ME 450 Winter 2024 Semester

FINAL DESIGN REPORT:

Bovine Biomimicry: Making Renewable Natural Gas from Food Waste, Wastewater, and Sludge

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# Table of Contents

Introduction 3
  Background Information 3
  Anaerobic Digestion 3
  Micro-aeration 3
  Current Bioreactor System 4
  Current Rumen Reactor Design 5
Benchmarking 7
  Discussion 10
Design Process 10
  Stakeholder Analysis 12
  Social Context Analysis 14
  The Role of Intellectual Property 14
  Sustainability Analysis 14
  Ethics 15
Requirements and Engineering Specifications 16
Concept Generation 17
  Functional Decomposition 17
  Concept Generation 18
Concept Selection Process 18
Concept Description 20
Engineering Analysis 21
  Fluid Mechanics 21
  Bubble Size 21
  Oxygen Transfer Rate 22
  Air Flow Rate 22
  Sparger Selection 23
Final Design Description 25
Description of Verification and Validation Approach 27
  Verification Testing 1: Air flow rate vs ORP reading 28
  Verification Testing 2: Sparger location versus uniformity of ORP levels 29
  Verification Testing 3: Bubble diameter 30
  Verification Testing 4: Time to remove the sparger 31
  Verification Testing Summary 31
  Validation Plans 32
Discussion 32
Reflection 34
Recommendations 36
Introduction

Background Information
Anaerobic Digestion

As of 2023, fossil fuel emissions have reached a record high [1]. To achieve the Biden administration’s goal of 50%-52% reduction in carbon emissions by 2030, alternative energy generation pathways need to be further understood [2]. Anaerobic Digestion (AD) is a potential solution to this energy crisis. The digestion process begins with bacterial hydrolysis of the input materials to break down insoluble organic polymers such as carbohydrates and make them available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. Acetogenic bacteria then convert these resulting organic acids into acetic acid, also known as volatile fatty acids or VFAs, along with additional ammonia, hydrogen, and carbon dioxide. Finally, methanogens convert these VFAs to methane and carbon dioxide [3]. By mimicking the natural AD process that occurs in ruminant animals, hoofed animals that are highly efficient at converting plant matter into energy (cows, sheep, goats, etc) [4], organic waste can be converted into a wide range of products ranging from fertilizer to fuel [5]. In particular, cows are highly adept at breaking down plant matter known as lignocellulose via hydrolysis through their unique AD process [6]. One of the cow’s four stomachs, the rumen, holds bacteria and fungi that convert the lignocellulose into volatile fatty acids (VFAs) which can later be converted into biogas [7]. VFAs are short chain acids composed of six or fewer carbon atoms in their molecular structure. VFAs are generally produced during anaerobic processes and carry a high energy density, making them very useful for producing high energy biogases [8]. The main fuel of interest produced from AD is methane gas which when burned generates heat and electricity. There even exist pathways that can convert the methane gas into hydrogen gas through pyrolysis to create a renewable clean burning energy source [9]. In this manner, anaerobic digestion is thought to be a path to reduce greenhouse gas emissions by producing clean renewable energy.

Micro-aeration

While AD is a strong solution to this crisis, it has drawbacks that need to be overcome. It remains inefficient as only 2% of the available energy has been harvested from available sources [10], it is unstable at high organic loading rates (OLR) [11], the accumulation of VFAs in the biofilm can create microbe hostile environments [12], and there is a low hydrolysis rate of the lignocellulosic feedstocks [13]. Research has been conducted to overcome these challenges through different organic waste pretreatment methods, altering the pH [14], adopting a two stage bioreactor solution [15], and more. Recent research has proposed a novel solution that can help hydrolytic fungi growth called micro-aeration. Micro-aeration is the introduction of small amounts of oxygen into an anaerobic digester. Micro-aeration attempts to emulate the ruminant animals' digestion process where they regurgitate food from their rumen stomach to their mouth exposing the food waste and rumen microbes to air [6]. The goal of implementing micro-aeration into a bioreactor is to achieve the benefits of both anaerobic and aerobic digestion without the drawbacks. Hydrolysis performed by extracellular hydrolytic enzymes, secreted by mainly aerobic or facultative and some anaerobic bacteria, is usually considered the rate-limiting step of AD, especially for lignocellulosic biomass [16]. Micro-aeration has been shown to improve hydrolysis efficiency and methane yield when using low concentrations of oxygen. This is because hydrolytic bacteria thrive when introduced to oxygen, however too much oxygen will
negatively impact the ability of methanogens, methane producing bacteria, as these are anaerobic microbes. Finding a balanced oxygen concentration will improve the hydrolysis reactions occurring in our reactor and keep the anaerobic methanogens intact, allowing for greater VFA and methane yield. Micro-aeration is a very promising technology, however there are challenges associated with its implementation. Uniformity of oxygen concentration inside the reactor is important because we don’t want over production of VFAs or methane in one area of the reactor that can lead to blockages in the dynamic membrane. The highest concentration of VFA producing bacteria are often found in the dynamic membrane, so aerating the bulk solution may be less efficient than aerating the dynamic membrane. However, aerating the bulk solution rather than the dynamic membrane often produces a more uniform oxygen concentration throughout the reactor which is also beneficial. Assessing these tradeoffs will be important for the team to determine the location at which oxygen will be introduced into the system. Oxidation reduction potential (ORP) is one method for controlling the oxygen levels precisely [17]. ORP is the measurement, in millivolts, of a solution’s capacity for electron transfer. When oxidation occurs inside the bioreactor, electrons are being freed from their associated molecules. An ORP probe is able to measure the potential difference these electrons create, allowing the user to indirectly measure the amount of oxygen inside the reactor. Figure 1 below shows a potential implementation of micro-aeration into a bioreactor and uses an ORP probe to measure the oxygen levels.

Figure 1: A potential implementation of micro-aeration into a bioreactor using an ORP probe to measure the oxygen levels. The SCADA system is a control system to monitor oxygen levels and modify those levels should they go outside of the desired range.

Current Bioreactor System

Renisha Karki, a PhD. Candidate in the Environmental Biotechnology Group at the University of Michigan, is interested in implementing micro-aeration in her current AD setup and has sponsored our ME 450 team to design a bioreactor that can achieve micro-aeration. Her current reactor setup is depicted in Figure 2 below.
Renisha uses a double bioreactor setup where one reactor converts organic waste into VFAs, and then the second bioreactor converts VFAs into biogas, primarily methane. This biogas is composed of 50%-75% methane with the remaining being CO₂ and other gasses [18]. Methane can then be separated and harvested using gas separation techniques. Organic waste, consisting of food waste and sludge, are fed into the first reactor. The first reactor is the rumen reactor that holds microbes found in the rumen stomach of a cow. Organic waste is fed into the system defined by an organic loading rate (OLR) which measures the amount or organic waste feed per volume per day [19]. The organic waste has a percentage of volatile solids (VS), solids that can easily be converted from solid to liquid or gas state [6]. Microbes inside this reactor convert the VS into VFAs [6]. VFAs are an intermediate from the organic food waste to biogas production cycle [20]. The most common VFAs are acetic acid, acetic acid, propionic acid, and butyric acid [21]. For Renisha’s research, she is mainly interested in acetate as it is used in the second bioreactor to generate biogas [6].

The VFAs produced from the first bioreactor are then manually transferred into the second bioreactor. Inside the second bioreactor, there are methanogens that convert the VFAs into biogas. The biogas is collected and filtered to produce methane gas. Renisha implemented a two bioreactor design because the rumen microbes in the first bioreactor outcompete the methanogens leading to a decrease in methane gas yield [6].

Renisha has indicated that the bottleneck of the system is the VFA production which occurs in the rumen reactor [6]. Therefore, if the methane gas yield is to increase, then the VFA production must increase, so we are focusing on designing a new bioreactor to replace the first bioreactor in the system. Renisha has also indicated that this new bioreactor must increase VFA production, increase the OLR, and add micro-aeration into the system. The project will be considered successful if a design for a new rumen reactor is created and tested.

**Current Rumen Reactor Design**

The rumen reactor currently used in Renisha’s lab is depicted in Figure 3 below.
Figure 3: (a) The currently used rumen reactor in the lab. (b) An inside view of the rumen reactor without any organic waste. The reactor’s notable components are labeled.

The bioreactor design has sensors and components designed to facilitate VFA production by promoting microbial growth [21]. While there are microbes floating around inside the organic waste medium, most of the microbes reside on the dynamic membrane along with solids. A dynamic membrane allows the produced VFAs to pass through without letting other organic waste and microbes to permeate through [22]. This membrane can develop a cake layer through microbe and solid accumulation on the biofilm in the membrane [18,23]. This cake layer can act as another form of filtration as it lowers the organic waste flux seen by the membrane [24]. However, too large of a cake layer can limit the flux of VFAs through the membrane, thus, the system has a mechanical stirrer that rotates a brush to remove this cake layer to maintain optimal flux. The stirrer operates on a 12, 4, 4 cycle defined as 12 minutes of settling time, where microbes and solids settle on the membrane, 4 minutes of draining, where the permeates, the VFA product, are drained from the top reactor section to the bottom reactor section and removed from the reactor, and then 4 minutes of stirring, where the motor oilers the brush to rotate and remove the biofilm that accumulated the microbes and waste. The microbes and waste are mixed into the rest of the waste medium to settle again after the mixing ceases [18].

