

## Uncertainties of laboratory and in-sewer measurements of COD in raw sewage

Incertitudes sur la DCO des eaux usées brutes mesurée en laboratoire et *in situ* en réseau d'assainissement

Bertrand-Krajewski J.-L.\* , Winkler S.\*\* , Saracevic E.\*\* , Torres A.\* and Schaar H.\*\*

\* LGCIE, INSA de Lyon, 34 avenue des Arts, 69621 Villeurbanne cedex, France. [jean-luc.bertrand-krajewski@insa-lyon.fr](mailto:jean-luc.bertrand-krajewski@insa-lyon.fr), [andres.torres@insa-lyon.fr](mailto:andres.torres@insa-lyon.fr)

\*\* Vienna University of Technology, Institute for water quality, Karlsplatz 13 / E226-1, A-1040 Vienna, Austria. [swinkler@iwag.tuwien.ac.at](mailto:swinkler@iwag.tuwien.ac.at), [erni@iwag.tuwien.ac.at](mailto:erni@iwag.tuwien.ac.at), [hschaar@iwag.tuwien.ac.at](mailto:hschaar@iwag.tuwien.ac.at)

### ABSTRACT

A comprehensive investigation of the uncertainty of different COD analysis methods (DIN, small tube tests (STT) and UV-(VIS) spectrometry methods) has been carried out on potassium hydrogen phthalate standard solutions and raw sewage samples from a large wastewater treatment plant. The UV-VIS method allows estimating COD equivalent concentration from the measured absorption spectra by means of site specific regression and correlation functions. COD<sub>DIN</sub> and COD<sub>STT</sub> methods showed equivalent global results but specific calibration relationships are necessary when high accuracy is required. The COD<sub>STT</sub> method is suitable for immediate COD analysis in the field. Sub-sampling is the main source of uncertainty. Spectrometry is able to estimate COD<sub>EQ</sub> with an uncertainty of the same order of magnitude than the uncertainty in COD<sub>DIN</sub>.

### RESUME

Une étude comparative détaillée de plusieurs méthodes de mesure de la DCO et des incertitudes associées (méthode DIN, micro-méthode STT et spectrométrie UV-visible) a été réalisée sur des solutions étalon d'hydrogénéphthalate de potassium et sur des échantillons d'eau usée d'une grande station d'épuration. La spectrométrie UV-visible permet d'estimer une concentration équivalente en DCO à partir des spectres d'absorbance, au moyen de relations de régression et de corrélations spécifiques au site étudié. Les méthodes DIN et STT fournissent des résultats globaux équivalents mais des relations d'étalonnage spécifiques sont nécessaires pour obtenir une grande précision. La méthode STT est parfaitement utilisable pour des mesures immédiates sur site. Le sous-échantillonnage est le facteur principal d'incertitude. La spectrométrie permet d'estimer une DCO équivalente avec une incertitude du même ordre de grandeur que celle obtenue avec la méthode DIN.

### KEYWORDS

COD analysis, in situ measurement, laboratory analysis, sewer system, uncertainty, UV-VIS spectrometry.

## 1 INTRODUCTION

The Chemical Oxygen Demand (COD) is a key parameter for the management of urban water systems, from sewers systems to receiving water bodies. For decades, COD concentrations have been evaluated by means of laboratory analyses, according to national and international standard methods (e.g. DIN 38409-H43, 1980; NF T-90-101, 2001), from samples collected manually and/or by automatic samplers. In the late 1980s - early 1990s, new analytical techniques appeared, based on ready-to-use small tube tests (STT), which have been standardised more recently (ISO 15705, 2002). Nevertheless, discrete samples are still required for small tube tests.

