24th EUROPEAN NITROGEN CYCLE MEETING

BOOK OF ABSTRACTS

11-13 September 2019
Lisbon - Portugal

24th European Nitrogen Cycle Meeting

Program & Abstracts

11 - 13 September 2019

Lisbon - Portugal

INDEX

INTRODUCTION

Welcome to the 24th **European Nitrogen Cycle Meeting**, which this year is held in Lisbon, Portugal. This is a European meeting that brings together researchers with complementary expertise in microbiology, physiology ecology, genetics, biochemistry, structural biology, biotechnology and engineers, with a shared interest in the Nitrogen Cycle. This is a short meeting, that cover the different aspects of the pathways that comprise the nitrogen cycle, with focus on new metabolic pathways, implications in health, biotechnology and climate change, without forgetting the basic science that lies behind.

This year the meeting is divided into five main Sessions that cover topics on *"Assembly of Nitrogen Cycle Enzymes"*, *"Denitrification and its Applications"*, *"Ammonium Oxidation"*, *"Other N-cycle Processes"* and "*Environmental Impact of N-cycle*". The meeting will have a total of 31 oral lectures, specially by young promising scientists and a poster session. The lectures are by both invited speakers and (the majority) those selected from submitted abstracts. One of the lectures is sponsored by the American Corners Portugal that enable us to bring Professor Markus Ribbe from USA.

The organisers take this opportunity to thank you for participating in the 24th European Nitrogen Cycle Meeting, and we hope that it will be a fruitful and pleasant scientific event. Enjoy the colourful and warm Lisbon and especially the science and please help to make this meeting a success!

Sofia Pauleta, UCIBIO-NOVA, Portugal
Isabel Moura, LAQV_NOVA, Portugal
David Richardson, UEA, UK
Maria J. Delgado, Estación Experimental del Zaidín, Spain
Rosa Maria Martínez-Espinosa, University of Alicante, Spain

CONFERENCE ORGANISERS

International Scientific Committee

Name	Email	Organization
Sofia R. Pauleta (Chair person)	srp@fct.unl.pt	UCIBIO, REQUIMTE, Universidade NOVA de Lisboa, Portugal
Isabel Moura (Co-Chair person)	isabelmoura@fct.unl.pt	LAQV, REQUIMTE, Universidade NOVA de Lisboa, Portugal
David Richardson	d.richardson@uea.ac.uk	University of East Anglia, United Kingdom
Maria J. Delgado	mariajesus.delgado@eez.csic.es	Estación Experimental del Zaidín, CSIC, Spain
Rosa Maria Martínez-Espinosa	rosa.martinez@ua.es	University of Alicante, Spain
Ascensão Reis	amr@fct.unl.pt	UCIBIO, REQUIMTE, Universidade NOVA de Lisboa, Portugal

Organizing Committee

Name	Email	Organization
Sofia R. Pauleta	srp@fct.unl.pt	UCIBIO, REQUIMTE, Universidade NOVA de Lisboa, Portugal
Cristina Cordas	Cristina.cordas@fct.unl.pt	LAQV, REQUIMTE, Universidade NOVA de Lisboa, Portugal
Catarina Magalhães	cmagalhaes@ciimar.up.pt	CIIMAR, Universidade do Porto, Portugal
David Richardson	d.richardson@uea.ac.uk	University of East Anglia, United Kingdom
Ascensão Reis	amr@fct.unl.pt	UCIBIO, REQUIMTE, Universidade NOVA de Lisboa, Portugal
Isabel Moura	isabelmoura@fct.unl.pt	LAQV, REQUIMTE, Universidade NOVA de Lisboa, Portugal

GENERAL INFORMATION

Venue - Conference Site

Reitoria da Universidade NOVA de Lisboa

Address: Reitoria da Universidade Nova de Lisboa, Campus de Campolide, 1099-085 Lisboa, Portugal

Metro Station: Blue or Red Line, S. Sebastião da Pedreira

Bus Line: Bus lines 701, 716, 726, 742, 758

Internet

Free wifi available in all the areas.

Welcome Reception, Lunches and Coffee-breaks

At the Conference Site. Reitoria da Universidade Nova de Lisboa

Conference Dinner

Hotel Sana Malhoa (10 min walking distance from Conference Venue)

Av. José Malhoa nº 8

Conference Photo

Thursday, 12th September, during Lunch Time.

Speakers

Presentations should be delivered at least in the prior coffee break/lunch before your schedule presentation time. Please check for further information in the reception desk at your arrival.

Posters

Poster should be on display in the Coffee-break area from Wednesday 11th September (16:00) and be removed after the coffee-break (16:00) on Friday 13th September. You need to use patafix to fix the poster at the designated area (ask at the registration desk).

TIMETABLE

Time	Wednesday September 11
15:00	**Registration**
16:30	**Welcome**
17:00	OL: Axel Magalon
18:00	**Welcome Reception**

Time	Thursday September 12
	Assembly of Nitrogen Cycle Enzymes
09:00	ACP: Markus Ribbe*
10:00	IL1: David Richardson
10:30	OC1: Lin Zhang
10:50	**Coffee-Break**
	Denitrification and its Applications (Part I)
11:30	IL2: Simone Morais
12:00	OC2: Javier Torregrosa-Crespo
12:20	OC3: Jose María Miralles Robledillo
12:40	OC4: Linda Bergaust
13:00	**Lunch/Posters**
	Denitrification and its Applications (Part II)
14:30	IL3: Rosa Martínez-Espinosa
15:00	OC5: Lea Wittorf
15:20	OC6: Cristina Cordas
15:40	OC7: Ricarda Kellermann
16:00	**Coffee-Break**
	Other N-Cycle Processes
16:30	IL4: Gottfried Unden
17:00	OC8: Serena Rinaldo
17:20	OC9: Eliane Meilhoc
17:40	OC10: Luísa Maia
18:00	OC11: Henrique Fernandes

*Sponsorded by American Corners Portugal

Time	Friday September 13
	Ammonium Oxidation
09:00	IL5: Boran Kartal
09:30	OC12: Hanna Koch
09:50	OC13: Justyna Barys
	Environmental Impact of N-Cycle (Part I)
10:10	**Coffee-Break**
10:40	IL6: Sara Hallin
11:10	OC14: Joana Costa
11:30	OC15: Yuan Gao
11:50	OC16: Aurélien Saghaï
12:10	OC17: Carlos Palacin-Lizarbe
12:30	OC18: Claire Brown
12:50	**Lunch/Posters**
	Environmental Impact of N-Cycle (Part II)
14:30	OC19: Viviane Figueiredo Souza
14:50	OC20: Víctor Luque Almagro
15:10	OC21: Miguel Semedo
15:30	OC22: Micaela Giani Alonso
15:50	**Coffee-Break**
16:30	CL: José Moura
17:30	**Closing Session**
20:30	**Conference Dinner**

EXTENDED PROGRAMME

Wednesday 11 September

Opening Lecture

Chair: Isabel Moura, LAQV, Universidade NOVA de Lisboa, Portugal

15:00-16:30	Registration
16:30-17:00	Opening & Welcome
17:00-18:00	Opening Lecture: What if clustering is a mean to maximize diffusion and handling of toxic intermediates? **Axel Magalon** Aix-Marseille Univ, CNRS, France, p. 18
18:00-19:30	Welcome Reception – "Wine and Cheese Tasting"

Thursday 12 September

Session I. Assembly of Nitrogen Cycle Enzymes

Chair: José Moura, LAQV, Universidade NOVA de Lisboa, Portugal

09:00-10:00	American Courner's Lecture*: Nitrogenase assembly mechanism **Markus Ribbe** University of California, USA, p. 22
10:00-10:30	Invited Lecture 1: Copper dependent assembly of the Nitrous Oxide Reductase **David Richardson** University of East Anglia, United Kingdom, p. 26
10:30-10:50	Oral 1: Functional assembly of Nitrous Oxide Reductase provides insights into copper site maturation **Lin Zhang** Albert-Ludwigs-Universität Freiburg, Germany, p. 40
10:50-11:30	Coffee break / Poster Session

* Sponsored by American Corners Portugal.

Thursday 12 September

Session II. Denitrification and its Applications (Part I)

Chair: Jörg Simon, TU Darmstadt, Germany

11:30-12:00	Invited Lecture 2: Nitric Oxide Reductase-based Biosensors **Simone Morais** LAQV, REQUIMTE, Universidade do Porto, Portugal, p. 28
12:00-12:20	Oral 2: *Haloferax mediterranei*, the saline model for denitrification **Javier Torregrosa-Crespo** Universidad de Alicante, Spain, p. 42
12:20-12:40	Oral 3: Haloarchaeal denitrification: A first look at its regulation **Jose Maria Miralles-Robledillo** Universidad de Alicante, Spain, p. 44
12:40-13:00	Oral 4: Nitric oxide regulates bet-hedging in *Paracoccus denitrificans* during the transition to anoxia **Linda Bergaust** Norwegian University of Life Sciences, Norway, p. 46
13:00-14:30	Lunch / Poster Session

Session III. Denitrification and its Applications (Part II)

Chair: Serena Rinaldo, Sapienza Univ. of Rome, Italy

14:30-15:00	Invited Lecture 3: Role of extreme microorganisms in the biogeochemical Nitrogen cycle **Rosa Martínez-Espinosa** Universidad de Alicante, Spain, p. 30
15:00-15:20	Oral 5: Differential expression of two copies of nitrite and nitrous oxide reductase genes in *Pseudomonas stutzeri* and *Thauera linaloolentis*, respectively **Lea Wittorf** Swedish University of Agricultural Sciences, Sweden, p. 48
15:20-15:40	Oral 6: Nitric Oxide Reductase – from fundamental studies to applied research **Cristina M. Cordas** LAQV-REQUIMTE, NOVA, Portugal, p. 50
15:40-16:00	Oral 7: Role of O_2 in recruitment to nitrite reduction in the bet-hedging denitrifier *Paracoccus denitrificans* **Ricarda Kellermann** Norwegian University of Life Sciences, Norway, p. 52
16:00-16:30	Coffee break / Poster Session

Thursday 12 September

Session IV. Other N-Cycle Processes

Chair: Stéphane Besson, Universidade Lusófona, Portugal

16:30-17:00	Invited Lecture 4: Joint sensing of O_2 and nitrate in a new type of O_2/nitrate sensor complex (NreA-NreB) of *Staphylococcus carnosus* **Gottfried Unden** University of Mainz, Germany p. 32
17:00-17:20	Oral 8: Sensing L-arginine in *Pseudomonas aeruginosa* links energy metabolism and *c*-di-GMP levels **Serena Rinaldo** Sapienza Unviersity of Rome, Italy, p. 54
17:20-17:40	Oral 9: *Sinorhizobium meliloti* nitrate assimilation pathway: contribution to NO production **Eliane Meilhoc** Université de Toulouse, France, p. 56
17:40-18:00	Oral 10: Lessons from denitrification to the human metabolism of signaling nitric oxide **Luísa B. Maia** LAQV-REQUIMTE, NOVA, Portugal, p. 58
18:00-18:20	Oral 11: Production of NO by Xanthine Oxidase – a QM/MM study **Henrique Silva Fernandes** UCIBIO-REQUIMTE, Universidade do Porto, Portugal, p. 60

Friday 13 September

Session V. Ammonium Oxidation

Chair: Eliane Meihoc, Université de Toulouse, France

09:00-09:30	Invited Lecture 5: Nitric oxide-dependent anaerobic ammonium oxidation **Boran Kartal** Max Planck Institute for Marine Microbiology, Germany, p. 34
09:30-09:50	Oral 12: Deciphering the metabolism of nitrite-oxidizing *Nitrospira* – a multi-omics approach **Hanna Koch** Radboud University, Netherlands, p. 62
09:50-10:10	Oral 13: In quest of ureolytic heterotrophs to pair with nitrifiers in a defined bacterial community for human urine treatment **Justyna Barys** Universitat Autonoma de Barcelona, Spain, p. 64
10:10-10:40	Coffee break / Poster Session

Friday 13 September

Session VI. Environmental Impact of N-Cycle (Part I)

Chair: Liz Baggs, University of Edinburgh, UK

10:40-11:10	Invited Lecture 6: Rewiring the microbial controls of N_2O emissions by soil management **Sara Hallin** Swedish University of Agricultural Sciences, Sweden p. 36
11:10-11:30	Oral 14: Evaluation of the denitrification efficiency of estuarine sediments exposed to Cu nanoparticles **Joana Faria da Costa** Universidade do Porto, Portugal, p. 66
11:30-11:50	Oral 15: Bradyrhizobia can act as strong sinks or sources of N_2O in agricultural ecosystems **Yuan Gao** Norwegian University of Life Sciences, Norway, p. 68
11:50-12:10	Oral 16: Changes in either plant or soil microbial diversity constrain plant microbiome selection and affect the potential for N_2O emissions **Aurélien Saghaï** Swedish University of Agricultural Sciences, Sweden, p. 70
12:10-12:30	Oral 17: Nitrogen-transforming guilds and denitrification rates in lakes of mountains affected by high atmospheric nitrogen deposition **Carlos Placin-Lizarbe** Universitat Autonoma de Barcelona, Spain, p. 72
12:30-12:50	Oral 18: Field-scale spatial variation in denitrifying microbial communities and the impacts on arable crop yield **Claire Brown** University of York, United Kingdom, p. 74
12:50-14:30	Lunch / Poster Session

Friday 13 September

Session VII. Environmental Impact of N-Cycle (Part II)

Chair: Åsa Frostegård, Norwegian University of Life Sciences, Norway

14:30-14:50	Oral 19: Evolution of nitrogen cycling in tropical forests **Viviane Figueiredo Souza** Federal University of Rio de Janeiro, Brazil, p. 76
14:50-15:10	Oral 20: Putative small RNAs controlling detoxification of industrial cyanide-containing wastewaters by *Pseudomonas pseudoalcaligenes* CECT5344 **Víctor Luque Almagro** Universidad de Córdoba, Spain, p. 78
15:10-15:30	Oral 21: Inhibited denitrification in tidal creek sediments impacted by poultry industry wastewater: activities, genes, and metagenomes **Miguel Semedo** Virginia Institute of Marine Science, USA, p. 80
15:30-15:50	Oral 22: Effect of nitrogen/carbon balance and pH value in bacterioruberin production in Haloferax mediterranei cultures **Micaela Giani Alonso** Universidad de Alicante, Spain, p. 82
15:50-16:30	Coffee break / Poster Session

Closing Lecture

Chair: Sofia Pauleta, UCIBIO, Universidade NOVA de Lisboa, Portugal

16:30-17:30	Closing Lecture Nitrate Reductases – Structural and mechanistic aspects **José J. G. Moura** LAQV-NOVA, Portugal, p. 20
17:30-18:00	Closing Remarks
20:30	Conference Dinner

Opening and Closing Lectures

American Corners Portugal Lecture

What if clustering is a mean to maximize diffusion and handling of toxic intermediates?

