Analysis of CDC Bioreactor Internal Thermal Measurements and Sample Coupon Temperatures

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The CDC Biofilm Reactor is an integral laboratory tool for the Environmental Control and Life Support Systems (ECLSS) biofilm formation and growth research program. Critical to this research is the need to adjust and maintain various surface temperatures of the coupons housed within the CDC Biofilm Reactor. The purpose of this study was to provide quantitative temperature gradient information when the CDC Biofilm Reactor was operating according to several process scenarios. Two primary process parameters were evaluated. For the first set of test parameters, the liquid level was maintained at 350 mL, with an inlet flowrate of 0.1 mL/min, 1 mL/min, 10 mL/min. The liquid was allowed to gravity drain out of the outlet spout. For the second set of test parameters, the liquid level within the reactor was maintained at 550 mL, with an inlet flow of 0.1 mL/min, 1 mL/min, 10 mL/min and draining intermittently controlled to 0.8 mL/min to maintain the 550 mL level. Due to the placement of the thermocouple in the reactor, a difference in temperature occurred between the coupon surfaces and target biofilm reactor temperature when operated according to the first set of test parameters. When the reactor was operated according to the second set of parameters, which resulted in the thermocouple being submerged, the temperature gradient was eliminated. The results demonstrated minimal temperature gradient between the top and bottom coupon surfaces for coupons placed in a single rod within the CDC Biofilm Reactor for both sets of test parameters evaluated. The collection of this information helped to explain previous ECLSS biofilm formation test runs, along with providing guidance on best operating practices for future ECLSS experiments. The placement of the thermocouple also helps to explain the challenge of achieving and maintaining bulk liquid temperatures when biofilm is grown according to the standardized test methods.

Nomenclature:

ASTM = American Society for Testing and Materials
CDC = Center for Disease Control
RPM = Rotations Per Minute

I. Introduction

CDC Biofilm Reactors were originally designed by researchers in the Centers for Disease Control and Prevention (CDC) biofilm laboratory to study *Legionella* biofilms[1, 2]. The CDC collaborated with researchers at the Center for Biofilm Engineering (CBE) and BioSurface Technologies to standardize and commercialize the reactor design[3] and to develop an approved standard test method for growing reproducible *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms that may be used for efficacy testing biocides[4,5]. These methods are referenced in US Environmental Protection Agency (EPA) guidance that companies must follow to make a "kills biofilm" label claim on their product[6]. In addition to being used for regulatory decision making, the CDC Biofilm Reactors have demonstrated their usefulness for the cultivation of various bacterial and fungal strains, and are often used for biofilm growth and mitigation research in academic, industrial, and pharmaceutical research[7,8,9,10]. Although numerous biofilm laboratories rely on the CDC Biofilm Reactor to grow a repeatable biofilm, none have characterized the internal thermal characteristics of the reactor throughout a test. The goal of the current study was to characterize the temperature profile within the CDC Biofilm Reactor.

II. Description of Test Setup and CDC Biofilm Reactors

The CDC Biofilm Reactor consists of a one-liter glass beaker that houses eight rods aligned with the inside of the beaker. Each rod holds three coupons. A baffle placed in the center of the reactor rotates at a speed set by a magnetic stir plate to maintain the fluid dynamics within the bulk liquid[11]. Inconel coupons were specifically tested in this scenario, although other coupon material options are available. The one-liter reactors were connected to an upstream peristaltic pump which feeds media from a carboy at a specified rate, depending upon the microorganisms being studied similar to the set up described in ASTM Methods E2562-22 and E3161-18. For this study, a float switch was included within the reactor, replacing one of the rods. This allowed for liquid level control feedback. A downstream peristaltic pump was included to control drain rates out of the reactor. By setting a high flow rate and ignoring level control feedback, the flow conditions within the reactor can simulate the protocols described in the ASTM methods. The peristaltic pumps utilized were Masterflex® L/S 100RPM 115/230 drives with a dual Pump Head L/S Easy-Load-II CRS. Inlet flow rates were verified through volumetric flow validation. Coupon temperatures were taken with thermocouples affixed on the top and bottom coupons of a single rod, Figure 1. Heating and stirring was provided by an IKA RET control-visc. A thermocouple installed in a center thermowell provided direct temperature feed back to the stir plate and controlled the temperature applied to the reactor. This thermocouple was used as the control feedback for the IKA stirring and heating plates.

