PTR-MS TECHNOLOGY FOR PROCESS MONITORING AND CONTROL IN BIOTECHNOLOGY

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ABSTRACT

Process monitoring is becoming increasingly relevant in biotechnology, especially in the manufacturing sector to promote cost-effective production. Proton transfer reaction mass spectrometry (PTR-MS) is a well established analytical tool for the measurement of volatile organic compounds (VOCs) and offers fast, real-time detection and quantification of VOCs at trace concentrations. In addition, this technique requires no pre-concentration or complex sample-handling prior to measurements. In a bioreactor, VOCs are emitted as by-products of microbial growth and development which may be used to gauge the activity of these micro-organisms. With real-time monitoring via the PTR-MS technique of the emitted VOCs in the bioreactor headspace, it may be possible to gain information for the control of the fermentation process. We have successfully developed a suitable sampling system that allows the PTR-MS measurement device to be coupled to a bioreactor in order to monitor fermentor off-gas in real-time. Using PTR-MS a large number of VOCs have been found which showed distinct variations over the course of a single fermentation bearing potential information for fermentation process control. Some of these marker masses have already been chemically identified and related to specific activity within the fermentation process.

INTRODUCTION

Biotechnical processes are currently monitored using different mainly off-line analytical procedures. In addition to assessments that rely on environmental parameters, such as fermentor temperature and pressure, microbiological activity can be derived from via physical and/or chemical analyses of the fermentation medium, e.g. by measurement of the pH or by quantification of solid or dissolved metabolites, products and by-products. To date, this generally involves regular liquid sampling and off-line analysis in order to gain the relevant information[1]. Such methods are invasive and considerable time delays between sampling and achieving the analytical results may be encountered, depending on the employed method.

An alternative approach is to exploit the headspace gas of a bioreactor. Presently, this is restricted to measuring a small number of components, for example oxygen (O_2) and carbon dioxide (CO_2) concentrations to ascertain the aerobic activity associated with micro-organisms. Although this is a very useful tool for monitoring microbial activity, it is very restrictive in terms of the amount of information it can provide.

By measuring the gas-phase composition of VOCs in the fermentor headspace gas, more in-depth information on the present fermentation status can be obtained. Although such analyses may be performed by conventional techniques such as gas chromatography mass spectrometry (GC-MS) or electronic noses, these invariably suffer from the instrumental constraints of either slow sampling or sample preparation requirements, or they are not sensitive or specific enough to detect the compounds of interest. These analyses therefore provide only restricted correlations with biological activity and are thus

not sufficient for complete mass balances and process simulations.

To aid in the understanding and control of production processes, which would allow for process improvements, new analytical tools are needed. These tools will enable the acquisition of on-line process parameters which directly or indirectly correlate with parameters such as production rate or product quality. The development of such on-line monitoring technologies is one of the major objectives of the process analytical technology (PAT)[2] initiative which was initiated by the FDA for the manufacturing of biopharmaceuticals. PTR-MS[2-4] has the potential to fulfill these requirements.

PROTON-TRANSFER-REACTION MASS SPECTROMETRY (PTR-MS)

PTR-MS is a chemical ionization MS (CIMS) method for on-line detection and quantification of VOCs down to parts per trillion, by volume (pptv) levels[3-5]. The technology offers fast, real-time detection and quantification of VOCs at trace concentrations and requires no pre-concentration or complex sample-handling procedures prior to measurement. PTR-MS has many advantages over other standard analytical gas-phase instruments. It has extremely low limits of detection (LOD) in the order of 5-500 pptv (depending on instrument model) and the time resolution (i.e. response time) is very high, with a measurement time of <1 s per compound. The method is quantitative, accurate and precise, and linear over six orders of magnitude (from the LOD to ~10 parts per million, by volume; ppmv). Furthermore, it is insensitive to changes in sample gas humidity, thus enabling direct analysis to be made without the need for prior sample treatment. These features offer many benefits for on-line process monitoring. The on-line capabilities of the instrument further allow a wide range of compounds to be measured in a short space of time, providing real-time measurements. Additionally, the fast time response also offers the possibility of multiplexing, i.e. monitoring many fermenters sequentially.

