
# Biotechnological process monitoring using a mid-infrared fiber probe

## Authors

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Process monitoring, fiber probe, mid-infrared, bioreactor, *Saccharomyces cerevisiae*

## Goal

Develop a mid-infrared spectroscopic technique for online-monitoring of biomass, ethanol and hydrocarbons (glucose and fructose) in course of the *Saccharomyces cerevisiae* yeast fermentation process using an ATR fiber probe.

## Introduction

Most biotechnological processes commonly applied worldwide are poorly regulated in terms of their chemical analysis, even those established in the industrial sphere. In order to determine the concentrations of nutrient components of the medium, such as glucose and other sugars, as well as the most desired organic products, it is necessary to retrieve samples from the bioreactor for a conventional, e.g. chromatographic analysis. Due to the samples' complexity, inevitable periodicity and slowness of the laboratory analysis as well as high process speeds, common analytical methods are often insufficient to assure the required product quality and production efficiency [1]. Furthermore, close monitoring of various process parameters in the course of fermentation may be critical for fast counter-steering in case of a deviation from the normal process conditions.

A leading role in the development of in-/ on-line analysis in biotechnological process monitoring belongs to optical spectroscopy in near infrared (NIR) range up to 2500 nm (4000 $cm^{-1}$) [2-7] and fluorimetry [8,9]. Perfect adaptability of the optical techniques to in-line process analysis is related to the application of fiber optics and flexible probes on its basis [9].

In actual fact, the data evaluation, particularly of NIR spectra, still remains a challenge that slows down the industrial distribution of spectroscopic systems, also in biotechnology [10-13]. Recent advances of multivariate data analysis (also known as chemometrics) made an essential contribution to the development of instrumental analytical chemistry in general. Multivariate modelling is an important science-intensive part of the analytical method development for process monitoring [1]. It includes several methodologies: design of experiment (DoE) [14], exploratory factor analysis, multivariate regression algorithms, such as partial least-squares (PLS) [3] in addition to others.

The spectroscopy of mid-infrared region (MIR) 4000–400 $cm^{-1}$ (2.5–25 µm) is rarely used for in-line analysis. Its application examples in biotechnology can be found in the following references [12,15-18]. This reluctance to use the highly sensitive and chemically specific MIR region in process analysis can be accounted for by high costs of general-purpose MIR spectrometers themselves and sometimes by a demanding handling under running process conditions that could not be avoided even in commercial systems. Advantages and drawbacks of MIR compared to NIR spectroscopy are considered in [19]. Novel IR-fiber materials available nowadays cover practically the whole mid-infrared range.

Attenuated Total Reflectance (ATR) is a measurement technique that is perfectly suited for MIR analysis of different materials. The total optical path of ATR analysis is formed by a typical penetration depth of ATR crystal's evanescent field (0.5–2 µm) times the number of internal reflections defined by the ATR element geometry. Short pathways are often advantageous considering that IR absorption coefficients of most substances are much higher than e.g. in the NIR range. The most common ATR crystals are made of $ZrO_2$, ZnSe, Ge, Si and diamond. The crystal shape (prism, cone, multi-bounce plate, etc.) can be varied depending on the probe type and sample nature. Over the last few decades a number of ATR probes for MIR analysis have been developed and manufactured based on CIR and PIR fiber technology [20-22]. Current advancements of optical technologies, in particular, budget techniques for high-resolution IR-detection [23-25] create prerequisites for the broadening of the application range of mid-infrared spectroscopy and for its increasing use in industrial and field applications.

Here we report on the results of a collaborative project [26] aimed at the development of a MIR sensor analyzer for in-line determination of ethanol, glucose and fructose using the ATR probe technology. It was tested and validated on a designed sample set under the conditions of a running S*accharomyces cerevisiae* fermentation process. Simultaneously, the measurements were performed with NIR spectroscopy using a transflectance probe. Multivariate prediction models built on spectra of the designed samples measured with a diamond ATR probe coupled with a high-end laboratory FT-IR spectrometer were used as a benchmark.

