MEASUREMENTS OF MULTIPLE CONSTITUENTS IN AN ACTIVATED SLUDGE PROCESS USING NEAR INFRARED SPECTROSCOPY – SOME PRELIMINARY RESULTS

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SAMMANFATTNING

Nära-infraröd (NIR) spektroskopi är en analysmetod som baserar sig på att ljusets växelverkan med molekylbindningar observeras. Ljus som träffar ett prov kan ge upphov till intermolekylära svängningar. Då detta inträffar absorberas det ljus som motsvarar energinivån i svängningen. På detta sätt kommer en viss svängning att svara mot en viss våglängd i ljusspektrat. I princip beskriver då våglängden som absorberas vilken typ av bindning som har absorberat ljuset och därigenom i förlängningen vilken molekyl som absorberat ljuset. Hur stor andel av det infallande ljuset som absorberas kan i sin tur relateras till hur många molekylbindningar som ljuset växelverkat med. Sammantaget genereras då både kvalitativ (vilken molekyl) och kvantitativ (hur många molekyler) information vid en mätning. Informationen i ett NIR spektrum kan i regel beskrivas som otydlig och överlappande, i synnerhet i spektrum som kommer från komplexa prov där många olika molekyler ingår. För att extrahera informationen krävs därför oftast kemometriska metoder som multivariat analys. Denna kännetecknas av att ett antal responser studeras simultant. I NIR spektroskopi utgörs responserna av för olika våglängder uppmätta amplituder i spektret och för kvantitativ modellering används oftast partiell minsta-kvadratregression (eng.: partial least squares (PLS) regression). Föreliggande rapport redogör för resultaten från en förstudie där ett flertal komponenter kvantifierades i industriellt avloppsvatten m.h.a. NIR spektroskopi och PLS modellering. Resultatet var mycket lovande, men måste samtidigt ses som väldigt prelimininärt mot bakgrunden att antalet prov var relativt lågt.

SUMMARY

This report comprises the results from a pre-study in which several components were quantified in industrial wastewater using near infrared (NIR) spectroscopy and partial least squares (PLS) model regression. In NIR spectroscopy the interaction between indecent radiation and molecular bonds is studied. This makes it a very versatile measurement technique, which enables both qualitative and quantitative measurements. However, the information in a NIR spectrum is generally characterised by broad and overlapping peaks. In order to extract useful information chemometrics in terms of multivariate analysis is therefore typically needed (in this case PLS regression). The results presented are very promising, but it should be pointed out that the number of samples was low and the results should therefore be seen as preliminary.

FOREWORD

INTRODUCTION

This work was carried out in order to evaluate the usability of near infrared (NIR) spectroscopy for quantitative measurements of the key constituents in wastewater from a pulp and paper mill. Vibrational spectroscopy, and in particular NIR spectroscopy, has emerged over the last years as a powerful tool for industrial quality control and process monitoring.[1] NIR spectroscopy covers the wavelength range from 750 to 2500 nm and its merits can be summarized as fast, non-destructive, non-invasive, with high penetration of the probing radiation, suitable for in-line use, and with little or no need for sample preparation.[2] Furthermore, the instruments can be said to be robust,[3] as well as low in maintenance.[4] Despite the fact that these seemingly appealing characteristics are associated with NIR spectroscopy, it seems that only a limited number of applications have been published within the field of wastewater measurements. A recent review on a subject closely related to wastewater treatment, i.e. anaerobic digestion, lists 9 publications describing applications of NIR spectroscopy in this context.[5] No review on NIR applications in aerobic wastewater treatment has been found, but it appears that the number of publications within this field is about the same as for anaerobic digestion.

Based on the applications found describing the use of NIR spectroscopy for wastewater measurements, this is a relatively new topic with the first publication within the field dating back to 2007. In this application, the concentration of urea, solids, and oil was determined with a satisfactory accuracy in samples of wastewater discharged from a biodiesel fuel production plant.[6] While some applications deal with fairly unusual constituents of wastewater such as glycerol and methanol, [7, 8] the most common constituent studied is still the chemical oxygen demand (COD). A number of publications focusing on this constituent solely can be found,[9-11] with reported accuracies expressed as root mean square errors of prediction (RMSEP)[12] ranging from 25 to 33 mg L^{-1}. Only one example of biological oxygen demand (BOD) measurements was found.[13] However, this could be a result of the time consuming reference measurements associated with BOD, rather than an indication that the NIR measurement itself would be difficult to implement. In the work presented here, COD, total suspended solids (TSS), phosphate phosphorus, and total phosphorus were measured off-line with a diode array instrument. Phosphate phosphorus and total phosphorus might represent new constituents in terms of NIR spectroscopy in wastewater measurements. With regard to the applicability of NIR spectroscopy as an on-line measurement technique within wastewater measurements, only a few in-situ measurement applications can be found.[4,14,15] However, in a broader perspective, on-line applications of liquid phase measurements utilising NIR spectroscopy can probably be described as an established method.

