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In-storage psychrophilic anaerobic digestion of swine manure: Acclimation of the microbial community

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ABSTRACT

Covering a concrete manure storage tank with an air-tight floating membrane should induce anaerobic digestion of the stored manure. If the microbial community in the manure can acclimate to the ambient conditions, then In-Storage Psychrophilic Anaerobic Digestion (ISPAD) could be used by Canadian livestock producers to produce methane and stabilize manure. The objective of this study was to determine whether the microbial community in swine manure can successfully acclimate to the psychrophilic operating conditions in ISPAD and develop robust anaerobic digestion. This was done in the laboratory by analyzing manure from a three-year old full-scale pilot ISPAD facility located in St. Francois Xavier, Quebec, Canada, along with fresh manure and manure from an uncovered storage tank. Biochemical methane production assays performed at the three temperatures were used to quantify the performance of the microbial community and its temperature dependence. The ISPAD microbial community produced methane, in terms of VS added, at rates of 44.6, 9.8 and 8.5 $dm^3\,kg^{-1}\,d^{-1}$, at 35, 18 and 8 °C, respectively. The ISPAD process reduced the organic matter content of the manure by 24% while releasing 63% of the potential methane in the manure, as opposed to the open storage tank where no measurable reduction in solids occurred, and only 15% of the potential methane was released. These results indicate that a robust, acclimated microbial community actively digests manure in the pilot ISPAD installation.

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1. Introduction

An increasing number of Canadian hogs, 33% in 1991 and 37% in 2001, are raised in regions of high livestock density [1] where manure production exceeds cropland nutrient requirements [2–4]. While management practices are used to control some environmental impacts [5], manure treatment strategies are not adapted to regional needs and remain unaffordable [6,7]. Manure methanization by In-Storage Psychrophilic Anaerobic Digestion (ISPAD) is proposed for livestock producers in Canada and other temperate regions because it is a passive ambient system [8]. ISPAD is expected to occur in manure storage tanks with an air-tight cover, once the anaerobic microbial community in the manure has acclimated to the ambient (psychrophilic) operating temperature.

Anaerobic digestion of swine manure at psychrophilic temperatures has been demonstrated previously. During an experiment using four 2.5 m^3 pilot scale reactors, digestion at 22.5 °C was stable; biogas production and solids reduction were comparable to those in the mesophilic range, when the

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retention time was doubled [9]. Laboratory digesters seeded with lagoon effluent successfully adapted to temperatures from 10 to 23 °C [10]. The importance of microbial acclimation has also been noted. Two full-scale accumulation digesters reached net biogas production rates comparable to mesophilic installations after 1.5 years of acclimation at 19–20 °C [11]. After 250 days of digestion at 20 °C, seeded 25-l sequencing batch reactors produced CH_4, removed COD and reduced odors as effectively as a mesophilic process [12]. Laboratory simulation of ISPAD concluded that the microbial community in swine manure could produce significant CH_4 at ambient temperatures following a 100-day mesophilic (35 °C) start-up period [8]. Acclimated microbial communities consume substrate without lag, and intermediate substrates do not accumulate [13]. Biochemical methane production (BMP) assays have been used to illustrate the progress of microbial acclimation to psychrophilic temperatures. When pig manure from an earthen lagoon was sampled at intervals over 2 years, BMP incubation initiated methane production at progressively lower temperatures (3–9 °C) [14]. Pig manure samples incubated for 15 months between 6 and 20 °C showed continuously increasing rates of methanogenesis at each temperature [15]. Because stored manure in the Canadian climate remains above freezing all year [16], acclimation should be possible in an ISPAD installation. However, it must be demonstrated that ISPAD does initiate microbial acclimation and develop robust anaerobic digestion.

The objectives of the present study were therefore to: confirm that the microbial community in swine manure does acclimate to the psychrophilic operating conditions in ISPAD; and to measure the resulting methane production and solids reduction. To accomplish these objectives, samples of manure from a three-year old full-scale ISPAD installation (X_f) were tested in the laboratory, along with two control materials: fresh manure (S_m) was used to evaluate the changes occurring in the ISPAD tank over time; manure from a standard uncovered storage tank (X_o) was used to evaluate the effect of residence in a storage tank separately from the treatment effect of the ISPAD cover. Organic matter fractionation and presence of intermediate products were evaluated by analyzing the composition of the three subject materials. Biochemical methane production assays performed at three temperatures of 8, 18 and 35 °C, were used to evaluate methane production rates, time required for acclimation, and methane potential.

