From fermentation control to protein structure monitoring: the value of Raman spectroscopy as an analysis tool in plant protein ingredients

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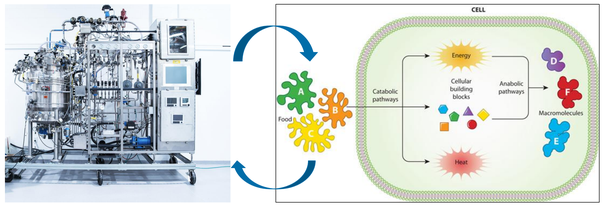
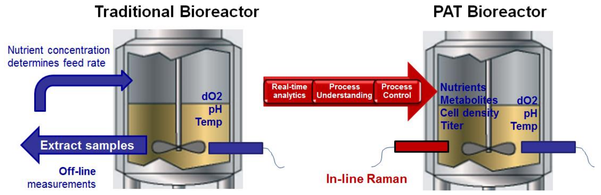
INTRODUCTION

Bioreactor parameters affect cellular metabolic processes and final product quality



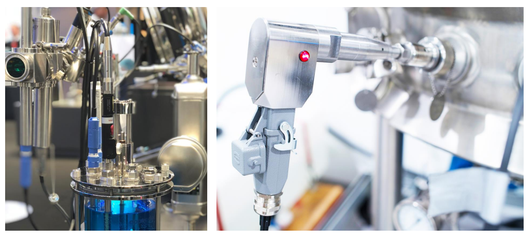
- Process Analytical Technologies (PAT) provide real-time, in situ process monitoring
- Optimized bioprocess require automated process control
- PAT-based monitoring of bioreactor parameters is the first step toward automated process control



EXPERIMENTAL

Raman spectroscopy has been an analysis technique for *in situ* fermentation monitoring since 1978 and for understanding biological molecules since 1937! **[1-4]**

In the studies described, a Kaiser Raman Rxn2 (λ=785 nm or 993 nm) was used to collect Raman spectra.

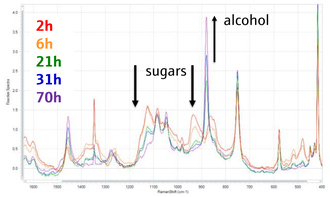


In situ bioprocess monitoring achieved using Kaiser probes for lab-scale/PD (bIO-Optic, left) or manufacturing scale (bIO-PRO, right) in single-use, glass or stainless steel bioreactors.

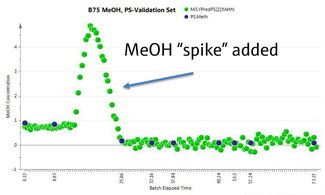
RESULTS AND DISCUSSION

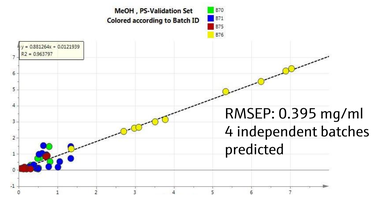
Raman spectroscopy: *in situ*, multi-attribute, real-time bioprocess monitoring

Fermentation Spectra



Monitoring and predicting methanol in *Pichia pastoris* fermentation



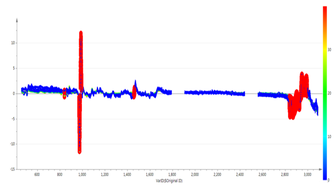


P. pastoris data courtesy of Juan Aon

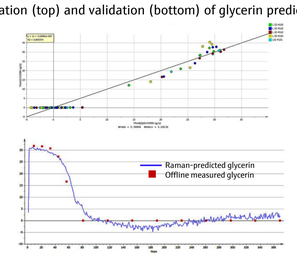
The specificity of *in situ* Raman spectroscopy in bioprocesses enables feedback control and model transfer without significant rework

In situ Raman-based predictions during yeast fermentation

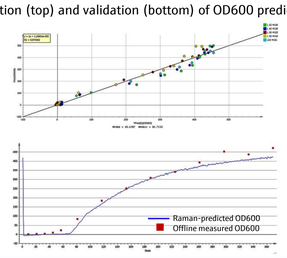
Variable weighing in glycerin spectra for PLS (SIMCA)



