Hoe ontdek je “onmogelijke” micro-organismen ?

Mike Jetten
NIBI Workshop
12 Januari 2013

INTRODUCTION@MICROBIOLOGY
Table of contents

INTRODUCTION@MIKE-ROBIOLOGY

Year		Position	University
1987	1991	PhD in Microbiology	WUR, NL
1991	1994	Post doc on Molecular Microbiology	MIT, USA
1994	2000	Assistant Professor in Microbiology	TU Delft, NL
2000	now	Full Professor in Ecological Microbiology	RU Nijmegen, NL
2002	now	Extra ordinary professor in Environmental Microbiology	TU Delft, NL
		AWARDS	
2008	2013	**ERC ADVANCED GRANT**	
2010	2027	**KNAW**	
2012	2022	**SPINOZAPREMIE**	

INTRODUCTION@MICROBIOLOGY

HOE GROOT ZIJN MICROBEN?

WELK DEEL VAN DE BIOMASSA OP AARDE IS MICROBIEEL?

HOELANG ZIJN ER AL MICROBEN OP AARDE?

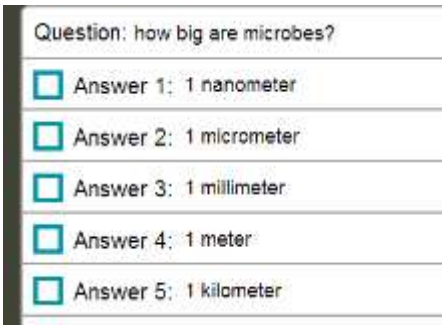
INTRODUCTION@MICROBIOLOGY

http://www.facebook.com/pages/Anammox/536394233052172

INTRODUCTION@MICROBIOLOGY

m.socrative.com student class # 286260

HOE GROOT ZIJN MICROBEN?



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socrative class # 286260
http://www.facebook.com/pages/Anammox/536394233052172

WELK DEEL VAN DE BIOMASSA OP AARDE IS MICROBIEEL?

INTRODUCTION@MICROBIOLOGY

socrative class # 286260

HOELANG ZIJN ER AL MICROBEN OP AARDE?

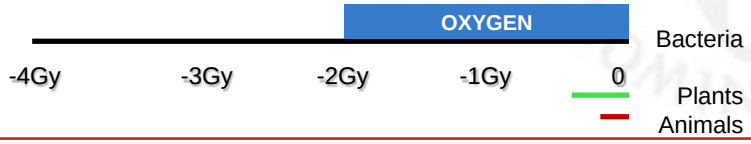
INTRODUCTION@MICROBIOLOGY
Microbial Planet

Small in size: **100 nm – 2 mm**

Large in numbers: **10^{30} microbial cells on Earth (50% of biomass)**

"The Earth is a **microbial** planet, on which macro-organisms are recent additions, highly interesting and extremely complex, but in the final analysis relatively unimportant in a global context."

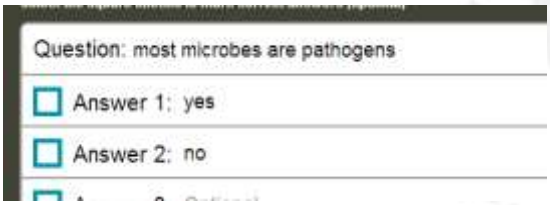
Wheelis et al. (1998) PNAS 95:11043-11046



INTRODUCTION@MICROBIOLOGY

STELLING DE MEESTE MICROBEN ZIJN PATHOGEEN

JUIST / ONJUIST



INTRODUCTION@MICROBIOLOGY
Microbial Planet

Many very useful microbes

- Wastewater treatment
- Oxygen production
- Nitrogen fixation
- Food and fermentation
- Drugs and Antibiotics
- Degradation of xenobiotics

Very few pathogens

INTRODUCTION@MICROBIOLOGY

DE MEESTE MICROBEN ZIJN AL BEKEND

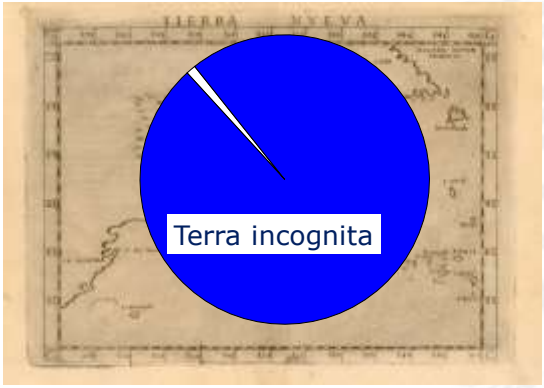
JUIST / ONJUIST

Select the square checks to mark correct answers (optional)

