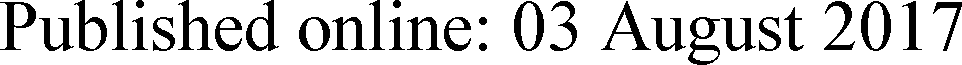
Monitoring biological wastewater treatment processes: recent advances in spectroscopy applications

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Abstract Biological processes based on aerobic and anaerobic technologies have been continuously devel- oped to wastewater treatment and are currently routinely employed to reduce the contaminants dis- charge levels in the environment. However, most methodologies commonly applied for monitoring key parameters are labor intensive, time-consuming and just provide a snapshot of the process. Thus, spec- troscopy applications in biological processes are, nowadays, considered a rapid and effective alternative technology for real-time monitoring though still lacking implementation in full-scale plants. In this review, the application of spectroscopic techniques to aerobic and anaerobic systems is addressed focusing on UV–Vis, infrared, and fluorescence spectroscopy. Furthermore, chemometric techniques, valuable tools to extract the relevant data, are also referred. To that effect, a detailed analysis is performed for aerobic and anaerobic systems to summarize the findings that have been obtained since 2000. Future prospects for the

application of spectroscopic techniques in biological wastewater treatment processes are further discussed.

Keywords Wastewater treatment · Ultraviolet– visible spectroscopy · Infrared spectroscopy · Fluorescence spectroscopy

Abbreviations

3D-EEM Three-dimensional excitation-emission matrix

AD Anaerobic digestion

AGS Aerobic granular sludge

ALE Alginate-like exopolysaccharides AMPTS Automatic methane potential test

system II

AnDMBR Anaerobic dynamic membrane

bioreactor

ANN Artificial neural networks

BA Bicarbonate alkalinity

BMP Biochemical methane potential BOD5 Biochemical oxygen demand CA Cluster analysis

CAS Conventional activated sludge CNOM Colored natural organic matter COD Chemical oxygen demand

DCOD Dissolved chemical oxygen demand DM Dry matter

DOC Dissolved organic carbon

DOM Dissolved organic matter

|  |  |  |  |
| --- | --- | --- | --- |
| EBPR | Enhanced biological phosphorus | SVM | Support vector machines |
|  | removal | SW-NIR | Short-wave near-infrared |
| EDAN | *N*-(1-naphthyl)ethylenediamine | SWR | Step-wise regression |
| EDTA | Ethylenediamine tetraacetic acid | TC | Total carbon |
| EEM | Excitation-emission matrix | TIC | Total inorganic carbon |
| EPS | Extracellular polymeric substances | TKN | Total Kjeldahl nitrogen |
| Ex/Em | Excitation/emission | TOC | Total organic carbon |
| FIA | Flow injection analysis | TS | Total solids |
| FIR | Far-infrared | TSS | Total suspended solids |
| FLC | Fluorescence | UV–Vis | Ultraviolet-visible |
| FRI | Fluorescence regional integration | VNIR | Visible-near-infrared |
| FT | Fourier-transform | VSS | Volatile suspended solids |
| FTIR | Fourier-transform infrared | WAS | Waste activated sludge |
| FT-NIR | Fourier transform near-infrared | WWT | Wastewater treatment |
| GC | Gas chromatography | WWTPs | Wastewater treatment plants |
| GC-MS | Gas chromatography-mass |  |  |
|  | spectrometry |  |  |
| GDA | Generalized discriminant analysis |  |  |

H2S Hydrogen sulfide

HS- Bisulfide ion

IR Infrared

KPLS Kernel partial least squares LC-MS Liquid chromatography-mass

spectrometry

LDA Linear discriminant analysis

LWR Locally weighted regression

MBR Membrane bioreactors

MIR Mid-infrared

ML-PLS Multi-layer partial least squares MLVSS Mixed liquor volatile suspended solids MMC Mixed microbial cultures

