Development of Resazurin-Based Method to Quantifying Dissolved Oxygen

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Abstract

Resazurin is a redox-active molecule with three states: a colorless reduced form, a reversibly-oxidized red-colored form, and an irreversibly-oxidized purple-colored form. Resazurin has long been used in anaerobic microbiology to test for oxygen in a qualitative fashion. In this work we present the initial development of a spectrophotometric method to quantify dissolved oxygen using resazurin in an anaerobic chamber. Preliminary experiments using different ratios of aerobic and anerobic water showed a classic spectrophotometric titration curve with a clear end-point, indicating that a titration approach instead of a standard curve approach was appropriate; keeping samples tightly closed was essential to maintaining consistency as it prevented the loss of oxygen to the anerobic chamber environment. Redox titrations of the resazurin form using sodium dithionite demonstrated the stepwise reduction of the purple resazurin form to the red resorufin form to the colorless dihydroresorufin form. These control experiments indicated that in our assay the dihydroresorufin is oxidized directly to the resorufin state. Acid-base titrations of the resazurin form using HCl indicated that color changes associated with the acid-base activity of resazurin produced spectra that were distinct from the spectra of the assorted redox states, indicating that pH had little influence on our spectrophotometric approach. Future work will be directed towards refining our method and determining its viability against complex mixtures.

Spectroscopic Studies

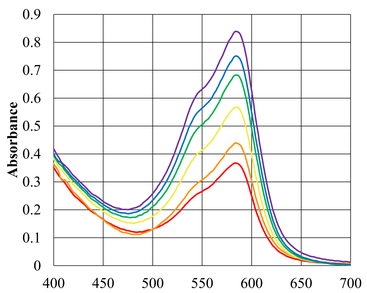

Figure 1: Absorbance spectra of dihidroresorufin containing solutions exposed to different amounts of oxygen. Traces are coordinated in a ROYGBIV arrangement based on the volume of oxic water from 100 µL, 150 µL, 200 µL, 260 µL, 300 µL, 350 µL. **These spectra are consistent with the presence of resorufin** (see appropriate spectra from figure 2).

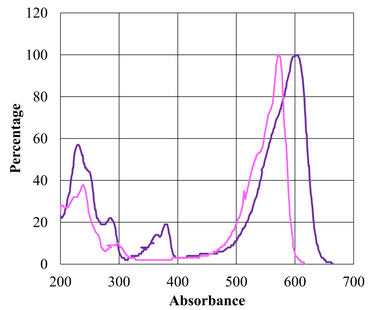

Figure 2: calculated absorbance spectra for resazurin and resorufin. Data was extracted from AAT Bioquest. **Notably the absorbance of resorufin is negligible at the resazurin peak allowing absorbance measurements to be deconvoluted for the generation of fractional composition diagrams.**

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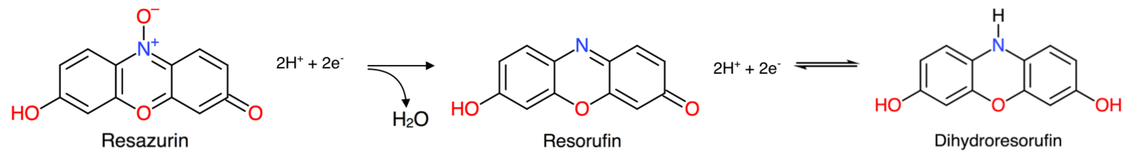
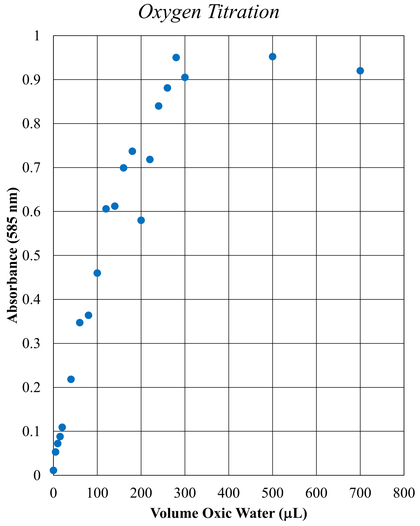
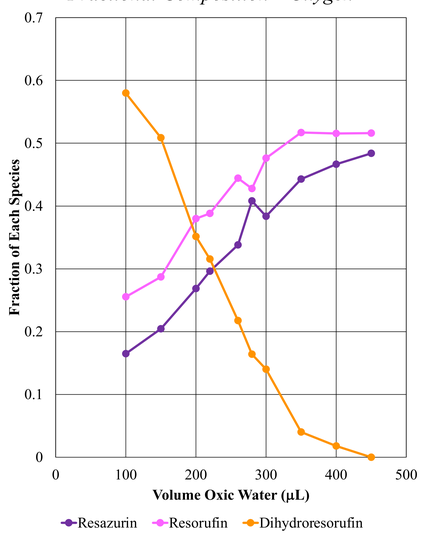
Resazurin can be used in a spectrophotometric titration to quantify the amount of dissolved oxygen in a sample. Spectroscopic studies indicate that the endpoint is a mixture of resazurin and resorufin forms.



