

# BATCH CO-DIGESTION OF MANURE, SOLID SLAUGHTERHOUSE WASTE, AND FRUIT & VEGETABLE WASTE

*René Alvarez<sup>a,\*</sup>, Victor Hugo Riera<sup>a</sup>, Gunnar Lidén<sup>b</sup>*

<sup>a</sup>IIDEPROQ, UMSA, Plaza del Obelisco 1175, La Paz, Bolivia; <sup>b</sup>Dept. of Chemical Engineering, Lund University, P.O.Box 124, 221 00, Lund, Sweden

\*Corresponding author: [rene.alvarez@iideproq.org](mailto:rene.alvarez@iideproq.org)

**Key words:** Co-digestion, anaerobic digestion, slaughterhouse waste, manure, fruit and vegetable waste

## ABSTRACT

Anaerobic co-digestion of different waste materials from the city of La Paz, Bolivia, was studied. The waste materials used were of three principal kinds; a) solid slaughterhouse wastes (blood, rumen, stomach and intestinal content), b) cow and swine manure, and c) fruit and vegetable wastes from a local market. An augmented simplex-centroid design for three components was used to evaluate the effects of mixing the different kinds of substrates. Anaerobic batch experiments were made in 2 l reactors at a temperature of 35°C. A positive synergistic effect was found for the binary mixture between manure and slaughterhouse waste. The highest methane yield based on added volatile solids (VS) was 0.29 m<sup>3</sup> CH<sub>4</sub> kg<sup>-1</sup> VS, and the obtained reduction of VS was 76 %. The positive mixture effect is most likely due to the balancing of nutrients and possibly also dilution of inhibitory material present in the different substrates.

In contrast, a strongly negative mixture effect of adding fruit and vegetable waste (FVW) was observed. The methane yield was decreased by 90% when 17% of the mixture consisted of FVW, and by 98% when 33% of the mixture consisted of FVW, in comparison to mixtures without FVW. The low methane yield from the FVW containing mixtures is probably an effect of the sensitivity of the methanogenic bacteria to changes in pH caused by the accumulation of volatile fatty acids.

## RESUMEN

La Co-digestión anaeróbica de diferentes residuos orgánicos de la ciudad de La Paz, Bolivia, fue estudiado. Los residuos utilizados principalmente fueron de tres tipos; a) residuos sólidos de matadero (sangre, rumen, y contenidos de

estómago e intestinos), b) estiércol de vaca y cerdo, y c) residuos de vegetales y frutas de un mercado local. Un diseño de mezcla mejorado simplex-centroide para tres componentes fue empleado para evaluar el efecto de mezclar los diferentes tipos de sustrato. Experimentos anaeróbicos en proceso batch fueron realizados en reactores de 2 litros a 35°C de temperatura. Un efecto sinérgico positivo se estableció para la mezcla binaria entre estiércol y residuos de matadero. El máximo rendimiento de metano en base a los sólidos volátiles (VS) agregados fue de 0.29 m<sup>3</sup> CH<sub>4</sub> kg<sup>-1</sup> SV, y la reducción de sólidos volátiles fueron del 76%. El efecto positivo de mezclar probablemente sea debido al balance de nutrientes y posiblemente también a la dilución de materiales inhibidores presentes en los diferentes sustratos.

En contraste, un fuerte efecto negativo de mezcla fue observado al adicionar los residuos de vegetales y frutas (FVW). El rendimiento de metano fue decreciendo hasta 90% cuando 17% de la mezcla consistió de FVW, y hasta 98% cuando 33% de la mezcla estaba compuesto de FVW, en comparación a mezclas sin FVW. El bajo rendimiento de metano de las mezclas conteniendo FVW probablemente es el resultado de la sensibilidad de las bacterias metanogénicas a los cambios del pH causado por la acumulación de ácidos grasos volátiles.

## INTRODUCTION

In many countries a reduction of deposited organic waste material is a stated governmental goal. This applies for example to the EU countries, where the deposited biodegradable material to landfills should be decreased by 65% in comparison to the amount of waste deposited in 1995 by the year 2014 (Council Directive 1999/31/EC). In other countries these goals are

less clearly stated, but the reduction of waste equally desirable. Anaerobic digestion is one way of decreasing the amount of solid waste, which also has the added benefit of methane production. The digestion is, of course, strongly affected by the type of raw material, and both the possible reduction of the solid contents and the methane yield depends on the composition of the waste material.

