How to discover and apply impossible anaerobic microbes to recycle C and N in a sustainable way

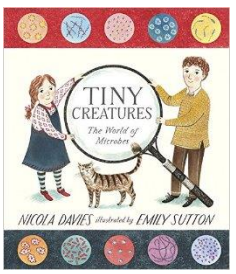
Melissa June 8th 2016

MIKE JETTEN

SOEHNGEN I Radboud University OBIOLOGY

www.anaerobic-microbiology.eu

Funded by
ERC AG 339880 EcoMoM 2014-2018
ERC PoC 713533 Initiator 2016-2017
STW 13184 CH_4 & N removal
Gravitation Grants NESSC & SIAM



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Introduction (anaerobic) microbiology

1. **Anaerobic oxidation of ammonium (anammox)**
2. **Anaerobic oxidation of methane (AOM)**
3. **Combinations & Take home message**

Many very useful (anaerobic) microbes

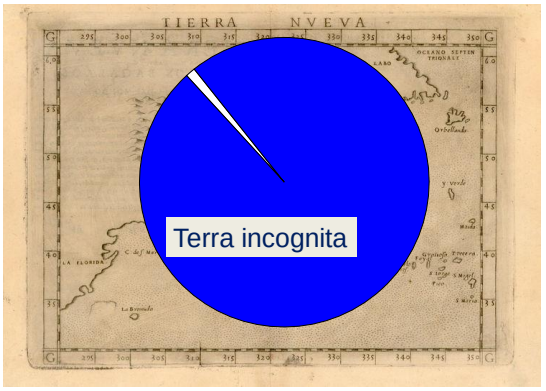
- Wastewater treatment
- Oxygen production
- Nitrogen fixation
- Fermentation
- Food digestion/Vitamins
- Drugs and Antibiotics
- Degradation of xenobiotics

Very few pathogens

How much do we know about the microbes on our planet?

Underexplored microbial diversity

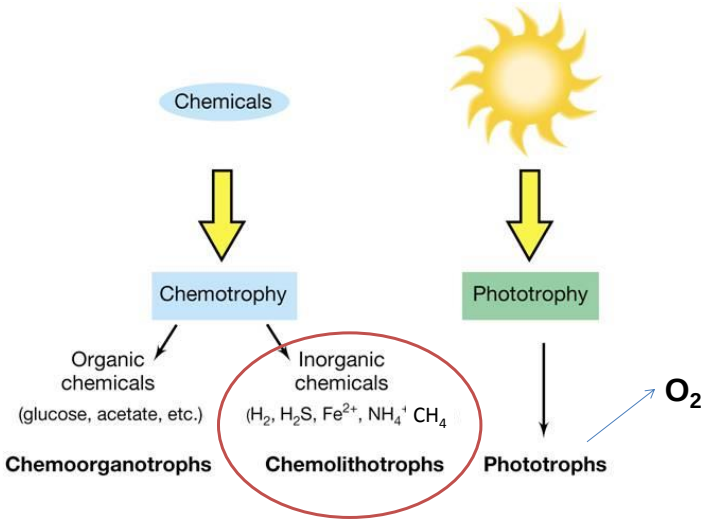
40,000	strains in DMSZ & ATCC
3,224,600	16S rRNA genes in RDP*
10,000,000,000,000,000,000,000,000,000,000	Nonillion microbial cells on Earth



New anaerobes for Health, Environment & Biobased economy

***RDP Release 11, Update 4 :: May 26, 2015**

Microbial Metabolic Diversity



The Quest for the "impossible" anaerobic microbes

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					

OXIC
ANOXIC

After 40 years of searching in vain
They were being called
"impossible" microbes

Raghoebarsing et al (2006)

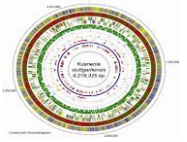
The Quest for the “impossible” anaerobic microbes

	H_2	CH_4
O_2		
NO_3^-		???
Fe^{3+}		???
SO_4^{2-}		
CO_2		

How to discover these “IMPOSSIBLE” microbes?

- Survey of selected ecosystems
- Bring the best samples to lab
- Design optimal bioreactors
- Enrichment under optimal conditions
- Grow enough cells
- **Use of the molecular toolbox to unravel their secrets**

- Back to the ecosystem
- Application of the new microbes

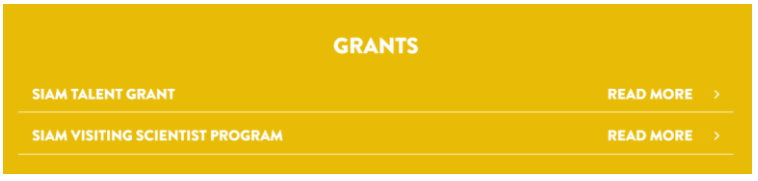


Pre requisite 1
Excellently educated & enthusiastic team members

www.ru.nl/microbiology/vacancies
www.ru.nl/masters/microbiology
www.anaerobic-microbiology.eu/scientists/jobs

(Inter)national Collaboration & Funding

Soehngen Institute of Anaerobic Microbiology
www.anaerobic-microbiology.eu



Pre requisite 3 State-of-the-art methods

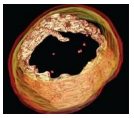
Bioreactors, Bioreactors, Bioreactors,
Bioreactors, Bioreactors, and more Bioreactors

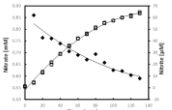
Metagenomics, Microscopy, new experiments

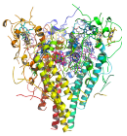
ion torrent

illumina MiSeq

CLC Genomics Workbench







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2. Surprise symbonts
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4. Combinations & Take home message

Funded by
ERC AG 232937 ANAMMOX 2009-2013
ERC PoC LTANITRO 2011-2012
STW & NWO 1995-2016

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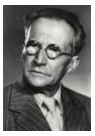
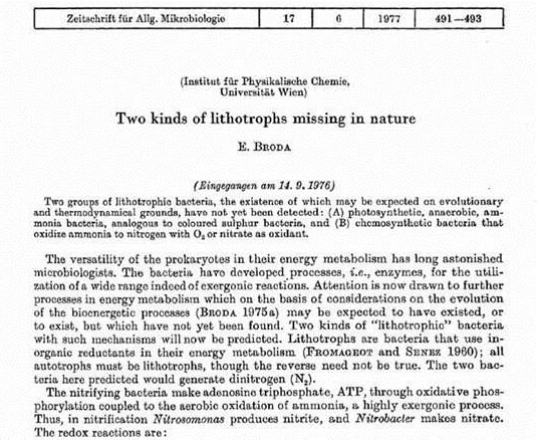
Calculations in the N cycle



Engelbert Broda 1910-1983



Zeitschrift für Allg. Mikrobiologie | 17 | 6 | 1977 | 491—493

(Institut für Physikalische Chemie, Universität Wien)

Two kinds of lithotrophs missing in nature

E. Broda

(Eingegangen am 14. 9. 1976)

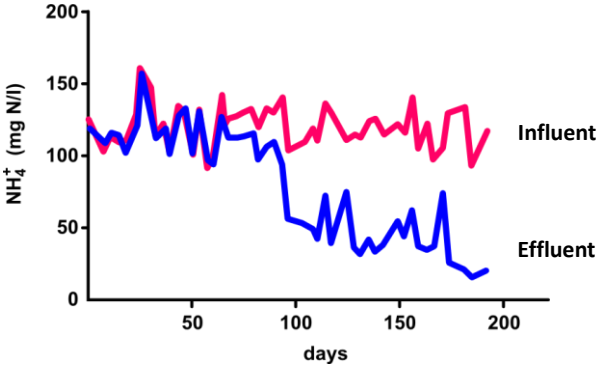
Two groups of lithotrophic bacteria, the existence of which may be expected on evolutionary and thermodynamical grounds, have not yet been detected: (A) photosynthetic, anaerobic, ammonia bacteria, analogous to coloured sulphur bacteria, and (B) chemosynthetic bacteria that oxidize ammonia to nitrogen 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 (Fromageot and Senez 1960); 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 exergonic process. Thus, in nitrification *Nitrosomonas* produces nitrite, and *Nitrobacter* makes nitrate. The redox reactions are:

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

Anaerobic pilot plant, TU Delft, the Netherlands



Mulder et al (1995) FEMS Microbiology Ecology

ANAMMOX MILESTONES

ANAEROBIC PILOT PLANT
Mulder et al FEMS 1995

SBR ENRICHMENT CULTURES
Strous et al AMB 1998

PHYLOGENETIC IDENTITY
Strous et al Nature 1999

LADDERANE LIPIDS
Damste et al Nature 2002

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ANAEROBIC PILOT PLANT
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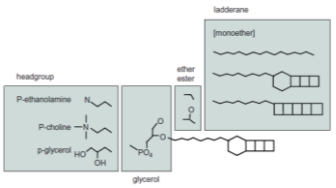
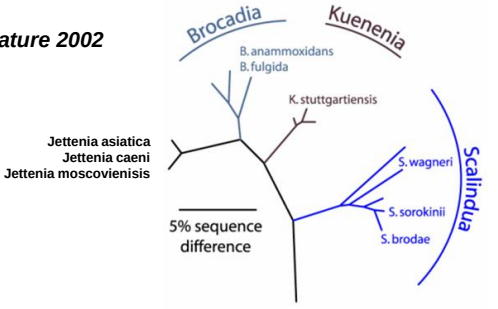




SIAM www.anaerobic-microbiology.eu

Anammox in the Ocean's oxygen minimum zones

PNAS

Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation

Marcel M. M. Kuypers*†, Gaute Lavik*, Dagmar Woebken*, Markus Schmid‡, Bernhard M. Fuchs*, Rudolf Amann*, Bo Barker Jørgensen*, and Mike S. M. Jetten§

*Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany; and §Department of Microbiology, Radboud University Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

Communicated by John M. Hayes, Woods Hole Oceanographic Institution, Woods Hole, MA, March 14, 2005 (received for review January 27, 2005)

PeerJ

Metagenomic analysis of nitrogen and methane cycling in the Arabian Sea oxygen minimum zone

Claudia Lüke[1,*], Daan R. Speth[1,*], Martine A.R. Kox[1], Laura Villanueva[1] and Mike S.M. Jetten[1,2,3]

SIAM www.anaerobic-microbiology.eu

Max-Planck-Institut für Marine Mikrobiologie

NIOZ

ANAMMOX MILESTONES

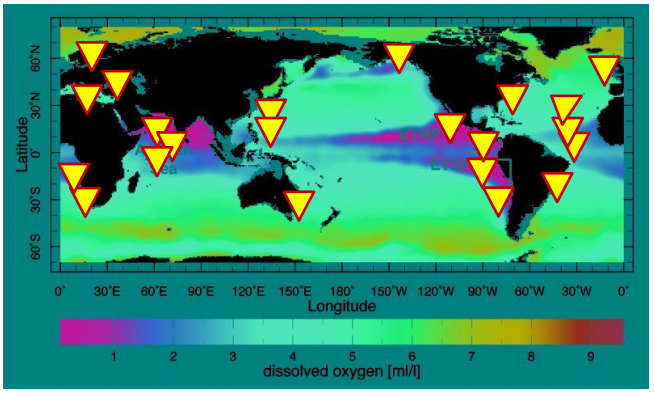
OMZs: *50% N loss*

Black Sea *Kuypers et al Nature 2003*

Namibia OMZ *Kuypers et al PNAS 2005*

Peru OMZ Lam *et al PNAS 2007; Stewart et al 2012*

Arabian Sea *Ward et al Nature 2008; Jensen et al ISME J 2011; Villanueva et al Frontiers Microbiology 2014; Lueke et al PeerJ 2016*



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ANAMMOX MILESTONES

2006 GENOME ASSEMBLY
Strous et al Nature

2008 CELL BIOLOGY
Van Niftrik et al Mol Mic

2011 HYDRAZINE & NO METABOLISM
Kartal et al Nature

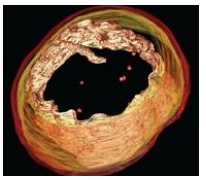
2013 CHINESE WETLANDS
Zhu et al Nature Geoscience

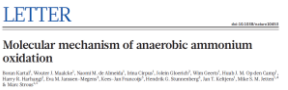
2014 ANAMMOXOSOME ISOLATION
Neumann et al Mol Micr

2015 PEPTIDOGLYCAN DETECTED
van Teeseling et al Ncomm

PROTEIN STRUCTURES
Dietl et al Nature

2016 FULL SCALE METAGENOME
Speth et al Ncomm




LETTER

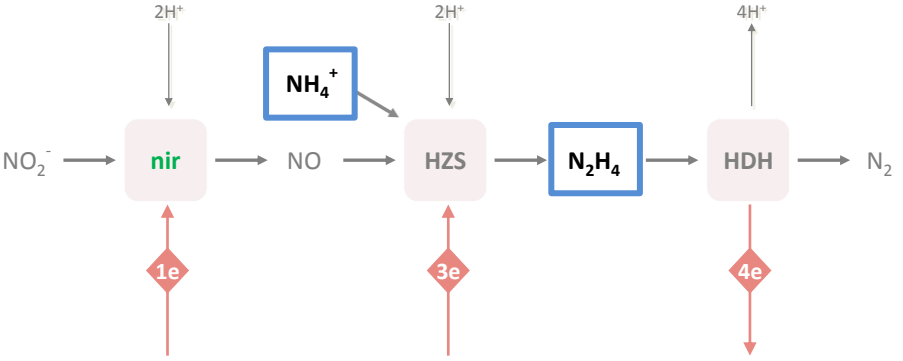
Molecular mechanism of anaerobic ammonium oxidation


LETTER

The inner workings of the hydrazine synthase multiprotein complex

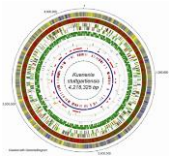
www.anaerobic-microbiology.eu

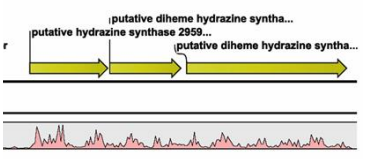
3-step anammox pathway



Nir = nirS in Scalindua
Nir = nirK in Jettenia
Nir = ? in Brocadia

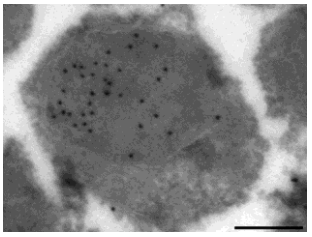
How does ANAMMOX make the rocket fuel hydrazine?