The microbes also need a specific temperature and pH range in order to survive. Thus, a pH and temperature probe are each submersed in the organic waste which records the pH and temperature. Since the VFAs being produced by the reactor are acidic, an alkaline solution is required to balance the pH. The current alkaline solution is NaOH which does not affect the microbes beyond altering the pH. The alkaline solution is pumped into the system through the hose in the top of the reactor. Heat tape is also used to create a positive growth environment by warming the organic waste if the temperature becomes too low.

Organic waste is loaded manually into the system through the top hole using a funnel. The organic waste needs to be tested for proper VS in the OLR, so tests are performed prior to
loading the food into the system. Additionally, the food waste is pretreated by blending the food with water [18]. After pretreatment, the solution is fed to the reactor.

Renisha follows Standard Methods [25], a standards organization which outlines testing and handling procedures, examination, and analysis techniques for water and waste. Therefore, any solution that our team generates, must allow for application of these standards.

**Benchmarking**

Micro-aeration will be a central function of our system, so understanding the different methods that are used to aerate different system’s is important. One of the typical uses of aeration occurs using a bubble aeration system. A typical bubble aeration system is a sparger system in pools to create bubbles on the water surface that creates a cushion for divers. The typical implementation of these systems involves the use of air compressors and air tanks to store and move the air throughout the system. The air is then released into the pool using spargers as seen below in **Figure 4**.

![Figure 4: A sparger system used in a pool to create large air bubbles for cushioning. The air tanks and compressors used to create the bubbles are placed in a separate room, away from the pool area.](image)

Spargers can be scaled down to a bioreactor where they can come in many different varieties which depend on the bubble size desired. If bubble size is not significantly important, stone diffusers such as ones found in aquariums can be used which are shown below in **Figure 5**.

These diffusers have small pores which air can permeate through and they release into the bulk solution. These can come in a large variety of shapes and sizes, but the main attraction to them is they are cheap, typically costing less than $15.

![Figure 5: A stone diffuser that is typical for an aquarium. Air enters through the large hole and then diffuses through the stone to bubble out into the bulk solution. [54]](image)
If the aim of the system is to use smaller bubbles that allow for greater oxygen diffusion throughout the system, then small bubble diffusers could be necessary. Again, they can offer a range in shapes and sizes, but the majority of the diffusers are flat, not cylindrical. These small bubble diffusers are often used in wastewater management for the removal of organic matter, similar to our project. Figure 6 below depicts a nanobubble sparger which is able to produce bubbles on the nano-level, but other varieties are offered such as microbubble (diameter \( \leq 50 \, \mu m \)) sparger.

![Figure 6: Shows a small bubble disk diffuser.](image)

Other geometries of spargers are depicted in Figure 7 below. Porous plates provide even gas distribution and are ideal for continuous ventilation. Perforated plates ensure even gas flow across the surface. The perforated ring, with its unique structure, is conducive to the diffusion of gas in the ring structure. Spyder style spreaders allow multi-directional airflow for enhanced coverage. Multi-hole nozzles adjust airflow to targeted areas, while single-hole nozzles sharply focus gas output. With all those six types of spargers, our team will find the best fit one to work for the whole system with a high efficiency on micro aeration process.

![Figure 7: Different geometries of small bubble spargers [53].](image)
Further investigation led to learning of designs that incorporate multiple spargers. The design by Thermo Fisher Scientific HyPerforma Single-Use Bioreactor depicted in **Figure 8** below has two spargers, one on the bottom of the tank and one on the side to provide a crossflow of air. This design aims to improve the uniformity, but also increases the complexity and cost of a single sparger system.

**Figure 8:** Image of Thermo Fisher Scientific HyPerforma Single-Use Bioreactor [30].

Opposed to a bubble aeration system, membrane aerated biofilm reactors (MABR) are a bubbleless aeration system because it uses a membrane to allow gas to diffuse directly to the microorganisms in the reactor. Typical bubble aerators use a lot of energy to produce their bubbles, so the MABR design strips away the need for high power compressors, reducing the overall energy usage. **Figure 9** below shows a typical MABR design. However it is difficult to regulate the uniformity of air after it diffuses to the other side of the membrane. A system such as this is used in many waste treatment plants throughout the nation.

**Figure 9:** This shows a typical MABR design with a fiber tube (the white cylinder in the middle) delivering oxygen to the biofilm (the orange surrounding the tube).
A system such as this is used in many waste treatment plants throughout the nation. One such example is a design by Fluence below in Figure 10. This design blows low pressure air through a sleeve of membranes which allow oxygen to diffuse to a biofilm on the other side. This design is then coiled to improve the uniformity, but it lacks a way to clean the biofilm if the caking is too much.

![Figure 10: A specific design of an MABR in wastewater treatment plants made by Fluence. [55]](image)

**Discussion**

After following the benchmarking process, we were able to analyze many different techniques used in solving variations of our own problems. Although some of these techniques are not directly transferable to our system, they can be useful for determining what existing solutions may or may not work for our project. For instance, the use of a sparger in a pool is very useful for divers so they do not get hurt on impact with the water. However, for our purposes, the bubbles released from the sparger would be too large and would not diffuse properly into our waste solution. Therefore, we may lean towards using a small bubble aerator as seen in Figure 6, or a MABR as seen in Figure 9. The benchmarking process is important because we can understand how existing solutions work and its associated benefits and drawbacks. Using this information to guide our design decisions, we are able to choose solutions that are more likely to succeed in our system.

**Design Process**

When working through complex design problems, it is crucial to provide a detailed structure to ensure that the activities and goals of the team are aligned for an efficient design process and to produce a quality end product. For this project, none of the team members came in with background knowledge about the biological processes necessary to explore the problem space. Due to this, the team spent an extensive amount of time and effort researching background information and engaging with stakeholders, such as Renisha Karki, our main sponsor and target user. Due to this, the team will likely have many future iterations as we continue to learn about the complexities of producing VFAs through the decomposition of biomass. For instance, when first completing our requirements and specifications, we required a certain level of fungi retention, but after speaking with Renisha, we realized that measuring this would be impossible. To fix this, we focused on the root of the requirement, VFA production, because fungi retention is a function of VFA production. The chosen design process should focus on problem definition and stakeholder engagement to give the team a strong understanding of what the primary
stakeholders want and how we can achieve a solution. Lastly, due to time constraints of the semester, the team needs to work efficiently through the design process. Due to this, the team may not reach a realization of our designs, which would result in detailed drawings and models of our system being the final output of the design process.

Due to the reasons mentioned above, the team has been following a stage-based design process, as pictured below in Figure 11. Some of the notable features of the chosen design process are the stage-based approach, the ability to iterate, constant analysis of the problem, a lengthy conceptual designing stage that focuses on divergence, and an ending point that doesn’t necessarily include realization. The team benefits from a stage-based process because we must have a strong understanding of the current stage before moving on to the next. If the team is able to thoroughly work through each stage, one at a time, there may be less iteration, streamlining the entire process. However, if iteration is needed, the feedback loop allows the team to revisit earlier stages, making iteration a principal aspect of our design process. This iteration is important because the team is constantly learning, so reflecting on previous work can ensure that any biases are addressed for a more inclusive and holistic design. Due to the complexity of the system we are designing, an emphasis on concept generation and concept selection stages is key to a strong final design. The chosen design process has three separate stages relating to the concept generation and selection periods that will allow the team to explore the solution space thoroughly. On top of this, the chosen design process incorporates divergence and convergence, designated by the use of three arrows in the conceptual design stage and one arrow after the stage. This signifies that we will be generating a broad variety of concepts that will be narrowed down using certain strategies such as pugh charts. As mentioned previously, the team will likely not reach the realization stage so the team’s design process ends at working drawings. This includes detailed drawings and models of our design so that our sponsor can easily understand and manufacture the team’s final design.

![Figure 11: The design process introduced by French. The model is based on design practice observed in industry [33].](image)

The team discussed using the design process presented on the first day of class but decided to use our design process for a few reasons. The first reason is that our group can benefit from a streamlined model due to the time constraints of the semester. The design process introduced in class is linear and very detailed, and while this is beneficial to ensure thoroughness during a longer design process, the team may not have the time to delve deeply into all the different activities. The design process chosen hits on all of the major design process stages as seen in the ME450 capstone design process like problem analysis, concept generation, and solution development and analysis, while leaving out some of the extra activities that may not be conducive during our process. The second reason is that some of the stages seen in the capstone design process are outside the scope of our project. For instance, the need identification and realization stages of the design process may not be used by the team during our design process, so choosing the design process seen in Figure 11 provides a more accurate representation of our specific project.
Stakeholder Analysis

The stakeholders of our project can be compiled into six categories: resource providers, supporters and beneficiaries of the status quo, complementary organizations and allies, beneficiaries and customers, opponents and problem makers, and affected or influential bystanders. Figure 12 below is a visual representation of our stakeholder list organized in terms of primary, secondary, and tertiary stakeholders. Primary stakeholders are those whose lives or work are directly impacted by the problem and/or the development of a solution [38]. Secondary stakeholders are people who are part of the problem context but may not experience the problem themselves and/or may not be directly impacted by a solution [38]. Tertiary stakeholders are those who are outside of the immediate problem context but may have the ability to influence the success or failure of a potential solution [38]. In Figure 12 below, the six groups are laid out in a stakeholder map showing each group’s relationship to the team’s project.

Resource providers are stakeholders that provide financial, human capital, knowledge/expertise, connections, and technological resources, and any brokers or intermediaries that channel these resources to those who want them [38]. A few of the team's resource providers include food waste providers, specifically the University of Michigan dining halls, that provide food waste for our system, chemical companies who provide sodium hydroxide to keep our system within the target pH range, and our sponsors, Renisha Karki, our main sponsor and target user, and Steven Skerlos, our section instructor and member of the project team, who provide the team with expert background knowledge and support [5]. The Department of Energy’s efficiency and renewable energy program (EERE) and the University of Michigan provide critical funding to support our research and design efforts [5].

