Optimizing Spectroscopic Detection of Formaldehyde in Seawater Medium

Keshav Srivenkatesh[1], Samantha Cerda[1], Marc Alperin[1], Scott Gifford[1]
[1]Department of Earth, Marine, and Environmental Sciences, University of North Carolina at Chapel Hill

Introduction

The bacterial family *Methylophilaceae*'s main carbon substrate is methanol.[1] *Methylophilaceae* grow at rates much higher than what is suggested by the ambient methanol in their environment.[1] Understanding the flux of transient methanol in cell models can elucidate how *Methylophilaceae* are able to grow. Sensitivity requirements for quantifying methanol in culture and the environment for the Gifford lab range from a low of ~70 nM to a high of ~100 uM.

Many methods for quantifying methanol use a two-step method where methanol is oxidized to formaldehyde via the enzyme alcohol oxidase and then quantified.[1] One reaction that uses relatively low-cost reagents to transform formaldehyde into a spectroscopically active compound is the Hantzsch ester synthesis, which reacts formaldehyde with ammonium and acetylacetone to form a Hantzsch ester.[2]

Goals and Hypothesis

As part of the optimization of a sensitive assay for methanol in seawater, I hypothesized that the synthesis of a spectroscopically active analyte, DDL, from formaldehyde could be detected at low concentrations via a low-cost but sensitive spectroscopic assay.

Materials and Methods

Preparation of Hantszch reagent: The reagents required for formaldehyde to undergo the Hantzsch reaction were 0.02 M acetylacetone, 0.05 M glacial acetic acid, and 2.0 M ammonium acetate. These were prepared fresh each time in ultrapure water.

Absorbance and fluorescence spectroscopy: Formaldehyde standards were prepared in MilliQ and filtered seawater from Morehead City (filters = 3 & 0.1 µm). 100 uL of Hantzsch reagent was added to 900 uL of each formaldehyde standard. Controls were prepared from the addition of 100 uL Hantzsch reagents to 900 uL of ultrapure or sea water. Samples were incubated for 40 minutes at 37C. **Absorbance** at 412 nm was read on Eppendorf UV/Vis Biospectrophotometer. **Fluorescence intensity** was read on Tecan F200 Fluorescence Infinite microplate reader; sample was excited with wavelength 400 ± 40 nm bandpass filter and emitted under wavelength 535 ± 45 nm bandpass filter.

Fluorescence Curve

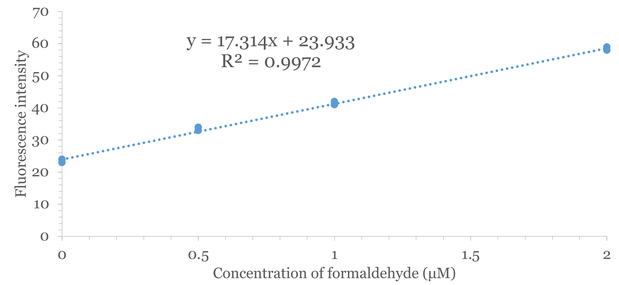

Figure 4. Preliminary **s**tandard curve of fluorescence intensity versus incubated concentration of formaldehyde in seawater. Not corrected for dilution. Gain = 30; flashes = 5; no shaking.

Comparing LODs

	Absorbance	Fluorescence
Average of Blanks	0.002157	23.67
Standard Deviation	7.8×10^{-4}	0.5773
LOD (blank + 3 STD)	**617 nM**	**65 nM**

Conclusion & Next Steps

Both methods worked in seawater: molar extinction of the absorbance curve approached literature value.[2] The fluorescent method had a superior limit of detection compared to the absorbance method. However, the plausible degradation of standards in seawater and quenching effects at lower concentrations merit further investigation of fluorometry. Future steps include increasing gain to optimize sensitivity and coupling the reaction to the enzymatic oxidation of methanol.

Reaction

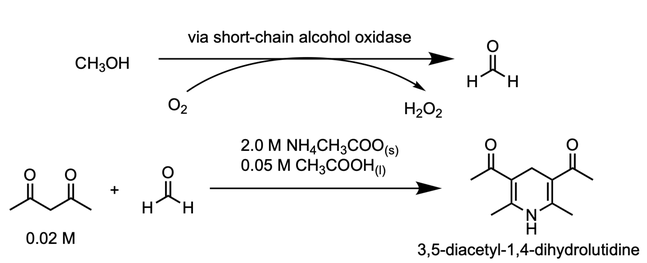

Figure 1. The proposed two-step transformation of methanol into a quantifiable and spectroscopically active analyte, DDL, using formaldehyde as an intermediate. The primary objective of this experiment was to evaluate a) the analytical sensitivity of quantification of the second step, and b) if high sensitivity could be maintained when formaldehyde was incubated in a purified seawater medium.

Absorbance Spectrum

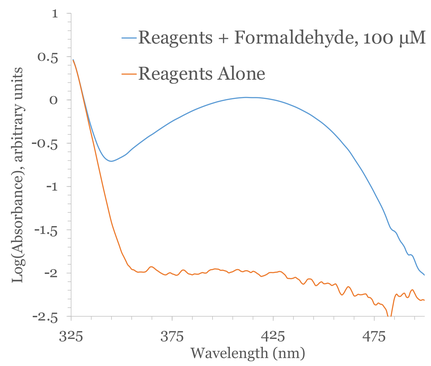

Figure 2. Log absorbance of acetylacetone, ammonium acetate, and acetic acid incubated with and without 10^{-4} M formaldehyde, showing smooth λ_{max} = 412 nm in agreement with Nash.[2]

Absorbance Curve

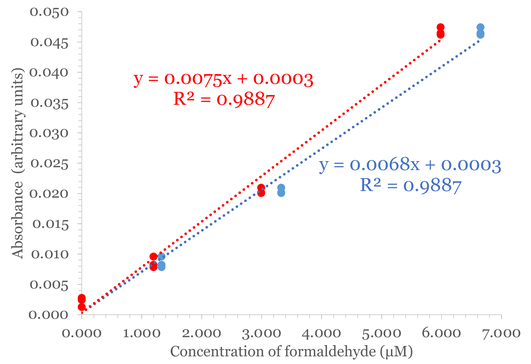

Figure 3. Standard curve of absorbance versus incubated concentration of formaldehyde, **corrected** for dilution (red) and **uncorrected** (blue).

References

1. Gifford, S. M.; Becker, J. W.; Sosa, O. A.; Repeta, D. J.; DeLong, E. F. Quantitative Transcriptomics Reveals the Growth- and Nutrient-Dependent Response of a Streamlined Marine Methylotroph to Methanol and Naturally Occurring Dissolved Organic Matter. *mBio* **2016**, *7* (6), 10.1128/mbio.01279-16. https://doi.org/10.1128/mbio.01279-16.
2. Nash, T. The Colorimetric Estimation of Formaldehyde by Means of the Hantzsch Reaction. *Biochemical Journal* **1953**, *55* (3), 416–421. https://doi.org/10.1042/bj0550416.