Analytica Chimica Acta 864 (2015) 39–47

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Rapid monitoring of intermediate states and mass balance of nitrogen during denitrification by means of cavity enhanced Raman multi-gas sensing

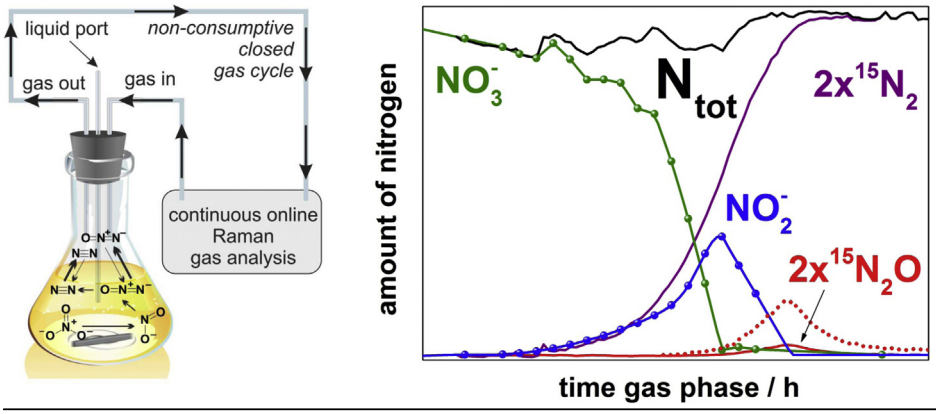
Robert Keiner [a,b], Martina Herrmann [c,d], Kirsten Küsel [c,d], Jürgen Popp [a,b,e,f], Torsten Frosch [a,b,e,*]

[a] Institute of Physical Chemistry, Friedrich Schiller University Jena, Jena 07743, Germany
[b] Leibniz Institute of Photonic Technology, Jena 07745, Germany
[c] Institute of Ecology, Friedrich Schiller University Jena, Jena 07743, Germany
[d] German Centre for Integrative Biodiversity Research (iDiv) Halle–Jena–Leipzig, Leipzig 04103, Germany
[e] InfectoGnostics Forschungscampus, Zentrum für Angewandte Forschung, Jena 07743, Germany
[f] Abbe School of Photonics, Friedrich Schiller University, Jena, Germany

HIGHLIGHTS

- CERS is a versatile new analytical methodology.
- Continuous online quantification of reduction of ^{15}N-labelled nitrate by *P. stutzeri* was demonstrated.
- The total nitrogen element budget was monitored online for the first time.
- Sterile online acquisition of the pH changes in the *P. stutzeri* culture was demonstrated.
- An increased slope of the pH value coincided with a temporary accumulation of N_2O.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 August 2014
Received in revised form 29 January 2015
Accepted 5 February 2015
Available online 12 February 2015

Keywords:
Cavity enhanced Raman spectroscopy
Gas sensing
Nitrogen cycle
pH monitoring

ABSTRACT

The comprehensive investigation of changes in N cycling has been challenging so far due to difficulties with measuring gases such as N_2 and N_2O simultaneously. In this study we introduce cavity enhanced Raman gas spectroscopy as a new analytical methodology for tracing the stepwise reduction of ^{15}N-labelled nitrate by the denitrifying bacteria *Pseudomonas stutzeri*. The unique capabilities of Raman multi-gas analysis enabled real-time, continuous, and non-consumptive quantification of the relevant gases ($^{14}N_2$, $^{14}N_2O$, O_2, and CO_2) and to trace the fate of ^{15}N-labeled nitrate substrate ($^{15}N_2$, $^{15}N_2O$) added to a *P. stutzeri* culture with one single measurement. Using this new methodology, we could quantify the kinetics of the formation and degradation for all gaseous compounds (educts and products) and thus study the reaction orders. The gas quantification was complemented with the analysis of nitrate and nitrite concentrations for the online monitoring of the total nitrogen element budget. The simultaneous quantification of all gases also enabled the contactless and sterile online acquisition of the pH changes in the *P. stutzeri* culture by the stoichiometry of the redox reactions during denitrification and the

* Corresponding author. +49 3641206221.
E-mail addresses: torsten.frosch@uni-jena.de, torsten.frosch@gmx.de (T. Frosch).

http://dx.doi.org/10.1016/j.aca.2015.02.007
0003-2670/© 2015 Elsevier B.V. All rights reserved.

