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**Use of Bradford and Quantigold Assays in Determining
Protein Content in Water Samples**

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Collegedale, TN 37315

Honors Project
Dr. Rhonda Scott-Ennis

3 May 2000

ABSTRACT

Water samples from seven different sites at the Amnicola marsh in Chattanooga, TN, were analyzed for the amount of protein present. Four sets of samples were concentrated using three methods—one using an ultrafiltration cell, one using a lyophilizer, and two utilizing a household food dehydrator. Two protein assays, the Bradford assay and the Quantigold assay (two sets of samples each), were used to determine the amount of protein present by measuring the absorbance of light at 595 nm. These data were compared with data from the standard curves of bovine serum albumin (BSA) to estimate the amount of protein in the water samples. Results showed that very little protein, if any, was present in the water.

INTRODUCTION

A biofilm is an organized community of microorganisms that adhere to solid surfaces when the materials are placed in aqueous environments, and easily seen on shower curtains, river rocks, and our teeth. These clusters of cells surrounded by a mesh of polysaccharides and proteins, which protect the bacteria from the environmental hazards. Biofilms can be both beneficial and detrimental to other organisms. For example, biofilms can be used in bioremediation of toxic compounds in water and soil, and in prevention of growth of damaging fungi on the roots of plants. However, biofilms are the key factor in bacterial resistance to disinfectants, antibiotics, and other germ-killing methods (Ben-Ari, 1999).

Biofilms form in three main steps: adsorption, adhesion, and adherence (Brisou, 1995). During the first few minutes of exposure, an organic monolayer made up of polysaccharides and/or proteins adsorbs onto the solid. This adsorption changes the chemical and physical properties of the solid's surface, making adhere to bacteria more readily. Due to Van der Waals

forces and electrostatic attraction, the bacteria begin a process of reversible adsorption. The longer this interaction occurs, the more irreversible the attachment becomes. Following the attachment or adhesion of the bacteria, extracellular polymer substances (EPS) are produced by the microorganisms. The EPS forms the sticky layer that traps nutrients, which are used by the growing biofilm (ibid.). The initial step of protein adsorption, however, is of primary concern when dealing with the chemical aspects of biofilm formation. What is the minimum protein concentration that must be present in the water before it begins to adsorb onto the solid surface and attract bacteria?

Two methods were used to determine the protein concentration of water samples from the Amnicola Marsh: the Bradford assay and the Quantigold assay. Both methods employ a reagent that binds to the protein in an aqueous solution to create a complex that can be detected spectrophotometrically at 595nm. These readings were then compared to a bovine serum albumin (BSA) protein standard to detect and quantify the protein in the test samples. Although the Quantigold assay is 25 times more sensitive than the Bradford assay, both methods were used to assure the presence and concentration of the protein the water samples (Stoscheck, 1987).

MATERIALS AND METHODS

Water Collection

Water samples were collected in 125-mL polypropylene bottles from the seven sites in the Amnicola marsh, located off the Amnicola Highway in Chattanooga, TN (see Appendix A). The absorbances of the water samples were taken at 280 nm on a UV-2101 PC UV-Vis Scanning Spectrophotometer (Shimadzu, Columbia, MD) before and after filtering through Whatman No.

1 filter paper (Fisher Scientific, Atlanta, GA) in order to assure that only larger contaminants, and not the protein itself, were removed.

Water Evaporation

The first set of 120-mL samples was quickly frozen using a mixture of dry ice and ethanol so that the water would freeze to the sides of the 300-mL LABCONCO lyophilization flasks (Fisher Scientific, Atlanta, GA), giving plenty of surface area. They were then freeze-dried on a LABCONCO lyophilizer (Kansas City, MO) with a Precision motor vacuum for 24 hours.

The second set of samples was concentrated using an Amicon Stirred Ultrafiltration Cell (Beverly, MA), which filtered the water through a Biomax-5 high-flux polyethersulfone membrane (Millipore, Bedford, MA) at 20 psi.

A Waring food dehydrator (~28°C) was used to concentrate the third and fourth groups of samples received. The samples were dehydrated for one week before testing.

