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The Potential of Mid- and Near-infrared Spectroscopy for Reliable Monitoring of Bioprocesses

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Abstract

The processes involved in bioreactors are complex and a detailed monitoring of the chemical composition of gaseous, liquid and solid matter involved in the bioprocess is of crucial importance for efficient operation. However, only a few parameters can be reliably monitored in real time with commercially available instrumentation, but there is a growing demand and need for effective and efficient process monitoring tools. Infrared (IR) spectroscopy has proved to be a powerful method for the study of various biomedical and bioanalytical applications. In this paper, applications of this analytical method for bioprocess monitoring have been discussed by reviewing the relevant literature from the last ten years. Furthermore, recent advances in our laboratory in the field of bio-reactor monitoring by use of mid-IR attenuated total reflection (ATR) spectroscopy and the development of mid-IR sensor systems for continuous biofluid monitoring based on transmission spectroscopy have also been addressed.

Keywords

Infrared spectroscopy, Bioprocess monitoring, Near-infrared spectroscopy, Mid-infrared spectroscopy

Introduction

Conventional pharmaceutical manufacturing involves the production of chemicals using batch processes, followed by laboratory testing of sampled specimens to analyse or verify their quality (1). The same strategy is also applicable to the food processing industry. A high efficiency in bioprocess operation can be achieved by careful monitoring and control of the nutrient concentrations, environmental factors and concentrations of products that inhibit the growth of the microorganisms within the reactor. For this purpose, several off-line or at-line monitoring systems based on gas chromatography, liquid chromatography and chemical or electrochemical biosensors have been developed (2). However, the bottleneck in biotechnological process control is the on-line measurement of process variables, by which a high level of quality, less end product variability and decreased production costs can be achieved. For such purpose, several spectroscopic methods have been developed and recent textbooks can provide the reader with a splendid overview on process analytics (3 – 5).

To support the innovation and efficiency in pharmaceutical research and development, manufacturing, and quality assurance, the Food and Drug Administration (FDA) in USA has proposed a scientific regulatory framework, known as Process Analytical Technology or PAT (6). The PAT initiative had recognised important tools that can be used for effective and efficient information acquisition to facilitate process understanding, continuous improvement, and development of risk-mitigation strategies. These include: 1) multivariate tools for design, data

acquisition and analysis, 2) process analysers, 3) process control tools and 4) continuous improvement and knowledge management tools. Detailed information on these subjects is beyond the scope of this article, but can be found elsewhere (6). However, an important aspect is the *off-line*, *at-line*, *on-line* or *in-line* measurement of the biological, chemical or physical process variables.

Off-line or at-line measurements are often using expensive, time consuming, error prone and laborious methods and require that the sample is removed, pre-processed and analysed at a separate laboratory or in close proximity to the process stream. On the other hand, most in-line or on-line process analysers are reliable, time saving and economical tools. The in-line system is an integrated real-time measurement device without sampling, but the process stream may be disturbed. In the on-line measurement system, the sample can be diverted from the manufacturing process and may be returned to the reactor. In recent years, infrared (IR) spectroscopy has gained prominence for such applications in the pharmaceutical or food processing industries. In this article, we review the state-of-the-art of the application of IR spectroscopy for bioreactor monitoring by assessing the published literature from the last ten years.

Infrared spectroscopy

IR-spectroscopy, either in its near-infrared (NIR) or mid-infrared (MIR) variant, is a mature analytical spectroscopic method, which is widely used by the analytical chemists for structural, qualitative and quantitative analysis (7, 8). While NIR spectroscopy, covering the spectral interval of 12000 – 4000 cm^{-1}, is mainly based on the overtones and combinations of the molecular vibrations of CH, NH, and OH groups of the molecules, MIR-spectroscopy (4000 – 400 cm^{-1}) provides important information on the fundamental vibrations of the molecules. The MIR fingerprint region (1300 – 900 cm^{-1}) in particular has significant importance with respect to continuous monitoring of the chemical components of interest.

These vibrational spectroscopic methods are flexible and can be used for the analysis of gaseous, liquid, solid or dry-film measurements. The industrial applications of this spectroscopy include raw material identification, formulations, in-line applications, blending applications, reaction monitoring etc. (8, 9). It has also been widely tested for clinical applications (8 – 11), for example, in biofluid characterisation, cancer diagnosis and therapy monitoring, continuous glucose monitoring, DNA structural elucidation etc. In this review, its application for bio-reactor monitoring is discussed by focusing mainly on MIR-spectroscopy.

The typical IR-measurement set-up for bio-reactor monitoring applications consists of a Fourier-Transform IR-spectrometer, a fibre-optic accessory containing radiation illumination and detecting fibres, and a computer (Fig. 1). The radiation is brought to the sample from a broad-band thermal, LED, or laser source via radiation wave-guiding fibres. Overviews on a multitude of options with different fibre-optic probes have been recently published (12, 13). For remote NIR spectroscopy, fused silica fibres have been routinely used with low OH-groups in the core material, which is not so relevant for the UV/Vis spectral range. For the MIR spectral range chalcogenide glasses are transparent between wavelengths around 4 to 11 μm, however the material has a problem with toxicity. The other materials preferred by us are polycrystalline silver halide mixtures of mainly chloride and bromide (14). Another alternative is the use of hollow optical fibre probes, which have been recently described by Kino and Matsuura for remote sensing applications (15).

With the use of fibre-optic probes, absorption measurements are usually carried out.

