EOLI
Efficient Operation of Urban Wastewater Treatment Plants

Work Package 3
Third year Mid Term Report

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WP3 - UNAM - Third year mid-term report

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Introduction

This report presents the activities of the partner 6 (UNAM) concerning WP3 of the EOLI project, regarding the use of spectrophotometric techniques to determine on-line measurements of concentrations during biodegradation of toxic (aromatic) substances. The report comprises activities between November 2004 and April 2005.

The report is divided as follows. First a brief description of the theoretical foundations of the measurement techniques is given. Then the methodology for conducting the experiments is described, followed by the results obtained. The conclusions, an outlook on future activities, as well as a list of publications, are given at the end of the report.

Theoretical foundations

Spectrophotometry is widely used to determine the concentration of certain species in samples. The technique is based on the well known Lambert-Beer law that states that the absorbance at a certain wavelength is directly proportional to the concentration if the path length traversed by the light remains constant. Commercial apparatus and standard methods using this technique are based on measuring absorbance at a certain wavelength and using this law to estimate the concentration, provided that a linear calibration curve has been determined beforehand (Jeffrey *et al.*, 1989). The advantage of spectrophotometry lies in the fact that once samples have been processed, they can be returned to the medium from which they were extracted, aside form the fact that usually the sample requires no pre-treatment and readout is quite fast. This makes is an ideal candidate for the construction of a hardware sensor for on-line concentration measurement.

Spectrophotometers operating in the ultraviolet and visible (UV/Vis) range are capable of reliably scanning the absorbance spectrum of a sample for a whole range of wavelengths. This multiwavelength data can be used to further enhance the concentration measurements, e.g. via *partial least squares* (PLS) regression (Dahlén *et al.*, 2000) or *spectral deconvolution* (SD) (Thomas *et al.*, 1996), which is based on expressing the measured absorbance spectrum as a linear combination of a few base spectra.

In course of the possible development of such a hardware sensor, the group has examined two possible concentration measurement techniques using data from a spectrophotometer to estimate the concentration of *4-chlorophenol* (4CP) during its biodegradation in a sequencing batch reactor (SBR) operating under a fixed-time strategy (see WP1 UNAM). On one hand, 4CP concentration was estimated using the absorbance at just one wavelength; this was dubbed the monowavelength technique (MWL). On the other, the concentration was also estimated using the SD technique for the same set of data. Both techniques were then compared. It must be noted that once the data has been collected, the SD technique is no more difficult than the other (simpler) technique if adequate software is available.

The multiwavelength data is given as a vector $\boldsymbol{s}$ of discrete absorbance data corresponding to certain wavelengths, whose resolution may be indicated by the user (in the case studied,

data was collected between 200 and 500 nm with a resolution of 1 nm, but just that between 205 and 300 nm was used). While the SD technique uses the whole spectral data, the other approach uses just one element of this vector.

The SD technique is based on finding a base spectrum vector $\boldsymbol{a}$ and then trying to find a scaling factor to match the measured spectrum, *i.e.* $\boldsymbol{s} \approx \beta \cdot \boldsymbol{a}$. If the base spectrum $\boldsymbol{a}$ is adequate, then essentially it happens that $\beta = x$, where x is the concentration that is sought (as a consequence of the Beer-Lambert law). To find such a β, usual least squares techniques can be used, since the relationship is linear, but there are as many "equations" as elements of $\boldsymbol{s}$ and just one parameter to look for (β). The advantage of the technique is that it can be extended to cope with a mixture of compounds in a sample, assuming that the measured spectrum is a weighted sum of individual spectra, and such weights are a function of the concentrations (Vargas and Buitrón, 2005). Calibration of the method is performed by taking the spectra of known concentration samples specially prepared to mimic the conditions in the reactor. For each calibration sample a spectrum is obtained. For each considered wavelength, a linear regression is performed and the slope of such a linear model is considered as the absorbance of the base spectrum at that wavelength. Of course, a correlation coefficient is obtained for each regression and such information can be used to instead perform a weighted least squares regression during concentration estimation.