MATERIALS AND METHODS

SAMPLES AND SAMPLE PREPARATION

The samples were collected from the influent and effluent stream of the activated sludge step in the wastewater treatment plant. During the time frame of the collection one sample was collected for each day from the influent and from the effluent, amounting to 12 samples in total. In order to

increase the number of samples, sample variance, and to some extent decrease constituent inter-correlations, these 12 samples were further diluted or mixed according to a scheme presented in figure 1. The reasoning behind the selected dilutions was to keep the mixtures closed to the actual sample in terms of concentration, but still make a significant enough impact on the variance. In figure 1 sample A represents the actual sample collected from the influent stream and sample B the actual sample collected from the effluent stream. Sample A was diluted by mixing it with either 10 volume-% water or 10% of sample B. Similarly, sample B was diluted by mixing it with 10% water and enriched by mixing it with 10% of sample A. This way 36 samples were obtained. In this report, the samples containing mainly A are considered to be influent samples and samples containing mainly B are considered to be effluent samples, i.e. no distinction is made between actual process samples and the mixtures based on these. The reference measurements were performed by the company operating the plant, and the accuracy and exact measurement methods of these are unknown.
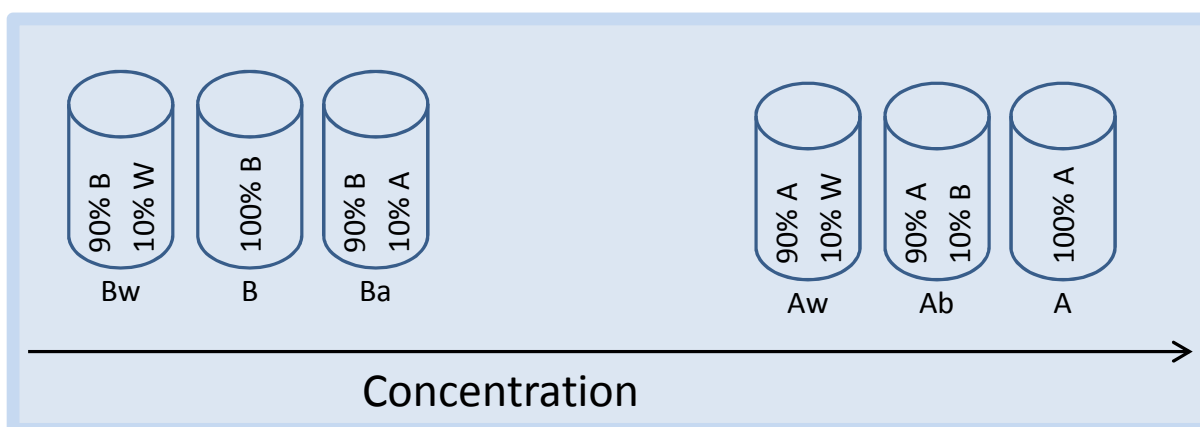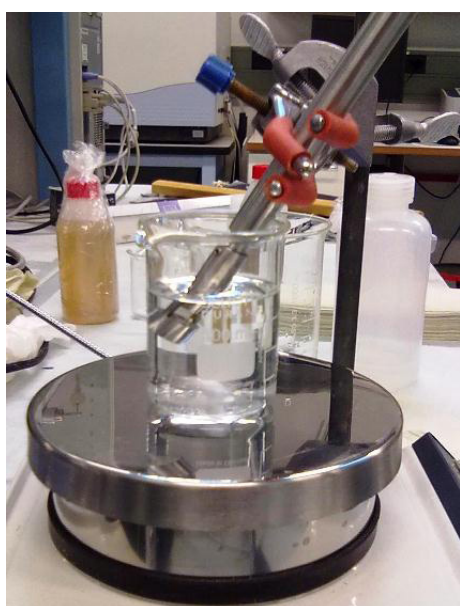

FIGURE 1 Influent, effluent, mixed and diluted samples schematically placed in concentration space.

SPECTROSCOPY AND MEASUREMENT SETUP

The NIR spectra were collected with a GetSpec spectrometer, model #: NIR-256L-1.7T1 (Sentronic GmbH, Gostritzer Str. 61-63, D-01217 Dresden, Germany). This diode array instrument has an Indium-Gallium-Arsenide (InGaAs) detector with 256 elements. The spectral range is 900-1700 nm. The spectrometer was equipped with a dip probe with variable path length (getSpec.com, Gostritzer Str. 61-63, D-01217 Dresden, Germany) and an AvaLight-HAL tungsten halogen light source (Avantes BV, Oude Apeldoornseweg 28, The Netherlands). In this case a fairly large gap size of 5 mm (i.e. a path length of 10 mm) was used in order to try to obtain information also about the suspended material. The spectra were collected as

PICTURE 1 The measurement setup during the collection of a reference spectrum, with the actual wastewater sample in the background.

