

DESIGN ANALYSIS AND VALIDATION OF AN
ALTERED GRAVITY BIOFILM REACTOR
FOR SIMULATING BIOFILMS IN SPACE

by
Matthew Joseph Culp

A thesis submitted in partial fulfillment
of the requirements for the degree
of
Master of Science
in
Mechanical Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2025

©COPYRIGHT

by

Matthew Joseph Culp

2025

All Rights Reserved

ACKNOWLEDGEMENTS

I have been blessed to have many friends, family, and mentors throughout my journey through graduate school. I would not have landed here if Glenn Foster and Joe Eldring had not taken me in at the machine shop here at MSU and hired me as a freshman. My time as a shop TA enabled me to develop as an engineer, machinist, and teacher. Aubrin Heinrichs also deserves thanks for taking me under his wing, showing me how to be a teacher, and showing me the beauty and joy of watching your students succeed. My advisor, Stephan Warnat, has also been instrumental in this process. Thank you for your patience and the immense respect that you have shown me. I could not ask for a better advisor. I would also like to thank Erick Johnson and Craig Shankwitz for being on my committee and for all your assistance throughout my education. My family, friends, and churches have also played crucial roles in allowing me to attend and endure throughout graduate school. Thank you to my department and the Center for Biofilm Engineering for all the support, resources, and opportunities you have provided. I also could not be here without NASA, which funded this work through the EPSCoR grant, 80NSSC20P0581.

TABLE OF CONTENTS

1. INTRODUCTION	1
Spacecraft Wastewater System Challenges.....	1
A Review of The Previously Developed AGBR System.....	4
Thesis Outline.....	5
Research Goals and Objectives.....	6
2. BACKGROUND	9
AGBR System Design Requirements	9
Altered Gravity Considerations.....	10
Fluid Shear	13
Altered Gravity Quality	14
AGBR System Introduction	15
Biofilm Growth and Detection	17
3. REACTOR DESIGN ANALYSIS AND ITERATION	20
Fluid Sealing	22
Reactor design and operating parameters	23
Sensor And Coupon Integration Methods	24
Reactor Size.....	25
Reactor Operating Parameters	27
Rotational Speed.....	27
Axial flow.....	28
Computational Fluid Dynamics	28
Effect of design changes on wall and fluid shear stress	31
Results And Discussion of Varying Size and Sensor Integration Method	31
Sensor Integration Method Changes	31

TABLE OF CONTENTS CONTINUED

Size Changes.....	35
Results And Discussion of The Effect of Operational Parameter Changes on Wall and Fluid Shear Stress.....	35
Rotational Speed.....	35
Axial Flow	37
Particle Tracking.....	42
Reactor Design Conclusions.....	44
4. CFD AND PIV VALIDATION OF A SIMULATED ALTERED GRAVITY BIOFILM REACTOR.....	48
Contribution of Authors and Co-Authors	48
Manuscript Information.....	49
Abstract.....	50
Introduction	50
Materials and Methods.....	54
Altered Gravity Biofilm Reactor	54
Computational Fluid Dynamics.....	55
Particle Imaging Velocimetry	57
Results.....	59
Discussion.....	65
Conclusions	67
5. CONCLUSIONS AND FUTURE WORK.....	69
REFERENCES CITED.....	73
APPENDIX	77
1-INCH TANGENTIAL AGBR DRAWING PACKAGE	77

LIST OF TABLES

Table	Page
1. Table 1: Design of experiment for evaluating design and operating parameters on the AGBR system	29
2. Table 2: CFD parameters for Design of Experiment Simulations	31
3. Table 3: CFD Mesh independence study comparing mesh base size to volume average fluid shear stress and surface averaged wall shear stress on the sensor and coupon surfaces	57

LIST OF FIGURES

Figure	Page
1. Figure 1: AGBR system, shown in the configuration used in the Fall of 2023, reprinted with permission (Ketteler et al., 2025) copyright Elsevier, 2025. The main components indicated are a) the centerline coupons, b) the sensor and biofilm coupon housing, c) the electrical slip ring, and d) the DC Gearmotor. Also shown are the inlet and outlet used for filling and emptying the reactor.....	17
2. Figure 2: Comparison of the sensor/coupon integration method, cross-sectional views of the AGBR coupon ring showing the a) original (flat/recessed) and b) tangential integration methods. 3-inch nominal reactor size. Recession is highlighted in red.	25
3. Figure 3: Comparison of the sensor/coupon integration method, cross-sectional views of the AGBR coupon ring showing the a) flat/recessed and b) tangential integration methods. 1-inch nominal reactor size. Recession is highlighted in red.	27
4. Figure 4: Set of bar graphs, showing the impact of three AGBR design Parameters: Sensor/coupon integration method, reactor diameter, and reactor RPM on wall and fluid shear stresses. Wall shear stresses are taken from a surface weighted average, and fluid shear stress is a volume weighted average.....	33
5. Figure 5: Plot of wall shear stress (mPa) on the coupon and sensor surfaces of the AGBR, shown at 24.8RPM, in a 3-inch reactor with tangential sensor and coupon integration.....	34
6. Figure 6: Reactor rotational speed (RPM) compared to the average wall shear stress at the sensor and coupon surfaces in mPa, expanded RPM values in a 1-inch recessed reactor.	37
7. Figure 7: Axial flow velocity in a) 1-inch, b) 3-inch tangential AGBR rotating at 24.8 RPM, with an axial flow rate of 62.5mL/min.	38
8. Figure 8: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 1-inch recessed AGBR.	40

LIST OF FIGURES CONTINUED

9. Figure 9: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 1-inch tangential AGBR.	40
10. Figure 10: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 3-inch tangential AGBR.	41
11. Figure 11: Simulated particle location over time within the 1-inch tangential AGBR. Particles enter from the AGBR end cap. The flow goes from left to right.	43
12. Figure 12: 3-inch tangential AGBR coupon ring with coupons and sensors mounted.	46
13. Figure 13: Tangential AGBR coupon ring with coupons-only design. Mounting locations for eight coupons.	46
14. Figure 14: AGBR setup for PIV measurements with clear end cap, laser sheet, and DSLR. The light enclosure is not shown.	58
15. Figure 15: Wall shear stress on coupon and sensor pair from CFD simulation of a 3-inch AGBR with clear end cap for PIV. CFD assumes no eccentricity in rotation. The spacing between the coupon and sensor matches the spacing on the AGBR coupon ring.	60
16. Figure 16: Velocity contour plot from PIV of the AGBR with the clear end cap. Data are shown from run 4. Vectors show the direction and magnitude of velocity. The black center mark shows the geometric center of the reactor	61
17. Figure 17: Velocity contour plot from CFD of the AGBR with the clear end cap. CFD with no eccentricity	61
18. Figure 18: Velocity contour plot from CFD of the AGBR with the clear end cap. CFD with 1.5mm eccentricity in rotation. Vectors show velocity magnitude and direction.	63

LIST OF FIGURES CONTINUED

19. Figure 19 – Velocity centerline plot comparing CFD and PIV velocity values along a centerline going through the geometric center and velocity minimum. CFD values use an eccentric axis of rotation.	63
20. Figure 20 – Sensor and coupon wall shear stress from CFD simulation using a 3-inch reactor with a clear end cap for PIV. CFD uses an eccentric axis of rotation.....	65
21. Figure 21 – Fluid shear stress plot of a section plane from CFD simulation of the 3-inch AGBR with clear end cap for PIV. CFD uses an eccentric axis of rotation.....	65
22. Figure A1: Tangential 1-inch AGBR Assembly Drawing Page 1	78
23. Figure A2: Tangential 1-inch AGBR Assembly Drawing Page 2	79
24. Figure A4: Tangential 1-inch AGBR End Cap Drawing	81
25. Figure A5: Tangential 1-inch AGBR Pulley Drawing	82
26. Figure A6: Tangential 1-inch AGBR Reactor Tube Drawing.....	83

GLOSSARY or NOMENCLATURE

CFD – Computational Fluid Dynamics

EIS - Electrochemical Impedance Spectroscopy

HARV – High Aspect Ratio Vessel

ISS – International Space Station

LEO – Low Earth Orbit

LSMMG – Low Shear Modeled Microgravity

PIV – Particle Imaging Velocimetry

RWPV – Rotating Wall Perfused Vessel

RWV – Rotating Wall Vessel

AGBR – Altered Gravity Biofilm Reactor

WSS – Wall Shear Stress

ABSTRACT

Biological growth within spacecraft has posed a challenge to human spaceflight since the first long-term missions to space began. Biofilms, a surface attaching biological mass of bacteria and fungi, have clogged space station water systems since we sent up the first space stations. Skylab, the Russian Mir program, and the International Space Station suffered system failures due to biofilm growth within water systems. These failures required system cleaning and filter replacement to remain functional, a solution that increases in cost and difficulty as humanity seeks to return to the moon and travel beyond it. To prevent biofilm growth within these systems and develop cleaning and operating procedures to manage growth, tools are needed to simulate these systems on the ground.

In this work, a novel tool is developed for simulating many of the conditions found within these water systems, such as altered gravity and nutrient limitation, while being able to evaluate the growth of these biofilms. This system is called the altered gravity biofilm reactor (AGBR). The AGBR system is designed to produce altered gravity while allowing biofilms to be grown and quantified. To evaluate if low-shear modeled microgravity is being produced, computational fluid dynamics (CFD) simulations were conducted to understand the fluid environment within the AGBR. These simulations were then compared to results obtained using particle imaging velocimetry (PIV) to capture velocity measurements from the AGBR experimentally. If too much fluid shear and mixing is present in the AGBR system, the device is not recognized as creating low-shear modeled microgravity. According to CFD simulations, the AGBR has low shear stress. Experimental PIV results validated these simulation results.

AGBR design and operational changes were evaluated to determine the effect on the shear stress within the AGBR. Optimized reactor designs are developed and manufactured for improved biological sensing and ease of use. The optimal AGBR designs are documented and ready to be used to develop biofilm control strategies for spacecraft wastewater systems.

CHAPTER ONE

INTRODUCTION

Spacecraft Wastewater System Challenges

Spacecraft wastewater systems are key to enabling long-term human spaceflight. Water is dense, and humans require significant amounts to survive. Launch costs to low Earth orbit run ~\$2,700 per kilogram, and costs to the moon are many times more (Jones, 2018). Reclaiming water on long-term missions is essential to reduce launch costs (Jones, 2018). Long-term missions to low earth orbit (LEO), such as the International Space Station (ISS) and Russian MIR program, have shown that biological growth within spacecraft wastewater systems can cause a variety of problems (Carter et al., 2013; Carter, 2010; Gu et al., 1998; Kim et al., 2013; Weir et al., 2012; Zea et al., 2020). Material degradation (Gu et al., 1998), biocorrosion, biofouling (Klintworth et al., 1998; Zea et al., 2020), and clogging of critical water systems (Weir et al., 2012) have been seen on these missions. These issues take time and money to resolve, requiring more replacement parts to be sent to space, incurring launch costs, and reducing crew time available for scientific activities. These issues can be solved quickly during resupply missions for space stations in LEO. Still, missions such as NASA's Artemis program and traveling to the moon compound these challenges, making them more expensive and difficult to solve. Artemis will involve a vehicle staying in space long term, with periods of shutdown (dormancy) where the vehicle will not be inhabited. Dormancy periods provide a prime time for biological growth, which could mean a nonfunctional water system for the next group of astronauts going

to the moon. To solve these biological issues, control methods for these systems must be developed to minimize biological growth and keep systems operational, even during dormancy (Ketteler et al., 2025; Zea et al., 2020).

The biological organisms that are relevant to these challenges are biofilms. Biofilms are surface-adherent accumulations of microorganisms embedded in an extracellular polymeric substance (Donlan & Costerton, 2002). Biofilms adhere, grow, and detach, with each stage creating possible problems within a wastewater system. Detachment can cause challenges as it propagates biofilms further downstream and provides a method for clogging filters with non-adhered biomass. Growth increases the biomass in the system, requiring more cleaning and filter replacement. Attached biofilms can also cause problems, as the buildup can reduce the effective pipe diameter and thus affect volumetric flow rates and system function. Adhesions can also cause material degradation in some materials due to biocorrosion (Klintworth et al., 1998). Biofilms can be harmful to humans, especially during spaceflight, due to their effect of weakening the immune system (Kim et al., 2013). Biofilm constituents can also cause humans to get sick, as the bacteria and fungi that make up the biofilm can include species such as *Escherichia coli* (*E. coli*). Biofilms must be filtered out of potable water to avoid these adverse effects. This combination of problems caused by biofilms can cause significant damage and disruption to wastewater system functions in spacecraft. Due to the severity of these problems, it is crucial to develop effective strategies to control biofilm growth.

Control strategies for biofilm involve a broad range of methods, such as preventing attachment with coatings, using biocides to kill biofilms, or limiting nutrients needed by the biofilm constituents to grow (Zea et al., 2020). Biofilms have been shown to behave differently under altered gravity (Zea et al., 2017), so testing for all these strategies should be conducted in altered gravity. The fluid environment within systems, such as the ISS water processor assembly, should also be simulated. This means testing the same biofilm constituents (bacteria and fungi), the altered gravity environment, and similar operating conditions (temperature, size, flow rate, etc.). All these parameters affect how the biofilm will behave, since factors like altered gravity and shear rate have been shown to affect biofilm growth (Zea et al., 2017; Zea et al., 2020). To develop and test these control strategies, a test apparatus is needed to simulate these factors, allowing for a close analog to spaceflight. Due to launch costs and the difficulty of testing in space, an on-ground simulation system is ideal. To evaluate the effectiveness of control strategies, quantifying biofilm growth is needed to determine how much growth has occurred. Traditional techniques to quantify biofilm accumulation include plate counts and a variety of microscopy techniques to evaluate the biofilm constituents. The fluid conditions within spacecraft water systems should also be simulated whenever possible to provide the most similar environment to spaceflight. In summary, a system is needed to simulate altered gravity, grow biofilms, quantify biofilm growth, and simulate the fluid conditions within a spacecraft wastewater system. The required system is an Altered Gravity Biofilm Reactor (AGBR). This system and its benefits are discussed further in Ketteler et al. (2025). This thesis

will discuss how to improve the AGBR system, to guide researchers toward the best design for their specific application and experimental goals.

A Review of The Previously Developed AGBR System

An AGBR system will provide a cost-effective method for simulating spacecraft wastewater systems, allowing biofilm control strategies to be tested on the ground. To enable this system to be functional, it must allow for traditional biofilm characterization methods such as microscopy and plate counts. These values provide a snapshot of a singular point in time, so live detection of the biofilm is also invaluable and is possible with Electrochemical Impedance Spectroscopy (EIS) (Ketteler et al., 2025; McGlennen et al., 2023a; McGlennen et al., 2023b). EIS sensors provide real-time measurement of biofilm growth, allowing for continuous measurement within a biofilm reactor. These sensors could also provide a method for quantifying biofilm growth on future spacecraft, assisting in knowing when control strategies need to be implemented and their effectiveness.

An initial design of an AGBR system was developed, where a 2D clinostat capable of growing biofilms and providing both continuous and traditional biofilm characterization methods was demonstrated to be biologically viable (Ketteler et al., 2025). This design is functional but leaves much room for improvement due to the high complexity, difficulty of manufacturing, and recession of the sensing surfaces into the main coupon ring. Improving these factors needs to be balanced with two factors: maintaining a low fluid shear environment required for simulating altered gravity with a fluid-filled clinostat (rotating wall vessel) and

minimizing forces that can cause the system to experience gravity-like forces, called altered gravity quality. This thesis will discuss how this system can be improved and showcase experimental validation of the fluid dynamics within this and similar reactors to validate the system as a valuable analog for spacecraft wastewater systems, such as the ISS's water processor assembly.

Thesis Outline

Chapter 2: Background. This chapter discusses the theoretical and practical background for understanding the AGBR system. It also discusses methods of altered gravity simulation on Earth for biological applications, fluid dynamics considerations of low-shear modeled microgravity, and design considerations affecting the functionality of the reactor, such as biofilm growth.

Chapter 3: Reactor Design and Optimization. A comprehensive review of design and operating parameters for the AGBR system. Factors such as sensor and biofilm coupon integration methods, reactor diameter, rotational speed, and axial flow are discussed. The effects of changing design and operating parameters on shear stress within the AGBR are shown, and trade-offs are explained. How to design the most suitable AGBR for experimental goals is discussed in the context of design trade-offs for biological testing

Chapter 4: "CFD and PIV Validation of an Altered Gravity Biofilm Reactor." This work shows experimental validation of the fluid dynamics within the AGBR system through particle

imaging velocimetry (PIV). CFD velocity values are compared to experimental velocities obtained through PIV to verify the accuracy of the CFD simulations. Low shear modeled microgravity (LSMMG) requires low shear within the fluid, which is shown in high fidelity CFD simulations, so proving the accuracy of the CFD simulations validates that LSMMG is produced.

Chapter 5: Conclusions and Future Work. This chapter showcases the key findings, such as the most suitable AGBR designs and operational parameters. Future work to improve the AGBR and further enable biofilm control strategies research is discussed.

Research Goals and Objectives

Improving the resilience of spacecraft wastewater systems requires interdisciplinary cooperation. To support this research, the AGBR system must be experimentally validated to produce low-shear modeled microgravity. Enhancements to the AGBR system are essential for positioning the sensing surfaces closer to the main reactor, which facilitates better quantification of the bulk fluid and the biofilm suspended within it. This work aims to explore the optimal design of the AGBR for developing biofilm control strategies for spaceflight; this will vary based on the requirements of biological testing, so the design will be examined in relation to biological objectives. The primary hypothesis of this work is to demonstrate that the tangential integration of coupons and sensors does not disrupt the AGBR's LSMMG regime. To test this hypothesis and support the objectives of this research, the following aims were established:

Objective 1: Use CFD to study the impacts of changing design and operating parameters of the AGBR system, such as rotational speed, reactor diameter, and sensor integration method. CFD simulations of varied parameters will help find the best operating and design parameters for the AGBR to provide the highest quality altered gravity environment while allowing for the best integration of sensing methods to quantify biofilm growth and environmental conditions.

Objective 2: Use Particle Imaging Velocimetry (PIV) to experimentally evaluate if CFD simulations show the same velocity profile as the physical AGBR system. This objective aims to validate the CFD simulations by comparing the velocity profiles of the simulations and the physical reactor. Shear stress is challenging to measure directly, so velocity was chosen since velocity can be quantified through PIV measurements. If CFD can be shown to accurately predict the velocity behavior within the AGBR, then low shear can be assumed to be present and validate the AGBR as a valid altered gravity analog.

Objective 3: Design a system for mounting biological test coupons and sensors closer to the main reactor chamber and use CFD to evaluate if new designs maintain sufficiently low shear. The distance from sensing and test coupons in initial AGBR designs creates a recess for biological growth in different fluid conditions than in the bulk fluid. The bulk fluid is considered a better quality LSMMG than the walls, so the less distance between sensing faces and the bulk fluid, the better.

Objective 4: Manufacture an optimized AGBR system using the tangential sensor and coupon mounting for biological testing. Collaborating microbiologists will use this system to

develop biofilm strategies for the biofilm constituents found in water flown down from the ISS. The system must be autoclavable and will include the ability to run coupon rings without sensors to allow for more test coupons for traditional microbiological characterization of biofilm growth.

