Product Test Procedure for E. Coli Reduction

HELIX FILTER

P/N: 10023-1-000

Revision F

Fabco Industries, Inc.
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Company: **Fabco Industries, Inc.**

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Agent
Title

Ana Rodríguez-Koster
Signature

12/9/14
Date

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GOOD LABORATORY PRACTICE STATEMENT

This study was not conducted in accordance with the Good Laboratory Practice (GLP) requirements set forth by 40 CFR Part 160. Although the study was not conducted in compliance with the GLP regulations, the design, performance and study conclusions are scientifically valid and accurate. Consequently, the Agency should consider this study to be reliable for decision-making purposes.

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Date: 12/9/14

Sponsor: _____

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1 Scope:

In general, this document defines the equipment, setup and procedures to be used during efficacy testing of the Fabco bacteria reducing helical filter configuration; otherwise known as the “Helix Filter”.

2 Purpose:

The purpose of this test procedure is to take a given bacteria concentration and first; verify the helix filter technology as a bacteria reduction device; second to develop performance curves based on varying flow rates; third and lastly to determine the static pressure head characteristics of the helical filters.

3 Objective:

Through third party testing, our main objective is to record, analyze and plot bacteria reduction data under at least three (3) different flow rates while using two (2) different microbial species namely, E. Coli and Enterococci, each seeded at a target concentration. This document will focus on E. Coli only.

4 References:

- Fabco Industries drawing number 10023-1-000, Helical Filter segment for bacteria reduction
- EPA 40 CFR Part 136
- Environmental Protection Agency (EPA), Tank Sampling SOP#: 2010 REV#: 0.0
- Industrial Stormwater Monitoring and Sampling Guide, March 2009, Doc#: EPA 832-B-09-003
- NPDES Stormwater Sampling Guidance Document, July 1992, Doc#: EPA 833-B-92-001

5 Test Facility and Equipment:

Testing shall be performed at the Fabco Industries facility located in Farmingdale NY. A project log book shall be designated and used to record all notes, testing, ambient conditions and observations throughout the test.

The following test equipment shall be used:

Description	Quantity
Supply Tank, 5000 Gallons	1
30 Gallon Open Head Graduated calibration Drum	1
Centrifugal Pump, 200 gpm @ 10-ft	1
Nyloplast Structure, Modified w/Staff Gauge	1
15-in Corrugated (ADS N-12) Polypropylene Pipe	1
Treated 15” Helical Filter Segments	5
Stop Watch	1
Butterfly Valve	2
Flow Meter w/Totalizer	1
Equipment Support Rack	1
Chlorine/pH test kit/Dechlorinator	1
Ice Packs/Coolers	As Needed

Note: Test modifications and equipment substitutions can be made only with permission from Fabco Industries and shall be duly noted in the project log book.

6 General Test Information

6.1 Responsibility:

Fabco Industries shall be responsible for all tests specified herein.

6.2 Laboratory Certification:

Unless otherwise specified or approved by Fabco, samples shall only be analyzed by a laboratory that is accredited in accordance with the National Environmental Laboratory Accreditation Conference (NELAC).

6.3 Ambient Test Conditions:

All tests specified herein shall be performed under the following conditions.

- Temperature: Above 40 ^{o}F (4 ^{o}C)
- Precipitation: N/A (indoor test)
- Humidity: < 85%

The average ambient test conditions shall be measured and recorded at the start of each days testing.

6.4 Test Fluid:

- Calibration: Dechlorinated city water.
- Concentrated Influent: Dechlorinated city water seeded with E. coli as specified.
- Chlorine Level: 0 PPM
- Water Temperature: Above 40 ^{o}F (4 ^{o}C)
- Water pH: 6 < X < 8

6.5 Witness Hold Point:

A Fabco representative shall be notified in advance and must approve any testing that includes any and all of the test equipment specified herein.

7 Acceptance Testing

Construct the test setup as illustrated in Figure 1 (see attached). The test setup shall be constructed using the materials and equipment supplied by Fabco Industries unless otherwise approved by Fabco or the designated test supervisor. Prior to testing, verify that the supply tank and the Nyloplast Structure are plumb and level and that the system is completely watertight. All electrical outlets used for the testing specified herein must have ground fault protection.

