An Updated Gas Distribution System for Cultivating Anaerobes

Seth Yoder

July 22, 2022

Abstract

Start-up company Buddy Enginner, formed by two working microbiologists, developed a new tool for cultivating anaerobic organisms. It is a gas mixing manifold has the ability to combine several gas sources at once, passes them through a high-quality oxygen scrubber, and distributes the mixed and treated gas through multiple output nozzles. This gas can then be used to sparge media or flush head space. The components are mounted to a compact and lightweight frame suitable for quick installation or transport to another laboratory.

1 Introduction

Anerobic microorganisms are becoming a hot topic of study in many industries and in many areas of clinical research. The gut microbiome, which is largely anaerobic, is a topic of interest due to its health effects.(1) The petroleum industry is interested in anaerobes to increase energy extraction.(2) Anerobic digestate can also play a role in producing organic fertilizers, wastewater treatment, among other uses.(3)

With this increasing interest in anaerobic organisms, an improvement in tools and techniques for cultivating these organisms is needed, particularly considering that cultivating anaerobic organisms is much more difficult than aerobic organisms. One of the aspects of cultivation that makes anaerobic microorganisms challenging to grow is that the growth medium that is used (such as a nutrient broth) needs to be have all of the oxygen removed and replaced with gases such as nitrogen or hydrogen.

Historically, this has been accomplished by reading previously published guides on anaerobic cell culture, purchasing individual components, and constructing your own gassing station.(4,5) However, we have developed a model gassing station that requires very little assembly and can work with most laboratory environments to cultivate a variety of microorganisms. This option of a near turnkey gassing station could prove to save laboratories time and effort from setting up their own from scratch.

2 Design

Following the flow of the gas through the manifold is as follows:

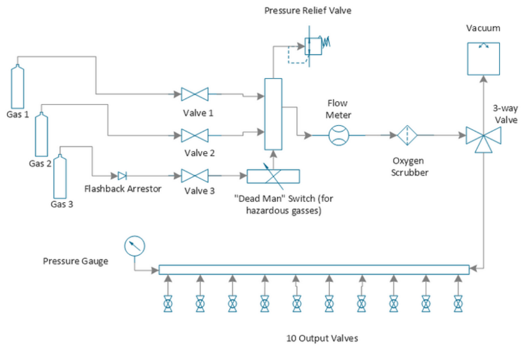

Figure 1: A simple drawing of the gas flow through the manifold

1. Gas enters the GDS (gas distribution system) through one or more input valves.
    (a) Typically, this would include nitrogen, carbon dioxide, hydrogen, or a mixture of gases.
    (b) Some GDS models include a "deadman switch" where hydrogen or another potentially flammable gas is used. This is a solenoid switch that actuates a valve for a specific period of time, such as 20 minutes, that will close the valve to the flammable gas after the programmed time period has elapsed. This is to reduce the risk of excess harmful gasses in the laboratory environment should valves be accidentally left in the open position.
    (c) Other models also have a flashback arrestor installed at this point for safety purposes.
2. The gas flows through tubing and into an aluminum or brass manifold where the gasses are mixed.
3. The mixed gas then flows through a flowmeter and into an oxygen scrubber (Vici Metronics, Poulsbo, WA, USA) to remove oxygen contamination and enhance gas purity.
4. For the organisms that are very strict anaerobes, such as methanogenic bacteria, a vacuum assembly that includes a three way valve and pressure gauge is installed at this point to help purge oxygen from the media.
5. Gas is then distributed via several needle valves with phenolic knobs (Swagelok, Solon, Ohio, USA). To these valves can be added additional material to help facilitate distribution, such as flexible tubing, Luer fittings, sterile 0.2 um membrane filters, and needles. In this way, multiple media receptacles can be sparged simultaneously.

The entire assembly is compactly assembled and mounted onto a lightweight aluminum frame. The framework is composed of rectangular aluminum tubing that is two feet tall and nearly three feet wide. The construction allows for easy mounting and dismounting to laboratory benchtops, as well as simply relocation to another laboratory.