These validations and improvements will validate the AGBR system, allowing for improved testing of biofilm control strategies. The biofilm control strategies developed using the AGBR system will reduce biofilm growth as a barrier to long-term spaceflight missions, minimizing another challenge prohibiting missions to the moon and Mars.

CHAPTER TWO

BACKGROUND

AGBR System Design Requirements

The AGBR system has three key functions: providing an altered gravity environment, growing biofilm, and providing methods to quantify biofilm growth. These functions are interdependent on design constraints. Biofilm growth requires a period of days to weeks to conduct, which influences the acceptable methods of simulating altered gravity. These interdependencies, along with the goal of developing biofilm control strategies, lead to the development of the following level one design requirements:

1. Provide an altered gravity environment for a minimum duration of seven days
2. The AGBR must be able to control the quantity and kind of fluids and gases present in the AGBR
3. The AGBR must be able to control the nutrients present in the AGBR
4. The AGBR must integrate standard biofilm test coupons to allow for traditional biofilm growth characterization
5. The AGBR must include electrochemical impedance spectroscopy sensors from the Warnat lab to provide in situ measurements
6. The AGBR must be made of components capable of being autoclaved
7. The AGBR must be able to be assembled and operated within a biosafety cabinet by a gloved user

The AGBR system is primarily designed to simulate components within the water processor system of the ISS, so whenever possible, the ability to model system operation, such as batched flow and component size, should be integrated. Design requires inherent trade-offs between factors such as cost, complexity, and functionality. The AGBR system is intended to be utilized by various end users from different disciplines with varying research projects and goals. These trade-offs must be considered in the context of these research goals and requirements, which will lead to different design choices and values. There is not one best AGBR design; individual designs need to be evaluated with the context of the application and testing goals.

Altered Gravity Considerations

The AGBR is intended to simulate the International Space Station, which is currently in low Earth orbit. With current orbital altitude, the ISS experiences ~88% of the gravitational acceleration present at sea level, due to the increased distance from the center of Earth's mass. Given this fact, why do astronauts float instead of walking around the ISS? The ISS is in a constant state of free fall, causing the phenomenon of weightlessness (Ferranti et al., 2020). The ISS would collide with Earth if it were not for the station's velocity. This means that the ISS experiences both weightlessness and altered gravity.

Weightlessness, as defined herein, occurs when the acceleration of an object is decoupled from its mass (Albrecht-Buehler, 1992). Altered gravity, or microgravity, is when a body experiences a reduced gravitational acceleration. Quality of altered gravity is measured by

the amount of gravitational acceleration experienced by an object, generally expressed in relation to the gravitational acceleration experienced on Earth.

Biofilm growth takes at least several days to reach the detachment stage and begin the attachment, growth, and detachment cycle. Weightlessness cannot be simulated for periods over an hour without spaceflight, but altered gravity can be simulated for lengths of days to weeks with a variety of methods. Due to this constraint, the AGBR system needs to provide an altered gravity environment, not a weightless environment, for at least 7 days. This time constraint narrows the acceptable methods of simulating altered gravity. The biofilms that are being studied exist in fluid environments, so the method of simulating altered gravity must also be compatible with a fluid-filled environment. These constraints are well-suited to a rotating wall vessel (RWV), a type of 2D clinostat. An RWV provides a fluid-filled, altered-gravity environment for a period of up to multiple weeks. The fluid environment is standard for biofilm formation and provides sufficient duration for biofilm growth experiments. Random positioning machines or spaceflight are the only other methods of altered gravity that provide sufficient duration for a fluid environment (Ferranti et al., 2020). Random positioning machines work on the same principles as a 2D clinostat, but rotate about another axis (Ferranti et al., 2020). This increased complexity makes system integration difficult, especially with a fluid-filled vessel. Spaceflight via the International Space Station or CubeSat would provide a better quality of altered gravity, but increase cost and complexity by multiple orders of magnitude (Ferranti et al., 2020; Jones, 2018).

A 2D clinostat is a device that simulates altered gravity by averaging the direction of the gravity vector to zero over time through rotation (Albrecht-Buehler, 1992; Ferranti et al., 2020). The device rotates about a horizontal axis, with speeds in the 10- 30 RPM range, causing a constant change in the direction that the gravity vector acts upon, averaging the direction to near zero over time. This averaging is dependent on multiple operating parameters: the device's rotational speed, the rotation consistency, and the system's rigidity. If a rotational speed gets too high, then a centrifugal acceleration will be introduced into the system, acting similarly to a constant direction of gravitational acceleration. If there is inconsistent rotation speed, the gravity vector's direction is not changing consistently, leading to a weighted average of the gravity vector, leaving a small amount of the gravity vector acting on the subject. Finally, system rigidity affects this averaging, as if the subject is not moving rigidly as a system, then a weighted average of the gravity vector will be experienced, leading to a small gravitational vector acting on the system.

A rotating wall vessel rotates a fluid-filled cylinder, operating on the principles of a 2D clinostat. Because an RWV has a fluid-filled vessel, there are two additional considerations: rigid body motion of the fluid and fluid shear. The fluid must rotate at the same rate as the wall. Otherwise, the gravity vector will not be averaged to near zero due to inconsistent rotation. The device must also operate in low shear conditions, as fluid shear will introduce forces that act upon the particles within the fluid. These forces affect the suspension of the particles, acting like gravity and leading to a less accurate simulated altered gravity analog. These

considerations are why well-designed RWSs are considered to produce low shear modeled microgravity. The definition of low shear in RWVs is not well defined, though it is agreed that lower shear is always better. A 1 mPa acceptable shear level was decided on for this work based on a literature review by a collaborator (Ketteler et al., 2025).

Fluid Shear

Low fluid shear stress is critical to producing a high-quality altered gravity analog. In true altered gravity, there is no shear or convection. Inside an RWV, convection and shear need to be minimized to prevent mechanical forces from acting upon the particles in suspension, forces that wouldn't be experienced in altered gravity. Fluid shear is derived from differences in velocity between layers of a fluid, causing layers to shear, or move, relative to one another. This shearing action causes stresses due to the viscous nature of fluids. At small shear stresses less than one mPa, these stresses will not cause a substantial change to particle motion. High shear stresses above one mPa add forces to the system that affect particle motion, increasing settling speed and decreasing the time until particles reach the vessel wall. High shear also creates stagnation zones and recirculating flow, further affecting particle suspension. These effects cause particles to no longer be in suspension and affect the constant change in the direction of the gravity vector. Two types of shear stress will be discussed: fluid shear stress and wall shear stress. Fluid shear stress, or intrafluid shear, is the shear stress within a fluid, while wall shear stress is the shear stress between a fluid and a solid object. Fluid shear is important for biofilm suspended in the fluid that has not reached the reactor wall, too high shear will lead to a force

that counteracts the biofilm's suspension in the fluid. Wall shear stress affects the biofilms that have attached to the walls of the reactor, which is most key at the sensing surfaces where changes in fluid shear could lead to differences in biofilm growth compared to the rest of the AGBR. Fluid shear has been shown to correlate with changes in the growth rate of biofilm, so maintaining similar wall shear stress throughout the AGBR is ideal.

Altered Gravity Quality

Altered gravity quality is the amount of gravitational acceleration experienced within a simulated altered gravity environment. Two mechanisms in a rotating wall vessel affect this altered gravity quality: sedimentation and centrifugal force. Sedimentation is caused by a combination of gravitational and buoyant forces acting to cause the particle to move closer or further from the center of rotation. Biofilms tend to have a specific gravity above one, being slightly denser than water. This higher density will cause particles in a water tank to sink slowly. Biofilm grows, even while detached, and so will tend to settle faster as the biofilm grows, as described by Stokes' law for small particles (Hammond & Hammond, 2001). Centrifugal forces exist in some reference frames due to the change in direction of the particles. Centrifugal force is proportional to the square of the angular velocity and linearly to the distance from the center of rotation. This means that faster rotation will lead to a higher centrifugal force, as will a larger vessel diameter. This balance of sedimentation and centrifugal forces is crucial for achieving the best altered gravity quality. A balance is crucial since both factors are coupled to rotation speed; slower speeds tend to increase sedimentation rates, while increased speeds introduce

increased centrifugal forces. Biofilms grow over time, and so particle sizes vary as well, leading to a single number for altered gravity being meaningless since it is dependent on factors that vary over time. Generally, these reactors are able to reduce gravity to $\sim 10^{-2}$ - $10^{-4}g$ in the best instances.

AGBR System Introduction

The version of the AGBR system used in Fall 2023 is shown in Figure 1, which is reproduced from a collaborator's publication (Ketteler et al., 2025). The AGBR fundamentally operates as a rotating wall vessel, integrating various sensors and biofilm coupons to allow for biofilm quantification. The electrical slip ring allows the sensor signals to be continuously received through rotation. The centerline coupons are no longer in use, but were necessary for early experiments. This system was initially designed and built by a capstone mechanical engineering team at Montana State University and has been improved continuously.

The system rotates with a motor at 24.8 RPM, about two Mosmatic rotary joints (PN 32.552), allowing fluid to flow through the axis of rotation while remaining sealed. The main reactor volume comprises two aluminum end caps with polycarbonate tubing (McMaster-Carr #8585K38) and a coupon ring for housing the sensors and coupons. The sensors and coupons are mounted with a 0.1-inch recession from the inside diameter of the coupon ring, discussed in more detail later. The connections at the coupon ring are sealed with O-rings that are compressed by threaded rods and thumb nuts to allow for tool-free assembly. The polycarbonate tubing is sealed to the end caps using two O-rings that function as piston seals.

The reactor end caps link to the rotary joints and other pipe components through National Pipe thread (NPT) connections. Flexible tubing can be connected to each rotary joint, allowing fluid flow in and out of the AGBR, which is generally performed with a peristaltic pump. An electrical slip ring is mounted to the reactor's influent side, continuously transmitting sensor signals throughout rotation. The primary internal diameter of the reactor is 2.75" (considered 3-inch nominal for later figures). This gives the main reactor chamber a volume of approximately 65.04 in³ (1065mL). The AGBR volume can be controlled by changing the length of the polycarbonate tubing used, allowing for increased or decreased volume and surface area. The AGBR is supported by a frame made from aluminum extrusion (McMaster-Carr #5537T101). The alignment of components on the frame is critical to ensuring smooth rotation of the AGBR system, including the location and alignment of the supports for the rotary joints and the location and angularity of the motor. The adjustability of the aluminum extrusion frame allows for the alignment of all components.

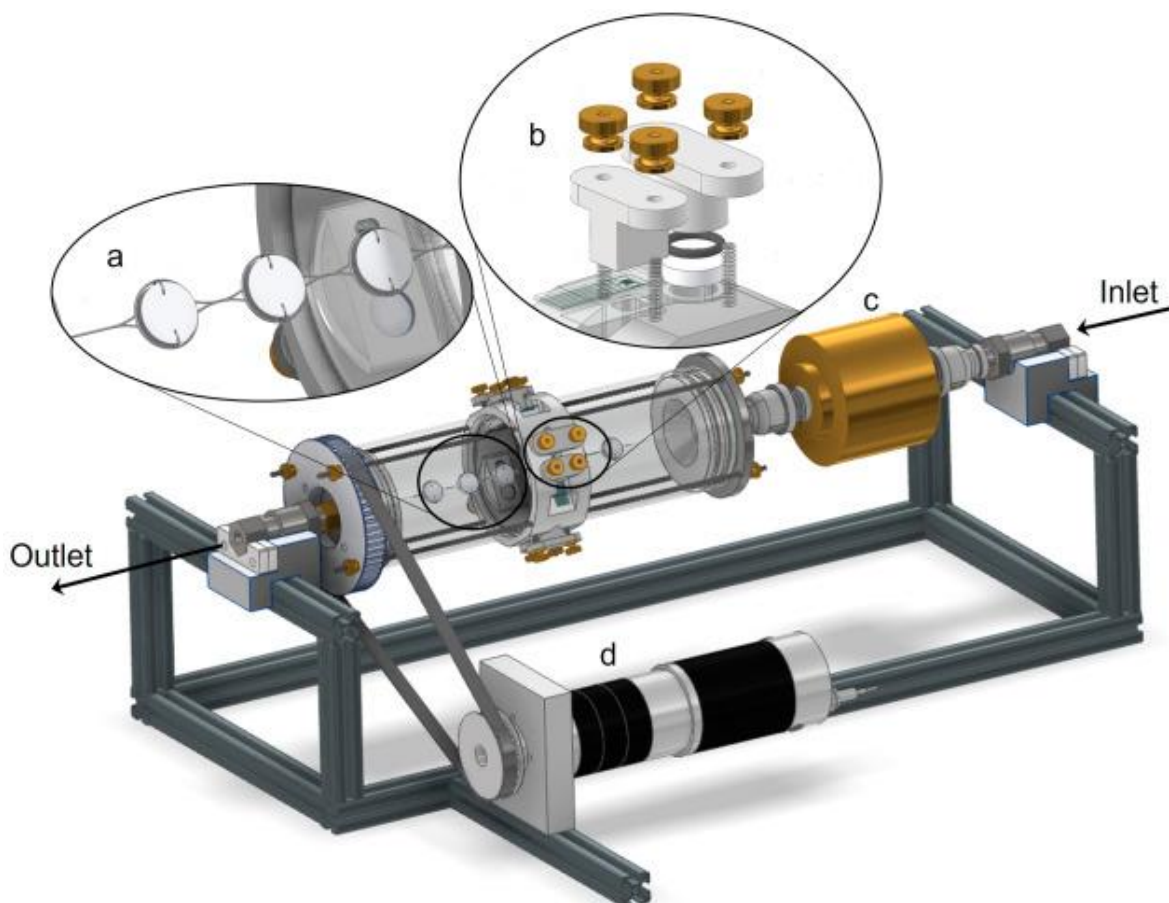


Figure 1: AGBR system, shown in the configuration used in the Fall of 2023, reprinted with permission (Ketteler et al., 2025) copyright Elsevier, 2025. The main components indicated are a) the centerline coupons, b) the sensor and biofilm coupon housing, c) the electrical slip ring, and d) the DC Gearmotor. Also shown are the inlet and outlet used for filling and emptying the reactor.

Biofilm Growth and Detection

Biofilm growth is generally conducted in the CDC reactor, a stirred vessel with a high fluid shear ideal for biofilm growth. These reactors use removable circular pucks to provide a surface that biofilm can attach to, on which microbiological characterization, such as microscopy and plate counts, can be performed. Biofilm grows faster in high-shear

environments (Characklis, 1984), which adds a layer of design difficulty since an RWV needs low shear to stay within the LSMMG regime. An AGBR system is expected to grow biofilm at a lower rate than a CDC reactor when considering microbiological applications. Since altered gravity affects the morphology of biofilms and has been shown to affect gene expression in organisms, it is necessary to diverge from the CDC reactor in design to a low-shear environment.

Nutrient and oxygen control are key to biofilm growth. Without nutrients, biofilms won't grow, and aerobic microbes cannot grow without oxygen. Because these factors affect biofilm growth, controlling them is needed to develop control strategies. Water samples from the ISS have shown a mix of anaerobic and aerobic bacteria (Weir et al., 2012). Being able to control the fluid and gas environment is important to match the conditions within the ISS water processor assembly. All seals on the AGBR system must prevent gas intrusion while retaining fluid to control the oxygen concentration. Nutrient input can be controlled by flowing nutrients through the reactor as part of AGBR operation.

Biofilm detection is conducted through electrochemical impedance spectroscopy (EIS) sensors integrated into the reactor coupon ring and coupons modeled after those used by Bioserve technologies (Zea et al., 2020), which can be used to conduct microscopy and plate counts. The EIS sensors used in the AGBR are made from gold and use a glass substrate and have been proven to detect biofilm growth (Ketteler et al., 2025; McGlennen et al., 2023a; McGlennen et al., 2023b). The Warnat lab at Montana State University manufactures and develops these sensors. The coupon material can be changed to model biofilm attachment on

different surfaces within a spacecraft, such as stainless steel, Inconel, and coated pipe surfaces.

The polycarbonate tubes can also be coated to simulate a coated pipe section.

CHAPTER THREE

REACTOR DESIGN ANALYSIS AND ITERATION

Reactor Design Considerations

The AGR is a biofilm reactor primarily used by microbiologists. It is assembled with nitrile gloves and can be autoclaved to kill any living organisms that are present after a biological test. All materials in the reactor need to be autoclavable and biologically compatible. This means they must survive temperatures of 250°F and pressures of up to 25psi for thirty minutes or more. Biological compatibility requires that the material does not actively hinder biological growth.

Most reactor components are made from polycarbonate, stainless steel, or aluminum. Polycarbonate is acceptable for 1-2 autoclaving cycles but will warp and lose optical clarity with multiple cycles, so polycarbonate components are sufficient for semi-disposable wear items. Stainless steel and aluminum are biologically compatible metals that can be autoclaved indefinitely. Aluminum is preferred for custom components due to the ease of machining and low cost. In contrast, due to their broad availability, stainless steel components are ideal for commercial off-the-shelf (COTS) components. Stainless steel components should not be threaded together whenever possible, as stainless-steel threads are prone to galling when connected to another stainless-steel fitting. Galling, also known as cold welding, is where the material on both components adheres at room temperature, damaging the threads and possibly causing seizing, which would prohibit disassembly. Generally, to prevent this, Nickel

tape is used, but nickel is not biologically compatible, so stainless steel components should not be threaded into other components made of stainless steel. This necessitates alternating stainless steel and aluminum components along the AGBR or using aluminum fittings wherever available.

AGBR Manufacturing and Assembly Methods

The AGBR system includes various fabricated components that are needed for operation. These components are made through a combination of machining and cutting processes. The coupon ring and end caps are machined, as are the rotary joint supports and coupon/sensor holders. All CNC programming was conducted in Autodesk Inventor CAM. The coupon ring is the most complex component and was made using a HAAS Mini Mill 2 or VF4 with an accompanying rotary table or trunnion, either an HRT160 or TRT200, respectively. The multi-axis nature of these machines allows for machining all sensor and coupon seats in a single operation, improving the speed of manufacturing and accuracy of the part. The end caps were turned on a HAAS TL1 lathe, with all operations conducted in a single setup to reduce runout of the AGBR. The rotary joint adapters and coupon/sensor holders were manufactured using a combination of manual machining and a HAAS mini-mill. The rotary joint holders were laser cut out of acrylic to allow for rapid changeover to different designs of rotary joints during testing. The polycarbonate tubes used to form the reactor body are cut on a miter saw to ensure sufficient surface finishes, allowing for a fluid and gas seal on the end faces in contact with the

coupon ring. All components underwent a manual inspection and deburring process to ensure that all components were glove-safe and free of burrs.

Reactor assembly was conducted after thoroughly cleaning all components to ensure no foreign debris would inhibit assembly. Since precise reactor rotation is desired, all components were assembled, and the runout of the AGBR system was measured. Adjustable rotary joint holders were made to allow system tuning to reduce runout. These blocks use two adjustment screws to allow for fine-tuning of the positioning of the rotary joints to minimize reactor runout and precession over more extended operating periods. Adjustment of the positions of the rotary joint holders and the motor to minimize runout was needed after each change to the reactor design to ensure smooth rotation.

Gloved users are less able to grip items, so the AGBR must be easy to assemble and operate without many tools. This is accomplished by having all assembly occur with thumb nuts, requiring no tools other than tweezers to aid with setting gaskets and sensors in place. All 3D-printed parts were also replaced with aluminum versions to allow for the assembled reactor to be autoclaved.