Axel Magalon

Aix-Marseille Univ, CNRS, Laboratoire de Chimie Bactérienne (UMR7283), IMM, Marseille, France.

magalon@imm.cnrs.fr

Keywords: Fluorescence microscopy, nitrate respiration, nitric oxide, microcompartmentation

Most bacteria rely on the redox activity of respiratory complexes embedded into the cytoplasmic membrane to gain energy in the form of ATP and of an electrochemical gradient established across the membrane. Nevertheless, production of harmful and toxic nitric oxide by actively growing bacteria either as an intermediate or side-product of nitrate respiration challenges how homeostasis control is exerted. Here, we show that components of the *E. coli* nitrate electron transport chain including two Mo-*bis*-pyranopterin guanine dinucleotide containing enzymes are clustered, likely influencing the kinetics of the process. Nitric oxide production by this respiratory chain is controlled and handled through a multiprotein complex including detoxifying systems [1]. In the same line of though, superoxide dismutases have been found associated with aerobic respiratory complexes, prone to reactive oxygen species generation in two model organisms, *Caenorhabditis elegans* and *Mycobacterium smegmatis* [2-4]. Altogether, our findings point to an essential role of compartmentalization of respiratory components in bacterial cell growth.

References:
[1] Bulot *et al*. (2019). *Under submission*.
[2] Suthammarak *et al*. (2013). *Aging cell,* 12:1132-1140.
[3] Gong *et al*. (2018). *Science*, **362**: 1020.
[4] Wiseman *et al*. (2018). *Nat Struct Mol Biol,* **25**: 1128-1136.

Nitrate Reductases (and Spin-offs)

José J. G. Moura

LAQV, REQUIMTE, Department of Chemistry, FCT-NOVA, Portugal.

jose.moura@fct.unl.pt

Prokaryotes use nitrate for dissimilatory and assimilatory processes and encode three distinct mononuclear molybdenum containing Nitrate reductases (Nar) enzymes present in different subcellular locations: (a) membrane-bound cytoplasm-faced respiratory NaR (Nar), associated with the generation of a proton motive force across the cytoplasmic membrane; (b) periplasmatic NaR (Nap), involved in the generation of a proton motive force or acting as an electron sink to eliminate excess of reducing equivalents; and (c) cytoplasmatic assimilatory NaR (Nas), involved in nitrogen assimilation. In response to their different biological roles/subcellular locations, these enzymes have different subunit organisations and cofactor compositions. Interestingly, in spite of catalysing the same reaction and having the molybdenum atom coordinated by the characteristic four sulphur atoms from two pyranopterin cofactor molecules (in the form of the guanine dinucleotide), the three types of NaR enzymes display significant differences in the remainder of the molybdenum coordination sphere. All these prokaryotic NaRs catalyse the two-electron reduction of nitrate to nitrite at their molybdenum centre, an oxygen atom transfer reaction. Similar to Nar, but different, are the Formate dehydrogenase (Fdh) enzymes, also structurally heterogeneous proteins, displaying diverse subunit organisations and cofactor compositions, but carrying out H transfer. Structural and mechanistic aspects are discussed for the two groups of enzymes. Artificial enzymes based on molybdenum substitutions on a sulphur rich protein template will be presented.

Acknowledgements:
Thanks are due to Isabel Moura, Luisa Maia and Cristina Cordas. This work was supported by the Associate Laboratory for Green Chemistry- LAQV, which is financed by national funds from Fundacão para a Ciência e a Tecnologia, MCTES (FCT/MCTES; UID/QUI/50006/2019).

References:
Maia and Moura, in Molybdenum and Tungsten Enzymes (2017) DOI: 10.1039/9781782623915-0000.
Cordas *et al*. (2019) *J. Inorg. Biochem.* DOI: 10.1016/j.jinorgbio.2019.110694.

Nitrogenase Assembly Mechanism *

Chi Chung Lee[1], Kazuki Tanifuji[1], Andrew J. Jasniewski[1], Yilin Hu[1], Markus W. Ribbe[1,2]

[1]Department of Molecular Biology and Biochemistry.
[2]Chemistry, University of California, Irvine, California 92697-3900, USA.

mribbe@uci.edu

Keywords: Nitrogenase, nitrogen fixation, assembly, M-cluster, [Fe_4S_4] cluster

The M-cluster is the active site of nitrogenase that contains an 8Fe-core assembled via coupling and rearrangement of two [Fe_4S_4] clusters concomitant with the insertion of an interstitial carbon and a '9^{th} sulfur' [1]. Combining synthetic [Fe_4S_4] clusters with an assembly protein template, we show that sulfite gives rise to the '9^{th} sulfur' that is incorporated in the catalytically important belt region of the cofactor after the radical SAM-dependent carbide insertion and the concurrent 8Fe-core rearrangement have already taken place [2]. This work provides a semi-synthetic tool for strategically labeling the cofactor—including its '9^{th} S' in the belt region—for mechanistic investigations of nitrogenase while suggesting an interesting link between nitrogen fixation and sulfite detoxification in diazotrophic organisms.

Acknowledgments: This work was supported by NIH-NIGMS grant GM67626 and DOE-BES grant DE-DC0014470 (to M.W.R. and Y.H.)

References:

1. Hu Y, Ribbe MW (2016). *Annu Rev Biochem* **85**: 455-483.
2. Tanifuji K, Lee CC, Sickerman NS, Tatsumi K, Ohki Y, Hu Y, Ribbe MW (2018). *Nat Chem* **10**(5): 568-572.

* Lecture sponsored by American Corners Portugal.

INVITED LECTURES

Copper dependent assembly of the Nitrous Oxide Reductase

David Richardson

University of East Anglia, Norwich, UK.

D.Richardson@uea.ac.uk

Keywords: Nitrous oxide reductase, copper centers assembly

Bacterial denitrification is a respiratory process which is a major source and sink of the potent greenhouse gas nitrous oxide. The destruction of this greenhouse gas yields the inert nitrogen gas.

Many denitrifying bacteria can adjust to life in both aerobic and anaerobic environments through differential expression of their respiromes in response to environmental electron acceptors such as oxygen, nitrate, nitric oxide and minerals such as copper.

Understanding the regulation of nitrous oxide production and destruction by denitrifying bacteria requires a deeper understanding of how bacteria integrate these different environmental signals into their metabolic regulation. The nitrous oxide reductase (Nos) is a copper-dependent enzyme so the regulatory effects of copper on the expression of the *nos* gene and assembly of active Nos enzyme are important to understand. Recent progress in this area will be presented.

Nitric Oxide Reductase-based Biosensors

Simone Morais

LAQV-REQUIMTE, Instituto Superior de Engenharia, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal.

sbm@isep.ipp.pt

Keywords: Direct electron transfer; Enzymatic biosensors; Nanomaterials; Nanocomposites; Nitric oxide

Electrochemical biosensors integrate the sensitivity of electrochemical transducers with the high selectivity of biological recognition. The biological element recognizes its analyte causing a biocatalytic or binding reaction, which yields an electrochemical signal (registered by a transducer), that is related with the analyte concentration. Third generation biosensors are based on the direct electron transfer of proteins, being the absence of mediator one of their main advantages [1]. Nitric oxide reductase (NOR), that can be isolated from the denitrifying organism *Marinobacter hydrocarbonoclasticus*, is a membrane-bound enzyme containing one heme c, two hemes b and one non-heme iron [2]. The specificity of NOR towards NO makes it an ideal biorecognition element to develop biosensing devices to monitor NO, and possibly other reactive nitrogen species, in biological systems [3-5]. The recent accomplishments of nanotechnology assure that good (immobilization) stability, ultrahigh sensitivity, good selectivity, low detection limit, fast response time, and miniaturization of the biosensing platform can be reached. Several case studies concerning the development of novel enzymatic biosensors based on NOR for NO detection will be discussed.

Acknowledgments:
I am grateful for the financial support of projects UID/QUI/50006/2019 and PTDC/ASP-PES/29547/2017 funded by FEDER funds through the POCI and by National Funds through FCT - Foundation for Science and Technology under the NORTE-01-0145-FEDER.

References:

1. Gomes *et al.* (2018). *Electroanalysis*, **30**: 2485-2503.
2. Duarte *et al.* (2014). *Biochim. Biophys. Acta*, **1837**: 375-384.
3. Gomes *et al*. (2019). *Bioelectrochemistry*, **125**: 8–14.
4. Gomes *et al.* (2019). *Bioelectrochemistry*, **127**: 76–86.
5. Gomes *et al*. (2019). *Sensors and Actuators B*, **285**: 445-452.

Role of Extreme Microorganisms in the Biogeochemical Nitrogen Cycle

Rosa Maria Martínez-Espinosa, Javier Torregrosa-Crespo, Jose Maria Miralles-Robledillo, E. Bernabeu, Micaela Giani-Alonso, Carmen Pire

Departamento de Agroquímica y Bioquímica, División de Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de Alicante, Carretera San Vicente del Raspeig s/n - 03690 San Vicente del Raspeig, Alicante, Spain.

rosa.martinez@ua.es

Keywords: nitrogen cycle, archaea, extremophile

Extreme microorganisms (extremophiles) are organisms that inhabit environments characterized by extreme temperatures, pH values, ionic strength, pressure or scarcity of nutrients. These physic-chemical conditions are detrimental to most life on Earth. In order to grow optimally, extremophiles have evolved molecular adaptations affecting physiology, metabolism, cell signaling, etc. Due to their unusual features, they have become good models for: i) understanding the limits of life on Earth: ii) exploring the possible existence of extra-terrestrial life (Astrobiology) or iii) to look for potential applications in biotechnology. Research carried out mainly during the last three decades have revealed that these microbes play key roles in all biogeochemical cycles on the planet. Nitrogen cycle is one of the most important where nitrogen is converted into multiple chemical forms, which circulate among atmosphere, terrestrial, and marine ecosystems. This work summarizes and highlights recent knowledge on the role of extreme microorganisms in the biogeochemical N-cycle, with special emphasis on members of the Archaea domain and denitrification as key pathway in salty environments with low oxygen availability [1-3].

Acknowledgments:
This work was supported by the University of Alicante (VIGROB-173 and VIGROB-309), the Spanish Ministerio de Ciencia, Innovación y Universidades (RTI2018-099860-B-I00).

References:
1. Torregrosa-Crespo J, Pire C, Martínez-Espinosa RM, Bergaust L. *Environ Microbiol*. 2019; **21**(1):427-436.
2. Torregrosa-Crespo J, Martínez-Espinosa RM, Esclapez J, Bautista V, Pire C, Camacho M, Richardson DJ, Bonete MJ. *Adv Microb Physiol*. 2016; **68**:41-85.
3. Martínez-Espinosa RM, Cole JA, Richardson DJ, Watmough NJ. *Biochem Soc Trans*. 2011; **39**(1):175-8.

Joint sensing of O_2 and nitrate in a new type of O_2/nitrate sensor complex (NreA-NreB) of *Staphylococcus carnosus*

Gottfried Unden, Robin Klein, Ann-Katrin Kretzschmar

Microbiology and Wine Research, University of Mainz, 55099 Mainz, Germany.

unden@uni-mainz.de

Keywords: Nitrate/O_2 co-sensing, NreA, NreB, *Staphylococcus*

The NreB-NreC two-component system of *Staphylococcus carnosus* for O_2-sensing [1] forms with the nitrate sensor NreA the NreA-NreB-NreC sensor complex for coordinated sensing of O_2 and nitrate [2, 3]. NreA-NreB-NreC controls expression of the nitrate respiratory system in Staphylococci. NreA controls NreB autophosphorylation (and activation) by direct interaction with NreB. Inhibition is found for the nitrate-free form of NreA and lost for the nitrate-bound form NreA·NO_3^- [2, 3]. The sensor kinase NreB contains a cryptic phosphatase site which is activated upon binding of NreA [4]. Activation of the phosphatase is missing in the nitrate bound form. As a result NreA represents a major regulator for modulating the O_2-sensor NreB and inhibits NreB in the absence of nitrate by lowering NreB autophosphorylation and by switching NreB from the kinase to the phosphatase state. In this way nitrate represents a major factor for controlling nitrate respiration via the joint O_2/nitrate sensor complex NreA-NreB-NreC.

Acknowledgments: We gratefully acknowledge funding by DFG (UN 49/18-1).

