Using PLS-DA to evaluate the quality of spore inoculum to optimize biotechnological process control

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INTRODUCTION

Efficient process control requires detailed knowledge about biochemical processes occurring in a bioreactor. Understanding the mechanisms and having access to the morphological state of the microorganisms - especially at an early stage - is the key for cost-efficient process control resulting in high product yields and operations at optimal workload.

The filamentous fungus *Penicillium chrysogenum* is famous for producing the β-lactam antibiotic penicillin [1]. Knowledge about the spore inoculum’s quality in terms of viability and germination ability is crucial for adapting process parameters needed for optimal growth, production condition and regulation.

Raman spectroscopy as label-free, non-invasive analytical tool was applied in combination with chemometrics to access this information. Finally, a PLS-DA model was established based on a calibration set of 170 single spectra (each spectrum representing one single spore).

pls-da model for life/dead - detection of *P. chrysogenum* spores

Spores of known viability:
- viable spores = freshly sampled spore inoculum
- dead spores = spore inoculum older than 1 year

PLS-DA model based on 260 Raman spectra each representing a single spore

Spectral features with significant influence on the class assignment such as the band at 1160 cm⁻¹ resulting from asymmetric C-C stretch and C-O ring breathing vibrations for determining the quality of the spore inoculum are indicated by the VIP scores.

RESULTS FOR LIFE/DEAD - DETECTION OF *P. CHRYSOGENUM* SPORES

Test set 1
52 Raman spectra of dead spores
Classification result:
2 % viable spores
98 % dead spores

Test set 2
50:50 dead spores:viable spores
Classification result:
43 % viable spores
57 % dead spores

Financial support was provided by the Christian Doppler Gesellschaft and the Austrian research funding association (FFG) under the scope of the COMET program within the research project „Industrial Methods for Process Analytical Chemistry – From Measurement Technologies to Information Systems (imPACts)” (contract # 843546).


Operational sequence

Sampling (1.5 ml of spore inoculum)
1:500 division with H₂O
3 aliquots on CaF₂ sample carrier

Parameter settings
- 785 nm Raman laser
- A. Littoe laser power
- 1064 nm diode laser (Dio, A. L. 0.5)
- 600 lines/mm grating
- 2100 cm⁻¹ range
- 10 x 10 s integration time per single spectrum
- 100 µm fiber diameter (confocal pinhole)
- Deep Deposition Charge-Coupled Device (DD CCD)

Ramification State

Classification: 95 % sensitivity and specificity
Validation Set: 90 Raman spectra
Calibration Set: 170 Raman spectra

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