Coadvise + Treasure programmes

Specialist Course

Tlemcen, 7th – 11th February 2010

POLITECNICO DI MILANO





Biomass activity measurements

Part 2 – Respirometry and Titrimetry

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General Index (2)

1) Fundamentals of Microbiology *(short hints)*

2) Main microbial substrates in wastewater: organic substances and nitrogen compounds

3) Bacterial activity assessment techniques

- Respirometry
- Titrimetry
- Manometry
- Calorimetry

Bacterial activity assessement

Bacterial activity can be evaluated in batch tests by tracking:

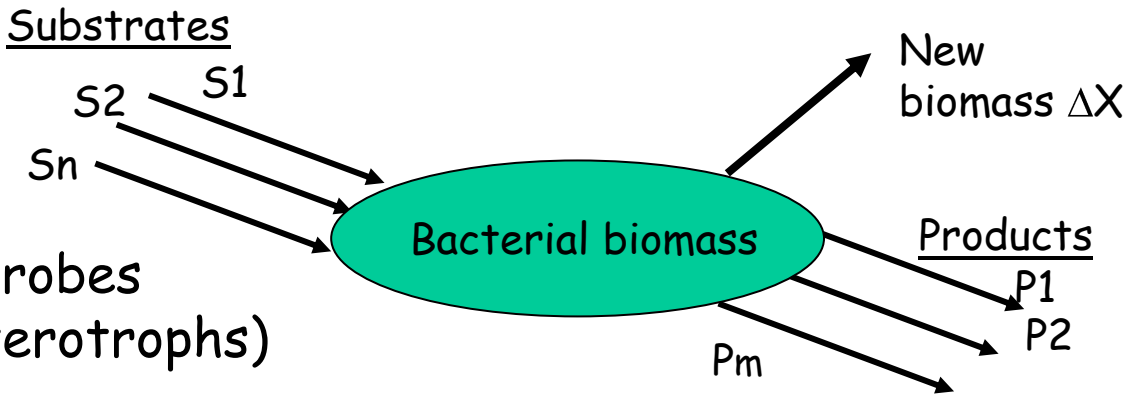
- The concentration of a substrate/product by:
  - manual sampling and analysis
    - ✓☺ simple and conventional
    - ✓☹ time consuming
  - using an on-line probe: titrimetry / respirometry
    - ✓☺ simple and convenient
    - ✓☹ dependent on probe availability/stability/reliability
  - measuring reaction by-products: manometry (gas production) and calorimetry (heat exchanged)
    - ✓☺ simple and convenient
    - ✓☺ simple and convenient (on-line data)
    - ✓☹ dependent on instrument reliability/sensitivity

RESPIROMETRY

Technique that draws information about aerobic biological reactions through the

analysis of the oxygen consumption rate

Applicable to aerobic microbes
(both autotrophs and heterotrophs)



Under ***aerobic conditions***, one of the substrates is dissolved oxygen (O_2, which acts as electron acceptor) :

$$S_1 + S_2 + \ldots + S_{n-1} + \boldsymbol{O_2} \xrightarrow{\text{rate, } r} P_1 + P_2 + \ldots P_m + \Delta X$$

reaction rate, ***r***, is proportional to consumption rate of oxygen:

$$r \propto r_{O_2} = d(O_2)/dt$$

Respirometry can be used to estimate:

1. **Biomass growth kinetic and stoichiometric parameters** such as:
   - ✓ maximum growth rate (μ, d^{-1})
   - ✓ decay rate (b_h, d^{-1})
   - ✓ half-saturation constant (K_s)
   - ✓ cell yield coefficient (Y)

$$-\frac{dS_O}{dt} = \hat{\mu}_H \cdot \left(\frac{S_S}{S_S + k_S}\right) \cdot \left(\frac{S_O}{S_O + k_{OH}}\right) \cdot X_{BH} \cdot \frac{1 - Y_H}{Y_H} + \hat{\mu}_A \cdot \left(\frac{S_{NH}}{S_{NH} + k_{NH}}\right) \cdot \left(\frac{S_O}{S_O + k_{OA}}\right) \cdot X_{BA} \cdot \frac{4{,}57 - Y_A}{Y_A}$$

2. **Organic substrate characteristics :**
   - ✓ Rapidly biodegradable fraction (rbCOD, mg L^{-1})
   - ✓ Slowly biodegradable fraction (sbCOD , mg L^{-1})
   - ✓ Toxicity and growth inhibition (fraction of μ_{max})

Respirometry (3)

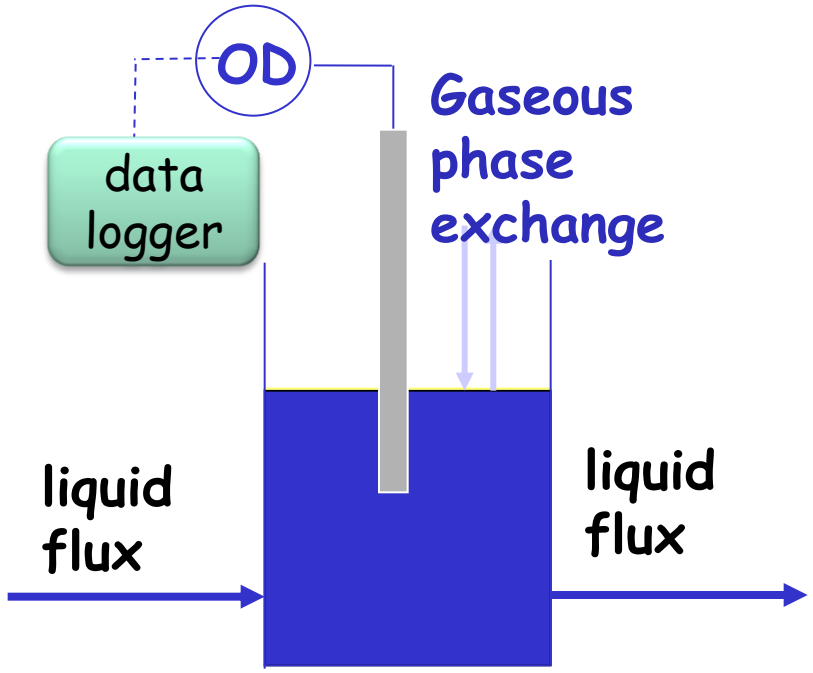
A **RESPIROMETER** measures oxygen concentration vs time and is capable of deriving the oxygen consumption rate (dO_2/dt). The output of a respirometer is a **RESPIROGRAM**

i.e.: graph of Oxygen Uptake Rate (r_o, or OUR, $mgO_2\ L^{-1}\ h^{-1}$) vs time

Once the concentration of VSS is known, then a specific OUR can be calculated (sOUR, $mgO_2\ gVSS^{-1}\ h^{-1}$)

A respirometer is made of:

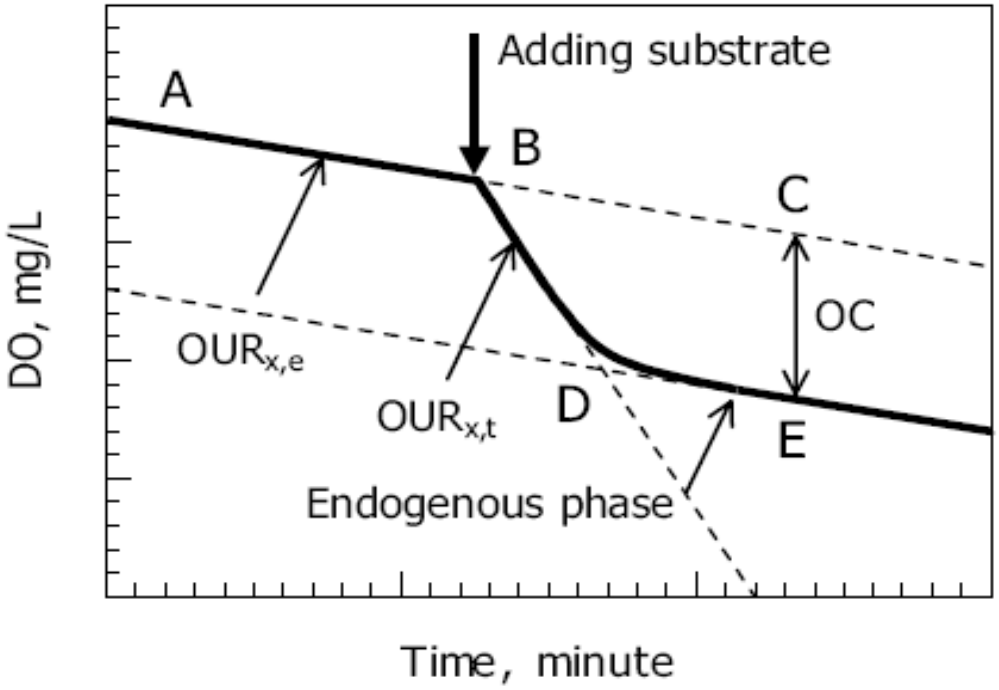
- A biological reactor (vessel)
- An O_2 concentration measuring probe
- A data acquisition and logging system

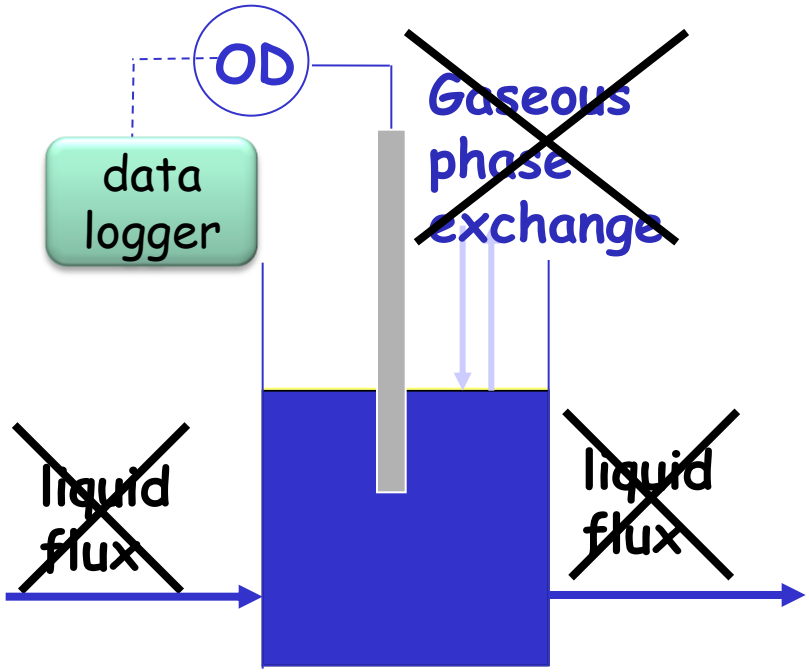


Oxygen can be measured in the gas phase or, more commonly, in the liquid phase

CLOSED RESPIROMETER

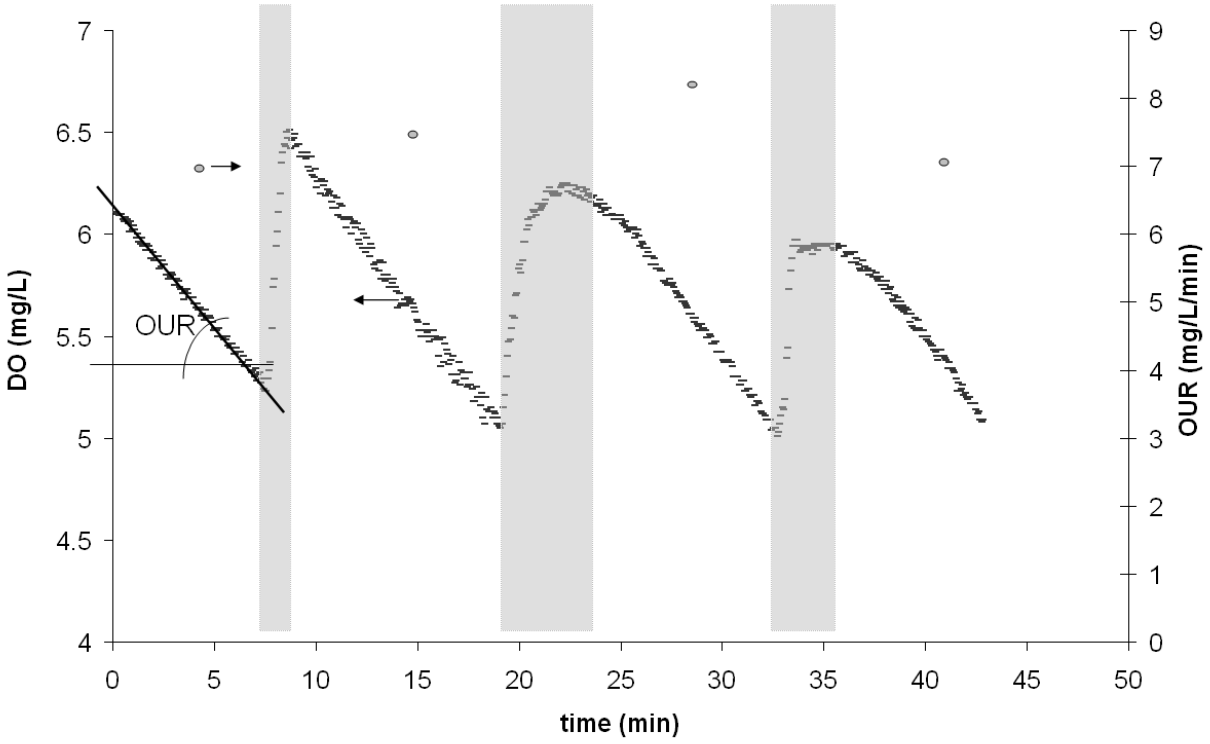
batch reactor – neither liquid, nor gaseous flux → Oxygen variation is due to biological consumption only





OPEN RESPIROMETER

Oxygen is not limiting: periodic aeration starts as O_2 concentration falls below a pre-set limit

