Goal: online glucose sensor for bioreactors

Microorganisms and sensitive cells that are grown in bioreactors need a well-regulated environment and a food supply in order to do what they are supposed to do: grow and produce biomass and metabolites. Many fermenters are equipped with sensors that continuously measure critical bioprocess parameters, including temperature, pH value and oxygen. Online glucose and ethanol sensors are not yet used in regulated bioreactors, even though they would make the bioprocess much quicker and more economical.

For more than 20 years, researchers and developers have been working on the development of methods to close this gap, without success. A consortium of research institutions and small companies is currently involved in the search for such methods. Financed with around one million euros from the German Federal Ministry of Economics and Technology’s “Zentrales Innovationsprogramm Mittelstand” (Central Innovation Programme for SMEs), the Ulm and Aalen Universities of Applied Sciences and the companies J & M Analytik AG (Essingen, Ostalbkreis), Faseroptik Henning (Allersberg close to Nuremberg) and EPIGAP Optronik (Berlin) have joined forces to develop a universal optical multiparameter sensor that enables the online monitoring of bioprocesses. The aim is to construct a system consisting of an optical sensor, a spectrometer and analysis algorithms that can be used for different bioprocesses and bioreactors. The new system also needs to tolerate sterilization temperatures of 121°C.

Highly confident: the time is right and the technologies mature

According to Martin Hessling from the Ulm University of Applied Sciences (Faculty of Mechatronics and Medical Technology), the collaborative project has rather ambitious objectives, although they are not totally unachievable. He believes that the conditions for such a project are a lot better than they were several years ago. Nowadays, biotechnologists have the device-related know-how required for such a project. State-of-the-art chemometric devices and software allow the complex and quick mathematical analysis of raw data produced by laboratory analysis devices. In addition, more effective spectrometers are also available. Unlike the scientists before them, the "BIOsens" project partners are now trying to base their measurements on several different spectral ranges (MIR, NIR, UV,
VIS) rather than just one.

Humans still rely on the enzymatic determination of their blood glucose level but bioreactors are quite different, as their contents are known, and hence reproducible. Nevertheless, there is one issue that can pose problems. Hessling knows from experience that although yeast (Saccharomyces cerevisiae) is one of the most intensively studied eukaryotic model organisms and the most common microorganism used for fermentation, it might produce side products that are not yet known. And this needs to be changed. The researchers ferment yeast in a 7-litre bioreactor that they have developed and optimized for their experiments.

The absorption spectrum in the NIR range is insufficient for the planned applications, which is why MIR is also taken into account.

Most measurements are time-delayed

It is known from scientific literature that the glucose concentration in bioreactors still cannot be measured online. What usually happens is that samples are removed from the fermentation broth and analyzed (often using enzymes). This all takes time during which the fermentation process in the bioreactor continues, so the information frequently fails to provide the researchers with a realistic picture of conditions in the reactor. Processes involving bioreactors fall into two categories, discontinuous processes and continuous processes. Discontinuous processes are still standard. The measurements take place outside the reactor and it takes a couple of minutes before the information about the glucose concentration becomes available. It is difficult to interfere and add glucose if the “offline” measurement indicates that the microorganisms do not have enough glucose, i.e. nutrients, available. The microorganisms no longer grow (they maybe even die) before the glucose concentration can be increased.

Larger amounts of glucose can of course be added to the fermentation broth in order to ensure the cells do not starve but this is not very economical. In addition, excess glucose can have deleterious effects and the cells might switch to a different metabolic pathway. Baker’s yeast (Saccharomyces cerevisiae) grows much slower than normal at glucose concentrations of 0.1 g glucose/litre. The fermentation of sugars to ethanol is an anaerobic process. However, in the presence of high external glucose concentrations Saccharomyces produces ethanol aerobically (Crabtree effect). It is difficult to equip reactor systems with enzyme sensors, as suitable enzymes do not tolerate high temperatures and do not survive the high temperatures (121°C) required for sterilization.

