CWA FOCUSED COMPLIANCE INSPECTION REPORT
U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 5

Purpose: Clean Water Act Focused Compliance Inspection – Milorganite® Biosolids Process

Facility: Milwaukee Metro Sewerage District (MMSD), Jones Island Biosolids Dewatering and Drying Facility

NPDES Permit Number: WI-0036820-04

Date of Inspection: June 4, 2021

MMSD Representatives:
Sid Arora, Contract Compliance Assistant Manager
Patrick Obenauf, Contract Compliance Manager
Steve Unger, Contract Administrator

EPA Inspector:
Dean Maraldo, EPA Region 5 Inspector; (312) 353-2098; maraldo.dean@epa.gov

Wisconsin Department of Natural Resources Representatives:
Frederick Hegeman, Wastewater Engineer
Jacob Wedesky, Wastewater Engineer

Report Prepared by:
Dean Maraldo, EPA Region 5

EPA Inspector Signature: Maraldo, Dean Digitally signed by Maraldo, Dean Date: 2021.06.09 17:19:31 -05'00'

Approver Name & Title:
Ryan Bahr, Chief, Section 2, Water Enforcement and Compliance Assurance Branch

Approver Signature/Date RYAN BAHR Digitally signed by RYAN BAHR Date: 2021.06.09 17:25:21 -05'00'

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I. INTRODUCTION

On March 14, 2017, I, along with scientists from EPA's Office of Research and Development (ORD), and inspectors from EPA Region 7, conducted a biosolids inspection at the Milwaukee Metro Sewerage District Combined's (MMSD), Jones Island EQ Biosolids Dewatering and Drying Facility ("D&D Facility"). The D&D Facility is owned and operated by MMSD. Veolia Water Milwaukee, LLC, provides operational management and support for the production of EQ biosolids at the D&D Facility (See Appendix A – Facility map). MMSD sells and distributes processed biosolids as EQ biosolids under the product name "Milorganite®." Once biosolids meet EQ requirements, they generally are not subject to further regulation under 40 CFR 503. As a result, 40 CFR 503 regulations include monitoring requirements to ensure EQ biosolids are safe for distribution to the public.

EPA assessed MMSD's compliance with 40 CFR Part 503 and the National Pollutant Discharge Elimination System (NPDES) permit for the D&D Facility. During the March 14, 2017 inspection, EPA identified the following areas of concern:

- **Heat drying temperature monitoring issues**. MMSD did not measure the temperature of the sewage sludge particles or the wet-bulb temperature of the gas in contact with sewage sludge, as required under 40 CFR Part 503 (Appendix B (B)(2). Instead, MMSD measured temperature approximately 19 feet away from dryer furnace outlet along dryer discharge screw chute.

- **Location of compliance sampling for fecal coliform and moisture content.** Fecal coliform samples were collected from the process distribution pile, not at the point of bagging or at the time of load out into trains or trucks for offsite shipping. 40 CFR Part 503.32(a)(7) Class A—Alternative 5 requires either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella, sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or given away in a bag or other container for application to the land, or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in §503.10(b), (c), (e), or (f).

  Product moisture samples were collected after temporary storage in silos, not at the point of bagging. 40 CFR Part 503.33(3) requires one of the vector attraction reduction requirements in §503.33 (b)(1) through (b)(8) shall be met when sewage sludge is sold or given away in a bag or other container for application to the land.

- **Fecal coliform sample preservation and holding time issues.** MMSD stored fecal coliform samples at room temperature until the end of the month, when all samples were analyzed. Fecal coliform samples were not refrigerated to <10°C and not analyzed within the standard holding time of 8 hours.

The purpose of the announced June 4, 2021 inspection was to follow up on MMSD's actions to address the above areas of concern and assess MMSD's compliance with 40 CFR Part 503 and the NPDES permit for the D&D Facility.

MMSD raised some concerns regarding COVID-19 and the face-to-face time needed to conduct the mainly indoor inspection of the D&D Facility. To address these concerns and limit face-to-face inspection efforts to only the physical inspection of the D&D Facility, we agreed to hold a pre-inspection teleconference on June 3, 2021, to discuss MMSD's efforts to address the above-referenced areas of concern. MMSD also presented a summary of recent biosolids compliance monitoring information (See

Appendix B "503 Compliance Update"). During the teleconference, MMSD described its new fecal coliform preservation and holding time protocols to address concerns raised during the March 2017 inspection. MMSD now refrigerates samples and analyzes samples within required holding times for fecal coliform. MMSD also described its new temperature monitoring system, including new temperature probes in each dryer and recycling bin. MMSD now utilizes two compliance methods to demonstrate Class-A biosolids compliance at any given time, including 40 CFR Part 503 Dryer Discharge Temperature method (Primary) and Recycle Bin Discharge Temperature method (40 CFR Part 503 Time and Temperature method)(Secondary).

MMSD describes the Class-A biosolids system overview and compliance methodology as follows (March 2021 Monthly Report, Appendix C):

> *The drying system consists of twelve Dryers, three Recycle Bins and several product conveyors. The dryers are laid out in two groups - North Side Dryers (1-6) and South Side Dryers (7-8). A recycling bin is at the end of each group of six dryers. The third recycle bin acts as redundant and can serve either side of dryers. From the recycle bin, most pellets return to the dryers. The remaining pellets go to classification and product screening. Figure 1* (below) *represents a typical day of biosolids processing with 8 dryers operating (4 Dryers on the North Side and 4 Dryers on the South Side) with Recycle Bins 1 and 2 routing dried product either back to the Dryers or allowing it to go to Classification and Screening through automatic slide gates. The dry mass values on Figure 1 represent annual averages as monitored over the years. Figure 1 also shows the approximate location of the temperature monitoring devices at the discharge end of each dryer and at the discharge end of the recycle bins.*
>
> [40 CFR Part 503] *Alternative 5-503.32(a)(7) and Appendix B.B.2 (Heat Drying) is used as the Primary means to demonstrate compliance with 503 Regulation for Class A. In this method, dryer discharge product temperatures for all in-production dryers stay above 176°F. Automatic controls in each dryer recycle all product leaving the dryer back to the inlet of the dryers if a discharge temperature falls below 180°F.* [40 CFR Part 503] *Alternative 1-503.32(a)(3) (Time Temperature) is used as the secondary means to demonstrate compliance. This method is only used when the primary method does not demonstrate compliance. In this method, we continuously monitor the recycle bin discharge temperature and determine detention time of the product through the recycle bin. The District continuously calculates a required temperature by using 40 CFR Part 503(a)(3) Equation 2 for the current detention time. Comparison between the measured recycle bin discharge temperature and the required temperature determines compliance with this method. If a 15-minute dryer discharge product temperature is below 176°F at any individual dryer discharge, then the District will use recycling bin time and temperature to demonstrate pathogen reduction. Use of time and temperature will continue until 40 minutes after all in-drum product temperatures are higher than the required temperature. 40 minutes is the worst-case product travel time from the dryer farthest from the recycling bin to the outlet of the recycling bin. If time and temperature does not show compliance, then the gates between the recycle bin and classification & screening will automatically CLOSE. In this configuration, all product will return to the dryers. These gates are reopened when time and temperature show compliance or Dryer discharge temperature show continuous compliance for at least 40 minutes. Figure 2* (below) *shows overall compliance strategy to demonstrate compliance with 503 Class A.*

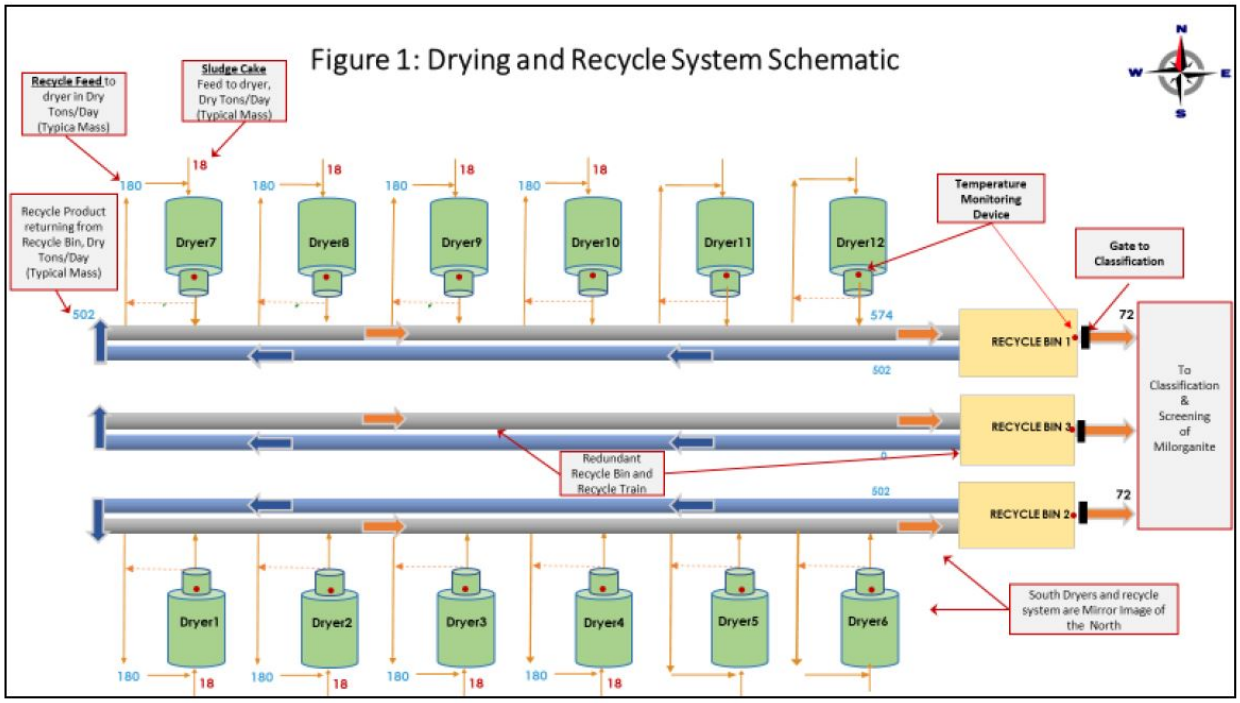


Figure 1: Drying and Recycle System Schematic
Recycle Feed to dryer in Dry Tons/Day (Typica Mass)
Sludge Cake Feed to dryer, Dry Tons/Day (Typical Mass)
Recycle Product returning from Recycle Bin, Dry Tons/Day (Typical Mass)
Temperature Monitoring Device
Gate to Classification
Dryer1
Dryer2
Dryer3
Dryer4
Dryer5
Dryer6
Dryer7
Dryer8
Dryer9
Dryer10
Dryer11
Dryer12
RECYCLE BIN 1
RECYCLE BIN 2
RECYCLE BIN 3
Redundant Recycle Bin and Recycle Train
To Classification & Screening of Milorganite
South Dryers and recycle system are Mirror Image of the North
180
18
502
574
72
N
S
W
E

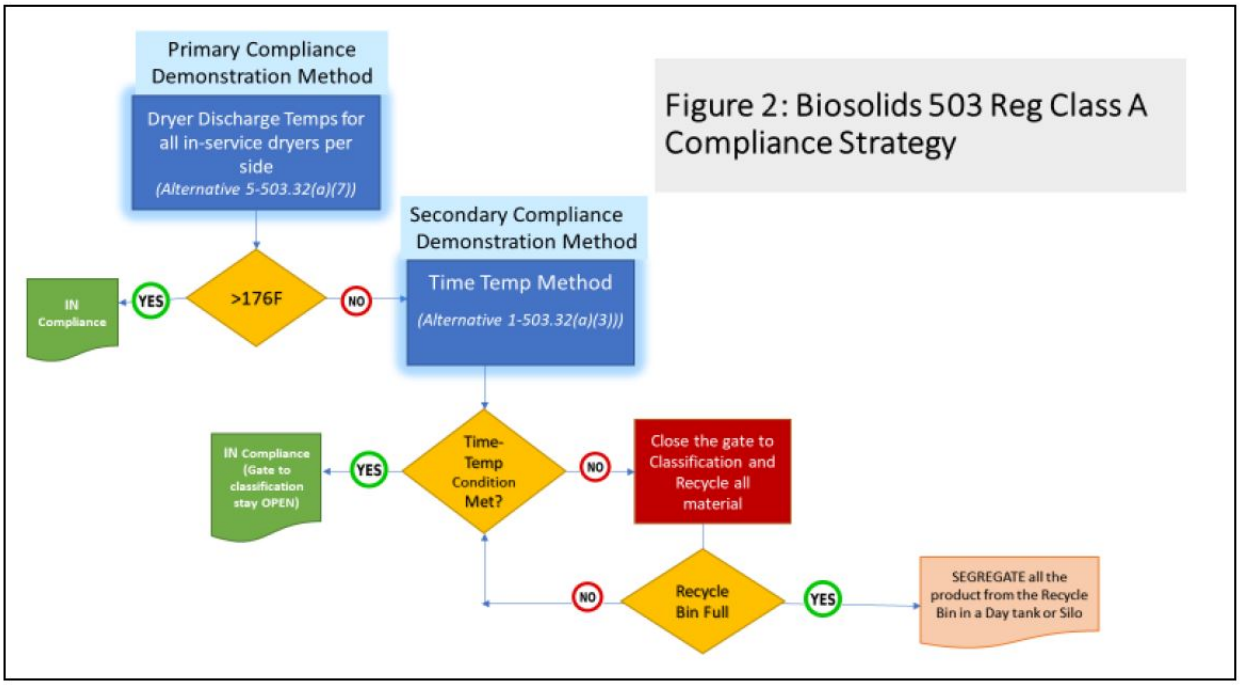


Figure 2: Biosolids 503 Reg Class A Compliance Strategy
Primary Compliance Demonstration Method
Dryer Discharge Temps for all in-service dryers per side
(Alternative 5-503.32(a)(7))
>176F
YES
NO
IN Compliance
Secondary Compliance Demonstration Method
Time Temp Method
(Alternative 1-503.32(a)(3)))
Time-Temp Condition Met?
YES
NO
IN Compliance (Gate to classification stay OPEN)
Close the gate to Classification and Recycle all material
Recycle Bin Full
NO
YES
SEGREGATE all the product from the Recycle Bin in a Day tank or Silo

According to MMSD, since implementing the new temperature monitoring system, dryer temperatures consistently meet the Dryer Discharge Temperature requirements. Before the pre-inspection teleconference, Wisconsin Department of Natural Resources ("WDNR") inspector Fred Hegeman provided monthly compliance reports for the D&D Facility for April 2020 thru March 2021. According to the reports, final biosolids product met temperature (40 CFR Part 503 (Appendix B (B)(2)), fecal coliform (40 CFR Part 503.32(a)(7)), and moisture content (40 CFR Part 503.33(3)) requirements.

II. BACKGROUND

MMSD is authorized to discharge treated wastewater from the Jones Island Water Reclamation Facility to Lake Michigan, Milwaukee Harbor, and tributary streams in Milwaukee County under NPDES permit number WI-0036820-04 ("Permit"). The Permit also includes land application requirements for EQ biosolids produced at the D&D Facility. The Facility's Operations Department is responsible for operating the D&D Facility and for compliance with the Permit. MMSD is responsible for conducting monitoring activities and reporting monitoring results to the WDNR and EPA.

MMSD's Jones Island Water Reclamation Facility (JIWRF) began operating in 1925. MMSD began producing Milorganite® at the JIWR Facility in 1926. The current sludge D&D Facility is located within the JIWR Facility and began operation since 1994. The D&D Facility treats sludge from the JIWR Facility and MMSD's South Shore Water Reclamation Facility. Milorganite® is produced at the D&D Facility from sludge collected at the two facilities. Milorganite® is marketed as commercial fertilizer and sold in bags at retail stores or shipped via rail and truck to commercial customers in the USA, Canada, and elsewhere.

III. INSPECTION ACTIVITY SUMMARY

The June 4, 2021 inspection consisted of the following activities:

- Inspection opening conference;
- Physical inspection of the D&D Facility; and
- Closing conference and area of concern review.

This report summarizes the results of the inspection. The list of attendees is included below. Note that numerous representatives attended on behalf of MMSD, and below I list only the MMSD representatives with key roles in leading the physical inspection or responding to questions:

MMSD:	Sid Arora, Contract Compliance Assistant Manager Patrick Obenauf, Contract Compliance Manager Steve Unger, Contract Administrator Tom Cox, Operator (Veolia Water Milwaukee) Zach Ream, Operator (Veolia Water Milwaukee)
EPA Region 5:	Dean Maraldo, Inspector/Enforcement Officer
WDNR:	Frederick Hegeman, Wastewater Engineer Jacob Wedesky, Wastewater Engineer

III. A. Opening Conference

I arrived at the MMSD Jones Island Water Reclamation Facility Operations Building at 8:45 am on June 4, 2021. I met WDNR engineers Frederick Hegeman and Jacob Wedesky and the MMSD representatives listed above and began the inspection opening conference with introductions at 8:57 am. Due to COVID-19 concerns, all participants wore face masks. I presented my EPA-issued Inspector credentials to Patrick Obenauf (MMSD). I discussed the intent and scope of the inspection. I mentioned my plans to take photographs during the inspection, focusing on the new temperature monitoring system and asked if there were any potential confidential business information concerns. Patrick Obenauf said they would have no concerns about taking photographs but asked to confirm before taking each photograph. I agreed, and no concerns were raised with any of the photographs taken during the inspection. Before starting the physical inspection, I asked the MMSD representatives if the new fecal coliform preservation and holding time protocols mentioned during the pre-inspection teleconference were available in written form, such as a standard operating procedure or memo. Sid Arora said they would relay the request to the laboratory manager.

III. B. Physical Facility Inspection – D&D Facility

We started the physical inspection of the D&D Facility at 9:04 am. Photographs referenced below are included in the Photo Log (Appendix D). Sid Arora, Patrick Obenauf, and Steve Unger led portions of the tour. To minimize concerns regarding COVID-19, we agreed to focus the inspection on the new temperature monitoring system. We began with an overview of the south side dryers (Photograph MMSD0001), then stopped at the three recycle bins (30-ton capacity each) which collect treated material from the dryers and directs material either back to recycling or to classification and screening. The recycle bins are now equipped with temperature probes to monitor for the secondary means to demonstrate compliance (Time and Temperature) as described on page 3, above, and in Appendix C. Sid Arora mentioned that MMSD installed three temperature probes per bin for redundancy (Photograph MMSD0002).

Next, we stopped at the product samplers station. Steve Unger explained that the sampler collects an aliquot for every four tons of material over 24 hours. This sampling location is known as WPDES Permit *Sampling Point (Outfall) 006 – Jones Island EQ Sludge – PRODUCTION*.

We walked to the south side dryer area and observed Dryer #1S, which was out of service at the time of inspection. Since the dryer was out of service, we were able to look inside the dryer and observe one of two new temperature probes (Photograph MMSD0004). Patrick Obenauf mentioned that each dryer has two new temperature probes on opposites sides of the dryer to measure temperature and each probe has a unique control signal. Photograph MMSD0005 captures the same probe from outside of Dryer #1S. I asked for copies of the specifications for the new temperature probes. Steve Unger said they would pull together the probe specifications and follow up after the inspection.

On the way to the operation control center, we stopped at the former temperature compliance monitoring location, approximately 19 feet away from the dryer furnace outlet along the dryer discharge screw chute (Photograph MMSD0006). Patrick Obenauf confirmed that the location is no longer used for 40 CFR Part 503 compliance monitoring.

The last stop in the D&D Facility was the operations control center (Photograph MMSD0007). The center is where operators monitor the treatment and control process, including the temperature monitoring system. Photograph MMSD0009 captures a control screen that monitors the temperature and status of all 12 dryers. Fred Hegeman asked the two operations control center operators, Zach Ream and Tom Cox, how the new temperature monitoring system is working. The operators described how the system improved control over drum temperature. Referring to the new temperature monitoring system, operator Zach Ream added that “monitoring is easier now” and “it’s awesome.”

On the way out of the D&D Facility, I asked Sid Arora if they've experienced any compliance issues since implementing the new temperature monitoring system. Sid Arora said they've had no compliance issues.

At the request of Fred Hegeman, Steve Unger led the group past the silos to observe the silo load-out facility. On the second floor of the load-out facility, we observed the belt conveyer which transports Milorganite® product from the silos to the load-out facility. Photograph MMSD0010 captures the belt conveyor and product autosampler. Steve Unger identified the product autosampler location as WPDES Permit *Sampling Point (Outfall) 008 – Jones Island EQ Sludge – SHIPPING*. Steve Unger added that all finished Milorganite® material, except for some special orders, is now loaded out to two offsite bagging facilities on Jones Island at Kinder Morgan and Spring Valley facilities, where compliance samples are collected at WPDES Permit *Sampling Points (Outfall) 009 – Jones Island EQ Sludge – BAGGING.*

We completed the physical inspection at 10:14 am.