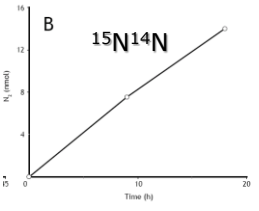


Expression of hzsABC

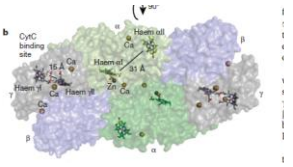
Protein purification
Hydrazine synthase



Immunogold labelling hzsA



Protein activity
Hydrazine and N_2 production



Crystalisation
$(hzsABC)_2$

Kartal et al (2011) Nature; Dietl et al (2016) Nature

From Discovery to Application

SIAM www.anaerobic-microbiology.eu

ERC PoC LTANITRO 2011-2012

ANAMMOX APPLICATION
Added Value

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


NRC HANDELSBLAD

Tomatensoep zuivert water

Hoe innovatief is Nederland? Deze week: een bacterie die de wereld moet veroveren.

Margriet van der Heijden

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

Form energy use to energy production


ENGINEERING

Sewage Treatment with Anammox

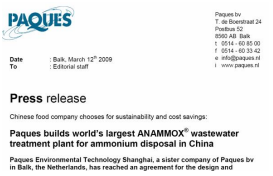
B. Kartal,[1] J. G. Kuenen,[2] M. C. M. van Loosdrecht[2]

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

TUDelft PAQUES stowa

ANAMMOX APPLICATION
Added Value

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



PAQUES

Paques bv
T. de Boerstraat 24
Postbus 52
8561 AB Balk
t. 0514 - 60 85 00
f. 0514 - 60 33 42
e. info@paques.nl
i. www.paques.nl

Date : Balk, March 12th 2009
To : Editorial staff

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

Energy from -44 to +24 (Wh p^{-1} d^{-1})

Savings NL 140 GWh =

-66 to + 33 Meuro a^{-1}

Meihua China 11 ton N/d
Rendac NL 15 ton N/d

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Introduction anaerobic microbiology

1. Anaerobic oxidation of ammonium (anammox)
2. **Anaerobic oxidation of methane (AOM)**
3. Combinations & home message

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ERC AG 339880 EcoMoM 2014-2018
ERC PoC Initiator 2016-2017
STW 13146 N & CH4 removal 2014-2017

SIAM www.anaerobic-microbiology.eu

Importance of methane

Green house gas

John Dalton (1766-1844)
collecting marsh fire gas (methane) with youngsters near Manchester

(***The Manchester Murals*** Ford Madox Brown)

Energy carrier

Statue by Marc Ruygrok celebrating 50 year of gas extraction in NL
(Photo: Reinier Treur)


HET ONTSTAAN EN VERDWIJNEN
van
WATERSTOF EN METHAAN
onder den invloed van het
ORGANISCHE LEVEN.
door
NICOLAAS LOUIS SÖHNGEN,
geboren te Utrecht.
DELFT — J. VIS Jr.
1906.

Aerobic methane oxidation (1906)

$$CH_4 + 2\ O_2 \longrightarrow CO_2 + 2\ H_2O$$

Quest for nitrite/nitrate/iron AOM

Nitrite dependent anaerobic oxidation of methane

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

Nitrate dependent anaerobic oxidation of methane

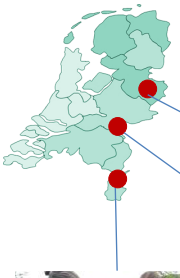
$$5CH_4 + 8NO_3^- + 8H^+ \longrightarrow 5CO_2 + 4N_2 + 14H_2O$$

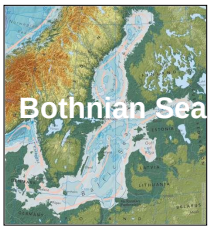
Iron dependent anaerobic oxidation of methane

$$CH_4 + 8Fe(OH)_3 + 7CO_2 \rightarrow 8\ FeCO_3 + 14H_2O$$

ERC AG 339880 EcoMoM 2014-2018
ERC PoC Initiator 2016-2017

Where do we find nitrate/nitrite-AOM?

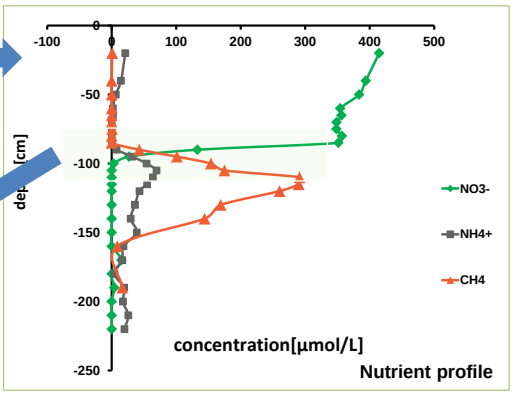




HIGH NO_3^- due to agricultural run-off /ground water

HIGH CH_4 production in the sediment

Suitable counter gradient profiles of nitrate & methane



Activity tests
qPCR
Stable isotopes
Enrichment
FISH
Metagenome

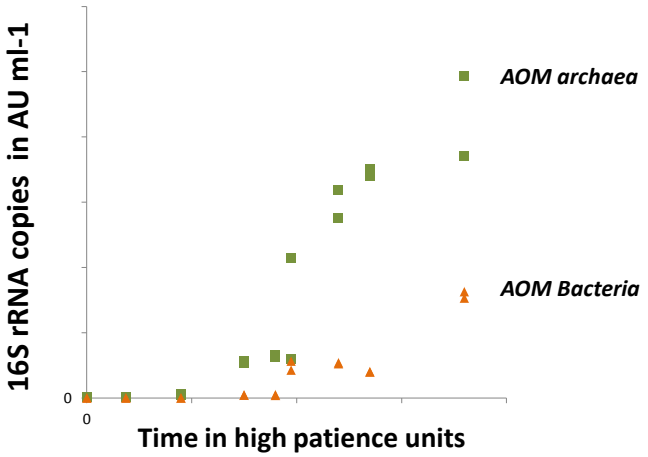
Enrichment Culture CH_4 & Nitrate

Annika Vaksmaa

Inoculum	Paddy field soil
Electron donor	CH_4
Electron acceptor	$NaNO_3$
Type	Continuous SBR
Time of operation	2 years
Monitoring	qPCR batch experiments nitrate, nitrite measurements Metagenome analysis

Vaksmaa et al (2016) in prep

Monitoring of enrichment culture for AOM archaea & bacteria



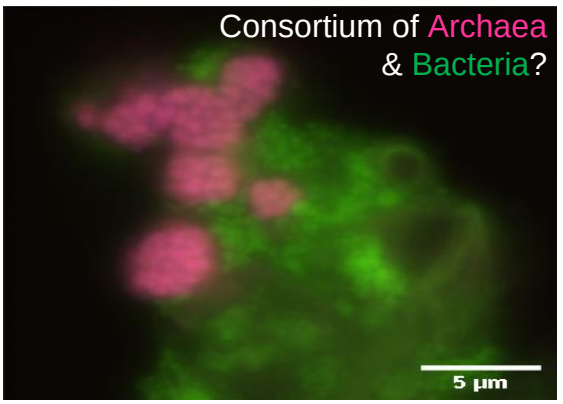
Vaksmaa et al (2016) in prep


nature Vol 440|13 April 2006|doi:10.1038/nature04617

LETTERS

A microbial consortium couples anaerobic methane oxidation to denitrification

Ashna A. Raghoebarsing[1], Arjan Pol[1], Katinka T. van de Pas-Schoonen[1], Alfons J. P. Smolders[2], Katharina F. Ettwig[1], W. Irene C. Rijpstra[3], Stefan Schouten[3], Jaap S. Sinninghe Damsté[3], Huub J. M. Op den Camp[1], Mike S. M. Jetten[1] & Marc Strous[1]



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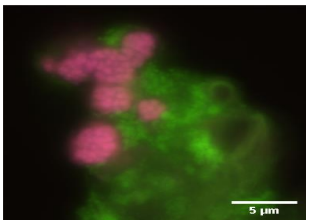
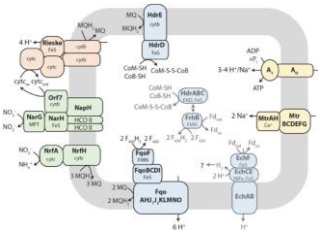
Raghoebarsing et al (2006) Nature;



+ Nitrate

+ Nitrite

Archaea
Methanoperedens
Metagenome extrapolations



Bacteria
Methylomirabilis
NO dismutase?