Co-digestion, i.e. the simultaneous digestion of a mixture of two or more substrates, is an attractive technique, by which the bioconversion rate as well as the methane yield can be increased. The process benefits of co-digestion lies in effects such as an improved nutrient balance, or improved rheological qualities of the substrate. There are also potential economic advantages such as the possibility of utilizing free capacity in digesters in sewage treatment plants, and the possibility of using energy crops as co-substrate [1, 2, 3].

The food industry generates substantial amounts of organic waste. The waste produced in slaughterhouses mainly consist of blood, washing water and the content of rumen, stomachs and intestines as well as droppings and manure from the delivery hall. The city of La Paz, Bolivia has a large meat industry. The size of the industry can be understood from statistics from the Centro de Promocion de Tecnologias Sostenibles (CPTS) of the National Chamber of Industry in Bolivia. In the period January to October 2003 the industry handled 19500 cattle (average weight 381 kg/animal, i.e. corresponding to a total weight of 7500 ton) and 17000 pigs (90 kg/pig, corresponding to a total weight of 1500 ton). The final recipient of untreated waste streams in La Paz is the Choqueyapu river. Approximately 137 m<sup>3</sup> of the slaughterhouse wastewater, containing 160 kg BOD content (mainly from blood) and 4.4 tons of solid material is discharged to the river daily. A reduction of the BOD containing waste from the meat industry is therefore highly desirable.

Rosenwinkel and Meyer [4] examined digestion of hog stomach contents and slaughter flotation tailings, both separately and using municipal sludge as a co-substrate in stirred 2 m<sup>3</sup> pilot plant reactors. In the case of hog stomach content, a gas production reported based on total solids (TS) of 0.16 m<sup>3</sup> kg<sup>-1</sup> TS with a methane content of 40% was obtained at a retention time of 44 days. The methanogenesis completely stopped when reducing the retention time to 25 days. Based on these results, the authors concluded that the sole digestion of the stomach content was not to be

recommended. On the other hand, the co-treatment of stomach content with sludge from a local wastewater treatment plant was successful, even when the stomach content part contributed with as much as 67% of the organic load. After an adaptation period, the reactor could be operated with a retention time of 17 days and a total organic volumetric loading of 2.9 kg TS m<sup>-3</sup> day<sup>-1</sup> with a methane yield of 0.229 m<sup>3</sup> kg<sup>-1</sup> TS.

The development a two phase anaerobic digestion system for the treatment of mixed slaughterhouse waste composed of mixture of cattle blood and cattle gut fill (rumen paunch content) was reported [5]. It was found that a process loading of 3.6 kg TS m<sup>-3</sup> day<sup>-1</sup> was achievable with a methane production rate of 0.27 m<sup>3</sup> kg<sup>-1</sup> TS. A 63% reduction of TS was obtained. Co-digestion of several different types of waste were examined [6]. Pig manure, slaughterhouse waste, vegetable waste and various kind of industrial waste were successfully co-digested. Although the conversion of volatile fatty acids (VFA) was incomplete, the process worked well with a methane yield of 0.56 - 0.70 m<sup>3</sup> kg<sup>-1</sup> VS at a load of 2.6 - 3.7 kg VS m<sup>-3</sup> day<sup>-1</sup> in parallel set-ups. The authors concluded that the mixture gave a highly buffered system as the manure contributed to high amounts of ammonia.

Fruits and vegetable waste (FVW) is another municipal organic waste material, which is a potential feedstock for anaerobic digestion. The biochemical methane potential (B<sub>0</sub>) of 54 different kinds of fruit and vegetable waste was reported [7]. Substantial differences were observed in both the methane yields and kinetics for the substrates studied. The B<sub>0</sub> of fruit wastes ranged from 0.18 to 0.73 m<sup>3</sup> (kg VS added)<sup>-1</sup>, and that of vegetable wastes ranged from 0.19 to 0.4 m<sup>3</sup> (kg VS added)<sup>-1</sup>. This feedstock is interesting as a co-feedstock, since the nitrogen and phosphorus content in FVW is normally low [8, 9, 10, 11]. This makes it tentatively suitable as a co-feed for e.g. nitrogen rich slaughterhouse waste. The methane yield reported in semi-continuous process using a tubular digester was between 0.25-0.45 m<sup>3</sup> (kg VS added)<sup>-1</sup> [12, 13]. With respect to co-digestion, a higher overall methane production was obtained when FVW was converted together with cattle slurry in a batch digester. However, the selectivity in the conversion (m<sup>3</sup> CH<sub>4</sub> (kg VS removed)<sup>-1</sup>) was somewhat lower the control digestion with cattle slurry alone [8].