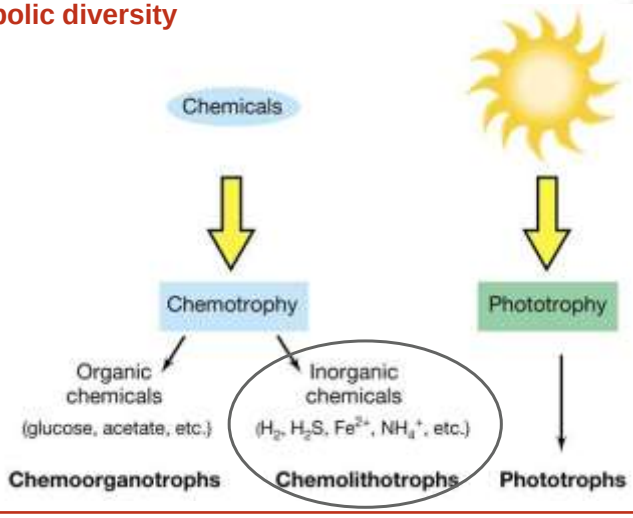
Question: most microbes are already known

- ☐ Answer 1: yes
- ☐ Answer 2: no

INTRODUCTION@MICROBIOLOGY
Unexplored biodiversity



INTRODUCTION@MICROBIOLOGY
Metabolic diversity



UNDISCOVERED CHEMOLITHOAUTOTROPHS

ELECTRON DONORS

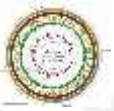
ELECTRON ACCEPTORS	H_2	CH_4	H_2S	NH_4^+	Fe^{2+}
O_2					
NO_3^-		???			
Fe^{3+}					
SO_4^{2-}					
CO_2					

After 4
They w
"imp

HOW TO DISCOVER THESE "IMPOSSIBLE" MICROBES?

- Survey of selected ecosystems
- Bring Best Samples to Lab
- Design optimal bioreactors
- Enrichment under optimal conditions
- Grow enough cells
- Use of the molecular toolbox

- Back to the ecosystem
- Application of the new microbes



WHAT DO YOU NEED TO DISCOVER THE “IMPOSSIBLE”?

- Adequate National and EU funding
- International Academic and Industrial Collaboration
- State of the Art Microbial Methods
- Skilled and Enthusiastic Team Members

STATE OF THE ART MICROBIOLOGY METHODS

Next Generation Sequencing
Structural Biology

Electron Microscopy
Physiology & Ecology

EXAMPLES of DISCOVERIES

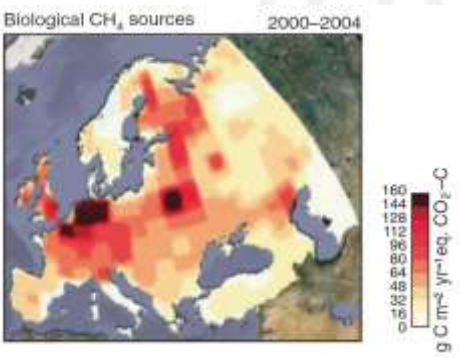
METHANE = GREENHOUSE GAS

[CH_4]$_{ATM}$ DOUBLED SINCE 1850

Do anaerobic methane oxidizing bacteria exist??

METHANE SOURCES

WETLANDS



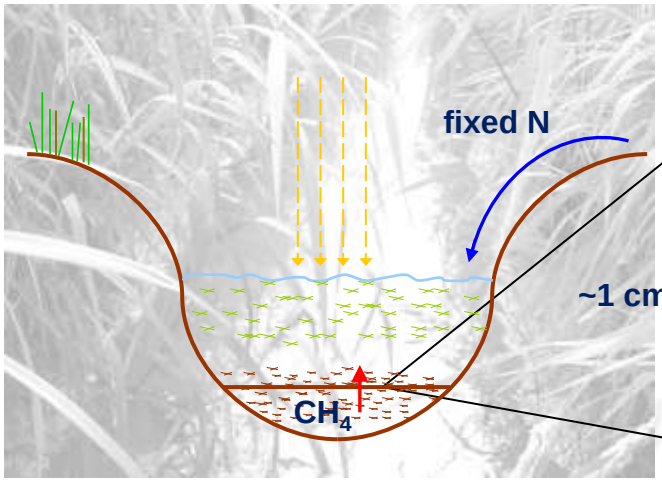
METHANE SINKS

METHANE OXIDIZING MICROBES

where to search?