Bradford Assay

The Protein Assay Dye Reagent Concentrate—containing Coomassie blue dye, phosphoric acid, and methanol—and the BSA standard were supplied by Bio-Rad Laboratories (Hercules, CA). According to a modified Bradford method, the reagent was diluted to a 1:4 concentration with distilled, deionized water (Bradford, 1976). The BSA standard was reconstituted with 20 mL deionized water, and had a protein concentration of 1.47 mg/mL. Portions of this standard were then diluted to eight different concentrations ranging from 0.034 mg/mL to 0.735 mg/mL. The remainder of the standard was then frozen at 0°C. For each assay, 100 μ L of each dilution was pipetted into an individual test tube, where it was mixed with 5.0

mL dilute dye reagent. The solutions were incubated at room temperature for 5 to 10 minutes before reading the absorbance at 595 nm.

Quantigold Assay

The Quantigold solution of colloidal gold and additional BSA standard were supplied by Diversified Biotech (Boston, MA). According to a modified method, the BSA, after reconstitution, had a concentration of 400 μ g/mL, and was diluted to five concentrations to attain a standard curve: 20 ng/ μ L, 2 ng/ μ L, 1 ng/ μ L, and 0.5 ng/ μ L (Stoscheck, 1987). Ten μ L of each sample was added to the 800 μ L Quantigold reagent in the microfuge tube. A blank was also prepared with 10 μ L of deionized water and 800 μ L Quantigold solution. The tubes were then shaken and placed in a water bath for incubation at 37°C for 45 minutes (ibid.).

Data Collection

The absorbances of the samples were measured at 595 nm on the UV-2101 PC UV-Vis Scanning Spectro-photometer by Shimadzu (Columbia, MD). Water sample absorbances were also measured on the UV-2101 PC UV-Vis Scanning Spectrophotometer at 280 nm.

RESULTS

The dilute water samples were tested before and after filtration. This trial demonstrated that the Whatman No. 1 filter paper retained mainly heavier dirt particles, and not a significant amount of protein.

Variation of BSA standard absorbances were taken into account and plotted using a best-fit line (see Figure 1). Results of the Bradford analysis showed very small absorbances covering a range of 0.3622 to 0.5456, typically less than absorbance of the smallest BSA concentration

(see Table 1). Samples collected at later dates (denoted 1st and 2nd Set) from the same region had an average absorbance variation of 0.0031.