Calibration (top) and validation (bottom) of glycerin predictions



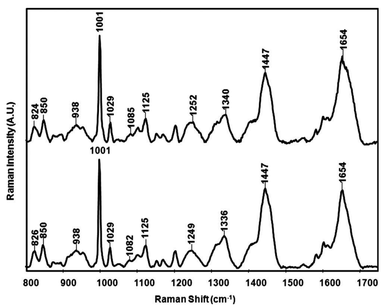
Calibration (top) and validation (bottom) of OD600 predictions



Raman: label-free measurements of protein composition and molecular structure

Raman spectroscopy of proteins **[4-5]**

Raman spectra of proteins is rich with information about the protein backbone and side chains

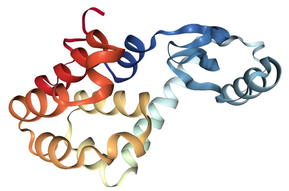


Protein secondary structure such as α-helix, random coil, and β-sheets are can be understood with a Raman spectrum

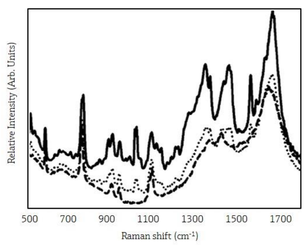
Raman shift (cm^{-1})	Band assignment	Component
620,640	Doublet	Tyrosine, Phenylalanine
940	C-C protein backbone	
1001	Ring breathing	Phenylalanine
1080	C-N, C-C stretch	Protein, polysaccharides
1125	C-C, C-OH C-N stretch C-O-C glycosidic linkage	Protein, polysaccharides
1235-1270	Amide N-H, α-helix	Protein structure
1270	Amide N-H, random coil	Protein structure
1340	CH_2/CH_3 wag	Protein
1446	CH_2/CH_3 deformation	Organic molecules
1655	Amide C=O, α-helix	Protein structure
1670	Amide C=O, random coil	Protein structure
1687	Amide C=O, β-sheet	Protein structure

Optimization of lysozyme crystallization **[6]**

Lysozyme was chosen as a model protein to optimize pH, ionic strength, and temperature conditions during crystallization

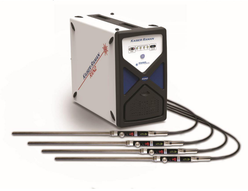


Raman spectra of lysozyme and the acetate buffer solution indicates that meaningful protein information could be obtained in an aqueous environment

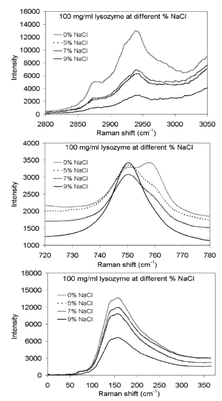


The 155 cm^{-1}, 750 cm^{-1}, and 2940 cm^{-1} bands were found to be responsive to supersaturation, aggregation, and eventual crystallization during the crystallization process

In the studies described, a Kaiser Raman Rxn1 (λ=785 nm) was used to collect Raman spectra directly in aqueous lysozyme solutions to monitor crystal growth. (current model: Raman Rxn2, shown below)



At varying salt concentrations, Raman spectra indicate various states in lysozyme molecular structure



CONCLUSIONS

Kaiser Raman is proven as a process analytical technology for industrial bioprocesses. The high chemical specificity of Raman to biological components such as alcohols, sugars, biomass and amino acids enables accurate bioprocess measurements and enables method transfer. *In situ* Raman probes are compatible with autoclave, SIP, CIP and gamma sterilization procedures. Raman spectroscopy has proven benefits for monitoring and control for industrial bioprocesses at the miniature bioreactor, benchtop, pilot, and cGMP manufacturing-scale scales **[7-12]** to achieve these goals:

Raman has also been demonstrated in characterizing proteins for these applications **[13-15]** and:

- Crystallization and aggregation
- Post-translational modifications
- Higher order structure
- Structure elucidation using isotope exchange
- Molecular response to mechanical stress

As a non-destructive, label-free, technique, a Raman-measured protein sample can also be examined by microscopy, mass spectrometry, x-ray crystallography, and rheology. Kaiser Raman is a valuable PAT throughout a product's lifecycle, enabling consistent product quality and process optimization.

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