DESIGN EVALUATION OF AN ANAEROBIC CHAMBER PROTOTYPE

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biomedical Engineering

by

Anthony Michael Kendig

June 2009

© 2009
Anthony Michael Kendig
ALL RIGHTS RESERVED

COMMITTEE MEMBERSHIP

TITLE: Design Evaluation of an Anaerobic Chamber
Prototype

AUTHOR: Anthony Michael Kendig

DATE SUBMITTED: June 2009

COMMITTEE CHAIR: Lanny Griffin, Biomedical Engineering Department
Chair

COMMITTEE MEMBER: Dan Walsh, Senior Associate Dean of Academic
Programs and Administration

COMMITTEE MEMBER: Robert Crockett, Director General Engineering
Program

ABSTRACT

Design Evaluation of an Anaerobic Chamber Prototype

Anthony Michael Kendig

Within the field of microbiology there is a group of bacteria termed, anaerobic, because of their aversion to oxygen. Clinical and industrial interest in these bacteria has made the use of anaerobic systems necessary for widespread testing and research. The glove-less anaerobic chamber has become the premier method for successful cultivation of anaerobic isolates. A prototype anaerobic chamber design contains new features and different anaerobic techniques formed around additional specifications given by microbiologists. Through testing and discussion of the anaerobic systems of this prototype, the design is evaluated based on its anaerobic performance. The three chamber systems that maintain the anaerobic environment are: the circulation system, the passbox, and the operator access armports. The prototype's techniques for these three systems are compared to a previous prototype's. The new prototype successfully meets the microbiologist's specifications but in doing so has significantly changed the systems of the chamber. This evaluation has shown that this chamber's unique systems successfully maintain anaerobic conditions using a new set of systems.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
1.0 INTRODUCTION	1
1.1 HISTORICAL REVIEW	2
1.1.1 Anaerobic Niches	2
1.1.2 Dangers of Oxygen	3
1.1.3 Endogenous and Exogenous Anaerobes	6
1.1.4 Basic Anaerobic Technique	8
1.1.5 Older Anaerobic Culturing Techniques	10
1.1.5.1 Inoculating Roll Tubes	10
1.1.5.2 Anaerobic Jar Incubators	12
1.1.5.3 Flexible Glove Chambers	12
1.1.5.4 Glove-Less Chambers	14
2.0 OBJECTIVE	17
2.1 SCOPE	18
3.0 MATERIALS AND METHODS	20
4.0 RESULTS	23
4.1 PASSBOX DATA	23
4.2 ARMPORT DATA	26
4.3 WORKING AREA DATA	29
4.4 CIRCULATION DATA	31
5.0 DISCUSSION	34
5.1 ANAEROBIC SYSTEMS	34
5.2 USER REQUIRMENTS	35
5.3 CONSTRUCTION REQUIREMENTS	37
6.0 CONCLUSION	39
7.0 REFERENCES	40

LIST OF TABLES

Table	Page
1. Oxygen Radicals Created by the Addition of Electrons	4
2. The Four Incubation Levels of Oxygen	5
3. Bacteria Groupings Based on Oxygen Tolerance	6
4. Comparison of Oxygen-Reduction Potentials	6
5. Examples of Anaerobic Bacteria Found in the Human Bowel	7
6. Examples of Human Diseases Which Commonly Involve Anaerobes	9
7. Identification of the Oxygen Removing Element Palladium	10
8. Prototype A Passbox Data	23
9. Prototype B Passbox Data	24
10. Comparison of Passbox Systems	25
11. Prototype A Armport Data	27
12. Prototype B Armport Data	27
13. Prototype B Sleeve Purging Data	27
14. Comparison of Chamber User Entry Systems	28
15. Prototype A Working Area Data	30
16. Prototype B Working Area Data	30
17. Prototype A Circulation Data	32
18. Prototype B Circulation Data	32
19. Comparison of Oxygen Removal Systems	33

1.0 INTRODUCTION

The identification of an unknown bacterial specimen requires growth inhibition tests to compare the sample against known species previously established reactions. Species that die or are inhibited by exposure to oxygen are called anaerobic bacteria. These bacteria first gained importance in the clinical field when identified as the source of many infections; isolating and identifying any pathogenic anaerobe is an important test during infection treatment. Anaerobes have also been used industrially, producing different chemicals, or digesting waste into less toxic end products.

Bacteria's handling in the clinical field follows a three-step procedure: Inoculation, Incubation, Inspection. When dealing with anaerobes the more steps that can be conducted away from oxygen increases the recovery of anaerobic species. Recording growth patterns on different mediums combined with other growth response tests can effectively determine the species of bacteria in a sample. These techniques require a repetitive approach by inoculating and treating flights of agar plates to get growth characteristics from the bacteria. After inoculation the bacteria require several days to produce growth that can be inspected to determine the outcome of the tests.

Various apparatus have been invented to limit a bacterial sample's exposure to oxygen. The most advanced and successful at achieving anaerobic growth are anaerobic chambers. These create an entire workstation with a controlled atmosphere. Necessary to the construction of an anaerobic chamber are several key

features that achieve the basic functions of the chamber. In addition to these necessities, chambers include other options to improve the ease of use and comfort of the microbiologist or other chamber user.

As microbiologists use and become familiar with anaerobic chambers certain features are disliked or identified as in need of improvement. Taking several suggestions a team has designed a chamber with updated features. These additional options were designed to meet further user specifications. Following a review of anaerobic bacteriology and earlier anaerobic methods the additions made to the Prototype A will be highlighted and their impact on chamber function defined.