2. Materials and methods

2.1. Experimental materials

In 2004, a full-scale ISPAD facility was established at a swine operation in St. Francois Xavier, Quebec, Canada. The circular concrete manure tank, 30 m in diameter by 3.7 m deep, was covered with an air-tight floating membrane (*GTI*, Fredericton, NB, Canada). Manure was regularly fed into the tank, and removed for land-spreading twice yearly, leaving a depth of 0.3–0.6 m. The facility had been in operation for three years when this study started in 2007, which should be sufficient for acclimation. Manure from this facility was used to represent ISPAD. The two controls, freshly produced manure and one year old manure contained in an uncovered storage tank, were obtained from the swine research facility of the McGill University Macdonald Campus Experimental Swine Centre in Montreal, Quebec, Canada. As they are produced by hogs fed a standard corn and soybean based ration, manures from the two locations were considered comparable [17].

All manure samples were collected in June 2007, using a sludge-judge apparatus to collect a composite sample representing the average of depths and locations within the tanks. The fresh manure samples were obtained after thorough mixing from the Mac piggery pre-pit. All collected manures were sieved to remove particles larger than 2 mm, transferred to glass bottles, flushed with N_2/CO_2 and stored at 4 °C.

2.2. Characterization of experimental materials

For all three materials, sub-samples were analyzed according to standard methods [18] to establish solids (TS, VS, TSS, VSS) and COD (total and soluble). The pH of all samples was determined using a pH meter (model 450, *Corning*, NY, USA). The quantity of active microbial biomass in each material was estimated using a wastewater ATP kit (*Luminultra*, NB, Canada) and a luminometer (*Sirius* V3.2, *Bethold Detection Systems*, TN, USA).

Supernatant from sub-samples centrifuged at 21,100 g was analyzed for VFA, anions and cations. Acetic, propionic and butyric acids were measured using a gas chromatograph (model 6890, *Agilent*, DE, USA) equipped with a flame ionization detector. Samples were fortified at a ratio of 1:1 by volume using an internal standard of *iso*-butyric acid dissolved in 6% formic acid. These were directly injected into a 1 m × 2 mm *Carbopack C* (60–80 mesh) glass column coated with 0.3% *Carbowax* 20 M and 0.1% H_3PO_4. The column was held at 130 °C for 4 min and helium as carrier gas was injected at a rate of 20 $cm^3\,min^{-1}$. The injector and the detector were both maintained at 200 °C. Anions (NO_2^-, NO_3^-, PO_4^{-3}, Cl^-) were analyzed using a polymer-based chromatography column, 250 mm × 41 mm OD, (model PRP-X100, *Hamilton*, NV, USA), on a high-performance liquid chromatograph (model P4000 & AS3000, *TSP*). Conductivity data were obtained by using a *Waters* Millipore detector model 432. The parameters were: mobile phase 4.0 mM p-hydroxybenzoic acid, pH 8.5 with 2.5% methanol, 100 μL injection, 1.8 $cm^3\,min^{-1}$ flow rate at 40 °C. Cations (Na^+, NH_4^+, K^+) were similarly analyzed on a cation resin-based chromatography column, 250 mm × 41 mm OD, (model PRP-X200, *Hamilton*) with: mobile phase 4.0 mM nitric acid with 30% methanol, 20 μL injection, and 1.8 $cm^3\,min^{-1}$ flow rate at 40 °C.

2.3. Biochemical methane production (BMP) assays

Two sets of BMP assays were performed: one using the ISPAD manure (X_f) and the other using the uncovered tank manure (X_o), as active biomass. The substrate tested was fresh manure (S_m). Each set comprised three batches, one each at 8, 18 and 35 °C. Each batch included three triplicate bottle-filling regimes: substrate alone, biomass alone and biomass with substrate. Substrate alone and biomass alone served as controls to separate the performance of manure-as-biomass from manure as substrate, and as self-fermentation for determining ultimate methane potential.

The tests were run based on established procedures [19,20]. For each batch, nine 160 cm^3 bottles were prepared. Defined media, sulphide solution and bicarbonate buffer were added to all the bottles in standard amounts. Biomass was measured into 6 bottles, for a VSS concentration of 10 $kg\,m^{-3}$. De-ionized water was added to all the bottles to provide 50.5 cm^3 total liquid after substrate addition. Bottles were capped, sealed and flushed with N_2/CO_2 gas and placed in a temperature-controlled shaker operating at 100 rpm, in the dark. After three or four days of adjustment, substrate was injected into 3 of the bottles containing biomass, and the 3 which contained no biomass, to result in a soluble COD loading of 4.5 $kg\,m^{-3}$. For each bottle-filling regime, a fourth bottle was prepared and analyzed to establish the initial conditions: solids, COD, VFA, anions and cations.