Anaerobic co-digestion has been demonstrated to be technically feasible in a number of cases. In

the present work, mesophilic anaerobic co-digestion was studied as a potential means to treat and decrease waste streams of significance in the city of La Paz, Bolivia. In particular, the mixture effects of different available feedstocks were evaluated using batch laboratory-scale experiments set-up according to a statistical mixture design. The waste feedstocks considered were solid wastes from slaughterhouses (blood, rumen, paunch waste), manure (cattle and swine), and fruit-vegetable market waste. The experiments were analysed with respect to gas production, methane yield and reduction of volatile solid in the substrate.

## RESULTS AND DISCUSSION

### Raw material

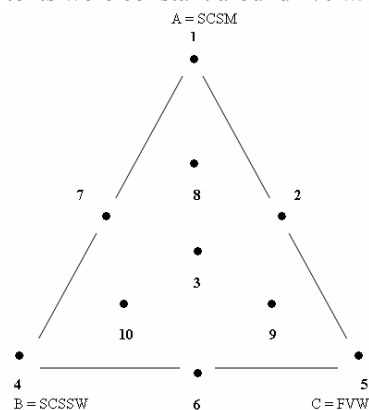
The components and physicochemical composition of the raw material used in the experiments are shown in Tables 1 and 3. The proportion of the components in FVW (table 1) is the result of the seasonal abundance of citrus fruits and onion in the La Paz - Bolivia markets between May to August. The physicochemical composition of the mixture of FVW (Table 3) is in the range of reported values of other studies ([8, 12, 17]). The pH-value of FVW was clearly lower (4.9) than that of the other materials. The swine manure had a particularly high pH-value (9.15). The composition of cow and swine manure obtained from the floor of the slaughterhouse (Table 3) agreed well with reported mean values for beef and swine manure [18], with the exception of the nitrogen content, which was low in both cases, 1.9 and 2.47 % of TS, respectively - less than 50% of reported values [18]. This most likely reflects the feed situation on the Altiplano. On the other hand, the mixture of slaughterhouse waste (SCSSW) from 57% of cow rumen, 9% of swine paunch and intestinal content, 29% of cow blood and 5% of swine blood had a higher nitrogen content (7.64 %TS) as a result of high nitrogen content in the blood of cow (15 %TS) and swine (8.32 %TS).

The sodium, potassium and calcium concentration in the substrate may be either stimulatory or inhibitory in the anaerobic digestion process, depending on the concentrations. Clearly, only the dissolved material contributes to the toxins [19]. The substrates formulated according Tables 2 and 3 had VS of 0.8-5.6 % wet weight. The concentrations of sodium, potassium and calcium

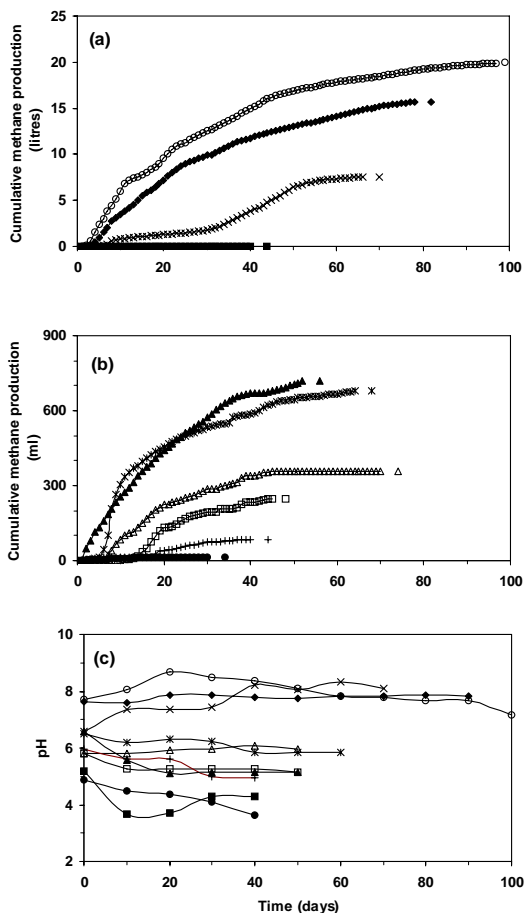
from the raw materials were all below the expected inhibitory values [19, 20].

### Mixture experiments

Co-digestion experiments were performed according to mixture design (Fig. 1) with the substrate formulation given by Table 2, in order to assess the influence of mixture composition on methane yield and volatile solids destruction. Mesophilic conditions were used and the initial VS contents were constant around 4% w/w.