1.1 HISTORICAL REVIEW

1.1.1 Anaerobic Niches

Although they may have blanketed the world at one time, today anaerobes are confined to specific habitats on the planet; they are found in soil, waterway sediments, and the bodies of animals. Anaerobes normally found in animal bodies are called endogenous, all others are termed exogenous (1). Anaerobic bacteria have become important to humans for two different reasons, they are involved in many human diseases, and their unique metabolism can create or eliminate many substances oxygen-utilizing species cannot.

Clinical work is done to identify bacteria aiding in the diagnosis of an infection. Clinical anaerobic microbiology focuses on the isolation of species,

determining anaerobes from aerobes and identifying all the bacteria present from a sample. Here the concern is identifying the bacteria as soon as possible, and possibly doing additional susceptibility testing to help determine the proper antibiotic regiment for the patient.

In industrial research anaerobic bacteria have been used to make a variety of chemicals, clean up wastewater, and ferment substrates into energy sources (1). The choice of bacteria to investigate can be based on many different aspects including: what they consume, what they produce, reaction to drugs, and the production of certain enzymes. The effective use of any bacteria requires an extensive evaluation of growing conditions to optimize growth and production for the task. Both of these anaerobic microbiology fields benefit if the samples are kept under strict anaerobic conditions.

1.1.2 Dangers of Oxygen

A two part theory explains the damaging effects of oxygen on anaerobes as bacteriostatic and bacteriocidal (1). If an initial exposure to oxygen occurs the bacteria must slow their growth giving up more energy to the removal of oxygen from their system. If only a brief exposure the bacteria may survive to eliminate the oxygen and return to a faster growth rate. If too much oxygen has been introduced they succumb to the damage inflicted by the toxic oxygen derivatives.

The chemical reduction of oxygen produces dangerous products. By the addition of electrons oxygen becomes damaging molecules such as hydrogen

peroxide. Enzymes protect creatures that use oxygen for aerobic metabolism; they break down the superoxide anion and the other toxic spinoffs described in Table 1. The superoxide anion exhibits highly reactive behavior and will inflict damage on enzyme systems as well as cell structure. It also has the capability to bond with hydrogen and hydrogen peroxide to produce more hydroxyl radicals. The enzymes, catalase and superoxide dismutases, are used to eliminate these dangerous molecules from cells (1). By combining efforts these enzymes can turn superoxide anions into oxygen and water. Some anaerobes lack these enzymes, a partial reason why oxygen can be especially damaging to them.

Clinical microbiology uses a bacterial classification system based on the organism's relationship to oxygen and carbon dioxide. Four different environments are used for comparison of growth patterns, as exhibited in Table 2. Depending on the growth in these four environments an initial identification can be made into one of the groups in Table 3.

Table 1. Oxygen Radicals Created by the Addition of Electrons (1)

Number of Electrons Combined with Oxygen	Resulting Molecules
1 electron	(-O ₂) Superoxide Anion
2 electrons	(H ₂ O ₂) Hydrogen Peroxide
3 electrons	(H ₂ O + OH ⁻) Hydroxyl Radical
4 electrons	(H ₂ O) Water

From this basic group determination the continuing trials to totally identify the organism can be conducted in the proper environment for optimal growth. The obligate anaerobes have no tolerance for, and may be killed by, minimal exposure to elemental oxygen (O₂). Working on the bench top and then transferring the samples to a controlled environment incubator is often sufficient for the other groups, but not obligate anaerobes. This makes an anaerobic chamber vital to anyone trying to isolate these bacteria. Inside such a chamber all the normal microbiology techniques may be performed in a controlled environment away from oxygen.

Table 2. The Four Incubation Levels of Oxygen (1)

Percentage Oxygen	Incubation Environment
21%	Air
15%	CO ₂ Incubator
5%	Microaerophilic
0%	Anaerobic

A chemically low oxidation-reduction potential at the sight of growth is another important factor when attempting to grow anaerobes. This redox potential measures the tendency of the system to give up or accept electrons. Low redox conditions have been measured in the body at the site of bacterial growth. As shown in Table 4 anaerobic media often attempts to imitate this by adding reducing agents like cysteine and thioglycollate, creating a pre-reduced media (1).

Table 3. Bacteria Groupings Based on Oxygen Tolerance (1)

Oxygen Tolerance	Group of Bacteria
Highest	Obligate Aerobe
	Microaerophilic Aerobe
	Facultative Anaerobe
	Aerotolerant Anaerobe
	Microaerotolerant Anaerobe
Lowest	Obligate Anaerobe

Table 4. Comparison of Oxygen-Reduction Potentials (2)

Oxidation-Reduction Potentials	Location
-150mv to -250mv	Intestinal Tract, Colon
-150mv or lower	Sites of Infection
-170mv	Pre-reduced Media

1.1.3 Endogenous and Exogenous Anaerobes

Some anaerobes play a beneficial role in keeping our body healthy. Most of these are in the gastrointestinal tract and aid in the transport of nutrients into our system. Starches, sugars and glycosides are digested by these bacteria, some species ferment xylan; a hemicellulose found in vegetable matter that remains undigested by

mammalian enzymes (3). Table 5 lists some common species found in the human bowel.

Other parts of the body are inhabited by specific species: the skin, oral cavity, upper respiratory tract, urethra, vagina, and colon (1); identifying a species can aid in diagnosing the origins of an infection. This indigenous microflora normally do not cause us problems; however if they are allowed access to normally sterile body sites, such as the blood, or healthy tissue, they can become opportunistic pathogens (1). Most endogenous species are obligate anaerobes, the most difficult to grow and isolate outside of the host body (4).

