

**Acoustic chemometric prediction of total solids in bioslurry:  
a full-scale feasibility study for on-line biogas process monitoring**

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## **Abstract**

Dry matter is an important process control parameter in the bioconversion application field. Acoustic chemometrics, as a Process Analytical Technology (PAT) modality for quantitative characterisation of dry matter in complex bioslurry systems (biogas fermentation), has not been successful despite several earlier dedicated attempts. A full-scale feasibility study based on standard addition experiments involving natural plant biomass was conducted using multivariate calibration (Partial Least Squares Regression, PLS-R) of acoustic signatures against dry matter content (total solids, TS). Prediction performance of the optimised process implementation was evaluated using independent test set validation, with estimates of accuracy (slope of predicted vs. reference values) and precision (squared correlation coefficient,  $r^2$ ) of 0.94 and 0.97 respectively, with RMSEP of 0.32 % w/w (RMSEP<sub>rel</sub> = 3.86 %) in the range of 5.8 – 10.8 % w/w dry matter. Based on these excellent prediction performance measures, it is concluded that acoustic chemometrics has come of age as a full grown PAT approach for on-line monitoring of dry matter (TS) in complex bioslurry, with a promising application potential in other biomass processing industries as well.

## **Keywords**

Acoustic chemometrics, partial least squares regression (PLS-R), Total Solids (TS), bioslurry, on-line monitoring, Process Analytical Technology (PAT)

## **1 Introduction**

### *1.1 Background*

Renewable energy is produced from biological conversion of a wide variety of biomass. However, apart from energy production other benefits from bioconversion processes are focused on organic waste handling and production of high-nutrient organic fertilisers, as emerging biorefining concepts will put biomass handling, utilisation, and processing into a whole new perspective at an industrial scale [1-5]. In recent years, renewable energy sources such as biogas have been considered important in order to address the global concern on energy sustainability, availability, and environmental impact of fossil fuels [5-7], but significant challenges still exist regarding diversification of feedstock especially by utilisation of various forms of dry matter [8]. In addition, providing economical competitive modalities for bioenergy production mostly from animal manure, organic waste and food crops is of critical importance [9,10]. This is because, in the bioenergy sector, the use of conventional methods remains the paradigm, despite several successes recorded by employment of new generation on-line process monitoring and analysis known as Process Analytical Technology, PAT [11]. This is far from routinely implemented however, because it is considered cost intensive, and therefore several technological challenges are experienced. For example, great uncertainty accompanies attempts to estimate and control the parameters of interests during biomass conversion processes owing to their inherent chemical and physical complexity. One of the most important process parameters concerns quantitative characterisation of the solid substrate in the bioslurry, because, optimal use of organic dry matter in anaerobic bioconversion is quite linked to improved quantity of biogas produced as well as increased stability of the process. Therefore, information about the contemporary amount of dry matter, as well as its state, is critical with respect to proper

control and optimisation of the biogas production process, and can lead to severe impairment, if not monitored with the accuracy and precision needed for effective process control [4,12]. For the interested reader, further background to bioconversion processes in general, and to the biogas production process in particular can be found in [1-10,12,13].

### *1.2 Process Analytical Technology*

Process Analytical Technology (PAT) modalities are more and more used in the process industries due to advantages over conventional process monitoring and data interpretation [11]. One main attraction for PAT lies in its applicability on-line, in-line or at-line, allowing for continuous monitoring of a process depending on the interest and nature of the system to be monitored. Real-time information is now routinely acquired, representing an approximation of the true nature of the process. Other benefits include low production costs, process optimisation and process status monitoring. Typically spectroscopic, chromatographic and other multi-signal sensors are utilised, generating multivariate data from processes/samples at various locations and time points. PAT applications were mostly introduced in the pharmaceutical sector and have been extensively explored [14]. There is now also, a wide-ranging interest in PAT in other types of industries such as food processing, chemical, and petrochemical [11]. The PAT concept involves use of problem-dependent measurement techniques, especially spectroscopy, image analysis and chromatography for characterisation of industrial processes. This is done on-line, usually in the process line, followed by multivariate data analysis or can be done in real-time using embedded software in dedicated controllers to obtain the process-related information of interest. In order to harness the full benefits of on-line analytical modalities, applications of statistics and chemometrics models as well as signal and data pre-processing are essential. Chemometric methods,

including multivariate calibration are employed to acquire information about processes such as the so-called early warnings of critical state or product conditions, which often involve optimisation of various process parameters and experimental designs [15].

### *1.3 Dry matter (TS) monitoring*

The organic substrates for biogas production are highly variable. Full development of biogas production will by necessity require a diversification of the feedstock basis [8]. For sustainability to be achieved, biogas production requires higher level of total solids in the bioslurry in order to boost the amount of organic carbon from carbohydrates, as well as reducing the economical cost of acquisition of the relevant biomaterials [8,16]. Use of maize silage as feedstock for biogas production has increased significantly in recent times [2,17]. Under anaerobic conditions, maize crops are known to improve the yield (determine by percentage of methane) of biogas [18], and methane production directly depends on the load of organic dry matter present in the bioslurry [2,4,11].

Determination of total solids in bioslurry is typically done off-line, which is laborious and do not depict the actual amount of TS present. Another downside is the disruption of the process during collection of the samples for analyses. Even more critical, there are few methods available for online monitoring of total solids. However, Luck et al. [19] and Nacke, et al. [20], reported on-line monitoring of solids in biogas plant using microwaves and NIR spectroscopy, respectively. On the other hand, one of the limitations in non-invasive NIR methodology is the need to construct optical aperture in the process line. The optical aperture is liable to fouling by microbial growth and may reduce effective process monitoring over a long period of time [4,18]. Also, in both studies [19,20] optimal validation with independent test set samples were lacking which is a cause of critical concern for future prediction purpose [21].