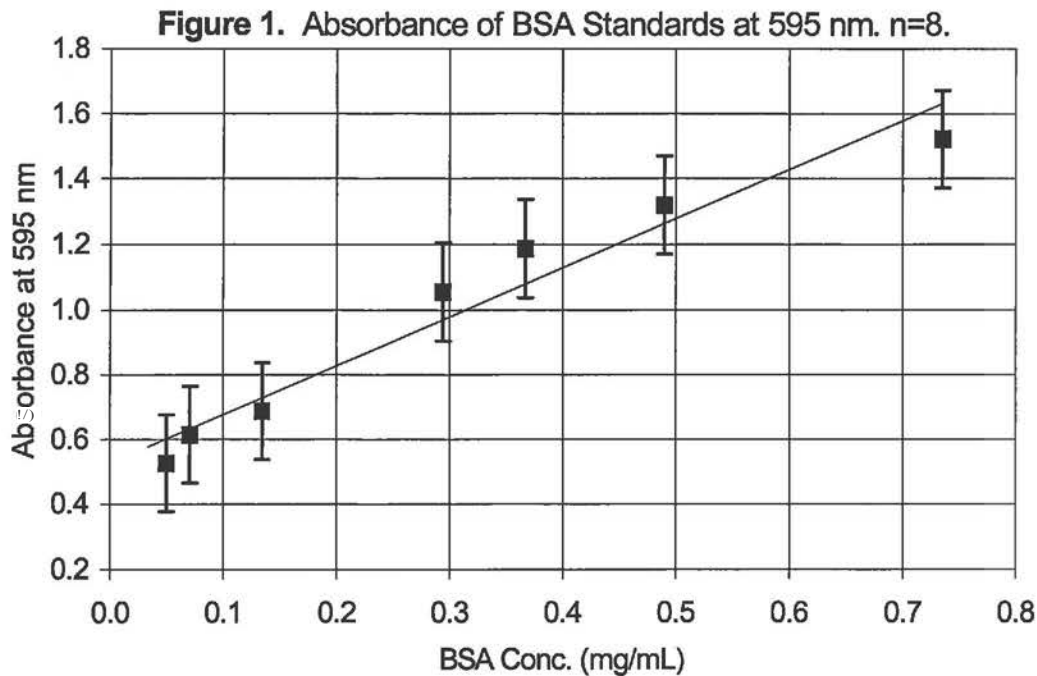


Table 1. Absorbance Readings of the Bradford Assay Samples at 595nm.

Region	Abs. 1 st Set	Abs. 2 nd Set
1	0.4176	0.4352
2	0.4196	0.4350
3	0.4172	0.4302
4	0.5307	0.4418
5	0.3758	0.4484
6	0.3846	0.4201
7	0.4816	--

For the Quantigold technique, the BSA standard absorbances and their variations were again plotted using a best-fit line (see Figure 2). Results of the water sample analysis, however, were slightly more ambiguous—an average absorbance variation of 0.1644 was demonstrated between different dates. While one sample had a higher absorbance than the smallest BSA concentration (0.5 ng/ μ L), many of the samples had absorbances below those created by the blank (see Table 2).

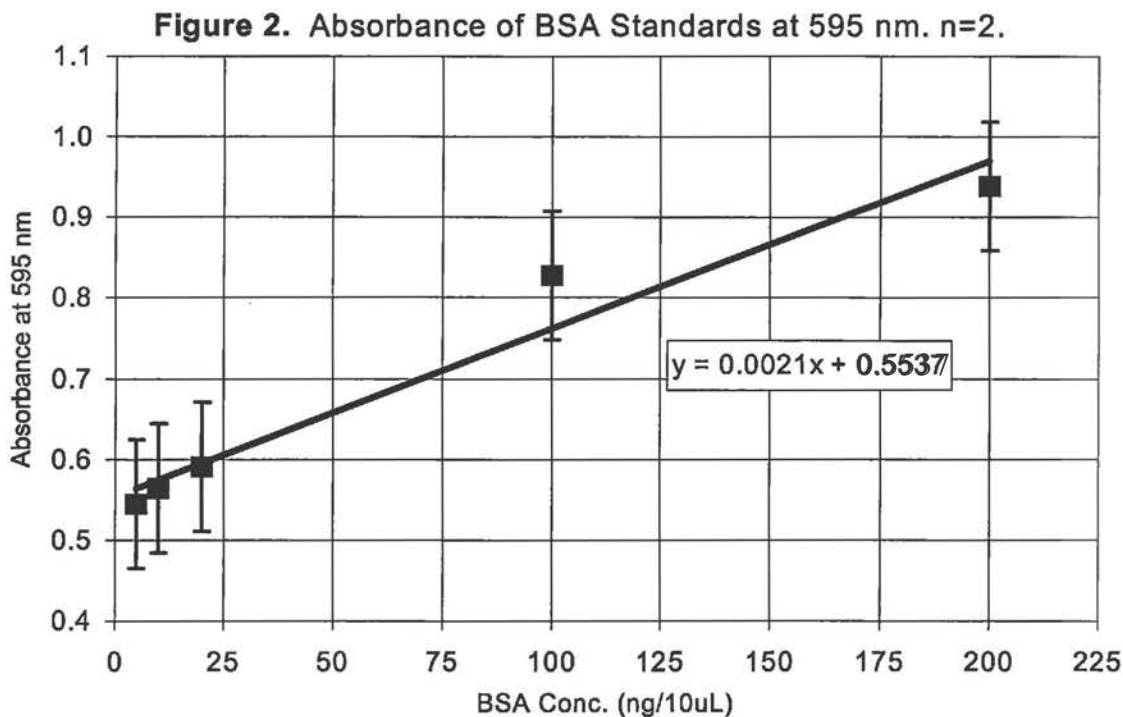


Table 2. Absorbance Readings of the Quantigold Assay Samples at 595nm

Region	Abs. 3 rd Set	Abs. 4 th Set
1	0.5276	0.6878
2	0.5266	0.6918
3	0.5264	0.7172
4	0.5232	0.7088

