

Introduction

The Colorado River Basin runs through multiple states in the West of the United States and is a prime water source for about 30 million people in our nation. The river basin is also used for much of the farmland and agricultural needs in this area. Throughout the river basin there has been some evidence of Ammonia Oxidizing-Archaea (AOA). AOA is not only unique in that it plays a crucial role in the nitrogen cycle, but is unique since previous research only revealed Ammonia-Oxidizing Bacteria were able to complete this process. Currently there is limited information about *Thaumarchaeota*, a family of Ammonia-Oxidizing Archaea. Although limited information is available, AOA is found under many different conditions including those of the river basin.

Objectives

Using cultures derived from the Colorado River Basin a multitude of tests were run in order to determine more about the microbes that are thriving within the river and the lab kept environments, specifically from archeal phylum *Thaumarchaeota*. If AOA is found, DNA could be isolated and sequenced in order to develop a thorough and effective research plan.

Methods



Figure 1. Autoclave



Figure 2. Sanitized work station



Figure 3. UV sanitation

➤ In figure 1 the autoclave machine is used to sterilize equipment including bottles and tubes used for all tests. After being autoclaved all instruments and materials are placed in the hood where they remain in this sanitary area as displayed in Figure 2. Another step in sanitation is the UV sanitation (Figure 3) this will eliminate any DNA that may be on the tubes prior to use.