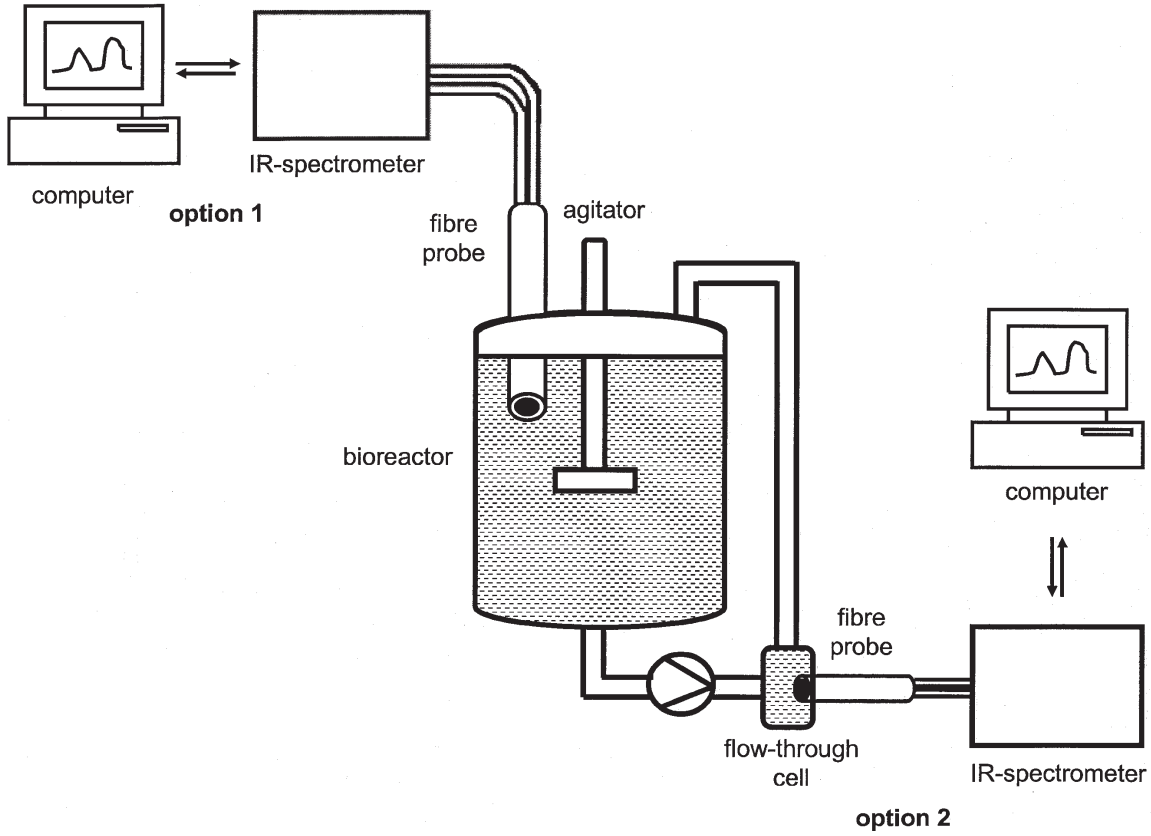

Fig 1: Schematic diagram of an IR-spectroscopic measurement system and its application in bioprocess monitoring.

The molecules within the sample absorb radiation at specific wavelengths, and the photons that are transmitted or diffusely reflected back from the sample are collected by the detecting fibres and will be forwarded to the spectrometer for analysis. While for NIR applications diffuse reflection and transmission modes of measurement are widely applied, the attenuated total reflection (ATR) technique has been commonly used for MIR-measurements. The advantages of fibre-optic probes include their inertness and optical stability to allow also high temperature applications as required for sterilisation.

Another promising option is based on exploiting the opportunities existing with today's microfluidics technology. A fluidic system can be used to load the sample, e.g., into a flow-through micro-transmission cell, which can be arranged within the conventional spectrometer's sample compartment. Microdialysis technology can also be implemented to simplify the sample matrix as the microdialysis process excludes molecules that exceed a certain threshold size, depending on the membrane characteristics. Recently, we have developed such a technology for continuous biofluid monitoring using the transmission technique (for the schematics, see Fig. 2), which can also be used to monitor cell-culture media or fermentation bioreactor broths (16 – 19).

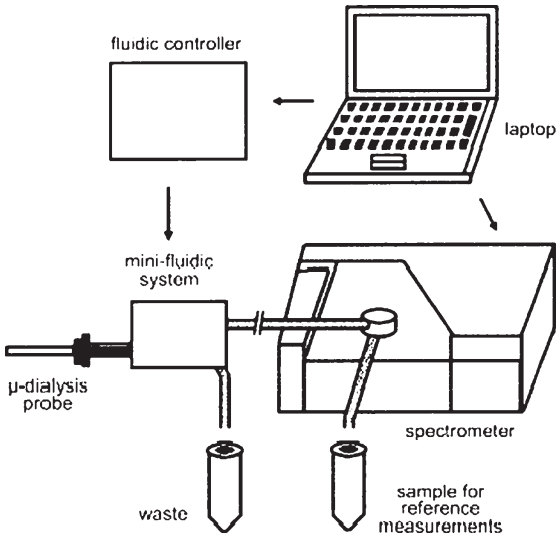

Fig 2: Schematics of a microdialysis-based MIR set-up developed for continuous or quasi-continuous online biofluid monitoring.

Chemometrics

Chemometrics is the discipline associated with the application of mathematical and statistical methods to chemical measurements. IR-spectroscopy has always been used in combination with chemometrics based on multivariate techniques covering a broad spectral interval, unless single isolated absorption bands are traceable, for which straight-forward photometry is available. For less complex mixtures to be analysed, band heights or integrals can be used. Such strategy could be applied for a volatile fatty acid assay applied for fermentation monitoring, preferably in combination with simple spectral pre-processing or disturbing component spectrum subtraction. For other applications with band overlap from other components multivariate techniques are indispensable.

