



Short Communication

FT-IR/ATR univariate and multivariate calibration models for *in situ* monitoring of sugars in complex microalgal culture media

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HIGHLIGHTS

- A new FT-IR/ATR technology is proposed for monitoring sugars in biological cultures.
- A univariate model allows quick and easy estimation of the sugar concentration.
- A multivariate model allows estimation of the proportion of each individual sugar.
- The presence of microalgal cells had no significant effect on the measurements.
- The approach was validated on a microalgal culture and complex media formulation.

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ABSTRACT

The objective of this work is to develop a quick and simple method for the *in situ* monitoring of sugars in biological cultures. A new technology based on Attenuated Total Reflectance–Fourier Transform Infrared (FT-IR/ATR) spectroscopy in combination with an external light guiding fiber probe was tested, first to build predictive models from solutions of pure sugars, and secondly to use those models to monitor the sugars in the complex culture medium of mixotrophic microalgae. Quantification results from the univariate model were correlated with the total dissolved solids content ($R^2 = 0.74$). A vector normalized multivariate model was used to proportionally quantify the different sugars present in the complex culture medium and showed a predictive accuracy of >90% for sugars representing >20% of the total. This method offers an alternative to conventional sugar monitoring assays and could be used at-line or on-line in commercial scale production systems.

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1. Introduction

Industrial microalgae cultivation for the production of valuable compounds has tremendous potential and offers many advantages over yeasts and bacteria. In particular, the endogenous ability of these photosynthetic microorganisms to produce high-value chemicals for pharmaceutical and biofuel applications (Spolaore et al., 2006). However, this potential is still largely under study and numerous cultivation processes are actually under development (Bumbak et al., 2011). Some of these processes include a mixo/heterotrophic cultivation stage where a dissolved organic carbon (DOC) source is added to the culture medium, in order to enhance productivity. Such DOC sources may even originate from

municipal or industrial waste (phytoremediation) or from low-cost residual matters (Liang et al., 2010; Mitra et al., 2012). As in the case of other microbiological cultures, process operation and optimization are largely dependent on the availability of efficient analytical tools and monitoring methods, ultimately leading to industrial applications.

The methods currently available for the tracking of dissolved sugars during microbial cultivation, such as gas and liquid chromatography (GC and LC) or colorimetric methods are time and energy consuming. Moreover, none of these methods are readily applicable for the on-line monitoring of sugars in biological cultures. Infrared (IR) spectrophotometry presents a simple and quick alternative, particularly in combination with Attenuated Total Reflectance (ATR) technology (Harrick, 1960; Hashimoto and Kam-eoka, 2000). Until recently, this technology was limited to offline measurements, as the samples were to be taken from the culture and introduced in a compartment of the spectrometer before the quantification would be performed. The recent advent of external

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probes in combination with optic fibers (1–4.5 m in length) now makes it possible to monitor the fate of various analytes *in situ* during laboratory or industrial scale biological/chemical processes.

In our preliminary work, *Scenedesmus obliquus* cultures productivity was shown to be highly stimulated by the addition of cheese whey permeate (WP) in the culture medium. WP is an abundant source of saccharides, mainly lactose (>95%). Monosaccharides like galactose and glucose and other oligosaccharides (3–10 monomers) are also present in small amounts (less than 3% of total sugars) in bovine milk and whey (Barile et al., 2009). Such experimental conditions are thus well adapted to test the potential of FT-IR/ATR technology for sugars monitoring in microalgae cultures (Solis-Oba et al., 2011). Our specific objectives were to (1) determine whether the presence of living cells in the medium influences the readings of the ATR probe and (2) monitor the sugars in a mixotrophic microalgae culture using FT-IR/ATR spectroscopy. We tested the hypothesis that univariate and multivariate calibration models built from the spectra of pure sugar solutions could be used to monitor the sugar composition of a complex culture medium.

2. Methods

2.1. FT-IR measurements

FT-IR absorbance spectra were acquired with a Bruker Matrix MF spectrometer equipped with an ATR diamond probe and a 1.5 m AgX optic fiber. All spectra were measured in the range between 4000 and 600 cm^{-1} , at resolution of 4 cm^{-1} and using 32 scans. Distilled water was used as background and to clean the diamond probe between each sample. All measurements were realized at room temperature. The data was treated with the OPUS 6.5 software.

2.1.1. Univariate calibration model

The calibration curve was realized using 8 different concentrations of pure D-lactose monohydrate dissolved in distilled water (5, 10, 20, 30, 40, 50, 60 and 70 g L^{-1}). Spectra were the average of three separate replicates ($n = 3$). Quantification of the lactose concentration was done from the integration of the absorption bands 1186–933 cm^{-1} . Integration represents the area above a line drawn between the intensity values of the frequency limits specified.