The situation is similar for ethanol, which is an important (intermediary) biotechnological product. Several methods to determine the concentration of ethanol in fermentation broths are available, but no online ones. With a few exceptions, samples of agricultural ethanol are removed from the fermenter and the ethanol concentration is subsequently determined manually in the laboratory. Valuable time is lost doing this, preventing any quick adjustment of the conditions in the fermenter. This is clearly uneconomical, especially considering the fact that the processes usually run without interruption for several days and weeks.

Greater spectral range

One of the project’s priorities is adsorption measurements in the mid infrared (MIR) range, where the strong, characteristic absorption bands of glucose and ethanol can be discerned much better than in the near infrared (NIR) range. These measurements will be complemented by fluorescence and absorption measurements in the UV/VIS (ultraviolet and visible light) and NIR range. It is expected that these additional spectral ranges will prevent overlay effects of water (usually used in 100 times higher concentrations) from occurring during the measurement at the same time as increasing the glucose detection limit.

The researchers will use light sources such as multispectral LEDs and optical fibres. J & M Analytik AG, the company that is coordinating the project, is faced with what is quite an ambitious challenge. The company’s task is to develop a sensor that is effective over a relatively wide spectral range (UV-VIS-NIR-MIR), can measure absorption and fluorescence in the culture medium, and be cleaned and sterilized after use. The biosensor has the potential to be used as immersion sensor in traditional reactors, and will most likely also be suitable for carrying out glucose/ethanol measurement through the plastic wall of the disposable reactor. Baden-Württemberg-based J & M Analytik AG and the nearby Aalen University of Applied Sciences are developing a system consisting of an MIR spectrometer and an absorption sensor which will soon be tested in a bioreactor operated by the Ulm University of Applied Sciences.

Chemometrics – obtaining information from chemical systems by data-driven means
On the Ulm University of Applied Sciences "Oberer Eselsberg" campus, researchers are working together with the coordinator of the project, J & M Analytik AG, with the goal of developing a robust mathematical process known as chemometrics. Chemometrics refers to the extraction of information from multivariate chemical data using statistical and mathematical tools. In their project, the researchers from Ulm will use the huge number of rapidly accumulating spectral data to calculate the concentration of glucose and ethanol. The conditions at the time of measurement will be simulated with simple transparent solutions consisting of glucose and ethanol dissolved in water. Before the concentration of analytes can be determined in new samples, the mathematical model needs to be calibrated with reference values for the properties of interest.

So how does this work? The two scientists from the Ulm University of Applied Sciences who are involved in the project somehow "explain" to the analysis software which spectrum (at presently done for the NIR range, in the future it will also be done for the MIR range) relates to which analyte concentrations. The spectra of the calibration samples are determined and compared with reference values for the properties of interest. At present, the simplified measurement conditions will gradually be adapted to the rather more complex reality in the bioreactor, where there are air bubbles, light scattering from cells moved around with a stirrer, and many other known and unknown substances. The chemometric model will thus be gradually adapted to more complex culture situations.

**Developmental work with well-studied yeast**

In summer 2012, the researchers hope to have improved hardware available for carrying out measurements in standard processes involving yeast. Although the project has a relatively short run time, Hessling nevertheless believes that the establishment of the envisaged chemometric method which allows measurements to be carried out under simple fermentation conditions (yeast, E. coli) and enable reliable statements on glucose concentration to be made, is a realistic goal. The researchers have not yet found an answer to the question as to whether the measurement is accurate enough to recognize or detect critical phenomena such as the aforementioned Crabtree effect.

In the second year of the project, the partners have plans to work on a sensor for disposable reactors. They expect to carry out the measurement through the plastic wall (of the disposable reactor) using attenuated total reflectance (ATR), a sampling technique that enables the measurement of absorption without requiring the sensor to be immersed in the culture broth.

The project partners do not really believe that the development of a multiparameter sensor that is able to measure the concentration of glucose in yeast, E. coli and CHO (Chinese hamster ovary) fermenters will be feasible within the next two years. However, all partners are convinced that an online multiparameter sensor of this kind will be of great interest in all areas where large product quantities need to be produced and processes need to be continuously monitored, for example in the biopharmaceutical and food industries, and in the future possibly also in the energy sector.