Methodology

A 6 liter laboratory biorreactor with an exchange volume of 4 liters was operated as an SBR. It was used to treat synthetic wastewater composed of 4-chlorophenol (4CP) as model substrate. Nutrients were added according to AFNOR (1985). The reactor was inoculated with activated sludge from a municipal wastewater treatment plant and acclimated according to the procedure by Melgoza and Buitrón (2001). Two peristaltic pumps (MasterFlex, Cole-Parmer) were used to feed and draw as fast as possible, while a mixing and aeration was carried out by diffusing air at the bottom of the reactor. The operating strategy was controlled through a programmable timer (Chrontrol XT), changing the reaction time depending on the observed kinetics, and allowing 45 min for sedimentation and 5 min of idle time. Initial concentration of 4CP was either 200 or 300 mg/L.

After acclimation, the samples for calibration were prepared by diluting influent with a known (high) concentration of 4CP with mixed liquor taken from the reactor during the idle phase. Such samples mimic samples that would be drawn during the reaction phase. The absorbance spectra of these samples were measured and the base spectrum was obtained.

Samples of 10 mL were taken at different times during the reaction phase, centrifuged at 2800 rpm (Sol-Bat J600) for 1 min and filtered through a 1.6 μm microfiber filter (Millex). The filtered samples were then measured by filling a continuous flow quartz cell in the spectrophotometer. This was done using a piston pump that will eventually be controlled by the computer. Distilled water was used as the reference. After measurement, the samples and the centrifuged sludge were returned to the reactor. For each kinetics the spectrophotometer was calibrated and the baseline corrected only once. It is expected in the future to fully automate these tasks so that the user can program the sampling rate.

On the other hand, concentration was also measured using the colorimetric technique of 4-aminoantipyrine established by Standard Methods (APHA et.al 1992), which is rather exact, but time-consuming. This was done to compare the three measurement techniques.

Results

To build the calibration spectra, two samples were prepared with concentrations 5, 10, 20, 25, 35, and 45 mg/L each. For the MWL technique, which uses absorbance at only one one wavelength, that where the peak of the absorbance spectrum occurs, *i.e.* at 225 nm, the calibration curve resulted in

$$x = 0.0557 s_{225} + 0.1518$$

where s_{225} is the absorbance at 225 nm, with a correlation coefficient of $R^2 = 0.9889$.

For the SD technique, the base spectrum is shown on figure 1. As explained, the concentration measurement is obtained from a least squares regression that minimizes the cost function

$$J(\beta) = \sum_{\lambda=205}^{300} \left((s_\lambda - f_\lambda) - \frac{1}{\sigma_\lambda} a_\lambda \beta \right)^2 = \left((\boldsymbol{s} - \boldsymbol{f}) - \boldsymbol{a}\Sigma^{-1}\beta \right)^T \left((\boldsymbol{s} - \boldsymbol{f}) - \boldsymbol{a}\Sigma^{-1}\beta \right)$$

Where $\boldsymbol{s}$ represents the measured absorbance spectrum vector, $\boldsymbol{f}$ is the effluent spectrum (*i.e.* the last measured spectrum in a kinetics), $\boldsymbol{a}$ is the base spectrum vector, and Σ is a diagonal matrix of variances or weights, obtained during calibration. I tmust be noted, however, that dilutions of the samples were necessary in order not to saturate the apparatus; however these were considered during off-line analysis.