3 Methods

Once the manifold is installed and flexible tubing is attached to the valves, the manifold can be used to prepare anoxic media for cultivating anaerobic organisms. One technique used in the authors' lab was the following:

1. Preparation of growth media that includes the redox indicator resazurin and cysteine to aid in media oxygen reduction as described by Fukushima and colleagues.(6)

2. Sterilize the media following preparation.

3. Place media into a 70 degrees C water bath to aid in the removal of dissolved oxygen.

4. Attach sterile Tuberculin syringes (without plunger) to the flexible tubing affixed to the output of each valve.

5. Place a disposable 0.2 um sterile membrane filter at the end of the syringe.

6. Attach another section of sterile tubing to the membrane filter and place aseptically into media container

7. Open output valves and the anoxic gas mixture will bubble through the media.

In a short time the rezasurin indicator will become colorless signifying a hypoxic environment. Multiple valves allow the sparging of multiple media vessels concurrently.

4 Conclusion

When cultivating anaerobic microorganisms, it is necessary to remove as much oxygen from the cultivation media as possible and replace it with a gas mixture that is conducive to the growth of organisms of study. A compact gassing manifold with most of the necessary components located on one panel to achieve this gas replacement is ideal in a laboratory environment. This type of compact gassing station can make cultivation of anaerobes less burdensome and more efficient.

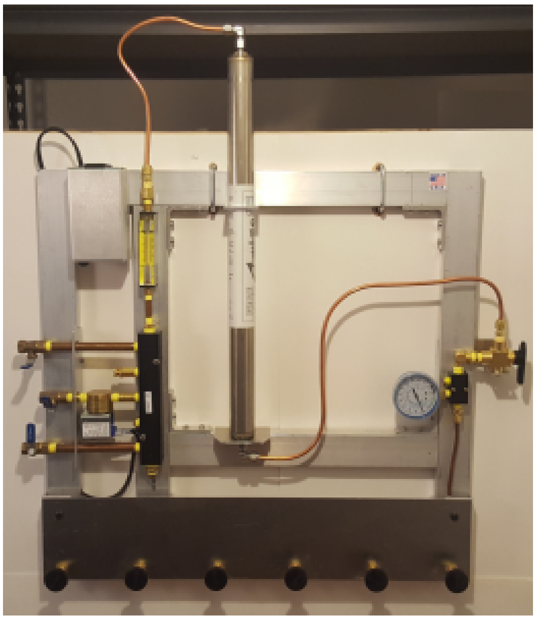

Figure 2: Example of the product customized for a university client

5 References

1. Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. Gastroenterology [Internet]. 2014 May [cited 2014 Jul 18];146(6):1564–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24503132 2.

2. de Rezende JR, Oldenburg TBP, Korin T, Richardson WDL, Fustic M, Aitken CM, et al. Anaerobic microbial communities and their potential for bioenergy production in heavily biodegraded petroleum reservoirs. Environ Microbiol. 2020;22(8):3049–65.

3. Anukam A, Mohammadi A, Naqvi M, Granström K. A Review of the Chemistry of Anaerobic Digestion: Methods of Accelerating and Optimizing Process Efficiency. Processes [Internet]. 2019 Aug 2;7(8):504. Available from: https://www.mdpi.com/2227-9717/7/8/504

4. Speers AM, Cologgi DL, Reguera G. Anaerobic cell culture. Curr Protoc Microbiol [Internet]. 2009 Feb;Appendix 4(February):Appendix 4F. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19235145

5. Wolfe RS. Techniques for cultivating methanogens. In: Methods in Enzymology [Internet]. 1st ed. Elsevier Inc.; 2011. p. 1–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21402207

6. Fukushima RS, Weimer PJ, Kunz DA. Photocatalytic interaction of resazurin N-Oxide with cysteine optimizes preparation of anaerobic culture media. Anaerobe. 2002;8(1):29–34.