For continuous COD monitoring, two principal techniques are available. The first one is represented by analysers, i.e. on site small scale laboratories based on standard methods. For practical and maintenance reasons, such analysers are very difficult to be operated at unmanned sewer monitoring stations. The second one is UV-(VIS) (ultraviolet-visible) spectrometry. *In situ* spectrometers are robust enough to be used directly in sewers and can deliver, after appropriate local calibration, an on-line COD-equivalent concentration (Langergraber *et al.*, 2003). If such sensors are promising for research and operational use, their results should be compared with standard and small tube tests methods, and uncertainties for all methods have to be evaluated and compared (Winkler, 2005). Within this respect, several aspects have to be considered. First, the representativeness of sampling is a very critical issue, especially for assuring similarity of the samples as 'seen' by the sensor and the sample used for laboratory analysis. Second, sample storage, transport and preparation can have a considerable impact. Studies with automated and temperature controlled samplers showed a degradation of COD in raw sewage of up to 15 % within 24 hours (Wandl *et al.*, 2001). Third, the uncertainty of the reference method needs also to be considered. For the COD laboratory analysis according to DIN 38409-H43 the main disturbance factors are e.g. chloride (if  $Cl^- > 1000$  mg/L), slowly biodegradable substances, nitrite and hydrogen sulphides. Small tube tests (STT) can be applied for fast on site analysis, eliminating uncertainties linked to sample storage and transport. However, as STT use a very small sample volume (typically 2 mL), sample homogenisation with high speed mixers is extremely important. Additionally, the oxidation at 148 °C requires two hours. In case the correct STT is chosen for the investigated concentration range, an uncertainty in the concentration of typically  $\pm 5-10$  % is claimed in manufacturers' specifications (e.g. Hach-Lange, Merck, etc.). Fourth, UV-VIS spectrometers require the development of site specific correlation models  $COD_{EQ} = f(Abs(\lambda_{ij}))$  with  $\lambda_{ij}$  being a set of wavelengths chosen from the complete absorbance spectrum. The achievable accuracy of any correlation model is implicitly limited due to the high number of possible disturbances such as turbidity, colour and variance of the wastewater composition. The spectrometer itself also introduces some uncertainty because of the variance of consecutively measured spectra due to some variance of the observed sample along the optical path (path length is typically 5 mm for raw wastewater) and variances of the optical properties (light source, detector, etc.) of the instrument.

## 2 OBJECTIVES

Considering the above aspects, a comprehensive study has been carried out investigating the uncertainties of both laboratory and field methods for COD analysis of raw sewage. COD concentrations of supposedly identical samples have been measured using four methods: i) DIN standard method, ii) small tube test method applying ii.1) a laboratory photometer and ii.2) a field photometer, and iii) *in situ* UV-(VIS) spectrometry. Redundant measurements have been carried out to evaluate the

reading errors and the sub-sampling errors. For all methods, uncertainties have been evaluated according to appropriate statistical and uncertainty propagation methods. Correlations between methods have been established. This paper will focus on three aspects only: i) uncertainties in COD concentrations measured with DIN and STT methods, ii) heterogeneity of sub-sampling, and iii) relationships between UV-(VIS) and  $COD_{DIN}$  concentrations.

### 3 MATERIAL AND METHODS

Two series of measurements have been carried out: i) a series with potassium hydrogen phthalate (PHP) standard solutions, ii) a series with raw wastewater collected at the Vienna, Austria 4 million p.e. wastewater treatment plant. For the COD laboratory analysis, the DIN 38409-H43 method was applied ( $COD_{DIN}$ ): it is applicable for a concentration range of 15-300 mg/L (for higher concentrations, raw sample needs to be diluted). In parallel, COD small tube tests ( $COD_{STT}$ ) according to ISO 15705 have been applied (Hach-Lange® LCI 500 [0-150 mg/L] and LCI 400 [0-1000 mg/L]). Two types of spectrometers have been used for UV-(VIS) spectrometry: an UV spectrometer (TRIOS® ProPS, 190-380 nm, optical path 10 mm) and a UV-VIS spectrometer (SCAN® Spectrolyser, 200-750 nm, optical path 5 mm).