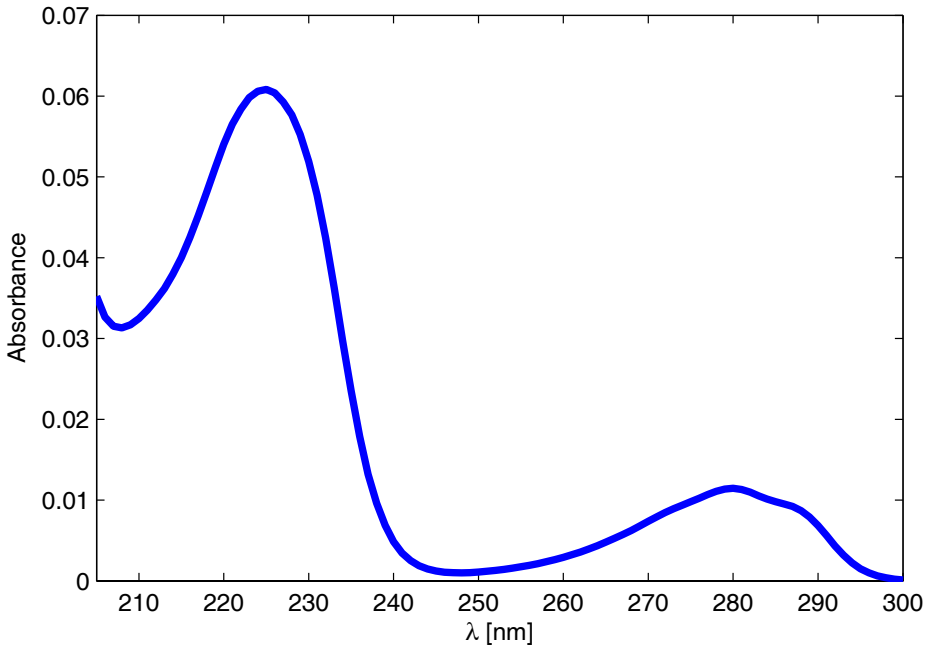

Figure 1. Base spectrum for the SD technique.

The results for two kinetics, one with an initial concentration of 200 mg/L and the other with an initial concentration of 300 mg/L, are shown on figures 2 and 3, respectively.

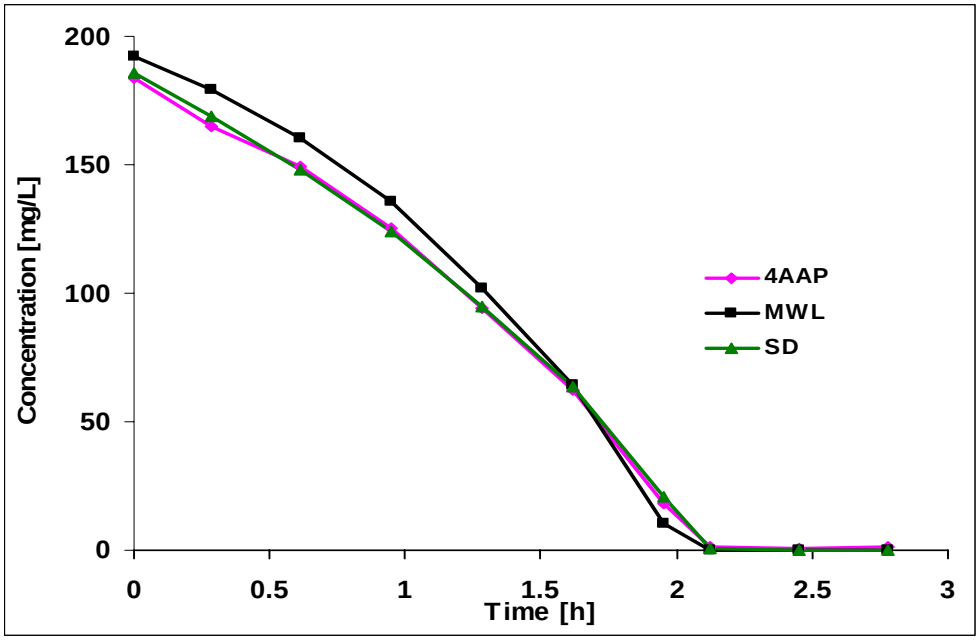

Figure 2. Concentration estimation for an initial concentration of 200 mg/L (4AAP = 4-aminoantipyrine, MWL = monowavelength, SD = spectral deconvolution)