In general, chemometrics will improve and enhance the understanding of the spectral information (20), giving much better opportunities also for outlier detection. While partial least squares (PLS), classical least squares (CLS), linear regression and principal component regression (PCR) have been implemented routinely for analyte quantification, spectral processing / data pre-treatment tools such as base-line correction and derivation are still used for improving the accuracy of the evaluation process.

Spectral processing

Spectral variations due to light scattering, pathlength variations, baseline drift, wavelength shifts, effects from detector non-linearity or stray light, or noise from the detector – rarely nowadays from amplifier and analogue-digital converters - have been often observed in IR-spectroscopic measurements. Spectral processing techniques are normally used to nullify some of these effects and are widely applied for clinical chemistry applications. However, in the field of bio-process monitoring only few papers (21 – 28) have reported their usage. These include application of baseline correction to the raw spectral data, or generation of first and/or second derivatives of the original spectral data. Through baseline correction the random offsets in spectra can be eliminated by shifting each spectrum, so that the signal value at a given wavelength becomes constant for all spectra.

Derivatives of spectral data are used to remove or suppress constant background signals and to enhance the visual resolution of the spectra. A constant background can be removed by transforming the original spectra into first derivative spectra, while the linear background can be removed by calculating second derivative spectra. However, as derivation amplifies the spectral noise, it is necessary to implement some smoothing into the derivation step. The most widely used algorithm for derivation and smoothing is the Savitzky-Golay algorithm.

Spectral evaluation

PLS or PCR is the most commonly applied tool for the quantitative evaluation of the analyte concentrations in bio-reactor monitoring. Both of these methods make use of an inverse

calibration approach, where it is possible to calibrate for the desired component while implicitly modelling or accounting for the other sources of variance. Both are multivariate techniques and a critical analysis of these methods for bioreactor monitoring applications have already been discussed elsewhere (29 – 31). Principal component analysis (PCA) involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called *principal components*. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. A PLS model will try to find the multidimensional direction in the *spectral* space that explains the maximum multidimensional variance direction in the *concentration* space. Alternatively, CLS can also be used, in which the measured spectra are modeled by individual component spectra. Nevertheless, the goal is to build a good calibration model by the use of *a priori* knowledge. A recent paper describes a simplified calibration technique that is based on a library of pure component spectra, assuming the underlying additivity of absorbances from the various components, as well as the linear relationship between absorbance and concentration as substantiated by Beer's law (28).

NIR-spectroscopy applications

Current status, trends and applications of NIR-spectroscopy (NIRS) in bioprocess monitoring were recently reviewed in detail by others elsewhere (29, 30). Therefore, less emphasis was given by us on this aspect, but the different applications are presented in Table 1 (21,25, 32 – 47). They can be mainly classified into two types: cell culture monitoring and biowaste degradation (biogas production in anaerobic digesters). Monitoring and determination of nutrient uptake (mainly glucose or lactate) and metabolite production (mainly ammonia or glutamate) in cell cultures, and monitoring the dynamics of volatile fatty acids (mainly acetate and propionate) during the biogas production are the widely applied areas.

Almost all authors reported satisfactory or identical performance of the spectroscopic method in the laboratory or industrial pilot studies when compared to the corresponding reference assay technologies. The standard errors of predictions tested for a wide range of concentrations for the different applications presented in Table 1 were as follows: for glucose 0.2 – 3.6 g/L; lactate 0.1 – 2.4 g/L; acetate 0.28 – 0.57 g/L; ethanol 5.0 %; biomass 0.2 – 1.2 g/L; glycerol 6.2 %; ammonia 0.036 – 2.1 mM; glutamine 0.3 – 1.1 mM; propionate 0.53 g/L; alanine 1.4 mM; and leucine 0.31 mM.

The advantages of NIRS include speed, high precision, online monitoring, low cost equipment, well developed accessories, absence of sample preparation or pre-treatment and simultaneous multi-analyte detection. In addition to these, an economical benefit of implementing the NIRS technology for online bioprocess monitoring has also been demonstrated by Ward et al. (48). Cost reduction by $ 500, 000 was estimated due to improved process stability and recovery when the on-line NIRS control system for water was applied for six months during a production campaign at Pfizer global manufacturing (CT, USA). Safety and environmental benefits were reported as additional assets. However, the major disadvantage with NIRS is that the spectral data in the NIR region contains overlapping features of several constituents, and it is often difficult to separate the information of one analyte from another. Furthermore, development of a good calibration model including validation, and the calibration transfer from one instrument to another are critical aspects.