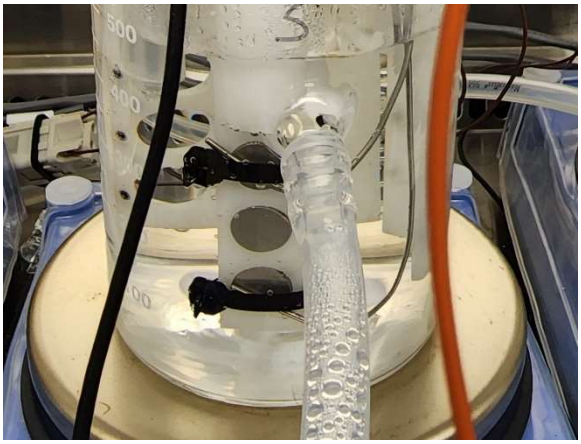

Figure 1: Coupon Thermocouple placement

III. Description of Testing Parameters

Two process parameters were examined during the acquisition of data. The first recreated the flow conditions defined in ASTM Methods E2562-22 and E3161−18. The first set of test parameters were as follows; liquid level in the reactor held at approximately 350 mL (contingent on where the effluent spout was placed on the one-liter beaker), inlet flow at 0.1, 1, and 10 mL/min, and a target temperature of 36°C. Level control was ignored, and the outlet drain rate matched a gravity drained reactor. The thermocouple well placement was not adjusted upon receiving the reactor from the manufacturer, and it should be noted ten other reactors had an identical thermowell position.

The second set of process parameters were adjusted to mirror how the MSFC's Biofilm Test Stand is run. The second test parameters were as follows; a consistent liquid level in the bioreactor held at 550 mL through float

switch control, inlet flow at 0.1, 1, and 10 mL/min, and a targeted temperature of 36°C. The outlet flow was toggled by the float switch to maintain the 550mL level. As with the first condition, the position of the thermocouple was not adjusted upon receiving the reactor from the manufacturer. This will be discussed further.

For both sets of parameters, RPM was held at 125 throughout the duration of the testing and deionized water was utilized for the process liquid.

IV. Methods

Three runs of each test case were conducted to ensure repeatability and accuracy of data. Each experiment was monitored for any anomalies. The data collection rate for all parameters was fixed at one reading every minute. Each run of the reactor encapsulated the initial heating phase, temperature ramp up, and stabilization of the bioreactor to an equilibrium. Data was taken for four cases, two with the old thermocouple and two with the new thermocouple provided by Biosurface. With both the old thermocouple and new thermocouples, each of the two test parameters outlined in the previous section were examined. However, with the final new thermocouple run following the first test parameters, the central baffle was removed.

V. Results

The results collected for the first set of test parameters are displayed in Figure 2. Each of the flow rates exhibited similar temperature behavior within the bioreactor. The temperature differential between the top and bottom coupons was very low. However, the temperature reading of the thermocouple located within the thermowell (labeled *Bioreactor Temp* in Figure 2) exhibited a dramatic temperature differential when compared to the temperature being recorded from both the top and bottom coupons throughout the test. Even after the reactor reached an equilibrium state, the temperature of the top and bottom coupons continued to read 5 to 7°C above the thermocouple reading. The most dramatic temperature differential was measured during initial ramp up, with a temperature differential up to 17.4°C (*Bioreactor 0.1 mL/min Run 3)* between the returned thermowell thermocouple value and the coupon surface temperatures. The biggest concern was how extensively the temperature deviated from the maximum and minimum bounds defined in ASTM E3161−18 for *Staphylococcus aureus*. ASTM E3161−18