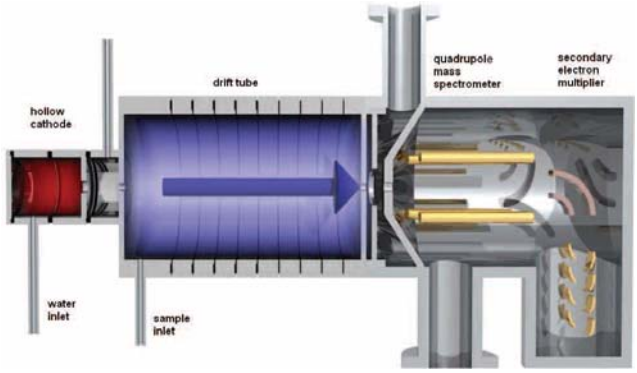

Figure 1. Schematic drawing of a PTR-MS apparatus. Hydronium (H_3O^+), produced in a hollow cathode discharge region of the instrument, protonate the target VOC in the drift tube. The product ion is subsequently guided through a quadrupole mass filter and counted by a secondary electron multiplier detector.

Since the operational principles of PTR-MS have been described extensively in literature[3], only a brief overview is provided here. Hydronium (H_3O^+), produced in a hollow cathode discharge region of the instrument, are used as proton donors in the proton transfer reaction ionization process. Upon collision of a VOC with hydronium an exothermic reaction takes place in which a proton is transferred from the hydronium to protonate the target VOC. These reactions occur only if the proton affinity (PA) of the VOC is greater than that of water (which is 165 kcal $mole^{-1}$). Provided this is the case, a proton transfer reaction occurs on every collision with a VOC. Using hydronium as the donor compound has two main advantages. All of the common constituents of air (e.g. N_2, O_2, Ar, CO_2) have PAs less than water and are therefore non-reactive with hydronium. Most VOCs, however, have PAs greater than water and therefore become protonated upon collision with H_3O^+. These proton transfer reactions are quantitative and offer reduced fragmentation (compared, for example, to other ionization techniques such as electron impact ionization). Examples of detectable compounds are alcohols, aldehydes, ketones, esters as well as sulphides, phosphines, amines, and amides. Following protonation the product ion is channelled into a detection region, which contains a quadrupole mass filter and a secondary electron multiplier

detector, where ions can be selected and their abundances counted and registered.

The air (to be analyzed) operates as the buffer gas. After entering the drift tube, proton transfer reactions occur between H_3O^+-ions and any molecules R_i present in the air which has a PA that exceeds that of water. Under typical operating conditions only a small fraction of the primary ions react with VOCs in the buffer gas, so that the density $[R_i]$ of the R_i molecules is obtained from the relation

$$[R_iH^+] = [H_3O^+]_0 (1-e^{-ki[Ri]t}) \approx [H_3O^+]_0[R_i]k_it$$

where k_i is the respective reaction rate constant for the proton transfer from H_3O^+ to R_i and t is the transient time for the H_3O^+ ions traversing the drift tube.

PTR-MS technology has been successful applied to the fields of environmental research, waste incineration, food and flavour science, biological research, (bio)-process monitoring, indoor air quality, medicine and biotechnology[4].

A short-coming of PTR-MS is its limited mass resolution, enabling only a measurement of the nominal mass of a compound. Thus, it is well suited for monitoring the components of the above mentioned compound groups, but generally it can not be used to identify a substance. For that reason a PTR-Time-Of-Flight (TOF) analyzer system with a mass resolution of $m/\Delta m = 5000$, enabling isobaric compounds like formic acid (HCOOH) and ethanol (C_2H_5OH) to be distinguished, has been developed. This system allows for a determination of the mass formula of a detected compound, and together with databases, aids in the identifying of the substance of interest.