absorbance spectra from 905 to 1682 nm, at a step size of 3 nm using the Spec32 v. 1.5.6.8 software as interface. The partial least squares (PLS)[16] models used to convert the spectral signal into quantitative constituent values were calculated using the PLS_Toolbox v. 7.3.1 (Eigenvector Research, Inc. 3905 West Eaglerock Drive Wenatchee, WA 98801, USA), together with MATLAB R2011b (The MathWorks AB, Kista, Sweden). The models were tested using cross validation, in which a number a number of spectra is removed from the calibration/model regression data and predicted with a model built on the remaining spectra repeatedly according to the specified number of splits. Here, cross validations were performed as 10-fold Venetian blinds splits. A first order Savitzky-Golay derivative, based on a three point second order polynomial, followed by auto scaling, was used as spectral preprocessing.
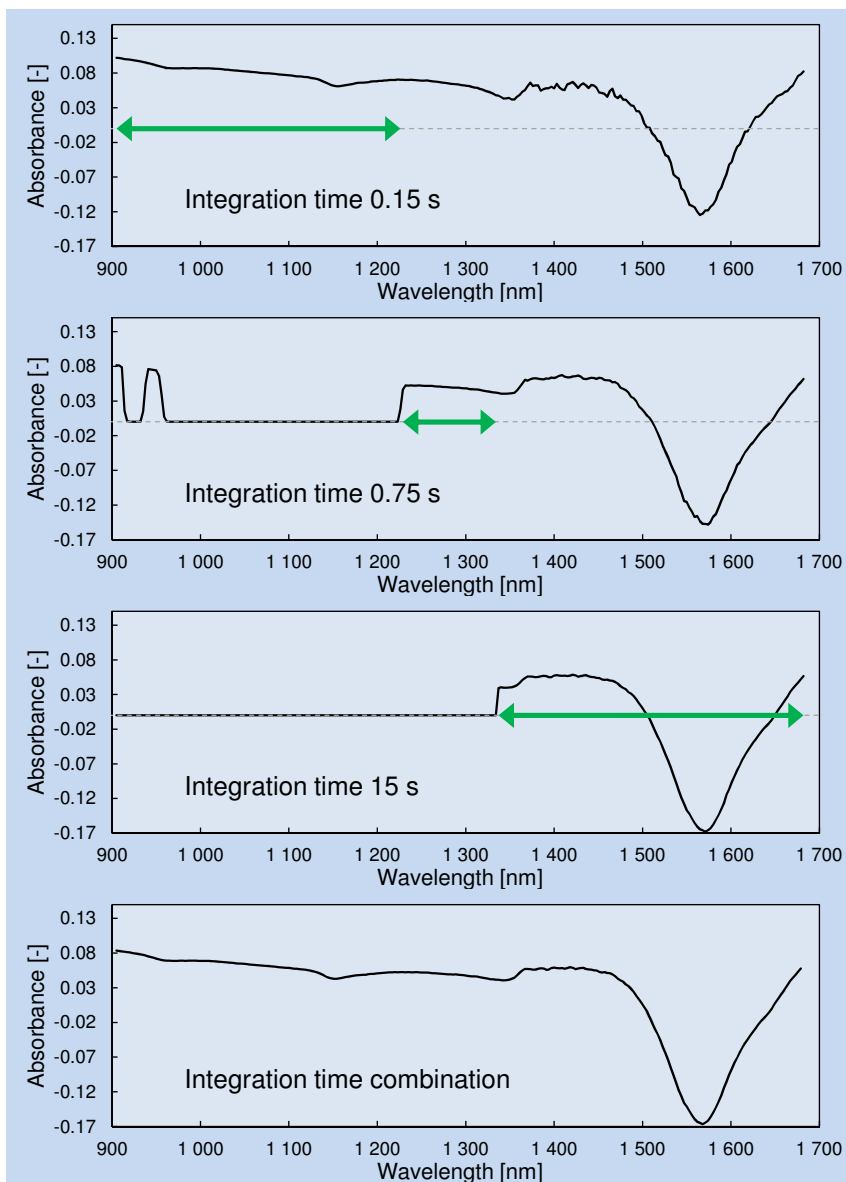
The measurement of the spectra was performed in a laboratory environment, and the temperature of the samples was allowed to reach room temperature before the spectra were collected. In order to get as representative a signal as possible from the solid phase, the samples were placed on a magnetic stirrer and continuously stirred during the collection of the spectra. Three different integration times (0.15, 0.75 and 15 s) were used in order to obtain a better signal quality throughout the whole wavelength region. For a given integration time, a reference spectrum was collected on water, and thereafter 3 spectra were collected from each of the 6 samples. The integration time was thereafter changed and the procedure repeated for the other two integration times. The spectra collected at 0.15 s integration time was averaged for 1024 scans, the spectra collected at 0.75 s integration time for 256 scans, and the spectra collected at 15 s integration time for 8 scans.

