

Hoe ontdek je “onmogelijke” micro-organismen ?



Mike Jetten
NIBI Workshop
12 Januari 2013



Radboud University Nijmegen



INTRODUCTION@MICROBIOLOGY

Table of contents

WAT IS MICROBIOLOGIE?

HOE ONTDEK JE “ONMOGELIJKE” BACTERIEN

VOORBEELDEN




METHAAN/AMMONIUM ZONDER ZUURSTOF

TOEGEVOEGDE WAARDE

Radboud University Nijmegen



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>



Radboud University Nijmegen 

INTRODUCTION@MICROBIOLOGY

m.socrative.com student class # 286260

HOE GROOT ZIJN MICROBEN?

Question: how big are microbes?

- Answer 1: 1 nanometer
- Answer 2: 1 micrometer
- Answer 3: 1 millimeter
- Answer 4: 1 meter
- Answer 5: 1 kilometer

A screenshot of a Socrative quiz interface. The question is "vragen voor nibi 12 januari hoe groot zijn microben?". The answer options are: "1 nanometer", "1 mikrometer", "1 millimeter", "1 centimeter", "1 meter", and "1 kilometer". There is a "Vraag aan opde toe..." button at the bottom.

Radboud University Nijmegen 

INTRODUCTION@MICROBIOLOGY

socrative class # 286260

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

WELK DEEL VAN DE BIOMASSA OP AARDE IS MICROBIEEL?

The image shows two screenshots related to a poll. On the left is a Socrative poll interface with the question 'Question: which part of the biomass on earth is microbial?' and five answer options: Answer 1: 1%, Answer 2: 10%, Answer 3: 50%, Answer 4: 99%, and Answer 5: Optional. On the right is a screenshot of a Facebook poll on the page 'Anammox' for the same question. The Facebook poll shows the same five options with radio buttons next to them, and a 'Polling' button at the bottom.

INTRODUCTION@MICROBIOLOGY

socrative class # 286260

HOELANG ZIJN ER AL MICROBEN OP AARDE?

The image shows two screenshots related to a poll. On the left is a Socrative poll interface with the question 'Question: How long ago did the first microbes appear on earth?' and five answer options: Answer 1: 4 Giga years ago, Answer 2: 2 Gy ago, Answer 3: 1 Gy ago, Answer 4: 100 My, and Answer 5: 1000 years ago. The 'Explanation' field is set to 'Optional'. On the right is a screenshot of a Facebook poll on the page 'Anammox' for the same question. The Facebook poll shows the same five options with radio buttons next to them, and a 'Polling' button at the bottom.

INTRODUCTION@MICROBIOLOGY

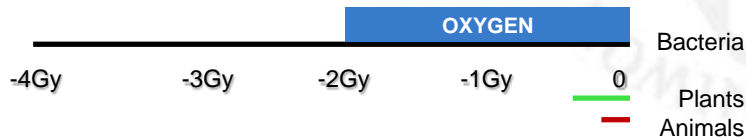
Microbial Planet

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.”

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



INTRODUCTION@MICROBIOLOGY

STELLING DE MEESTE MICROBEN ZIJN PATHOGEEN

JUIST / ONJUIST

Question: most microbes are pathogens

Answer 1: yes

Answer 2: no



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

Question 6 (Multiple Choice):

Select the square checks to mark correct answers (optional)