Fluid Sealing

All fluid seals were designed using the Parker Hannifin O-ring design guide for vacuum seals. Vacuum seals ensure no gas intrusion into the reactor system and operate at a higher compression than liquid O-ring seals. Standard circular profile O-rings are used for the end cap

seals to the polycarbonate tubing, with dual O-rings to ensure alignment. Square profile O-rings are used to seal the coupon ring to the polycarbonate tubing, giving more sealing surface area to enable a better seal under small amounts of misalignment that can occur during assembly. As the manufacturer allows, the polycarbonate tubing used has a high degree of variability in dimensional tolerances. Polycarbonate tubing was inspected to ensure the proper inner diameter to prevent oversized tubing from leaking at the end caps.

Sensors and coupons are sealed using custom 0.01" gaskets made from laser-cut silicone. Two thumb nuts with a spreader bar are pressed into place, distributing the load. O-rings can be placed above coupons to ensure an even load distribution and help prevent leaks. All threaded fluid connections are sealed using PTFE pipe tape to ensure gas-tight seals and prevent gas ingress.

Bubbles formed after one day of operation and grew over time; it was discovered that this was due to the tubing used on the inlet pump, which was found to be oxygen permeable. These bubbles created a higher shear area and prevented oxygen concentration control within the AGBR. Tubing that is not air permeable was substituted, and when combined with O-ring seals designed for vacuum, the number of bubbles was minimized. Reactor filling is done by pumping the fluid in with the reactor held vertically, with the rotational axis, and pumping fluid in from the bottom. This reduces the amount of air trapped in the reactor during filling.

Reactor design and operating parameters

Sensor And Coupon Integration Methods

The initial reactor design developed by a capstone team at Montana State University used a coupon ring with internal flat faces, giving a constant amount of recess for the sensors and coupons, which were mounted in pairs on each flat. These flats reduce the inner diameter of the coupon ring and simplify the manufacturing of the coupon ring. It was decided to mount coupons and sensors individually tangentially to a cylindrical center bore, allowing for a significant decrease in the sensor/coupon recession, as seen in Figure 2. The decreased amount of recess provides less crevice for the biofilm to grow in and allows for better access to the bulk fluid, allowing the sensors to measure closer to the main reactor volume. An additional benefit is maintaining the circularity of the internal reactor volume, which means near-constant cross-sectional area. Changes in the cross-sectional area can cause changes in flow velocity when fluid is pumped through the AGRB; reducing changes in the cross-sectional area should reduce changes in fluid velocity along the length of the reactor. While marginally more challenging to manufacture, these changes are biologically beneficial and worth the additional manufacturing difficulty.

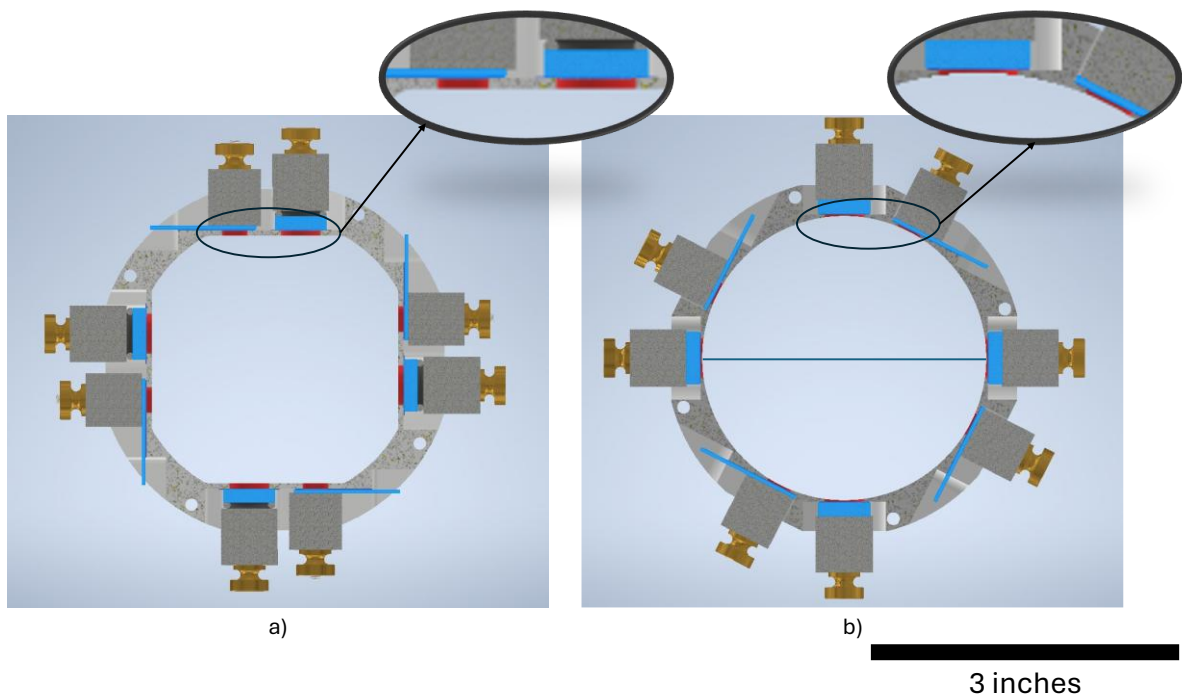


Figure 2: Comparison of the sensor/coupon integration method, cross-sectional views of the AGBR coupon ring showing the a) original (flat/recessed) and b) tangential integration methods. 3-inch nominal reactor size. Recession is highlighted in red.

In the tangential design, the sensors and coupons are mounted between 0 and 0.01 inches away from the point of tangency on the inner wall of the AGBR coupon ring, allowing for manufacturing variation while maintaining functionality. CFD analysis was performed to evaluate whether the sharp edges of this design strategy will cause a rise in shear stress. If sharp corners cause problems, filleting can be performed in a 5-axis CNC machine to round these sharp corners.

Reactor Size

The water processor assembly on the ISS uses pipes around 0.25 inches in diameter. To better match these components, a 1-inch inner diameter AGBR was developed, the smallest

reactor size determined to be manufacturable with equipment at Montana State University due to minimum feature sizes and tolerances. These reactors have two coupons and two sensors mounted tangentially or with a flat and recessed surface, as shown in Figure 3. For the same reactor length, this reactor offers a much lower volume, which is a benefit or challenge depending on experimental goals. Residence time, how long a particle, such as an agglomeration of biofilm, resides within the reactor, is decreased when under the same flow rate as a larger reactor. The time for biofilm to reach the reactor wall and attach is also decreased, which can be advantageous in allowing shorter experiments due to a shorter time till biofilm attachment. These factors are advantageous for shorter experiments, but offer challenges in longer experiments where it is ideal for the biofilm to remain suspended in the fluid. This reactor design was manufactured and is fully functional, but has not been tested biologically.

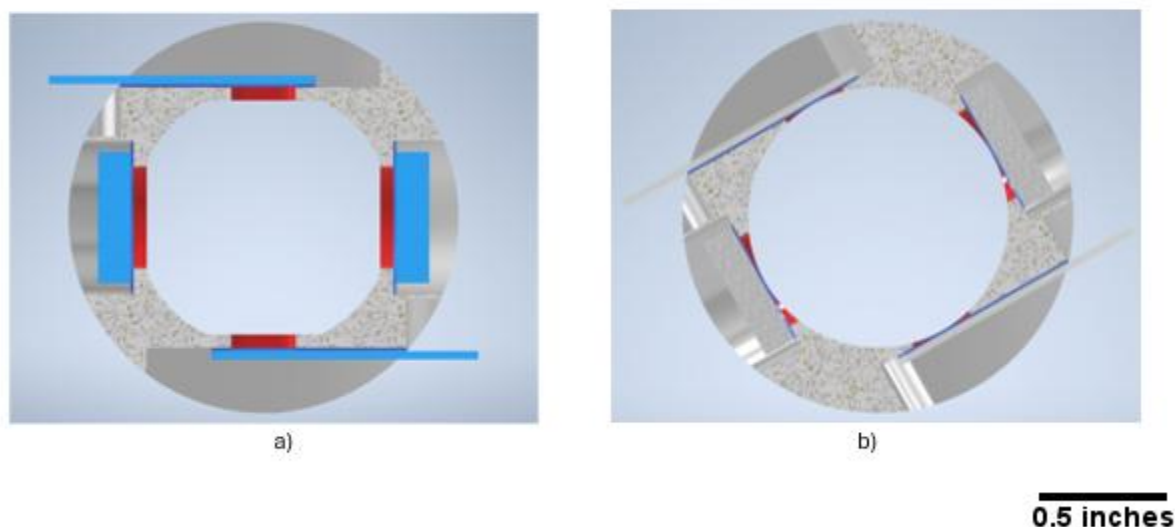


Figure 3: Comparison of the sensor/coupon integration method, cross-sectional views of the AGBR coupon ring showing the a) flat/recessed and b) tangential integration methods. 1-inch nominal reactor size. Recession is highlighted in red.

Reactor Operating Parameters

Rotational Speed

The rotational speed of a rotating wall vessel is important because it affects the amount of particle settling and centrifugal acceleration experienced within the vessel, as well as the shear. Finding the optimal speed for the AGBR is difficult because the size and shape of particles within the AGBR are constantly changing. As biofilm grows, it will grow larger and thus be affected by settling and centrifugal forces differently. Reactor size also plays a role in these factors since the distance from the point of rotation plays a role in both settling and centrifugal acceleration (Albrecht-Buehler, 1992; Ferranti et al., 2020; Hammond & Hammond, 2001). A

higher rotational speed of 32 RPM was considered, primarily for the smaller diameter reactor, which generally should be spun faster to better balance settling and centrifugal forces (Ferranti et al., 2020). Understanding the effects of shear caused by a higher rotational speed was also of interest since at a higher rotational speed, there was expected to be increased shear, since shear is derived from the gradient of velocity.

Axial flow

Simulating biofilm formation in pipes with flow is an area of interest for biofilm researchers. The pipes on the ISS operate in batch flow conditions where fluid will stagnate for hours and then operate with flow for a shorter period. Both states are of research interest, but one advantage of the AGBR system is the ability to simulate flow within a pipe under flow conditions. Low flow rates were desired to be explored since high flow rates were likely to wash biofilm out of the system before it could reach the walls and attach. Understanding the velocity profile within the AGBR was also of interest since if biofilm and nutrients stayed near the center of the reactor, biofilm may not be able to attach. Axial flow is also the primary mechanism for adding nutrients and controlling the gas environment within the system, so understanding the effect of these actions on the shear within the AGBR system is needed to understand if the shear caused by flow will interrupt the LSMMG environment.

Computational Fluid Dynamics

Fluid shear is extremely difficult to measure directly. The amount of shear stress within the AGBR is critical to ensuring the device operates within the LSMMG regime, so the shear

stress needs to be quantified. Due to the difficulty in directly measuring shear, numerical simulations were used to estimate the shear stress within the system. Computational Fluid Dynamics (CFD) software was utilized to estimate the shear stress and evaluate the effects of changing design and operational parameters on the AGBR, as shown in Table 1.

	Design Parameter		Operating Parameter	
	Sensor/Coupon Integration Method	Reactor diameter (in)	Rotational speed (RPM)	Axial flow rate (mL/min)
Level 1	Flat/Recessed	2.75" (3" nominal)	24.8	0
Level 2	Tangential	1"	32	62.5

Table 1: Design of experiment for evaluating design and operating parameters on the AGBR system

All CFD was performed using Siemens Star CCM+ version 2306. All 3D models were first created in Autodesk Inventor; depending on the geometric complexity, one of two processes was used to import the geometry into Star CCM+. For geometry with less sharp corners, the solid model of the AGBR was exported from Inventor as a .STEP file and then imported into Star CCM+, and then a surface wrapper was used to derive the fluid volume. For geometry with sharper corners, such as tangential sensor integration methods, the liquid volume was derived in Inventor, exported as a .STEP file, and imported into Star CCM+. The fluid volume was limited to the main reactor volume, covering the fittings mounted to the endcaps rather than simulating the entire rotating assembly. This reduced the element count and allowed for faster

simulation convergence. Most simulations were run with the parameters listed in Table 2. The simulations were run as implicitly unsteady due to a lack of stability when running simulations under a steady assumption; this is theorized to be due to the time-dependent effects seen in rotating horizontal cylinders (Seiden & Thomas, 2011; Seiden et al., 2005, 2007). An initial velocity was assumed to be equivalent to an AGBR that is already in rotation. These simulations do not mimic the startup of the reactor from rest. This initial velocity speeds up the time to model convergence. The time step of 0.006667 seconds was chosen to limit the rotation of the AGBR to less than 3 degrees per second. The automated mesh tool created the mesh with the surface, polyhedral, and prism lasher meshers. A uniform mesh refinement maintained the mesh size constant throughout the reactor volume. Prism layers were utilized to provide better refinement around walls and thus provide more accurate wall shear stress values. An average element size of 0.020 inches was used for most simulations to allow for faster CFD analysis. It was shown that in higher fidelity models, lower shear will be seen (Ketteler et al., 2025). Most CFD simulations aim to evaluate if design changes create significant increases in shear stress within the AGBR, not assess the actual amount of shear seen in the reactor.

The physics models within Star CCM+ were chosen as follows. Water was modeled as incompressible. Reynolds-Averaged Navier Stokes with K-Omega turbulence models were used. All outer walls were treated with a no-slip boundary condition. The inlet utilizes a uniform volumetric flow rate when modeled with through flow. The outlet acts as a pressure outlet. Rotation was performed about the geometric centerline, rotating at 24.8PM.

Parameter	Value
Average mesh base size	0.020 in (0.5 mm)
Prism layers	2 layers with a thickness of 0.0065in
Time step	0.006667 seconds
Water density	997.561kg/m ³
Water dynamic viscosity	.887 mPa-s

Table 2: CFD parameters for Design of Experiment Simulations

On all runs, the average fluid shear stress within the AGBR and wall shear stress on all coupon and sensor faces were tracked. All models were run until a 1% or less difference was seen in all residuals, though much lower values were often achieved.

Effect of design changes on wall and fluid shear stress

Changes in reactor diameter (size) and sensor integration method (SIM) were evaluated for their impact on fluid and wall shear stress. The same settings, rather than a similar element count, allowed for a better comparison of shear values across the different volumes since shear is directly related to element size.

Results And Discussion of Varying Size and Sensor Integration Method

Sensor Integration Method Changes

The previously discussed sensor integration methods were evaluated in a series of CFD models that applied the same setting across various operational and design factors. In addition

to sensor integration methods, the reactor size and rotational speed were changed across all permutations to allow for an understanding of how all factors affect shear stress within the AGBR. The sensor integration methods will be referred to as tangential or flats/recessed. Due to the larger element size used in this study, these values do not reflect actual shear values. Shear is known to decrease with smaller element sizes, so these values are used purely to evaluate the difference between reactor types.

Figure 4 shows that tangential sensor integration generally shows similar or higher shear stress than the recessed method. These shear stresses all remained small, at or below 10 mPa. The rise in shear was substantially more pronounced at 32 RPM, where a 315% and 469% increase in wall shear stress was seen in the 3-inch reactors, respectively. This rise is most likely due to the combination of larger size and RPM, which contribute to higher velocities, and since shear is derived from changes in velocities, a higher shear is expected. At 24.8 RPM, 24% and 42% increases in wall shear stress were substantially lower. Fluid average shear stress saw similar trends, with tangential sensor integration showing an almost identical shear as the recessed method at 24.8 RPM, with less than 1% shear change. Higher shear increases were seen at 32 RPM, with 121% and 160% increases for the 1-inch and 3-inch reactors, respectively.

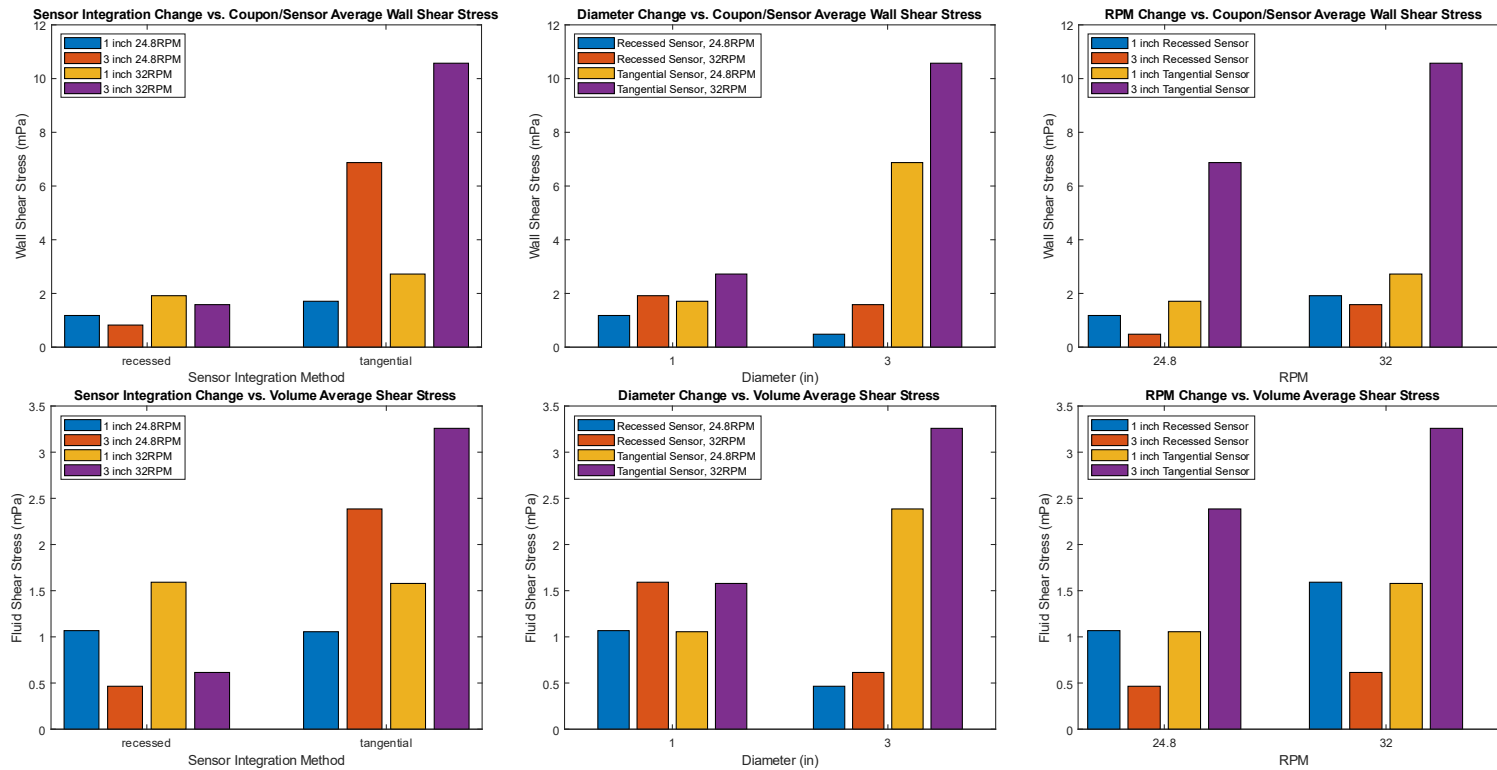


Figure 4: Set of bar graphs, showing the impact of three AGBR design Parameters: Sensor/coupon integration method, reactor diameter, and reactor RPM on wall and fluid shear stresses. Wall shear stresses are taken from a surface weighted average, and fluid shear stress is a volume weighted average

Based on the shear profile seen across the sensor and coupons, shown below in Figure 5, most of the high shear regions exist in the center of the coupons and sensors. The 1-inch reactor showed smaller increases in shear and lower shear than the 3-inch reactor with the same sensor integration method changes. The lower changes track with the theory that smaller-diameter vessels will have a lower velocity near the outer wall than larger-diameter vessels, since the velocity is expected to change as a function of angular velocity times the radius. The increase in shear stress by switching to a tangential integration method for sensors and coupons showed an increase of much less than an order of magnitude for current operation conditions. Given the biological benefits and sensing benefits of moving to this design, it was decided to utilize this design moving forward.