Spectra built up from spectra collected for different integration times were used to create calibration models for COD, phosphate-phosphorus and total phosphorus on all the samples, the influent samples (A, Aw, and Ab), and the effluent samples (B, Bw, and Ba). For TSS a calibration model was only created for the influent samples (TSS was not measured in the effluent samples). These combination spectra were obtained by combining the spectral region of 905-1226 nm from the spectra collected at 0.15 s integration time, the spectral region of 1229-1334 nm from the spectra collected at 0.75 s integration time, and the spectral region from 1337-1682 nm from the spectra collected at 15 s integration time. The wavelength segments were aligned by removing the offset between the 1226 nm point in the spectra collected at 0.15 s integration time and the 1229 nm point in the spectra collected at 0.75 s integration time for the lower wavelength region, and with the same approach for the higher wavelength region. Due to the limited amount of data available, no external data set was extracted for model validation. Instead the accuracies for the models are given as root mean square error of calibration (*RMSEC*) and as root mean square error of cross validation (*RMSECV*) values,[12] in combination with coefficients of determination (R^2) and the range error ratio (*RER*)[17.] The latter describes the ratio between the constituent range in the validation data and the *RMSEP*. However, computed here using the *RMSECV* instead of the *RMSEP*.

RESULTS AND DISCUSSION

SPECTRAL INFORMATION AND CONSTITUENT INTER-CORRELATION

The hardware used in this study can perhaps be described as less than optimally configured for liquid phased measurements, at least with the path length of 10 mm that was used. Figure 2 shows the impact of the integration time on the spectral information. With an integration time of 0.15 s, all of the available wavelengths can be recorded, but as can be seen in figure 2 the signal around the water peak at 1380 nm appears to be quite noisy. This is the combined result of using water as reference medium (making the low signal obvious) and the high absorbance of the water (resulting in the low signal). By increasing the integration time to 0.75 s, there is a visually observable decrease in the noise level around the water peak. However, at the same time most of the detectors below 1200 nm become saturated. In the equipment used, the maximal integration time is limited to 17 s. Therefore the maximal integration time used here was 15 s. As can be seen in figure 2, roughly half of the detectors are still not saturated with this long integration time. Figure 2 also contains the combination spectra described in the materials and methods section. At least by a visual assessment this spectrum does not contain any clearly noisy region.

FIGURE 2. Absorbance spectra of a single sample collected using 3 different integration times (0.15, 0.75 and 15 seconds), and an absorbance spectrum created by combining the spectra collected using different integration times. The arrow indicates the spectral interval from each integration time that that was used in the combination spectrum.

Due to the nature of the wastewater treatment process, which leads to the fact that there is always less of basically everything in the effluent sample than in the influent sample, there is an obvious risk

for creating calibration models based on random inter-correlation rather than reproducible correlation. Table 1 contains inter-correlation matrices for the four known (measured) constituents. As can be seen, the inter-correlation is very high for COD, phosphate phosphorus and total phosphorus when computed on the full data set (all samples). This is basically unavoidable with this type of samples, and there are probably many more strongly inter-correlated constituents in the samples that are simply not measured. However, the observable inter-correlation when computed on the influent samples only can perhaps be described as less than alarming. Hence, models built on this data are at least more reliable than the corresponding calibration models built on the whole data set. In the case of the effluent samples, strong inter-correlation is again present. On the other hand, the concentration levels and concentration span in the effluent samples can be described as somewhat unfavourable for NIR spectroscopic measurement in any case (narrow span, very low concentrations). Hence, many of the observations presented here are based only on the results obtained from the influent data set.

TABLE 1 Correlation matrices for the constituents studied in the whole data set, the influent samples, and the effluent samples.

All samples	COD	Phosphate-P	Phosphorus	TSS
COD	1.00	0.76	0.97	0.40
Phosphate-P		1.00	0.80	-0.28
Phosphorus			1.00	0.63
TSS				1.00
Influent	COD	Phosphate-P	Phosphorus	TSS
COD	1.00	-0.55	0.16	0.40
Phosphate-P		1.00	-0.20	-0.33
Phosphorus			1.00	0.63
TSS				1.00
Effluent	COD	Phosphate-P	Phosphorus	TSS
COD	1.00	0.56	0.91	-
Phosphate-P		1.00	0.71	-
Phosphorus			1.00	-
TSS				1.00

Since it was acknowledged that inter-correlation is present in the data, it was considered to be of interest to try to interpret the information in the PLS components for the constituents studied, or at least to verify that the information is not the same for all the constituents. Figure 3 shows the two first PLS components for each of the four constituents, together with the percentage of variance explained by each component. In this case, mean centering was used instead of auto scaling in order to facilitate easier component interpretation and the models were built on the influent samples only. According to figure 3, the information is basically the same in the first component for all the four constituents. On the other hand, the first component only explains only 9% of the constituent variance for COD, while the corresponding number for the other constituents is about 40%. In the case of COD, it is instead the second component that is particularly important, explaining 66% of the constituent variance but only 2% of the spectral variance. The actual loadings of the second components