MWPLS Moving window partial least squares NH4–N Ammonia nitrogen

NIR Near-infrared

NMR Nuclear magnetic resonance NNPCR Nonlinear principal components

regression

OLR Organic loading rate

OM Organic matter PARAFAC Parallel factor analysis

PCA Principal component analysis

PHA Polyhydroxyacanoates

PHB Polyhydroxybutyrates

PLS Partial least squares

RF Random forest

RVM Relevance vector machines

SBR Sequencing batch reactor

SG Savitzky–Golay

1. Introduction

The arrival of new technologies and products over the past decades, promoted not only progress but several environmental problems. It is known that living organisms’ activities, urban demand, domestic con- sumption and industrial operations, including wash- ing, rinsing, and cleaning equipments, generate high amounts of effluents. From the above, the industry activities are the most prominent factor of water contamination, exceeding the environment regenera- tive capacity and self-purification, causing imbalances in the aquatic ecosystems when an appropriate treat- ment is not taken into account. Thus, environmental concerns, associated with sustainable development, led to the appearance of restrictive legislation, limiting the levels of contaminants discharge in watercourses. Generally, effluents are composed by organic and inorganic substances, including nutrients and aromatic compounds, and feasible treatments are commonly required.

Aerobic systems are, nowadays, frequently estab- lished in wastewater treatment plants (WWTPs) worldwide, with conventional activated sludge (CAS) systems, a classical technology, still adopted for its convenience and simplicity (Tandoi et al. [2006](#_bookmark138)). However, these systems are also prone to be affected by a multiplicity of malfunctions, as reported by Mesquita et al. ([2016](#_bookmark91)). In addition, the use of membrane bioreactors (MBR) has been increasingly

applied in WWTP over the years, providing many advantages over CAS, such as a small footprint and high effluent quality (Van de Staey et al. [2015](#_bookmark123)). On the other hand, despite extensive research efforts, the fouling process still remains one of the main problems and concerns of MBR research within the academic community (Krzeminski et al. [2012](#_bookmark76)). Aerobic pro- cesses are quite dependent on the operating conditions, and, therefore, can be unstable, particularly when subjected to changes in the environment. This, in turn, leads to a negative effect on the bacterial metabolic activity and consequently on the process efficiency. On the basis of these considerations, the sequencing batch reactor (SBR) technology has been increasingly developed taking into account the early experiences gained with CAS systems. SBRs which are flexible systems where both nutrients and organic matter (OM) are removed in the same unit, have been arising with a variety of several attractive properties presented elsewhere (Tchobanoglous et al. [2003](#_bookmark141)).It is well- known that in flocculent activated sludge systems, like CAS, the sludge aggregation is essential for the solid/ liquid separation, and poor sludge aggregation leads to an increase in the effluent turbidity and biomass washout (Li and Yuan [2002](#_bookmark85); Bitton [2005](#_bookmark20); Jenkins et al. [2003](#_bookmark66)). An attractive low-cost and low-footprint alter- native to the flocculent CAS process for WWT is the aerobic granular sludge (AGS) (Lou et al. [2014](#_bookmark101)). AGS systems have been developed in SBRs and have demonstrated excellent settling capabilities, due to their self-immobilization formation process, which increases their density (Adav et al. [2008](#_bookmark18)). Neverthe- less, several key factors have been already described as responsible for the loss of the structural long-term stability (Liu and Liu [2006](#_bookmark77); Tay et al. [2002](#_bookmark139); Zheng et al. [2006](#_bookmark159); Lemaire et al. [2008](#_bookmark84)).

In the past few years, with the increase of contam- inants complexity, anaerobic technology also emerged as a means to improve WWT. Indeed, anaerobic digestion (AD) is commonly used as a pre-treatment of agro-food industrial wastewaters containing high levels of biodegradable organic compounds. As Alves et al. ([2009](#_bookmark5)) points out, AD should be applied to concentrated effluents allowing energy production and nutrient redistribution. However, a post-treatment step is quite always necessary in order to meet the required discharge criteria in surface waters.

Monitoring biological systems is an important task for the performance enhancement of WWT processes.