7.1 Test Runs

Table 1

Target Influent Concentration	50-GPM	10-GPM	40-GPM	100-GPM
E. Coli @ 350,000 MPN/100mL	Influent Sample (Raw)	Test 1	Test 2	Test 3

Note: A total of 20 discrete samples shall be required to complete the testing outlined in this document. Before beginning this test procedure, verify that sufficient sample bottles, ice packs and coolers are clean and readily available.

7.2 Sampling Methods

Since the seeded influent concentration is fed from a thoroughly (and continuously) mixed supply tank, composite sampling of the effluent is not necessary. Grab sampling of the effluent from the discharge port of the Nyloplast Structure accurately represents a composite of the seeded concentration in the supply tank. The following step-by-step procedures can be modified as necessary to suit site conditions or limitations with approval of the testing supervisor. Any deviation from this procedure shall be noted in the test log book and in the final test report. Each sample shall be clearly labeled and fill a 100 ml glass or plastic jar and be preserved with ice packs in a covered cooler. After completing the planned test runs, the samples shall be delivered under chain of custody to an approved laboratory for analysis. The hold-time on the

samples must not exceed 4 hours from when the samples are taken to when the samples are inoculated by the laboratory.

Test Precautions:

Proper eye protection and disposable powder-free gloves shall be worn when collecting samples. The sample bottles and lids shall be sterilized. To prevent contamination, never touch the inside of the lid or bottle.

7.2.1 Supply Tank (Influent) Sampling, Grab

Untreated samples from the supply tank shall be collected using a standard manual grab sampling technique. Before taking the first sample, verify that all the sampling equipment is clean, labeled and readily at hand. Influent grab samples shall be collected from the pump discharge feed pipe opening that supplies the Nyloplast Structure (see Figure 1, Q_{in}). Each influent sample shall be collected in a 100 mL bottle directly from the feed pipe opening before the sample contacts or mixes with the water in the Nyloplast Structure. A total of five (5) influent grab samples shall be collected. To collect samples, hold the 100 mL sample bottle securely so not to drop due to the force of the fast flowing discharge stream of the supply pump.
Carefully tilt the open mouth of the sample bottle in the flow stream and fill the 100 mL sample bottle to the bottle fill line, Cover the sample bottle immediately, dry any excess water from the outside of the bottle and store the bottle in a cooler with ice packs.

7.2.2 Effluent Sampling, Grab

The treated effluent samples shall be collected using the manual grab sampling technique outlined below. The grab sample must be collected as a free falling sample from the end of the 15-in corrugated (ADS N-12) polypropylene pipe, subsequently herein referred to as the "Nyloplast discharge pipe". Do not lay the sample bottle horizontally inside the Nyloplast discharge pipe to collect the samples. Before taking

the first sample, verify that all the sampling equipment is clean, labeled and readily at hand. Also, allow the treated helical filters to be wetted before sampling. Unless otherwise specified, a total of five (5) effluent grab samples per test shall be collected from the Nyloplast discharge pipe.

1. With the effluent flowing at the rate specified in Table 1, and the prepared 100 mL sample bottle at the ready, remove the lid from the sample bottle and carefully place the mouth of the sample bottle directly into the falling stream of effluent and collect the sample. Be sure not to touch the Nyloplast discharge pipe with the mouth of the sample bottle. Fill the 100 mL sample bottle as above. Extreme care must be used when handling an open sample bottle to prevent contamination from environmental factors. Airborne dust, insects, blades of grass or any material coming in contact with the sample bottle or cap, other than the free falling effluent, will contaminate the sample and render it useless.
2. Once the sample volume has been collected, carefully remove the bottle from the flow stream again making sure not to touch the mouth of the bottle, or underside of the cap, against any other surfaces. Immediately cap and store the sample bottle in a cooler with ice or ice packs.

7.3 Performance Test

Performance testing shall be done for each test specified in Table 1 and in accordance with the concentration and flow rates listed for the microbial species, namely E. coli.

7.3.1 Procedure, Performance Test

1. Install five (5) treated helical filters segments into the Nyloplast discharge pipe. Take all necessary precautions to limit the exposure of the helical filter segments to potentially high bacteria laden surfaces.