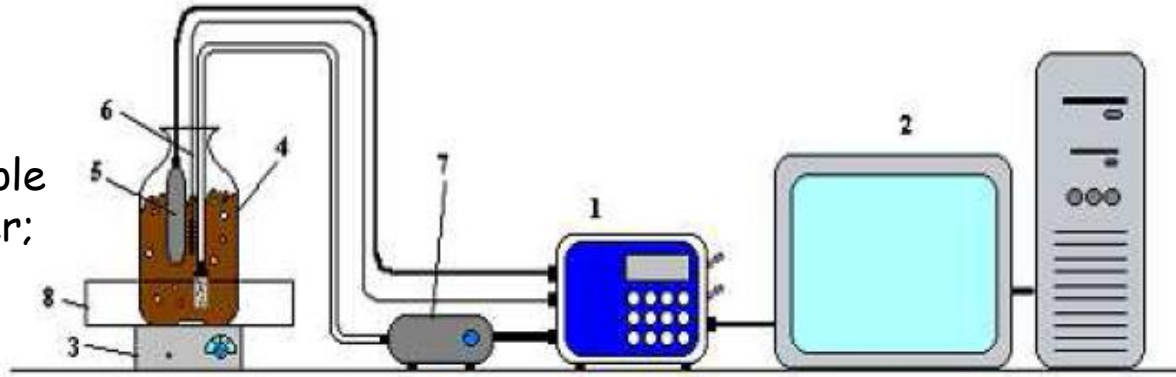


OUR is determined as the slope during non-aerated periods

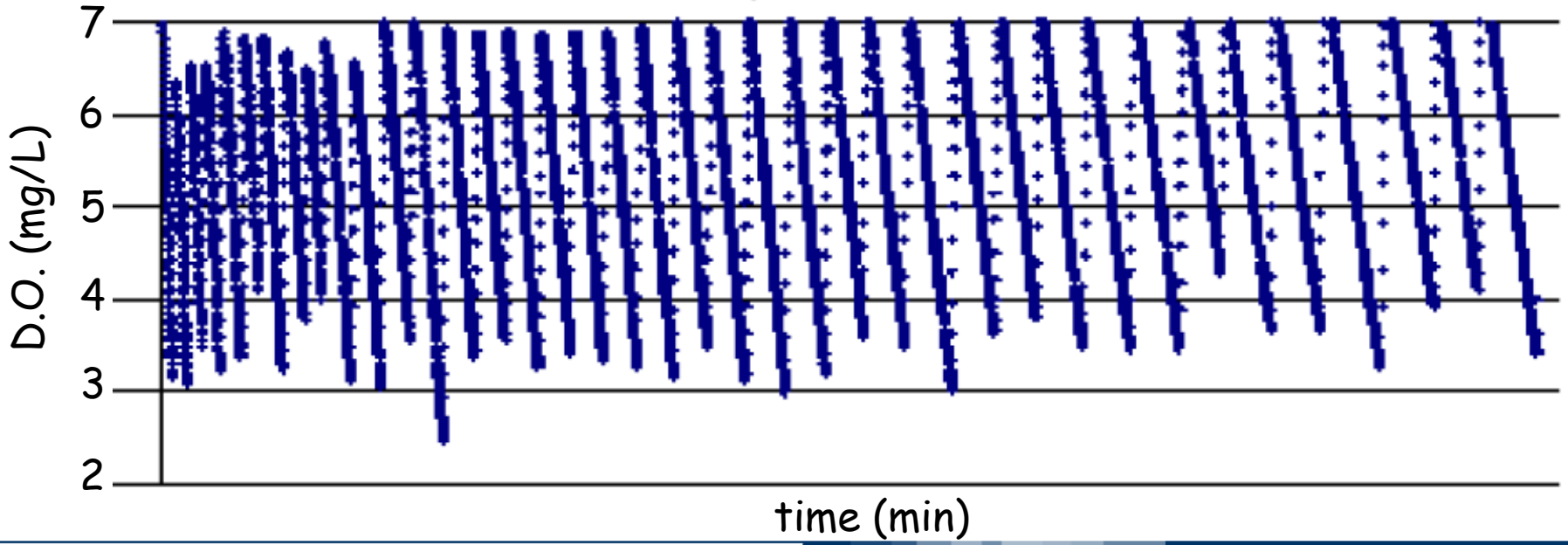
Respirometry (6): respirometer and respirograms - OUR analysis (1)

A Lab respirometer:

1: DO recorder; 2: PC; 3: magnetic stirrer; 4: Serum bottle with sample and activated sludge ; 5: DO-meter; 6: aerator (porous stone); 7: air compressor; 8: thermostatic bath

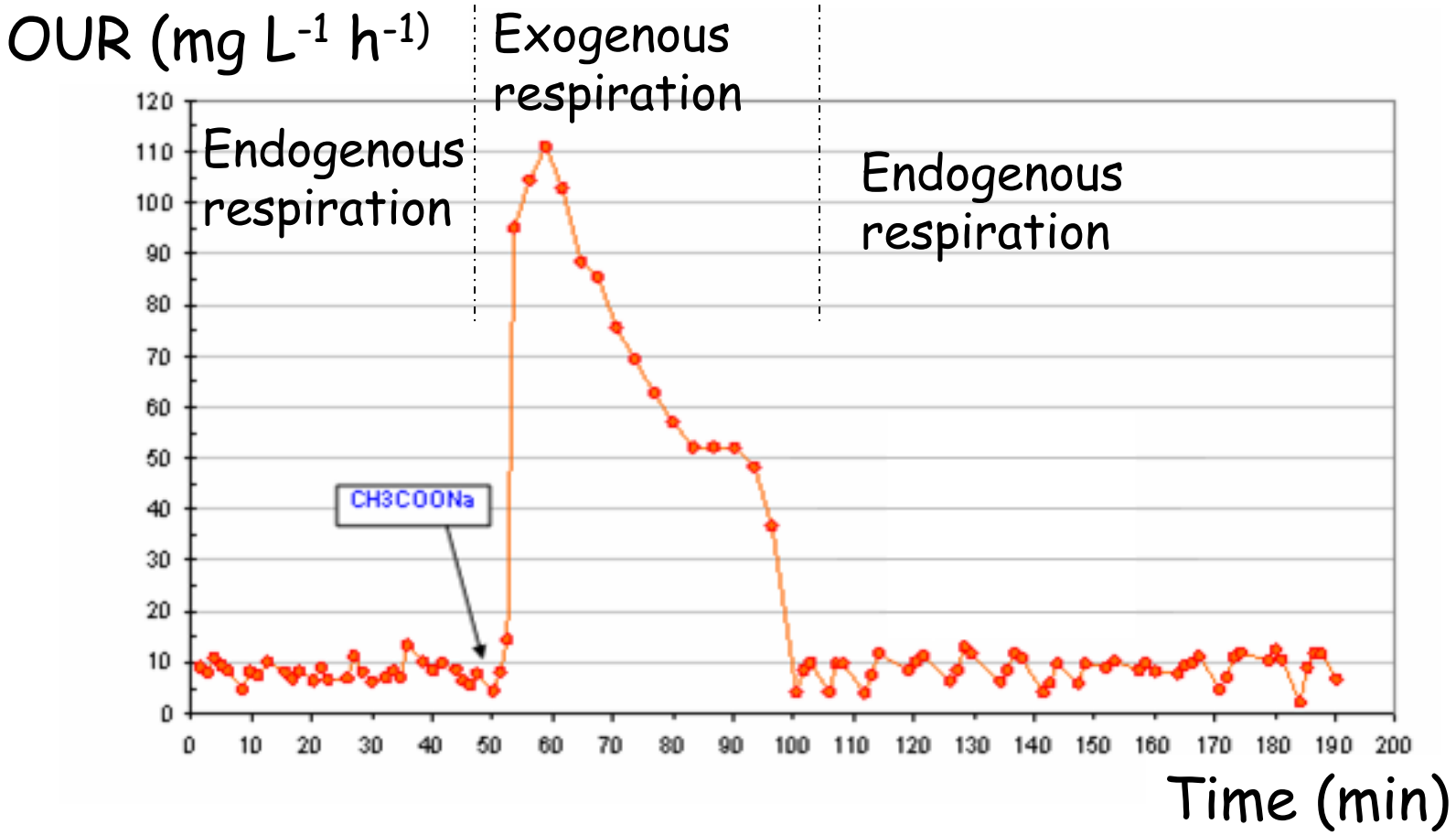


raw D.O. measurements: **respiration** (slopes) and re-aeration

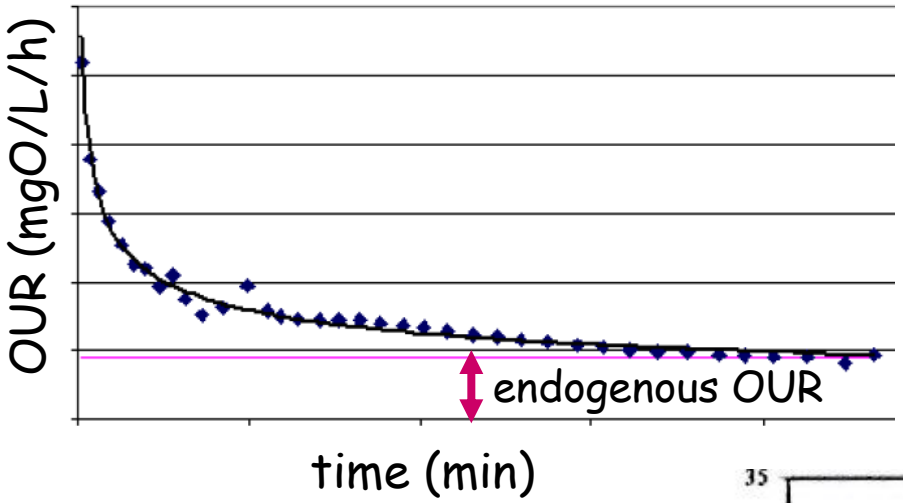


Respirometry (7): respirometer and respirograms - OUR analysis (2)

Example of respirogram: acetate (rbCOD) is added to an activated sludge sample

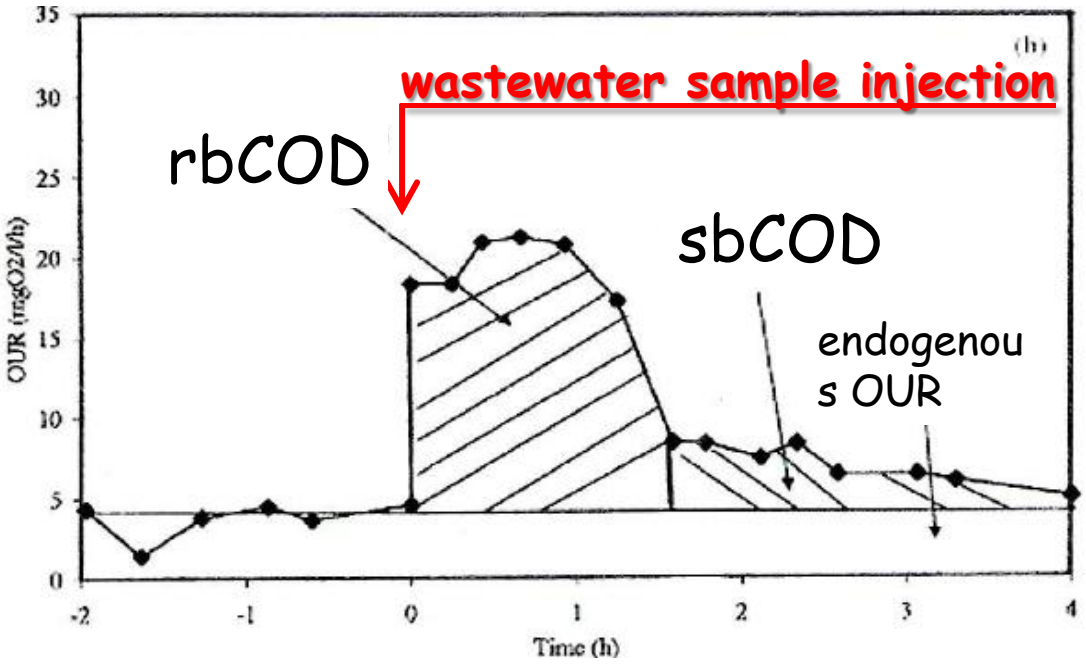


Respirometry (8): respirometer and respirograms - OUR analysis (3)



OUR chart (respirogram)

each point is the value of "1 slope" taken from the raw data in the previous graph



OUR chart (respirogram)

for the determination of rbCOD and sbCOD; care must be taken to choose the right F/M ratio (usually F/M = 0,1 to 0,2 gCOD/gVSS)

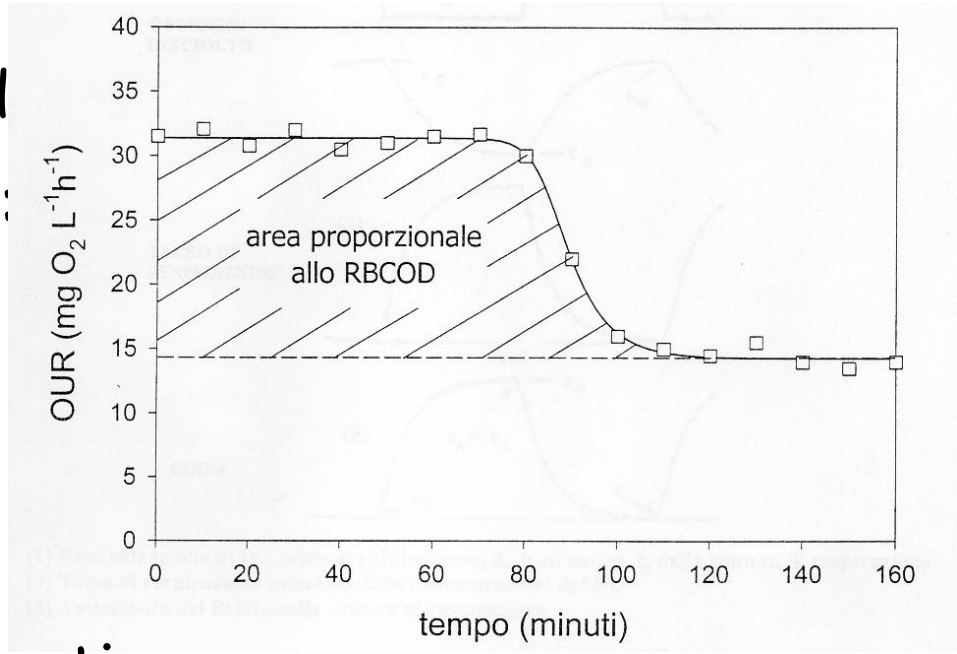
Respirometry (9): rbCOD measurement

1) a wastewater sample is added

2) r_o (OUR) vs time is measured

3) Oxygen consumed is calculated (dotted area)

$\Delta O_2 = \int r_o \, dt$



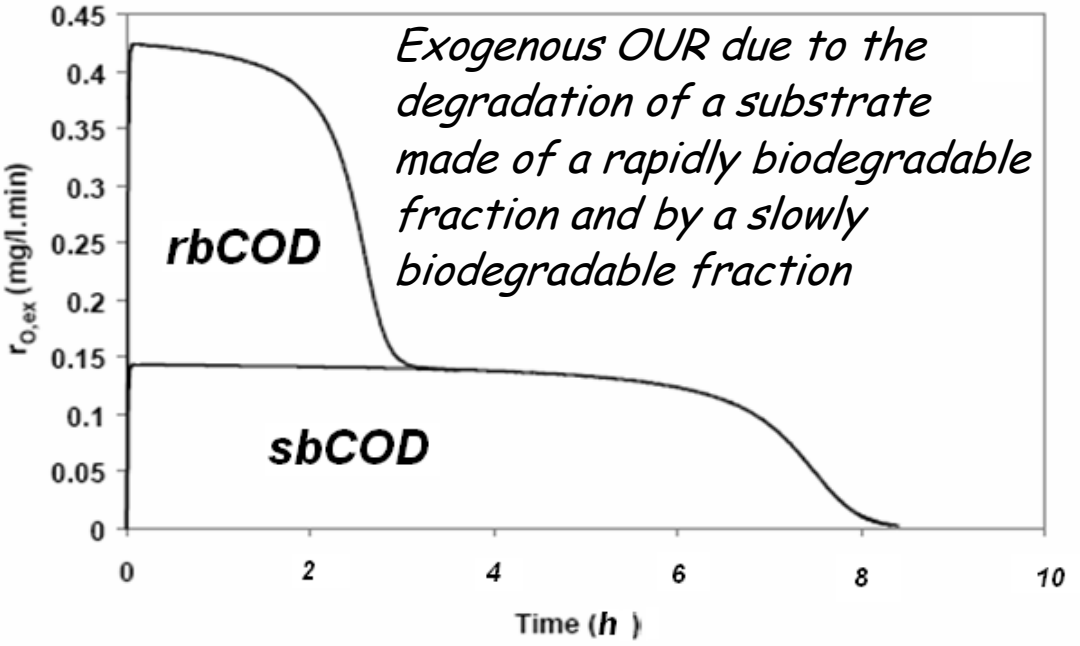
4) rbCOD is calculated by subtracting the fraction used for growth