The increasing demand for biological processes tech- nologies requires the development of adequate mon- itoring and control techniques. Several physical, chemical and operational parameters are generally monitored in WWTP for assessing wastewater and sludge quality, being of the utmost importance to meet the required discharged values. However, most tradi- tional methodologies for determining these parame- ters are costly, labor intensive and time-consuming, and a few may even present environmental risks associated to end products. Therefore, a preferable alternative would be to continuously monitor the key variables within the process and to use this informa- tion to make educated decisions. Thus, a great deal of attention has been recently given to different moni- toring strategies to help clarifying the behavior of WWT biological processes. Moreover, it is known that waste sludge is a by-product of the biological processes, and its treatment and disposal represents up to 50% of the running operating costs of a WWTP (Guo et al. [2014](#_bookmark64)). Thus, it is quite imperative to also monitor the waste sludge post-treatment which gen- erally includes stabilization, drying or composting stages.

Recently, high significance has been attributed to the technological evolution and advances in spectro- scopic methods to investigate complex samples. In fact, over the last years, spectroscopic techniques have gained a relevant interest within the biotechnology field. With these technologies, absorption, reflectance, transmission, or vibrational properties of chemical species can be measured in order to determine the concentration or composition of a sample. Once implemented, and optimized, these methods are fast, non-destructive and user friendly, allowing rapid inference of the process state.

Furthermore, spectrometry combined with multi- variate statistical analysis has been shown to be a valuable tool for monitoring physico-chemical param- eters associated to water quality, being the analysis performed without the need of any special reagent or solvent, both offline and online, with potential to be applied in situ or *in*-*line*. Among the spectroscopy techniques, ultraviolet–visible (UV–Vis), infrared (IR), and fluorescence (FLC) have lately attracted substantial attention in WWT monitoring. It should be noticed, though, that other spectroscopy techniques (Raman spectroscopy, NMR spectroscopy, Terahertz spectroscopy) are distinguished by specific

applications and implementations which are beyond the scope of this article.

In conclusion, this review outlines recent pro- gresses in UV–Vis, IR and FLC spectroscopy, related to WWT monitoring. An in-depth analysis is per- formed to summarize the many new findings that have been obtained in the last years, and future develop- ments for the application of spectroscopy techniques in WWT biological processes.

1. Monitoring aerobic and anaerobic systems

Traditionally, the biochemical oxygen demand (BOD5), chemical oxygen demand (COD) (including filtered and dissolved—DCOD), turbidity, total organic carbon (TOC) (including dissolved organic carbon—DOC), and volatile fatty acids (VFA) are considered key monitoring parameters and have been widely employed for assessing the wastewater quality in aerobic and anaerobic systems. In the particular case of AD systems, the monitoring of alkalinity, biochemical methane potential (BMP), gas production rate and hydrogen sulfide (H2S) production (Pontoni et al. [2015](#_bookmark117)) is also essential for the evaluation of a good performance. Furthermore, BOD5, COD, and TOC have also been used to characterize various complex compositions known as OM, and namely dissolved organic matter (DOM). DOM is a heteroge- neous mixture of aromatic, amino and aliphatic organic compounds containing oxygen, nitrogen and sulfur functional groups (Chen et al. [2003](#_bookmark39)). However, the above parameters do not provide information on the composition of the DOM, and in addition, their analysis is rather tedious and time consuming, and sometimes requires expensive equipment and instru- mentation (Janhom et al. [2009](#_bookmark65)).

Additionally, in recent years, the development of new and more sensitive methods of analysis has made possible the detection of other potentially harmful contaminants, globally referred to as emerging con- taminants, and present in trace amounts, in both aerobic and anaerobic systems. The most common methods include the use of gas chromatography-mass spectrometry (GC–MS) and/or liquid chromatogra- phy-mass spectrometry (LC–MS). Traditionally, these chromatography techniques, coupled with mass spec- trometry, have been used for the identification and quantification of trace compounds (Aguera et al. [2006](#_bookmark6);

Afonso-Olivares et al. [2012](#_bookmark7); Feng et al. [2015](#_bookmark51)). However, this methodology typically requires exten- sive off-line sample preparation, and since the com- pounds of interest are generally present at trace levels, the sample preparation method requires a prior concentration step. It is known that MS always requires the use of GC and LC equipments, thus difficult to implement online (in situ or *in*-*line*) towards the monitoring of WWT. Thus, the present review will focus on the use of fast, non-destructive and user friendly spectroscopic techniques (UV–Vis, IR and FLC) without extensive sample preparation, thus not encompassing MS spectroscopy.