IV. CLOSING CONFERENCE

I began the closing conference at the Jones Island Water Reclamation Facility office at 10:20 am with the WDNR and MMSD representatives in attendance. I mentioned that I did not observe any preliminary areas of concern, and added that areas of concern may be identified after reviewing information collected as part of the inspection. I asked the group if they had any questions. There were no questions. However, Steve Unger mentioned that the new temperature monitoring system and strategy was "overall a good thing for us."

I asked if MMSD could provide the two items requested earlier in the inspection (fecal sampling procedures and temperature probe specifications) within one week. Patrick Obenauf agreed with the requested timeframe.

I provided the group with an estimated timeframe for completing the inspection report, and we concluded the closing conference at 10:25 am. I departed the Jones Island Water Reclamation Facility at 10:30 am on June 4, 2021.

V. DOCUMENTS RECEIVED

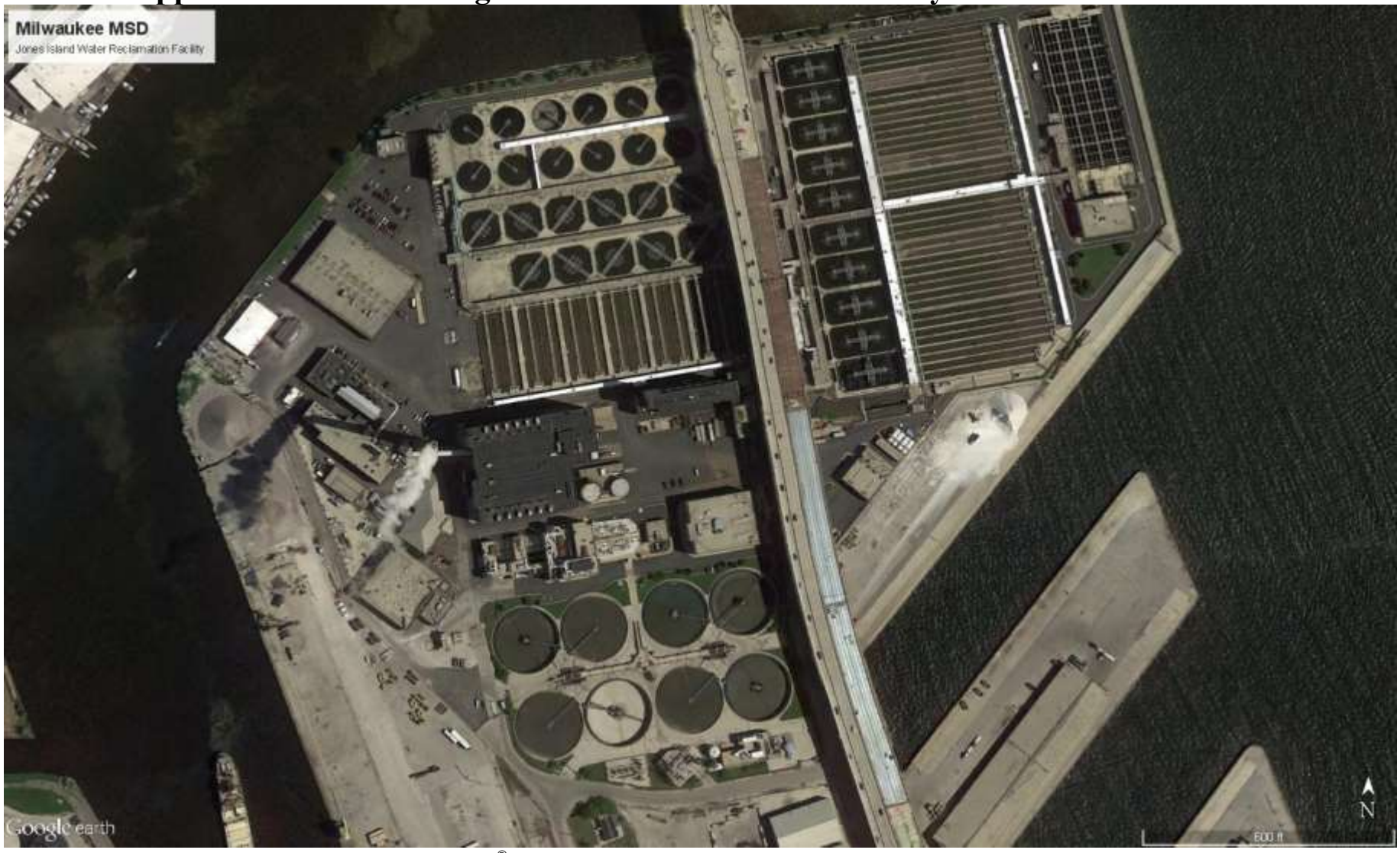
Documents received after the inspection (included in Appendix E):

- MMSD LAB-031 Quantification of Fecal Coliform and Total Coliform Bacteria in Biosolids by MPN REV.0.4 (1/4/2021)
- Milorganite® Fecal Coliform Chain of Custody Form
- Thermowell and Bosset Fitting Data Form
- Wireless Transmitter Equipment Data Form

Specific resource included by reference:

- Milwaukee Metropolitan Sewerage District Combined WPDES Permit# WI-0036820-04; issued by WDNR on March 27, 2019; expiration date March 31, 2024.

Appendix A: Aerial Image of MMSD Jones Island Facility



***Google Earth*® aerial image of the Facility, dated June 18, 2015.**

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Appendix B: Facility Presentation "503 Compliance Update"

503 Compliance Update

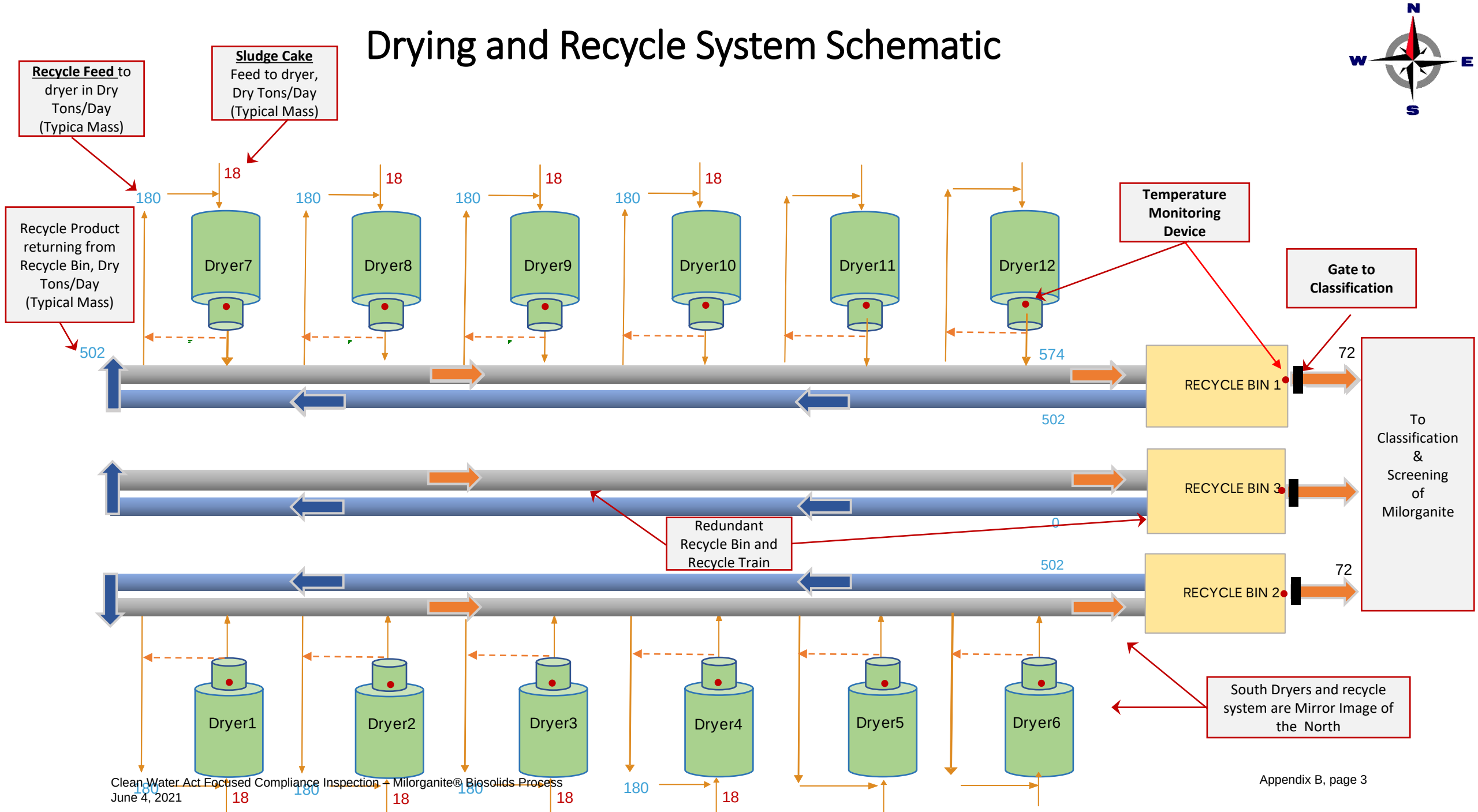
EPA WDNR WDNR Visit

June 2021

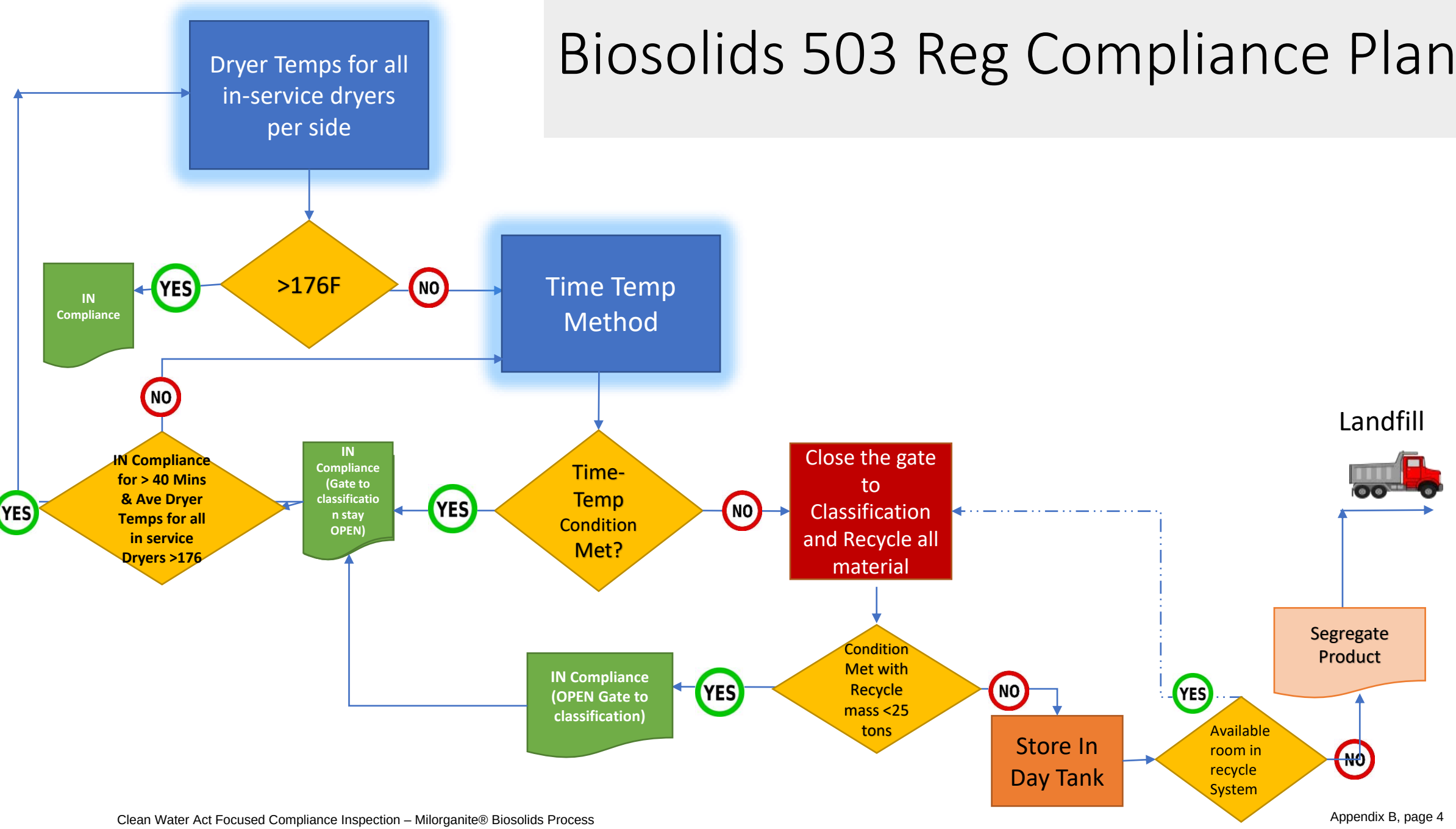
Presentation Outline

- Overview of the drying system
- MMSD 503 Regulation Compliance Plan
- Dryer Discharge Temperature Mode- Controls Strategy
- Recycle Bin Temp Mode- Controls Strategy
- Construction Sequence
- Operations Summary 2019-Present

Drying and Recycle System Schematic



Biosolids 503 Reg Compliance Plan



503 Regulation Compliance Strategy

We can use any one of the compliance methods to demonstrate **CLASS-A** compliance in any given time:

- Dryer Discharge Temperature (*Primary Method*)

- Recycle Bin Discharge Temp (Time Temperature)(*Secondary Method*)

- ~~Helminth Ova & Virus Testing (*Emergency Method*)~~

  Only Few States Including WI Allow this.
  Product Produced by this method will stay in WI

Primary Method- Dryer Temperature

WPDES Permit Requirements

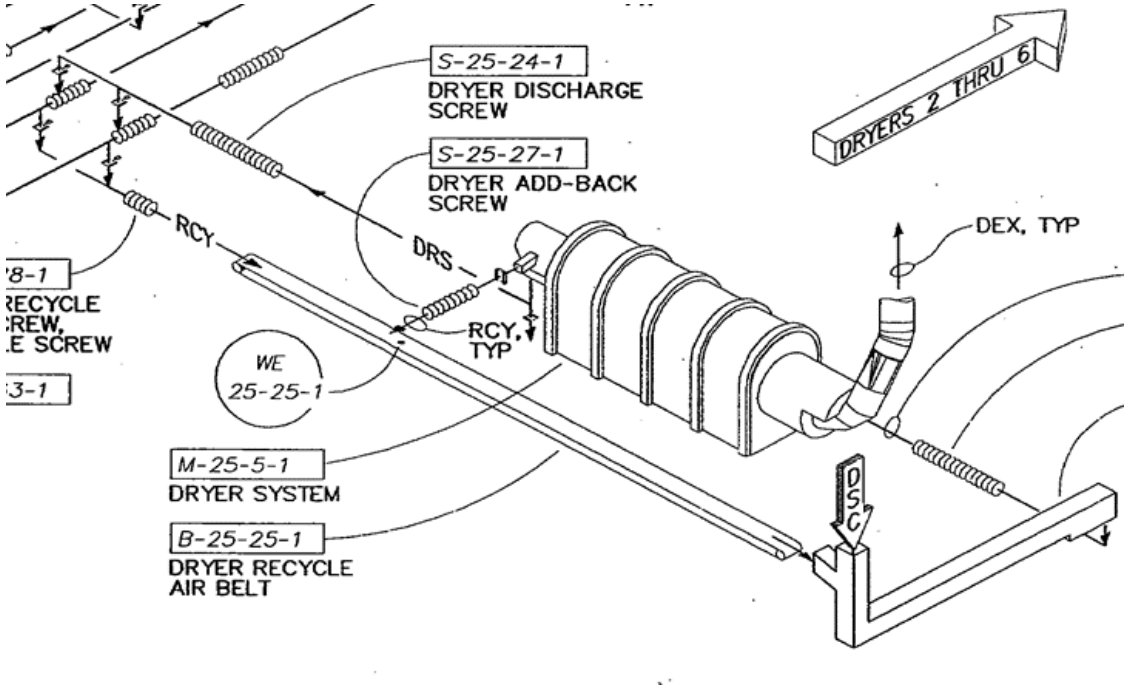
Dryer Drum Product Temp for all dryers **In-Production >176** on **continuous basis**

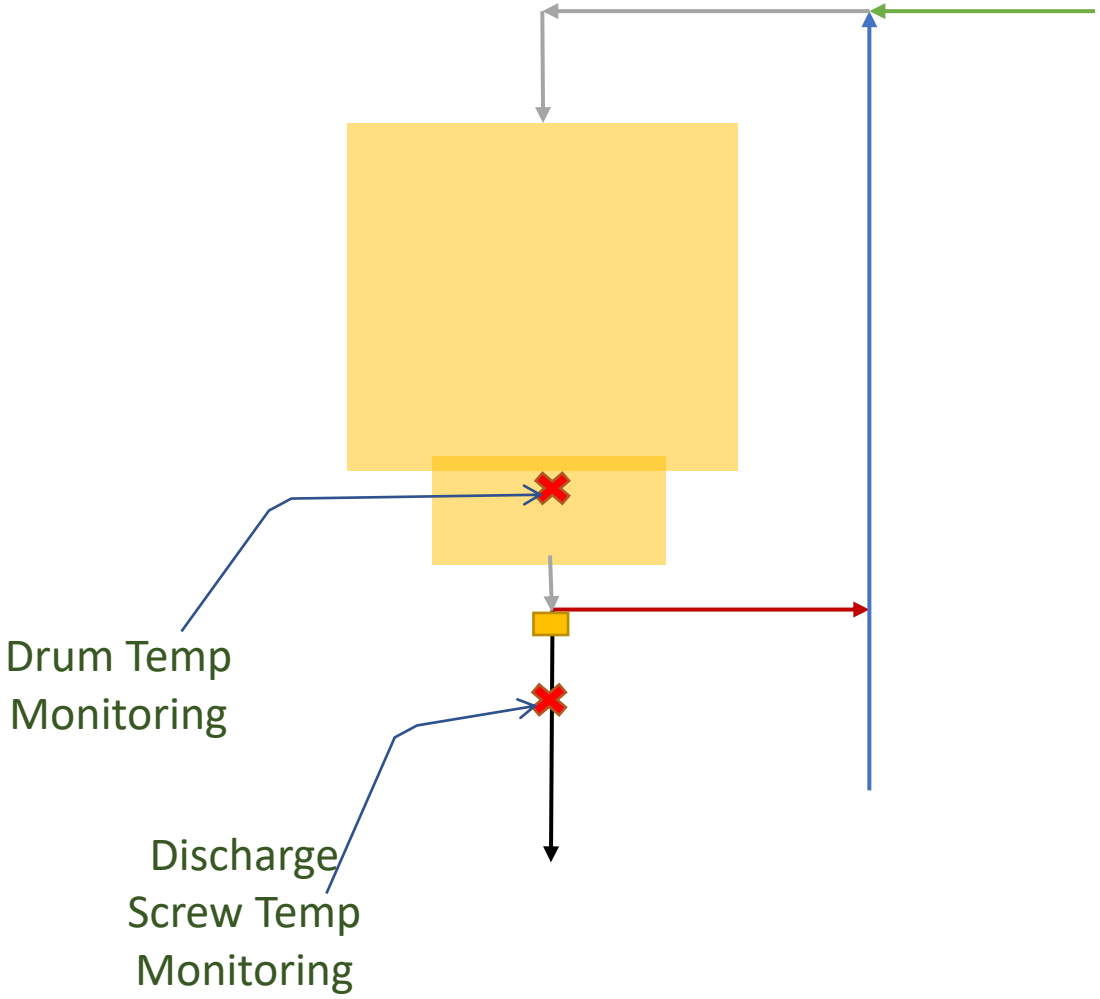
IN-Production: Dryer **IN Service** and **NOT** in **ADDBACK**