Individual bottle biogas production was monitored until production ceased, following a schedule based on the assay temperature. The 35 °C assays were sampled every day for one week to establish the initial rate of methane production, then every 2–3 days until a near-plateau was reached and from then on every 2–3 weeks. At 18 and 8 °C respectively, the sampling period was doubled and quadrupled. Each sampling measured the volume of biogas accumulated in the headspace of each bottle using the U-tube water displacement method. The biogas composition (H_2, $N_2 + O_2$, CH_4, CO_2) was determined by injecting 300 μL of the bottle headspace gas into a gas chromatograph (68900 series, *Hewlett Packard*, DE, USA) equipped with a thermal conductivity detector and a 0.9 m × 3 mm 60/80 mesh *Chromosorb* 102 column (*Supelco*, PA, USA). The carrier gas was argon. The column temperature was held at 50 °C for 3.9 min. All gas quantities were corrected to standard temperature and pressure of 0 °C and 101 kPa according to the ideal gas law, and normalized in terms of VS as $m^3\,kg^{-1}$.

2.4. *Calculations*

For each microbial community, substrate and temperature combination, the specific methane production rate was determined from the steepest CH_4 accumulation slope during the initial period of the BMP assay. This represents the activity of the original (extant) community with excess substrate [21]. The initial period used was the doubling time for the methanogen population, adjusted exponentially for temperature: 4 days at 35 °C, 14 days at 18 °C and 28 days at 8 °C [22]. Since the methanogens are slowest to acclimate, the development of methane production is a good indicator of development of the community as a whole [13]. Therefore, when the steepest slope occurred within the initial period, the extant community was presumed to have entered the BMP acclimated to the bottle temperature. A longer lag period illustrated acclimation occurring in the assay bottles, and defined the time required for acclimation to the bottle temperature.

The ultimate methane potential (B_0) is a property of a substrate, independent of the biomass used. It represents the CH_4 released by digestion of the substrate under ideal conditions for an infinite time period. After 150 days, the 35 °C BMP self-fermentation bottles were still producing traces of CH_4. The total CH_4 produced in this period was defined as B_{150} and a value for B_0 was calculated using *Matlab* (®2008a, *the Mathworks*, Natick, MA) to fit the data to a first-order curve of the form: $B = B_0\,(1 - e^{-kt})$ where t = time (days), B = methane released up to time t ($m^3\,kg^{-1}$) and k = rate constant ($days^{-1}$) [23]. Both B_0 and k were estimated in the fitting process. The coefficient of determination, R^2, was used to evaluate the goodness of fit.

2.5. *Statistical analysis*

All statistical analyses were performed using *SAS* 9.0 (®2009, *SAS Institute inc.*, Cary NC, USA). The composite manure samples were characterized in triplicate based on a completely randomized design. The biochemical methane production assays used a randomized complete block design (RCBD), with microbial community type as treatment factor and temperature as block factor. Treatments were assigned randomly to experimental units (bottles), and all treatment-block combinations were completed in triplicate. The significance of differences between measured values was evaluated by the Student–Newman–Keuls method in ANOVA.

3. Results and discussion

3.1. *Characterization of experimental materials*

The analyzed characteristics of the three study manures are presented in Table 1. Differences in total solids, anion and cation concentrations are effects related to water content. The TS data indicate that X_f and X_o are respectively 19 and 52% less concentrated than S_m, reflecting the inclusion of wash water in both tanks, as well as rain and snow in X_o. Higher Cl^- in S_m and X_o and SO_4^{-2} in X_f are explained by the use of municipally treated water at the control location and untreated well water in the ISPAD installation. Both storage tanks (X_o and X_f) contain less PO_4^{-3} due to settling. There is more variability in cations due to a combination of water source, pH and ionization. Note that these concentrations are expressed in terms of VS to eliminate the effect of dilution.

Comparing characteristics related to the organic matter content of manure reveal the effects of microbial conversion processes occurring in both storage tanks. For both S_m and X_o, VS represent 72% of TS, while X_f shows a reduction of VS to 66% of TS, due to organic matter leaving the ISPAD tank as CH_4 from anaerobic digestion. Values of COD were lower in both X_o and X_f, as compared to S_m, as COD is reduced by anaerobic processes in X_f and by anaerobic, aerobic and volatilization processes in X_o. Because VFA are the immediate precursors of CH_4 production, the presence of 243.0 and 182.5 $mg\,g^{-1}$ total VFA, in X_o and S_m respectively, indicate acidogenic activity in X_o with little methanogenesis to consume the products. Conversely, VFA of 0.3 $mg\,g^{-1}$ in X_f, indicate that any VFA produced are rapidly consumed, suggesting robust anaerobic digestion in the ISPAD tank.