**Fig. 1.** Experimental mixture design. Augmented simplex-lattice for three components, • Design points,  $x_1 = A$ : SCSM (Solid cattle and swine manure),  $x_2 = B$ : SCSSW (Solid cattle and swine slaughterhouse wastes),  $x_3 = C$ : FVW (Fruit and vegetable wastes). The initial pH of the ten experiments were between 4.9 and 7.7. The mixtures with high proportion of FVW (exp. number 5, 9) showed the most acid character, whereas experiments number 1 and 7 with a high fraction of SCSM (mixture of cattle and swine manure) had an initial pH > 7. The measured pH-values and the cumulative methane production over time are shown in Fig. 2. Methanogenic bacteria are sensitive to low pH and the satisfactory pH range for the methane production was in the current study found to be between 6.6 and 7.8 (exp. 1 and 7). Significant inhibition of the methanogenic bacteria was found below a pH-value of 6.6 (exp. 2, 3, 8 and 10). However, the production of volatile acids (VFA) continued. Acidogenic bacteria will produce VFA and  $\text{CO}_2$  until the pH drops below 5 (exp. 5 and 9). In exp. 4 - one of the corner points containing only SCSSW - the pH-value gradually increased from 6.6 to 8.08, which resulted in a gradual inhibition of methanogenesis. This may be attributed to the accumulation of high levels of ammonia resulting from the degradation of nitrogen rich protein components of blood.

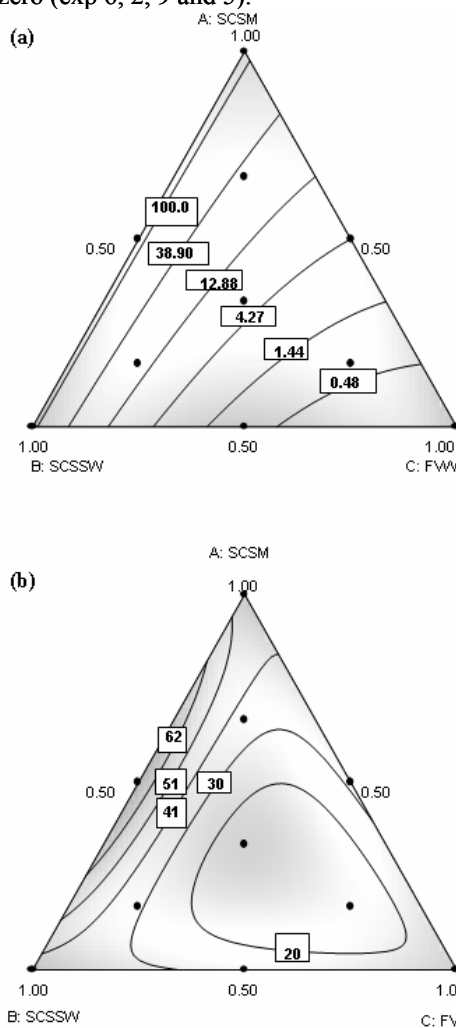


**Fig. 2.** Cumulative methane productions (a and b) and pH (c) from 10 batch assays. Symbols indicate experiment numbers; 1 (♦), 2 (□), 3 (Δ), 4 (×), 5 (●), 6 (+), 7 (○), 8 (▲), 9 (■), 10 (⋈)

The maximum methane yield,  $0.29 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$  (cf. Table 4), was obtained in exp. 7, which was a mixture of manure (SCSM) and the slaughterhouse waste (SCSSW). Also with respect to removal of volatile solids in the final slurry this mixture gave the best result, with a 75% reduction of VS.

**Methane yield.** Response surfaces for the methane yield ( $\text{m}^3 \text{ kg}^{-1} \text{ VS}$  added) and the reduction of VS were fitted to the data points from the simplex-centroid design. The methane yields obtained ( $0.0002\text{-}0.29 \text{ m}^3 \text{ kg}^{-1} \text{ VS}$ , Table 4) vary over several orders of magnitude. For this reason, a logarithmic transformation was made to the data to allow a parameter estimation covering the entire range.

Fig. 3(a) shows that the ternary contour lines with higher methane yield are close to the A-B axes (i.e. the mixture of manure and slaughterhouse waste) and the maximum methane yield is found at the mixture  $\text{SCSM:SCSSW:FVW} = 0.5:0.5:0.0$ . The methane yield dramatically decreases when FVW is incorporated, i.e. when the composition approaches corner C. The high inhibition effect of the FVW was seen as a 90% decrease in the methane yield already at a fraction of 17% FVW in the mixture. Higher proportions of FVW practically reduces the methane yield to zero (exp 6, 2, 9 and 5).



**Fig. 3.** Ternary contour plots from the fitted model equations listed in Table 7 for (a) Methane yield ( $\text{L kg}^{-1} \text{ VS added}$ ), (b) %VS reduction.