### *1.3.1 Acoustic chemometrics monitoring of TS*

On-line monitoring of TS in bioslurry requires a simple, non-invasive, cost effective and robust technique to improve overall production of bioenergy. Acoustic chemometrics [22,23,24,25,26,27], fits into this picture, and it has also proven suitable in various other, closely related process industries. This study does not present the application of acoustic chemometrics for TS monitoring in biogas process for the first time; a similar approach for monitoring of dry TS was explored initially by Lomborg et al. [18]. However, it was here reported that more work was needed regarding reliable TS prediction, due to concern about the applicability in full-scale bioconversion processes. Lomborg et al. [18] investigated a biogas production in a 1 L volume reactor only (small volume, from which up-scaling of prediction models may not be possible). In addition, the developed model was not properly validated (not test set-validated). The use of independent test set for model validation is imperative in that this ensures the most complete, realistic estimate of the total prediction error [21]. Specifically, the present study was done at a full-scale industrial biogas plant, LinkoGas A.m.b.a, Lintrup, Denmark (reactor volume of 2 400 m<sup>3</sup>). The intent of this investigation was to upgrade the methodology and solve the central problems encountered during the initial study by Lomborg et al. [18]. Thus, the authors here present a complete feasibility study of acoustic chemometrics for real time analysis of TS in a full-scale industrial biogas process plant.

## **2 Materials and methods**

### *2.1 The recirculation loop*

A recirculation loop (Figure 1) was constructed and connected to one of three full-scale anaerobic digestion reactors at LinkoGas A.m.b.a. Two modes of operation was

designed and implemented, a) operating as an *open loop* modus in which bioslurry flows continuously to/from the main reactor, and b) *closed loop* configuration, in which a dedicated flow was only allowed to flow through the loop's measurement sections at controlled conditions. This design permit experiments to be made using the closed loop configuration for on-line characterisation in a monitoring setup which is identical to open loop flow. The recirculation loop includes a pump (EH1024, Bornemann GmbH, Obernkirchen, Germany) controlled by a frequency inverter (Omron VZA V-1000, YASKAWA Europe GmbH, Eschborn, Germany) allowing adjustment of the flow rate, a measurement cell for both optical and acoustic monitoring of the flowing biomass, and a three-way valve arrangement (consisting of two valves (EVBS-BE, Armatec, Glostrup, Denmark) equipped with a fast (6 s) acting actuator (OA6, Armatec, Glostrup, Denmark). For the present work, the loop was operated as a closed system, whereby the measurement section was connected to a barrel of 40 L volume, used as the as collection, feeding and mixing tank. Bioslurry was let into the 40 L tank via the full reactor outlet valve and pumped through the closed loop system at a flow rate of  $3.4 \text{ L s}^{-1}$ . Insulation of the system made the temperature constant and the temperature of the system near the acoustic sensor were noted on each experimental run. Bioslurry of various TS compositions were obtained by spiking the bioslurry with required quantities of dry matter in a standard addition manner (more description below).

## 2.2 Acoustic measurement

Two industrial type piezoelectric accelerometers (DeltaTron® 4396, Brüel & Kjær Sound & Vibration Measurement A/S, Nærum, Denmark) were used in order to evaluate the signal/noise-ratio in two different sensor locations. The accelerometers were mounted on glue studs (10-32 cement stud DB0756, Brüel & Kjær Sound & Vibration A/S, Nærum, Denmark), which had been deployed onto the process line using

cyanoacrylate glue. Signal cable lengths were kept as short as possible (maximum 1 m) to reduce electromagnetic interference.

A two-channel Process Signature Analyser (PSA-100i, Applied Chemometrics Research Group (ACRG), Porsgrunn, Norway) was used for data acquisition and signal conditioning which involved amplification, filtering, analogue-to-digital conversion, Blackman Harris window transformation, Fast Fourier Transformation (FFT), and averaging [24,28]. FFT data representing the acoustic signatures emitted from the flows in the recirculation loop were transferred from the PSA-100i to a standard portable personal computer. Signals from both accelerometers were amplified with a gain equal to 15 (23.5 dB) along with low pass filtering (4<sup>th</sup> order) cut off frequency of 150 kHz (maximum). The PSA unit recorded signals from the accelerometers in time domain with a sampling rate of 375 kHz, followed by relevant signal processing, including transformation from time domain to frequency domain (acoustic spectra) and averaging. A window size of 1024 was used with a resolution of 0.18 kHz. Each acquired acoustic spectrum covered the frequency range 0 – 187.5 kHz representing an average of 250 FFT spectra, which corresponds to a compound sampling duration of approximately 60 seconds. This is considered to result in a precise physical average characterisation of the bioslurry flow, in effect producing a composite sample with a high number of increments; full compliance with the Theory of Sampling (TOS) is a critical success factor for dealing with systems as complex as flowing bioslurry [29,30].

### *2.3 Maize silage preparation*

Maize silage was collected from a local farmer and dried for minimum 40 h at 105°C in a convection oven (ED-115, Binder, Tuttlingen, Germany). The lengths of the biomass particles after drying were 10-15 mm. The resulting dry maize silage in conjunction



with solids present in the manure slurry is referred to as total solids, *TS*, in this work.

Dry matter doses were weighed out on an analytical balance (Sartorius type 1574, Bie & Berntsen A/S, Rødovre, Denmark) and stored in sealed polyethylene bags until added to the experimental system.

#### *2.4 Optimisation of process conditions*

Optimisation step involved series of experimentations. These include optimisation of process parameters such as the effect of flow rate and sensor location (see Figure 2) on the acoustic, as well as quantitative analysis of the amount of added maize silage doses in the bioslurry (“reference measures”). More discussions on these are presented below. The feedstock used by LinkoGas A.m.b.a for biogas production principally was made up of 75 % cow and pig manure and 25 % organic industrial wastes measured on a dry matter basis. This resulted therefore, in a bioslurry with about 5.5 % w/w *TS*. The necessity to evaluate previous work on the subject arose because of the poor results attained in the initial trials [18] and the desire to unravel the underlying cause(s). In this work, necessary measures were taken including acquiring reference samples for calibration and validation purposes from the same protocol, carefully insulating the process line to ensure stable temperature, and maintaining a constant flow rate throughout the duration of the investigation.

#### *2.5 Acoustic data acquisition procedure*

Bioslurry was fed from the biogas plant reactor into a holding and mixing vessel (40 L) connected in series with the flow cell and the slurry was allowed to circulate at flow rate of  $3.4 \text{ L s}^{-1}$  and mix thoroughly for 3 minutes (Figure 1b) after which data acquisition was initiated. Then, the bioslurry was spiked to a maximum of roughly 12 % w/w from the base natural dry matter level of 5.5 % w/w with the required doses, and allowed to

mix properly by extensive recirculation in the loop for 2 minutes on each dry matter feeding. Thereafter, acoustic signals (5 spectra) were recorded for the duration of approximately 5 min at each discrete level of dry matter. Significant efforts were made throughout the entire project to ensure that the pipe line was not clogging with dry matter and to maintain constant temperature as mentioned earlier (experiments were conducted at relatively stable temperature, with a fluctuation set point of no more than  $\pm 0.5^{\circ}\text{C}$ ). Subsequently, acquired data were subjected to multivariate calibration (PLS-R) of the measured acoustic data and TS (reference).

