Coadvise + Treasure programmes

Specialist Course

Tlemcen, 7th – 11th February 2010

POLITECNICO DI MILANO





Biomass activity measurements

Part 3 – manometry and microcalorimetry

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General Index (3)

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**

MANOMETRY

Manometry (1)

- Manometry: generalities
  - Principle of the method
  - Potential applications in sanitary engineering
- Materials and methods
  - Apparatus
  - Sludge samples
  - Procedures
- Experimental validation on:
  - Low F/M tests
  - High F/M tests
  - Decay tests
- Conclusions

Manometry (2) - Principle of the method

- **Manometry:** technique of measuring the pressure variation due to gas production/consumption caused by biochemical reactions or physical changes

- Applicable to any bioprocess implying the production/consumption of a poorly soluble gas.

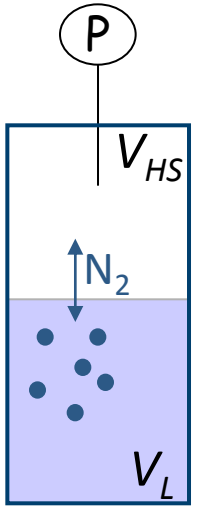
- Main applications:
  - aerobic processes (oxygen consumption)
  - anoxic processes (N_2 production)
  - anaerobic processes (biogas production)

Manometry (3) - Principle of the method: *monitoring a bioreaction by manometric data*

Manometric tests

A batch test is performed in a closed reactor (no gas/liquid exchanges, constant volume) at constant temperature.

In the liquid phase a bioreaction produces (consumes) a poorly soluble gas: *e.g.* N_2



Under the following assumptions:

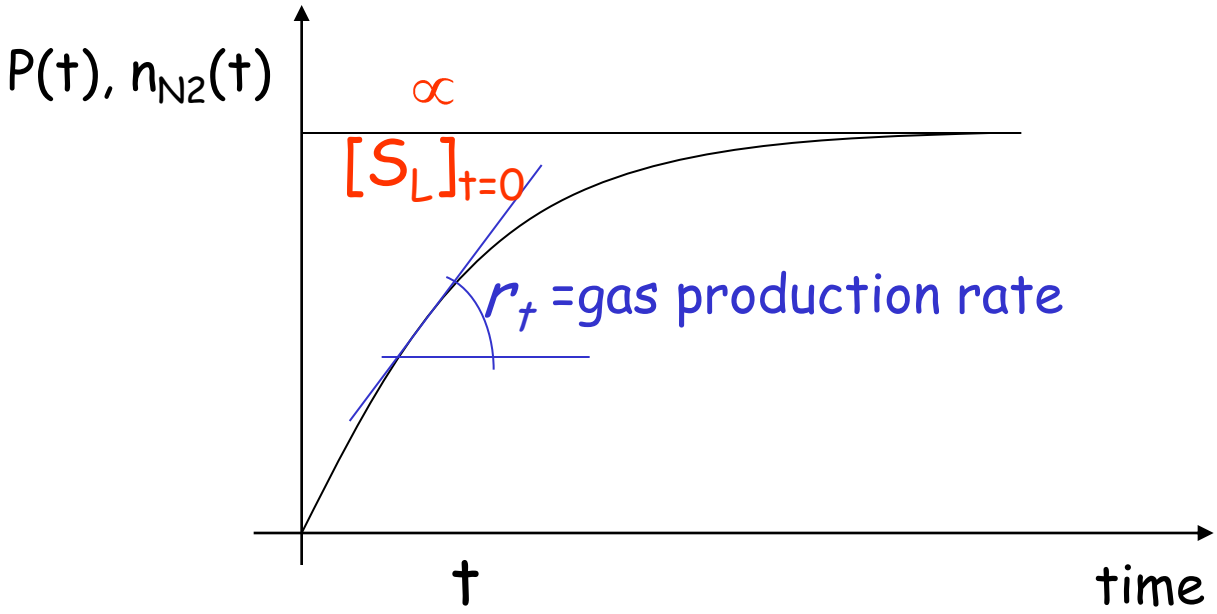
1. N_2 is the main gaseous product
2. N_2 in the liquid phase is always at its saturation concentration: no accumulation/decumulation and liquid/gas N_2 transfer is not kinetically limiting

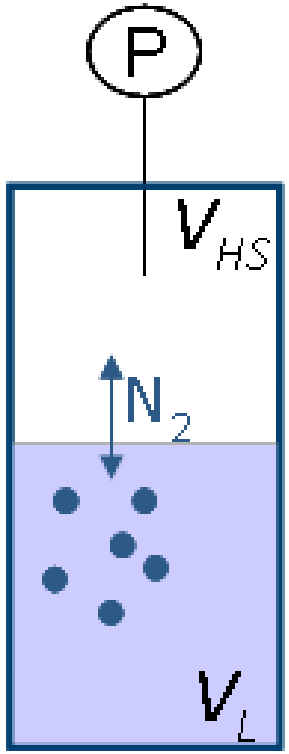
N_2 production can be estimated from PRESSURE data, by the *ideal gas law*:

$$P(t) = n_{N_2}(t) \cdot \frac{R \cdot T}{V_{HS}}$$

Manometry (4) - Principle of the method: *monitoring a bioreaction by manometric data*

Typical output of a manometric test





denitrification tests under limiting nitrate concentration → $[S_L] = [NO_3^-]$

Manometry (5) - Materials and Methods (1)

Sludge origin

Sludge sampled from two large WWTPs in Lombardy:

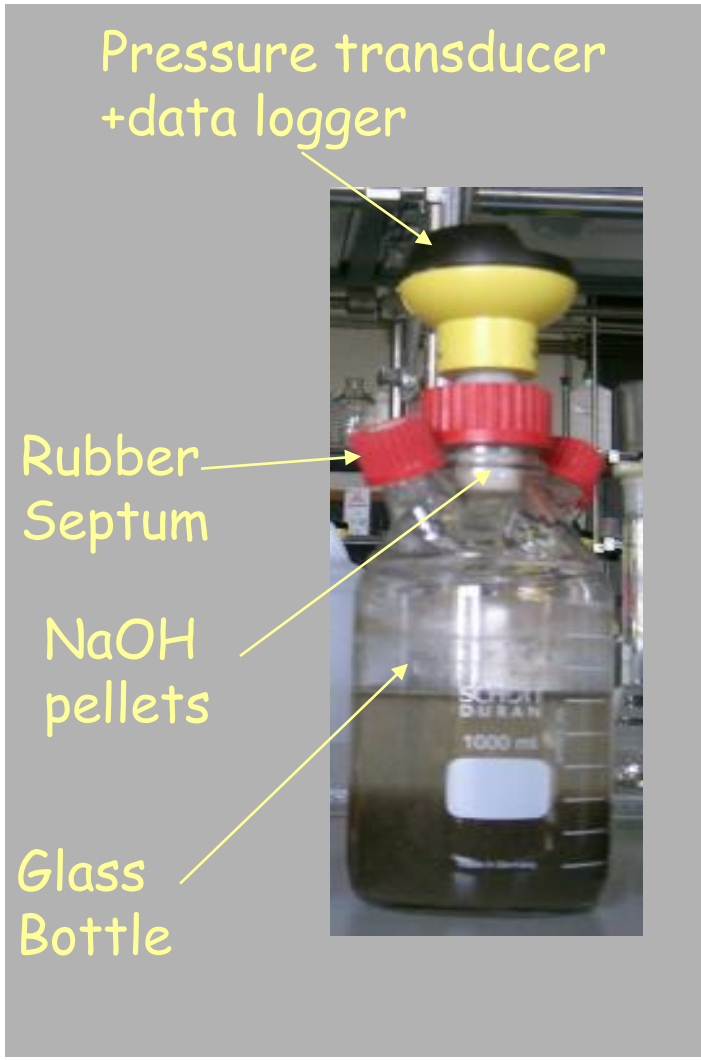
- WW1: flow rate= $3.7 \cdot 10^5$ m^3 d^{-1}, mainly urban wastewater;
- WW2: flow rate= $2.7 \cdot 10^5$ m^3 d^{-1}, about 80% of which from industrial origin;

Both with conventional nitrification/denitrification biological treatment

After collection: sludge samples were kept at 4°C for no more than 15 days before use.

Apparatus

- Glass bottle:
  - Total volume = 1160 mL
  - Head space volume = 160-560 mL
- Magnetically mixed
- Located in a thermostatic chamber at 20°C
- Up to 6 tests in parallel



Manometry (7) - Materials and Methods (3)

Test Procedure

(1) Sludge preparation:

- Sludge pre-conditioning (sludge washing with NO_3-free physiological saline solution, dilution to desired MLVSS, ATU addition, pH verification)
- Transfer to test-bottle
- Deoxygenation by N_2 flushing in the bottle headspace, bottle sealing
- temperature stabilisation in thermostated chamber

(2) Addition of a known volume of a stock solution of nitrate (nitrite) and, if required, of organic carbon source by injection through the rubber septum

(3) Pressure data collection

Manometry (8) - Materials and Methods (4)

Test conditions:

(1) Tests at Low Food to Biomass (F/M) ratio:

- VL = 1000 L
- MLVSS = 2-5 g/L
- N-NOx = 15-30 mg/L (limiting substrate)
- Acetate = 200-600 mgCOD/L
- Average test duration = 3-12 h

(2) Tests at High Food to Biomass (F/M) ratio:

- VL = 600 L
- MLVSS = 0.7-2 g/L
- N-NOx = 150-600 mg/L
- Acetate = 800-1000 mgCOD/L (limiting substrate)
- Average test duration = 2-7 d

Test conditions:

(3) Decay Tests

- VL = 1000 L
- MLVSS = 2-10 g/L
- N-NOx = 150 mg/L
- Acetate= 0 mgCOD/L (endogenous respiration)
- Average test duration = 5-7 d

Manometry (10) - denitrification activity (1)

As we assume that:

1. Gaseous N_2 is always at its saturation concentration (obtained by initial N_2 flushing)
2. Gaseous N_2 transfer from sludge to head-space gas is never kinetically limiting

N_2 can be estimated in the same way O_2 was in the BOD test, but in this case an overpressure is measured.

The link is the Gay-Lussac equation for a perfect gas:

$$P(t) = n_{N_2}(t) \cdot \frac{R \cdot T}{V_{HS}}$$

Manometry (11) - denitrification activity (2) - objectives

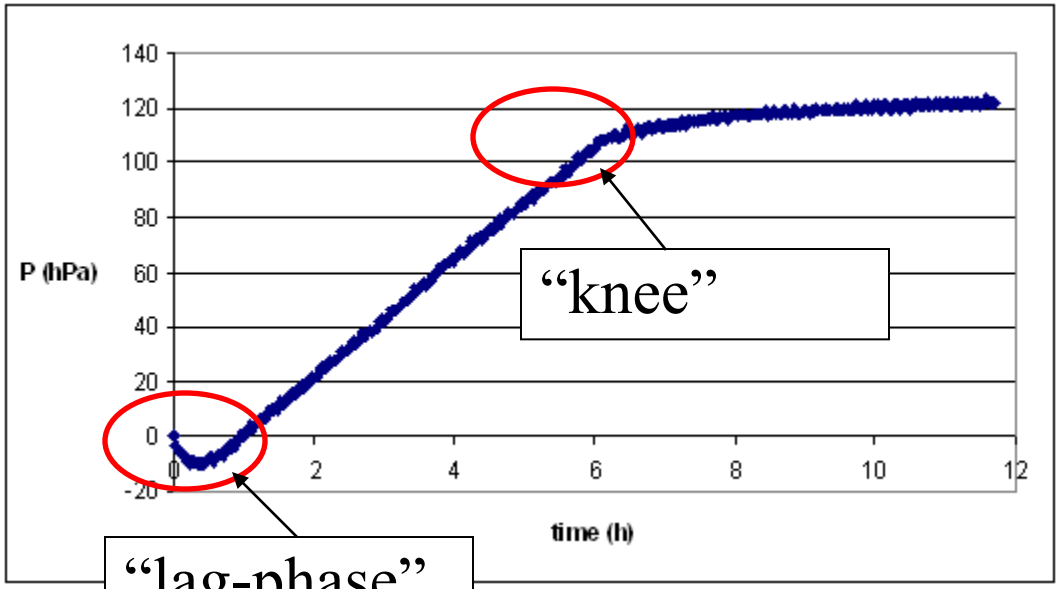
Assessment of

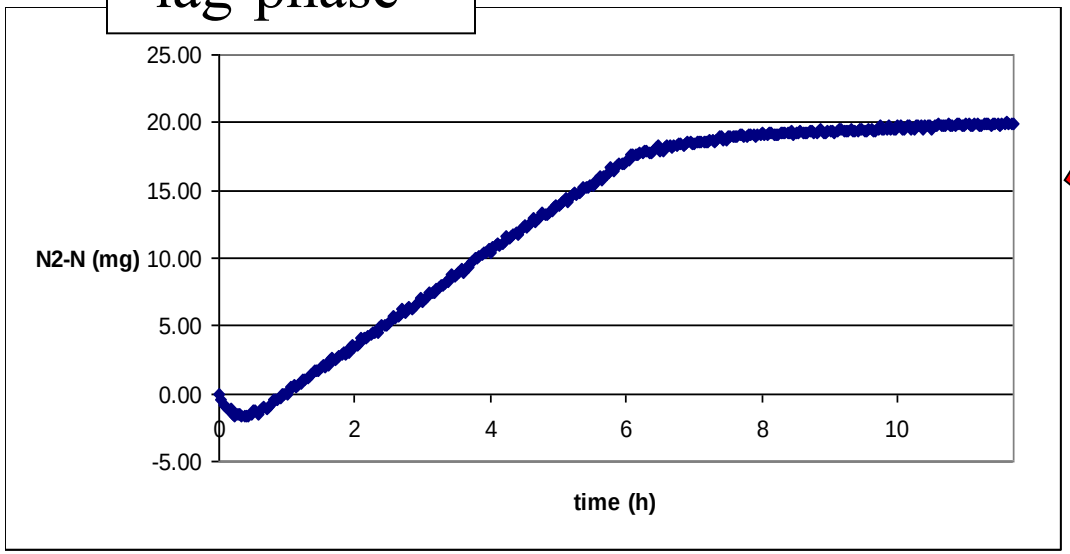
- **accuracy**
- **reproducibility**
- **specific denitrification rate r_d** [mgN g^{-1}SSV h^{-1}] with different organic substrates (acetate, ethanol, hydrolised urban wastewater, decaying biomass) and different oxidising ions (NO_3^- e NO_2^-)
- **decay tests**

Test conditions (@20°C):

V_L [L]	1000
MLVSS [g L^{-1}]	2÷5
N-NO_x [mgN L^{-1}]	15÷30
Acetate [mgCOD L^{-1}]	200÷600
Test duration (avg)	12 h

Manometry (12) – Denitrification: Tests at Low Food to Biomass (F/M) ratio (1)



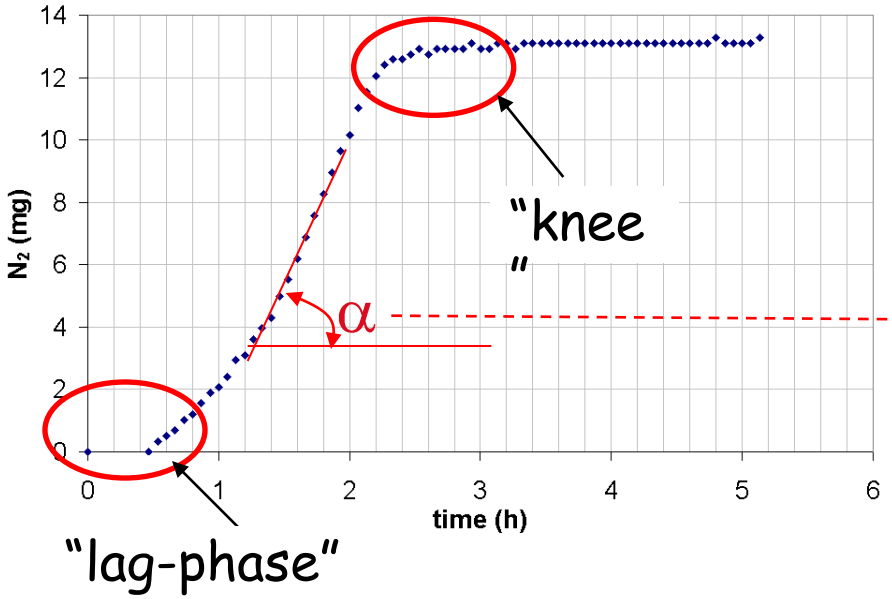


$$P(t) = n_{N_2}(t) \cdot \frac{R \cdot T}{V_{HS}}$$

Initial lag-phase...why?

Manometry (13) – Denitrification: Tests at Low Food to Biomass (F/M) ratio (2)

- **Typical output:**



$$v_d = \frac{\alpha}{MLVSS \cdot V_L}$$

Initial lag-phase may be due to:

- Residual oxygen in head space
- "diauxic growth"

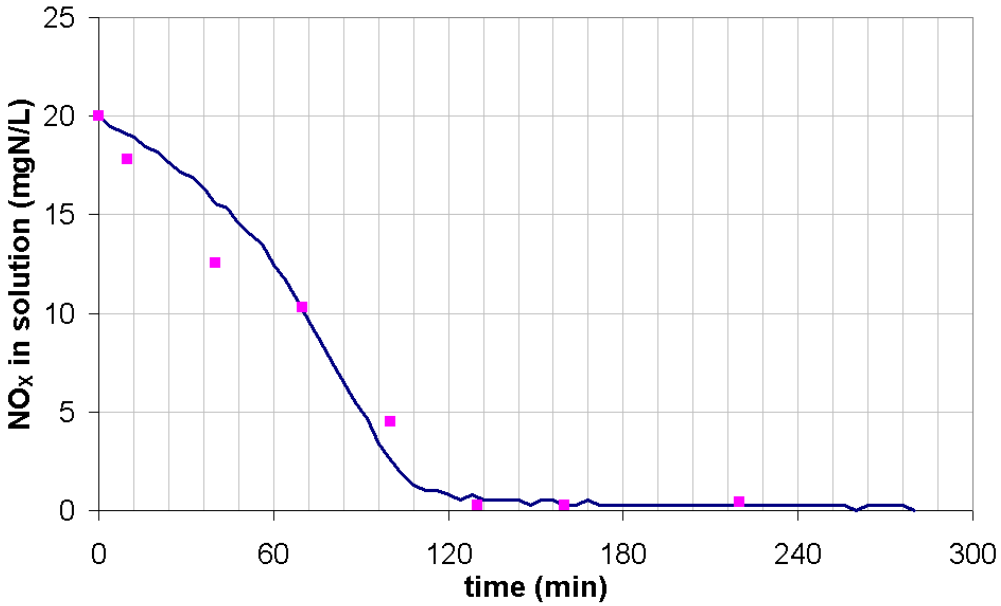
Manometry (14) – Denitrification: Tests at Low Food to Biomass (F/M) ratio (3)

Comparison between in-solution NOx concentration:

measured by analytical methods (solid squares)

vs

back-calculated from N_2 evolution data (solid line)

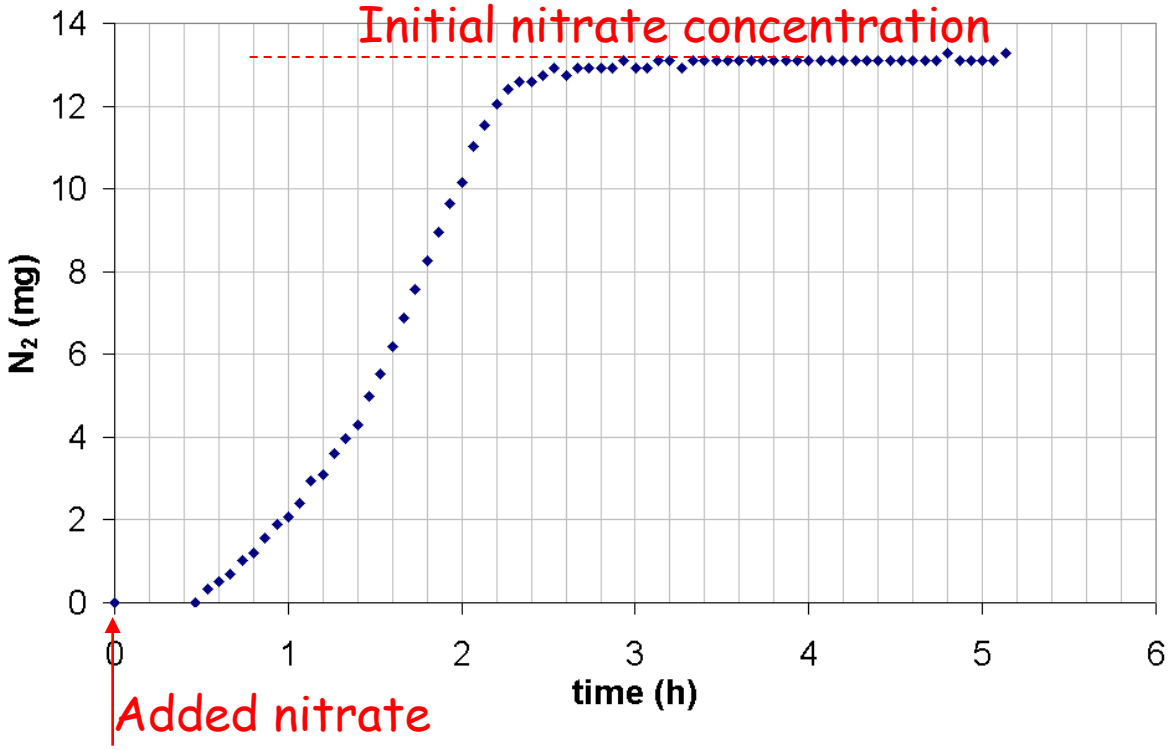


- **Nitrogen mass balance** for nitrate-limiting tests:

added nitrate vs initial nitrate concentration

In 165 tests: *average error = -22%*

→ Why?



Manometry (16) – Denitrification: Tests at Low Food to Biomass (F/M) ratio (5)

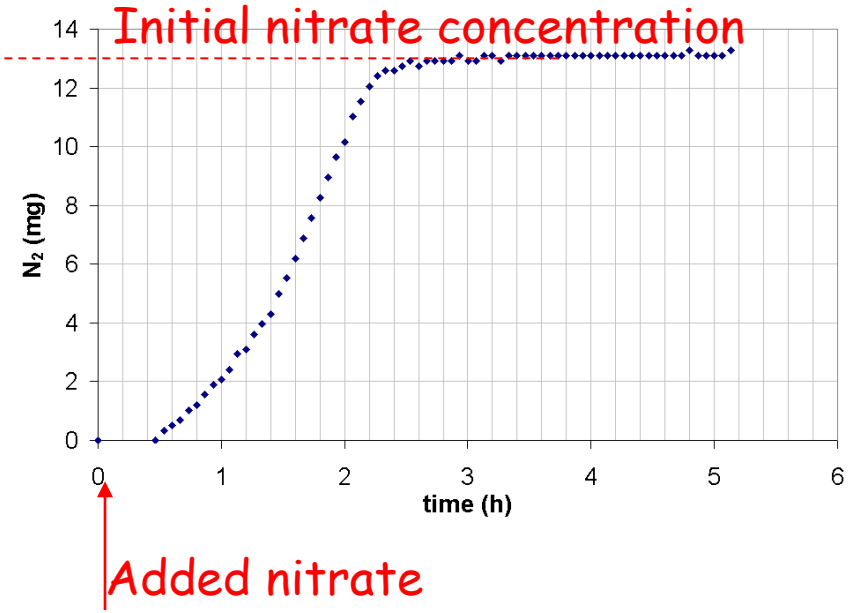
Why?

Residual O_2 in the head space:

O_2 uptake for biomass respiration = pressure decrease (lag-phase).

pressure decrease would mask the concomitant nitrogen production,

underestimation of the total overpressure due to N_2 production.