Beneficiaries and supporters of the status quo are stakeholders that benefit or would continue to benefit if the status quo is maintained [38]. If the project was a failure, the stakeholders that would benefit are natural gas distributors like DTE and fracking companies like Shell and BP because it would maintain their market share in the energy industry. Fracking currently accounts for 67% of the natural gas production in the United States, so introducing a renewable natural gas source could greatly impact their overall market share [34]. On top of a reduction in market share, increased supply would likely lead to a decrease in price, negatively affecting the revenues of natural gas distributors.

Similar to the supporters and beneficiaries of the status quo, opponents and problem makers are stakeholders who contribute to the problem, undermine the ability of others to achieve solutions and sustain intended impacts, or otherwise oppose any efforts to develop a solution [38]. Fossil fuel producers and suppliers and Organization of the Petroleum Exporting Countries (OPEC) fall into this category because they oppose new methods of natural gas production. Fracking companies that produce natural gas and fossil fuel products are a strong example of stakeholders belonging to multiple categories, as they are a supporter of the status quo and an opponent of our solution because of the negative impact our solution would have on the company's sales.
Complementary organizations and allies may facilitate your ability to create an impact in the problem space, such as stakeholders who support the same cause and/or provide important complementary services [38]. A few of these organizations and individuals include other bioreactor researchers, the Department of Energy, and environmental activist groups. Other researchers, such as Dianna Kitt, are allies to our project because they use similar technology that can provide insights into our technology. While the research goals are not the same, we can implement technological advancements, like improved dynamic membrane structure, by fitting them to our design. The Department of Energy provides resources like supplementary research databases and data analytics software that are helpful when determining optimal values for pH, temperature, and VFA production [35]. Environmental activist groups are also trying to achieve the overarching goal of helping the environment through activist efforts and are a notable opponent of fracking companies.

Beneficiaries and customers are stakeholders who may directly or indirectly benefit from the development of a solution in the problem space [38]. Private homeowners, enterprises, and the general population would benefit from our product because an increase in natural gas production could mean a price decrease, benefiting homeowners and companies who pay for natural gas to heat their properties. Our solution also provides a renewable source of natural gas, which could lead to a decrease in other methods of natural gas production that are harmful to the environment, benefitting the general population.

Affected or influential bystanders are stakeholders who have no current direct impact but who could become affected by efforts to develop a solution or who could influence the success of a potential solution [38]. A few affected bystanders of our project are local communities and other renewable energy providers, like solar and wind energy vendors. Renewable energy sources, such as solar and wind energy, perform the functions of heating and electricity generation, similar to natural gas, so the successful integration of our product could outproduce our renewable energy counterparts, leaving their technology obsolete. Lastly, if the team can produce a commercializable product, mass production of renewable natural gas would require...
large power plants. Local communities would be affected by this and would need a voice to maintain the livability of their community.

**Social Context Analysis**

One of the significant issues society faces due to its growing population is fossil fuel abundance. There are a finite amount of natural resources, and at current usage levels, these resources, like coal, oil, and natural gas, will be depleted in less than a century [36]. Our project aims to provide a renewable energy resource that would allow humans to produce fuel even if the natural resources run out, which is essential for the progression of civilization. However, in the context of our problem, Renishaw and our other sponsors are more worried about environmental implications. Our project is one subsystem of a clean energy system with the hopes of producing clean fuel products. The team's project aims to increase VFA and methane production using a ruminant bioreactor so that the methane can later be converted into hydrogen using pyrolysis technology. Hydrogen is the desired end product because it does not produce harmful emissions when burned for fuel. Due to this, our design will focus more on solving the environmental issues associated with our problem. However, the final solution will involve renewable energy capabilities that can positively impact society.

**The Role of Intellectual Property**

The University of Michigan has been researching and developing solutions to this problem for many years. As a result, faculty members involved in this research have published scholarly papers describing the function and use of the system as well as detailed experimental trials. Although the University of Michigan does not currently have a patent for their design, they have established documentation showcasing their technology, giving them full ownership of the intellectual property rights. As a result, when the team began working on this project, all members signed a contract assigning their intellectual property rights to the University of Michigan. This allows the university to use the team's intellectual property in future patents or copyrights. However, the team still retains the right of an “employee inventor”, so in the case that one or more of the team members makes a contribution to the conceptualization of a design that is commercialized, the team members involved will receive compensation.

**Sustainability Analysis**

Sustainability is instrumental in limiting negative societal and environmental impacts our product may have. One of the key contributors to the sustainability of the current design is the use of abundant and renewable biomass and waste resources. The bioreactor uses food waste provided by the University of Michigan dining halls and sludge and water waste from local waste collection companies. This food waste consists of uneaten or partially eaten food and other organic materials thrown out during cooking. The sludge consists of water runoff and sewage components that occur naturally. The waste used in our bioreactor is renewable and globally available, allowing for the widespread adoption of our technology. Another critical contributor to the sustainability of our design is the limited production of waste or pollutants. Our current system produces small amounts of hydrogen and carbon dioxide that is contained and stored. Beyond that, the system does not produce significant emissions that are harmful to the environment. One of the current system’s unsustainable practices is the usage of sodium hydroxide to maintain a proper pH level. This issue can be solved by the introduction of oxygen using micro-aeration, as small amounts of oxygen have been observed to increase carbon dioxide production which aids in maintaining an acidic pH [39]. However, micro-aeration is a technology
currently undergoing extensive research, making it difficult to estimate if the introduction of oxygen can replace the use of acidic chemicals. Continued research and testing are needed to determine if this could be a permanent solution. The final unsustainable feature of our system is the need for high energy usage to maintain the system's optimal temperature of 37°C and the mechanical energy via the stirring phase. The team plans to lower the current energy consumption by providing optimal thermal conditions using insulating materials. Ideally, this will reduce operational costs through less thermal energy consumption.

Ethics

One of the ethical dilemmas the team faces involves the manufacturing and production costs of the system. The product is highly beneficial for the global population because it provides a source of clean renewable energy. However, the cost of producing the bioreactor system could prove to be high which can price out impoverished communities. The team will manage this dilemma using cheap and readily available materials while maintaining the system's integrity, enabling the team to maximize the communities that can realistically afford and implement the technology.

Most people are instilled with personal values and ethics that they maintain throughout their lives. Certain values like truthfulness, fidelity, integrity, and responsibility are crucial for maintaining strong relationships among peers, teachers, and employers. The University of Michigan expects all members of its community to uphold certain professional standards, such as possessing personal integrity both as students and as professionals. Students must be honorable people to ensure safety, health, fairness, and the proper use of available resources in their undertakings. Members of the College of Engineering community are honorable and trustworthy persons [40]. All members of the team are committed to upholding these standards to provide a safe environment for the project to be completed. These values align with the team’s because each member is committed to doing good, honest work to create a solution to our problem. However, it is important to uphold your personal ethics, so understanding when an organization may be violating your ethics is critical. If a future employer does not respect the ethics one holds, then it may be worthwhile searching for a new employer.

One power dynamic that exists between Renisha and the team is the difference in academic background and experience. While Renisha’s background focuses on the biological aspects of the project, the team is primarily focused on the mechanical engineering aspects. Therefore, when disputes come up dealing with biology and chemical aspects of our project, we may feel intimidated to suggest ideas in these areas. Similar issues can arise when dealing with mechanical engineering aspects of the project. Due to this, it is important for the team to make sure we create and maintain a welcoming environment where everyone feels comfortable sharing their ideas.

Another important power dynamic lies between our section instructor and sponsor, Steven Skerlos, and the team. Steven is the team's professor and section instructor, but he also plays a role in leading the project team that we are sponsored by. This can lead to a conflict of interest because as a leader of the project team, Steven has done extensive research on the project topic, leading to potential biases when discussing things like a final solution. As our professor, Steven is supposed to provide unbiased advice and guidance, so his role as both our professor and project team leader could be contradictory. As a result of this, the team must make sure that they are adequately challenging Steven’s perspective when receiving guidance. This will ensure that the team is considering different perspectives other than our sponsors, ultimately leading to a less biased and more robust design.
The last power dynamic is within our team stemming from three members being Seniors and one being a Junior. As the only Junior in the group, Anthony may not be open to share his ideas as he may feel intimidated or unvalued due to his age. He may also believe that since he has less experience than the other team members, his ideas are not as valid. It is important for us as a team to be aware of these power dynamics and always keep a friendly environment that is welcoming to every and all ideas everyone has to offer. When all team members are sharing equally, there is greater diversity in thoughts and ideas, ultimately leading to a stronger team chemistry and solution.

The team strives to create an open and inviting environment so the members involved feel comfortable contributing during meetings and conversation. A few ways the team is enabling this is maintaining a relaxed and friendly atmosphere. The team members understand that people come from different backgrounds that can make them feel less qualified than others, so to combat this, the team puts an emphasis on open communication. By prompting team members to contribute in all conversations, diverse perspectives are being shared and overall team confidence improves as a result. Additionally, each team member is committed to keeping an open and curious mind. New information often comes to light, especially during a project like this where the team is constantly iterating on their background knowledge, so being open to new ideas is crucial to promoting a healthy learning environment. The team is committed to promoting a healthy professional environment for all participants.