Total solids (TS), volatile solids (VS), suspended solids (SS), comprising total suspended solids (TSS) and volatile suspended solids (VSS) have been of utmost significance for monitoring purposes. Total nitrogen, including nitrate, nitrite, and ammonia and phosphorus concentration is also widely assessed, reflecting the extent of nitrification, denitrification, and phosphorus removal processes. The sludge quality and stability, which can be related to the extracellular polymeric substances (EPS) matrix role, is also an important factor to take in consideration, but still not implemented in real WWTP.

Globally, the wastewater quality is generally assessed using physical, chemical and microbiological tests. However, these parameters depend on expen- sive, labor intensive and/or time-consuming methods, offering only snapshots of moments in time, which makes them unsuitable for real-time monitoring (Carstea et al. [2016](#_bookmark32)). Direct and rapid measurements of the parameters previously presented would provide close monitoring of WWTP quality and allow for a real-time process diagnosis and control. Thus, in the next sections, the most promising and recent available spectroscopic techniques for aerobic and anaerobic processes monitoring are described. Figure [1](#_bookmark0) provides brief information about the evaluated parameters in the biological processes for each spectroscopy tech- nique discussed in this review.

1. Aerobic systems

Taking into account the advantages of the spectro- scopic techniques above mentioned, it comes as no surprise that their use in WWT processes has been exponentially growing in the last decades. In this

**Spectroscopy**

Aerobic processes

Anaerobic processes

UV-Vis

IR

FLC

UV-Vis

IR

FLC

BOD5

PHA

BOD

VFA

COD

COD

COD

EPS

COD

H2S

TOC

VFA

Ammonia

BOD

TOC

COD

TIC

EPS

Total phosphate

COD

EPS

TOC

BMP

DOM

TSS

TSS

Nitrogen

TS, VS

TOC

Nitrate

Phosphorus

VFA

Nitrate

BA

Ammonia

Fig. 1 Schematic representation of the spectroscopy techniques discussed in this review with the relevant parameters evaluated in each case

sense, the recent progresses in UV–Vis, IR and FLC spectroscopy monitoring applications regarding aero- bic systems are next described.

* 1. UV–visible spectroscopy

UV–Vis light absorption has been used since the 1930s for wastewater characterization (Pons et al. [2004](#_bookmark115)). There are also a great number of works dealing with natural waters including rivers and drinking waters

which are outside the scope of this review. Table [1](#_bookmark1) shows the most relevant published studies in the use of solely UV or UV–Vis spectroscopy in wastewater. The first clear evidence provided by Table [1](#_bookmark1) is related to the huge application of this spectroscopy technique in full-scale WWTPs. UV–Vis refers to the interaction between samples and radiation in the 200–780 nm wavelength range (Lourenc¸o et al. [2012](#_bookmark81)), at single or multiple wavelengths to estimate a number of param- eters. Several works have been already performed

using UV absorbance at 254 nm (A254). In this case, right after the discharge of treated effluents, a linear regression was found between A254 and COD considering the effect of a single rainfall event (Stumwohrer et al. [2003](#_bookmark136)). To account for the effects of particles (turbidity) for each type of sample in a set of rain dilutions, they considered A350 as a corrective parameter. The use of solely A254 was lately studied by Kwak et al. ([2013](#_bookmark80)). These authors developed a multiple linear regression model for predicting BOD5. However, they found that combining DOC and A254 provided the best model results. Wu et al. ([2006](#_bookmark149)) confirmed that A254 is a good surrogate parameter to monitor wastewater pollution, mainly DCOD, COD, ammonia, and turbidity when samples are collected in a short period of time in the same WWTP. However, two more WWTPs were reported with lower correla- tion coefficients due to higher pollutants and episodes of pollutants discharge. Also the A280 (280 nm absorbance) was used to estimate the BOD5 in wastewater resulting in: (1) a reasonable prediction in the reactor entrance and an inaccurate prediction on the final effluent for pulp and paper mill wastewaters (Muzio et al. [2001](#_bookmark97)); and (2) a linear regression for raw, primary, and final effluent from full-scale WWTP (Nataraja et al. [2006](#_bookmark99)).

