Surveying an Activated Sludge Reactor using Online UV-Visible and NIR Spectroscopy and Chemometrics

A. Paulo*, A.M.A. Dias*, M.C. Sarraguça, J.A. Lopes**, M.M. Alves*, E.C. Ferreira*

* IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
(E-mail: *ana_paulo@deb.uminho.pt*)
** REQUIMTE, Serviço de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha, 164, 4099-030, Porto, Portugal

Abstract
The performance of activated sludge reactors can be enhanced by the ability to monitor the status of the process without the need for chemicals addition or complex calibration procedures. Nowadays automation is still limited by poor sensor performance and high maintenance costs. Spectroscopic methods associated with chemometrics are being presented as a powerful tool for process monitoring and control. Once implemented, the method is fast, non-destructive and it can be implemented online, permitting to rapidly infer about the status of the process being monitored.
In this work, UV-Visible and Near Infra-Red (NIR) Spectroscopy were used to monitor an activated sludge reactor using immersion probes that were connected to the respective spectrophotometers using optical fibbers. During the monitoring period, changes were induced in the system to test the ability of the monitoring scheme to detect them. The results obtained so far show that it is possible to clearly detect changes in the influent composition as well as the effects of a sudden increase in the influent flow which are among the most common problems that can disturb a biological WWT system. The use of the NIR range for this application is not as common as the UV-Visible range and a direct comparison will allow taking conclusions about the advantages and/or disadvantages of one compared to the other.

INTRODUCTION

Any biological process is, due to its inherent characteristics, very sensible to external and internal variations affecting its optimum working conditions. Some processes are more sensitive than others and different perturbations actuate in different manners depending on the process but in the worse cases, some changes can even lead to the total lost of the microorganism's community. In most of the cases this can represent extra costs and the failure in the attendance of the quality and safety limits imposed by the regulatory entities. In the case of biological wastewater treatment systems in particular, these limits are getting more severe nowadays. Wastewater biological processes are among the few bioprocesses which are run in most of the cases in a continuous mode, although not at steady-state. Furthermore, instead of using a single biomass, these processes involve complex microbial consortia which have to be kept in good balance. However, the large number of uncontrolled variables that characterize this type of systems and the lack of reliable online sensors makes process control extremely difficult. In spite of that, often, trained operators know the action(s) to take when they are confronted with a given perturbation. Their knowledge would be more valuable and efficient if fast detection is provided. In this way, the development of online, fast, simple and intuitive methods for wastewater process monitoring would have a significant impact on the way wastewater treatment plants are monitored and operated.
Traditional monitoring of wastewater treatment systems include among others, the analysis of COD, TSS, BOD_5, TN, $N-NO_3^-$, $N-NO_2^-$, $N-NH_4^+$, etc according to standardized methods. The main problems associated with these methods include the use of chemical solvents and the fact that they can not be used for online monitoring due to the delay between sampling and quantifying the sample. In some cases, as for nitrates, nitrites and ammonium quantification, there are some online sensors commercially available that would permit to rapidly access the status of the system however, these sensors are often disturbed by matrix effects that can hardly be avoided in the case of wastewater treatment systems (Capelo *et al.*, 2007).

Spectroscopic methods are being proposed as a valuable alternative for online system's monitoring. Among the many advantages of the technique, the fact that it ensures rapid dynamic responses being non-destructive and providing the opportunity to measure several constituents simultaneously are some of the most important.

Different spectroscopic ranges were already proposed and tested for bioprocesses monitoring being the ultra-violet–visible (UV-Vis), infra-red (mid or near) and the fluorescence among the most common ranges reported in the literature (Pons et al., 2004). The development of robust array double beam spectrophotometers associated with the advanced low cost technology in the optical fibre field was crucial to the success of the modern optical sensing with the consequent development of immersion probes for online monitoring, helping in the notoriety of the method (Grattan, 1998).

