IN-SITU, REAL-TIME BIOREACTOR MONITORING BY FIBER OPTICS SENSORS

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Abstract: One of the most studied bioprocesses is fermentation by yeasts. Although this is true, there is still the lack of real-time instrumentation that is capable of providing detailed information on metabolic state of fermentations. In this research we explore the possibility of using UV-VIS-SWNIR spectroscopy as a high-output, non-destructive and multivariate methodology of monitoring beer fermentation. We herein report the implementation of the a fibber optics sensor and the capacity for detecting key parameters by partial least squares regression for biomass, extract, pH and total sugars. Results show that UV-VIS-SWNIR is a robust technique for monitoring beer fermentations, being able to provide detailed information spectroscopic fingerprinting of the process. Calibrations were possible to obtain for all the studied parameters with R2 of 0.85 to 0.94 in the UV-VIS region and 0.95 to 0.97 in the VIS-SWNIR region. This preliminary study allowed to conclude that further improvements in experimental methodology and signal processing may turn this technique into a valuable instrument for detailed metabolic studies in biotechnology.

1 INTRODUCTION

The most common bioprocess in bioengineering is fermentation. Fermentation is the result of plant physiology, yeast physiology, metabolomics and quimiomics, and as well as of physical phenomena, such as fluid flow, heat and mass transfer. Because this complex system is auto-organized inside a bioreactor during fermentation, fermentations have been controlled using only macroscopic variables, such as, temperature, pH and CO_2 pressure. Nevertheless, these provide low discrimination and information on how to control the metabolic transformations, especially because fermented products differ not in macroscopic chemical composition, but rather in micro quantities which are especially relevant, such as key odorants in beer or wine, or in the production of pharmaceutical highly valued drugs.

The metabolome has been mostly studied by targeted approaches in analytical chemistry techniques. These are not capable to obtain in real time the metabolomics inside bioreactors and are usually destructive methodologies. Therefore, various on-line analytical methods, such as flow injection analysis (FIA), liquid chromatography (HPLC), infrared spectroscopy (IR), gas chromatography (GC) and mass spectrometry (MS), become more and more popular in metabolic studies (Schugerl, 2001). Although these are highly accurate methods for metabolic studies, these are difficult to be implemented in-line, not only due to their high price, but because they need special and highly controlled conditions to operate.

Spectroscopy is a powerful multivariate methodology that have great potential for the metabolomics study in biological systems. It provides a convenient method for analysis of liquids, solutions, pastes, powders, films, fibres, gases and surfaces, and making possible to characterize proteins, peptides, lipids, membranes, carbohydrates in pharmaceuticals, foods, plants or animal tissues. It can also provide detailed information about the structure and mechanism of action of molecules (Stuart, 1997). Over the past decade, interest in bioprocess monitoring using non-invasive infrared spectroscopic sensors has increased. This is mainly due to their rapid simultaneous multivariate determination ability, in situ sterilizability, and low need for maintenance during operation. Among the various spectral regions, near-infrared (NIR) spec-

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troscopy has been used widely due to ease of sampling using fiber optics, and inexpensive and robust instrumentation (Cooper et al., 1997; Yano et al., 1997; Majara et al., 1998; Yeung et al., 1999; Alison et al., 2000; Rhiel et al., 2002).

UV-VIS spectroscopy has not been used in bioprocess engineering, where the use of near infrared (NIR) has been preferred due to the association to specific vibrations of molecular groups (Workman, 1993). It has been widely accepted that vibrational spectroscopy is more adequate for organic chemistry measurement than transitional spectroscopy (Faust, 1992). However, NIR spectroscopy has as high inconvenience the high absorbance of water in this region, which greatly decreases the sensitivity, being more difficult to detect metabolites in lower concentrations. Furthermore, NIR and MIR equipments are more expensive, and today's cheap miniaturized spectrometers are capable to detect with high precision in the UV-VIS-SWNIR region.

UV-VIS-SWNIR has not been used for a fermentation monitoring. This is perhaps attributed to the fact that UV-VIS spectroscopy records transmitions between electron energy levels from molecular orbitals, instead of vibrational or structural oscillation of molecular groups as in the infrared region. Electronic transitions in the UV-VIS region depend upon the energy involved. For any molecular bound (sharing a pair of electrons), orbitals are a mixture of two contributing orbitals σ and π, with corresponding anti-bounding orbitals σ^* and π^*, respectively (Bruice, 2006). Many organic molecules present conjugated unsaturated and carbonyls bounds, such as aminoacids, phospholipids, free fatty acids, phenols and flavonoids, peroxides, peptides and proteins, sugars and their polymers absorbance in these bands. Furthermore, many biological molecules present chromophore groups, which increase the absorption in the UV-VIS region, such as: nitro, nitroso, azo, azo-amino, azoxy, carbonyl and thiocarbonyl, which can be used to characterize the different metabolomic stages that occur on a bioprocess. Furthermore, UV-VIS-SWNIR registers many features such as fluorescence and vibrational resonances due to energy decay of exited electrons, which may provide highly accurate fingerprinting of metabolites and metabolic state of the fermentation.