$$3\ CH_4 + 8\ NO_2^- + 8\ H^+ \rightarrow 3\ CO_2 + 4\ N_2 + 10\ H_2O$$

where to search?



sampling sites nitrite dependent anaerobic methane oxidation

$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 4N_2 + 3CO_2 + 6H_2O$

Ooijpolder

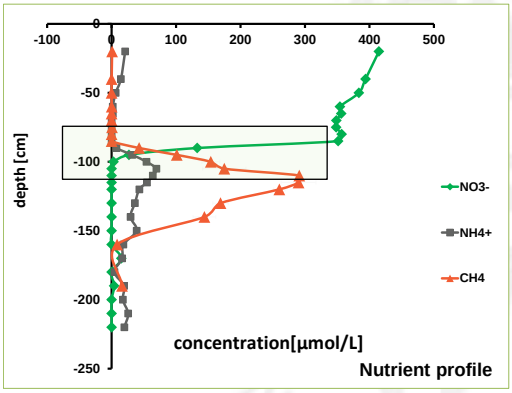
Brunsummerheide

Characteristics:

- HIGH NO_3^- due to agricultural run-off /ground water
- HIGH CH_4 production in the sediment

METHYLOMIRABILIS OXYFERA

Unique Twente Canal Bacteria; gradient profile Nitrate/Methane



METHYLOMIRABILIS OXYFERA
Unique Twente Canal Bacteria

- strictly anoxic conditions
- 25 C, pH 7,3
- Only CH_4 and CO_2
- Mineral medium with NO_3^- & NO_2^-
- NO_2^- consumption 1 mM day^{-1}

PATIENCE

Activity fully established only after 16 months

NOS Journaal

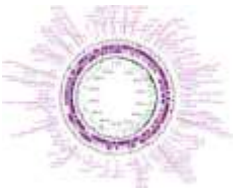

LETTERS

A microbial consortium couples anaerobic methane oxidation to denitrification

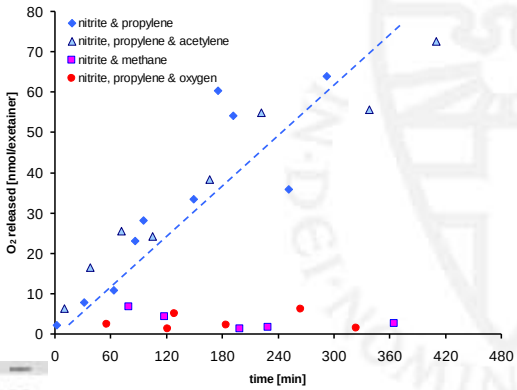
METHYLOMIRABILIS OXYFERA
Unique Twente Canal "miracle" Bacteria

Genome Sequencing

ion torrent



^{18}Oxygen experiments show: Oxygen Production




ARTICLES

Nitrite-driven anaerobic methane oxidation by oxygenic bacteria

Radboud University Nijmegen

METHYLOMIRABILIS OXYFERA
Unique Twente Canal Bacteria

Unknown enzyme

$$NO_2^- \rightarrow NO \rightarrow N_2$$

$$NO_2^- \rightarrow NO \rightarrow O_2$$

frontiers in MICROBIOLOGY

HYPOTHESIS AND THEORY ARTICLE

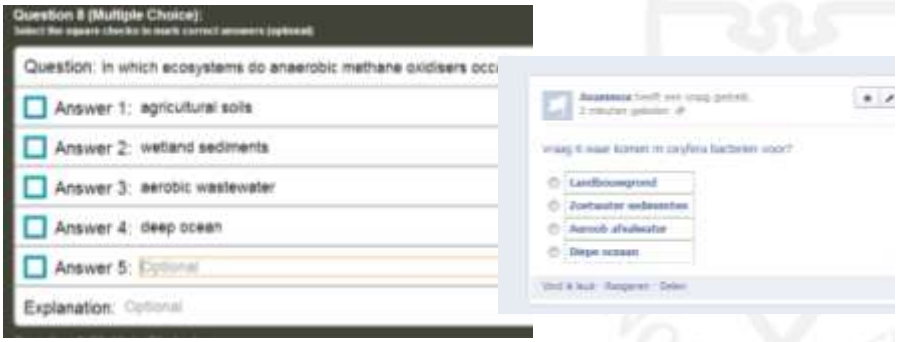
Bacterial oxygen production in the dark

Katharina F. Ettwig, Daan R. Speth, Joachim Reimann, Ming L. Wu, Mike S. M. Jetten and Jan T. Keltjens*