Physiology
Genome
Stable isotopes

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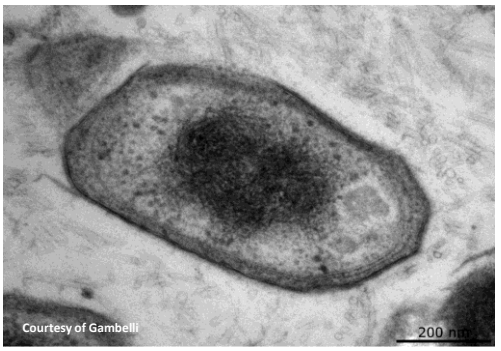
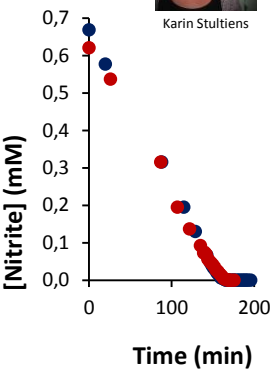
Raghoebarsing et al (2006) Nature; Ettwig (2010) Nature; Haroon et al (2013) Nature

Methylomirabilis oxyfera




Karin Stultiens



- Doubling time of 2 weeks
- Ecophysiology Ks & Yield?
- Enrichment >80 % *M. oxyfera*
- Polygonal shape
- Rubisco

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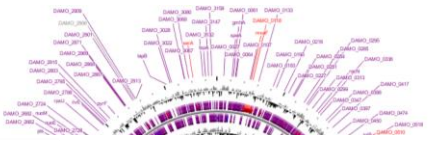
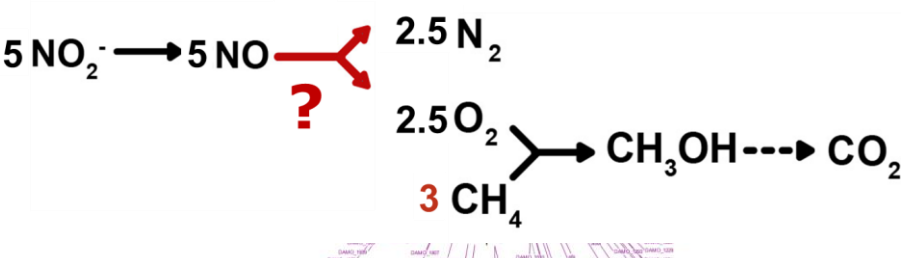
Genome of *Methylomirabilis oxyfera*





2010

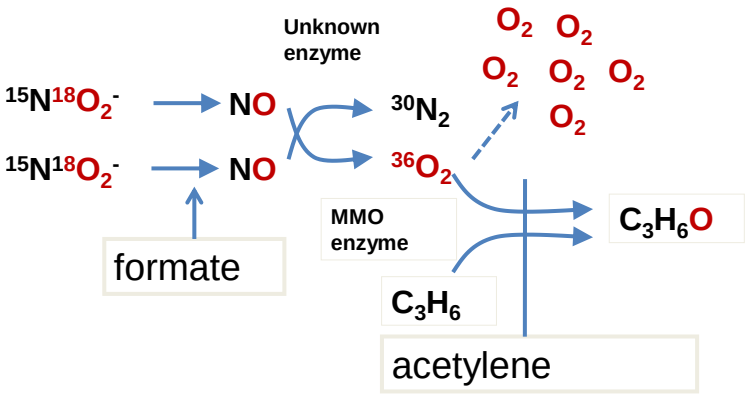
pathway of (aerobic) methane oxidation
Incomplete denitrification
Putative NO dismutase

www.anaerobic-microbiology.eu

Ettwig (2010) Nature

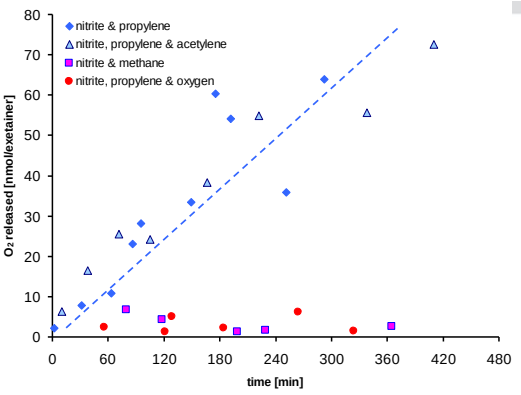
Possible mechanisms of *M. oxyfera*

Use of suicide substrates, inhibitors and $^{15}N^{18}O$ nitrite



Ettwig (2010) Nature

Methylomirabilis oxyfera putative NO dismutase
2NO ➔ N_2 +O_2




ARTICLES

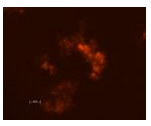
Nitrite-driven anaerobic methane oxidation by oxygenic bacteria

$^{15}N^{18}O$ experiments show: Oxygen Production

Ettwig (2010) Nature

New impossible anaerobes can be discovered

- **Hydrazine synthase in anammox**
- ***M oxyfera* makes O_2 from NO**
- **Novel nitrate reducing AOM archaea**
- **AOM present in many ecosystems**
- **Combinations for application in WWTP**



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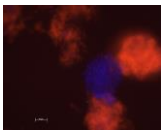
Moxy193 = red
Amx368= blue



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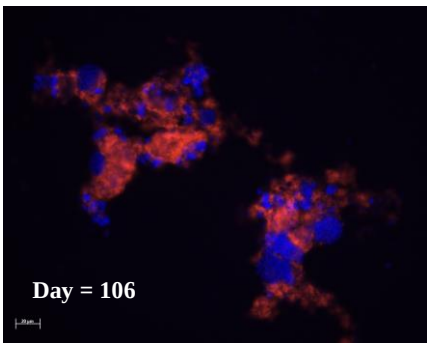


SIAM www.anaerobic-microbiology.eu

FOX NEWS.com

Anammox, Moxyfera, Methanoperedens and other new (an)aerobic microbes could save the world

By Jeanna Bryner

LIVE SCIENCE

Unique bacteria hiding out in a witches' brew of anoxic water not only thrive in cold wetlands and oceans but also chow down its ammonium and methane

Thank you !

www.anaerobic-microbiology.eu

Materials not shown at melissa

Anammox and *M. oxyfera*

M. oxyfera and anammox bacteria use the same electron acceptor and live under similar environmental conditions

It should be possible to enriched co-cultures in the same bioreactor if nitrite, ammonium and methane are sufficiently present.