Table 5. Examples of Anaerobic Bacteria Found in the Human Bowel (3)

Anaerobic Species
<i>Bifidobacterium bifidum</i>
<i>Fusobacterium symbiosum</i>
<i>Veillonella</i>
<i>Acidaminococcus fermentans</i>
<i>Fusobacterium necrophorum</i>
<i>Bacteroides fragilis</i>
<i>Peptostreptococcus productus</i>
<i>Ruminococcus bromii</i>

Some exogenous species find their way into human bodies with a range of impacts on the host. The most common of the exogenous anaerobes are *Clostridium*

species, found in soil. These bacteria or their spores may enter through the mouth. One problem they can create is antibiotic-associated diarrhea. This happens after a patient has had antibiotic treatment killing off much of the gastrointestinal tract microflora. *Clostridium difficile* can then move in and produce abundant amounts of toxin harming tissues (1).

Anaerobes can be involved in serious and fatal infections and intoxications in any part of the body. Isolation and identification of the bacteria present at the site of infection leads to effective treatment. Examples of infections and diseases commonly involving anaerobes are listed in Table 6.

1.1.4 Basic Anaerobic Technique

In order to successfully grow anaerobic bacteria three separate factors must be controlled: the gaseous atmosphere, the temperature of the environment, and the growth surface's contents and characteristics. All three must be tailored to the needs of the bacteria for optimal growth.

The anaerobic gas is usually a blend of nitrogen, carbon dioxide, and hydrogen, although other gasses may be used. Nitrogen because it is less expensive and inert, carbon dioxide is required by some species metabolism, and hydrogen is used primarily because it reacts with a palladium catalyst effectively removing trace amounts of oxygen from the atmosphere. Palladium is a metal element in the Platinum metals group its elemental information is in Table 7.

Table 6. Examples of Human Diseases Which Commonly Involve Anaerobes (1)

Brain abscess
Infectious Processes of the Eye
Sinusitis
Oral and Dental Infectious Processes
Complications of Vincent's Angina
Actinomycosis
Pleuropulmonary Infectious Processes
Endocarditis
Liver Abscess
Bacteremia
Peritonitis
Infectious Processes of the Female Genital Tract
Intraabdominal Infectious Process
Pseudomembranous Colitis
Antibiotic-Associated Diarrhea
Perineal and Perirectal Infectious Processes
Myonecrosis

The most common incubation temperature for anaerobes is thirty-seven degrees Celsius. To achieve this a temperature controlled incubator is used to store the samples when not being inoculated or inspected.

Table 7. Identification of the Oxygen Removing Element Palladium (6)

Palladium (Pd)	
Atomic Number	46
Atomic Weight	106.42

Anaerobic bacteria media is constructed to mimic conditions in the body for growth. Going beyond this more specialty medias are being produced that grow only certain species by matching their metabolic needs or antibiotic resistances. By streaking across a series of pre-reduced selective growth plates the family of bacteria can be identified in a short number of days for many groups (5).

1.1.5 Older Anaerobic culturing techniques

The anaerobic chamber is based on methods developed earlier for separately inoculating, incubating, and inspecting anaerobic bacteria. Earlier techniques involved many small anaerobic vessels handled on the bench top. Many of the technologies and methods of these older techniques have been improved upon for how anaerobes are currently handled in chambers.

1.1.5.1 Inoculating Roll Tubes

A prominent bench top procedure for recovering anaerobes was published in 1950 by Hungate. This technique and other similar alterations produced anaerobic

growth without an anaerobic chamber (7). An anaerobic gas mixture containing a mix of several of these gases: CO₂, N₂, H₂, argon; was directed into small vessels containing the media, or samples, when being used. For instance if mixing up a batch of media in a flask, it would be boiled, near the end of the boiling a nitrogen gas line would be inserted into the flask, creating a nitrogen purge forcing out any oxygen in the vessel. The vessel would be stopped up until fully mixed and ready for transfer to growth vessels for solidification using a pipette (7). The tubes used to store media and for growth tests had rubber stopper lids, creating an airtight seal. The rubber stoppers can either be penetrated with a needle delivering a sample or twisted off and recapped after delivery of the sample, again with anaerobic gas flowing into the open tube. This technique could produce anaerobic results, but did require a dexterous lab technician to maintain sterile and anaerobic conditions simultaneously.

Roll tubes were used with this technique to obtain isolated colonies in the small vessels. Well before inoculation molten agar media would be spun horizontally in a tube so that it solidified coating the round inner wall. An inoculation loop with the specimen was placed into the bottom of the tube; then it was drawn out while spinning the tube creating a spiral streaking effect, ideally delivering isolated colonies near the top of the tube. Colonies would have to be picked off the inside walls of the tube to be transferred to different medias. Flat plates are easier to test and investigate; roll tubes were limited to translucent medias so that colonies could be identified through the media.

1.1.5.2 Anaerobic Jar Incubators

Jars provide an anaerobic environment in which flat Petri plates may be incubated. These would be streaked on the bench top or in a chamber then locked into a jar. Commonly a vacuum pump or other device would remove the air from the jar and replace it with anaerobic gas. Various systems for removing oxygen and excess moisture were used inside the jars as well; desiccants for water absorption and catalysts for chemical oxygen removal. Jars can be used in conjunction with chambers for additional incubation space, transport, or as a double seal against oxygen. The major drawback when using jars without a chamber is the exposure to oxygen on the bench top, which may prevent some anaerobic growth. This can be minimized using similar techniques as with roll tube inoculation.

1.1.5.3 Flexible Glove Chambers

To overcome the weaknesses of the two popular and successful anaerobic bacteriology techniques, simple glove boxes began being used. The Hungate technique of small vessels kept anaerobic with a stream of gas on the bench top, limited the options of the microbiologist to roll tubes, which could be difficult to identify and pick colonies from. Additionally some tests call for serial dilutions, which adds up to a large number of tubes, complicating the managing of so many small sealed containers.