In this research we explore the use of fiber optics sensors for monitoring the yeast metabolic activity during beer fermentation. Beer its the final result of wort fermentation with selected yeast. As the metabolism is dependent on several internal and external variables the main objectives of this work were: i) implement a fibber optics sensor capable of reproducibility and immunity to processing conditions; ii) determine the capacities of UV-VIS-SWNIR spectroscopy in capturing detailed metabolic features during fermentation; and iii) determine the capacity for detecting key metabolites by partial least squares regression of the signal (biomass, extract, pH and sugars).

2 MATERIALS AND METHODS

2.1 Fermentation

Two complete fermentations were monitored in this exploratory study. The conical cylinder fermentor, with maximum volume 1.9 L and a work volume of 1.4 L in Figure 1 (a) was used. To maintain a constant temperature of 18^oC a refrigeration serpentine involves the cylindrical part of the bioreactor, being possible to achieve a complete fermentation in 5 days. The fermentation starts by adding the inoculum to the malt wort (13.4^o Brix) previously put inside the bioreactor. The inoculum is composed of *Saccharomyces pastorianus (carsbergensis)* (brewing yeast) with a concentration of 12 to $16x10^6$ cells / ml of wort. An inoculum stock was stored in a freezer at -80^oC in order to obtain reproducibility between batches. Inoculum was prepared in the following fashion: the microorganisms were incubated in wort (previously aired) under anaerobic conditions on a rotary shaker (120 rpm) at 27^oC for 72h. After this period of time the yeast were collected from the wort by centrifugation (5000 rpm for 10 minutes) in Sigma 4K15 (Sigma, 2008), and then diluted in NaCl (0,9% v/v) in the reason of 4 ml/g of yeast, to form a paste. Once obtained this paste it's necessary determine, by direct counting in the microscope of the yeast, the correct volume of inoculum, to ensure the necessary initial concentration of yeast on the fermentor (12 to $16x10^6$ cells / ml). After this, the required volume of inoculum is calculated and added to the wort to begin fermentation. Monitorization was performed at-line by removing 15 ml samples with a frequency of three samples per day during the consecutive five days of fermentation. These samples were thereafter subjected to both spectroscopy and physical-chemical measurements.

2.2 Spectroscopy

UV-VIS-SWNIR spectroscopy measurements were performed using the fiber optic spectrometer AvaSpec-2048-4-DT (2048 pixel, 200-1100nm)(Avantes, 2005). Standard Transmition

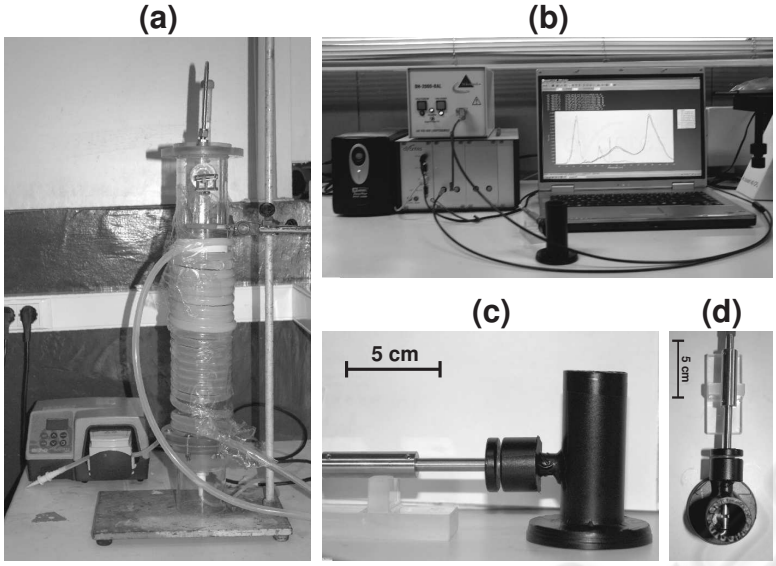

Figure 1: Experimental setup: (a) Fermentor; (b) Equipment for spectroscopy analysis (Spectrometer, Light source, Computer with spectroscopy software, Transmition dip probe and Probe holder); (c) Probe holder, side view and corresponding dimensions and (d) Probe holder, up view and corresponding dimensions).