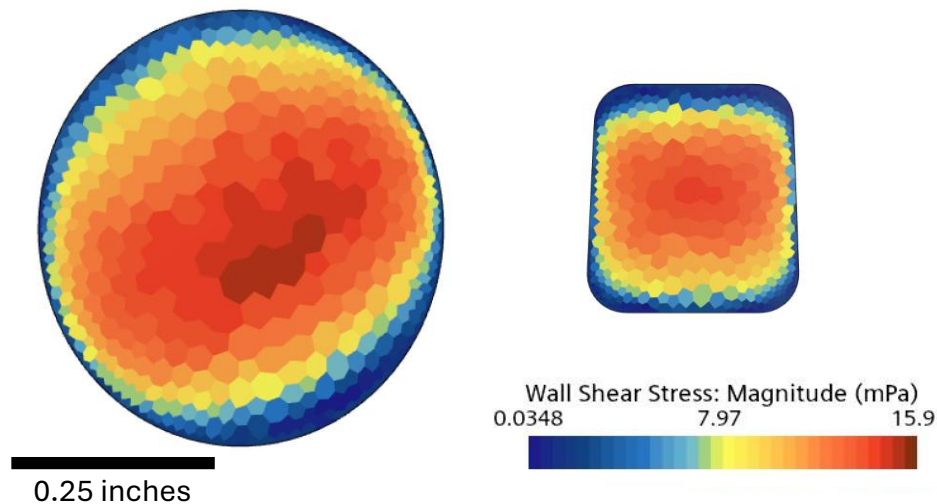


Figure 5: Plot of wall shear stress (mPa) on the coupon and sensor surfaces of the AGBR, shown at 24.8RPM, in a 3-inch reactor with tangential sensor and coupon integration.

Size Changes

Reactor size was evaluated as a design factor since component sizes in spacecraft tend to be much smaller than the 2.75-inch inner diameter (3-inch nominal diameter) of the larger AGBR designs. A 1-inch inner diameter reactor was evaluated. The 1-inch and 3-inch reactors were evaluated with tangential and recessed sensor/coupon integration methods.

As shown in Figure 4, changing the diameter was coupled with the sensor/coupon integration method in evaluating the change in shear stress. For a recessed sensor and coupon integration, an increase in diameter causes a decrease in shear stresses, while for tangential integration, an increase in diameter causes an increase in shear stresses. For the recessed sensors, a decrease in surface-averaged wall shear stress was 26% and 12% for 24.8 RPM and 32 RPM, respectively. For volume-averaged shear stress, the reductions were 190% and 288%, respectively. For tangential integration, an increase in reactor diameter was correlated to an increase in surface-averaged wall shear stress of 26% and 12%, respectively, for 24.8 RPM and 32 RPM. For volume-averaged shear stress, the increases were 84% and 106%, respectively. The decrease in shear stress for the recessed reactors is surprising, considering that the fluid near the outer wall is moving at a higher velocity with a larger reactor. Since shear depends on velocity, an increase in shear is expected with an increase in reactor diameter.

Results And Discussion of The Effect of Operational Parameter Changes on Wall and Fluid Shear Stress

Rotational Speed

Changes in the rotational speed of the AGBR were evaluated because, as rotational speed increases, the quality of altered gravity should increase due to improved averaging from faster rotation. While this improvement in altered gravity quality is expected, increasing rotational speed is expected to cause an increase in shear stresses, which could cause the flow to leave the low-shear modeled microgravity regime. This trade-off means an AGBR should spin as fast as possible while staying in a low-shear regime. For 3-inch reactors, that size was evaluated to be around 24.8 RPM, which should decrease for smaller reactors. A higher rotational speed of 32 RPM was assessed to see if shear stresses increased substantially, showing if an RPM increase could be viable to achieve higher quality simulated altered gravity.

As shown in Figure 4, higher RPMs showed an increase in shear stress, matching the trend expected. For the recessed sensor/coupon integrated reactors, an increase in surface-averaged wall shear stress was 11% and 16% for 1-inch and 3-inch AGBRs, respectively. For volume-averaged shear stress, the increases were 22% and 6%, respectively. For the tangential sensor/coupon integrated reactors, an increase in surface-averaged wall shear stress was 15% and 54% for 1-inch and 3-inch AGBRs, respectively. For volume-averaged shear stress, the increases were 22% and 37%, respectively. These increases in shear stress are low and show that, as long as true shear is low enough to allow for an increase, running at a higher RPM could be a valid method to improve simulated altered gravity quality.

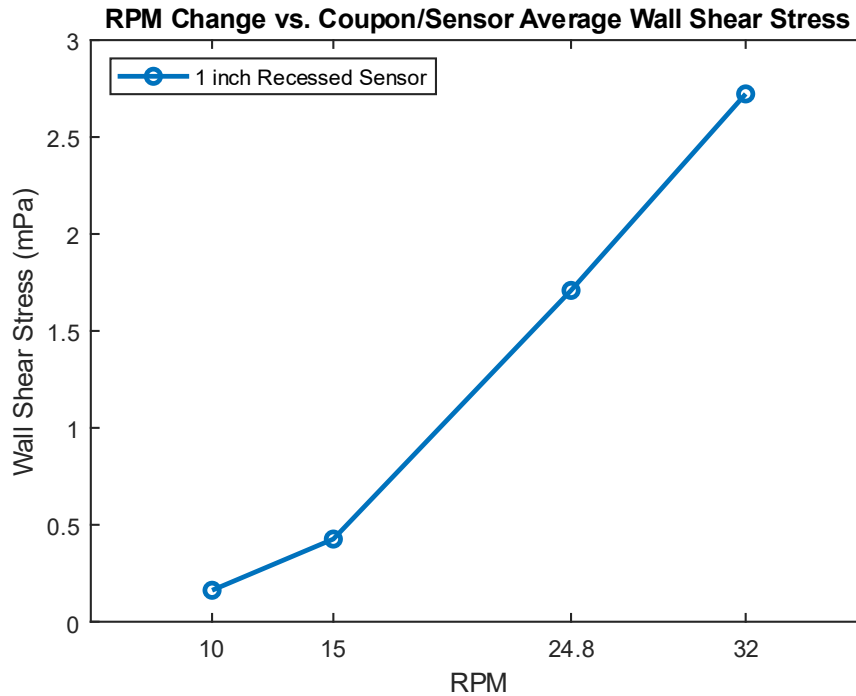


Figure 6: Reactor rotational speed (RPM) compared to the average wall shear stress at the sensor and coupon surfaces in mPa, expanded RPM values in a 1-inch recessed reactor.

An expanded RPM study on the 1-inch recessed reactor was conducted to evaluate whether the wall shear stress trend maintained linearity. This study is shown in Figure 6 above. Linearity is expected because shear is derived from differences in velocity, and with a linear increase in velocity, shear is also anticipated to increase linearly. These studies were all performed with the same mesh and time step. Linearity was seen from 15-32 RPM, but not from 10-15 RPM.

Axial Flow

The axial flow rate was considered since real spacecraft wastewater systems, such as the ISS water processor assembly, operate under batched flow conditions. This batched flow means that the system is stagnant for some time, then it will activate flow and process

wastewater for some time. These cycle times and flow rates vary between and sometimes within systems as fluid is split into different processing steps or types. Axial flow was evaluated for the effects on shear stress to ensure that flow does not cause a substantial rise in shear stress and cause the AGBR to leave the LSMMG regime. Axial flow is necessary during AGBR operations to add nutrients if simulating an active system without nutrient limitation, such as when personnel are aboard a spacecraft. Research collaborators explored axial flow in a 3-inch reactor, so it will not be discussed here since higher fidelity simulations of that use case have been published (Ketteler et al., 2025).

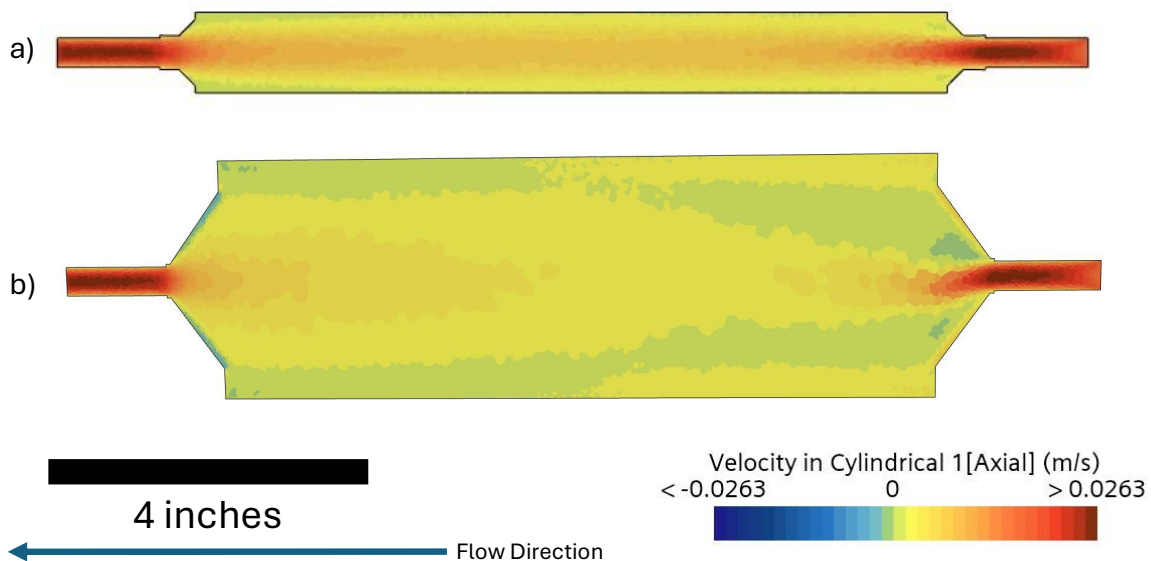


Figure 7: Axial flow velocity in a) 1-inch, b) 3-inch tangential AGBR rotating at 24.8 RPM, with an axial flow rate of 62.5mL/min.

Axial flow is expected to be more impactful in smaller reactors due to the smaller volume and distance to the wall. The 3-inch tangential reactor has a volume of 1066mL, the 1-

inch tangential reactor has a volume of 128mL, and the 1-inch recessed reactor has a volume of 127mL. As shown in Figure 7, the center of the reactor maintains the highest axial velocity, with a center jet surrounded by areas of recirculation near the end caps. This recirculation zone matches a rise in vorticity seen in the same area, which is seen to a lesser degree in simulations with no through flow. The wall shear stress is also at its highest at the angled expansion zone on the end caps, regardless of the reactor diameter or sensor/coupon integration method. The rise in wall and fluid shear stress shows increases of an order of magnitude in 3-inch designs compared to the average wall and fluid shear stresses across multiple simulations with and without through flow. This high shear region is unideal but necessary due to the need to expand reactor fittings from rotary joints and to allow space for components such as an electrical slip ring for AGBR sensor data output. Researching ways to decrease this rise in shear could be a good direction for future research.

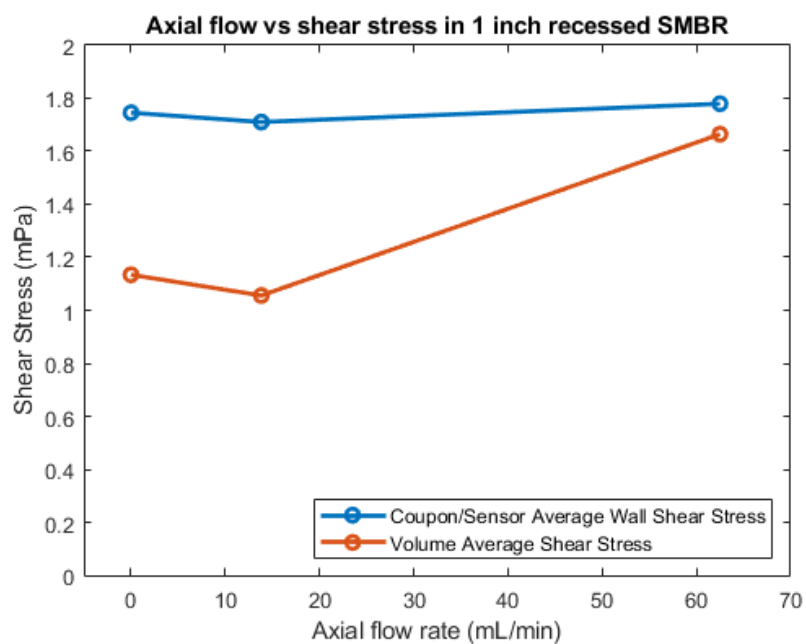


Figure 8: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 1-inch recessed AGR.

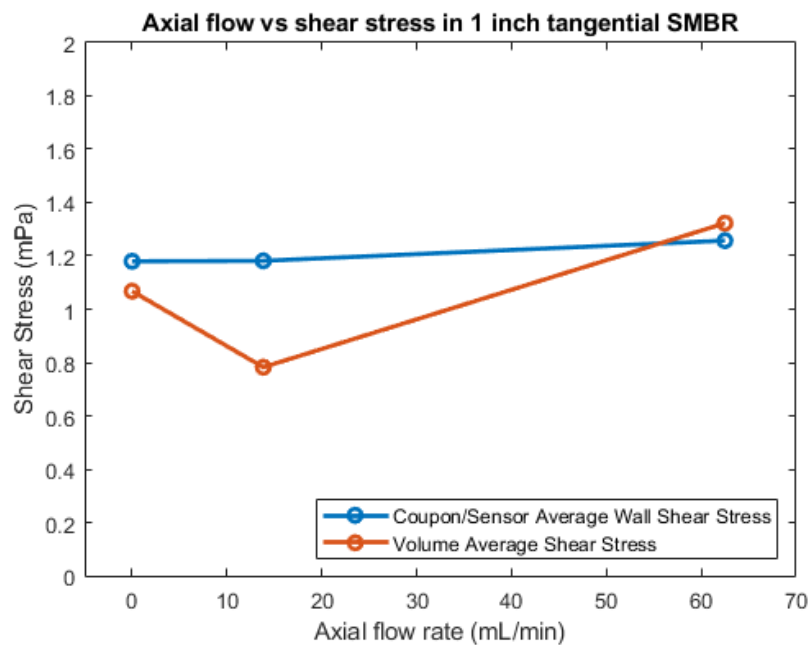


Figure 9: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 1-inch tangential AGR.

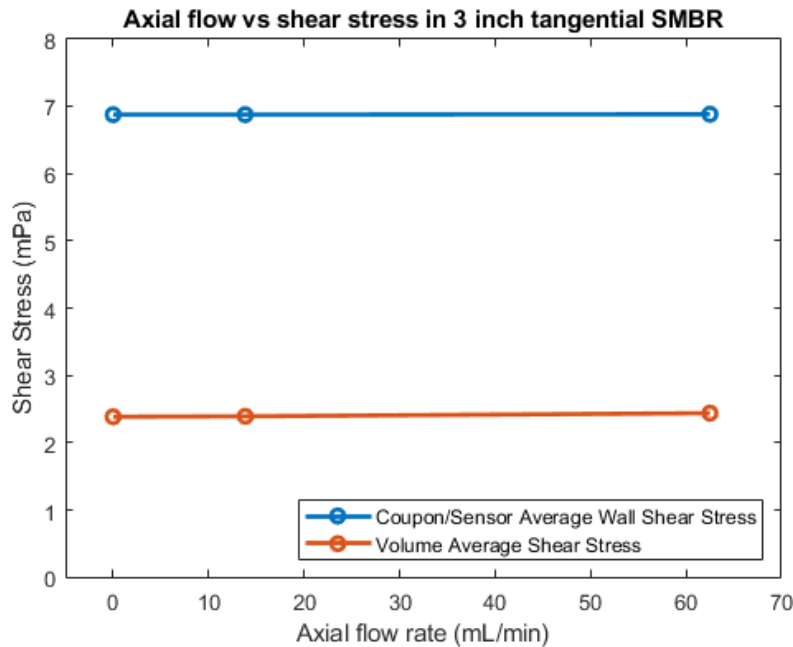


Figure 10: Axial flow rate (mL/min) versus shear stress (mPa), volume-averaged fluid shear stress, and coupon/sensor surface average wall shear stress within 3-inch tangential AGR.

Axial flow rates cause a minimal rise in the average shear stress in the reactors on key surfaces and the bulk fluid volume, as shown in Figures 8, 9, and 10. The most significant increase in shear stress is seen in the 1-inch tangential reactor, where the fluid average shear stress rose 69%, with an increase in shear of less than 1 mPa. In this case, the average coupon and sensor wall shear stress rose only 6%. Based on the significant change in shear stress in the bulk fluid and the known high shear near the inlet and outlet, it is anticipated that these concentrated regions are the main factor raising the volume average fluid shear stress; the area near the coupon ring shows a marginal rise in fluid shear stress. The 1-inch recessed reactor showed an increase in average fluid shear stress of 58% and surface average wall shear stress on the coupon and sensor faces of 4%. The 3-inch tangential reactor saw almost no change in

shear stress, with changes accounting for a less than 1% difference under the same range of flow conditions. The low change in the 3-inch reactor and higher change in the 1-inch reactors matched the expected trends.

Particle Tracking

Biofilm entering the AGBR was modeled as suspended particles in the tested fluid, generally soy broth or water. The amount of time it takes for these particles of biofilm constituents to reach the reactor wall is vital in determining if particles will reach the reactor wall during flow. Suppose the flow is too fast and particle settlement is too slow. In that case, these particles can get pushed through the center of the AGBR without having the opportunity to attach to the walls and go through the biofilm life cycle of attachment, growth, and detachment. Generally, the reactor wall of a rotating wall vessel is not considered a simulated altered gravity environment. Still, biofilm grown within the AGBR has shown the same shape as in space, spherical shapes instead of long, slender rods (Ketteler et al., 2025). Biofilm reaching the walls too quickly could be a sign of low-quality simulated altered gravity, since the fluid suspension helps maintain the averaging of the gravity vector to zero over time. Biofilms have a specific gravity averaging around 1.05 (Characklis, 1984), meaning they tend to be around 5% heavier than water, which will tend to draw the particles toward the reactor's center.

Biofilm was chosen to be simulated as 2 μ m and 500 μ m diameter spherical particles for testing. The 2 μ m size was selected to be approximately the size of a single bacterium, and 500 μ m was used to approximate a small agglomeration of biofilm. Particles were simulated

both along the reactor centerline and entering via axial flow. The axial flow particle simulations provided a more realistic representation, and it was shown that particles generally reached the reactor wall over a period of 2-3 minutes after insertion with $2\mu\text{m}$ particles, with the radial distance from the center of rotation affecting how far along the reactor the particle would reach the wall. A typical particle track is shown in Figure 11 below. The particle collisions were modeled as pure elastic, and no adhesion was modeled. However, particles tended to stay near the wall after contact. $500\mu\text{m}$ particles showed similar behavior. However, the time to the wall was shorter, generally measured in dozens of seconds, as drag and buoyancy forces sped up the precession to the wall.

