then, through a second gas line, the slurry was gently bubbled for 10 min with N<sub>2</sub> to remove CH<sub>4</sub> from the liquid phase; this was done to reduce the background of dissolved CH<sub>4</sub> in the aliquots subsequently conditioned for incubation in the TGI. While continuously flushing slurry and headspace with N<sub>2</sub>, subsamples of ~3-mL were transferred to 28-mL test tubes ( $n = 70$ ) using a 5-mL pipette with a cut-off plastic tip while also gas flushing the recipient test tube to avoid oxygen contamination (Macy et al., 1972). Following slurry addition, each test tube was immediately closed (under N<sub>2</sub> headspace) with a butyl rubber stopper (1 cm thick) and placed on ice to temporarily arrest methanogenesis. Stoppers of the test tubes were secured with crimp seals, and the tubes were evacuated and refilled with He three times; they were then left at atmospheric pressure on ice until all samples were ready for incubation (within 1–2 h). A total of 60 test tubes were placed in the TGI according to a randomization scheme, with triplicate samples for each of 20 different temperatures covering the range from 5 to 52 °C. The last ten test tubes were used for determination of background CH<sub>4</sub> concentrations and were processed for CH<sub>4</sub> measurements at the time of starting the TGI incubation.

Three different incubation periods were evaluated, i.e., 3 h (short-term), 17 h (over-night) and 41 h (over-night + 24 h). Methane production rates after 3 and 17 h were compared for the cattle slurry, and CH<sub>4</sub> production rates after 17 and 41 h were compared for fresh digested slurry. By the end of an incubation period, gas samples were taken from the headspace of each test tube in the TGI. The pressure inside test tubes was expected to vary, partly because test tubes were all at room temperature when closed, but at different temperatures when sampled, and partly because of temperature effects on gas production during incubation. In order to avoid pressure deficits at sampling, the test tubes were all pressurized by injecting between 2 and 5 mL He (5 mL at the lowest temperatures); this was done 0.5 h prior to gas sampling to allow the gas phase temperature to re-adjust to the specific incubation temperature. A 10-mL glass syringe was then used to determine gas volumes at atmospheric pressure; this was done by inserting the glass syringe (with a hypodermic needle) through the stoppers while the test tubes were still in the incubator. After reading the gas volume, a 3-mL sample of the headspace gas was transferred to a 6-mL Exetainer (Labco Inc., Lampeter, UK) previously equilibrated to atmospheric pressure with He.

## 2.4. Analytical methods

Slurry dry matter (DM) was determined after drying of samples at 105 °C for 24 h, and volatile solids (VS) was determined after combustion of dry samples at 450 °C for 5 h. Total and ammoniacal N was determined by Kjeldahl digestion (Kjeltec 1030, Höganäs, Sweden). Ammonia trapped in 20 mM H<sub>3</sub>PO<sub>4</sub> was determined colorimetrically (Keeney and Nelson, 1982). Slurry pH was analysed with a Sentron 3001 pH-meter (Roden, The Netherlands), and electrical conductivity (EC) with a Radiometer conductivity-meter (Copenhagen, Denmark). Concentrations of CH<sub>4</sub> were analysed on an Agilent 7890 gas chromatograph with a CTC Combipal autosampler (Agilent, Nærum, Denmark). The instrument was configured with a 2-m backflushed pre-column with Hayesep P and a 2-m main column with Poropak Q connected to a flame ionization detector (FID). The carrier was N<sub>2</sub> at a flow rate of 45 mL min<sup>-1</sup>. The FID received 45 mL min<sup>-1</sup> H<sub>2</sub>, 450 mL min<sup>-1</sup> air and 20 mL min<sup>-1</sup> N<sub>2</sub>. Temperatures of injection port, columns, and FID were 80, 80 and 200 °C, respectively.

## 2.5. Data analysis

The exact weight of slurry samples in individual test tubes was determined by the end of incubation, and test-tube headspace volumes could then be detailed assuming a slurry bulk density of 1.0 g mL<sup>-1</sup> (unpublished data). Total gas phase volume was calculated by adding the volume determined with the glass syringe and correcting for incubation temperature using the ideal gas law. Corrections in the final calculation of specific CH<sub>4</sub> production rates (g CH<sub>4</sub> kg VS<sup>-1</sup> d<sup>-1</sup>) were made for background CH<sub>4</sub> concentrations and dilutions associated with gas sampling (Supporting Information, S1).

Relationships between specific CH<sub>4</sub> production rates and incubation temperature were derived from Arrhenius plots, i.e., exploiting the linear relationship between ln (rate) and 1/T as predicted from the natural log-transformed Arrhenius equation (e.g., Elsgaard and Jørgensen, 2002):

$$\ln(\text{rate}) = \ln A - E_a/R \times 1/T. \quad (1)$$

Linear regression parameters and associated 95% confidence intervals were calculated in R version 3.1.0 (R Core Team, 2014) using the *lm* and *confint* functions; tests of normal distribution of residuals (Shapiro–Wilks test) were done using the *shapiro.test* function and tests of heteroscedasticity (non-constant variance score test) was done using *ncvTest* in the R package ‘car’ (Fox and Weisberg, 2011).