UV multiwavelengths have also been used in wastewater monitoring due to the current extensive use of mathematical modeling (Thomas et al. [1996](#_bookmark144)). Several authors used UV-deconvolution methods based on least square regressions mainly to: (1) estimate the DOC (Escalas et al. [2003](#_bookmark41)); (2) provide information about the suspended solids (mostly on soluble and colloidal fractions) (Azema et al. [2002](#_bookmark12)); and (3) estimate organic sulfur compounds such as mercaptans, in urban and industrial wastewaters (Roig et al. [2002](#_bookmark111)). On the other hand, Vaillant et al. ([2002](#_bookmark124))

used simple methods developed for UV spectra exploitation, namely direct comparison, spectra dif- ferences and normalization to understand the UV physical response of TSS and fractions typology. The use of artificial neural networks (ANN) was also previously reported as a good strategy to estimate COD, total nitrogen, total phosphate, and TSS (Fo- gelman et al. [2006](#_bookmark54); Jeong et al. [2007](#_bookmark69)). Influent and treated wastewater samples, collected during the week and weekend, were clearly distinguished using prin- cipal component analysis (PCA) and cluster analysis (CA) (Lourenc¸o et al. [2006](#_bookmark79)). Moreover, nitrification and denitrification processes monitoring have also been a field of interest, since most of the available methods are expensive, complicated, labor intensive, time consuming and may require large sample volumes. For instance, the work of Ferree and Shannon ([2001](#_bookmark52)) used a second derivative of the UV spectra to quantify total nitrogen and nitrate. Also an in situ UV spectrometer has already been used to evaluate the nitrite, nitrate, COD, DOC and TSS based on a multivariate calibration algorithm (partial least squares—PLS regression) (Rieger et al. [2004](#_bookmark107)), result- ing in good and acceptable precisions for nitrite, nitrate, and COD and low for DOC. The evaluation of online UV spectra was also performed using three different types of wastewater (municipal, landfill, and industrial wastewaters) with acceptable results to estimate the sum of nitrite and nitrate (Drolc and Vrtovsˇek [2010](#_bookmark34)).

Up until quite recently, research activities were mainly focused on offline spectral acquisition (Tho- mas et al. [1993](#_bookmark142)). A clear example of this was the monitoring of slaughterhouse wastewater biodegrada- tion in a lab-scale SBR using offline UV–Vis spectra, relating A416 (416 nm absorbance) with COD during wastewater biodegradation (Louvet et al. [2013](#_bookmark82)). Also,

TOC estimation in a WWTP using offline UV–Vis spectra was performed by Lourenc¸o et al. ([2008](#_bookmark80)) through the use of PLS regression models.

However, over the last years, online UV–Vis spectrophotometric data has been increasingly applied in situ to monitor WWT processes in different types of wastewaters. For instance, UV–Vis spectrometry was studied regarding its application for the evaluation of winery WWT and monitoring rain events (Carvallo et al. [2007](#_bookmark34); Maribas et al. [2008](#_bookmark86)). Mathematical modelling has also been applied to UV–Vis data and PLS models have been developed to: (1) predict COD (Langergraber et al. [2003](#_bookmark81)); (2) calibrate COD, filtered COD, nitrate, and TSS concentrations (Bertrand- Krajewski et al. [2007](#_bookmark19)); (3) calibrate the previous parameters in paper mill wastewater and in a pilot- scale SBR with acceptable results (Langergraber et al. [2004a](#_bookmark82), [b](#_bookmark83)); (4) detect outliers and predict COD and TSS (Zamora and Torres [2014](#_bookmark152)), and (5) predict COD, nitrate, and TSS in a lab-scale CAS (Sarraguc¸a et al. [2009](#_bookmark116)).