Table 1 : Near infrared spectroscopy applications of bio-processes monitoring

Application	Objective	Bio-processes	Analytes monitored	Ref.
Off-line	Quantifying cellular nutrients and wastes at concentrations below 1 mM	Animal cell culture medium	Ammonium, glucose, glutamate, glutamine, and lactate	32
	Monitoring dynamics in anaerobic digesters	Human prostate cancer cells growth in the serum-based animal cell culture medium	Glucose, lactate, glutamine, and ammonia	33
	Develop calibration models for different analytical parameters involved in alcohol fermentation	Alcoholic fermentation using *Saccharomyces cerevisiae*	Glucose, ethanol, acetic acid, glycerine and biomass	23
At-line	Removing the contaminants with minimum product loss	Flocculation process	Cell-debris, proteins and RNA	34
	Monitoring biomass in an industrial fed-batch *Escherichia coli* process	Recombinant protein production	Biomass	35
	Development of robust near-infrared calibration models for monitoring of industrial bioprocesses with multiphase, chemically ill-defined, complex media.	Clavulanic acid production by *Streptomyces clavuligerus*	Clavulanic acid	24
In-line	Monitoring and automatic control of batch, repeated batch, and continuous fermentations by interfacing the NIR to the bioreactor control unit	Fermentations of *Staphylococcus xylosus* ES13	Glucose, lactic acid, acetic acid and biomass	36
	Multiplexed NIRS to monitor key analytes in multiple bioreactor vessels	Antibody manufacture using CHO cell cultures	Glucose and lactate	37
In-situ	Monitoring biomass in an industrial fed-batch *Escherichia coli* process	Recombinant protein production	Biomass	35
	Monitoring key analytes in mammalian cell cultivation	Cultivation of CHO-K1 animal cells	Glucose, lactate, glutamine and ammonia	38
	Monitoring the submerged fermentation processes	Submerged fermentation of *Staphylococcus* and *Lactobacillus*	Glucose, lactic acid, acetic acid and biomass	39
	Real time multi-parameter analysis during laboratory and industrial fed-batch cultivation	Vibrio cholerae fed-batch cultivation	Glucose, acetate and biomass	40
On-line	Maintaining nutrient and waste levels within fairly tight tolerances	Insect cell culture bioreactor	Alanine, glucose, glutamine, and leucine	41
	Monitoring dynamics in anaerobic digesters	Biogas production	Acetate, propionate, glucose, phospholipid fatty acids	42
	Monitoring dynamics in anaerobic digesters Monitoring and quality control of yoghurt fermentation	Biogas production Yoghurt fermentation	Acetate and propionate Dynamics in yoghurt formation	43 21
	Improving process control strategies by near real time simultaneous monitoring of gellan and biomass	Biopolymer production by *Sphingomonas paucimobilis*	Gellan and biomass	22
	Monitoring dynamics in anaerobic digesters	Anaerobic treatment of municipal solid waste	Propionate	44
	Monitoring and determination of metabolite uptake and production	PC-3 human prostate cancer cell cultures in a perfusion rotating wall vessel	Glucose, lactate glutamine and ammonia	45
	To extract important process dynamic features such as physiology phase transitions	-	-	25
	Real-time monitoring of algal biomass density	Micro-algal (*Nannochloropsis oceanica*) production in photobioreactors	biomass	46
Closed-loop system	Fully automatic NIR-based control system	L-(+)-Lactic acid production using *Lactobacillus caseii* DSM	Glucose, lactic acid and biomass concentration	*47*

MIR-spectroscopy applications

Unlike NIRS, MIR-spectroscopy (MIRS) covers the range of the fundamental vibrations, which provide substantial information both on the concentration and chemical composition of the sample. However, the strong absorption of water in the MIR region, especially when applied to aqueous biosystems is a major disadvantage for transmission measurements. A reduced transmission equivalent optical sample pathlength can be achieved using different ATR accessories. An example is presented in Fig. 3, the spectra of which have been recorded using a fibre-coupled diamond micro-prism with two reflections at 45°.

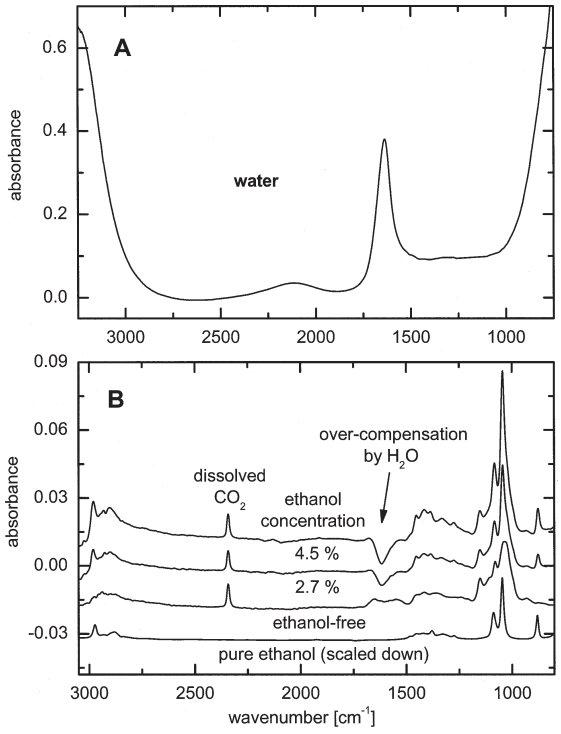

Fig 3: Mid-infrared ATR-spectrum of water measured with a fibre-coupled diamond micro-prism (**A**); difference spectra of different beer brands measured versus water as background spectrum, except for the pure ethanol spectrum shown for comparison (**B**); note: the individual spectra have been offset for clarity.

Except for the conventional ATR accessories, fibre-optic probes will need further development, although our recent studies have shown fine long-term and high-temperature stability of recently fabricated ATR-probes (Infrared fiber sensors, Aachen, Germany; http://www.ifs-aachen.de), for which silver-halide fibres were coupled to a diamond prism as described above. Nevertheless, MIR-spectroscopy has several advantages over NIRS, such as the ability to quantify analytes present at lower levels, to distinguish between analytes of very similar structure (see our examples in Figs. 4 and 5), identification of unexpected compounds, good calibration models and easy

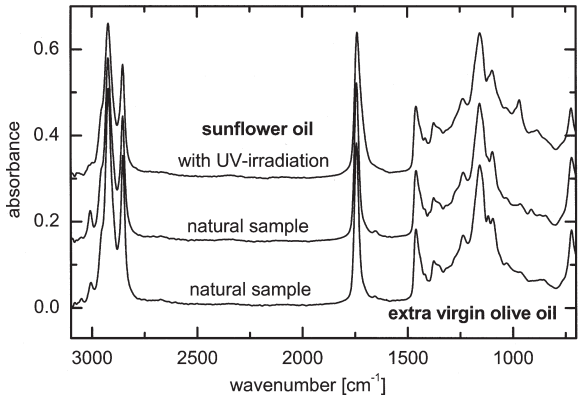

Fig 4: Spectral changes observed for similar composition of fatty acid esters in vegetable oils [49]; structural cis-trans changes and polymerisation effects under UV radiation exposure have also been detected; for clarity individual spectra have been offset.

transfer of calibration models from one instrument then another due to matching instrumental line functions and high wavenumber reproducibility. The potential, advantages and disadvantages of this technique have been critically discussed by Roychoudhury et al. (31), and the authors stated that MIR-spectroscopy has matured enough to be routinely applied also for the monitoring of complex bioprocesses.