Region	Abs. 3 rd Set	Abs. 4 th Set
5	0.5240	0.7067
6	0.5440	0.6922
7	0.5380	0.6932

DISCUSSION

The variation observed in the absorbances of the first set of samples may be due to using freshly prepared reagent, differing incubation lengths, and differing lyophilization lengths, as well as the microorganism growth and population present in the marsh water itself. The differences may also be due to variations in the cuvettes used for readings. Two BSA trials were duplicated using the same cuvettes to correct this. These trials demonstrated that the error was most likely due to differences in the cuvettes, and that minor scratches or discoloration on the

cuvettes can alter the measurements of absorbance of the samples. Therefore, to maintain consistency, the absorbances of all duplicate samples were measured using the same cuvettes. This resolved any major differences that were observed. The second set of samples, which were concentrated using the ultrafiltration cell, also differed due to older reagent. This, however, should not be considered a major source of error.

Variance in the Quantigold analysis was more intriguing. Sets 3 and 4 of samples varied, demonstrating the water's heterogeneous nature, and also that a rainstorm prior to collection of Set 4 may have diluted the protein concentration. More protein seemed to be present at site 6 located near a beaver dam (most likely due to organic material in food, fecal matter, etc.), whereas less was present in other sites. The colored microfuge tubes may also have been a source of error if the solution absorbed dye from the tubes, altering the spectrophotometric readings (Stoscheck, 1987). Although each water sample was mixed in the same color tube, the blank and BSA solutions were mixed in different colored tubes in order to distinguish them from the water samples. This may have caused an error in reading if the Quantigold solution happened to pull any dye out of the tubes. Re-analyzing the samples using clear tubes eliminated major variation.

The range of absorbances recorded with either the Bradford or the Quantigold analyses demonstrated that if any protein was present in the water samples, the concentration was so low that it could not be detected using these techniques. The protein concentration, therefore, should not be considered a significant factor in the adsorption of bacterial colonies to solid surfaces. Further research would likely include tests to determine the polysaccharide/carbohydrate concentration in the water in order to establish the origin of the monolayer adsorption, and therefore, the biofilm formation.