One raw wastewater grab sample has been collected manually in the WWTP influent channel (*raw sample*, 10 L). It was immediately transported to and processed in the laboratory. From the raw sample, five dilutions were prepared using a graduated cylinder and tap water (*dilution samples*, 5 x 3.5 L). Each diluted sample was diverted into three bottles (*sub samples*, 5 x 3 x 1 L). From each sub sample, 250 mL were diverted and homogenized with an Ultra-Turrax® mixer for subsequent processing with DIN and STT methods (*homogenized sub samples*, 5 x 3 x 0.25 L). The rest of each sub sample (750 mL) was used for i) TSS (Total Suspended Solids) analysis according to the EN 12880 (2000) method and ii) both UV and UV-VIS spectrometry.

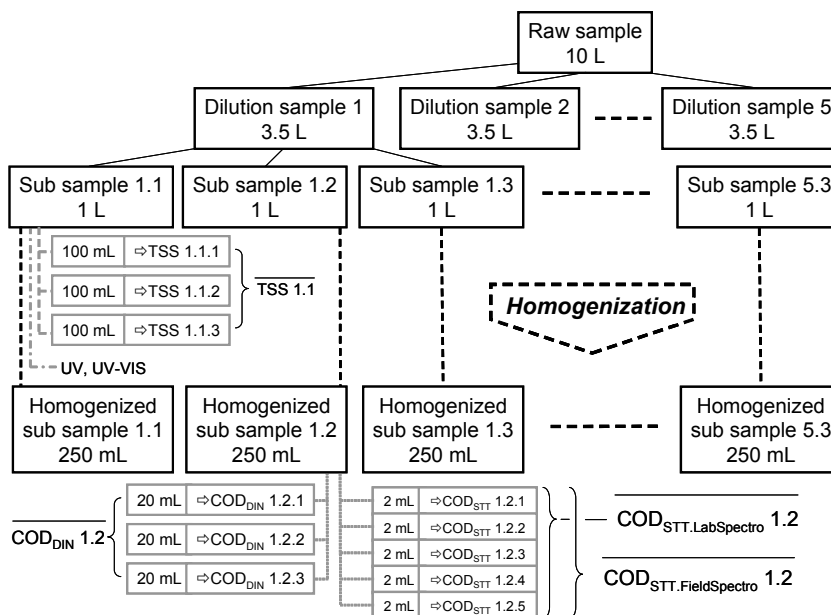


Figure 1 : Sampling and measurement scheme

Each homogenized sub sample was analyzed using the DIN method (3 sub samples x 3 replicates = 9 COD<sub>DIN</sub> values per dilution sample). In parallel, five tests using the STT method have been analyzed for each sub sample, and 5 repeated readings were made for each tube (3 sub samples x 5 replicates x 5 readings = 75 COD<sub>STT</sub> values per dilution sample).

This sampling scheme, summarized in Figure 1, was designed in order to minimize the overall processing time of all samples. To ensure sample identity, all analyses of sub samples (TSS, spectrometry) would have been required to be completed before the sub sample could have been homogenized for COD analysis. By splitting the sub samples (1 L each) into a raw (750 mL) and a homogenized part (250 mL), effects due to sample storage were minimized; but COD and TSS analyses were consequently based on different samples (raw and homogenized sub samples).

## 4 RESULTS

### 4.1 COD analyses using PHP calibration standard solutions

Considering the measurement ranges of DIN and STT methods, a calibration experiment using PHP standard solutions has been carried out. Nine different standard solutions were prepared and analysed applying both i) the DIN method (6 replicates for the 25, 50, 75 and 100 mg/L standards; 3 replicates for the 200, 500, 750 and 1000 mg/L standards) and ii) the STT method (4 to 6 replicates).