Furthermore, a number of other mathematical strategies, encompassing the use of PLS models, were also investigated for online prediction of several key parameters from wastewaters. Nonlinear UV–Vis deconvolution and PLS models were used during nitrophenol biodegradation (Vargas and Buitro´n [2006](#_bookmark127)). Different types of calibration methodologies using PLS models were developed for COD, filtered COD, nitrate, nitrite, and TSS (Rieger et al. [2006](#_bookmark109)). More recently, newer modeling strategies were devel- oped for online COD monitoring. A variable path- length approach, combined with PLS, was developed by Chen et al. ([2014](#_bookmark42)), and a Boosting-Iterative Predictor Weighting-PLS model was developed to handle the noise to information unbalance (Qin et al. [2012](#_bookmark122)). In the latter case, also TSS, oil and grease were monitored.

* 1. Infrared spectroscopy

The IR spectrum can be divided into three main energy (wave number) regions: the far-infrared (FIR) of less than 400 cm-1 (\25,000 nm), the mid-infrared (MIR) of 4000–400 cm-1 (2500–25,000 nm) and near-in- frared (NIR) of 13,000–4000 cm-1 (750–2500 nm) (Stuart [2004](#_bookmark134)). The IR spectrum can be obtained using absorbance, transmittance, and reflectance methods. These are considered simple techniques to obtain

spectra from liquid, solid or gaseous samples. Trans- flectance combines the transmittance and reflectance measurements and can be used to acquire spectra from turbid or transparent liquids (Lourenc¸o et al. [2012](#_bookmark81)). In WWT processes the band intensity measurements could range from the three types described above depending on the sample type. Some of the works described below have been referred as using Fourier- transform infrared (FTIR) spectroscopy, mostly on the MIR spectrum region and using samples in the solid state (lyophilized or dried).

The application of IR spectroscopy to aerobic WWT processes was, until very recently, less common than UV–Vis, nevertheless, it has gain lately a greater emphasis as reported by the works presented below, namely on WWT process performance, PHA quan- tification, EPS characterization and excess sludge post-treatment processes.

Lab-scale experiments have been performed to evaluate the feasibility of FTIR spectroscopy to investigate WWT processes. Campos et al. ([2014](#_bookmark29)) performed experiments with CAS systems fed with landfill leachate/domestic wastewater and found that FTIR analyses were able to demonstrate that most of the pretreated leachate OM was removed rather than diluted. The prediction of intracellular PHA in mixed microbial cultures (MMC) from different sources has been also studied by FTIR spectroscopy, indicating that this methodology significantly reduces the ana- lytical time needed for polyhydroxyacanoates (PHA) quantification compared to the gas chromatography (GC) analytical technique (Khardenavis et al. [2009](#_bookmark74); Arcos-Hernandez et al. [2010](#_bookmark8)).

A large variety of methods for the extraction of EPS in activated sludge are now available, and a comparison of the efficiency of eight EPS extraction methods has been already conducted based on IR spectra analysis. It was found by Comte et al. ([2006](#_bookmark46)) that IR spectra demonstrated EPS contamination by identifying reagents, and possible products of reac- tions between EPS and reagents. Furthermore, EPS characterization based on FTIR spectroscopy has expanded a few years ago mainly to study: (1) the influence of antibiotics on activated sludge composi- tion (Avella et al. [2010](#_bookmark13)); (2) the bioflocculation and settling properties of the sludge using different reactors configuration (Ehlers and Turner [2011](#_bookmark40); Ehlers et al. [2012](#_bookmark43)); (3) the nanoparticles effects on the physicochemical stability of activated sludge

providing information about the major functional groups (Hou et al. [2015](#_bookmark73)); (4) the distribution of heavy metals in microbial aggregates (Sheng et al. [2013](#_bookmark119)); (5) the effect of salinity in activated sludge (Wang et al. [2013](#_bookmark135)); (6) the effect of carbon/nitrogen (C/N) ratio in microbial aggregates functional groups (Wang et al. [2014a](#_bookmark137)); (7) the phosphorus transfer process in the enhanced biological phosphorus removal (EBPR) system (Wang et al. [2014b](#_bookmark138)); (8) nitrogen rich wastewaters from a mixed culture predominated by ammonia-oxidizing bacteria (Yin et al. [2015](#_bookmark160)); (9) the importance of aromatic protein-like substances (espe- cially tyrosine) in maintaining the stability of AGS (Zhu et al. [2012](#_bookmark166)); (10) the detection of EPS proteins and polysaccharides in colloidal form in AGS (Tu et al. [2012](#_bookmark147)); and (11) the EPS functional constituents on bioflocculation in activated sludge processes (Badireddy et al. [2010](#_bookmark15)). Lin et al. ([2010](#_bookmark94)) used FTIR to study alginate-like exopolysaccharides (ALE) to improve the understanding of AGS formation and stability, concluding that a large proportion of the AGS dry weight is ALE, being the dominant exopolysaccharides in AGS. Afterwards, Lin et al. ([2013](#_bookmark96)) were able to investigate, by FTIR spectroscopy, differences between the gel matrix of AGS and normal aerobic flocculent sludge.