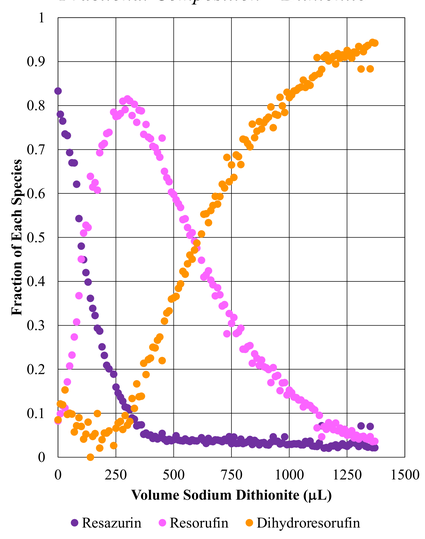
Oxygen Titration



Fractional Composition – Oxygen



Fractional Composition – Dithionite



Method Development: pH, sample conditions, color stability

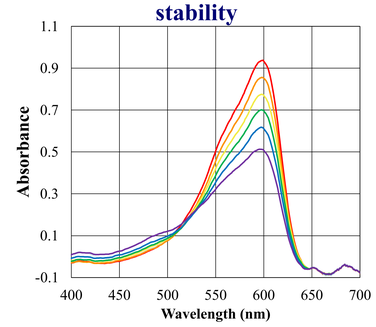


Figure 3: Absorbance spectra of resazurin titrated with 5.46 µM HCl. Traces are coordinated in a ROYGBIV arrangement based on increasing total volume of HCl. **Shifts in pH have limited effects of shape indicating limited effects on our assay.**

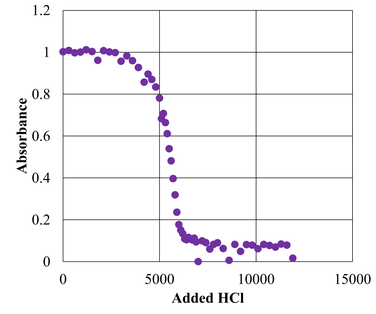


Figure 4: Spectrophotometric titration curve of the titration performed in figure 3**.** Note the absence of a clear equivalence point and the presence of a clear end point at approximately 6,000 µL. **Resazurin follows typical acid-base indicator behavior.**

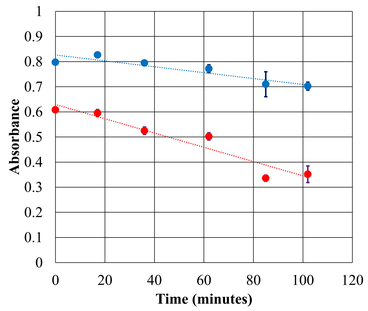


Figure 5: Time course comparing oxic water stock solutions left open (red markers) and closed (blue markers) in anaerobic chamber. Open stock data was fit with the following trendline y = -0.0026x + 0.6292. Closed stock data was fit with the following trendline y = -0.0012x + 0.8263. **Note the steady loss of oxygen in the open container compared to closed container.**

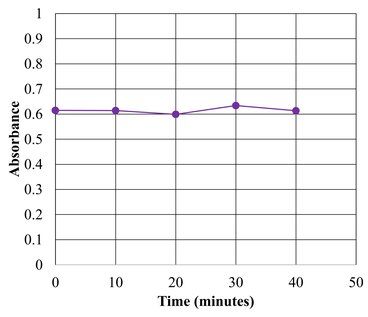


Figure 6: Time course to determine color stability of resazurin indicator mixed with oxygen. Samples were left open: **once resorufin has formed, the color remains stable.**