## Experimental

### Designed sample sets

Two sample sets containing binary aqueous solutions ethanol–glucose (EG) and glucose–fructose (FG) were prepared by dissolving water-free pure ethanol (containing 1% of methyl ethyl ketone), glucose and fructose both obtained from Merck KGaA (Darmstadt, Germany) in de-ionized water. Each set was composed in accordance with the scheme of the so-called diagonal design [14]. It provides independent (i.e. uncorrelated) variability of both component concentrations and their uniform distribution over 25 levels of the chosen concentration ranges. The diagonal experimental design has been developed for the multivariate calibration of multi-component mixtures and includes an integrated validation subset (Fig. 1).

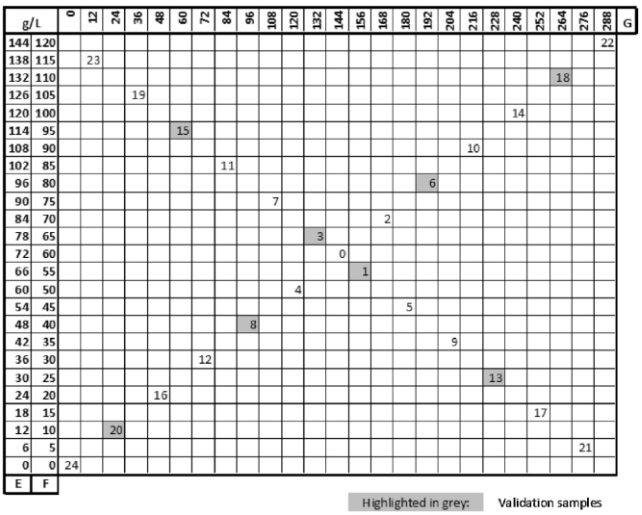


**Figure 1.** Diagonal experimental design of ethanol-glucose (EG) and fructose-glucose (FG) sample sets.

In the following text the samples are referred to by a prefix of the sample set (EG or FG) followed by the sample number indicated in the corresponding cell of the chart.

The solution samples were stored in tightly closed falcon tubes in a refrigerator at 4±1°C.

### Fermentation process

An anaerobic *Saccharomyces cerevisiae* fermentation was chosen for the testing of developed analyzers under process conditions. Two processes were carried out in a bench- scale bioreactor system with an amateur control unit provided by Ulm University of Applied Sciences.

Total durations of first and second fermentations were 5 h and 32 min and 5 h and 52 min, respectively (Table 1).

Samples were periodically retrieved from the bioreactor and glucose and ethanol concentrations were determined by enzymatic UV tests [27,28] by R-BIOPHARM AG (Darmstadt, Germany) at 340 nm using a Büchi 900 photometer (Büchi Labortechnik GmbH, Essen, Germany).

After the fermentation process completion, the resulting bioreactor broth was modified by adding of calculated quantities of glucose solution and ethanol in order to extend the content regions for a more robust calibration. The quantities of added glucose and ethanol were adjusted to minimize the correlation between the component concentrations in the whole data set. The resulting compositions of 41 process and post-process samples (Table 1) were used as reference values in subsequent multivariate calibration on spectral data.

**Probes**

Two types of ATR fiber probes by art photonics GmbH (Berlin, Germany) were used (Fig. 2). Both probes were based on PIR fibers as light guides, but used different ATR elements: a diamond crystal (hereinafter this probe is referred to as D-ATR) in Fig. 2a and detachable PIR loop (L-ATR) in Fig. 2b. The L-ATR probe avoids using any expensive materials and technologies and thus is much cheaper than the D-ATR probe.


**Figure 2.** ATR infrared fiber probes: (a) with diamond crystal and (b) with a detachable PIR loop.

For the in-line NIR measurements in the fermentation broth an Avantes transflectance dip probe (Apeldoorn, The Netherlands) with an adjustable gap was used.

The optical path length was set to the minimal value of 0.5 mm to compensate for high NIR absorption of water.

**Full-range MIR and NIR spectroscopic analysis**

To provide benchmark models for the subsequent performance testing of the developed sensor systems full-range MIR and NIR spectra of the designed EG- and FG-sample sets have been measured. MIR spectra were acquired with a MatrixMF FT-IR spectrophotometer (Bruker AG, Ettlingen, Germany) with a D-ATR probe. NIR spectra in the wavelength range 1100–2100 nm were obtained using TIDAS S-1000 MS-T50/16 by J&M Analytik AG (Essingen, Germany).