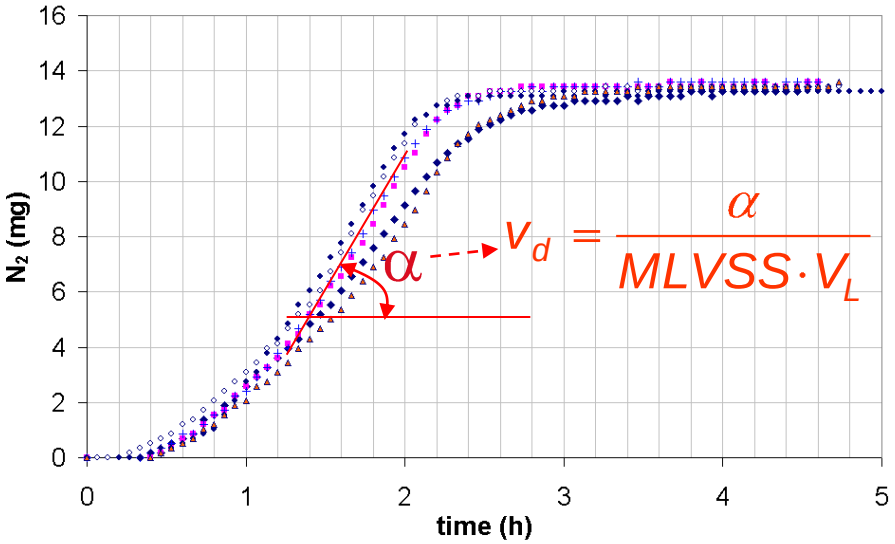


V_{HS} = 140 mL initial nitrate concentration = 20 mgN/L;

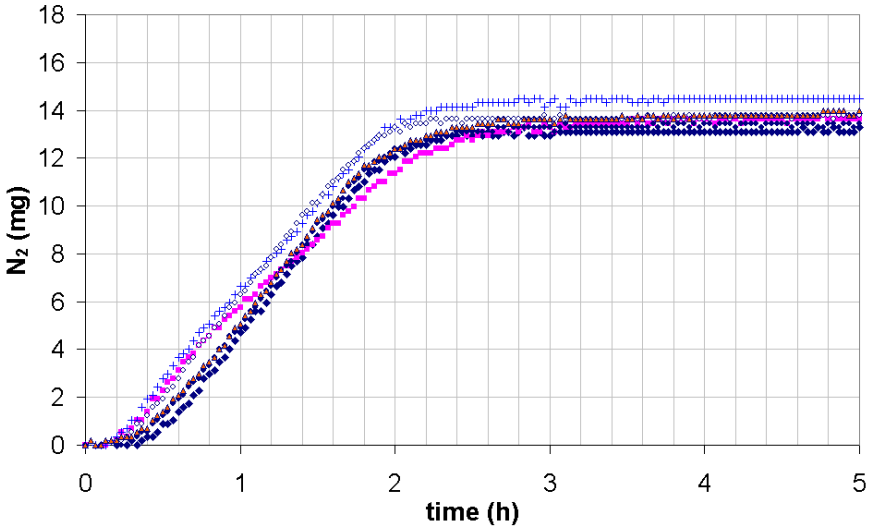
the observed average error of -22% = pO_2 of 2 to 3%

- **Reproducibility**

Replicates of denitrification tests on WW1 sludge samples



Acetate+nitrate



Acetate+nitrite

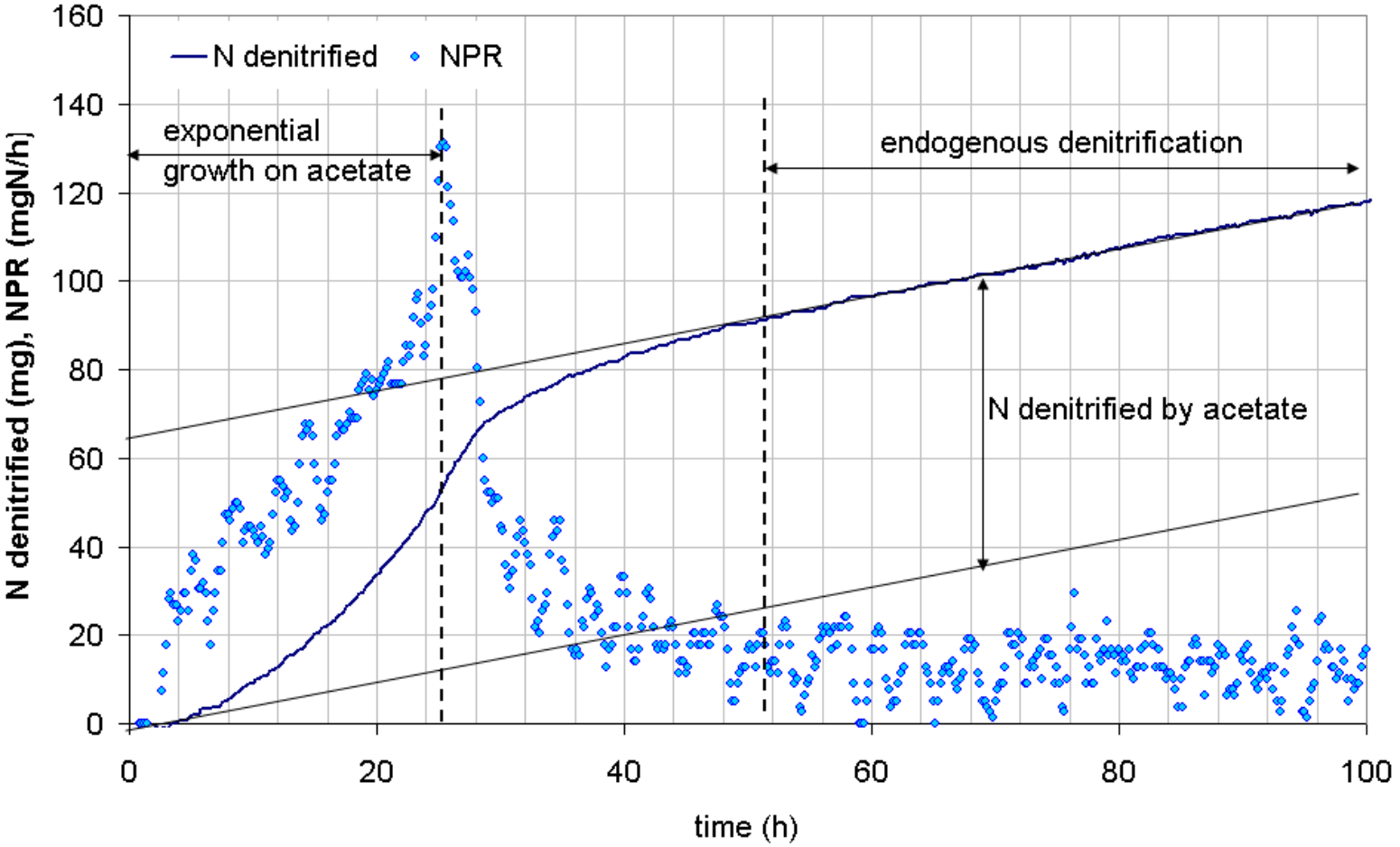
Manometry (18) – Denitrification: Tests at Low Food to Biomass (F/M) ratio

Reproducibility of **specific denitrification rate** estimates (mgN/gVSS/h)

Electron donor	Electron acceptor	WW1				WW2			
		mean	st. dev.	CV (%)	# of repetitions	Mean	st. dev.	CV (%)	# of repetitions
Acetate	$N\text{-}NO_2^-$	5.00	0.46	9	6	2.18	0.72	33	29
	$N\text{-}NO_3^-$	2.61	0.77	29	9	1.76	0.48	28	47
Endogenous organic matter	$N\text{-}NO_2^-$	1.58	0.28	17	7	n.a.	n.a.	n.a.	5
	$N\text{-}NO_3^-$	1.70	0.13	8	9	0.66	0.21	31	6

Manometry (19) – Denitrification: Tests at High Food to Biomass (F/M) ratio

Typical output

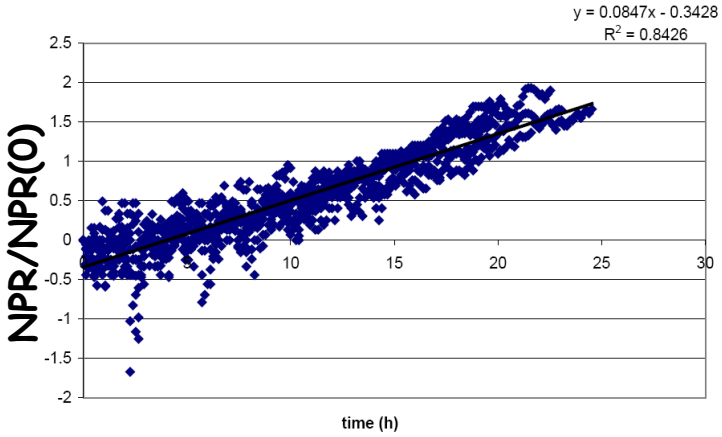


NPR = N_2 Production Rate $$NPR(mgN/h) = \frac{1 - Y_{HD}}{2.86 \cdot Y_{HD}} \cdot \mu_{HD} \cdot X_{BH,t=0} \cdot e^{(\mu_{HD} - b_{DH}) \cdot t}$$

Manometry (20) – Denitrification: Tests at High Food to Biomass (F/M) ratio

From these data the following parameters can be assessed:

-(μ-b) Gross maximum growth rate
linear fitting of NPR data in a semi-log graph

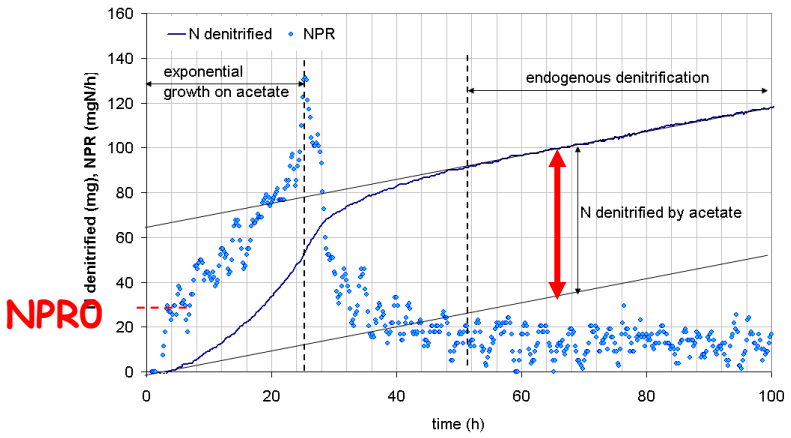


-Y_{HD} Anoxic growth yield

$$Y_{HD} = 1 - \frac{N_{denitrified} \cdot 2.86}{COD_0}$$

-$X_{HD}(0)$: initial concentration of denitrifiers

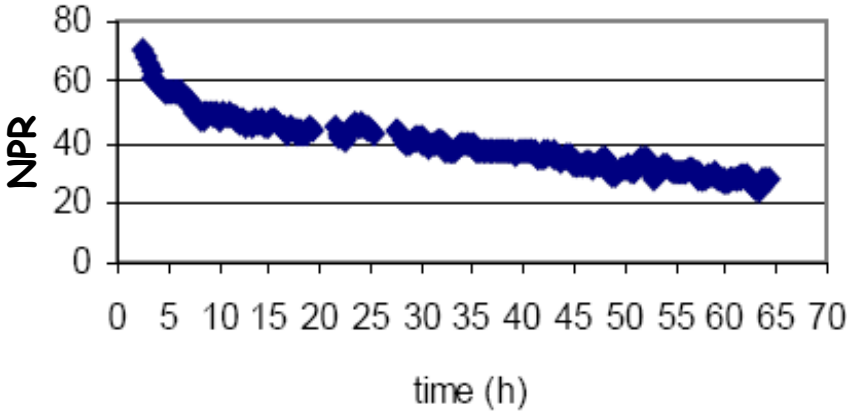
$$X_{BH,t=0} = NPR_0 \cdot \frac{2.86 \cdot Y_{HD}}{\mu_{HD} \cdot (1 - Y_{HD})}$$



Manometry (21) – Denitrification: Decay tests

- No external carbon source

→ slow decrease in the NPR



- linear fitting of NPR data in a semi-log graph

→apparent decay rate constant (b'_{HD})

- b'_{HD} is then corrected for cryptic growth to get the net anoxic decay rate (b_{HD}) according to:

$$b_{HD} = \frac{b'_{HD}}{1 - Y_H(1 - f_p)}$$

f_p = cell debris from biomass decay

Manometry (22) – Denitrification: biomass parameter estimates (1)