2. For test 1 only, start by adjusting the supply tank volume down to 4000-gallons of seeded dechlorinated water in accordance with Table 1. With valves (V1) and (V3) open and valves (V2) and (V4) closed, turn on the pump and allow the system to recirculate at approximately 100-gpm for at least one (1) hour. Slowly open and regulate valve (V2) to the required flow rate specified in Table 1. Sample the influent in accordance with paragraph 7.2.1.
3. Maintain the required influent flow rate by regulating valve (V2) as necessary and verify that the flow meter registers a steady flow in accordance with the corresponding test value specified in Table 1. Effluent from the supply tank should now be flowing through the Nyloplast discharge pipe and the treated helical filters. If necessary, adjustments to valve (V1) can be made to help stabilize the flow rate.
4. With the flow rate stabilized, measure and record the vertical static head (in inches) of water in the Nyloplast Structure. The static head height is measured from the invert of the Nyloplast discharge pipe that houses the helical filters, and can be easily read directly from the staff gauge mounted to a sight tube on the Nyloplast Structure.
5. For the initial test run (Test 1), allow at least one (1) minute of flow through the helical filters before taking a sample. This will ensure that the helical filter media is completely wetted. Then, sample the effluent in accordance with the grab sampling procedure outlined in paragraph 7.2.2 allowing approximately 30 seconds between each sample collected.
6. Repeat steps 3 through 5 for each test specified in Table 1.

7.4 Reporting

The performance test results are to be documented in a final test report which shall include a table of reduction efficiencies as well as plotted performance curves. As a minimum, these performance curves should include percent removal efficiencies and concentration vs. flow rates. Additionally, the final report should include a record of

the vertical static head for each flow rate and photographs of the test setup and a record of the ambient weather conditions for each test day.

8 Results

8.1 Ambient Conditions

Ambient Conditions		
Air Temp.:	78	Deg F
Water Temp.:	80	Deg F
Water pH:	7.6	N/A
Chlorine:	0	PPM

Table 8.1: Ambient conditions for air and water, recorded at start of testing.

At the start of the test the ambient conditions for the air and water were recorded to ensure that the testing parameters resembled real world conditions. All of the ambient conditions tested normal and fell within the acceptable ranges to perform the test.

8.2 Dosage Statistics

Dosage Statistics		
E. Coli OD:	0.293	4 x Dilution
Target Conc.:	350000	MPN/100mL
Calc. Broth Vol.:	238.3	mL
Act. Broth Vol.:	250	mL

Table 8.2: Measurements and calculations required for introducing bacteria to the system.

The calculated broth volume was found for the target concentration by using the optical density of the E. Coli, which was at a 4x dilution. The calculations done are as follows:

Concentration of bacteria in the 4x dilution (MPN/mL):

$$COB_{4x\ Dilution} = \left((2 * 10^7) * OD_{E.Coli\ @\ 4x\ Dilution}\right) - 3{,}000{,}000$$

$$COB_{4x\ Dilution} = \left((2 * 10^7) * (0.323)\right) - 3{,}000{,}000$$

$$COB_{4x\ Dilution} = 60{,}800{,}000\frac{MPN}{mL}$$

Concentration of bacteria in the original broth (MPN/mL):

$$COB = COB_{4x\ Dilution} * 4$$

$$COB = 60{,}800{,}000 \frac{MPN}{mL} * 4$$

$$COB = 243{,}200{,}000 \frac{MPN}{mL}$$

Volume broth to add to 5000 GAL tank (mL):

$$V_{broth} = \frac{COB_{target} * V_{water} * \left(3785 \frac{mL}{GAL}\right)}{COB}$$

$$V_{broth} = \frac{\left(3500 \frac{MPN}{mL}\right)(4{,}500\ GAL)\left(3785 \frac{mL}{GAL}\right)}{243{,}200{,}000 \frac{MPN}{mL}}$$

$$V_{broth} = 245.1223\ mL$$

To implement a 10% reduction:

$$V_{broth\ 10\%} = V_{broth} - (V_{broth} * 0.1)$$

$$V_{broth\ 10\%} = 245.1223\ mL - (245.1223\ mL * 0.1)$$

$$V_{broth\ 10\%} = 220.6101\ mL$$

Based on previously calculated experimental results, it was determined that the Actual broth volume to add to the 5000 GAL tank should be reduced to 250 mL.