are very hard to interpret, because the information seems very noisy. Thus, the loading plots gave very little indication as to what information the PLS models utilise for each of the constituents. However, the conclusion may be drawn that the information is not exactly the same for all the constituents.
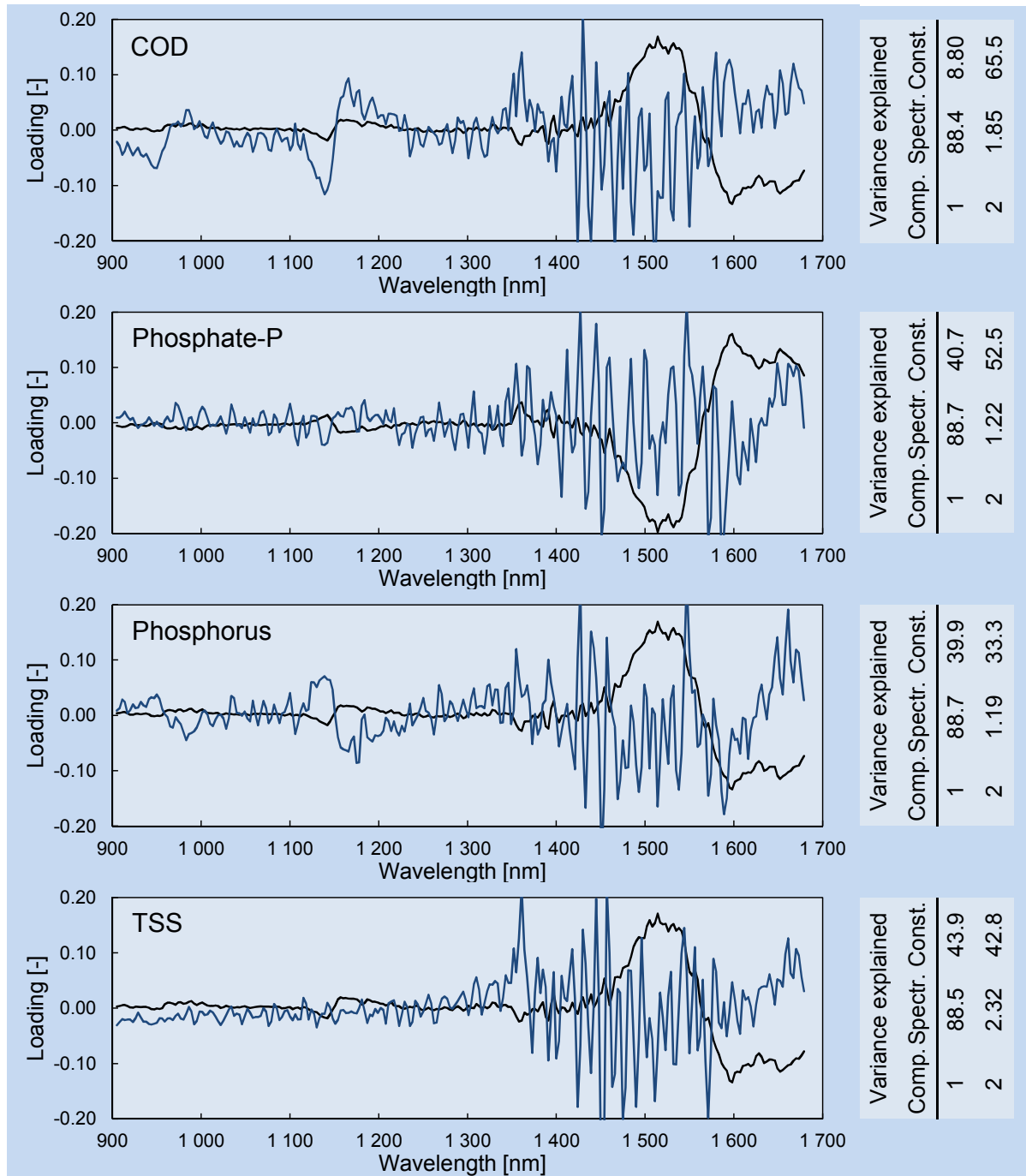

FIGURE 3 The first and second PLS component for the constituents studied, when calibrating on the influent spectra and using a first order Savitzky-Golay derivative with 3 points in a second order polynomial, followed by mean centring, as spectral pre-processing.

CALIBRATION MODEL EVALUATIONS

The calibration results presented in this section are given for models built on the combination spectra described in the materials and methods chapter. For COD, phosphate phosphorus, and total phosphorus, calibration models were built for all the samples, the effluent, and the influent samples. For TSS, reference measurements were available only for the influent samples. Thus, only one model was built for this constituent. When building these models, an in-house script was utilised for parameter selection. This script was applied to the full data set, which means that another selection of regions could have been favourable in the split sets.

TABLE 2. PLS model parameters and accuracies for the chemical oxygen demand.