IMPLEMENTATION OF PTR-MS TECHNOLOGY FOR BIOPROCESS MONITORING

Of central importance for monitoring and control of biotechnological processes is a fast and quantitative transfer of the sample gas to the PTR-MS measurement device. In addition, the measurements have to be made non-invasively, so there is no interference with cell metabolism and sterility is maintained. We have developed a suitable sampling system that enables PTR-MS to be coupled to a bioreactor, enabling cultivation phases to be monitored for microbiological activity, thereby providing specific information of the status and development of a production batch. The inlet system was conceived to minimize loss during transfer of the sample gas between the fermentor and the PTR-MS device. It consists of a special piping, a sterility barrier for the bioreactor and a number of additional safety measures reducing the likelihood of fermentation broth from accidentally passing through the system to the PTR-MS device.

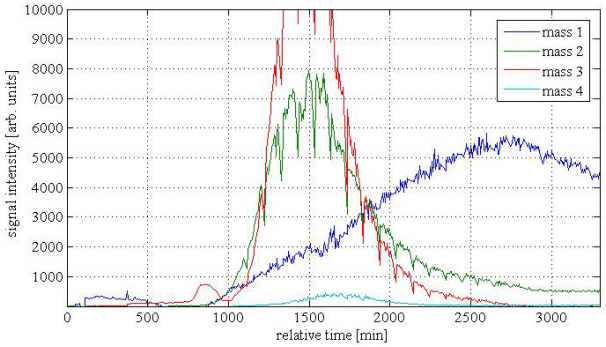

Figure 2. Typical fermentation progression, different masses show distinct variations over the course of an *E.coli* fermentation.

In addition to the PTR-MS' sensitivity to VOCs the whole system, consisting of the PTR-MS and the purpose-built coupling system, proved to be very robust and has been up and running on an industrial test site for more than two years. In order to enable an efficient use of the PTR-MS technology for industrial applications, a multiport-system was developed for switching between different bioreactors.

In the course of the Austrian Center of Biopharmaceutical Technology (ACBT) PTR-MS measurement devices have been installed at the Sandoz research center in Kundl, Tyrol and at the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna, both in Austria[6]. Both of these PTR-MS devices are remotely controlled. Series of recombinant *E.coli* fed-batch cultivations with different levels of induction were performed to generate

data sets from different stages of recombinant protein expression. In the first measurements of *E.coli* fermentations it became clear that the ammonia concentration is higher than 10 ppmv in the bioreactor off-gas, due to the applied process conditions. Because such high concentrations of ammonia affect the quantitative quality of the PTR-MS measurements, the inlet system was also equipped with a device which allows for the dilution of the bioreactor headspace gas sample with clean air. Using this modified coupling system, the off-gas from a bioreactor can be diluted as desired.

For the above mentioned fermentation measurements the PTR-MS instrument was used in scanning mode. All masses from 18 to 200 amu (atomic mass units) were measured with a one second integration time for each mass. A complete scan took therefore approximately three minutes. Figure 2 shows the concentration variation of typical VOCs during a fermentation.

In order to evaluate the possible information on VOC emanation, the data gathered by PTR-MS measurements are correlated with other relevant fermentation parameters. These parameters are for instance content of biomass dry matter (BDM) or the recombinant protein production rate (qP). More than 20 masses of a single fermentation exhibit distinct variations in time, bearing information on the current status of the fermentation process. Some of these masses have already been chemically identified and were assigned to specific fermentation states. This information can be used to incorporated into a process control system for the development of advanced control regimes.

We expect to verify the role of other marker masses by monitoring and evaluation of additional fermentation processes, as well as to chemically identify additional marker compounds in the near future.

CONCLUSION

Improving product yield and quality is a major goal of all fields in industrial biotechnology. To aid in the understanding and control of production processes, which allow for such improvements, new analytical tools are needed. Improvements will be found by better understanding the bioprocess which will most likely be achieved by real-time monitoring of a broader spectrum of process variables. For this purpose, we incorporated the PTR-MS technology to investigate its usefulness as an analytical tool to monitor the VOC emissions of *E.coli* fermentations to determine those which correlate with process parameters e.g. BDM and qP. More than 20 masses have been found which are correlated with fermentation parameters and can be used for process control. The PTR-MS technology proved to be well suited for the real-time monitoring of bioprocesses and can contribute to the implementation of PAT-compliant process development.

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