During the fermentation process NIR measurements were repeated at 5-min intervals in a time scan mode. Each time-scan measurement consisted of single spectra taken with 1-second intervals over three minutes. This acquisition technique was chosen to apply the “5%-quantile” pre-processing method [13]. Dark current and reference spectra of deionized water at 30°C were measured once right before the fermentation start.

**Table 1.** Process and post-process sample composition [26].

| Samp. No | Time h:mm | Biom. g/L | Gluc. g/L | Eth. g/L | Cal/ Val[a] | Remarks | |
|---|---|---|---|---|---|---|---|
| *Fermentation 1* | | | | | | | |
| - | 00:00 | - | - | - | - | Inoculation | |
| 1 | 0:02 | 7.39 | 67.88 | **0.28** | 0 | Fermentation | |
| 2 | 0:32 | - | 63.74 | 2.84 | 1 | | |
| 3 | 1:02 | 8.33 | 54.76 | 6.10 | 0 | | |
| 4 | 1:32 | - | 48.02 | 9.16 | 0 | | |
| 5 | 2:02 | 9.78 | 38.69 | 13.48 | 1 | | |
| 6 | 2:32 | - | 30.75 | 16.7 | 0 | | |
| 7 | 3:02 | 11.02 | 22.11 | 20.62 | 0 | | |
| 8 | 3:32 | - | 14.42 | 24.19 | 1 | | |
| 9 | 4:02 | 12.4 | 5.52 | 26.61 | 0 | | |
| 10 | 4:32 | - | 0.40 | 28.68 | 0 | | |
| 11 | 5:02 | 12.72 | 0.00 | 28.56 | 1 | | |
| 12 | 5:32 | 12.36 | **0.00** | 28.56 | 0 | | |
| 13 | 0:00 [b] | 11.92 | 0.00 | 30.75 | 0 | Add | - |
| 14 | 0:26 | 11.44 | 0.00 | 36.4 | 1 | | ethanol |
| 15 | 0:45 | - | 36.10 | 34.9 | 0 | | glucose |
| 16 | 1:02 | - | 35.50 | 42.38 | 0 | | ethanol |
| 17 | 1:37 | 10.04 | 65.12 | 39.16 | 1 | | glucose |
| 18 | 1:52 | - | 61.84 | 48.37 | 0 | | ethanol |
| 19 | 2:11 | - | 100.62 | 45.84 | 0 | | glucose |
| 20 | 2:24 | - | 97.16 | 56.44 | 1 | | ethanol |
| 21 | 2:42 | 8.125 | 135.59 | 52.98 | 0 | | glucose |
| 22 | 2:56 | - | 128.68 | 67.26 | 1 | | ethanol |
| 23 | 3:15 | **7.09** | **166.68** | 61.96 | 0 | | glucose |
| *Fermentation 2* | | | | | | | |
| - | 0:00 | - | - | - | - | Inoculation | |
| 24 | 0:22 | 7.41 | 76.35 | 2.08 | 1 | Fermentation | |
| 25 | 0:52 | 8.23 | 67.19 | 4.77 | 0 | | |
| 26 | 1:22 | 8.95 | 59.94 | 7.97 | 1 | | |
| 27 | 1:52 | 9.74 | 52.68 | 11.52 | 0 | | |
| 28 | 2:22 | 10.39 | 42.49 | 16.64 | 0 | | |
| 29 | 2:52 | 11.06 | 34.72 | 18.95 | 1 | | |
| 30 | 3:22 | 11.90 | 23.32 | 22.11 | 0 | | |
| 31 | 3:52 | 12.57 | 14.94 | 24.99 | 0 | | |
| 32 | 4:22 | 13.11 | 5.83 | 28.33 | 1 | | |
| 33 | 4:52 | **13.80** | 0.32 | 31.21 | 0 | | |
| 34 | 5:22 | 13.52 | 0.00 | 30.41 | 0 | | |
| 35 | 5:52 | 13.39 | 0.01 | 30.52 | 1 | | |
| 36 | 6:17 | 13.28 | 0.00 | 46.30 | 0 | Add | ethanol |
| 37 | 6:27 | 11.54 | 52.68 | 40.77 | 0 | | glucose |
| 38 | 6:37 | 11.80 | 45.95 | 55.74 | 1 | | ethanol |
| 39 | 6:53 | 10.84 | 104.07 | 49.99 | 0 | | glucose |
| 40 | 7:21 | 11.11 | 98.02 | **67.95** | 0 | | ethanol |
| 41 | 7:33 | 9.81 | 157.62 | 58.51 | 1 | | glucose |

