

INTRODUCTION

Ethanol and methanol are products of various microorganisms. Depending on the process they can be main product, by-product, or can be used as inducer. In a lot of processes it is important to monitor the amount of ethanol or methanol in order to know the end point of the fermentation or to avoid a toxic concentration inside the fermentation broth. For some organisms methanol is used as an inducer, where the concentration must be controlled in a very tight range. BioPAT® Trace is a new on-line analyzer for measuring methanol and ethanol in fermentation processes.

BioPAT® Trace on-line analyzer

BioPAT® Trace is an on-line analyzer for measuring ethanol or methanol (Figure 1). Both substances can be detected continuously.



Figure 1. BioPAT® Trace on-line analyzer

The range of methanol concentration is 0.5 - 20 g/L and the range of ethanol concentration is 1 - 40 g/L. The measurement frequency is about 25 per hour. Because of the internal temperature correction coefficient a temperature range from 15°C to 25°C is possible.

Enzymatic reaction and detection

In the presence of oxygen the immobilized enzyme alcoholoxidase (AOD) catalyses the transformation of alcohol (e.g. methanol or ethanol) to the corresponding aldehyde and hydrogen peroxide. The alcohol content is measured indirectly on a platinum electrode via the formed hydrogen peroxide, which is oxidized to water and oxygen. In an amperometric measurement the electrical current is proportional to the amount of oxidized alcohol.

- Safe and economic operations
- Low installation size and less maintenance effort
- Assays of ethanol and methanol
- Disposable consumables (tubing set, sensor, reactors and transport solution)
- Enzyme reactors with long-term stability and low drift
- No sample volume required
- Connection to different fermenter types

Enzyme reactor

The enzymatic bioreactor has life time of 5000 measurements or 14 days.

The reactor is very small, reusable, and easy to connect with a Luer connector (Figure 2).



Figure 2. Enzyme reactor

Analytical performance

The linearity of the measurements was proven by changing the concentrations of methanol in the range between 0 and 20 g/L (Figure 3) and by changing the concentrations of ethanol in the range between 0 and 40 g/L (Figure 4).

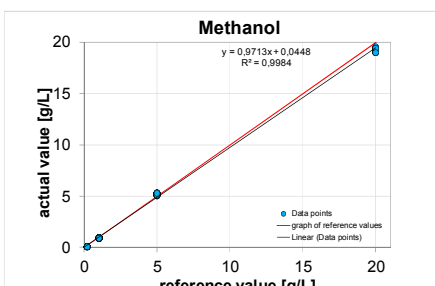


Figure 3. Linearity of methanol measurements

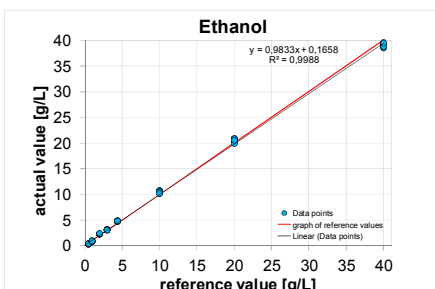


Figure 4. Linearity of ethanol measurements

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Ethanol monitoring in an industrial fermentation process

A fed-batch fermentation of a recombinant yeast was performed. This process run over 70 hours. The yeast was cultivated on a glucose medium.

During the first 20 hours ethanol was produced and consumed. After this period the ethanol concentration stayed at a very low level near 0 g/L. With the BioPAT® Trace it was possible to monitor this trend (dark green line in Figure 5).

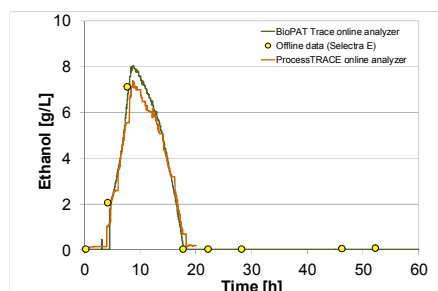


Figure 5. Ethanol concentration in a fermentation run with a recombinant yeast.

The ethanol concentration data from the BioPAT® Trace were proven with offline data (yellow points in Figure 5) and with online data from another alcohol analyzer (brown line in Figure 5).

It was possible to show the robustness of the process and of the BioPAT® Trace in three fermentation runs (Figure 6).

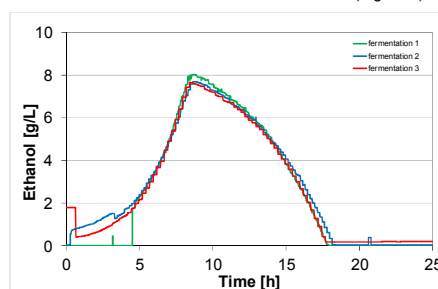


Figure 6. Ethanol profiles of three fermentation runs.

Ethanol concentrations can be reliably monitored in fermentation processes with the BioPAT® Trace.

CONCLUSION

The BioPAT® TRACE is a new online alcohol analyzer for measuring ethanol and methanol.

During an industrial fermentation process it was demonstrated that the dynamic profile of ethanol production and consumption can be reliably monitored with the BioPAT® Trace analyzer.