Detecting inhibition of nitrogen removal

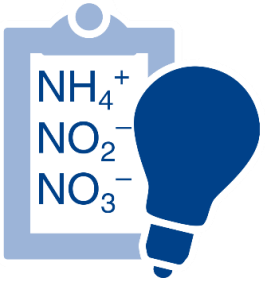
Handling interferences

Dr. Achim Leitzke, 11.08.2019

Agenda

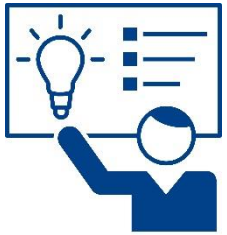
Company

Nutrient Removal



Nitrification-inhibition tests

Results



Case studies

Tips and Tricks

Summary

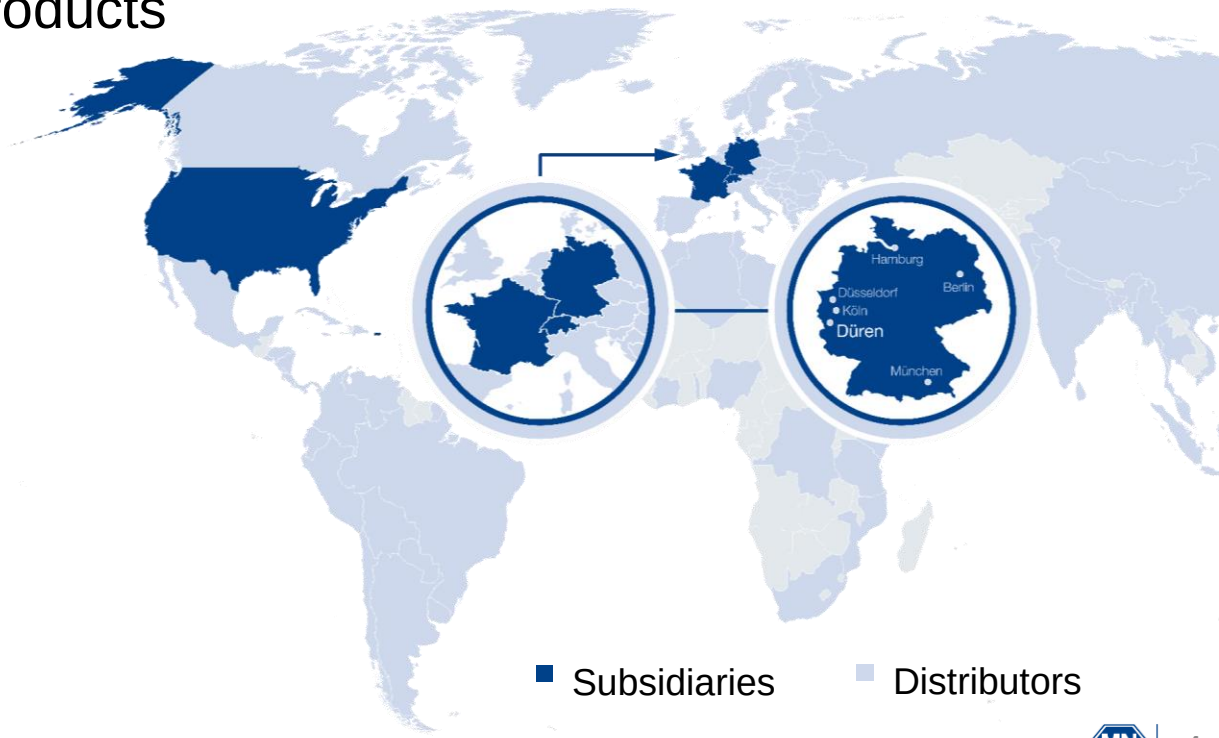
Company

MN Water Analysis

Company

MN today

- 4th Generation family owned
- More than 700 employees
- More than 25.000 products
- Turnover 120 Mio. €