**Volatile solids reduction in the substrate.** The maximum reduction of volatile solid was also obtained with mixtures of manure-slaughterhouse waste (see Fig 3b, Table 4). A positive synergistic co-digestion was found also here from mixing manure (SCSM) and slaughterhouse waste

(SCSSW). The highest VS reduction, 76%, should be compared to the corner points of 52% and 35% for CSSM and SCSSW respectively. The inhibitory effect of FVW, although not as dramatic as in the case of methane yield, was found also in reduction of VS. The volatile solids reduction in the absence of methane production is a result of acidogenesis and was seen as an increased content of CO<sub>2</sub> in the produced gas (not shown).

The results obtained in the present study show that mesophilic anaerobic treatment of a mixture of manure and slaughterhouse waste in a batch process leads to an increased methane yield, and improves the reduction of volatile solids. In contrast, anaerobic batch digestion of fruit and vegetable waste gives a strong inhibition of the methane yield and removal of VS, both in co-digestion with slaughterhouse waste and manure as well as by itself.

#### **Co-digestion of manure and slaughterhouse waste**

The digestion experiments with manure (cattle and swine) (SCMS) and the manure-slaughterhouse waste (SSCW) showed good stability in the evolution of methane production. The obtained methane yield was 0.20 for SCMS and 0.29 m<sup>3</sup> (kg VS added)<sup>-1</sup> for a mixture of SCMS and SSCW. The digestion of SCSSW alone (exp 4, Table 4) showed a gradual increase in pH from 6.6 up to 8.08. The production of biogas was lower than for manure, with a low fraction of methane in the biogas. Consequently, the methane yield was lower (0.11 m<sup>3</sup> (kg VS added)<sup>-1</sup> and also the reduction of VS was lower (35%). The yield (or selectivity) with respect to removed VS was 0.31- 0.39 m<sup>3</sup> CH<sub>4</sub> (kg VS removed)<sup>-1</sup> for these experiments.

These results could be explained by ammonia inhibition. Similar patterns of inhibition in digesters working with mixtures of swine and cattle manure were found [5] working with cattle blood and gut fill (3 parts gut fills to 1 part blood by weight) in a continuous digester. These authors reported that the process was prone to failure and attributed this to the accumulation of high level of ammonia resulting from the degradation the protein rich blood. The authors obtained a gas production of 0.17 m<sup>3</sup> (kg TS)<sup>-1</sup> and a 50% average TS reduction from the single pass reactor at 30 days of HRT.

The methane yield (0.20 m<sup>3</sup> (kg VS added)<sup>-1</sup>) obtained in experiment 1, i.e. with only the

manure, and the removal of VS (52%) agrees well with yields previously reported for cow and swine manure. For cow manure a methane yield of 0.2 m<sup>3</sup> (kg VS added)<sup>-1</sup> was reported [21], and a yield of 0.148 m<sup>3</sup> (kg VS added)<sup>-1</sup> [22]. For swine manure, a yield of approximately 0.3 m<sup>3</sup> (kg VS added)<sup>-1</sup> [23], and 0.275 m<sup>3</sup> (kg VS added)<sup>-1</sup> [22] was reported. Batch experiments with mixtures of swine and cattle manure have been reported to be inhibited for a swine-to-cattle manure ratio higher than 25:75, corresponding to a free ammonia concentration of approximately 1.1 g N l<sup>-1</sup> [23]. Experiment 1 had a swine-to-cattle manure ratio of 29:79, i.e. close to the limit indicated. However, the pH profile did not suggest any inhibition (cf. Fig 2c).

In the present study a positive synergistic effect of co-digestion between manure and slaughterhouse waste was observed. This applied both to the methane yield and the reduction of VS (cf Table 4). The methane yield was almost 90% higher than the mean of the two sources, whereas the removal of VS was 100% higher than the mean. The reason for this may be a balancing of nutrients and/or dilution of inhibitory compounds present in one or the other of the materials due to the mixing. The obtained methane yields in the present study may appear low compared to previous reported values (0.6-0.7 m<sup>3</sup> CH<sub>4</sub> (kg VS)<sup>-1</sup> [6], 0.7 m<sup>3</sup> CH<sub>4</sub> (kg VS)<sup>-1</sup> [24]. However, in several cases with continuous or semi continuous anaerobic digestion processes, the material has been pre-treated (heat-treated) which increases the methane yield substantially (Edstrom et al., 2003). An increase of methane yield in batch digestion of pasteurised animal by-products from 0.056 m<sup>3</sup> kg<sup>-1</sup> to 0.225 m<sup>3</sup> kg<sup>-1</sup> was reported by [24]. Co-digestion of pasteurised (70°C, 1h) animal by-products resulted in a fourfold increase in biogas yield (1.14 m<sup>3</sup> (kg VS)<sup>-1</sup>) compared with non pasteurized animal by-products (0.31 m<sup>3</sup> (kg VS)<sup>-1</sup>) [25]. The increased degradability after thermal pretreatment has been explained by an increased solubility [26, 27], or an increased accessibility of lipids for the microorganisms [25].