5) Y_H has been previously calculated with calibration tests, where a known amount of sodium acetate is added, assuming that rbCOD = sodium acetate

$$rbCOD = \frac{\Delta O_2}{\left(-Y_H\right)}$$

Respirometry (10): slowly biodegradable COD (sbCOD) measurement (1)

Total biodegradable COD = bCOD = rbCOD + sbCOD



Test conditions :

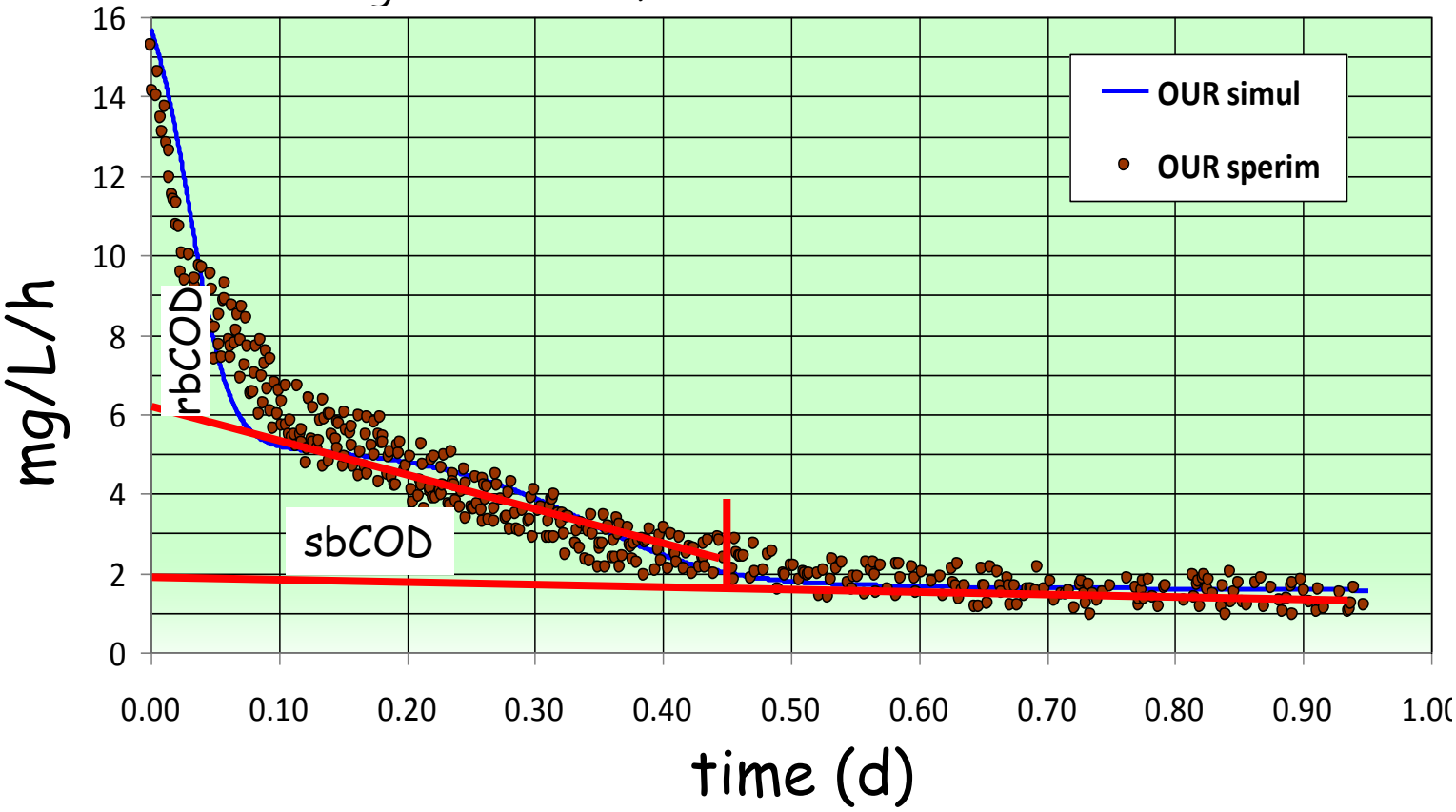
- Biomass concentration: high enough so that r_O is clearly measurable (0,8-2 gVSS/L)
- F/M ($bCOD_{ww}/COD_{biomass}$) high enough (0,2-0,4) to ensure at least 2 hours at sustained OUR values
- Nitrification is suppressed by ATU addition

Respirometry (11): slowly biodegradable COD (sbCOD) measurement (2)

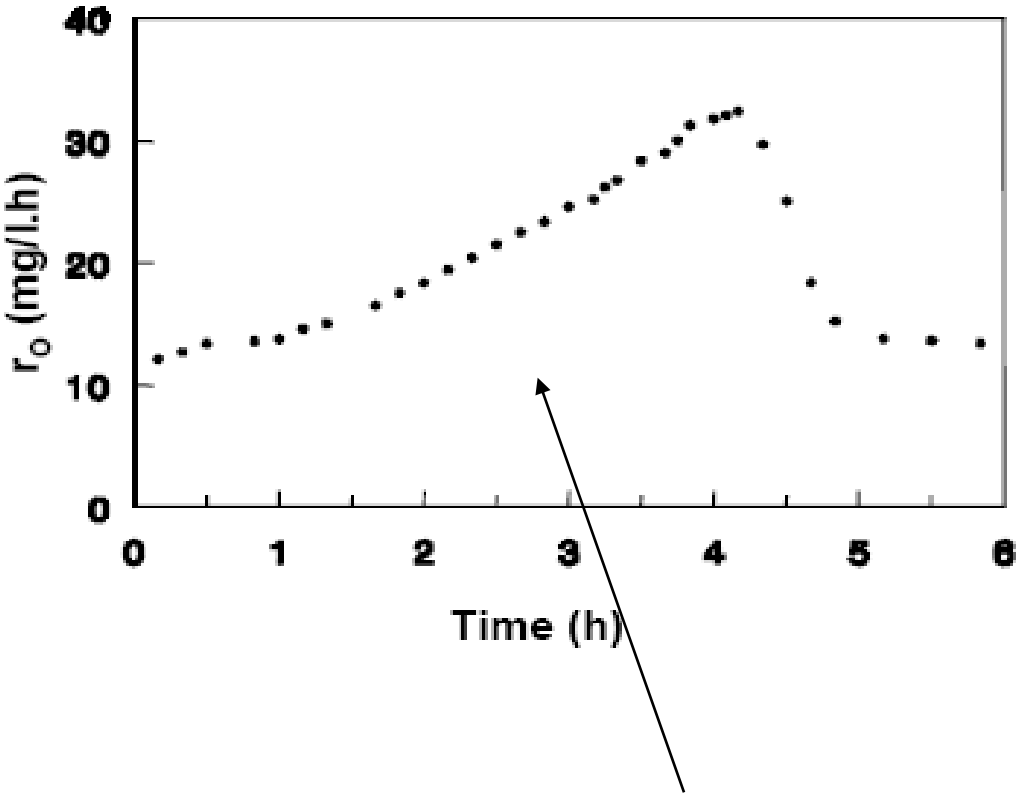
Actual example of an experiment with real sewage

rbCOD is almost absent and sbCOD is made of a mixture of substrates)

Te blue line is an ASM1 simulation of the respirogram : sbCOD and rbCOD have been chosen as the values that give the best fit.



Respirometry (12): maximum growth rate (μ_{max}) and active heterotrophic biomass (X_{bh}) - 1



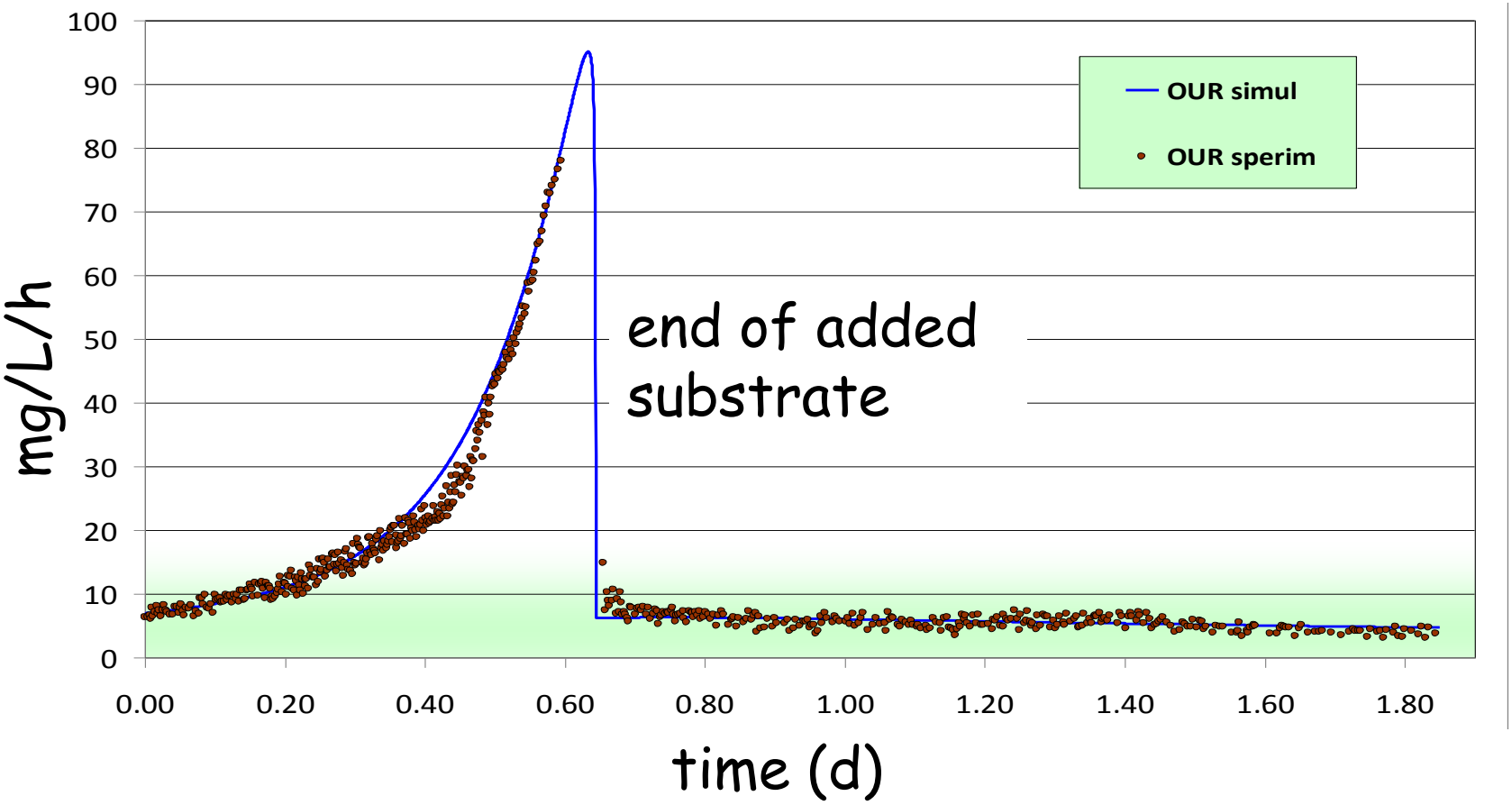
Only data in the exponential growth phase are considered

Test conditions :

- High F/M (2-4) so that $S_s >> K_S$ and X_{bh} growth can be observed during the experiment
- Nitrification is suppressed by adding ATU

Respirometry (13): maximum growth rate (μ_{max}) and active heterotrophic biomass (X_{bh}) - 2

Actual experimental test with ASM1 interpretation



As for Oxygen, it can be written

$$r_O = \frac{1-Y_H}{Y_H} \cdot \left(\hat{\mu} \cdot \frac{S_S}{S_S + K_S} \cdot \frac{S_O}{S_O + K_{OH}} \right) \cdot X_{BH}$$

As $S_S >> K_S$, $S_O >> K_{OH}$

$$\frac{S_S}{S_S + K_S} \cong 1; \quad \frac{S_O}{S_O + K_{OH}} \cong 1$$

$$r_O = \frac{1-Y_H}{Y_H} \cdot \hat{\mu} \cdot X_{BH}$$

As for biomass, it can be written:

$$r_{X_{BH}} = \frac{dX_{BH}}{dt} = (\hat{\mu} - b) \cdot X_{BH}$$

And, integrating:

$$X_{BH} = X_{BH}(0) \cdot e^{(\hat{\mu}-b)t}$$

$$r_O(t) = r_O(0) \cdot e^{(\hat{\mu}-b) \cdot t}$$

$$r_O(t) = r_O(0) \cdot e^{(\hat{\mu}-b)\cdot t}$$

or, in logarithms $\ln(r_o(t)) = (\hat{\mu} - b) \cdot t + \ln(r_o(0))$

which is a straight line y = mx + q where:

$q = \ln(r_o(0))$ and $m = \hat{\mu} - b$

$x = t, \quad y = \ln(r_o(t))$

Therefore it can be written $e^q = (\frac{1 - Y_H}{Y_H} \hat{\mu}) \cdot X_{BH}(0)$

As Y_H is known and assuming $\hat{\mu} \gg b$, $X_{BH}(0)$ can be estimated

$X_{BH}(0)$ = active biomass in the original activated sludge sample

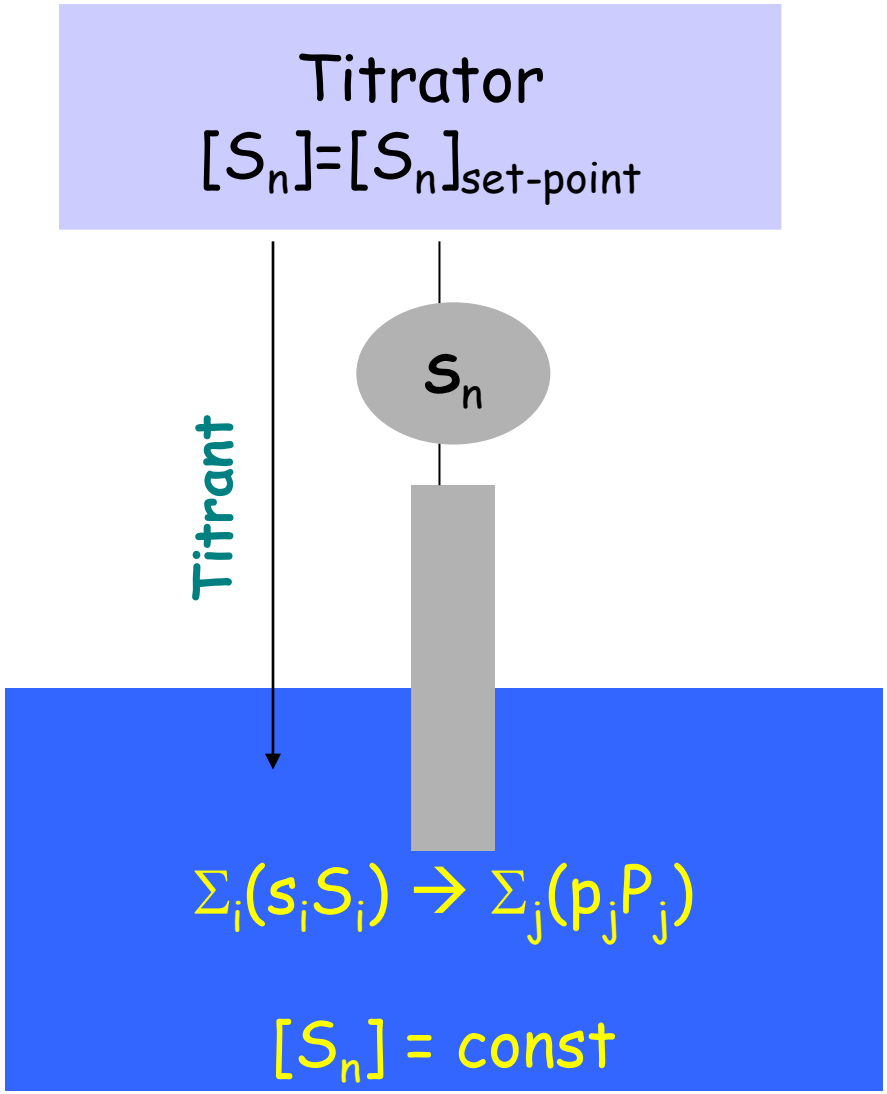
TITRIMETRY

A bioreaction to be monitored takes place in a fed-batch reactor.