Company

Business units

1911

Filtration

1959

Rapid Tests

1961

Chromatography

1970

Water Analysis

1989

Bioanalysis

Nutrient removal

Nutrient removal

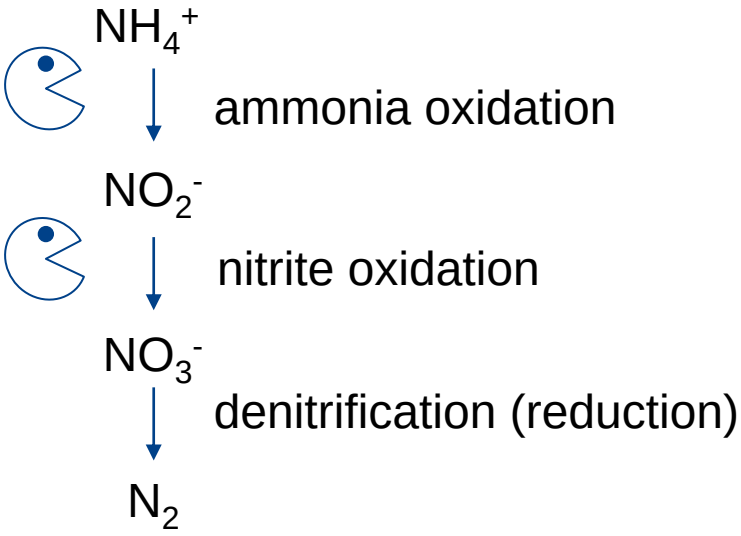
EPA Fact Sheet

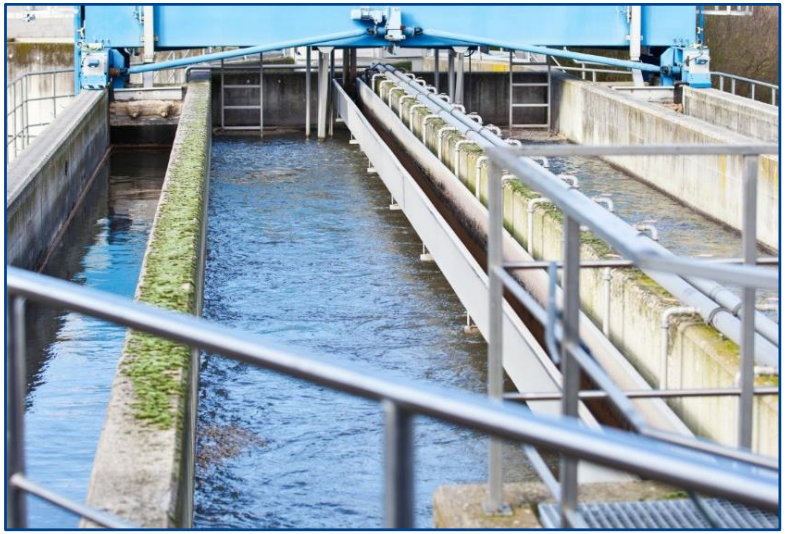
- nitrogen and phosphorus are the primary causes of cultural eutrophication
- approximately 25% of all water body impairments are due to nutrient-related causes
  - oxygen depletion
  - algal growth
  - ammonia

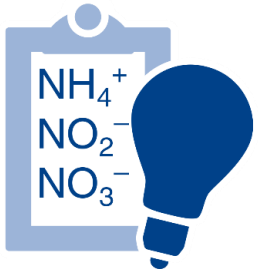
- more stringent effluent limits lead to
  - P-removal
  - N-removal