dip probes T300 - RT-5mm UV-VIS and VIS-NIR (200-1100 nm) for UV-VIS and VIS-SWNIR (Ocean-Optics, 2008) were adapted to a special probe container designed to isolate the environmental light and maintain the probe at the horizontally to prevent the deposition of debris in the mirrored surface, as presented in Figures 1 (b) to 1 (d). A balanced deuterium tungsten Light Source - DH2000-Bal was used for UV-VIS and VIS-SWNIR transmission measurements (Ocean-Optics, 2008); and recorded using AvaSoft 7.0 software (Avantes, 2007). All measurements were performed at the room temperature of 18 ± 2^oC. The deuterium lamp (UV-VIS) was let to stabilize during 20 min; and the tungsten lamp (VIS-SWNIR) was let to stabilize during 15 min. The dark spectra was recorded and the spectra measurements were taken with linear and electric dark correction. Both light spectra were monitored by statistically assessing the reproducibility of the light source with measurements of light during the several days of the experiment. The spectra were acquired three times a day to follow the different stages of the fermentation. Ten spectra replicates were recorded of UV-VIS and VIS-SWNIR measurement for each sample of the fermentation to study scattering effects.

2.3 Physic-chemical Analysis

Cell Concentration: Samples were centrifuged (5000 rpm for 5 minutes) to remove the yeast from beer; To determine the concentration of yeast cells. The yeast removed by centrifugation was resuspended in 15 ml of NaCl (0.9% v/v), and after performing the appropriate dilution, cell concentrations were determined with a Neubauer improved counting chamber at the microscope. **pH determination**: 2 ml of each centrifuged beer were analyzed in triplicate in a Metrohm 691 pH meter following the instructions from (Metrohm, 2008); **Total sugars**: sugar weight percentage in solution was obtained through refractometry, by placing 1 ml of sample in the AR12 SCHMIDT+HAENSCH ABBE refractometer (Schemidt-haensch, 2008); **Dry extract**: three samples of 1 ml each were placed in the oven at 105^oC for 24 h and the dry weight of this samples was calculated matching the dissolved solids concentration in the original samples .

2.4 Analysis and Multivariate Calibration

2.4.1 Spectra Pre-processing

The large biochemical transformations of fermentation makes impossible use the same integration time for all the fermentation sampling times. Under these circumstances, all the collected spectra were transformed into intensity (I) to remove this effect ($I = \mathit{Raw\ Signal}/\mathit{Integration\ Time}$) and smoothed by using the Stavisky-Golay filter (length = 15, Order= 2) (Stavitzky and Golay, 1964). Afterwards, spectra was transformed into absorbance (Figure 2): $\mathbf{Abs}_i = \mathbf{log}\frac{\mathbf{I_0}}{\mathbf{I}_i}$; where $\mathbf{Abs}_i$ is the absorbance spectra for the

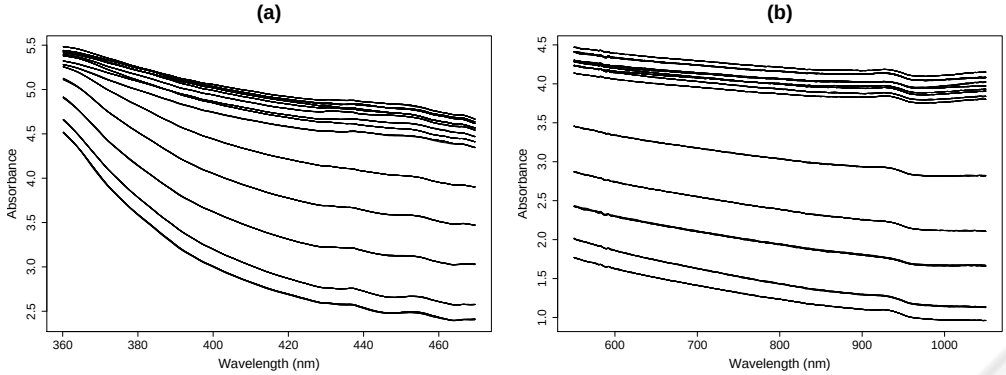

Figure 2: Absorbance spectra: (a) UV-VIS; (b) VIS-SWNIR.

different samples, $\mathbf{I_0}$ is the light spectra and $\mathbf{I}_i$ sample spectra intensities, respectively. Furthermore, small scattering artifacts were corrected using a modified robust multiplicative scatter correction algorithm (RMSC) (Martens and Stark, 1991; Martens et al., 2003; Gallager et al., 2005): $\mathbf{x}_{corr} = \mathbf{xb} + \mathbf{a} = \mathbf{x}_{ref}$. The **a** and **b** are computed by minimizing the following error: $\mathbf{e}_j = \mathbf{bx}_j + \mathbf{a} - \mathbf{x}_{ref}$; where the $\mathbf{x}_j$ is the j sample spectrum and $\mathbf{x}_{ref}$ is a reference spectrum.