A sensor measures the concentration of one among Si or Pj (e.g. [Sn]) and a titrator keeps $[S_n]$ constant by addition of an appropriate titrant.

Normally the titration experiment stops when one of the substrates is used up (e.g. S_L)

If S_n = [H+] → pH = constant



Biomass activity tests with pH-stat titration

Generic biological reaction **involving protons**

a) stoichiometry

$$\Sigma_i(s_i S_i) \rightarrow \Sigma_j(p P_j) + b HCO_3^- + c CO_2 + d CO_3^= + h H^+$$

b) reaction rate $r_{p,i}$ *can be measured by the titration rate* r_t

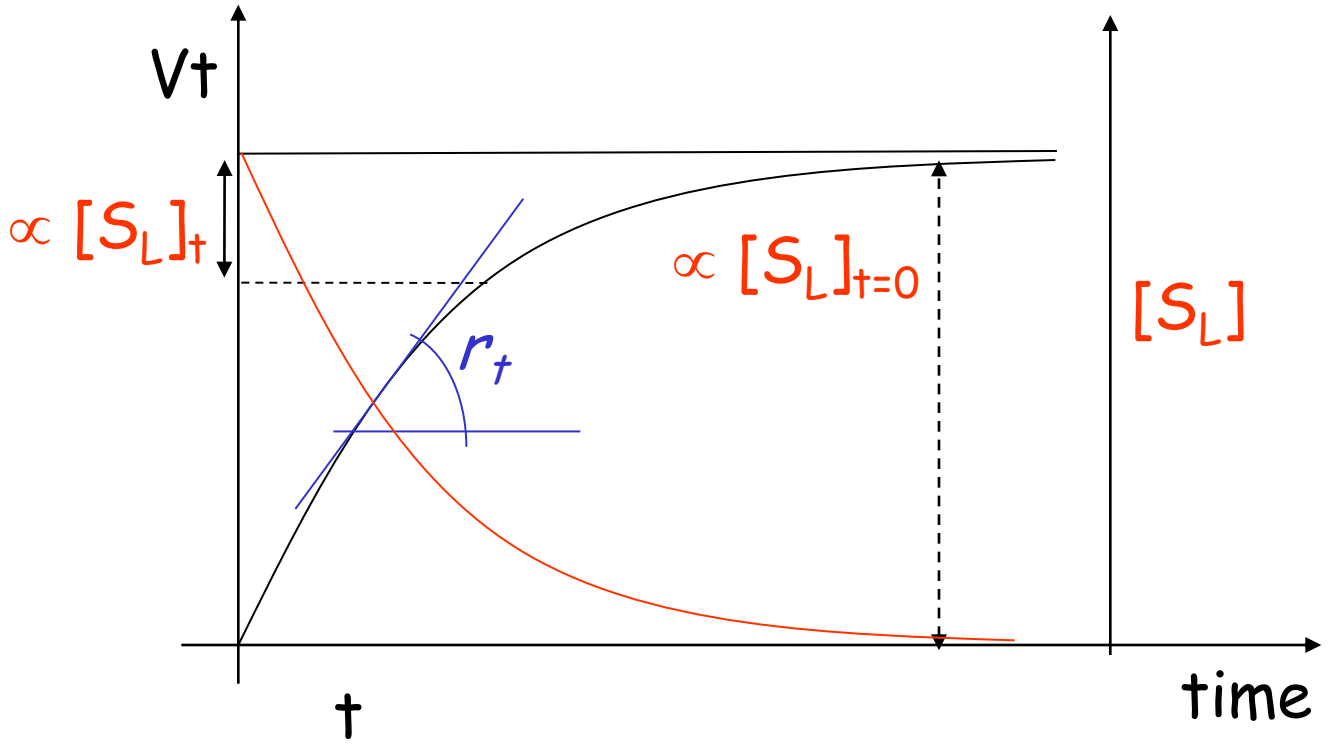
$$r_{p,i} = d[P_i]/dt \propto r_t = r_t\,(1) + r_t\,(2)$$

(1) accounts for products formation;

(2) accounts for gas transfer in open systems

*Titration **curve***: volume of titrant added (Vt) vs time

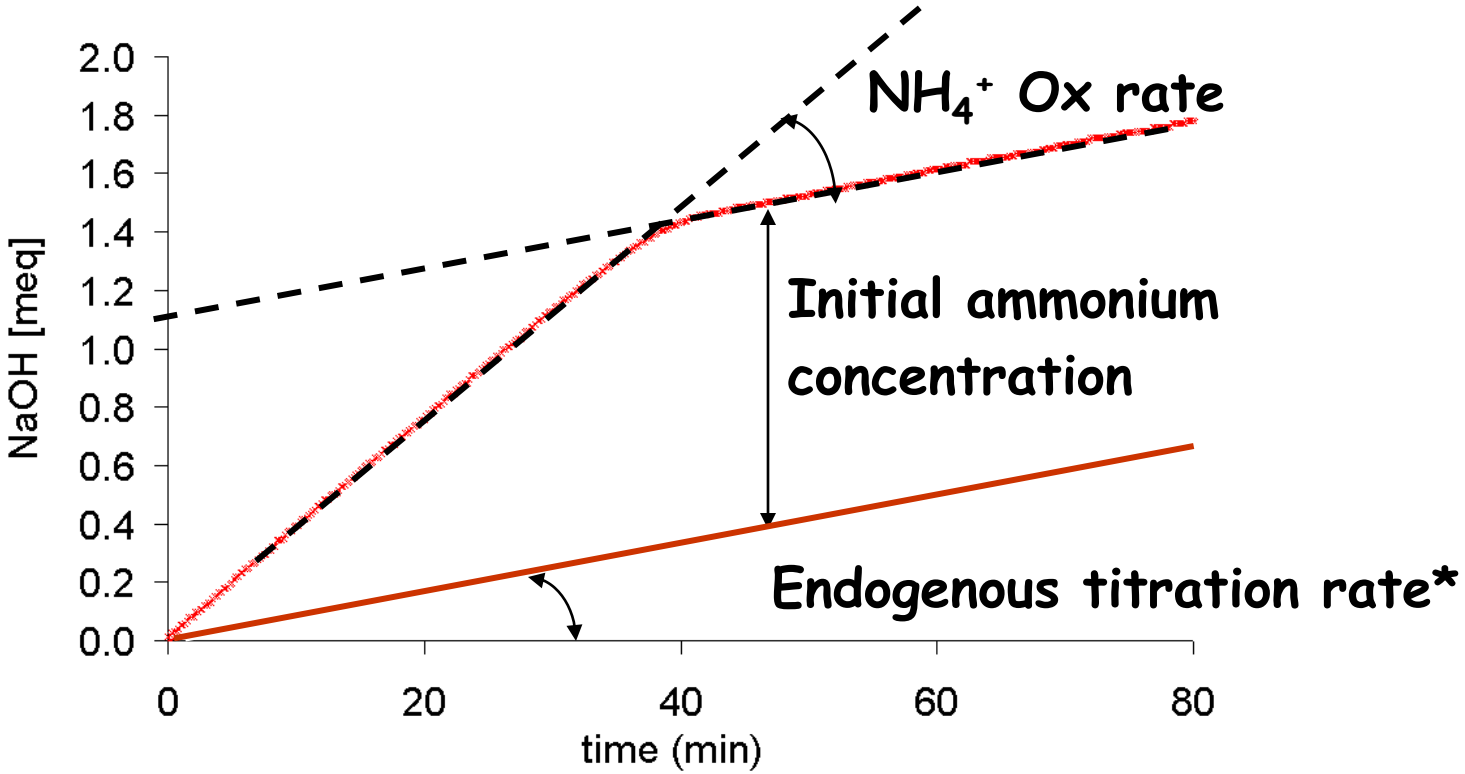
*Titration **rate***: $r_t = \frac{dS_n}{dt}$



Application of the pH-DO stat titrator to assess:

- maximum and actual **nitrification activity**
- detection of the **end of nitrification** during the aeration phase of the SBR cycle
- **denitrification activity** of SBR sludge

$$NH_4^+ + 1.5\ O_2 \rightarrow 2\ H^+ + NO_2^- + H_2O$$

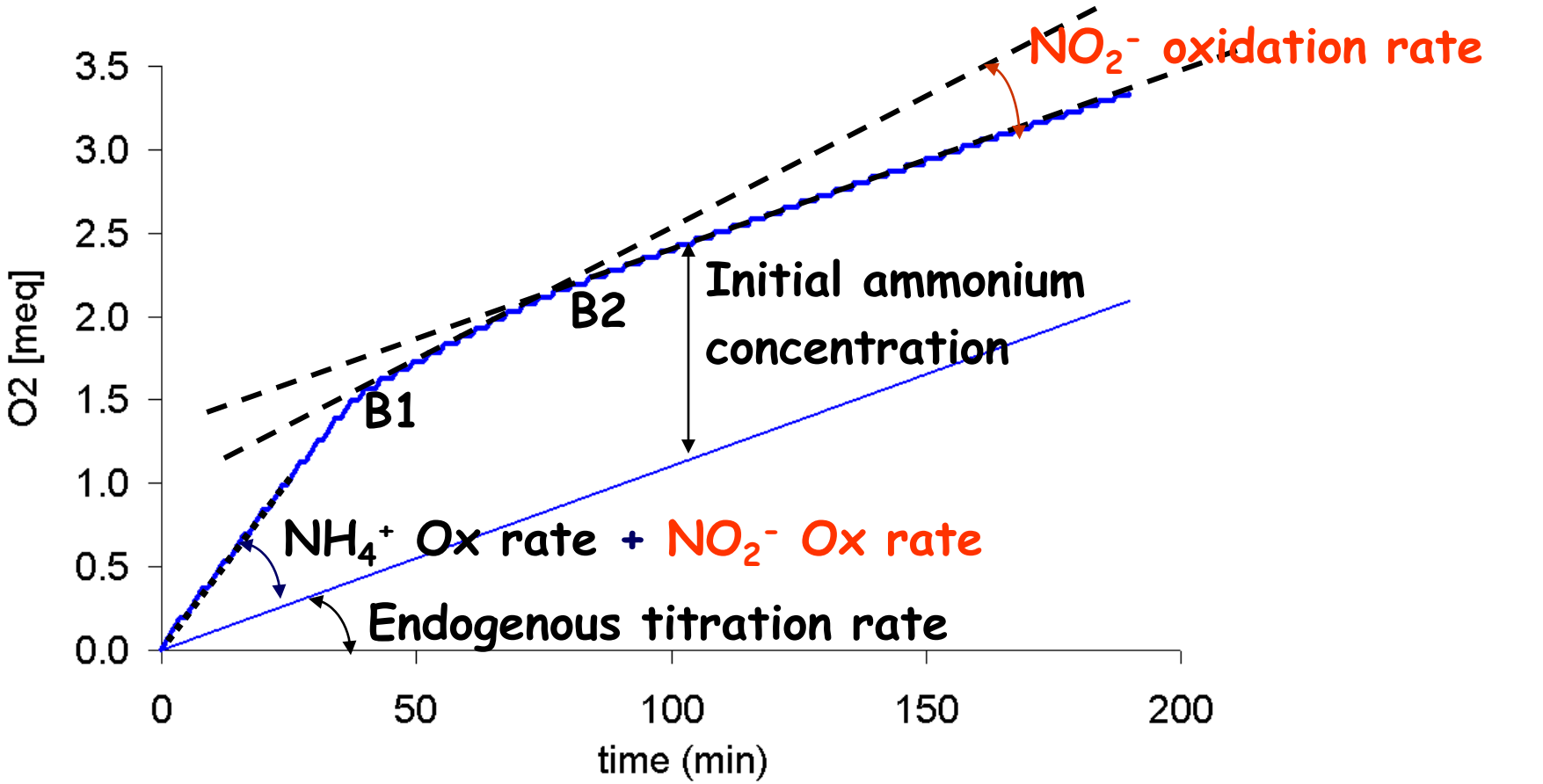


Stoichiometric factor: 2 mol OH^- = 1 mol NH_4^+

* Due to heterotrophic respiration producing CO_2 which absorbs NaOH

Titrimetry (6) – e.g.: DO-stat titration for nitrification monitoring

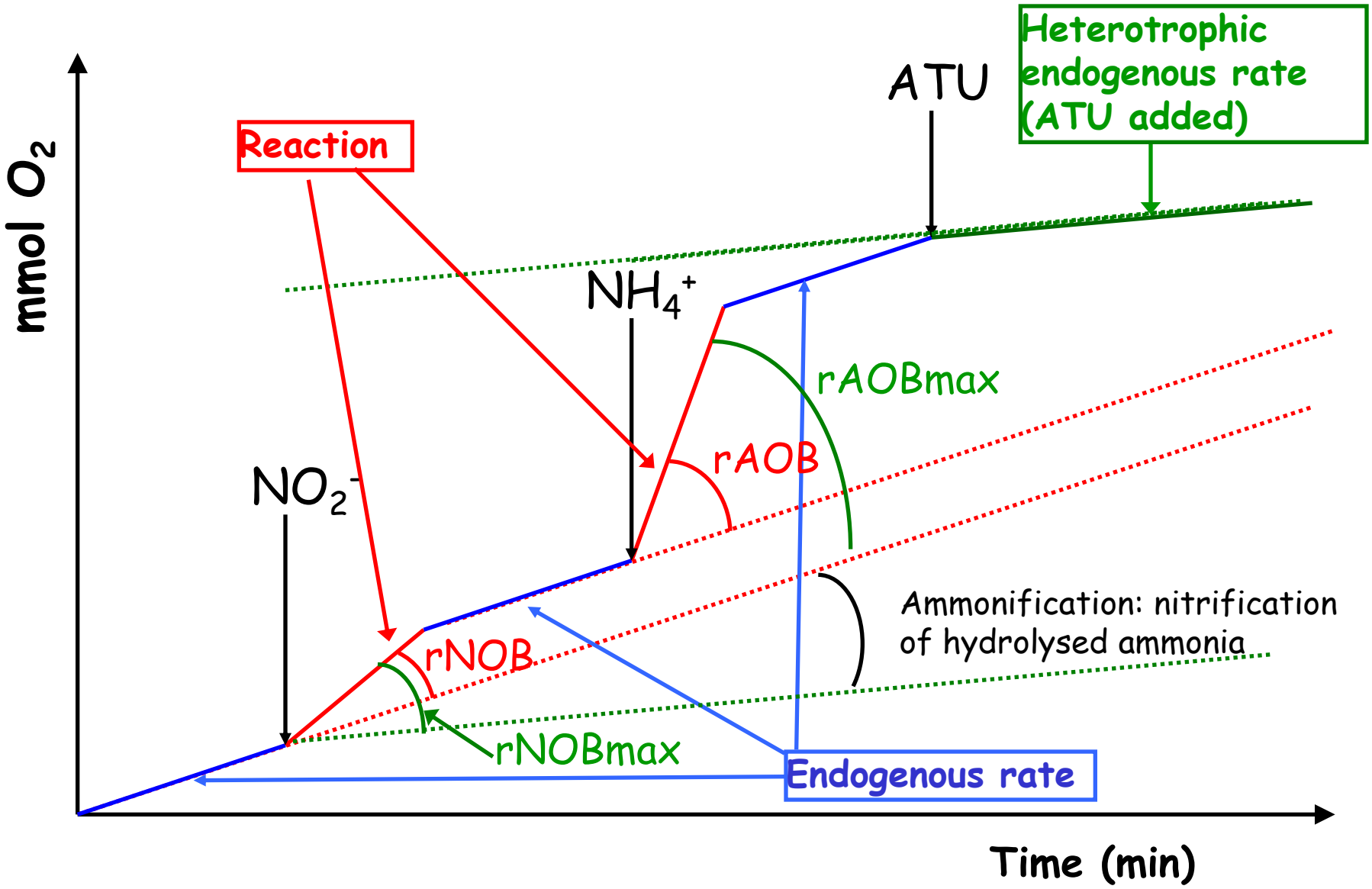
$NH_4^+ + 1.5\ O_2 \rightarrow 2\ H^+ + NO_2^- + H_2O$; $NO_2^- + 0.5 O_2 \rightarrow NO_3^-$