Nutrient removal

Denitrification process



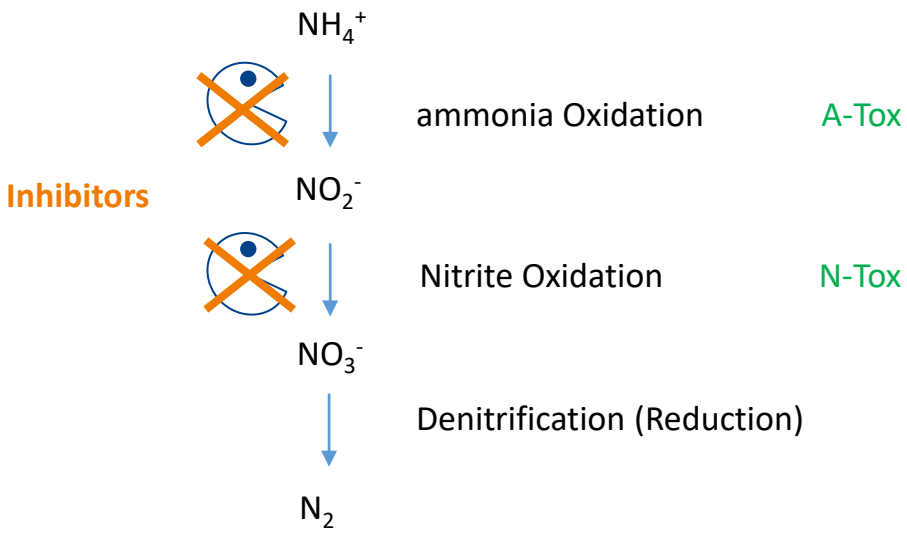




Nitrification-inhibition tests

Nitrification-inhibition tests

Inhibition



“(de)nitrification-inhibition” results in increased total N in outflow or down-time of plant

Nitrification-inhibition tests

- ISO 9509
  - use active sludge
  - add sample
  - incubate 4-24 h
  - measure nitrate and ammonia

Or…

Nitrification-inhibition tests

How to detect inhibition?

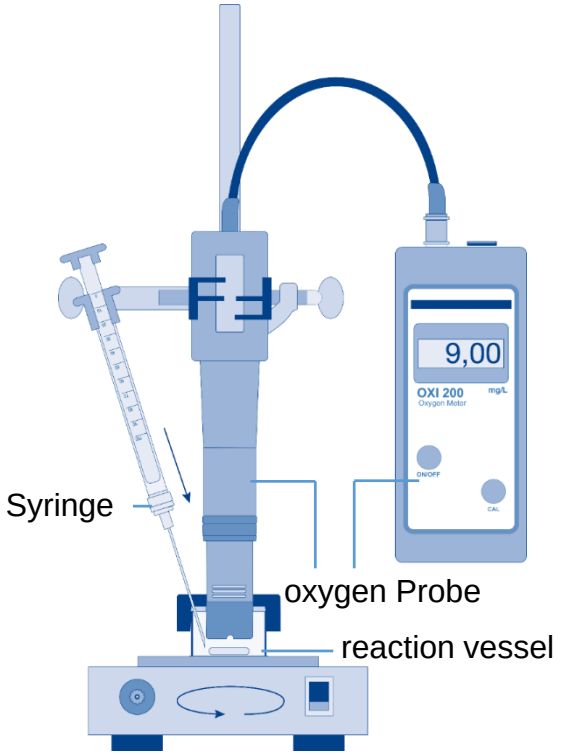
- use ready-to-use kit
  - lyophilized bacteria
  - includes all necessary solutions…
  - … and feed for bacteria
- How it works?
  - bacteria oxidize ammonia and nitrite
  - oxygen is consumed
  - oxygen consumption correlates with bacteria activity