8.3 Test Data and Calculations:

8.3.1 Calculations:

The Filter media used was double thick polyether foam, 15" helical configuration. 5 filter segments total. Each sample was done by taking grab samples of the treated water were taken 30-sec apart for each flow rate. The following method used for determining outliers is the Modified Z-Score (Iglewicz and Hoaglin) method, which is sited below.

> U.S. COMMERCE DEPARTMENT: NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY-INFORMATION TECHNOLOGY LABORATORY, 2003, "ENGINEERING STATISTICS HANDBOOK: DETECTION OF OUTLIERS", WWW.ITL.NIST.GOV (ACCESSED 09.24.2014)

The mean ($\bar{x}$) of the raw data (x) samples was calculated separately for each flow rate:

$$\bar{x} = \frac{\sum x}{N}$$

The median (M) of the raw data samples (x) was then calculated separately for each flow rate:

$$M_{odd\ \#} = \left(\frac{n+1}{2}\right)^{th} term$$

$$M_{even\ \#} = \frac{\left(\frac{n}{2}\right)^{th} term + \left(\frac{n}{2}+1\right)^{th} term}{2}$$

The Median Absolute Deviation (MAD) was then calculated for each sample set:

$$MAD = \frac{1}{N}\sum |x - M|$$

A check for outliers was then performed on each sample set using the following criterion:

$$|\bar{x} - x| > (3.5 * MAD)$$

After all outliers were removed from the sample sets the mean ($\bar{x}_c$) was recalculated for each flow rate:

$$\bar{x}_c = \frac{\sum x_c}{N_c}$$

*Note that the subscript c signifies that the value has been modified based on the previously stated criterion.

8.3.2 Raw Test – 50 GPM

Raw Test: 50 GPM (Untreated)			
Flow Rate:		50	GPM
Volume In Tank:		4000	GAL
Sample Number	**Client ID**	**Result [MPN/100mL]**	**Outlier Check [MPN/100mL]**
1	SIEU 1	250,000	250,000
2	SIEU 2	450,000	450,000
3	SIEU 3	450,000	450,000

4	SIEU 4	250,000	250,000
5	SIEU 5	120,000	120,000
	Mean:	**304,000**	**304,000**
	Median:	**250,000**	
	MAD:	**130,000**	

Table 8.3: Five untreated test samples taken at a flow rate of 50 GPM.

8.3.3 Performance Test – 10 GPM

Performance Test: 10 GPM (Treated)			
Flow Rate		10	GPM
Static Head		6.5	IN
Sample Number	**Client ID**	**Result [MPN/100mL]**	**Outlier Check [MPN/100mL]**
6	SIET L1	40,000	40,000
7	SIET L2	17,000	17,000
8	SIET L3	120,000	OUTLIER
9	SIET L4	25,000	25,000
10	SIET L5	15,000	15,000
	Mean:	**43,400**	**24,250**
	Median:	**25,000**	
	MAD:	**10,000**	
Percent Change [%]:		**-92.02%**	

Table 8.4: Five treated samples taken at a flow rate of 10 GPM

8.3.4 Performance Test – 40 GPM

Performance Test: 40 GPM (Treated)			
Flow Rate		40	GPM
Static Head		10.5	IN
Sample Number	**Client ID**	**Result [MPN/100mL]**	**Outlier Check [MPN/100mL]**
11	SIET M1	40,000	40,000
12	SIET M2	65,000	OUTLIER
13	SIET M3	40,000	40,000
14	SIET M4	40,000	40,000
15	SIET M5	65,000	OUTLIER

Mean:	**50,000**	**40,000**
Median:	**40,000**	
MAD:	**0**	
Percent Change:	**-86.84%**	

Table 8.5: Five treated samples taken at a flow rate of 40 GPM

8.3.5 Performance Test – 100 GPM

Performance Test: 100 GPM (Treated)			
Flow Rate		100	GPM
Static Head		28.5	IN
Sample Number	**Client ID**	**Result [MPN/100mL]**	**Outlier Check [MPN/100mL]**
16	SIET H1	15,000	15,000
17	SIET H2	55,000	55,000
18	SIET H3	140,000	140,000
19	SIET H4	140,000	140,000
20	SIET H5	65,000	65,000
Mean:		**83,000**	**83,000**
Median:		**65,000**	
MAD:		**50,000**	
Percent Change:		**-72.70%**	