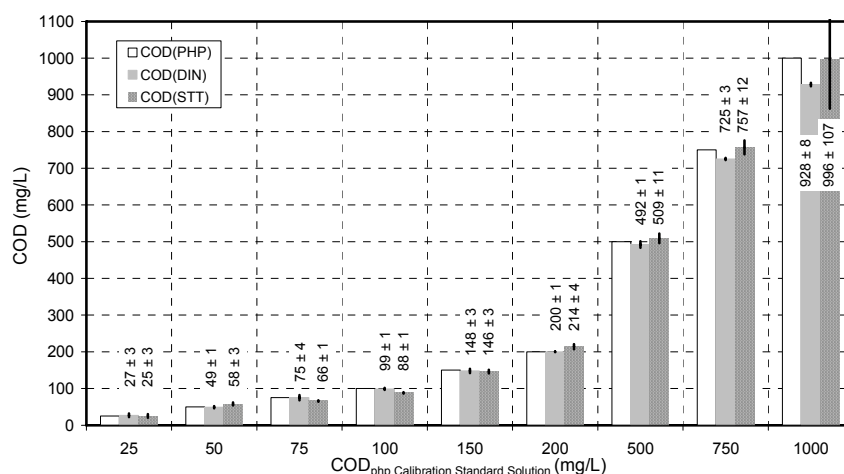


Figure 2 : Calibration experiment: mean concentrations and their 95 % confidence intervals

Figure 2 shows the calibration results: for each standard solution COD<sub>PHP</sub>, mean COD values and their 95 % confidence intervals are given for both DIN and STT methods. Orders of magnitude with both methods are globally compatible, but mean values are statistically significantly different. Thus, if high accuracy is required, raw results can not be directly compared: specific calibration relationships have to be established to convert both DIN and STT values into most likely best estimates PHP equivalent concentrations (results not shown in this paper). The two highest standards solutions (750 and 1000 mg/L) are underestimated by the DIN method: the reason for this is still under investigation and could be linked to dilution. The high uncertainty in the STT value at 1000 mg/L is due to one high value of 1097 mg/L (compared to the other ones equal to 966, 957 and 966 mg/L) which is probably an outlier.

## 4.2 COD and TSS analyses of raw sewage samples

According to Figure 1, three sub samples (1 L) were diverted for all five dilutions: 250 mL were then homogenized for COD<sub>DIN</sub> (3 replicates) and COD<sub>STT</sub> (5 replicates x 5 repeated readings) analyses, while the remaining 750 mL were used for TSS analyses (3 replicates). In the left columns, Figure 3 shows the mean TSS concentration and the 95 % confidence intervals for replicates measurements for the five dilution samples. The relative uncertainty in the mean TSS concentrations (not shown in Figure 3) is ranging from 3.5 to 7 %. The central and right columns show respectively the original COD<sub>DIN</sub> and COD<sub>STT</sub> results, including all measurements without any outlier removal: i) mean COD concentrations and ii) 95 % confidence intervals for replicates measurements for the five dilution samples. Uncertainties in mean COD values (not shown in Figure 3) range from 2 to 12 % for COD<sub>DIN</sub> and from 1 to 3.5 % for COD<sub>STT</sub>. Uncertainties in mean COD<sub>STT</sub> values are lower because 75 instead of 9 replicates have been measured, and thus should not be compared directly. Globally, DIN and STT methods confidence intervals overlap, but mean values are significantly different: below approx. 250 mg/L, COD<sub>DIN</sub> values are greater than COD<sub>STT</sub> values, and reciprocally above 250 mg/L. This is also observed for the COD:TSS ratios given in Table 1. These observations confirm that specific calibration relationships should be established for both methods to evaluate their most likely 'COD PHP equivalent' concentrations. Moreover, the variance analysis (see section 4.3) indicates that outliers exist in data sets of dilution samples 1, 3 and 4, due to heterogeneity in sub-sampling. Table 1 allows comparing results with and without outliers: significant differences may be observed.

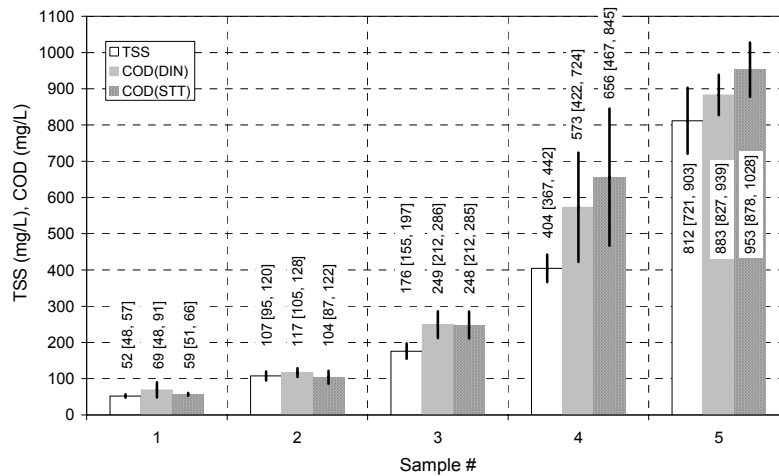


Figure 3 : TSS and COD: mean concentrations and 95 % confidence intervals of replicates