Stoichiometric factors: 1.5 mol O_2 = 1 mol NH_4^+

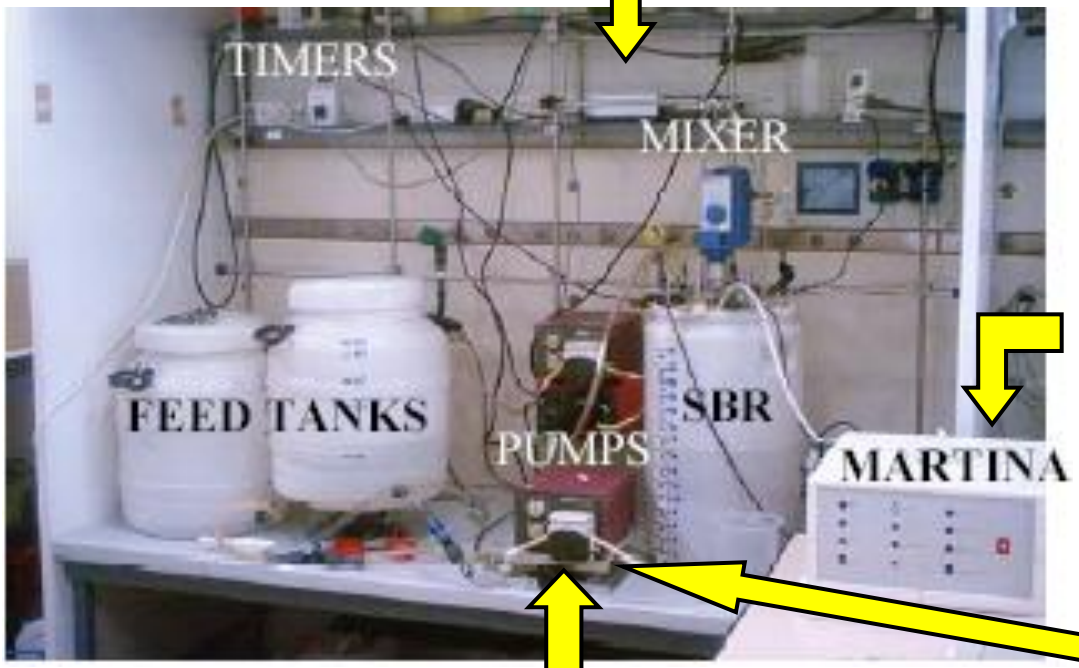
0.5 mol O_2 = 1 mol NO_2^-

Titrimetry (7) - pH/DO-stat titration for nitrification monitoring - methodology



Lab- scale SBR (30 L cylindrical tank – working volume 20 L)

aeration system



controlled heating system T = 25° C

Online monitoring unit (pH, DO, ORP, T)

peristaltic pumps for effluent discharge
- 1 connected to the floater
- 1 safety pump at the maximum level 20 L

peristaltic pumps for feeding
- 1 for concentrated influent (10X)
- 1 for dilution water

Titrants:

-NaOH 0.05 M
-H_2O_2 0.1 - 0.3 M
-HNO_3 0.05 M

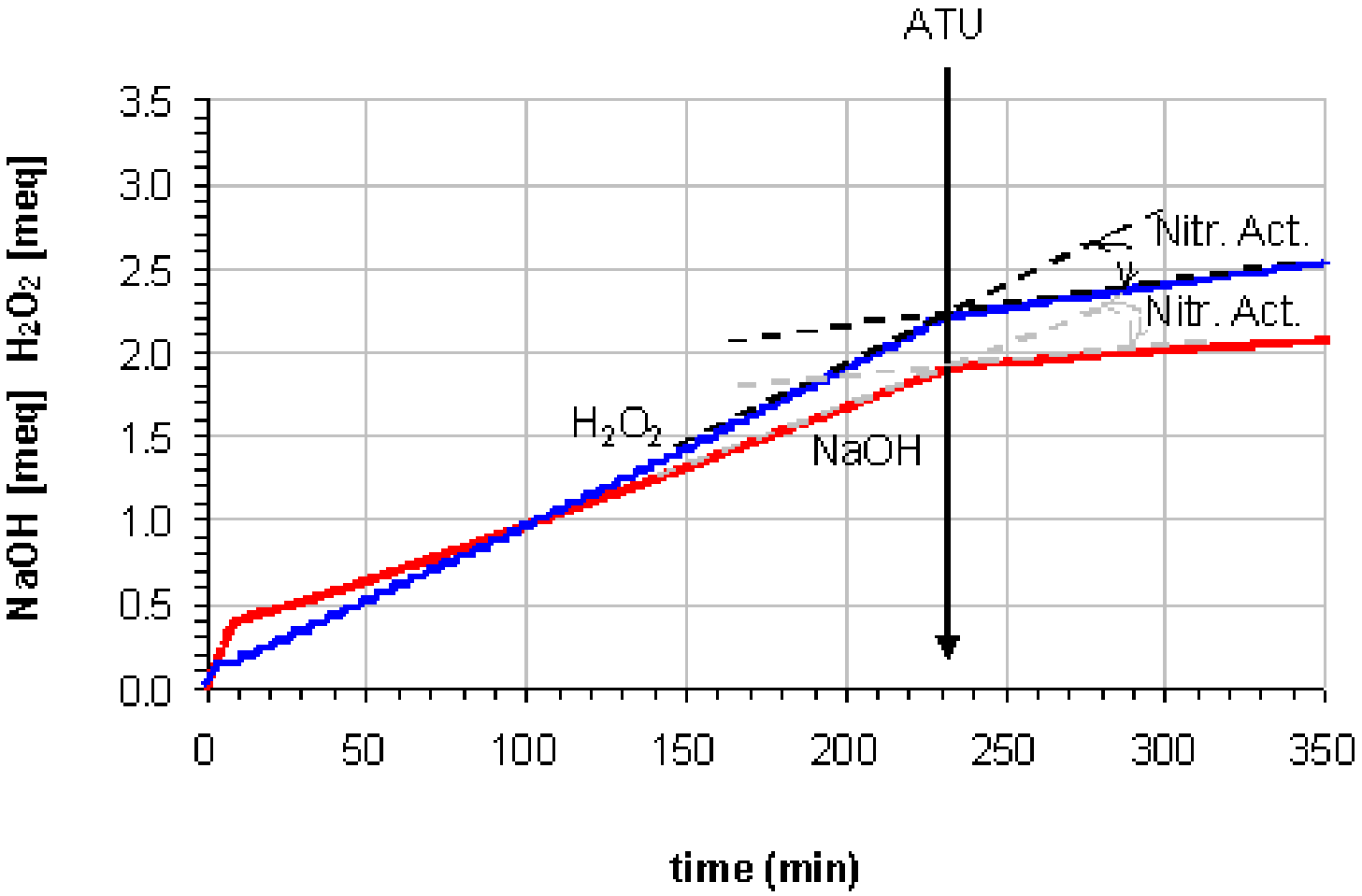
Set-point values:

-DO_{sp} = 8 mg/L
-pH_{sp} = 8.3

} for nitrification tests

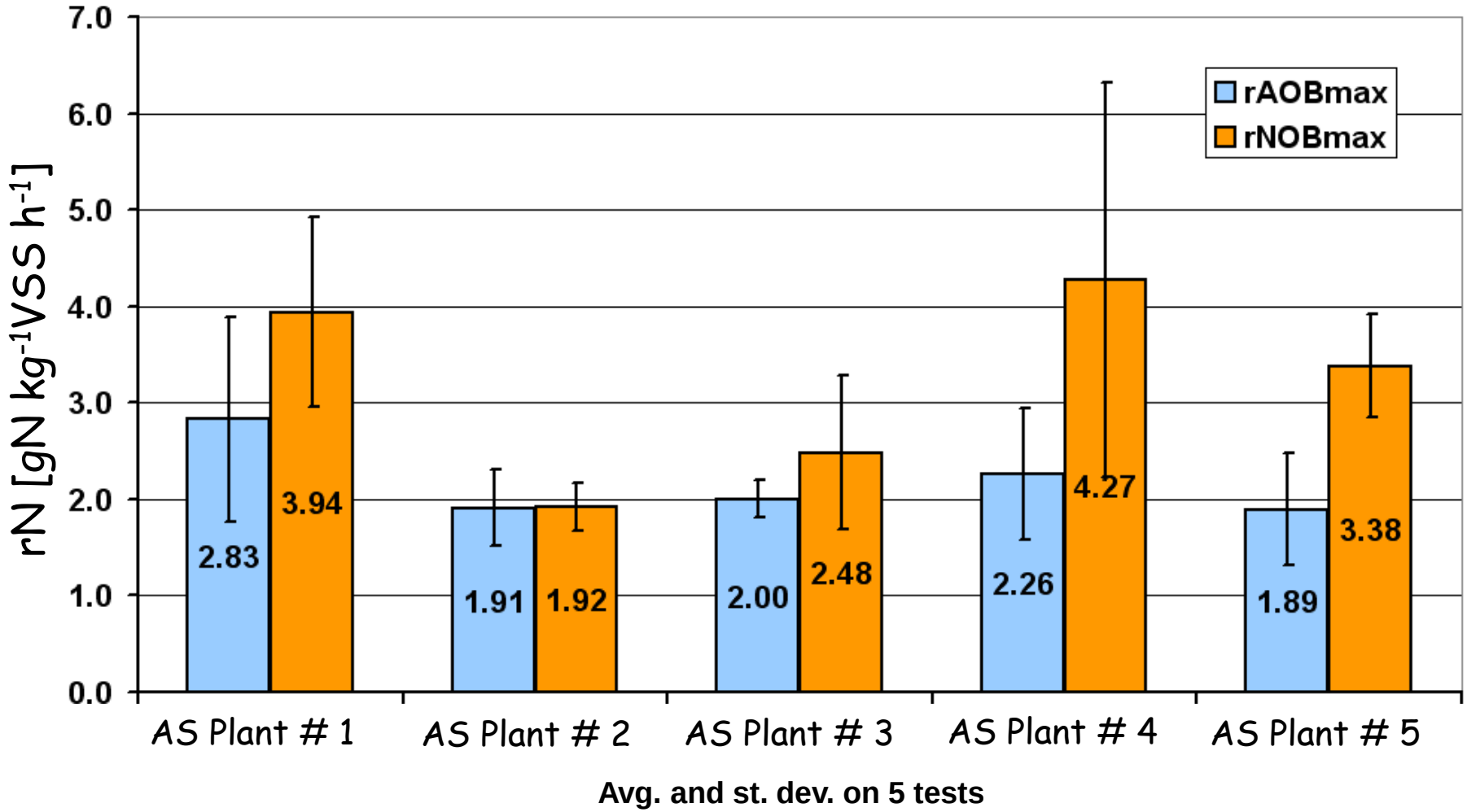
(pH_{sp} = 7.4 - 7.9 for denitrification tests)

Real output with freshly sampled activated sludge



Titrimetry (11) - pH/DO-stat titration for nitrification monitoring – results (2)

Seasonal monitoring in 5 full-scale WWTPs (activated sludge process)

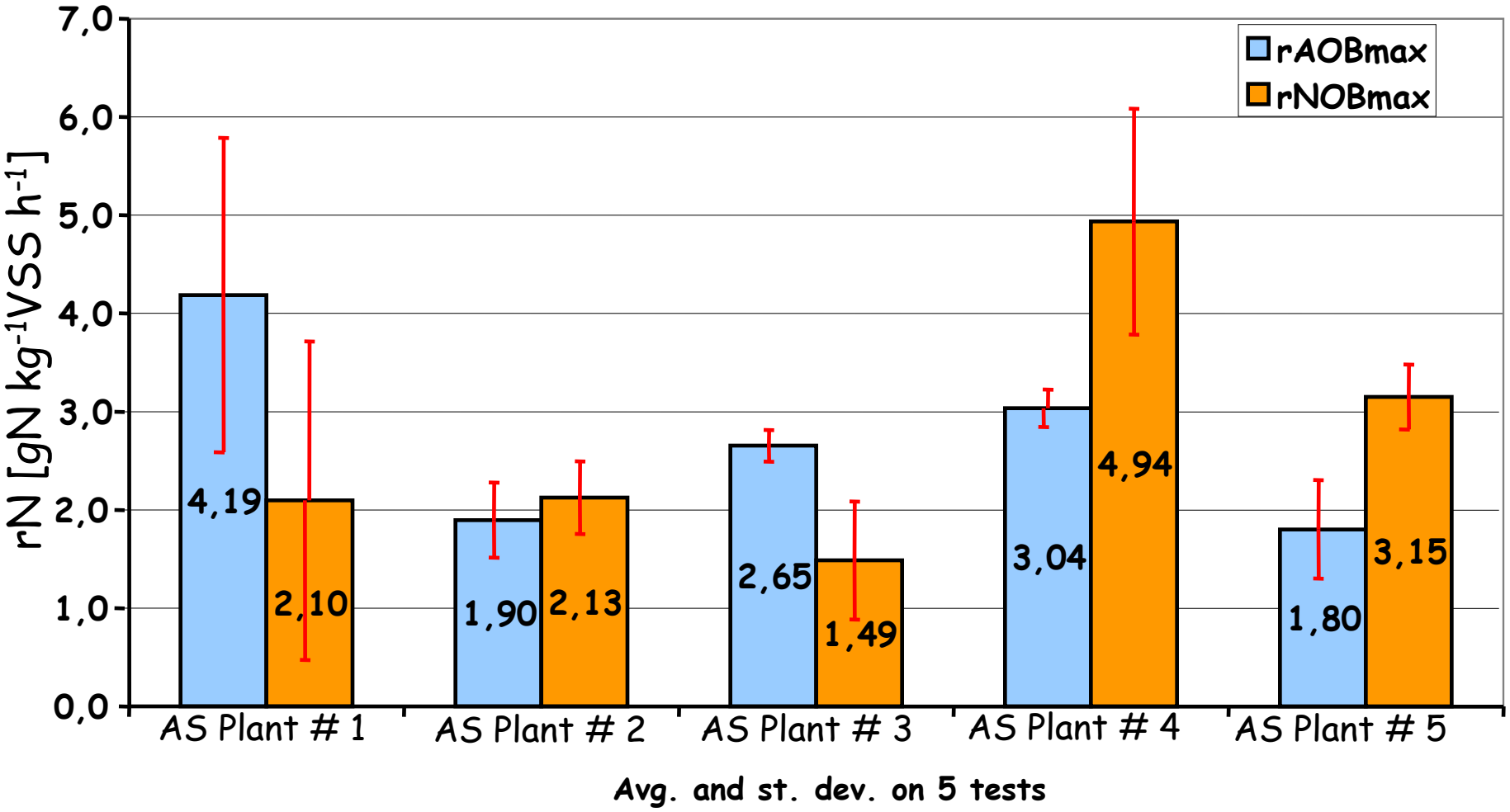


Avg. and st. dev. on 5 tests

All WWTP have rAOBmax ≤ rNOBmax, i.e.: no nitrite build-up

Titrimetry (12) - pH/DO-stat titration for nitrification monitoring – results (3)