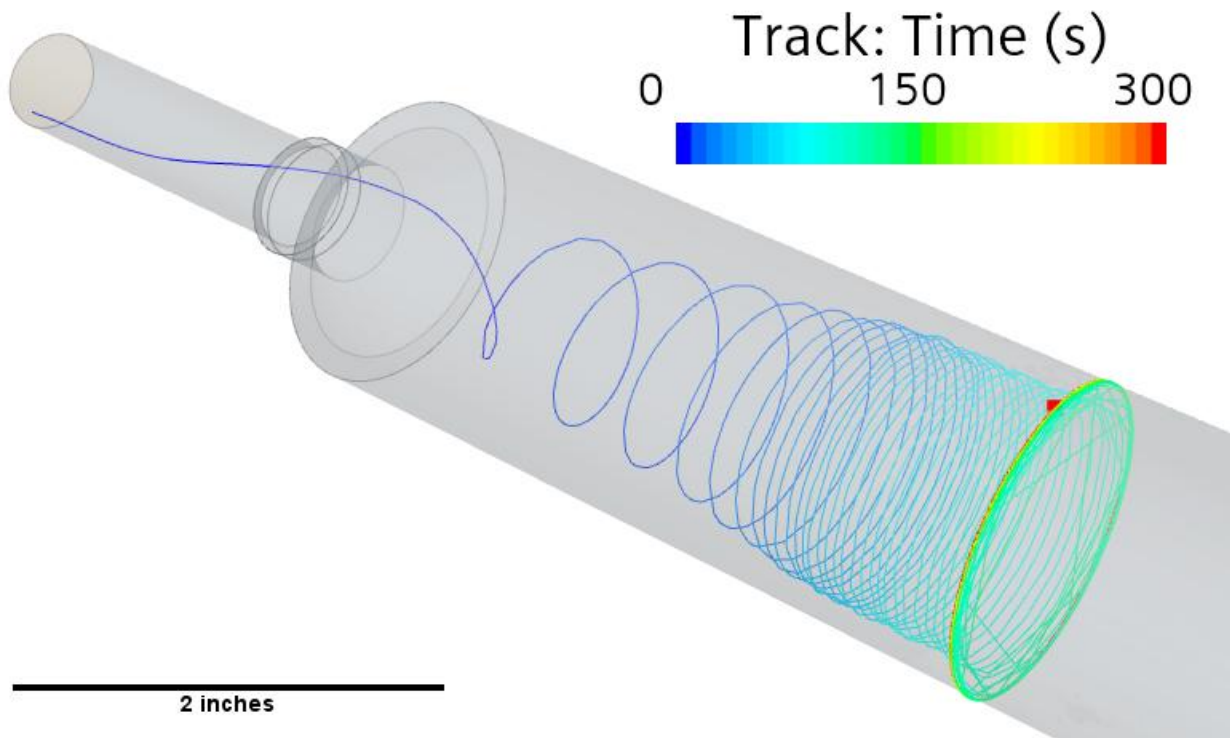


Figure 11: Simulated particle location over time within the 1-inch tangential AGBR. Particles enter from the AGBR end cap. The flow goes from left to right.

Other methods of estimating particle time to reach the wall, such as PDE solutions from first principles, were explored and provided longer time-to-wall estimates. However, substantial assumptions had to be made that limited confidence in these values. Existing literature on rotating wall vessel time-to-wall estimates varies significantly from seconds to days, depending on the assumptions used to model particle settling (Bluemink, 2008; Zea et al., 2020).

Reactor Design Conclusions

The best reactor design for most biological testing will be the 3-inch tangential reactor. This reactor provides sensor and coupon surfaces much closer to the bulk reactor fluid than a recessed design, allowing for better biological characterization. The four coupons offer enough data for traditional characterization methods. The rise in shear stress seen with the 3-inch tangential reactor over the recessed design is minimal enough that it should not cause the AGBR to leave the LSMMG regime, so biological benefits should be prioritized. The larger surface area and volume make it less likely to flush out biofilm when pumping new fluid and nutrients into the reactor. All designs of the AGBR, except the tangential 3-inch at 32 RPM, maintained shear within an order of magnitude of the original AGBR designed, which was modeled with a 14.5 million element simulation that showed shear below 1 mPa (Ketteler et al., 2025). The shear seen in these simulations gives confidence that these designs will also remain below the 1 mPa limit of LSMMG. High fidelity simulations of all design parameters were not feasible to perform, so the comparison to one high fidelity simulation was utilized.

The 1-inch tangential reactor offers a compelling system for shorter biological experiments focused on attached biofilm. However, the lower fluid volume and higher shear from axial flow need to be considered when designing experiments that use this platform. Lower axial flow speeds must be used to prevent biofilm from being washed out. Multiple coupon rings may also be needed to have enough replicates for biofilm quantification to be done confidently.

The 3-inch AGBR with tangential sensor and coupon integration was manufactured, as well as a version with only test coupons, to allow for more plate counts and microscopy samples. These designs are pictured below in Figures 12 and 13. The tangential reactor with only coupons has been used for approximately five months to conduct experiments within the Center for Biofilm Engineering at Montana State University for the NASA EPSCOR project. The higher number of test coupons allows for more certainty when using traditional biofilm characterization methods, since more replicates are possible with eight coupons than with four. This comes at the cost of real-time sensing via EIS sensors. Data can only be collected at the end of the experiment since removing coupons is impossible without introducing air into the system, preventing further use. During continuous flow, a flow cam can characterize microbes outside the microgravity environment by sampling the effluent, allowing for characterization during operation. Due to the low flow rates, these organisms will spend minutes outside the microgravity environment, making this an imperfect characterization method, but it is the most practical way to characterize growth with a traditional biofilm method.



Figure 12: 3-inch tangential AGBR coupon ring with coupons and sensors mounted.

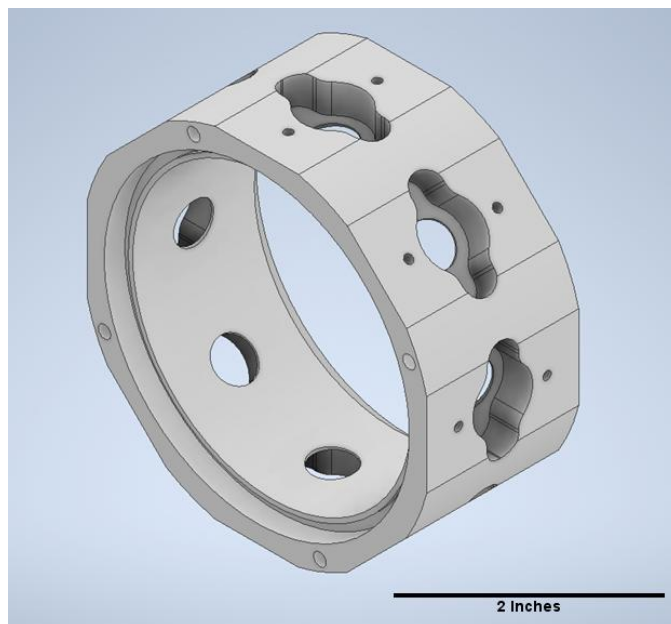


Figure 13: Tangential AGBR coupon ring with coupons-only design. Mounting locations for eight coupons.

A concept worth exploring is using multiple coupon rings in AGBR systems to allow for more experimental data without requiring more AGBR runs. Since experiments can range from days to weeks, more experimental replicates could provide more data promptly, supporting research in biofilm control strategies. The change in reactor surface area could be counteracted by changes in the length of the polycarbonate tubing, making up part of the main AGBR chamber. Multiple coupon rings may require some additional adjustable tensioning screws to ensure proper O-ring compression at all fluid seals, especially with three or more coupon rings. CFD simulations of reactors with multiple coupon rings should be performed to validate that these reactors maintain LSMMG. High fidelity simulations of the 1-inch and 3-inch tangential AGBR systems to quantify wall and fluid shear stress with higher accuracy should also be performed if sufficient computing power is available.

CHAPTER FOUR

CFD AND PIV VALIDATION OF A SIMULATED ALTERED GRAVITY
BIOFILM REACTOR

Contribution of Authors and Co-Authors

Manuscript in Chapter: 4

Author: Matthew Culp

Contributions: Investigation, Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft.

Co-Author: Erick Johnson

Contributions: Conceptualization, Formal analysis, Methodology, Data curation, Writing – review & editing.

Co-Author: Sarah Morris

Contributions: Formal analysis, Methodology, Data curation, Writing – review & editing.

Co-Author: Stephan Warnat

Contributions: Conceptualization, Supervision, Funding acquisition, Writing – review & editing

Manuscript Information

Matthew Culp, Erick Johnson, Sarah Morris, Stephan Warnat

Status of Manuscript:

- ☒ Prepared for submission to a peer-reviewed journal
- ☐ Officially submitted to a peer-reviewed journal
- ☐ Accepted by a peer-reviewed journal
- ☐ Published in a peer-reviewed journal

Abstract

Biofilms behave differently under altered gravity than under gravity on Earth (Nickerson et al., 2003; Nickerson et al., 2004; Zea et al., 2017). An Altered Gravity Biofilm Reactor (AGBR) was developed to study biofilms in this environment. The AGBR is a type of rotating wall vessel designed to provide an altered gravity environment capable of simulating the environment within the water processor assembly of the International Space Station. The AGBR is intended to grow biofilm and characterize this growth with electrochemical impedance spectroscopy sensors during operation, then with biofilm coupons for traditional microbiological methods. The AGBR has been shown to maintain sufficiently low shear in computational fluid dynamics (CFD) simulations to fall within the low-shear modeled microgravity regime. Still, low shear has not been proven experimentally. Particle imaging velocimetry (PIV) was used to capture the velocity profile within the AGBR. The velocity profiles from PIV and CFD were compared and showed agreement, allowing for validation of the CFD simulations. This confirmation confirms the AGBR system's ability to produce low-shear modeled microgravity. This validation makes the AGBR a functional and valid altered gravity analog, which will help conduct research into biofilm control strategies for spaceflight.

Introduction

Altered gravity simulation has been an ongoing field of study since humanity sent biological organisms into space. Sending biology to space, in addition to the means to keep it alive and study it, is an expensive endeavor. Various systems for simulating this altered gravity

environment have been developed and utilized, including random positioning machines and 2D clinostats (Ferranti et al., 2020).

To understand how these systems function, it is necessary to define altered gravity and weightlessness. These two inertial acceleration states are experienced in spacecraft in low Earth orbit (LEO) and beyond and are demonstrated by the astronauts aboard the International Space Station (ISS) (Albrecht-Buehler, 1992). Weightlessness, commonly associated with spaceflight, involves floating around and being free to accelerate equally in any direction. This differs from altered gravity, where a small amount of gravity still acts upon a body, such as motion within a spacecraft. Weightlessness, a complex topic, is best defined as “the condition where the acceleration a of an object is independent of its mass m ” (Albrecht-Buehler, 1992). Altered gravity occurs when one experiences a reduced gravitational acceleration. Based on this definition, ground simulation methods will not perfectly simulate weightlessness but are acceptable analogs to simulate altered gravity experienced during spaceflight.

The goal of the system discussed herein is to simulate the wastewater recovery system of a spacecraft to study biofilm growth within a controlled, simulated altered gravity environment. To do this, a few key capabilities are required: creating simulated altered gravity, growing biofilm, and characterizing biofilm growth.

To achieve the simulated altered gravity environment, a rotating wall vessel (RWV), a version of a two-dimensional clinostat, will be used. A clinostat uses rotation about a horizontal axis to average the gravity vector to zero over time by constantly changing the direction in

which gravity acts on an object from the object's reference frame (Hammond & Hammond, 2001). These devices allow for long-term altered gravity simulation, on the order of hours to weeks, and are relatively low cost but do not simulate weightlessness (Ferranti et al., 2020). A rotating wall vessel uses the principle of clinorotation to simulate altered gravity in a fluid-filled vessel. By rotating the outer wall of an RWV, the fluid inside is also rotated so long as sufficiently low shear characteristics are present, creating what is known as low-shear modeled microgravity (LSMMG). This LSMMG environment means that particles are experiencing some small amount of gravity, around 10^{-2} to $10^{-3}g$ (Albrecht-Buehler, 1992; Ferranti et al., 2020). Though not as high fidelity as other methods, this is the only method that is feasible for ground-based biological experiments lasting for periods longer than the scale of an hour, which is necessary for biofilm growth experiments (Ferranti et al., 2020).

Biofilms are of concern in spacecraft as they can cause a variety of issues for spacecraft, including material degradation (Gu et al., 1998), biocorrosion and fouling (Klintworth et al., 1998; Zea et al., 2020), and clogging of critical water systems (Weir et al., 2012). These biofouling issues are of vital importance to the water systems of long-term spacecraft, where these issues can interfere with the ability of a craft to provide potable water (Zea et al., 2020). While resupplying water and system components is an expensive but feasible solution in LEO, this task becomes increasingly complex for missions further away with less resupply capability (Zea et al., 2020). These issues and the goals of spaceflight necessitate research into biofilm control methods to minimize fouling and corrosion within critical life support systems. Cost

dictates that performing this research in a real altered gravity environment is prohibitively expensive (Ferranti et al., 2020). However, the question remains of how the bacteria that make up these biofilms grow differently in altered gravity than in normal gravity. Studies suggest that altered gravity and Earth's gravity affect various biological aspects of bacteria (Kim et al., 2013; Nickerson et al., 2003; Nickerson et al., 2004). While these aspects are not fully characterized, it suggests that altered gravity should be simulated for the most accurate modeling of spacecraft water systems to best model the actual system.

A key addition to help prevent loss of system function is the real-time detection of biofilm growth within these systems, allowing for the proper application of control techniques. Biological characterization of biofilm growth on the ground utilizes a variety of methods such as confocal laser scanning microscopy, colony forming unit enumeration, or cell staining processes (Funari & Shen, 2022). These tools are good at characterizing growth but lack real-time measurement capability for this application (Funari & Shen, 2022). A promising real-time measurement system for characterizing biofilm growth using electrochemical impedance spectroscopy sensors has shown success within fluid systems (McGlennen et al., 2023a; McGlennen et al., 2023b). With this approach, it is possible to add sensors to an existing system and evaluate biofilm growth in real-time in a variety of fluids, allowing for measurements in different aspects of water and wastewater systems with varying contaminants (McGlennen et al., 2023a).

To summarize what has been identified so far, a system for growing and quantifying biofilms in altered gravity without requiring spaceflight is needed to explore further how to prevent biofouling issues that plague long-term spaceflight. Real-time sensing is also a key technology that would be beneficial in assessing the current level of fouling within a system and allowing for the implementation of control strategies for these biofilms. An RWV would allow for the simulation of altered gravity at a reasonable cost while also being able to support biofilm growth within the fluid. To assess the biofilm growth, this system would also require the ability to characterize the bacterial growth through traditional biofilm measurement methods. It should also incorporate EIS sensors to evaluate the growth in real time. This system can be described as a simulated altered gravity biofilm reactor (AGBR), as discussed herein. The goal of this system is to support research into biofilm control methods, allowing for longer-term spaceflight by helping to limit the issues of biofouling and biocorrosion caused by these biofilms.

Materials and Methods

Altered Gravity Biofilm Reactor

The AGBR system was used in two configurations, one as previously used to test biofilm growth with centerline coupons and one with a clear end cap to perform particle imaging velocimetry (PIV) (Ketteler et al., 2025). A clear end cap was necessary to image the reactor to perform PIV. This setup involved using a set of two bearings to support the end cap externally since the rotary joint used in biological testing needed to be removed.

Computational Fluid Dynamics

CFD was performed using Star CCM+ and based upon a CAD model of the system using a clear acrylic end cap, which allows for PIV measurements of the system. The internal volume of the reactor was derived from a CAD model of the system, and then a mesh was created of this volume. A 0.5mm average base size was used to make a polyhedral mesh with two prism layers at 33% of the base size total thickness to better capture wall shear stress. A uniform mesh size refinement was utilized to ensure a consistent mesh size across the entire part. A 0.5mm average mesh was used due to computational constraints; while this will not fully capture the actual shear stress, it provides an adequate resolution to capture the velocity profile compared to PIV data. Shear stress from higher element simulations showed shear stress below 100 mPa (Ketteler et al., 2025). The fluid volume was rotated at 24.8 RPM to match the AGR's rotation under operating conditions and an initial velocity to match the reactor having been rotated consistently; reactor startup was not modeled. An implicit unsteady time step was utilized due to instability in steady simulations. A time step of 0.006667 seconds was used to keep the rotation per time step to under 3 degrees. A constant density was utilized to simulate water, with a 997.561kg/m³ water density. Water was modeled as incompressible due to the low pressures within the system. A turbulent model was utilized as an energy sink to dissipate non-rigid body turbulence in the model. A K omega turbulence scheme was used because of the low complexity of the vortices observed during simulations. Gravity was added to the simulation to simulate the effects on the system. In addition to the base evaluations, fluid shear stress was derived via the Navier-Stokes equations utilizing field functions within the simulation.

The reactor was not quartered during the CFD simulations because the entire velocity plane from PIV had to be simulated. Based on the literature, the possible existence of oscillatory patterns, as seen in rotating drum flows like the AGBR system (Seiden & Thomas, 2011; Seiden et al., 2005, 2007). These flows could have a period greater than $\frac{1}{4}$ of a reactor and wouldn't be captured in a quartered simulation, so a complete reactor was chosen to be simulated.

A mesh independence study was performed with mesh sizes ranging from 0.75 mm to 0.25 mm, as shown in Table 3. This showed consistently decreasing shear stresses on the wall shear stress. Volume average shear stress shows a rise with mesh size. This is likely due to artifacts resulting from meshing around corners. These results show that fluid shear is not accurately captured, since shear is changing with mesh size. Based on the CFD of the AGBR system in biological operations configurations, shear stress will decrease to adequate levels with finer meshes (Ketteler et al., 2025). Velocity profiles within the reactors do not show meaningful differences between these models, so the coarser mesh size of 0.5mm was deemed sufficient for comparison to PIV data. Mesh size was chosen as the comparison since a variety of sizes of AGBR are considered, with large volume changes. Since shear values in CFD are element size-dependent, it was decided to use a consistent mesh size rather than element count.

Mesh base size (mm)	Volume average fluid shear stress (mPa)	Sensor/coupon Wall Shear Stress Surface Average (mPa)
0.75	2.061	3.798
0.5	2.064	3.600
0.25	2.206	3.562

Table 3: CFD Mesh independence study comparing mesh base size to volume average fluid shear stress and surface averaged wall shear stress on the sensor and coupon surfaces

Particle Imaging Velocimetry

Particle Imaging Velocimetry (PIV) was performed to quantify the fluid dynamics within the AGBR system. A clear, acrylic end cap and roller bearing support system were fitted onto the AGBR system to allow viewing into the reactor, replacing an aluminum end cap and rotary joint. The test setup is shown below in Figure 14. Images were sampled at 60 HZ with a resolution of 1280x720 pixels on a Canon T8i. Six hundred image pairs were used to create a time average of each run due to noise from reflections and shadows caused by metal components within the AGBR. The tracer particles were hollow glass spheres with a mean diameter of 10 μ m and a density of 1.1g/mL (Potters' Industries Spheric 110P8). A 532nm continuous wavelength laser and a convex cylindrical lens created a one mm-thick laser sheet 30 mm away from the coupon ring. The reactor was run for 15 minutes before rotation to ensure steady-state conditions. Cross-correlation of image pairs was performed using a multi-pass interrogation method in the open-source software PIV lab (Thielicke, 2014, 2022; Thielicke & Sonntag, 2021; Thielicke & Stamhuis, 2014). A field of view of 153.6x86.4mm was used to capture the reactor's ~70mm internal diameter with a final window size of 32x32 pixels and 50% overlap. Once masked to remove areas not within the AGBR and remove the top and

bottom of the reactor due to reflections causing poor results, this left a 36×24 vector space with a spacing of 1.92mm. The time spacing between image pairs was 0.0167 seconds. Four runs were collected at this location, with each run performed sequentially. Post-processing was performed in MATLAB using data output from PIVlab.