bacteria inhibition leads to lower Oxygen Consumption

Nitrification-inhibition tests

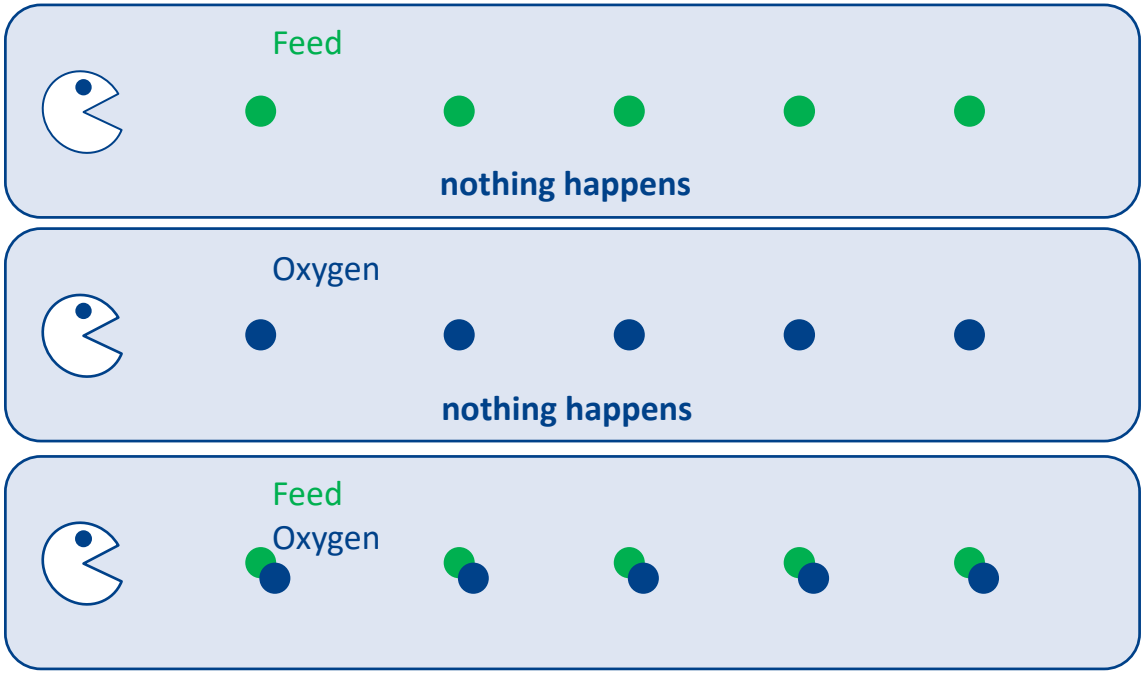
How the test is done?

- use oxygen probe
- measure
  - Control
  - Sample
- calculate inhibition from difference



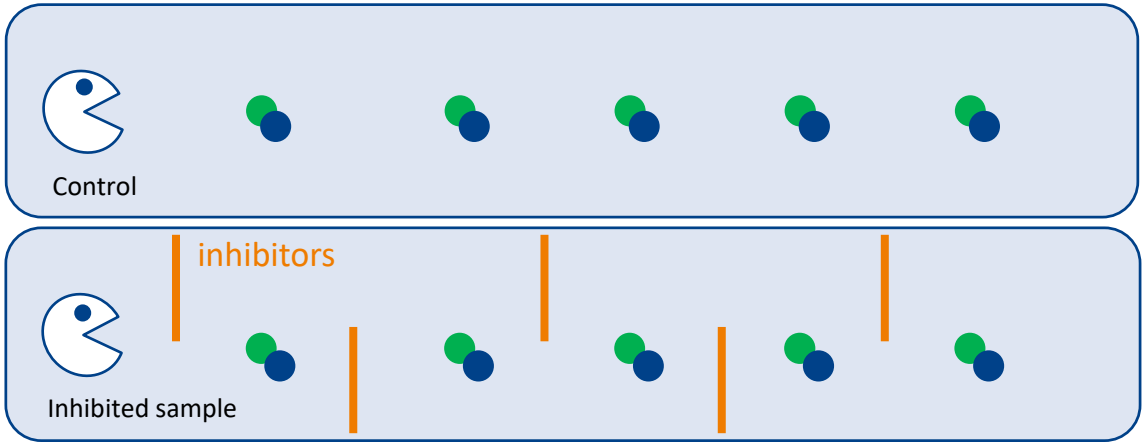
Nitrification-inhibition tests

How the tests work?