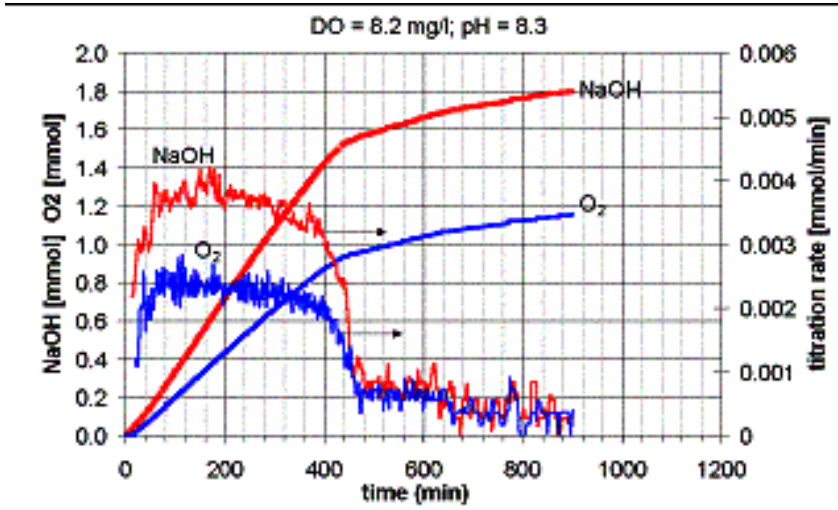
Seasonal monitoring in 5 full-scale WWTPs (activated sludge process)

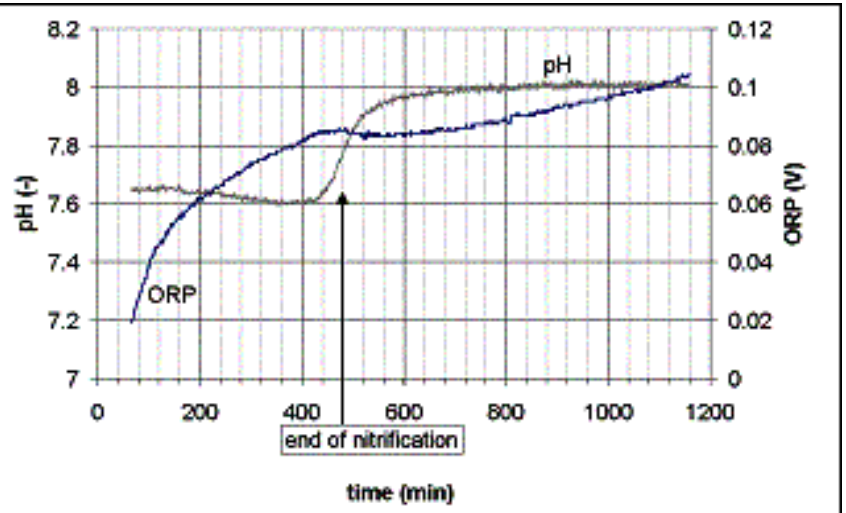


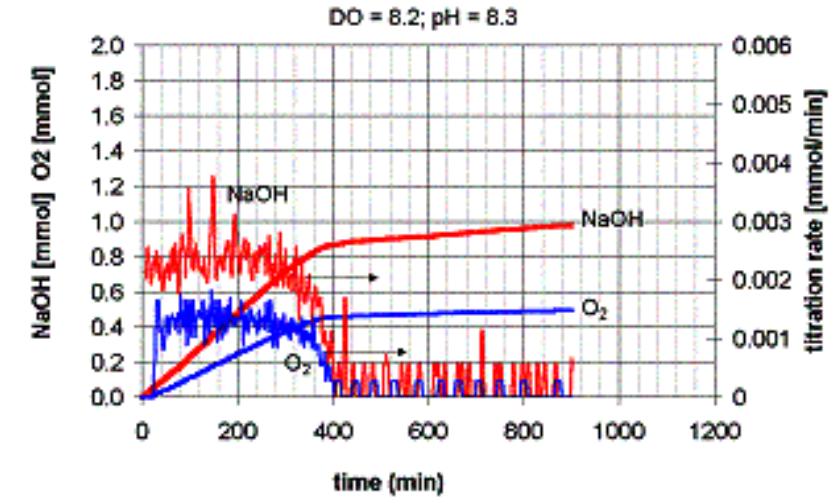
WWTP #1 and 3 have rAOBmax > rNOBmax, i.e.: risk of nitrite build-up

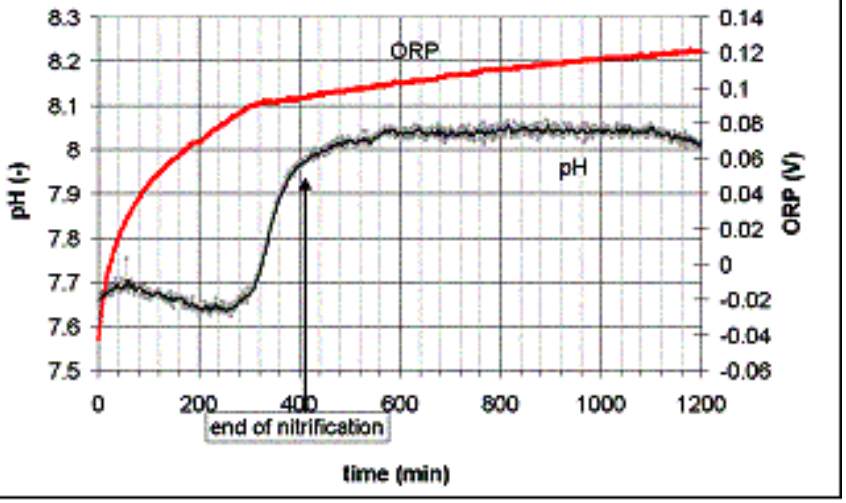
Titrimetry (13) - nitrification activity in an SBR - 1