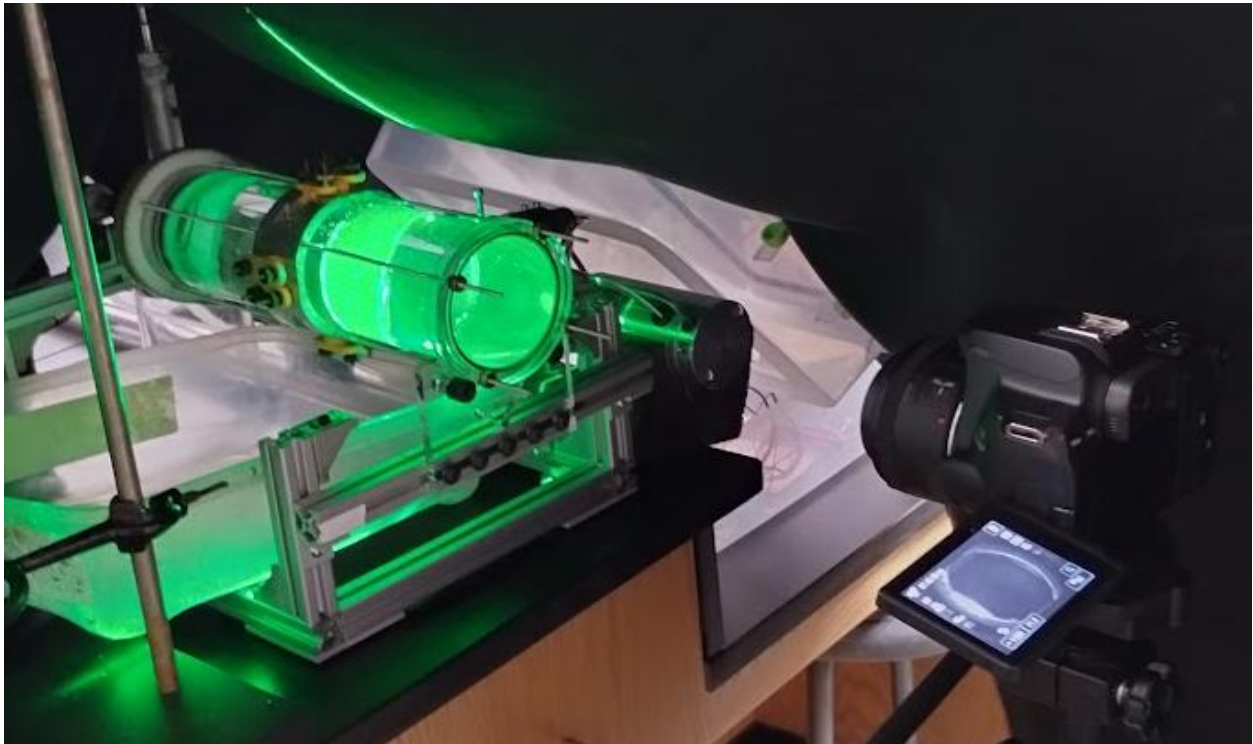


Figure 14: AGBR setup for PIV measurements with clear end cap, laser sheet, and DSLR. The light enclosure is not shown.

Based on PIV videos, the reactor's runout was quantified by comparing video frame by frame and comparing the location of the reactor wall at the maximum and minimum values throughout multiple rotations in ImageJ. This image analysis showed a 0.961° difference between the geometric centerline and the centerline of rotation. Eccentric motion in the PIV images was found to be approximately 1 mm. This axis of rotation was then used to perform

revised CFD simulations that better reflect the experimental setup. These CFD simulations used the same settings as prior simulations, with a changed axis of rotation to reflect the experimental axis of rotation.

Results

During CFD simulations, shear stress within the AGBR system in the PIV configuration remained low, with a surface weighted average of 3.56 mPa on the sensor and coupon faces, as shown in Figure 15, and 2.21 mPa volume average fluid stress in the bulk fluid. Stress concentrations in the fluid and at the wall occur near any change in geometry, with the largest occurring on the expansion area of the aluminum end caps. The clear end cap showed lower shear stress than the aluminum end cap, with no new stress concentrations. Higher fidelity modeling is expected to yield decreasing shear values since fluid shear values depend on element sizes due to larger elements having larger velocity differences between elements. Shear is difficult to measure directly, so CFD simulations will be compared to the velocity fields derived from PIV measurements.

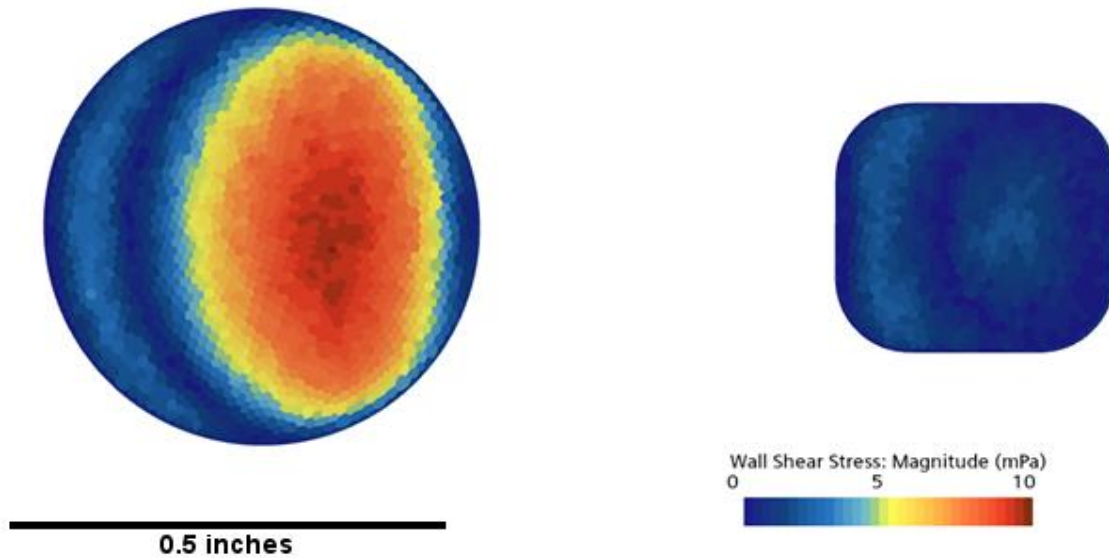


Figure 15: Wall shear stress on coupon and sensor pair from CFD simulation of a 3-inch AGBR with clear end cap for PIV. CFD assumes no eccentricity in rotation. The spacing between the coupon and sensor matches the spacing on the AGBR coupon ring.

PIV measurements were processed into velocity fields, as shown below in Figure 16. A comparable plot from CFD is shown below in Figure 17. The PIV plots showed expected behavior of increasing velocity further from the center of the AGBR, but eccentricity was noticed of $\sim 1.5\text{mm}$. This eccentricity caused the minimum velocity to fall away from the geometric center of the AGBR. It was decided to run CFD simulations that included this eccentricity, which accounts for $\sim 1^\circ$ of eccentricity over the length of the reactor, quantified from videos taken looking at the length of the reactor on the day of the PIV measurements. This simulation will allow for the best possible analog to predict the physical AGBR system and validate the CFD simulation's low shear.

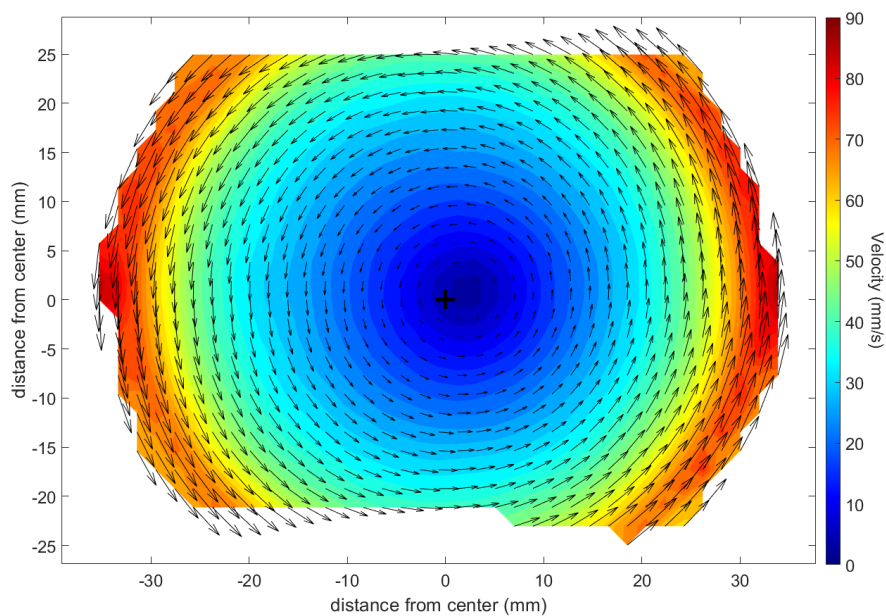


Figure 16: Velocity contour plot from PIV of the AGBR with the clear end cap. Data are shown from run 4. Vectors show the direction and magnitude of velocity. The black center mark shows the geometric center of the reactor

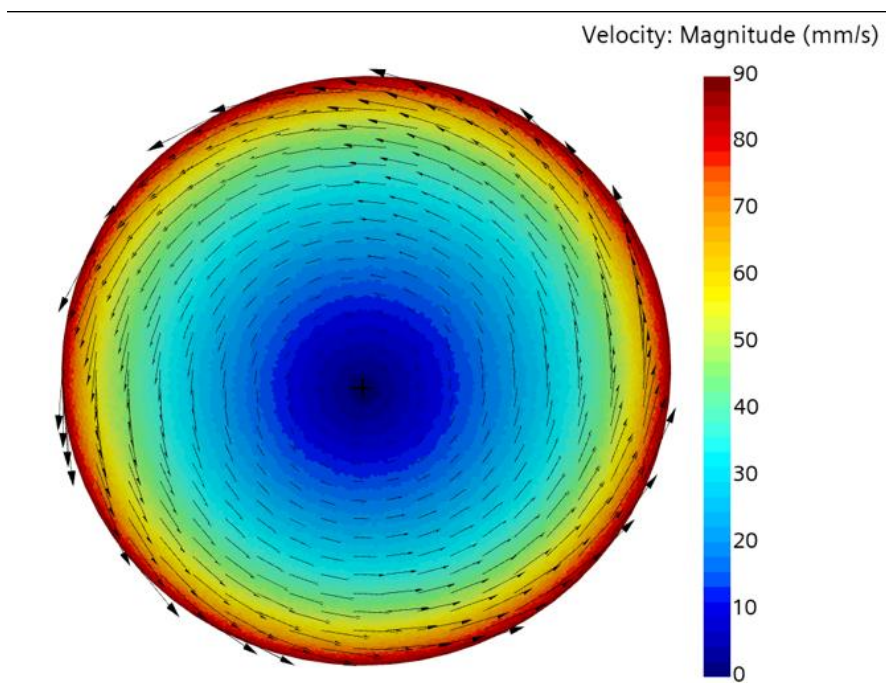


Figure 17: Velocity contour plot from CFD of the AGBR with the clear end cap. CFD with no eccentricity

With Eccentric CFD performed, the distance between the geometric center and minimum velocity was ~1.5mm, as shown in Figure 18. Velocity measurements along a centerline, including the geometric and velocity centers, were taken from the PIV measurements and CFD simulations to compare the velocity profiles, yielding a centerline approximately 30 degrees to the right of the vertical centerline. The velocity profile along this centerline is shown in Figure 19. CFD error was calculated using four revolutions (~10) seconds of outputting the velocity along this line to find the standard deviation. The PIV line used four runs of PIV, combining the standard deviation within the time averaging on each run and the standard deviation from averaging the four runs together. The PIV data holds a high degree of uncertainty, primarily coming from reflections of the laser on the aluminum components of the AGBR.

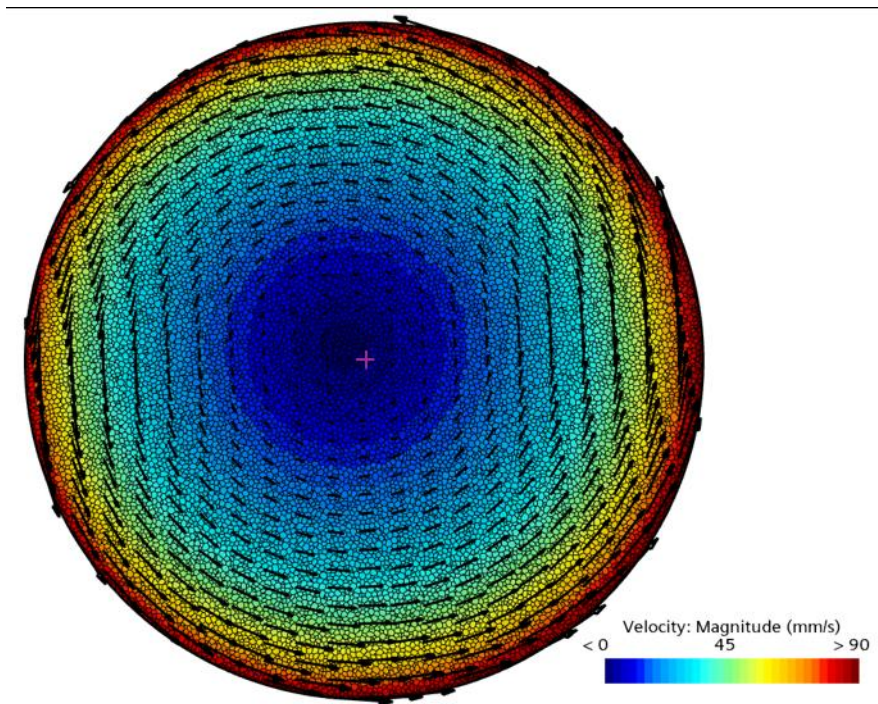


Figure 18: Velocity contour plot from CFD of the AGBR with the clear end cap. CFD with 1.5mm eccentricity in rotation. Vectors show velocity magnitude and direction.

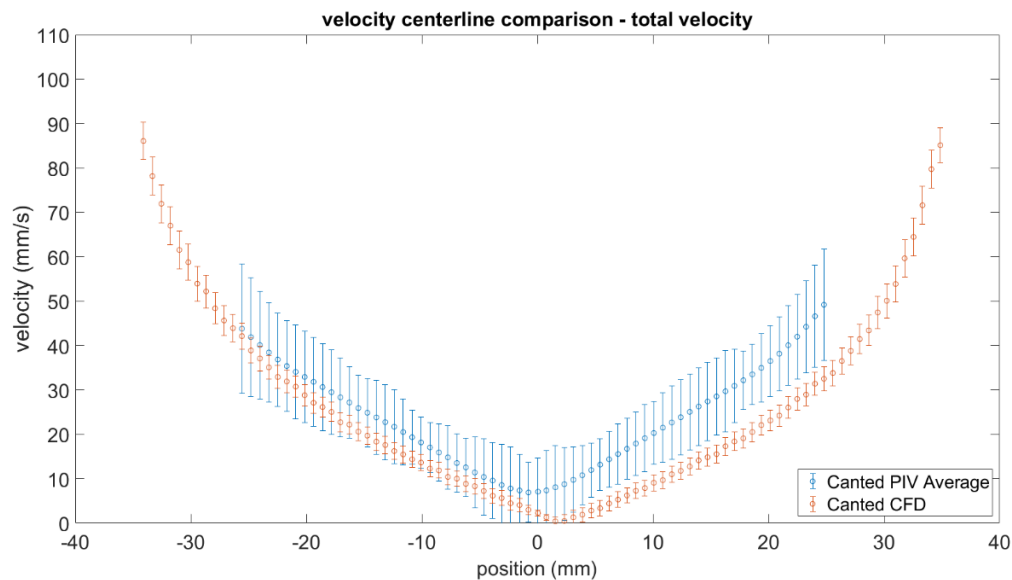


Figure 19 – Velocity centerline plot comparing CFD and PIV velocity values along a centerline going through the geometric center and velocity minimum. CFD values use an eccentric axis of rotation.

CFD and PIV show a quadratic trend in velocity measurements, not the expected linear trend due to further distance from the center of rotation. Due to cropping, the PIV measurements along this line do not reach the reactor wall. The PIV velocities do not reach zero but get a minimum of 6.9 mm/s. The nonzero velocity is theorized to be due to a combination of factors: eccentric rotation, spatial resolution of the PIV, and PIV image resolution. The eccentric rotation, as predicted by the CFD, shows a non-zero velocity minimum. The spatial resolution of the PIV also contributes, with a vector spacing of 1.92mm. Hence, the location of the velocity value is an average of the area around that point rather than a discrete value from that exact location. This averaging would introduce averaging within an area expected to have non-zero velocities, contributing to the non-zero velocity at the velocity center. Finally, the image resolution limits the spatial resolution and the ability to distinguish small velocities due to the inability to quantify small motions in the PIV images.

Eccentric CFD shows minimal changes to shear compared to the non-eccentric CFD simulations. High shear concentrations remained in the same areas, and shear values remained similar, as shown in Figures 20 and 21.

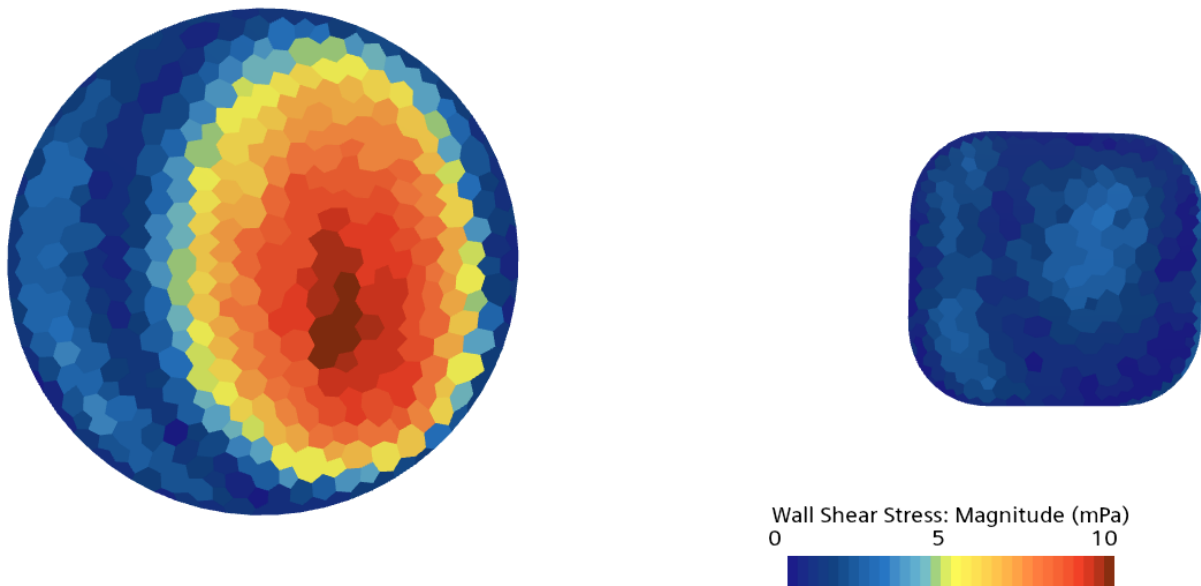


Figure 20 – Sensor and coupon wall shear stress from CFD simulation using a 3-inch reactor with a clear end cap for PIV. CFD uses an eccentric axis of rotation.