### *2.6 Process sampling and reference samples preparation*

Proper techniques for representative process sampling based on Theory of Sampling (TOS), as have been described in-depth in literatures [30,31,32] were employed. Representative samples are devoid of a structural sampling bias, while the remaining “imprecision” sampling errors are reduced to minimum. In depth description of unbiased process sampling approaches have been previously provided in many scientific literatures [18,29,30,31,32]. In order to implement correct sampling protocol in this work, before addition of dry maize silage bioslurry in the recirculation loop was allowed to circulate a minimum five looping times and thereby well mixed. As the material flowed from the hose, 20m above a holding and mixing tank, a fast delineation of a full cross section of the moving stream were done using a 1L volume plastic container. The increment collected (about 200 mL) was mixed thoroughly, divided into two portions, and poured into two 100 mL plastic containers. Ten such increments were sampled in five steps over equidistant time points of 1 minute. Thereafter, the bioslurry was spiked with the required doses dried maize supplied in a standard addition approach, which in this context implies that equal amounts of dry maize silage were added to the bioslurry in the mixing and holding tank such that the concentration of dry matter in the bioslurry

increased on each addition. This was performed five times, to obtain six different concentration levels, and the same procedure of mixing, data recording (as stated in section 2.5), and sampling were executed. These samples were kept at freezing temperature if not analysed same day. The reference samples were used for determining the exact content of dry matter (TS) at each level. A full detailed documentation of representative process sampling on bioslurry systems, the methods of which were followed in the present study, can be found [29,31].

Ten increments of 100 mL each were sampled at each level of dry matter, then weighed individually on an analytical balance (Sartorius type 1574, Bie & Berntsen A/S, Rødovre, Denmark), then dried at 105°C in 2.2 L foil trays for 24 h in a convection oven (ED-115, Binder, Tuttlingen, Germany). After drying, increments were again weighed individually and the total solid present was calculated according to the standard method for determining amount of water and dry residue in sludge, Danish Standard DS/EN 12880 “Characterization of sludges - Determination of dry residue and water content”. For each TS level, the *average* of each set of the dried ten increments was used as the reference sample ( $\mathbf{y}$ ) value (corresponding to TOS’ prescription for a composite sample), to be used in the ensuing multivariate calibration stage.

## *2.7 Multivariate data analysis*

Multivariate calibrations by Partial Least-Squares regression (PLS-R) were carried out using Unscrambler v. 9.8 (CAMO Software AS, Oslo, Norway). Relevant pre-processing, i.e. auto-scaling (mean-centering and variance scaling) were applied to the data. (refs. given below)

## **3 Theory/calculation**

### *3.1 Acoustic chemometrics*

Acoustic chemometrics is an emerging PAT modality for non-invasive online process monitoring. Passive acoustic emission analysis was developed based on the relationship between vibration generated by some systems and their physico-chemical properties. In acoustic chemometrics, accelerometers are used to capture the system's vibrations generated by an industrial process. An accelerometer is principally made of a piezo electric material e.g. quartz crystal that on perturbation generates an electric signal corresponding to the vibration dynamics of the system. Acoustic signals are sampled in time domain and can be converted to frequencies domain using digital signal processing, for instance Fast Fourier Transformation (FFT). The acoustic spectra are then subjected to multivariate data analysis (chemometrics) to extract information about the system under study. Detailed and extensive documentation of the principle of acoustic sensors and signals pre-processing have been done earlier [23,25,24,26,27,28,33].

The location of the sensor and the dynamics of flow [34] in the system is a critical factor regarding the quality of information acquired. Kupyna et al. [35] have reported in literature, the dependency of acoustic signal on certain process condition such as temperature, flow rate and location of the sensor. At present, acoustic chemometrics approach for process analysis span from monitoring particle and fluid flow behaviour in pipes, chemical reaction monitoring, to process failure detection etc. [23,25,24,27].

Previous studies have shown that acoustic behaviour of liquid flow [25,35,36] is dependent on the physico-chemical properties such as temperature, pressure, viscosity, flow rate, composition and phase. The methodology (acoustic chemometrics) is unfortunately not fully appreciated yet, thus more applications need to be explored.

The key advantages of acoustic measurement lie in the simplicity of non-invasive deployment of the sensors, since the process remains undisturbed while information is

being acquired. This fully eliminates the possibility of materials clogging/sticking onto the sensors. Also, from economic point of view, virtually no maintenance cost is needed once the sensor is effectively deployed, which makes it attractive for process monitoring. Acoustic sensors can also withstand harsh industrial environments. Furthermore, the sensors are easily deployed onto any process line using glue, glue studs or screws and modification of the system under study is required.

### *3.2 Process sampling*

Representative sampling should be seen as integral part of any process optimisation approach because of the inherent heterogeneity of materials and flows-of-matter, slurries etc. In most cases, however, too much emphasis is placed on specific utilisation of the sensor signals for process characterization (data analysis, process chemometrics) but too little attention is directed to controlling the relevant process sampling errors. Only Theory of Sampling, TOS [29,30,31,32], enable reliable characterisation of the heterogeneity of process materials. Typical examples of implementation of TOS in this study involved thorough mixing of the materials under study (in the loop, extracted increments, sub-samples etc.), cross-sectional delineating of materials from the flowing stream while maintaining same sampling frequency, extraction of a sufficient number of increments at each concentration/level of the bioslurry, among others (see section 2.6). Representative sampling controls the accuracy and precision in process sampling procedures. Sampling errors originate from both materials and from the specific sampling technique employed. By TOS-correct sampling, the errors due to heterogeneous materials or sensor signals are eliminated or reduced. It is crucial that the reference samples used for calibration purposes are representative of the substances under investigation, as was outlined in detail above.