In what concern to wastewater treatment systems, the UV-Vis range (200 to 700nm) is the one that has been most tested. The technique is being used for many purposes being the most common the detection of the appearance or disappearance of a detectable compound (Manani et al., 2002; Pinheiro et al., 2004; Sahin et al., 2007), the characterization and classification of wastewater effluents (Azema et al. 2002; Langergraber et al., 2004; Lourenço et al., 2006; Kotti et al., 2007) and the efficient online quantification of quality criteria parameters by means of pre-defined calibrations between these parameters and the acquired spectra (Escalas et al., 2003; Langergrabe et al., 2004; Vargas et al., 2006; Fogelman et al., 2006). One of the main disadvantages reported for the UV-Vis range is the fact that saturated hydrocarbons and sugars cannot be detected. When monitoring samples where these substances are present, as happens in a large number of cases in wastewater systems, the use of the Infrared range can be much more accurate. The NIR region (780 to 2500 nm) is absorbed only by molecules with covalent bonds where vibrations or rotations can change the dipole moment being essentially useful to detect components that contain hydrogen atoms bound to a heteroatom. Moreover, the NIR spectrum provides chemical information of the compounds, not just concentrations what can be an extra advantage for monitoring/detection purposes. This spectroscopic range (working in transmission, diffuse reflectance or transflectance modes) is being largely used to monitor purposes in the pharmaceutical and food industries (Roggo et al., 2007). As in the case of the UV-Visible range, NIR is being increasingly applied to process qualitative and quantitative monitoring and to process diagnosis, to reveal sudden changes in the process that can be harmful for the bioproduct quality (Lim et al., 2007; Veale et al., 2007). The appliance of the NIR range to wastewater treatment processes is less common and only few, although successful, approaches have been reported in this field (Steyer et al., 2002; Sousa et al., 2007). This can be due to the fact that NIR spectra are affected by changes of the matrix chemical and physical properties (temperature, viscosity, turbidity, refractive index, ionic strength, etc.) as well as by its morphology (Vaidyanathan et al., 2003).

In this work, the capacity of the UV-Visible and NIR spectroscopic ranges to detect changes induced to a lab scale wastewater treatment system was tested. A typical spectrum includes information regarding almost all the constituents of the sample matrix and because of that such information would be invaluable in providing operator-level assistance on the progress of a given process due to the large amount of information involved. However, when associated with chemometrics, changes in the spectral information corresponding to variations in the process can be identified. Principal component analysis (PCA) can be used to identify the most relevant patterns among the data.. The online acquired spectra are analysed using PCA to reduce the superfluous information and to rapidly and clearly identify the status of the system at any time. The main purpose is to present the information as clearly as possible allowing this technique to be used even by those that are not familiar with chemometrics methods.

In this work, the UV-Vis and NIR spectroscopic ranges were used to characterize and analyse the feed and the effluent of a lab-scale activated sludge system trying to simulate the most interesting monitoring points in real systems either to avoid system's damage and to guarantee effluent's quality.

METHODOLOGY

Experiments were carried out in an activated sludge plant consisting of a 25 L tank with 17 L of suspended biomass and a cylindrical settler of 2.5 L, with recirculation of biomass from the

settler to the tank using an air pump. The reactor was inoculated with biomass collected from a municipal WWTP. Synthetic wastewater was prepared with a mixture of peptone and meat extract as carbon source, urea, K_2HPO_4, NaCl, $CaCl_2$ and $MgSO_4$. The system was monitored for influent flow and COD and effluent COD, $N-NO_3^-$, TSS and VSS. The MLTSS and MLVSS were also measured during the monitoring period. $N-NO_3^-$ was measured using HPLC and the remaining parameters were analyzed according to Standard Methods. The bioreactor was further equipped with a TFK 325 thermometer (WTW, Weilheim, Germany), a SensoLyt pH electrode connected to a 296 R/RS monitor (WTW) and a TriOxmatic 690 dissolved oxygen probe (WTW). The concentration of dissolved oxygen was maintained in excess during the entire monitoring period. The efficient agitation of the system is guaranteed by the aeration process with an air diffuser that covers the bottom of the reactor.