Using anaerobic jars to incubate streaked plates was improved ease of viewing and picking the colonies. However it lacked the ability to grow some strict anaerobes (8), even with active catalyst in the jars to remove trace oxygen. The problem was the initial inoculation carried out in air on the bench. This short exposure could be enough to inhibit growth.

Simple glove boxes were constructed of thin clear flexible vinyl plastic with Neoprene gloves, sealed all around except for a dock up port for an airlock (9). Inside this anaerobic tent, through the gloves many agar plates could be used to test anaerobic bacteria samples. Simple effective microbiology techniques could be carried out without the worry of oxygen intrusion. A catalyst and fan system: alumina pellets coated with palladium had chamber air blown over them chemically removing any oxygen in the chamber.

Simple flexible chambers were set up by first vacuuming out the air; and then refilling the chamber with a sterile anaerobic gas mixture with some hydrogen (10). The catalyst would scrub trace amounts of oxygen out in a few days. That gas would remain in the chamber for the duration of the experiments. A small amount is refreshed every time supplies are brought in through the airlock.

Flexible chambers laid out all the essential elements of a successful anaerobic chamber: positive pressure versus the atmosphere, palladium catalyst, circulation system, operator arm access, and a passbox. These elements are the basic and most important pieces of any anaerobic chamber. Improvements on anaerobic workstations after these focus on ease of use, reliability, and efficiency. The more

advanced solid chambers put all the elements into one simple to operate machine that is easy for the user to manage.

1.1.5.4 Glove-Less Chambers

Rigid chambers produced another advancement, a glove-less chamber. Instead of working through gloves, where the hands remain in the outside atmosphere, gloveless chambers have sleeves that seal around the forearm allowing the hands direct access to the inside of the chamber. This provides the user with much higher dexterity making the handling of many samples quicker and easier. However this design does create two large openings in the front of the chamber that have to be filled by two arms or something else at all times. Most chambers use a door that locks the sleeves off from the rest of the chamber. Closed off from the chamber the sleeves can be taken off and replaced, cleaned, or repaired. When getting into the chamber the user puts their arms into the sleeves and then cycles a system to remove the air in the sleeves around their arms and replace it with anaerobic gas before entering the chamber.

A whole spectrum of advanced rigid and flexible chambers allows the specific demands of any lab to be sufficiently met. All these chambers combine the basic elements described by these earlier techniques with new designs and a variety of incubator sizes. The basic elements of any chamber are the passbox, chamber circulation, and the armports.

All supplies, samples and tools are brought into the chamber through the passbox; this is a controlled compartment between the chamber and the outside atmosphere. It simply creates a buffer area so that the chamber atmosphere, and outside air are kept strictly separate. Supplies are loaded into the passbox from the outside. Then a cycle is triggered that will exchange the air for the anaerobic gas mix used in the chamber. After this all the supplies can be brought into the chamber proper without sacrificing the anaerobic atmosphere.

Even with an effective passbox and overall positive pressure versus the outside atmosphere, small amounts of oxygen will find their way into the chamber. The continuous removal of this damaging element is absolutely necessary for a successful anaerobic chamber. The tool used for this is a palladium catalyst. A thin coating of palladium on the outside of pellets gives the best surface area ratio of the expensive metal. Because of its chemical properties palladium will eliminate gaseous oxygen in the presence of hydrogen. Circulating the chamber atmosphere onto the palladium will allow this reaction to occur, removing trace oxygen in the gas mixture. This system removes oxygen as long as the hydrogen and oxygen can reach the palladium, over time other chemicals given off by growing bacteria will attach to the catalyst and inhibit its ability to contact oxygen. For this reason the catalyst needs to be routinely cleaned by heating it to nearly 200 degrees Celsius. This cleans off any debris allowing for optimal oxygen removal again. Anatox activated charcoal may be used as a preventative technique to absorb some of the chemicals given off by the bacteria before they attach to the catalyst (11).

With a passbox for bringing in supplies, and a circulation system to remove oxygen the main factor left is user access to the chamber. Sleeves attach to the front of the chamber surrounding the two access ports. At the end of the sleeve a stretchable cuff creates the seal around the user's arms. When in use the sleeves become inflated by the positive pressure of the chamber and are pushed and pulled in and out of the chamber by the operator's arms.

2.0 OBJECTIVE

In an effort to improve upon anaerobic chambers for clinical and industrial laboratories a team designed a new chamber, Prototype A. This chamber incorporates new features to a construction model identical to Prototype B chambers, an earlier design. Three areas for change were identified in the original chambers: the requirement of a vacuum pump, working area access restrictions, and the manual palladium catalyst replacement schedule. The purpose of this study is to evaluate the design changes that allowed the exclusion of a vacuum pump, the improvement of working area access, and an automatic catalyst regeneration system. The evaluation covers aspects of the systems individually and their interactions that affect the anaerobic chamber as a whole.

2.1 SCOPE

The Prototype A design meets its new specifications while remaining a viable anaerobic chamber. The replacement systems have a new set of challenges for the manufacture and use of the new design.

The removal of the vacuum pump required the passbox and sleeves of the chamber to operate in new ways. The vacuum pump was used to pull air out of the sleeves and passbox as the first part of a purging system to replace air with anaerobic gas. The passbox now uses positive pressure to purge out the oxygen. The sleeves must be left inflated at all times to eliminate the need to purge them before entry. These changes have increased the gas usage of the chamber as well as putting additional requirements on the two major sealing areas of the chamber, the passbox doors and sleeves.

To improve the user's comfort and maneuverability inside the chamber the armports were enlarged and the inside passbox door was converted to a sliding door which takes up less of the working area. The larger armports allow the user more flexibility to move their arms inside the chamber; an effect of this is an increase in sleeve size to fit the armports. Changing the inside passbox door to a sliding model required a new door seal design.