Parameter	Sludge from WW1				Sludge from WW2			
	Mean	St. dev	CV(%)	# repetitions	Mean	St.dev	CV(%)	# repetitions
μ_{HD} (d^{-1})	2.26	0.03	2	8	2.74	0.21	8	8
Y_{HD} ($gCOD\ g^{-1}COD$)	0.56	0.03	6	4	0.66	0.02	3	4
X_{BH}/X_T (g/g)	0.15	0.01	6	8	0.16	0.06	36	8
b_{HD} (d^{-1})	0.76	0.06	9	6	0.67	0.02	3	2

Manometry (23) – Denitrification: biomass parameter estimates (2)

Estimated parameters can be used to compute the **specific denitrification rate** (SDR):

$$SDR\left(\frac{mgN}{gVSS \cdot h}\right) = \frac{\mu_{HD} \cdot (-Y_{HD})}{2.86 \cdot Y_{HD}} \cdot \frac{1000}{24} \cdot \left(\frac{X_{BH}}{X_T} \cdot 1.42\right)$$

The standard deviation for each parameter was propagated according to error propagation

$$\Delta\phi = \Delta\phi(x_1, x_2, \ldots x_n, \Delta x_1, \Delta x_2, \ldots \Delta x_n) = \left(\sum_{j=1}^{n}\left(\frac{\partial\phi}{\partial x_j}\Delta x_j\right)^2\right)^{\frac{1}{2}}$$

The following results were obtained:

From low F/M tests:

SDR_{WW1} = 5.5 ± 0.9 mgN/gVSS/h — 2.61 ± 0.77

SDR_{WW2} = 4.8 ± 1.8 mgN/gVSS/h — 1.76 ± 0.48

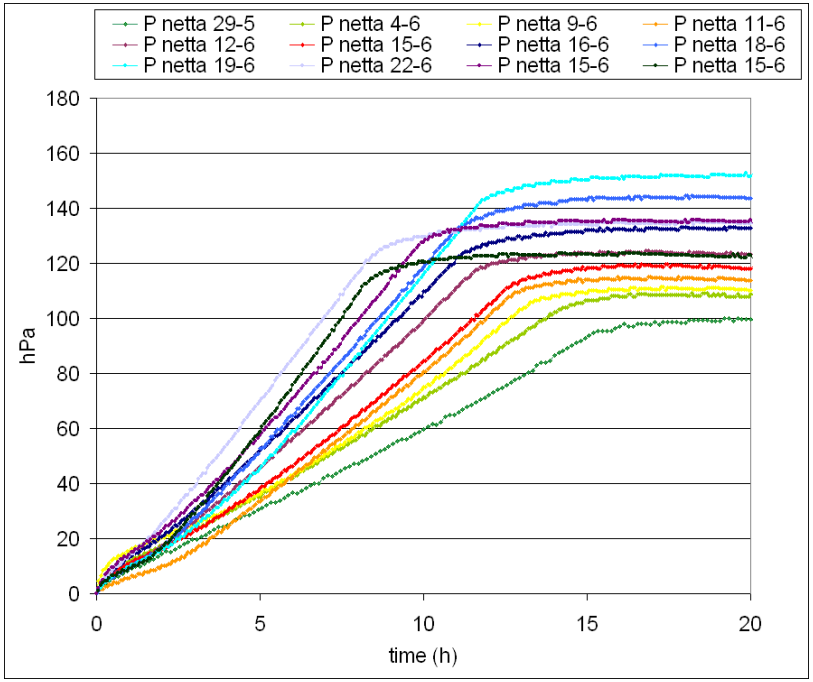
further investigation is required:

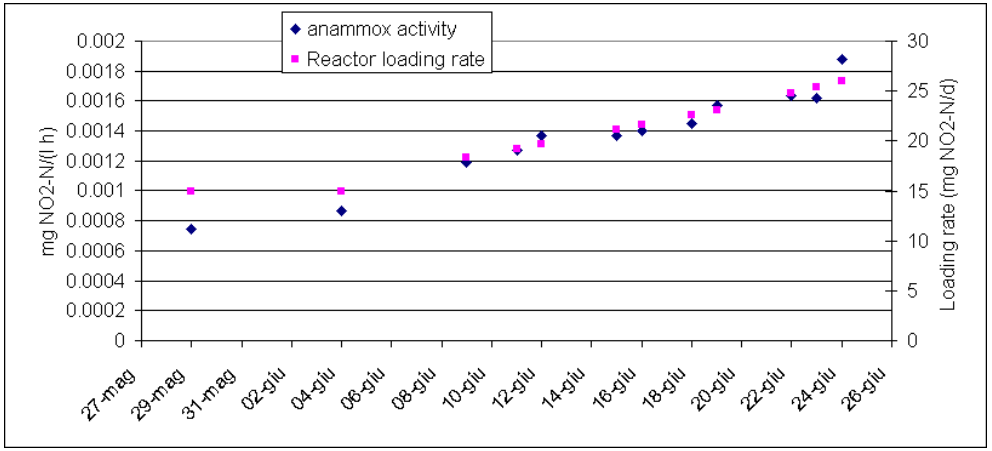
Inert accumulation? viable biomass…

Manometry (24) – ANAMMOX activity

Batch activity tests on **ANAMMOX bacteria** under enrichment

→ Allows verification of the loading pattern

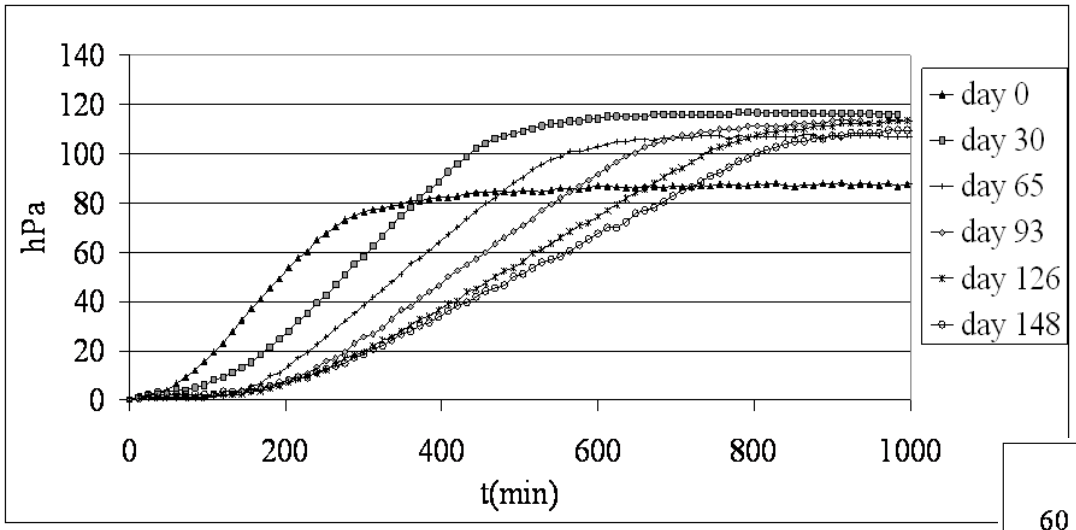


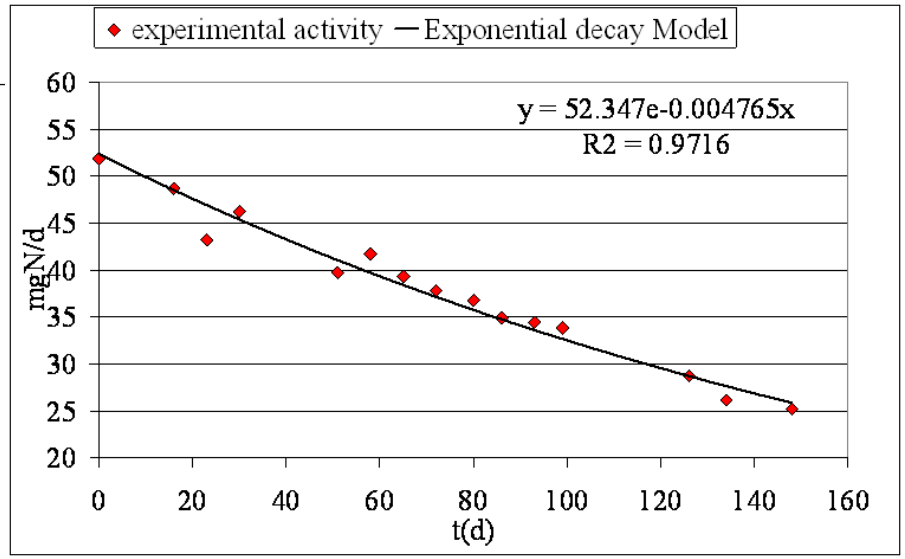


Manometry (25) – ANAMMOX activity

Batch activity tests on ANAMMOX bacteria under decaying conditions

Scaglione et al. J Chem Technol Biotechnol (2009)





Manometry (26) – Conclusions

- The proposed manometric batch bioassay proved to be simple and reliable
- It is convenient as it uses simple and widely employed manometric devices.
- Estimated specific rates were reproducible
- For Anammox and denitrification tests, accuracy can be improved by completely depleting oxygen at the beginning of the tests.
- High and Low F/M tests can be used for estimation of the main stoichiometric and kinetic denitrification parameters, *but these may not correspond to those of biomass at full scale WWPTs*.

Manometry seems promising for anoxic process monitoring and control, but needs further investigation.

MICRO-CALORIMETRY

Microcalorimetry (1): what is microcalorimetry ?

Calorimetry = *Technique of measuring the heat produced by chemical reactions or physical changes*

Microcalorimeter: Instrument able to measure really small heat exchange (5-10 mW L^{-1})

- sensitivity up to 0.001°C
- equivalent to 3-5 mg BCOD L^{-1}

It can measure any temperature change associated to biochemical reactions

Universal tool for the study, optimisation and modelling of bioprocesses (substrates consumption as well as biomass adaptation, inhibition, growth)

Microcalorimetry (2): Advantages and disadvantages

☺

- ✓ **Universal**
- ✓ Non intrusive
- ✓ No interferences with the studied system
- ✓ Immediate response
- ✓ No sample pretreatment required

☹

- ❖ **Necessity of a finely controlled environment**
- ❖ Global data → Difficult to differentiate subsequent reactions
- ❖ Costly
- ❖ Difficult to use if
  - concentration is low
  - biodegradation kinetic is slow
  - metabolism dissipates little energy (little ΔH)

$$\Delta G = \Delta H - T\Delta S$$

$\Delta G < 0$ (exergonic reaction)

Biomass metabolism : ΔH usually < 0 (**exothermic reaction**)

EXCEPTION: methanogenic processes (e.g. acetoclastic methanogenic biomass) can be endothermic

at constant temperature and pressure: $\Delta H = \int Q_R \cdot dt$

Ref.:

Daverio E., Aulenta F., Ligthart J., Bassani C., Rozzi A. (2003) Application of calorimetric measurements for biokinetic characterisation of nitrifying population in activated sludge, Water Research, 37, 2723-2731

Scaglione D., Buttiglieri G., Ficara E., Caffaz S., Lubello C., Malpei F. (2009) Water Science and Technology, vol. 60, n°10, pp. 2705-2711

$\Delta H°$ is calculated from standard enthalpy formation values of reaction reagents and products

Examples:

1) Anammox reaction

$NH_4^+ + 1{,}32\ NO_2^- + 0{,}066\ HCO_3^- + 0{,}13\ H^+ \rightarrow$

$\rightarrow 1{,}02\ N_2 + 0{,}26\ NO_3^- + 0{,}066\ CH_2O_{0.5}N_{0.15} + 2{,}03\ H_2O \quad \Delta H° = -318$ J/mmol NH_4