Nitrification-inhibition tests

Control and sample



ΔO_C : **4 O_2**

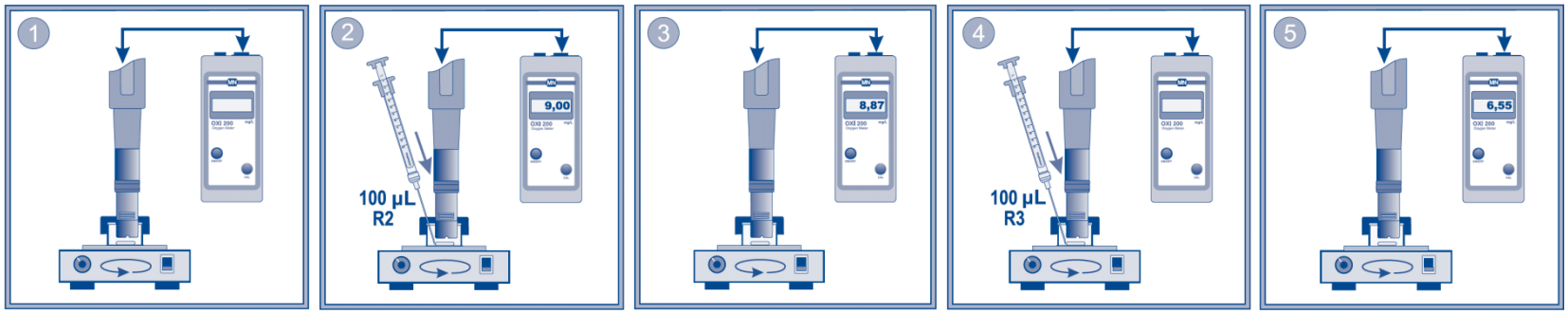
$\Delta O_C - \Delta O_S = 1\ O_2$

ΔO_S: **3 O_2**

inhibition: $\frac{\Delta O_C - \Delta O_S}{\Delta O_C} * 100 = \frac{1}{4} * 100$ **= 25 %**