DRYER Temperature MODE for North or South Side

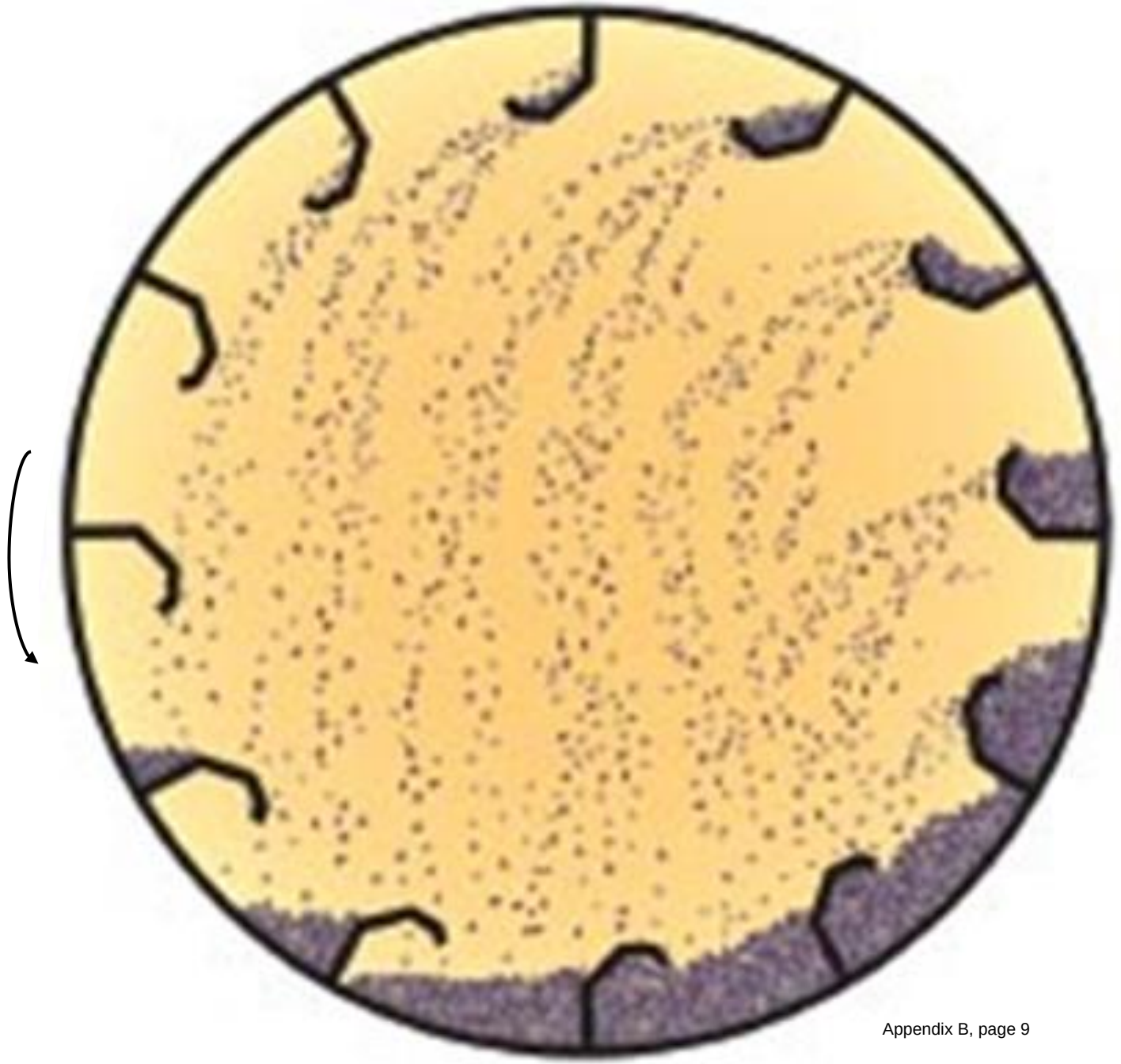
- This is the Primary Method to demonstrate compliance
- This Mode is Active per Side (North or South) when:
  - Drum Temperatures > 176F for all Inservice Dryers which are not in Addback
  - If a Dryer is in Addback there is no product leaving the system, hence is not included to determine the Active Mode per side.

Auto Add Back Mode ACTIVE

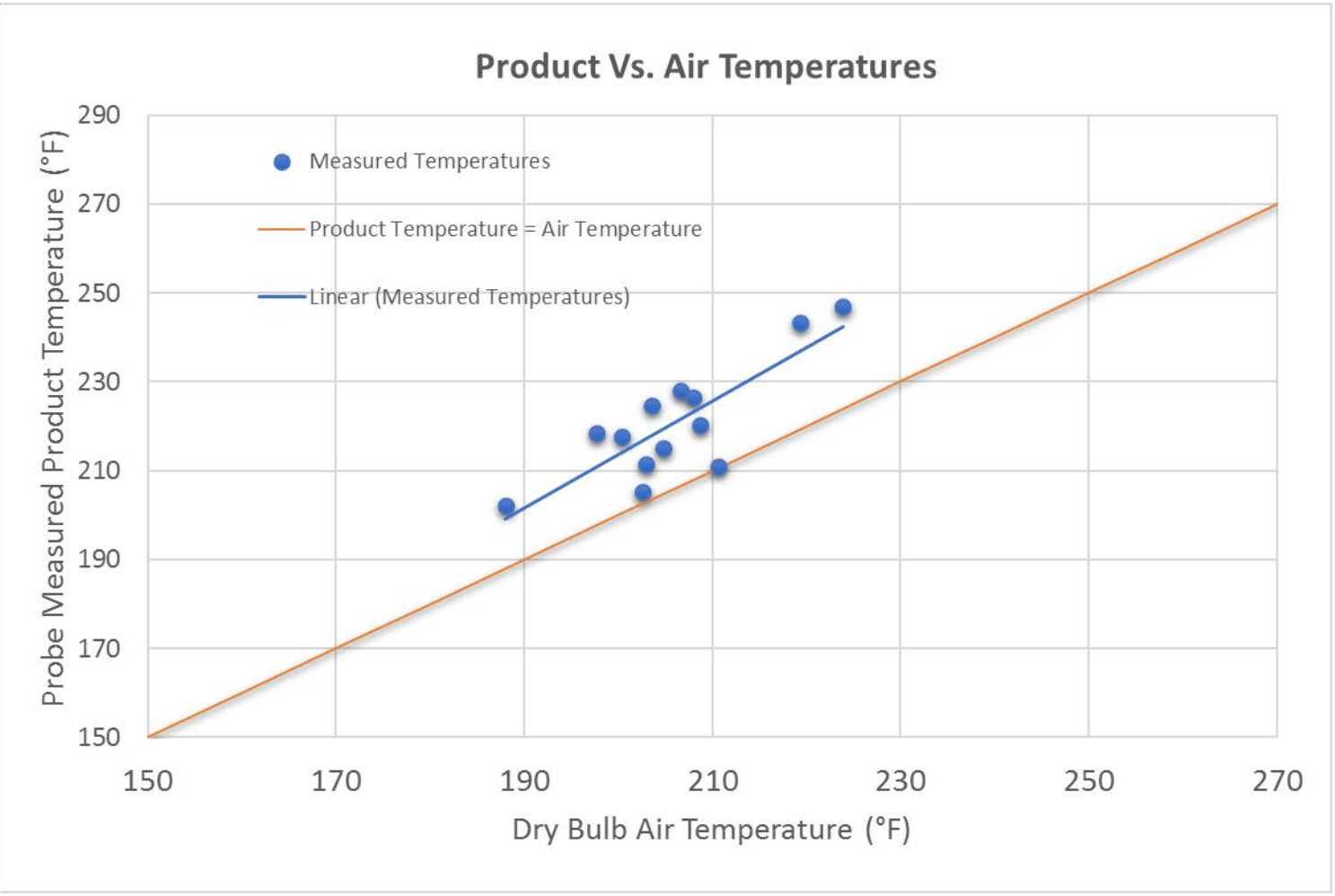




Product Mapping

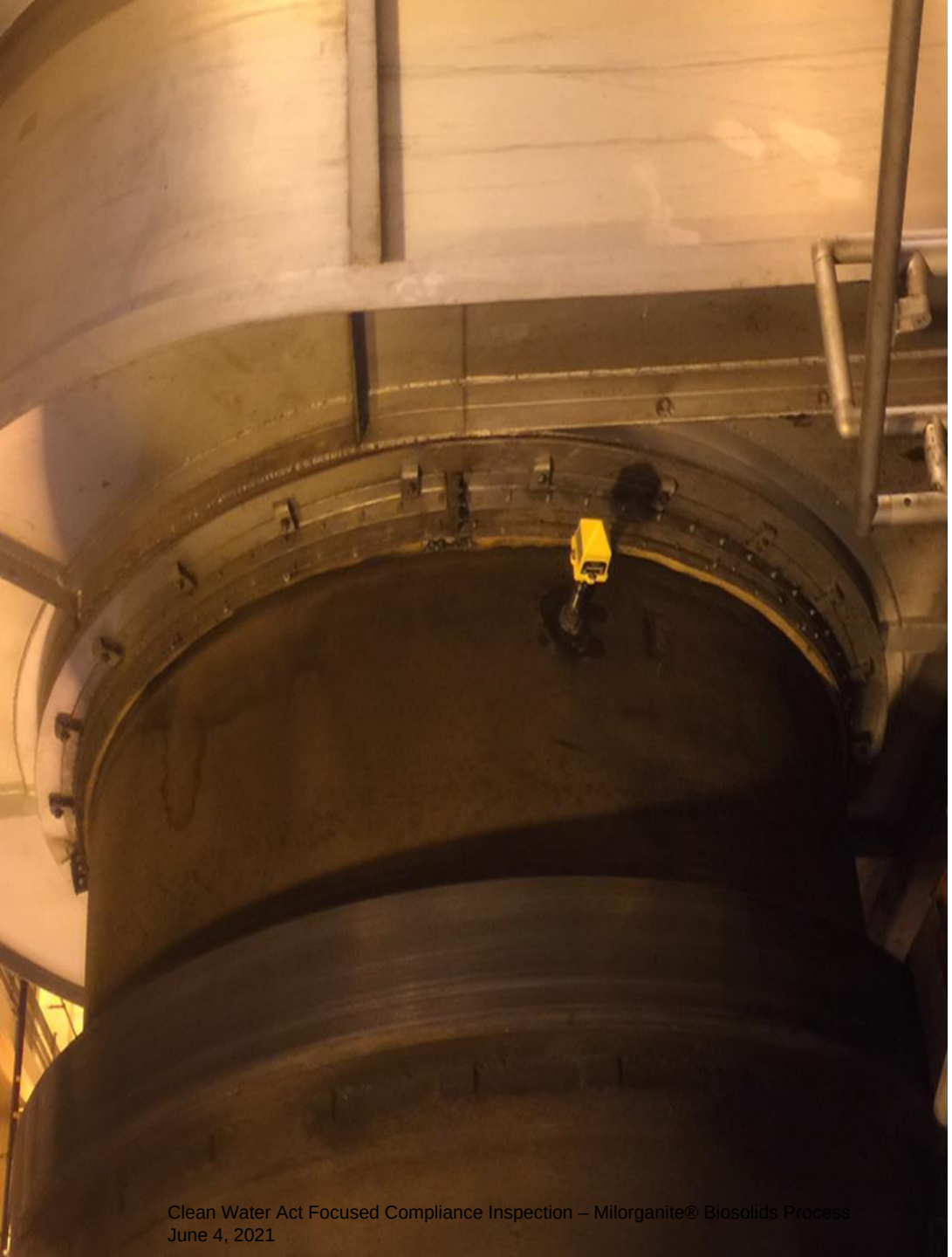


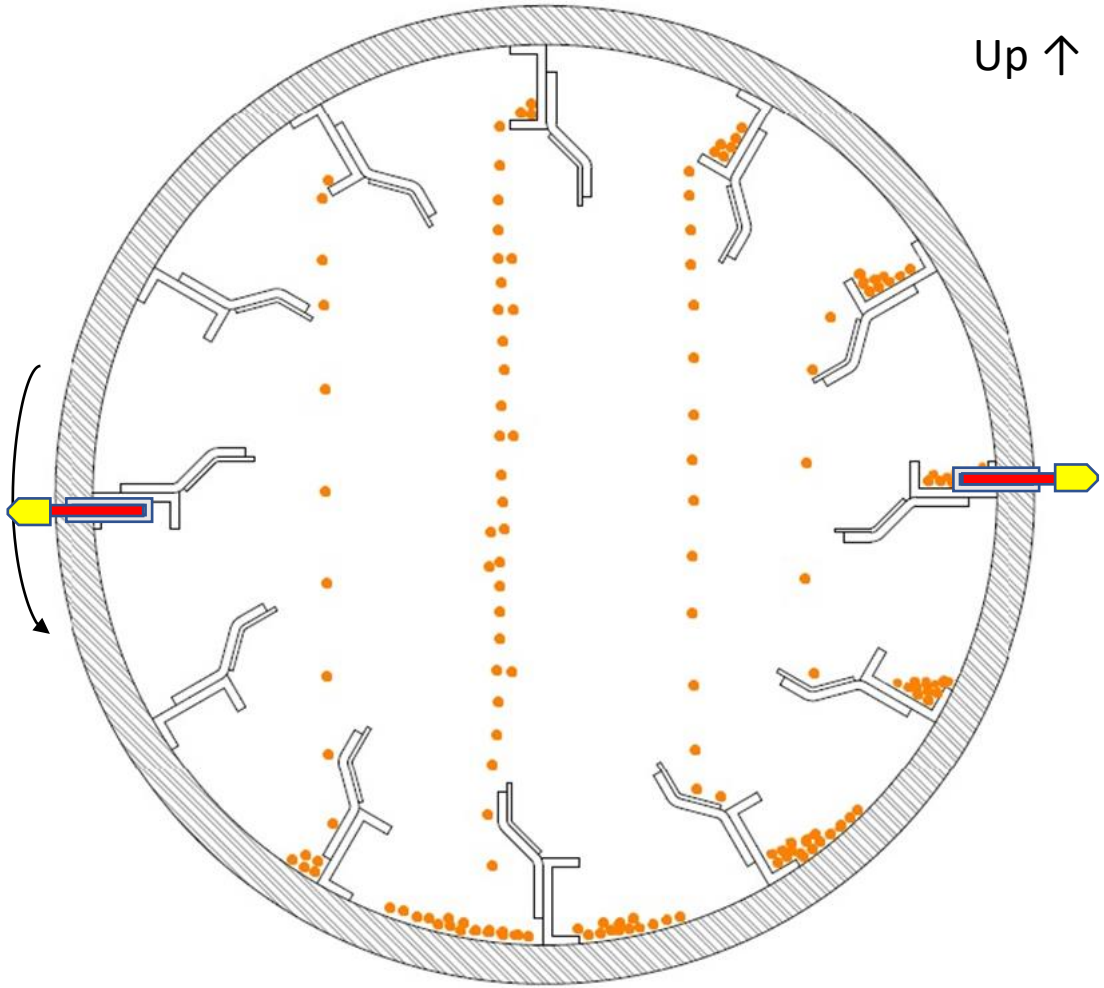
Dryer Temperature Study Report

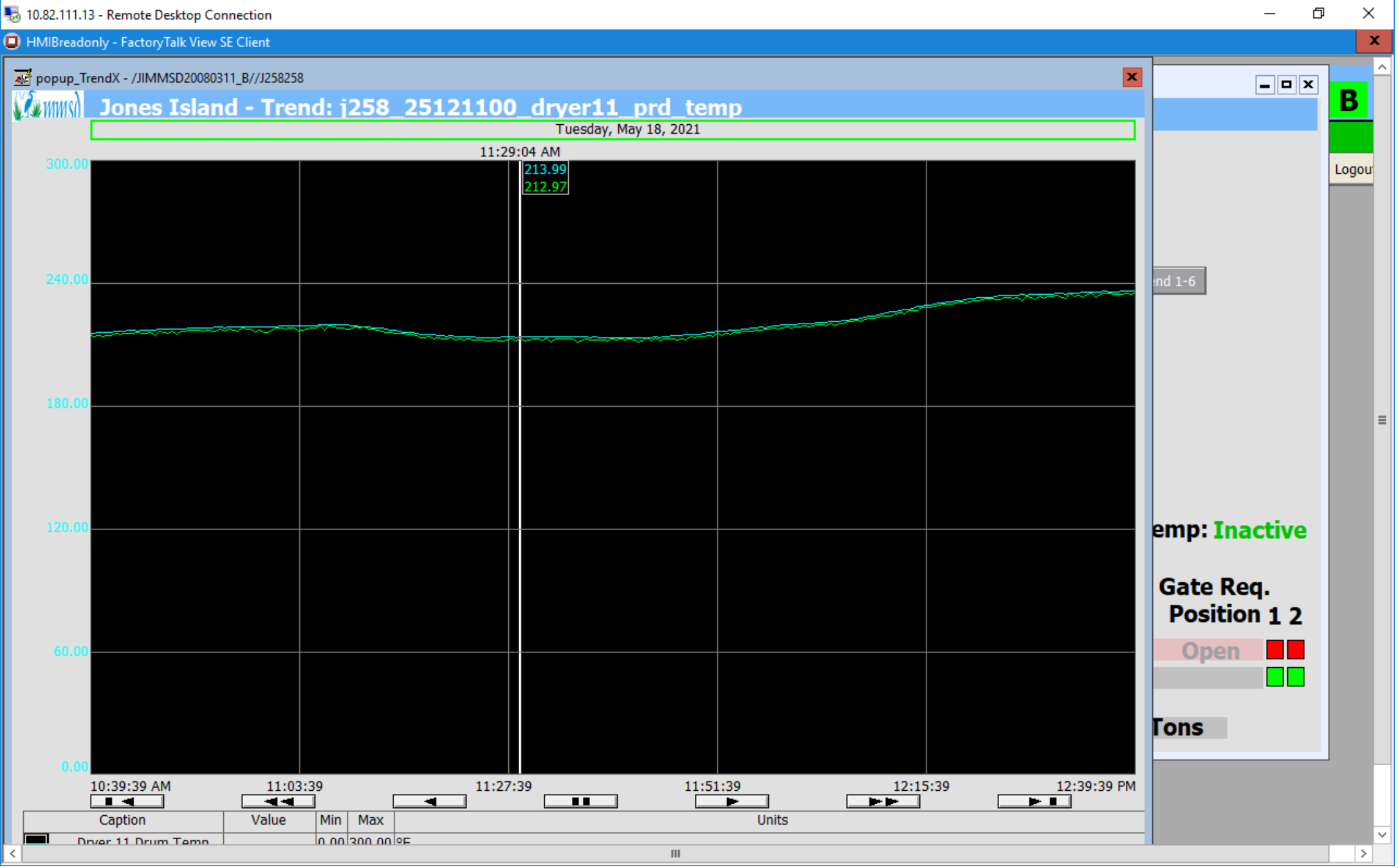


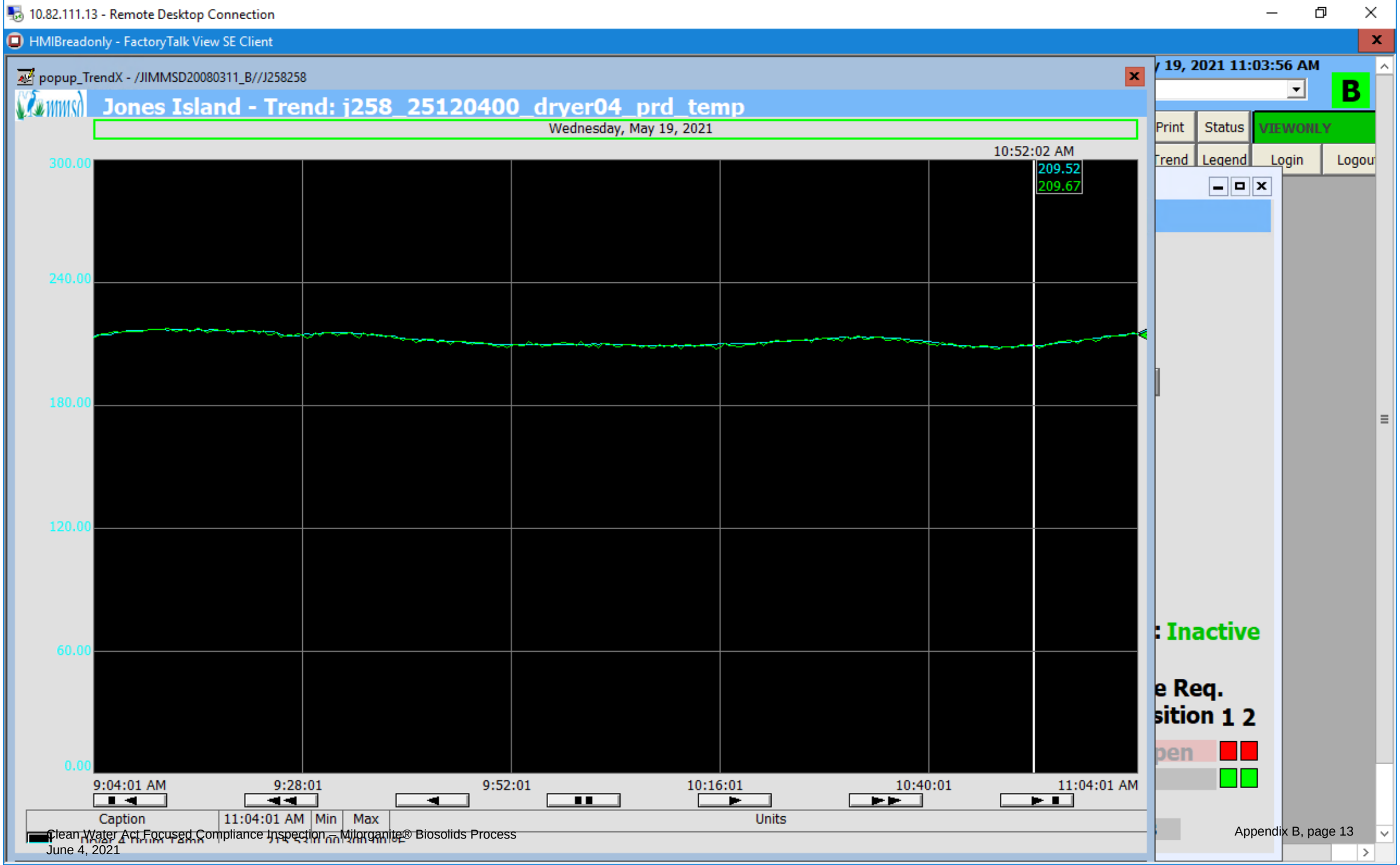
Dry Bulb Air Temps are more conservative measurements as compared to Product Temps