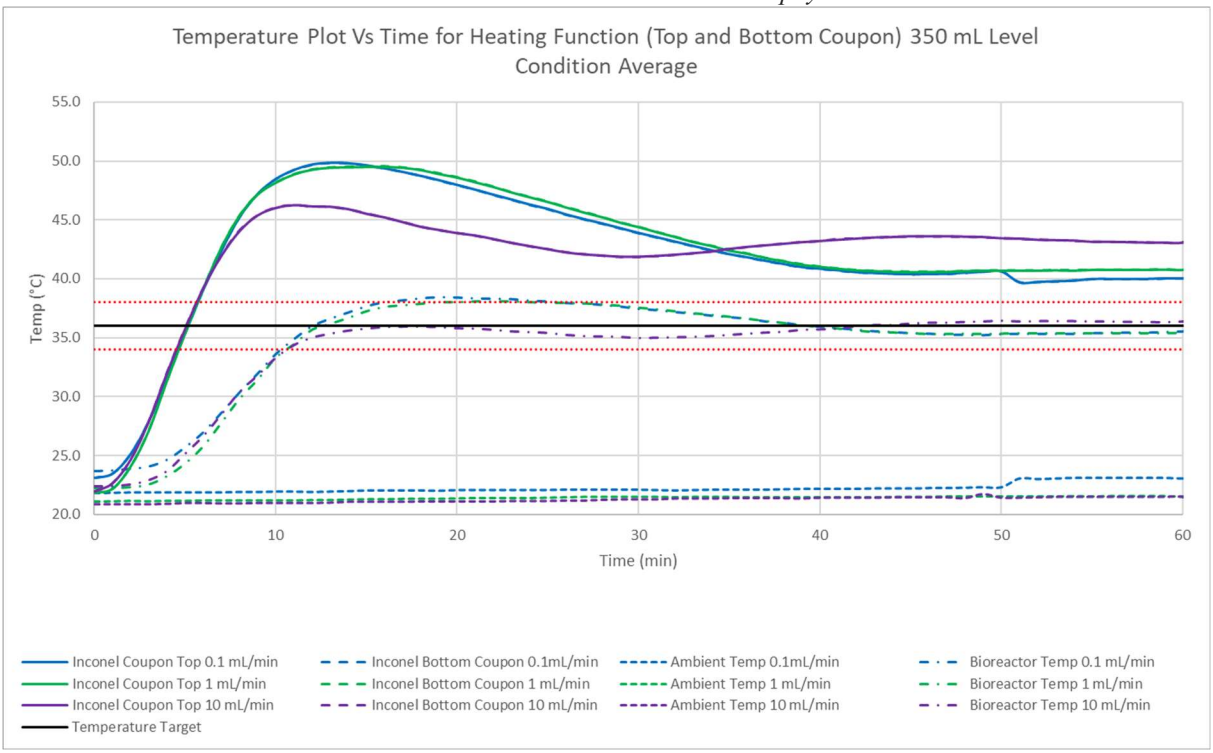

Figure 2: Results of 350 mL level temperature tests at 0.1, 1, and 10 mL/min flow rates

specifies growing *S. aureus,* at 36±2 °C for 24±2 hours in batch and 24±2 hours in continuous flow. These bounds are represented by the two red dotted lines in Figure 2. Although the *Bioreactor Temp* for each flow rate stays between these bounds once the bioreactor reaches equilibrium, the surface temperature of the top and bottom coupons never falls within the recommended range, illustrating that this setup is unable to reach the ASTM E3161−18 targeted growth temperatures for the coupons.

The results found using the second set of test parameters were different, Figure 3. As expected, based on the results from testing the first set of parameters, there was little temperature differential between the top and bottom coupon thermocouple readings. Conversely, where there was significant difference between thermocouple thermowell reading and the surface of the coupons for the first set of test parameters, for the second set of test

