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# Representative sampling for process analytical characterization of heterogeneous bioslurry systems—a reference study of sampling issues in PAT

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**Abstract**

Extensive experimentation and evaluation of alternative sampling methods of highly heterogenous bioslurries are carried out in the context of the theory of sampling (TOS) in order to delineate optimal, *representative* sampling procedures in PAT (process analytical technologies). The analytical methods investigated are at-line NIR and image analysis (IA) for monitoring of an industrial bioenergy anaerobic digestion processes (AD), subject to stringent economic bracketing: PAT solutions have to be both *practical* and *inexpensive* in the bioenergy sector where cost efficiency of instrumentation and monitoring systems is an absolute must. Focus is on development of minimum expenditure methods for sufficient characterization of the very heterogeneous types of biomass feedstock as used in industrial scale, continuously stirred tank reactors (CSTR). Product and process characterization necessarily involves a chemometric multivariate calibration predictor (PLS regression). The general goal is development of appropriate sampling/PAT facilities for at-line/on-line process monitoring in typically low-tech bioenergy, agro-industrial sectors. Experimental laboratory reactor evaluations, based on biomass feedstock and digested products from a full-scale biogas plant, were initially run in batch mode for a week, followed by fed-batch addition of maize silage, introducing a systematic increase in total solids allowing properly spanning multivariate calibration models. Measurements on 55 laboratory reactor samples taken during a complete 14-day fermentation cycle included three key process parameters: total solids (TS), volatile solids (VS) and chemical oxygen demand (COD), representing difficult-to-sample analytes. NIR spectroscopy and image analysis, including the angle measure technique transform (AMT), were evaluated for characterization of different feedstocks as well as continuously extracted process samples with respect to selected chemistry and dry matter characteristics. Optimized sampling on four different scale-levels allowed acceptable PLS prediction models for TS and VS for both NIR and image analysis compared to chemical reference analysis, while it was not possible to predict the COD levels satisfactorily due to large uncertainties in mandatory reference measurement protocols. This feasibility study is promising for NIR as at-line prediction of TS and VS content as well as other AD parameters, which can be measured on the same sample types, while image analysis is currently too complex and expensive for these industry sectors. The findings in the present bioslurries study have a considerable generalization potential: all PAT approaches are critically dependent on *representative* reference calibration sampling, which has to be fully compliant with the theory of sampling (TOS).


*Keywords:* PAT (process analytical technologies); Representative sampling; TOS (theory of sampling); Chemometrics; AMT (angle measure technique); Image analysis; PLS regression; Anaerobic digestion; Bioslurry; Biogas production

## 1. Introduction

Fermentation processes at large-scale biogas plants are sensitive to sudden changes in feedstock composition, which cause significant variability in the process conditions. Today, fermentation process control is achieved through manual sample extraction (shown below to be highly problematic due to the very heterogenous nature of the fermentation media) with off-line (sometimes even off-site) analysis of a few key process parameters such as total solids (TS), volatile solids (VS) and chemical oxygen demand (COD), as well as volatile fatty acids (VFA), total-N and ammonia content. However, other parameters, e.g., process temperature, pH, volumetric biogas yield as well as methane, carbon dioxide and hydrogen sulfur content

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in the gaseous headspace of the reactor, are also monitored more-or-less on-line as control parameters in more advanced plants [1,2].

Even though time-constants involved in anaerobic digestion (AD) are not critically short (average hydraulic retention time of feedstock in a semi-continuously fed-batch digestion system is of the order of 10 to 25 days), there is nevertheless a desire for implementation of on-line technologies, of a distinctly inexpensive, robust types. The perspective is to develop real-time process monitoring data at full-scale commercial biogas plants above a certain minimum size to be used for improved routine managing tools, i.e., for identification of critical process state parameters, upsets and for monitoring when unavoidable deviations in feedstock composition occurs. By combining low-tech, but reliable sensors with critical at-line facilities if/where/when physical samples must be examined, the goal is to contribute towards a new generation of fully integrated process management systems for the daily operating plant staff [3,4]. This endeavor fits well with current EU renewable energy initiatives for considerably increased utilization of biofuels including biogas, integrated in the overall energy as electricity, heat and in the transportation fuel sectors.