Department of Microbiology, Institute for Water and Wetland Research, Radboud University Nijmegen, Nijmegen, Netherlands

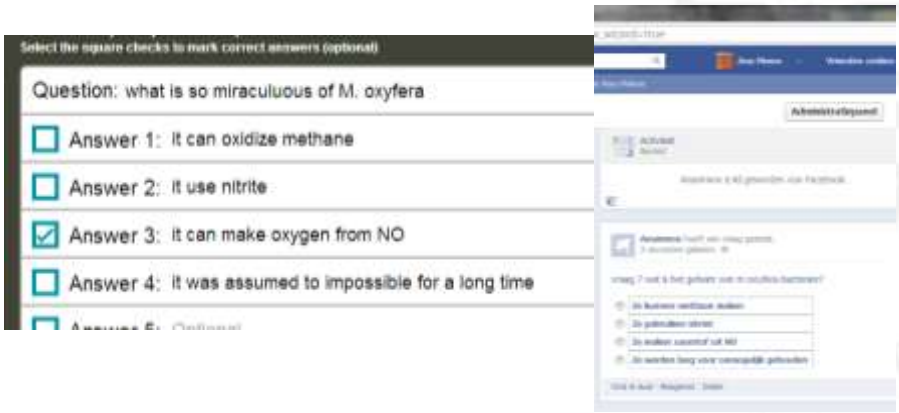
METHYLOMIRABILIS OXYFERA
Unique Twente Canal Bacteria

Waar komen *M oxyfera* bacterien voor?



METHYLOMIRABILIS OXYFERA
Unique Twente Canal Bacteria

Wat is het geheim van *M oxyfera* bacterien?



EXAMPLES of DISCOVERIES

AMMONIUM = WATER POLLUTANT

[NH_4] TOO HIGH

SOURCES

MANURE/FERTILIZERS
SEWER SYSTEMS

SINKS

AMMONIUM OXIDIZING MICROBES

ERC Anammox project : Unique prokaryotes

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ $\Delta G'_0 = -358$ kJ/mol

ONTDEKKING

CELL BIOLOGIE

RAKETBRANDSTOF

ROL IN DE MONDIALE N KRINGLOOP

TOEPASSING IN AFVALWATERZUIVERING

Engelbert Broda
1910-1983

Calculations in N cycle

Zeitschrift für Allg. Mikrobiologie | 17 | 9 | 1977 | 491—498

(Institut für Physikalische Chemie, Universität Wien)

Two kinds of lithotrophs missing in nature

E. Broda

(Eingegangen am 14. 8. 1976)

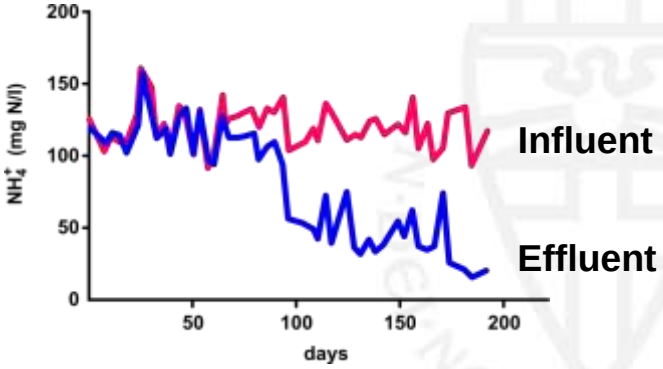
Two groups of lithotrophic bacteria, the existence of which may be expected on evolutionary and thermodynamical grounds, have not yet been described: (A) photosynthetic, anaerobic, ammonia bacteria, analogous to coloured sulphur bacteria, and (B) chemosynthetic bacteria that oxidize ammonia to nitrogen with O_2 or nitrate as oxidant.