Figure 4. Culture samples

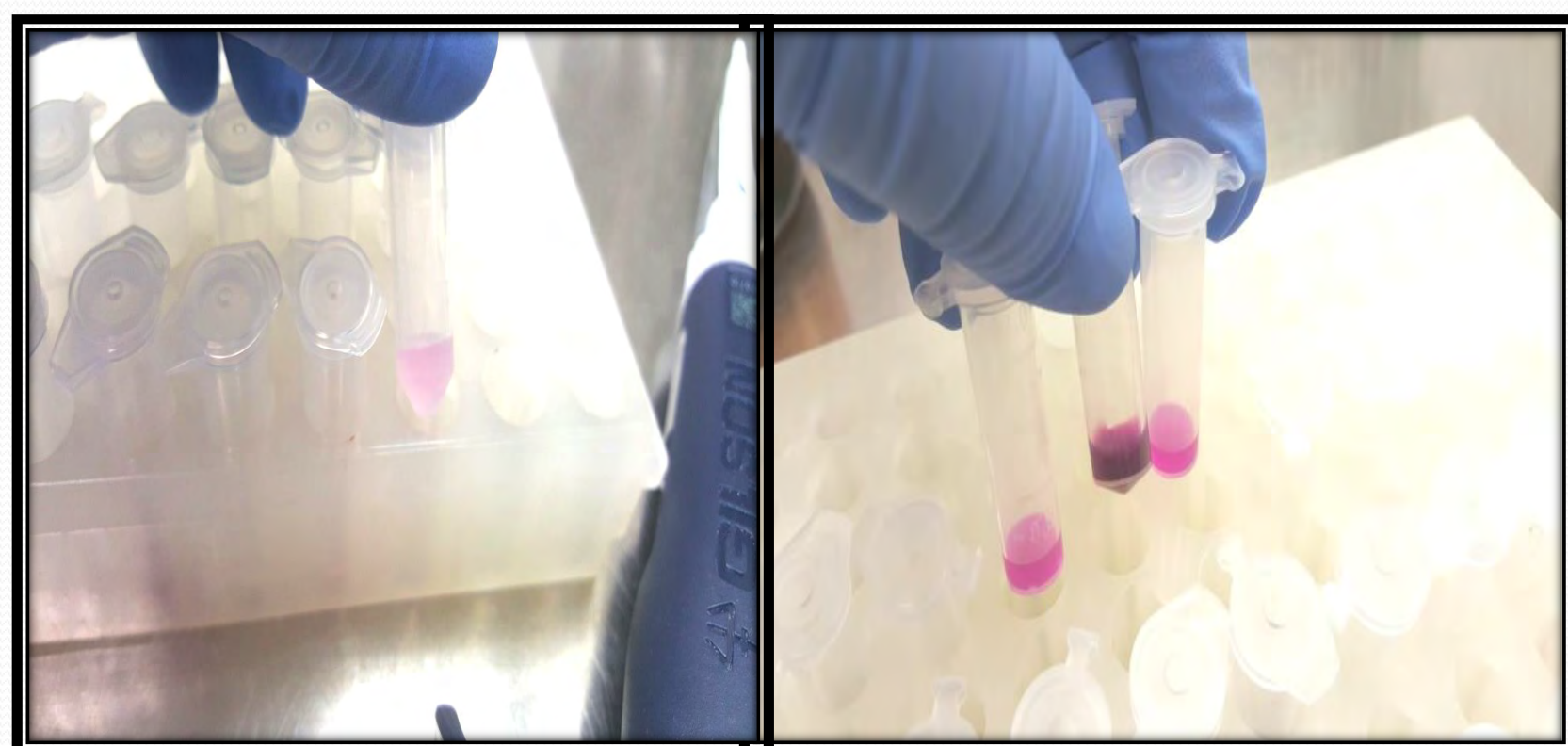


Figure 5 & 6. Nitrite activity levels 2, 3, & 4 low-high activity

➤ The first test administered is a nitrite activity test, this will determine if AOA or another microorganism is performing the nitrification process in which ammonia is converted into nitrite ions. Clear to very light pink is no activity while light pink, magenta, and dark purple indicate higher levels of activity and are ranked 0-4 respectively.

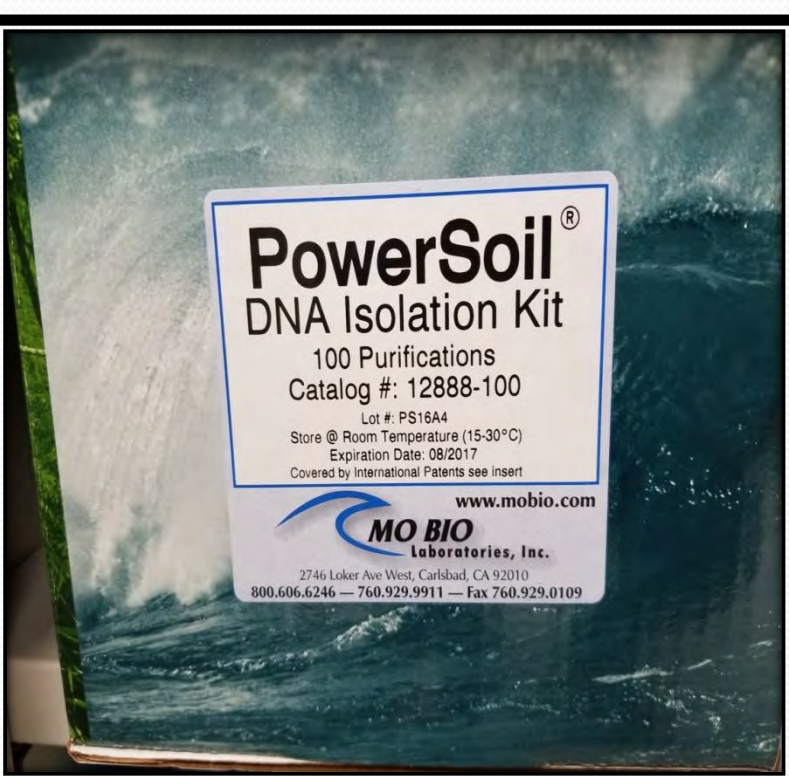


Figure 7. DNA Isolation Kit



Figure 8. Filtration apparatus



Figure 9. Isolated DNA on filter

➤ After nitrite activity is determined, the DNA isolation kit (Figure 7) is used. Culture samples must be filtered using a pump and the DNA is then caught in a small filter placed between the flask and small glass holding culture sample above as shown in Figure 8. The filter will then show either a dark or light circle in the middle of the filter paper which indicates DNA is on the filter (Figure 9).