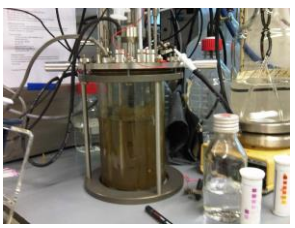
Universidad del Valle

UNIVERSIDAD DE LOS ANDES VENEZUELA

Luesken, sanchez et al unpublished

European Research Council erc

Anammox and *M. oxyfera*



Use a stable *M. oxyfera* reactor

- check if anammox 16s rRNA genes are still present
- start adding ammonium
- monitor
  - FISH, 16S rRNA, activity

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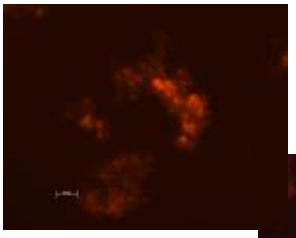
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2011, p. 000 Vol. 77, No. 19
0099-2240/11/$12.00 doi:10.1128/AEM.05539-11

Simultaneous Nitrite-Dependent Anaerobic Methane and Ammonium Oxidation Processes[▽]

Francisca A. Luesken,[1†] Jaime Sánchez,[1,2,3†] Theo A. van Alen,[1] Janeth Sanabria,[2] Huub J. M. Op den Camp,[1] Mike S. M. Jetten,[1,4] and Boran Kartal[1]*

erc



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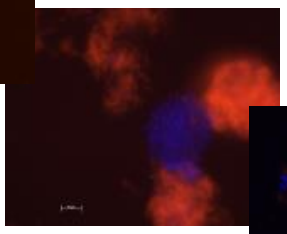
Moxy193 = red
Amx368= blue

Day = 61
Moxy193 = red
Amx368= blue



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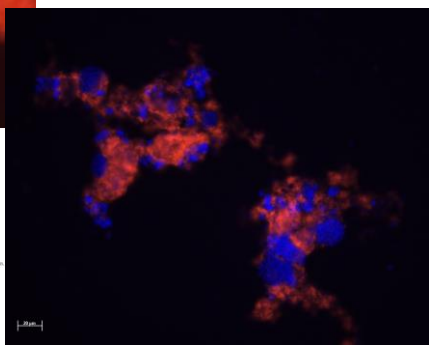

European Research Council

erc

Appl. and Environmental Microbiology, Oct. 2011, p. 000
1240/11/$12.00 doi:10.1128/AEM.05539-11
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Vol. 77, No.

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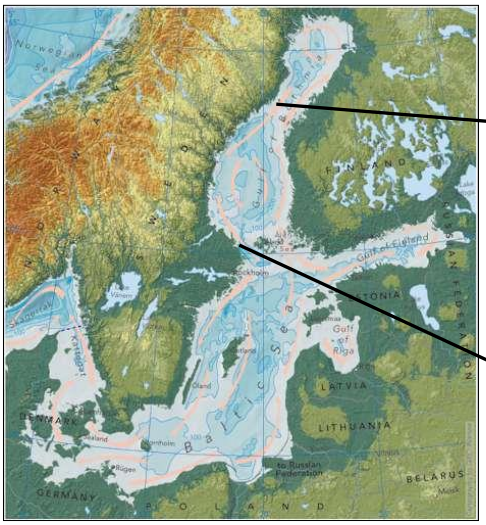
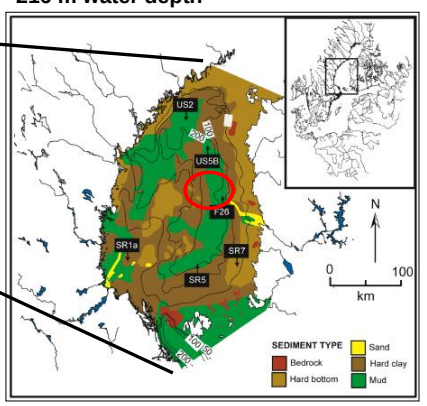
Sampling site: Bothnian Sea

Olivia Rasigraf



216 m water depth



Matthias Egger & Caroline Slomp

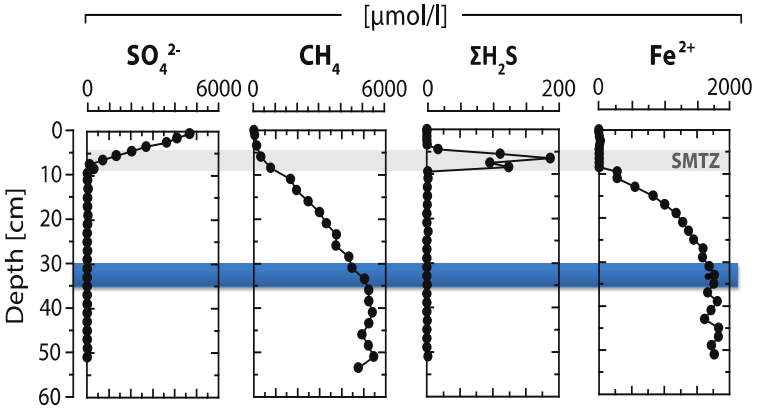
SIAM www.anaerobic-microbiology.eu

NESSC NETHERLANDS EARTH SYSTEM SCIENCE CENTRE

Universiteit Utrecht

Sediment biogeochemistry

Olivia Rasigraf



iron-AOM

$CH_4 + 8Fe(OH)_3 + 7CO_2 \rightarrow 8\ FeCO_3 + 14H_2O$

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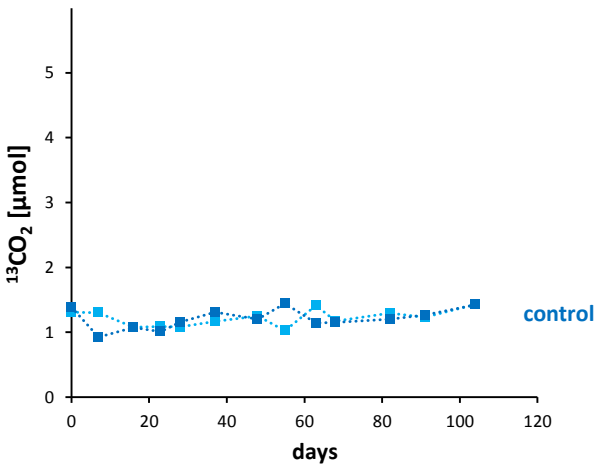
$^{13}CH_4$ oxidation to $^{13}CO_2$ coupled to Fe^{3+} reduction



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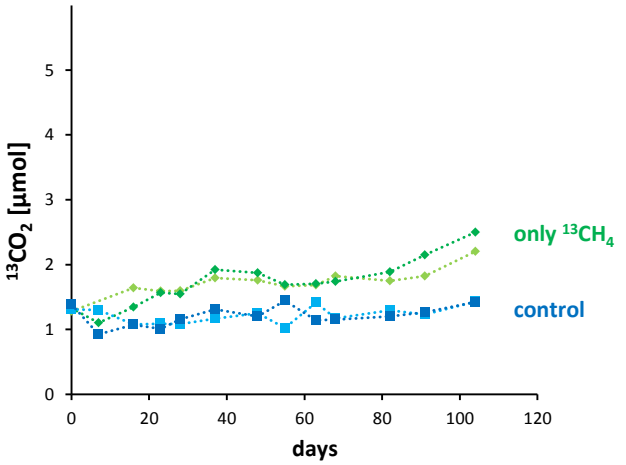
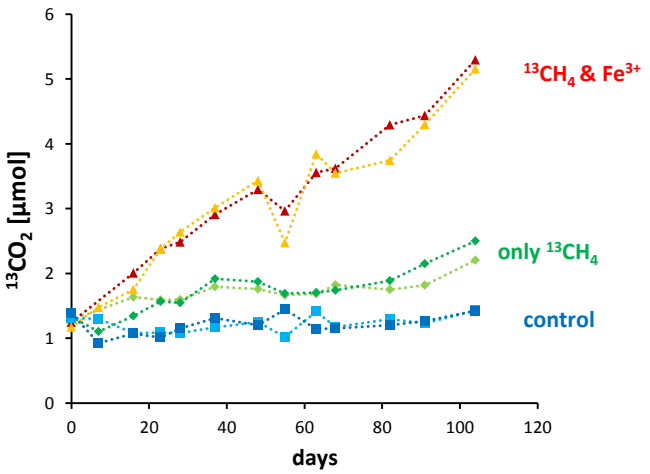
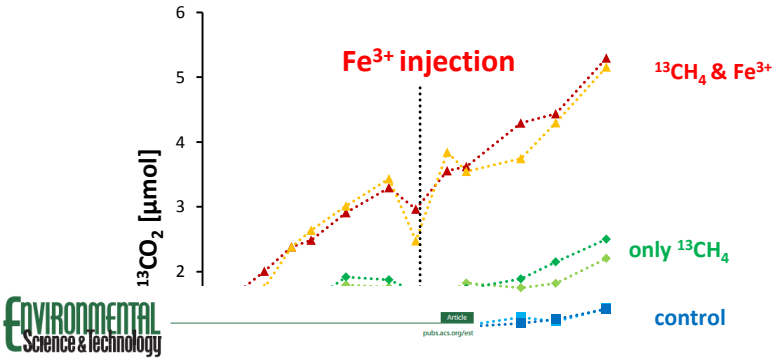
$^{13}CH_4$ oxidation to $^{13}CO_2$ coupled to Fe^{3+} reduction