Question: most microbes are already known

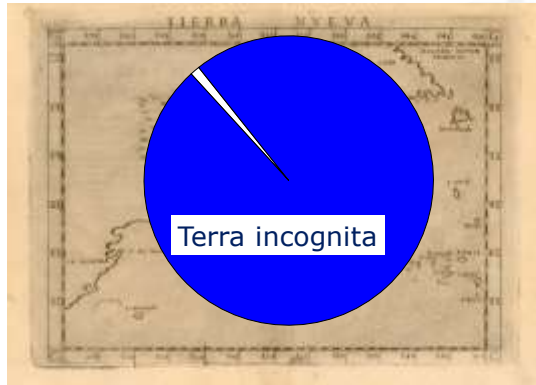
Answer 1: yes

Answer 2: no



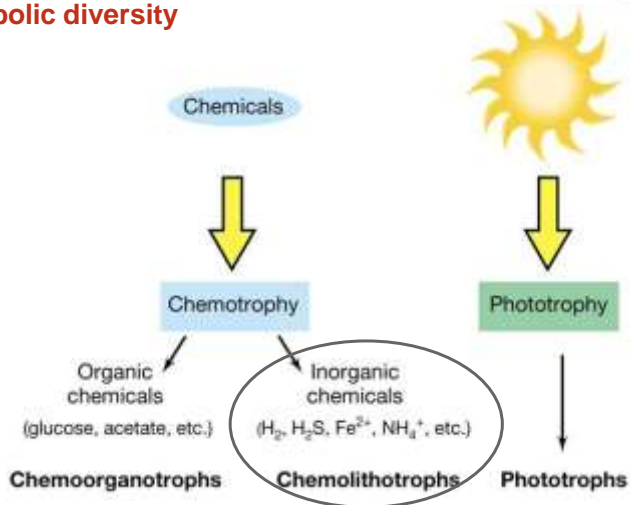
INTRODUCTION@MICROBIOLOGY

Unexplored biodiversity



INTRODUCTION@MICROBIOLOGY



Metabolic diversity



UNDISCOVERED CHEMOLITHOAUTOTROPHS

ELECTRON ACCEPTORS	ELECTRON DONORS				
	H ₂	CH ₄	H ₂ S	NH ₄ ⁺	Fe ²⁺
O ₂					
NO ₃ ⁻		???			
Fe ³⁺					
SO ₄ ²⁻					
CO ₂					

After 4
They v
"imp

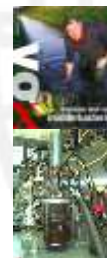


Radboud University Nijmegen

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



Radboud University Nijmegen



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

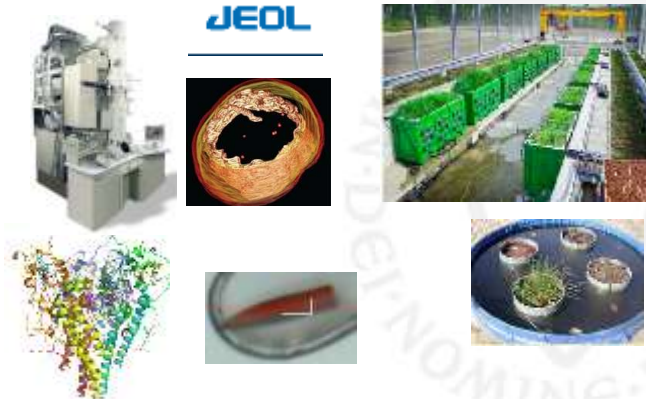


STATE OF THE ART MICROBIOLOGY METHODS

Next Generation Sequencing
Structural Biology



Electron Microscopy
Physiology & Ecology



EXAMPLES of DISCOVERIES

METHANE = GREENHOUSE GAS

[CH₄]_{ATM} DOUBLED SINCE 1850

Do anaerobic methane oxidizing bacteria exist??



METHANE SOURCES WETLANDS

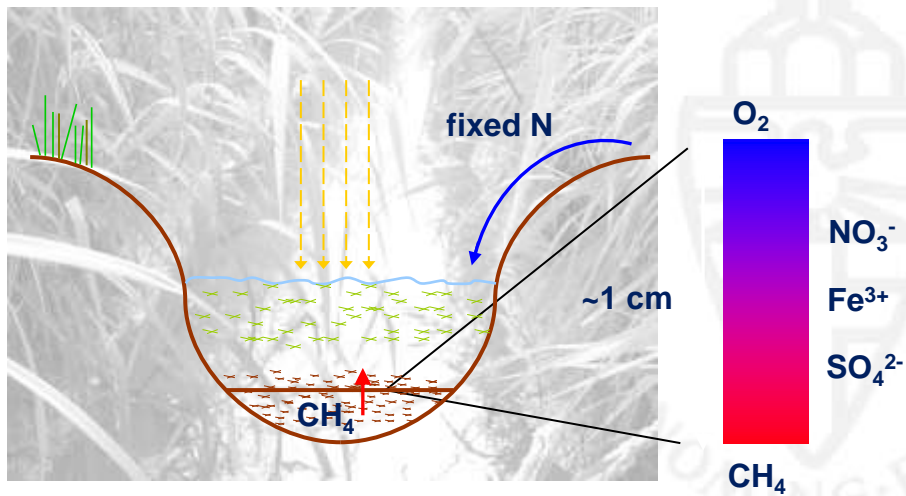


METHANE SINKS METHANE OXIDIZING MICROBES

where to search?