### 3.3 Partial Least Squares Regression (PLS-R)

PLS-R involves projection of independent  $\mathbf{X}$  variables onto lower dimensional underlying structures (latent variables). It provides the best possible approximation of the systematic variation in both  $\mathbf{X}$  and  $y$ , and is obviously the approach of choice for calibration purposes for systems as complex as bioslurry. Several documentations on the principles and theory of PLS-R are available references herein [37, 38, 39,40].

The  $\mathbf{X}$  variables are here the instrument measurements (FFT acoustic spectra) and the TS reference is the  $y$  variable. A completely representative training- and test set is both a necessary and sufficient condition for the ultimate purpose of optimal prediction of the amount of dry matter in bioslurry. The driving force behind application of multivariate calibration methods is to reduce the effort (and cost) of performing actual  $y$  measurements on the full-scale processes. The training set and test set used in this study were obtained from independent set of experiments in accordance with the requirements stipulated by Esbensen and Geladi [21] regarding realism of independent test set validated models. Generally, visualisation plots such as the scores, loading weights, explained variances, residual matrices etc. are used for interpretation of the model, and explicitly for the purpose of determining the optimal number of PLS-components to be retained in the prediction model. Theory and interpretation of these model diagnostic plots will not be presented in this work, consult instead [37,38,39,40] for more detail.

Test set RMSEP estimation is carried out simultaneously with determination of the optimal number of PLS components [21]; the optimal model complexity is achieved at minimum RMSEP. RMSEP gives the average prediction error in the same unit as  $y$ .

$$\text{RMSEP} = \text{Sqrt} \left( \left[ \sum (y_{\text{predicted}} - y_{\text{reference}})^2 \right] / n \right)$$

where  $n$  is the number of reference samples in the independent test set.

The relative RMSEP ( $\text{RMSEP}_{\text{rel}}$ ) is also used as a convenient measure, where RMSEP is assessed in relation to the *average y*-predicted level.  $\text{RMSEP}_{\text{rel}}$  allows comparison between predictions models irrespective of the levels, and units, of the measurement.

$$\text{RMSEP}_{\text{rel}} = \text{RMSEP}/\text{average}_{\text{Y-predicted}}$$

### 3.4 Data pre-processing

Before any multivariate analysis modelling task, it is *always* important to check for the need to subject the raw data set to some form of *pre-treatment*, which is done e.g. to remove interferences (noise) and thereby better locate the ‘real’ *y*-correlated information in the data - information, that is otherwise dampened or interfered with in the original **X** data. There are numerous pre-processing tools available but this is a complex issue and is related to the specific nature of the data. Application of too rigorous pre-processing can also dampen the valuable information in data, there are no guarantees, and thus should be done with informed experience and care. However, pre-processing approach such as auto-scaling [37,40], Multiplicative Scatter Correction, MSC [37,40], and filtering such as moving average smoothening [37], are commonly used for complex systems, but it is generally not possible to infer which and how much pre-processing will be necessary in specific cases. For auto-scaling, variables are mean-centred, and scaled to unit variance such that they have equal variances, allowing the multivariate data model to be based on correlations between all **X**- and **Y**-variables. Both mean-centering and variance scaling are variable-wise operations. Mean-centering, involves calculation of mean value of each of the variable and subsequently subtracting the value from the data, whilst in variance scaling; the data is standardised (multiplied by the reciprocal of the standard deviation,  $1/\text{SD}$ ). Variance scaling is a conjecture that

all variables are *a priori* equally important. More detailed information on these pre-processing tools are documented in literatures [37,40]. MSC can compensate effectively for additive and multiplicative effects in data whilst moving average smoothing involves filtering of data to remove spectral random noise.

The acoustic data used in this investigation were found only to need auto-scaling (mean-centering and variance scaling) however.

## **4 Results**

### *4.1 Process optimisation*

Optimal location of the acoustic sensors for dry matter monitoring was determined by comparing the amplitude of generated acoustic signals. The acoustic signals from sensor 1 and sensor 2 were analysed. The signals from these acoustic sensors were amplified to the maximum possible digital resolution. The amplitude of the time series signals, of sensor 1 and sensor 2 were found to be  $\pm 0.25$  and  $\pm 0.5$ , respectively. This indicated that sensor 2 was better. Futhermore, the acoustic spectra from both sensors were also plotted and it was observed that sensor 2 generated signal with higher amplitude as well as more peaks than sensor 1, as depicted in Figure 3. Therefore, sensor 2 was used for this present study. The observations are in line with the extensive studies documented by Esbensen et al [25] and Kupyna et al [35,36]. In addition, effect of flow rate on acoustic signal was monitored and is herein shown in Figure 4. In summary, the result confirms that high flow velocity generated more vibration in the system. See instead in-depth studies on this in [25,35,36].

### *4.2 Multivariate data analysis*



In the multivariate calibration step only auto-scaling and frequency range reductions were necessary before the final modelling. The model, comprised of acoustic spectra calibrated against the doses of TS (reference samples) using PLS-R, and validated by predicting the total empirical levels of TS in independent test set samples. A comparison of relevant model diagnostics for four regression models at different frequency ranges is presented in Table 1. The best model, based on the results presented in Table 1 will from this point be discussed, wherein the frequencies range of 9.3 – 98.3 kHz was used. The criterion used for the choice of optimal number of PLS-components in the regression is the RMSEP, and three PLS-components were required. This is evident in the regression results in Table 1, as the application of the frequency range, 9.3 – 98.3 kHz, gave the lowest RMSEP value. For model validation, use of independent test set from an experimental run different from that of training data set, whilst maintaining the same measurement and sampling protocols, demonstrated the reliability of the optimal number of PLS components. It is also interesting to state that there were no outliers present during the modelling step. Satisfactory results were achieved as can be seen in the predicted versus plot (Figure 5, lower plot). In multivariate modelling, explained variance plots are important indicator since the percentages of modelled  $\mathbf{X}$ - and  $\mathbf{y}$ - data can be deduced visually (quantitative measures are of course also produced). An optimal prediction model was produced with 96 % of the total  $\mathbf{X}$ - variables used in the calibration step (Figure 5, upper right). Considering the  $\mathbf{Y}$ - validation variance plot in Figure 5 (upper left), the prediction model requires only three PLS components for optimal TS prediction. Therefore, utilisation of PLS component less (under-fitting) or more (over-fitting) than three would have resulted in not modelling all the systematic information in the  $\mathbf{X}$  data (under-fitting), or in modelling of noise/error in  $\mathbf{X}$  (over-fitting). In Figure 5 (lower plot), predicted and measured reference samples exhibited

high correlation with each other (squared value of correlation coefficient,  $r^2 = 0.97$ ). The accuracy (slope of fitted regression line) of 0.94 and an estimate of RMSEP of 0.32 % w/w is also highly satisfactory. This translates into a relative RMSEP of 3.86 %, which is considered an acceptable precision, indeed excellent, for a system as complex as bioslurry – especially also as compared to previous acoustic chemometrics attempts (refs. given above).