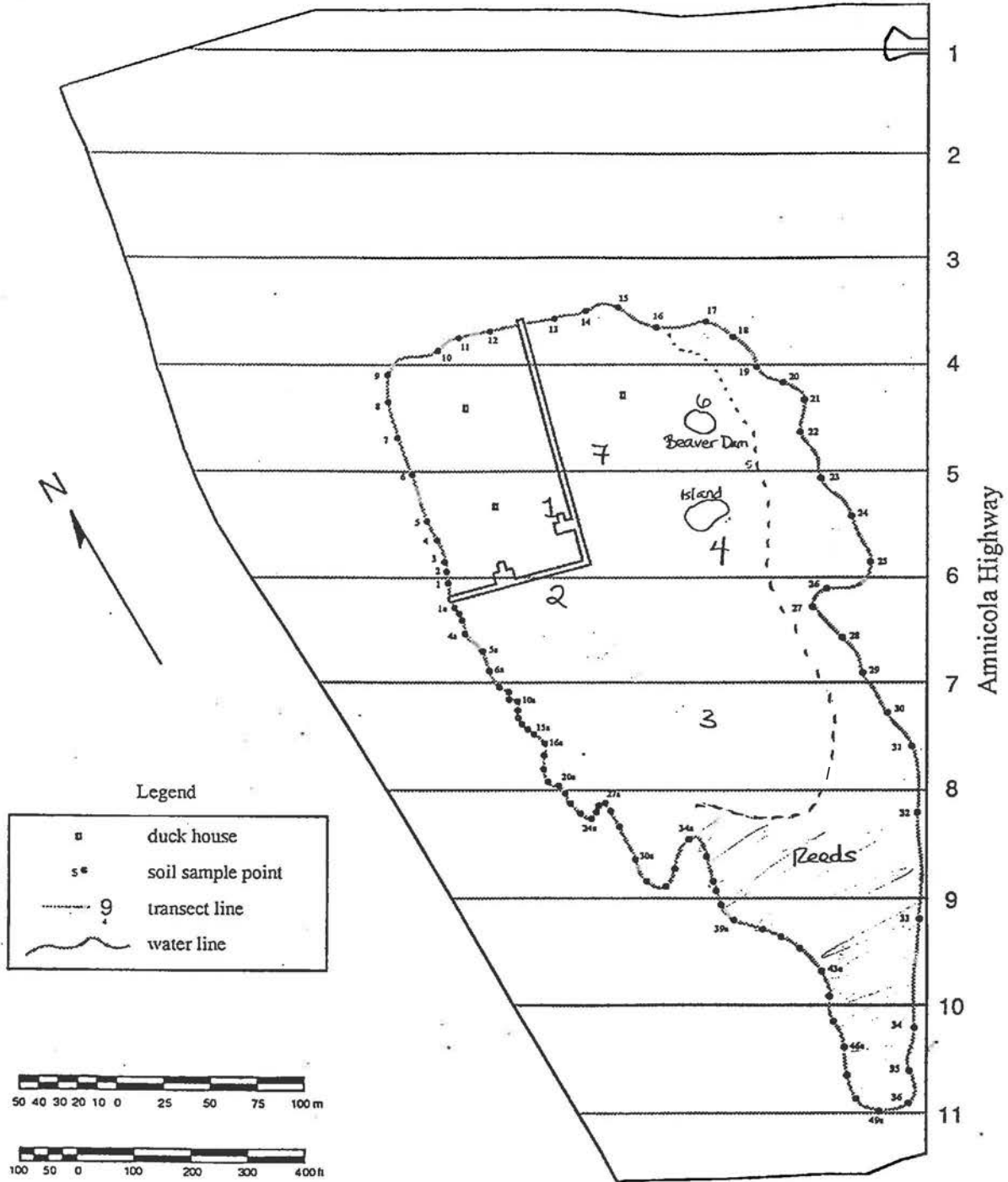
ACKNOWLEDGMENTS

I would like to thank Dr. Ann Foster and the Introduction to Biology Research class at Southern Adventist University for collecting water samples and preparing them for analysis, and Dr. Joyce Azevedo for assistance with lyophilization. I am also indebted to Dr. Rhonda Scott-Ennis for demonstrating and assisting in the Bradford, Quantigold, and ultrafiltration techniques. This work was supported by the Chemistry Department at Southern Adventist University.

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Appendix A. Map of the Amnicola Marsh and Water Sample Collection Regions



Scale 1: 3,000
 1 cm = 30 m
 1 in = 250 ft

Amnicola Marsh

A-4
 mapped February 23-25, 1996 by ESC 340 class, UTC

SOUTHERN SCHOLARS SENIOR PROJECT

Name: Kati Chaffin

Date: 12/10/99

Major: Chemistry

Senior Project

A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. Ideally, this project will demonstrate an understanding of the relationship between the student's major field and some other discipline. The project is expected to be of sufficiently high quality to warrant a grade of A and to justify public presentation. The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor three weeks prior to graduation. The 2-3 hours of credit for this project is done as directed study or in a research class.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake:

Dr. Foster in the Biology department is studying the possibility of biofilm formation due to possible protein found in water samples. These biofilms can form infections when bacteria grow on the site. I am doing two different protein assays, the Bradford assay and the Quantigold assay, to determine the amount of protein present (if any) in water samples from the Amnicola Marsh. Using concentration techniques such as lyophilization, ultrafiltration, and dehydration, I will compare these results with a standard curve of bovine serum albumin, to determine the amount of protein.

Expected date of completion 3/00

Signature of faculty advisor Rhonda J Scott-Lewis

Approval to be signed by faculty advisor when project is completed:

This project has been completed as planned:

This is an "A" project:

The project is worth 2-3 hours of credit:

Advisor's Final Signature Rhonda J Scott-Lewis

Chair, Honors Committee _____ Date Approved: _____