where to search?





sampling sites nitrite dependent anaerobic methane oxidation



Twentekanaal



Ooijpolder



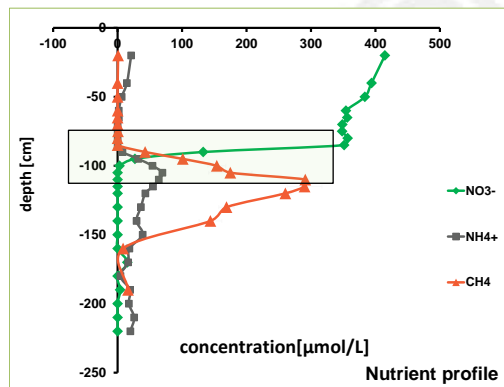
Brunsummerheide

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 CH₄ and CO₂
- Mineral medium with NO₃⁻ & NO₂⁻
- NO₂⁻ consumption 1 mM day⁻¹

PATIENCE

Activity fully established only after 16 months



LETTERS

A microbial consortium couples anaerobic methane oxidation to denitrification

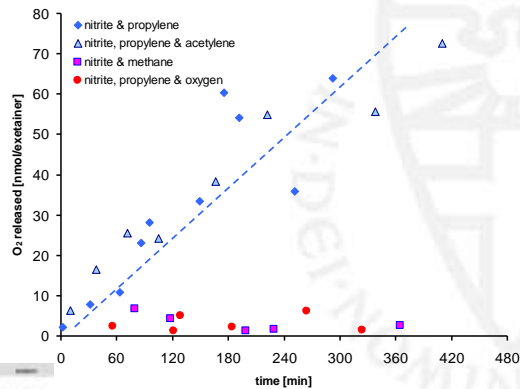
Kathrin A. Rapphuberung¹, Arjan P. de Zeeuw¹, Estelle T. van de Paschelen¹, Marco J. P. van den Broek¹, Sathish T. Eswari¹, M. Yusef C. Rijssen¹, Willem S. de Boer¹, Bas S. de Boer¹, Mark J. M. de Boer¹, M. S. M. de Boer¹ & M. S. M. de Boer¹

METHYLOMIRABILIS OXYFERA Unique Twente Canal “miracle” Bacteria

Genome Sequencing



¹⁸Oxygen experiments show: Oxygen Production



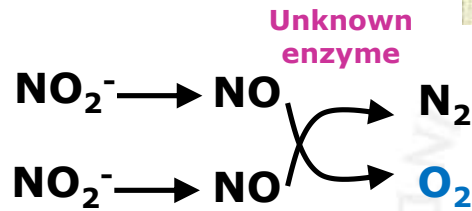
ARTICLES

Nitrite-driven anaerobic methane oxidation by oxygenic bacteria

Kathrin A. Rapphuberung¹, Arjan P. de Zeeuw¹, Estelle T. van de Paschelen¹, Marco J. P. van den Broek¹, Sathish T. Eswari¹, M. Yusef C. Rijssen¹, Willem S. de Boer¹, Bas S. de Boer¹, Mark J. M. de Boer¹, M. S. M. de Boer¹ & M. S. M. de Boer¹



METHYLOMIRABILIS OXYFERA Unique Twente Canal Bacteria



Frontiers in
MICROBIOLOGY

HYPOTHESIS AND THEORY ARTICLE
published: 07 August 2012
doi: 10.3389/fmicb.2012.00273

Bacterial oxygen production in the dark

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

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

Radboud University Nijmegen



METHYLOMIRABILIS OXYFERA Unique Twente Canal Bacteria

Waar komen *M oxyfera* bacterien voor?

Question 8 (Multiple Choice):
Select the square checks in each correct answers (optional)

Question: In which ecosystems do anaerobic methane oxidisers occur?

- Answer 1: agricultural soils
- Answer 2: wetland sediments
- Answer 3: aerobic wastewater
- Answer 4: deep ocean
- Answer 5: Optional

Explanation: Optional

Answered: 100% van vraag getikt.
2 minuten geleden

Vraag 6 waar komen *M oxyfera* bacterien voor?