To determine whether the ISPAD activity was simply due to the presence of a larger microbial community, the concentration of active microbial biomass in relation to the total VSS

Table 1 – Characteristics of experimental manures.

		Fresh manure S_m		Uncovered tank X_o		ISPAD tank X_f	
		Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Solids							
TS	$kg\,m^{-3}$	48.01	0.26	22.71	0.18	38.71	0.35
VS	$kg\,m^{-3}$	34.34	0.22	16.45	0.14	25.40	0.15
FS	$kg\,m^{-3}$	13.67	0.04	6.25	0.05	13.31	0.20
VSS	$kg\,m^{-3}$	27.38	1.22	11.17	0.57	24.01	0.19
VDS	$kg\,m^{-3}$	6.96	0.72	5.29	0.35	1.38	0.17
pH							
pH		6.90	0.03	7.33	0.04	7.46	0.05
COD[a]							
Total	$kg\,kg^{-1}$	2.43	n/a	2.07	0.05	1.99	0.10
Soluble	$kg\,kg^{-1}$	0.88	n/a	0.67	0.01	0.08	0.02
VFA[a]							
Acetic	$g\,kg^{-1}$	142.04	13.30	29.66	0.87	0.33	0.15
Propionic	$g\,kg^{-1}$	60.31	2.93	152.83	4.11	0.00	0.00
Butyric	$g\,kg^{-1}$	40.69	1.70	0.00	0.00	0.00	0.00
Anions[a]							
Cl^-	$g\,kg^{-1}$	33.81	0.54	40.69	0.11	21.33	0.18
NO_2^-	$g\,kg^{-1}$	2.93	0.04	0.00	0.00	0.04	0.03
NO_3^-	$g\,kg^{-1}$	0.00	0.00	0.00	0.00	0.00	0.00
PO_4^{3-}	$g\,kg^{-1}$	15.61	0.24	6.88	0.20	6.94	0.05
SO_4^{2-}	$g\,kg^{-1}$	0.00	0.00	0.00	0.00	17.38	0.29
Cations[a]							
Na^+	$g\,kg^{-1}$	19.19	0.23	24.05	0.32	13.43	0.29
NH_4^+	$g\,kg^{-1}$	108.88	0.23	111.37	0.65	79.16	0.19
K^+	$g\,kg^{-1}$	70.23	0.38	99.82	0.45	34.51	0.24
ATP[b]							
ATP	$\mu g\,g^{-1}$	12.0	n/a	10.7	n/a	16.7	n/a
Active	% VSS	0.4–1.2	n/a	0.3–1.0	n/a	0.5–1.6	n/a

a In terms of VS.
b In terms of VSS.

in the manure was estimated by measuring adenosine triphosphate (ATP), which is present only in viable microbial cells [24,25]. Because microbial species contain varying concentrations of ATP, the conversion of ATP data to % VSS was estimated based on an empirical cellular ATP concentration range of 2–6 $\mu mol\,g^{-1}$, dry weight [26]. This analysis of the study materials indicates that the three active microbial communities are similar in size and account for less than 2% of the VSS, while the remaining 98% of VSS represents organic matter available as substrate. Therefore the reduction of organic matter in X_f can be ascribed to the activity resulting from acclimation of the microbial community to the tank conditions, rather than the presence of a larger community. Conversely, it may be inferred that, although the size of the community is similar, conditions in X_o are less conducive to such acclimation.

3.2. *Intermediate products*

The hypothesis of active anaerobic digestion in the ISPAD tank may be further investigated by examining the concentrations of intermediate products, which are known to accumulate in the presence of a non-acclimated community [13,27]. These products represent the sequential conversion of VSS through several stages to form methane, with each conversion performed by a specific population within the anaerobic microbial community. Considering that fresh manure is the feed for both the uncovered and ISPAD tanks, characteristics of both digested materials, X_f and X_o, are presented in Table 2, in terms of the VS of fresh manure (S_m), namely VS_i. The conversion uses the fixed solids (FS or ash) as reference because they are not lost during anaerobic digestion and are similar for all manures due the use of standard diets [17,28]. Therefore $FS_j = FS_i$ where j denotes either X_f or X_o and i denotes S_m. Thus X_f and X_o characteristics expressed in terms of VS are multiplied by the ratio $(VS_j/FS_j)*(FS_i/VS_i)$, to be presented in terms of VS_i.