The loading weights of the  $\mathbf{X}$ - variables for the three-component model, as well as a spectrum comprising of the frequencies 0- to 98.3 kHz of the highest TS content (10.8) predicted is shown in Figure 6. The spectrum comprise of the frequencies 0- to 98.3 kHz of the highest TS content (10.8) predicted. Variables with weights significantly different from zero influence the model most because these are the frequencies constituting the  $\mathbf{X}$ -data that form the basis for the quantitative relation with  $\mathbf{y}$ . In other words, high weights are assigned to  $\mathbf{X}$ -variables, in this context; frequencies that are highly correlated with  $\mathbf{Y}$ -variable (see refs. for PLS-R in section 3.3). For the investigated spectral range (9.3 – 98.3 kHz), first-, second-, and third- PLS-component comprised of 80%, 12%, and 4% of the spectral information ( $\mathbf{X}$ ) used for modelling 84%, 8%, and 4% of the TS ( $\mathbf{y}$ ), respectively, in the bioslurry. Close inspection of the final model showed that the most influential variable intervals are distributed across all frequencies, as shown by the horizontal disposition of the first loading weight,  $W_1$  (which mainly accounted for the changes in concentration level), with well-defined, narrow specific intervals laid out to be the details of second loading weight,  $W_2$ , and third loading weight,  $W_3$ ; which also added to the final model, as seen by the ‘Y-variance validation’ plot in Figure 5 (upper left).

## 5 Discussion

### *5.1 Substrates for biogas production*

The biogas plants are forced to investigate new options in order to maintain stable operation and sufficiently high biogas process yield. Agricultural biomass wastes are not fully utilised at present and these materials include various types of straw, grasses, silages, and other plant residues. In the future also, energy crops such as maize, sugar beets, and grasses, which are abundant in most part of the world, can dominate in the biogas material portfolio. Hence, biogas production concepts will be based on either increasing the dry matter fraction of manure or introducing dry matter in the form of dedicated energy crops [8,13]. The change has to most likely include discovering of easily degradable organic matter such as lipids, sugars, or protein that can be added to the process as by-products from food industries or as food leftover.

### *5.2 Bioconversion process monitoring*

The quality of measurements from a PAT system is a direct result of the compatibility of the analytical strategy employed to elucidate the information of interest. For experiments involving sensors, a thorough understanding the technological features as well as determining the relevant process and analyte factors that influence the output signal is of paramount importance. The bioconversion sector (and other process industries) requires full knowledge of the behaviour and composition of critical components. Some improvements have been recorded in this sector over the last ten years and more researches are ongoing with specific aim of harnessing the most relevant real-time monitoring information [1,10]. If bioconversion processes are not well understood and managed, the economic viability of bioenergy production will be compromised. An early attempt to develop appropriate monitoring tools to address the challenges associated with bioconversion employing acoustic chemometrics was applied

for dry matter determination in bioslurry as reported by Lomborg et al. [18]. However, this study, as well as several others (partially unpublished M.Sc. studies) suffered from incomplete optimisation of the system under study. Sub-optimal sensor deployment location [25, 27, 35], low flow speed [35,36], or insufficient experimentation to generate enough data for full elucidation and modelling of the systems involved (test set validation) are the likely culprits, or this may have been as a result of calibrating the acoustic spectra against the nominal volumes of the added dry matter only, not the real concentration with respect to the complexity of the bioslurry.

### *5.3 Process control and optimisation strategies*

Initial experiments were conducted on the experimental loop to become familiar with the behaviour of the process. For acoustic measurement, the temperature of the measurement system, flow rate, and sensor location have profound effects on the spectra. Temperature gradient in a system for acoustic chemometrics monitoring will definitely affect the nature acquired spectra even if the same component/analyte is studied. This generally, is one of the specific problems that need much attention when using acoustic sensors for process monitoring. Kypyna et al. [35], among other authors, have carried out research to investigate this phenomenon. In study presented herein, the temperature of the system was stable due to insulation of the loop, basically to eliminate temperature fluctuation generated problems. In real world processes though, maintaining constant temperature is a herculean task and might not always be perfectly achievable. There is always a possibility for ‘internal’ temperature compensation based on the multivariate full-spectrum advantage, but this is mainly a system-dependent issue, which must be investigated in each particular case.

Optimization step geared towards determination of the best sensor location on the system was executed. Vibration gradient detected at specific locations on the recirculation line can be argued to result from changes in the rheological properties of flowing stream stemming from difference in the configuration of the recirculation line (as can be seen Figure 2). Extensive studies on finding optimal sensor location for acoustic measurement have been carried out by Esbensen et al. [25] and Kupyna et al [35]. The authors concluded that the existence of a pressure drop following modification of the transport line by introducing constriction, such as use of orifice plate with smaller dimension, resulted in more vibrations.

It was not, however, reasonable to use orifice on the recirculation line under investigation because the slurry contain dry particles that would have resulted in clogging of the pipeline. Therefore, sensors were deployed at definite locations (as shown in Figure 2) based on their features, for instance, the tapered nature of the pipe where sensor 1 was mounted (lower dimensionality of the pipe at this juncture as compared to lower part of the pipeline) can be argued to create similar effect as a dedicated constriction. On the other hand, sensor 2 was deployed close to a bend on the pipeline on the ground that some degree of perturbation occurred due to the change in flow pattern of the flowing medium, resulting most likely in pressure drop that in turn produced more vibration energy at the region immediately after the bend. An example from this work is presented in Figure 3, where higher signal and more peaks were observed for acoustic spectrum of sensor 2 compared to sensor 1. Generally, the most sensitive sensor locations for a fixed flow regimen are not necessarily the optimal locations; there are several hydro dynamical issues to consider in ducted multi-phase flow, as have been documented earlier [25,34,35]. The change in characteristic signal of acoustic spectra due to use of different flow velocities on the system is presented in

Figure 4, which conforms with studies done earlier [25,35] showing increase in the magnitude of acoustic signal due to more vibrations as flow rates increase in a moving a flowing medium. Therefore, keeping all these parameters constant at optimal settings or configurations is crucial for PAT to succeed.