Requirements and Engineering Specifications

The team determined the requirements and specifications by consulting our sponsor, Renisha Karki. Renisha is the main sponsor and target user of the design and was able to provide expert insight for the team’s guidance. Through a set of interviews, the team was able to determine a list of wants and needs Renisha envisioned for her new design. She also provided the team with her preliminary research proposal and other research articles, which included metrics for determining safe oxygen levels for micro-aeration and information about the performance of her current rumen reactor. After redefining the scope of the project to solely focus on micro-aeration, not the entire bioreactor system, the team was able to convert this information into the requirements and specifications seen in Table 1 below.

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Priority</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reactor Receives Enough Air</td>
<td>High</td>
<td>Introduce 0.005-5L of O\textsubscript{2} per L\textsubscript{reactor} per day</td>
</tr>
<tr>
<td>2. Release Air into the Bulk Solution</td>
<td>High</td>
<td>Must aerate more than 2in vertically from membrane</td>
</tr>
<tr>
<td>3. Aeration Will Maintain a Consistent Range of Values</td>
<td>High</td>
<td>Oxygen levels must be within 0-300mV measured by ORP referenced to a standard hydrogen electrode</td>
</tr>
<tr>
<td>Requirement</td>
<td>Difficulty</td>
<td>Specification</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Air is Uniformly Distributed in Solution</td>
<td>High</td>
<td>Standard deviation of air measured by ORP must be &lt;1 z score</td>
</tr>
<tr>
<td>Design must be affordable</td>
<td>Moderate</td>
<td>Aeration device must cost less than $100</td>
</tr>
<tr>
<td>Aeration Device is Easy to Remove and Clean</td>
<td>Moderate</td>
<td>Aeration device can be removed within 5 minutes</td>
</tr>
<tr>
<td>Diffusion of Air is Optimal</td>
<td>Low</td>
<td>Air bubble size must not exceed 0.2 mm</td>
</tr>
</tbody>
</table>

The requirements are ranked in terms of their priority, and the specifications delve further into the specific criteria that is needed in order to verify the requirement. Most of these requirements (requirements 1-4) are focused on attempting to optimize aeration efficiency and usability such as maintaining a consistent range of aeration, receiving enough air, and uniformly distributed because they all pertain to keeping the microbes inside the reactor alive and optimizing their production rates. These requirements were given the highest priority as they are the focus of the project and what will ultimately lead to its success because if these are not met, the device will not work at all for the intended purposes. To maintain the sparger as cleaning off any biofilm will be necessary, specification 6 was created. 5 minutes was decided as a threshold as any time above may be very inconvenient especially as this solely pertains to removing the aeration device, not cleaning. Its lower priority reflects the idea that the time it takes to remove the device is not integral to the functionality of the system, but rather a requirement made for convenience for the user. This justification extends to requirement 5 as it also is for the user’s experience, not for the functionality of the system. The cost was set to have a $100 maximum as this will provide room to explore options for devices which may be more costly without exceeding an enormous budget. This will solely include the device and not any sensors such as a dissolved oxygen (DO) probe or an ORP probe, devices that measure the amount of oxygen in the liquid medium, or mechanical devices such as a pump. Lastly, requirement 5 is based on background research where it is stated that the smaller the volume to surface area ratio of a bubble, the more diffusion of air into the solution will occur. Larger diffusion indicates that less pumping power will be used as less air is needed to enter the system. This will rescue the energy cost of the system. Because we will be working at such low levels of aeration, it is expected that the pumping cost to be minimal, so minimizing the size of bubbles, while important, is not the highest priority.

As mentioned previously, the scope of the project used to be larger where the project was to create a full bioreactor, not add micro-aeration to a current bioreactor. This refinement of the project adjusted our specifications and requirements as previously, there was only one micro-aeration requirement and 10 more for other aspects of the bioreactor. Limiting the scope allowed the project to be more focused and develop stronger and more developed requirements that are shown in Table 1.

**Concept Generation**

**Functional Decomposition**

Since DR2 when the concept generation phase took place, the team narrowed the scope of our project to focus solely on micro-aeration. The team’s original concept generation phase
used a functional decomposition of the entire system to produce subsystems for individual concept generation as seen below in Figure 13. This analysis allowed the team to understand exactly what the inputs of the system are, what has to happen in the system, and what the outputs of the system are. The red arrows follow the energy input and loss throughout the system and the orange arrows represent the material inputs and outputs. Since aeration was already a subsystem of the larger bioreactor, the team already generated 20 unique aeration strategies, so when switching the scope of our project, the team did not think it was necessary to perform another concept generation phase. Additionally, the team performed extensive research and found few strategies for effectively aerating a bioreactor, so we felt that the 20 concepts generated earlier in the semester plus the research encompassed the entire solution space.

![Functional decomposition chart](image)

**Figure 13**: Functional decomposition chart.

**Concept Generation**

The main techniques that we used when generating concepts were design heuristics and focusing on radical concepts to increase the number of generated concepts because when generating design concepts quantity is important to get a broad variety of ideas. As mentioned earlier there aren’t a lot of viable strategies for aerating a bioreactor so using these strategies forced the team to think outside of the box. One design heuristic we used was “add motion”. We applied this heuristic to create a design that distributed air uniformly to create a design that adds pumps to the system to move the waste around while air is being added. One way we used radical design is by using a two vat system that we had not observed in our research to introduce oxygen to the food and sludge waste. Although these concepts may not have been picked as the final design, they still ensured that the team considered the entire solution space. The designs for our micro-aeration system are depicted in Appendix A.3.

**Concept Selection Process**

After generating many concepts in the previous section, we needed to be narrowed down in a scientific manner. One method of doing so is by using a Pugh Chart. A Pugh Chart allows for multiple ideas to be compared based on design criteria and their associated weights. Because we broke down our designs by subsystems we were able to create design criteria specific to each
subsystem. Our group determined that this is the best option in determining the optimal bioreactor design as it allowed us to focus on choosing the best design for each subsystem and then combining the best subsystems to create the alpha design. In comparison, if designs that incorporate multiple subsystems were compared in the same Pugh Chart, some lower performing individual systems may be hidden because other systems in the group outperformed. Thus, breaking down the Pugh Chart to be personalized to each subsystem allowed the best design to be chosen.

To determine the design criteria, we analyzed the requirements and specifications as well as used our engineering background to determine which subsystem will have the largest impact on each specification. Then, a design criteria was created to rank the designs based on how well they will accomplish the specifications. The weights provided to the design criteria are indicative of the importance of each design criteria.

To create a Pugh Chart, a baseline design is set by choosing any design that will be compared against all other designs. This baseline design will score a “0” in every design criteria score. When comparing a design directly to the baseline design, the product will receive a “1” if the compared design improves upon the baseline, a “0” if the compared design is similar in quality to the baseline, or a “-1” if the compared design is worse than the baseline. In rare instances a score besides the “1”, “0”, or “-1” will be used only if the design is exceptionally poor at completing the design criteria or if the design performs exceptionally well at completing the design criteria. The weights of each design criteria are then multiplied by the score the design received where, finally, the score is totaled for a final score. The best scores will be the most positive while the worst scores will be the most negative. It is not impossible that the best design is the baseline, and if so, the best score will be a “0”.

The second subsystem that was analyzed was the micro-aeration subsystem as shown in Table 2.1 below. Adding this subsystem to the bioreactor design is one of the main goals of our project, so choosing a good design is important. After narrowing down the initial concepts, the five methods of adding the air into the system are spargers which generate bubbles inside the bioreactor, a double vat that moves the waste from one anaerobic reactor to a separate aerobic reactor, an external cross flow device that moves the waste outside the reactor to a device that puts the waste in contact with air via a cross flow of device, an air tube that blows air into the system similar to a engine injector, and a MABR device on the walls of the bioreactor. The most important aspects of micro-aeration is the control of air concentration and where air is dispersed into the system, so these weights are given higher values. Additionally, because of the specifications to deliver the air to the bulk system, not the membrane, the larger the distance to the membrane, the better. As our main project’s goal is to generate energy via methane production, the less energy consumed by the process is important, so it was also chosen as a design criteria. Finally, simplicity of the design tends to lead to easier maintenance, but since the maintenance requirement is lower on our requirement list, it has a lower weight.

After ranking all the devices, the sparger was determined to be the best option for micro-aeration. It excels at adjusting the flow by simply changing the speed of a pump, it can be moved away from the membrane, and is relatively simple as there are many benchmarks for spargers as highlighter previously. However, it lacks the uniformity of air concentration as there will be a larger concentration of dissolved air around the sparger. Despite this downside, it outperformed the other designs and will be considered for the alpha design.
Table 2.1: Micro-aeration Pugh Chart

<table>
<thead>
<tr>
<th>Design Criteria</th>
<th>Weight</th>
<th>Design 1 (Sparger)</th>
<th>Design 2 (Double Vat/Regurgitation)</th>
<th>Design 3 (External Cross Flow)</th>
<th>Design 4 (Air Tube Directly into Membrane)</th>
<th>Design 5 (MABR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance to Dynamic Membrane</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Adjustable Flow</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uniformity of Air Concentration</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>Energy Cost</td>
<td>2</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Simplicity</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>0</td>
<td>-1</td>
<td>-2</td>
<td>-2</td>
<td>-1</td>
</tr>
</tbody>
</table>

**Concept Description**

The final design concept consists of a sparger capable of producing bubbles as the form of aeration. The sparger will be used with an air pump connected to a power generator to supply oxygen to the bioreactor. Additionally, the team plans to implement PID control through the use of Labview software and a DO/ORP probe. The schematic for how this will all work together can be seen below in Figure 14.

