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Developing Two-Dimensional Ammonium Sensors for use in Marine Sediments

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Abstract

Nitrogen spatial distribution and denitrification rates are not currently well understood in marine sediments. Both nitrogen distribution and denitrification rates vary widely. Better understanding these processes and the factors that impact them could have a variety of applications, from providing us with a foundation for determining any potential impacts of anthropogenic nitrogen to restoring eel grass beds. This project focused on the development of a two-dimensional ammonium sensor using diffusive equilibrium thin films. We successfully created and calibrated our sensor before deploying it at Padilla Bay to produce a two-dimensional image of the spatial distribution and concentrations of ammonium in the sediment. The final image suggests that ammonium is produced in the sediment at depth, with concentrations decreasing closer to the surface as ammonium diffuses into the water column or is oxidized to nitrite and nitrate. Our image also showed the presence of a microsite with higher concentrations of ammonium in the middle of the column.

Introduction

Nitrogen is a crucial nutrient for marine organisms. It acts as a limiting factor for primary productivity in estuaries, coastal waters, and some areas of the open ocean (Moore et al. 2013). As a limiting factor for primary production, nitrogen can also impose greater limitations on atmospheric CO₂ sequestration through biological productivity (Zhang et al. 2020). It is present in marine systems in several species, both inert and bioavailable and is controlled by multiple processes. Nitrogen is available in the atmosphere as inert N₂ gas. It can be converted to more bioavailable forms such as nitrite, nitrate or ammonium through nitrogen fixation and back again through denitrification (Zhang et al. 2020).

One of the current questions facing scientists and legislators in Washington State is whether anthropogenic nitrogen, especially from wastewater treatment plants, is impacting the
nitrogen budget in the Salish Sea. The global marine nitrogen budget is currently not well-understood, especially concerning the rate of nitrogen loss from marine systems through denitrification. While global marine nitrogen fixation has been studied extensively, current estimates of denitrification rates vary widely. This is primarily due to large uncertainties in denitrification in sediment (Zhang et al. 2020). Any impacts from anthropogenic nitrogen sources will remain unclear until we have a better understanding of the current rates and drivers of different nitrogen processes in marine systems.

David Shull’s lab ultimately aims to locate and quantify the various processes that affect denitrification rates in marine sediment. Denitrification is the main process governing the removal of nitrogen from marine systems (Devol 2015) and developing a deeper understanding denitrification in sediment could have broader applications, ranging from aiding in eelgrass bed restoration to understanding the impacts of anthropogenic nitrogen.

As the first step in this process, we are building on prior use of diffusive equilibrium thin films to develop sensors for nitrate, nitrite and ammonium in sediment. These sensors will provide us with a 2-dimensional image of nitrogen concentrations. While the nitrite and nitrate sensors are currently also in development, this report will focus on the agarose ammonium sensor.

As my capstone research, I assisted David Shull and his graduate student, Jessica Scotten, in developing the ammonium sensor. I was responsible for molding, pipetting and calibrating the gels, and I refined the molding process. Our molds went through several iterations as we tried different using types of rubber gaskets, determining what volume of agarose to prepare for different numbers of gels and pipetting the agarose as different temperatures to avoid trapping air bubbles in the gels. I also prepared the ammonium standards and reagent gels.

Development and Methods

Gel Preparation

Our ammonium DET methods are adapted from Metzger et al. (2019). I prepared the agarose gel solution by mixing 1.5 g agarose powder in 100 ml MilliQ water and microwaving the solution in 20 second intervals until completely dissolved. This volume was sufficient for three gels, so one batch of agarose can be used to mold the sample or calibration gel and two reagent gels. I initially prepared this solution in a 150 ml Erlenmeyer flask, but the solution boiled over. The solution at the bottom of the flask was significantly more viscous and the spilled liquid did not set immediately, so I assumed that the boiled over liquid was primarily water and added more MilliQ water to the flask to bring the total volume back up to 100 ml. This resulted in gels that took longer than normal to set, were thinner than usual and were ultimately unusable. To avoid compromising the consistency of the gels as well as cleaning agarose out of a
microwave, this volume of agarose solution should be microwaved in a 250 ml Erlenmeyer flask or larger.

Once boiled and mixed, I pipetted the agarose into the prepared molds using a 1000 mL micropipette. Through multiple trials, I determined that pipetting the solution while it is as hot as possible reduces the formation of air bubbles in the set gels. I also noticed that lines were forming between each layer of added gel as they cooled. I was again able to reduce this by pipetting the gel while hot and by filling the mold as quickly as possible to allow new additions of gel to mix before they could set and form layers. Reducing both the formation of air bubbles and layering created gels that were more uniform and less likely to contain imperfections that could show up as false microsites in the final nitrogen image.

I assembled the molds by clipping two glass panes together and running a length of 1 mm rubber tubing around the bottom and side edges to create a seal. We added two plastic 1.5 mm spacers to ensure even thickness across the gel. The glass panes were stored in a 10% HCL solution and rinsed and dried before use.

