Application report: Methanol

Method: Dialysis
Measurement range: Methanol 0.5 – 20 g/L

INTRODUCTION

During the last years, the methylotrophe yeast *Pichia pastoris* gained a reputation as an ideal expression system for heterologous proteins. Usually, the target gene integrated into the host chromosome is under the control of a strong alcoholoxidase-1-promotor. The expression is tightly controlled by the promoter and only induced in the presence of methanol as carbon source.

Cultivation of the modified organism commonly starts with a batch phase, during which glycerine serves as the carbon source. This is followed by a fed-batch phase to achieve higher cell densities for the induction period, which is initiated by the addition of methanol. A precise control of the methanol concentration is essential during the production period to guarantee a constant induction of the recombinant protein gene while preventing the accumulation of cytotoxic amounts of methanol. The control range is relatively narrow, a methanol concentration of 0.3 to 0.5 % (v/v) is recommended in the „Pichia expression kit"(Invitrogen, San Diego, USA) to achieve good productivity. Analysis methods like HPLC and GC are capable of measuring these methanol concentrations, but are relatively expensive and problematic for on-line analysis.

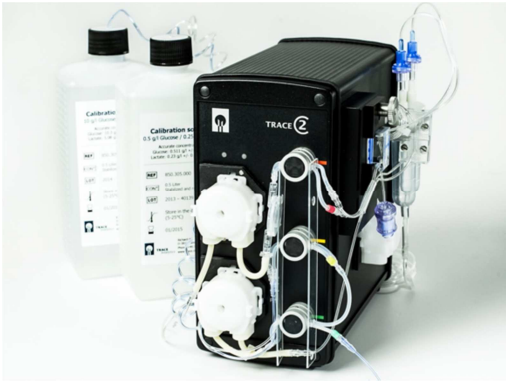

Figure 1. Trace C2 Control

Released: 03/2016

The online analyzer TRACE C2 Control (Figure 1) allows a rapid and precise determination of the methanol concentration in the fermenter and provides the tools for a quick set-up of a feed control strategy.

MEASUREMENT PRINCIPLE

Methanol
The enzyme alcohol oxidase (AOD) is used for the detection of methanol.

In presence of oxygen, alcohol oxidase (Figure 2) catalyses the transformation of alcohols, mostly methanol and ethanol, to the corresponding aldehydes and hydrogen peroxide. The alcohol content is measured indirectly via the formed peroxide, which is oxidized to water and oxygen during the amperometric measurement. The resulting electrical current at the electrode is directly proportional to the amount of oxidized alcohol.

Figure 2. Enzyme reactor alcohol oxidase (AOD)

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SYSTEM PERFORMANCE

These data were compiled in order to give an overview of the system- and sensor-performance in the normal concentration range using the dialysis sampling method.

Linearity

By comparing the actual value with the set value a regression coefficient R^2 of not less than 0,999 will be obtained (Figure 3).

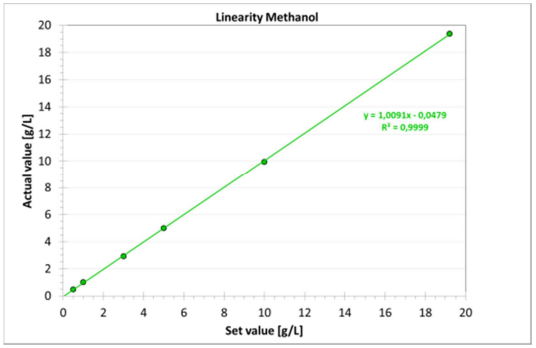

Figure 3. Linearity of Methanol (R^2=0,9999)

Precision

The typical variation about the mean value is below 1,5% (Figure 4).

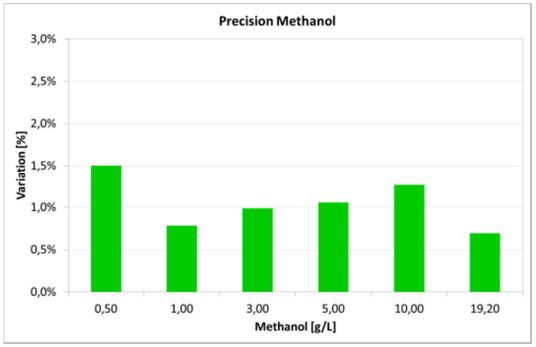

Figure 4. Precision of Methanol

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Recovery

The recovery of the Methanol values is shown in figure 5.

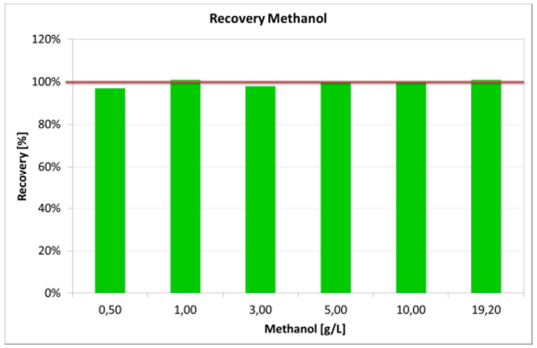

Figure 5. Recovery of Methanol

Operational stability

Long term stability for the application Methanol is guaranteed for 5.000 measurements or 14 days.

Shelf life

Alcohol oxidase enzyme reactors have a shelf life of at least 6 months at 4-10°C.

Consumables

Consumables for the application Methanol are listed in the following table:

Part	Part number
Tubing set dialysis (Ethanol/Methanol)	130.200.020
Transport Buffer conc. 5x (Ethanol/Methanol)	850.300.613
Enzyme reactor for application Ethanol/Methanol	811.100.120
Calibration Standard 20 g/L Methanol	850.301.008
Calibration Standard 5 g/L Methanol	850.301.005
Calibration Standard 1 g/L Methanol	850.301.004
Cleaning solution (Ethanol/Methanol)	850.300.711