COD	All	Influent	Effluent
Spectral range [nm]	962-1373, 1508-1679	962-1373, 1508-1679	962-1373, 1508-1679
# PLS components	11	9	7
R^2 Cross validation	0.993	0.965	0.968
$RMSEC$ [mg L^{-1}]	16.7	9.24	3.45
$RMSECV$ [mg L^{-1}]	31.8	22.5	10.4
Ratio Error Range	34.4	21.2	18.8

Table 2 shows the calibration results for COD. In this case the highest RER was obtained for the full data set, whereas the lowest $RMSECV$ was obtained for the effluent samples. The latter was the case also for the other constituents studied, which can be seen as an indirect result from the narrow concentration span in the data. For COD, regardless of the set of samples that is evaluated, the accuracy cannot be described as anything less but excellent. There is a discrepancy between the $RMSEC$ and the $RMSECV$ values for all the data sets. However, this is not surprising considering the limited amount of data available. The scatter plots presented in figure 4 reveal no obvious outliers.
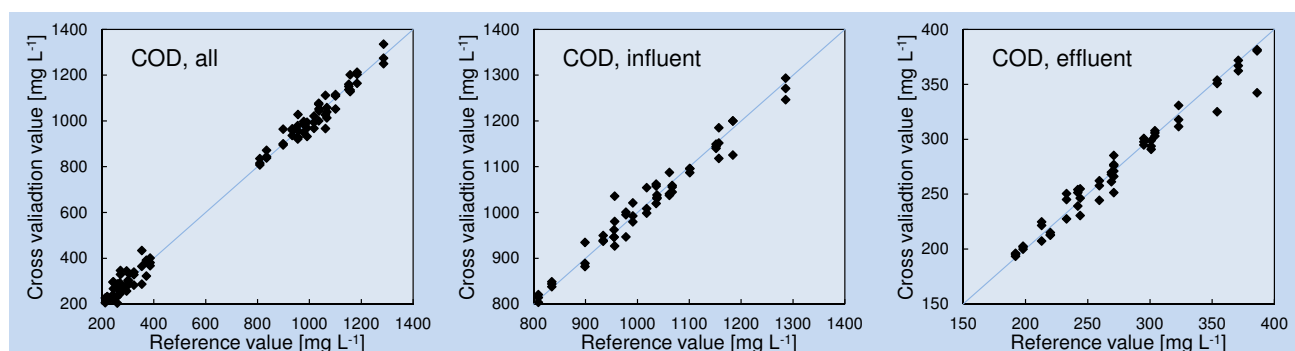

FIGURE 4 Cross validation predictions of COD vs. reference values for all the samples, the influent samples, and the effluent samples.

In the case of phosphate phosphorus, the accuracy of the models built was even more striking. For the influent samples, an *RER* of 50 was obtained. However, this is partly due to the fact that the concentration in one sample was considerably higher than in the other samples, extending the concentration range by approximately a factor of 2. Here, the *RMSECV* value for the effluent samples is only 7 μg L⁻¹. It seems very unlikely that concentrations or accuracies this low could be obtainable with a diode array instrument in aqueous samples. However, for the samples studied, useful spectral to constituent correlation is found, and the result is therefore valid in this sense. Like the COD models, the scatter plots presented in figure 5 for phosphate phosphorus do not reveal any clear outliers.

TABLE 3 PLS model parameters and accuracies for phosphate phosphorus.

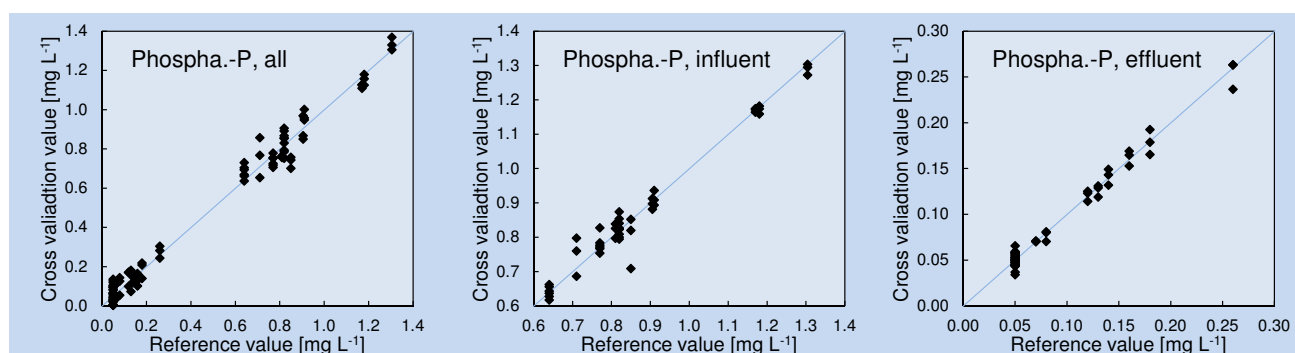| Phosphate-P	All	Influent	Effluent
Spectral range [nm]	905-1352 1502-1679	905-1352 1502-1679	905-1352 1502-1679
# PLS components	11	9	8
R^2 Cross validation	0.992	0.981	0.985
RMSEC [mg L⁻¹]	0.0184	0.0085	0.00329
RMSECV [mg L⁻¹]	0.0377	0.0260	0.00731
Ratio Error Range	34.4	49.9	29.6

FIGURE 5 Cross validation predictions of phosphate phosphorus vs. reference values for all the samples, the influent samples, and the effluent samples.