Volatile solids are the substrate initially consumed during anaerobic digestion. The ratio $VS:VS_i$ of 1.05 for the uncovered tank manure (X_o), is not statistically different from the fresh manure, indicating that no VS were consumed in X_o. However, the corresponding value for ISPAD, at 0.76, indicates that 24% of VS_i has been consumed by the X_f community. Within the VS, volatile suspended solids (VSS) are subjected to hydrolysis, and its product, volatile dissolved solids (VDS) are subjected to acidogenesis and acetogenesis. In terms of VS_i, the VSS fraction in fresh manure was 0.80, compared to 0.71 for both X_o and X_f, indicating the same rate of hydrolysis in both tanks. However, the uncovered tank accumulated the products of hydrolysis, with a VDS fraction of 0.34, increased from 0.20 for fresh manure, implying that acidogenesis and acetogenesis are slower than hydrolysis. In contrast, the VDS fraction of X_f, at 0.04, demonstrated that acidogenesis and acetogenesis balance hydrolysis in ISPAD.

The VFA, which are substrate to methanogenesis, provided similar conclusions. In X_o, propionic acid had accumulated along with a moderate decrease in acetic and butyric acids, as compared to S_m, suggesting limited methanogenesis. Traces

Table 2 – Intermediate products of anaerobic digestion in experimental manures.

		Fresh manure, S_m		Uncovered tank manure, X_0		ISPAD manure, X_f	
		Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Solids							
VS/FS	$kg\,kg^{-1}$	2.51	0.01	2.63	0.01	1.91	0.02
VS/VS_i	$kg\,kg^{-1}$	1.00	0.004	1.05	0.004	0.76	0.008
VSS/VS_i	$kg\,kg^{-1}$	0.80	0.04	0.71	0.04	0.71	0.016
VDS/VS_i	$kg\,kg^{-1}$	0.20	0.04	0.34	0.04	0.04	0.008
VFA[a]							
Acetic	$g\,kg^{-1}$	28.4	2.66	6.2	0.18	0.05	0.02
Propionic	$g\,kg^{-1}$	12.1	0.59	32.0	0.86	0.00	0.00
Butyric	$g\,kg^{-1}$	8.2	0.34	0.0	0.00	0.00	0.00

a In terms of VS_i.

of acetic acid and no measurable propionic or butyric acids in X_f indicated strong methanogenesis from these intermediate products in ISPAD.

Accordingly, the ISPAD microbial community in X_f demonstrated acclimation to its operating conditions, with balanced hydrolysis, acidogenesis, acetogenesis and methanogenesis [13]. In the uncovered tank community of X_o, hydrolysis was equivalent to that of ISPAD, but the corresponding acidogens, acetogens and methanogens were not as well developed, resulting in accumulation of VDS and VFA.

3.3. Initial BMP methane production rates

The initial rates of CH_4 production in the BMP assays are presented in Fig. 1. The control rates for fresh manure, S_m, are taken from the substrate-only bottles and represent the seed microbial community present in fresh manure. The rates for both the ISPAD and uncovered tanks, X_f and X_o, represent CH_4 produced by these communities with fresh manure as substrate, subtracting CH_4 produced in the biomass-only, self-fermentation controls. All 3 microbial communities had measurable initial CH_4 production rates at all three assay temperatures, increasing with temperature, being highest at 35 °C. Thus the communities are mesophilic overall. The low rates produced by S_m at all temperatures indicate the presence of a viable methanogenic seed community, with the potential for activity under both psychrophilic and mesophilic conditions. Although both X_f and X_o had experienced long-term exposure to psychrophilic conditions, both retained this extended range of activity. However, values for X_f were 3–12 times higher than those for X_o, and 25–85 times those for S_m. Considering the slightly larger size of the ISPAD community, possibly 50% based on ATP data, cannot account for these increases. Rather, it may be inferred that differences in community structure are the dominant factor. In addition, the largest increases, at 8 °C, suggest the disproportionate development of psychrophilic populations.

The relationships between CH_4 production rate and temperature, which appear linear for X_o and S_m though not for X_f, were subject to statistical RCBD analysis. Neither community type (S_m, X_o, X_f) nor temperature alone were found to significantly affect the relationship between methane production rate and temperature ($P = 0.19$ and $P = 0.16$, respectively), but the interaction between these two factors was significant ($P < 0.001$). Accordingly, the three communities react differently to temperature, indicating that the original community had changed in acclimating to the tank environments.