The versatility of the prokaryotes in their energy metabolism has long astonished microbiologists. The bacteria have developed processes, i.e., enzymes, for the utilization of a wide range indeed of exergonic reactions. Attention is now drawn to further processes in energy metabolism which on the basis of considerations on the evolution of the bioenergetic processes (Broda 1975a) may be expected to have existed, or to exist, but which have not yet been found. Two kinds of "lithotrophic" bacteria with such mechanisms will now be predicted. Lithotrophs are bacteria that use inorganic reductants in their energy metabolism. (Pringsheim and Suzuki 1966): all autotrophs must be lithotrophs, though the reverse need not be true. The two bacteria here predicted would generate dinitrogen (N_2).

The nitrifying bacteria make adenosine triphosphate, ATP, through oxidative phosphorylation coupled to the aerobic oxidation of ammonia, a highly energonic process. Thus, in nitrification *Nitrosomonas* produces nitrite, and *Nitrobacter* makes nitrate. The redox reactions are:

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ $\Delta G'_0 = -358$ kJ/mol

Anaerobic pilot plant, TU Delft, the Netherlands



Mulder , van de Graaf et al FEMS Ecology 1995

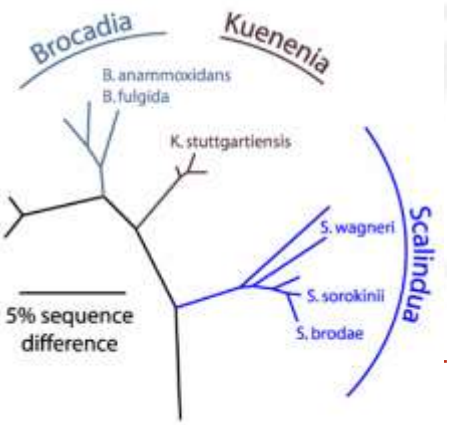
1995 Anaerobic pilot plant

1997 VERRIJKINGSCULTUUR

1999 STAMBOOM

Anammoxoglobus propionicus

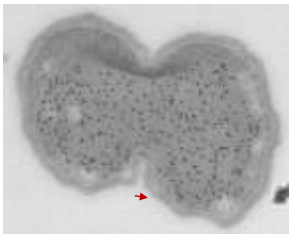
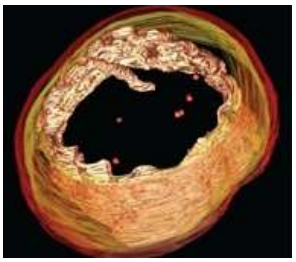
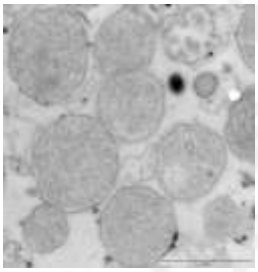
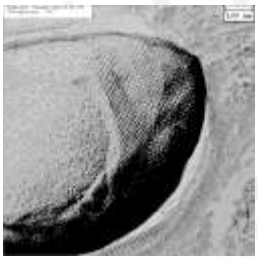
Jettenia asiatica