Results

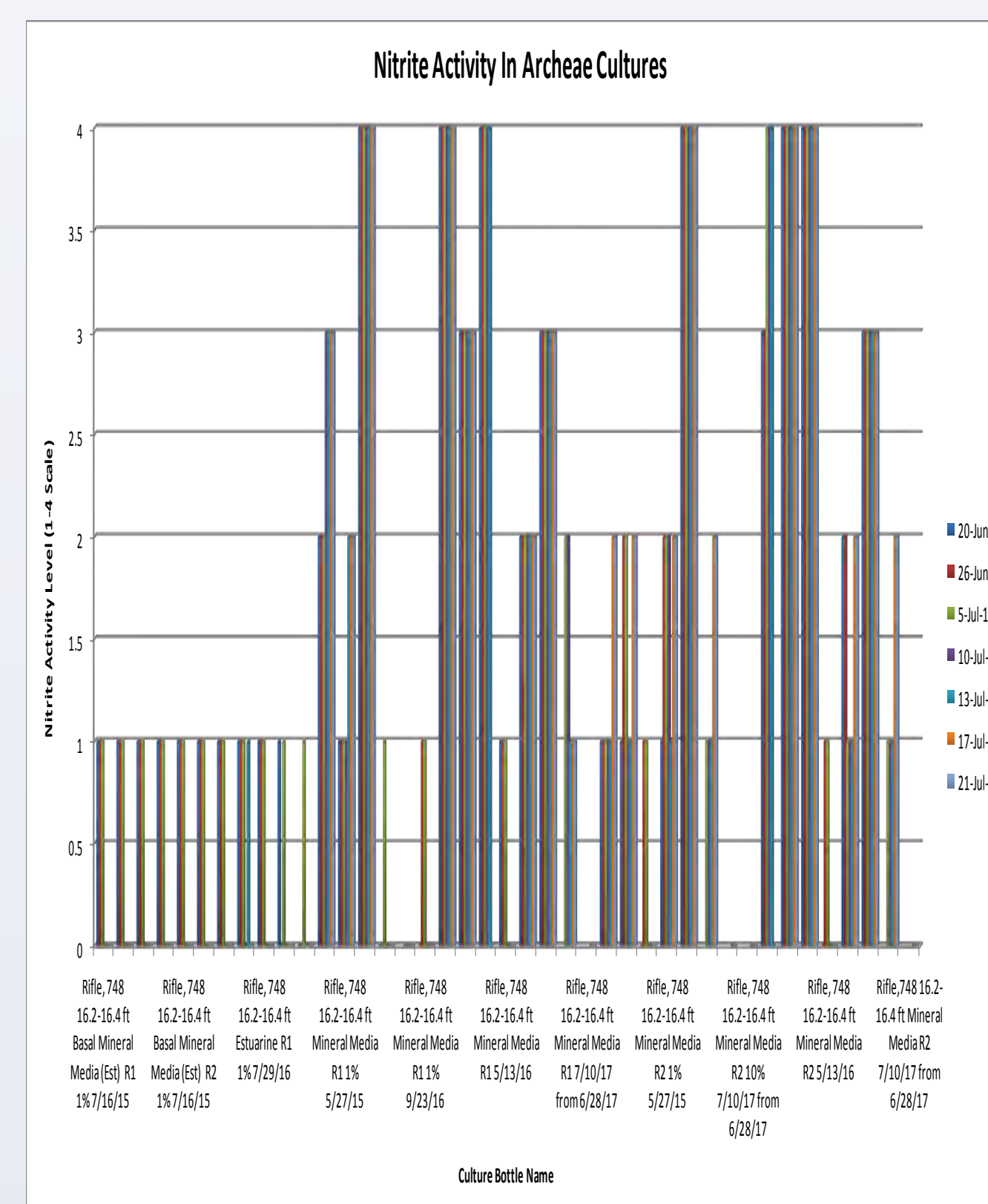


Figure 10. Nitrite activity Rifle samples.