2.4.2 Qualitative Spectral Analysis

It is well known that beer fermentation has: i) initial lag phase where the yeast initiates its metabolism for budding; ii) exponential phase, where the yeast reproduces at high rates increasing biomass and metabolites such as alcohol and CO_2; and iii) floculation - when the yeast forms colonies that are became denser than the wort and start to flocculate; and iv) decrease in metabolic activity. As the physical-chemical composition was followed, it is possible to perform a qualitative spectral analysis to these important steps, identifying important spectral zones which correspond to characteristic absorbance bands. Such allows to comment on qualitative increases and decreases on groups of compounds which are formed or degraded during fermentation, providing information about the wavelengths and the corresponding compounds that are suffering transformations over the process time (Ozaki et al., 2001).

2.4.3 Multivariate Regression Methods

Partial least squares regression (PLSR) is a multivariate regression method, which is used to relate multivariate data set **X** to a reference value **y** (Wold et al., 1983; Geladi and Kowalsky, 1986; Martens and Naes, 1989):

$$\mathbf{y} = \mathbf{X} \cdot \mathbf{b} + \mathbf{e} \qquad (1)$$

where, **b** represents the regression coefficient and **e** the error. Typically, **X** is a low-cost and high-output multivariate method, such as UV-VIS-SWNIR measurements, whereas **y** are often time-consuming and expensive reference methods, such as metabolomic data. The overall purpose of PLSR is to interpret the relationship between the two data sets, and being able to predict **y** in order to use spectroscopy as a software sensor. Partial least squares regression (PLSR) is a well established regression methodology applying least squares regression on a small number of orthogonal factors (the scores or latent variables) if both dependent (**Y**) and independent (**X**) variables. PLSR algorithms maximize the covariance between **Y** and **X** ($cov(\mathbf{Y}, \mathbf{X})$) (Denham, 1995; Denham, 1997; Jong, 2003). PLSR can cope with the non-linear features of spectra, because regression is performed in the latent variables obtained by orthogonal decomposition of both dependent and independent data sets (Geladi and Kowalsky, 1988).

In order to avoid over-fitting, the number of selected PLS factors was performed by using cross validation on the n-1 blocks (90% of data), and tested on the remaining block (10% of total data) the to calculate the predicted sum of squares criteria (PRESS). PRESS is obtained by $\sum_{i=1}^{n} \left(\mathbf{Y_j} - \hat{\mathbf{Y}}_\mathbf{j}\right)^2$; where $\mathbf{Y_j}$ is the data left-over from the original dataset after subtracting validation sample i, and $\hat{\mathbf{Y}}_\mathbf{j}$ the model prediction for the j sample, so that it is possible to derive for each model the PRESS criteria statistical distribution and select the minimum number of PLS factors. By performing The PLSR confidence intervals were thereafter determined according to Denham (1997), and the limit of quantification was assumed ten times the regression standard error (SE). All statistical computing analysis were performed using R (R-Project, 2008)

3 RESULTS AND DISCUSSION

3.1 Qualitative Analysis

For each fermentation sample it were determined: i) number of yeast cells; ii) Extract; iii) Sugar weight percentage in solution; and iv) pH. This results are shown on Figure 3. In the first 7h, after yeast addition, the lag phase occurs and a rapid absorption of the oxygen contained in the wort occurs. Therefore, a significant decrease in pH and absorption of free amino nitrogen, indicating the adjustment of the yeast to the nutrient medium. Followed by an exponential growth phase with rapid decrease in medium density, abundant consumption of wort sugars and consequent abundant production of alcohol, marked fall in pH and nitrogen absorption of the wort. After 50h yeast flocculation occurs, greatly decreasing the ethanol production as the nitrogen source also begins to scare. This phase continues until the end of the fermentation, whereby sugar consumption begins to decrease until it stabilizes at the 3rd day of fermentation. At the end of fermentation pH is about 3.7, the sugar weight percentage falls 50% to (from 13.4% to 6.5%) and dry extract the value of 0.05g/ml.

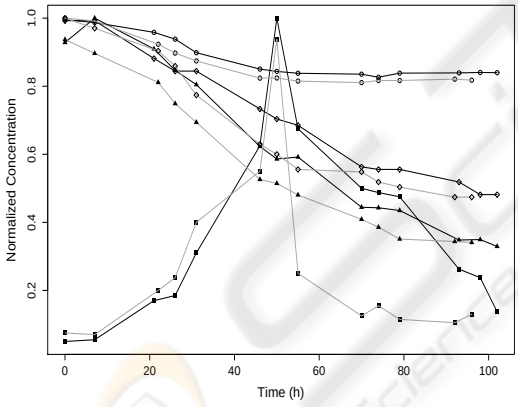

Figure 3: Normalized chemical data over the fermentation time (■ Biomass x $2x10^8$ cells/ml of wort; ▲ Extract x 0.1382 g/ml of wort, ○ pH; and ◊ sugar weight percentage in the wort x 13.5). Black lines represents Fermentation 1; and grey lines represents Fermentation 2.

