Multi-wavelength fluorometry for anaerobic digestion process monitoring

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Abstract Applicability of multi-wavelength fluorometry for anaerobic digestion process monitoring was investigated in a 3.5 L upflow anaerobic sludge bed (UASB) lab-scale reactor. Both off-line and on-line monitoring of key process parameters was tested. Off-line emission spectra were measured at an angle of 90° to the excitation beam using a cuvette. On-line measurements were carried out using a fiber optic probe in the external recirculation line of the digester. Fluorescence spectra were correlated to available analytical measurements to obtain partial least square regression models. An independent set of measurements was used to validate the regression models. Model estimations showed reasonable agreement with analytical measurements with multiple determination coefficients ($R^2$) between 0.6 and 0.95. Results showed that off-line fluorescence measurements can be used for fast estimation of anaerobic digestor effluent quality. At the same time, the on-line implementation of multi-wavelength fluorescence measurements can be used for real-time process monitoring and, potentially, for on-line process control.

Keywords Multi-wavelength fluorometry; process monitoring; partial least square regression; anaerobic digestion

Introduction

Numerous demonstrations of successful anaerobic treatment of various wastes suggest, that anaerobic digestion can be incorporated in a broad variety of wastewater treatment technologies (Foresti, 2001; van Lier et al., 2001). Nevertheless, aerobic treatment is the preferred design option for new wastewater treatment facilities, especially in North America. Instability of the anaerobic digestion is often cited as one of the main hurdles for implementation of the anaerobic digestion technology (Dupla et al., 2004). Indeed, the anaerobic digestion process is very sensitive to organic load disturbances, which can cause uncontrolled removal efficiency variations, biomass washout, and even process failure (Pullammanappallil et al., 1998). Consequently, process monitoring is of paramount importance in stabilizing anaerobic digestors. While biogas monitoring provides important information on process state and dynamics, it only serves as an indirect indication of the composition of the digester effluent. Direct measurements of parameters such as chemical oxygen demand (COD), volatile fatty acids (VFAs), and volatile suspended solids (VSS) would provide close monitoring of effluent quality and allow for a real-time process diagnosis and control.

In this paper, application of multi-wavelength fluorometry for anaerobic digestor monitoring is discussed. The use of fluorescence for rapid detection of fermentation imbalances and metabolic activities of the anaerobic digestion process has previously been demonstrated (Peck and Chynoweth, 1992). Also, NADPH-dependant fluorometry was used for monitoring an aerobic wastewater treatment process (Farabegoli et al., 2003). However, wastewaters contain large amounts of proteins, amino acids, and other fluorescent compounds that interfere with NADPH-related fluorescence. The quality of monitoring can be improved by using multiple-excitation multiple-emission fluorescence...
measurements (Hagedorn et al., 2003; Lindemann et al., 1998; Tartakovsky et al., 1996). In this technique both excitation and emission wavelengths are varied to obtain two-dimensional spectra, which are processed using multivariate statistical analysis methods, such as partial least square (PLS) regression (Skibsted et al., 2001). The PLS algorithm decomposes spectra into latent variables, which are then correlated with analytical measurements using linear dependencies. In comparison to standard linear regression methods, PLS is a suitable method in analysis of highly correlated and noisy data (Wold et al., 2001).

In this paper, both off-line and on-line fluorescence measurements of the anaerobic digester effluent were carried out. While the off-line fluorescence measurements can be seen as a fast method for estimating effluent quality and troubleshooting, the on-line process monitoring not only provides fast process diagnosis but also enables real-time feedback process control.