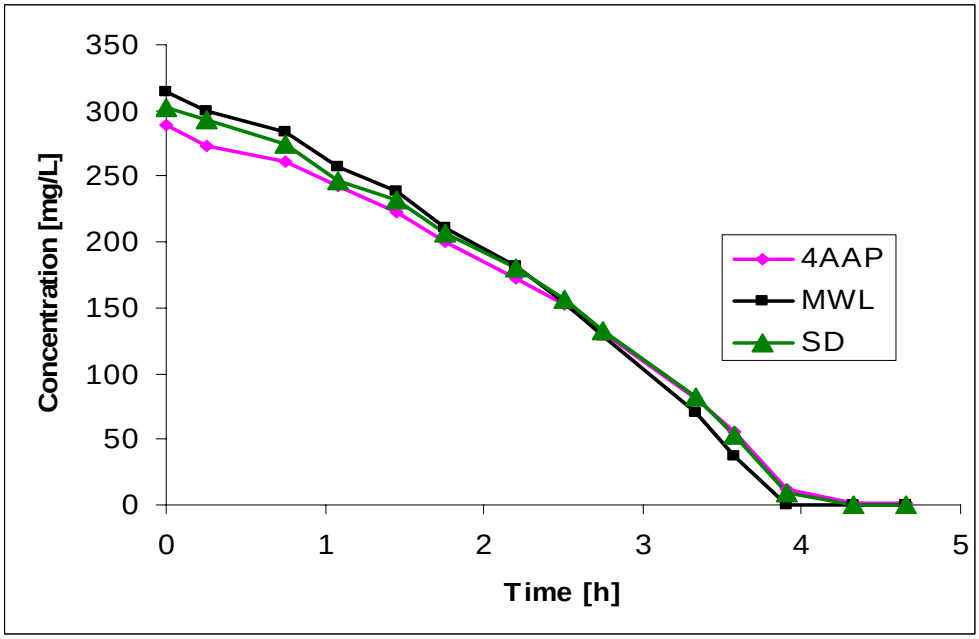

Figure 3. Concentration estimation for an initial concentration of 300 mg/L (4AAP = 4-aminoantipyrine, MWL = monowavelength, SD = spectral deconvolution)

Discussion and future work

As it can be seen from the results presented, both methodologies seem to give acceptable results, comparable with those obtained for the standard technique. Both techniques are suitable for the construction of a hardware sensor, which would only need an automated sampling device and proper interconnection with the computer. This is precisely where the challenge now stands, since solids must be separated from the liquid, either by sedimentation or centrifugation, and possibly also filtered. Future work is aimed in this direction, both to try some techniques to separate solids fast and efficiently, and to evaluate the possibility of measuring spectra even with suspended solids.

An advantage of the SD technique, which is not evident with this bioreactor, is the possibility to extend the results to measure concentrations in a mixture. In this sense, future work aims at determining also suspended solids concentration or a metabolite that could be formed during the reaction due to deficient aeration.

Publication list

So far this project has only one related publication, but as soon as new results are available, another one will be submitted:

- Vargas, A. and Buitrón, G. (2005). *On-line concentration measurements in wastewater using nonlinear deconvolution and partial least squares of spectrophotometric data*, 2nd IWA Conference on Instrumentation, Control and Automation, Busan, South Korea, May-June, 2005.

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- Melgoza, R. and Buitrón, G. (2001). Degradation of p-nitrophenol in a batch biofilter under sequential anaerobic/aerobic environments, *Wat. Sci. Tech.,* **44**(4), 151–157.
- Thomas, O., Theraulaz, F., Agnel, C., and Suryani, S. (1996). Advanced UV examination of wastewater, *Environ. Technol.*, **17**, 251–261.