*Notes: [a] Calibration and validation data subsets: 0 – calibration sample; 1 – validation sample; [b] next day morning. Minimal and maximal values of analyzed components are marked in bold.*

**Custom MIR detection systems**

In the developed sensor system a MIR spectrometric technology with pyroelectric detectors was tested [26] (supplementary materials).

The system was a grating spectrometer (GrS) built on a PY128LA line sensor by PYREOS (Edinburgh, Scotland, UK) with 128 sensing elements. The spectral region of the sensor was tuned to cover two intensive ethanol peaks between 9 and 10 µm (approximately 1100–1000 $cm^{-1}$).

**Data analysis**

Partial least-squares (PLS) regression used for quantitative modelling is a multivariate calibration method described elsewhere [29].

Prior to the ethanol, glucose and fructose calibration NIR spectra were converted into their first or second derivatives using Savitzky-Golay's [30] algorithm with second-order polynomial and a window width individually adjusted to the modelled data. This pre-processing method was found to be advantageous for the modelling of spectral data of both designed sample sets and fermentation process.

The model performance was characterized by the root mean-square error of calibration (RMSEC), prediction (RMSEP, for a validation subset) and full cross-validation (RMSECV) as well as by the respective coefficients of determination $R^2$. Further experimental details can be found in [26].

## Results and Discussion

**Mid-infrared sensor development**

Fig. 3 presents MIR spectra of EG- and FG-sets of the design samples (Fig. 1) in the full range 2999‑700 $cm^{-1}$ obtained with the "gold standard" method including the high-resolution FT-IR spectrometer and a diamond ATR element that is the most preferred material for precise analysis. PLS regression modelling and validation statistics for full-spectrum MIR data is presented in Table 1.

Generally, the spectral similarity of EG and FG samples is accounted for by their structural similarity in terms of functional groups composing the molecules of ethanol, glucose and fructose. Thus, similar broad signals located between 2800 and 3000 $cm^{-1}$ in Fig. 3a and b are basically assigned to different C‑H stretching vibrations of aliphatic groups that are present in all three components of the studied mixtures. Because of the low signal intensity and selectivity, this spectral region can hardly be useful for the present analytical purpose. The same conclusion can be made for the adjacent range from 1800 $cm^{-1}$ toward higher wavenumbers also containing minor and poorly characteristic absorption bands. Negative signals at 1570 $cm^{-1}$ are related to the water content reduction compared to the reference.

The most intense absorbance in both datasets is observed in the "fingerprint" region, hence the choice of the PIR-fiber probe. Ethanol exhibits here two distinct peaks at about 1090 $cm^{-1}$ and 1050 $cm^{-1}$ related to C‑O and C‑C stretching vibrations that are supported by a less intensive C‑H bend signal at 880 $cm^{-1}$. Glucose and fructose in this region are represented by a complex combination of poorly resolved and hardly interpretable bands (Fig. 3a). Two most intensive distinguishing peaks observed at about 1060 and 1030 $cm^{-1}$ (1057 and 1025 $cm^{-1}$ in [31]) presumably belong to C‑O stretching vibrations of fructose and glucose, respectively. This assignment follows from the experimental and calculated data reported in the literature [31,32]. In terms of quantitative analysis, it is very important that despite general similarity, ethanol, glucose and fructose have distinguishable spectral signatures clearly observed in the fingerprint region by FT-IR spectroscopy with the D-ATR probe (Fig. 3a). The region 1150‑950 $cm^{-1}$ seems to be the most suited for quantitative analysis performed in the present study.