$^{13}CH_4$ oxidation to $^{13}CO_2$ coupled to Fe^{3+} reduction



$^{13}CH_4$ oxidation to $^{13}CO_2$ coupled to Fe^{3+} reduction



Environmental Science & Technology
Article
pubs.acs.org/est

Iron-Mediated Anaerobic Oxidation of Methane in Brackish Coastal Sediments

Matthias Egger,*[†] Olivia Rasigraf,[‡] Célia J. Sapart,[§,∥] Tom Jilbert,[†,⊥] Mike S. M. Jetten,[‡] Thomas Röckmann,[§] Carina van der Veen,[§] Narcisa Bândă,[§] Boran Kartal,[‡,#] Katharina F. Ettwig,[‡] and Caroline P. Slomp[†]

[†]Department of Earth Sciences - Geochemistry, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands
[‡]Department of Microbiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
[§]Institute for Marine and Atmospheric Research Utrecht (IMAU), Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands
[∥]Laboratoire de Glaciologie, Université Libre de Bruxelles, 50 Avenue F. D. Roosevelt, B-1050 Bruxelles, Belgium
[⊥]Department of Biochemistry and Microbiology, Laboratory of Microbiology, Ghent University, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

More Evidence for iron AOM

Microbial Cell Factories
RESEARCH
Open Access

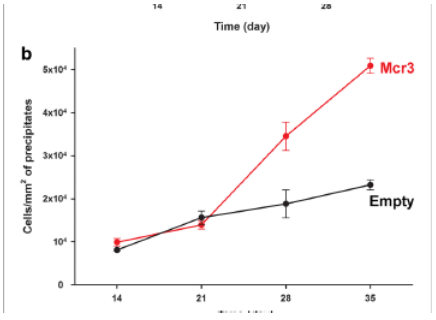
Reversing methanogenesis to capture methane for liquid biofuel precursors

Valerie W.C. Soo[†], Michael J. McAnulty[†], Arti Tripathi[†], Fayin Zhu[†], Limin Zhang[†], Emmanuel Hatzakis[‡], Philip B. Smith[‡], Saumya Agrawal[†], Hadi Nazem-Bokaee[†], Sarabram Gopalakrishnan[†], Howard M. Salis[†], James G. Ferry[§], Costas D. Maranas[†], Andrew D. Patterson[‡] and Thomas K. Wood[†,#]*

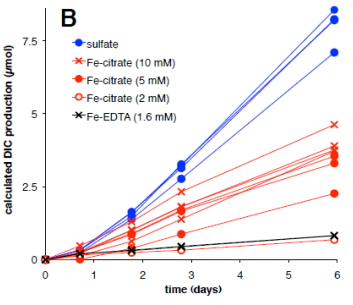
MICROBIAL PHYSIOLOGY

Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction

Silvan Scheller,[a] Hang Yu, Grayson L. Chadwick, Shawn E. McGlynn,[b] Victoria J. Orphan[a]



Growth of engineered Methanosarcina
On Methane & 10 mM $FeCl_3$



Activity of AOM
On Methane & 10 mM Fe-Citrate

www.anaerobic-microbiology.eu

Improvement of N removal

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

7 MAY 2010 VOL 328 **SCIENCE** www.sciencemag.org

stowa

stw

IcoN

TU Delft

Radboud Universiteit Nijmegen



Improvement of N removal

- **Less oxygen demand**
- **No COD use**
- **Less biomass production**

ENGINEERING

Sewage Treatment with Anammox

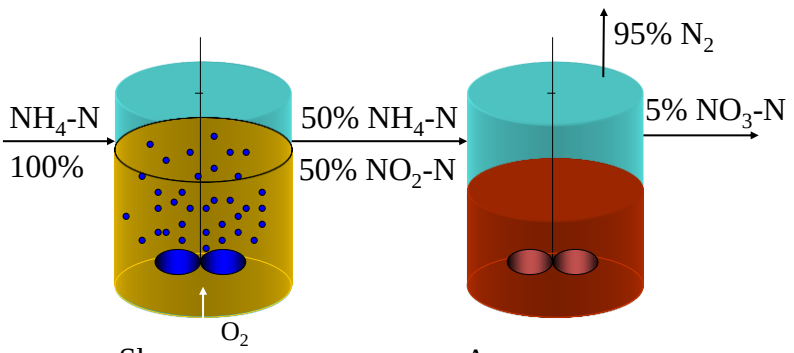
B. Kartal,[1] J. G. Kuenen,[2] M. C. M. van Loosdrecht[2]

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

7 MAY 2010 VOL 328 SCIENCE www.sciencemag.org

stowa stw IcoN TU Delft Radboud Universiteit Nijmegen

SHARON & ANAMMOX



No Chemicals; No CO_2 emission;

Energy from -44 to +24 (Wh p^{-1} d^{-1})

Savings NL 140 GWh = -66 to + 33 Meuro a^{-1}

ANAMMOX APPLICATION
Added Value

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


NRC HANDELSBLAD

Tomatensoep zuivert water

Hoe innovatief is Nederland? Deze week: een bacterie die de wereld moet veroveren.

Margriet van der Heijden

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

stowa stw PAQUES

ENGINEERING

Sewage Treatment with Anammox

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

TUDelft

ANAMMOX APPLICATION
Added Value

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

PAQUES

Paques bv
T. de Boerstraat 24
Postbus 52
8560 AB Balk
t 0514 - 60 85 00
f 0514 - 60 33 42
e info@paques.nl
i www.paques.nl

Date : Balk, March 12th 2009
To : Editorial staff

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 15 ton N/d

ENGINEERING

Sewage Treatment with Anammox

B. Kartal,[1] J. G. Kuenen,[2] M. C. M. van Loosdrecht[2]

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

TUDelft

surprise symbionts

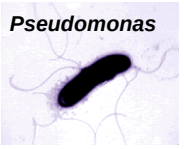
environmental microbiology reports
Environmental Microbiology Reports (2016) 00(00), 00–00 doi:10.1111/1758-2229.12407