References:
1. Müllner *et al.* (2008). *Biochemistry,* **47**: 13921-32.
2. Nilkens *et al.* (2014). *Molec. Microbiol.* **91**: 381-93.
3. Niemann *et al.* (2014). *J. Mol. Biol.,* **426**: 1539-53.
4. Klein *et al.* (2019). *submitted.*

Nitric oxide-dependent anaerobic ammonium oxidation

Ziye Hu[1], Boran Kartal[2]

[1]Radboud University, Nijmegen, The Netherlands, [2]Max Planck Institute for Marine Microbiology, Bremen, Germany.

bkartal@mpi-bremen.de

Keywords: anammox, nitric oxide, ammonium

Nitric oxide (NO) has important functions in biology and atmospheric chemistry as a toxin, signaling molecule, ozone depleting agent and the precursor of the greenhouse gas nitrous oxide (N_2O). Although NO is a potent oxidant, and was available on earth earlier than oxygen, its direct use by microorganisms for growth was not demonstrated before. Anaerobic ammonium-oxidizing (anammox) bacteria couple nitrite reduction to ammonium oxidation with NO and hydrazine as intermediates, and produce N_2 and nitrate. Here we show that anammox bacterium Kuenenia stuttgartiensis grew in the absence of nitrite by coupling ammonium oxidation to NO reduction, and produced only N_2.

Physiological experiments, metatranscriptomics, metaproteomics revealed that the transcription of proteins necessary for NO generation were downregulated. NO-dependent ammonium oxidation could have existed on early earth, and has implications in controlling N_2O and NO emissions from natural and manmade ecosystems, where anammox bacteria contribute significantly to N_2 release to

the atmosphere.

Rewiring the microbial controls of N_2O emissions by soil management

Sara Hallin, M. Putz, A. Maheshwari, M. Emmerich, L. Wittorf, C. M. Jones

Swedish University of Agricultural Sciences, Uppsala, Sweden.

Sara.Hallin@slu.se

Keywords: arable soil, denitrifiers, DNRA bacteria, microbial community ecology, N_2O reducers

Arable soil is a major source of N_2O, controlled by microbial communities involved in inorganic N cycling. Dissimilatory nitrate reduction to ammonium (DNRA) competes with denitrification for NO_3^- and result in N conservation, whereas denitrification leads to losses (N_2, N_2O). N_2O reduction is further controlled by denitrifiers and non-denitrifying N_2O reducers. Using microcosms and 16 long-term field trials, we addressed if changes in the proportion of these functional groups and the overall bacterial community indicate loss or retention of soil N and net N_2O emission. Fertilization rewired the communities in a predictive way across a range of soils with effects on soil N_2O production and consumption. The shifts in abundances of functional groups are in line with theoretical predictions on their taxonomic coherence, allowing further insight of ecological strategies of denitrifiers under elevated N levels and genetic controls of N_2O. Fertilization selected for denitrifiers rather than non-denitrifiying N_2O reducers, in line with increased N_2O production. In unfertilized soils, N_2O was mainly controlled by biotic factors, whereas abiotic factors were more important in fertilized soils. Further, we conclude that soil C/N ratios determines the genetic potential of denitrification and DNRA, with consequences for N_2O emissions. Although N_2O reducers are important, the balance between DNRA and denitrification can be more determinant for net N_2O emissions. Overall, conservation of soil N and mitigation of N_2O can be mediated by the soil microbiome by soil management.

ORAL COMMUNICATIONS

Functional Assembly of Nitrous Oxide Reductase provides Insights into Copper Site Maturation

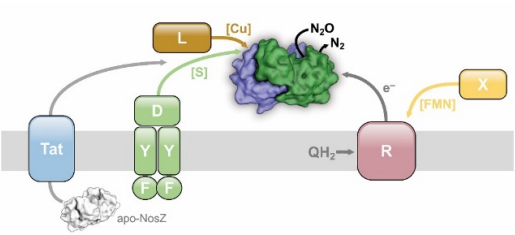
Lin Zhang, Anja Wüst, Benedikt Prasser, Christoph Müller, and Oliver Einsle

Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Albertstrasse 21, 79104 Freiburg, Germany.

zhang@bio.chemie.uni-freiburg.de

Keywords: nitrous oxide reductase, denitrification, enzyme refactoring, cofactor biogenesis, structural biology

The multicopper enzyme nitrous oxide reductase reduces the greenhouse gas N_2O to uncritical N_2 as the final step of bacterial denitrification. Its two metal centers require an elaborate assembly machinery that so far has precluded heterologous production as a prerequisite for bioremediatory applications in agriculture and wastewater treatment. Here, we report on the production of active holoenzyme in *Escherichia coli* using a two-plasmid system to produce the entire biosynthetic machinery as well as the structural gene for the enzyme [1]. Using this recombinant system to probe the role of individual maturation factors, we find that the ABC transporter NosFY and the accessory NosD protein are essential for the formation of the [4Cu:2S] site CuZ, but not the electron transfer site CuA. Depending on source organism, the heterologous host *E. coli* can, in some cases, compensate for the lack of the Cu chaperone NosL, while in others this protein is strictly required, underlining the case for designing a recombinant system to be entirely self-contained.



Acknowledgments: We thank Prof. Dr. Peter Kroneck for stimulating and informative discussions; the staff of beamline X06DA at the Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland) for providing excellent support during data collection; Dr. Anton Brausemann for assistance with X-ray dataset processing; and Dr. Julia Netzer for EPR measurements. This work was supported by the European Research Council Grant 310656.

References:

1. Zhang *et al*. (2019) *Proc Nat Acad Sci USA*, **116**(26): 12822-12827.

Haloferax mediterranei, the saline model for denitrification

Javier Torregrosa-Crespo[1], Linda L. Bergaust[2], Carmen Pire[1], David J. Richardson[3], Rosa Maria Martínez-Espinosa[1]

[1]Dpto Agroquímica y Bioquímica, Facultad de Ciencias, Universidad de Alicante, Carretera Sant Vicent del Raspeig s/n - 03690. Sant Vicent del Raspeig, Alicante, Spain, [2]Faculty of Chemistry, Biotechnology and Food Science. Norwegian University of Life Sciences. Chr M Falsens vei 1 - 1430, Ås, Norway, [3]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK.

javitorregrosa@ua.es

Keywords: Haloarchaea, denitrification, extremophiles

Haloferax mediterranei (R4) belongs to the group of halophilic archaea, one of the predominant microbial populations in hypersaline environments. In these ecosystems, the low availability of oxygen and the increasing concentrations of N-oxyanions pushes the microbial inhabitants towards denitrification. In a recent study comparing three *Haloferax* species carrying N-oxide reductases, *H. mediterranei* showed promise as a future model for archaeal denitrification [1]. In this sense, the last results about nitrate/nitrite respiration by this haloarchaeon are here presented.
First, *H. mediterranei* was grown under different concentrations of nitrates/nitrites in order to test its ability to tolerate/reduce high concentrations of N-oxyanions. Although it was able to grow in media with up to 2 M of nitrate, it could only sustain a few generations of exponential anaerobic growth, apparently requiring micro-oxic conditions for *de novo* synthesis of denitrification enzymes.
Second, under anaerobic conditions, the membrane fraction of *H. mediterranei* was solubilised and analysed by LC-MS/MS to identify the respiratory proteins associated to it. It could be identified proteins involved in denitrification, electron transfer processes as well as ATP synthesis. This is the first characterisation of the denitrifying machinery in haloarchaea using proteomics.

Acknowledgments: this work was supported by the University of Alicante (VIGROB-309), the Spanish Ministerio de Ciencia, Innovación y Universidades (RTI2018-099860-B-I00), the European Molecular Biology Organization (EMBO – 331-2016) and Generalitat Valenciana (ACIF2016/77).
References:
1. Torregrosa-Crespo *et al*. (2019). *Environ Microbiol*, **21**(1):427-36.

Haloarchaeal denitrification: A first look at its regulation

Jose Maria Miralles-Robledillo, Javier Torregrosa-Crespo, Rosa Maria Martínez-Espinosa, Carmen Pire

Department of Agrochemistry and Biochemistry, Faculty of Science, University of Alicante, Alicante, Spain.

jmmr19@alu.ua.es

Keywords: haloarchaea, denitrification, promoter activity, beta-galactosidase assay, regulation

Haloferax mediterranei is a halophilic archaeon that can completely reduce nitrate (NO_3^-) to dinitrogen (N_2) in a series of reactions catalysed by four metalloenzymes under anoxic conditions [1]. This ability has allowed us to use it as model for investigations about the role of haloarchaea in the denitrification pathway and their potential use in wastewater treatments.

Information about denitrification in the *Archaea* domain is still scarce. Its biochemical bases are known, but there is only one report about its regulation [2]. In order to elucidate how environmental signals and transcriptional factors regulate denitrification in this kind of microorganisms, we are characterizing the promoter region of the genes that encode the N-reductases from *H. mediterranei*. This characterization has been performed by bioinformatic analysis and by fusion of the respiratory nitrate (*nar*) and nitrous oxide reductase (*nos*) operon promoters to the reporter gene *bgaH* (β-galactosidase) from *Haloferax lucentense.*

On the one hand, bioinformatic analysis have identified a palindromic motif that could be a potential target of regulation from genes related to denitrification. On the other hand, reporter gene assay results display that denitrification pathway does not require a drastic oxygen depletion to become activated. Furthermore, the *nos* promoter is activated before the *nar* promoter, suggesting being more sensitive to oxygen drop.

Acknowledgments: This work is funded by the Spanish Ministry of Science, Education and Universities (RTI2018-099860-B-I00) and the University of Alicante (VIGROB-309).

References:

1. Torregrosa-Crespo J, Pire C, Martínez-Espinosa RM, Bergaust, L. (2019) *Environ Microbiol*. **21**(1): 427-36.

2. Hattori T, Shiba H, Ashiki K, Araki T, Nagashima YK, Yoshimatsu K, Fujiwara T. (2016). *J Bacteriol*. **198**(7): 1077-86.

Nitric oxide regulates bet-hedging in *Paracoccus denitrificans* during the transition to anoxia

Linda L. Bergaust[1], Santosh Kumar[2], Alexander Jarvis[3], Andrew Gates[3], Stephen Spiro[2], and Lars Bakken[1]

[1]Norwegian University of Life Sciences, Faculty of Chemistry, Biotechnology and Food Science, Ås, Norway. [2]University of Texas at Dallas, Department of Biological Sciences, Richardson, Texas, USA. [3]School of Biological Sciences, University of East Anglia, Norwich, United Kingdom.

linda.bergaust@nmbu.no

Keywords: denitrification, nitric oxide, bet-hedging

When switching from aerobic respiration to denitrification, clonal populations of *Paracoccus denitrificans* (Pd1222) display phenotypic diversification: a minority of cells synthesize the nitrite reductase NirS, whereas all cells produce the N_2O reductase NosZ. This bet-hedging phenomenon has been demonstrated by tracking NirS and NosZ in a strain carrying a chimeric *mCherry-nirS* fusion gene. However, there is still a way to go to understand the regulatory mechanisms involved [1]. Nitric oxide (NO) and oxygen (O_2) are both central, and opposite, players in controlling denitrification and their respective regulatory pathways are likely to modulate cell differentiation in *P. denitrificans*. We monitored NirS activity and expression during oxygen depletion and subsequent denitrification onset in a collection of strains with altered propensities for NO release: a *norCB* knockout, and strains lacking or overexpressing *hmp*. Hmp is a NO dioxygenase primarily active under oxic conditions. Its absence likely leads to a less-stringent regulation of NO during the oxic-anoxic transition, and thus a higher probability of *nirS* expression. In line with this, the *hmp* deletion strain displayed less pronounced bet-hedging than wild-type, whereas Hmp overproduction resulted in a decrease in the Nir positive fraction. Thus, Hmp appears to promote bet-hedging by limiting the positive signal for induction of Nir and thus initiation of denitrification. The NO-scavenging enzyme Nor is crucial during denitrification in closed systems where its absence leads to the generation of lethal concentrations of NO. Thus, the Δ*norCB* strain was grown in co-culture with FITC stained Pd1222. When facing anoxia, all Nor deficient cells synthesized Nir, whereas the Pd1222 subpopulation appeared to diversify. This illustrates that the intracellular feedback loop with NO is critical for full expression of *nirS*, at least in liquid cultures with nM concentrations of NO, where intercellular induction of *nirS* appeared not to take place.

References: 1. Lycus *et al*. (2018). Proc Natl Acad Sci, **115**(46):11820-11825.

Differential expression of two copies of nitrite and nitrous oxide reductase genes in *Pseudomonas stutzeri* and *Thauera linaloolentis*, respectively

Lea Wittorf[1], Miguel A. Semedo[2], Christopher M. Jones[1], Germán Bonilla-Rosso[1], Bongkeun Song[2], Sara Hallin[1]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[2]Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, USA.

Lea.Wittorf@slu.se

Keywords: Denitrification, *Pseudomonas stutzeri*, *Thauera linaloolentis*, gene co-occurrence

Denitrification is a stepwise respiratory pathway in which nitrate is reduced to dinitrogen. The reactions are catalyzed by different enzymes or versions of the same enzyme. Two examples are the reduction of nitrite to nitric oxide by the copper or iron dependent nitrite reductases, encoded by the genes *nirS* and *nirK*, and the reduction of nitrous oxide to dinitrogen, encoded by the gene *nosZ*, which has two distinct genotypes, *nosZ*I and *nosZ*II. The two nitrite reductases have long been considered mutually exclusive and functionally redundant in denitrifying bacteria. Similarly, we only begin to understand the differences in the functionality and ecology of bacteria carrying *nosZ*I and *nosZ*II genes. Here we show that *nirS* and *nirK* [1] co-occurring in two strains of *Pseudomonas stutzeri* and *nosZ*I and *nosZ*II in *Thauera linaloolentis* can be expressed simultaneously. The differential expression patterns within and between strains in relation to oxygen, nitrate and nitrous oxide, and their different denitrification phenotypes, suggest that *nirS* and *nirK*, as well as *nosZ*I and *nosZ*II can have different roles in organisms in which they co-occur. Dissimilar gene arrangements and transcription factors in the *nir* gene neighborhoods could explain the observed differences in *nir* gene expression and denitrification activity.