The construction of a three-dimension graph of UV-VIS and VIS-SWNIR spectra (Figure 4) allows to observe that the previous phases described on the chemical results is discriminated in the spectra. We observed that there is high similarity between the VIS-NIR and UV-VIS surfaces in both fermentations, which is an indication of good process reproducibility. In each graph of Figure 4 we flagged the spectra with letters from “a” to “n” as being the different sampling times for better qualitative spectra interpretation.

The confrontation of Figure 4 information with the data contained on Figure 3 makes possible observe that: “a” corresponds to the fermentation start; from “a” to “b” there is a stabilization in both absorbance spectra indicating that lag phase is taking place; “b” to “g” corresponds to the exponential growth of the yeast, in this phase organic acids are produced and buffering compounds (basic amino acids and primary phosphates) are consumed, this phenomenon is observed in Figure 4 by an increase of the spectra absorbance; “g” point corresponds to the maximum number of yeast cells and to a peak in all 3D graphs; from point “g” to “n” the number of yeast cells decrease, which leads to a decrease of number of reactions, leading to a metabolic modification in the process, this is perfectly identified in both 3D graphs of Figure 4.

Although the different stages of beer process being well identified in both spectra regions, the UV-VIS and the VIS-SWNIR regions are quite different on its behavior. On the lag phase (points “a”-“b”) the UV-VIS spectra have a slope in the absorbance, in UV wavelengths the absorbance is more high than VIS region; while VIS-SWNIR spectra its all on its minimum absorbance. Once started the exponential phase (from point “b” to “g”) the absorbance increases in different proportions until achieve a similar level in all wavelengths; In the same phase the VIS-SWNIR spectra suffer a large increase in all wavelengths until its maximum spectra (point “g”) where the fermentation reaches the flocculation point; The following spectra (“h”) suffers a decrease in the absorbance for both spectra regions (UV-VIS and VIS-SWNIR). Then the spectra absorbance increase again until point “i” reaching the absorbance peak, after this the absorbance stabilizes in the next four points (“j”, “k”, “l” and “m”) for the entire region under study (UV-VIS and VIS-SWNIR); The end of the fermentation is identified in both spectra by a decrease in the last point (“n”) absorbance. This analysis show that UV region can be a very important region for the characterization of the compounds that are changing along the fermentation, once that is the region where the absorbance have more difference between the wavelengths over the time.

3.2 Multivariate Calibrations

Beer wort is highly dispersive and scattering artifacts are significant. The undesired scattering occurs because of the particles in wort and accumulation of solids in the dip probe. Light scattering does not

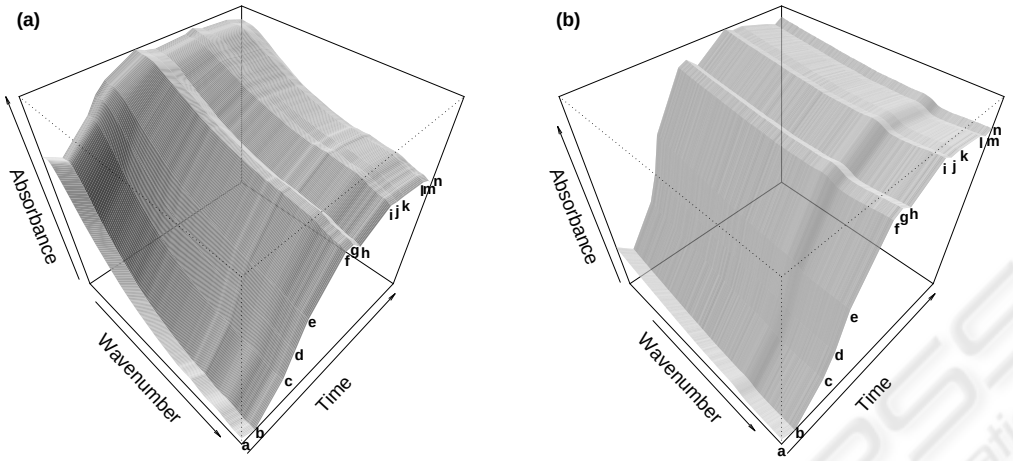

Figure 4: Spectra evolution during fermentation: (a) UV-VIS and (b) VIS-SWNIR; where: **a** - 0 hrs; **b** - 7 hrs; **c** - 21 hrs; **d** - 26 hrs; **e** - 31 hrs; **f** - 46 hrs; **g** - 50 hrs; **h** - 55 hrs; **i** - 70 hrs; **j** - 74 hrs; **k** - 79 hrs; **l** - 93 hrs; **m** - 98 hrs; and **m** 102 hrs.

Table 1: Partial least squares regression model estimates for both fermentations UV-VIS spectra.