Given that the waste sludge contains a large part of biodegradable OM, an arbitrary discharge of such sludge to the environment would present a pollution problem. Hence, such WWT sludge usually undergoes a post-treatment process such as stabilization, drying or composting. Again, FTIR spectroscopy has been conducted regarding waste sludge post-treatment processes, as follows: (1) determination of different functional groups, able to react with dye molecules in aqueous solution, in dried activated sludge used for reactive dyes adsorption (Gulnaz et al. [2006](#_bookmark62)); (2) study of humic acid structures change, and release of aliphatic compounds, during activated sludge com- posting (Amir et al. [2010](#_bookmark9)); (3) evaluation of sludge post-stabilization, and identification of specific func- tional groups, for a number of sample sources (Smidt and Parravicini [2009](#_bookmark121)); (4) characterization of ligno- cellulosic substances, proteins and polysaccharides in a pulp and paper mill secondary sludge (Edalatmanesh et al. [2010](#_bookmark37)); (5) characterization of macromolecular OM hydrolysis, and polysaccharide-like and protein- like materials degradation, in sludge vermicomposting (Yang et al. [2014a](#_bookmark156)).

It is clear from the literature survey that it is not straightforward to use FTIR as a quantitative analyt- ical technique, due to the difficulties related to sample preparation. For instance, samples need to be dried or lyophilized, to eliminate water masking the FTIR spectrum, leading to the production of artifacts (Kunacheva and Stuckey [2014](#_bookmark79)). It is also clear that FTIR data does not allow estimating key parameters (COD, BOD5, etc.) regarding the process behavior. Thus, it cannot be used for real-time and in situ monitoring due to its technical requirements and limitations (Galinha et al. [2012](#_bookmark61)). Therefore, it can be concluded that, while FTIR spectroscopy is useful for predicting compound structures, it is not suitable for compound identification, which most times would be advantageous in monitoring WWT.

NIR spectroscopy has been widespread applied in the pharmaceutical industry for quality control and process monitoring (Lourenc¸o et al. [2012](#_bookmark81)). Currently, great importance is given to NIR techniques in aerobic WWT according to the works presented below. Because of overlapping bands, NIR information must be extracted by chemometric techniques, including the frequently used PLS regression to estimate the key parameters. Mathematical treatment of spectral data is a common way to extract the most relevant informa- tion. In 2002, Stephens and Walker used offline visible-NIR (VNIR) spectroscopy to develop a pre- dictive model for rapid measurement of BOD5 in a large number of wastewater samples. The COD was investigated by Dahlbacka et al. ([2014](#_bookmark20)), but in this case the COD was predicted online in pulp and paper mill wastewater. Different strategies were conducted by Pan et al. ([2011](#_bookmark108), [2012](#_bookmark109)), Pan and Chen ([2012](#_bookmark106)) to estimate COD offline. For that purpose, the NIR spectra waveband selection by moving window PLS (MWPLS) method was performed. Then, the opti- mization of Savitzky–Golay (SG) smoothing modes was applied to optimize the model of NIR spec- troscopy analysis. Finally, the authors found that the short-wave NIR (SW-NIR) region presented the best stable results in sugar refinery wastewaters. The SW- NIR region was also studied by Melendez-Pastor et al. ([2013](#_bookmark87)), combining SW-NIR region with the visible region (V/SW-NIR). PLS models were established for the full spectra and for the visible and NIR spectral ranges separately.