Table 8.6: Five treated samples taken at a flow rate of 100 GPM

8.3.6 Analysis

Once all the outliers were removed and the corrected means recalculated, the percent change in bacteria was found for each flow rate (10 GPM, 40 GPM and 100 GPM). The calculation for Percent Change (%Δ) was done using the following formula for each performance test:

$$\%\Delta = \frac{(\bar{x}_c)_{Performance} - (\bar{x}_c)_{Raw}}{(\bar{x}_c)_{Raw}}$$

As seen in Table 8.4, 8.5 and 8.6 the calculations have been performed for each performance test. The yielded results are as follows:

- Performance Test 1: An E. Coli reduction of 92.02% for a flow rate of 10 GPM

- Performance Test 2: An E. Coli reduction of 86.84% for a flow rate of 40 GPM
- Performance Test 3: An E. Coli reduction of 72.70% for a flow rate of 100 GPM

8.4 E. Coli Concentration vs. Flow Rate

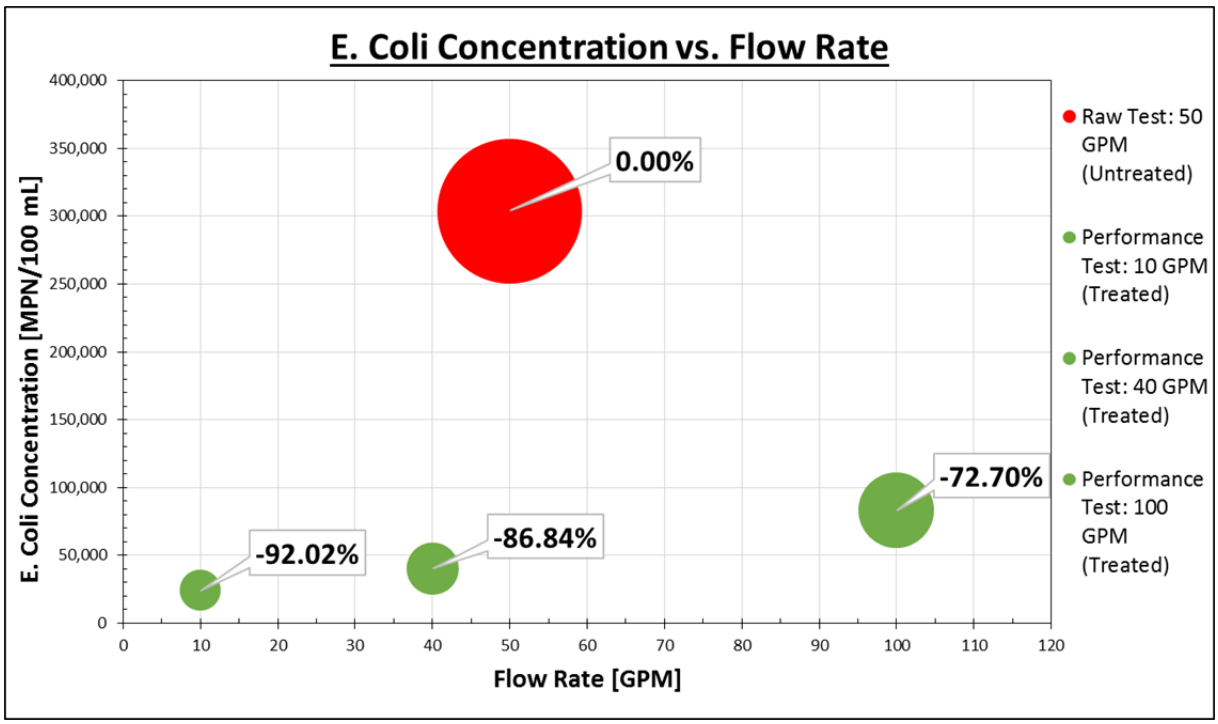
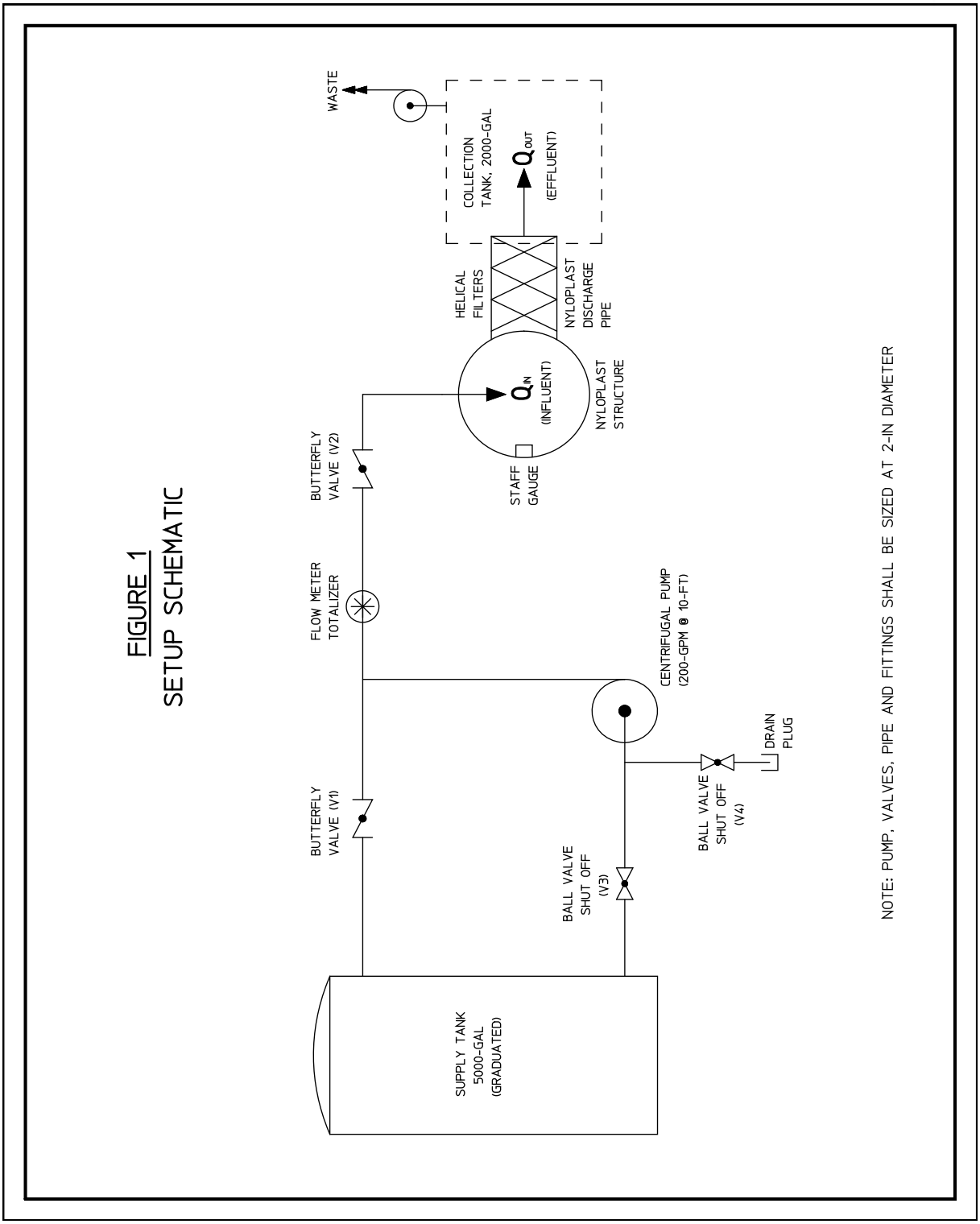


Figure 2: E. Coli concentrations plotted for varying flow rates, including percent reduction labels.

The concentrations and the percent reductions in E. Coli from both the performance tests and the raw test were plotted against flow rate as shown in Figure 2. The red circle shows the untreated raw test which was performed at 50 GPM and is untreated (0% reduction) having an E. Coli concentration of 304,000 MPN/100 mL. The green circles show the performance tests at 10, 40 and 100 gallons per minute with the percent reductions being 92.02%, 86.84% and 72.70% consecutively and the concentration of E. Coli after treatment averaging approximately 49,000 MPN/100 mL.