Spectra acquisition
UV-Visible and NIR spectra were acquired with immersion probes in the ranges from 240 to 700nm and 900 to 1700nm, respectively. The operating mode is the same in both cases. Light travels from the light source through a lens near the end of the probe. The light then transmits through the sample compartment to a second-surface mirror. The light reflects and travels back through the sample compartment a second time and is then focused by the lens onto the read fibber and through the spectrometer. The returned beam is sent to the portable detectors connected to a PC allowing the immediate spectra visualization and acquisition.
The Ocean Optics USB4000 portable dispersive UV-Vis equipment was used. The instrument is a high-performance 3648-element linear CCD-array detector equipped with a grating for the UV range, connected to a DH-2000 Deuterium Tungsten Halogen Light Source that combines the continuous spectrum of deuterium and tungsten halogen light sources in a single optical path. The immersion probe is connected to the light source and to the spectrometer by means of two TP300-UV-VIS solarization-resistant optical fibers.
The Ocean Optics NIR 512 portable dispersive NIR equipment including a PDA cooling detector was used. A transflectance probe (Ocean Optics/T300RT) is connected through optical fibbers (OceanOptics/QP400-2-VISNIR) to a light source (Stellarnet/SL1) and to the NIR spectrometer which in turn is connected to a PC by a USB 2.0 cable. In both cases spectra are acquired using the OOIBase32/Ocean Optics software. This software allows the configuration of certain parameters like the integration time, average spectra, filter type (to avoid noise mostly when low integration times are used) and the temperature of the detector in the case of the NIR probe. All these parameters were previously optimized in order to improve the quality of the spectra acquired. When measuring for the first time, a reference spectrum is taken as in traditional spectroscopic methods. The reference spectrum is checked regularly and if changes occur, the above mentioned parameters may be adjusted to fix the deviations.
Wastewater treatment systems are usually 'hostile' environments making their continuous and dynamic monitoring extremely difficult to achieve due to problems associated with fouling. As the systems used in this work do not have a cleaning-in-place system incorporated, it was decided to immerse the probes only at the moment at which the spectra are to be saved. An average of 15 spectra was saved with each probe.


RESULTS AND DISCUSSION
One of the main advantages of an online monitoring system applied to wastewater treatment systems would be to detect changes in the influent before it enters the system being it an increase in concentration or a change in the influent composition. As mentioned in the introduction, the UV-Vis range can not detect saturated hydrocarbons or sugars which are usually present in wastewater effluents. In order to compare the performance of both probes in detecting changes in feed concentrations, different feed solutions, with compositions as indicated in Table 1, were prepared and analysed together with the feed used in this work.
Different concentrations with averaged COD between 500 and 1300 mg/L were prepared. Starting from a stock solution, dilutions were made to average concentrations of 1000, 800 and 500 mg/L.

Table 1. Composition of the different feed solutions prepared.

	Feed composition
Solution #1	Peptone, meat extract, urea, micronutrients
Solution #2	Glucose, meat extract, urea, micronutrients
Solution #3	Skim milk

For each concentration, three independent dilutions were prepared and both NIR and UV-Vis spectra were acquired. The first approach was to apply the PCA to differentiate among the different solutions. Figure 1 presents the score plot obtained when the analysis is applied to the entire set of spectra collected with the NIR and the UV-Vis probes. Before applying PCA the spectra were pre-treated by applying the first derivative according to the method of Savitzky-Golay (15,2,1) (Savitzky and Golay, 1964) in the case of the NIR spectra and the standard normal variate method (Barnes *et al.*, 1989) in the case of the UV-Vis spectra. As shown in the figure, in both cases, a clear differentiation among the different solutions is observed with the formation of three independent clusters in the score plot.
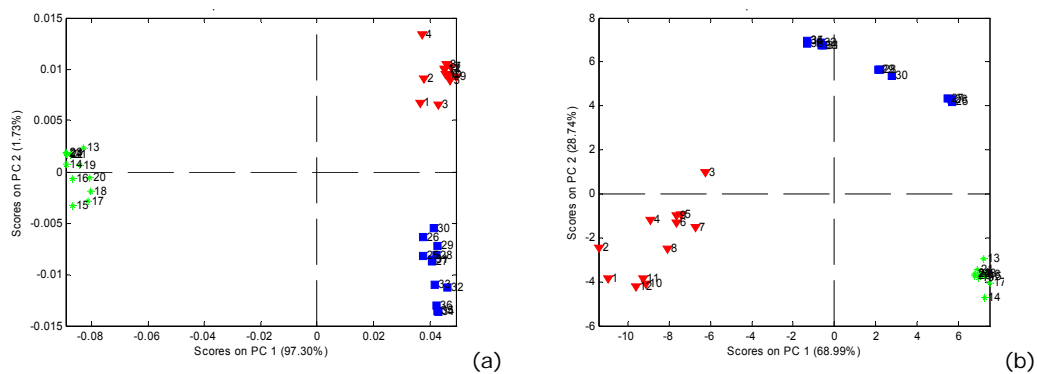