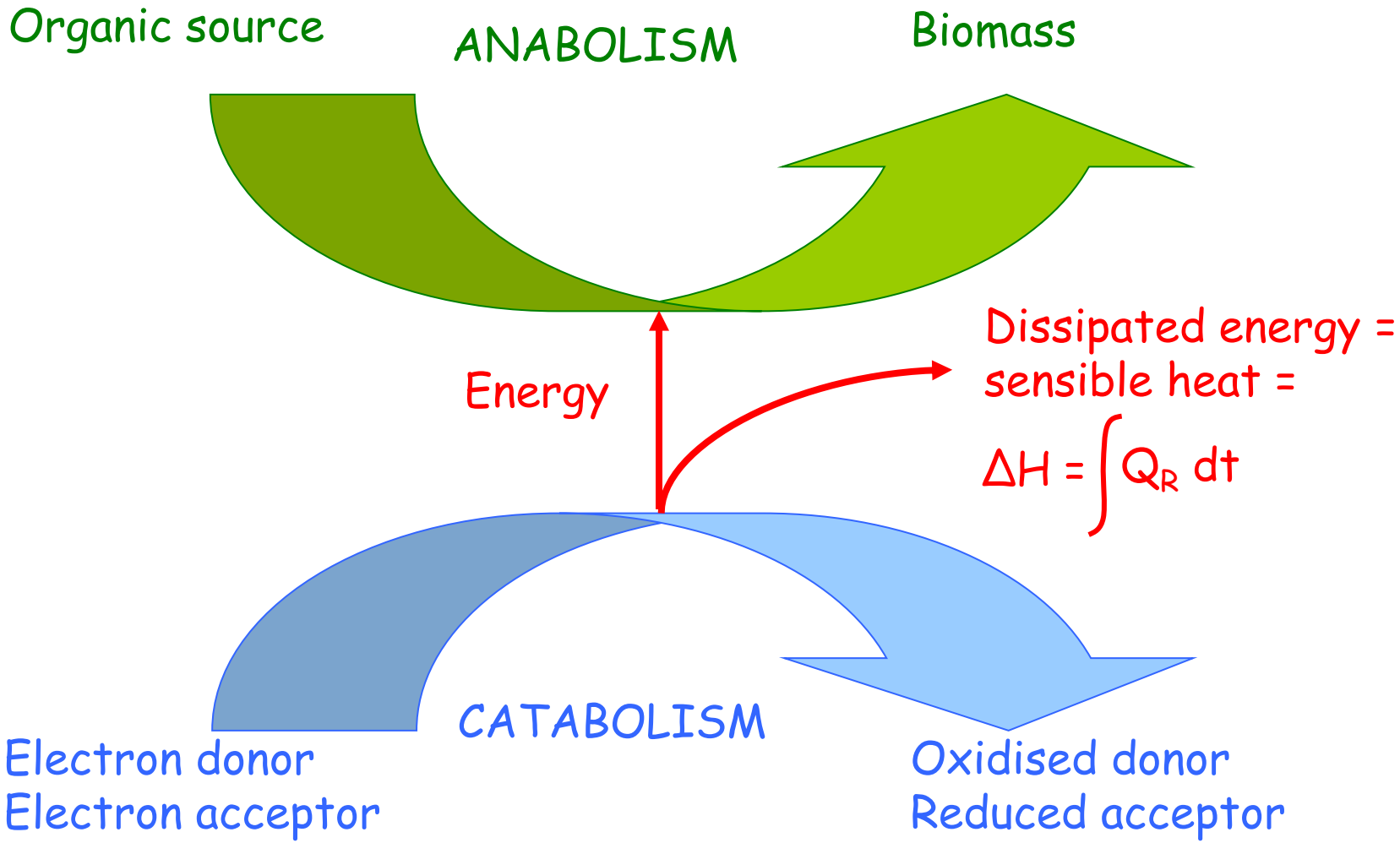
2) Ammonia oxidation into nitrite

$NH_4^+ + 3/2\ O_2 \rightarrow 2\ H^+ + NO_2^- + H_2O \quad \Delta H° = -259$ J/mmolN or $\Delta H° = -18.5$ J/mgN

3) Nitrite oxidation to nitrate

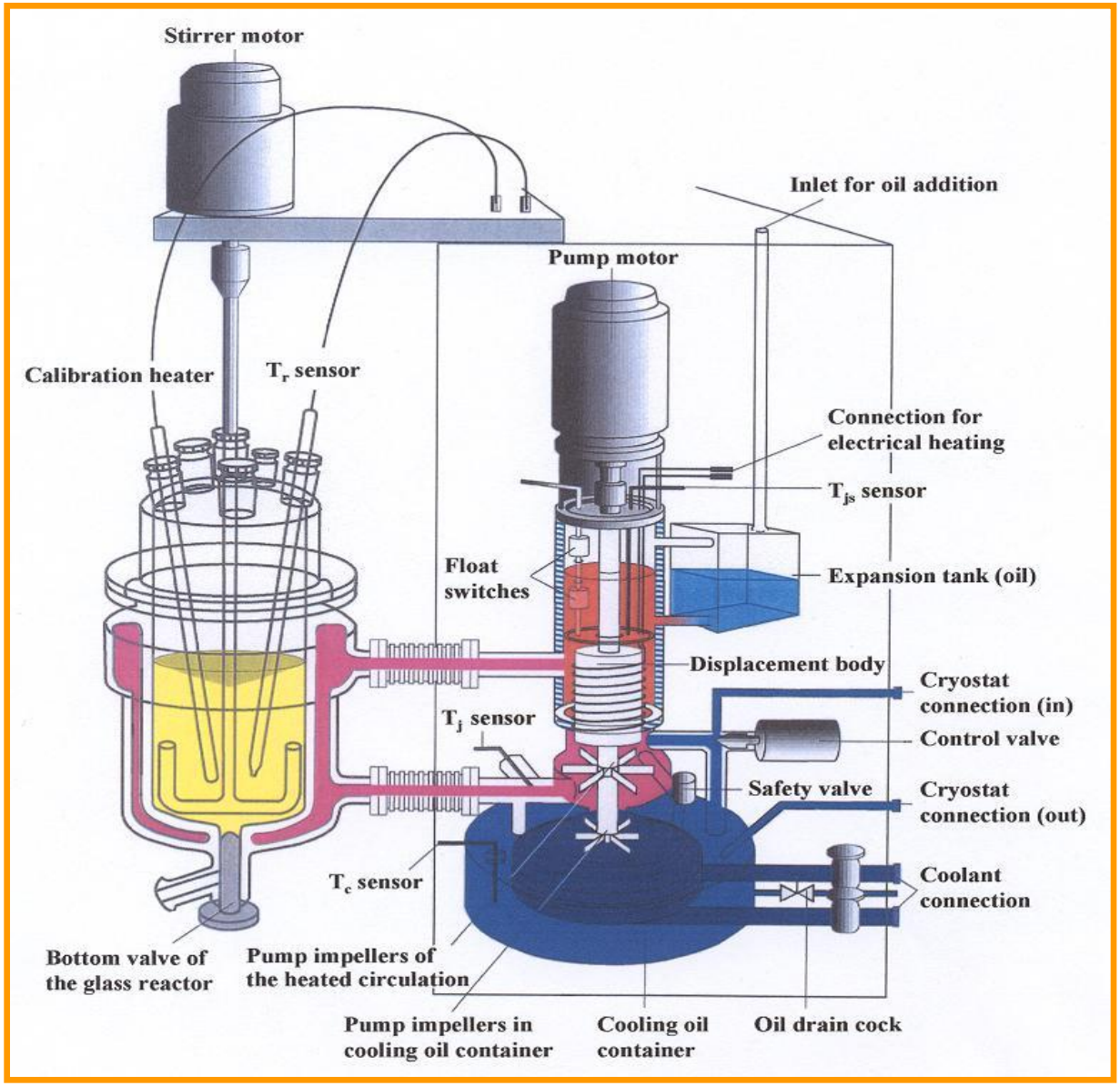
$NO_2^- + \frac{1}{2}\ O_2 \rightarrow NO_3^- \quad \Delta H° = -99.4$ J/ mmolN or $\Delta H° = -7.1$ J/mgN

Microcalorimetry (5): Basics of thermodynamics (3)



Microcalorimeter (6): how does it work? (1)

Microcalorimeter (7): how does it work ? (2)



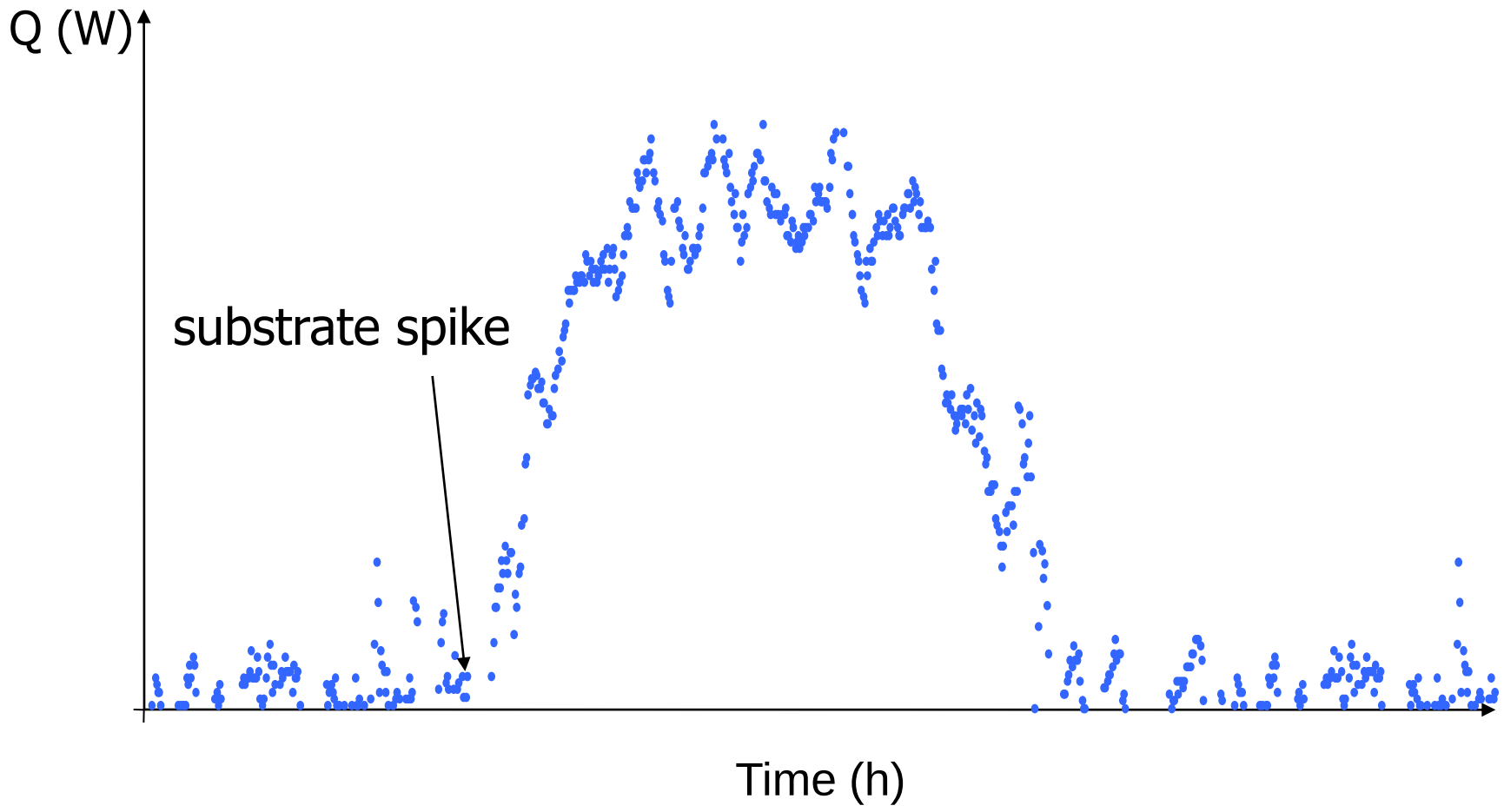
Microcalorimeter (8): how does it work ? (3)

- Q_R, Heat flux, W
- U, Overall heat-transfer coefficient, W m^{-2} K^{-1} } Calibration

- A, Heat transfer area, m^2
- T_R, Temperature of the reactor content, K
- T_j, Temperature of the jacket, K

$$Q_R = U \cdot A \cdot (T_R - T_j)$$

Microcalorimetry (9): Thermogram (1)