In order to relocate the catalyst into a cartridge that could be regenerated within the chamber the plumbing of the chamber was completely redesigned. A completely different system using pumps to move chamber gas through a plumbing network. Additional components were also added to control the flow of gas this led

to a more complex control system. Evaluating these effects of the design improvements is the purpose of this project.

3.0 MATERIALS AND METHODS

In order to describe and evaluate the design of the Prototype A anaerobic chamber it must be referenced to the Prototype B chambers, which were the basic construction blueprint and subject of the study identifying areas for improvement. Information about the Prototype B chambers was obtained from physical specimens, as well as basic information available in their operating manuals. Information about Prototype A was obtained in the same way.

Through simple tests of both of these chambers the changes made to the anaerobic systems can be examined through functional and physical data. The achievement of the three chamber operational changes required a redesign of all the major systems of the chamber. The major aspects of each system were documented in the following ways.

The removal of a vacuum pump changed the technique used to remove oxygen from the access areas of the chamber: the passbox and the armports. The method to evaluate this involves a look at how the new system functions as well as the changes to the parts and control of that system. Instead of pulling out the passbox air with the vacuum pump and then replacing that gas with an anaerobic mix, a more complex system was created that incorporates the addition of anaerobic gas with the use of palladium catalyst for a two pronged oxygen removal system. To eliminate the purging of the sleeves a simpler system leaves them inflated eliminating the purge altogether.

The passbox systems will be described and evaluated based on seven topics concerning their operation and construction: speed, speed consistency, oxygen removal, sealing requirements, gas usage, plumbing, and replacement parts. The speed and speed consistency were measured by timing the cycles running with an empty passbox. The oxygen removal is gauged by using an anaerobic indicator strip the speed of any color change indicates the presence and relative amount of oxygen. The sealing requirements are illustrated by measuring the working pressure during a passbox cycle. The gas usage will be determined by comparing the times that the gassing valves are activated during a cycle. The plumbing comparison is based on the number and nature of its parts. The replacement parts comparison is based on ease of removal and replacement of the gaskets that will receive most of the wear.

The impact of the armport system requiring that the sleeves remain attached and inflated is evaluated on eight factors: size/comfort, speed of entry, speed of exit, plumbing, gas usage, replacing/repairing sleeves, and strength of seal.

The size and comfort aspect is evaluated by comparing the size, shape and construction of the armports. Speed of entry and exit are timed for comparison as well as the differing techniques explained highlighting user controlled factors. The external plumbing that is required for the Prototype B chambers sleeve purging system is absent from Prototype A. Gas usage will be determined by timing the use of the gas addition valves. Replacing and repairing data concerns the operations of removing any one of the different parts of the sleeves for both systems, the steps involved and the chance of allowing oxygen into the chamber. Strength of seal is

determined with a close look at the two different sealing systems, their technique and what size leaks could occur.

The evaluation of the sliding inside passbox door is based on how wide of a sweep the swinging model has compared to the slider. Also considered are the sealing requirements brought about by the differently constructed door seals and unique passbox cycles. The vacuuming, and pressurizing, techniques provide very different stresses on the doors; this required a new design for the Prototype A passbox doors. Working area access is further exhibited by measuring common reaches necessary when working in the chamber.

The chamber circulation changes are evaluated on three factors: oxygen removal speed, regeneration technique, and plumbing. Each of these has several factors that influence each other and the overall statistics of the system. The quantity of catalyst pellets as well as the geometry of the cartridge were weighed and measured. The flow created by the circulation fan or circulation pumps are listed as well as measured circulation flow information. Requirements of the regeneration cycle are very different for the models and describing all that is involved for each. The plumbing is evaluated on number of parts, size, and complexity of control.

4.0 RESULTS

4.1 PASSBOX DATA

The technique developed to eliminate oxygen from the passbox in a cycle without a vacuum pump changed many aspects of the passbox. Quantitative data was obtained for all relevant factors and is accented with qualitative comparison of the methods in question. The operating data for both models is displayed in Tables 8 and 9. Table 10 lists seven comparison factors and summarizes that factor for each chamber.

Table 8. Prototype A Passbox Data

Feature Characteristic	Measured Value
Cycle Time	
Working Pressure of Cycle	
Gassing Valve Time	
Plumbing Components	

Prototype A has a consistent three and a half minute cycle that is timed within the unit. The gassing valve is on for the entire cycle, which uses gas to pressurize the passbox. Oxygen is vented out as well as scrubbed using a catalyst as part of the system. This two-part system excellently removes oxygen at a cost of using more anaerobic gas. The pressurizing technique also adds complications to the door seals.

First they must maintain a seal while being pushed away from the sealing surface by the positive pressure. Second if the inside door leaks, air from the passbox will be given access to the chamber damaging the anaerobic environment. A more complex control system is required to heat and monitor the catalyst cartridge during the cycle.

Table 9. Prototype B Passbox Data

Feature Characteristic	Measured Value
Passbox Time Average (3 Cycles)	
Average Gassing Valve Time per Cycle	
Average Vacuum Valve Time per Cycle	
Working Pressure of Cycle	
Plumbing Components	

The Prototype B chambers use a vacuum pump to pull air out of the passbox that is then replaced with an anaerobic gas. This system involves the repetition of this vacuuming and gassing procedure. The control is based on vacuum switches, they activate when certain levels of vacuum are reached. With differing circumstances such as: full or empty passbox, older vacuum pump, vacuum plumbing leaks, adjusted switches, these set-points can take longer or shorter to reach changing the overall time of the cycle. This technique is repeated three times can only remove an adequate percentage of oxygen; therefore some remainder will be introduced into the