sample #	mean COD <sub>DIN</sub> (mg/L)		mean COD <sub>STT</sub> (mg/L)		mean TSS (mg/L)	COD <sub>DIN</sub> : TSS	COD <sub>STT</sub> : TSS
	all data	outliers removed	all data	outliers removed			
1	69 ± 8	63 ± 3	59 ± 0.9	57 ± 0.6	52 ± 2	1.20 ± 0.08	1.09 ± 0.04
2	117 ± 4	117 ± 4	104 ± 2	104 ± 2	107 ± 5	1.09 ± 0.06	0.97 ± 0.05
3	249 ± 14	238 ± 6	248 ± 4	256 ± 4	176 ± 8	1.35 ± 0.07	1.45 ± 0.07
4	573 ± 58	524 ± 16	656 ± 22	598 ± 13	404 ± 15	1.29 ± 0.06	1.48 ± 0.06
5	883 ± 22	883 ± 22	953 ± 9	953 ± 9	812 ± 57	1.09 ± 0.08	1.17 ± 0.08
mean COD:TSS						1.20 ± 0.03	1.23 ± 0.03

Table 1 : COD, TSS and COD/TSS: mean values and 95 % confidence intervals

### 4.3 Variance analysis

According to Figure 1, for each dilution  $COD_{DIN}$  values were measured 9 times (3 sub samples  $\times$  3 repetitions) and  $COD_{STT}$  values 75 times (3 sub samples  $\times$  5 replicates  $\times$  5 readings). A variance analysis (ANOVA) was carried out, for one and two factors respectively. As all data and results cannot be given in this paper, only very synthetic results for  $COD_{STT}$  values are given in Figure 4. It appears clearly that i) sub sampling and ii) replicates correlated with sub sampling are the main contribution (55 to 90 %) of the total variance. Replicates have a smaller contribution, ranging from 5 to 40 %, while repeated readings of  $COD_{STT}$  values for each small tube represents only approx. 4 to 7 % of the total variance. Sub-sampling also appeared as the main source of variance with the DIN method. The sub sampling variance was so high that one sub sample among the three ones for dilution samples 1, 3 and 4 was considered as an outlier: the corresponding results have been removed from the data set used to establish the correlation between  $COD_{DIN}$  and  $COD_{EQ}$  (paragraph 4.4). This confirms that manual sub sampling, as frequently made in laboratories, is by far the main source of uncertainty, and that analytical methods, replicates and repetitions have a significantly lower influence on the final uncertainty if they are carried out rigorously.

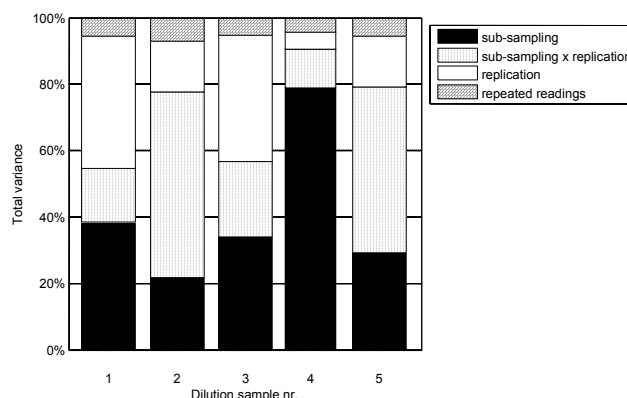


Figure 4 : Synthetic results of the variance analysis for  $COD_{STT}$  measurements