- Landbouwgrond
- Zuiverwater sedimenten
- Aerobisch afvalwater
- Diepe oceanen

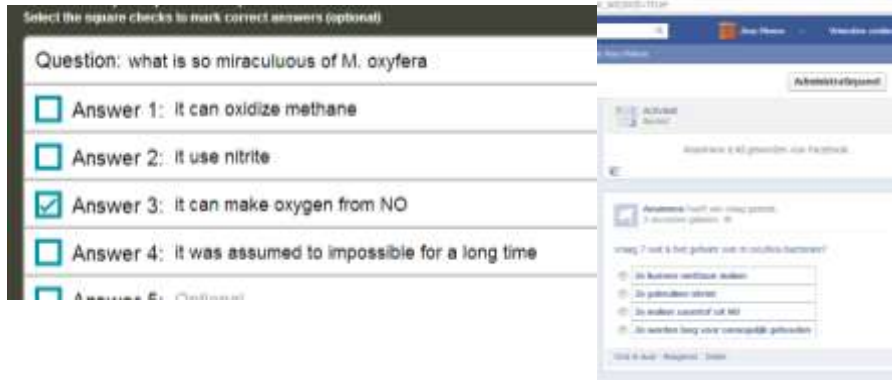
Vind ik leuk · Reageren · Delen

Radboud University Nijmegen



METHYLOMIRABILIS OXYFERA Unique Twente Canal Bacteria

Wat is het geheim van *M oxyfera* bacterien?



The image shows a quiz interface with a question and five multiple-choice answers. The question is: "Question: what is so miraculoous of M. oxyfera". The answers are: "Answer 1: It can oxidize methane", "Answer 2: It use nitrite", "Answer 3: It can make oxygen from NO", "Answer 4: It was assumed to impossible for a long time", and "Answer 5: O2". Answer 3 is selected with a checkmark. To the right, a partial view of a forum post is visible, showing a question about "MethyloMirabilis oxyfera" and several replies.

Radboud University Nijmegen 

EXAMPLES of DISCOVERIES

AMMONIUM = WATER POLLUTANT
[NH₄] TOO HIGH

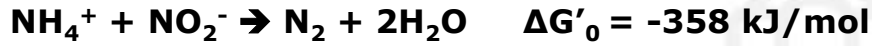
SOURCES

MANURE/FERTILIZERS
SEWER SYSTEMS

SINKS

AMMONIUM OXIDIZING MICROBES

ERC Anammox project : Unique prokaryotes



ONTDEKKING CELL BIOLOGIE RAKETBRANDSTOF ROL IN DE MONDIALE N KRINGLOOP TOEPASSING IN AFVALWATERZUIVERING

European Research Council



Engelbert Broda
1910-1983



Calculations in N cycle

Zeitschrift für Allgemeine Mikrobiologie	47	9	1971	491-503
--	----	---	------	---------

Institut für Experimentelle Chemie,
Universität Wien

Two kinds of lithotrophy missing in nature

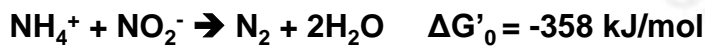
E. BRODA

(Biographien aus 11. 5. 1976)

Two groups of lithotrophic bacteria, the existence of which may be expected on energetic and thermodynamic grounds, have not yet been detected: (A) photolithotrophic, anaerobic, anoxygenic bacteria, analogous to cultured sulphur bacteria, and (B) chemolithotrophic bacteria that oxidize ammonia to nitrogen with O_2 or nitrate as oxidant.

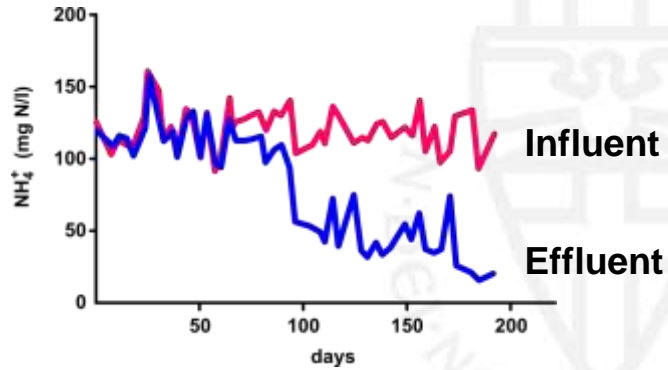
The versatility of the prokaryotes in their energy metabolism has long astonished microbiologists. The bacteria have developed processes, i.e., enzymes, for the utilization of a wide range instead of catabolic reactions. Attention is now drawn to further processes in energy metabolism which on the basis of considerations on the evolution of the biotrophic processes (Broda 1973a) 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. Lithotrophic are bacteria that use inorganic substrates in their energy metabolism (Frothingham and Sumner 1960); all autotrophs must be lithotrophic, though the reverse need not be true. The two bacterial lines 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 energetic process. Thus, in nitrification *Nitrosomonas* produces nitrite, and *Nitrobacter* makes nitrate. The redox reactions are:





Anaerobic pilot plant, TU Delft, the Netherlands



Mulder, van de Graaf et al FEMS Ecology 1995



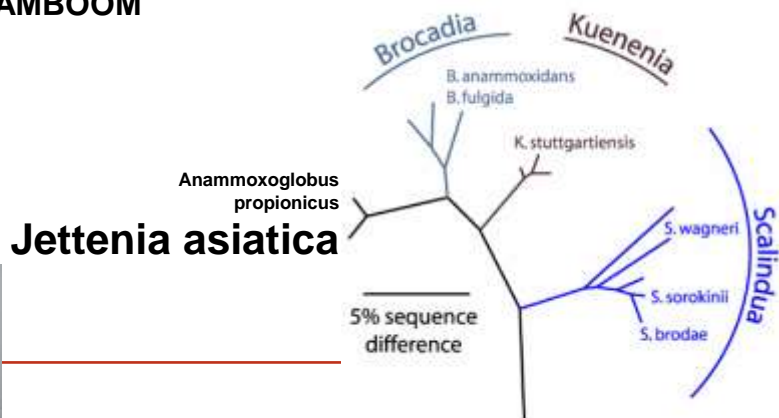
1995 Anaerobic pilot plant



1997 VERRIJKINGSCULTUUR



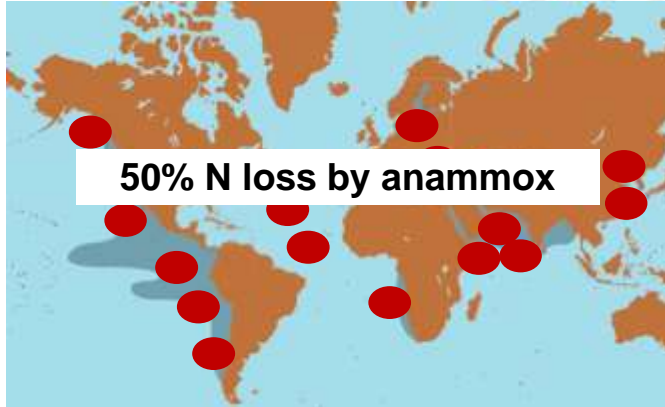
1999 STAMBOOM



ANAMMOX
Global Significance



Ocean expeditions



Anammox in wetlands

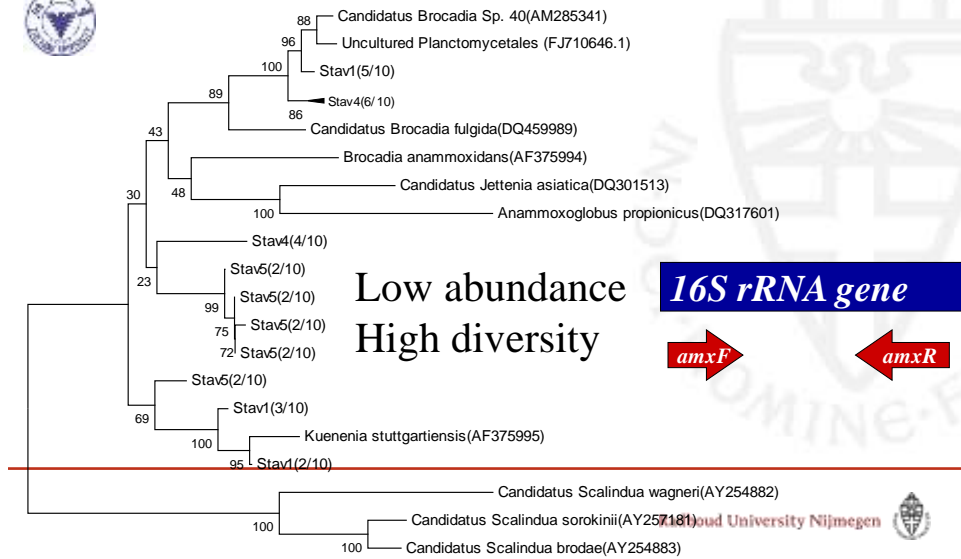
Staverden sampling site
Aquatic Ecology / B-ware