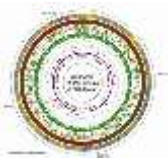




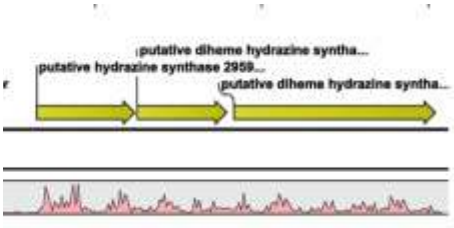
Anammox Cell Biology

ANAMMOX
Unique Properties

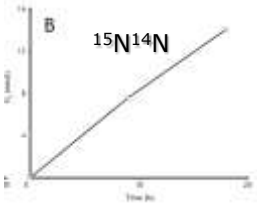


Genome sequencing



Protein purification

ROCKET FUEL



Hydrazine and N_2 production

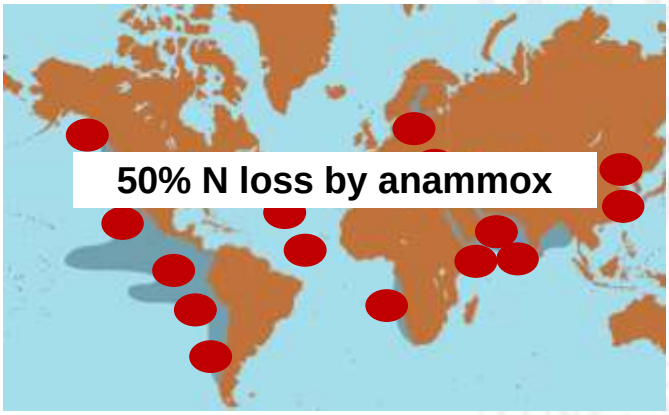
LETTER

Molecular mechanism of anaerobic ammonium oxidation

ANAMMOX
Global Significance

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Ocean expeditions



Max-Planck-Institut für Marine Mikrobiologie

NIOZ

Radboud University Nijmegen

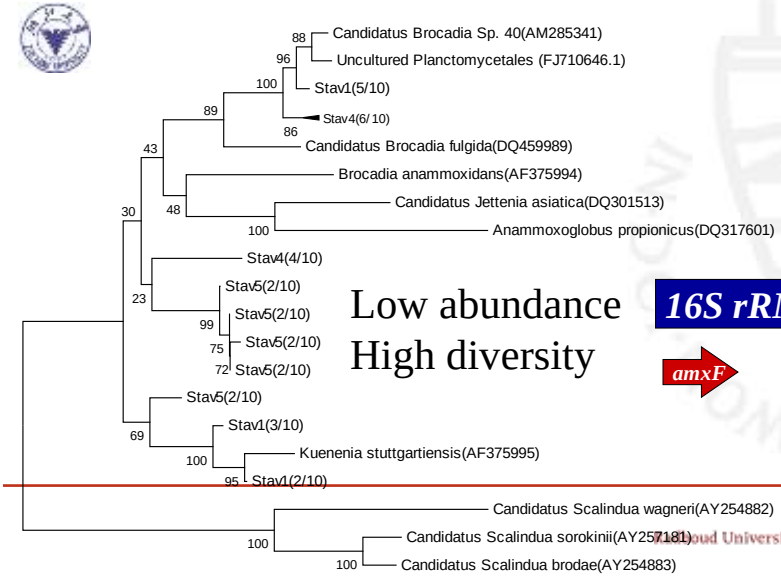
Anammox in wetlands

Staverden sampling site
Aquatic Ecology / B-ware

Number	Depth (m)	pH	Nitrate (µmol^{-1})	Ammonium (µmol^{-1})
Stav1	0.1	6.0	12.9	37
Stav2	2	5.6	330	2.5
Stav3	1		1.6	8.1
Stav4	2	6.8	603	43.4
Stav5	1			

Radboud University Nijmegen

Anammox 16S rRNA phylogenetic tree of different original Staverden wetland samples