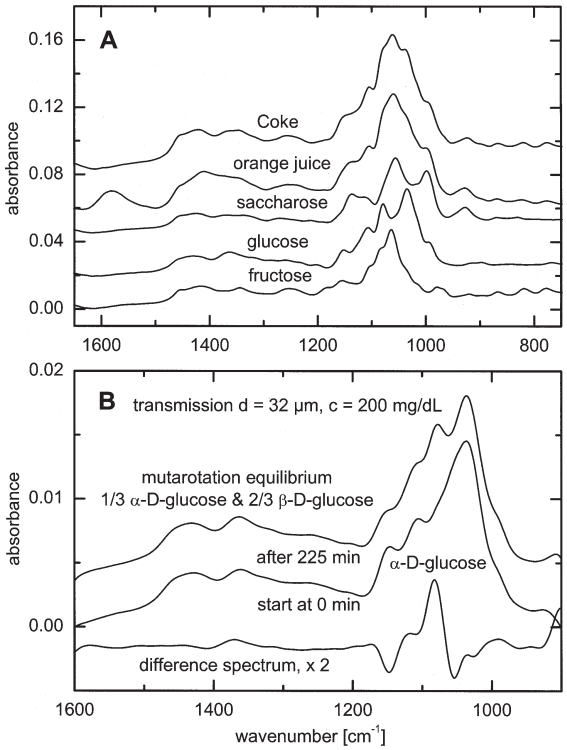

Fig 5: Mid-infrared ATR spectra of different sugars at 5 % concentration and soft drinks (**A**) and absorbance spectra with involvement of the two glucose anomers in aqueous solution at pH = 7.0, measured in a transmission micro-cell (half-life time of the mutarotation experiment starting from pure α-D-glucose was 45 min) (**B**); individual spectra have been offset for clarity.

Although MIR-spectroscopy is more sensitive to NIRS, only few applications have been reported so far in the literature (Table 2). These studies include mainly cell culture monitoring (nutrient uptake and metabolite production) and waste water treatment (anaerobic digestion processes). The standard error of predictions reported for the applications presented in Table 2 [26 – 28, 50 – 62] were as follows: glucose 0.27 – 1.39 g/L; fructose 1.0 – 2.2 g/L; gluconacetan 0.8 – 2.2 g/L; acetate 0.1 – 0.9 g/L; ethanol 0.1 – 1.0 g/L; glycerol 0.32 g/L; methanol < 0.12 g/L; methyl oleate 0.38 g/L; biomass 0.39 g/L; ammonium 0.01 – 0.22 g/L; phosphate 0.45 g/L; trichloroethylene 0.61 ppm; tetrachloroethylene 0.79 ppm; and carbon tetrachloride 0.96 ppm.

Recent advances in our laboratory

Our research group has specialised in the developments of IR-spectroscopy for biological and medical applications. NIRS has been applied mainly for non-invasive dermal diabetes screening, characterisation of human skin and study of glycation effects from hyperglycaemia, whole blood and plasma measurements and cancer diagnostics (9, 10, 63). MIR-spectroscopy has been used for food processing, bioreactor monitoring (51 – 53) and continuous bed-side blood glucose or biofluid monitoring (16 – 19). The state-of-the-art developed in our laboratory is able to utilise nano-litre sample volumes (e.g., down to 1 nl) for off-line dried sample measurements (64) or micro-litre volumes (e.g., 8 µl and less) for liquid samples (16 – 19, 65). Chemometric methods involving Tabu search, variable selection, CLS and PLS have also been developed for accurate measurements of bioanalytes. Advantages from other technology branches, especially aimed at micro-technologies, e.g., for micro-fluidics and micro-accessories (e.g., transmission micro-cell, micro-ATR fibre probes) have been exploited and successfully implemented. The state-of-the-art MIR-spectroscopic technologies developed by us can be applied for bioreactor monitoring and will be presented in the following sections.

Bioreactor monitoring with mid-IR ATR spectroscopy

ATR fibre-optic probes are important and well suited accessories for the MIR-spectroscopic monitoring of bio-technological processes. Development of micro-accessories for micro- or-nano-sample volumes is a challenge, which is required for the growing needs for on-line and low-cost technology. Silver halide fibres of

