Effect of Media on Cellular Metabolism

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2013 Co-op Scholars Student Poster Exposition

UMASS University of Massachusetts Lowell — Learning with Purpose

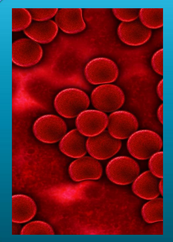
UML Biomanufacturing

CURRENT CHALLENGES

Next-generation drugs are grown in live cell cultures
- Batch and fed-batch cultures are increasingly common
- Cell culture manufacturing allows for synthesis of novel proteins and larger molecules

FDA encourages Quality by Design and Multivariate Data Analysis
- Design manufacturing to guarantee product quality
- Implement process analytical technology to monitor processes
- Improve reliability of products

Increased competition between companies
- Approval process for new drugs is slower
- Biosimilars and generics make competition fierce
- Need exists for very efficient manufacturing



Next-Gen Medicines

WHY MULTIVARIATE DATA ANALYSIS?

Handle Large Datasets
- Include extensive process data
- Non-linear and very multivariate datasets
- Use modern sensors

Meet Regulatory Standards
- Implement QbD and PAT
- Reduce final product assays
- Improve product uniformity

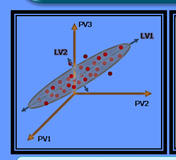
Metabolic Engineering
- Optimize cellular metabolism
- Increase production efficiency
- Process cultures more easily

MACROSCOPIC MODEL: MULTIVARIATE DATA ANALYSIS

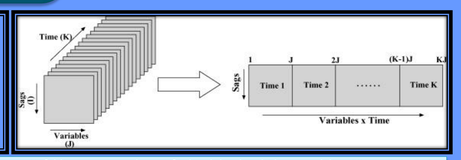
Principal Component Analysis
- See covariance in X-variables
- See relationships between data
- Reduce dimension of X-data

Partial Least Squares
- Create predictive models
- PCA analogue of multiple regression analysis
- What factors affect output

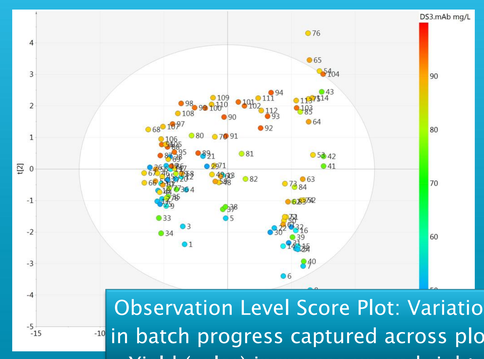
SIMCA Data Analysis Package
- Create charts and models
- Transform datasets for analysis
- Handle large volumes of data
- *Simca v. 13, MKS Umetrics AB, Sweden



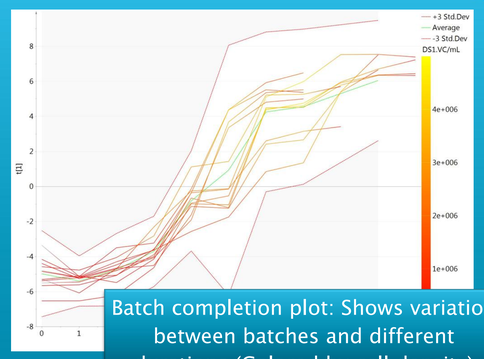
Reducing 3D data into 2 Principal Components



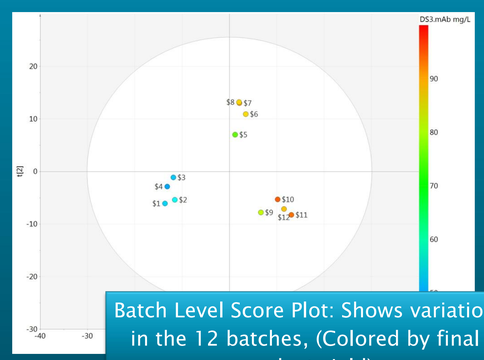
Unfolding a process dataset for batch level multi layer modeling (Khosravi, A, et al, Classification of sags gathered in distribution..., Electric Power Systems Research, 2009.)