Statistical performance indicators of the applied Arrhenius model were calculated according to Mayer and Butler (1993) including the modelling efficiency (MEF) and the mean absolute error relative to the observed mean (*rMAE*). Further model validation parameters were calculated from regression analysis of observed versus predicted (1:1) data plots, including the paired *t*-test, the significance of the correlation coefficient, and the simultaneous *F*-test for unit slope and zero intercept (Haefner, 2005). All statistical performance indicators were calculated for observed versus back-transformed predicted rates, cf. Eq. (1).

Differences between elevations (ln *A*) and slopes (*E<sub>a</sub>/R*) of Arrhenius plots for the four slurry materials were tested using the analysis of covariance (ANCOVA) procedure described by Zar (2010).

The temperature sensitivity of CH<sub>4</sub> production, as indicated by the *E<sub>a</sub>*, was expressed also as the *Q<sub>10</sub>* value, which is the relative change in rate associated with a 10 °C increase in temperature from *T* to *T* + 10 (e.g., Schipper et al., 2014):

$$Q_{10} = \exp(10E_a/RT(T + 10)). \quad (2)$$

Calculations of *Q<sub>10</sub>* were done for the temperature increase from 5 to 15 °C.

## 3. Results

### 3.1. Slurry characteristics

Slurry materials differed in age, but all except fresh digested slurry had been stored for at least several weeks at ambient temperature when sampled (Table 1). Dry matter content was similar in cattle slurry and the two digestates (55–59 g kg<sup>-1</sup>), but lower in the pig slurry (29 g kg<sup>-1</sup>). Volatile solids constituted 66–79% of DM, also with the lowest proportion observed for pig slurry. Ammoniacal N constituted 43–71% of total N, with the highest concentration (2.61 g NH<sub>4</sub>-N kg<sup>-1</sup>) and proportion (71%) in pig slurry and the lowest concentration (0.78 g NH<sub>4</sub>-N kg<sup>-1</sup>) and proportion (43%) in cattle slurry. The differences in NH<sub>4</sub>-N were also reflected in the EC values (Table 1).

### 3.2. Temperature responses of CH<sub>4</sub> production

Methane production rates in slurry materials incubated for 3 to 41 h consistently showed a low variability among the three test tube replicates (Fig. 1). Most variation was observed for the incubation period of 3 h (Fig. 1a), which also had less consistent CH<sub>4</sub> production rates between adjacent incubation temperatures and yielded lower specific rates than after 17 h of incubation. This indicated incomplete temperature equilibrium of the microbial CH<sub>4</sub> production for the 3-h incubation period. Incubations at 17 and 41 h resulted in similar responses of CH<sub>4</sub> production rate to temperature (Fig. 1c) except at the highest incubation temperatures (47 and 52 °C) where the rates after 41 h were slightly lower. Based on these results, the 17-h incubation period was selected as basis for comparing CH<sub>4</sub> production rates in the four slurry materials.

The temperature responses of CH<sub>4</sub> production in cattle and pig slurry were similar, with an optimum temperature close to 37 °C, and there was evidence for methanogenic activity also at 5–10 °C (Fig. 1a, b). Maximal rates observed with cattle slurry were approximately twice as high as those for pig slurry. Methane production rates in fresh and stored co-digested slurry showed a different (thermophilic) temperature response with higher optimum temperatures (>47 °C) and very little or no CH<sub>4</sub> production at temperatures below 10–15 °C (Fig. 1c, d). Higher specific CH<sub>4</sub> production rates were observed for the stored digestate, whereas higher optimum temperature (>52 °C) was observed for the fresh digestate.

### 3.3. Arrhenius parameters and *Q<sub>10</sub>*

Arrhenius parameters for the four slurry types were derived using all data points (*n* = 16) below 37 °C, which was the lowest temperature optimum for CH<sub>4</sub> production observed and expected to encompass all environmentally realistic storage temperatures. Linear relationships between log-transformed CH<sub>4</sub> production rates and the inverse temperature were indicated for all four slurry materials (Fig. 2). In accordance with this, model performance indicators for back-transformed predicted rates showed high MEF (93.4–98.1%) and low *rMAE* (8.2–10.3%) for all slurry types except the stored digestate, and only weak, though significant, biases occurred (Table 2). Resulting estimates of ln *A* and *E<sub>a</sub>* are shown in Table 3. Differences among estimates of ln *A* were highly significant (*P* < 0.001); in contrast there were no significant differences among estimates of *E<sub>a</sub>* (*P* = 0.849) which ranged from 79.2 to 83.3 kJ mol<sup>-1</sup>. These inferences were upheld even though a preceding Shapiro–Wilks test showed that residuals for stored digestate (*P* = 0.026) and cattle slurry (*P* = 0.002) were only approximately normally distributed (see Discussion). Thus, a common regression coefficient was calculated for all data to estimate the common *E<sub>a</sub>* representing all four slurry types (Zar, 2010). This value corresponded to 81.0 kJ mol<sup>-1</sup> with a common standard error (SE) of 3.6 kJ mol<sup>-1</sup> as calculated from the four individual SEs.