Martina vs. SBR

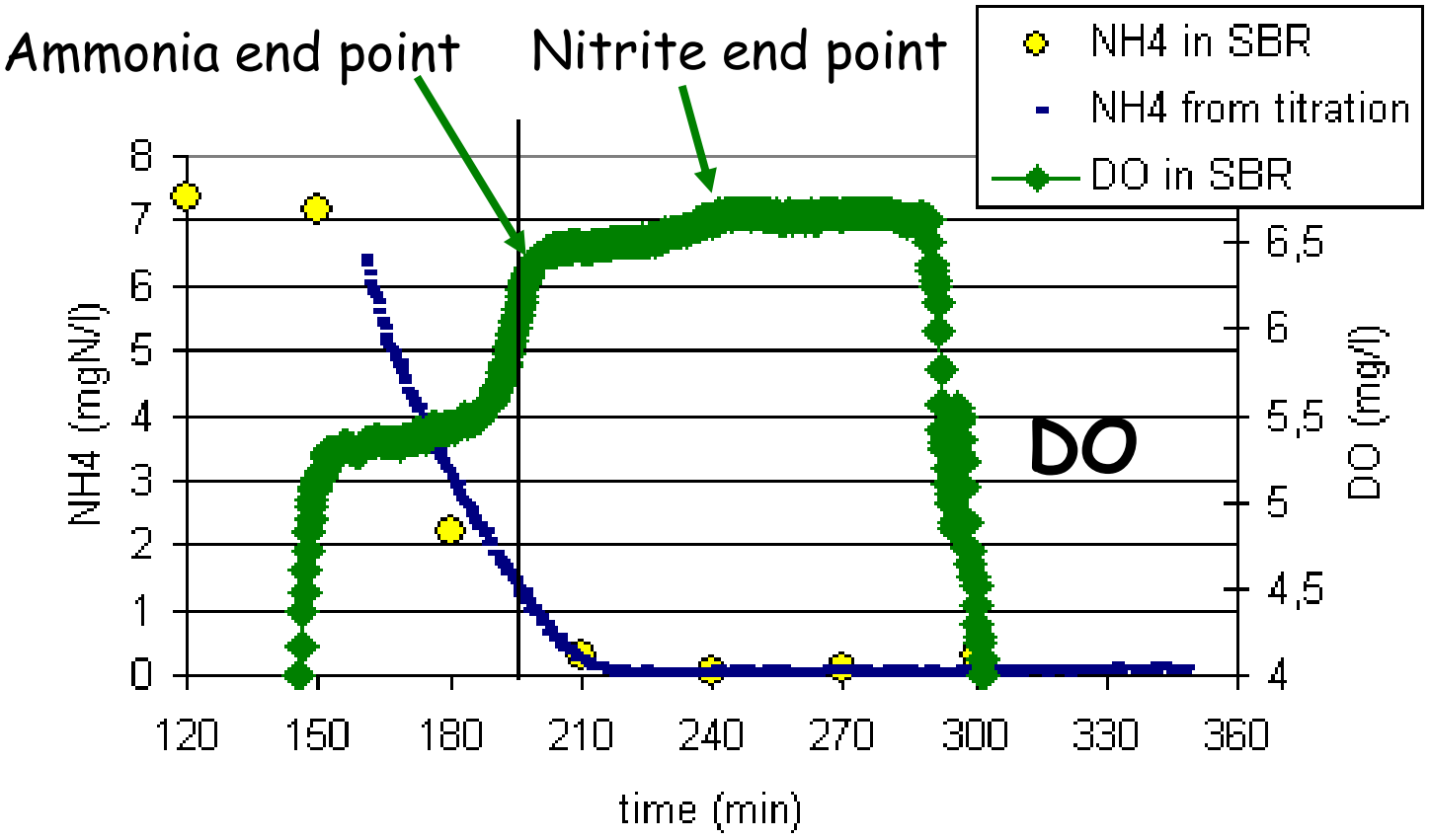




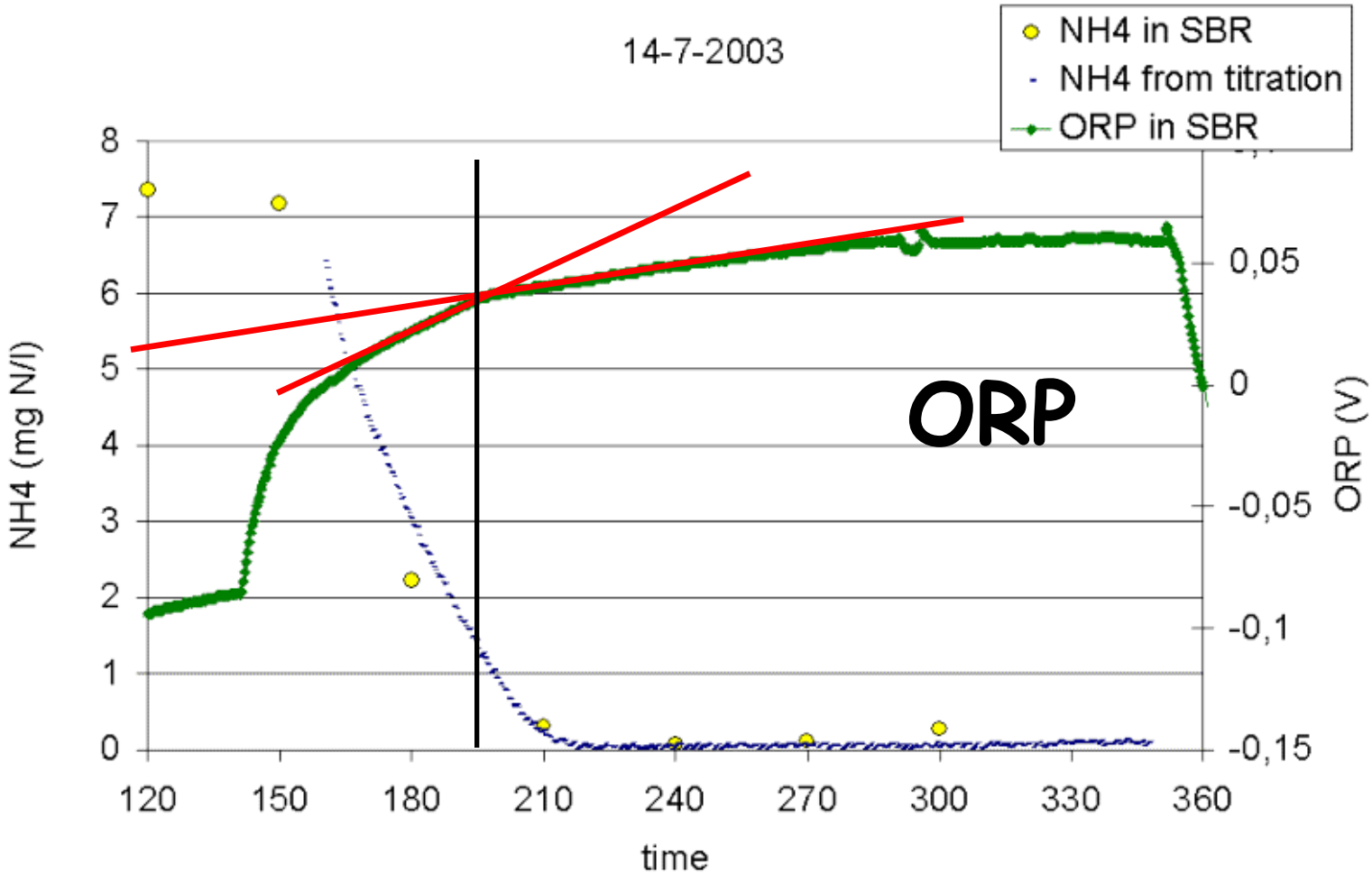




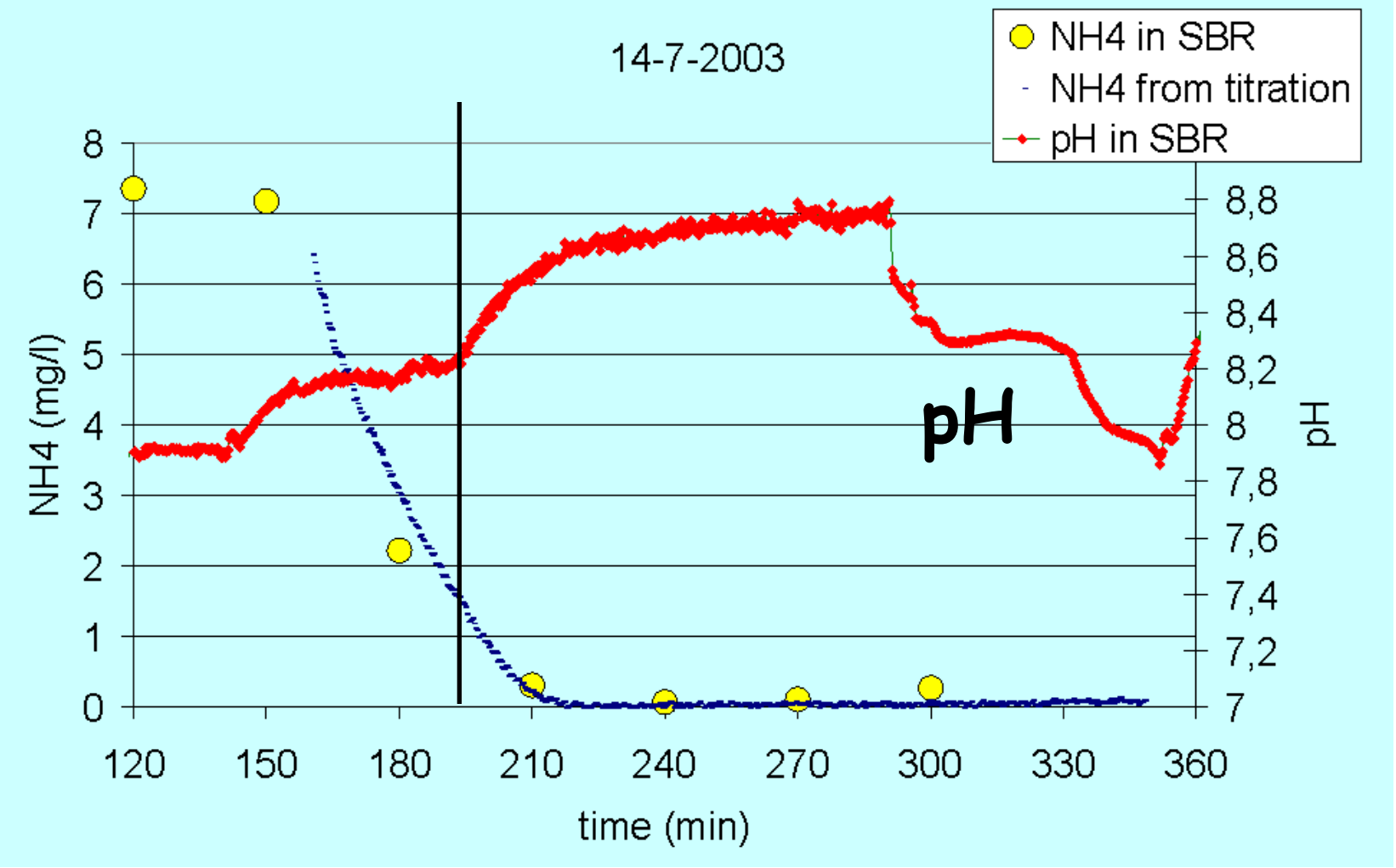
Comparison between Lab-Titrator (Martina) and SBR



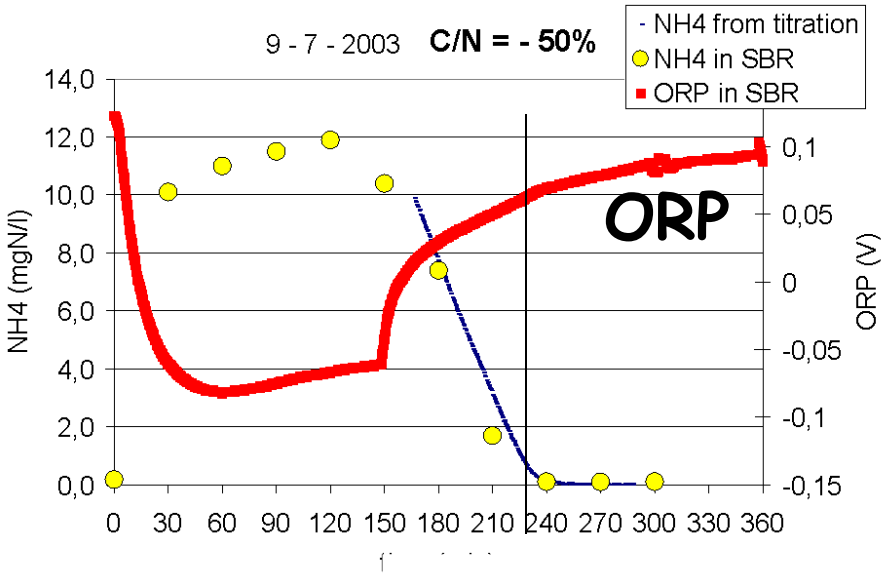
Comparison between Martina and SBR - ORP

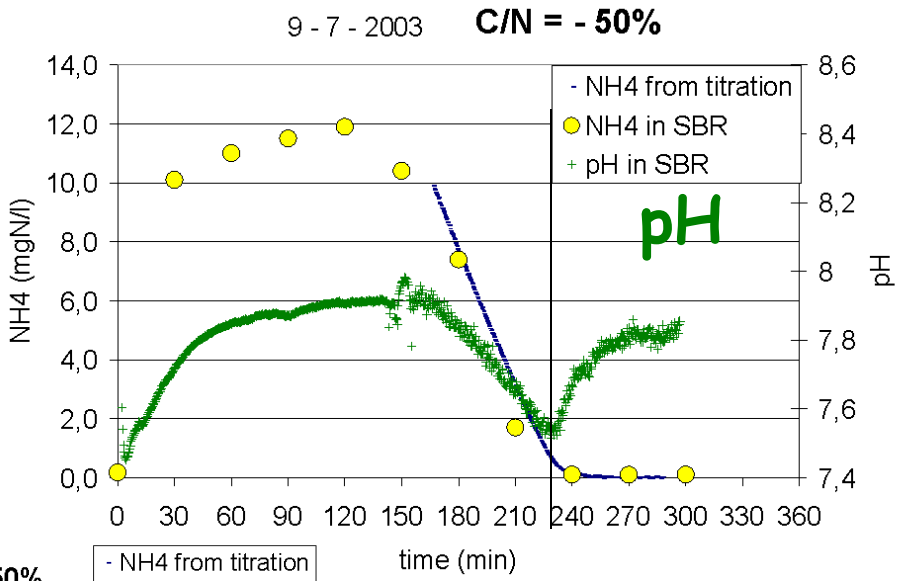


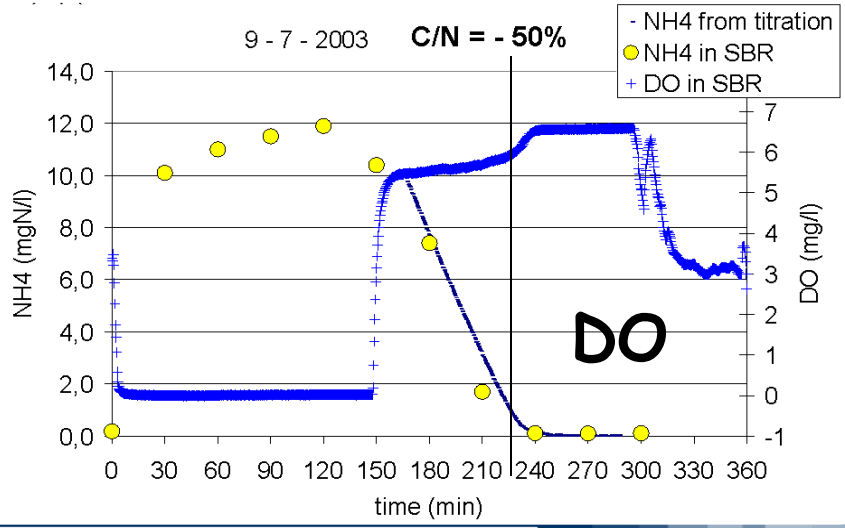
Comparison between Martina and SBR



Comparison between Martina and SBR







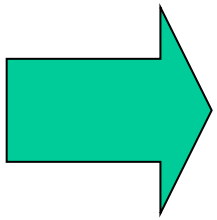
Heterotrophic denitrification activity tests
with **pH-stat titration**

$$P\,NO_3 + 0.625\ CH_3COONa \rightarrow$$
$$\rightarrow 0.5\ N_2 + 1.25\ CO_2 + 0.125\ H_2O + 0.625\ NaOH + P\,OH$$

P cation (P^+) not influencing pH (i.e.: Na^+)

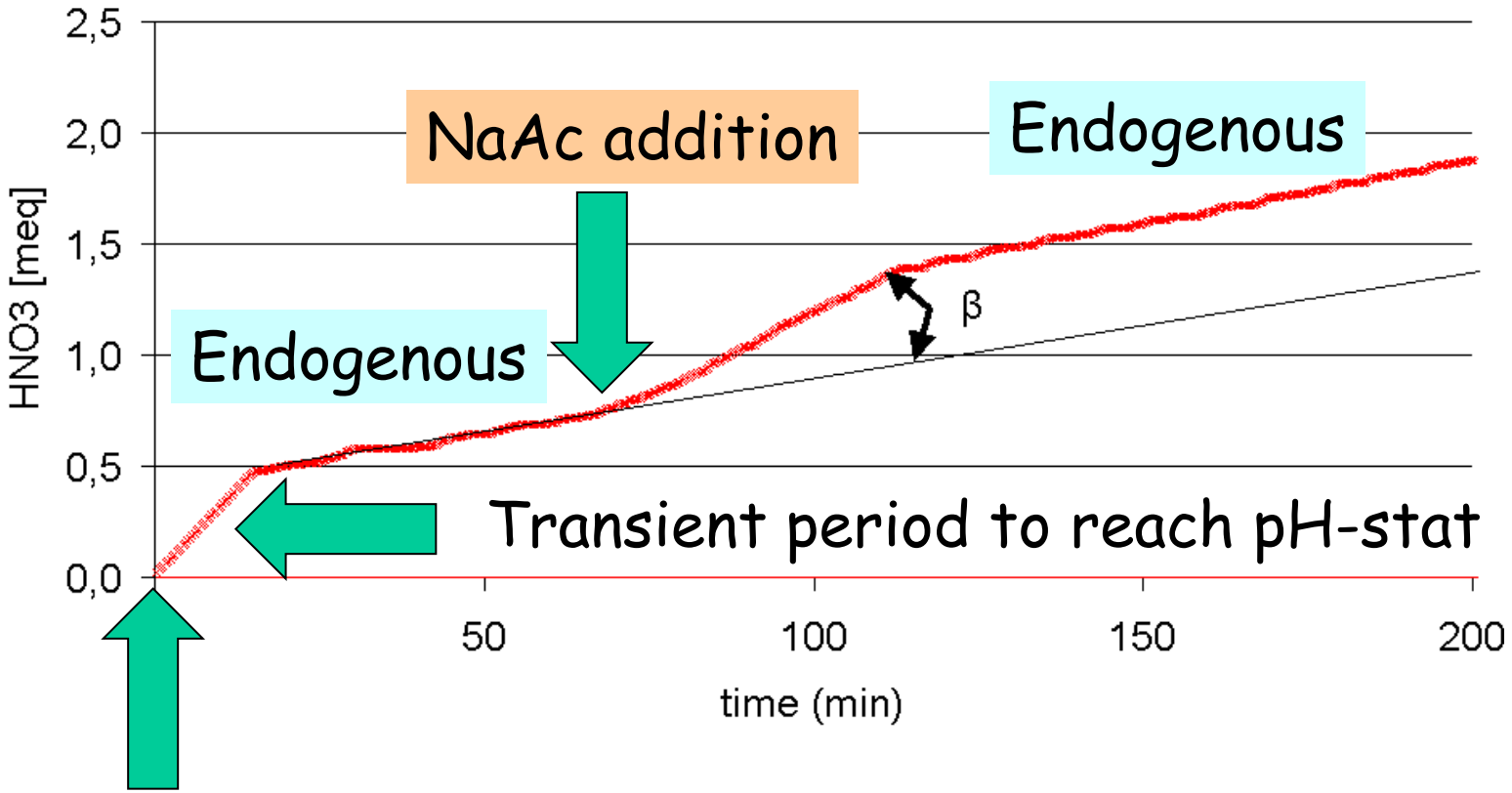
CH_3COONa
as carbon source

HNO_3
Titrant



COD-limited reaction
NO_3^- not limiting

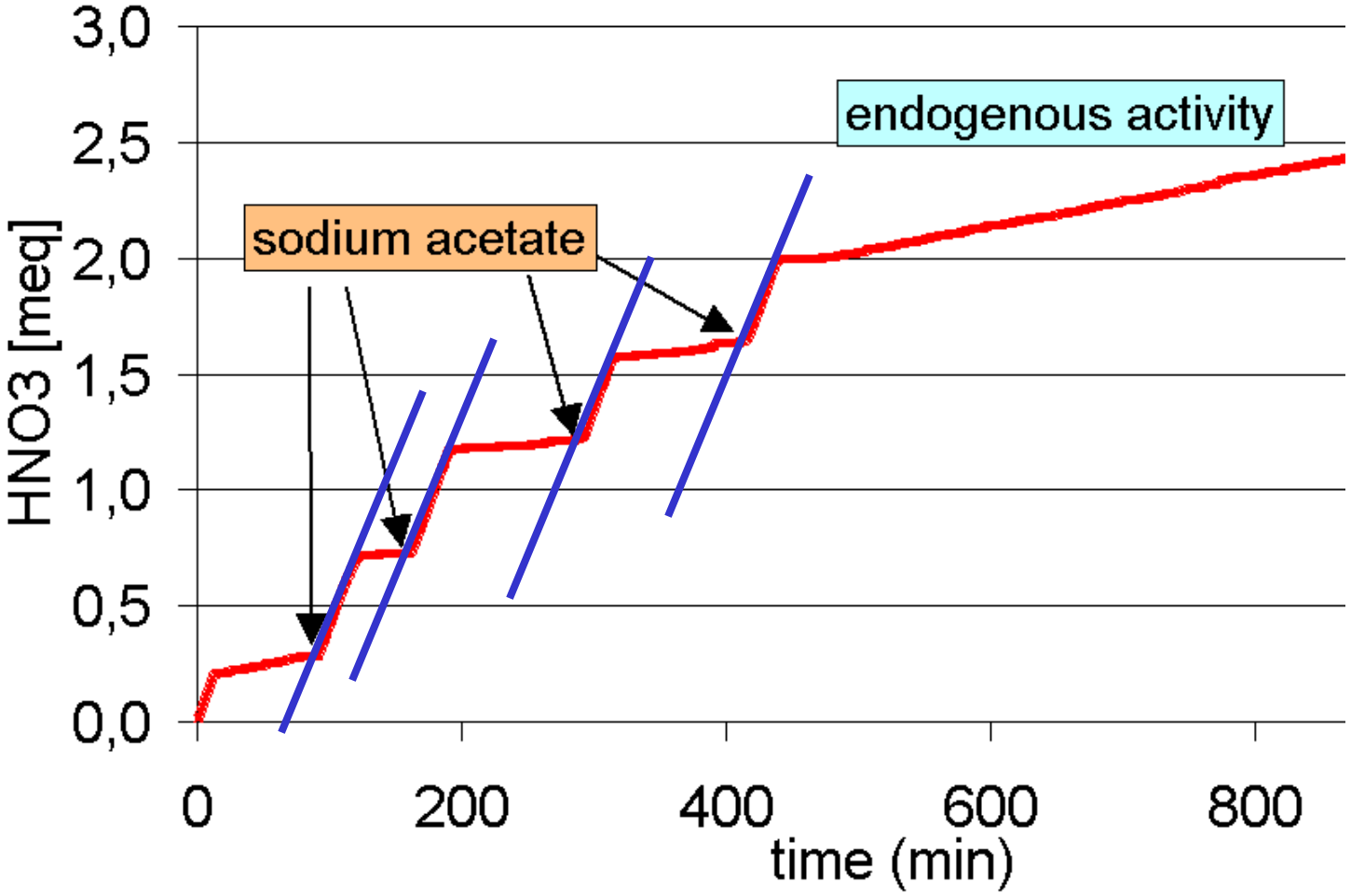
Denitrification activity tests with acetate