#### **FVW effect in the co-digestion**

Fruit and vegetable waste (FVW) was found to exhibit a strong inhibitory effect in the batch co-digestion process (cf Table 4 and Fig 2). The low pH of FVW, and the high content of easily biodegradable carbohydrates, gives a rapid acidification from the formation of volatile fatty acids (VFA). In the absence of a continuous pH

regulation this gives a fast inhibition of methanogenic bacteria [28].

In the current work, the methane formation does not proceed at all below a pH value of 5, whereas in the range 5 to 6.5 the methane yield and the reduction of VS are low. The observed reduction of VS in these experiments is explained by the destruction of organic matter in non-methanogenic reactions. The process imbalance between acidogenic reaction and methanogenic reaction could be seen not only in the drop in the pH-value, but also in the composition of the generated biogas. For example, in the experiments 5 and 9 the gas generated has a high CO<sub>2</sub> content (about 67% in both cases) and a negligible methane concentration due to lack of methanogenesis. A similar problem of inhibition by accumulation of VFA and a thereby associated irreversible decrease of pH in batch digestion at 8% TS was described [12]. The observed toxicity under low pH conditions is likely associated with the presence of undissociated volatile fatty acids [29] and the inhibition is therefore dependent on the buffering capacity of the medium. The microbiology of digesters fed with tomato processing waste was studied [30], they observed that in batch digestion, the population of methanogens was low due to the drop in pH of slurry. However, in semi-continuous digestion, the population of methanogens (together with cellulolytic, proteolytic, lypolytic organisms) increased with increase in hydraulic retention times (HRT). Therefore, a semi-continuous process might allow methane production at a higher FVW content than in the present batch conditions.

## EXPERIMENTAL

### Raw material

The FVW were obtained from the vegetable market, La Paz, Bolivia. Each item of waste was separated and weighed before being placed in the bin so that the overall composition was known (Table 1). All the samples were mixed and minced into smaller pieces with a domestic electric mincer for further homogenized with a domestic electric blender (Hamilton beach 908, Hamilton beach commercial, USA); the samples were packed into polyethylene bags and stored at -10°C in a freezer until used. Solid cattle and swine slaughterhouse waste (SCSSW) were obtained from a local municipal slaughterhouse in the proportion in which they were produced. The minced and mixed fractions of rumen cow (57.1 weight %), stomach content and gut fill of swine (9.4 weight %), blood cow (28.6 weight %), and

blood swine (4.9 weight %) was used as co-substrate in the essays.

Solid cattle and swine manure, SCSM (collected from the same slaughterhouse) in the proportion cow manure 71 weight % and swine manure 29 weight % were mixed, and the samples were packed into polyethylene bags and stored in a freezer until used.

The inoculum used to seed the reactors was obtained from an active 4 l mesophilic biogas digester of cow manure.

### Experimental set-up

The digester experiments were carried out in four identical, semi-continuously stirred stainless steel digesters, each with a total volume of 2 l. The cylindrical vessel was equipped with a flanged top to which a flange plate with stoppered ports was fitted. This allowed gas collection, substrate sample removal, and the mounting of a geared motor drive unit for the reactor mixer. The contents of the reactor were semi-continuously mixed controlled by a timer. The digesters were operated at 35 ± 1°C by immersion in a water bath. Gas collection from the reactor was made via a flexible PVC tube to a separate water displacement glass bottle filled with water acidified to pH 2. The positive pressure in the bottles allowed the gas to be transferred to a measuring gas cylinder.

### Experimental procedure

FVW, SCSSW and SCSM were taken out of the freezer and allowed to thaw overnight. The substrates were weighed and diluted with tap water to the desired solid-content in each experiment (Table 2) and the contents was homogenized with a domestic electric blender. The mixed substrate was charged to the reactor, and finally inoculum slurry was added corresponding to 10 weight%.