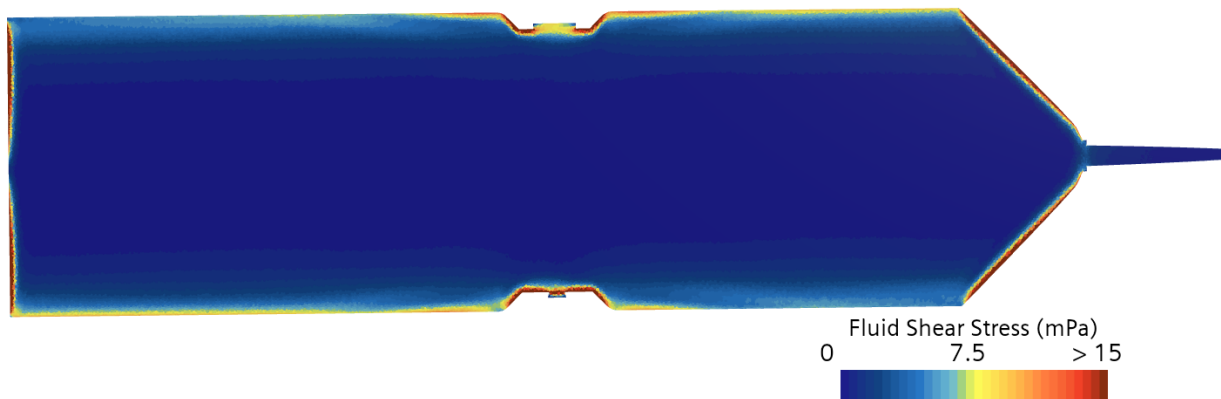


Figure 21 – Fluid shear stress plot of a section plane from CFD simulation of the 3-inch AGBR with clear end cap for PIV. CFD uses an eccentric axis of rotation.

Discussion

The differences in velocity profile between the CFD and PIV are of note and have a likely cause. As with any system, manufacturing tolerances play a role in the operation of these

systems; with the current design and manufacturing methods used for purchased components of these systems, eccentricity in the rotation of the AGBR system is to be expected. This eccentricity in the reactor will be highest near the middle of the reactor due to tolerance stacking further from the rotational supports. PIV velocity measurements were taken between approximately 40-60mm from the end cap of the reactor, so it would not have the maximum amount of eccentricity possible within the AGBR system. The roller support needed to utilize a clear end cap also does not provide the same support as the rotary joint used in the AGBR system for biotic testing. Modeling the exact eccentricity of the reactor will be nearly impossible since the buildup of components along the rotational axis can cause misalignment at every joint in different directions. The eccentric CFD uses a center axis that matches an approximately 1-degree offset, which will not exactly match the experimental conditions. This helps explain some of the differences between CFD and PIV. The high uncertainty of the PIV measurements shown from the time averaging and averaging multiple runs also indicates the possibility of error, causing some deviation between the PIV and CFD. Generally, the trend of both PIV and CFD velocity measurements shows a quadratic profile with differences averaging under 10%.

Understanding and validating the fluid environment within the AGBR system is a critical step in verifying the usefulness of the AGBR system for the intended application, simulating the environment within spacecraft wastewater systems on Earth. Of vital importance is the shear stress within the reactor, as too high shear stress will cause the reactor to no longer create low-

shear modeled microgravity. Fluid shear stress is not easy to directly measure within the AGBR system, but through PIV, the velocity profile was measured and showed good agreement with the simulated velocity profile. Since fluid shear is derived from the gradient of the velocity field, we have chosen to validate these simulations by comparing the velocity profiles. With the low levels of shear shown in higher fidelity CFD simulations of the AGBR system seen in other work of less than 1 mPa, we believe that this reactor system can be determined to have sufficiently low shear to create low shear modeled microgravity (Ketteler et al., 2025).

Little simulation has been done of rotating wall vessels, and what work has been done has been primarily on high aspect ratio vessels (HARV) or rotating wall perfused vessels (RWPV) (Begley & Kleis, 2000), which have significant differences in design and operation compared to the AGBR system. HARVs are much shorter than the AGBR system and have significantly different axial flow characteristics and concerns. RWPVs have a rotating inner wall and outer walls and work to create a recirculating flow field between those walls, as needed for tissue engineering work (Begley & Kleis, 2000). Despite these differences, all reactors operate on the same principles, using rotation about a horizontal axis to average the gravity vector to zero over time. Critical factors for these reactors vary significantly, so the work done quantifying these other reactor designs has focused on different critical factors or is being run with such different operating parameters as not to provide helpful comparisons.

Conclusions

The AGBR system offers a solution for developing biofilm control strategies for current and future spaceflight. The velocity measurements from experimental and CFD simulations show similar trends, within 10% of the simulation results. These results give confidence that the CFD models' low fluid and wall shear stresses are accurate. With the low shear stress being valid, LSMMG is considered present in the AGBR system, making it a viable altered gravity analog.

The eccentricity seen in the physical system could be minimized by machining more of the AGBR components to ensure better concentricity between them. Tighter tolerances will raise the cost of the device but will improve the quality of microgravity produced by the AGBR. Future work with the AGBR should focus on developing biological experiments with it and evaluating existing biofilm control strategies. Reactor design optimization should also continue, emphasizing improving the ease of use of the AGBR and the sensor and coupon integration strategies.

CHAPTER FIVE

CONCLUSIONS AND FUTURE WORK

The design of AGBRs has been analyzed from a machine design and fluidics perspective to validate these systems as a viable analog for spacecraft wastewater systems. This work's conclusions correlate with the research objectives listed in Chapter 1.

Objective 1: CFD was performed to evaluate the effects of changes to operating speed, reactor diameter, and sensor/coupon integration method. These results showed that there is frequently a trade-off between design parameters. Higher reactor speed provides a better averaging of the gravity vector to zero but causes increased fluid shear. This means that acceptable shear stress should be chosen, and the AGBR run at the maximum speed allowed by that shear rate. Larger reactor diameter correlates to higher shear in some configurations, but smaller reactors come at the cost of manufacturing complexity and fewer sensing surfaces. The biological needs of the experiment should inform which reactor size is practical to use, since larger reactors may have higher shear, but not by an order of magnitude. Larger reactors will have longer times for biofilm to reach the wall and attach, so experiments about biofilm attachment will need to run longer. Shorter experiments will benefit from smaller reactors where particles will reach the walls and sensing surfaces faster. The challenges with smaller reactors come with the lower fluid volume, meaning that biofilm is more likely to get washed out of the reactor when flowing nutrients, necessitating lower nutrient flow, which could cause a nutrient limitation. Tangential sensor integration shows higher shear in coarse CFD

simulations but offers substantial advantages to sensing within the reactor. The slight increase in shear is worth the benefits of sensing within the bulk fluid and reducing crevasses for biofilm to adhere to. The recommended AGBR uses a tangential sensor and coupon integration and should run as fast as possible while remaining under the shear limit. Reactor size should be dictated by organism requirements and experiment length, with longer experiments able to utilize larger reactors.

Objective 2: Particle imaging velocimetry was performed on the original AGBR system. The velocity measurements from the PIV showed significant noise but similar trends to those in CFD. The similarities validate that CFD can predict the fluid dynamics within the AGBR system. Error is still present, but the velocity measurements give sufficient confidence to trust that shear values within the AGBR system remain low, since shear is derived from velocity.

Objective 3: The tangential sensor and coupon integration method offers a solution for solving integration challenges present in the recessed design. CFD analysis shows slightly higher wall and fluid shear stress in coarse models, but remains within an order of magnitude of existing designs. Shear decreases by orders of magnitude with higher element models, so while the shear is higher than the recessed design, it should fall within the realm of LSMMG during operation. Higher fidelity modeling would be a good way to validate this if sufficient computing power is available to run a ~15 million element simulation of the tangential 1-inch and 3-inch designs.

Objective 4: A tangential AGBR was manufactured in 3-inch and 1-inch versions. The 3-inch version includes a coupon ring with only coupons, allowing for more biofilm growth characterization with traditional microbiological methods such as plate counts. The system has been used in biological experiments and received positive feedback from research collaborators specializing in microbiology.

The primary hypothesis was validated, and an improved AGBR coupon ring design that remains within the LSMMG regime was developed. A rise in shear was seen, but it remains within less than an order of magnitude of existing designs. The AGBR system was also validated by experiments, giving confidence in the low shear seen within CFD simulations. The AGBR system provides value to researchers with a platform offering the best analog for simulating altered gravity and spacecraft wastewater systems. This tool will enable further research into biofilm control strategies for human spaceflight, helping enable long-term human spaceflight, especially outside of low Earth orbit.

For future work on the AGBR system, it is recommended that the AGBR system be utilized to facilitate space science research into how best to control biofilms in the context of new spacecraft. Each spacecraft offers unique challenges and design considerations and should be updated to match the systems being designed by space agencies. After testing different control methods for biofilms under altered gravity, these measures should be implemented and applied to the next generation of spacecraft serving long-term missions. The EIS sensors being utilized in this reactor should be within these systems to allow for live quantification of the

biofilm growth within these systems, providing feedback on the control methods developed using the AGBR system. Operational changes, such as utilizing multiple coupon rings in series to offer more sensing locations, should be studied to allow for more data capture on these systems. Novel sensor integration methods enabled by advanced manufacturing methods, such as electrode discharge machining, could also be examined to allow for more sensor integration methods to better quantify the bulk media with sensors.

REFERENCES CITED

- Albrecht-Buehler, G. (1992). The Simulation of Microgravity Conditions on the Ground. *ASGSB bulletin : publication of the American Society for Gravitational and Space Biology*, 5(2), 3-10.
- Begley, C. M., & Kleis, S. J. (2000). The fluid dynamic and shear environment in the NASA/JSC rotating-wall perfused-vessel bioreactor. *Biotechnol Bioeng*, 70(1), 32-40.
[https://doi.org/10.1002/1097-0290\(20001005\)70:1<32::aid-bit5>3.0.co;2-v](https://doi.org/10.1002/1097-0290(20001005)70:1<32::aid-bit5>3.0.co;2-v)
- Bluemink, J. (2008). *Bubbles and particles in a cylindrical rotating flow* University of Twente].
- Carter, D. L., Tobias, B., & Orozco, N. Y. (2013, 2013-07-14). Status of ISS Water Management and Recovery. 43rd International Conference on Environmental Systems,
- Carter, L. (2010). *Status of the Regenerative ECLS Water Recovery System* 40th International Conference on Environmental Systems, <https://arc.aiaa.org/doi/abs/10.2514/6.2010-6216>
- Characklis, W. G. (1984, 1984//). Biofilm Development: A Process Analysis. Microbial Adhesion and Aggregation, Berlin, Heidelberg.
- Donlan, R. M., & Costerton, J. W. (2002). Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. *Clinical Microbiology Reviews*, 15(2), 167-193.
<https://doi.org/doi:10.1128/cmr.15.2.167-193.2002>
- Ferranti, F., Del Bianco, M., & Pacelli, C. (2020). Advantages and Limitations of Current Microgravity Platforms for Space Biology Research. *Applied Sciences*, 11(1).
<https://doi.org/10.3390/app11010068>
- Funari, R., & Shen, A. Q. (2022). Detection and Characterization of Bacterial Biofilms and Biofilm-Based Sensors. *ACS Sensors*, 7(2), 347-357.
<https://doi.org/10.1021/acssensors.1c02722>
- Gu, J.-D., Romanb, M., Esselmanc, T., & Mitchell, R. (1998). The role of microbial biofilms in deterioration of space station candidate materials. *International Biodeterioration &*

Biodegradation, 41(1), 25-33. [https://doi.org/https://doi.org/10.1016/S0964-8305\(98\)80005-X](https://doi.org/https://doi.org/10.1016/S0964-8305(98)80005-X)

Hammond, T. G., & Hammond, J. M. (2001). Optimized Suspension Culture: The Rotating Wall Vessel. *American Journal Physiological Renal Physiology*, 281(1), F12–F25. <https://doi.org/https://doi.org/10.1152/ajprenal.2001.281.1.F12>

Jones, H. (2018, 2020/07/08). The recent large reduction in space launch cost. 48th International Conference on Environmental Systems, Albuquerque, NM.

Ketteler, H. M., Johnson, E. L., McGlennen, M., Dieser, M., Foreman, C. M., & Warnat, S. (2025). A simulated microgravity biofilm reactor with integrated microfabricated sensors: Advancing biofilm studies in near-space conditions. *Biofilm*, 9, 100263. <https://doi.org/https://doi.org/10.1016/j.biofilm.2025.100263>

Kim, W., Tengra, F. K., Young, Z., Shong, J., Marchand, N., Chan, H. K., Pangule, R. C., Parra, M., Dordick, J. S., Plawsky, J. L., & Collins, C. H. (2013). Spaceflight Promotes Biofilm Formation by *Pseudomonas aeruginosa*. *PLoS ONE*, 8(4), e62437. <https://doi.org/10.1371/journal.pone.0062437>

Klintworth, R., Reher, H., Viktorov, A., & Bohle, D. (1998). BIOLOGICAL INDUCED CORROSION OF MATERIALS II: NEW TEST METHODS AND EXPERIENCES FROM MIR STATION. *Acta Astronautica*, 44, 569-578.

McGlennen, M., Dieser, M., Foreman, C. M., & Warnat, S. (2023a). Monitoring biofilm growth and dispersal in real-time with impedance biosensors. *J Ind Microbiol Biotechnol*, 50(1). <https://doi.org/10.1093/jimb/kuad022>

McGlennen, M., Dieser, M., Foreman, C. M., & Warnat, S. (2023b). Using electrochemical impedance spectroscopy to study biofilm growth in a 3D-printed flow cell system. *Biosensors and Bioelectronics: X*, 14, 100326. <https://doi.org/https://doi.org/10.1016/j.biosx.2023.100326>

Nickerson, C. A., Ott, C. M., Wilson, J. W., Ramamurthy, R., LeBlanc, C. L., Honer zu Bentrup, K., Hammond, T., & Pierson, D. L. (2003). Low-shear modeled microgravity: a global environmental regulatory signal affecting bacterial gene expression, physiology, and

- pathogenesis. *J Microbiol Methods*, 54(1), 1-11. [https://doi.org/10.1016/s0167-7012\(03\)00018-6](https://doi.org/10.1016/s0167-7012(03)00018-6)
- Nickerson, C. A., Ott, C. M., Wilson, J. W., Ramamurthy, R., & Pierson, D. L. (2004). Microbial responses to microgravity and other low-shear environments. *Microbiol Mol Biol Rev*, 68(2), 345-361. <https://doi.org/10.1128/MMBR.68.2.345-361.2004>
- Seiden, G., & Thomas, P. J. (2011). Complexity, segregation, and pattern formation in rotating-drum flows. *Reviews of Modern Physics*, 83(4), 1323-1365. <https://doi.org/10.1103/RevModPhys.83.1323>
- Seiden, G., Ungarish, M., & Lipson, S. G. (2005). Banding of suspended particles in a rotating fluid-filled horizontal cylinder. *Phys Rev E Stat Nonlin Soft Matter Phys*, 72(2 Pt 1), 021407. <https://doi.org/10.1103/PhysRevE.72.021407>
- Seiden, G., Ungarish, M., & Lipson, S. G. (2007). Formation and stability of band patterns in a rotating suspension-filled cylinder. *Phys Rev E Stat Nonlin Soft Matter Phys*, 76(2 Pt 2), 026221. <https://doi.org/10.1103/PhysRevE.76.026221>
- Thielicke, W. (2014). *The flapping flight of birds Analysis and application* University of Groningen].
- Thielicke, W. (2022, 2022/09/06). Pulse-length induced motion blur in PIV particle images: To be avoided at any cost? Fachtagung Experimentelle Strömungsmechanik, Ilmenau, Germany.
- Thielicke, W., & Sonntag, R. (2021). Particle Image Velocimetry for MATLAB: Accuracy and enhanced algorithms in PIVlab. *Journal of Open Research Software*, 9(1), 12. <https://doi.org/10.5334/jors.334>
- Thielicke, W., & Stamhuis, E. J. (2014). PIVlab – Towards User-friendly, Affordable and Accurate Digital Particle Image Velocimetry in MATLAB. *Journal of Open Research Software*, 2. <https://doi.org/10.5334/jors.bl>
- Weir, N., Wilson, M., Yoets, A., Molina, T., Bruce, R., Sitler, G., & Carter, L. (2012). *Microbiological Characterization of the International Space Station Water Processor*

Assembly External Filter Assembly S/N 01. 42nd International Conference on Environmental Systems (ICES), San Diego, CA.