Table 10. Comparison of Passbox Systems

Aspect of Passbox System	Prototype B	Prototype A
Speed	Time needed to vacuum passbox to 18 inHg three times	3 minute 30 seconds cycle
Speed consistency	Can vary because of vacuum pump and vacuum switch settings	Consistent, no external influences
Oxygen removal	Small amount introduced to chamber	Excellent, eliminates to equivalent level as present in the chamber
Sealing requirements	Vacuuming technique pulls glass doors down onto gaskets creating seal	Pressurizing technique creates additional stress on door seals, imperative that inside door not leak air into chamber
Gas usage	Minimal, only enough to refill passbox three times after vacuuming cycles	Gas valve from tank is open for entire cycle
Plumbing	Simple, one gas line, one vacuum line, and a gage. Vacuum plumbing requires check valve	More complex requiring institution of a catalyst cartridge
Replacement parts	Door Gaskets simple to install	Seal is attached to door

chamber. This technique is gas efficient, by pulling the air out instead of using additional gas to purge the passbox. The working pressure being in the vacuum range helps the passbox doors to create a good seal. Their design takes advantage of this by creating a floating smooth sealing surface that can take up variations and still make good contact with the thick gasket preventing air entry into the chamber. The plumbing is simple but is mostly vacuum lines, which are larger than the gas lines and must be connected to the vacuum switches as well as the vacuum pump outside the chamber.

4.2 ARMPORT DATA

The newly designed armports of the Prototype A were enlarged and the sleeves are left attached to them and inflated at all times. The enlarged oval armports are designed to allow the user easier access to the chamber working area. The removal of the vacuum pump eliminated the purging mechanism that allowed the sleeves to be locked off from the chamber by armport doors, therefore they are left inflated with plugs at the end in the cuffs. The size measurements for the chambers' armports are in Table 11 and Table 12. Prototype B sleeve purging data is in Table 13. A summary of the two chambers armport systems is available in Table 14.

Prototype A's chamber entry system is simple and allows quick access. Along with the speed and ease of entering the chamber this system uses the chamber's positive pressure effectively to prevent oxygen entry. This system does sacrifice the solid mechanical seal provided by armport doors. Another issue with

lacking armport doors is the inability to remove a sleeve and repair or replace any part of it. This can be important as the sleeves and cuffs flex continually with the users arms and are a common area of wear on any chamber. The strength of seal depends highly on the cuffs and the users placement of the plug in that cuff.

Table 11. Prototype A Armport Data

Armport Shape	Oval
Armport Cutout Dimensions	
Height	
Width	

Table 12. Prototype B Armport Data

Armport Shape	Circular
Armport Cutout Diameter	

Table 13. Prototype B Sleeve Purging Data

Total Entry Time (3 Cycles)	
Average Vacuum Valve Time of Cycle	
Average Gassing Valve Time of Cycle	

Table 14. Comparison of Chamber User Entry Systems

Aspect of Chamber Entry System	Prototype B	Prototype A
Size/comfort	Smaller round ports that have grooves for the doors to lock into	Large oval ports with smooth sides
Speed entry	Slow, must vacuum and gas sleeves at least three times, takes over one minute	Very fast, just push plugs inside and follow with arms
Speed exit	Quick, just lock doors into place and pull arms out	Quick, pull arms out with plugs in hands to plug cuffs as arms are released
Plumbing	Requires foot pedals with vacuum and gas plumbing	No additional plumbing
Gas usage	Uses enough gas to fill sleeves three times	Once familiar with system can get into chamber without using any gas, requires enough gas to fill sleeves on exit.
Replacing/repairing	Locking doors allow sleeves to be removed easily and safely when user is not in chamber	Difficult to replace sleeves as they are left as part of chamber when not in use
Strength of seal	Very strong well sealing doors effectively lock off chamber	Cuff plugs effectively close off chamber but do not make a mechanical seal
Oxygen entry	Minimal after vacuuming cycles	Positive pressure prevents it as long as the plugs are replaced quickly with arms and a good seal is made.

The Prototype B chamber entry system can be restrictive to the user but provides a solid sealing system that has an effective purging technique to prevent air entry. This more complex system requires external plumbing controlled by foot pedals. Requiring a purge before entry lengthens the time spent accessing the chamber. The armport doors create a good seal and in the event that one is left loose or does not create a perfect seal the leak will be small as the locking bar must be rotated a full ninety degrees to allow the door to fall back into the chamber to create a large opening. Replacing and repairing sleeves is very easy as they are cut off from the chamber except when in use so may easily be removed for maintenance.

4.3 WORKING AREA DATA

Common reaching distances inside the Prototype A are listed in Table 15, Table 16 has this information for Prototype B. Reaching distances in both the chambers were measured from the center of the bottom rim of the closest armport, in a straight line terminating at the listed chamber component.

Creating a sliding door moved the latch to the rear of the door in the Prototype A. Separate from the latch a handle in the front is used to pull the door open or closed. When closed this handle is in the extreme front of the left wall of the chamber, when open it is underneath the latch near the middle of the left wall.

The swinging door of the Prototype B passbox latches in the front of the left wall of the chamber. It swings outward in the center to a right angle with the wall.

Table 15. Prototype A Working Area Data

Chamber Component	Reaching Distance
Passbox Door Latch	
Passbox Door Handle Closed	
Passbox Door Handle Open	
Front of Incubator	
Rear of Incubator	
Electrical Outlet	
Rear of Chamber	

Table 16. Prototype B Working Area Data

Chamber Component	Reaching Distance
Door Handle Closed	
Door Handle Open	
Front of Incubator	
Rear of Incubator	
Electrical Outlet	
Rear of Chamber	
Catalyst Basket Handle	

The catalyst basket is placed above this door. The actual basket sits in a mount in front of the circulation fan in the top rear corner of the left wall. A handle extends

out of this basket along the wall and then is bent down at the end; the handle is pressed into a spring-loaded clamp to hold the cartridge in place.