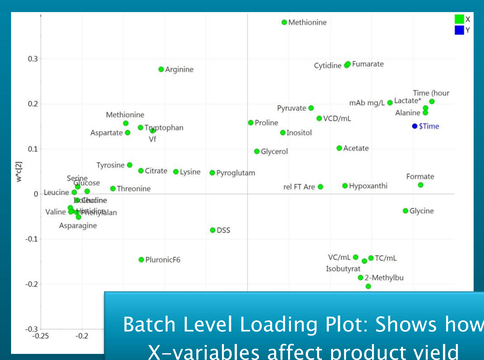
Observation Level Score Plot: Variation in batch progress captured across plot. Yield (color) increases up and right.



Batch completion plot: Shows variation between batches and different durations (Colored by cell density)



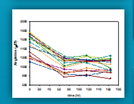
Batch Level Score Plot: Shows variation in the 12 batches, (Colored by final product yield)



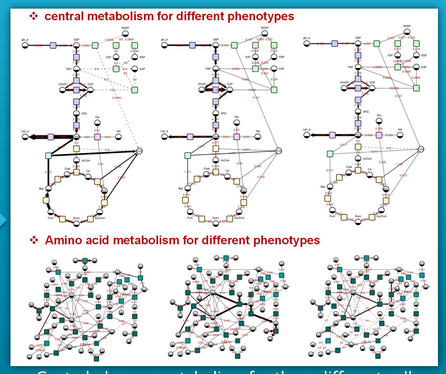
Batch Level Loading Plot: Shows how X-variables affect product yield

MICRO MODEL (PLANNED): FLUX BALANCE ANALYSIS

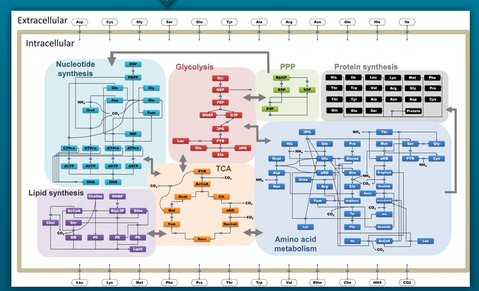
A method to determine reaction fluxes in cellular metabolism



1. Track changes in extracellular analyte concentration during batch
2. Calculate uptake/excretion rates for analytes based on run data
3. Set up mass balance system for cell metabolic network
4. Use optimization function to calculate biochemical reactions fluxes



Central glucose metabolism for three different cell culture phenotypes: 1 shows high antibody production, 2 shows high growth rate, and 3 shows neither

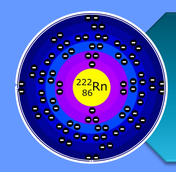


Example diagram of central cellular metabolism showing biochemical pathways

Compare reaction flux with cell behavior to find critical conditions

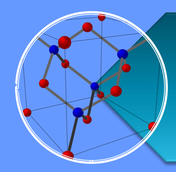
- Correlate flux variation to growth and product yield
- Describe the effects of media and environmental conditions on metabolism
- Explain macroscopic changes on a metabolic level
- Find genes responsible for reactions

EXTENSIONS TO F.B.A.



Use radioactive isotopes to trace compounds through cellular metabolism

Include thermodynamic constraints to fix direction of reversible reactions



Use reaction kinetics to extend model to dynamic flux balance analysis and metabolomics

Employ HPLC and Mass Spectroscopy techniques to track more analytes in and out of the cell

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Flux diagrams are from Lee, H. "Accessing impacts of raw material variability on cellular metabolic status using flux balance analysis of antibody-producing CHO cell culture." 2012.