Drum Temp Probes






10.82.111.13 - Remote Desktop Connection
HMIBreadonly - FactoryTalk View SE Client
popup_TrendX - /JIMMSD20080311_B//J258258
Jones Island - Trend: j258_25121100_dryer11_prd_temp
Tuesday, May 18, 2021
11:29:04 AM
213.99
212.97
300.00
240.00
180.00
120.00
60.00
0.00
10:39:39 AM
11:03:39
11:27:39
11:51:39
12:15:39
12:39:39 PM
Caption
Value
Min
Max
Units
Dryer 11 Drum Temp
0.00
300.00
°F
B
Logou
nd 1-6
emp: Inactive
Gate Req.
Position 1 2
Open
Tons


10.82.111.13 - Remote Desktop Connection
HMIBreadonly - FactoryTalk View SE Client
popup_TrendX - /JIMMSD20080311_B//J258258
Jones Island - Trend: j258_25120400_dryer04_prd_temp
Wednesday, May 19, 2021
10:52:02 AM
209.52
209.67
300.00
240.00
180.00
120.00
60.00
0.00
9:04:01 AM
9:28:01
9:52:01
10:16:01
10:40:01
11:04:01 AM
Caption
11:04:01 AM
Min
Max
Units
19, 2021 11:03:56 AM
B
Print
Status
VIEWONLY
Trend
Legend
Login
Logou
: Inactive
e Req.
sition 1 2

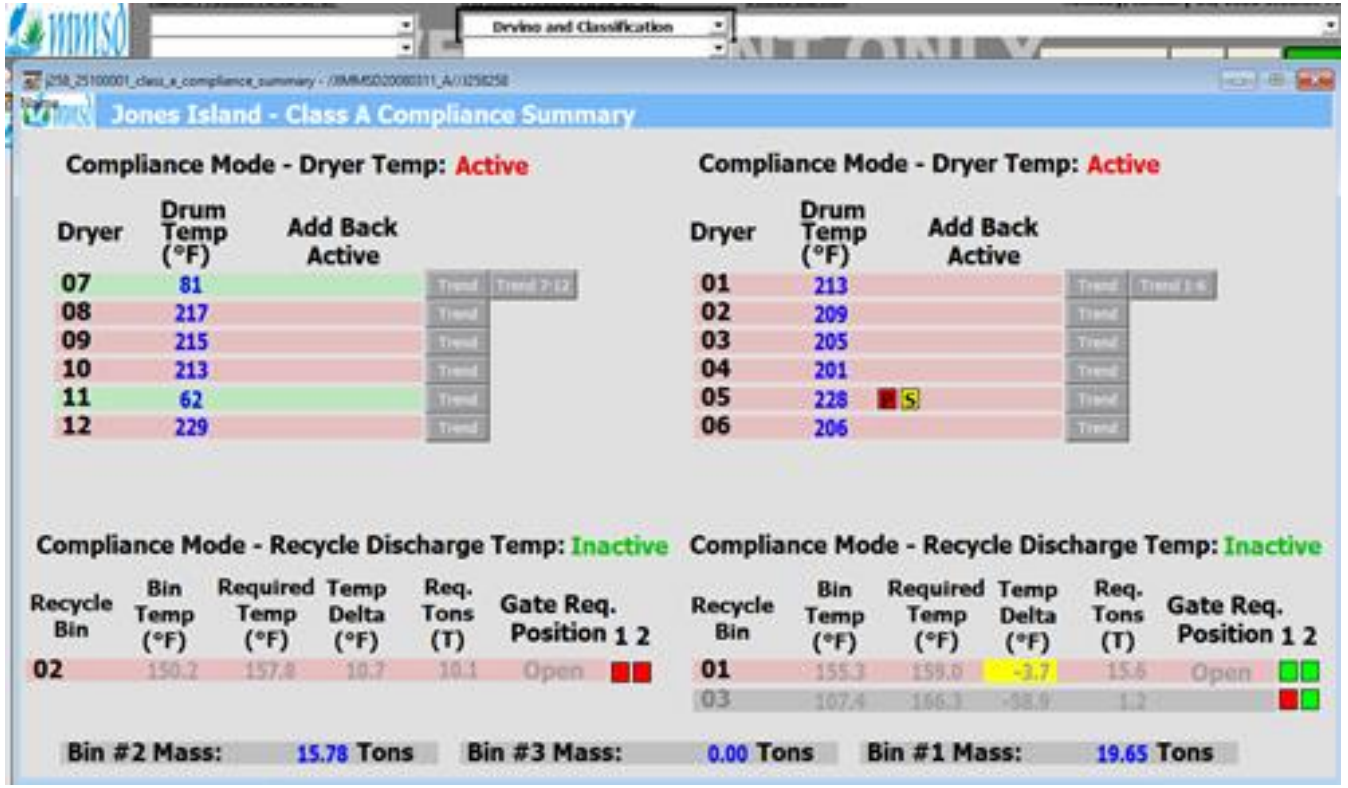
Primary (P) and Secondary (S) Temperature Sensors

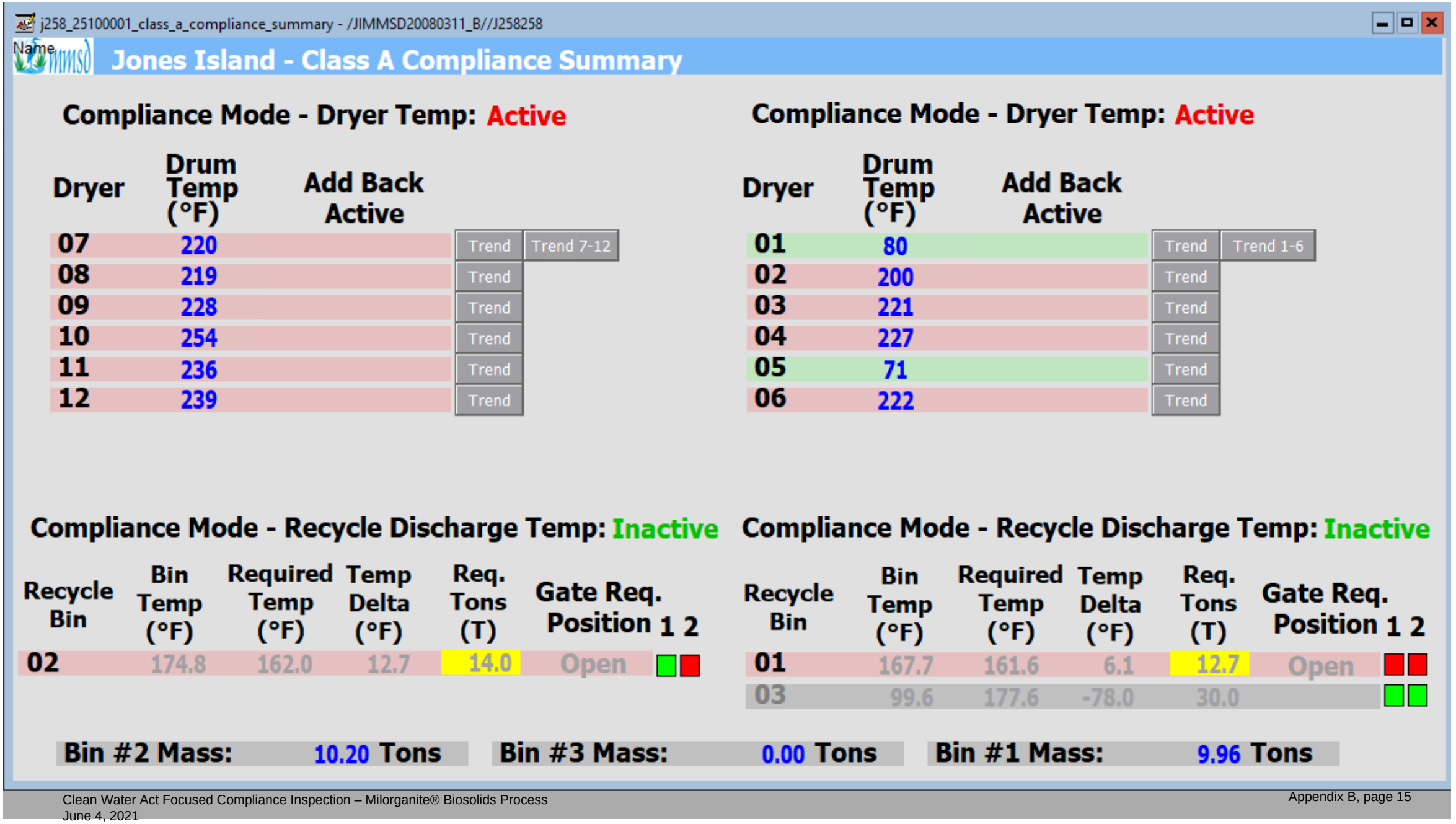
During normal operation (P) is used for compliance demonstration and nothing is displayed next to drum temperature.

Normal operation is defined as when the difference between temperatures measured from Primary and Secondary sensors (Δ T) is less than 10°F.

If Δ T is greater than 10°F, letters (P) and (S) will be displayed next to the drum temperature. Sensor being used will be highlighted Red and the other sensor will be Flashing Yellow highlight.

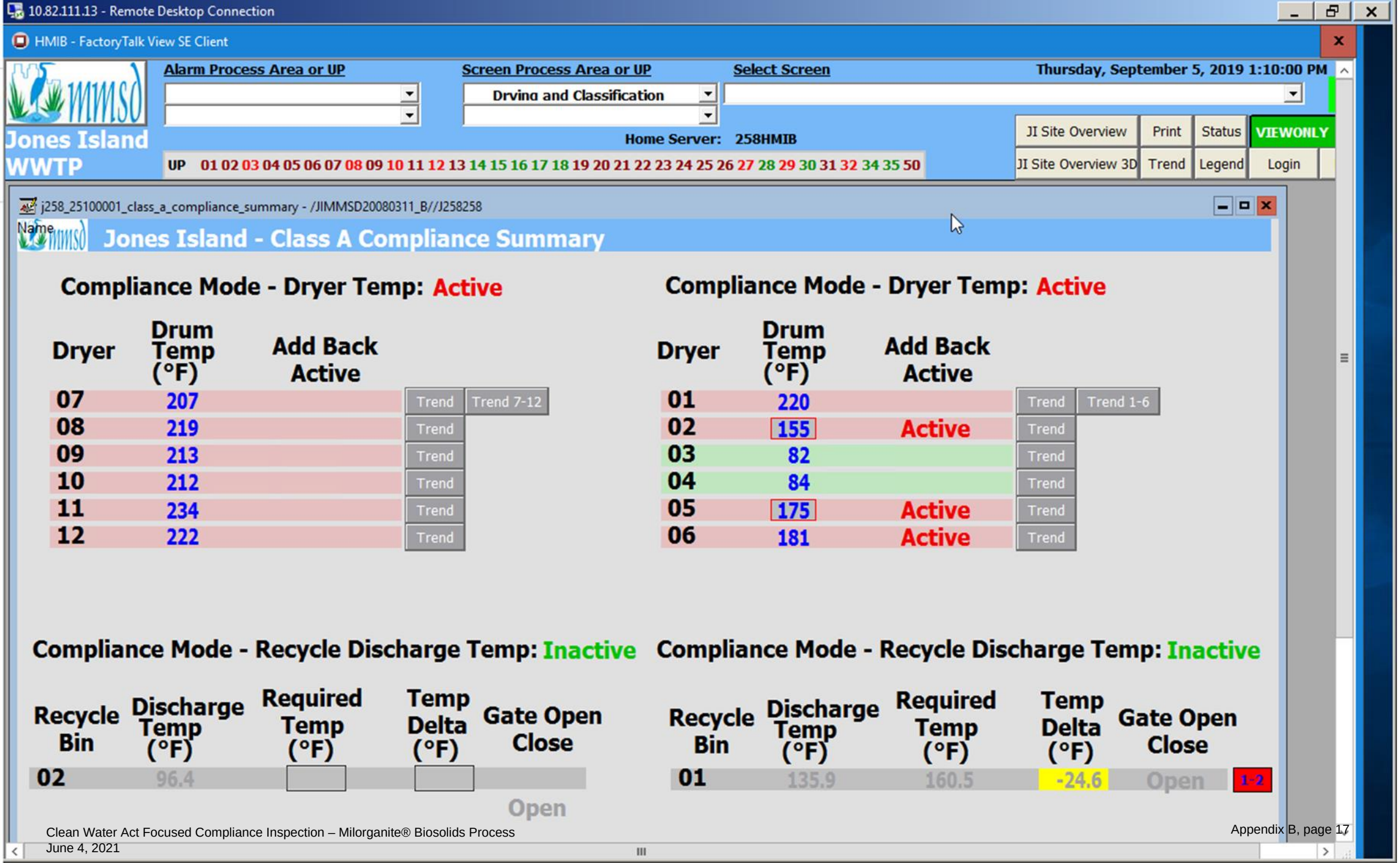
For example, PS indicates that sensor (S) is being used and (P) needs repair. Hence operator should put in the work request for sensor (P).




j258_25100001_class_a_compliance_summary - /JIMMSD20080311_B//J258258
Name
Jones Island - Class A Compliance Summary
Compliance Mode - Dryer Temp: Active
Dryer
Drum Temp (°F)
Add Back Active
07 220 Trend Trend 7-12
08 219 Trend
09 228 Trend
10 254 Trend
11 236 Trend
12 239 Trend
Compliance Mode - Dryer Temp: Active
Dryer
Drum Temp (°F)
Add Back Active
01 80 Trend Trend 1-6
02 200 Trend
03 221 Trend
04 227 Trend
05 71 Trend
06 222 Trend
Compliance Mode - Recycle Discharge Temp: Inactive
Recycle Bin
Bin Temp (°F)
Required Temp (°F)
Temp Delta (°F)
Req. Tons (T)
Gate Req. Position 1 2
02 174.8 162.0 12.7 14.0 Open
Compliance Mode - Recycle Discharge Temp: Inactive
Recycle Bin
Bin Temp (°F)
Required Temp (°F)
Temp Delta (°F)
Req. Tons (T)
Gate Req. Position 1 2
01 167.7 161.6 6.1 12.7 Open
03 99.6 177.6 -78.0 30.0
Bin #2 Mass: 10.20 Tons
Bin #3 Mass: 0.00 Tons
Bin #1 Mass: 9.96 Tons

Alarms and Warnings

	Normal Operation	Warning Alarm	Add-Back Mode ACTIVE	ALARM	When Add-Back ACTIVE Dryer Discharge Screw Starts IF
Dryer Drum Temperature	≥ 180°F	176°F < T < 180°F	<180°F	≤176°F	≥ 180°F for 5 min OR > 200°F
HMI Drum Temperature Data Display	**Blue Text**	Yellow	**"ACTIVE"**	FLASHING RED	**Blue Text**
Discharge Screw Temperature					> 220°F


10.82.111.13 - Remote Desktop Connection
HMIB - FactoryTalk View SE Client
Jones Island WWTP
Alarm Process Area or UP
Screen Process Area or UP
Drying and Classification
Select Screen
Thursday, September 5, 2019 1:10:00 PM
Home Server: 258HMIB
JI Site Overview
Print
Status
VIEWONLY
JI Site Overview 3D
Trend
Legend
Login
UP 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 34 35 50
j258_25100001_class_a_compliance_summary - /JIMMSD20080311_B//J258258
Name
Jones Island - Class A Compliance Summary
Compliance Mode - Dryer Temp: Active
Dryer
Drum Temp (°F)
Add Back Active
07 207 Trend Trend 7-12
08 219 Trend
09 213 Trend
10 212 Trend
11 234 Trend
12 222 Trend
Compliance Mode - Dryer Temp: Active
Dryer
Drum Temp (°F)
Add Back Active
01 220 Trend Trend 1-6
02 155 Active Trend
03 82 Trend
04 84 Trend
05 175 Active Trend
06 181 Active Trend
Compliance Mode - Recycle Discharge Temp: Inactive
Recycle Bin
Discharge Temp (°F)
Required Temp (°F)
Temp Delta (°F)
Gate Open Close
02 96.4 Open
Compliance Mode - Recycle Discharge Temp: Inactive
Recycle Bin
Discharge Temp (°F)
Required Temp (°F)
Temp Delta (°F)
Gate Open Close
01 135.9 160.5 -24.6 Open 1-2

Secondary Means of Demonstrating Compliance with 503 Regulation

Alternative I: Time-temperature Regime

Meet the Established relation between Detention Time and Product Temperature

Alternative 2: Biosolids Treated In A High Ph-high Temperature Process

Alternative 3: Biosolids Treated In Other Processes

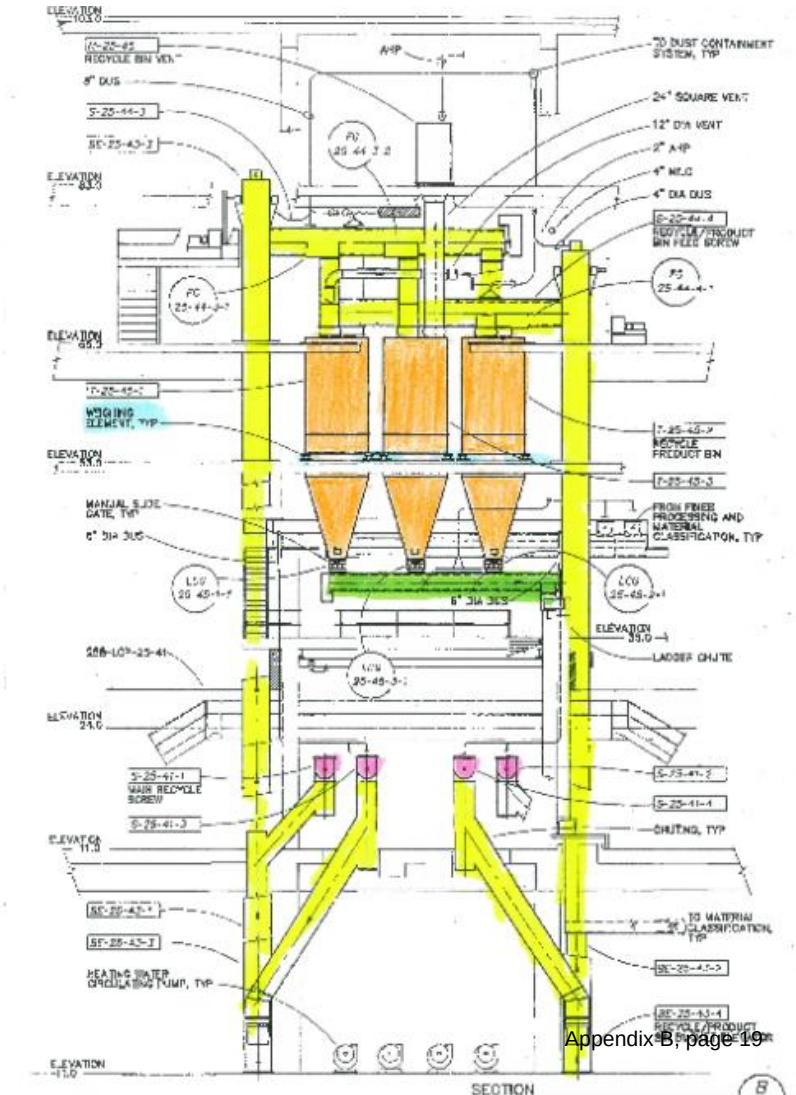
Alternative 4: Biosolids Treated In Unknown Processes

