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Review

The potential of mid infrared spectroscopy (MIRS) for real time bioprocess monitoring

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Abstract

The need for effective bioprocess (fermentation) monitoring is growing in importance due to the rapid pace of change in the fermentation industry, and attendant financial pressures. Vibrational spectroscopy has shown great promise in bioprocess monitoring. In particular, recently attention has been focused on the capability of mid infrared spectroscopy (MIRS) to monitor multiple analytes in highly complex fermentation fluids. The potential of this powerful analytical technique is critically evaluated by discussion of relevant studies. The advantages and limitations of MIR are discussed in the context of "rival" technologies, such as near infrared, focusing especially on employing such techniques in bioprocesses for real time (either *in situ* or *ex situ*) measurements. The potential barriers to the development of MIRS for real time monitoring are identified and further research directions highlighted.

Keywords: Bioprocess monitoring; Fermentation; Mid infrared spectroscopy

Contents

1. Introduction

The past decade has been a period of unparalleled change and development in the fermentation industry. As the nature of fermentation industry evolves, and in particular, with the increasing prominence of the new biopharmaceuticals (therapeutic proteins, diagnostic enzymes and monoclonal antibodies) the need for effective bioprocess monitoring grows in importance [1]. In order to deliver the "revolution in clinical medicine" promised by this new range of therapeutic agents, there is a pressing need to develop effective bioprocess monitoring techniques/technologies which can deliver robust, reproducible, stable manufacturing processes, and high quality data, not just about product levels but also about product authenticity and purity. This latter point is especially important, since the new biopharmaceuticals can often exist in many different versions or variations of a molecule (e.g. different glycoforms, different folding patterns).

The characteristics of the "ideal" bioprocess monitoring technology are usually accepted as including the following: rapid, non-destructive, generating multi-analyte data, operable

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in (near) real time, capable of automation, robust, sensitive, and the data should be amenable to integration with information from other sensor types [2].

At first sight, few technologies seem capable of delivering all of these characteristics of the "ideal", however, perhaps vibrational spectroscopy is the technology, which most closely approaches the ideal. Both near (NIR) and mid infrared (MIR) spectroscopy offers simultaneous, multi-analyte information within minutes (less than 2 usually), and since information about all analytes in the fermentation fluid is usually included in the spectra, potentially a "metabolic snapshot" is achievable using these technologies. However, the two techniques clearly diverge in the areas of sensitivity and accuracy and in terms of ease of use *in situ* [3].

However, from first principles, discussed in more detail below, MIR should be a more sensitive technique than NIR. This has very significant implications for the use of MIR in bioprocess monitoring.

1.1. Bioprocess monitoring

Although all modes of bioprocessing, batch, fed-batch and continuous processes are in current industrial use, most industrial fermentation processes are fed-batch. The typical fed-batch bioprocess usually involves very close control of one or more nutrients at very low (limiting) levels. This is also characteristic of chemostat continuous culture systems. This is an area where the much stronger absorbances in the MIR spectra may provide much more useful information than the correspondingly weaker NIR absorbances. NIR generally struggles in very complex matrices to generate useful information about analytes in sub $g\,L^{-1}$ levels [4].

Similarly, information about product identity or authenticity is potentially more readily derived from MIR spectra than from NIR. For these reasons, and because of recent advances in both hardware (optics and probe technologies) and software systems (chemometrics principally, but also instrumental operating system software) MIR is rapidly gaining new applications in areas of bioprocessing [1,5]. The technical details of these developments, and their impact upon the use of MIR in bioprocessing, are discussed in more detail below.

2. Theory and instrumentation

In order to understand the origin of MIR spectra to be able to interpret it and to implement MIRS as an important tool in analytical model development, one should be familiar with the fundamentals of vibrational spectroscopy. Although there are a number of excellent textbooks on this area, Chalmers and Griffiths [6] compendium is an accessible, useful introduction.

Vibrational spectroscopy deals with measurements in the infrared region of the electromagnetic spectrum. The infrared (IR) region can be divided into NIR (750–2500 nm), MIR (2500–40,000), and far-infrared (FIR) (40,000–60,000 nm) zones. MIR spectra originate from transitions between quantised vibrational energy states. Several organic and inorganic compounds have spectral signatures in the IR region, as a result of molecular vibrations. In comparison to the NIR spectrum, the corresponding MIR spectrum of a sample exhibits a high degree of spectral resolution; as a result, peaks can be effectively assigned to specific chemical entities or individual product constituents [7]. Application of the MIR to quantify multiple components in aqueous solutions has not been common until recently, for a number of reasons, such as very pronounced water absorptions, laborious sample preparation involving Nujol mulls or potassium bromide (KBr) disks, and instrumentation that was designed more for laboratory analysis than for "on-line" measurements [2]. The development of optical conduit/fibre optic probes, coupled with developments in electronics and mathematical/computational techniques is making measurements "on-line" [8], even in aqueous solutions, feasible.

Problems associated with signal stability, and measurement noise encountered in employing conventional MIR for process measurements have been minimised by the application of Fourier transformation (FT). FT-IR spectrophotometers using interferometers, rather than conventional monochromators, are useful in measuring concentrations of components in complex mixtures such as the typical fermentation fluid [7]. The advent of attenuated total reflectance (ATR) technique has liberated users of MIRS from laborious sample preparation techniques for obtaining high-quality spectra from aqueous samples with multiple analytes present [9].

However, before the development of the ATR sampling accessory, the strong absorption coefficient of mid-IR, required the material of interest to be diluted in another infrared-transparent material, such as KBr or Nujol, before making a transmission measurements. With the development of FT-IR and the ATR sample presentation accessory, it is now much easier to acquire the MIR spectra of multiple compounds in aqueous mixtures.

2.1. Fourier transform infrared (FT-IR) with attenuated total reflectance (ATR) sample presentation accessory

FT-IR spectrometers along with ATR sample presentation accessory have revolutionised the use of MIR spectroscopy, and have allowed mid-IR spectroscopy to be used in many areas that are difficult to analyse by other instruments. FT-IR is a powerful tool for identifying types of chemical bonds in a molecule by producing an infrared absorption spectrum similar to a molecular "fingerprint" [10].

The basic configuration of an FT system is simple, and consists of the following components: a radiation source, interferometer and detector [11]. A simplified layout of a typical FT-IR spectrometer is illustrated in Fig. 1.

The technique of ATR was developed by Harrick [12] and Fahrenfort [13], respectively. ATR is based on the transmission of IR radiation through a crystal, which is in contact with the sample as observed in Fig. 2 [14]. The effective pathlength experienced by the light depends on the probe geometry, the refractive indices of the ATR element and the sample, the number of internal reflections and the wavelength of light being considered [15]. ATR requires little or no sample preparation for most samples, and is one of the most versatile sampling