Acoustic chemometrics, in this study, demonstrated the feasibility of characterising TS in bioslurry in real-time which led to the present completion of this line of approach. More efforts were directed towards optimisation of the process and process sampling which leads to accurate and precise results (Figure 5) from the test-set validated data analysis.

## **6 Conclusions**

On-line methodologies are necessary for monitoring and for improving the yield of bioenergy production while maintaining control over production costs. For this to be achieved, diversification of substrates for bioenergy production must be considered, which leads to a critical focus on methods for easy and reliable dry matter determination. Application of a new Process Analytical Technology (PAT) modality, acoustic chemometrics, was proven feasible for prediction of dry matter (TS) in bioslurry in the interval 5.8–10.8 % /w/w, in a field study carried out in a full-scale biogas plant. A series of optimisation strategies were explored to master the often erratic behaviour of highly complex bioslurry mixture. Acoustic sensors are sensitive to changes in flow rate and temperature (but can also withstand high temperature). After suitable initial system choices, and subsequent modifications, prediction of independent test set samples resulted in a validated measure of accuracy (slope = 0.94), precision ( $r^2 = 0.97$ ) and RMSEP of 0.32 w/w % ( $RMSEP_{(rel)} = 3.86$  %) over the range of 5.8 – 10.8 % w/w dry matter content in the bioslurry. These excellent performance statistics

confirm the feasibility of calibrating satisfactory prediction models for monitoring of TS in complex bioslurry systems. Figure 6 (upper plot) in particular shows a specific frequency interval (9.3 kHz – 93.8 kHz) that carried the dominating information from **X** to **y**, but the main information carrier is clearly a level effect distributed across all frequencies, which manifested itself as a highly dominating first PLS-component.

Acoustic chemometrics owes a great debt to chemometrics, allowing for efficient full-spectrum advantage in modelling acoustic (Fast Fourier Transformed) spectra and calibrating these against high quality Y reference measurements. A.c. can be seen as a particularly effective merging of dedicated sensor technology, signal analysis and the full swing of multivariate calibration/validation; as ever against a backdrop of mandatory representative sampling (TOS).

Acoustic chemometrics, which in several earlier dry matter attempts, never really fully realised its potential, has now come of age and can now be considered to be a matured PAT modality. There is no doubt a significant carrying over potential to other biomass (TS) systems within the biogas and bioconversion sectors as well.

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## Figure captions

**Figure 1.** Process instrumentation diagram of the process loop used in the study. The recirculating loop can be set, using a three-way valve, in both “open (a)” as well as “closed (b)” configurations, allowing for realistic flow and stream composition experimentation at the measurement interface (acoustic sensor) with input either from reactor vessel or from the mixing tank (the latter filled with realistic bioslurry from the reactor). Closed loop mode was used in this study: bioslurry from the reactor was pumped into a mixing tank via the bypass line (dotted arrow), and allowed to circulate in the loop (full arrow) while spiking and sampling for calibration were carried out.

**Figure 2.** Acoustic sensors deployed at two different bypass string locations. Sensors 1 and 2 were investigated, sensor 2 was found to be optimal for the present purpose.

**Figure 3.** Choice of optimal sensor location for the study: amplitude of the signals was the basis for choice of sensor location. The acoustic spectrum from Sensor 2 was considered better based on higher amplitude signals and more peaks generated compared to sensor 1, and thus was used for the study.

**Figure 4.** Effect of flow rate changes on the acoustic signal. The amplitude of acoustic signal increases with increase in flow rate hence flow rate of  $3.4 \text{ L s}^{-1}$  was used.

**Figure 5.** Final PLS prediction model evaluation. No outliers deleted. Test set validation: 3 PLS-components.  $\text{RMSEP}_{(\text{rel})} = 3.86\%$ .

**Figure 6.** The loading weights plot (upper plot) and the corresponding spectrum of the frequencies (lower plot) used for final modelling. From loading weight, the most influential frequency regions can be observed at 9.3 kHz – 93.8 kHz. The acoustic spectrum indicate that the most distinct peak at 37 – 39 kHz is particularly relevant for all three PLS components.

**Table 1**

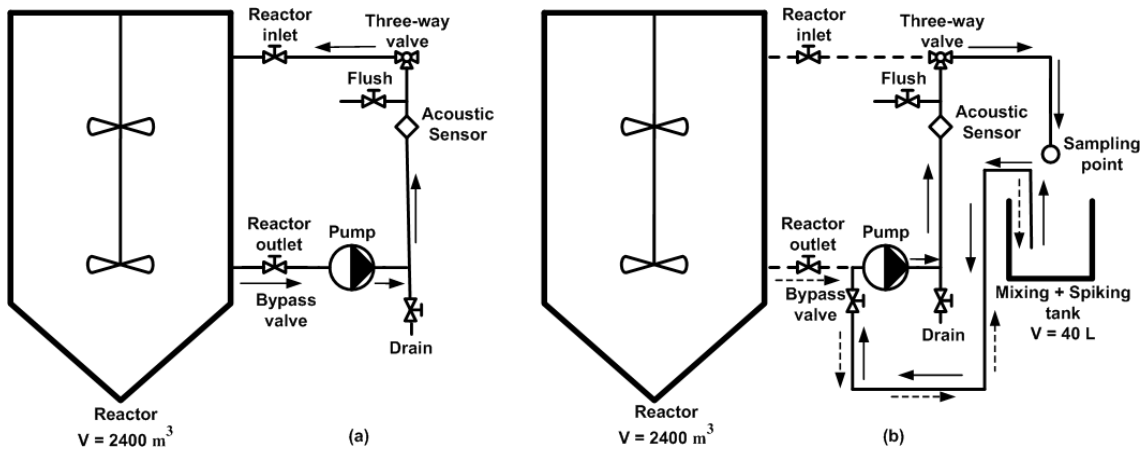
**Test set prediction results from PLS regression for full and reduced acoustic spectra**