Alternative 5: Biosolids Treated In Processes To Further Reduce Pathogens Such As Heat Drying

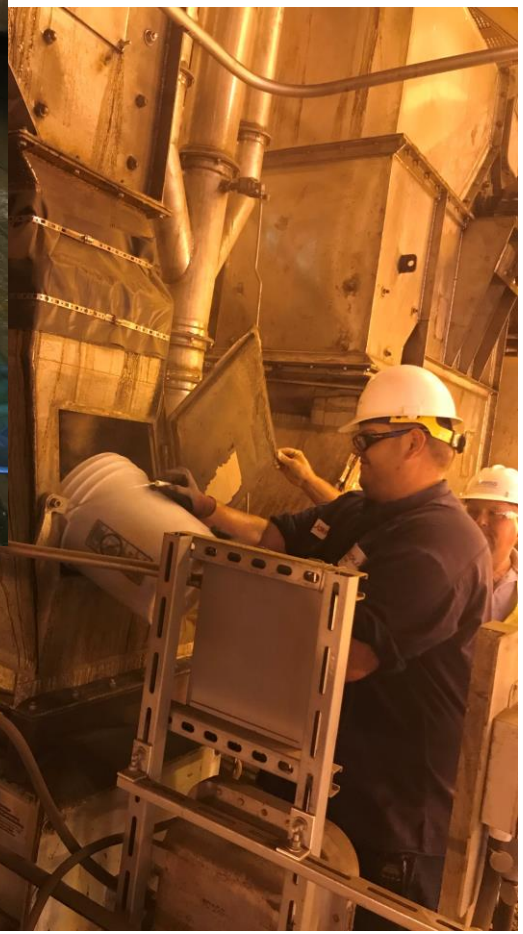
Alternative 6: Biosolids Treated In A Process Equivalent To A PFRP

Evaluation of Time Temperature Method

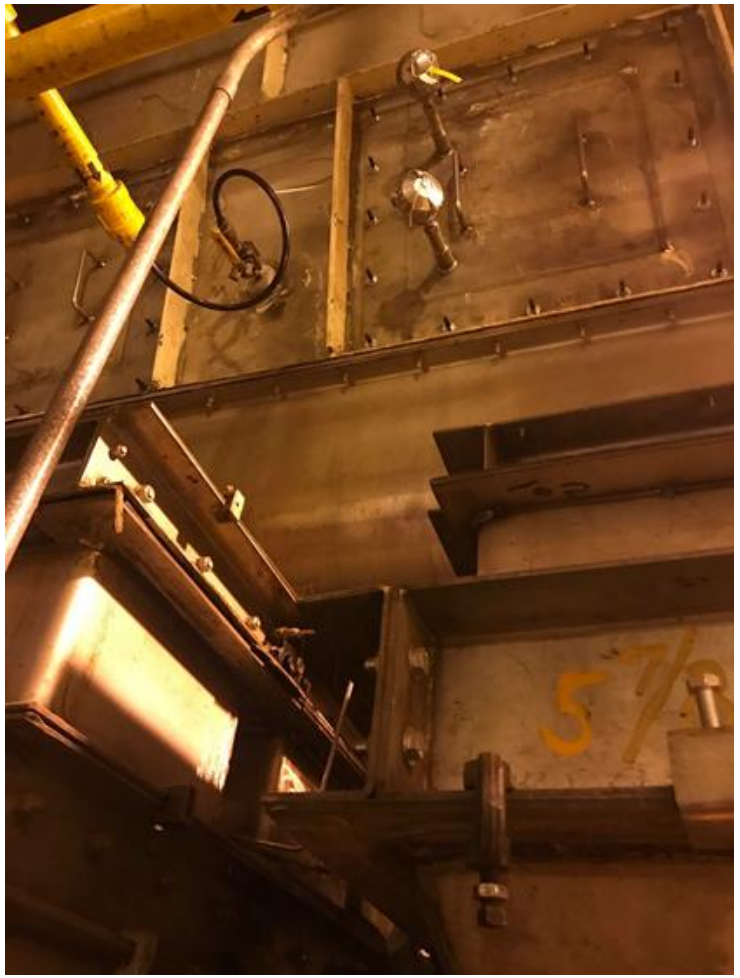
- 3 Recycle Bins with capacity of 30 tons each
- Product discharging the recycle bin approx. 160F
- Retention time in each recycle bin approx. 20 minutes



TRACER Study



Recycle Bin-Time Temperature



- 3 Temp Probes Installed in each Recycle Bin
- Using Existing Parameters monitored in HMI added calculation for Retention Time in Recycle Bin
- Used **Safety Factor** in Retention Time Calculation based on Continual evaluation of **Tracer Study**
- **Automatic closure of discharge gate** and recycle all material when time temperature requirements are not met
- Operator verify visually and document gate closed

Recycle Bin Display Data

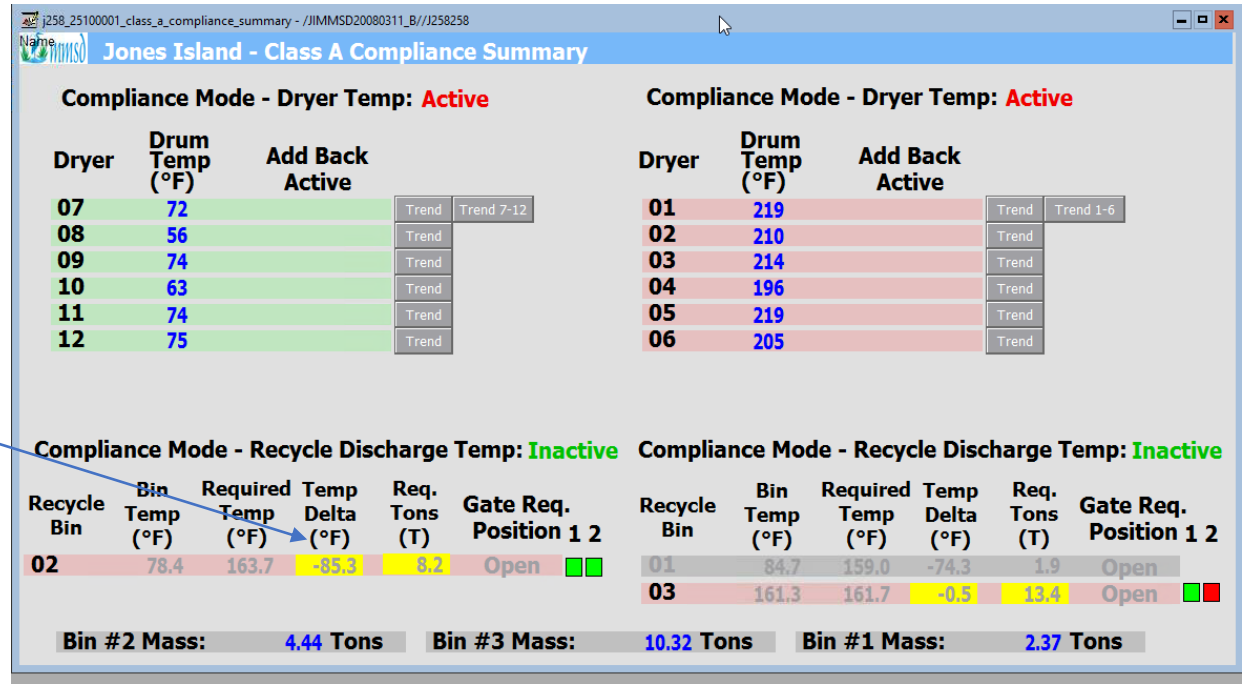
Recycle Bin Tonnage and Δ T (Required- Actual Bin Temperatures) display YELLOW, if minimum requirements for time-temperature are not being met.

Out of Service Bin Shown in Gray

Gate Requested Position

Actual Gate Position

Actual Bin Mass



Condition for Switching Compliance Mode – Dryer Temp to Recycle Bin Temp

1. In-service dryer Drum T ≤ 176 F and add-back fails to engage for that dryer for **15 seconds**

2. If Operator MANUALLY takes in-service dryer out of add back mode (manually starts discharge screw) and drum T ≤ 176°F
    - Dryers Getting too full
    - Temperature Rising

Recycle Bin Temp MODE for North or South Side

This Mode is Active per Side (North or South) when any Drum Temperature <176F for all Inservice Dryers which are not in Addback

Permit Conditions:

- Measured Recycle Bin Discharge Temperature should be > **Required Temperature** AND
- The detention time through the bin should be 20 minutes or greater.

"Required Temperature": Calculated based on the Detention Time in the Recycle bin. Higher the Detention Time in the Bin lower the required temperature and vice versa.

- Recycle Bin Discharge Temp > Required Temperature then gate to Classification is **OPEN** (Auto)
- Recycle Bin Discharge Temp < Required Temperature then gate to Classification is **CLOSED**(Auto) (**OPERATORS VERIFY THAT GATES ARE CLOSED**)

Alarms and Warnings

	Normal Operation	Warning Alarm	ALARM & Gates to Classification Closes	Outlet Gate Reopens
HMI Data	Blue Text	Yellow	FLASHING RED	Blue
Residence time			≤ 20 min OR	> 20 min AND
Bin Temp – Req Temp = Temp Delta (Δ T)	≥ 3°F	1°F ≤ ΔT < 3°F	< 1° F	≥ 3°F

*** NOTE: OPERATOR MUST PHYSICALLY VERIFY AND DOCUMENT THAT GATES TO CLASSIFICATION ARE CLOSED FOR THE NON-COMPLIANT BIN.**

Condition for Switching Compliance Mode- Recycle Bin Temp to Dryer Temp

- When in Recycle Bin Mode for North or South Side and the Dryer Drum Temperatures for all in-production dryers for that side become greater than 176°F, a countdown timer is displayed on HMI.

- When the timer countdowns 40 minutes, the compliance mode is switched to **Dryer Compliance Mode**.

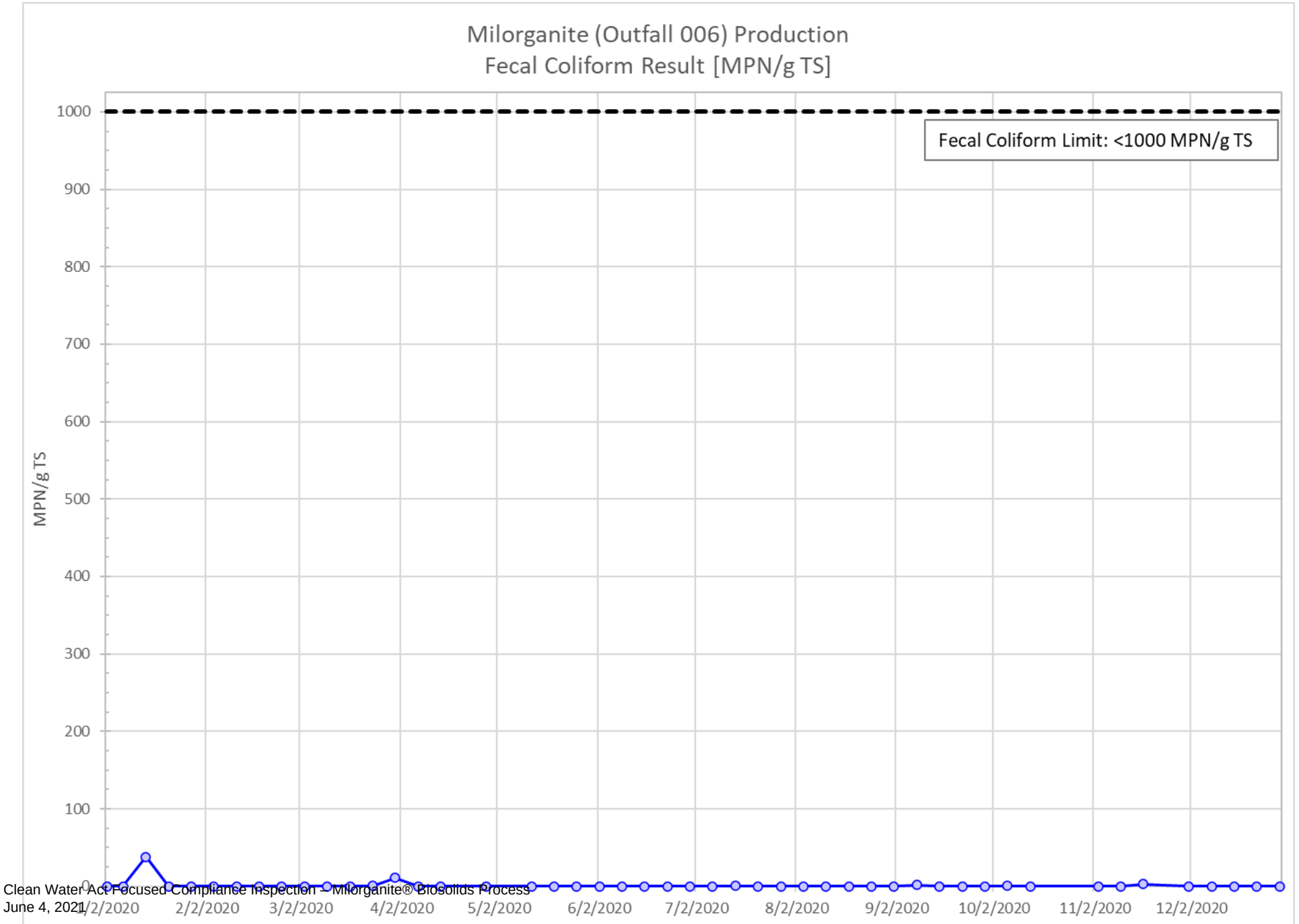
- Why 40 minutes?

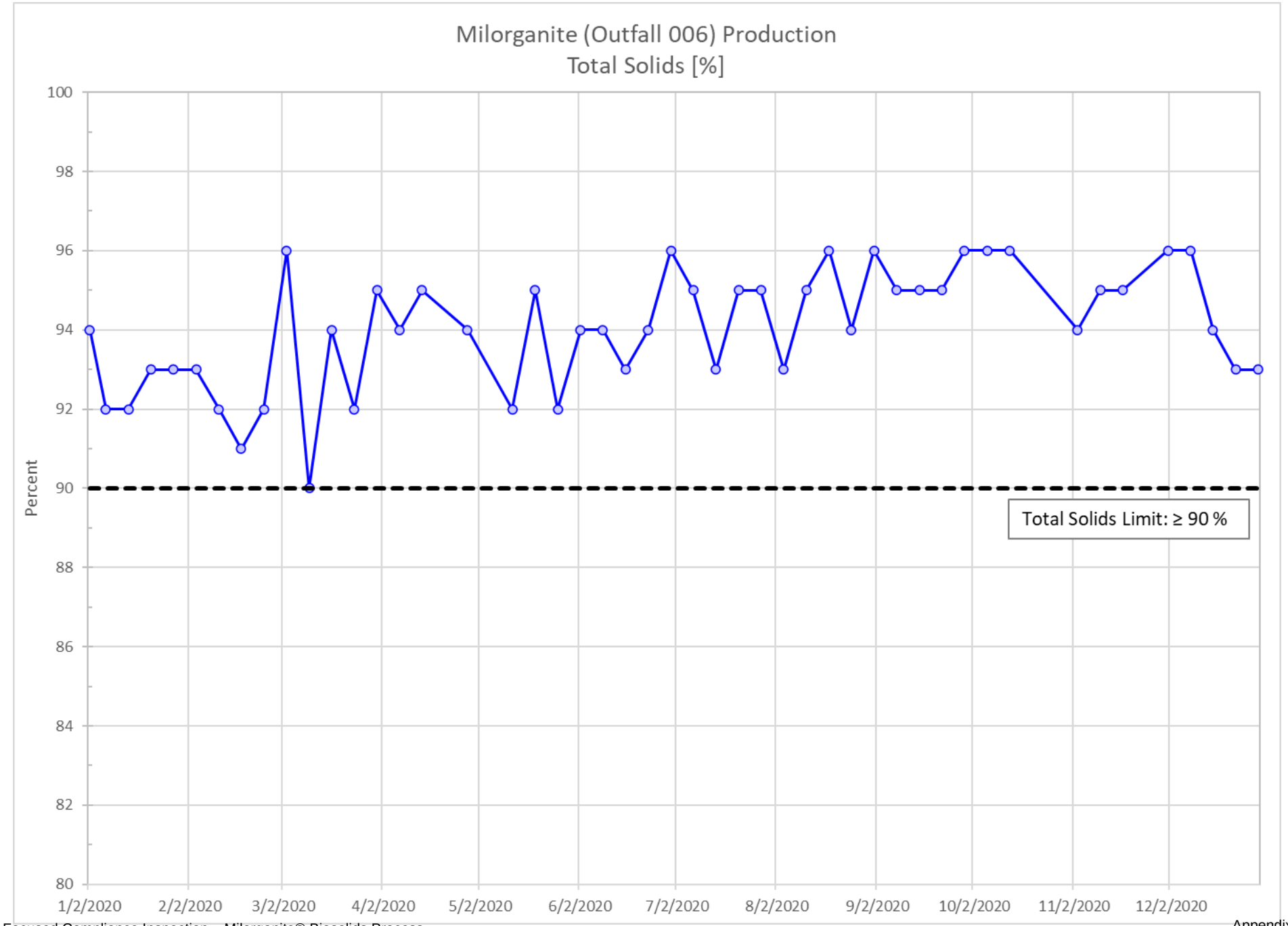
Construction Sequence

Activity	Completion Date
Dryer Drums	
Tested Accutech wireless transmitter for continuous temperature monitoring	7/26/2018
Installed first thermowell and temperature sensor (RTD) with wireless transmitter	10/16/2018
Installed thermowells and RTD with wireless transmitters in all 12 dryer drums	2/28/2019
Installed and tested two redundant RTDs with wireless transmitter	7/9/2019
Completion adding a dedicated power supply for each RTD receiver radio	10/8/2019
Ready to test second RTD installed in Dryers 6 and 12	10/25/2019
Completed installation of redundant RTDs in all 12 dryers	11/19/2019
Recycle Bins	
Installed and tested first hardwired RTD at a recycle bin	9/24/2018
Completed recycle bin tracer testing to establish residence time	10/22/2018
Installed and collecting data from two hardwired RTDs in each of three recycle bins	11/15/2018
Installed a third hardwired RTD in two active recycle bins	1/24/2019
Installed a third hardwired RTD in redundant recycle bin	4/5/2019
Completed rebuilding all 6 recycle bin outlet gates with SS material to ensure proper	8/31/2019
Installed and tested 6 inductive sensors to confirm recycle bin outlet gates "closed"	9/17/2019
Milorganite Temperature Monitoring and Compliance Programming	
Completed initial PLC programming to control processes to ensure compliance	12/21/2018
Revised control program to use dryer drum temperature as primary compliance meas	3/1/2019
Completed and tested programming to record compliance and place data to historian	3/22/2019
Installed compliance monitor in operator control room (HMI Screen)	4/5/2019
Programmed automatic addback at each dryer to recycle product if temp. <176^{o} F	4/26/2019
Implemented full operation of compliance program for Milorganite production	4/26/2019
Generated first monthly compliance report from Hach WIMS	5/3/2019
Added 20 min. minimum residence time to time-temperature compliance program	9/5/2019
Added 15 sec. delay when switching to time-temperature mode from dryer mode	9/20/2019
Completed programming for redundant RTDs at all 12 dryer drums	10/31/2019
Completed FATs for all compliance modes	2/14/2020
Training, O&M Manual and Spare Parts	
Completed initial operator and maintenance training on new system	11/28/2018
Completed operator training on revised compliance program	3/19/2019
Transferred first shipment of spare instrument parts to Veolia	5/30/2019
Anticipate completing second version of O&M manual	10/31/2019
Transferred final shipment of spare instrument parts to Veolia	12/10/2019
Conducted second training session with Veolia operators, 2 days	2/12/2020

Summary - April 2019 through Present

- All stored and shipped product complied with Dryer Temp or Time Temp
- Most of time complied with Dryer Temperature Mode
- Isolated events in Time Temp Mode
  - Example May 28, 2020
- One event – had to send product from day tank back to drying system
  - May 31, 2021
  - Communication failure caused gate control issue
- No events where we had to send product to landfill


Milorganite (Outfall 006) Production
Fecal Coliform Result [MPN/g TS]
Fecal Coliform Limit: <1000 MPN/g TS
MPN/g TS
1000
900
800
700
600
500
400
300
200
100
0
1/2/2020
2/2/2020
3/2/2020
4/2/2020
5/2/2020
6/2/2020
7/2/2020
8/2/2020
9/2/2020
10/2/2020
11/2/2020
12/2/2020