**Table 1**

Characteristics of the four slurry types. Digestates were from cattle and pig slurry anaerobically co-digested at 52 °C mainly with maize silage. DM, dry matter; VS, volatile solids; TAN, total ammoniacal N, EC, electric conductivity.

Type	Origin and sampling month	Age (months)	DM (g kg <sup>-1</sup> )	VS (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	TAN (g kg <sup>-1</sup> )	pH	EC (mS cm <sup>-1</sup> )
Cattle slurry	Beef cattle (Apr 2013)	0–6	55	40	1.80	0.78	7.7	3.4
Pig slurry	Finishers, farrowing sows (Oct 2013)	>6	29	19	3.67	2.61	7.7	10.1
Fresh digestate	Cattle, pigs, w/ maize silage (Oct 2013)	0	59	43	3.91	1.90	8.3	7.3
Stored digestate	Cattle, pigs, w/ maize silage (Mar 2014)	>1	57	45	3.21	1.72	7.9	7.4

The temperature sensitivity of CH<sub>4</sub> production in the four slurry materials corresponded to Q<sub>10</sub> values ranging from 3.3 to 3.5 (Table 3); the common temperature sensitivity for all materials corresponded to a Q<sub>10</sub> of 3.4 ± 0.2 (mean ± SE), i.e., signifying a 3.4-fold increase in CH<sub>4</sub> production rate for a temperature increase from 5 to 15 °C. Q<sub>10</sub> values of CH<sub>4</sub> production in fresh digestate incubated for 17 and 41 h (3.4 and 3.5, respectively) were equal, substantiating the robustness of the experimental approach to characterize the temperature response of CH<sub>4</sub> production in liquid manure and co-digestates. Q<sub>10</sub> values for cattle slurry incubated for 3 and 17 h were 2.6 and 3.5, respectively, aligning with incomplete temperature equilibrium of the microbial CH<sub>4</sub> release within the short duration of 3 h.

## 4. Discussion

### 4.1. Slurry characteristics

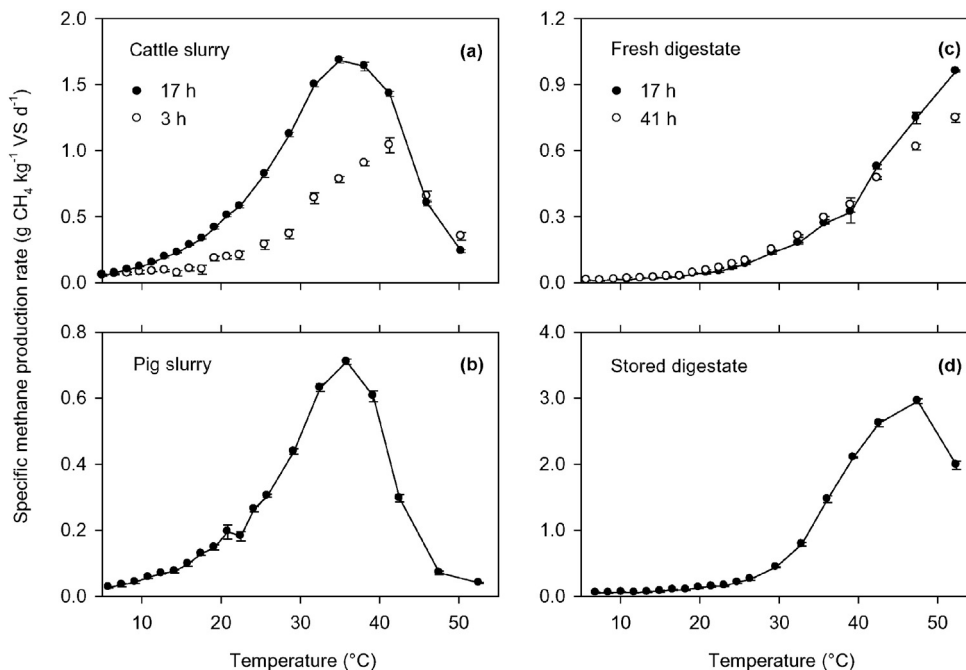
A national report on slurry characteristics in Denmark reported that DM in cattle slurry, pig slurry and digestates averaged 73, 44 and 46 g kg<sup>-1</sup> (Hansen et al., 2008). Compared to these numbers, the cattle and pig slurries used in this study contained 25–34% less DM, and co-digestates contained 24–28% more DM. The lower values, at least in untreated cattle slurry, was related to use of water to flush slurry from barn to the outside storage tank and dilution by rainfall during storage, as also reflected in low concentrations of total and ammoniacal N. The higher DM in the two digestates reflected the use of fibre-rich maize silage as co-digestate. Deviations between materials used in this

study and other slurry materials could influence the absolute methanogenic activity (and therefore ln A), but not necessarily the microbial temperature response.