<b>Acoustic spectra</b>	<b># PLS components</b>	<b>RMSEP (% w/w)</b>	<b>Slope</b>	<b>r<sup>2</sup></b>
Full <sup>a</sup>	1	0.37	0.87	0.96
Full <sup>b</sup>	1	0.37	0.88	0.96
Reduced <sup>c</sup>	3	0.35	0.92	0.96
Reduced <sup>d</sup>	3	0.32	0.94	0.97

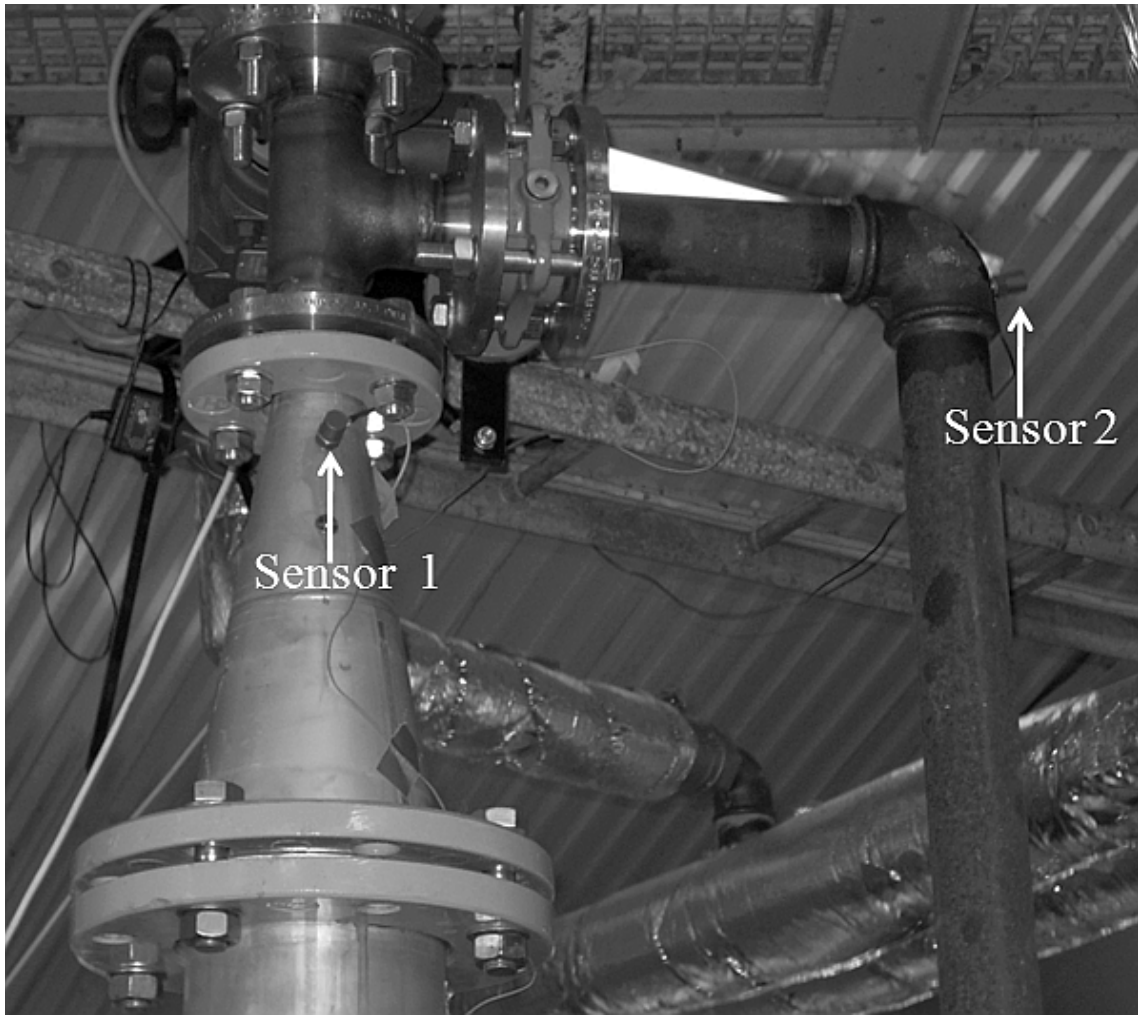
a: 0-187.5 kHz, b: 9.3-187.5 kHz, c: 0-93.8 kHz, d: 9.3-93.8 kHz.



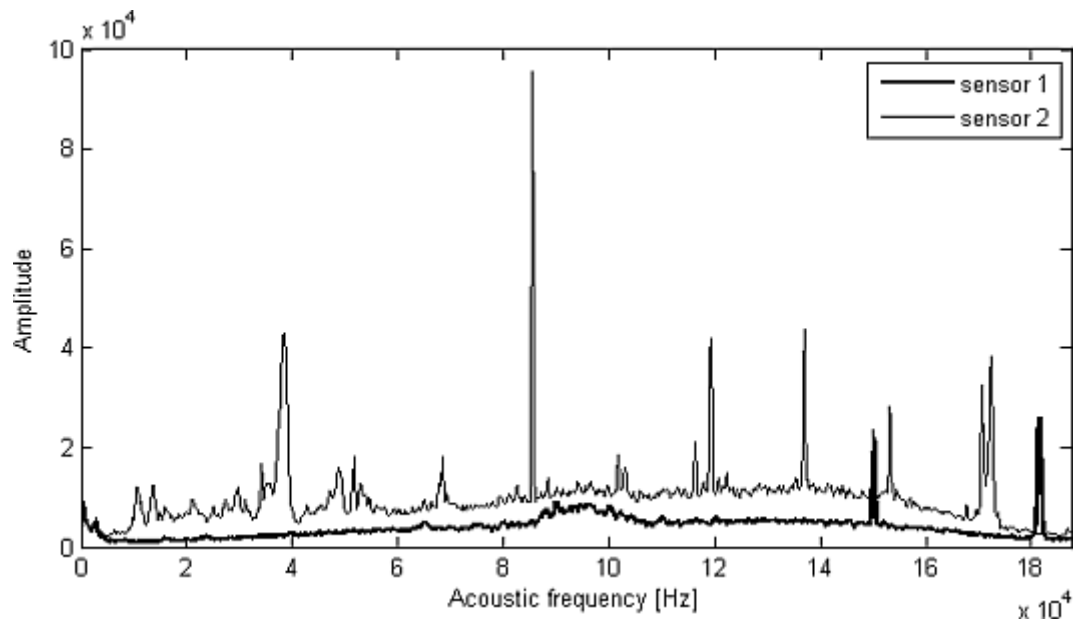
Figure 1.