Table 2 : Mid-infrared spectroscopic applications of bio-processes monitoring

Application	Objective	Bio-processes	Analytes monitored	Ref.
Off-line	Complementary to magnetic resonance microscopy	Cultivation of bone in a bioreactor	Phosphate and collagen	50
At-line	Rapid monitoring of the key analytes in a *Streptomyces clavuligerus* bioprocess	Clavulanic acid production by *Streptomyces clavuligerus*	Ammonium, glucose, methyl oleate and biomass	26
	Analysis of substrate and reaction products	Anaerobic digestion process	Acetic, propionic and butyric acid	51 52 53
In-line	Development of a functional monitoring system to facilitate advanced control of anaerobic treatment plants	Anaerobic digestion process	Volatile fatty acids, chemical oxygen demand, sulphate, ammonium, and total Kjeldahl nitrogen	54
Real-time in-situ	On-line monitoring of freely suspended and immobilised cell cultures	Cultivation of Chinese hamster ovary cells	Glucose and lactate	55
	Multi-analyte monitoring of fermentations	Production of gluconacetan by use of *Gluconacetobacter xylinus*	Glucose, fructose, sucrose, gluconic acid, acetic acid, ethanol, 2-ketogluconic acid, 5-ketogluconic acid, glucon-acetan	56
On-line	Development of a robust on-line monitoring method for fermentation monitoring	Baker's yeast fermentation	Glucose and ethanol	57
	Simple calibration model for on-line fermentation monitoring	Batch-fermentations of *Gluconacetobacter xylinus*	Fructose, acetate, glucon -acetan	58
	Development of a simple method to monitor and control bioprocesses	Methanol feeding by *Pichia pastoris*	Methanol	27
	Real-time monitoring of process constituents such as product and substrate	Ethanol fermentation using *Saccharomyces cerevisiae*	Glucose, ethanol and optical density	59
	Development of a simplified calibration model	Aerobic *Saccharomyces cerevisiae* fermentation	Glucose, ethanol, glycerol, ammonium and acetate	28
Continuous on-line	Continuous on-line monitoring of the toxic compounds in the dechlorinating bioreactor	Anaerobic dechlorinating using methogenic microbes	Tri- and tetra - chloroethylene, carbon tetrachloride, hexachlorobutadiene	60
	Continuous on-line monitoring of the dechlorinating process	Anaerobic dechlorinating using methogenic microbes	Tri- and tetra - chloroethylene, carbon tetrachloride	61
Closed-loop system	Implementation of on-line spectroscopic data in a closed-loop control strategy for fed-batch fermentation	Production of gluconacetan by use of fed-batch cultures of *Gluconacetobacter xylinus*	Fructose, ethanol, acetate, glucon-acetan phosphate and ammonium	62

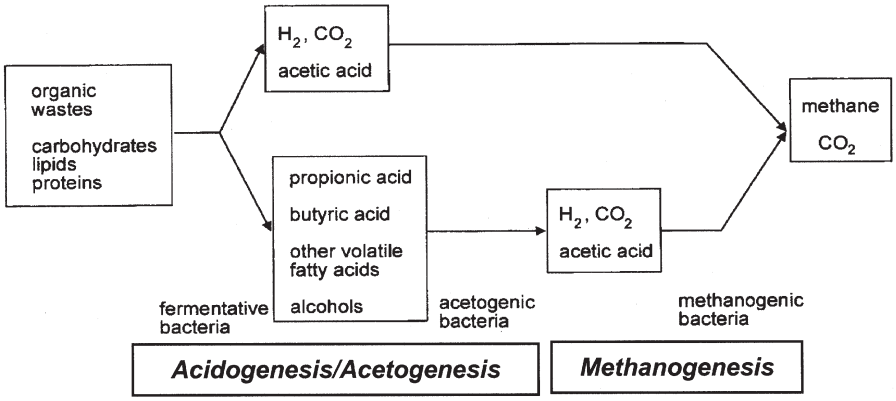

Fig 6: Biochemical transformations during anaerobic digestion processes for biogas production.