Slurry materials were sieved to <2 mm in order to allow reproducible subsampling of 3-g aliquots for the TGI incubations. Rico et al. (2007) sieved cow manure to <1 mm and examined the composition and CH<sub>4</sub> production at 35 °C of untreated and sieved manure. Mainly cellulose and hemicellulose was removed by sieving, while the relative proportions of fat and protein increased; accumulated CH<sub>4</sub> production per g VS after 45 d was 20% greater in the sieved manure, indicating a modest change in CH<sub>4</sub> production potential as a result of sieving. However, shifting the balance between dissolved and particulate organic matter could change the immediate degradability and hence the short-term dynamics of CH<sub>4</sub> production. A recent study by Witasara and Lansing (2015) examined the time course of CH<sub>4</sub> production in untreated and screw-press separated dairy manure at 14 and 24 °C. They found greater long-term production of CH<sub>4</sub> from VS in untreated manure, but no significant difference in CH<sub>4</sub> production during the first 16 d at either temperature. This implies that for determination of CH<sub>4</sub> production rates in short-term incubations (here, <2 d), sieved manure is an acceptable representation of the intact sample.

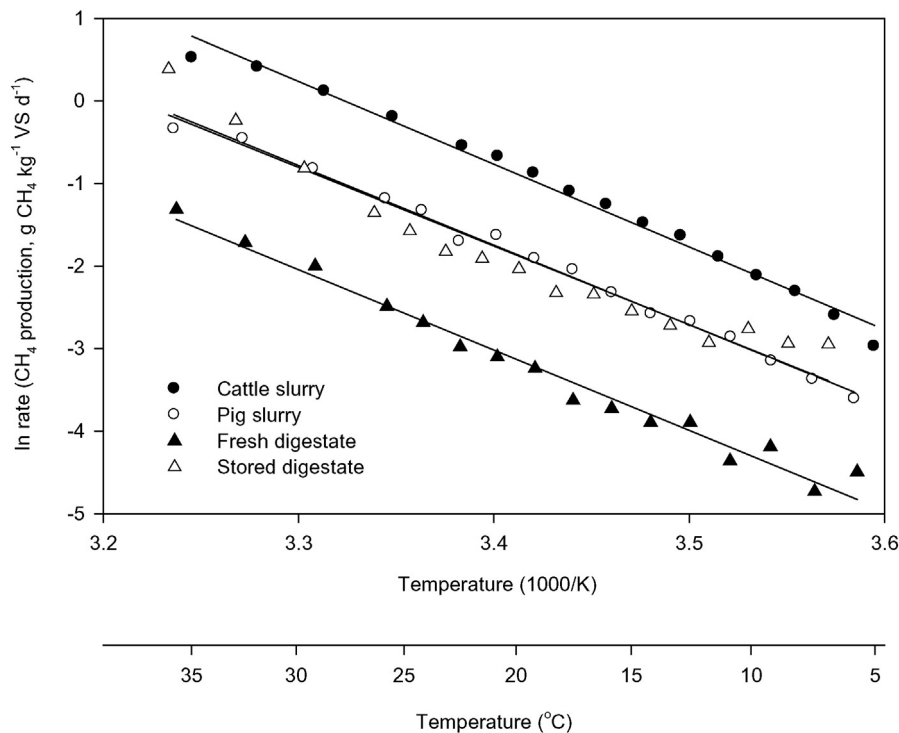
### 4.2. Temperature responses of CH<sub>4</sub> production

Given the incubation periods of <2 d and the relatively high levels of organic substrates in all four slurry types, the rates of CH<sub>4</sub> production were considered to represent the temperature response of methanogens rather than up-stream processes delivering substrates for methanogens,



**Fig. 1.** Temperature response of specific methane production rates in the four slurry types. Data are shown as mean ± standard error of three replicates. The common incubation period was 17 h (closed symbols); in panels a and c methane production rates after 3 and 41 h, respectively, are also indicated (open symbols).





**Fig. 2.** Arrhenius plots (ln rate versus inverse temperature, K) of the temperature response of  $\text{CH}_4$  production in the four slurry types at 5–37 °C. Lines show least squares linear regression. Offset x-axis shows the corresponding temperatures in °C.

i.e., acetate and/or  $\text{CO}_2/\text{H}_2$  (Conrad, 2007). However, it should be acknowledged that, during long-term storage of typical slurry materials,  $\text{CH}_4$  production and emission will reflect the temperature response of a series of integral processes.