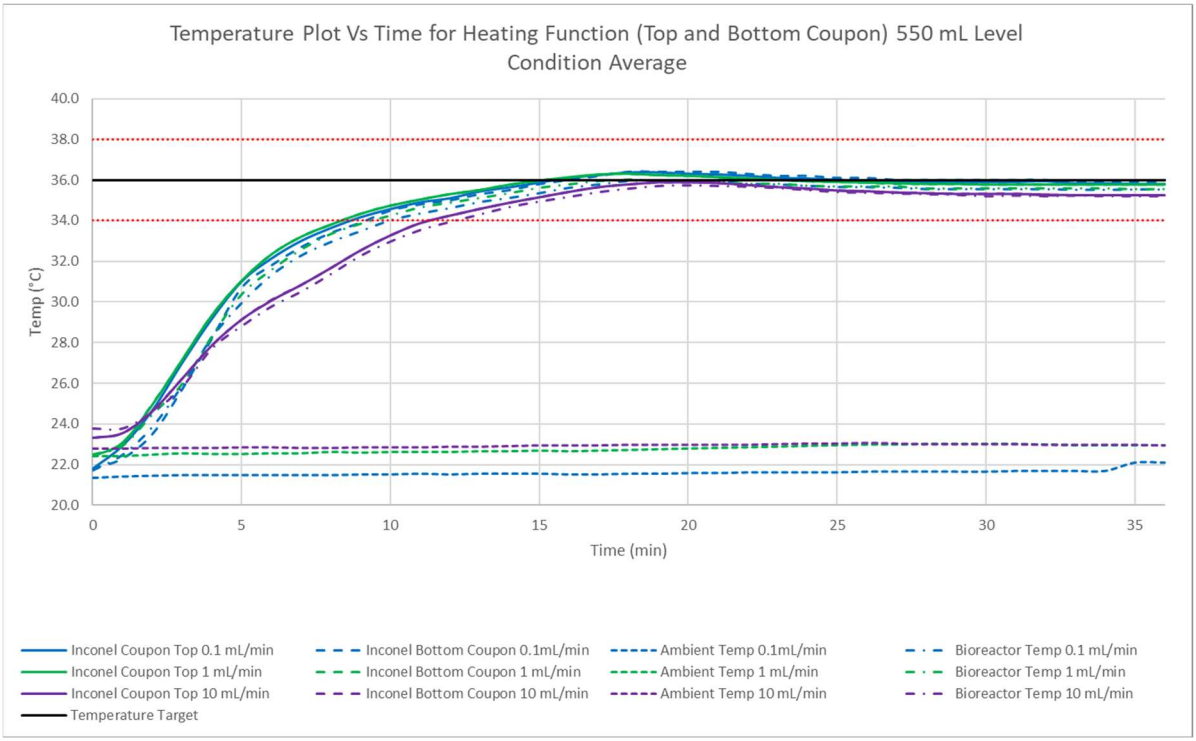

Figure 3: Average results of 550 mL level temperature tests at 0.1, 1, and 10 mL/min flow rates

conditions the readings were very close. The maximum temperature differential exhibited in these tests was 1.2°C, in comparison to the up to 17.4°C temperature differential measured in the first test parameter results. In direct contrast to the first test results, this second test's thermocouple readings also fell within the 36±2°C after the heating phase and settled in that range after the reactor reached equilibrium. These bounds are represented by two dotted red lines in Figure 3. The top and bottom coupons also remained tightly around the 36°C target after initial heating was completed. These results illustrate that the ASTM E3161−18 targeted growth temperatures can be reached and maintained, but not with the exact setup described by the standard for this configuration.

These conditions were then repeated with the newer thermocouple provided by Biosurface. For the first test parameter, even with the submerged thermowell, results were similar to previous 350mL level test runs, as seen in

Figure 4. Temperature overshot up to 9.5°C, well over the 36±2°C. With the baffle removed however, even the first

