

**UNIVERSITEIT GENT**  
**BIOMATH**  
 Department of Applied Mathematics,  
 Biometrics and Process Control

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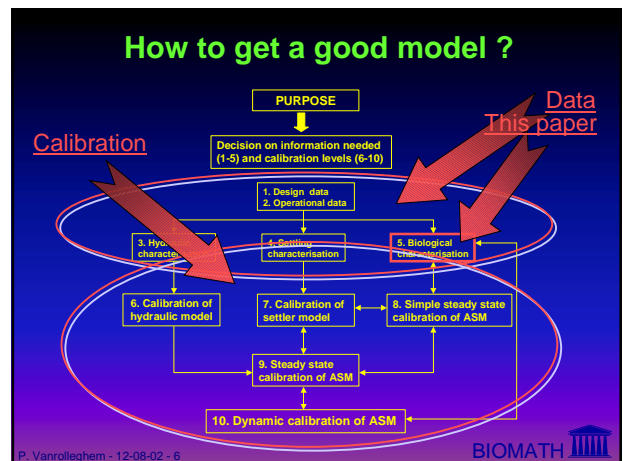
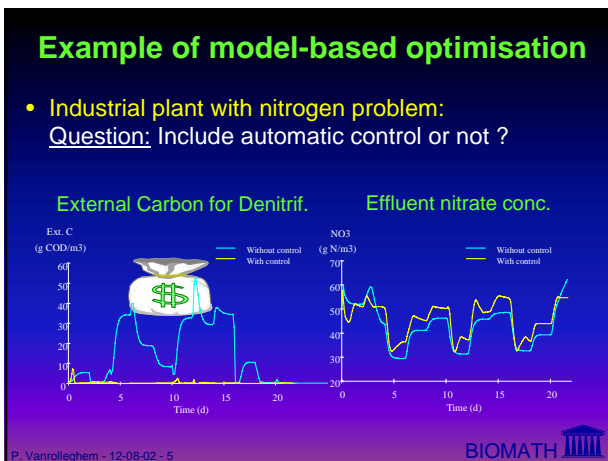
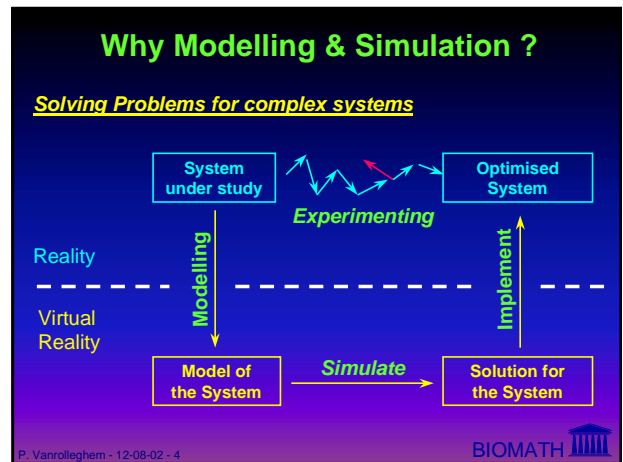
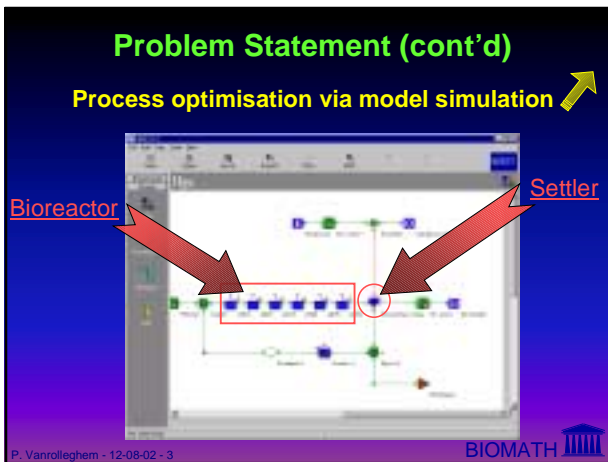
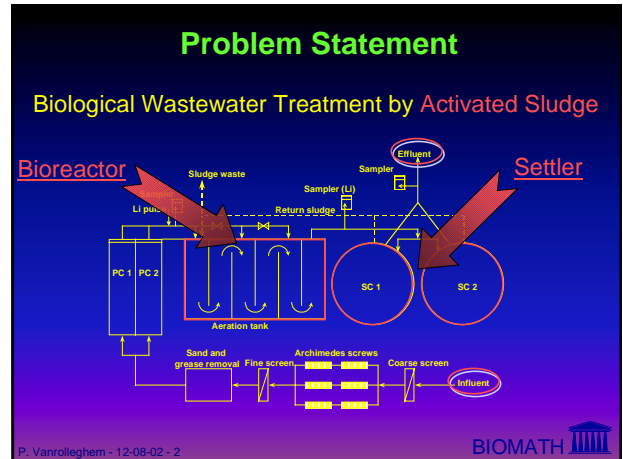
**On the limitations of short term experiments  
 for the calibration of dynamic models of  
 activated sludge wastewater treatment**