Methane production in cattle and pig slurry showed a typical mesophilic temperature response with an optimum around 36 °C after 17 h of incubation. The shorter 3-h incubation resulted in a slightly higher optimum temperature (41 °C); this was interpreted as a transient metabolic response of mesophiles to temperatures above their normal optimum, a phenomenon observed also for other metabolic types of microorganisms (Harder and Veldkamp, 1968; Isaksen et al., 1994). Methane production in fresh digestate had a thermophilic temperature response with an optimum temperature exceeding the highest temperature employed (52 °C), which was also the operating temperature of the digester, and thus, a thermophilic methanogenic community clearly predominated at this time. Yet, there was also  $\text{CH}_4$  production at around 15 °C even though a typical span between minimum and optimum temperatures for growth of most microorganisms, including methanogens, is 20–30 °C (Zinder et al., 1984; Wiegel, 1990). While it has been documented that microorganisms may show activity at temperatures somewhat below the minimum temperature for growth (Wiegel, 1990), the response of  $\text{CH}_4$  production in the fresh digestate

could also reflect the presence of both mesophilic and thermophilic methanogenic populations. In accordance with this interpretation,  $\text{CH}_4$  production in stored digestate showed an optimum temperature at 43–47 °C and a steep rate increase with temperature in the range from 30 to 40 °C, suggesting that successional changes took place during post-digestion storage favouring mesophilic populations of methanogens. Accordingly the temperature response of stored digestate apparently had a higher contribution from mesophilic populations of methanogens than fresh digestate.

#### 4.3. Arrhenius parameters and $Q_{10}$

Arrhenius parameters were in this study derived using the conventional procedure of linear regression of log-transformed  $\text{CH}_4$  production rates versus inverse temperature (Arrhenius plots). Tests of variance homogeneity were satisfied ( $P > 0.05$ ) for the log-transformed rates, whereas residuals for cattle slurry and stored digestate showed some deviation from normal distribution. Yet, the parameters derived from Arrhenius plots were considered to be sound as linear regression is known to be robust to deviations from normality unless they are severe (Box, 1953; van Belle, 2002; Zar, 2010). The modelling efficiency of the regression for stored digestate was only 79.2%, and a better MEF of 92.9% could be obtained by including all data ( $n = 19$ ) below the optimum temperature for this material (47 °C) in the regression. However, this only changed  $Q_{10}$  from 3.3 to 3.6, and therefore it was decided to

**Table 2**

Modelling efficiency (MEF), relative mean absolute error (rMAE), and 1:1 model performance statistics based on observed and predicted (back-transformed) methane production rates from Arrhenius plots. Data were analyzed for similar incubation periods (17 h).

Type	Data (n)	MEF (%)	rMAE (%)	Paired t-test	Correlation (r)	Mean bias <sup>a</sup> (g $\text{CH}_4$ kg $\text{VS}^{-1}$ d $^{-1}$ )
Cattle slurry	16	93.4	10.3	ns	0.986	−0.020***
Pig slurry	16	97.3	8.2	ns	0.989	0.003 <sup>ns</sup>
Fresh digestate	16	98.1	9.3	ns	0.997	0.003***
Stored digestate	16	79.2	27.4	ns	0.963	0.036***

<sup>a</sup> Data given as mean bias with indication of the significance of the simultaneous  $F$ -test for unit slope,  $a$ , and zero intercept,  $b$  (i.e.,  $H_0: a = 1$  and  $b = 0$ ).

\*\*\*  $P < 0.001$ .

<sup>ns</sup> Not significant.

**Table 3**

Arrhenius parameters (activation energy,  $E_a$ , and log-transformed frequency factor,  $\ln A$ ) and temperature sensitivity ( $Q_{10}$ ) of methane production for the four slurry types. The 95% confidence intervals are shown in parentheses. Data were analyzed for similar incubation periods (17 h).

Type	$E_a$ (kJ mol $^{-1}$ )	$\ln A$	$Q_{10}$ (5–15 °C)
Cattle slurry	83.3 (78.2–88.4)	33.3 (31.2–35.4)	3.5 (3.2–3.8)
Pig slurry	80.2 (76.5–83.9)	31.1 (29.5–32.6)	3.3 (3.2–3.5)
Fresh digestate	80.9 (74.1–87.7)	30.1 (27.3–32.9)	3.4 (3.0–3.7)
Stored digestate	79.2 (66.7–91.7)	30.6 (25.5–35.8)	3.3 (2.7–3.9)

focus on a common, and environmentally realistic, temperature range for parameter estimation.

