Fed-batch Control of *S. cerevisiae* and *E. coli* at Critical Point Using mid-FTIR Spectroscopy

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Introduction

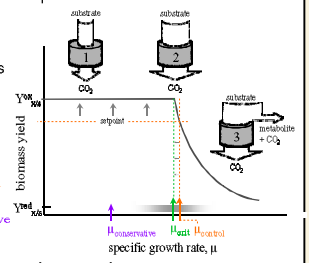
Many microorganisms exhibit an overflow type metabolism whereby the most efficient production pathway(s) become(s) saturated, and less efficient ones must be utilized. Often, the by-products from the "overflow" path accumulate in the reactor and reach inhibitory levels. This may be avoided by limiting the substrate concentration in the reactor, though control based directly on the limiting substrate concentration will often be very unstable or sub-optimal.

Optimal productivity achieved at μ_{crit}

Critical point at which metabolism overflows **is NOT** fixed; dependent upon...
- cell strain
- innoculum
- inhibitory components

Proposed control strategy fixes μ at $\mu_{control}$

Traditional approach operates at $\mu_{conservative}$



Optimal control of fed-batch fermentation requires:
- controller which can handle exponential biomass growth
- on-line measurement of overflow metabolite

Example organisms with (overflow metabolite):
- *S. cerevisiae* (ethanol): experimental results shown below, Sc9914
- *E. coli* (acetate): experiments in progress
- mammalian cells (lactate): experiments scheduled

Controller design

Based on two simple models:

1. Exponential cell growth: $VX = V_0 X_0 e^{\mu^* t}$

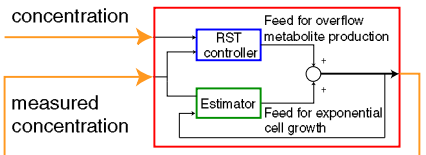
2. Overflow metabolite production: $\frac{dP}{dt} = K_a(Y_{P/S}) \cdot [F_{in} - K_b(VX)]$

Tuning is quick, simple, and requires only the overflow metabolite yield, **Yp/s**

Feed rate calculated to minimize error between the desired and the measured overflow metabolite concentration

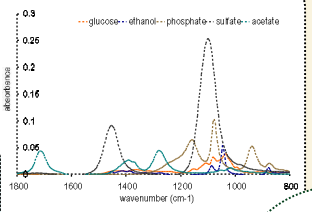
Growth rate is recursively identified

Proposed Adaptive Controller

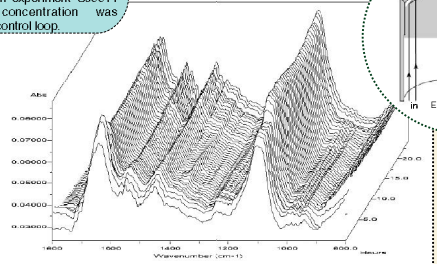


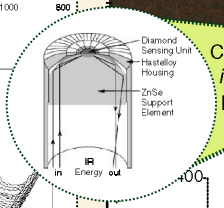
Mid-FTIR spectroscopy

Online mid-FTIR monitoring
- rapid (< 2 min)
- non-invasive
- flexible ("all-in-one" sensor)
- measures "fingerprint" region

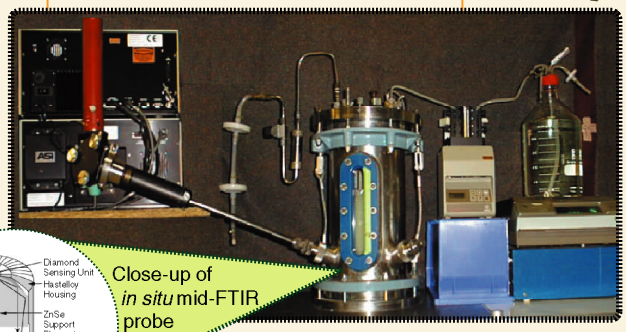


To your upper right are the pure component spectra of the principal medium components and metabolites for *S. cerevisiae* culture. These data (plus additional spectra) were used to build the Partial Least Square (PLS) model shown below. When the model was applied to the reaction spectra from experiment Sc9914 (right), ethanol concentration was estimated for use in control loop.



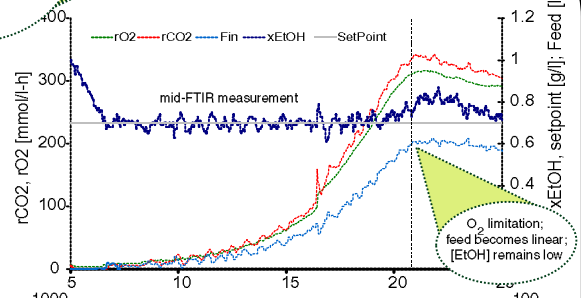


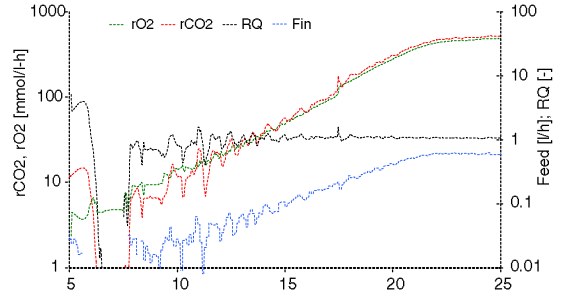
Analyte	Calibration spec. range [cm-1]	PLS factors [-]	SEC [g/L]	SEE validation [g/L]	SEE interferent [g/L]
glucose	1500-950	19	0.14	0.22	0.11
ethanol	1150-950	8	0.11	0.13	0.08
glycerol	1500-950	15	0.18	0.21	0.21
acetate	1500-950	11	0.09	0.08	0.08
sulfate	1500-950	11	1.00	2.14	0.98
phosphate	1500-950	11	0.61	0.20	0.65
PPG2000	1150-950	10	1.97	3.21	1.78

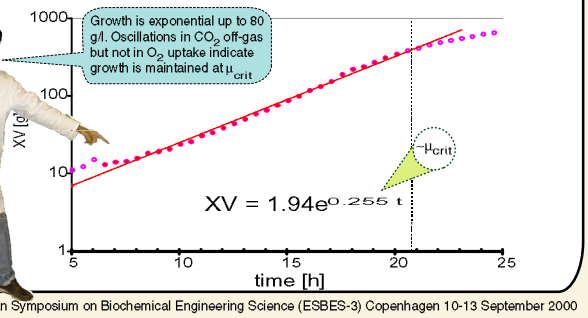


Close-up of *in situ* mid-FTIR probe

S. cerevisiae (Sc9914) fed-batch experiment







Conclusions

- Methodology enables exponential biomass growth with constant overflow metabolite production
- High yield maintained for duration of process
- Little knowledge of metabolism needed. Only Yp/s required
- Automatic compensation for:
  - biological disturbances (e.g. changing μ_{crit})
  - physical limitations (e.g. oxygen, heat)
- Online mid-FTIR measurement was successfully incorporated into control loop. One of very first applications!
- For more information, visit either of our websites

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