PLS-1 Model	Data	Variance (%)	PRESS	R^2	Err
Number of yeast cells	X-block	89.34	$3.7478x10^{16}$	0.9471	$1.1782x10^{7}$
(nPC=15)	Y-block	98.47			
Extract	X-block	79.24	$3.4708x10^{-2}$	0.8632	$1.3380x10^{-2}$
(nPC=10)	Y-block	92.31			
pH	X-block	72.58	3.2430	0.8539	0.1095
(nPC=10)	Y-block	92.62			
Sugars weight percentage	X-block	79.52	235.4118	0.8565	0.9337
(nPC=10)	Y-block	92.27			

$^{(a)}$Limit of quantification = 10 x Err (10:1 Signal to noise ratio)

affect the the chemical information contained in the spectra. Neverthenless, if not corrected, it influences the interpretation of the SVD and microorganisms classification. Scattering is mostly of multiplicative matter (Gallager et al., 2005), and the robust mean scattering correction (RMSC) was able to minimize this effect. Direct comparison of the corrected spectra (figure not shown) leads to the conclusion that scattering is obtained in both light sources evenly spread over all wavelengths. As the absorbance has an extremely high variation when compared to the scattering effect, it expected that scattering even if not corrected totally, will not significantly influence most of PLS regressions. Such observation gives a good indication for fiber optics sensors robustness, which may work in very difficult conditions of bioprocesses, such as in this case with high particle densities and cellular concentrations.

PLS regression results are presented in Tables 1 and 2 and calibrations are presented in Figure 5. Calibrations were obtained using the 2nd derivate which allows the direct background and baseline correction. A direct comparison of the R^2 allows to conclude that VIS-NIR calibrations are better than the calibrations in UV-VIS spectra; all VIS-NIR calibrations have $R^2 \geq 0.95$ and only need $\simeq 58\%$ of X-block variance to explain 99% of Y-block variance; while all UV-VIS calibrations have $R^2 \leq 0.95$ and need at least 79% of X-block variance to explain 92% of Y-block variance. Furthermore, VIS-SWNIR have lower detection limits for the studied parameters than with the UV-VIS wavelengths, especially for dry extract, pH and total sugars. Nevertheless, interesting detection limits are observed for all the studied parameters: i) cell density: UV-VIS ($1.1782x10^8$ cells/ml) and VIS-SWNIR ($1.0259x10^8$ cells/ml); ii) dry extract: UV-VIS ($1.3380x10^{-1}$ g/ml) and VIS-SWNIR ($4.8024x10^{-2}$ g/ml); iii) pH: UV-VIS (1.095) and VIS-SWNIR (0.416); and iv) total sugars UV-VIS (9.337 % w/w) and VIS-SWNIR (3.565 % w/w).

Furthermore, VIS-SWNIR wavelengths allowed to obtain robust calibrations with less number of spectral decompositions. In all UV-VIS PLS regressions were obtained with 10 to 15 factors, where as, VIS-