Maartje van Kessel

Branchial nitrogen cycle symbionts can remove ammonia in fish gills

NH_4^+

NO_2^-



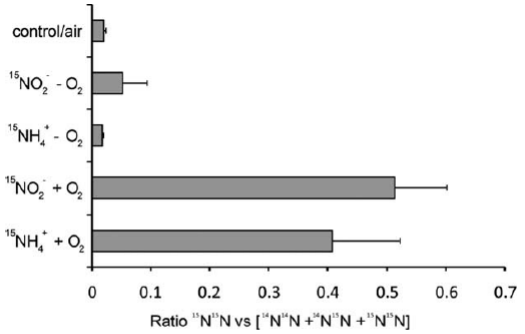
N_2

Funded by ERC AG 232937 ANAMMOX 2009-2013

environmental microbiology reports
Environmental Microbiology Reports (2016) 00(00), 00–00 doi:10.1111/1758-2229.12407

Branchial nitrogen cycle symbionts can remove ammonia in fish gills

Maartje van Kessel



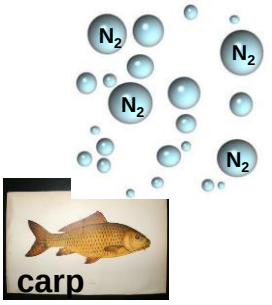


Fig. 1. Nitrogen gas production by dissected gills of common carp. Dissected gills were incubated for 90 min with ^{15}N-labelled ammonium or nitrite in the presence or absence of oxygen. Production of labelled nitrogen gas is shown by an increase in the ratio of ^{30}N to total nitrogen gas. Values are the mean ± SD ($n = 6$).

European Research Council
ERC AG 2008 anammox

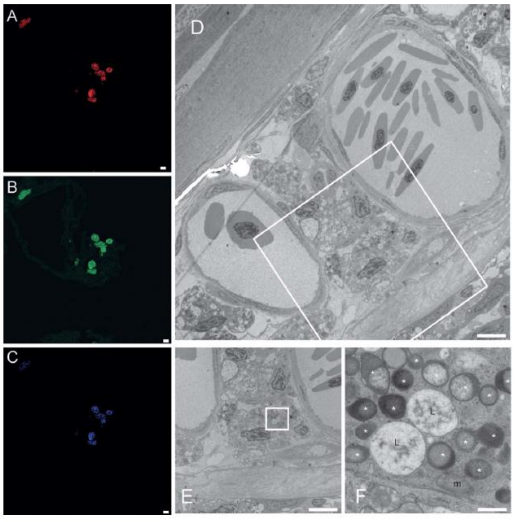
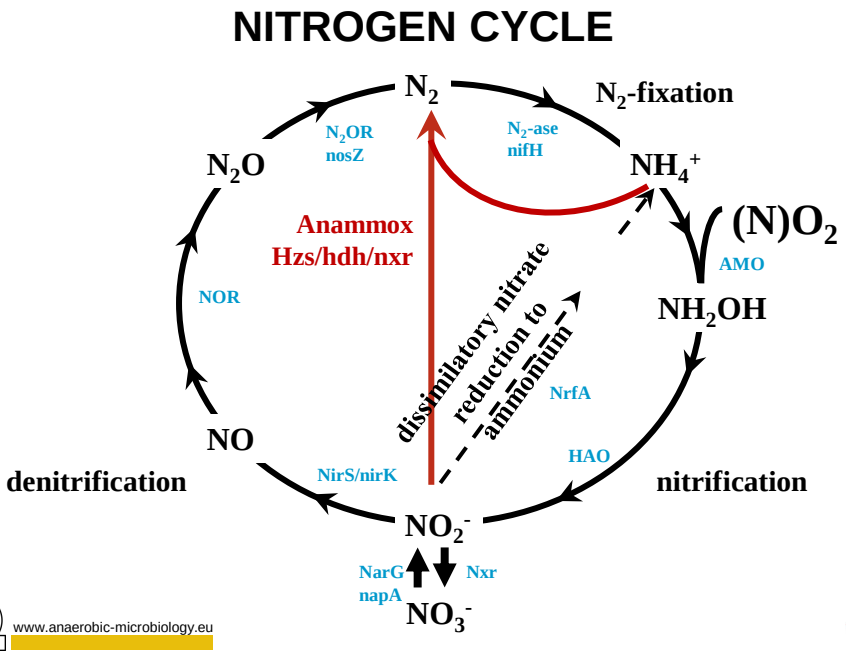


Fig. 2. Microscopic observations of gill tissue. Fluorescence *in situ* hybridization (FISH) on the gills of common carp (A., B., C.). A. FISH using eubmix (Eub I, II, III, labelled with Cy3) targeting all bacteria. B. FISH targeting beta-proteobacteria (bet42a + competitor, labelled with FLUOS). C. FISH targeting beta-proteobacterial ammonia-oxidizing bacteria (NSO1225, labelled with Cy5). The bacterial clusters are targeted by the probes. Many bacteria within the clusters are ammonia-oxidizing bacteria. Scale bar represents 10 μm. Transmission electron microscopy on gills of common carp (D., E., F.). D. Clusters of bacteria are found close to blood vessels within the carp gill. E. Bacteria reside in host cells. F. Magnification of the bacterial cells in the carp gills. Scale bars represent 5 μm, 5 μm and 500 nm for panels D, E, and F, respectively.


NITROGEN CYCLE
N2
N2-fixation
N2OR
nosZ
N2-ase
nifH
N2O
NH4+
Anammox
Hzs/hdh/nxr
(N)O2
AMO
NOR
NH2OH
dissimilatory nitrate
reduction to
ammonium
NrfA
NO
HAO
denitrification
NirS/nirK
nitrification
NO2-
NarG
napA
Nxr
NO3-

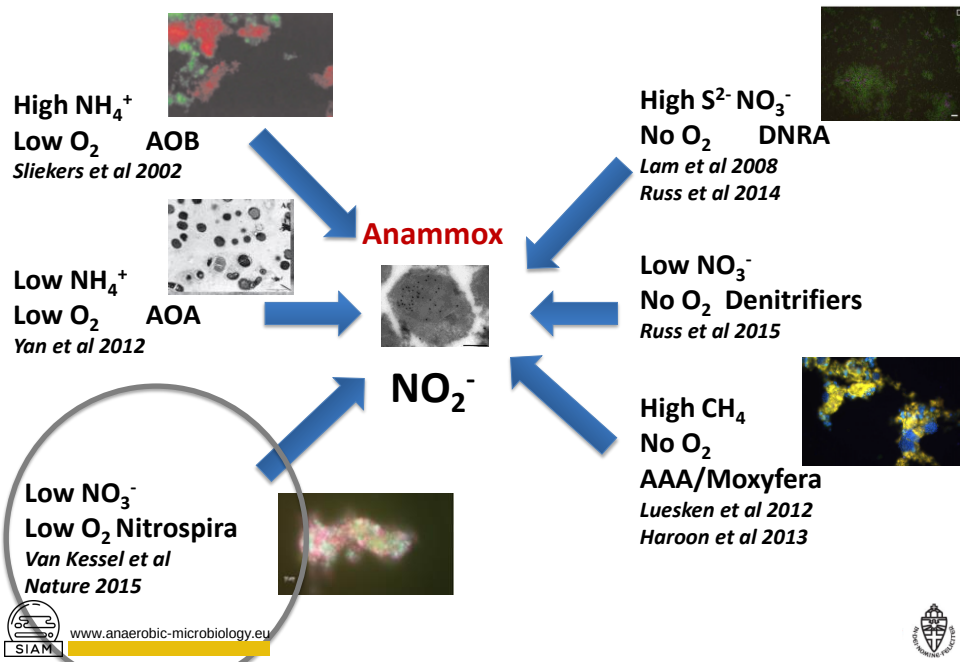
HOW DOES ANAMMOX GET NITRITE?