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			





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



Anammox in wetlands



erry

Nijmegen



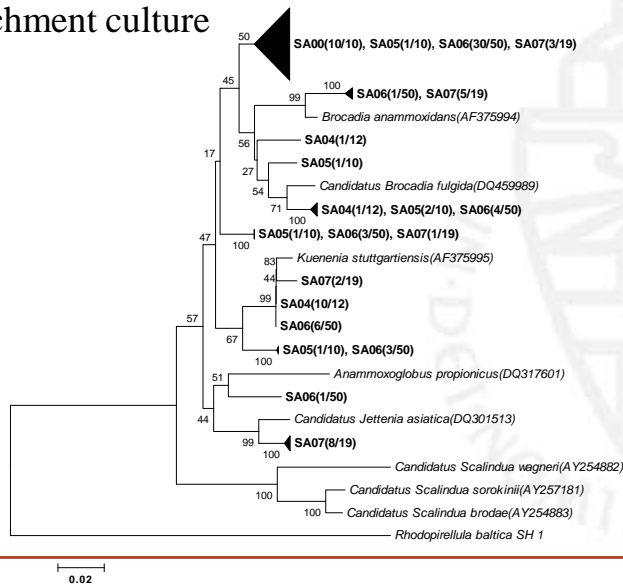
Anammox in wetlands



Fluorescence in situ hybridization with oligonucleotide AMX368



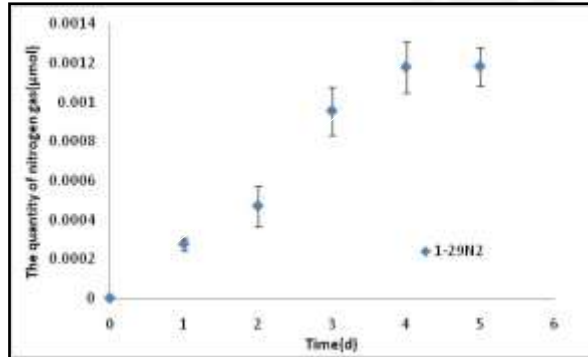
Anammox 16S rRNA phylogenetic tree of Wetland enrichment culture





Anammox Activity

7 nmol $^{29}\text{N}_2$ g dry soil $^{-1}$ d $^{-1}$
 $^{15}\text{NH}_4^+ + \text{NO}_2^-$



$^{15}\text{NO}_2^- + \text{NH}_4^+$ 12 nmol $^{29}\text{N}_2$ g dry soil $^{-1}$ d $^{-1}$ denitrification + amx
 6 nmol $^{30}\text{N}_2$ g dry soil $^{-1}$ d $^{-1}$ denitrification

Radboud University Nijmegen



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2011, p. 900
 0099-2240/11/\$12.00 doi:10.1128/AEM.02462-10
 Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Vol. 77, No. 2

New Anaerobic, Ammonium-Oxidizing Community Enriched from Peat Soil^{2,3}

Bao-Lan Hu,^{1,2} Danci Rush,³ Erwin van der Biezen,² Ping Zheng,¹ Mark van Mullekom,⁴
 Stefan Schouten,³ Jaap S. Sinninghe Damsté,¹ 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,³ IWR, and B-WARE, Research Centre,⁴ Radboud University Nijmegen, 6525 AJ Nijmegen, Royal Netherlands Institute for Sea Research,⁵ Department of Marine Organic Biogeochemistry, 1790 AB Den Burg, Texel,⁵ 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 2011 | DOI: 10.1038/ngeo1488

Hotspots of anaerobic ammonium oxidation at land-freshwater interfaces

Guibing Zhu^{1*}, Shanyun Wang^{1,2}, Weidong Wang¹, Yu Wang¹, Leiliu Zhou¹, Bo Jiang¹,
 Huub J. M. Op den Camp³, Nils Risgaard-Petersen⁴, Lorenz Schwark⁵, Yongzhen Peng²,
 Mariet M. Heffling⁶, Mike S. M. Jetten² and Chengqing Yin¹



European Network of Excellence



Conclusions

anammox is also important in wetlands

High diversity

Low abundance

Contribution 12-38%



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

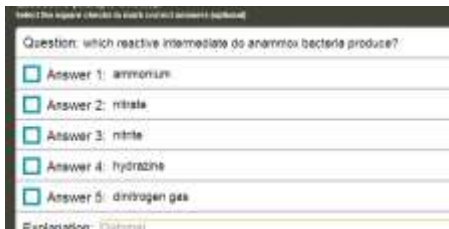


European Network Co-vent



ANAMMOX Unique Properties

WELK REACTIEF TUSSENPRODUKT MAKEN ANAMMOX BACTERIEN?