Ten batch experiments were made according to the mixture design (see below). 1800 g of substrate was charged in each reactor (Table 2), the reactors were placed in the temperature controlled water bath at 35°C, and allowed to ferment for periods between 40 to 100 days. The reactors were semi-continuously mixed. The stirrer was controlled by a timer and operated for 15 minutes at 30 rpm, followed by a period of 105 minutes without stirring. The atmospheric pressure during the experiment was 495 mmHg (the mean atmospheric pressure in La Paz, Bolivia). Samples of the slurry (40 ml) were taken from the reactor through the sample removal port with a syringe every 10 days and the

pH and solid content were analysed. Biogas was collected and measured by displacement of water once a day at zero gauge pressure and ambient temperature. The volumes were corrected to normal temperature and pressure conditions (0°C, 760 mmHg).

### Analytical methods

Methane and carbon dioxide concentration in the biogas were measured with a gas chromatograph (Shimadzu Model GC14B, Japan) equipped with a thermal conductivity detector (TCD) and Carboxen-1010 plot Capillary column 0.32 mm ID (Supelco, USA), Helium served as the carrier gas. The injector, detector and oven temperatures were 150°C, 200°C and 120°C, respectively.

The total solids (TS), volatile solids (VS), pH, total Nitrogen and total phosphorous, in the feed and samples of the substrates were all analysed using standard methods [14]. The total solids content (TS) was determined after a repeated heating (105°C for 1 h), cooling, desiccating, and weighing procedure until the weight change was less than 4%. Volatile solids (VS) were determined by ignition of the residue produced in TS analysis to constant weight in a muffle furnace at a temperature of 550°C. Total Kjeldahl nitrogen (TKN) was measured by semi-micro-Kjeldahl method as described in Standard methods [14]. Potassium and phosphorus were measured by spectrophotometry (Method 3500-K and 4500-P, respectively). Calcium was estimated by titration with EDTA (Method 3500-Ca) and sodium was measured by flame emission photometry (Method 3500-Na).

### Experimental design

The co-digestion of organic wastes depends on the relative proportion of the components, the amount of the mixture, and other physical process variables such as temperature and pressure. In the current design, the same temperature and pressure was maintained in all experiments. An augmented simplex-centroid design for three mixture components was used. This design gives 10 treatments (Fig 1), and provides the minimum number of points needed to estimate a quadratic (second-degree) response surface and provide some ability to detect lack-of-fit [15, 16]. In the mixture experiments, the independent factors are the fractions of the three components of a blend. Since the fractions of the different components always sum to 1 (100% total of volatile solids), the factor space is a two-dimensional plane represented by an equilateral triangle whose vertices are the three pure compounds A: SCSM

=  $x_1 = 1$ , B: SCSSW =  $x_2 = 1$ , and C: FVW =  $x_3 = 1$ . These points have the coordinates (1,0,0); (0,1,0) and (0,0,1) in a 3D space. The points along the edges of the triangle represent mixtures of only two components, whereas points in the interior ( $x_1, x_2, x_3$ ) represent mixture of all three components. The response surfaces (i.e. the methane yield and the percentage VS removed) depend on the substrate composition. Since the feed-stock fractions are not linearly independent, there are in fact only two degrees of freedom for the regressor variables.

### CONCLUSION

In anaerobic co-digestion it is important to consider the effect that the composition of the incoming substrate will have on the digester performance. The co-digestion effect between mixture of cattle and swine manure together solid slaughterhouses wastes (Rumen, paunch content and blood of cattle and swine) enhance the methane yield and volatile solids removal. The synergistic effect obtained may be attributed to the presence of nutrients and dilution of inhibitory materials present in the co-substrates due to mixing and benefits the overall process.

In the present study, a maximum methane yield of 0.29 m<sup>3</sup> kg<sup>-1</sup>VS with a reduction of volatile solids of 76% was obtained in batch anaerobic co-digestion of mixture (4% w/w SV) of manure and solid slaughterhouse waste. Batch co-digestion of fruit and vegetable waste with either manure or slaughterhouse waste was, however, not found possible. Including FVW resulted in a drop in pH and a strong reduction of methane yield and volatile solids removal.

### ACKNOWLEDGEMENTS

This work was financially supported by SIDA, the Swedish International Development Cooperation Agency.