Peter Vanrolleghem  
 12-Jul-2000

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*Cairns, 2000 Joint Scientific Meeting  
 The Millenium for Microbiology*

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## Good quality experimental data for biological characterisation

- **Process related information**
  - nitrification ( $\text{NH}_4 \rightarrow \text{NO}_3$ )
  - oxidation of organic pollutants
- **Fast:** experiments cannot take too much time
- **Cost-effective**
  - simple experimental set-ups & little labour involved
- **Accurate:** little error in raw data
- **Sensitive:** data quickly reflect change in characteristics
- **Relevant**
  - organisms behave similarly in experiment as in full-scale

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## Respirometry as preferred method

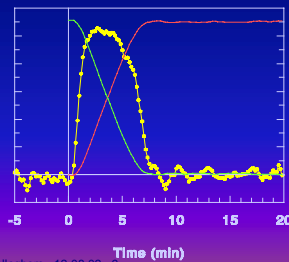
- **Process related information**
  - respirometry = measurement of biological oxygen uptake
  - oxygen consumption for nitrification & organics oxidation
- **Fast:** experiments take approx. 1-2 hr
- **Cost-effective**
  - aerated vessel + dissolved oxygen probe
  - no lab analysis; automatic data collection on PC
- **Accuracy:** < 1% error on respiration rates
- **Sensitivity:** 0.01 mg  $\text{O}_2/\text{l}$  = 10 ppb !
- **Relevant data ???**

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## Batch respirometry: a typical data set

- Inject a given amount of substrate to endogenous sludge
- Substrate concentration decreases as it is degraded
- Oxygen consumed  $\approx$  substrate degraded
- Oxygen uptake rate (OUR)  $\approx$  slope of degradation



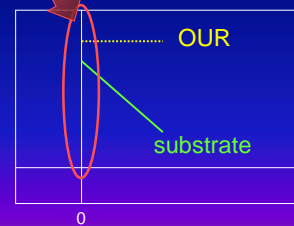
OUR measurements tell us something about degradation characteristics of sludge !

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## Problem 1 with the data set

- Classic (Monod) biodegradation models predict instantaneous start of substrate degradation

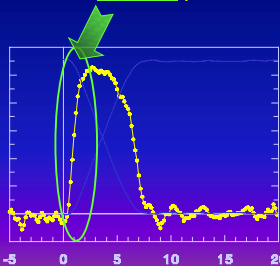


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## Problem 1 with the data set

- Classic (Monod) models predict instantaneous start of substrate degradation
- Observations show a start-up phenomenon



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## Understanding of Problem 1

### Three hypotheses:

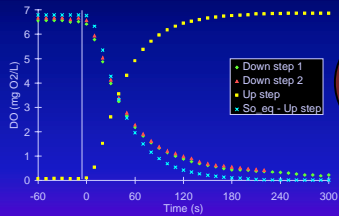
- Dissolved oxygen electrode ?
- Mixing of the substrate in the reactor ?
- Diffusion of the substrate in the sludge floc ?

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### Hypothesis 1

OUR is calculated from raw dissolved oxygen data  
 Thus: DO probe dynamics will influence resulting OUR data



Experiment:  
 Step response to characterise DO probes

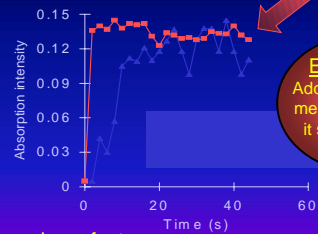
Effect of slow DO probe dynamics cannot be neglected but is insufficient to explain findings!