References:

1. Wittorf *et al.* (2018). *Res Microbiol* **169**:343–347.
4. Oliveri *et al*. (2014). *Chem.-Eur. J.*, **20**(29): 8954−64.
5. Oliveri *et al*. (2015). *ChemPlusChem*, **80**(4), 762-70.

Nitric Oxide Reductase – from fundamental studies to applied research

Cristina M. Cordas[1], Sofia R. Pauleta[2], Luísa B. Maia[1], José J. G. Moura[1], Isabel Moura[1]

[1]LAQV, Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.
[2]UCIBIO, Requimte, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.

cristina.cordas@fct.unl.pt

Keywords: Denitrification, Nitric Oxide Reductase, Enzymatic catalysis, Electrochemistry

Nitric Oxide Reductase (NOR) is a relevant enzyme in the denitrification pathway, catalysing the reduction of NO to N_2O. The enzyme isolated from *Marinobacter hydrocarbonoclasticus* (a marine bacterium) comprises two subunits (NORB and NORC) harbouring an electron transfer *c*-type heme (on the NORC subunit) and a catalytic site consisting of a b_3-type heme linked to a non-heme Fe atom and a *b*-type heme (on the NORB subunit). The physiological redox partner was demonstrated to be cytochrome c_{552} (Cyt c_{552}) [1, 2]. The NOR (and its subunit NORC) electrochemical behaviour was well characterized and its catalytic properties/mechanisms were extensively studied [2-5]. The accumulated knowledge allowed the development of the first third generation biosensor for NO [6]. This well-succeed history of research (more than one decade) encourage us to further develop fundamental structural and mechanistic studies towards outreaches that can be beneficial to the Society.

Acknowledgments: This work was supported by the Associate Laboratory for Green Chemistry-LAQV, with national funds from FCT/MCTES (UID/QUI/50006/2019) and by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). Also, the authors acknowledge FCT-MCTES for funding through Project PTDC/BBB-BQB/0129/2014 (IM).

References:
1. Timóteo *et al.* (2011). *Biochemistry*, **50**(20): 4251-62.
2. Duarte *et al.* (2014). *BBA-Bioenergetics*, **1837**(3): 375-84.
3. Cordas *et al.* (2006). *ChemBioChem,* **7**(12): 1878-84.
4. Cordas *et al.* (2013). *BBA-Bioenergetics,* **1827**(3): 233-8.
5. Ramos *et al.* (2017). *J. Inorg. Biochem.,* **177**: 402-11.
6. Gomes *et al.* (2019). *Sensors&Actuators B: Chemical,* **285**: 445-52.

Role of O_2 in recruitment to nitrite reduction in the bet-hedging denitrifier *Paracoccus denitrificans*

Ricarda Kellermann[1], Kjetil Hauge[1], Rannei Tjåland[1], Sebastian Thalmann[2], Linda L. Bergaust[1], Lars Bakken[1]

[1]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Ås, Norway.
[2]Supervisor Field Applications Luminex, Het Zuiderkruis 1, 5215 MV 's-Hertogenbosch, Netherlands.

ricarda.kellermann@nmbu.no

Keywords: denitrification, bet-hedging, flow cytometry

When anoxia is imminent, denitrifying organisms synthesize four N-oxide reductases (NAR, NIR, NOR and NOS) using the remaining O_2 as electron acceptor. In *Paracoccus denitrificans* these enzymes are not uniformly produced in all cells. Instead, isogenic cultures diversify into active (NIR + NOR) and inactive (no NIR + NOR) subpopulations [1]. This "bet-hedging" is likely driven by low probability, stochastic induction of *nirS,* which increases with hypoxia and then becomes autocatalytic [2]. Investigation of O_2 as a critical factor for recruitment to denitrification has been challenging, as it requires complete removal of O_2 from cultures. Moreover, although evidence suggest that inactive cells remain viable during prolonged anoxia, their response to short oxic spells has not been tested.

Glucose oxidase (GOX) was used to remove all traces of O_2 from the medium. A *P. denitrificans* strain carrying a *mCherry-nirS* fusion gene was raised aerobically and stained with FITC before transfer to anoxic vials with nitrite in the medium. Subsequent gas kinetics were monitored using a robotized incubation system. Single-cell FITC and mCherry fluorescence was monitored by flow cytometry, showing complete entrapment in anoxia in GOX-treated cultures: mCherry remained below detection, no nitrite reduction or dilution of FITC stain was observed. A short oxic spell after 72 h resulted in mCherry-NIR synthesis and growth in a subpopulation, demonstrating the robustness of arrested cells on the one hand, and the importance of O_2 for energy conservation during adaptation to anoxia on the other.

References:

1. Lycus *et al.* (2018). *PNAS*, **115**(46):11820-11825.
2. Hassan *et al.* (2014). *PLoS Comput Biol,* **10**(11): e1003933.

Sensing L-arginine in *Pseudomonas aeruginosa* links energy metabolism and C-di-GMP levels

Giorgio Giardina[1], Alessandro Paiardini[1], Federico Mantoni[1], Adele Di Matteo[2], Leoni L[3], Rampioni G[3], Francesca Cutruzzolà[1], Serena Rinaldo[1]

[1] Laboratory affiliated to Istituto Pasteur Italia - Fondazione Cenci Bolognetti – Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza Unviersity of Rome, Rome, Italy
[2] Istituto di Biologia e Patologia Molecolari, Consiglio Nazionale delle Ricerche, Rome, Italy.
[3] Department of Science – University Roma Tre, Rome, Italy.

serena.rinaldo@uniroma1.it

Keywords: C-di-GMP, Arginine, FAD, metabolism

Bacterial biofilms are difficult to be eradicated since this lyfestyle confers to bacteria resistance to antimicrobial treatments and host defences. The second messenger c-di-GMP controls the metabolic re-programming required for biofilm formation and dispersion (the latter upon decrease of c-di-GMP) in response to environmental cues, including NO and nutrients [1]. Among nutrients, arginine represents one key metabolite in biofilm formation being at the crossroad of many metabolic processes and acting as a substrate for NO production by the host immune system. Indeed, in *P. aeruginosa*, arginine is associated to chronic infections, biofilm/virulence and antibiotic resistance.

We recently found that *P. aeruginosa* is able to perceive environmental arginine to decrease the intracellular levels of c-di-GMP *via* the RmcA (Redox regulator of c-di-GMP) protein [2, 3], a multidomain membrane protein. Here we show that RmcA may perceive the metabolic status of the cell *via* FAD/$FADH_2$ sensing and consequently affects the central metabolism in response to a nitrogen nutrient such as arginine. Therefore, arginine can modulate the metabolic fate of the bacterial cell through the c-di-GMP messenger, thus widening the impact of this nucleotide also in the re-shaping of the central metabolism.

References:

1. Rinaldo *et al*. (2018). *FEMS Microbiol Lett.*, **365**(6).
2. Paiardini *et. al.* (2018) *Proteins.* **86**(10): 1088-1096.
3. Mantoni *et al.* (2018) *FEBS J.* **285**(20): 3815-3834.

Sinorhizobium meliloti nitrate assimilation pathway: contribution to NO production

Bryan Ruiz, Alexandre le Scornet, Laurent Sauviac, Antoine Rémy, Claude Bruand and Eliane Meilhoc

Laboratoire des Interactions Plantes-Microorganismes (LIPM), Université de Toulouse, INRA, CNRS, INSA, 31326 Castanet-Tolosan, France.

Eliane.Meilhoc@inra.fr

Keywords: nitric oxide, denitrification, *Sinorhizobium meliloti*, *Medicago truncatula*, nitrogen fixing symbiosis

The interaction between rhizobia and their legume host plant conduces to the formation of specialized root organs called nodules in which differentiated endosymbiotic bacteria (bacteroids) fix atmospheric nitrogen to the benefit of the host plant. Interestingly, nitric oxide (NO) has been detected at different steps of the rhizobium-legume symbiosis where it has been shown to play multifaceted roles [1]. Both bacterial and plant partners are involved in NO synthesis. On the plant side, nitrate reductase appears as a major player in NO synthesis. On the bacterial side, *S. meliloti* does not possess any NO synthase, instead, the denitrification pathway is often described as the main driver of NO production. In *Bradyrhizobium japonicum* and *Rhizobium etli* the nitrate assimilatory pathway was also shown to participate to NO production [2, 3].

S. meliloti possesses a putative nitrate assimilation pathway, encoded by the *narB* and *nirBD* genes, which has not been characterized so far. In the work presented here, we investigated the involvement of NarB and NirB in nitrate assimilation and NO synthesis. Our results support the hypothesis that in *S. meliloti*, denitrification is the main enzymatic way to produce NO while the assimilatory pathway involving NarB and NirB participates indirectly to NO synthesis by cooperating with the denitrification pathway. We found that both pathways are subjected to different regulation. Finally, we found that *narB* and *nirB* are not essential to maintain an efficient nitrogen fixing symbiosis with *Medicago truncatula* even though they are expressed in the nodule nitrogen fixation zone [4].

References:
1. Hichri et al. (2016). *Adv. Bot. Res*., **77**: 213-218.
2. Cabrera et al. (2016). *Biochem. J*., **473**(3): 297-309.
3. Hidalgo-García et al. (2019). *Front. Microbiol.,* **10**: 980.
4. Ruiz et al. (2019) *Front. Microbiol*., July 3.

Lessons from Denitrification to the human metabolism of signaling nitric oxide

Luísa B. Maia, José J. G. Moura

LAQV, REQUIMTE, FCT, NOVA, Portugal.

luisa.maia@fct.unl.pt

Keywords: nitrite, nitric oxide, human metabolism, signaling, xanthine oxidase

Nitric oxide radical, •NO (NO), is a signaling molecule involved in several physiological processes in humans, including vasodilation, immune response, neurotransmission, platelet aggregation, apoptosis or gene expression. Undue normal conditions, NO synthases catalyse the formation of NO from L-arginine and dioxygen. Yet, upon a hypoxic event, when the decreased dioxygen concentration compromises the NOS activity, cells can generate NO from another source: nitrite! Since late 2000s, it became clear that nitrite can be reduced back to NO under hypoxic/anoxic conditions. Simultaneously, it was realized that nitrite can exert a significant cytoprotective action *in vivo*, during ischemia and other pathological conditions. Presently, blood and tissue nitrite are recognized as a NO "storage form" that can be made available to maintain NO formation and ensure cell signaling and survival under challenging conditions. To reduce nitrite to NO, human cells can use different metalloproteins, present in cells to carry out other functions, including several heme proteins and molybdoenzymes, forming what we denominated as "non-dedicated nitrite reductases" [1-4]. In this communication, some non-dedicated nitrite reductases, including xanthine oxidase, aldehyde oxidase (molybdoenzymes) and myoglobin (heme protein), will be described and the human nitrate/nitrite/NO signaling pathway will be discussed within the cellular context and the nitrogen cycle big scenario.

Acknowledgments: This work was supported by the Associate Laboratory for Green Chemistry-LAQV, which is financed by national funds from Fundacão para a Ciência e a Tecnologia, MCTES (FCT/MCTES; UID/QUI/50006/2019). LBM also thanks to FCT/MCTES, for the CEEC-Individual 2017 Program Contract.

References:

1. Maia *et al.* (2018). *Redox Biol.*, **19**: 274-289.
2. Maia *et al.* (2015). *J. Biol. Inorg. Chem.*, **20**: 403-433.
3. Maia *et al.* (2015). *Biochemistry*, **54**: 685-710.
4. Maia *et al.* (2014). *Chem. Rev.*, **114**: 5273-5357.

Production of NO by Xanthine Oxidase – a QM/MM study

Henrique S. Fernandes[1], Sérgio F. Sousa[1], Luísa B. Maia[2], José J. G. Moura[2], Nuno M.F.S.A. Cerqueira[1]

[1]UCIBIO, REQUIMTE, BioSIM, Dpt. Biomedicina, Faculdade Medicina, Universidade do Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal.
[2]LAQV, REQUIMTE, FCT, NOVA, Campus de Caparica, 2829-516 Caparica, Portugal.

hfernandes@med.up.pt

Keywords: xanthine oxidase, nitric oxide, molybdenum, mechanism, QM/MM

Xanthine Oxidase (XO, EC 1.17.3.2) is a molybdenum-containing enzyme that under hypoxic conditions can catalyze the reduction of NO_3^- to NO_2^- and of NO_2^- to $^\bullet$NO.[1] $^\bullet$NO is an important signaling molecule and its deficiency in humans has been associated with some disease conditions such as myocardial infarction, systemic and pulmonary hypertension and gastric ulceration.[2] In this work, the reaction mechanism of XO-catalyzed NO_3^- and NO_2^- reduction to $^\bullet$NO was studied, with an atomic level of detail, using computational means. The ONIOM QM/MM methodology was employed to characterize all the minima and transition-state structures using the B3LYP/6-31G(d,p):ff99SB scheme with the LanL2dz as pseudo-potential for molybdenum. The results obtained support the $^\bullet$NO-forming nitrate and nitrite reductase activities of XO observed experimentally.[1] The reaction mechanism of NO_3^- reduction involves three-sequential steps: NO_3^- binding to the molybdenum ion (at the enzyme active site); heterolytic cleavage of N-O bond; and the enzymatic turnover. In this process, the metal is oxidized from Mo(IV) to Mo(VI), making it ready to start a new catalytic cycle. The reduction of NO_2^- follows the same type of mechanism, but the N-O bond undergoes a homolytic cleavage. Consequently, the metal is oxidized to Mo(V) in the first cycle and then, a new NO_2^- molecule is reduced to $^\bullet$NO leading to oxidation of the metal to Mo(VI), thus closing the enzymatic turnover. These results support the catalytic ability of XO to form $^\bullet$NO from NO_3^- and NO_2^-.