Nitrification-inhibition tests

Control

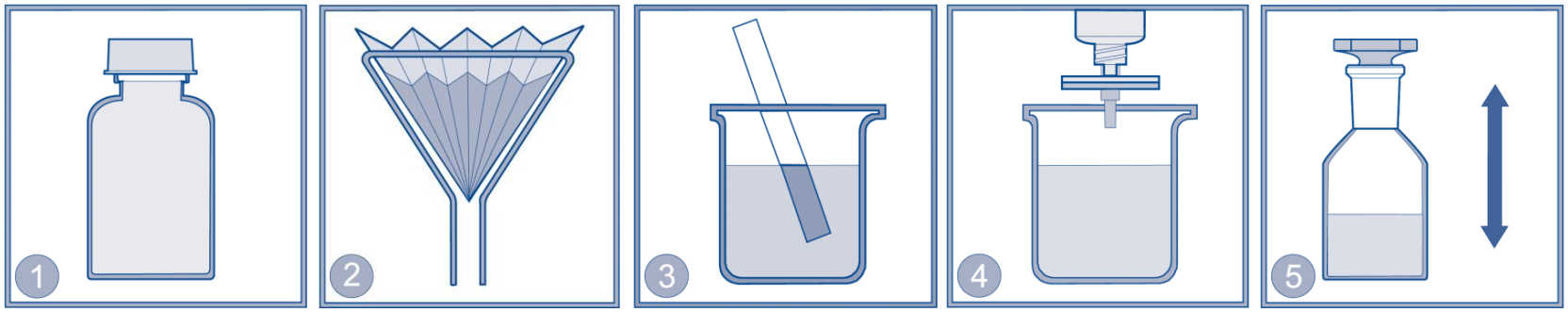


solution R1 | O_{C0} | feed | O_{C10}

calculate Oxygen Consumption in control as : $\Delta O_C = O_{C0} - O_{C10}$

Nitrification-inhibition tests

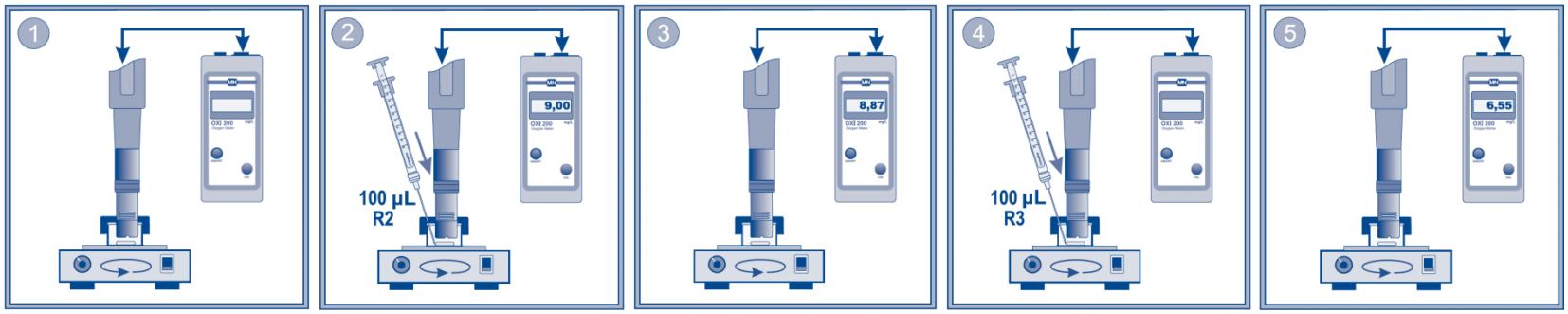
Sample preparation



reactivate *A-Tox*-Bacteria by shaking 4-6 time one day before performing test

Nitrification-inhibition tests

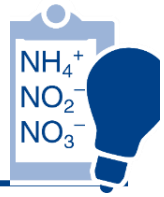
Sample



sample | O_{S0} | feed | O_{S10}

calculate Oxygen Consumption in sample as : $\Delta O_S = O_{S0} - O_{S10}$

Nitrification-inhibition tests

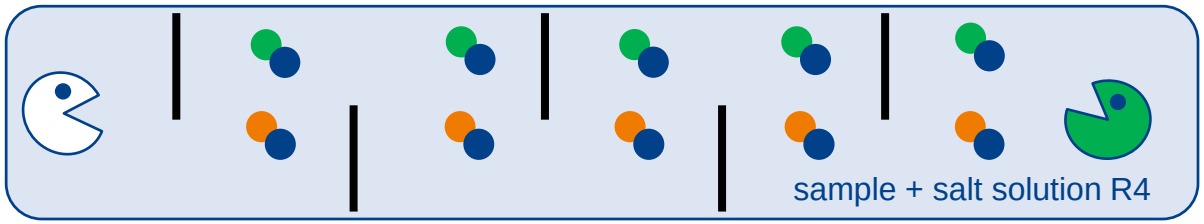


Calculate inhibition

- oxygen demand Control: ΔO_C
- oxygen demand Sample: ΔO_S
- inhibition [%] = $\frac{\Delta O_S - \Delta O_C}{\Delta O_C} * 100$

Nitrification-inhibition tests

Correct for other oxygen consuming processes



other processes

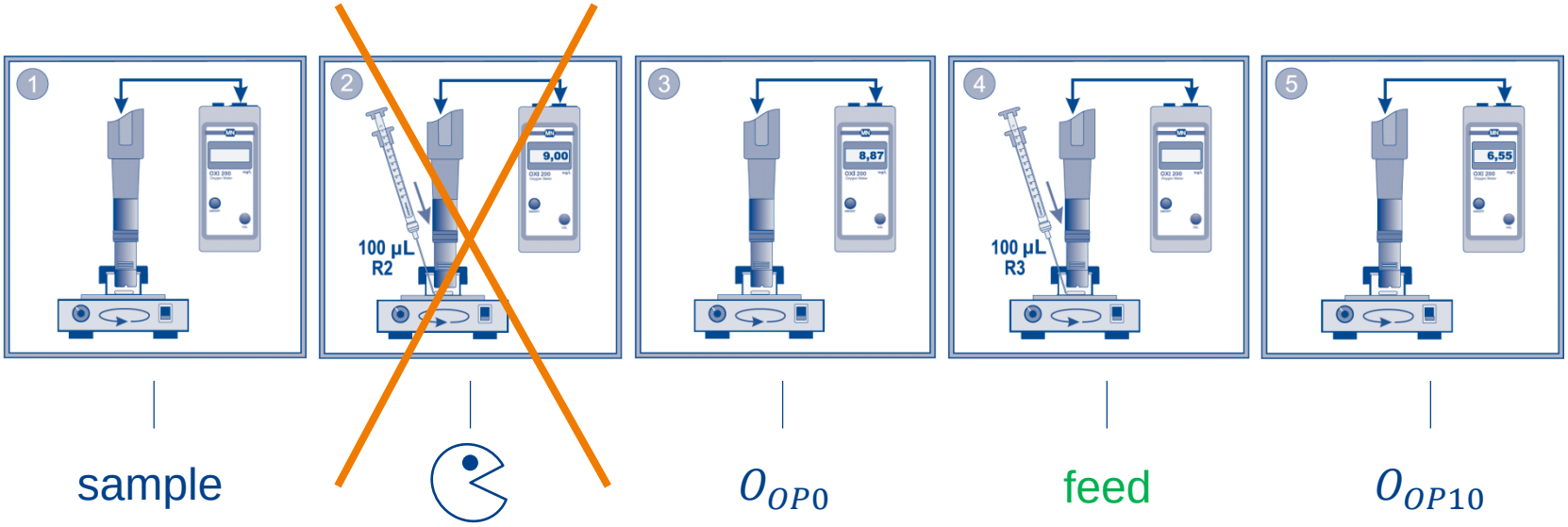
nitrification + other processes = **8 O_2**