4.4 CIRCULATION DATA

Chamber circulation is the system that exposes chamber gas to the palladium catalyst, achieving the desired effect of removing trace amounts of oxygen from the atmosphere. To analyze the effect of changing the circulation system to allow a self-regenerating catalyst the following data, Table 17 and Table 18, was obtained for each of the chamber models. The relevant factors include: the amount of catalyst, the geometry of the catalyst cartridge and the characteristics of the flow onto the catalyst. Following this data is Table 19, which summarizes the circulation system differences of the compared chambers.

In order to automatically and safely heat the catalyst for regeneration it had to be moved to the control compartment where it could easily be cut off from the chamber atmosphere. First pumps had to be used to move chamber gas into the control compartment. This flow must travel through tubing and then through cutoff valves that separate the catalyst from the chamber for regeneration. This circulation flow passes through a string of components. The addition of the necessary valves and heating elements of the catalyst cartridge created more control requirements, a more complex program and additional relays and sensors to monitor and control the catalyst.

Table 17. Prototype A Circulation Data

Catalyst in Circulation System	
Shape of catalyst cartridge	Cylindrical
Flow	Gas pumped through length of cylinder
Dimensions of cartridge	
Length	
Inner Diameter	
Flow rating of pumps	
Measured flow through system	
Chamber volume	
Time required to pump entire chamber volume through catalyst (chamber volume / flow rate)	

Table 18. Prototype B Circulation Data

Catalyst in Circulation System	
Shape of catalyst cartridge	Square Box
Flow	Fan blows chamber gas onto catalyst basket
Dimensions of Cartridge	
Length / Width	
Length / Width of Opening	
Depth	
Flow rating of fan	
Measured flow through system	
Chamber volume	
Time required to pump entire chamber volume through catalyst (chamber volume / flow rate)	

Prototype B's circulation is both simple and effective. It does place one large responsibility on the user, the regeneration of the catalyst basket, this must be

exchanged daily with a freshly regenerated one. The high output fan blowing chamber gas directly onto the catalyst basket quickly removes trace oxygen. To prevent needing additional circulation equipment the condensate plate is behind the fan where it will experience some flow. The gas addition port is also behind the fan delivering hydrogen near the catalyst. The simple system introduces very little restriction creating an effective oxygen removal system.

Table 19. Comparison of Oxygen Removal Systems

Circulation Aspect	Prototype B	Prototype A
Oxygen removal speed	Very quick because of high flow fan directly onto large catalyst basket	Slower because of use of pumps instead of fan
Regeneration	Done outside chamber with oven, user must swap catalyst baskets daily	Internally regenerated twice-daily keeping the catalyst very clean.
Plumbing	Very simple	Complex

5.0 DISCUSSION

The data in the results sections illustrates the changes in function using the physical data of the system. To further evaluate the merits of the Prototype A design, additional characteristics and interactions need to be examined: beginning with an explanation of how the different aspects of the chamber: passbox, armports, circulation, work together to maintain an anaerobic environment; secondly an evaluation of the requirements this design places on the chamber user; lastly the design's construction requirements, and component assembly are evaluated.

5.1 ANAEROBIC SYSTEMS

Prototype A's automated catalyst regeneration cycle required circulation pumps instead of a fan. This can permit oxygen that has gotten in through the passbox or sleeves to remain in the chamber longer before being pumped through the palladium. However the efficient passbox cycle of Prototype A prevents any noticeable amount of oxygen from entering the chamber.

The larger sleeves of Prototype A would also have been a potential area for oxygen intrusion, however by leaving them inflated as part of the chamber atmosphere they remain anaerobic at all times. In this way the circulation system may function at a slower rate without allowing intruding trace oxygen to damage growing conditions.

The Prototype B chambers have a highly effective circulation system that will quickly eliminate any oxygen let into the chamber; therefore using the vacuuming technique in both passbox and sleeves is viable. Once inside the main chamber the catalyst quickly eliminates the minor amount of oxygen left over from those systems. This system is more forgiving of oxygen intrusion.

5.2 USER REQUIREMENTS

The regeneration of the catalyst basket was the major factor left up to the user in the Prototype B chamber system. It required an appropriate temperature external oven and daily replacement by the user. The automation of this process in the Prototype A removed the user from any tasks the chamber required to make itself anaerobic.

In order to improve the user's ability to reach all the components in the chamber working area, these reaches were not reduced in the Prototype A design. Instead the armports were enlarged which facilitates the user comfortably reaching more of their arm inside the chamber to make the required reaches. This was a simple technique that prevents closing in the working area, by opening up the user's access ports instead.

One area that the system of the Prototype A can add cost is gas usage. The passbox uses more gas. The catalyst cleaning cycles also use a small amount of gas to purge the catalyst plumbing before returning circulation through it.

The main place where the demands on the user have increased are the sleeves. Leaving the sleeves inflated is necessary without a vacuum pump to purge them, this has created an area of wear the user must manage.

When the user breaks the seal created by the cuff plugs the chamber immediately expels anaerobic gas because of its positive pressure, an arm must be quickly inserted and sealed against the cuff. If not quickly plugged with an arm the sleeve will deflate. Entering the chamber should be done one arm at a time so that the other hand can position the cuff on the arm making the proper seal. Exiting the chamber is somewhat easier. Both arms may exit simultaneously by pulling the plugs into the cuffs with the hands. If a mistake occurs the chamber must simply be purged for a period of time to accelerate the removal of oxygen. A small amount of training and experience is necessary for any user to efficiently manage these tasks.