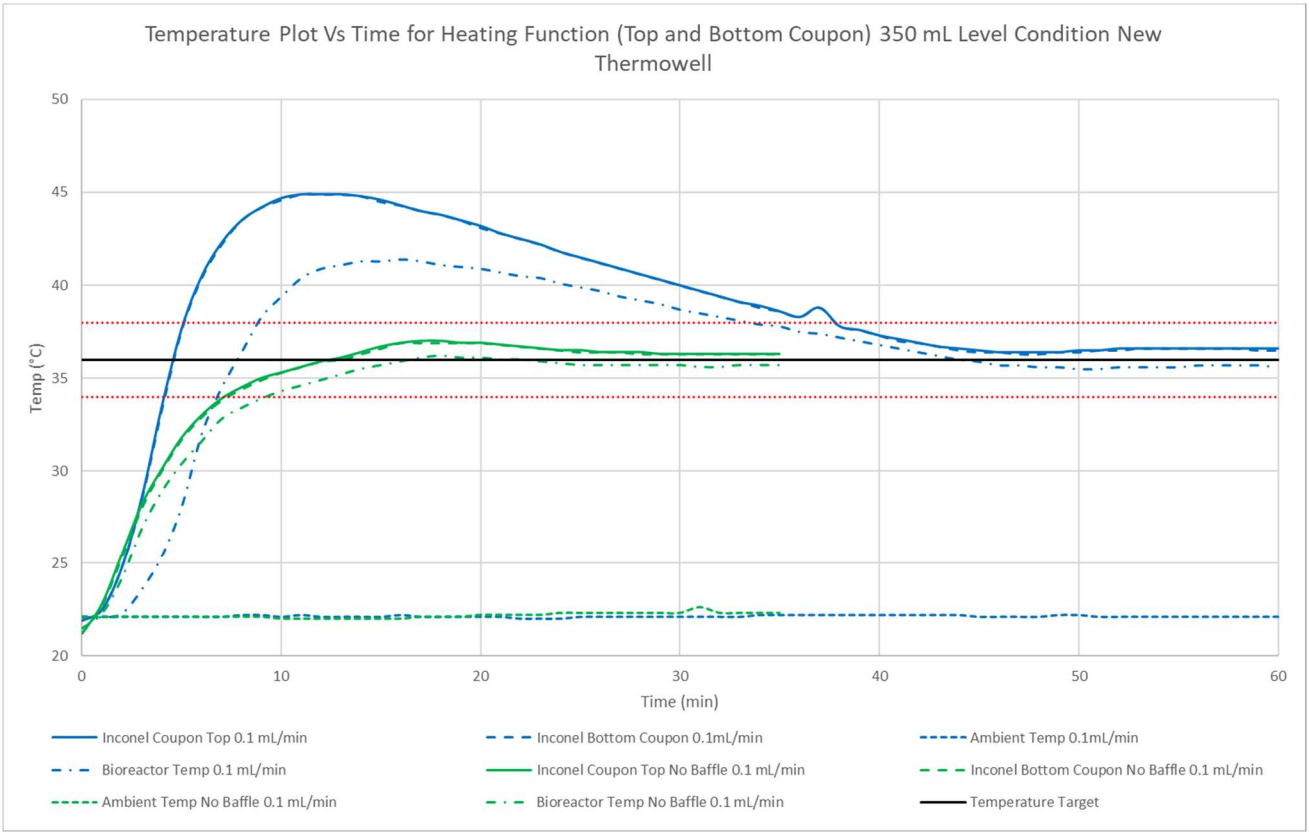

Figure 4: New thermocouple 0.1 mL/min 350 mL level run with and without the central baffle

test parameter results mirrored the shorter thermocouple second test parameter results.

VI. Discussion of Results

Evaluating the significant difference in performance for the first two test parameter suggested that the water level within the reactor was impacting temperature control. With that in mind, the reactor was disassembled to determine the thermowell thermocouple position and it was found that the thermocouple would be above the waterline within the bioreactor if the reactor was operated according to ASTM- E3161−18. The location of the thermocouple is shown in Figure 4 for the two test scenarios. Therefore, the thermowell thermocouple for the first test parameters read the temperature of the air headspace in the reactor, not the liquid temperature. The vastly higher differential temperature between the headspace gas and submerged top and bottom coupons is to be expected, as the bioreactor is not jacketed and only heated from the bottom hot plate. A higher water level led to a

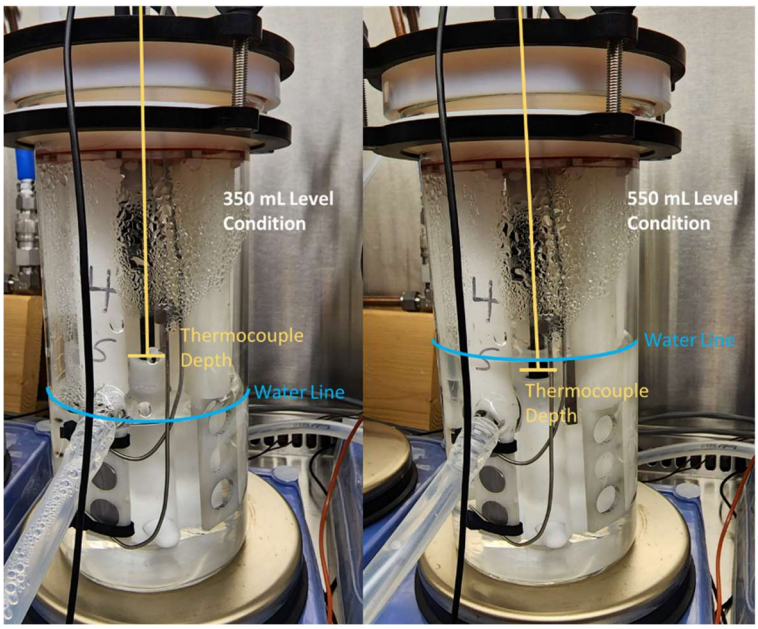

Figure 5: Comparison of thermowell thermocouple position and water line

submerged thermocouple, which explains the tight control achieved in the 550 mL level conditions used in the second parameter testing. With accurate feedback of real time temperatures, the IKA RET control-visc could accurately hit and maintain the targeted temperature.