Few estimates of the temperature dependency of methanogenesis in livestock manure have been published. Several studies modelling CH<sub>4</sub> production in stored manure (Safley and Westerman, 1990; Mangino et al., 2001; Wood et al., 2012) all used an  $E_a$  value of 63 kJ mol<sup>-1</sup> based on a relationship that was originally derived from nine separate studies with (mostly) agricultural waste materials (Ashare et al., 1977; reproduced in Ashare et al., 1979). The individual studies represented between one and six incubation temperatures (20 observations in total) and together covered a temperature interval from 15 to 60 °C (Ashare et al., 1979). Sommer et al. (2004), in a desk study investigating manure management strategies, adopted a temperature relationship derived from three studies of slurry storage;  $\ln A$  was adjusted to arrive at the annual emission predicted by the IPCC Tier 2 methodology. The  $E_a$  derived by Sommer et al. (2004) corresponded to 112.7 kJ mol<sup>-1</sup>. It is difficult to judge how well such compiled data characterize methanogenesis in individual manure (or waste) materials, but during the present study comparable  $E_a$  values of 79.2–83.3 kJ mol<sup>-1</sup> were obtained with slurry-based materials and pointing at a common  $E_a$  of 81.0 kJ mol<sup>-1</sup> (95% confidence interval, 74.9–87.1 kJ mol<sup>-1</sup>). This robust estimate based on four individual slurry materials was thus intermediate when compared to the  $E_a$  values of Ashare et al. (1979) and Sommer et al. (2004). This also translated into an intermediate  $Q_{10}$  value of 3.4 for CH<sub>4</sub> production as compared to  $Q_{10}$  values of 2.6 and 5.4 as calculated from the studies of Ashare et al. (1979) and Sommer et al. (2004), respectively.

The frequency factor  $A$  (and  $\ln A$ ) varied between treatments with the sequence: cattle slurry > pig slurry = fresh digestate = stored digestate. This parameter, which has the same unit as the modelled variable (here, g CH<sub>4</sub> kg VS<sup>-1</sup> d<sup>-1</sup>), can be interpreted as the theoretical rate assuming infinite temperature or zero activation energy, neither of which have biological meaning. For the presented specific CH<sub>4</sub> production rates, the size of the frequency factor probably reflected different potentials for methanogenesis in the slurry materials. This is determined by the composition of residual organic matter and/or the size and composition of the methanogenic community at the time of measurement, as shown in manure storage experiments (Massé et al., 2008; Barret et al., 2013; Petersen et al., 2014) and discussed above.

#### 4.4. Implications for modelling of methane emissions

An important application of temperature response functions is for the prediction of CH<sub>4</sub> emissions from manure during storage (Sommer et al., 2009; Wood et al., 2012). The chemical Arrhenius relationship, as presently used, has been found to successfully apply to various integral biological processes, although some studies have noted a tendency of overestimation of reaction rates at low temperatures (Elsgaard and Vinther, 2004; Portner et al., 2010). The parameter estimates presented here were based on a diverse selection of slurry materials, but still indicated a common temperature sensitivity of CH<sub>4</sub> production, represented by an  $E_a$  value of 81.0 kJ mol<sup>-1</sup>. Methane production in liquid manure may not scale directly with CH<sub>4</sub> emissions during storage as bacterial methanotrophy can partly intercept emissions in the presence of a surface crust (Petersen et al., 2005; Duan et al., 2014). However, in a meta-analysis of CH<sub>4</sub> fluxes at microbial to ecosystem scales, Yvon-Durocher et al. (2014) found that the average temperature dependency of CH<sub>4</sub> emissions at ecosystem level (0.96 eV) was very similar to that of CH<sub>4</sub> production by anaerobic microbial communities (0.93 eV) across a range of aquatic ecosystems, wetlands and rice paddies. Since 0.93 eV is equivalent to 89.3 kJ mol<sup>-1</sup>, the average  $E_a$  estimate for CH<sub>4</sub> production at community level reported by Yvon-Durocher et al. (2014) is in good agreement with the temperature response of microbial consortia in liquid manure and co-digestates presented in this study. This supports the applicability of the derived temperature sensitivity parameters for estimating CH<sub>4</sub> emissions from manure. The present estimate of  $E_a$

was derived from rate measurements over a temperature range that includes the average annual temperature in different climate zones, making this value of potential interest for national inventories of CH<sub>4</sub> emission from livestock manure.

In conclusion, the Arrhenius temperature dependency of CH<sub>4</sub> production in liquid cattle and pig manure (slurry), and in fresh and stored co-digestates showed no significant differences in activation energy ( $E_a$ , 81.0 kJ mol<sup>-1</sup>; 95% confidence interval, 74.9–87.1 kJ mol<sup>-1</sup>), whereas there were significant differences in the frequency factor ( $30.1 < \ln A < 33.3$ ; mean, 31.3) suggesting that origin and age of the manure will affect this parameter. The Arrhenius parameters were determined using a fine-scale temperature gradient and a rigorous procedure for rate determinations. The parameter estimates should be substantiated using a wider range of manure materials, but we propose that the current estimates may be applicable to modelling of CH<sub>4</sub> emissions from liquid manure. The temperature sensitivity parameters ( $E_a$  and  $Q_{10}$ ) can be applied in a generic sense whereas the  $\ln A$  parameter is specific for the materials and units from which it is derived, here g CH<sub>4</sub> kg VS<sup>-1</sup> d<sup>-1</sup>.