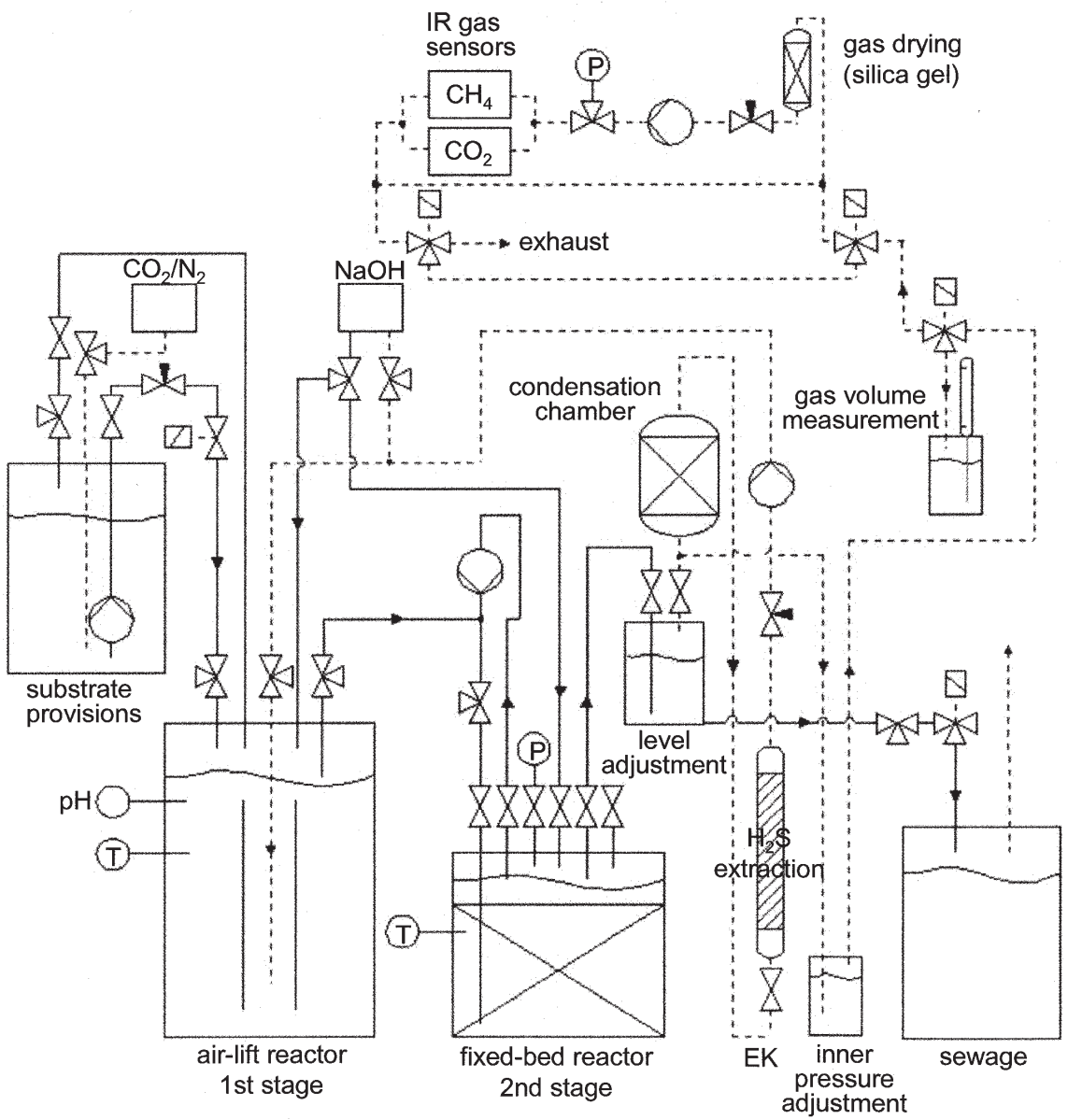

Fig 7: Schematics of the continuous two-stage anaerobic biomass fermenter (52).

different geometries were already developed by our collaborators and applied by us for biomedical monitoring (51. One of those studies includes the application of a remote-sensing ATR-probe for the surveillance of a two stage anaerobic bio-fermentation reactor at the South Westfalia University of Applied Sciences in Iserlohn, Germany (51 – 53); see also Figs. 6 and 7.

The focus was on processing harvested biomass and its further conversion into biogas within a two-stage anaerobic fermentation process. The processes of hydrolysis and acidification have been separated from the stage of methane production. For the on-line monitoring of the reactor broth media and the cellular material a flow system was developed

with a flow-through cell that could be flushed, cleaned and loaded for background measurements. By using the flow-system, several weeks of operation of the bioreactor were followed with extensive analytical characterisation of the aqueous media containing the substrate, and first and second stages for the monitoring of the acido- and methanogenesis status. The biogas production and composition was also measured for assessing the biomass utilisation rates.

In addition to the process engineering parameters, biochemical variables such as short chain carboxylic acids and bicarbonate concentrations are the critical parameters for achieving a better process management. The concentrations of several carboxylic acids (e.g., acetic, propionic and butyric acid) within the bioreactor broth media were analysed using a routine HPLC assay and compared with the infrared spectrometric analysis calculated from the 1740 – 1550 cm^{-1} band areas after baseline correction. Exemplary spectra are shown in Figs. 8 and 9. It was observed that as long as bicarbonate buffers the biodigester broth, organic compounds will be degraded, but if the carboxylic acid concentration continues to increase, and especially after bicarbonate exhaustion, the pH of the aqueous media will drop rapidly with the consequence of the destruction of the bacterial eco-system. In particular, the mortality of methanogenic bacteria due to extreme pH variations can have the consequence of a very slow recovery of the bioreactor over months.

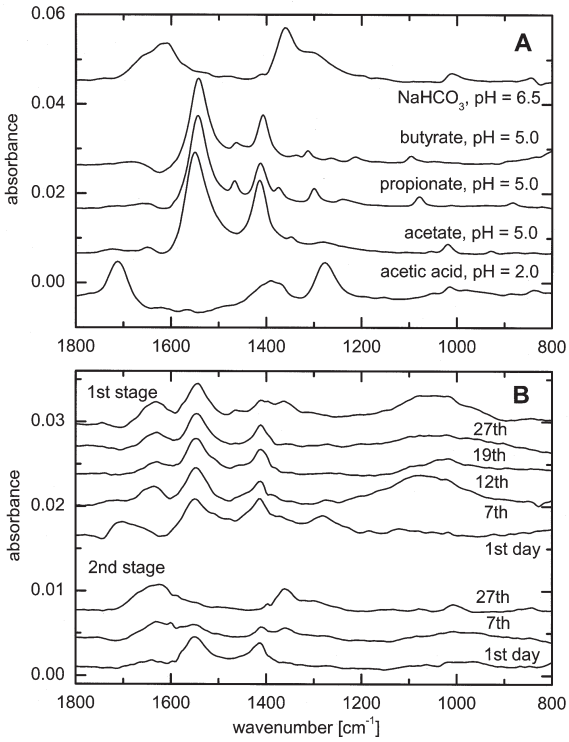

Fig 8: Mid-infrared ATR-spectra of components found within the bio-fermenter as measured by using a fibre-coupled diamond-prism (**A**) and exemplary broth spectra recorded during one month of operation (**B**); individual spectra have been offset for clarity.

Continuous biofluid monitoring with transmission MIR-spectroscopy

As a part of the European Commision's research project on Closed Loop Insulin Infusion for Critically Ill Patients (CLINICIP), we developed a mid-infrared sensor in combination with a subcutaneous or a vascular body interface for continuous glucose monitoring with the utmost reliability needed for the intensive care environment (16 – 19). The developed prototype (Fig. 2) is based on a microdialysis approach and has been tested successfully for online monitoring of blood glucose in several healthy and type 1 diabetic subjects including the further exploration of its multi-component capability. The applicability of the developed sensor towards closed loop control had also been tested by feeding the sensor readouts into the eMPC algorithm developed by the Cambridge University partners and controlling the patient glucose levels manually by appropriate insulin dosage. Good performance was achieved with the mid-infrared spectrometric sensor readings for controlling the insulin infusions (results to be published).