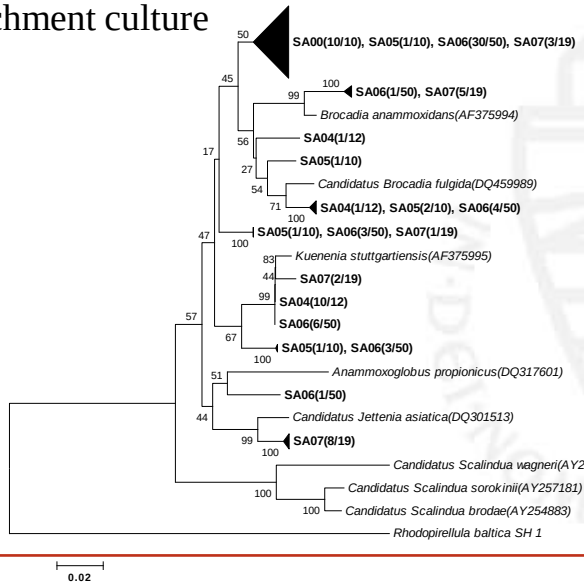
Low abundance
High diversity

Anammox in wetlands

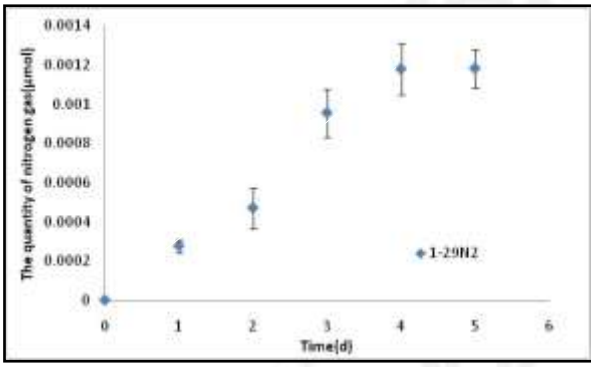
Anammox in wetlands

Fluorescence in situ hybridization with oligonucleotide AMX368

Anammox 16S rRNA phylogenetic tree of Wetland enrichment culture



Anammox Activity



7 nmol $^{29}N_2$ g dry soil^{-1} d^{-1}

$^{15}NH_4^+ + NO_2^-$

$^{15}NO_2^- + NH_4^+$ 12 nmol $^{29}N_2$ g dry soil^{-1} d^{-1} denitrification + amx

6 nmol $^{30}N_2$ g dry soil^{-1} d^{-1} denitrification

Radboud University Nijmegen

Applied and Environmental Microbiology, Feb. 2011, p. 000 Vol. 77, No. 3
0099-2240/11/$12.00 doi:10.1128/AEM.02402-10

New Anaerobic, Ammonium-Oxidizing Community Enriched from Peat Soil[▽]

Bao-Lan Hu,[1,2] Darci Rush,[3] Erwin van der Biezen,[2] Ping Zheng,[1] Mark van Mullekom,[4] Stefan Schouten,[3] Jaap S. Sinninghe Damsté,[3] Alfons J. P. Smolders,[4,5] Mike S. M. Jetten,[2,6] and Boran Kartal[2]*

Department of Environmental Engineering, Zhejiang University, Hangzhou, 310029 China,[1] and Department of Microbiology[2] and Department of Aquatic Ecology and Environmental Biology,[4] IWWR and B-WARE Research Centre,[5] Radboud University Nijmegen, 6525 AJ Nijmegen, Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, 1790 AB Den Burg, Texel,[3] and Delft University of Technology, Department of Biotechnology, 2628 BC Delft,[6] Netherlands

Received 11 October 2010/Accepted 30 November 2010

nature geoscience LETTERS
PUBLISHED ONLINE: 6 JANUARY 2013 | DOI: 10.1038/NGEO1685

Hotspots of anaerobic ammonium oxidation at land–freshwater interfaces

Guibing Zhu[1]*, Shanyun Wang[1,2], Weidong Wang[1], Yu Wang[1], Leiliu Zhou[1], Bo Jiang[1], Huub J. M. Op den Camp[3], Nils Risgaard-Petersen[4], Lorenz Schwark[5], Yongzhen Peng[2], Mariet M. Hefting[6], Mike S. M. Jetten[3] and Chengqing Yin[1]

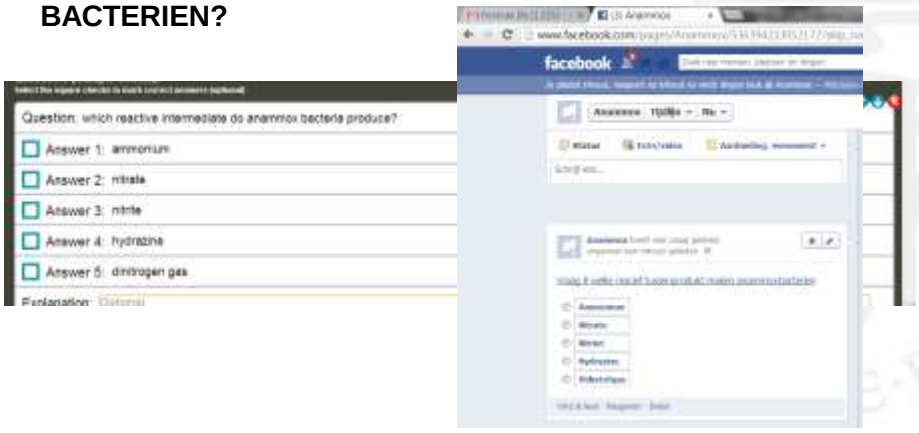
Conclusions

anammox is also important in wetlands
High diversity
Low abundance
Contribution 12-38%

Special thanks to Baolan Hu (Zhejing University) and Guibing Zhu Chinese (Academy of Sciences)