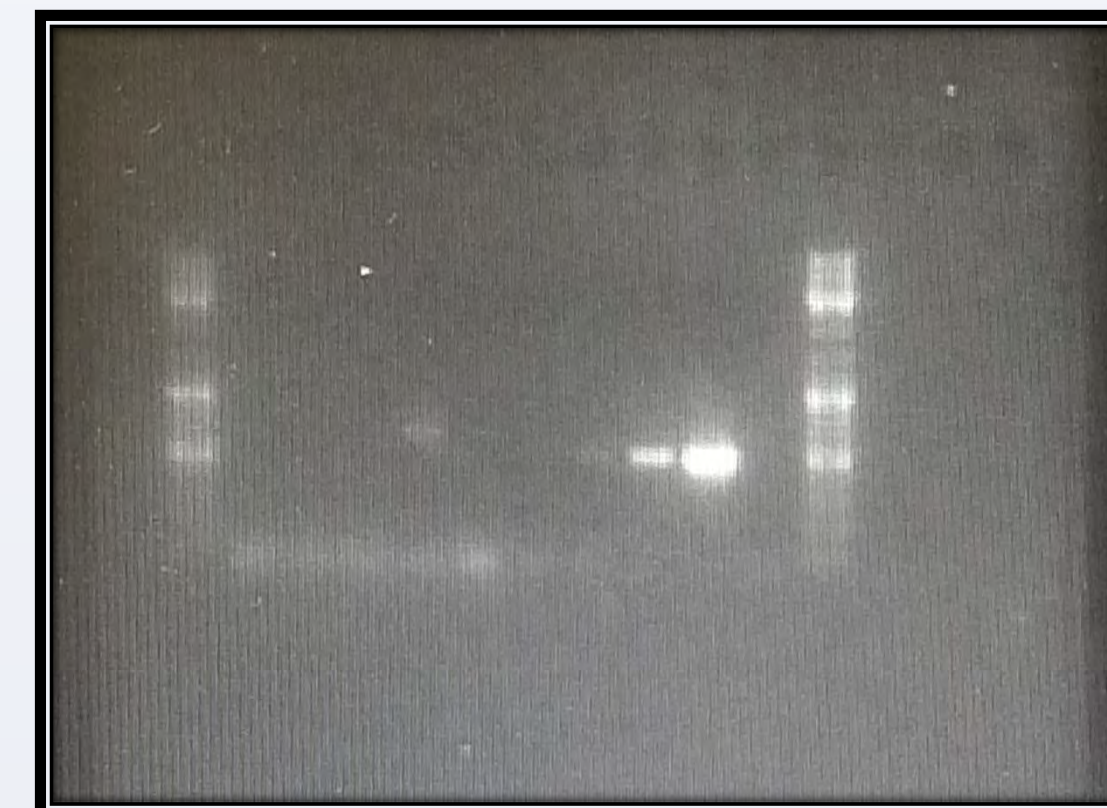


Figure 11. PCR gel indicating samples with bacteria.



Figure 12. Qubit Analysis

➤ Once the DNA was isolated it was run through PCR and found that only bacteria was in the samples and not AOA (Figure 11). Although we see from Figure 10 that there was nitrite activity in the cultures, none were active AOA.
➤ Qubit Analysis was run to verify high enough DNA concentration of the sample was obtained.

Conclusions

- No AOA found but bacteria surviving strenuous conditions.
- Ammonia concentration too high for cultures.
- New cultures using different media and ammonia concentration.
- Growth and activity tests more frequently.
- Cross contamination considered.
- Possibility of competition between AOA and AOB.

Acknowledgements

This material is based upon work supported by the National Science Foundation through the Robert Noyce Teacher Scholarship Program under Grant # 1340110. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The research was also made possible by the California State University STEM Teacher and Researcher Program, in partnership with Chevron (Chevron.com), the National Marine Sanctuary Foundation (marinesanctuary.org), SLAC and Stanford University. The Francis Lab is greatly appreciated along with the STAR Bay Area Cohort of 2017 and mentors Larry Horvath and Analise Elliot Heid.



**NATIONAL
MARINE
SANCTUARY
FOUNDATION**



CAL POLY
SAN LUIS OBISPO