### 4.4 UV-(VIS) spectrometry in raw sewage samples

Absorption spectra with both UV and UV-VIS spectrometers have been recorded in each sub-sample, using respectively a 10 mm quartz cuvette and a Teflon<sup>®</sup> reference tube. The laboratory tests showed low uncertainty (on average  $\pm 3\%$ ) and similar absorption spectra for both spectrometers if operated under comparable conditions. Figure 5 shows the mean absorption spectra measured with both spectrometers for two dilution samples 2 and 4 (for legibility in black and white, 95 % confidence intervals are not shown in Figure 5). Both instruments use a 256-channel photo detector, thus the theoretical resolution of the UV spectrometer is higher (190-380 nm, theoretical resolution = 0.75 nm) than to the UV-VIS spectrometer (200-750 nm, theoretical resolution = 2.2 nm). Also the light sources of the two spectrometers are different with the UV lamp showing a higher intensity in the lower wavelength range. Thus, the UV spectrometer shows a higher sensitivity in the lower wavelength range, while the UV-VIS spectrometer has advantages with respect to estimation of the TSS content of the sample – since it also covers the VIS range.

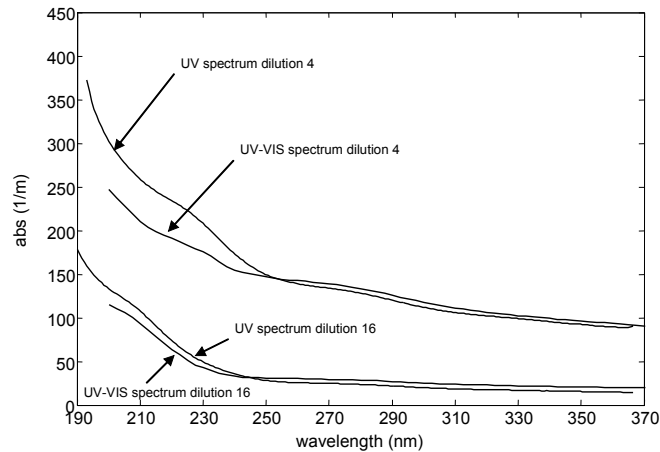


Figure 5 : Mean absorption spectra for dilution samples 2 (i.e. dilution 16) and 4 (i.e. dilution 4) measured with UV and UV-VIS spectrometers

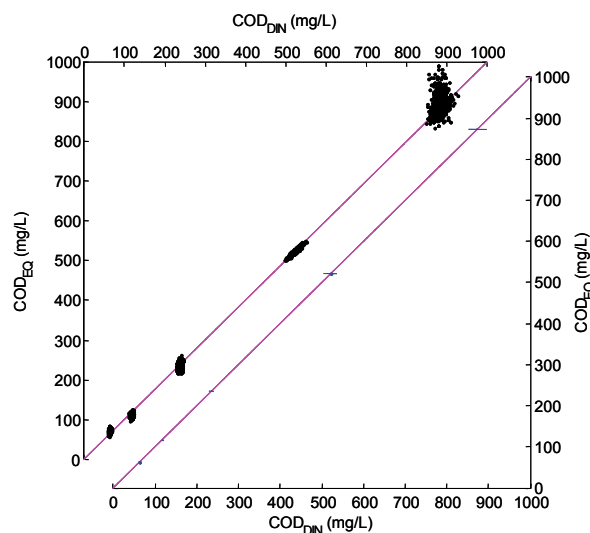


Figure 6 : left:  $COD_{EQ}$  vs.  $COD_{DIN}$  values for 1000 MC simulations considering the uncertainty in both the absorption spectra and the corresponding  $COD_{DIN}$  values; right: optimal PLS regression  $COD_{EQ} = f(Abs(\lambda_i))$  and  $COD_{DIN}$ , with their respective 95 % confidence intervals