Milorganite (Outfall 006) Production
Total Solids [%]
Percent
100
98
96
94
92
90
88
86
84
82
80
1/2/2020
2/2/2020
3/2/2020
4/2/2020
5/2/2020
6/2/2020
7/2/2020
8/2/2020
9/2/2020
10/2/2020
11/2/2020
12/2/2020
Total Solids Limit: ≥ 90 %

Appendix C: March 2021 Monthly Report

Milorganite® Fertilizer
503 Regulation Class A
Compliance Monitoring Report

Milorganite® Fertilizer

503 Regulation Class A Compliance Monitoring Report

March 2021

Table of Contents

Section 1:

Milorganite® Solids and Fecal Coliform

Results at Various Outfalls

March 2021 Results

			Fecal Coliform		Solids, Total	
Sample Collection Date	Outfall	Sample Location	Limit MPN/gTS	Sample Results MPN/gTS	Limit % TS	Sample Results % TS
3/9/21	009	BAGGING - Kinder Morgan	*<1000*	**< 0.19**	*≥90*	**95**
3/9/21	009	BAGGING - Spring Valley	*<1000*	**< 0.19**	*≥90*	**95**
3/9/21	008	SHIPPING	*<1000*	**< 0.19**	*≥90*	**95**
3/2/21	006	PRODUCTION	*<1000*	**< 0.19**	*≥90*	**96**
3/9/21	006	PRODUCTION	*<1000*	**< 0.19**	*≥90*	**94**
3/16/21	006	PRODUCTION	*<1000*	**< 0.19**	*≥90*	**96**
3/23/21	006	PRODUCTION	*<1000*	**< 0.19**	*≥90*	**96**
3/30/21	006	PRODUCTION	*<1000*	**< 0.19**	*≥90*	**94**

Milorganite Production = 3,555 ton/month
Milorganite to Kinder Morgan Bagging = 3,574 tons/month
Milorganite to Spring Valley Bagging = 332 tons/month
Milorganite Shipped = 3,959 tons/month
Note:

- The results shown above are for grab samples collected at Outfalls 6, 8, 9 as defined in the WPDES Permit WI-0036820-04-0
- For all samples shown in the table above, fecal coliform analyses are started within 8 hrs. of collection, as per EPA Method 1680
- All Milorganite Produced in a month is not necessarily bagged or shipped the same month

Section 2:

Drying System Temperature Monitoring

Section 2.1: System Overview and Compliance Methodology

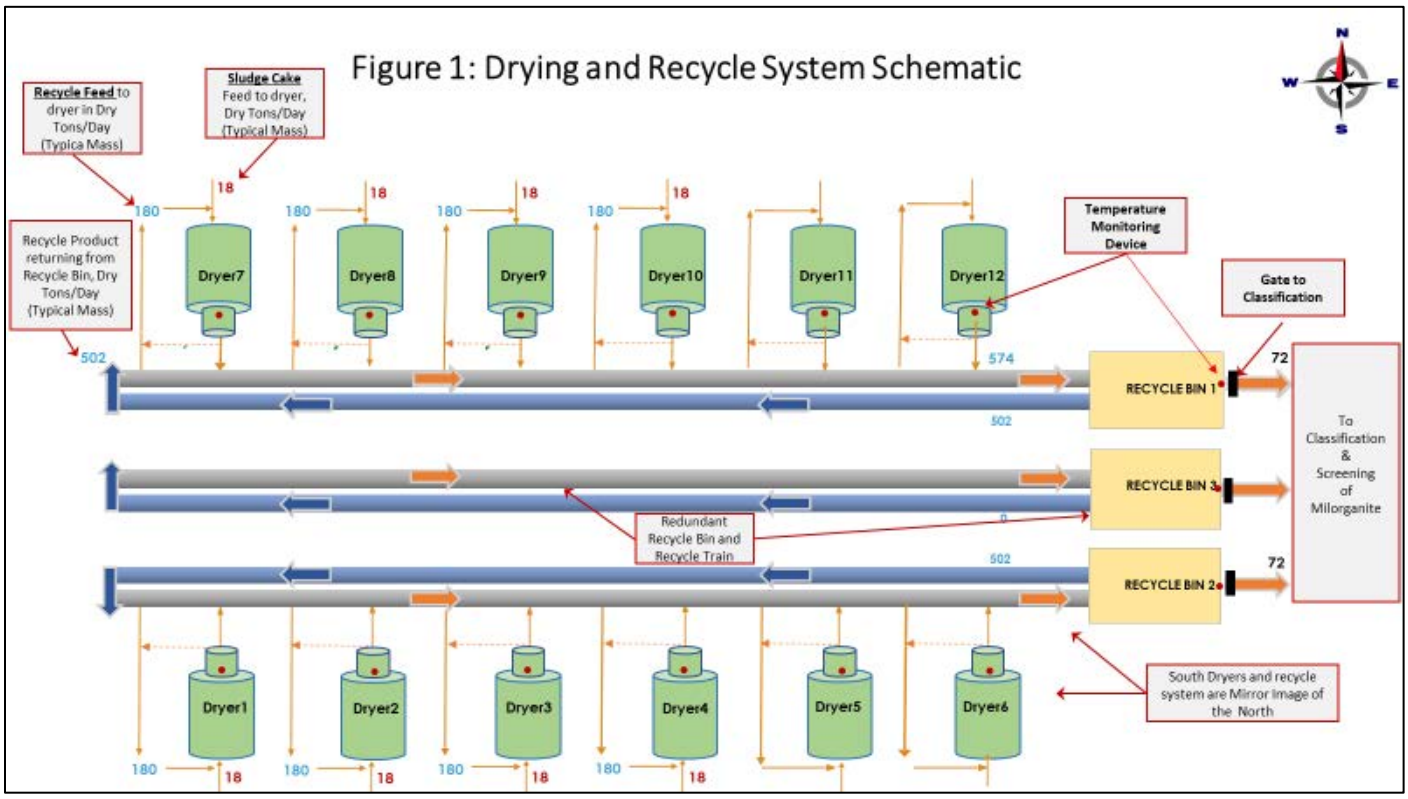
The drying system consists of twelve Dryers, three Recycle Bins and several product conveyors. The dryers are laid out in two groups - North Side Dryers (1-6) and South Side Dryers (7-8). A recycling bin is at the end of each group of six dryers. The third recycle bin acts is redundant can serve either side of dryers. From the recycle bin, most pellets return to the dryers. The remaining pellets go to classification and product screening. Figure 1 represents a typical day of biosolids processing with 8 dryers operating (4 Dryers on the North Side and 4 Dryers on the South Side) with Recycle Bins 1 and 2 routing dried product either back to the Dryers or allowing it to go to Classification and Screening through automatic slide gates. The dry mass values on Figure 1 represent annual averages as monitored over the years. Figure 1 also shows the approximate location of the temperature monitoring devices at the discharge end of each dryer and at the discharge end of the recycle bins.

Alternative 5-503.32(a)(7) and Appendix B.B.2 (Heat Drying) is used as the Primary means to demonstrate compliance with 503 Regulation for Class A. In this method, dryer discharge product temperatures for all in-production dryers stay above 176°F. Automatic controls in each dryer recycle all product leaving the dryer back to the inlet of the dryers if a discharge temperature falls below 180°F.

Alternative 1-503.32(a)(3) (Time Temperature) is used as the secondary means to demonstrate compliance. This method is only used when the primary method does not demonstrate compliance. In this method, we continuously monitor the recycle bin discharge temperature and determine detention time of the product through the recycle bin. The District continuously calculates a required temperature by using 40 CFR Part 503(a)(3) Equation 2 for the current detention time. Comparison between the measured recycle bin discharge temperature and the required temperature determines compliance with this method.

If a 15-minute dryer discharge product temperature is below 176°F at any individual dryer discharge, then the District will use recycling bin time and temperature to demonstrate pathogen reduction. Use of time and temperature will continue until 40 minutes after all in-drum product temperatures are higher than the required temperature. 40 minutes is the worst-case product travel time from the dryer farthest from the recycling bin to the outlet of the recycling bin. If time and temperature does not show compliance, then the gates between the recycle bin and classification & screening will automatically CLOSE. In this configuration, all product will return

to the dryers. These gates are reopened when time and temperature show compliance or Dryer discharge temperature show continuous compliance for at least 40 minutes. Figure 2 shows overall compliance strategy to demonstrate compliance with 503 Class A.



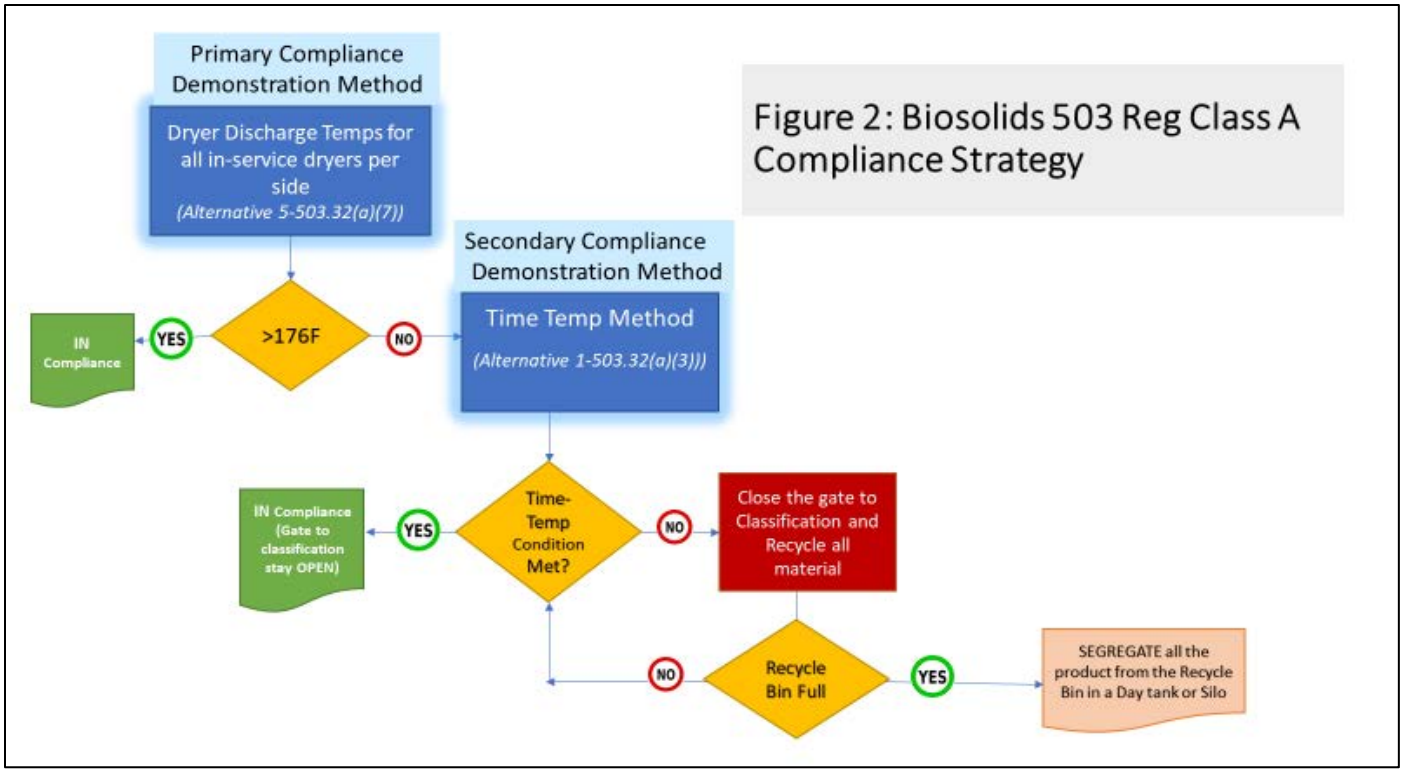


Figure 2: Biosolids 503 Reg Class A Compliance Strategy

Section 2.2 Operations Notes- March 2021

- Dryer discharge product temperatures for all in-production South Side Dryers (Dryers 1-6) were greater than 176°F for the entire month, except on March 3, 2021 at 04:29 hours for Dryer 1, and on March 14, 2021 at 05:01 hours for Dryer 3.
- Dryer discharge product temperatures for all in-production North Side Dryers (Dryers 7-12) were greater than 176°F for the entire month.

DRYER 1 – March 3, 2021, 04:29 hours

- At 04:29 hours on March 3, 2021 Dryer 1 was in add back mode due to product temperature less than 176F. The dryer was in add back mode for a prolonged time and eventually became too full. Hence the Operator manually took Dryer 1 out of add back mode. To take the dryer out of add back mode, the Operator manually turned on the discharge screw. At that time, the Dryer 1 discharge product temperature was below 176F, therefore the system controls for South Side Dryers immediately shifted to the Time Temperature mode for compliance demonstration. While in Time Temperature mode, the Recycle Bin discharge temperature and detention time did not meet the required conditions of Time Temperature. Hence the gates to classification and screening were automatically closed and all product was recycled back to the South Side Dryers. Additionally, Operators visually verified that no product was going to classification - the gates to classification were closed. About 1 hour later, Dryer 1 was in compliance and compliance strategy switched back to dryer discharge product temperature mode.

DRYER 3 – March 14, 2021, 05:01 hours

- At 05:01 hours on March 14, 2021 Dryer 3 was in add back mode due to product temperature less than 176F. It was in add back mode for a prolonged time and eventually became too full. Hence the Operator took Dryer 3 out of add back mode. To take the dryer out of add back mode, the Operator turned on the discharge screw. At that time, the Dryer 3 discharge product temperature was below 176F, therefore the system controls for South Side Dryers immediately shifted to the Time Temperature mode for compliance demonstration. When the south dryers first shifted to Time Temperature mode, the Recycle Bin discharge temperature and detention time did indeed meet the required conditions of Time Temperature. Hence the gates to classification and screening were initially left open as shown on Table #3. However, shortly after the shift to Time Temperature mode, the Recycle Bin temperature dropped and no longer met the required conditions of Time Temperature. Hence the gates to classification and screening automatically closed and all product was recycled back to the South Side Dryers. At this time, Operators visually verified that no product was going to classification while the gates to classification were closed. About 1 hour later, Dryer 3 was in compliance and compliance strategy switched back to dryer discharge temperature mode.

Section 2.3. Dryer Discharge Product Temperature Monitoring

Graph 1: South Side Dryer Discharge Product Temperature Monitoring

Graph 2: North Side Dryer Discharge Product Temperature Monitoring

Table 1: South Side Dryers Daily Minimum Discharge Product Temperatures

Table 2: North Side Dryers Daily Minimum Discharge Product Temperatures

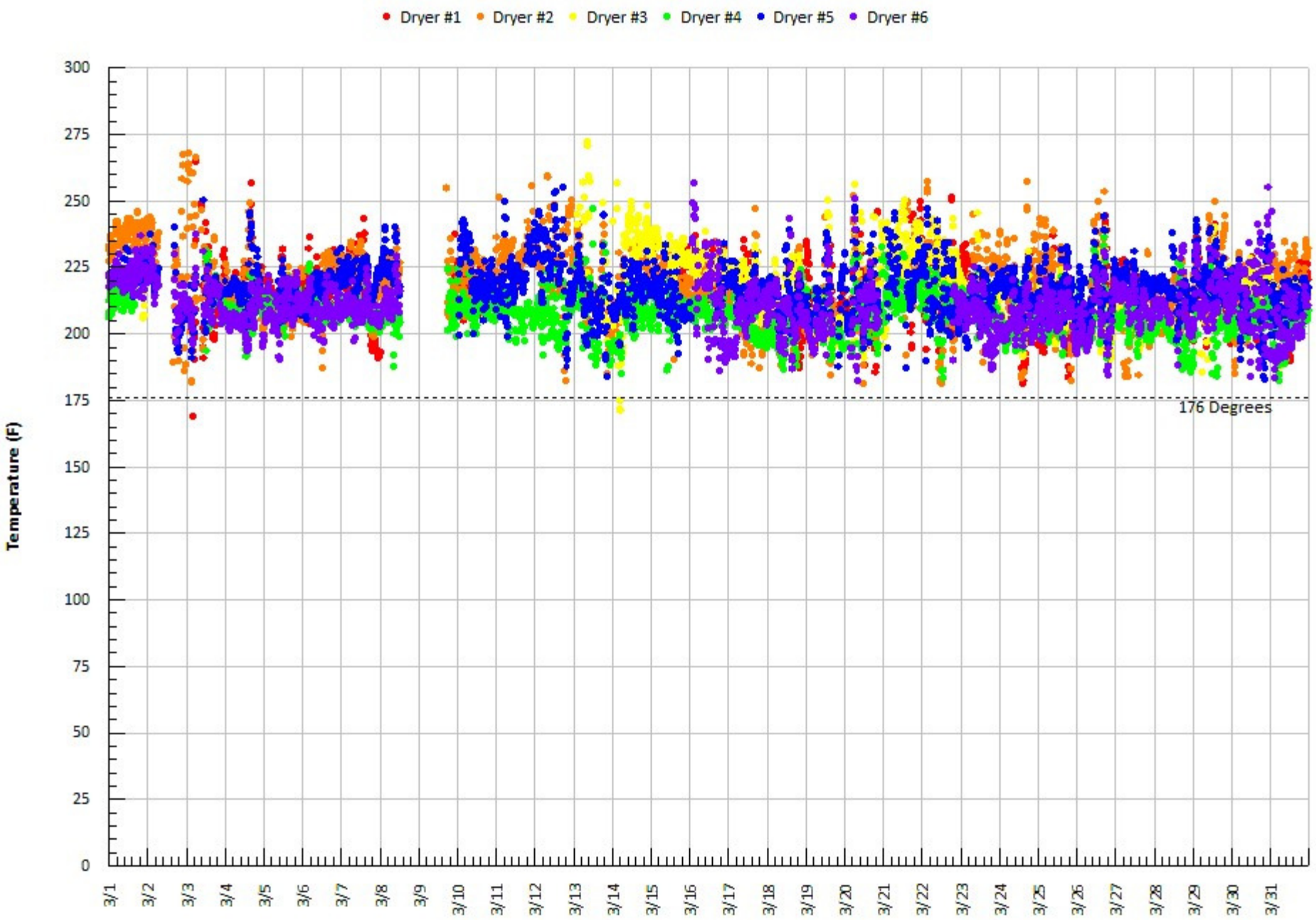
Notes for Graphs 1 and 2

1. Temperature data is presented on these graphs as 15-minute average when the dryer is in production.
2. Temperatures are measured at end of heated zone of each dryer by a resistance temperature detector (RTD) thermometer located in a thermowell on the interior wall of dryer drum.
3. Dryer discharge temperatures are measured continuously and are recorded in reporting database as a 15-minute average.
4. If any dryer temperature drops below 176°F, the system control shifts to time temperature control and the data is presented in the Time-Temperature Mode Table.

Notes for Tables 1 and 2

1. Data represents the daily minimum 15-minute average temperatures when the dryer was in production.
2. Blank spaces represent that the dryer was out of service the entire day.