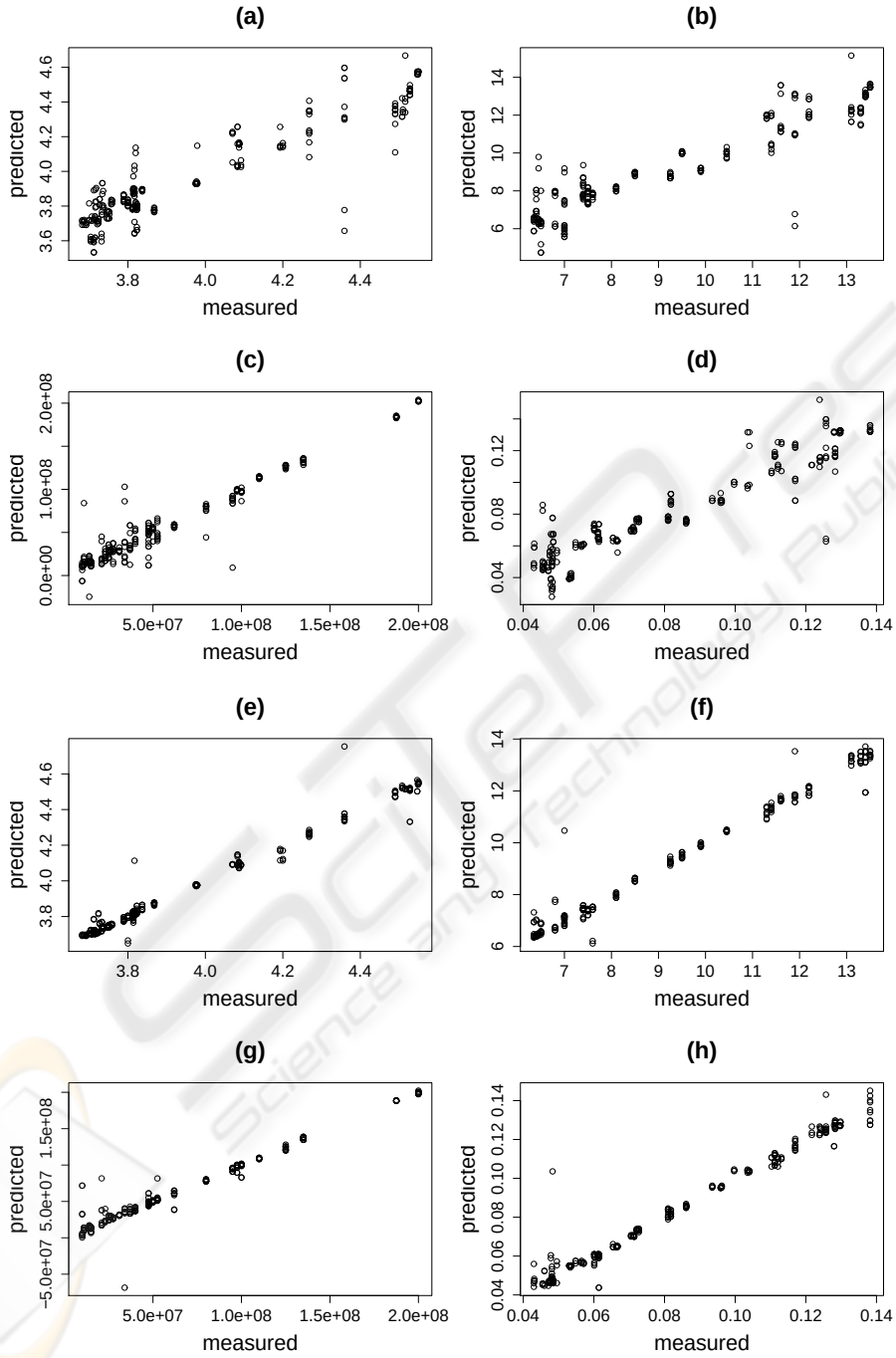

Figure 5: PLS-R Calibrations for UV-VIS: (a) pH; (b) sugar weight percentage; (c) Biomass; (d) extract; and for VIS-SWNIR: (e) pH; (f) sugar weight percentage; (g) Biomass; (h) extract.