**Materials and methods**

Experiments were carried out in a 3.5 L upflow anaerobic sludge bed (UASB) reactor with an external recirculation loop. The reactor was inoculated with 1 L of granular anaerobic sludge (A. Lassonde Inc., Rougemont, Quebec, Canada) with an average volatile suspended solids content of 50 g L\(^{-1}\). A temperature of 26\(^\circ\)C was maintained by a water jacket. The reactor was fed continuously with a synthetic medium and a trace metal solution, combined with a bicarbonate buffer. The stock solution of synthetic wastewater had a COD content of 315 g COD L\(^{-1}\) and contained (in g L\(^{-1}\)): sucrose 99, butyric acid 48, yeast extract 60, ethanol (95%) 35, KH\(_2\)PO\(_4\) 3, K\(_2\)HPO\(_4\) 3.5, and NH\(_4\)HCO\(_3\) 34. The stock solution of trace metals contained (in g L\(^{-1}\)): AlK(SO\(_4\))\(_2\)-12H\(_2\)O 0.0006; H\(_2\)BO\(_3\) 0.001; Ca(NO\(_3\))\(_2\)-4H\(_2\)O 0.5351; Co(NO\(_3\))\(_2\)-6H\(_2\)O 0.0075; Cu(SO\(_4\)) 0.0003; Fe(SO\(_4\))-7H\(_2\)O 0.0546; MgSO\(_4\) 0.1973; Mn(SO\(_4\))-H\(_2\)O 0.0151; Na\(_2\)(MoO\(_4\))-2H\(_2\)O 0.0023; NiSO\(_4\)-6H\(_2\)O 0.0007; NaSeO\(_3\) 0.0013; and ZnSO\(_4\)-7H\(_2\)O 0.0035. The stock solutions of synthetic wastewater and trace metals were continuously added to the bicarbonate buffer stream to obtain the desired organic load. The bicarbonate buffer was composed of 1.36 g L\(^{-1}\) of NaHCO\(_3\) and 1.74 g L\(^{-1}\) of KHCO\(_3\) and was fed into the reactor at a rate of 4.8 L d\(^{-1}\). In addition, a 0.5 N solution of NaOH was used by a pH controller to ensure a pH of 7 within the reactor.

Samples of reactor effluent (40 mL) were taken periodically from the recirculation loop. Volatile fatty acids (VFAs) were determined by gas chromatography (Sigma 2000, Perkin-Elmer, Norwalk, Connecticut, USA); chemical oxygen demand (COD) and volatile susped solids (VSS) were determined according to Standard Methods (1995). Total VFA concentration was calculated by a summation of propionate, butyrate, and acetate concentrations.

Off-line fluorescence spectra were acquired using a spectrofluorometer, which consisted of a DT-1000 Deuterium Tungsten Halogen light source (Ocean Optics Inc, Dunedin, Florida, USA) equipped with a cuvette holder. Fluorescence emission was measured at an angle of 90\(^\circ\) to the excitation beam using a USB2000 fiber optic spectrometer (Ocean Optics Inc, Dunedin, Florida, USA) interfaced with a personal computer. The spectrometer integration time was 1.000 ms.

Fluorescence spectra acquired off-line were regressed with analytical measurements of CODs, VFAs and volatile suspend solids (VSS) using PLS regression. The PLS regression is a common method of data processing in multivariate statistical analysis (Langergraber et al., 2004). The PLS algorithm reduced spectra into several latent variables. These latent variables were correlated to analytical measurements using linear regression. All available experimental data were divided into training and validation sets.
First, PLS regressions models were calibrated using the training data set. Next, prediction accuracy of the regression models were evaluated using the validation data set. Statistical parameters, such as $R^2$ (multiple determination coefficient) and RMSE (Root Mean-Square Error) were employed to assess accuracy of PLS regression models. All computations were done using PLS Toolbox 3.0 (Eigenvector Research Inc., Manson, Washington, USA) and MATLAB 6.5 (MathWorks Inc., Natick, Massachusetts, USA).

The on-line monitoring experiment was carried out using a R400-7 (Ocean Optics Inc, Dunedin, Florida, USA) reflection probe inserted in the external recirculation line of the reactor. The reflection probe contained six illumination fibers and one read fiber. Fluorescence was measured from the front surface of the sample, using a 380 nm UV light source (LS-450 with a UV LED) and a USB2000 fiber optic spectrometer (both from Ocean Optics Inc, Dunedin, Florida, USA). The spectrometer integration time was 5,000 ms. Fluorescence spectra were acquired in intervals of 10 min with background acquisition prior to each fluorescence measurement. As in off-line measurements, fluorescence spectra were regressed with available experimental data using PLS regression models. Then, PLS regression models were applied to predict (interpolate) key process variables (e.g. CODs, VFAs, and VSS) between analytical measurements.