2.1.2. Multivariate calibration model

Duplicate spectra of 46 aqueous standard solutions (e-supplement) made from various mixtures of pure D-lactose, D-glucose and D-galactose (Sigma–Aldrich) were obtained with proportions varying from 0% to 100%, 0% to 50% and 0% to 50%, respectively. In these samples, the total sugar concentration was kept constant at 100 g L^{-1} . These spectra were used to build the partial least square (PLS-1) regression model (see Section 3.2).

2.2. Experimental procedure

The experiment was conducted in triplicate ($n = 3$) and lasted for 13 days. At day 0, media (74 mL) were inoculated with a green microalga *S. obliquus* (CPCC 5) culture in exponential growth phase (6 mL) for an initial volume of 80 mL at an initial cell concentration of 1×10^6 cells mL^{-1} in 250 mL culture flasks. Initial composition of the complex culture medium was 60% (v/v) Bold's basal medium (BBM) + 40% (v/v) cheese whey permeate (WP). BBM (Stein, 1973) was heat sterilized (121 °C, 15 min), while WP was sterilized by filtration (0.2 μm). Cultures were kept on an orbital shaker at 120 rpm (C1 platform shaker, New Brunswick Scientific, Edison,

NJ, USA) at 22.5 °C under constant illumination and light intensity of 100 $\mu\text{E m}^{-2} \text{s}^{-1}$, as measured at the surface of the flasks using a Q201 quantum radiometer (Macam Photometrics Ltd., Livingston, Scotland). All manipulations were realized in a laminar flow hood and axenic culture conditions were confirmed by flow cytometry (Epic Altra, Beckman Coulter Inc., Fullerton, BC, CA) at the beginning and end of each experiment.

Medium sugars and total dissolved solids content (DSC) were analyzed daily by sampling 5 mL of culture. Triplicate FT-IR/ATR spectra were acquired directly for each sample, then centrifuged at 1500g for 5 min and another triplicate FT-IR/ATR spectra was acquired. Fixed volume (4 mL) of this supernatant was taken and weighed. The culture medium sample was then freeze-dried, and proportion of the total DSC determined (mg g^{-1} of culture medium). The weight average of all 4 mL samples was 4.07 ± 0.04 g for a culture medium density of 1017 ± 11 g L^{-1} . Total DSC (in mg g^{-1}) was converted in g L^{-1} by multiplication of culture medium density.

2.3. Sugar analysis using the GC–MS method

Total DSC were silylated prior to gas chromatography. Lyophilized material (3 mg) and arabinose (1 mg) were dissolved in anhydrous pyridine obtained by distillation over calcium hydride (0.3 mL), and were treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.3 mL) containing 1% (v/v) of chlorotrimethylsilane under nitrogen atmosphere. The solution was heated to 70 °C during 4 h. The solution was then allowed to reach room temperature and was evaporated to dryness with a nitrogen flow. The resulting white solid was dissolved in hexane (4 mL) and the solution was filtered before analysis by GC–MS (Agilent Technologies, GC model 6850 series II and MS model 5975B) equipped with a HP-5MS capillary column (30 m $\times$ 250 μm $\times$ 0.25 μm film thickness) with 5% phenyl methyl siloxane. Initial oven temperature was 80 °C for 5 min and was increased at a rate of 4 °C min^{-1} until it reached 290 °C followed by a post run at 300 °C. Injector temperature was 250 °C and a constant helium flow of 1.2 mL min^{-1} was used. A volume of 1 μL of sample was injected. Silylated arabinose was utilized as internal standard. Commercial sugars were used for calibration after silylation as described above.

2.4. Statistical analysis

A repeated measures ANOVA with time between days 0 and 7 as repeated measure was performed to determine differences between sugar concentrations obtained with or without microalgae cells (before and after the centrifugation). The normality was verified by a Shapiro–Wilk test and the variances homoscedasticity through direct observations of residuals using the expected normal probability plot. Analyses were carried out using the software R (version 2.15.1).

3. Results and discussion

3.1. Sugar concentration monitoring using the univariate model

As lactose is the major sugar (>95%) present in our complex culture medium, the linear regression made from the integration of the region between 1186 and 933 cm^{-1} obtained from the IR spectra of pure lactose aqueous solutions (Fig. 1) was used as univariate model for the daily monitoring of the sugar concentration during the first 7 days of microalgae cultivation. Relative intensities and wavenumbers of the C–O, C–C and C–OH stretching bands (1186–933 cm^{-1}) were unchanged during this period of time (e-supplement).

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