Another complication of leaving the sleeves attached and inflated at all times is replacing any part of the sleeve system, sleeve, ring, cuff. Without an armport door separating the sleeve from the chamber the options in these cases are limited. One: remove any samples from the chamber, allow it to become aerobic and repair the sleeve then. Two: attempt to replace the sleeve part with the chamber still running. In this case if a cuff or ring at the end of the sleeve is broken it would be possible and not too difficult. Simply pinch off the sleeve shorter with a clip or rubber band and then replace the piece on the end quickly.

By providing an armport door with the chamber, necessary repairs can be completed without making the chamber aerobic. Not to be used everyday, but if a sleeve needs repair a door could block up the armport and facilitate removal of a

sleeve while leaving the chamber anaerobic. After returning the sleeve to the chamber a careful purge by opening the door slightly could effectively purge the repaired sleeve before removal of the door. This simple addition would effectively allow repairing of any sleeve problem without allowing oxygen into the chamber.

Prototype A's sleeve system is very convenient for the user as far as accessing the chamber, this design does rely more on the user to create the airtight seal and maintain the sleeves in proper working condition.

5.3 CONSTRUCTION REQUIREMENTS

The construction requirements of Prototype A are largely the same as its predecessors. There are three areas that require additional specifications and more elaborate control systems. These are the passbox doors, the circulation pumps, and the circulation plumbing system.

During Prototype A's passbox cycle the addition of gas pressurizes the passbox slightly. The danger here is that the passbox will leak air into the chamber. For this reason the doors are sturdy, solid plastic with a compressible gasket attached to the door to be pressed against the chamber walls. These doors require a smooth and flat sealing surface along the walls surrounding the passbox openings. This has added requirements to the cabinet construction. Slight variances occurring from the welding of the cabinet must be minimized. The application of the inside sliding door complicates these sealing issues as it required a new design for the sliding door clamping system.

In the top right corner of the working area the circulation pumps are mounted on a shelf next to the incubator. More so than the fan circulation in the Prototype B chambers the pumps give off vibration. Normally this can be corrected with a solid mounting system, but is another area that requires additional consideration in the chamber design.

The automation of the catalyst regeneration has the largest impact on the chamber. More valves and components are required to manage this gas flow. The chamber now functions in one of two modes at all times, regular operation dominates for twenty-two hours a day, for two hours the chamber is in a cleaning cycle. These dual modes prevent any component from being hard wired in the on position; everything must be turned on or off depending on the chamber mode. This required a larger controller with more outputs to control the additional valves and other components. A more complex program is needed to run this system in both modes, especially the monitoring of the catalyst heating during regeneration cycles. This all adds in cost of parts, complexity of construction and assembly, while creating more testing and validation requirements.

6.0 CONCLUSION

The ideal anaerobic chamber would blend optimal growing conditions with ease of access and comfort for the microbiologist. Optimal growing conditions require an anaerobic environment, an incubator, and suitable growth media. The oxygen content of the chamber depends on how much oxygen the passbox, and the armports let into the chamber, and how fast the circulation system removes it.

The three goals of the Prototype A design project, removing the vacuum pump, improving user access to the working area, and internally regenerating the catalyst, have been successfully implemented. Each has altered how the chamber functions, removing the vacuum pump required that the sleeves be left inflated, and created a passbox cycle that used more gas. Improving working area access enlarged the sleeves and instituted a sliding passbox door that requires a precise sealing surface. The internal regeneration of the catalyst required pumps to force gas through the plumbing network.

These new systems work together well to create and maintain an anaerobic environment. The passbox and sleeves systems assist the circulation system by preventing oxygen entry. In this way the efficiency of one system makes up for the limitations of another. The main concern with the Prototype A as with all anaerobic chambers, is recovery from a sizeable oxygen intrusion. Proper training and a diligent microbiology user is what are needed to prevent that issue.

7.0 REFERENCES.

1. Engelkirk, Paul G., Janet Duben-Engelkirk, and V.R. Dowell Jr., Principles and Practice of clinical anaerobic bacteriology. Belmont, CA: Star P, 1992
2. Holderman, Lillian V., and W. E. C. Moore. "Roll-tube technique for anaerobic bacteria." American Journal of Clinical Nutrition 25 (December 1972): 1314-1317
3. Bryant, M. P. "Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract." American Journal of Clinical Nutrition 27 (November 1974): 1313-1319.
4. Moore W.E.C., and Lillian V. Holdeman. "Identification of anaerobic bacteria." American journal of clinical nutrition 25 (December 1972): 1306-1313
5. Jousimies-Somer, Hannele, et al. Wadsworth-KTL Anaerobic Bacteriology Manual: Sixth Edition. Belmont, CA: Star P, 2002
6. Bailey, Philip S. Jr., Christina A. Bailey, Organic Chemistry Sixth Edition. Upper Saddle River, NJ: Prentice Hall, 2000
7. Bryant, M. P. "Commentary on the Hungate technique for culture of anaerobic bacteria." American Journal of Clinical Nutrition 25 (December 1972): 1324-1328
8. Aranki, Alexander, and Rolf Freter. "Use of anaerobic glove boxes for the cultivation of strictly anaerobic bacteria." American journal of Clinical nutrition 25 (December 1972): 1329-1334.
9. Aranki, Alexander, et al. "Isolation of Anaerobic Bacteria from Human Gingiva and Mouse Cecum by means of a simplified glove box procedure." Applied Microbiology 17 (April 1969): 568-576
10. Dowell, V.R. Jr. "Comparison of techniques for isolation and identification of anaerobic bacteria" American journal of clinical nutrition 25 (December 1972): 1335-1343
11. Brazier, JS. "appraisal of anotox, a new anaerobic atmospheric detoxifying agent for use in anaerobic cabinets." Journal of Clinical Pathology 35 (1982): 233-238