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**Table 1.** Composition of the FVW

Sample	Part used as feedstock	Percentage (w/w) <sup>a</sup>
Banana	Whole rotten fruit	2.23
Carrot	Leaves, roots	2.34
Cassava	Peels, roots, whole plant	5.00
Eggplant	Whole fruit	6.20
Grapefruit	Whole rotten fruit	6.80
Lacayote (squash)	Whole rotten fruit	6.26
Lemon	Whole rotten fruit	1.50
Lettuce	Leaves	0.92
Locoto (chile pepper)	Whole rotten fruit	3.79
Onion	Exterior peels, leaves	11.50
Orange	Whole rotten fruit	21.60
Peas	Pods, leaves	0.72
Pineapple	Peels of fresh riped fruit	1.50
Potatoes	Peels	2.74
pumpkin	peels, seeds	5.15
Radish	Leaves, whole plant	0.64
Sugar beet	Leaves, roots, whole plant	5.85
Sweet pepper	Whole rotten fruit	7.66
Tomato	Whole rotten fruit	5.84
Turnip	Leaves, whole plant	0.88
Watermelon	Whole rotten fruit	0.88

<sup>a</sup>Wet weight basis.

**Table 2.** Mixture design and substrate composition in the anaerobic digestion of slaughterhouse and fruit & vegetable wastes.

Exp. nr.	Fraction of solid part			Substrate formulation (%)				
	$x_1 = \text{SCSM}$	$x_2 = \text{SCSSW}$	$x_3 = \text{FVW}$	SCSM	SCSSW	FVW	Water	Inoculum
1	1,00	0,00	0,00	20	0	0	70	10
2	0,50	0,00	0,50	10	0	14	66	10
3	0,33	0,33	0,33	7	7	9	67	10
4	0,00	1,00	0,00	0	21	0	69	10
5	0,00	0,00	1,00	0	0	27	63	10
6	0,00	0,50	0,50	0	11	14	66	10
7	0,50	0,50	0,00	10	11	0	69	10
8	0,67	0,17	0,17	14	4	5	68	10
9	0,17	0,17	0,67	3	4	18	65	10
10	0,17	0,67	0,17	3	14	5	68	10

**Table 3.** Characterization of slaughterhouses wastes and FVW

Analysis	Cow	Swine	Cow	Swine paunch	Cow	Swine	Mixtures		
	manure	manure	rumen	wastes	blood	blood	FVW	SCSM	SCSSW
pH	7.1	9.15	6.1	5.95	7.4	7.25	4.9	8.25	7.11
Total solids (% w,w)	14.14	37.88	13.42	13.52	23.03	22.28	14.52	23.49	18.33
Volatile solids (% of TS)	77.45	67.77	86.55	85.53	96.36	95.56	92.01	75.96	91.74
Total nitrogen (% of TS)	1.9	2.47	2.2	1.85	15	8.32	2.1	2.2	7.64
Total Phosphorus <sup>a</sup> (mg kg <sup>-1</sup> )	9200	21647	6600	4510	870	494	3100	15703	3771
Total sodium <sup>a</sup> (mg kg <sup>-1</sup> )	1100	2772	20000	19890	12000	7406	2700	1974	16000
Total potassium <sup>a</sup> (mg kg <sup>-1</sup> )	15000	10612	8800	8790	2900	10682	27000	12707	6584
Total calcium <sup>a</sup> (mg kg <sup>-1</sup> )	23000	13886	2100	1922	130	90	9100	18238	1175

<sup>a</sup> TS basis

SCSM means Solid Cattle and Swine Manure

SCSSW means Solid Cattle and Swine Slaughterhouse Wastes they're composed of Rumen, blood and Pig's paunch wastes

**Table 4.** Initial and final pH, VS of the digester contents, and methane production during essays

Exp. no	Mixture design			pH		VS (% wet weight)		VS reduction	Cumulative	Methane yield
	x <sub>1</sub>	x <sub>2</sub>	x <sub>3</sub>	Initial	Final	Initial	Final	(%)	methane (l)	(m <sup>3</sup> kg <sup>-1</sup> VSadded)
1	1,00	0,00	0,00	7,64	7,83	4,43	2,13	51,94	15,72	0,1971
2	0,50	0,00	0,50	5,79	5,16	3,84	2,79	27,37	0,25	0,0036
3	0,33	0,33	0,33	5,80	5,96	3,64	2,75	24,37	0,36	0,0055
4	0,00	1,00	0,00	6,60	8,08	3,85	2,50	35,02	7,55	0,1090
5	0,00	0,00	1,00	4,86	3,62	3,69	2,80	24,00	0,01	0,0002
6	0,00	0,50	0,50	5,95	4,95	3,82	2,66	30,24	0,08	0,0012
7	0,50	0,50	0,00	7,70	7,15	3,87	0,93	75,89	19,98	0,2868
8	0,67	0,17	0,17	6,58	5,15	3,71	2,85	23,09	0,72	0,0108
9	0,17	0,17	0,67	5,10	3,61	3,72	3,31	11,10	0,04	0,0006
10	0,17	0,67	0,17	6,49	5,84	3,79	2,80	26,04	0,68	0,0100