Graph #1 - Dryer Discharge Temperature - South Dryers - March 2021



Graph #2 - Dryer Discharge Temperature - North Dryers - March 2021

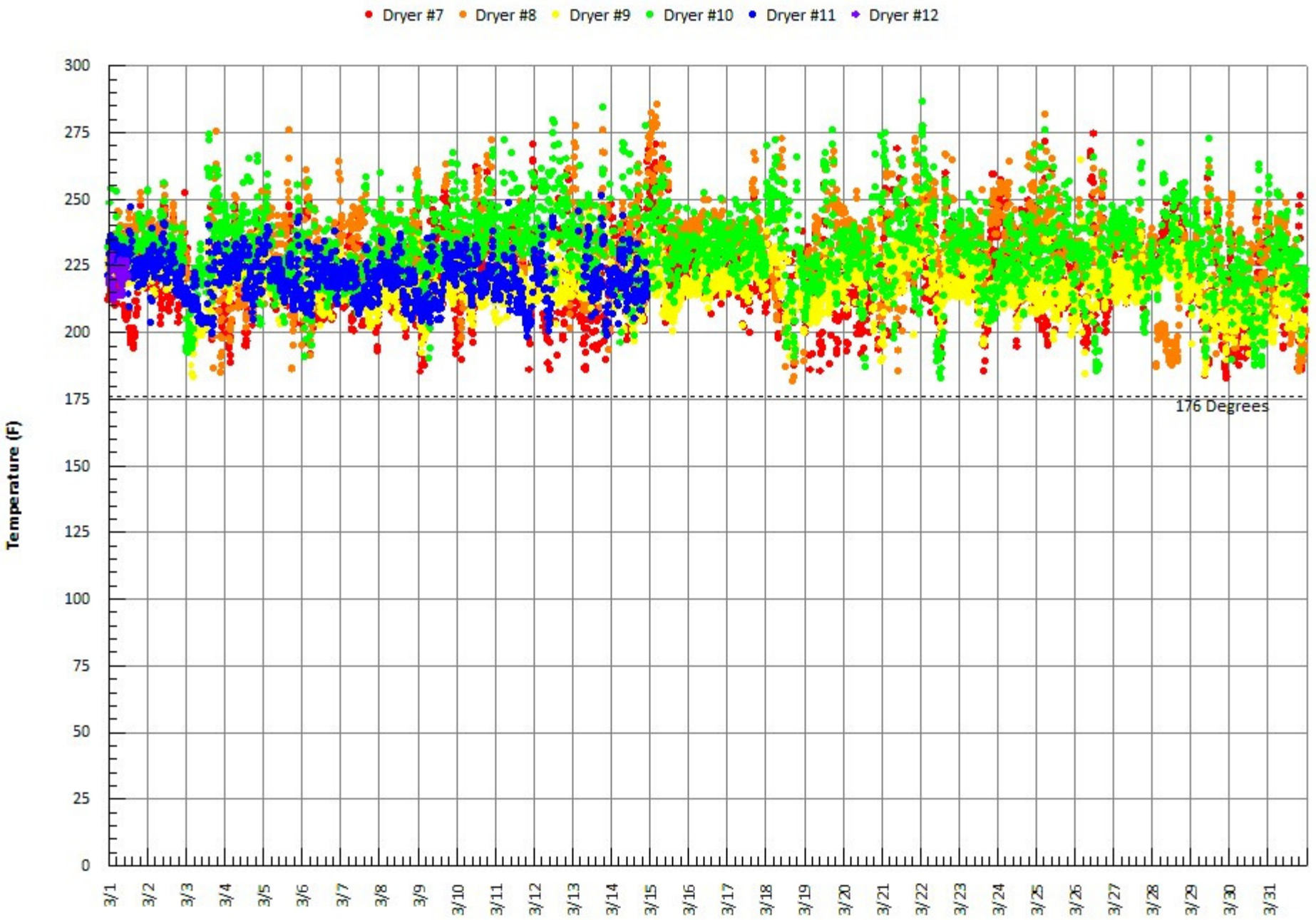


Table #1 Daily Minimum Discharge Product Temperature - South Dryers - March 2021

Daily Minimum Discharge Product Temperature

Date	Dryer #1	Dryer #2	Dryer #3	Dryer #4	Dryer #5	Dryer #6
03/01/21		227.30	205.62	205.93	213.85	213.41
03/02/21		185.81			192.83	196.88
03/03/21	**168.90**	181.85		193.44	190.54	195.95
03/04/21	205.39	201.82		191.42	202.30	193.22
03/05/21	202.98	198.77		199.01	203.72	190.16
03/06/21	203.68	187.11		202.87	205.25	196.66
03/07/21	190.54	198.87		200.32	210.28	197.18
03/08/21	191.99	210.35		187.70	214.09	203.53
03/09/21	217.11	206.36		198.58		
03/10/21	204.96	205.29		199.70	199.50	
03/11/21		214.73		196.69	198.92	
03/12/21		182.23		191.58	187.54	
03/13/21		184.97	190.89	187.93	183.90	
03/14/21		192.99	**170.78**	184.93	195.55	
03/15/21	201.10	190.09	204.95	186.15	192.32	
03/16/21	208.39	210.03	217.58	201.37	190.67	185.97
03/17/21	203.92	187.00	199.93	195.16	206.05	191.25
03/18/21	186.87	187.87	196.46	186.34	197.69	186.70
03/19/21	193.59	184.24	193.40	190.38	187.45	191.70
03/20/21	185.60	181.09	187.67	192.35	194.49	182.34
03/21/21	194.37	191.67	195.09	203.81	186.87	
03/22/21	193.61	181.25	206.66	183.12	189.70	189.97
03/23/21	217.72	189.27	203.23	198.23	194.63	186.40
03/24/21	180.99	183.07	191.03	193.17	204.81	194.95
03/25/21	183.51	182.29	195.66	191.94	203.40	193.30
03/26/21	193.74	194.51	189.56	190.62	195.81	184.48
03/27/21	199.48	183.71	193.25	191.05	208.92	199.70
03/28/21	198.37	199.67	199.28	185.61	197.43	188.09
03/29/21	194.98	202.53	185.21	183.85	209.19	200.64
03/30/21	197.37	185.05	209.14	198.21	182.77	183.63
03/31/21	189.45	203.68	187.71	182.05	190.88	183.34

Table #2 Daily Minimum Discharge Product Temperature - North Dryers - March 2021

Daily Minimum Discharge Product Temperature

Date	Dryer #7	Dryer #8	Dryer #9	Dryer #10	Dryer #11	Dryer #12
03/01/21	193.75	221.39	215.84	221.29	209.46	212.32
03/02/21	203.10	217.72	212.96	212.39	203.67	
03/03/21	196.99	185.00	183.28	192.20	199.14	
03/04/21	188.73	196.51	211.49	202.81	204.39	
03/05/21	203.12	186.21	202.83	203.69	208.29	
03/06/21	191.73	191.34	202.68	190.82	206.03	
03/07/21	192.66	209.01	201.76	206.91	206.58	
03/08/21	198.11	220.71	201.80	218.14	204.83	
03/09/21	185.12	210.76	192.31	190.29	203.64	
03/10/21	189.54	197.21	202.52	217.47	210.02	
03/11/21	186.06	212.22	203.62	218.75	198.27	
03/12/21	185.93	201.55	203.22	218.31	200.37	
03/13/21	185.94	193.50	203.62	199.85	198.76	
03/14/21	197.27	202.20	196.44	195.87	203.04	
03/15/21	203.66	220.13	200.40	210.95		
03/16/21	206.72	225.18	208.72	215.40		
03/17/21	199.96	227.77	202.72	216.28		
03/18/21	187.50	181.61	190.81	190.79		
03/19/21	185.52	192.54	199.87	204.42		
03/20/21	191.56	195.16	200.24	187.13		
03/21/21	198.84	185.62	189.31	197.45		
03/22/21	189.90	201.64	198.94	182.47		
03/23/21	185.31	195.66	195.33	204.06		
03/24/21	194.64	211.18	201.71	206.45		
03/25/21	195.05	211.34	197.09	207.83		
03/26/21	193.17	211.12	184.25	185.28		
03/27/21	209.01	221.78	211.05	199.58		
03/28/21	206.73	186.80	211.56	211.95		
03/29/21	182.93	191.56	184.11	190.59		
03/30/21	187.65	193.84	192.05	187.39		
03/31/21	185.94	185.32	196.32	192.79		

Section 2.4 Time Temperature Monitoring

Table 3: South Side Time-Temperature Mode Temperatures

Table 4: North Side Time-Temperature Mode Temperatures

Notes for Tables 3 and 4

1. Recycle bin discharge temperature is measured continuously and is reported on this table as a 15-minute average.
2. Time temperature control is active only if any dryer discharge temperature per side is less than 176°F.
3. No values in the table (blank table) represents that the dryer recycle system never went to Time Temperate Mode for the month
4. Once the time temperature control is active, it stays active for a minimum of 40 minutes.
5. Recycle bin discharge temperature is measured using RTD probes located in a thermowell on the interior wall of each recycle bin.
6. The required temperature is calculated with 40 CFR 503(a)(3) Equation 2
7. If the recycle bin discharge temperature is less than the required temperature, then gates to classification close and all product is recycled back to the dryers.
8. If the recycle bin discharge temperature is greater than required temperature, then gates to classification stay open and some product goes to classification and the remaining product is recycled back to the dryers.

Table #3 Time-Temperature Mode - South Dryers - March 2021

South Dryers (Recycle Bin #1 or Bin #3)

Date/Time	Required Temp.	Recycle Bin Discharge Temp	Gates to Classification	Recycle Bin Discharge To:
03/03/21 04:30	160.14	153.03	CLOSED	All Recycled to Dryers
03/03/21 04:45	159.67	152.46	CLOSED	All Recycled to Dryers
03/03/21 05:00	159.42	153.77	CLOSED	All Recycled to Dryers
03/03/21 05:15	159.13	157.67	CLOSED	All Recycled to Dryers
03/14/21 05:00	159.56	162.56	OPENED	Classification & Recycled to Dryers
03/14/21 05:15	159.50	160.28	CLOSED	All Recycled to Dryers
03/14/21 05:30	159.15	156.78	CLOSED	All Recycled to Dryers
03/14/21 05:45	158.85	158.66	CLOSED	All Recycled to Dryers
03/14/21 06:00	158.69	158.42	CLOSED	All Recycled to Dryers
03/14/21 06:15	158.43	157.82	CLOSED	All Recycled to Dryers

Table #4 Time-Temperature Mode - North Dryers - March 2021

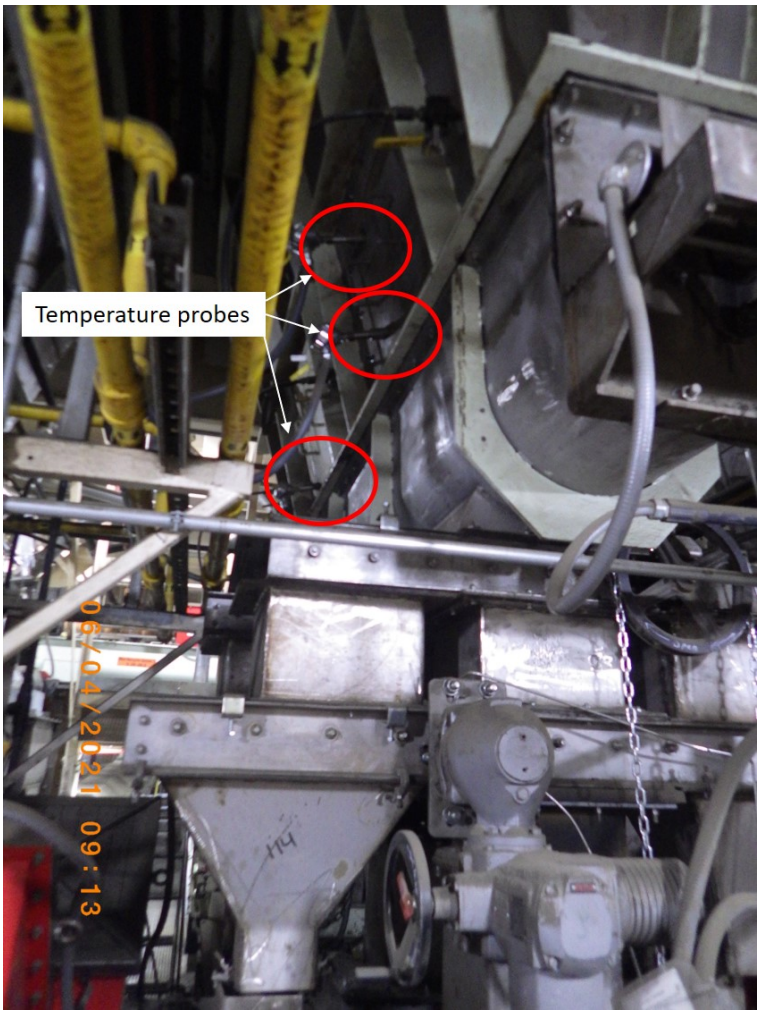
North Dryers (Recycle Bin #2 or Bin #3)

Date/Time	Required Temp.	Recycle Bin Discharge Temp	Gates to Classification	Recycle Bin Discharge To:

Appendix D: Photo Log

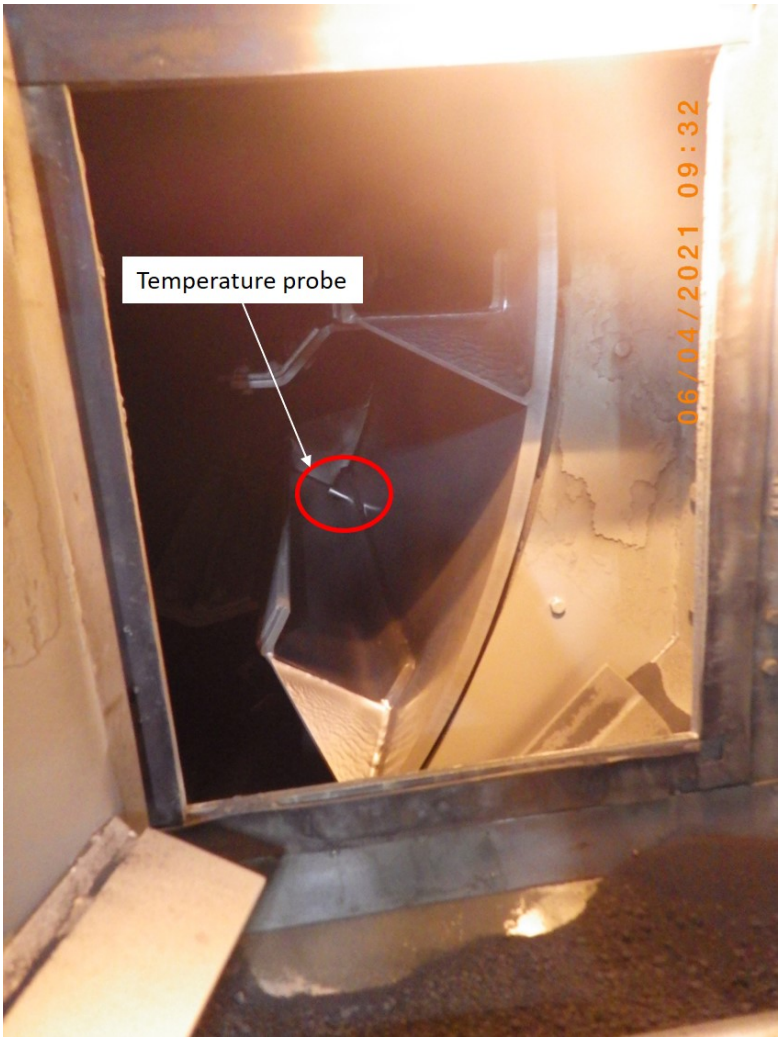
Milwaukee Metro Sewerage District (MMSD)
Jones Island Biosolids Dewatering and Drying Facility
EPA Inspection June 4, 2021
All photos taken by Dean Maraldo, Inspector, U.S. EPA
Camera: Ricoh WG-4

1: MMSD0001
Description: Overview of the south side dryers.
Location: MMSD D&D Facility
Camera Direction: 46°
Date/Time: June 4, 2021; 09:06



2: MMSD0002
Description: new temperature probes (3) in recycling bin #3.
Location: MMSD D&D Facility
Camera Direction: 349°
Date/Time: June 4, 2021; 09:13

3: MMSD0003
Description: Product composite sampler.
Location: MMSD D&D Facility (WPDES Permit Sampling Point (Outfall) 006 – Jones Island EQ Sludge – PRODUCTION).
Camera Direction: 70°
Date/Time: June 4, 2021; 09:24

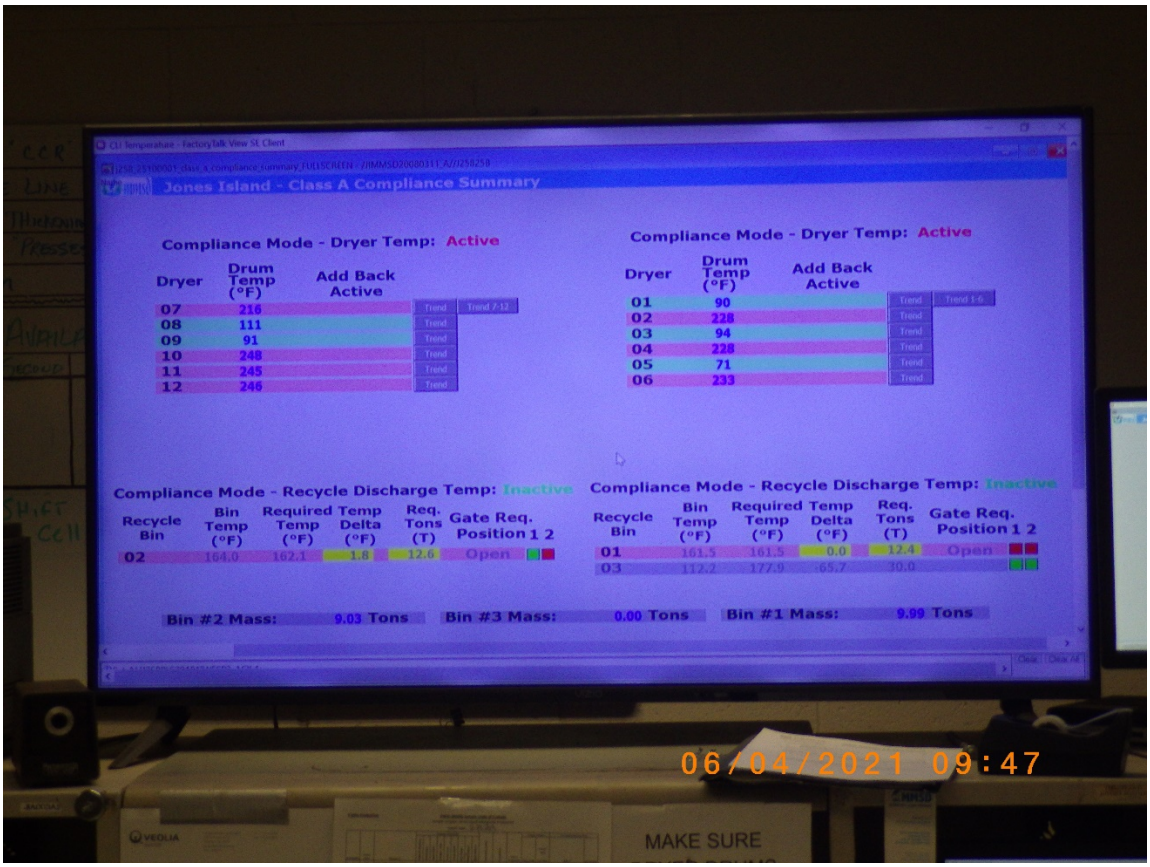


4: MMSD0004
Description: One of two new temperature probes installed within the Dryer #1S.
Location: MMSD D&D Facility
Camera Direction: 198°
Date/Time: June 4, 2021; 09:32

5: MMSD0005
Description: The same probe shown in Photograph MMSD0004, viewed from outside of Dryer #1S.
Location: MMSD D&D Facility
Camera Direction: 105°
Date/Time: June 4, 2021; 09:34

6: MMSD0006
Description: The former temperature compliance monitoring location (vicinity of steel plate on the screw chute), measured approximately 19 feet away from dryer furnace outlet along dryer discharge screw chute.
Location: MMSD D&D Facility
Camera Direction: 262°
Date/Time: June 4, 2021; 09:40

7: MMSD0007
Description: D&D Facility operations control center.
Location: MMSD D&D Facility
Camera Direction: 240°
Date/Time: June 4, 2021; 09:46



8: MMSD0009
Description: Close up view of dryer temperature control screen.
Location: MMSD D&D Facility
Camera Direction: 251°
Date/Time: June 4, 2021; 09:47

9: MMSD0010
Description: Silo load out belt conveyor and product auto sampler.
Location: MMSD Silo Load Out Facility
Camera Direction: 232°
Date/Time: June 4, 2021; 10:05

Appendix E: Documents Received

- MMSD LAB-031 Quantification of Fecal Coliform and Total Coliform Bacteria in Biosolids by MPN REV.0.4 (1/4/2021)
- Thermowell and Bosset Fitting Data Form
- Wireless Transmitter Equipment Data Form

MMSD CENTRAL LABORATORY
STANDARD OPERATING PROCEDURES

Quantification of Fecal Coliform and Total Coliform Bacteria in Biosolids by the Most Probable Number Method

Procedure Number:	LAB-031	Creation Date:	10/20/2017	Revision Date:	1/4/21
Prepared By:	Kori Kenney		Reviewed By:	J. Nanes, L. Fischer	
Laboratory Manager Approval:			Quality Assurance Specialist Approval:		
Alfredo DeTomaso, Jr			Kim M Walden		

PROPRIETARY INFORMATION STATEMENT

This document has been prepared by MMSD solely for its use and the use of MMSD's customers in evaluating its qualifications and capabilities in connection with a particular project.

TABLE OF CONTENTS

1.0 IDENTIFICATION THE METHOD

1.1 This method can be used to determine the fecal coliform (FC) and total coliform (TC) density in Class A and Class B biosolids. The method describes analysis of biosolids by two procedures: LTB/EC procedure by EPA Method 1680 and an adaptation of method 9221 B Standard Total Coliform Fermentation Technique from the *Standard Methods for the Examination of Water and Wastewater* (2006) to estimate coliform density of both fecal and total respectively.