For the second new thermowell provided, the thermocouple was submerged below the water line even in the first test parameter case, as seen in Figure 6. However, a similar temperature separation was denoted between the coupon readings and bioreactor thermocouple readings. This is due to an additional design flaw found within the bioreactor. Due to the small form factor desired, the thermowell is utilized as a support for the spinning baffle assemble. The unforeseen consequence of this is approximately a quarter inch insulating layer of polypropylene between the thermowell and water (Figure 7). This issue is bypassed when the baffle is removed, as seen in the results in Figure 4, or when the water level is above this insulting layer as with the second set of test parameters.

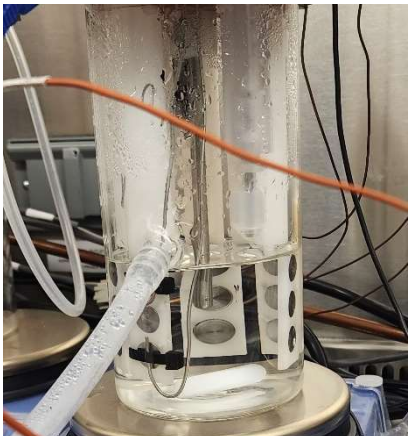

Figure 6: New thermocouple submerged below the 350 mL water line

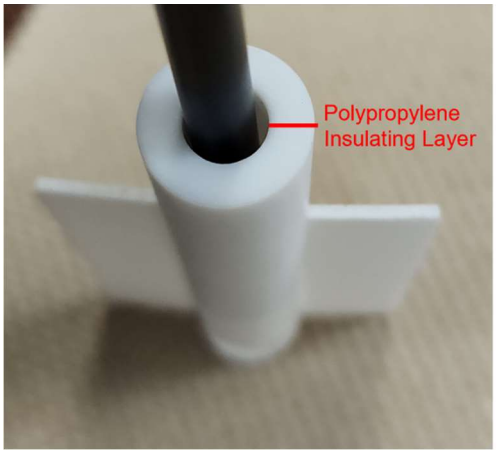

Figure 7: Baffle providing polypropylene insulting layer

VII. Impacts on Research

The impacts on both ECLSS and other related research have already occurred. For ECLSS specific research, the CDC Biofilm Reactors are used to grow a biofilm. The Biofilm Test Stand at Marshall Space Flight Center currently utilizes eleven CDC Biofilm Reactors that experienced temperature control issues when testing thermal shock treatments for future biofilm mitigation. Five reactors conducting thermal shock treatment tests experienced unstable conditions when operated according to the first set of test parameters. The data from this test is unable to be considered, due to the thermowell thermocouple measuring the headspace gas as opposed to the liquid resulting in the actual coupon temperatures being unknown.

Researchers operating the CDC Biofilm Reactor at temperatures other than room temperature also need to be aware of the location of the thermowell thermocouple. While an extensive evaluation of the temperature gradients has not been conducted before now, researchers have reported challenges with achieving the biofilm density required by the EPA guidance documents. To circumvent this challenge, ASTM Method E3161 recommends placing the entire reactor in an incubator rather than using the heating option on the stir plate. Another commonly used solution was to place the reactor in a water bath. Lowering the thermowell thermocouple position within the reactor or raising the liquid level within the reactor are both viable choices to maintain a consistent reactor temperature which will promote better growth of the bacteria requiring higher temperatures. Researchers should also be aware of the insulting effect the included baffle provides to the thermocouple inside the bioreactor thermowell shown in Figure 7. If this control scheme is utilized even with a thermocouple submerged below the water line, time is needed to allow this baffle insulating material to absorb heat and equalize with the liquid within the bioreactor.

VIII. Conclusion

Several solutions are available to remedy the thermocouple thermowell positional issues. After discussion and assistance from BioSurface, adjustments were made to the reactor design which allow for the thermocouple and thermowell position within the reactors to reach the liquid level on the majority of the bioreactors. Due to the position of the drain spout deviating slightly from reactor to reactor, there is still some risk of the liquid dipping below the thermocouple in the thermowell, so perhaps including a warning statement to check the thermowell position with each reactor is merited. BioSurface is in the process of delivering a deeper thermowell to remedy the situation. An additional solution lies in the float switch control setup utilized by the Biofilm Test Stand at Marshall to introduce level control. With this introduction of level control, the water line can be raised above the drain spout and thermocouple tip, allowing for the submergence of the thermocouple in the thermowell. This setup is what enabled the second set of test parameters to be conducted, and as seen in the results section, allows for precise temperature control. Finally, the reactor may also be placed in an incubator or water bath negating the need to use the thermocouple.

IX. References

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