Results and discussion

Applicability of multi-wavelength fluorometry for anaerobic digestion monitoring was tested off-line and on-line on three consecutive reactor runs. In the first two reactor experiments fluorescence spectra were acquired off-line, while on-line measurements were carried out in the third run.

Off-line fluorescence measurements

In the first experiment, the organic load was gradually increased from 13 to 78 g COD d$^{-1}$ over a period of 400 hours. In the second experiment, which lasted 200 hours, organic load varied from 6.5 g COD d$^{-1}$ at startup to 32.5 g COD d$^{-1}$ at the end. Over the course of the experiments, analytical measurements of CODs, VFAs, and VSS were carried out along with fluorescence spectra acquisition. In the first (training) and second (validation) runs 27 and 23 samples were acquired, respectively.

While fluorescence spectra were acquired in the 300–800 nm range, preliminary tests showed that the accuracy of model predictions can be enhanced by narrowing the range of wavelengths used in the regression analysis. Vaidyanathan et al. (2001), showed that truncating spectrum range reduces noise and consequently improves model quality. Therefore, fluorescence spectra were truncated to 400–600 nm.

As mentioned above, PLS regression method was used for data processing. The results of the first reactor run were used for PLS model training, while the second experiment results were used for model validation. For the sake of comparison, all regression models were computed with five latent variables. A summary of model training results for total CODs, VFAs, and VSS models are given in Table 1. Overall, model calibrations yielded

| Table 1 | $R^2$ and RMSE values for key process parameters estimated using off-line fluorescence spectra |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Constituent | Range | Calibration | Validation |
| | $R^2$ | RMSE | $R^2$ | RMSE |
| Total CODs (g L$^{-1}$) | 0.15–4.2 | 0.92 | 0.34 | 0.74 | 0.69 |
| VFAs (g L$^{-1}$) | 0.01–0.9 | 0.89 | 0.09 | 0.75 | 0.13 |
| VSS (g L$^{-1}$) | 0.7–3.1 | 0.97 | 0.22 | 0.63 | 0.81 |
a multiple determination coefficient ($R^2$) of 0.9 or better for all process parameters (Figure 1A).

After successful calibration of the regression models, fluorescence spectra acquired in the validation run were used to predict concentrations of total CODs, total VFAs, and VSS in the effluent. An acceptable agreement between fluorescence-based estimations and analytical measurements was found (Figure 1B and Table 1). Notably, fluorescence-based predictions were in good agreement with analytical measurements during the period of high organic load, which lasted from 150 to 200 hours (Figure 1B). Model validation yielded an $R^2$ of 0.75 for total CODs and total VFAs. The accuracy of VSS predictions was somewhat lower ($R^2 = 0.6$, Table 1). Standard deviation of the VSS measurements, however, was significantly higher than that of CODs and VFAs.

CODs, VFAs and VSS content in the reactor effluent are key parameters for assessing efficiency and the state of the anaerobic digestion process. Standard methods for determination of these parameters are time consuming, and may lead to significant delays in process diagnosis. An express method for assessing effluent quality would enable faster process diagnosis (Hagedorn et al., 2004; Tartakovsky et al., 1996). Most importantly, multi-wavelength fluorometry requires no reagents and minimal sample preparation is involved (Langergraber et al., 2003). It is worth noting that the synthetic wastewater used in this study contained low levels of fluorescent components. Consequently, the estimation of COD concentration was based on the correlation of analytically measured values with spectral components linked to cell metabolic state (e.g. NADPH pool) and degradation intermediates such as VFAs. This indirect correlation, obviously, reduced the prediction accuracy. Real wastewaters contain a significant amount of fluorescent compounds, such as proteins. Consequently, higher COD prediction accuracy might be expected when applying this method at an industrial scale.