Figure 14: PID control of the sparger air flow.
Engineering Analysis

Fluid Mechanics
To move fluid around, either a liquid or gas, there needs to be some energy input into the system. As our system is designed to generate energy, the lower the energy input, the larger the net energy yield will be. The fluid is flowing internally in pipes, so the mechanical energy model can be used to determine the required pressures, pumping work, energy losses, etc while moving these fluids. The mechanical energy equation is depicted below in Equation 1 where $P$ is the pressure at, $a$ is a correcting factor depending on the type of flow, $\rho$ is the density of the fluid, $g$ is the gravitational constant, $v$ is the velocity of the flow, $z$ is the change of height of the flow, $h_s$ is the shaft work, and $h_L$ is the head loss. This equation is used between two different points labeled 1 and 2.

$$\frac{P_2}{\rho g} + \alpha \frac{v_2^2}{2g} + z_2 = \frac{P_1}{\rho g} + \alpha \frac{v_1^2}{2g} + z_1 + h_s - h_L$$  \hspace{1cm} (1) [48]

Equation 2 is used to determine the shaft work where $\dot{m}$ is the mass flow rate and $\dot{W}$ is the shaft power. Equation 3 is used to determine the head loss made up of both major and minor losses where $f$ is the friction factor, $D$ is the diameter of the tube, and $K$ is a loss factor dependent on the setup.

$$h_s = \frac{\dot{W}}{\dot{m}g}$$  \hspace{1cm} (2) [48]

$$h_L = h_{L, major} + h_{L, minor} = f \frac{v^2 L}{2Dg} + \sum K \frac{v^2}{2g}$$  \hspace{1cm} (3) [48]

Using Equations 1-3, required energy input can be found based on the mass flow rate for specification 3.

Bubble Size
In most studies, bubbles are classified into nanobubbles (diameter <200 nm), fine bubbles (diameter 200–10 µm), microbubbles (diameter ≤50 µm), and macrobubbles (diameter 2–5 mm). Under scientific standards, size-based bubble terminology is defined as micron, fine and ultrafine bubbles/nanobubbles, ranging from 1 to 100 µm, less than 100 µm, and less than 1 µm respectively [50]. For our micro-aeration system we are focused on producing fine bubbles.

Since it is crucial to understand the influencing factors of oxygen transport in the overall system, our team prefers to start with the analysis of bubble size because it is a key factor. Larger bubbles produce greater buoyancy, which affects their ability to move in liquid media. Bubble size is inherently related to various distributor parameters such as nozzle size and tube length. Based on our subsequent experimental settings for various distributors, the following formulas are mainly related to the distributor hole size and bubble size shown in Equation 4.

$$\frac{d_b}{d_0} = 1.8 \times \left[ \frac{\sigma}{\rho g d_0^2} \right]^{1/3}$$  \hspace{1cm} (4) [51]

where $d_b$ represents the bubble diameter, $d_0$ represents the nozzle diameter, $g$ is the gravitational acceleration, $\rho$ is the density of the liquid, and $\sigma$ is the surface tension of the bubble. These factors are critical for optimizing the amount of oxygen injected into the solution, directly affecting the efficiency of the micro aeration process. Using Equation 4, we can determine the best fit sparger with the most efficient nozzle size in our experiment 1 during verification.
**Oxygen Transfer Rate**

By decomposing the influencing factors of bubble size, we further understand the relevant background of the required experiments, and then explore the relationship between bubble size and the production efficiency of micro-aeration reaction. Interface area, denoted as, plays an important role in the oxygen transfer rate within our micro aeration system. Smaller bubbles have higher interfacial areas, allowing for more efficient oxygen mass transfer rates, which is important for biological reaction processes that require large amounts of dissolved oxygen. The smaller the bubbles, the more efficiently oxygen is distributed throughout the biofilm, thereby enhancing ammonia degradation. Equation 5 shows the relationship between the specific interface area a of the bubble and the Sauter mean diameter d_b. In this case, reducing the bubble size will increase the gas-liquid interface area, thereby improving the efficiency of the micro-aeration reaction. The gas content rate is $\varepsilon_g$.

$$ a = \frac{6 \varepsilon_g}{d_b(1 - \varepsilon_g)} \quad (5)[51] $$

Equation 6 represents the relationship between mass conversion efficiency. The mass transfer rate affected by the interface area a depends on the oxygen transfer rate (OTR), and the formula is $k_{La}(T)(C^*-C)$

$$ dC/dt = k_L a_{(T)} (C^* - C) \quad (6)[51] $$

The equation $k_La_{(T)}(C^*-C)$ explains how the specific interface area a affects the overall mass transfer rate by affecting the oxygen transfer rate (OTR), where $k_La_{(T)}$ is the temperature-dependent volumetric mass transfer coefficient, $C^*$ represents the saturation concentration, and $C$ is the dissolved oxygen concentration at time t. Oxygen transfer rate is a portion of the system's efficacy and can be further explained by the differential rate of change in oxygen concentration over time $dC/dt$, as shown in Equation 7 [51].

$$ ln(1 - \frac{C}{C^*}) = k_L a_{(T)} t \quad (7)[51] $$

Using those equations, we can connect the bubble size with the changed concentration which shows the efficiency of oxygen input.

**Air Flow Rate**

The relationship between gas content rate and specific interface area mentioned above can help us obtain the change pattern of specific interface area to further analyze the efficiency value of micro-aeration reaction. Then the gas content rate here is related to the air flow rate of our sparger by Equation 8. The gas content determines the gas-liquid interface area and mass transfer rate, and is an important fluid dynamics parameter of the airlift reactor. From our background study, the gas holdup ratio of different distributors increases with the increase of air flow rate. The overall relationship can be expressed by Equation 17.

$$ \varepsilon = \alpha U_g^{\beta} \quad (8)[52] $$

where $\varepsilon$ is the gas content rate, $U_g$ is the air flow rate, and two parameters $\alpha$ and $\beta$ related to the properties of the sparger system shown in Table 3 below [52]. Especially for $\alpha$ and $\beta$, the experiments can be set up to measure the gas holdup for all tested spargers and their matched air flow rate $U_g$, then using a math model to fit the experimental data to finalize the values of $\alpha$ and $\beta$ for different types of spargers.
Table 3: Empirical correlation regarding different sparger type

<table>
<thead>
<tr>
<th>Sparger Type</th>
<th>Correlation</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate</td>
<td>$\varepsilon = 0.76U^{0.66}$</td>
<td>$R^2 = 0.97$</td>
</tr>
<tr>
<td>Ladder</td>
<td>$\varepsilon = 0.83U^{0.72}$</td>
<td>$R^2 = 0.99$</td>
</tr>
<tr>
<td>Tri-nozzle</td>
<td>$\varepsilon = 0.64U^{0.57}$</td>
<td>$R^2 = 0.97$</td>
</tr>
</tbody>
</table>

With all those, calculated air flow rate by the pressure differential through Equation.18 will help us fully prepare for the experiment 1. Here Equation 9 shows the relationship between air flow rate and the pressure differential

$$P = kQ^2$$  \hspace{1cm} (9)

where $P$ is the pressure differential and $Q$ is the flow rate, meanwhile $k$ is constant unit conversion value. Using Equation 8 and Equation 9, we get the air flow rate and help further calculate the specific interface area working for the final efficiency determination.

Sparger Selection

Our project has multiple stone diffuser sparger geometries that need to be tested. In total we limited the number of spargers to test to five designs to try as these were readily available to us at a low cost. The designs consisted of two circular designs, one large and one small, and three cylindrical designs, one large, one medium, and one small. All these designs are depicted in Figure 15 below.

![Figure 15: Stone diffuser sparger designs.](image)

These designs needed to be compared to determine which would work ideally for the system. It was chosen to compare the range of ORP values to the at different flow rates as well as the average ORP reading at multiple flow rates. This same test is the foundation of later verification testing where the proper methods to determine the data will be discussed. However, the results for the average ORP reading as a function of airflow are depicted in Figure 16 below.

23
Figure 16: Average airflow reading for different stone diffuser spargers.

Analyzing the data, the large cylindrical sparger was determined to have the largest average ORP reading for the majority of the airflow rates. It is especially important that this sparger has large airflow rates at low airflows because that is the region where micro-aeration will occur. This data also enables the creation of an empirical model which relates flow rate to ORP. This empirical model equation can be used for an open-loop control system.

Additionally, the range of ORP values were also compared as seen in Figure 17 below. This would indicate which sparger would offer a more consistent range of aeration as the larger the range, the more inconsistent the ORP readings were. The large cylindrical sparger and the small cylindrical sparger both offered good consistent ORP readings that either choice would be ideal for the bioreactor,

Figure 17: Range of ORP values are varying airflow rates for different stone diffuser spargers.
The large cylindrical stone diffuser sparger performed well in both metrics and was decided upon as the final design.

Final Design Description

The final design was the large cylindrical stone diffuser sparger because it outperformed other stone diffusers. The design functions as a porous stone where air enters through the open hole in the top. This section will need to be connected to an air supply tube. Once the air enters, it will diffuse through the porous stone and leave through the sides. This design has multiple benefits such as higher uniformity of aeration as the air can evenly leave on all sides, a low cost as the diffuser was $5.99, and its cylindrical shape which helps it fit through small holes in the bioreactor so it can be removed quickly. In testing, the sparger was able to be removed in less than 3 minutes. The chosen sparger is depicted below in Figure 18.

Figure 18: Chosen final sparger: cylindrical stone diffuser.