other processes alone (ΔO_{OP}) = **5 O_2**

corrected consumption (ΔO_S) = **3 O_2**

Nitrification-inhibition tests

Sample



calculate consumption by other processes as: $\Delta O_{OP} = O_{OP0} - O_{OP10}$

Results

Nitrification-inhibition tests

ISO 9509 vs. BioFix® Tox Tests, IC_{50} values

Substance	ISO 9509	A-Tox	N-Tox
Pure Substances and Mixtures			
N-Allylthiourea	0,5	0,2	16
Cu-Sulfate	0,02 g/L	0,4 g/L	0,04 g/L
Palegal SF	0,1 ml/L	3 ml/l	0,15 ml/l
Dishwashing liquid	0,1 ml/l	1,6 ml/l	0,3 ml/L
Real Samples			
Metall industry	0,004 ml/l	0,005 ml/l	0,05 ml/L
Tannery	105 ml/l	>125 ml/l	75 ml/l
Food industry	2 ml/l	4 ml/l	77 ml/l

Tox-tests compare very well to ISO 9509

Nitrification-inhibition tests

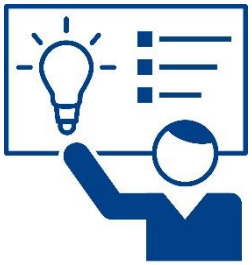
IC_{50} values for A-Tox / N-Tox

Classification	Reference	IC_{50}-Values	
		BioFix® A-Tox	BioFix® N-Tox
Nitrification inhibitors in the chemical industry	*N*-Allylthiourea	0,89 mg/L NATU	19,8 g/L NATU
	Thiourea	0,99 mg/L TU	48,8 g/L TU
	Potassium chlorate	185 g/L $KClO_3$	42,5 g/L $KClO_3$
Nitrification inhibitors in agriculture	„*N*-Serve“ / „Nitrapyrine“ 2-Chloro-6-(trichloromethyl)pyridine	72 mg/L *N*-Serve	80 mg/L *N*-Serve
	„Didin“ (Cyanoguanidine)	450 mg/L Cyanoguanidine	>100 g/L Cyanoguanidine
Chlorophenols	2-Chlorophenol	2,9 mg/L 2-Chlorophenol	71 mg/L 2-Chlorophenol
	3,5-Dichlorophenol	7,1 mg/L 3,5-DCP	6,3 mg/L 3,5-DCP
Heavy Metals	Cu^{2+} (as copper(II)sulfate x 5 H_20)	407 mg/L $CuSO_4$ x 5 H_2O 104 mg/L Cu^{2+}	52 mg/L $CuSO_4$ x 5 H_2O 13,2 mg/L Cu^{2+}
	Cd^{2+} (as cadmium chloride x H_2O)	246 mg/L $CdCl_2$ x H_20 137 mg/L Cd^{2+}	190 mg/L $CdCl_2$ x H_20 106 mg/L Cd^{2+}
	Zn^{2+} (as zink sulfate x 7 H_2O)	1,2 g/L $ZnSO_4$ x 7 H_2O 0,27 g/L Zn^{2+}	430 mg/L $ZnSO_4$ x 7 H_2O 98 mg/L Zn^{2+}