Based on repeated measurements of the five dilution samples, a set of calibration data including the mean spectra and the corresponding  $COD_{DIN}$  values was set up. Considering the uncertainty in both absorption and  $COD_{DIN}$  values, 1000 Monte Carlo (MC) simulations including a specific PLS based calibration algorithm (Torres and Bertrand-Krajewski, 2006) were carried out (Figure 6, up left corner). Among the corresponding 1000 correlation functions  $COD_{EQ} = f(Abs(\lambda_i))$ , the optimal one was chosen according to the minimum sum of squared errors (Figure 6, bottom right corner). This optimal regression gives very good results, which means that COD may be estimated with rather high accuracy from absorption spectra. Notwithstanding, the highest  $COD_{EQ}$  values (approx. 900 mg/L) show a higher uncertainty, which is

assumed to be due to the higher TSS content of this sample and to a subsequently higher uncertainty in the absorption spectra (in terms of absolute values). In addition, it should be noted that in this experiment i) all available data points were used in developing the correlation function and ii) that the data set does not include a considerable change of the wastewater composition (dilution of one single raw grab sample).

## 5 CONCLUSIONS

COD<sub>DIN</sub> and COD<sub>STT</sub> methods showed globally similar results for both calibration standards and raw sewage samples when considering the dispersion of all replicates. However, mean values are different and specific calibration functions are necessary to correct systematic gaps and bias when high accuracy is required and/or when methods are compared. Referring to PHP standard solutions, both methods tend towards underestimation of the highest concentration (1000 mg/L), which is not yet fully explained. Overall, the COD<sub>STT</sub> method is suitable for immediate COD analysis in the field. Its uncertainty has to be assessed considering the uncertainty introduced by transport and storage of samples for later laboratory COD<sub>DIN</sub> analysis. For both DIN and STT methods, sub-sampling is absolutely critical and is the main source of variance and uncertainty in final results. UV-(VIS) spectrometry combined with site and wastewater matrix specific PLS regression is able to estimate COD<sub>EQ</sub> with an uncertainty of the same order of magnitude like the uncertainty in COD<sub>DIN</sub>. This is only achievable under strict conditions: i) the spectrometer should be correctly installed, ii) the representative sampling location should be identical to the position of the spectrometer in the medium to be analysed. Changes and variations in the wastewater matrix to be analyzed can only be accounted for with this method if the calibration data set used to determine the correlation functions includes these changes and variations. This usually leads to higher uncertainties in estimated equivalent COD.

## LITERATURE

- Bertrand-Krajewski J.-L. (2004). TSS concentration in sewers estimated from turbidity measurements by means of linear regression accounting for uncertainties in both variables. *Wat. Sci. Tech.*, 50(11), 81-88.
- DIN 38409-H43 (1980). *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung - Gruppe H: Bestimmung des chemischen Sauerstoffbedarfs (CSB) - Kurzzeitverfahren (H 43)*. Berlin (Deutschland): Deutsches Institut für Normung.
- EN 12880 (2000). *Characterization of sludges – Determination of dry residue and water content*. Paris (France): AFNOR, November 2000, 12 p.
- ISO 15705 (2002). *Water quality - Determination of the chemical oxygen demand index (ST-COD) - Small-scale sealed-tube method*. Geneva (Switzerland): ISO, 18 p.
- Langergraber, G., Fleischmann, N. and Hofstaedter F. (2003). A multivariate calibration procedure for UV/VIS spectrometric quantification of organic matter and nitrate in wastewater. *Wat. Sci. Tech.*, 47(2), 63-71.
- NF T 90-101 (2000). *Détermination de la demande chimique en oxygène*. Paris (France): AFNOR, Février 2001, 7 p.
- Torres A., Bertrand-Krajewski J.-L. (2006). Mesurage en continu des polluants dans les effluents urbains: amélioration des algorithmes d'étalonnage d'un spectromètre UV/visible in situ. 24<sup>e</sup> *Rencontres Universitaires de Génie Civil*, La Grande Motte, France, 1-2 juin, 8 p.
- Wandl G., Winkler S., Mueller H. and Svardal K. (2001). *Report on the operation of the pilot plant for the extension of the main Vienna WWTP*. Vienna (Austria): University of Technology, Institute for Water Quality [in German].
- Winkler S. (2005). Field properties and accuracy of in-situ water quality sensors. *Proceedings of the 2<sup>nd</sup> ICA conference on 'Instrumentation, control and automation'*, Busan, Korea, 29 May-2 June, 8 p.