Zea, L., Larsen, M., Estante, F., Qvortrup, K., Moeller, R., Dias De Oliveira, S., Stodieck, L., & Klaus, D. (2017). Phenotypic Changes Exhibited by *E. coli* Cultured in Space. *Frontiers in Microbiology*, 8. <https://doi.org/10.3389/fmicb.2017.01598>

Zea, L., McLean, R. J. C., Rook, T. A., Angle, G., Carter, D. L., Delegard, A., Denvir, A., Gerlach, R., Gorti, S., McIlwaine, D., Nur, M., Peyton, B. M., Stewart, P. S., Sturman, P., & Velez Justiniano, Y. A. (2020). Potential biofilm control strategies for extended spaceflight missions. *Biofilm*, 2, 100026. <https://doi.org/10.1016/j.biofilm.2020.100026>

APPENDIX

1-INCH TANGENTIAL AGBR DRAWING PACKAGE

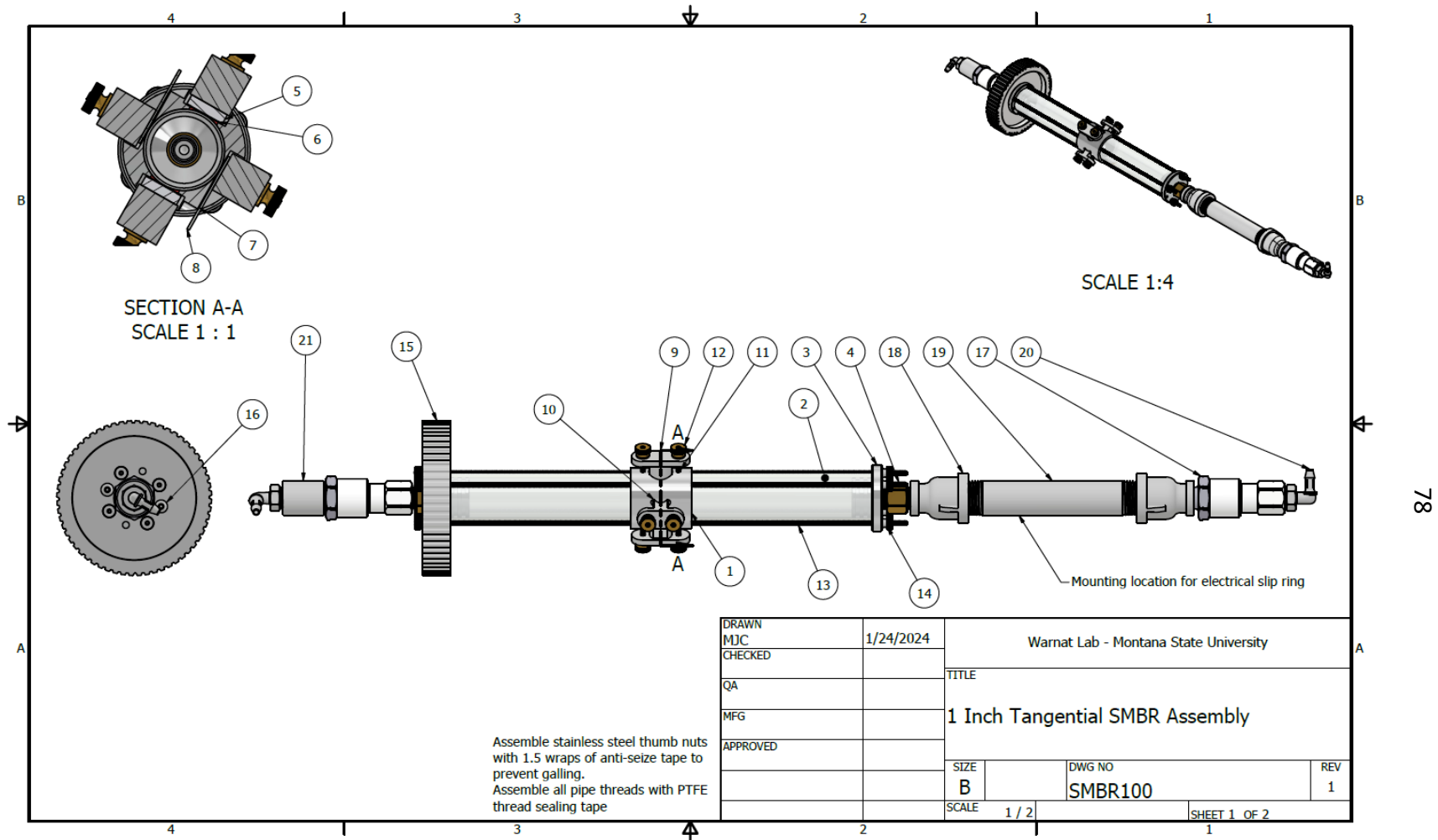


Figure A1: Tangential 1-inch AGBR Assembly Drawing Page 1

PARTS LIST		
ITEM	QTY	PART NUMBER
1	1	1in_CouponRing
2	2	Reactor_Tube
3	2	1in_endCap
4	2	4464K721_304 Stainless Steel Threaded Pipe Fitting
5	2	Coupon
6	2	gasket_coupon
7	2	gasket_6.4x5.3hole
8	2	sensor9x26mm
9	2	couponclampv1
10	2	sensorclampv1
11	8	92311A113_18-8 Stainless Steel Cup-Point Set Screw
12	8	92741A100_Brass Flanged Knurled-Head Thumb Nut
13	8	98790A401_Low-Strength Steel Threaded Rod
14	8	95150A103_18-8 Stainless Steel Flanged Knurled-Head Thumb Nut
15	1	57105K33_og
16	2	97763A178_18-8 Stainless Steel Button Head Hex Drive Screws
17	2	32
18	2	4464K524_304 Stainless Steel Threaded Pipe Fitting
19	1	4830K177_Standard-Wall 304 304L Stainless Steel Pipe Nipple
20	2	2808K52_Super-Flow Barbed Fitting for Food and Beverage
21	1	4452K112_316 Stainless Steel Threaded Pipe Fitting

DRAWN MJC	1/24/2024	Warnat Lab - Montana State University		
CHECKED		TITLE		
QA		1 Inch Tangential SMBR Assembly		
MFG				
APPROVED				
		SIZE B	DWG NO SMBR100	REV 1
		SCALE	SHEET 2 OF 2	

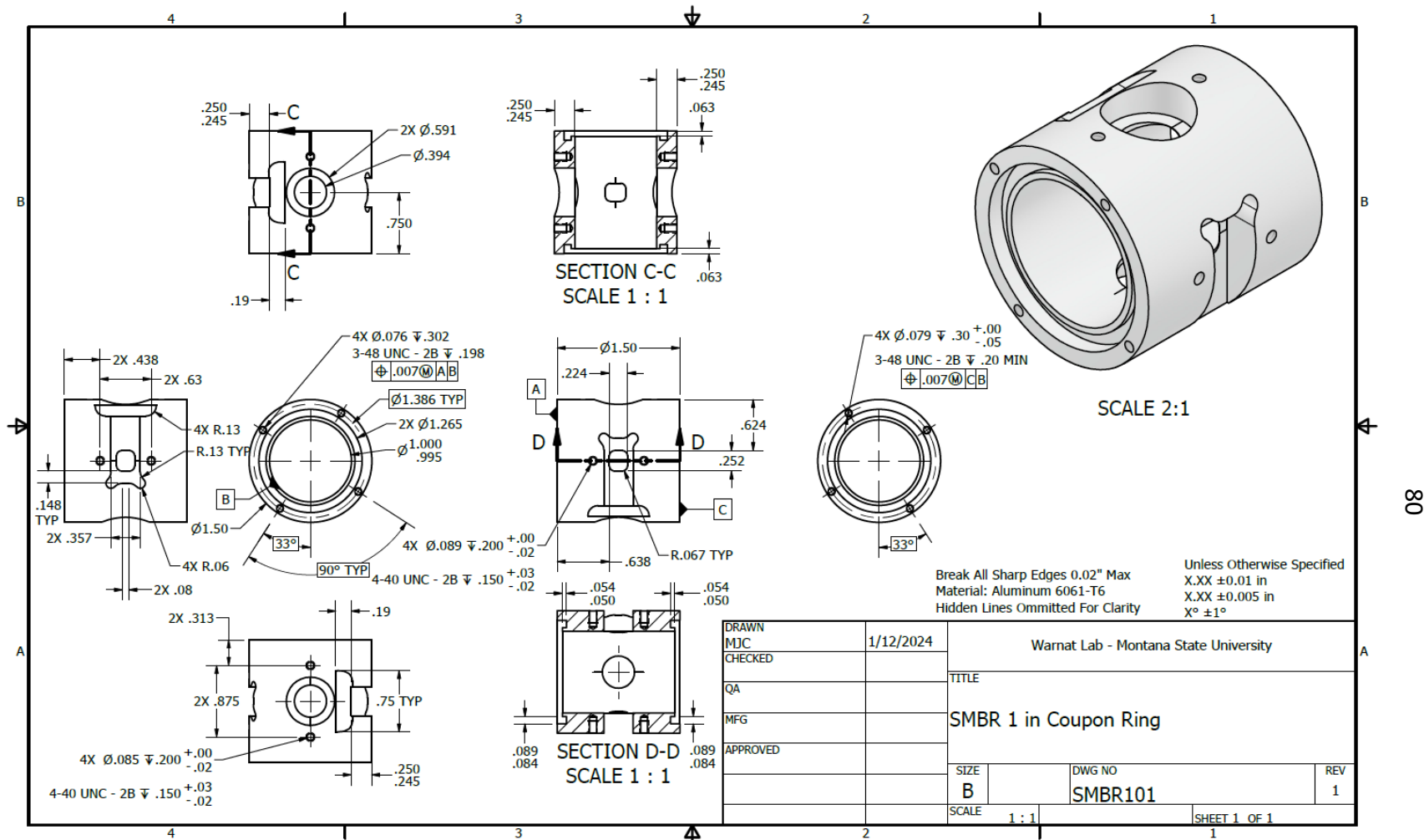


Figure A3: Tangential 1-inch AGBR Coupon Ring Drawing

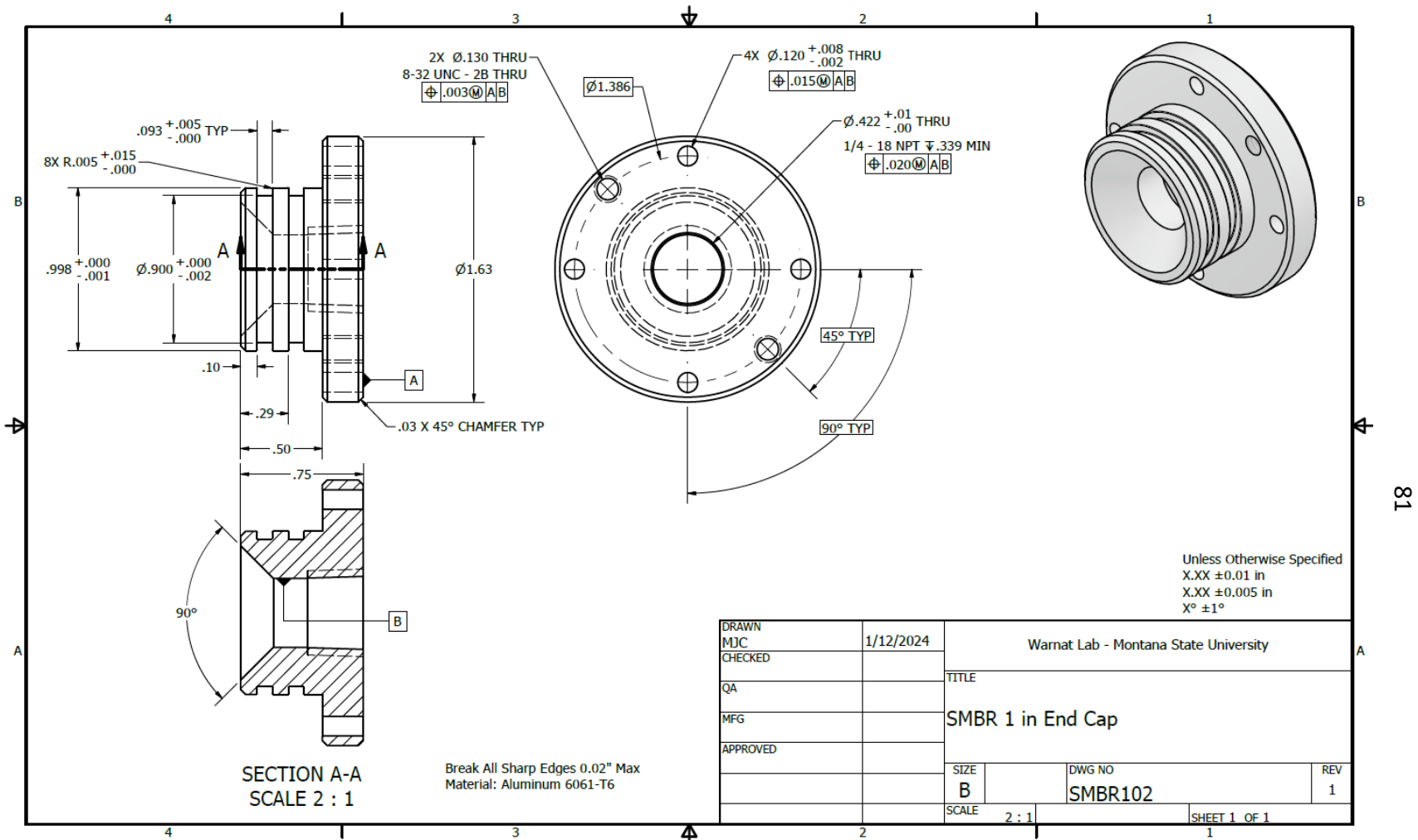


Figure A4: Tangential 1-inch AGBR End Cap Drawing

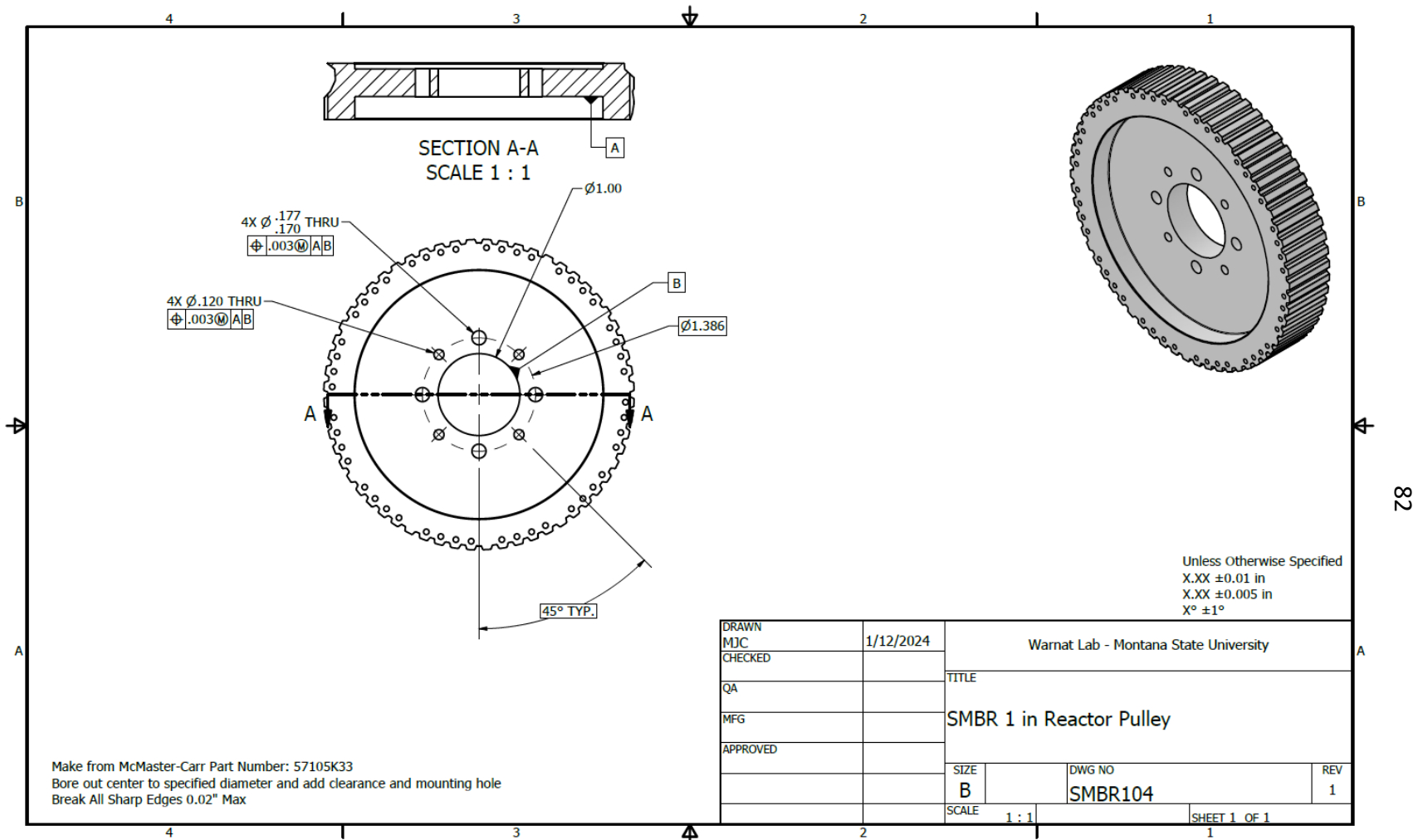


Figure A5: Tangential 1-inch AGBR Pulley Drawing

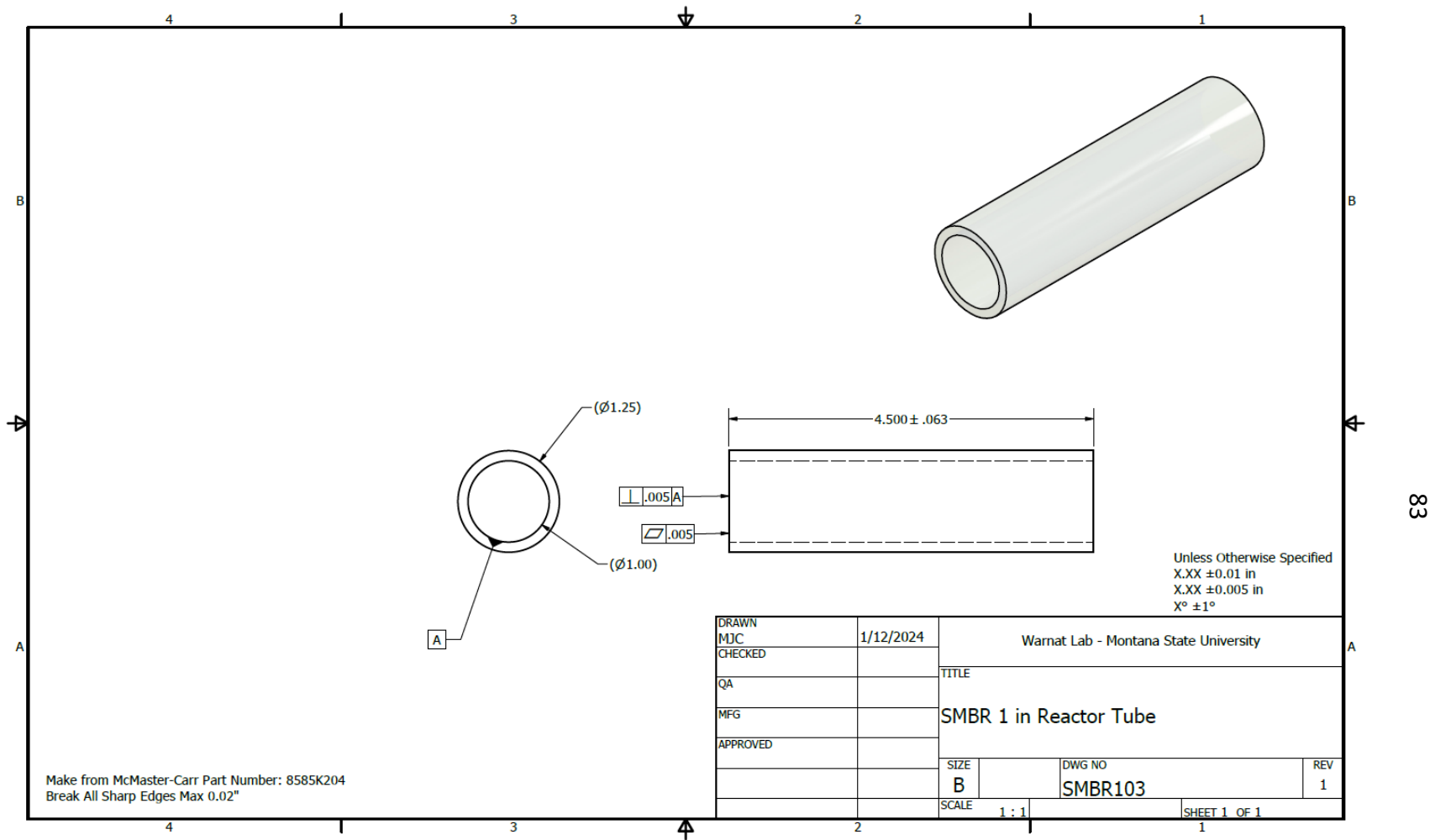


Figure A6: Tangential 1-inch AGBR Reactor Tube Drawing