3.4. Acclimation during BMP assays

Figs. 2–4 illustrate the complete progress of CH_4 production in the BMP assays. Again, the control series, S_m, represent the substrate-only bottles, while those of X_f and X_o represent CH_4 produced by these communities with fresh manure as substrate, subtracting CH_4 produced in the biomass-only, self-fermentation controls. Note that slight decreases seen in some of these cumulative curves reflect experimental error generated by subtracting control values.

The slow initial CH_4 production by S_m (Fig. 2) increased to a maximum in 15 and 60 days respectively, at 35 °C and 18 °C, demonstrating acclimation to the BMP bottle conditions. However, in nearly 500 days at 8 °C, an exponential growth phase was not reached, and methane was produced at a minimal rate only, with little, if any, acclimation.

In contrast, the X_o community (Fig. 3) developed its maximum CH_4 production rate within the initial BMP period at both 35 °C and 18 °C, indicating that this community had acclimated in the tank environment to the range of 18–35 °C. However, at 8 °C the initial low CH_4 production rate was maintained for over 200 days, increasing around 300 to reach another steady rate, still lower than that of X_f, for the rest of the assay. This demonstrates that acclimation to the 8 °C conditions occurred in the X_o BMP bottles during the first 300 days. Because this is different from the behavior of the equivalent S_m bottles, it suggests that processes occurring in the X_o tank environment predisposed this inoculum to acclimate in the ideal bottle conditions.

The X_f community (Fig. 4) demonstrated an immediate maximum CH_4 production rate at all 3 temperatures, confirming its full acclimation in the range of 8–35 °C. Taken

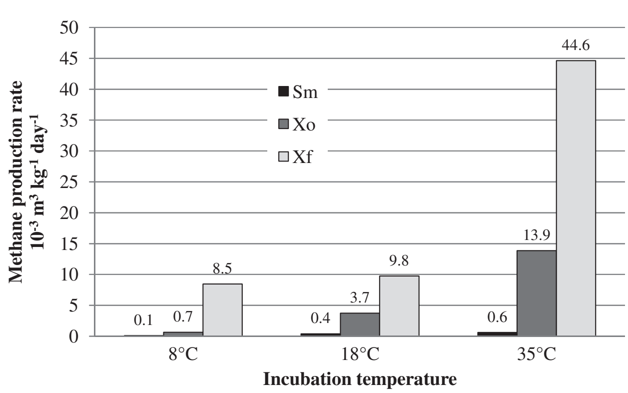

Fig. 1 – Methane production rates during the initial period of BMP incubation, by the microbial communities in fresh manure (S_m), uncovered tank manure (X_o) and ISPAD tank manure (X_f), with fresh manure as substrate.

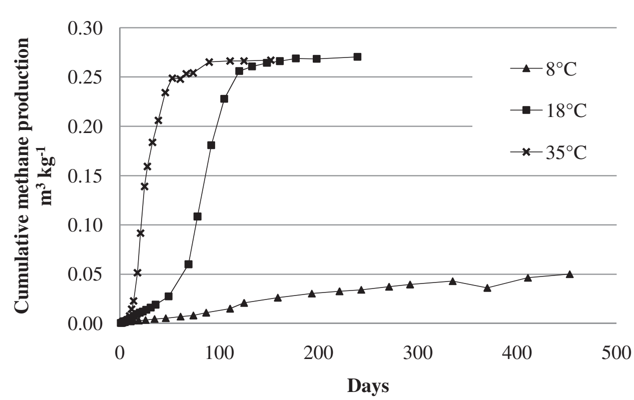

Fig. 2 – Development of methane production by the microbial community in fresh manure (S_m) during BMP incubation with fresh manure as substrate.

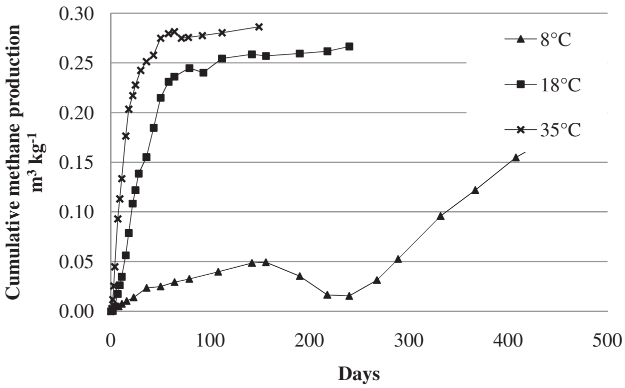

Fig. 3 – Development of methane production by the microbial community in manure from an uncovered storage tank (X_o) during BMP incubation with fresh manure as substrate. (control subtracted).

together, these results confirm that a manure microbial community can acclimate to the anaerobic conditions in ISPAD, developing robust psychro-active digestion, while an uncovered storage tank, though partially acclimated, is not an effective anaerobic digester.