Improved control of this type of fermentation processes especially calls for more controllable raw materials characterization throughout, which today are often based on slow off-line measurements. Improved optimization of anaerobic digestion processes can only be achieved by incorporating new, relevant sensor technologies for semi-continuous at-line/on-line measurements of the composition of both raw materials and processed biomass at important process stages. The most relevant sensor deployment locations with this scope include:

- *Feedstock* arrival depositories (raw materials)
- *Inlets* (feed-points) for fermentation reactors
- *In-process deployments* (in reactors and transportation pipelines)
- *Outlets* (exit-points) for the fermentation reactors

Any process analytical technology (PAT) is critically dependent on the representativity of the signals obtained from on-line or in-line probes or sensors in pipelines or reactor tanks as well as representativity of the samples analysed for calibration. For example, what is the relationship between a sensor or probe's field-of-view (a few mm$^2$) in relation to the entire cross-section of a transportation pipeline or the entire reactor volume in question? What with TOS is termed "incorrect sampling procedures", which are legion in the AD regimen, will always result in biased, non-representative and hence unreliable analytical results [5–9]. All traditional sampling procedures involved in the general AD process flow are therefore carefully evaluated in the present study in the light of TOS (theory of sampling) and the specific sampling errors are estimated where needed in order to put these hitherto largely neglected sampling issues on a firm quantitative footing.

Sampling is a critical component in any analytical procedure and must always ensure representativity of the primary, secondary and tertiary sampling steps. Total sampling errors typically can be in the order of 100+ times the specific analytical error. "Incorrect sampling" always results in non-representative samples because of an uncontrollable bias even though all subsequent steps have been performed in a correct way [5–8].

Animal manure forms a highly complex and heterogeneous suspension because of a significant proportion of solids, "dry matter" (3–12%): straw, grass, undigested lignocellulosic fiber particles, besides not yet digested macromolecule aggregates such as starch, sugars and proteins. Such suspensions are therefore always prone to segregation on several scales, which sets in immediately after agitation has been terminated. Sampling from such systems consequently will introduce a significant sampling bias if not properly counteracted, wherefore this study will deal exhaustively with all relevant sampling issues using the complete TOS toolbox [8]. In this context, focus will be on a general sampling hierarchy, covering all aspects from primary field sampling to the ultimate, apparently insignificant, mass reduction involved in securing the often minute analytical volume.

This study also evaluates the potential of an imaging technique in characterizing important physical and chemical parameters. Since NIR reflectance/transmission has shown good potential in mixed liquids in linked fermentations sectors, a NIR reflectance system was employed for reference comparison and/or as the main PAT technique. NIR has been tested extensively with varying degrees of success as a spectroscopy tool for on-line bioslurry monitoring of continuous AD systems and similar [2,10–13].

We here combine image analysis (IA) and the angle measure technique (AMT), a powerful texture extraction technique [14–19], with chemometric multivariate modeling (PLS-R) in a pilot study with a potential for on- or at-line monitoring and prediction of visible features such as the surface manifestation of bioslurries and the TS content. By virtue of stoichiometric closed array relationships, there is also a possibility for *indirect* multivariate calibration modeling for correlated parameters, VS and perhaps even COD a.o. Such methods constitute a potential for monitoring and optimization of full-scale production plants.

## 2. Sampling in AD systems for multivariate calibration

Procurement of feedstock as well as digested biomass in preparation for AD trials and testing took place at a full-scale industrial biogas plant, the Ribe Biogas plant, Denmark. Primary and secondary sampling and preparation will be described in detail below. Experimental fermentation trials–including a tertiary sampling step–is a commonly used procedure both in academic studies as well as in commercial laboratories to study biomass feedstock compositions and to characterize their biogas potentials [1,2]. This is followed by a quaternary sampling procedure for securing representative analytical samples for the final NIR and IA analysis. Fig. 1 is a schematic illustration of the entire AD process and the attendant sampling hierarchy as examined in this study.