Indeed, limiting the modelling to the chosen range does not lead to any essential loss in the model performance compared to the full-range data and in some cases even results in a reduction of the validation errors (Table 1). In general, the prediction accuracy achieved by this analytical method is to be taken as the ultimate case. RMSE validation accuracies of 4‑6 g/L and 1‑2 g/L for EG- and FG-sets respectively can be characterized as very high and suitable for a wide range of practical analytical needs. Somewhat lower accuracy in the case of EG-set, in particular for the ethanol model (RMSECV=5.79) is accounted for by the presence of an outlying sample.

The full spectral range is rarely necessary for industrial process monitoring tasks. It has already been suggested here that determination of the main nutrients and products in the course of fermentation or another biotechnological process may be based on a relatively narrow spectral range 950‑1150 $cm^{-1}$. For such a narrow spectral range, simpler grating spectrometers free of any moving parts are much more efficient and robust than FT-IR devices.

Therefore, it can be concluded, and this is confirmed by test results [26] that multivariate regression is capable of handling the reduction of spectral selectivity introduced by the downgrading of sensor parts.

The results obtained by MIR spectroscopic analysis of the same designed samples were also compared to the measurements by NIR spectroscopy. Although NIR spectroscopy does not belong to the most precise spectroscopic methods in aqueous media, its wide application to the analysis of biotechnological objects and processes [2-7] makes this comparison interesting and methodologically useful.

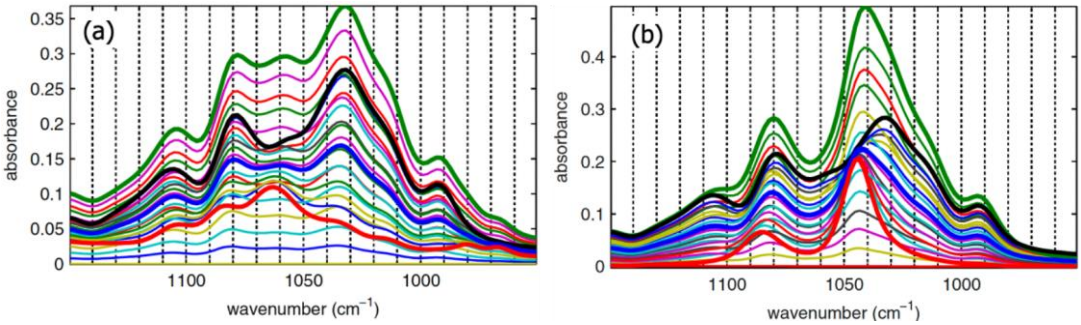


**Figure 3.** Full MIR spectra of EG- (a) and FG-sets (b). The following demonstrative samples are highlighted with a bold line and color: green – 22; black – 21; blue – 0 and red – 23 (see Fig. 1 for sample numbers).