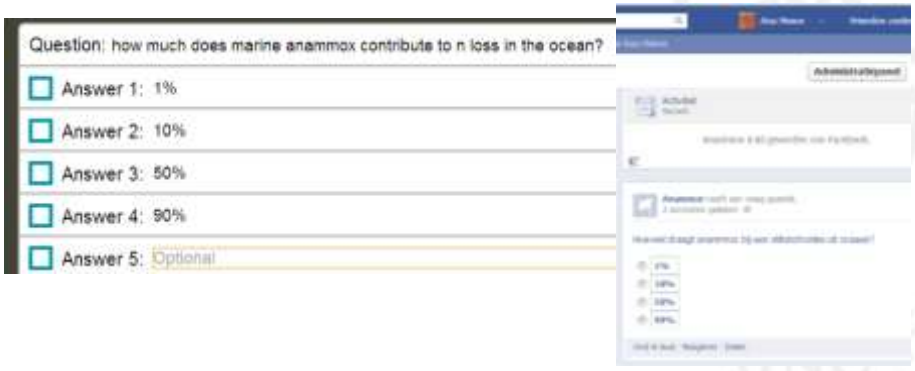
ANAMMOX
Unique Properties

WELK REACTIEF TUSSENPRODUKT MAKEN ANAMMOX BACTERIEN?



ANAMMOX
Global Significance

HOEVEEL DRAAGT ANAMMOX BIJ AAN DE MONDIALE STIKSTOFCYCLUS?



ANAMMOX APPLICATION
Added Value

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$


NRC HANDELSBLAD

Tomatensoep zuivert water

- **Less oxygen demand**
- **No COD use**
- **Less biomass production**
- **No emission of CO_2 and N_2O**

stowa stw PAQUES

ANAMMOX APPLICATION
Added Value

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

PAQUES

Press release

Chinese food company chooses for sustainability and cost savings

Paques builds world's largest ANAMMOX® wastewater treatment plant for ammonium disposal in China

Paques Environmental Technology Shanghai, a sister company of Paques bv in Balk, the Netherlands, has reached an agreement for the design and

Meihua China 11 ton N/d
Rendac NL 6 ton N/d

Sewage Treatment with Anammox

TU Delft Radboud University Nijmegen

Anammox and *M. oxyfera*

M. oxyfera en anammox bacterien gebruiken allebei nitriet en leven onder dezelfde milieuomstandigheden

Is het mogelijk om stabiele co cultures te verkrijgen en toe te passen?

erc

Universidad del Valle

UNIVERSIDAD DE LOS ANDES VENEZUELA

Radboud University Nijmegen

Anammox and *M. oxyfera*

Use a stable *M. oxyfera* reactor
- check if anammox 16s rRNA genes are still present
- start adding ammonium
- Monitor
  - Cells by FISH
  - count 16S rRNA
  - measure activity

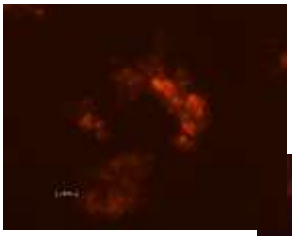
Universidad del Valle

UNIVERSIDAD DE LOS ANDES VENEZUELA

Luesken, sanchez et al unpublished

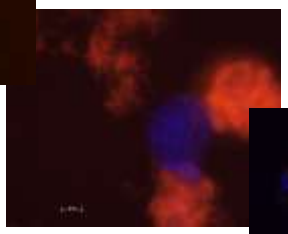
erc

Anammox and *M. oxyfera*

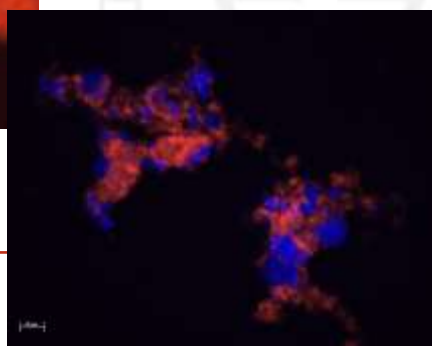


Day = 0
Moxy193 = red
Amx368= blue

Day = 61
Moxy193 = red
Amx368= blue



Day = 106
Moxy193 = red
Amx368= blue



Simultaneous Nitrite-Dependent Anaerobic Methane and Ammonium Oxidation Processes

FOX NEWS.com

“Impossible” bacteria could save the world

Wednesday, November 14, 2007

By Jeanna Bryner

LIVE SCIENCE

THANK YOU!

erc

NWO

Radboud University Nijmegen