ANAMMOX Global Significance

HOEVEEL DRAAGT ANAMMOX BIJ AAN DE MONDIALE STIKSTOF CYCLUS?

Question: how much does marine anammox contribute to n loss in the ocean?

- Answer 1: 1%
- Answer 2: 10%
- Answer 3: 50%
- Answer 4: 90%
- Answer 5: Optional

Results: 100% correct, 1 incorrect answer. The correct answer is 90%.

Radboud University Nijmegen 

ANAMMOX APPLICATION Added Value



- Less oxygen demand
- No COD use
- Less biomass production
- No emission of CO₂ and N₂O



INNOVATIEF
Sewage Treatment with Anammox
B. Kartal, J. A. Klok, M. C. M. van Loosdrecht

Wastewater treatment including high-rate processes presents a great potential to become energy-neutral or even energy-producing.

 **TU Delft** Radboud University Nijmegen



ANAMMOX APPLICATION
Added Value



Date: Balk, March 12th 2008
To: External staff

Paques by
T. H. Overmaat 24
Postbus 912
6500 AH, Nijmegen
t. 0514 - 60 85 00
f. 0514 - 60 35 42
a. info@paques.nl
i. www.paques.nl



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 by in Balk, the Netherlands, has reached an agreement for the design and

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

INNOVATION

Sewage Treatment with Anammox

B. Kart, J. A. Kuenen, M. C. M. van Loosdrecht

Wastewater treatment including high-rate processes has the potential to become energy-neutral or even energy-producing.



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?

Rijksuniversiteit Nijmegen



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

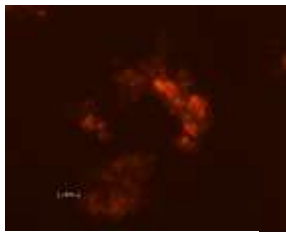


Luesken, sanchez et al unpublished

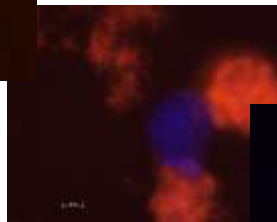
European Research Council



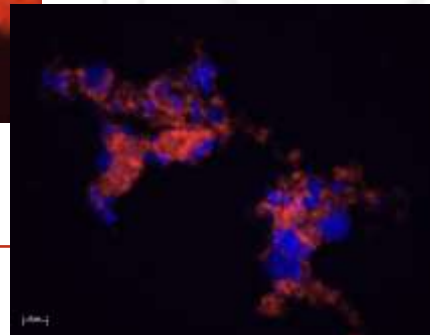
Anammox and *M. oxyfera*



Day = 0
Moxy193 = red
Amx368= blue



Day = 61
Moxy193 = red
Amx368= blue



Day = 106
Moxy193 = red
Amx368= blue

Journal of Environmental Microbiology, Vol. 76, No. 10, pp. 3015-3020, October 2010

Simultaneous Nitrite-Dependent Anaerobic Methane and Ammonium Oxidation Processes¹

Francois A. Luesken,¹ Anne Steyer,^{1,2,3,4} Thoralf van Alen,¹ Anker Schloter,¹ Hans-J. M. Op den Camp,¹ Mike S. M. Jetten,^{1,2} and Basim Kana¹

¹Department of Microbiology, Wageningen University, Wageningen, 6500 ZB, The Netherlands; ²Centraal Bureau voor de Statistiek, The Netherlands; ³Department of Microbiology, Wageningen University, Wageningen, 6500 ZB, The Netherlands; ⁴Department of Microbiology, Wageningen University, Wageningen, 6500 ZB, The Netherlands

Received 19 May 2010; accepted 1 August 2010



“Impossible” bacteria could save the world

Wednesday, November 14, 2007

By Jeanna Bryner



THANK YOU!