CO_2-bicarbonate equilibrium. Continuous pH monitoring – without the need to insert an electrode into solution – elucidated e.g. an increase in the slope of the pH value coinciding with an accumulation of nitrite, which in turn led to a temporary accumulation of N_2O, due to an inhibition of nitrous oxide reductase. Cavity enhanced Raman gas spectroscopy has a high potential for the assessment of denitrification processes and can contribute substantially to our understanding of nitrogen cycling in both natural and agricultural systems.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The global nitrogen cycle has been highly impacted by humans and understanding rates and processes of microbial N-transformation, particularly those involving N-containing gases, is of great importance in environmental chemistry research. Nitrogen is a fundamental component of living organisms and human activities, including fertilizer use, which has altered the global cycling of N between inorganic and organic forms [1]. Of particular environmental interest are N-containing gases like N_2 and N_2O. N_2 is the largest reservoir of N, and needs to be transformed to forms that can be taken up by organisms via nitrogen fixation. N_2O is a greenhouse gas as well as a gas involved in stratospheric ozone depletion, and is produced by microbial nitrification and denitrification. Understanding of all these transformations and how they vary with environmental conditions is the subject of much research [2–4], and has been hampered by the difficulty of measuring simultaneous changes in these gases. In this study we demonstrate a novel methodology to measure N_2 and N_2O. These two gases are released during denitrification, the reduction of nitrate with the contemporary oxidation of organic or inorganic electron donors [5]. The ability to perform denitrification is widely distributed within the domain bacteria [5] and has also been described for archaea [6] and eukaryotic microorganisms [7,8]. Common for all prokaryotic denitrifiers is the stepwise reduction of nitrate via nitrite, nitric oxide, and nitrous oxide, to dinitrogen, employing four different enzymes: nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase [5]. Intense research regarding the biochemistry and genetics of denitrification has been carried out in the past by using *Pseudomonas stutzeri* as a model organism, one of the most active denitrifying heterotrophic bacteria [9–12].

Denitrification plays a crucial role in agricultural systems since up to one third of the nitrogen applied as fertilizer may be lost in gaseous form as a result of denitrification processes. Another environmental problem is leaching of excess nitrate into the groundwater in intensively managed farmlands and in forests that are nitrogen saturated due to high atmospheric nitrogen input [13,14]. In these systems, denitrification processes in the soil contribute substantially to the reduction of the amount of nitrate that percolates into the groundwater, resulting in a reduced nitrogen eutrophication of downstream ecosystems and in improved water quality. The impact of nitrous oxide as a potent greenhouse gas [15] and the decomposition of nitrogen containing waste [16] are further important issues.

The diversity of gases that need to be monitored for the investigation of the denitrification process include: N_2, N_2O, CO_2, and O_2, as well as their isotopic labels ($^{15}N_2$ and $^{15}N_2O$) for the evaluation of metabolic pathways. In addition, previous studies showed the release of significant amounts of NO and NO_2 from co-cultures of the nitrifier *Nitrosomonas* and the denitrifier *Paracoccus* while only trace amounts were produced during the denitrification stage alone [17]. The reduction of nitrite to NO is central for energy conservation in denitrification [9]. Due to its high reactivity and fast turnover, concentrations of the intermediate NO are often low in natural environments [18]. Expecting a similarly rapid turnover of NO and, consequently, very low concentrations, we did not include NO in our analysis. The rapid and simultaneous quantification of these gases is hard to accomplish with the currently available technologies. The quantification of dinitrogen is often accomplished by indirect or volumetric methods [19] due to a lack of available gas sensors. It can be quantified with thermal conductivity detectors, but these suffer from poor signal to noise ratios [20]. The detection with special columns in gas chromatography [21] is more sensitive, especially when combined with isotope ratio mass spectrometry [22]. Mass spectrometry enables the quantification of the relevant gases and their isotopes simultaneously and sensitively, but it is rather expensive and its application is restricted to laboratory environments and further can only be carried out on individual samples (not continuously). Gas chromatography has been used to follow the microbial formation and consumption of CH_4, CO_2, and H_2 in incubation experiments [23–25]. However, all gas chromatographic methods suffer from atmospheric contamination and the miniaturisation potential is limited. Rates of N_2 production by denitrification are mostly determined indirectly, using the acetylene blocking method [26]. Acetylene inhibits the last step of denitrification, the reduction of N_2O to N_2, resulting in the formation of N_2O as the only end product. The difference between the rate of production of N_2O with and without acetylene thus is used to estimate the fraction of N_2O that is fully denitrified to N_2. Blocking the last step of denitrification may also interfere with the metabolism of the denitrifying bacteria, and may therefore alter the rates of the process in an unwanted way.

A novel approach for simultaneous quantification of gaseous nitrogen isotopes that does not interfere with the bacterial metabolism is non-destructive Raman spectroscopy, which has emerged as a powerful method in various natural science disciplines [27–30]. This technique is based on the characteristic inherent vibrations [31–35] of the molecules and is widely established for the analysis of liquid and solid samples [36,37]. Innovative techniques based on optical cavities [38–41] and hollow-fibres [42–45] provide a strong signal enhancement for sensitive, simultaneous and non-consumptive quantification of multiple gases. In this study we demonstrate the rapid and continuous monitoring of all the above mentioned gases in batch incubation experiments with *P. stutzeri* DSM5190 as a model denitrifier in order to: (i) follow the formation of N_2, N_2O, and CO_2 during denitrification, depending on temperature and initial nitrate concentrations, (ii) analyse the interdependence of nitrite accumulation, pH value, and the formation dynamics of gaseous nitrogen compounds, and (iii) use mass balance of ^{14}N and added ^{15}N label to close the total nitrogen budget.

2. Experimental methods

2.1. Preparation of cultures of P. stutzeri in the batch experiments and chemical analysis

Cultures of *P. stutzeri* (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), strain DSM No. 5190) were maintained under aerobic conditions on peptone–meat extract

Download English Version:

https://daneshyari.com/en/article/1163589

Download Persian Version:

https://daneshyari.com/article/1163589

Daneshyari.com