Non-Invasive In-Situ Fluorescence Spectroscopy: Flexible Monitoring of Complex Bioprocesses in Stirred Tank Fermenters

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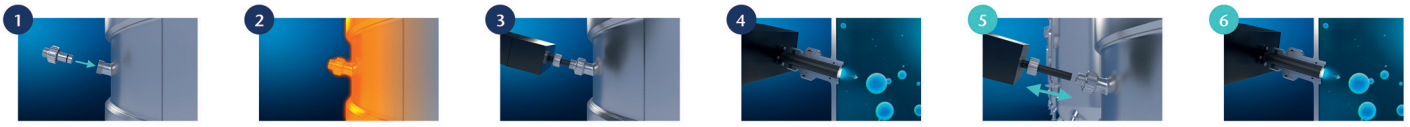
1. INTRODUCTION

- **Challenge:** With its efficiency-boosting potential, **biocatalysis** is becoming more important to **pharma and biotech industries. Economically making the needed enzymes** is difficult because insoluble media components hamper **continuous process control.**
- **Approach: In-situ fluorescence spectroscopy** can analyze **biomass growth, substrate consumption** and **product formation** during bioprocesses and is **less effected by insoluble particles** compared to optical density or scattered light measurements, which cannot distinguish between light absorption by cells and cellulose fiber. However, the practicality of fluorescence spectroscopy depends on the **ability to mount and unmount the spectrometer probe** during cultivation without compromising sterile integrity.
- **Enabler:** To overcome this challenge, SCHOTT developed the **ViewPort®**, which provides a **sterile-safe interface** that enables in-situ bioprocess monitoring through a **hermetically sealed optical window.**

2. PRINCIPAL SETUP and ASSEMBLY



1 Spectrometer interface connected to bioreactor port

2 Sterilizable with bioreactor

3 Spectrometer interface provides sterile integrity throughout the cultivation

4 Real-time monitoring through optical window

5 Spectrometers exchangeable any time while running cultivation

6 **Continue** real-time monitoring

3. USE CASE: IN-SITU MONITORING of ENZYME PRODUCTION in a SUSPENSION

- We used a **stirred-tank fermenter** (2.5 L fermentation volume), **ViewPort®** optical interface (SCHOTT, Germany) and **Carry Eclipse Fluorescence spectrometer** (Agilent, USA) with fiber optic probe (Fig. 1).
- The fungus ***Trichoderma reesei*** was cultivated in a medium containing 5 g/L glucose, 30 g/L alpha cellulose as carbon sources and 2 g/L peptone.
- The fungus was **tagged** to **express mCherry fluorescent protein** as marker to follow growth.
- We **compared** in-situ fluorescence data with data from online **off-gas monitoring** (non-optical approach, real-time estimates of cell growth and substrate consumption).
- An **excitation vs. emission scan** (Fig. 2) was used to detect **biomass autofluorescence signals.**

Fig. 1: Experimental setup

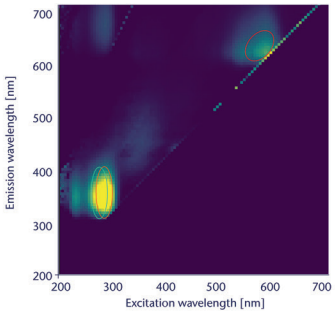


Fig. 2: Spectrally resolved in-situ fluorescence measurement for discrimination

4. RESULTS

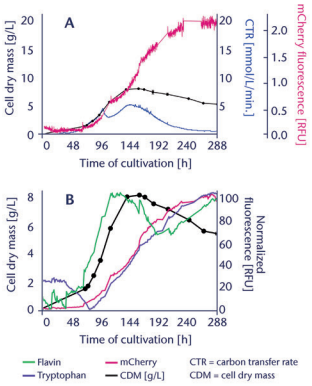


Fig. 3: Fluorescence-based characterization of growth and protein formation of *T. reesei* RUT-C30 mCherry

- Standard **off-gas analysis parameter CTR** correlated with **CDM** until **100 h** of cultivation (exponential cell growth); in-situ **mCherry fluorescence signal** correlated until **144 h** (Fig. 3A); difference is likely due to metabolism change.
- Recording **autofluorescence signals** for tryptophan and flavin; **flavin correlated better** with cell growth **than mCherry** or tryptophan (Fig. 3B); setup's **sensitivity** allowed detection of autofluorescence signals with **low response** (flavin).

5. SUMMARY

Autofluorescence of flavin is a **suitable process parameter** (vs. mCherry fluorescence)

The in-situ set-up is **sensitive enough to detect** autofluorescence signals

Autofluorescence detection enables **marker-free** bioprocess monitoring – a prerequisite for production scales

ViewPort® enables **sterile-safe, sensitive and flexible** in-situ monitoring

Link to TechnoPharm article (in German):

schott.com/viewport