Acknowledgments: Fundação para a Ciências e Tecnologia (SFRH/BD/115396/2016, IF/01310/2013, IF/00052/2014, UID/Multi/04378/2019, PTDC/QUI-QFI/31689/2017).

References:

1. Maia *et al*. (2018). *Redox Biology*, **19**:274.89.
2. Lundberg *et al*. (2008). *Nat Rev Drug Discov*, **7**(2):156.167.

Deciphering the metabolism of nitrite-oxidizing *Nitrospira* – a multi-omics approach

Hanna Koch[1], Mundinger A.B.[1], Lawson C.E.[2], Pabst M.[3], Jetten M.S.M[1], Lücker S. [1]

[1]Department of Microbiology, Institute for Water and Wetland Research, Radboud University, Nijmegen, Netherlands.
[2]Department of Civil and Environmental Engineering, University of Wisconsin–Madison, Madison, United States.
[3]Department of Biotechnology, Delft University of Technology, Delft, Netherlands.

H.koch@science.ru.nl

Keywords: nitrification, nitrite oxidation, *Nitrospira*, transcriptomics, proteomics

Nitrite oxidizing bacteria (NOB) catalyze the second step in nitrification, nitrite oxidation to nitrate, and are important drivers of the global biogeochemical nitrogen cycle. Members of the genus *Nitrospira* are known key NOB that show a high metabolic and phylogenetic diversity. Notably, genomic analyses [1, 2] revealed major differences in the core metabolism of *Nitrospira* compared to other NOB, including e.g. the nitrite oxidation and carbon fixation machineries. However, we still lack profound insights into the physiology of *Nitrospira*.

In this study, we established a continuous stirred tank reactor system to cultivate *Nitrospira moscoviensis* [3], which enabled cultivation under stable growth conditions by accurate control of incubation parameters. A multi-omics approach using transcriptomic and proteomic profiling allowed a comprehensive expression analysis under nitrite-oxidizing conditions.

Transcriptome and proteome data supported the genome-based metabolic predictions, including expression of proteins required for carbon fixation, assimilatory nitrite reduction, and the respiratory chain. Additionally, the transcriptome data indicated that the nitrite oxidoreductase (NXR), the enzyme catalyzing nitrite oxidation, is formed by three soluble periplasmic subunits. Contrastingly, preliminary complexome analysis indicated a co-migration pattern of the soluble alpha and beta subunits with a membrane-bound subunit, which might anchor the NXR to the cytoplasmic membrane. Overall, this multi-omics approach greatly refined our understanding of the metabolism of *Nitrospira*.

References:
1. Koch, H.*, et al.* (2015). *Proc Natl Acad Sci U S A*, **112**(36): 11371-11376.
2. Lücker, S.*, et al.* (2010). *Proc Natl Acad Sci U S A*, **107**(30): 13479-13484.
3. Mundinger, A.B.*, et al.* (2019). *Front Microbiol*, **10**: 15.

In quest of ureolytic heterotrophs to pair with nitrifiers in a defined bacterial community for human urine treatment

Justyna Barys[1,2], Carolina Arnau[1], Francesc Godia[1], Nico Boon[2], Siegfried E. Vlaeminck[2,3], Peter Clauwaert[2]

[1]MELiSSA Pilot Plant – Laboratory Claude Chipaux, Universitat Autònoma de Barcelona, Spain; [2]Center for Microbial Ecology and Technology, Faculty of Bioscience Engineering, Ghent University, Belgium; [3]Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering, Faculty of Science, University of Antwerp, Belgium.

justynaanna.barys@e-campus.uab.cat

Keywords: Urine, Urea hydrolysis, Nitrification, Synthetic bacterial community

Human urine treatment will be needed in a future space life support systems to provide stable nitrogen (N) fertilizer for a phototrophic food production. Human urine contains 85% of the crew's N uptake, mainly present as urea. To enable food production, it is desirable to convert urea to nitrate, which can biologically be achieved through combining ureolysis and nitrification, based on a consortium of three functional groups of bacteria: i) urea hydrolyzing bacteria (heterotrophs and/or AOB), ii) ammonium oxidizing bacteria (AOB) and iii) nitrite oxidizing bacteria (NOB).

Nitrosomonas europaea and *Nitrobacter winogradskyi* have been previously selected as AOB and NOB representatives respectively. The main goal of conducted research was to develop a synthetic microbial consortium able to perform ureolysis and nitrification in the synthetic urine (SU) and real urine (RU). Selected heterotrophs needed to meet specific requirements: nonpathogenic, non-spore forming, ureolytic, active in a consortium with nitrifiers. Cultures of selected single heterotrophs (*Pseudomonas fluorescens, Comamonas testosteroni, Acidovorax delafieldii, Delftia acidovorans, Cupriavidus necator*) were cultivated in SU and RU to evaluate their urea hydrolysis. Based on highest urea hydrolysis rates *C. testosteroni* (SU=57.02±10.28 mg N/L*d; RU=35.80±9.52 mg N/L*d), *P. fluorescens* (SU=58.07±13.23 mg N/L*d; RU=41.30±0.83 mg N/L*d) and *A. delafieldii* (SU=44.28±3.05 mg N/L*d, RU=17.20±4.05 mg N/L*d) were selected to be tested in the consortium with nitrifiers in SU and RU. Nitrate production rates in the co-cultures equaled 25.11±9.96 mg N/L*d for SU and 16.51±4.47 mg N/L*d for RU. Performed research proved that selected microbial consortium is able to convert urea from human urine to nitrate, without ammonium and nitrite accumulation.

Evaluation of the denitrification efficiency of estuarine sediments exposed to Cu nanoparticles

Joana Faria da Costa[1], António G.G. Sousa[1], Ana Carolina Carneiro[1,2], Marisa Almeida[1,3], Catarina Magalhães[1,2], Mafalda S. Baptista[1,3]

[1] CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros de Leixões, Matosinhos, [2]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre s/n, 4169-007, Porto, Portugal; [3]Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua Campo Alegre s/n, 4169-007, Porto, Portugal.

jcosta@ciimar.up.pt

Keywords: microcosms, bacteria, gene expression, aquatic environment

Despite the use of copper nanoparticles (Cu-NPs) having increased in absolute values and in number of applications, current knowledge about their effects on environmental microbial communities is still scarce. Aquatic environments are prone to be the final destination of several types of xenobiotic compounds, such as Cu-NPs, not efficiently removed from water treatment plants. The goal of our study was to assess the impact of environmentally relevant concentrations of Cu-NPs in the denitrifying bacterial communities of estuarine sediments (Douro river, Portugal). In laboratory microcosms two Cu-NPs sizes (50 and 150nm) and three concentrations (0.01, 0.1 and 1µg g^{-1} of sediment) of Cu-NPs were tested up to 6 days. Expression of nitrite (*nirS*) and nitrous oxide reductase (*nosZ*) genes and denitrification rates were assessed. For 50 nm Cu-NPs, an upregulation of the expression was seen for both genes up until 24 h; however, for 150 nm Cu-NPs, after 7 h a downregulation of the expression could be seen; and moreover, the production of N_2 was inhibited by 20%. After 6 days both genes were again up-regulated when exposed to 50 nm Cu-NPs, while they remained down-regulated after exposure to 150 nm Cu-NPs.

Our results suggest that denitrifying communities are immediately activated after Cu-NPs exposure, followed by a period of diminished activity. After one week of exposure, signs of recovered activity where observed only for 50nm NPs. These findings point to an interference of Cu-NPs on the denitrification process, and consequently on the nitrogen cycle in estuarine sediments.

Bradyrhizobia can act as strong sinks or sources of N_2O in agricultural ecosystems

Yuan Gao[1,2,3], Daniel Mania[1], Seyed Abdollah Mousavi[2], Kedir Woliy[1], Lars Bakken[1], Kristina Lindström[2], Åsa Frostegård[1]

[1]Faculty of Chemistry, Biotechnology and Food Sciences, Norwegian University of Life Sciences, Norway.
[2]Faculty of Biological and Environmental Sciences, University of Helsinki, Finland. [3]International Research and Development Center, Kingenta Ecology Engineering Group Co. Ltd., China.

yuan.gao@nmbu.no

The legume-rhizobium symbiosis accounts for the major part of the biological N_2 fixation in agricultural systems, reducing the need for synthetic nitrogen fertilizers. The N_2 fixation efficacy varies strongly between combinations of plant cultivar and rhizobial strains, and to utilize the full potential, legumes therefore often have to be inoculated with compatible rhizobia. Several economically important legumes such as peanut, soybean and cowpea fix N_2 in symbiosis with bacteria belonging to the genus *Bradyrhizobium.* Many bradyrhizobia are also denitrifiers, and some studies with greenhouse and field experiments show that inoculation with some strains of bradyrhizobia can mitigate N_2O emissions. Yet, there is limited knowledge on how widespread the trait is within this genus. We recently found [1] that half of the strains in one collection of bradyrhizobia could reduce N_2O, while the others lacked this capacity and thus instead would act as sources for this greenhouse gas. We also found that the strains that expressed N_2O reductase showed a strong preference for N_2O over nitrate as electron acceptor, making them potentially strong N_2O sinks, and hypothesized that the electron pathway to N2OR competes very efficiently for electrons compared to that of Nap. Here, we present results from another collection of bradyrhizobia, isolated mostly from peanut nodules, which support our hypothesis. We also extended our earlier study to include quantifications of the denitrification reductases by proteomics and, further, we present how the phenotype is affected by starvation.

Reference:
1. Daniel Mania, Kedir Woliy, Tulu Degefu, Åsa Frostegård (2019). *Environ. Microbiol. In Press*.

Changes in either plant or soil microbial diversity constrain plant microbiome selection and affect the potential for N_2O emissions

Aurélien Saghaï[1], Daniel Graf[1], Lea Wittorf[1], Ming Zhao[2], Georg Carlsson[3], Christopher Jones[1], Sara Hallin[1]

[1]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology.
[2]Swedish University of Agricultural Sciences, Department of Plant Biology, Sweden.
[3]Swedish University of Agricultural Sciences, Department of Biosystems and Technology, Sweden.

aurelien.saghai@slu.se

Keywords: intercropping, soil microbial diversity, rhizosphere, N_2O

Agriculture intensification reduces the complexity of plant communities and is characterized by high N losses, especially through large emissions of the greenhouse gas N_2O. Increasing both above and belowground biodiversity is often presented as a solution to mitigate such losses. The rhizosphere is a hotspot for N cycling, and harbors microbial communities that are mainly shaped by a combination of edaphic and plant-derived factors. However, the interactions between roots of different plant species and the community composition and structure of the surrounding soil can also influence plant microbiome selection. Understanding how they affect the establishment of the microbial communities is crucial, as the only known biological sink for N_2O is its reduction to nitrogen gas by bacteria and archaea.

Here, we present data from two studies looking at the effect of (i) plant diversification, in terms of intercropping between a legume and a grass species, and (ii) soil bacterial diversity loss on the potential for N_2O emissions in the rhizosphere. We show that the presence of the legume influenced the composition of the grass rhizosphere, which led to decreased genetic potential for N_2O reduction and higher N_2O production rates in the rhizosphere of the grass species during intercropping compare to sole cropping. In barley, the reduction in bacterial diversity promoted the assembly of rhizosphere communities with the genetic potential to act as N_2O sinks. Our findings indicate that there is no straightforward relationship between biodiversity and N_2O emission potential.

Nitrogen-transforming guilds and denitrification rates in lakes of mountains affected by high atmospheric nitrogen deposition

Carlos Palacin-Lizarbe[1], Sara Hallin[2], Luís Camarero[3], Christopher M. Jones[2], Jordi Catalan[1]

[1]CREAF, Campus UAB, Cerdanyola del Vallès, Spain.
[2]Swedish University of Agricultural Sciences, Department of Forest Mycology and Plant Pathology, Uppsala, Sweden.
[3]Center for Advanced Studies of Blanes, (CEAB–CSIC), Girona, Spain.

cpalacli7@gmail.com

Keywords: remote ecosystems, genetic potentials, activation energy

The nitrogen (N) cycle has been profoundly anthropogenically modified, doubling the reactive nitrogen (N_r) available in the biosphere. Denitrification is the way back by eliminating N_r from the system. However, there is limited knowledge on field denitrification rates, as well as of the microbial guilds involved in the related N transformations. Some mountains have been particularly affected by N deposition with lakes increasing N_r levels. We aimed to investigate how denitrification could have been affected. We studied the benthic habitats of eleven mountain lakes in the Pyrenees with different productivity features. We found a complex composition of N-transforming guilds deeply embedded in the general prokaryotic community. The dominant N transformation pathway changed depending on the habitat and lake productivity. The DNRA-Denitrification dichotomy was the main difference, with a predominance of nitrite-reducing denitrifiers (*nirS*) in the upper sediment layers of shallow, warmer and more productive lakes [1]. An average denitrification rate of 1.5 µmol N_2O m^{-2} h^{-1} was estimated in the lake sediments, with greater activity in the littoral zone. Current potential denitrification rates were controlled by the nitrate availability and secondarily by temperature; while potential rates were determined by the system productivity, sulphate content and DNRA-denitrification (*nrfA*-*nirS*) competition [2]. Denitrification temperature dependence increased with nitrate limitation [3].