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### Hypothesis 2

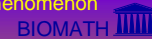
Is mixing of the injected substrate sufficiently fast?  
 => Mixing characterisation with fluorescent tracer test



Experiment:  
 Add fluorophore & measure how fast it spreads in the reactor

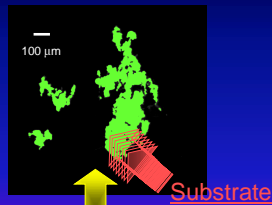
Mixing is nearly perfect and, thus, cannot be the cause of start-up phenomenon

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### Hypothesis 3

Is the substrate diffusion rate into the floc limiting?



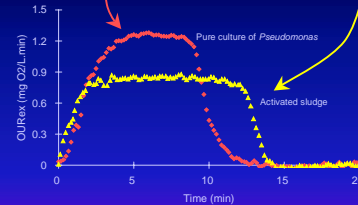
How to test this? Via single cell culture!

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### Hypothesis 3 testing

Pure culture of *Pseudomonas* vs. Activated sludge



The same behaviour was observed  
 Thus, no diffusion limitation occurs in sludge!

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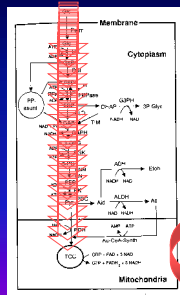


### Last hypothesis!

Metabolism of substrates induces delay?

Inspired by work in group of M. Reuss:

"In vivo analysis of metabolic dynamics of *S. cerevisiae*"



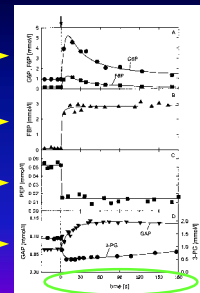
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### Last hypothesis: Experimental Evidence

Dynamic evolution of intermediates inside yeast cell after addition of a pulse of glucose

- G6P, F6P
- Fruc-1,6-bis-P
- PEP
- GAP, 3-PG



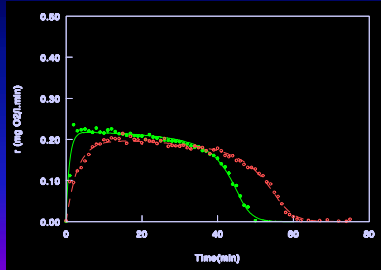
Timing (2') looks appropriate!

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## Nitrifiers start-up depends on history

Immediate  $\text{NH}_4$  addition vs. addition after 1 famine day

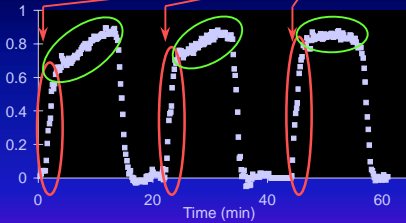


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## Problem 2: Another phenomenon...

Respirometric response after consecutive acetate additions:



Response is not reproducible !!

Approx. 1 hr needed to stabilise, but returns after 1 famine day



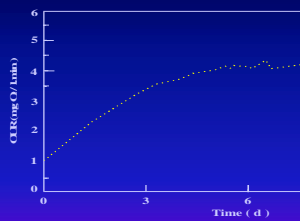
Enzyme activation ?

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## And yet another one ...

Long term evolution of maximum OUR of a sludge  
( $\approx$  max. substrate degradation rate) :



Evolution over days  $\implies$  change in microbial population

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## Take home

- Why do we model ?  $\implies$  complex system optimisation
- Biological characterisation of activated sludge (1/10)
  - respirometry as preferred method
  - fast, cheap, cost-effective, accurate, sensitive
  - process related information, but is it relevant information ?
- Start-up phenomena triggered our attention (2 min)
  - 3 physical hypotheses turned out not to be valid
  - metabolic phenomena seem to be a good hypothesis
- Further, slower, start-up phenomena were observed
  - enzyme activation (1 hr)
  - microbial population dynamics (5 d)

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## Take home

Consider both physical and microbial properties when designing experiments to characterise the biological processes occurring in activated sludge

Serious limitations have (still) to be overcome when performing lab scale experiments to obtain relevant information on full-scale behaviour

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