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

COOPERATION WITH OTHER N-CYCLE MICROBES

AOB	$NH_4^+ + O_2^- \rightarrow NO_2$
AOA	$NH_4^+ + O_2^- \rightarrow NO_2$
NOB	Competition?
DENITRIFIERS	$NO_3 + ORG \rightarrow NO_2$
DNRA	$NO_3 + ORG \rightarrow NO_2 / NH_4$
AOM-Archaea	$NO_3 + CH_4 \rightarrow NO_2$

Microbial interactions of ANAMMOX bacteria

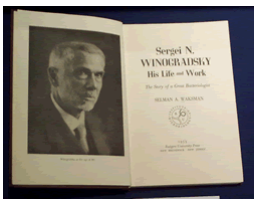


www.anaerobic-microbiology.eu

Opinion TRENDS in Microbiology Vol.14 No.5 May 2006
Full text provided by www.sciencedirect.com
16459

Why is metabolic labour divided in nitrification?

Engràcia Costa[1], Julio Pérez[1] and Jan-Ulrich Kreft[2]



$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^-$ AOB/AOA
$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ NOB
$NH_4^+ + 2\ O_2 \rightarrow NO_3^-$?

Does a Complete ammonium oxidiser (comammox) exist?

Funded by ERC AG 232937 ANAMMOX 2009-2013

www.anaerobic-microbiology.eu

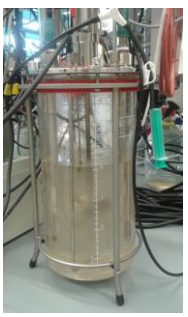
European Research Council
ERC AG 2008
anammox

LETTER

doi:10.1038/nature16459

Complete nitrification by a single microorganism

Maartje A. H. J. van Kessel[1], Daan R. Speth[1], Mads Albertsen[2], Per H. Nielsen[2], Huub J. M. Op den Camp[1], Boran Kartal[1,3], Mike S. M. Jetten[1,4] & Sebastian Lücker[1]



Inoculum: Biofilm from aquaculture biofilter

Medium: Aquaculture water with

low [NH_4^+, NO_2^-, NO_3^-] < 0.5 mM

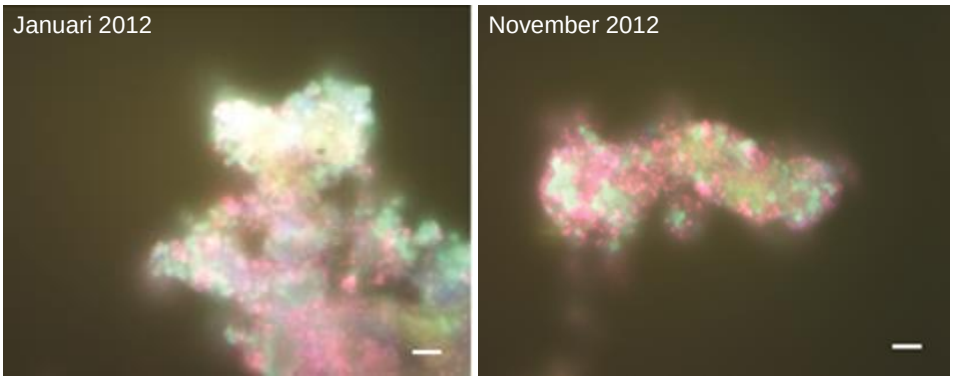
No extra carbon source; No O_2 supply

➔ Conditions for anammox & comammox

➔ After 1 year NH_4^+ plus NO_2^- consumption

www.anaerobic-microbiology.eu

Fluorescence *in situ* hybridization of commamox & anammox



pink = anammox; green = *Nitrospira*; blue= all bacteria

***Nitrospira* is always present in flocs with anammox**

Stable coculture; cross feeding? What does Nitrospira do?

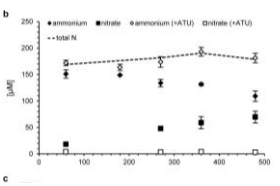
What does Nitrospira do in this culture?

ion torrent

illumina MiSeq

AALBORG UNIVERSITY DENMARK

CLC Genomics Workbench

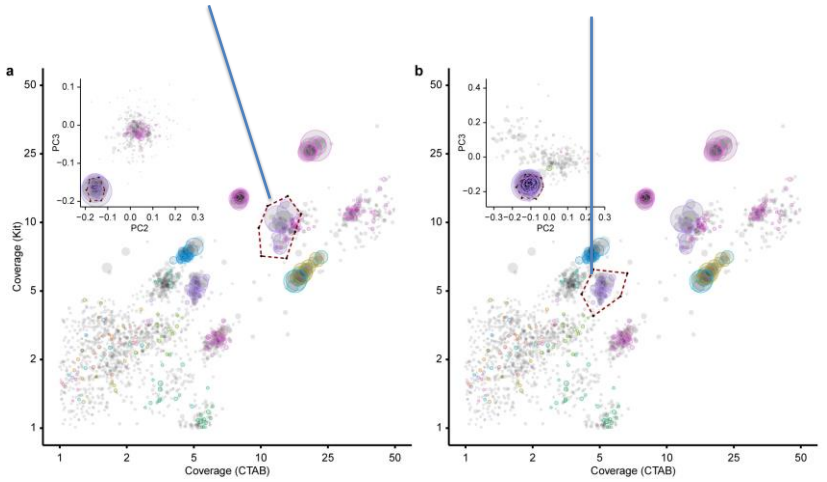


Extract DNA
Sequence DNA by high trough put
Assemble contigs & bin genomes
Analysis of genomes
Design new experiments

Metagenome sequencing, assembly & coverage binning

Two high quality *Nitrospira* genomes

Nitrospira nitrificans & *Nitrospira nitrosa*



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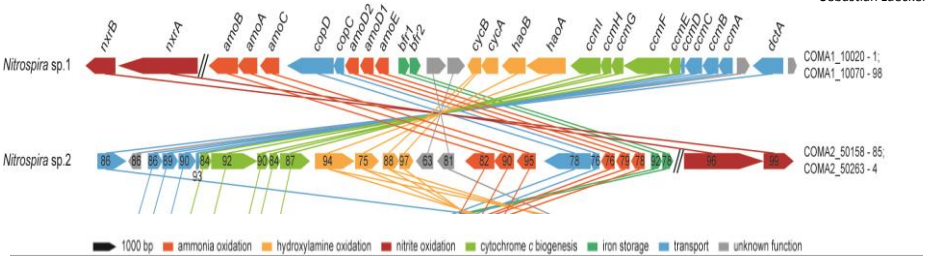
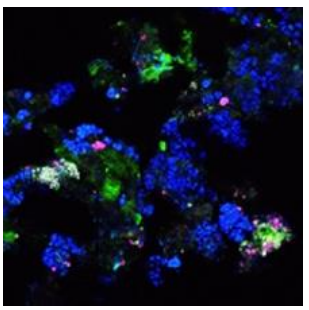
Nitrospira genomes do contain amoA & hao!

Sebastian Luecker



Experimental validation
with ATU = amo inhibitor
with FISH MAR
with specific AMO labeling



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Excellent example of synergy & collaboration

LETTER

DOME Divison of Microbial Ecology

AALBORG UNIVERSITY DENMARK

ARTICLE

Complete nitrification by a single microorganism

Complete nitrification by *Nitrospira* bacteria

Comammox does exist = *Nitrospira* species unusual/novel amoA gene

Future research:
Isolation & defined co-cultures


Dimitra Sakoula


Maartje van Kessel

Environmental relevance & detection


Lianna Poghosyan


Sebastian Luecker

SIAM www.anaerobic-microbiology.eu