In addition to deciding upon the best aeration device, a CAD model of the bioreactor that the sparger system will be implemented into was created shown in Figure 19 below. This model highlights how and where the sparger could be included into the reactor to not interfere with the other devices inside.
Figure 19: CAD of the bioreactor that the chosen sparger will be implemented into.

Lastly, a potential control system diagram was created which depicts how a control system can be implemented to control the airflow rates into the system which is shown in Figure 20 below. Currently, our system can be run preliminarily on an open-loop control system. The equation for the sparger open loop control is shown in Equation 10 where DO is the dissolved oxygen reading and AFR is the airflow rate. This equation is based on the empirical model depicted in Figure 16.

\[
DO = -6.59 \cdot 10^{-6} (AFR)^2 + 0.0059AFR + 6.6385 \tag{10}
\]

Ideally, this will be altered to a feedback closed-loop control system as they are more adept at reacting to the systems current state and adjusting. The setup for a PID, feedback control system, works as follows: an ORP probe sends a reading to an Arduino (this could also be a LabView computer setup), where the Arduino interprets the signal using PID control to adjust the air pumps power to increase or decrease the sparger airflow rates. The user interface can be used to set desired parameters.

Description of Verification and Validation Approach

When creating verification and validation strategies we decided to follow guiding questions that allow us to characterize our system’s performance based on the requirements and specifications laid out in Table 1. Using these requirements as our starting point, we designed experiments to help answer each question and determined statistical analyses to help understand the data we collect. In total, four verification testing methods were used to test all the specifications which will be outlined later on in this section.

After conducting literature review, we have determined that air flow rate, nozzle size, sparger pressure, uniformity of dissolved air, sparger location, sparger orientation, surface roughness of the holes on a sparger, sparger tube diameter, and sparger tube length all can have an effect on DO levels in the reactor. Some of these parameters are dependent on Rensiha’s setup such as sparger tube length and diameter, so we will not be investigating any further effect of tube length. Additionally, we will not be focusing on the surface roughness of the sparger’s holes for our experiments.
The experimental setup depicted below in Figure 21 is the experimental setup used for all 3 verification testing experiments. A reactor in similar shape and size to the reactor which the aeration will be implemented into was used to control against the geometry of the system. The reactor was also filled with a height of 12in of water. The sparger is connected to an air supply and inserted at specific locations inside the reactor and which can be measured with the ruler. A pump will circulate the water while the sparger is running. This is to mimic the design of the brush that will mix the solution when implemented into the system. While this does not perfectly encapsulate the fidelity of mixing the brush will provide, it will be assumed that it works well for this purpose. In addition to the sparger in the system, a DO sensor and probe were used to determine the DO content of the water in the solution.

Figure 21: Experimental setup for verification testing.

The DO was converted into ORP using Equation 11 below where DO is dissolved oxygen reading and ORP is the ORP value.

\[
DO = 0.457e^{0.04\text{ORP}}
\]  

(11) [55]

As seen in Figure 21, the system we used was aerobic. To set up an anaerobic system would be a significant time investment which would not be possible in the given time. Thus, an aerobic system is used for verification assuming that an anaerobic system will provide similar results. For further verification and validation, these experiments should be run in an anaerobic environment.

Verification Testing 1: Air flow rate vs ORP reading

The purpose of this experiment is to understand how airflow rate affects ORP to determine if the system can validate requirements 1 and 3. This experiment was also used in the engineering analysis section as it helped inform the final design solution. The only significance was that the test was used for all 5 sparger types and not solely the chosen sparger.

The experiment was set up as shown in Figure 21. Because there was no air flow sensor, the air flow rates from the air hose were calculated by attaching the air hose to a catch bag and measuring the volume of air inducted after a certain period of time. During the experiment, various airflow rates were used to determine the DO values which were converted into an ORP reading. The sparger and DO probe were held at a fixed location to mitigate the effect the
locations may have on DO readings. The experiments were conducted multiple times creating an average ORP reading. An empirical model was then created to use in an open-loop control system that relates airflow rate to ORP. The data from this experiment is shown in **Figure 22** below.

![Figure 22](image)

**Figure 22**: The data for the relationship between airflow rate and ORP levels

When analyzing the ORP value it was found that the average ORP readings at very low to very high airflow rates were all between the range required for requirement 3. Additionally, the airflow rates measured were all higher than that of requirement 1 as the airflow rate could reach about 650mL/min which is much larger than

**Verification Testing 2: Sparger location versus uniformity of ORP levels**

Verification test 2 focuses on uniformity as a function of sparger location and ORP level uniformity to verify requirement 2 and 4. During the test, the sparger was moved to different heights indicated in **Figure 23** where for example, 0in VFB means the sparger was 0in vertically from the bottom (VFB) of the reactor. The test reactor had no membrane, so the bottom of the reactor was assumed to operate similarly to the glass bottom of the reactor. When the sparger was moved to different heights, the DO probe was moved radially from the sparger at varying heights, to reduce the effect of height dependence of the probe, and the DO levels were recorded. Multiple readings at the same radial distance were measured and averaged. After the DO was moved to the four locations, 0in, 1in, 2in, and 3in, the standard deviation was calculated. The standard deviation would be a strong indicator of uniformity as the standard deviation is a measure of spread for a data set. **Figure 23** depicts all the data recorded during these experiments.
The data in Figure 23 indicates that the majority of the ORP readings at different sparger locations are relatively the same. This contradicts previous research as it was believed that the lower the sparger in the reactor, the higher the ORP values would be. However, this could be an artifact of the experiment as we are using an aerobic reactor, not an anaerobic one.

In addition to all the ORP values being relatively similar, the standard deviations are also similar with 0in having the highest uniformity (a low standard deviation indicates that the spread of the data is low which means the solution is uniform). Regardless, all the locations can verify requirement 4 as all the standard deviations are below 1 z score.

It was decided that because the experiment did not encapsulate anaerobic conditions, the data may be skewed. So, based on prior research, the location of the sparger, 3in VFB, will optimize ORP and as shown above have a z score, so the design verifies requirements 2 and 4.

0.005-5L of O\textsubscript{2} per L\textsubscript{reactor} per day.

**Verification Testing 3: Bubble diameter**

As discussed previously, decreasing bubble diameter is an important factor for increasing the efficiency of a micro-aeration system. To verify requirement 7, the first experimental setup was used. Using a high-resolution camera, images of the bubbles were taken. When compared to a known distance in an image processing software, the diameter of the bubble is able to be measured. Figure 24 below shows a depiction of the process with some labeled bubbles diameters.
The max diameter measured with the chosen sparger was 10.6mm which exceeds the specification. This failure is due to the priority of cost and time to get the sparger. While this requirement is not met, it was a low priority and the system will still function, although not as efficiently, as before.

**Verification Testing 4: Time to remove the sparger**

To test requirement 6, the same experimental setup was used. The time to remove the sparger when it was implemented was recorded through multiple trials. The timing included removing the lid of the bioreactor and removing the sparger from the solution which resulted in an average time of 2 minutes, which is less than the required time indicated necessary to pass requirement 6. However, the validity of this time is speculative because the bioreactor that was tested is not anaerobic, so it is difficult to determine if this time will carry over into an anaerobic system. In addition, other parts of the reactor may need to be removed such as a motor or other probes which will delay the measured time. Thus, while the recorded time does pass, it is tentative and should be tested further.

**Verification Testing Summary**

Through the verification testing, the design was iterated and finalized to meet most of the requirements. The only requirement that was not met was the lowest priority dealing with optimal diffusion of air into the system. Table 4 below highlights the requirements compared to their past verification, in green, or the failed verification, in red. The cost of the sparger was previously mentioned in the final design description which will verify requirement 5.
Table 4: Requirements compared to the verifications. Colored in green indicates the requirement was satisfied while red indicates the requirement failed.

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Priority</th>
<th>Specification</th>
<th>Verification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reactor Receives Enough Air</td>
<td>High</td>
<td>Introduce 0.005-5L of O₂ per L&lt;sub&gt;reactor&lt;/sub&gt; per day</td>
<td>Achieved measured Airflow Rate: 60mL per minute</td>
</tr>
<tr>
<td>2. Release Air into the Bulk Solution</td>
<td>High</td>
<td>Must aerate more than 2in vertically from membrane</td>
<td>Final Location: 3in from membrane (9in from top liquid level)</td>
</tr>
<tr>
<td>3. Aeration Will Maintain a Consistent Range of Values</td>
<td>High</td>
<td>Oxygen levels must be within 0-300mV measured by ORP referenced to a standard hydrogen electrode</td>
<td>ORP Max: 16.6mV</td>
</tr>
<tr>
<td>4. Air is Uniformly Distributed in Solution</td>
<td>High</td>
<td>Standard deviation of air measured by ORP must be &lt;1 z score</td>
<td>ORP z score: 0.65</td>
</tr>
<tr>
<td>5. Design must be affordable</td>
<td>Moderate</td>
<td>Aeration device must cost less than $100</td>
<td>Final Sparger Cost: $5.99 Other sources used</td>
</tr>
<tr>
<td>6. Aeration Device is Easy to Remove and Clean</td>
<td>Moderate</td>
<td>Aeration device can be removed within 5 minutes</td>
<td>Tested Time: 2min</td>
</tr>
<tr>
<td>7. Diffusion of Air is Optimal</td>
<td>Low</td>
<td>Air bubble size must not exceed 0.2 mm</td>
<td>Max bubble size: 10.6 mm</td>
</tr>
</tbody>
</table>

Validation Plans

As mentioned previously, these verification tests were run in an aerobic environment. Performing similar tests in an anaerobic environment or even in the final bioreactor will lead to further validation. Final implementation of the sparger into Rensiha’s system, with testing will confirm that the design works and is validated. Ideally, when testing a PID controller can be created and implemented as this is the last major step into a complete integration of the design into the final system which will conclude validation.

