

On-line Glucose Measurement in Disposable Bioreactors



TRACE
Analytics

www.trace.de

INTRODUCTION

The use of disposables in biopharmaceutical processes has increased significantly during the last few years, both for improved performance and for the economic advantages they offer. However, there is a need for additional sensors that will enable the disposable process to be integrated in compliance with the latest Process Analytical Technologies. TRACE on-line analyzers are well established for continuous process monitoring in conventional bioreactors connected via rugged sterile filtration and dialysis probes, respectively. For disposable bioreactors a new dialysis probe was designed and characterized in connection with a novel single-use bioreactor system.

TRACE C2 on-line analyzer

TRACE C2 is a dual-channel on-line analyzer. The substances glucose and lactate can be detected continuously. The analyzer was specially developed for cell culture fermentations and disposable technologies. The range of glucose concentration is 0.01 – 10 g/L and the frequency is 30 measurements per hour. The internal temperature correction coefficient makes a temperature range from 15 °C to 30 °C possible.



The detection is performed using a combination of an enzymatic reaction with an amperometric detection. Glucose is converted via glucose oxidase to gluconolactone and hydrogen peroxide. This is measured at a platinum electrode. The biosensors have life time of 5000 measurements or 21 days.

- Safe and economic operations
- Low installation size and less maintenance effort
- Simultaneous assay of glucose and lactate
- Especially suitable for cell cultures
- Sterile consumables (tubing set, sensor and transport solution)
- Disposable biosensors with long-term stability and low drift
- External sensor unit to be placed directly at the process
- No sample volume required
- Connection to different fermenter and single-use reactors

Disposable Sampling

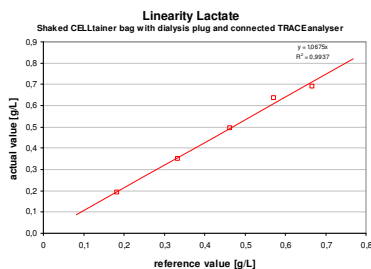
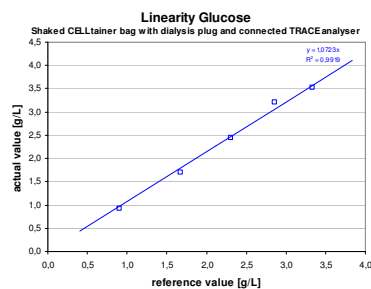
Based upon the patented dialysis sampling technique a special single-use plug for disposable bioreactors was constructed as continuous sampling device. A cellulose-acetate-membrane is fixed inside the plug (picture below).



The membrane acts as a sterile barrier and is used for the separation of the analytes of interest. The dialysis plug is installed at the bottom side and sterilized together with the disposable bag.

Analytical performance

The linearity of the measurements was proven by changing the concentrations of glucose and lactate inside the bag. The results of linearity are shown in figures below.



Michael Hartlep and Wolfgang Künnecke

TRACE Analytics GmbH
Richard-Wagner-Straße 1-2
38106 Braunschweig (Germany)

Nico Oosterhuis

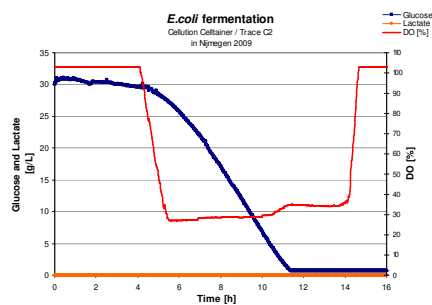
CELLution Biotech BV
Dr. A.F. Philipsweg 15A
9403 AC Assen (The Netherlands)

Online-Glucose measurement in batch fermentation of *E.coli* in a bag fermenter

The disposable bioreactor is placed inside the CELLtainer system from CELLution. Sterile medium and glucose solution (30 g/L) are filled into the bag under sterile conditions. The tubing of the dialysis plug is connected to the TRACE analyzer. The bag is shaken with the CELLtainer system (see picture below).



The TRACE analyzer was calibrated and the continuous on-line measurement of glucose started. The disposable bioreactor was inoculated with *E.coli* cells. After a lag-period of several hours the cells start to consume glucose (blue line in figure below). At the same time the dissolved oxygen level inside the medium decreased (red line in figure below). After eleven hours of cultivation glucose was completely consumed and the dissolved oxygen increased again.



Acknowledgements

The authors would like to thank the European Union and the BMBF for supporting this project (Eurotransbio, proposal no. ETB-2008-6, reference no. 0315558).

CONCLUSION

Disposable on-line sensing of glucose and lactate using a single-use sampling probe can be provided with similar performance compared to conventional PAT systems. This probe can be custom made for different bioreactor designs and special applications.

During an *E.coli* fermentation it was demonstrated that the dynamics of glucose consumption can be reliably monitored with the TRACE C2 analyzer under real conditions.