Figure 1. Score plots representing the two principal components used to differentiate among the different feed solutions. Figures (a) and (b) present the results obtained with the NIR and the UV-Vis probes, respectively for solutions #1 (♦), #2 (▼) and #3 (■).
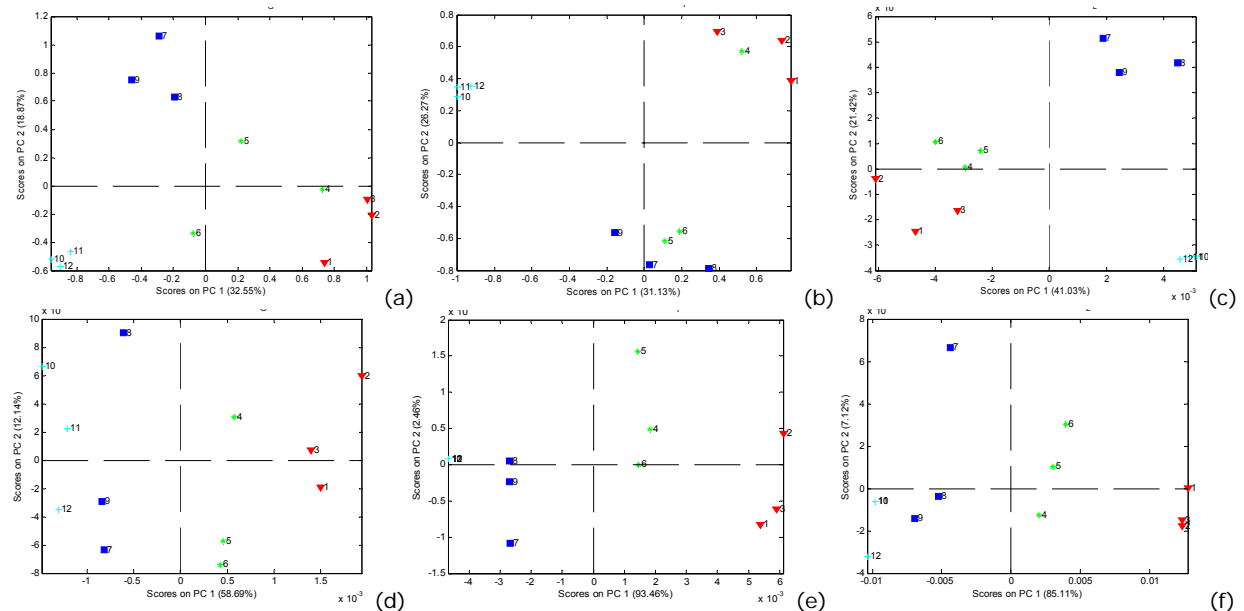
Figure 2. Score plots representing the two principal components used to differentiate among the different concentrations in the solutions with glucose (a,d), peptone (b,e) and skim milk (c,f). The upper and lower figures correspond to the results obtained with the NIR and the UV-Vis probes. For each feed solution, three samples were measured at averaged concentrations of 500mg/L (▼), 800mg/L (♦), 1000mg/L (■) and 1300mg/L (+).

The second step was to analyse each solution independently and to test the probes to differentiate among the different concentrations prepared. As shown in Figure 2, in both cases it is possible to discriminate the different concentrations although, in the case of the peptone and milk solutions, a more explicit differentiation is given by the UV-Vis range. According to the results, it is the first principal component (PC) that mainly accounts for the changes in the solution's concentrations while the second PC mainly accounts for differences among replicas of the same concentration. The variations observed in the concentrations of each set of replicas were planed to further test the accuracy of the probes. It is also interesting to mention that the differentiation along the first PC is more explicit in the case of the UV-Vis range confirming that in this range the 'concentration effect' is more important than the 'interaction effect' which is mainly captured by the NIR range.