Discussion

For the overall project, our aim is to introduce the micro-aeration system into the original bioreactor. One of the largest challenges that had to be overcome was the time that was allotted to solve the problem. Because of the lack of time to understand the background of the project thoroughly and the lack of time to run tests, large assumptions were made such as assuming the aerobic system will correlate to an anaerobic system during the validation tests. If more time was allotted, more in depth testing would have occurred such as setting up an anaerobic system. The
lack of time also hindered the creation of a control mechanism as we are unable to test on a real anaerobic system to tune the control parameters.

In subsequent studies, we will try to test the micro-aeration system in an anaerobic bioreactor environment, paying special attention to oxygen levels. The time required to achieve the set target is used to further test the design parameters of the micro-aeration system so that it can better adapt to different anaerobic biological reaction systems. With more time and research resources in the future, we also plan to understand and test more different operating parameters, such as the impact of microbial population density in the bioreactor on oxygen saturation. In subsequent experiments, we plan to use DO probe for continuous oxygen monitoring and fluid dynamics software for computational simulation to adjust and analyze the function of the micro-aeration system in the anaerobic environment bioreactor, and test the system under the most basic testing conditions to find out some unrevealed problems.

Overall, the stone diffuser sparger is not the ideal choice to solve this solution. It may have the functionality to work in this scenario, but it lacks in its efficiency because price and time to get the sparger was prioritized when purchasing a sparger. Because our project changed drastically with only 4 weeks left before the design expo, design generation and concept selection were not as in depth as they needed to be for a specific micro-aeration subsystem. With more time, acquiring a more robust sparger such as a microbubble or nanobubble sparger could prove to be a better option as it is expected to reduce bubble size without compromising verification of other requirements. Then, redoing the validation tests in an anaerobic environment with an improved sparger will likely solve most of the expected issues.

At this stage of the design we are using commercially available spargers, which limits our experimental variables to a few sparger geometries and sizes of our choice. When analyzing the oxygen dissolution efficiency of the system, the insensitivity of the second experiment to position changes may be because the parameters of the distributor itself are not diverse, resulting in the selected results being insensitive to the position test. If there is an opportunity for redesign, we will use the optimal geometric and dimensional parameters derived from our experiments to tailor the sparger to the requirements of our bioreactor, thereby increasing micro aeration efficiency. If more resources are used, we also plan to use CAD software to simulate and calculate the final distributor design to verify the change in oxygen dissolution efficiency of the design itself.

A significant challenge we faced during our experiments was that our secondary experimental observations deviated from information we gathered in our literature survey, which showed that placing the sparger at the bottom of the bioreactor is the most efficient location for dissolved oxygen, however, our research results show that the position of the sparger does not significantly affect the dissolved oxygen level. This difference may make our final sparger position slightly different from the optimal dissolved oxygen position, resulting in sub-optimal efficiency. When faced with this discrepancy, we follow the results from the literature without support from experimental results because our experimental testing was done on a small scale reactor in aerobic conditions. In the future design and experiment process, combined with the customized design of the distributor, we can repeat the second experiment to test the position of the distributor to mitigate this risk. This kind of system testing will provide data for the design of the overall system to ensure that the location of the distributor is optimized to achieve the maximum efficiency of the final oxygen delivery of the distributor and avoid adding uncontrollable factors in the next step of anaerobic environment testing.
There are not any significant health risks associated with this project currently, but there can be in the future. The air supply to the sparger will likely come from a compressed air tank that will need to be properly stored and maintained so it will not explode. This can be done through a fairly standard procedure set by the EPA. Similarly, there are health risks when working with biological material. For example, all of the team needed to go through lab training to work in the Environmental Biotechnology Group’s lab. If proper training is given and standards are followed, these risks are also mitigated.

**Reflection**

Our design deals with an issue that is relevant to the whole world, energy and power demand. One of the significant issues society faces due to its growing population is overreliance on fossil fuels for energy. There are a finite amount of natural resources, and at current usage levels, these resources, like coal, oil, and natural gas, will be depleted in less than a century [36]. In addition, the extraction of fossil fuels through processes like mining or fracking can destroy ecosystems and increase pollution. Our project aims to provide a renewable energy resource that would allow humans to produce fuel even if the natural resources run out, which is essential for the progression of civilization. One way in which public health safety and welfare are impacted by our design is that the ultimate bioreactor’s byproduct is methane which is a natural gas that could be harmful to the environment if not handled properly. The team's project aims to increase methane production by implementing micro-aeration so that the methane can later be converted into hydrogen using pyrolysis technology. Green hydrogen is the desired end product because it does not produce harmful emissions when burned for fuel and there are no harmful extraction processes.

Since renewable natural gas from something that would normally go to waste is desirable by any society, our design is beneficial in a global marketplace. Increasing the efficiency of producing methane from food waste and sludge could provide a beneficial economic impact by repurposing waste into something that could be used as an energy source which would otherwise cost more money to acquire.

To characterize and organize the potential societal impacts of our project we used a stakeholder map. The stakeholders of our project can be compiled into six categories: resource providers, supporters and beneficiaries of the status quo, complementary organizations and allies, beneficiaries and customers, opponents and problem makers, and affected or influential bystanders. **Figure 12** is a visual representation of our stakeholder list organized in terms of primary, secondary, and tertiary stakeholders. Further discussion of stakeholders is done in the introduction.

One power dynamic that exists between Renisha and the team is the difference in academic background and experience. While Renisha’s background focuses on the biological aspects of the project, the team is primarily focused on the mechanical engineering aspects. Therefore, when disputes came up dealing with the biology and chemical aspects of our project, the team often felt less willing to suggest ideas in these areas. The opposite could have happened when dealing with mechanical engineering aspects of the project. Due to this, the team made sure we created and maintained a welcoming environment where everyone feels comfortable sharing their ideas so that we could get a variety of ideas and opinions to influence and improve our design.

Another important power dynamic was between our section instructor and sponsor, Steven Skerlos, and the team. Steven is the team's professor and section instructor, but he also
plays a role in leading the project team that we are sponsored by. This led to a conflict of interest because as a leader of the project team, Steven has done extensive research on the project topic, which led to potential biases when discussing things like a final solution. As our professor, Steven is supposed to provide unbiased advice and guidance, so his role as both our professor and project team leader could be contradictory. As a result of this, the team made sure that they were adequately challenging Steven’s perspective when receiving guidance. This ensured that the team considered different perspectives other than our sponsors, ultimately leading to a less biased and more robust design.

The last power dynamic within our team stemmed from three members being Seniors and one being a Junior. As the only Junior in the group, Anthony sometimes felt less open to share his ideas due to feeling intimidated or unvalued because of his age. He also sometimes believed that since he had less experience than the other team members, his ideas were less valid. The team was aware of these power dynamics and always kept a friendly environment that was welcoming to every and all ideas everyone had to offer. Creating an environment where all team members are sharing equally, led to greater diversity in thoughts and ideas, ultimately leading to a stronger team chemistry and solution.

When there were multiple different viewpoints between team members and our stakeholders, most notably when the viewpoints of our sponsor Renisha were involved, we used our best judgment when deciding how to proceed with the project. This often resulted in us taking the recommendations from Renisha because the ultimate goal of our project was to help her improve her system. For example when we were narrowing the scope of our project there was some debate over how we could best provide benefit to her system. Through discussions with Renisha we ultimately decided on focusing on incorporating the micro aeration system. We also often valued Renisha’s input and advice because she has much more experience and knowledge in the field, especially when it came to the biology and chemical background that is important to the project.

In our team, Allen, as a Chinese individual, has some cultural differences with most of the team members, but it is this cultural difference that also creates mutual complementation and collaboration in teamwork. Influenced by American culture, everyone enriched the multi-directional and comprehensive thinking in the overall team collaboration through active discussions and the courage to put forward opinions. Then for Allen, influenced by Chinese culture, he often spoke less in discussions and listened more. After everyone has expressed their opinions, he organizes and filters the opinions on his own, then relates his, so that the overall team discussion can eventually narrow down and stay on the focus. Such different team communication methods promote the efficiency of problem solving and the establishment of the final solution. These differences help us achieve a comprehensive and inclusive design philosophy.

One of the ethical dilemmas the team faced involved the manufacturing and production costs of the system. The product is highly beneficial for the global population because it provides a source of clean renewable energy. However, the cost of producing the bioreactor system is high which can price out impoverished communities. The team managed this dilemma by trying to use cheap and readily available materials while maintaining the system's integrity, enabling the team to maximize the communities that can realistically afford and implement the technology.

Most people are instilled with personal values and ethics that they maintain throughout their lives. Certain values like truthfulness, fidelity, integrity, and responsibility are crucial for maintaining strong relationships among peers, teachers, and employers. The University of
Michigan expects all members of its community to uphold certain professional standards, such as possessing personal integrity both as students and as professionals. Students must be honorable people to ensure safety, health, fairness, and the proper use of available resources in their undertakings. Members of the College of Engineering community are honorable and trustworthy persons [40]. All members of the team were committed to upholding these standards to provide a safe environment for the project to be completed. These values aligned with the team’s because each member was committed to doing good, honest work to create a solution to our problem. However, it is important to uphold your personal ethics, so understanding when an organization may be violating your ethics is critical. If a future employer does not respect the ethics one holds, then it may be worthwhile searching for a new employer.