References:
1. Palacin-Lizarbe *et al*. (2019) *Front Microbiol*, **10:** 1229.
2. Palacin-Lizarbe *et al*. (2019) *In prep.*
3. Palacin-Lizarbe *et al*. (2018) *Water Resour Res,* **54:**1161-1173.

Field-scale spatial variation in denitrifying microbial communities and the impacts on arable crop yield

Claire E. Brown[1], C. Blacker[2], S. Leese[2], T. Helgason[1], C. Dytham[1], J.W.B. Moir[1]

[1]Biology, University of York, York, YO10 5DD, UK.
[2] Precision Decisions Ltd. Shipton by Beningbrough, York, YO30 1BS, UK.

ceb561@york.ac.uk

Keywords: Denitrifying communities; Soil microbiology, Agroecosystems, Spatial mapping, Yield

Denitrification results in loss of available nitrogen from terrestrial ecosystems through the microbially-mediated reduction of nitrate to nitrogen gases. Despite nitrogen being the most limiting nutrient in agro-ecosystem productivity [1], little is currently known about the effects of denitrifying microbial community structure on crop yield. Furthermore, since microbial processes, such as denitrification, can vary significantly over field scale [2], it is important to understand the spatial distribution of the microbial guilds which mediate these processes. Using a spatially-structured, stratified sampling regime we used amplicon sequencing and novel methods of abundance measurement of 16S rRNA and nitrite reductase genes *nirK* and *nirS* to investigate how denitrifying communities vary between high and low yield soils with the same physico-chemical footprint.

We found clear distance-decay relationships over the field scale, with denitrification genes more strongly affected than the overall microbial population. We also found elevated *nirK* numbers in low yield soils. Additionally, yield could account for approximately 5% of the variation in nitrite reductase diversity but not in 16S rRNA diversity, suggesting that denitrifying community composition may be implicated in the loss of yield in otherwise favourable soils. Altogether, these results lay the foundations for a better understanding of the role of soil microbial communities on agricultural productivity and how it may ultimately be targeted by farming practices to meet the global food demand.

Acknowledgments: This work was funded by a BBSRC NPIF studentship. We thank C. Blacker and S. Leese of Precision Decisions for permission to work at Harold Smiths field.

References:

1. Rütting *et al*. (2018). *Nutr Cycl Agroecosys*, **110**(1):1-5.
2. Philppot *et al*. (2009). *Environ Microbiol*, **11**(6):1518-1526.

Evolution of nitrogen cycling in tropical forests

Viviane Figueiredo[1], Alex Enrich-Prast[2], Tobias Rütting[3]

[1]Federal University of Rio de Janeiro, Department of Botany, Rio de Janeiro, Brazil, [2]Department of Environmental Change, Linköping University, Linköping, Sweden, [3]University of Gothenburg, Department of Earth Science, Gothenburg, Sweden.

vivi.bioufrj@hotmail.com

Keywords: Nitrogen, soil, Amazon forest, Atlantic forest

Extensive regions of tropical forests are subjected to deforestation and forest regrowth and both strongly affect soil nutrient cycling [1]. Nitrogen (N) dynamics changes during forest regrowth and functioning similar to pristine conditions depends on N availability. We evaluated gross N processes using ^{15}N pool dilution in Amazonian and Atlantic forest. We show that, in a chronosequence of Amazonian forests, gross nitrification was lower in all stages of regrowing (10, 20, 40 yrs) compared to pristine forest. This indicates the evolution of a more conservative and closed N cycle [2] with reduced risk for N leaking out of the ecosystem, a robust pattern around the world [3]. In Atlantic forest, an opposite pattern was found in the two studied forests, with higher gross mineralization in pristine than in a 10 years regrowth forest, while gross nitrification was higher in regrowth forest, suggesting an open N-cycle in recovery forest. These unexpected findings were associated to the recent management in restored forest, which alters the soil characteristics and causes depletion of soil SOM and TN. Our findings suggest an N limitation in both forests, but mainly in pristine with a very low nitrification rate compared to mineralization, highlighting other processes as immobilization. It shows the importance of biogeochemical studies in a long-term scale in restored areas, to evaluate the mechanisms of and time needed for forest recovery [3].

Acknowledgments: VF and AEP thanks for CNPq, Capes, FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro) and STINT (The Swedish Foundation for International Cooperation in Research and Higher Education) for travel support from Brazil to Sweden for sample analyses. V.F. has a post-doctoral fellowship from FAPERJ NOTA 10 program. T.R. is supported by the Strategic Research Area BECC (Biodiversity and Ecosystems services in a Changing Climate; www.becc.lu.se).

References:

1. Barona *et al*. (2010). *Environ Res Lett*, **5**:024002.
2. Davidson et al. (2007). *Nature*, **447**(7147): 995–98.
3. Figueiredo *et al*. (2019). *Sci Rep*, **9**:8538.

Putative small RNAs controlling detoxification of industrial cyanide-containing wastewaters by *Pseudomonas pseudoalcaligenes* CECT5344

Víctor M. Luque-Almagro[1], Alfonso Olaya-Abril[1], M. Dolores Pérez[1], Francisco Amil[2], Purificación Cabello[3], Lara P. Sáez[1], Conrado Moreno-Vivián[1], Mª Dolores Roldán[1]

[1]Departamento de Bioquímica y Biología Molecular, Edif. Severo Ochoa, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain, [2]Servicio Central de Apoyo a la Investigación, Unidad de Proteómica, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain, [3]Departamento de Botánica, Ecología y Fisiología Vegetal, Edif. Celestino Mutis, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain.

b42lualv@uco.es

Keywords: cyanide, ferric uptake regulator, industrial wastewater, *Pseudomonas*, small RNAs

The alkaliphilic bacterium *Pseudomonas pseudoalcaligenes* CECT5344 uses free cyanide and several metal−cyanide complexes present in the jewelry wastewaters as the sole nitrogen source. To understand the regulatory mechanisms involved in the assimilation/detoxification of cyanide from cyanide-containing wastewaters in *P. pseudoalcaligenes* CECT5344, RNA-Seq has been performed from cells cultured with the cyanide-containing residue. In total 20 sRNAs were identified that may have potential regulatory functions under cyanotrophic conditions in the strain CECT5344. From these, 16 sRNAs could be amplified successfully by RT-PCR. As predicted targets of these sRNAs were several components of the *nit1C* gene cluster encoding the nitrilase NitC essential for cyanide assimilation, the *cioAB* gene cluster that codes for the cyanide-insensitive cytochrome *bd*-type terminal oxidase, and gene clusters involved in the synthesis of polyhydroxyalkanoates, the global nitrogen limitation response, such as those coding for glutamine synthase and urease, metal resistance and iron acquisition like some metal extrusion systems and the ferric uptake regulatory (Fur) protein. Induction or repression of several genes targeted by sRNAs, which were found affected by the jewelry residue in previous omic studies, was demonstrated by qRT-PCR.

Acknowledgments and Funding: This work was funded by Ministerio de Ciencia, Innovación y Universidades, Spain (RTI2018-099573-B-100, also supported by FEDER, UE). The companies Gemasur FCC-Ambito, Saveco, S.L., Avenir S.L. and Magtel S.A., are acknowledged.

1.

Inhibited denitrification in tidal creek sediments impacted by poultry industry wastewater: activities, genes, and metagenomes.

Miguel Semedo, Bongkeun Song

Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, VA 23062, USA.

masemedo@vims.edu

Keywords: denitrification, nitrate, wastewater, pollution, poultry industry

In the past decades, the intensification of the poultry industry has led to an increase in the number of animal processing plants discharging wastewater to waterbodies. Despite the high levels of nitrogen and contaminants associated with the wastewater, knowledge about its impacts on denitrification, a vital ecosystem function in the aquatic environment, is scarce.

The objective of this study was to determine the impacts of wastewater discharge from a poultry processing plant on denitrification and microbial community structure in tidal creek sediments.

We conducted a field survey and a microcosm experiment in two tidal creeks (impacted vs. reference) of the Virginia Eastern Shore, USA, where poultry industrial farms are abundant. The “impacted” creek has received direct discharges of treated wastewater from a poultry processing plant, while the “reference” creek has no wastewater discharge. Denitrification was measured using slurry incubations and the microbial community was examined with qPCR of denitrification genes, 16S rRNA gene sequencing, metabolic inference, and shotgun metagenome sequencing.

Denitrification rates were lower in the impacted creek, especially near the wastewater discharge. Denitrification inhibition by impacted creek water was clearly observed in the microcosm experiment. Denitrification rates were associated with changes in the microbial community composition and denitrification gene abundance. This research demonstrates denitrification inhibition by wastewater discharge from a poultry processing plant with potential consequences to eutrophication.

Effect of nitrogen/carbon balance and pH value in bacterioruberin production in Haloferax mediterranei cultures

Micaela Giani-Alonso, Rosa Maria Martínez-Espinosa

Biochemistry and Molecular Biology Division, Agrochemistry and Biochemistry Department, Faculty of Sciences, University of Alicante, Alicante, Spain.

micaelagiani25@gmail.com

Keywords: archaea, bacterioruberin, pH, nitrate, nitrite

The halophilic Archaeon *Haloferax mediterranei* was grown under aerobic conditions in a non-buffered minimal medium containing 25% w/v inorganic salts, glucose (0.5% w/v) and nitrate (0.5-100 mM) or nitrite (0.5-10 mM). Changes on pH value were monitored during growth. In the case of culture media with KNO_3/glucose, final pH was 6.9-8.2; although in the culture with nitrite, final pH was around 5.9. Acetate was found into the cultures as the sole acid formed via modified Entner-Doudoroff pathway. Maximal acetate concentrations detected in 100 mM KNO_3/glucose and 2 mM KNO_2/glucose were 1.30 mM and 1.28 mM, respectively. However, final pH value of both cultures was notably different (8.20 *vs*. 5.90). This data suggest that acid production is not the only cause of pH reduction. Data allow us to propose that nitrate and nitrite transport mechanisms might be associated to pH changes observed. Also, bacterioruberin levels in the cells increased with higher pH values and therefore, we can conclude that the uptake of different nitrogen sources led to an important variation of pH (causing stress), as well as variation of C/N balance, which affected bacterioruberin synthesis.

References:

Montero-Lobato *et al*. (2018). *Mar. Drugs*. **16**(10): 372
Rodrigo-Baños *et al*. (2015). *Mar. Drugs*. **13**(9), 5508-32
Sowmya *et al*. Sachindra (2015). *Biocatal. Agric. Biotechnol*. **4**(4):559–567
Torregrosa-Crespo *et al.* (2018). *Mar. Drugs*. **16**(6): 203

POSTER PRESENTATIONS

List of Posters

P1 **Renée Isabel Bakkemo** Inter-strain variation in denitrification phenotype and potential bet-hedging in *Pseudomonas stutzeri*

P2 **Daniela Barreiro** Biochemical characterization of *Neisseria gonorrhoeae* cytochrome c_2 involved in hydrogen peroxide detoxification and denitrification

P3 **Stephane Besson** Phylogenetic analysis of heme d_1 domain provides evidence of horizontal gene transfers between Bacteria and Archaea superkingdoms

P4 **Marta S. P. Carepo** Effect of exogenous ligands on N_2O reductase

P5 **Lukas Denkhaus** An octaheme cytochrome form *Geobacter metallireducens*, a novel nitric oxide reductase GmNOR

P6 **Victoria Grießmeier** Microbial and ecological principles of the functionality of field denitrification beds

P7 **Kerstin Leberecht** The *Hells Bells* from Yucatán – unique calcite formations that grow through microbial nitrate-driven sulfur oxidation?

P8 **Wouter Lenferink** Physiology of heterotrophic nitrifiers

P9 **Maria João Nunes** Analytical chemistry as tool for understanding of the nitrogen cycle

P10 **Sofia R. Pauleta** An electrochemical study of nitrous oxide reductase from *Marinobacter hydrocarbonoclasticus*

P11 **Niek Stortenbeker** Enrichment of nitrous oxide converting microorganisms in a continuous bioreactor

Inter-strain variation in denitrification phenotype and potential bet-hedging in *Pseudomonas stutzeri*

Renée I. Bakkemo, Lars R. Bakken, Åsa Frostegård, Linda L. Bergaust

Norwegian University of Life Science, Faculty of Chemistry, Biotechnology and Food Science, Norway.

renee.isabel.bakkemo@nmbu.no

Keywords: *Pseudomonas stutzeri*, denitrification phenotypes, bet-hedging

Full-fledged denitrifiers share many similarities with respect to the N-oxide reductases they carry. However, subtle regulatory differences, even between closely related organisms result in great phenotypic variation during transition to anoxia and subsequent denitrification. Until now, we have characterized and compared prokaryotes within the same genus, but recently we investigated the respiratory phenotypes of strains belonging to the same species, *Pseudomonas stutzeri.* In a collection of 18 *P. stutzeri* strains, two distinctive groups appeared: group one transiently accumulated high concentrations of nitrite after oxygen depletion and group two did not. Some of the nitrite accumulating strains displayed gas kinetics indicative of bet-hedging as previously seen in *Paracoccus denitrificans,* where only a fraction of cells synthesize NIR (nitrite reductase) before oxygen depletion [1]. Currently we only have circumstantial evidence for bet-hedging in *P. stutzeri*, thus further investigation is needed. As a first approach, we will combine detailed gas kinetics with staining techniques (e.g. FITC) to screen potential bet-hedgers with respect to phenotypic diversification. Our results illustrate the danger of using phylogeny as a proxy for function, even at species-level. Moreover, the collection of *P. stutzeri* strains sharply contrasting with respect to apparent cell diversification, represents a uniquely powerful perspective for unpicking the regulatory mechanisms underlying bet-hedging in denitrifiers.