3.5. Estimating ISPAD acclimation time

The BMP assays indicate the time required for acclimation at three constant temperatures, while in practice, manure storage tank temperatures vary through the seasons, remaining 4 °C warmer, 60 cm below the surface, than the average air temperature [16]. Thus, temperature acclimation for an ISPAD tank can be estimated from the time to maximum CH_4 production in the BMP, if 35 °C represents summer, 18 °C spring and fall, and 8 °C winter. Accordingly, an existing tank similar to X_o, when converted to ISPAD, will acclimate to summer temperatures within a week, to spring and fall within a month, and to winter conditions within the first year. A completely new installation fed fresh manure will adjust to summer temperatures in a month, spring and fall-within 100 days and winter temperatures in more than 500 days.

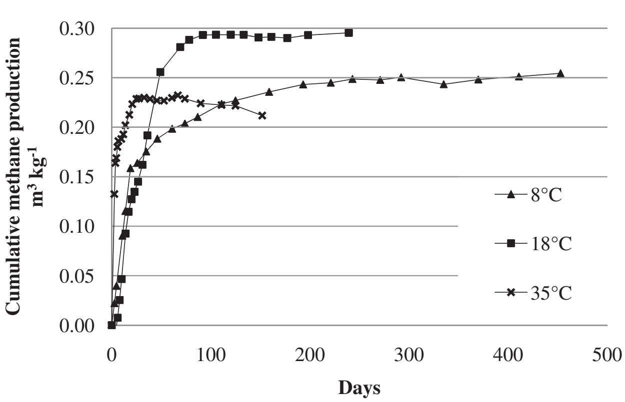

Fig. 4 – Development of methane production by the microbial community in manure from an ISPAD covered storage tank (X_f) during BMP incubation with fresh manure as substrate. (control subtracted).

3.6. Ultimate methane potential (B_0)

Substrates for anaerobic digestion are characterized by their ultimate methane potential, B_0. Table 3 presents B_0 from first-order curve fitting, as well as B_{150} from the assay data, for S_m, X_o and X_f. Fig. 5 illustrates the self-fermentation data used to determine these values. The fresh manure, S_m, exhibited a significant lag phase prior to reaching first-order exponential growth. Using the data points from the first-order region gave a good fit between the experimental and calculated curves (R^2 of 0.99) and equal values for B_0 and B_{150}, of 0.27 $m^3 kg^{-1}$ in terms of VS_i. For X_o, the entire data set was used, as it followed a first-order form with a good fit (R^2 of 0.99) and values of 0.22 and 0.23 for B_0 and B_{150}. For X_f, the data followed a first-order form, but the curve fitting showed that the initial maximum rate region entered a prolonged and flattened transition phase, and did not reach a clearly defined asymptote. This gave a poorer fit (R^2 of 0.87) with B_0 of 0.08 underestimated by 20% compared to B_{150} at 0.10. Thus in this case, B_{150} was a better estimator of ultimate methane potential than mathematical evaluation which does not consider the recalcitrance of some portions of VS, especially in previously digested materials such as X_f. Overall B_{150} gave a good estimate of B_0 for all three manures without the need to assume first-order behaviour.

The ultimate methane potential, B_0, and its indicator, B_{150}, are expected to be constant for a specific material [29], and thus all three swine manures should have presented results similar to S_m, at 0.27 $m^3 kg^{-1}$. Significant differences in the B_{150} values for X_f and X_o, respectively 0.10 and 0.23, are explained by the release of methane from the tanks. Thus, robust digestion in the ISPAD covered tank X_f had released 63% of its potential methane compared to 15% for the uncovered tank X_o. A review of swine manure anaerobic digestion research reports mesophilic CH_4 production of 0.26–0.35 $m^3 kg^{-1}$, and B_0 values of 0.32–0.48 $m^3 kg^{-1}$ [30]. An average mesophilic methane release of approximately 75% of B_0 compares favorably to the 63% released by ISPAD.