Recommendations
Sparger Recommendations

The spargers used in testing were lower quality stone diffusers as opposed to industrial grade spargers typically used in a bioreactor setup. This was a result of the large purchasing expense of better quality spargers and timing issues of purchasing an industrial grade sparger. As a result, the spargers used during the verification and validation were unable to meet all of the specifications, in specific the bubble size specification. To achieve all of the specifications we will recommend a few industrial spargers that will produce bubbles smaller than 200 microns.

When looking to reduce the size of air bubbles, a key factor is reducing the size of the pores. Mott Corporation offers a wide variety of porous metal sparger tips for laboratory and pilot scale bioreactors and fermenters. With pore sizes ranging from 2 μm to 15 μm, the porous spargers offer flexibility to generate bubble sizes that are optimal for specific media, organism and mass transfer requirements. Sparger tips come with a M5 thread, 10-32 UNF thread, weld stubs, or an adapter kit with other common connection styles and sizes for flexible integration strategies. The sparger can be seen below in Figure 25.

Figure 25: Micro Spargers made by Mott.

Another producer of industrial micro spargers is Hengko. The micro spargers they make are made of 316L stainless steel and are available in pore sizes ranging from 1 μm to 15 μm. The spargers are able to produce bubbles 10-100 times larger than the pores of the sparger, which will result in bubbles less than 200 μm meeting the bubble size specification. The spargers are configured with a uniquely designed adapter to allow easy assembly to the mating sparger tip and easy removal for replacement after each batch. This eliminates the need to re-weld the tip or clean the entire assembly. The spargers are durable and corrosion-resistant, and the porosity of the media provides exceptional mass transfer efficiency throughout the tank. The spargers also
perform in high temperatures, and corrosive environments and can survive an almost unlimited number of sterilization cycles or can be discarded after each campaign. The sparger can be seen below in Figure 26.

![Figure 26: Micro Sparger made by Hengko.](image)

These two sparger systems are the best performing spargers in the industry and produce bubbles in the correct range to meet all of the specifications. To obtain the price of the spargers, a sales representative must be contacted or a request form must be filled out; contact information for both companies can be found in Appendix A.1 and A.2.

**Necessary Further Testing**

When purchasing a sparger, it is crucial to understand how different air flow rates will affect the DO concentration as this will impact how much oxygen will be present in the system. It is also important to understand how fast the system will aerate to specific DO levels from an anaerobic state to avoid any overshoot in DO value resulting from a time delay of the DO meter readings. Knowing this information will allow Renisha to control the aeration of her bioreactor in a precise and easy manner.

To determine the behavior of a sparger, initial testing must be done. Ideally, testing would be completed in a medium similar to that of the bioreactor, however water can be used to understand trends of the behavior. First, the DO levels must be recorded without any aeration to set a baseline measurement. Next, the sparger needs to be tested at multiple airflow rates to determine the average DO readings at each airflow rate. Since the DO probe is very sensitive, the readings often did not maintain a constant value and fluctuated heavily. Due to this, keeping track of the minimum and maximum DO values observed at a certain airflow may be useful to understand the variability of the sparger as well. This can help reduce error and ensure that DO levels are in the desired range.

Lastly, to understand the time aspect of the control system, the sparger must be tested in an anaerobic environment. To start, the DO value should be recorded without aeration to develop a baseline measurement. Next, the user must pick a few desired DO values that may be used in the actual bioreactor system. This is to ensure the PID control system is designed specifically for the chemical reaction taking place in the bioreactor, since different reactions require different levels of oxygen. Next, the user must choose airflow rates that are able to achieve the desired DO values based on the earlier testing of airflow rates. After finding airflow rates for each DO value,
Implementing PID Control

The basic idea behind a PID controller is to read a sensor, then compute the desired actuator output by calculating proportional, integral, and derivative responses and summing those three components to compute the output. In this case, the controller will monitor the DO or ORP probe and based on the desired range, will control the air pump used to supply oxygen to the system. By doing this, the user can simply control the system using a software like labview by inputting the desired parameters. This will allow the researchers using this PID system to easily control the airflow and oxygen concentration in the system.

Conclusion

Our goal was to implement a micro-aeration device into a bioreactor. After generating concepts and narrowing down through engineering analysis, it was decided to use a stone diffuser. Some benefits offered by the diffuser is that it is cost efficient, easy to obtain, and has the ability to deliver uniform ORP readings. Some notable drawbacks included the size of bubbles it creates as well as the lack of control to specific areas that need aeration. Testing the diffuser, it satisfied 6 out of 7 of the requirements, only not satisfying the requirement that attempts to optimize diffusion of air by controlling bubble volume. Our verification techniques involved two experiments where the majority of the requirements were validated, but these experiments used major assumptions such as claiming the aerobic testing conditions would emulate the anaerobic conditions in the real bioreactor. This is likely not the case and it is important that further testing goes into ensuring these are verified and validated in an anaerobic system. During these tests an open-loop control equation that relates DO to airflow was developed, but since this was for aerobic conditions, the validity needs to be tested further.

The future work of the project aims to fix the areas in the project where the solution lacks. Returning to a concept selection phase and selecting a different sparger such as a nanobubble sparger could potentially prove useful as these are more adept, although more costly, than the stone diffuser. Additionally, an experiment will also be conducted to attempt to create a closed loop control system under anaerobic conditions as this will be more convenient for the sponsor. Overall, the project would be considered a moderate success given the background the group comes from as well as the time the group had to accomplish this goal.

Acknowledgements

The team would like to thank the contributors who helped lead to the success of our project. We would first like to thank our sponsor, Renisha Karki who provided us with background information as well as a large amount of guidance with the direction of our project. We would also like to thank our section instructor, Steven Skerlos, for helping us work through the design process for our project and giving us feedback and direction. Lastly we would like to thank the Environmental Biotechnology Group which allowed us to use their space for our experimental testing.
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Paul J. Weimer, Mary Beth Hall, The potential for biomimetic application of rumination to bioreactor design, Biomass and Bioenergy, Volume 143, 2020, 105822


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“Heat Loss through Wall Equation and Calculator.” Engineers Edge - Engineering,


[56] Duc Nguyen, Zhuoying Wu, Shilva Shrestha, Po-Heng Lee, Lutgarde Raskin, Samir Kumar Khanal, Intermittent micro-aeration: New strategy to control volatile fatty acid accumulation in high organic loading anaerobic digestion, Water Research, Volume 166, 2019, 115080, ISSN 0043-1354,

Appendix A

A.1
Mott Corporation
Phone Number: 860.891.2652
Website for request form: https://mottcorp.com/product/spargers/micro-spargers-for-bioreactors/

A.2
Hengko
Phone Number: +86.755.88823250
Email: ka@hengko.com

A.3
Below in Figures 27, 28, and 29, the concept generation for the micro-aeration were concepts used in the concept generation phase are depicted.
Figure 27. Chris’s concepts for micro-aeration
Figure 28. Allen’s concepts for micro-aeration

Figure 29. Anthony’s concepts for micro-aeration
Build Design Bill of Materials

Below in Table 5 is the bill of materials with some associated costs and contact information.

Table 5: Bill of materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Source</th>
<th>Catalog Number</th>
<th>Cost</th>
<th>Contact</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro Sparger</td>
<td>1</td>
<td>Mort Corporation</td>
<td>1242439-01-020-H</td>
<td>Need to contact</td>
<td>860-864-4927</td>
<td></td>
</tr>
<tr>
<td>Stainless Steel 316 Micro Sparger</td>
<td>1</td>
<td>Hengko</td>
<td>N/A</td>
<td>Need to contact</td>
<td><a href="mailto:ka@hengko.com">ka@hengko.com</a></td>
<td></td>
</tr>
<tr>
<td>Pneumatic Tubing 32.8ft</td>
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<td>Quickun</td>
<td>1-3001-pu-4clear</td>
<td>$12.59</td>
<td>amazon.com</td>
<td></td>
</tr>
<tr>
<td>Air Compressor</td>
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<td>Husky</td>
<td>3320445</td>
<td>$219.00</td>
<td>1-888-HUSKY</td>
<td></td>
</tr>
<tr>
<td>DO Benchtop Meter and Probe</td>
<td>1</td>
<td>Thermo Fisher Scientific</td>
<td>STAR2135</td>
<td>$2,720.00</td>
<td><a href="mailto:customers@thermo.com">customers@thermo.com</a></td>
<td></td>
</tr>
<tr>
<td>ORP Probe</td>
<td>1</td>
<td>Atlas Scientific</td>
<td>#KIT-1030</td>
<td>$979.99</td>
<td>718-387-2075</td>
<td></td>
</tr>
</tbody>
</table>

Manufacturing/Fabrication Plan

The process of assembling the components of the bioreactor (as shown in the Figure 30 below) starts with the sparger, which is placed at the bottom with a long L-shaped air tube that introduces air to the sparger to ensure optimal gas distribution for the micro aeration process. The mixing brush is connected to the bottom of the reactor through a rod in the middle, and the motor fixed on the top provides power to the mixing mechanism to maintain uniformity of the mixture in the reactor. pH and ORP probes extend internally for continuous monitoring of the system's biochemical parameters. The NaOH tube is a pH adjustment component to achieve real-time control of the pH value inside the reactor. The sample tube provides ready delivery of experimental samples without the need to open the entire reactor each time to add. Finally, a pressure reliever is installed as a safety feature to maintain the system pressure at a certain level and prevent the biological reaction process from producing gasses that could overpressure the reactor.

Figure 30. CAD file for bioreactor