Nitrate 60 mgNO_3^-/L (13,5 mg/L as N)

Denitrification activity tests with acetate

Tests are fully repeatable



Denitrification activity tests with acetate

Run n°	denitrification rate [mgN -NO $_3$/gVSS*h]
1	12,68
2	15,95
3	18,68
4	19,76
5	17,50
6	17,58
7	20,35
8	19,97

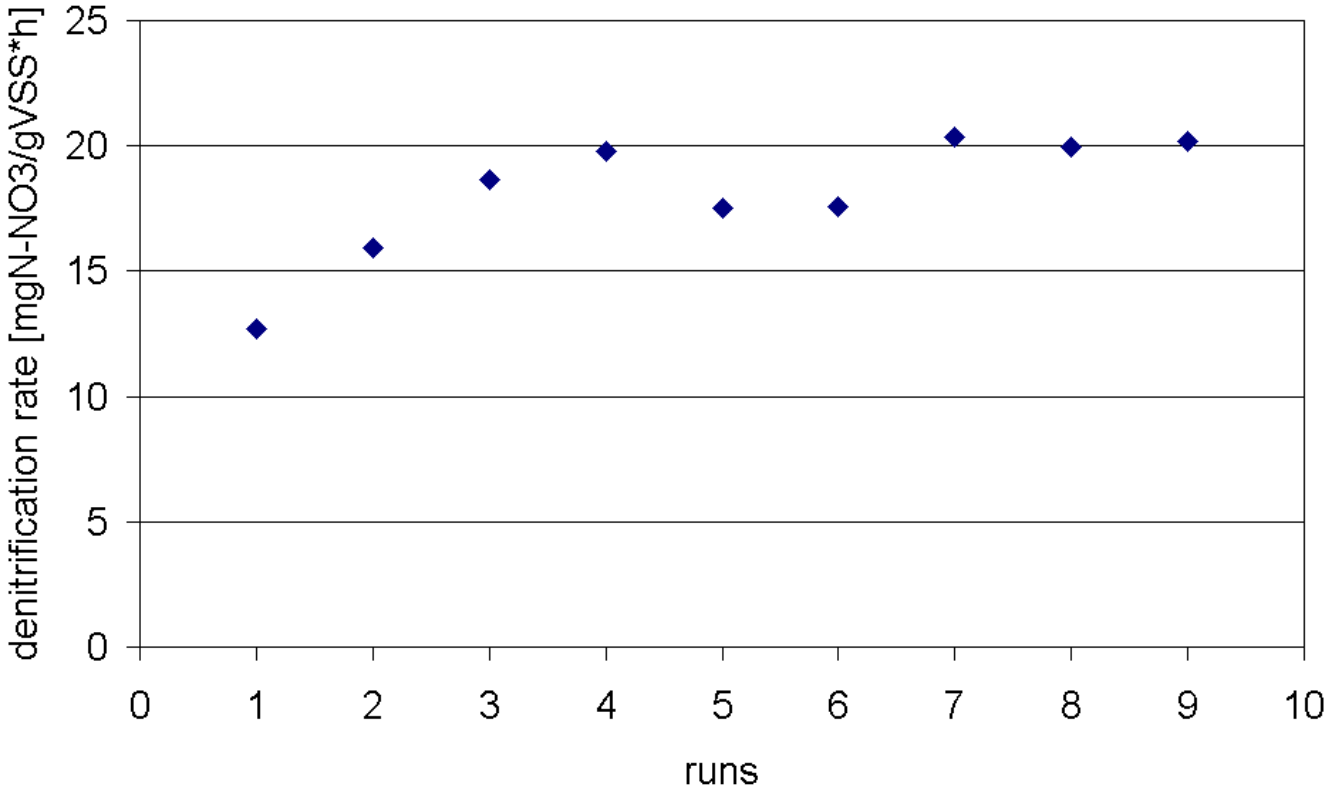
Max denitrification rate: 20 mgN-NO_3^- g^{-1}VSS h^{-1}

Endogenous rate: 1.12 – 1.94 mgN-NO_3^- g^{-1}VSS h^{-1}

(biomass grown on synthetic sewage, high % active biomass, low particulate organic debris)

Denitrification activity tests with acetate

Denitrifying activity in SBR increased in the first 4 tests (confirmed by nitrate analysis)



Titrimetry (23) - TITAAN (TITrimetric Automated Analyzer - 1)

Sludge+Influent storing tanks

Probes pH/DO (ORP, T)

Titration reactor

Pump +EV for titrant dosage

Titrant tanks

User interface

Titration MODEs for the assessment of:

1. Acute toxicity of autotrophic biomass
2. Nitrifiable nitrogen in the influent
3. Maximum nitrifying activity
4. End of nitrification process (SBR only)
5. Residual nitrates at end of anoxic/aerobic phase

Titrimetry (25) – TITAAN – 3

File in analisi: C:\Archivio\Installazioni\2008 Titaan ImolaSanterno\Dati\T1_20090108_115121\T1_20090108_115121.xls

Dati

Volume Fango [L]	1.2
Conc. Fango	gSSV/L 3.47
Titolo NaOH [mmol/mL]	0.10021
Titolo H2O2 [mmol/mL]	0.34965
Elettrovalvola EV 1	NaOH
Elettrovalvola EV 2	H2O2
T.Rif.=T.Media [°C]	13.2
Vol. Inoculo [mL]	

Calcola | Modifica

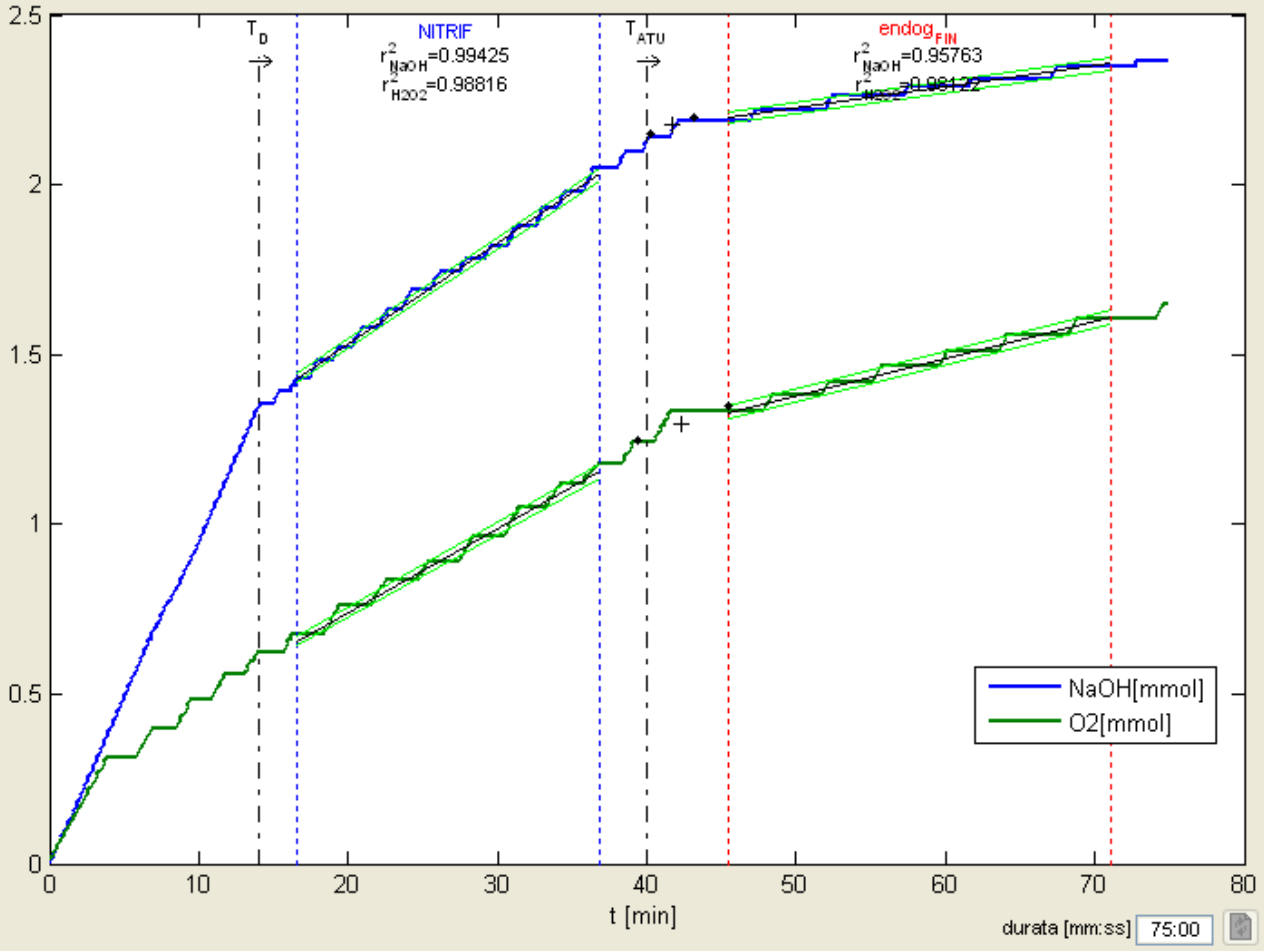
Intervalli [mm:ss]

end. iniziale	tD	Attività nitrificante		tATU	end. finale	
	14:03	16:33	36:47	40	45:31	71:06

	EndogINI	Nitrif	EndogFIN	Gin. [min]
NaOH		29.65	6.14	42
i.c.95%		0.01%	0.02%	1
H2O2		24.69	11.05	42
i.c.95%		0.01%	0.02%	3

Attività

	mgN / (L*h)	mgN / (gSSV*h)	Errore
AOB	8.2	2.4	0.98%
NOB	0	0	
AOB_NOB	4.8	1.4	0.96%

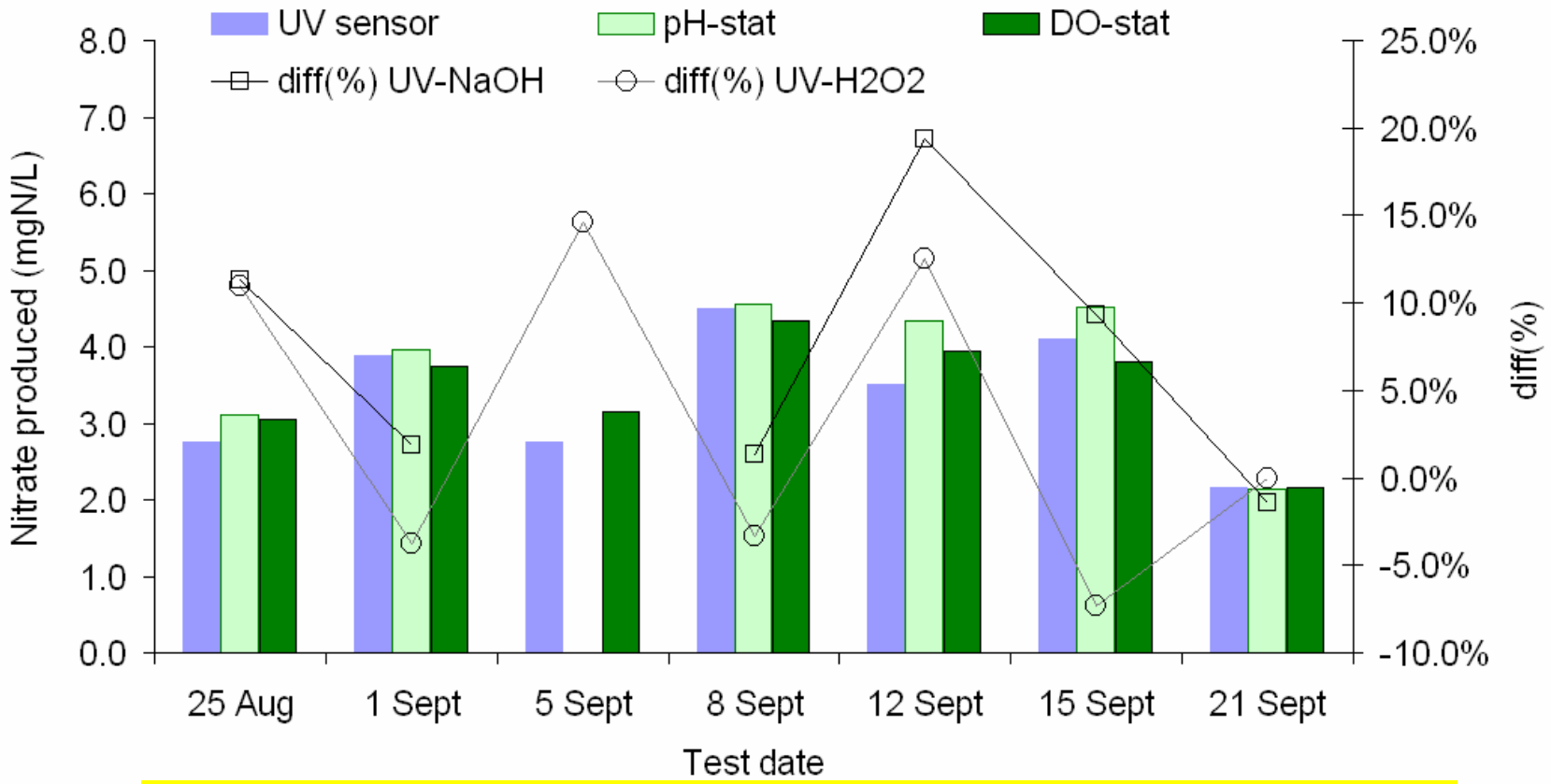


On-line nitrification activity data validation in SBR:

Production of N-NO_3^- during aerobic phase

1) estimated on-line by TITAAN-Mode 1 pHstat and DOstat

2) measured by on-line UV sensor and validated with lab analysis (HPLC)



satisfactory correspondence: avg. error 8%