The *RER* values for total phosphorus given in table 4 suggest that this constituent is more difficult to quantify by means of NIR spectroscopy and PLS models than the constituent phosphate phosphorus. One explanation for this could be that the matrix that makes up the total phosphorus is chemically more complex than is the case for phosphate phosphorus. The scatter plots presented in figure 6 also suggest a lower reproducibility for the predictions of total phosphorus than of phosphate phosphorus, i.e. a fairly large spread in the predictions of the three replicate spectra of a single sample is noticeable. Nevertheless, also all the models built for this constituent performed very satisfactorily.

TABLE 4 PLS model parameters and accuracies for total phosphorus.

Phosphorus	All	Influent	Effluent
Spectral range [nm]	905-1352 1502-1679	905-1352 1502-1679	905-1352 1502-1679
# PLS components	9	8	7
R^2 Cross validation	0.983	0.931	0.958
RMSEC [mg L^{-1}]	0.0628	0.0254	0.00706
RMSECV [mg L^{-1}]	0.109	0.0561	0.0209
Ratio Error Range	21.6	14.5	18.3
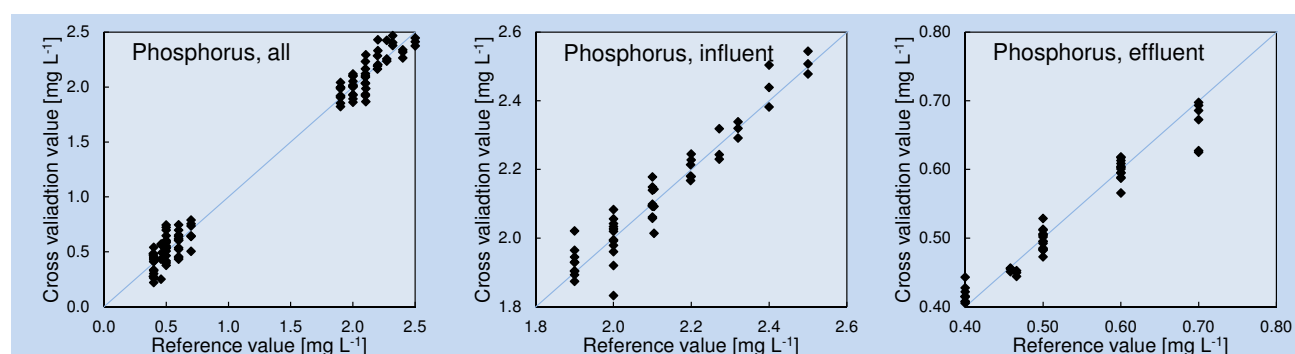
FIGURE 6 Cross validation predictions of total phosphorus vs. reference values for all the samples, the influent samples, and the effluent samples.

TABLE 5 PLS model parameters and accuracies for total suspended solids.

TSS	Influent
Spectral range [nm]	905-1532
# PLS components	6
R^2 Cross validation	0.988
RMSEC [mg L^{-1}]	1.41
RMSECV [mg L^{-1}]	2.68
Ratio Error Range	28.5

Modelling the TSS concentration differs somewhat from the other three constituents studied, since the information that this model utilises ideally should originate from the solid phase. In the case of suspensions, an impact from the solid phase can be expected through the multiplicative scattering effect and the baseline drift that it causes. However, in this case a first order derivative was applied on the spectra before the model was built. Hence, the modelling cannot be based on a "simple" baseline drift. The information (if it represents the solid state) should come from diffuse reflectance originating from the suspended particles. According to table 5 and figure 7, the calibration model for TSS performed very well, even though the derivative was used as spectral pre-processing. Although this is somewhat speculative, the result could be an indication that chemical information can be obtained also from the solid phase with the equipment used.

IMPACT OF THE INTEGRATION TIME ON QUANTITATIVE MEASUREMENTS

The impact on the integration time during the collection of the spectra was evaluated by comparing the performance of PLS models regressed on and cross validated against data sets containing only
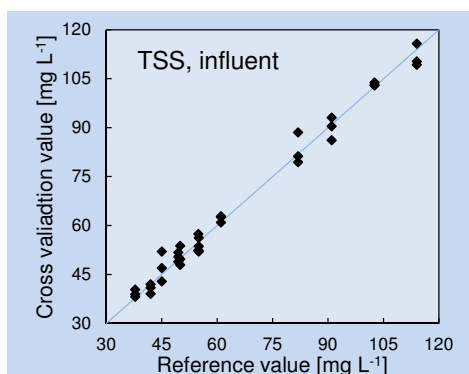
FIGURE 7 Cross validation predictions of TSS vs. reference values for the influent samples.

spectra collected at a single integration time. The performance of these models was also compared to the PLS models regressed and cross validated on the spectra obtained by combining spectra of different integration times. For these models, no variable selection was performed, which is why the results for the combined spectra differ somewhat from those seen in tables 2-5. In order to avoid the observed inter-correlation of the full data set, only the influent data was used in the calculations. The number of PLS components to be used in the models was selected according to the lowest RMSECV value obtained, regardless of how much additional information the last selected PLS component contained. Table 6 shows the model performance for the constituents studied at different integration times, and the combined spectra.