As mentioned above the probes were also placed in the settler to study their response to detect the changes that a sudden increase in the influent flow rate can induce in the system. Spectra were acquired before and after increasing the influent flow. Again, they were pre-processed before applying PCA. In this case, the first derivative according to the method of Savitzky-Golay (15,2,1) were applied in both cases. A very intuitive and user friendly way to analyse the results obtained from PCA is through the analyses of the residues (Q) and the Hotelling's T^2 statistics. The first measures how well each sample conforms the PCA model while the latest measures the variation in each sample within the PCA model. In this way these parameters permit to identify when a new sample is deviating from the regular working conditions or when, due to different reasons, samples start to differentiate among them.
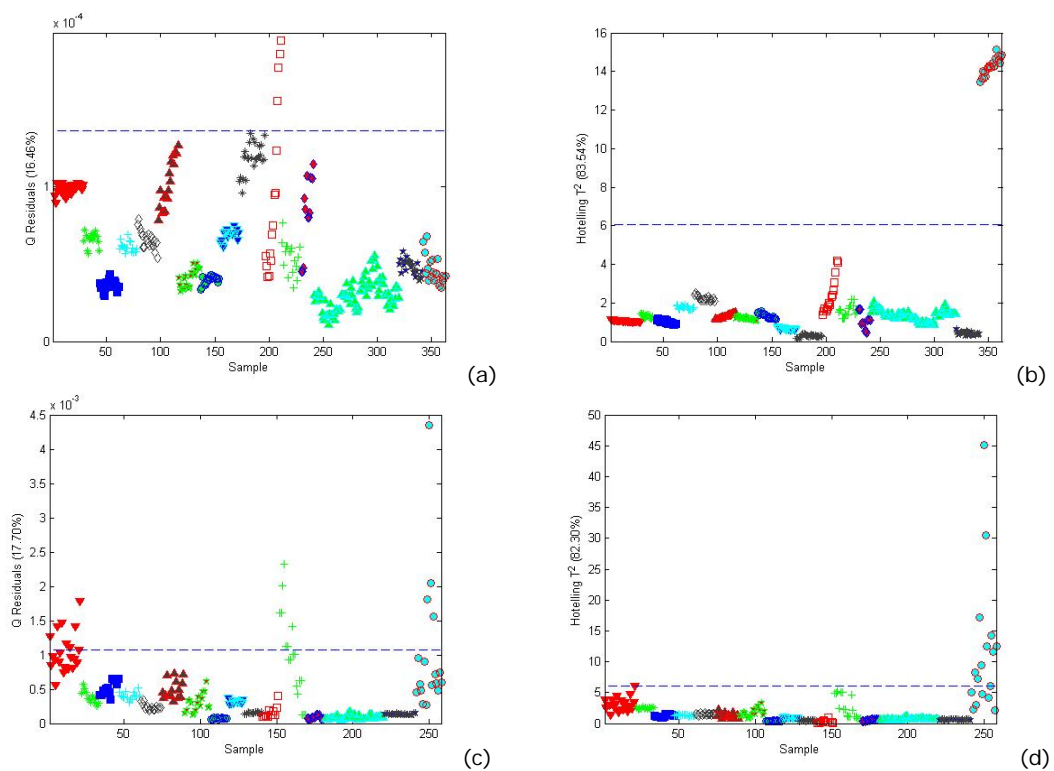
Figure 3. Residuals (a,c) and Hotelling's T^2 statistics (b,d) obtained when PCA is applied to spectra acquired with the probes immersed in the settler. The upper and lower figures refer to the results obtained with the NIR and the UV-Vis probe, respectively. The different symbols used in the figures intend to differentiate among the different days where spectra were measured. The dashed line represents the 95% confidence limit.

The residuals and Hotelling's T^2 statistics obtained when PCA is applied to spectra acquired with the probes immersed in the settler are given in Figure 3. In this case the residuals permit to clearly identify the moment when the influent flow was increased, corresponding to the samples represented by crosses and non-filed squares. Taking into account the residuals obtained with the NIR and the UV-Vis probes (Figures 3a and 3c), it is possible to see that the

UV-Vis probe only detects the perturbation some time after it really occurred while the NIR probe gives a more 'on time' identification. This can again be justified by the fact that the UV-Vis range is detecting changes in concentration originated by the increase in the influent flow rate which will only be detected in the settler after the hydraulic residence time period. In the case of the NIR range, the probe is detecting the disturbance that the increase in the influent flow promotes on the interaction among the constituents of the system.

After the perturbation, the system seems to return to the original conditions but, at the end of the monitoring period another change is detected. In this case, the change is clearly detected with the Hotelling's T^2 statistic indicating that, in this case, what is being detected is a difference among the samples. In fact it was observed that the spectra acquired during this period had a slightly different shape, but to date there is not a clear justification for the fact. It is believed that at this point the system is probably evolving to a new 'pseudo-equilibrium' state. Further tests including extended monitoring periods, are being planned and will help to elucidate this occurrence. It is however important to point out that the results show that changes of different origins can be detected using simple and intuitive analysis.

CONCLUSIONS

This work compared the performance of two portable immersion probes for spectra acquisition in the UV-Vis and NIR ranges. The probes where used to monitor influent and effluent characteristic parameters of a lab-scale wastewater treatment system. The results obtained so far are promissory, showing that in both cases it is possible to differentiate among different inlet compounds and concentrations and to detect disturbances in the system promoted by a sudden increase in the influent flow.

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