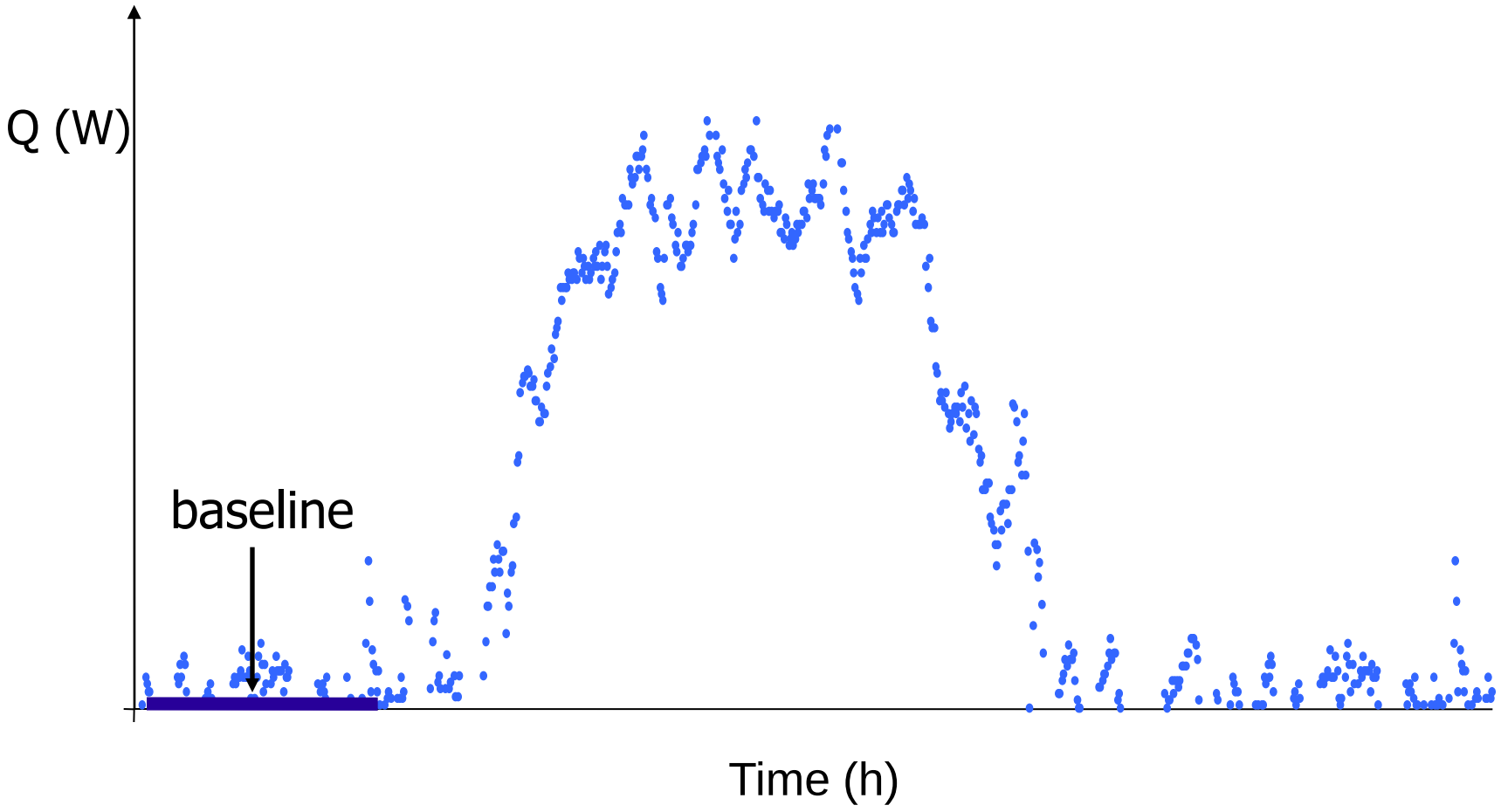


Microcalorimetry (10): Data elaboration (1)

Estimation of the baseline

→ average of the Q_R value before the spike

Microcalorimetry (11): Thermogram (2)



Microcalorimetry (12): Data elaboration (2)

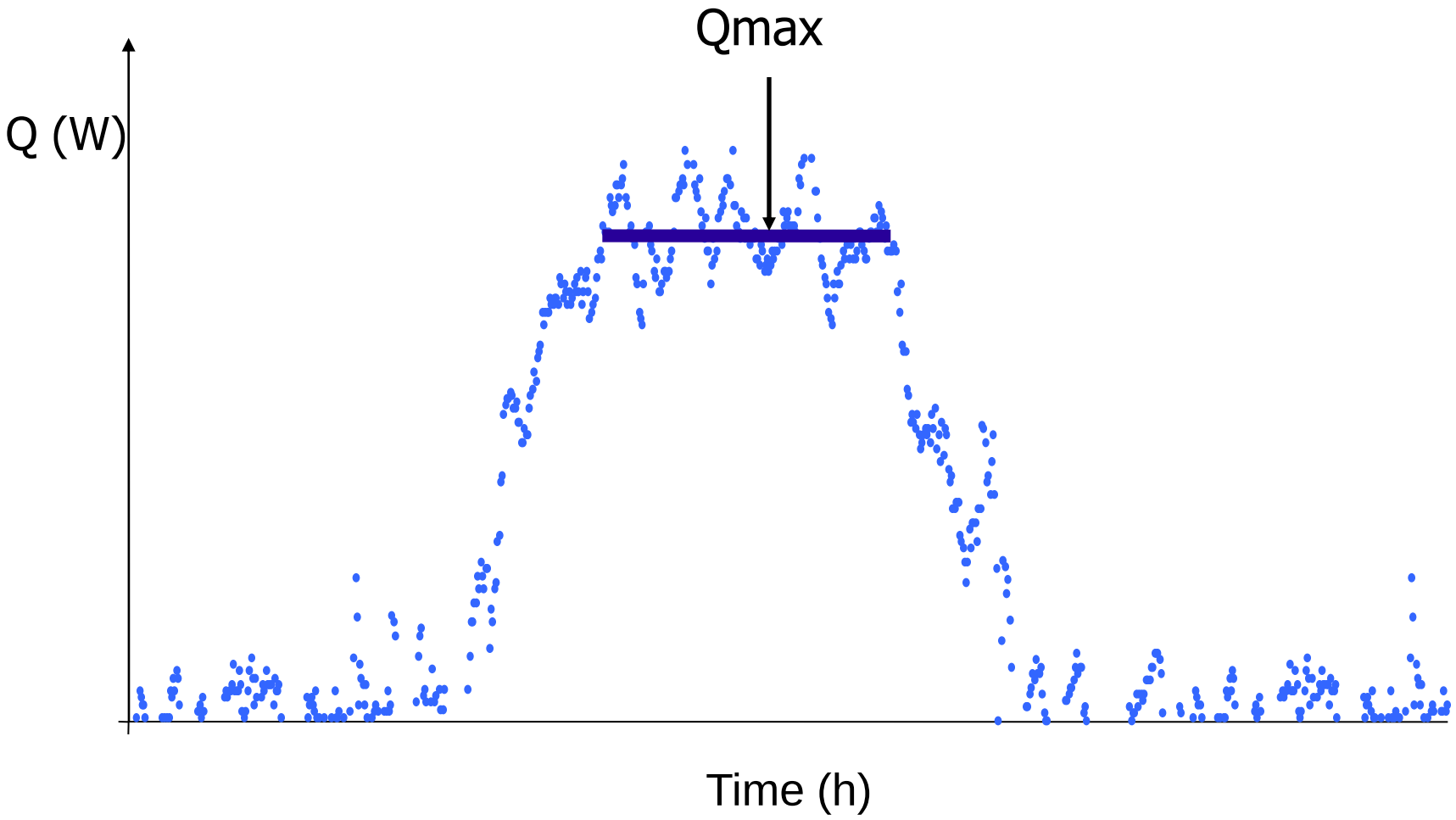
Estimation of the baseline

→ average of the Q_R value before the spike

Calculation of Qmax

→ average of the Q_R value at the maximum value

Microcalorimetry (13): Thermogram (3)



Microcalorimetry (14): Data elaboration (4)

Estimation of the baseline

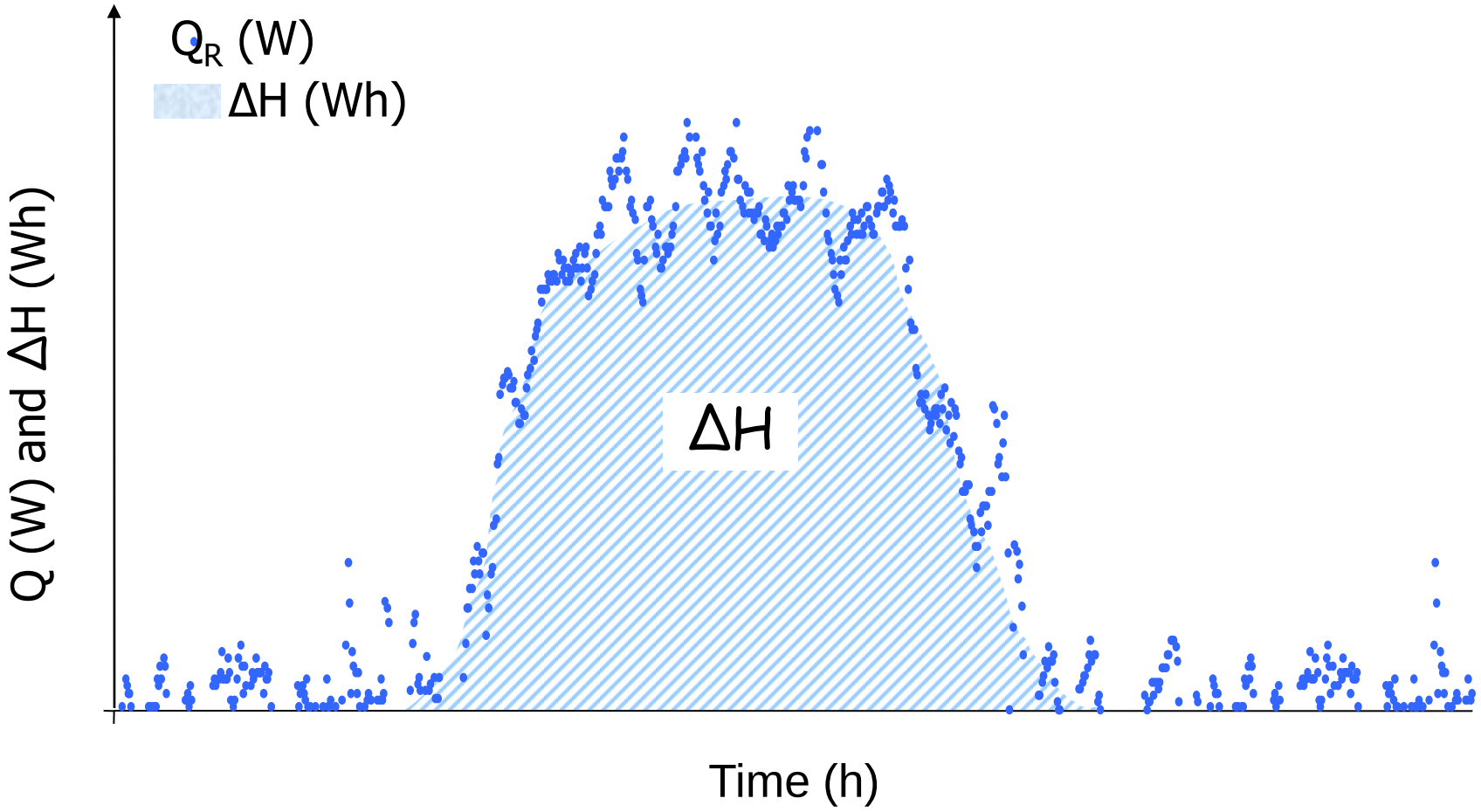
→ average of the Q_R value before the spike

Calculation of Qmax

→ average of the Q_R value at the maximum value

Calculation of ΔH: $\Delta H = \int Q_R.dt$

Microcalorimetry (15): Thermogram (4)



Microcalorimetry (16): Data elaboration (5)