COD	Integration time [s]			
	0.15	0.75	15	Combined
# PLS components	6	10	6	10
R^2 Cross validation	0.930	0.960	0.683	0.955
RMSEC [mg L^{-1}]	13.9	5.91	27.19	5.83
RMSECV [mg L^{-1}]	31.7	23.9	69.0	25.6

Phosphate-P	Integration time [s]			
	0.15	0.75	15	Combined
# PLS components	7	7	8	9
R^2 Cross validation	0.963	0.990	0.700	0.981
RMSEC [mg L^{-1}]	0.01077	0.00324	0.0128	0.00846
RMSECV [mg L^{-1}]	0.0365	0.0193	0.0472	0.0260

Phosphorus	Integration time [s]			
	0.15	0.75	15	Combined
# PLS components	8	10	8	10
R^2 Cross validation	0.905	0.923	0.698	0.940
RMSEC [mg L^{-1}]	0.0198	0.0143	0.0470	0.0146
RMSECV [mg L^{-1}]	0.0663	0.0604	0.119	0.0527

TSS	Integration time [s]			
	0.15	0.75	15	Combined
# PLS components	6	4	5	6
R^2 Cross validation	0.958	0.955	0.910	0.975
RMSEC [mg L^{-1}]	1.74	2.81	3.10	1.23
RMSECV [mg L^{-1}]	5.10	5.18	7.46	3.86

TABLE 6 The impact of the integration time on the PLS model accuracy on the four constituents studied with calibrations made on the influent samples.

In general, the models based on spectra collected by using an integration time of 0.75 s outperformed the models based on the shorter and the longer integration times. The only exception to this is found in the results for TSS, where the shorter integration time resulted in the most accurate model. Table 6 also shows that by using an integration time of 15 s, the number of wavelengths lost in the spectra becomes too great and the result is a significant decrease in PLS model accuracy for any of the constituents studied. It can also be pointed out that the models regressed on spectra collected at 0.75 s integration times are essentially as accurate as the models regressed on the combination spectra. The only exceptions are phosphate phosphorus, where the model based on spectra with an integration time of 0.75 s is actually slightly better, and TSS, where the model based on the combined spectra is slightly better. Thus, for this application, using this instrument and this path length, an integration time of 0.75 s can readily be recommended, despite the fact that many of the lower wavelengths are lost.

SUMMARY

These preliminary results cannot be described as anything else but excellent. In fact, they seem too good to be true. With the highest obtained cross validation accuracy obtained for COD, phosphate phosphorus, total phosphorus, and TSS at 10, 0.007, 0.02 and 2.68 mg L^{-1}, it seems unlikely that the correlation between the constituents and the spectral signal is directly related to all of the constituents. For an instrument of the type that was used, the accuracy could be expected to be in the range of g L^{-1}, rather than mg L^{-1}. However, the fact still remains that the correlation existed in the samples measured and that this correlation was utilised to produce calibration models with remarkably high accuracies. Thus, if the case is that this correlation between the constituents and the spectral signal is always present in the streams in the wastewater treatment facility, this is a relatively inexpensive and very rapid way to obtain simultaneous quantification of numerous constituents. In the samples studied here, the constituent inter-correlation is very strong if the effluent and influents samples are explored as a combined data set. However, this is not the case if the data is split into separate sets of only influent and only effluent samples. Thus, apart from the correlations arising when comparing untreated samples and spectra of these to treated samples and spectra of these, other correlations between constituents and spectral data do exist.

The issue regarding which integration time to use in the collection of the spectra is to some extent an instrument specific question and to some extent not. Since the longer integration times studied fully saturate some of the detectors, the results obtained also indirectly indicate the wavelengths that are important for the determination of the studied constituents. In this case only indirectly, because a longer integration time improves the signal to noise ratio for the higher wavelengths at the same time as the information in the lower wavelengths is lost. For the instrument used here, the conclusion was that some of the lower wavelengths can readily be sacrificed in order to get a better signal in the higher wavelengths (i.e. using an integration time of 0.75 seconds). As a contrast to this, virtually no improvement was obtained by trying to improve the signal above 1350 nm with a significantly longer integration time (i.e. a 15 s integration time).

In conclusion, the obtained results are very intriguing and (further) indicate that high precision on-line measurements using NIR spectroscopy could be an option for the process water streams in the pulp and paper industry.

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