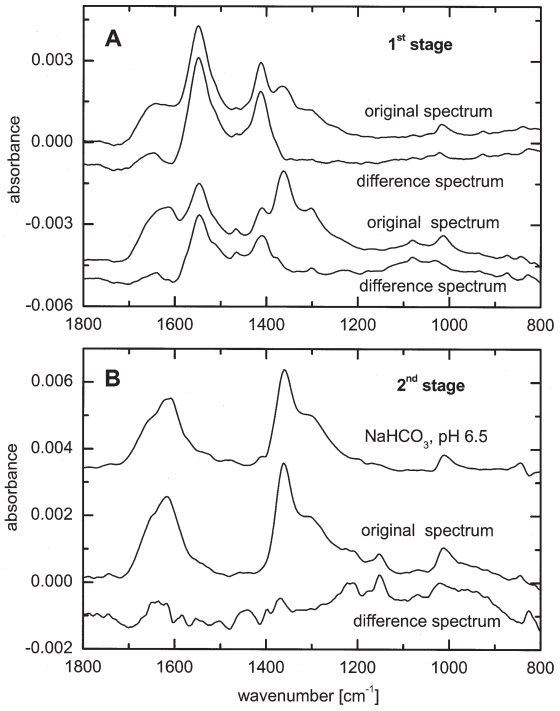

Fig 9: Mid-infrared raw ATR absorbance spectra from first and second stage bioreactor broths and difference spectra obtained after bicarbonate spectrum subtraction; individual spectra have been offset for clarity; difference spectrum in B was scaled by a factor of 2.

The fully automated system has been explored for its multi-component capability for glucose, urea, lactate, acetate, bicarbonate, pCO_2 and pH of the interstitial buffer system; it further includes accurate simultaneous microdialysis recovery rate measurements by the use of acetate in the ELO-MEL perfusate as a marker, air bubble detection and removal by using ancillary electronics and software. Besides the clinical use, applications in other areas such as optimal dialysis dose quantification, bioreactor monitoring (fermentations and cell-cultures) and chemical or biochemical reaction dynamics can also be envisaged. One of the examples included its application for the continuous monitoring of the structural changes between α -and β -anomers of glucose (53).

Glucose in aqueous solution is known for its mutarotation, i.e., a structural change between α -and β -anomers in aqueous solution. The developed automated set-up was used to continuously monitor the mutarotation up to 5 hours. The bands at 1081 and 1055 cm^{-1} are of greatest significance for this study (Fig. 5B). The other bands of interest are located at 1165, 1148, 1119, 1043, 1028 and 990 cm^{-1}. The transformation rate between the anomers has been investigated by an exponential fit, which yielded a half-life time of $t_{1/2}$ = 45 min in water at a pH-value of 7. The quantification of freshly prepared substrate solutions, which is important for bioreactor monitoring, needs this variability to be taken into account when evaluating the infrared spectra. Similar precautions have to be implemented for the pH dependency of bioreactor component spectra, as demonstrated for the volatile fatty acids (Fig. 8A).

Conclusions

Improvements during the past 10 years in IR-instrumentation, fibre-optic accessories and chemometric algorithms have matured this technique to be applicable for off-line, at-line, in-line, on-line or even monitoring of bio-reactors with closed loop control. Its advantages or superiority over other commercially available techniques have been demonstrated by several research groups world wide. As listed in Tables 1 and 2, the spectrometric assay is often used for reliable multi-component analysis from a single spectral measurement. In comparison to other on-line tools, the accuracy of the concentrations measured either by NIRS or MIR-spectroscopy is sufficiently high to allow application in bio-process monitoring. Although there is a need to further improve the instrumental accessories and develop stable and robust calibration models, the inherent advantages of this spectroscopy, e.g., simplicity, rapidity, multiplicity, flexibility, sterility etc. has a great potential for implementation in bioprocess analytical chemistry.

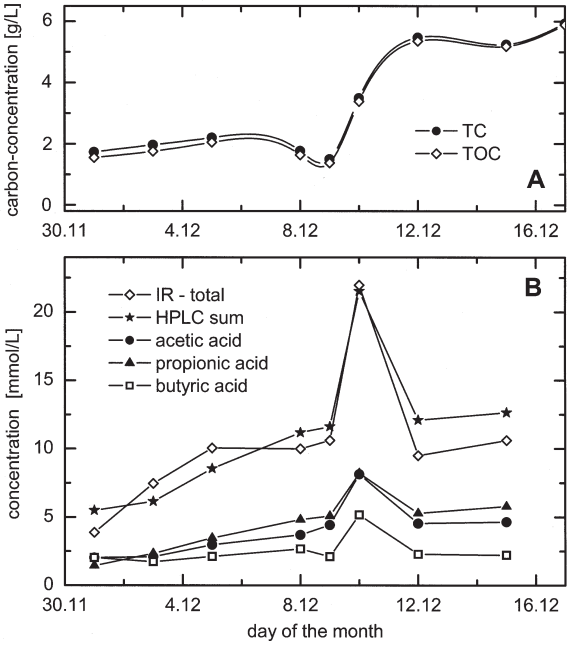

Fig 10: Temporal concentration profiles of several parameters: total carbon (TC), total organic carbon (TOC) using Dr. Lange photometry tests (**A**) and volatile fatty acids measured by HPLC and MIR-spectroscopy (**B**).

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