**Table 2.** PLS regression statistics for designed EG- and FG-sets [26].

| Method and Dataset | Range | nLV[a] | Calibration | | Cross-validation | | Prediction[b] | |
|---|---|---|---|---|---|---|---|---|
| | | | RMSE | $R^2$ | RMSE | $R^2$ | RMSE | $R^2$ |
| *Ethanol (EG-set)* | | | | | | | | |
| NIR_SG2D13.2[c] | 1127–2090 nm | 3 | 3.93 | 0.992 | 5.09 | 0.987 | 3.96 | 0.990 |
| FT-IR&D-ATR | 2999–700 cm$^{-1}$ | 2 | 5.52 | 0.984 | 7.16 | 0.975 | 5.41 | 0.980 |
| FT-IR&D-ATR | 1049–951 cm$^{-1}$ | 2 | 4.55 | 0.989 | 5.79 | 0.983 | 5.10 | 0.983 |
| *Glucose (EG-set)* | | | | | | | | |
| NIR_SG2D13.2 | 1127–2090 nm | 3 | 6.29 | 0.995 | 8.06 | 0.992 | 5.79 | 0.994 |
| FT-IR&D-ATR | 2999–700 cm$^{-1}$ | 2 | 3.71 | 0.998 | 4.38 | 0.998 | 3.58 | 0.998 |
| FT-IR&D-ATR | 1049–951 cm$^{-1}$ | 2 | 5.47 | 0.996 | 6.66 | 0.995 | 5.53 | 0.995 |
| *Fructose (FG-set)* | | | | | | | | |
| NIR_SG1D15.2[d] | 1132–2086 nm | 4 | 11.99 | 0.886 | 19.47 | 0.725 | 14.57 | 0.797 |
| FT-IR&D-ATR | 2999–700 cm$^{-1}$ | 3 | 0.79 | 1.000 | 1.81 | 0.998 | 1.41 | 0.998 |
| FT-IR&D-ATR | 1049–951 cm$^{-1}$ | 3 | 0.96 | 0.999 | 1.14 | 0.999 | 1.11 | 0.999 |
| *Glucose (FG-set)* | | | | | | | | |
| NIR_SG1D15.2 | 1132–2086 nm | 4 | 12.83 | 0.977 | 20.80 | 0.946 | 15.12 | 0.962 |
| FT-IR&D-ATR | 2999–700 cm$^{-1}$ | 3 | 2.17 | 0.999 | 3.50 | 0.998 | 1.78 | 0.999 |
| FT-IR&D-ATR | 1049–951 cm$^{-1}$ | 3 | 2.35 | 0.999 | 2.92 | 0.999 | 1.82 | 0.999 |

*Notes: [a] the number of latent variables used in the PLS model; [b] validation dataset by design; [c] NIR spectra transformed by Savitzky-Golay 2nd derivative algorithm with 2nd order polynomial and window of 13 points; [d] NIR spectra transformed by Savitzky-Golay 1st derivative algorithm with 2nd order polynomial and window of 15 points*

In NIR-spectra of EG- and FG-sets of samples absorption of water strongly complicates the analysis of other constituents [26]. The main spectral variance here is due to the changing water content. This indirect correlation with dissolved component concentration is not helpful for their quantitative analysis, because their characteristic features are strongly masked with water bands making them hardly distinguishable from one another, and therefore, influenced by the dynamic range of spectral measurement. Application of spectral derivatives of second order in EG-data and of first order for FG-set serves to emphasize the chemical differences and hence to improve the model performance. An extensive pretreatment applied makes the models for EG-samples comparable to the FT-IR&D-ATR method, but at the expense of an additional LV, pointing at possible non-linearity and related robustness issues. Calibration models for the FG-set of samples (Table 1) exhibit impracticable performances (RMSECV about 20 g/L at four LVs) evidencing the failure of the NIR system to distinguish glucose and fructose from their fine spectral differences in the presence of dominating water.

The above observations evidence that ATR MIR spectroscopy is generally preferred to NIR transmittance (transflectance) spectroscopy in the analysis of ethanol, glucose and fructose at concentrations that typically occur in the fermentation processes.

ATR probe with a PIR fiber loop as a measurement unit is an important component of a sensor system. The previous publication on the probe development [22] is mainly devoted to a discussion of signal strength and sensitivity of differently shaped loops. The ATR probe design described in the present work is of particular interest for modern biotechnology. Its detachable head allows using the ATR element in disposable bioreactors as a small flange mounted in the reactor wall. By itself, the present

combination of a fiber-based ATR probe with an infrared sensor is a new type of the process analytical device. The probe with a PIR-loop element is three to five times cheaper than a diamond probe at the same or similar signal performance.

**In-line monitoring of a fermentation process with NIR and MIR sensors**

A previously developed sensor system [26] based on a grating spectrometer with a PIR-loop ATR probe, was tested under the conditions of a running *Saccharomyces cerevisiae* fermentation process. In parallel, NIR-spectra were acquired using a transflectance probe. Two fermentation processes were analyzed. Along with the spectroscopic analysis, 41 samples were taken from the fermentation broth in the process course and during its post-process modifications (Table 1). This data was used to build predictive models for ethanol, glucose and biomass content. Both traditional NIR analyzer and developed MIR sensor show satisfactory validation statistics. Generally, the accuracy of ethanol and glucose prediction are somewhat lower than those of laboratory analysis (Table 2; $R^2_{CV}$ values should be compared because of the difference in concentration ranges). PLS models were validated using both full cross-validation and prediction on a subset of 15 process samples, basically. The sequence of test samples was selected and slightly modified in order to span the component concentration ranges adequately but avoiding their minimum and maximum values (Table 1). The analysis has shown that PLS successfully captured both in- and inter-process variability.