References:

1. Lycus, P. *et al*. (2018) *PNAS* **115**(46): 11820-11825.

Biochemical characterization of *Neisseria gonorrhoeae* cytochrome c_2 involved in hydrogen peroxide detoxification and denitrification

Daniela Barreiro[1], Simona Incorvaia[1], Marta S. P. Carepo[2], Sofia R. Pauleta[1]

[1] UCIBIO, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.

[2] LAQV, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.

d.barreiro@campus.fct.unl.pt

Keywords: Denitrification, *Neisseria*, nitrite reductase, bacterial peroxidase, cytochrome *c*

Neisseria gonorrhoeae is an obligate human pathogen that causes the sexually transmitted disease gonorrhoea, that infects each year millions of people worldwide.

During infection, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by host defence mechanisms, and in order to cope with the oxidative stress, *N. gonorrhoeae* uses the cytochrome *c* peroxidase (*Ng*CCP) and nitrite reductase (AniA), two outer membrane lipoproteins, that catalyses the reduction of hydrogen peroxide to water and the reduction of nitrite to nitric oxide. *N. gonorrhoeae* has also a membrane nitric oxide reductase, constituting with AniA a truncated denitrification pathway.

A putative electron donor to *Ng*CCP and AniA, the periplasmic cytochrome c_2, was heterologously expressed in *E. coli* BL21(DE3) and isolated from its periplasm through two different chromatographic purification procedures. The isolated cytochrome c_2 was biochemically and spectroscopically characterized using visible, nuclear magnetic resonance and circular dichroism.

The ^{1}H-NMR spectrum assessed the folding of protein and the coordination sphere of the haem, with a resonance at -3.39 ppm being attribute to the ε-methyl of a methionine residue (assigned to Met71). The circular dichroism allowed to assess the secondary structure and the thermostability of the protein. The melting curve was fitted with a two-state model, with denaturation enthalpy (ΔH) and melting temperature (T_M) estimated to be 157 kJ/mol and 61 °C, respectively.

Kinetic assays with *N. gonorrhoeae* AniA and *Ng*CCP are being carried out to assess the ability of this small cytochrome *c* to donate electrons to these two enzymes.

Acknowledgments: We thank the financial support from Fundação para a Ciência e Tecnologia, Portugal, through PTDC/BIA-BQM/29442/2017 (SRP) and UID/Multi/04378/2019.

Phylogenetic analysis of heme d_1 domain provides evidence of horizontal gene transfers between Bacteria and Archaea superkingdoms

Stéphane Besson[1,2]

[1]Escola de Psicologia e Ciências da Vida, Departamento de Ciências da Vida, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande, 1749-024 Lisboa, Portugal.
[2]LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

stephane.besson@ulusofona.pt

Keywords: Cytochrome d_1, Phylogeny, Horizontal Gene Transfer

A phylogenetic analysis of bacterial sequences of cytochrome cd_1 published in 2006 [1] showed evidence of horizontal gene transfer (HGT) between bacterial taxa. The present analysis was restricted to heme d_1 domain since heme d_1 and *c* domains are likely to have followed different evolutionary pathways and should be considered separately. Furthermore, 6 archaeal sequences were included in the study, along with 23 bacterial sequences. Trees were constructed by applying Maximum Parsimony and Maximum Likelihood methods (Phylip package) to an aminoacid sequence alignment and tree topology was checked through Complete-and-Partial Bootstrap (1000 replicates) [2]. The trees exhibit phylogenetic discrepancies compatible with multiple HGTs, including "long-range" transfers between Bacteria and Archaea superkingdoms. These results thus confirm, and extend to Archaea, earlier conclusions by Heylen *et al.* [1]. These findings do not challenge an earlier hypothesis by Mat *et al.* [3]: the Last Universal Common Ancestor, L.U.C.A., would have lacked genes to synthesize cytochromes, and such genes would have appeared later and spread to most Bacteria and Archaea taxa through HGT. However, precisely because of multiple HGT, the taxonomic origin of cytochrome d_1 remains unclear. More sequences in poorly represented ancient taxa, for instance Planctomycetes, might help.

1. Heylen *et al.* (2006). *Environ Microbiol*, **8**(11):2012-21.
2. Felsenstein (1989). *Cladistics*, **5**:164-6.

Effect of exogenous ligands on N_2O reductase

Marta S. P. Carepo[1], Cintia Carreira[1,2], Sofia R. Pauleta[2], Isabel Moura[1]

[1] LAQV, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.
[2] UCIBIO, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.

marta.carepo@fct.unl.pt

Keywords: *Marinobacter*, nitrous oxide, denitrification, nitrous oxide reductase, CuZ center

N_2O is a powerful greenhouse gas that can only be detoxified by nitrous oxide reductase (N_2OR), an enzyme that catalyses the last step of denitrification pathway. Incomplete denitrification in bacteria results in substantial nitrous oxide (N_2O) emissions to the atmosphere. N_2OR contains two distinct copper centers per monomer: CuA, the electron transferring center and "CuZ", a tetranuclear copper-sulfide center. The CuZ center can exists in two forms CuZ(4Cu2S) and CuZ*(4Cu1S) [1]. The effect of exogenous ligands, such as halides on both forms of the "CuZ center" was studied in particularly for iodide. Spectroscopy studies, such as UV-visible, EPR and circular dichroism, as well as steady- state kinetics were performed.

In the absence of substrate or electrons a novel "CuZ center" intermediate species is formed with a maximum absorption band at 617 nm, with a [$1Cu^{2+}$-$3Cu^{1+}$] oxidation state. This intermediate was spectroscopically and kinetically characterized.

Acknowledgments:This work was supported by the Associate Laboratory for Green Chemistry-LAQV, with national funds from FCT/MCTES (UID/QUI/50006/2019) and by the Applied Molecular Biosciences Unit-UCIBIO which is financed by national funds from FCT/MCTES (UID/Multi/04378/2019). The authors acknowledge FCT-MCTES for funding through Project PTDC/BBB-BQB/0129/2014 (IM). MSPC acknowledges FCT/MCTES for funding her "Research Position" (signed with FCT NOVA in accordance with DL.57/2016 and Lei 57/2017).

References:

1. Pauleta, SR, Carepo MSP, Moura, I (2019) *Coord. Chem. Rev*, **387**, 436-449.

An octaheme cytochrome form *Geobacter metallireducens*, a novel nitric oxide reductase GmNOR

Lukas Denkhaus[1], Melanie Dietrich[1], Grietje N. Kuipers[1], Bianca Hermann[1,2] and Oliver Einsle[1,3]

[1] Institute for Biochemistry, Albert-Ludwigs-University Freiburg, Freiburg, Germany.
[2] Institute for Physical Chemistry, Albert-Ludwigs-University Freiburg, Freiburg, Germany.
[3] BIOSS Centre for Biological Signalling Studies, Freiburg, Germany.

denkhaus@bio.chemie.uni-freiburg.de

Keywords: Multiheme cytochromes, evolution of multihemes, NO-reduction

Multiheme cytochromes play versatile roles in microbial metabolism including enzymatic activity and electron transfer [1]. In the biological nitrogen cycle various reactions are catalysed by multiheme cytochromes such as cytochrome *c* nitrite reductase (NrfA, 5 heme groups), hydrazine hydrolase, hydrazine oxidoreductase and hydroxylamine oxidoreductase (HAO, 8 heme groups) [2,3]. Based on phylogenetic analysis, cytochrome *c* nitrite reductase was postulated as an evolutionary ancestor for octaheme cytochromes in the nitrogen cycle [4]. By addition of three more heme groups, a class of enzymes emerged, capable of the detoxification of nitrite. One of these enzymes, still a nitrite reductase, was characterized previously [5]. As another potential member of these evolutionary transition enzymes from NrfA to HAO, we identified an octaheme cytochrome in the anaerobe *Geobacter metallireducens*. The 45 kDa protein was heterologously expressed in *E. coli* and the structure of the enzyme was determined by X-ray crystallography to a resolution of 2.3 Å, revealing a trimeric arrangement, typically for octaheme cytochromes. The active site at the 5-coordinated heme 4, is covered by another monomer but there is no covalent attachment of a Tyr as found in HAO. The surrounding of the active site is very hydrophobic with a small hydrophobic channel leading to the surface of the protein. This enables the protein to reduce NO to ammonia with high specificity, utilising a reaction mechanism that is likely closely related to the one of NrfA.

References:

1. Bewley, K. D. *et al*. (2013) *Biochimica et biophysica acta* **1827:** 938–948.
2. Lukat, P. *et al.* (2008) *Biochemistry* **47:** 2080–2086.
3. Igarashi, N. *et al*. (1997) *Nature structural biology* **4:** 276–284.
4. Klotz, M. G. *et al*. (2008) Environmental microbiology **10**: 3150–3163.
5. Tikhonova, T. V. *et al*. (2006) *Biochim Biophys Acta – Proteins Proteomics* **1764:** 715-723.

Microbial and ecological principles of the functionality of field denitrification beds

Victoria Grießmeier[1], Johannes Gescher[1,2]

[1]Institute for Applied Biosciences, Department of Applied Biology, Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany.
[2]Institute for Biological Interfaces, Karlsruhe Institute of Technology (KIT), Eggenstein-Leopoldshafen, Germany.

victoria.griessmeier@kit.edu

Keywords: denitrification, denitrification bed, microbial diversity

Field denitrification beds containing polymeric plant material are increasingly used to eliminate nitrate from agricultural drainage waters and provide a tool for a decentralized water treatment. This study investigates the nitrate reducing capacity, the environmental sustainability as well as the microbial interactions of field denitrification beds. The overall knowledge regarding the microbial composition, potential synergistic effects and the overall carbon degradation process in these beds is sparse. Therefore, we analyzed the microbial wood chip degradation and nitrate elimination process via metagenomic- and metatranscriptomic sequencing. The microbial key players in the denitrification and carbon degradation process were identified and revealed Proteobacteria with the orders Burkholderiales, Rhodospirillales, *Ca. Accumulibacter*, Rhodocyclales and Nitrosomonadales as the main contributors to the nitrate removal process in the planktonic phase, whereas Bacteria and Fungi were equally involved in the hydrolysis of the wood material.

Moreover, several nitrogen parameters were measured with an installed online monitoring station and thus the functionality of the bed evaluated, which reached its limits in the cold, humid winter months. Also the greenhouse gas emission was analyzed regularly and revealed a CH_4 production predominately in the dry summer months Although the nitrate removal efficiency often reached 100%, the nitrate elimination process needs to be improved as some conditions lead to an outflow of nitrite. Potential reasons for this result will be discussed.

The *Hells Bells* from Yucatán – unique calcite formations that grow through microbial nitrate-driven sulfur oxidation?

Kerstin Leberecht[1], S. Ritter[2], M. Isenbeck-Schröter[2], J. Gescher[1,3]

[1]Karlsruhe Institute of Technology, Institute for Biological Interfaces, Eggenstein-Leopoldshafen, Germany. [2]University of Heidelberg, Institute of Earth Sciences, Heidelberg, Germany. [3]Karlsruhe Institute of Technology, Institute for Applied Biology, Applied Biology, Karlsruhe, Germany.

kerstin.leberecht@kit.edu

Keywords: calcite precipitation; metagenomic/transcriptomic study; Sox system

Extraordinary, bell-shaped calcite formations, so-called 'Hells Bells', were discovered in the subaquatic system of the cenote *El Zapote* on the Yucatán Peninsula, Mexico. Above a sulfidic halocline, these speleothems cover the cave ceiling in the water depth of 28-38 m. Previous studies of our cooperation partners indicated that the Hells Bells must have grown underwater and potentially through the known process of microbially induced or influenced organomineralization [1, 2]. To determine if the development of Hells Bells is related to microbial activity, we analyzed the microbiome throughout the complex water system (fresh water, redoxcline, salt water) and focused on the microbiology of the redoxcline where the calcite formations still grow. Metagenomic- and transcriptomic profiling of the community in the turbid layer revealed the key metabolic pathways. In the nitrogen cycle, the ammonia oxidation by Thaumarchaeota appears to be dominant. Throughout the nitrogen, sulfur and methane cycle, the SoxZY heterodimer as central protein of the Sox pathway showed by far the highest expression. The sulfur-oxidizing bacteria can be assigned to the Hydrogenophilales and unclassified Betaproteobacteria which seem to use nitrate as alternative electron acceptor in the hypoxic environment. The metatranscriptome indicates that the oxidation of reduced sulfur species mainly halts after production of elementary sulfur particles instead of sulfate. Here, we hypothesize that the microbial process of nitrate-driven anaerobic sulfur oxidation may promote organomineralization by increasing the local pH in the redoxcline through the depletion of sulfide, nitrate and carbon dioxide.