9 Appendix

Figure 1: Schematic of testing structure.

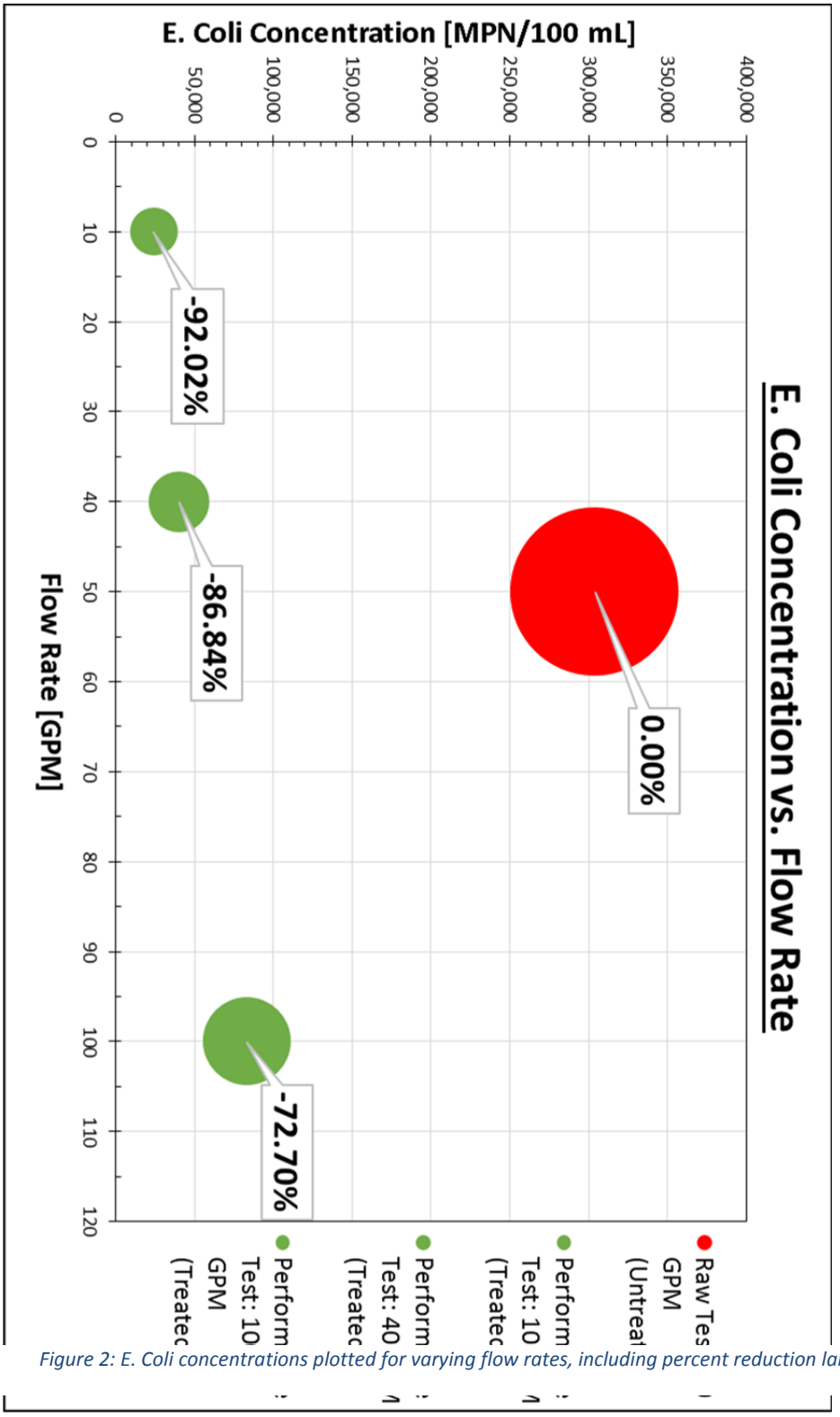


Figure 2: E. Coli concentrations plotted for varying flow rates, including percent reduction labels.