Estimation of the baseline

→ average of the Q_R value before the spike

Calculation of Qmax

→ average of the Q_R value at the maximum value

Calculation of ΔH → $\Delta H = \int Q_R . dt$

Calculation of substrate degradation

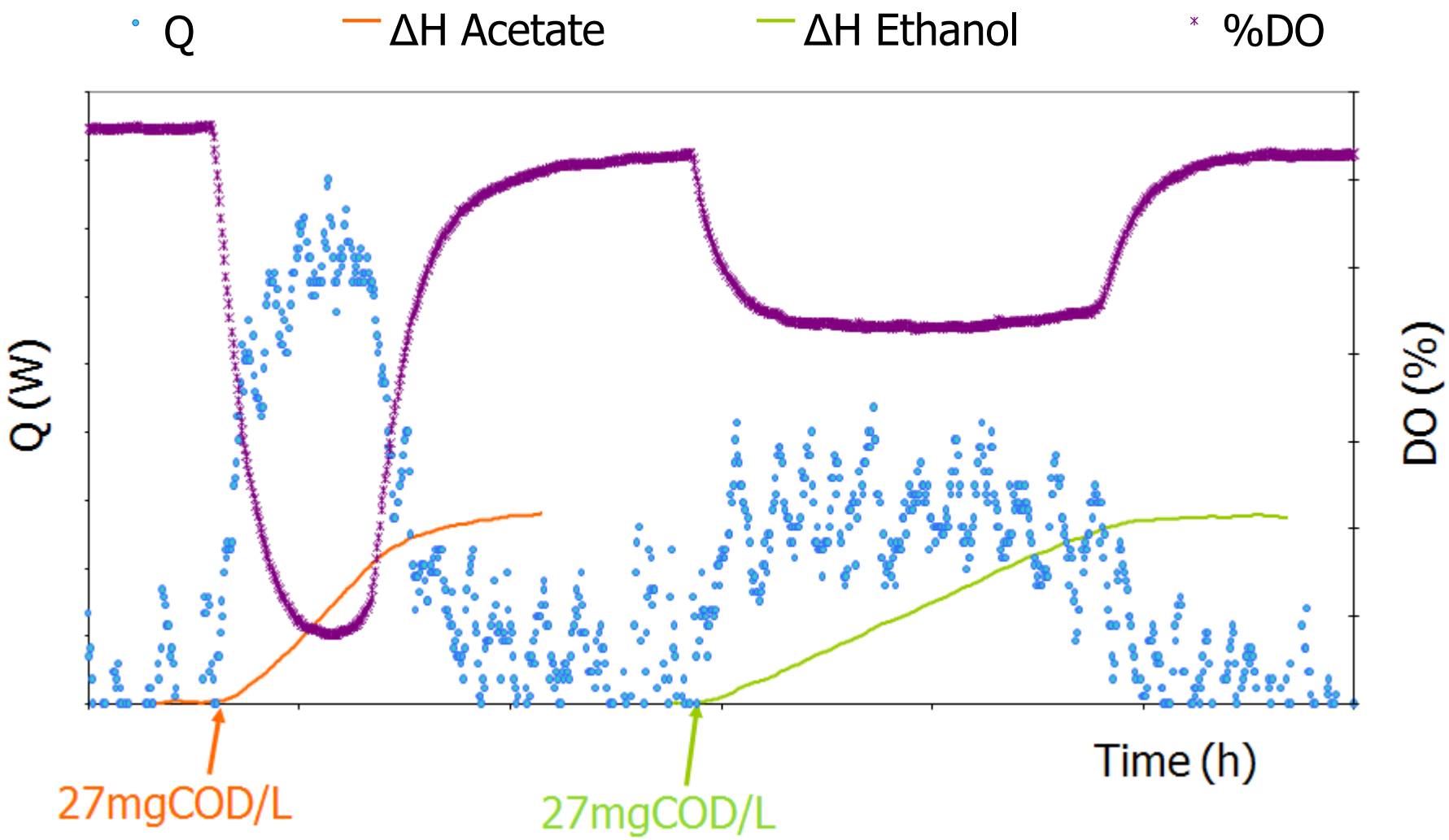
$$\Delta S = \frac{\Delta H}{Y_{q/s} \cdot V_S}$$

ΔS: is the substrate degradated (mg/L)

$Y_{q/s}$: is the energy generated for the unit of substrate degraded (mJ/mg)

V_S: is the volume where reaction takes place (L)

Microcalorimetry (17): Example - Heterotrophic activity measurements



Microcalorimetry (18): Anammox inoculum

40-L pilot-scale SBR (Caffaz et al., 2008)

Fed with the effluent from a moving bed biofilm pilot-scale reactor operating the partial nitrosation of the **anaerobic supernatant** coming from a wastewater treatment plant (WWTP)

Reactor loading rate: 0.044 kg N (kg_{SSV})$^{-1}$ d^{-1}

Average hydraulic retention time: 8 d

Nitrogen removal efficiency: 99 %

Influent characteristics:

NH_4^+-N [mg/l]	NO_2^--N [mg/l]	NO_2^--N/NH_4^+-N	COD[mg/l]
319 ± 51.4	311 ± 58.5	0.98 ± 0.12	444 ± 223

Microcalorimetry (19): Experimental procedure

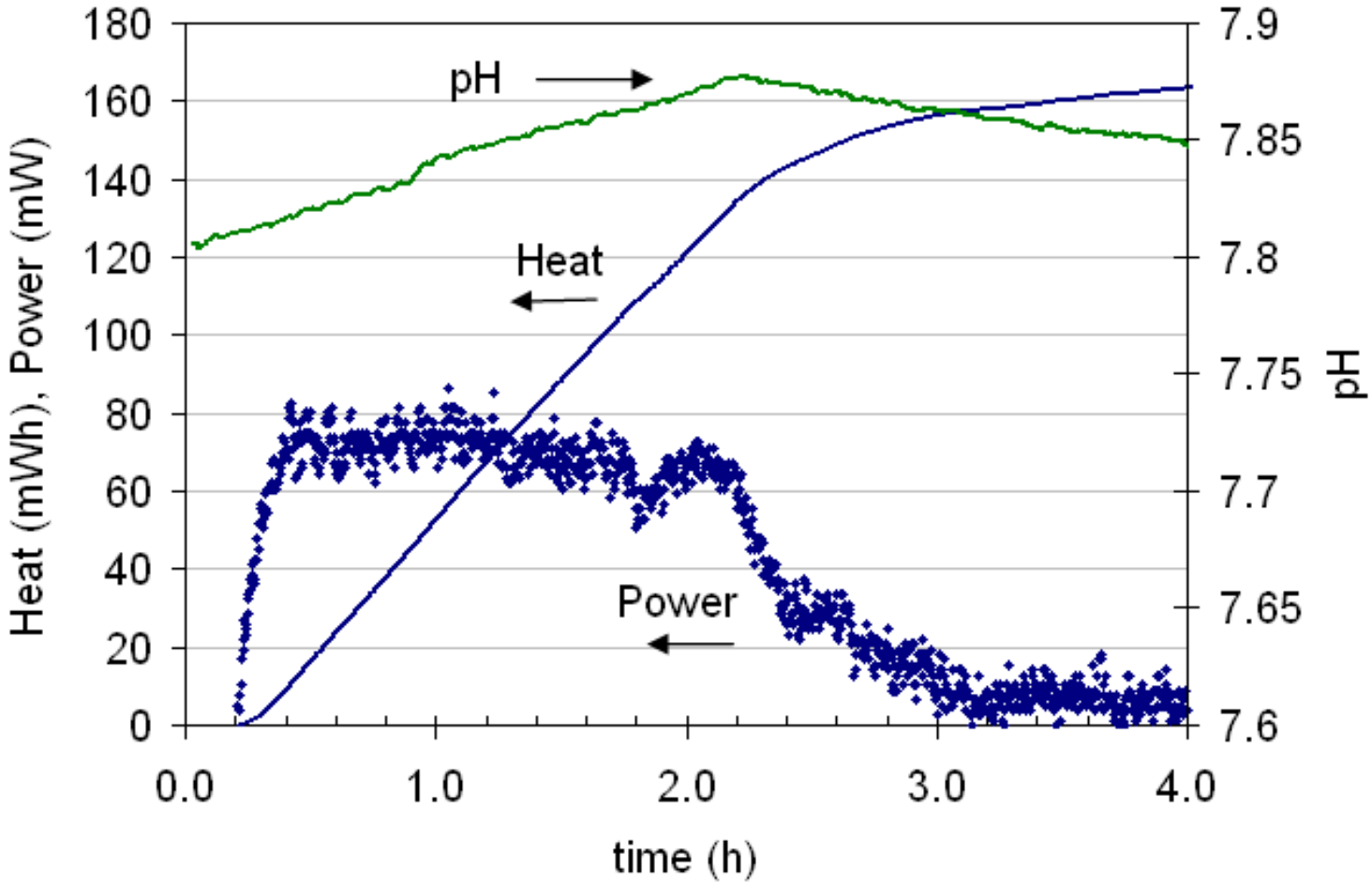
Manometric and calorimetric determinations of the Anammox activity performed in parallel under similar conditions.

injections of 5-10 mg/L NO_2-N and NH_4-N

Sludge sample		Manometric tests			Microcalometric tests		
Sampling time	SSV (g/L)	Test	Sludge volume (L)	NO_2 added (mgN)	Test	Sludge volume (L)	NO_2 added (mgN)
25-lug-07	2	1	1	10	1a	2	20
					1b	2	10
30-gen-08	1.7	2	1	10	2a	2	7
					2b	2	20
					2c	2	10
					2d	2	20

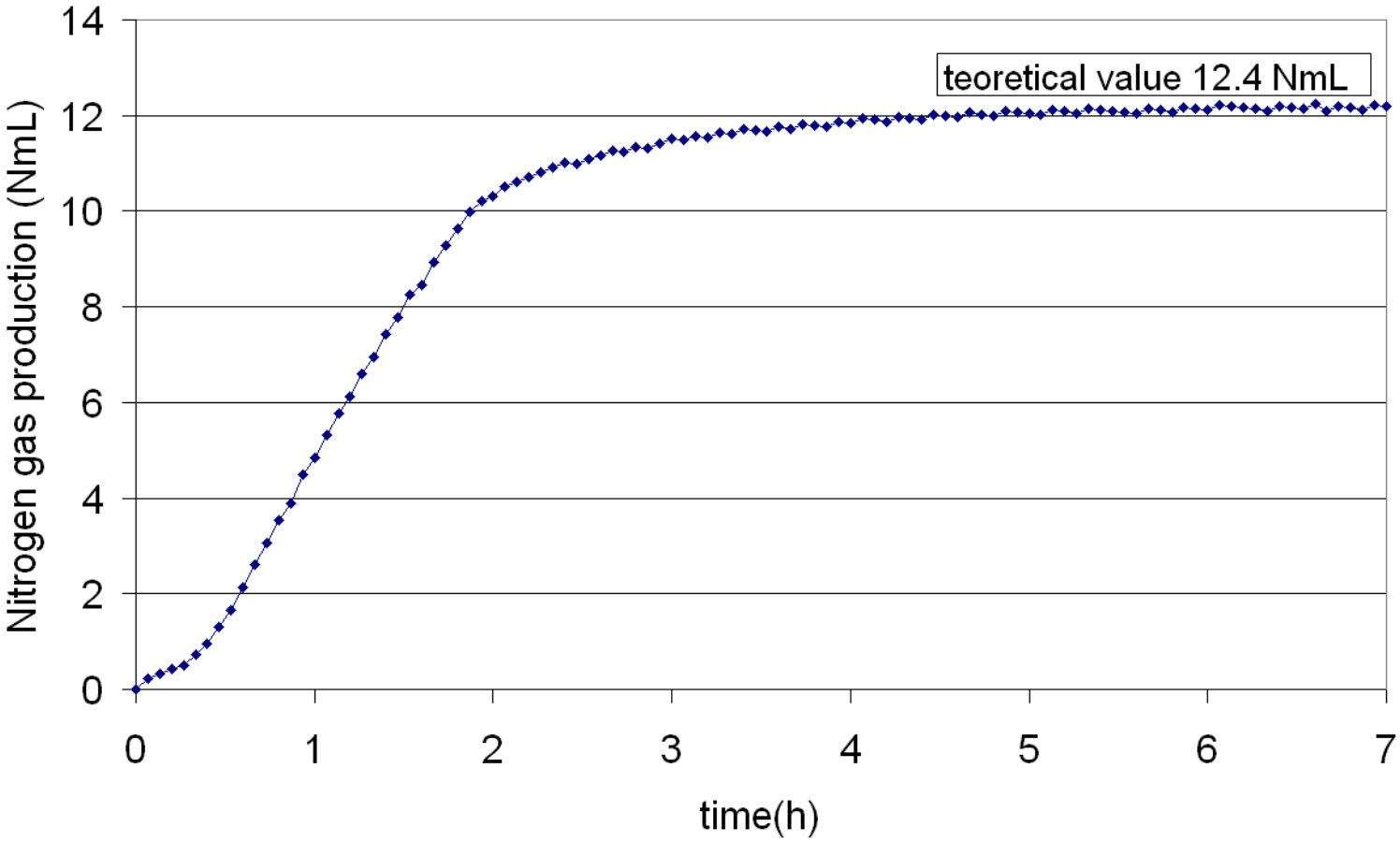
Microcalorimetry vs Manometry (1): calorimetric results