**On-line fluorescence measurements**

While off-line fluorescence measurements offer a tool for prompt evaluation of effluent quality, an on-line sensor would allow for automated process diagnosis and control. Consequently, in the third reactor experiment feasibility of on-line fluorescence measurements was examined. The on-line monitoring experiment was carried out over a period of 25 days. In this experiment, continuous measurements were archived by attaching a fiber optic reflection probe to the external recirculation loop of the reactor. In this experiment the organic load was progressively increased from 6 g COD d$^{-1}$ to 30 g COD d$^{-1}$, then it
was decreased to 6 g COD d⁻¹ at day 17. A second increase in the organic load from 6 to 18 g COD d⁻¹ was carried out between days 22 and 27. Each change in the organic load was followed by a corresponding change in the effluent concentrations of CODs, VFAs and VSS (Figure 2). In addition to organic load variations, at day 12 of the experiment the pH control loop was disabled and bicarbonate buffer concentration in the influent stream was diluted by a factor of two. In response, the pH value progressively declined from 7 to 6.4, while effluent concentrations of CODs and VFAs remained high (Figure 2A). Biogas production was also affected by the low pH value. The pH control system was reactivated and the bicarbonate buffer concentration was returned to its previous value at day 17.

Throughout the experiment, effluent samples were collected two to three times a day and concentrations of CODs, VFAs, and VSS were determined by appropriate analytical methods. Overall, a total of 57 samples were collected and analyzed. During the experiment, liquid in the recirculation line remained mostly clear with the presence of some granules of biomass. Fluorescence spectra as well as methane and pH values were acquired at 10 min intervals by the data acquisition system. While fluorescence spectra were acquired in the 380–800 nm range, as with off-line fluorescence measurements, the range of wavelengths was narrowed to improve the accuracy of PLS models. For on-line measurements, a range of 400–600 nm was used. While gas bubbles present in the recirculation line were found to interfere with the fluorescence measurements, once again an acceptable agreement between analytical measurements and fluorescence-based estimations of CODs and VFAs was found. To reduce the noise, 500 fluorescent spectra were averaged for each data acquisition point and a digital filter was applied.

Once data acquisition was completed, PLS regression models, which related fluorescence spectra to analytical measurements, were developed. All PLS models used three latent variables, which captured 69%, 75%, and 27% of variance for COD, VFAs, and VSS measurements, respectively. A multiple determination coefficient $R^2$ of 0.49, 0.63, and 0.43, was obtained for total CODs, VFAs, and VSS estimations, respectively.

Predictions of all process parameters have been realistic with respect to the imposed organic load (Figure 2). Each variation in the influent content was readily detected by fluorescence-based measurements. Moreover, fluorescence-based measurements demonstrated lower levels of noise particularly in comparison to analytical measurements of VSS. The use of on-line measurements enabled extrapolation of key process parameter dynamics between limited analytical measurements for an anaerobic digestion process.

![Figure 2](https://iwa.silverchair.com/wst/article-pdf/52/1-2/465/433910/465.pdf)

Figure 2 On-line monitoring of total COD and VFAs (A) and VSS (B). Notation: (■) total CODs, analytical; (—) PLS model predictions for total CODs, (▲) total VFAs, analytical; (—) PLS model predictions for VFAs. (★) analytical VSS, (---) PLS model predictions for VSS.
Conclusions
Overall, the study demonstrated the feasibility of multi-wavelength fluorometry as a method for assessing composition of anaerobic digestor effluent. Off-line acquisition of fluorescence spectra allowed for rapid estimation of key process parameters, such as COD, total VFAs, and VSS in the reactor effluent. A comparison of analytical measurements for these parameters with fluorescence-based predictions in the validation experiment showed an acceptable agreement. Thus, off-line acquisition of fluorescence spectra can be used as an express method for analyzing the effluent quality and deliver prompt process diagnosis. In addition, analysis of anaerobic digestor influent would allow for timely detection of strong variations in the influent composition.

Moreover, on-line monitoring of the digestor effluent was successfully demonstrated. In this demonstration, fluorescence measurements were carried out using a UV LED light source and a reflection probe inserted in the recirculation line. This setup allowed for real-time process monitoring, which suggests an avenue for on-line process diagnosis and control. Notably, on-line estimations of effluent parameters showed lower noise levels in comparison with the analytical measurements.

Overall, a number of applications of multi-wavelength fluorometry including measurements of key effluent parameters such as CODs, VFAs and biological oxygen demand (BOD) in a broad range of wastewater treatment processes, both anaerobic and aerobic, can be envisioned. Fiber-optic based fluorometers are much less expensive than near-infrared (NIR) and mid-infrared (MIR) spectrometers thus allowing for a transfer of fluorescence-based technology to an industrial scale in the not so distant future.

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References