#### Acknowledgements

We thank Bodil Stensgaard for skilled laboratory assistance and Rodrigo Labouriau for discussions on statistical interpretation of temperature responses. The work was financially supported by the Danish Energy Agency (Grant No. Agr-2014-4734), and by the project “Optimisation of value chains for biogas production in Denmark (Bio Chain)” funded by the Danish Strategic Research Council (Grant No. 12-132631).

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2015.07.145>.

#### References

- Ashare, E., Wise, D.L., Wentworth, R.L., 1977. Fuel gas production from animal residue. Dynatech Report No. 1551, issued on January 14, 1977 to U.S. Energy Research and Development Administration under Contract No. EY-76-C-02-2991. Dynatech R/D Company, Cambridge, Massachusetts.
- Ashare, E., Wentworth, R.L., Wise, D.L., 1979. Fuel gas production from animal residue. Part II. An economic assessment. Resour. Recover. Conserv. 3, 259–286.
- Barret, M., Gagnon, N., Topp, E., Masse, L., Massé, D.I., Talbot, G., 2013. Physico-chemical characteristics and methanogen communities in swine and dairy manure storage tanks: spatio-temporal variations and impact on methanogenic activity. Water Res. 47, 737–746.
- Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H.W., Van Vuuren, D.P., Willems, J., Rufino, M.C., Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. Proc. Natl. Acad. Sci. U. S. A. 110, 20882–20887.
- Box, G.E.P., 1953. Non-normality and tests on variances. Biometrika 40, 318–335.
- Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.
- Chianese, D.S., Rotz, C.A., Richard, T.L., 2009. Simulation of methane emissions from dairy farms to assess greenhouse gas reduction strategies. Trans. ASABE 52, 1313–1323.
- Conrad, R., 2007. Microbial ecology of methanogens and methanotrophs. Adv. Agron. 96, 1–63.
- Cullimore, D.R., Maule, A., Mansuy, N., 1985. Ambient temperature methanogenesis from pig manure waste lagoons: thermal gradient incubator studies. Agric. Wastes 12, 147–157.
- Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
- Duan, Y.F., Abu Al-Soud, W., Breyndrod, A., Elsgaard, L., Sørensen, S.J., Petersen, S.O., Boon, N., 2014. Community structure of methane-metabolizing bacteria and archaea in livestock slurry surface crusts. J. Appl. Microbiol. 117, 1066–1078.
- Elsgaard, L., Jørgensen, L.W., 2002. A sandwich-designed temperature-gradient incubator for studies of microbial temperature responses. J. Microb. Methods 49, 19–29.
- Elsgaard, L., Vinther, F.P., 2004. Modeling of the fine-scale temperature response of arylsulfatase activity in soil. J. Plant Nutr. Soil Sci. 167, 196–201.
- Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression. 2nd ed. SAGE Publications, Thousand Oaks, California.
- Haefner, J.W., 2005. Modelling Biological Systems: Principles and Applications. 2nd ed. Springer Science & Business Media, New York, New York.