References:

1. Ritter et al. (2019). *Biogeosciences*, **16.11**: 2285-2305.

2. Stinnesbeck *et al.* (2018). *Palaeogeography, palaeoclimatology, palaeoecology,* **489**: 209-229.

Physiology of heterotrophic nitrifiers

Wouter B. Lenferink, Mike S.M. Jetten, Maartje A.H.J. van Kessel, Sebastian Lücker

Radboud University, Nijmegen, The Netherlands.

w.lenferink@science.ru.nl

Keywords: heterotrophic, nitrification, ammonia, oxidation

Nitrification is a key factor controlling the amount of bioavailable nitrogen in natural and man-made ecosystems. In soils, positively charged ammonium is retained while the negatively charged nitrification products nitrite and nitrate leach into the groundwater. Nitrification also controls the electron acceptor availability for denitrifying microorganisms. Together, the interplay of nitrification and denitrification determine how much nitrogen is retained in an ecosystem. The ecological importance of autotrophic nitrifying archaea and bacteria is generally recognized, which is in stark contrast to our understanding of the role of heterotrophic nitrifiers. Previous studies on *Paracoccus* spp. indicate that no energy conservation takes place during heterotrophic nitrification [1] and that nitrification rates are generally low [2]. However, this may be compensated by higher growth rates on organic substrates and cell numbers. Also, little is known about the proteins involved in heterotrophic nitrification which puts a limit on how they can be detected in the environment This study aims to elucidate the mechanism underlying these phenotypic differences. Activity experiments were performed on *Alcaligenes faecalis*. This organism was found to consistently produce nitrite when ammonium was present. Furthermore, we have started to explore the effect of carbon source, oxygen concentration, and pH on heterotrophic nitrification.

References:

1. Robertson *et al.* (1988) ApplEnvironMicrobiol, **54**(11):2812–2818.
2. Arts *et al.* (1995). FEMS Microbiol Ecol. **18**(4):305–315.

Analytical chemistry as tool for understanding of the nitrogen cycle

M. João Nunes, José J. G. Moura, Cristina M. Cordas

LAQV, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.

mjm.nunes@fct.unl.pt

Keywords: Nitrogen, methodologies, measurement

The knowledge/understanding of the nitrogen cycle is crucial for management improvement to protect Earth's natural resources. For this, the five main processes concerning nitrogen cycle need to be assessed: nitrogen fixation, nitrogen uptake through organismal growth, nitrogen mineralization through decay, nitrification, and denitrification. Currently, much research is devoted to understanding the effects of nitrogen enrichment [1].

Scientific progress provided rigor leading to the identification of nitrogen compounds, their reactions and their biological relevance. This conducted to an intensive development of analytical chemistry protocols for the measurement of nitrogen reactive forms [2, 3]. Some of the most frequently analytical methodologies used in the assessment of N reactive forms are spectrophotometric, chemiluminescent, electrochemical, chromatographic, capillary electrophoresis, spectrofluorimetric and electrochemiluminescent. Each method has its own merits and drawbacks and is selected depending on availability, sensitivity and selectivity, limits of detection and cost but also depending on matrix and subject of measurement. When comparing data results, the relationship between analytical methodologies must be accounted and evaluated. This work aims to present the advantages and disadvantages of analytical chemistry methodologies as a tool for the traceability of data and results comparison.

Acknowledgments: This work was supported by the Associate Laboratory for Green Chemistry-LAQV, with national funds from FCT/MCTES (UID/QUI/50006/2019). The authors also acknowledge the Fundação para a Ciência e Tecnologia for financial support through Project PTDC/SAU-SOC/28390/2017 (STRESSENSE).

References:

1. Nunes *et al*. (2015). *Analytical Methods*, **7**: 2219-2228.
2. Wang *et al*. (2017). *Talanta*, **165**: 709-720.
3. Fowler *et al*. (2013). *Phil Trans R Soc B*, **368**: 20130164.

An electrochemical study of nitrous oxide reductase from *Marinobacter hydrocarbonoclasticus*

Cintia Carreira[1,2], Margarida M. C. Romão[3], Isabel Moura[2], Sofia R. Pauleta[1]

[1] UCIBIO, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.
[2] LAQV, REQUIMTE, Depart. de Química, FCT, NOVA, 2829-516 Caparica, Portugal.
[3] Centro de Química Estrutural, IST, Universidade de Lisboa, 1049-001 Lisboa, Portugal.

srp@fct.unl.pt

Keywords: *Marinobacter*, denitrification, nitrous oxide reductase, electrochemistry

Bacterial reduction of N_2O to N_2 is catalyzed by nitrous oxide reductase in the last step of the denitrification pathway. This multicopper enzyme has an electron transferring center, CuA center, and a tetranuclear copper-sulfide catalytic center, "CuZ" center, which can exist as CuZ*(4Cu1S) or CuZ(4Cu2S).

The redox behavior of *Marinobacter hydrocarbonoclasticus* nitrous oxide reductase metals centers was investigated by potentiometry and for the first time by direct electrochemistry, using cyclic voltammetry. The reduction potential of CuA and CuZ(4Cu2S) centers is pH dependent, with a value of + 272 ± 5 mV and + 65 ± 5 mV *vs* SHE, at pH 7.6, respectively, determined by potentiometry. The two redox pairs were unequivocally identified in the cyclic voltammograms, with their redox potentials being controlled by a proton-coupled electron transfer process in the pH range studied, as observed by potentiometry. Two different mechanisms for the pH dependence on the reduction potential of CuZ(4Cu2S) center were identified: at high pH, the e^-/H^+ transfer pathway is preferred for the oxidized microstates, while at low pH, the reduced state of CuZ(4Cu2S) center is stabilized, with its reduction potential being explained by two pK_a values, 6.0 ± 0.1 and 9.4 ± 0.1. No additional voltammetric signals were detected that could be attributed to "CuZ" center as CuZ*(4Cu1S). However, an enhanced cathodic signal for the pre-activated enzyme was observed under turnover conditions that is explained by the binding of nitrous oxide to CuZ^0(4Cu1S), an intermediate species involved in the catalytic cycle of N_2OR.

Acknowledgments: We thank the financial support from Fundação para a Ciência e Tecnologia, Portugal, through PTDC/BBB-BQB/0129/2014 (IM) and SFRH/BD/87898/2012 (CC). This work was supported by the UCIBIO, LAQV and IST-ID which are financed by national funds from FCT/MCTES, UID/Multi/04378/2019 and UID/QUI/50006/2019, respectively and UID/QUI/00100/2013, respectively.

Enrichment of nitrous oxide converting microorganisms in a continuous bioreactor

Niek Stortenbeker, Boran Kartal

Microbial Physiology Group, Max Planck Institute for Marine Microbiology, Bremen, Germany.

nstorten@mpi-bremen.de

Keywords: denitrification, nitrous oxide

Nitrous oxide is a very potent greenhouse gas. The main biological process responsible for nitrous oxide turnover is denitrification, the stepwise reduction of nitrate to nitrite, nitric oxide, nitrous oxide and dinitrogen gas. The complete denitrification process can be achieved by generalist microorganisms that perform the reduction of nitrate all the way to dinitrogen gas. Alternatively, it could be performed as a group effort by several nitrogen oxide reducing specialists, which carry out a part of the pathway. Studies focusing on natural and manmade environments indeed suggest that denitrification is carried out by a diverse group of microorganisms acting together, rather than a small group of generalists that perform the whole pathway. To further our understanding of nitrous oxide turnover, and to study the responsible microorganisms, we enriched for nitrous oxide reducing specialists in a bioreactor using continuous cultivation techniques. In the bioreactor nitrous oxide was used as the sole electron acceptor, and formate was used as the electron donor and carbon source. We operated the bioreactor for over a year and monitored substrate and biomass concentrations. The enriched community grows stoichiometrically by coupling formate oxidation to nitrous oxide reduction to dinitrogen gas.

AUTHOR INDEX

LIST OF PARTICIPANTS

Name	Email	Institution	City Country
Åsa Frostegård	asa.frostegard@nmbu.no	Norwegian University of Life Sciences	ÅS Norway
Aurélien Saghai	aurelien.saghai@slu.se	Swedish University of Agricultural Sciences	Uppsala Sweden
Axel Magalon	magalon@imm.cnrs.fr	Aix-Marseille University, CNRS, IMM	Marseille France
Boran Kartal	bkartal@mpi-bremen.de	Max Planck Institute for Marine Microbiology	Bremen Germany
Carlos Palacin-Lizarbe	cpalacli7@gmail.com	CREAF	Barcelona Spain
Carmen Pire	carmen.pire@ua.es	Universidad de Alicante	Alicante Spain
Claire Brown	ceb561@york.ac.uk	University of York	York UK
Conrado Moreno Vivián	bb1movic@uco.es	Universidad de Córdoba	Córdoba Spain
Cristina M. Cordas	cristina.cordas@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Daniela Barreiro	d.barreiro@campus.fct.unl.pt	UCIBIO, REQUIMTE, FCT-NOVA	Caparica Portugal
David Richardson	d.richardson@uea.ac.uk	University of East Anglia	Norwich UK
Eliane Meilhoc	Eliane.Meilhoc@inra.fr	LIPM	Toulouse France
Gottfried Unden	unden@uni-mainz.de	University of Mainz	Mainz Germany
Hanna Koch	h.koch@science.ru.nl	Radboud University	Nijmegen Netherlands
Henrique Silva Fernandes	hfernandes@med.up.pt	BioSIM, F. Medicina, Univ. Porto	Porto Portugal
Isabel Moura	isabelmoura@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Javier Torregrosa-Crespo	javitorregrosa@ua.es	University of Alicante	Alicante Spain
Joana Faria da Costa	joafarcos@gmail.com	CIIMAR, Univ. Porto	Porto Portugal
Jörg Simon	simon@bio.tu-darmstadt.de	TU Darmstadt	Darmstadt Germany
José J. G. Moura	jose.moura@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Jose María Miralles Robledillo	jmmirallesrobledillo@gmail.com	University of Alicante	Alicante Spain

Name	Email	Institution	City Country
Justyna Barys	justynaanna.barys@e-campus.uab.cat	Universitat Autonoma de Barcelona	Barcelona Spain
Kerstin Leberecht	kerstin.leberecht@kit.edu	IAB/KIT	Karlsruhe Germany
Lars Bakken	lars.bakken@nmbu.no	Norwegian University of Life Sciences	ÅS Norway
Lea Wittorf	lea.wittorf@slu.se	Swedish University of Agricultural Sciences	Uppsala Sweden
Lin Zhang	zhang@bio.chemie.uni-freiburg.de	University of Freiburg	Freiburg Germany
Linda Liberg Bergaust	linda.bergaust@nmbu.no	NMBU	ÅS Norway
Liz Baggs	liz.baggs@ed.ac.uk	University of Edinburgh	Edinburgh UK
Luísa Maia	luisa.maia@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Lukas Denkhaus	denkhaus@bio.chemie.uni-freiburg.de	Albert-Ludwigs-Universität Freiburg	Freiburg Germany
María Dolores Roldán Ruiz	bb2rorum@uco.es	Universidad de Córdoba	Córdoba Spain
Maria João Nunes	mjm.nunes@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Markus Ribbe	mribbe@uci.edu	University of California	Irvine USA
Marta S. P. Carepo	marta.carepo@fct.unl.pt	LAQV, REQUIMTE, FCT-NOVA	Caparica Portugal
Micaela Giani Alonso	micaelagiani25@gmail.com	University of Alicante	Alicante Spain
Miguel Semedo	masemedo@vims.edu	Virginia Institute of Marine Science	Gloucester Point USA
Niek Stortenbeker	nstorten@mpi-bremen.de	Max Planck Institute for Marine Microbiology	Bremen Germany
Purificación Cabello de la Haba	bv1cahap@uco.es	Universidad de Córdoba	Córdoba Spain
Renée Isabel Bakkemo	renee.isabel.bakkemo@nmbu.no	Norwegian University of Life Sciences	ÅS Norway
Ricarda Kellermann	ricarda.kellermann@nmbu.no	NMBU	ÅS Norway
Rosa María Martínez-Espinosa	rosa.martinez@ua.es	University of Alicante	Alicante Spain
Sara Hallin	sara.hallin@slu.se	Swedish University of Agricultural Sciences	Uppsala Sweden
Serena Rinaldo	serena.rinaldo@uniroma1.it	Dept. of Biochemical Sciences - Sapienza Univ. of Rome	Rome Italy
Simone Morais	sbm@isep.ipp.pt	LAQV, REQUIMTE, Univ. Porto	Porto Portugal

Name	Email	Institution	City Country
Sofia R. Pauleta	srp@fct.unl.pt	UCIBIO, REQUIMTE, FCT-NOVA	Caparica Portugal
Stéphane Besson	stephane.besson@ulusofona.pt	Departamento de Ciências da Vida, Universidade Lusófona	Lisbon Portugal
Stuart John Ferguson	stuart.ferguson@bioch.ox.ac.uk	Oxford University	Oxford UK
Víctor M. Luque Almagro	b42lualv@uco.es	Universidad de Córdoba	Córboba Spain
Victoria Grießmeier	victoria.griessmeier@kit.edu	KIT/IAB	Karlsruhe Germany
Viviane Figueiredo Souza	vivi.bioufrj@hotmail.com	Universidade Federal do Rio de Janeiro	Rio de Janeiro Brazil
Wouter Bernardus Lenferink	w.lenferink@science.ru.nl	Radboud University	Nijmegen Netherlands
Yuan Gao	yuan.gao@nmbu.no	Norwegian University of Life sciences	ÅS Norway

SPONSORS


UCIBIO
Applied Molecular Biosciences Unit
@REQUIMTE


FCT
FACULDADE DE
CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA


LAQV
requimte
LABORATÓRIO ASSOCIADO
PARA A QUÍMICA VERDE


UNIVERSIDADE
NOVA
DE LISBOA


AMERICAN
CORNERS
PORTUGAL
BIBLIOTECA FCT UNL
CAMPUS DE CAPARICA


Turismo
de
Lisboa

SOCIEDADE PORTUGUESA DE QUÍMICA

ChemPubSoc Europe · EuChemS · IUPAC International Union of Pure and Applied Chemistry · IMSF · EFMC European Federation for Medicinal Chemistry

FCT Fundação para a Ciência e a Tecnologia · FUNDAÇÃO CALOUSTE GULBENKIAN · GOVERNO DE PORTUGAL · MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA · direção-geral educação