Tipical output of a microcalorimetric test



Microcalorimetry vs Manometry (2): manometric results

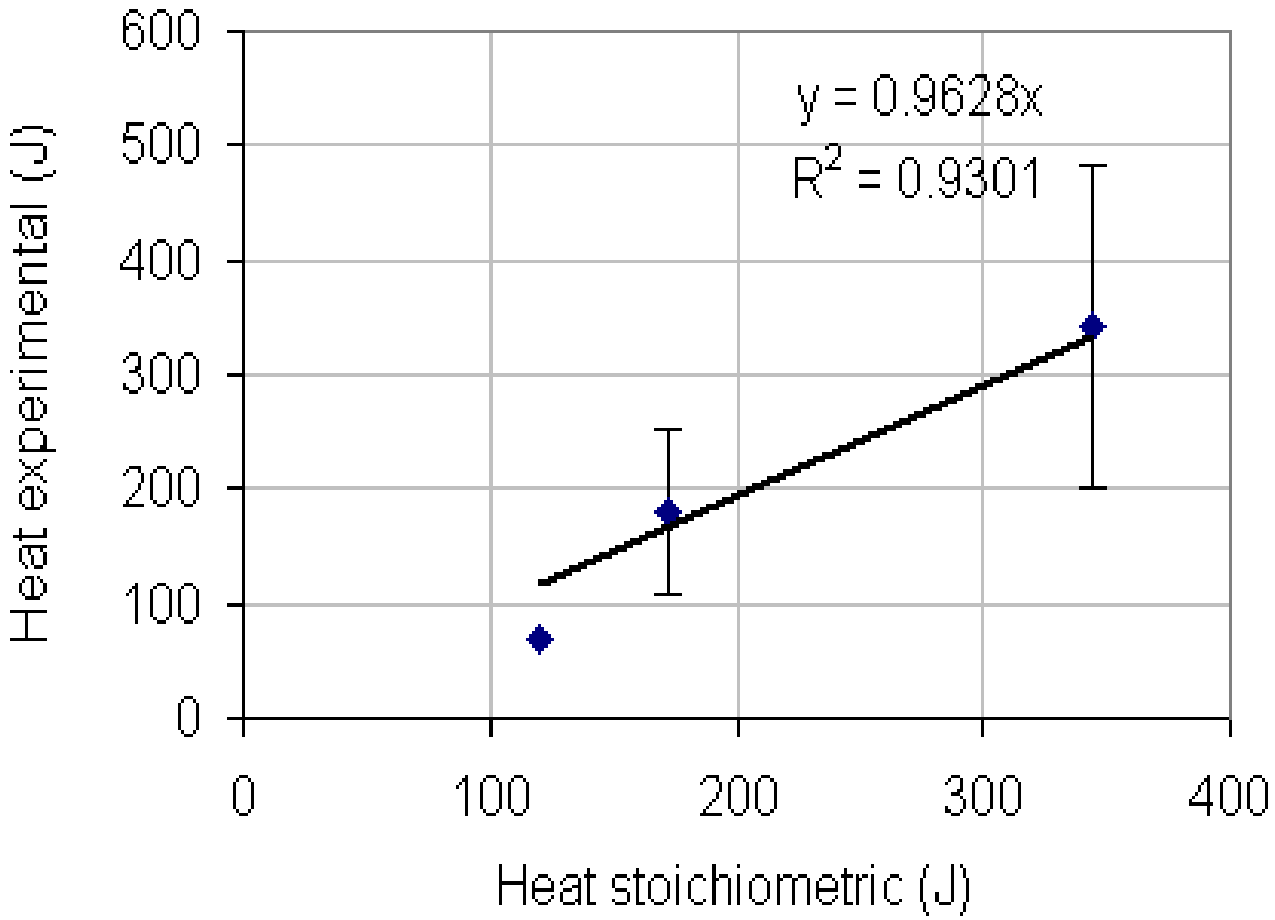
Tipical output of a manometric test



Small understimation due to N_2 solubility

Microcalorimetry vs Manometry (3): theoretical vs measured ΔH

Comparison between the heat exchanged and the heat expected from the enthalpic variation



Microcalorimetry vs Manometry (4): comparison of results

Comparison between the **maximum specific Anammox activity** estimated by microcalorimetric and manometric tests.

Sludge sample	From microcalorimetry	From manometry
	SAA_{max} ($mgNO_2$-N/gSSV/h)	
1	3.39 ± 0.05	2.74
2	3.37 ± 0.54	2.58

Microcalorimetry vs Manometry:
+24% difference

Microcalorimetry vs Manometry (5) conclusions

- ✓ **Manometry confirm its applicability** to monitor biological reactions, including Anammox

- ✓ **Microcalorimetry: applicable alternative** even for poorly active Anammox biomass.

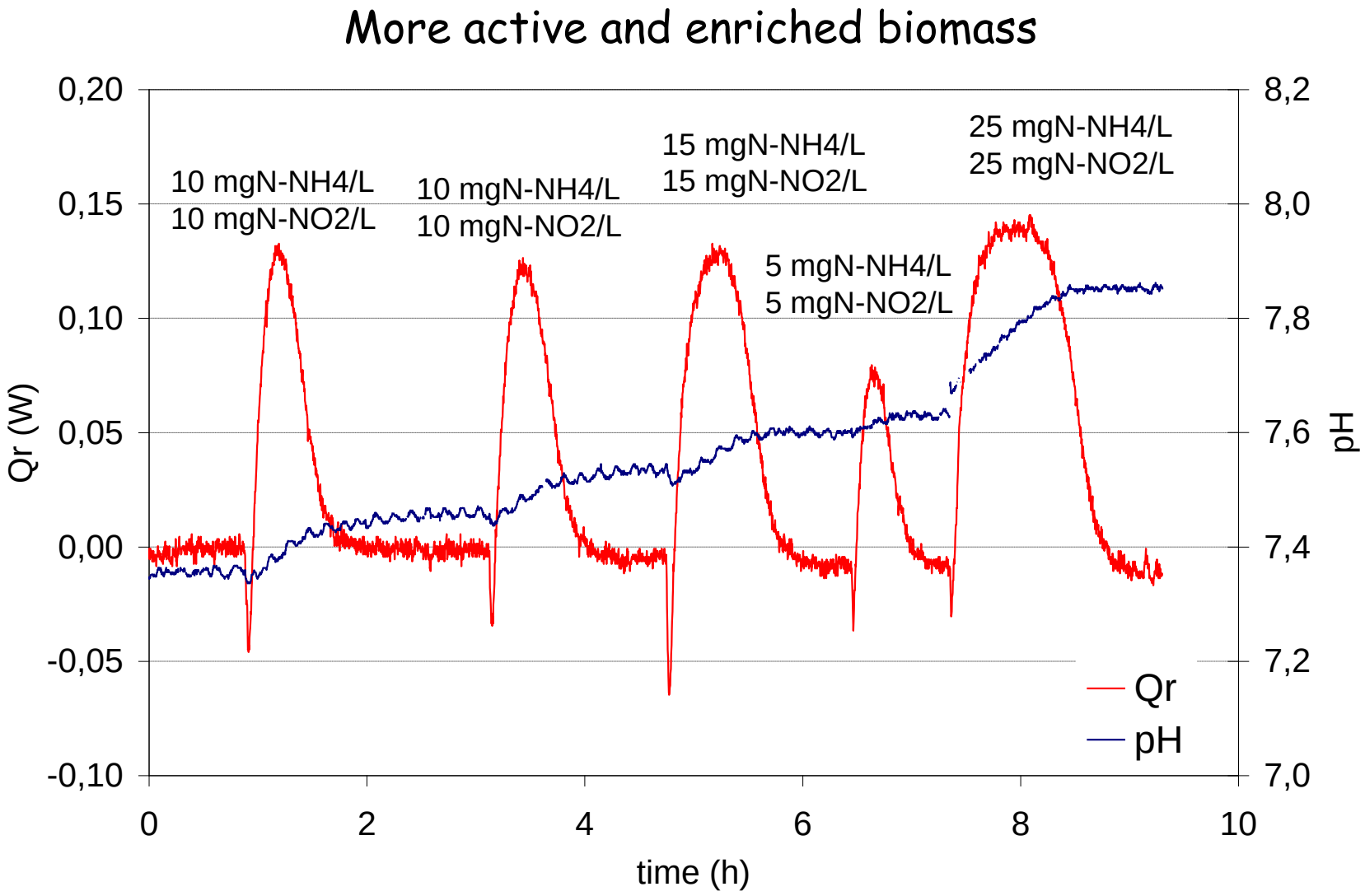
- ✓ **Experimental heat exchanged averagely comparable with what expected from the theoretical reaction enthalpy.**

  → Variability and differences are still quite high.
  Further research is needed.

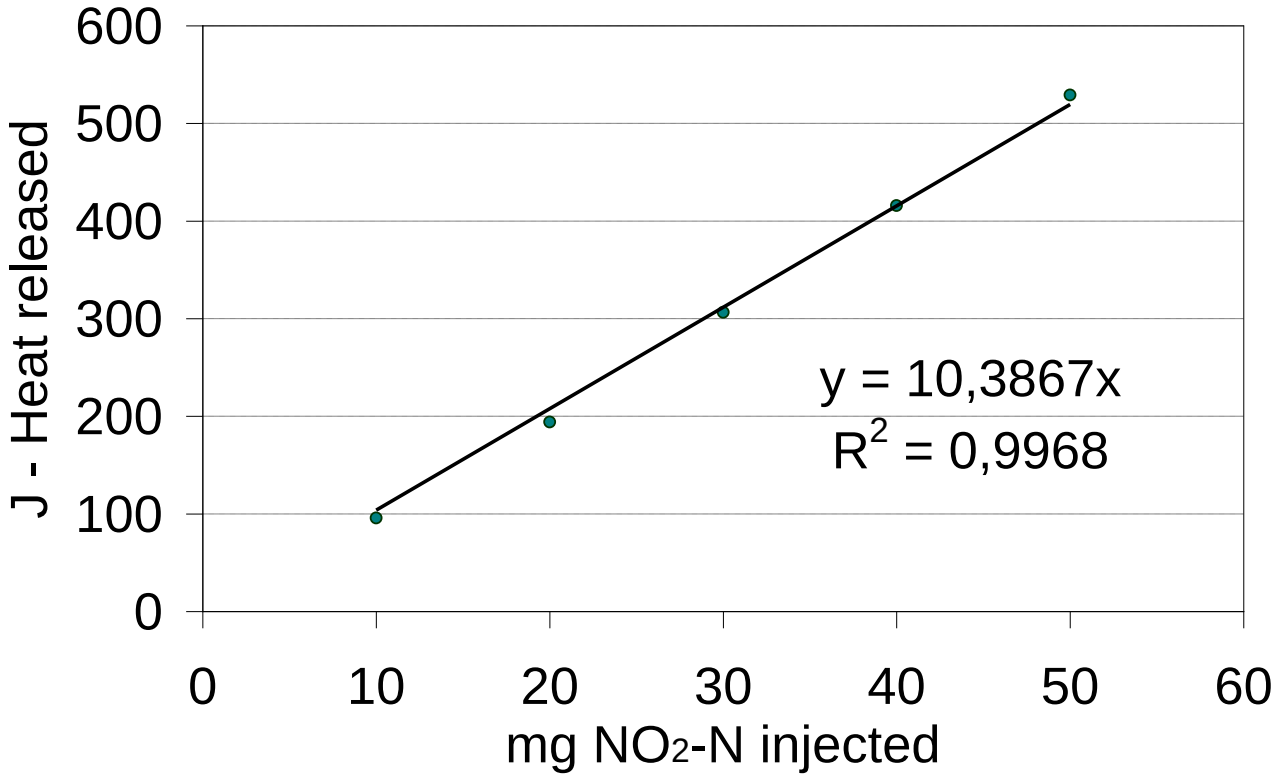
- ✓ Microcalorimetric estimates were found to be slightly higher (+23.7% on average) then values obtained in manometric tests.

- ✓ **Preliminary results are encouraging** and further research will be devoted to elucidate differences on the observed specific Anammox activity estimates.

ON-GOING RESEARCH…



ON-GOING RESEARCH…



Better corrispondence of specific activity between manometric and calorimetric measurements

-40% of ΔH still some open questions…