- Hansen, M.N., Sommer, S.G., Hutchings, N.J., Sørensen, P., 2008. Emission factors for calculation of ammonia volatilization by storage and application of animal manure. DJF Report Animal Production No. 84. Aarhus University, Denmark.
- Harder, W., Veldkamp, H., 1968. Physiology of an obligately psychrophilic marine *Pseudomonas* species. *J. Appl. Bacteriol.* 31, 12–23.
- IPCC, 1997. Revised 1996 IPCC guidelines for national greenhouse gas inventories. In: Houghton, J.T., Meira, L.G., Filho, L.G., Lim, B., Treanton, K., Mamaty, I., Bonduki, Y., Griggs, D.J., Callender, B.A. (Eds.), Intergovernmental Panel on Climate Change. Hadley Centre, Bracknell, UK.
- IPCC, 2006. 2006 IPCC guidelines for national greenhouse gas inventories. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.), Prepared by the National Green-house Gas Inventories Programme. IGES, Hayama, Japan.
- Isaksen, M.F., Bak, F., Jørgensen, B.B., 1994. Thermophilic sulfate reducing bacteria in cold marine sediment. *FEMS Microbiol. Ecol.* 14, 1–8.
- Keeney, D.R., Nelson, D.W., 1982. Nitrogen – inorganic forms. In: Page, A.L., Miller, T.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis, Part 2, 2nd ed.* Agronomy Monographs 9. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, pp. 643–693.
- Knapp, J.R., Laur, G.L., Vadas, P.A., Weiss, W.P., Tricarico, J.M., 2014. Enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci.* 97, 3231–3261.
- Le Mer, J., Roger, P., 2001. Production, oxidation, emission and consumption of methane: a review. *Eur. J. Soil Biol.* 37, 25–50.
- MacLeod, M., Gerber, P., Mottet, A., Tempio, G., Falcucci, A., Opio, C., Vellinga, T., Henderson, B., Steinfeld, H., 2013. Greenhouse Gas Emissions From Pig and Chicken Supply Chains – a Global Life Cycle Assessment. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Macy, J.M., Snellen, J.E., Hungate, R.E., 1972. Use of syringe methods for anaerobiosis. *Am. J. Clin. Nutr.* 25, 1318–1323.
- Mangino, J., Bartram, D., Brazy, A., 2001. Development of a methane conversion factor to estimate emissions from animal waste lagoons. U.S. EPA's 17th Annual Emission Inventory Conference, Atlanta, Georgia.
- Massé, D.I., Masse, L., Claveau, S., Benchaar, C., Thomas, O., 2008. Methane emissions from manure storages. *Trans. ASABE* 51, 1775–1781.
- Mayer, D.G., Butler, D.G., 1993. Statistical validation. *Ecol. Model.* 68, 21–32.
- Opio, C., Gerber, P., Mottet, A., Falcucci, A., Tempio, G., MacLeod, M., Vellinga, T., Henderson, B., Steinfeld, H., 2013. Greenhouse Gas Emissions From Ruminant Supply Chains – A Global Life Cycle Assessment. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
- Petersen, S.O., Amon, B., Gattinger, A., 2005. Methane oxidation in slurry storage surface crusts. *J. Environ. Qual.* 34, 455–461.
- Petersen, S.O., Højberg, O., Poulsen, M., Schwab, C., Eriksen, J., 2014. Methane mitigation and methanogen community changes with acidification of pig slurry. *J. Appl. Microbiol.* 117, 160–172.
- Portner, H., Bugmann, H.K.M., Wolf, A., 2010. Temperature response functions introduce high uncertainty in modelled carbon stocks in cold temperature regimes. *Biogeosciences* 7, 3669–3684.
- R Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (URL <http://www.R-project.org/>).
- Rico, J.L., García, H., Rico, C., Tejero, I., 2007. Characterisation of solid and liquid fractions of dairy manure with regard to their component distribution and methane production. *Bioresour. Technol.* 98, 971–979.
- Safley, L.M., Westerman, P.W., 1990. Psychrophilic anaerobic digestion of animal manure: proposed design methodology. *Biol. Wastes* 34, 133–148.
- Schipper, L., Hobbs, J., Rutledge, S., Arcus, V., 2014. Thermodynamic theory explains the temperature optima of soil microbial processes and high  $Q_{10}$  values at low temperatures. *Glob. Chang. Biol.* 20, 3578–3586.
- Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L., Luna-Guido, M., 2014. Methanogenesis and methanotrophy in soil: a review. *Pedosphere* 24, 291–307.
- Sommer, S.G., Petersen, S.O., Møller, H.B., 2004. Algorithms for calculating methane and nitrous oxide emissions from manure management. *Nutr. Cycl. Agroecosyst.* 69, 143–154.
- Sommer, S.G., Olesen, J.E., Petersen, S.O., Weisbjerg, M.R., Valli, L., Rohde, L., Béline, F., 2009. Region-specific assessment of greenhouse gas mitigation with different manure management strategies in four agroecological zones. *Glob. Chang. Biol.* 15, 2825–2837.
- van Belle, G., 2002. *Statistical Rules of Thumb*. John Wiley & Sons, New York, New York.
- Wiegel, J., 1990. Temperature spans for growth: hypothesis and discussion. *FEMS Microbiol. Rev.* 75, 155–170.
- Witarsa, F., Lansing, S., 2015. Quantifying methane production from psychrophilic anaerobic digestion of separated and unseparated dairy manure. *Ecol. Eng.* 78, 95–100.
- Wood, J.D., Gordon, R.J., Wagner-Riddle, C., Dunfield, K.E., Madani, A., 2012. Relationships between dairy slurry total solids, gas emissions, and surface crusts. *J. Environ. Qual.* 41, 694–704.
- Yvon-Durocher, G., Allen, A.P., Bastviken, D., Conrad, R., Gudas, C., St-Pierre, A., Thanh-Duc, N., del Gioglio, P.A., 2014. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature* 507, 488–491.
- Zar, J.H., 2010. *Biostatistical Analysis*. 4th ed. Prentice-Hall, Upper Saddle River, New Jersey.
- Zeeman, G., 1994. Methane production/emission in storages for animal manure. *Fertil. Res.* 37, 207–211.
- Zinder, S.H., Anguish, T., Cardwell, S.C., 1984. Effect of temperature on methanogenesis in a thermophilic (58 °C) anaerobic digester. *Appl. Environ. Microbiol.* 47, 808–813.