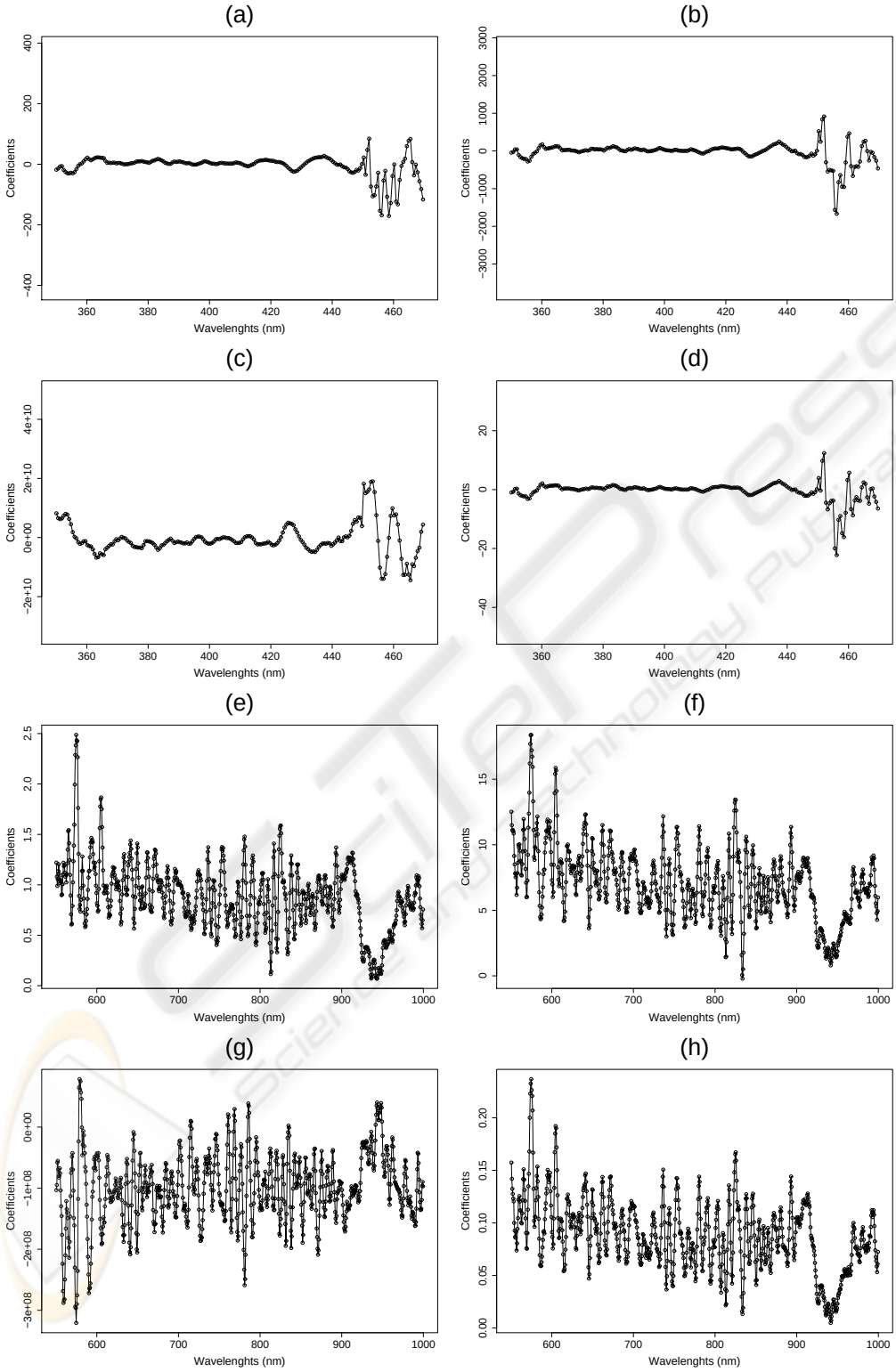

Figure 6: PLS-R Coefficients for UV-VIS: (a) pH; (b) Sugar weight percentage; (c) Number of yeast cells; (d) Extract; and for VIS-SWNIR: (e) pH; (f) Weight sugar percentage; (g) Number of yeast cells; (h) Extract.

Table 2: Partial least squares regression model estimates for both fermentations VIS-SWNIR spectra.

PLS-1 Model	Data	Variance (%)	PRESS	R^2	Err
Number of yeast cells	X-block	58.63	$2.8417x10^{16}$	0.9599	$1.0259x10^{7}$
(nPC=8)	Y-block	99.51			
Extract	X-block	58.46	$6.2269x10^{-3}$	0.9755	$4.8024x10^{-3}$
(nPC=8)	Y-block	99.48			
pH	X-block	58.43	0.4675	0.9789	0.0416
(nPC=8)	Y-block	99.63			
Sugars weight percentage	X-block	58.49	34.3200	0.9790	0.3565
(nPC=8)	Y-block	99.61			

$^{(a)}$Limit of quantification = 10 x Err (10:1 Signal to noise ratio)

SWNIR region were obtained with 8. Such discrepancy is not yet fully explainable, being only possible to affirm so far that perhaps the UV-VIS region may contain less information correlated to the studied parameters. This is not an indication that this region has less information than the VIS-SWNIR region, because absorbance in this region may be more well correlated with other chemical and biochemical compounds. Figure 6 presents the coefficients in the 1st spectral decomposition during PLSR. It is observable that the UV-VIS coefficients present higher autocorrelation than the VIS-SWNIR coefficients. Furthermore, for all the studied parameters the UV-VIS region presents the highest coefficients in the region of 420 to 480 nm. Interpretation in this region is however not straight forward, because coefficients present high fluctuations, not being possible to interpret a well defined peak. In the VIS-SWNIR region most of the coefficients are high for biomass, increasing from 600nm to 1000nm and showing a peak at 900nm. The contrary is observed for the rest of the studied parameters, where all the coefficients decrease with the wavelength, with a lower peak at 900nm. Therefore, one can conclude that higher absorbancies are generally correlated with higher biomass, with special relevance in the SWNIR region. High absorbances in this region is correlated with lower sugar, pH and dry extract concentrations.

3.3 Conclusions

This exploratory research shows the feasibility of UV-VIS and VIS-SWNIR spectroscopy for monitoring bioprocesses. Nevertheless, both experimental and signal processing techniques can be improved to take advantage of the high-output information contained in the UV-VIS-SWNIR spectra.

Improvements on the experimental methodology can improve the quality of bioreactor monitoring in the studied region of the spectra. For example, a better location inside the bioreactor can be found to place the probe, or the use of an attenuated total reflection probes can improve the signal quality in high-density wort or high number of yeast cells. Furthermore, the measurement of more detailed chemical and biochemical composition of the wort during fermentation, may help in the future the development of precision fermentation monitoring and control systems.

The data pre-treatment successfully remove the small noise and scattering artifacts present in the spectra; being possible to achieve high-quality and resolution in the final spectra before signal treatment. Improvements also should be performed in order to better understand the relationship between the chemical composition and the information contained in the spectra. Methods such as the combination of the two spectral regions using with n-way and multiblock PLS-R (e.g. UV-VIS + VIS-SWNIR spectra) as well as using wavelets or Fourier transformate for compressing and modelling the spectra may provide better interpretation of spectra variance during bioprocesses.

In the near future, these improvements on both experimental and data treatment may turn UV-VIS-SWNIR a feasible technique for high-output metabolomic studies and monitoring of Bioprocesses.

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