Nitrification-inhibition tests

IC_{50} values for A-Tox / N-Tox

Classification	Reference	IC_{50}-Values	
		BioFix® A-Tox	BioFix® N-Tox
Additives in textile industry / textile finishing	Palegal SF	3,8 mL/L Palegal SF	0,23 mL/L Palegal SF
	Glyezin A	15,3 mL/L Gleyzin A	223 mL/L Gleyzin A
Disinfectants, Detergents, Preservatives	Hydrogen peroxide (H_2O_2)	5,5 mg/L H_2O_2	4,7 mg/L H_2O_2
	Benzyldimethyl-dodecylammonia chloride	45,2 mg/L	76,2 mg/L
	4-Dodecylbenzenesulfonic acid	0,55 mL/L	0,84 mL/L
	Dimethyldioctadecylammonia chloride	18,6 mg/L	5,0 g/L
Solvents	DMSO	4,68%	(> 100%)
	Ethanol	0,74%	43,0%



Case studies

Case study 1

Indirect discharger in Germany

- Discharge of sludge waters
- Discharger needs to check with BioFix® A-Tox
- Inhibitions >20% → Discharge is not permitted
- Waste must be disposed at costs of 35.000 EUR (39.000 USD)
- 1 mg/L NATU is used regularly to check the status of the bacteria

Case study 2

Sewage plant in Dresden, Germany

- Analysis of waste waters from industrial indirect dischargers
- Food industry, galvanics, chemical industry
- Water of each discharger is analyzed every 4-6 weeks
- Determination of the concentration with non significant inhibition
- Inhibition of >50% is considered as toxic
- One discharger creates a stimulation through a high NH_4-load

Tips and Tricks

Tips and Tricks

When doing the test…

Be carefull to treat control and sample identically

Tips and Tricks

Recommended Oxygen Level

> 8 mg/L O_2

increase oxygen level by shaking of air flow through sample

Tips and Tricks

Ensure Minimum Oxygen Consumption of Control

> 1,0 mg/L O_2

- Depends on fitness of bacteria
- Initial Consumption when shipped:
  - *A-Tox*: > 3,0 mg/L O_2
  - *N-Tox*: > 1,5 mg/L O_2

Tips and Tricks

Take care of stirring speed

- Optimal 200 – 400 /min
- Use same speed for all tests

Tips and Tricks

Storage of bacteria

A-Tox

+ 2 to + 8 °C
+35 to +46 °F

N-Tox

-20 ± 2 °C
- 7 ± 0 °F

take care of different storage conditions for different parts of the kit

Tips and Tricks

Take of reagent temperature prior to use

- Bring all reagents to room temperature before use
- Shake salt solutions well before use

Tips and Tricks

Reactivate bacteria

Shake well before use to reactivate and homogenize

Tips and Tricks

Look at reagent color!

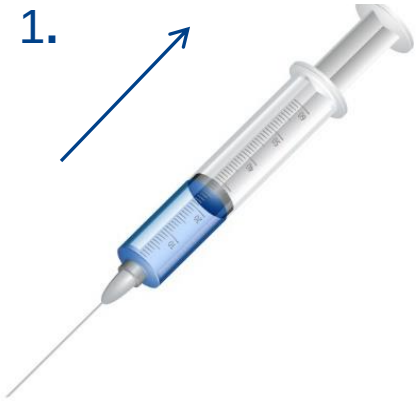
Activity correlates with color

- Red-brown: fresh bacteria
- Dark-brown - grey: getting older
  - Still working well
- Black: inactive / dead bacteria
  - Not usable anymore
  - Take care of storage

Tips and Tricks

Bacteria activity a probelm?

1.



2.

NO – as long as sample and control are done identical

Tips and Tricks

Remove air bubbles

- Take care when assembling
- Take care to carefully wet the adaptor

Summary

Summary

Comparison ISO 9509 – BioFix®

	DIN EN ISO 9509	BioFix®	
Preparation	Active sludge clean-up	Lyophylized bacteria	✓
Incubation time	4 h	12 min	✓
Solutions	Prepare	Ready to use	✓
Detection	NH_4^+ / NO_3^- (any method)	Oxygen probe	✓

BioFix® *A-Tox/N-Tox* – Sold in Europe for more than 15 years

MACHEREY-NAGEL

Thank you for your attention!

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