3.7. Monitoring protocol

This study pointed to microbial acclimation indicators that could be used to monitor the process in ISPAD installations. The simplest indicator is VS, and its variation over time, which requires a simple standard analytical procedure. The ultimate methane potential, B_0, could be evaluated seasonally using BMP at 35 °C for 75 days. Fig. 5 shows that 75-day incubation gives a good approximation of B_0. Incubations of 15 days or less, at temperatures lower than 35 °C could also be done seasonally to evaluate the initial methane production rates. These 3 combined procedures could give a clear picture of the development of acclimation in a new ISPAD microbial community, with all values reported based on VS_i. Reporting CH_4 production based on B_0 is also more meaningful than an absolute value, allowing installations with different manure compositions to be compared.

Table 3 – Methane potential of experimental manures.

		Fresh manure, S_m		Uncovered tank manure, X_0		ISPAD manure, X_f	
		Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
B_{150}	$m^3 kg^{-1}$	0.27	0.01	0.23	0.004	0.10	0.01
		Average	R^2	Average	R^2	Average	R^2
B_0	$m^3 kg^{-1}$	0.27	0.99	0.22	0.99	0.08	0.87

Notes: B_{150} is the total methane released during anaerobic incubation in a BMP assay at 35 °C for 150 days.
B_0 is the maximum possible methane release from the same manure, calculated by fitting a first-order equation to the data from the same BMP assay.

3.8. Further investigation

This study demonstrated that a swine manure microbial community can acclimate to psychrophilic ISPAD operating conditions, resulting in significant anaerobic digestion. However, to develop an ISPAD design model, further studies are needed. Using specific activity tests (SAT) with individual pure substrates representing each stage in the digestion process would define the kinetics of each population [31] and provide parameters for modeling and optimal design and operation of ISPAD systems [32]. If these assays were repeated at different temperatures, the results could describe the relationship between ISPAD CH_4 production and temperature, another essential feature of the successful model. The present study indicates differences in this relationship for acclimated and non-acclimated communities, as previously reported [33,34]; however, the form of the relationship has not been confirmed.

The essential role of H_2 could also be investigated in this way, as the H_2 pathway is expected to dominate methanogenesis during low-temperature acclimation, with the acetate pathway producing 95% of CH_4 once acclimation is complete [13]. The data obtained from X_o in the current study support this hypothesis with CH_4 produced concurrently with VFA accumulation, suggesting that H_2 is the major source of CH_4 in this partially acclimated system.

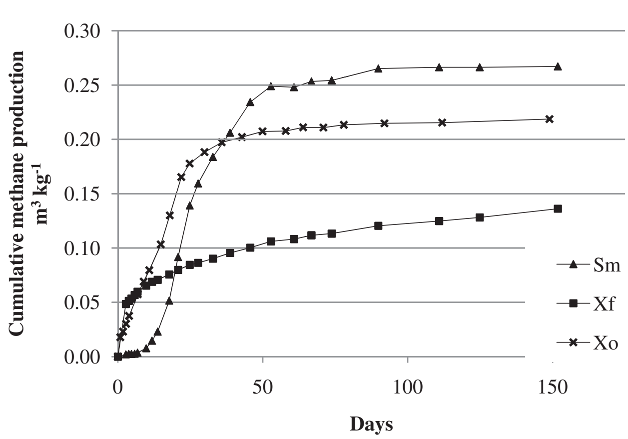

Fig. 5 – Cumulative methane production during self-fermentation BMP incubation at 35 °C, with no added substrate, for fresh manure (S_m), uncovered tank manure (X_o) and ISPAD tank manure (X_f).

4. Conclusion

The objectives of this study were to confirm the psychrophilic acclimation of the In-Storage Psychrophilic Anaerobic Digestion (ISPAD) microbial community, and to quantify the extent of the resulting digestion process.

The results of the study confirm that swine manure treated using ISPAD develops an acclimated microbial community within 1–2 years, depending on the prevailing climatic conditions. Volatile solids are rapidly consumed without lag, and intermediate products do not accumulate. The resulting anaerobic digestion process can transform 24% of the manure organic matter (as VS) into methane, representing 63% of the ultimate methane potential, B_0.

The ISPAD acclimation process may be monitored using standard laboratory analyses conducted on manure samples collected periodically, such as VS and B_0. Monitoring other installations in this way could allow the results of this study to be generalized to all ISPAD facilities. Future laboratory studies are required to investigate the microbial kinetics and develop a model capable of optimizing both the design and operation of ISPAD systems. The treatment effects including ammonia release, phosphorus separation and odor reduction could also be evaluated.

Once fully described, ISPAD could find other application such as the digestion of waste activated sludge from small and medium-sized waste water treatment operations, producing methane and stabilizing biosolids for land application, possibly combined with struvite precipitation for nutrient removal and revenue generation.

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