Organic soil samples were taken from 2 locations along Otter Creek East Middlebury and from the Winogradsky columns made in week 1.

One sample was from a drainage pond close to the Addison Country Transit building, which will be called the ACTR location, and the other was further south, from the bank of the creek. This will be called the East Middlebury location. Both mud and sediment were obtained from each location.

SRB use sulfate to produce H2S, which reacts with iron and forms a black precipitate FeS. (1) (2)

[Choose one: TSA and LB] plates were supplemented with iron sulfate. This compound was chosen because the iron could act as an indicator with the byproducts of SRB if present, while the sulfate could act as an electron acceptor. It was sterilely filtered into media after autoclaving to prevent the precipitate from forming prematurely.

SRB are strict or facultative anaerobes. (3) Therefore, GasPaks and anaerobic jars were used to obtain anaerobic conditions.

Each sample was also treated with either a 25 or 37 °C incubation. These temperatures were chosen because the optimum temperature for *Desulfovibrio* species was found to be between 20 and 40 degrees. (4)

A 1:100 dilution was also performed. These two dilutions were chosen to hopefully increase the growth rate of the microbes, as many papers state that the growth takes between 5 and 7 days. (3) (5) (6)

However, this high concentration of microbes increased the need for monitoring for contamination and the outcompeting of SRB by other species.

(Precautions to preserve anaerobic conditions?)  (2) (6)

(NaSO3 to isolate colonies and reduce competition?)

Presence of a black precipitate and the distinctive rotten eggs scent were used to identify the presence of SRB. Black colonies were isolated and replated with the same conditions from which they appeared.

The colonies were confirmed using a gram stain, catalase test, and aerobic profile. SRB are gram negative, strict or facultative anaerobes, and catalase negative. (Other tests from Bergeys?)

(7)

1. Gibson GR. 1990. A Reveiw: Physiology and Ecology of the Sulphate-Reducing Bacteria. J Appl Bacteriol 69:769–797.

2. van der Hoeven JS, van den Kieboom CWA, Schaeken MJM. 1995. Sulfate‐reducing bacteria in the periodontal pocket. Oral Microbiol Immunol 10:288–290.

3. Shukla SK, Reed KD. 2000. Desulfovibrio desulfuricans bacteremia in a dog. J Clin Microbiol 38:1701–1702.

4. Gilmour CC, Elias DA, Kucken AM, Brown SD, Palumbo A V., Schadt CW, Wall JD. 2011. Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. Appl Environ Microbiol 77:3938–3951.

5. Costinar L, Herman V, Pascu C. 2010. The Presence of Sulfate-Reducing Bacteria in Dog’s Oral Cavity XLIII:128–131.

6. Keith SM, Herbert RA, Harfoot CG. 1982. Isolation of new types of sulphate-reducing bacteria from estuarine and marine sediments using chemostat enrichments. J Appl Bacteriol 53:29–33.

7. Warren YA, Citron DM, Merriam CV, Goldstein EJC. 2005. Biochemical Differentiation and Comparison of Desulfovibrio Species and Other Phenotypically SImilar Genera. J Clin Microbiol 43:4041–4045.