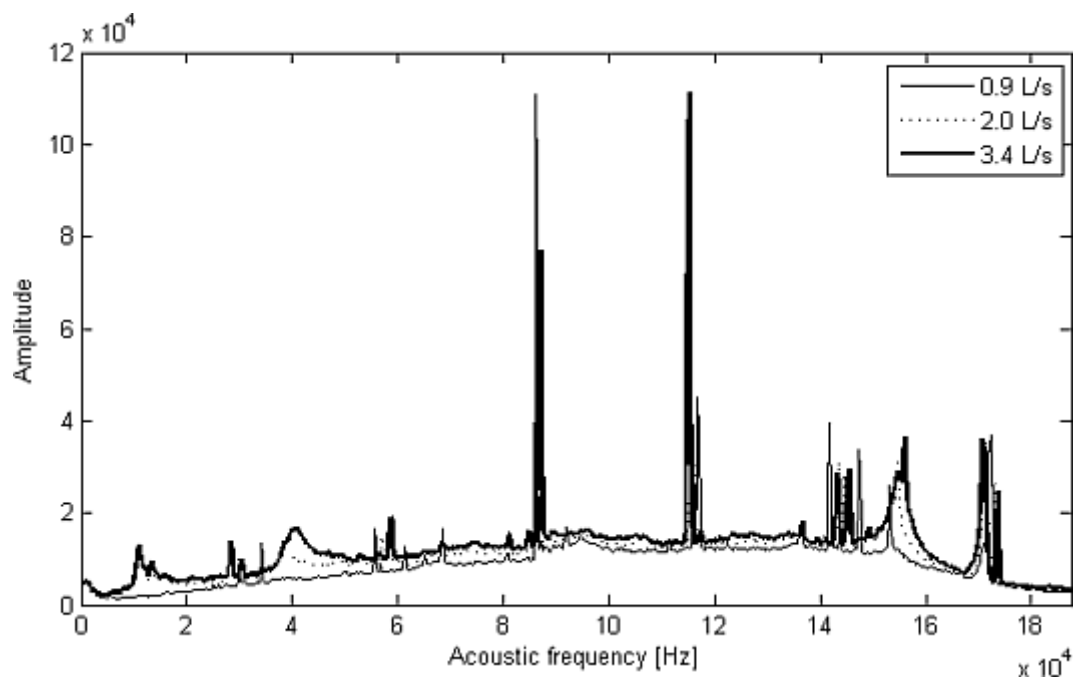
**Figure 2.**



**Figure 3.**



**Figure 4.**



**Figure 5.**

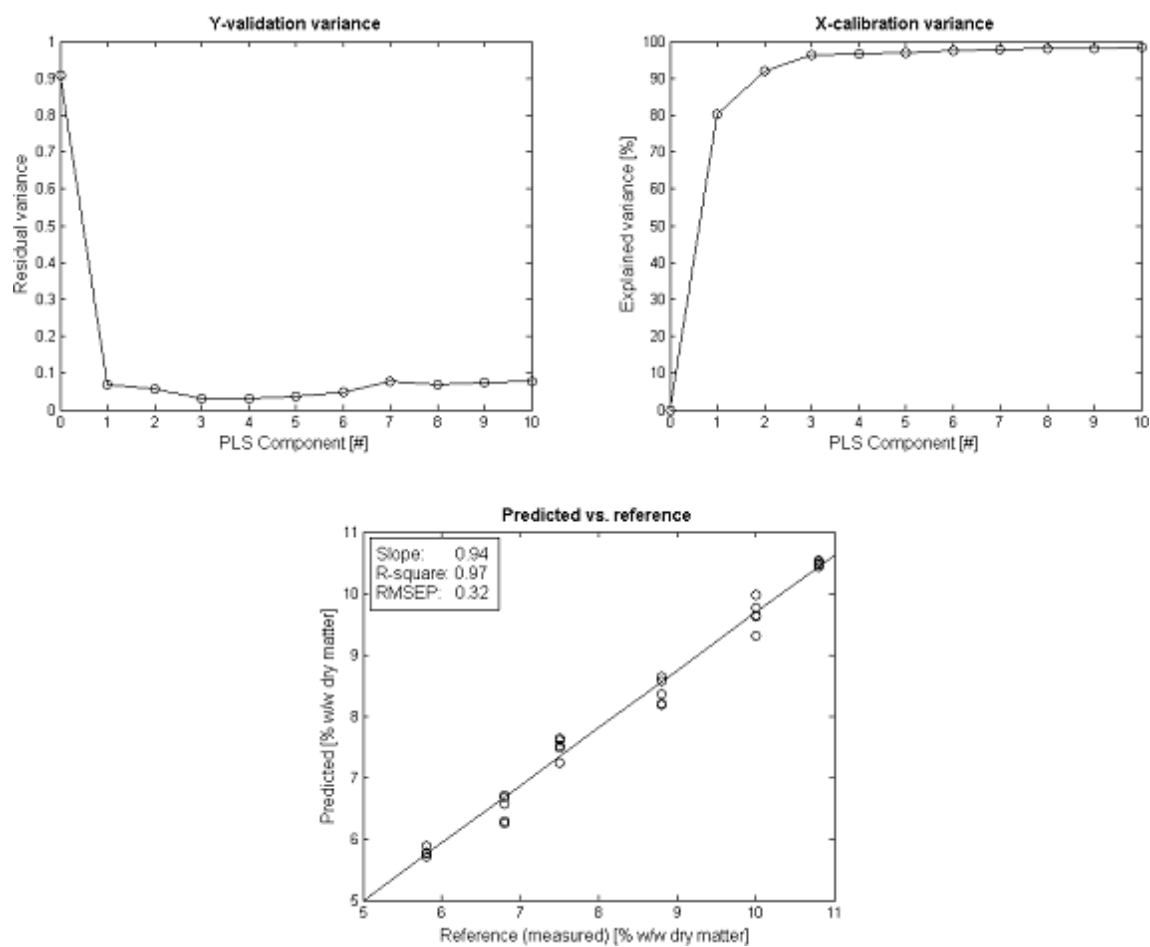


Figure 6.