The models for ethanol and glucose included an additional LV, compared to the designed EG-set and to the models based on a single fermentation process, which is an expected complication at the model transfer into real-life conditions. General consistency of different modelling and validation statistics to each other (cross-validation $R^2_{CV}$ of 0.972 and 0.962 for ethanol and glucose, respectively [26]) and to previously reported analysis of designed EG-mixtures (Table 2) supports our conclusion about feasibility of using the developed low-range MIR analyzer with a loop ATR probe for process monitoring in biotechnology as a viable alternative to relatively well established NIR spectroscopy (Fig. 4).

The ATR spectra have no sensitivity to the biomass content because of IR-light's low penetration depth into the sample. The biomass prediction model based on NIR spectra is reasonably simple (only two LVs) and the accuracy it provides (RMSECV=0.47 and $R^2_{CV}$=0.946) evidences of the method's practical usability, e.g. as an additional option at in-line analysis of components dissolved in the fermentation broth.

In general, the budget combination of the grating-based PYREOS sensor with the ATR loop-probe still loses the accuracy competition to the more traditional wide-range NIR spectroscopy, especially in the case of glucose analysis. However, the much lower price of the MIR sensor makes the methods competitive in a practical sense, in particular, considering that the technical advancement of the MIR sensor technology is only beginning. A convincing example of this kind is a fast and super sensitive NLIR spectrometer for mid-IR, which has recently appeared on the market [33]. The ability of budget ATR sensors to distinguish different hydrocarbons in a typical fermentation mixture is another significant advantage compared to traditional NIR-spectroscopy method.

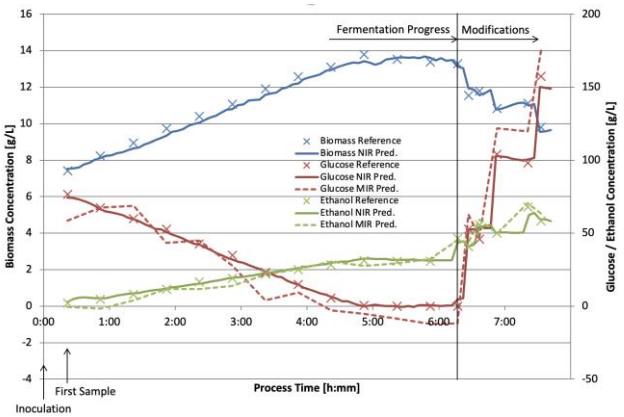


**Figure 4.** Biomass (blue), glucose (brown) and ethanol (green) concentration profiles during the fermentation process and post-process modifications.

## Conclusion

Functioning prototype of a mid-infrared sensor systems, consisting of a simplified spectrometer equipped with an ATR probe, for simultaneous determination of ethanol, glucose and fructose (and potentially, other carbohydrates) have been developed, and their prediction performances were critically assessed in a systematic fashion.

The technological simplification and hence price reduction of the developed sensor systems compared to the full-range laboratory MIR or NIR spectroscopic analysis was achieved at the expense of spectral resolution reduction and by limiting of the wavelength range to a narrow interval containing the most informative responses of the studied components. This modification has been possible due to the application of up-to-date pyroelectric detectors. Additionally, the application of PIR fibers and ATR probes on their basis enables the application of MIR sensors for in-line process monitoring in the biotechnology.

The sensor prototype developed in the present study can be transformed into a full-featured analytical system capable of solving a wide range of analytical problems in biotechnological process monitoring. The methodical scrutiny, with which this investigation was performed here, provides a proof of general feasibility of suggested approach to the MIR sensor development on the basis of up-to-date detection and light guiding technologies.

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