We tried using two different rubber gaskets. One was thicker and wrapped around the bottom of the glass, creating a better seal. I found that with these gaskets, we only needed to run them along the bottom edge and the spacers could be pressed into the gasket until watertight. This made assembling the molds easier and more efficient, however, we only had two lengths of the thinker gasket and were unable to source more. While the thinner tubing (Fig. 1) was more time consuming to assemble and needed to be run up the sides to create a seal, we had plenty of it and were able to mold more than two gels at once.

I left the gels to set at room temperature and stored the in a sealed container with DI water following demolding. We originally stored the gels in an uncovered container but learned that the water will evaporate if uncovered and the gels will dry out. We successfully rehydrated our dried gels, but they hyperhydrated, compromising the structural integrity of the gel. When I clamped the calibrated well plate over one of our rehydrated gels, it crushed immediately.

Figure 1. The assembled gel molds in a modified rack.
**Gel Calibration**

We established our standard curve using ammonium concentrations of 0 uM, 10 uM, 30 uM, 50 uM, 70 uM, and 100 uM. I calibrated the gels by clamping a well plate to the sample gel and allowing the standards to equilibrate for one hour. I then layered the calibrated gel between two reagent gels to develop the indicator pigment. The first reagent solution contained 0.65 M NaOH, 0.12 M NaClO and 0.12 M etridonic acid. The second reagent gel contained 0.027 M Sodium nitroprusside and 0.12 M thymol. I exposed the reagent gels to their respective solutions for 30 minutes in Tupperware containers. I found that the containers weren’t quite big enough for the gels to lie completely flat or be completely covered by the reagent solution, so I placed them on a shaker table on a low setting to ensure that the reagents were distributed evenly thoroughly the gel. Once equilibrated, I layered the reagent gels with the calibration gel to develop and scanned them after 20 minutes.

**Field Validation**

Following several successful gel calibrations in the lab, we performed a field validation. We deployed the sensor in the Padilla Bay eelgrass beds, a few hundred feet offshore at low tide. The gels were bubbled with nitrogen gas for at least two hours prior to deployment to deoxygenate the water. This prevented the introduction of oxygen into the anoxic sediment environment which could change the sediment chemistry. We placed the gel on a hard plastic backing and covered it with a 0.2-μm Durapore PVDF membrane to protect the gel from physical damage while allowing pore water from the sediment to equilibrate with the gel. We secured the membrane with PVC tape and buried the sensor to 3/4 the depth of the plastic backing in the sediment. After allowing the gel to equilibrate for 1.5 hours, I collected the sensor using a sediment core tube. We left the sensor in the core tube during transportation back to the lab and did not remove it until we were ready for analysis. Collecting the sensor in a sediment core allowed the gel to continue to equilibrate with pore water during transportation and will allow for MIMS and QPCR analysis of the sediment in later research.

I processed the gel on site at the Padilla Bay Estuarine Research Reserve Laboratory approximately half an hour following collection from the field. I followed our gel calibration procedure to establish an ammonium calibration curve. We removed the Durapore membrane from the sample gel using a knife to cut the tape along all four edges. I removed from the probe and sandwiched it between the two reagent gels on a clean transparency sheet. The use of clean transparency sheets between gels ensured that there was no cross contamination between the calibration gels or between nitrite, nitrate and ammonium sample gels. We placed the sample gels in a dark space to react for 20 minutes before the reagent gels were removed and the sample gel was scanned on a flatbed scanner.
Sample Analysis

Our sample analysis methods are currently still in development. After scanning, the gel on a flatbed scanner, the image is uploaded into ImageJ and the red channel is separated and converted into grayscale. The absorbance of the grayscale image can then be converted to concentration values using the established calibration curve in R Studio.

Results

As a result of our efforts, we were able to successfully create and calibrate our agarose gels, deploy the ammonium sensor in the field and determine the spatial distribution of ammonium concentrations in a sediment core taken from Padilla Bay.

Figure 2. The scanned calibration gel (left) and sample gel (right) from the Padilla Bay field deployment.
Figure 3. The calibration curve created from the calibrated field deployment gel (Fig. 2)

Figure 4. Processed scans of the field calibration (left) and sample (right) gels. The scanned images were converted from absorbance to concentration values using Image J and R Studio.

The processed field gel (Fig 3) shows a difference in ammonium distribution with depth. Ammonium concentrations are higher below 50 mm and increasing further at the bottom of the gel, around 170 mm. There is also a concentrated spot of ammonium around 75 mm. This is consistent with other research suggesting that nitrogen reduction is not spatially consistent throughout sediment and that microsites could be responsible for inconsistencies in current models of sediment diagenesis (Brandes and Devol 1997). This distribution also suggests that ammonium is produced in the sediment at depth by organic material. Ammonium concentrations
decrease closer to the surface as it diffuses into the water column. This exchange is likely facilitated by bioturbation from animal burrows.

Further development of these ammonium, nitrate, and nitrite DETs could have broad applications for understanding nitrogen cycling in marine systems. Measurement of the ammonium, nitrate and nitrite concentrations in a sediment core could be paired with membrane-inlet mass spectrometry to measure fluctuations in N2 gas and determine the overall rate of denitrification. QPCR could also be used to investigate the relationship between nitrogen concentrations in microsites and select genetic markers for microbial denitrification processes.

References