2.0 APPLICABLE MATRIX OR MATRICES

2.1 This method covers the determination of fecal coliform and total coliform in biosolid samples.

3.0 LIMITS OF DETECTION AND QUANTIFICATION

3.1 The limit of detection and quantification for this method is determined by the lowest most probable number (MPN) index value 0.1803 divided by the percent total solids (TS) of a sample expressed as a decimal.

4.0 SCOPE AND APPLICATION

4.1 This method may be used for the successive determination of fecal coliform and total coliform or each coliform type individually.

4.2 This method describes the multiple-tube fermentation procedure (also called MPN) using culture specific media and elevated temperatures to isolate and enumerate coliforms from biosolid samples using the EPA MPN table. Fecal coliform and total coliform results are reported as MPN/g TS by a dry weight basis.

4.3 Total coliform density is expected to correlate with the probability of fecal coliform presence. Fecal coliform density is expected to correlate with the probability of pathogen presence.

4.4 EPA Method 1680 is adapted from 9221E in *Standard Methods for the Examination of Water and Wastewater* (1994) for fecal coliform density enumeration and is designed to meet the survey and monitoring requirements of the EPA in regulating the use and disposal of biosolids under 40 CFR Part 503 Subpart D.

5.0 SUMMARY OF METHOD

5.1 Fecal coliform and total coliform densities of Class A and Class B biosolids can be determined by the MPN procedure.

5.2 EPA Method 1680 provides for the enumeration of fecal coliforms in Class A and Class B biosolids by the LTB/EC MPN procedure.

5.3 An adaptation of Standard Method 9221 B (2006) provides for the enumeration of total coliforms in Class A and Class B biosolids by the MPN procedure.

5.4 A weighed sample is combined with peptone dilution water and homogenized.

5.5 Lauryl Tryptose Broth (LTB) is used as a presumptive media as required for optimum recovery of both fecal coliform and total coliform. LTB sample tubes are incubated at 35.0°C ± 0.5°C. Inverted tubes are assessed for growth and gas after 24 ± 2 hours. Simultaneous inoculation of positive LTB tubes first to EC broth for fecal coliform and then Brilliant Green Bile (BGB) broth for total coliform takes place after the prior enrichment step. Negative LTB sample tubes are returned to the same incubation conditions for an additional assessment and any positive LTB tubes are transferred at 48 ± 3 hours to EC and BGB broth.

5.6 Positive results in LTB, EC, and BGB broth are characterized by the presence of growth and gas in the inverted tube.

5.7 Negative results in LTB, EC, and BGB broth are characterized by the lack of gas in the inverted tube.

5.8 EC broth sample tubes are incubated in a water bath at 44.5°C ± 0.2°C. Positive tubes are scored on the bench sheets as a "+" and are used for the enumeration of fecal coliform presence in the biosolid sample tested. Negative tubes are scored on the bench sheets as a "-".

5.9 BGB broth sample tubes are incubated at 35.0°C ± 0.5°C. BGB sample tubes are assessed for growth and gas in the inverted tubes after 24 ± 2 hours. Positive tubes are scored on the bench sheets as a "+" and are used for the enumeration of total coliform presence in the biosolid sample tested. Negative BGB sample tubes are returned to the same incubation conditions for an additional assessment of positive tubes at 48 ± 3 hours. Negative tubes are scored on the bench sheets as a "-".

5.10 Neither EC nor BGB broth may be used to directly isolate fecal or total coliform from a biosolid sample.

5.11 Total solids determination by SM 2540 G (1997) is performed on a portion of the biosolids sample being tested for fecal and total coliform and is used to calculate MPN/g TS (dry weight basis).

6.0 DEFINITIONS

6.1 Refer to the glossary in the current revision of the Laboratory Quality Manual (LQM) for additional information.

6.2 Fecal coliform bacteria: Gram-negative, non-spore-forming rods that are found in the intestines and feces of humans and other warm-blooded animals. In this method, fecal coliform are those bacteria that grow and produce gas in LTB within a total of 48 ± 3 hours after incubation at 35°C ± 0.5°C, and that subsequently ferment lactose and produce gas within 24 ± 2 hours in EC broth after incubation at 44.5°C ± 0.2°C. Since coliforms from other sources often cannot produce gas under these conditions, this criterion is used to define the fecal component of the coliform group.

6.3 Total coliform bacteria: Facultative anaerobes, gram-negative, non-spore forming, rod shaped bacteria that ferment lactose. In this method, total coliforms are those bacteria that grow and produce gas in LTB broth within a total of 48 ± 3 hours after incubation at 35°C ± 0.5°C, and that subsequently ferment lactose and produce gas within 48 ± 3 hours in BGB broth after incubation at 35°C ± 0.5°C.

6.4 Class A biosolids: Biosolids that contain a fecal coliform density below 1,000 MPN/g of TS (dry weight basis).

6.5 Class B biosolids: Biosolids that contain a geometric mean fecal coliform density of less than 2×10^6 MPN/g of TS (dry weight basis).

6.6 Most probable number (MPN) method: A statistical determination of the number of bacteria per weight or volume of sample. It is based on the fact that the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in a dilution series.

6.7 Selective media: A culture media designed to suppress the growth of unwanted microorganisms and encourage the growth of desired ones.

6.8 Solid samples: Samples that generally contain greater than seven percent total solids.

7.0 INTERFERENCES

7.1 Since the MPN tables are based on a Poisson distribution (random dispersion), if the "homogenized" samples are not adequately shaken the bacterial cells will clump and the MPN value will be a misrepresentation of the actual bacterial density.

7.2 If the proper sample collection procedure is not followed there will be a misrepresentation of the bacterial densities of the final product.

7.3 If the sampling containers, media, or any equipment and glassware is not adequately sterilized prior to use contamination may cause a misrepresentation of bacterial densities.

8.0 SAFETY

8.1 Procedures must be performed in accordance with the MMSD Chemical Hygiene Plan.

8.1.1 Work surfaces are cleaned with bench top disinfectant at least twice per workday, and after any spill of concentrated viable cultures.

8.1.2 Mouth pipetting is prohibited.

8.1.3 All procedures are performed carefully to minimize the creation of aerosols. Pipets are not to be "blown-out".

8.2 Personal protective equipment, such as eye protection (must satisfy ANSI Z87.1), laboratory coat, hearing protection, and appropriate gloves must be worn according to the requirements of the particular job function. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

8.3 Smoking, eating and drinking are prohibited in the laboratory.

8.4 The health and safety hazards of many of the chemicals used in the procedure have not been fully defined. Additional health and safety information can be obtained from the Safety Data Sheets (SDS) maintained in the laboratory.

8.4.1 Handling of concentrated bacterial cultures occurs in this test. Good microbiological laboratory practices must be followed. These would include washing hands before

and after analysis, keeping hands away from mouth and eyes during analysis, and proper disposal of pipets, used tubes, and bottles.

8.4.2 All samples, media, cultures, and equipment used in the analysis should be treated as if they contain pathogenic organisms. All media, cultures, and pipets used in the analysis must be autoclaved prior to disposal.

8.4.3 All work must be stopped in the event of a known or potential compromise to the health and safety of employees. The situation must be reported immediately to a laboratory supervisor. Work surfaces are decontaminated before and after analysis, and after any spill of concentrated viable cultures. If a spill of a concentrated bacterial culture occurs the area should be saturated with benchtop disinfectant and allowed to sit for five minutes, minimum. Using gloved hands, discard the paper towel and clean the benchtop top with bench top disinfectant.

8.5 Flame sterilization of an inoculating loop is part of this method. Take steps to guard against fire, including:

8.5.1 Keep flammable items away from flame.

8.5.2 Locate nearest fire-arresting equipment prior to analysis.

9.0 EQUIPMENT AND SUPPLIES

9.1 Pipets: 1.0 mL and 10 mL, wide-bore 10 mL, sterile, T.D.

9.2 20 x 150 mm test tubes, Pyrex, or equivalent, with autoclavable caps

9.3 16 x 150 mm test tubes, Pyrex, or equivalent, with autoclavable caps

9.4 10 x 75 mm insert tube

9.5 Pipetting bulb or pipetting aid

9.6 Dilution bottles containing 99 ± 2 mL sterile peptone dilution water

9.7 Test tube racks

9.8 Jacketed incubator capable of maintaining 35.0 ± 0.5°C

9.9 Water bath capable of maintaining 44.5 ± 0.2°C

9.10 Autoclave capable of maintaining 15 psi in the chamber, with appropriate controls

9.11 pH meter

9.12 Automated pipettor/dispenser

9.13 Inoculating loop, 3 mm diameter, nichrome alloy, 26 B & S gauge

9.14 Fisher burner or equivalent

9.15 Top loading balance, capable of accurately weighing to the closest 0.1 g over a range of 10 g to 150 g

9.16 Large (4" x 4") weigh boats

9.17 Sterile porcelain dishes

9.18 Blender base

9.19 Sterile blender vessels

9.20 Sterile bacti cell spreaders or bent glass rod

9.21 Sterile 500 mL graduated cylinder

9.22 Aluminum foil

9.23 Sterile, 100 mm x 15 mm, plastic petri dishes with tight-fitting lids

9.24 Control culture of *Escherichia coli* (*E. coli)* ATCC 25922

9.25 Dehydrated media:
9.25.1 LTB (also known as Lauryl Sulfate Broth).
9.25.2 EC.
9.25.3 BGB.
9.25.4 Heart Infusion Agar (HI agar).
9.25.5 Trypticase Soy Broth (TSB).
9.25.6 Peptone.

10.0 REAGENTS AND STANDARDS

10.1 Use ASTM Type I water for all solutions and whenever "DI water" is indicated in the procedure (see LQM for specifications).

10.2 LTB: Dehydrated medium can be purchased commercially.

10.2.1 Single Strength (1X) LTB: Combine 35.6 g medium with 1.0 L DI water. Mix on stir plate until dissolved, apply low heat; do not boil. Dispense 10 mL into 16 x 150 mm tubes containing 10 x 75 mm inverted insert tubes. Loosely cap tubes and autoclave at 121°C for 15 minutes. Medium must be removed from autoclave immediately after a cycle and the full cycle shall not exceed 45 minutes. Cool quickly, and discard any tubes containing air bubbles. Refrigerate at 2 - 8°C until use. Shelf life of prepared tubes is two weeks. Acceptable pH range is 6.8 ± 0.2 after sterilization.

10.2.2 Double Strength (2X) LTB: Combine 71.2 g medium with 1.0 L DI water. Mix on stir plate until dissolved, apply low heat; do not boil. Dispense 10 mL into 20 x 150 mm tubes containing 10 x 75 mm inverted insert tubes. Loosely cap tubes and autoclave at 121°C for 15 minutes. Medium must be removed from autoclave immediately after a cycle and the full cycle shall not exceed 45 minutes. Cool quickly, and discard any tubes containing air bubbles. Refrigerate at 2 - 8°C until use. Shelf life of prepared tubes is two weeks. Acceptable pH range is 6.8 ± 0.2 after sterilization.

10.3 EC broth: Dehydrated medium can be purchased commercially.

10.3.1 Combine 37.0 g medium with 1.0 L DI water. Mix on stir plate until dissolved while applying low heat; do not boil. Dispense 10 mL into 16 x 150 mm tubes containing 10 x 75 mm inverted insert tubes. Loosely cap tubes and autoclave at 121°C for 15 minutes. Medium must be removed from autoclave immediately after a cycle and the full cycle shall not exceed 45 minutes. Cool quickly and discard any tubes containing air bubbles. Refrigerate at 2 - 8°C until use, shelf life of prepared tubes is two weeks. Acceptable pH range is 6.9 ± 0.2 after sterilization.

10.4 BGB broth: Dehydrated medium can be purchased commercially.

10.4.1 Combine 40.0 g medium with 1.0 L DI water. Mix on stir plate until dissolved while applying low heat; do not boil. Dispense 10 mL into 16 x 150 mm tubes containing 10 x 75 mm inverted insert tubes. Loosely cap tubes and autoclave at 121°C for 15 minutes. Medium must be removed from autoclave immediately after a cycle and the full cycle shall not exceed 45 minutes. Cool quickly and discard any tubes containing air bubbles. Refrigerate at 2 - 8°C until use, shelf life of prepared tubes is two weeks. Acceptable pH range is 7.2 ± 0.2 after sterilization.

10.5 HI agar: Dehydrated medium can be purchased commercially.

10.5.1 Combine 40.0 g dehydrated medium with 1.0 L DI water in a sterile Erlenmeyer flask. Mix by swirling. Heat to dissolve agar. Pour 250 - 300 mL of the liquid agar into 500 mL glass jar. Cap loosely and autoclave at 121°C for 15 minutes. Medium must be removed from autoclave immediately after a cycle and the full cycle shall not exceed 45 minutes. Allow the agar to cool to room temperature, tighten caps. Shelf life of prepared bottles is three months at 2 - 8°C. Acceptable pH range is 7.3 ± 0.2 after sterilization.

10.5.2 HI spread plate preparation: Liquify the media and dispense 20 - 25 mL into 100 X 15 mm sterile petri dishes then allow to solidify. Cover, invert, and leave at room temperature for at least a day prior to analysis. Refrigerate any plates that will not be used for the current analysis. Shelf life of prepared plates is two weeks if kept in airtight container and refrigerated at 2 - 8°C.

10.6 Peptone dilution water: Dehydrated medium can be purchased commercially.

10.6.1 Combine 1.0g of dehydrated media with 1.0 L DI water. Mix on stir plate until dissolved while applying low heat; do not boil. Dispense 103 mL laboratory reagent water containing 0.1% peptone into standard 99 mL dilution bottles. Loosely cap bottles and autoclave at 121°C for 15 minutes. Remove the dilution bottles from the autoclave immediately after the cycle. Allow to cool to room temperature before tightening caps. Shelf life of prepared bottles is one month at room temperature. Acceptable pH range is 7.0 ± 0.2 after sterilization

10.7 Control culture of *E. coli* ATCC 25922: can be purchased commercially. Refer to MMSD SOP LPROS-014 Bacteriological Reference Cultures.

10.8 Preparation of *E. coli* undiluted spiking suspension:

10.8.1 Prepare a 1% solution of single-strength LTB using lab prepared materials, 1 mL of LTB plus 99 mL of peptone dilution water. Shake to mix.

10.8.2 Inoculate the 1% LTB solution with working bacteriological culture of *E. coli* 25922 using a sterile loop's worth of culture. Shake the bottle vigorously a minimum of 25 times. Incubate at 35°C ± 3°C for 20 ± 4 hours. This is the undiluted spiking suspension.

10.9 Record the corresponding lot numbers to bench sheets for all materials used.

11.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT, AND STORAGE

11.1 Samples must be collected in sterile glass or plastic bottles. A minimum of 200 grams or 200 mL of sample is required.

11.2 Samples must not be exposed to direct sunlight.

11.3 A headspace of 2.5 to 5 cm must be left in all sample containers containing liquids.

11.4 Samples must be analyzed within 8 hours from the collection time; 6 hours maximum transport time and 2 hours for processing.

11.4.1 The hold time for Class A composted, Class B aerobically or anaerobically digested samples is extended to 24 hours from the sample collection time.

11.4.2 Maintain bacteriological samples at <10°C during transport.

11.4.3 Do not allow samples to freeze.

11.4.4 Refrigerate samples upon arrival and analyze as soon as possible.

12.0 QUALITY CONTROL

12.1 Established control limits for quality control parameters can be found in the QC Requirements Summary Sheets in the LQM.

12.2 All consumables are subject to QA/QC testing prior to analysis. Refer to LPROS-013, Microbiology Support Activities for supplemental microbiological QC procedures including but not limited to the broth media lot-lot comparison test, spore sterility tests, and media sterility tests. Annual and monthly tests are performed on the water used to prepare media. Refer to LPROS-007, Microbiology Water Verification Testing, for reagent water testing.

12.3 The analytical QC requirements for biosolids fecal coliform and total coliform analysis include satisfactory initial precision and recovery (IPR), ongoing precision recovery (OPR), matrix spike (MS), matrix spike duplicate (MSD), routine analysis of positive and negative controls, method blanks, and media sterility checks.

12.4 IPRs are used to demonstrate acceptable method performance per analyst prior to monitoring field samples; this is equivalent to an initial demonstration of capability (IDOC) analysis.

12.4.1 Prepare four, 30 g samples of Milorganite® and spike them with the current laboratory working control culture of *E. coli* ATCC #25922.

12.4.2 Process and analyze each IPR sample per the matrix spike procedure. IPR analysis must be accompanied appropriate media QA, sterility checks, and the analysis of a blank.

12.4.3 Calculate the fecal coliform and total coliform densities MPN/g TS. Total coliform results should be equivalent to fecal coliform result for these IPR samples.

12.4.4 Calculate the percent recovery for each IPR sample.

12.4.5 Using the percent recovery results for all four IPR samples calculate the mean percent recovery and the relative standard deviation (RSD).

Table 1. IPR and OPR Acceptance Criteria.

Performance Test	LTB/EC Acceptance Criteria
IPR	
• **Mean percent recovery**	65 - 221%
• **Precision (as a maximum RSD)**	84%
OPR as percent recovery	37 - 391%

12.5 OPR control of the analytical system is demonstrated by analyzing a matrix spike sample with each analytical batch. OPR analysis must be accompanied by appropriate media QA and sterility checks.

12.5.1 Spike a 30-g sample of Milorganite® with 3 mL of the *E. coli* spiking suspension dilution "B" (10^{-3} dilution) created with the current laboratory working control culture of *E. coli* ATCC #25922. Homogenize with 270 mL of peptone dilution water.

12.5.2 Process and analyze the OPR sample per the matrix spike procedure.

12.5.3 Calculate the fecal coliform and total coliform densities MPN/g TS. Total coliform results should be equivalent to fecal coliform result for these OPR samples.

12.5.4 Calculate the percent recovery for each OPR sample.

12.5.5 OPR and IPR sample results should be charted and updated records maintained to monitor ongoing method performance.

12.6 Method Blank: A method blank (consisting of peptone dilution water) is run at a minimum of one each day of analysis. The method blank should not contain the target organisms. Failure of a method blank may indicate contamination during analysis. Absence of growth and gas indicates media is free of contamination from the target organism.

12.7 Matrix Spike/Matrix Spike Duplicates (MS/MSD): A sample spiked, in duplicate, with viable *E. coli* is run at a minimum of once per 20 samples and included with each sample run. A spiking suspension is also analyzed. Results of the spiked sample are compared to the spiking suspension concentration to determine percent recovery of *E. coli* in the matrix of interest.

13.0 CALIBRATION AND STANDARDIZATION

13.1 Balance shall be checked each day of use with three weights within the normal range of use.

13.2 The pH meter shall be calibrated with three buffers that bracket the expected pH of the solution being tested. Buffer aliquots are poured fresh daily.

13.3 The temperature of water baths and incubators used in this analysis are determined twice a day with readings separated by a minimum of 4 hours.

13.3.1 Water baths are checked annually for temperature distribution characteristics. See the "Equilibrium Checks" section of SOP LPROS-003, "Oven, Refrigerator, Incubator, Water Bath Temperature Checks" for further details.

13.4 The Filamatic Automatic Dispensing device is calibrated before each use. Verification of calibration is performed quarterly.

14.0 PROCEDURE

14.1 Set jacketed incubators at 35.0°C ± 0.5°C and water baths at 44.5°C ± 0.2°C. Allow media to come to room temperature prior to use.

14.2 Solid sample homogenization:

14.2.1 Weigh 30 g ± 0.1 g of a well-mixed biosolid sample into a sterile dish. For samples with high solids content, use a sterile spatula to transfer material in and out of the sterile dish to reach desired weight.

14.2.2 Combine the weighed portion of biosolid sample and 270 mL of peptone dilution water in a sterile blender. Use a portion of peptone dilution water to rinse the contents of the dish into the blender jar.

14.2.3 Homogenize sample and peptone dilution water on high for one minute to create the "homogenized" sample.

14.2.4 Immediately pour the "homogenized" sample into a sterile sample bottle. Rinse the blender jar with cold tap water to prevent etching of the glass by organic acids. Refrigerate the "homogenized" sample if analysis of inoculation into presumptive media is not to occur within 30 minutes.

14.2.5 After a sample is spiked, no longer than an hour may elapse between initial unspiked sample homogenization and analysis of spiked sample.

14.2.6 Do not suspend bacteria in peptone dilution water for more than 30 minutes at room temperature. Fill a small plastic bin with ice to chill the spiking solution upon removal from the incubation temperature. This is also used to keep the MS and MSD cold in the time between blending and inoculating the spiked sample.

14.3 Serial dilution of Class A Biosolids solid samples:

14.3.1 The "homogenized" sample contains 10^{-1} g of the original biosolid sample.

14.3.2 Shake the "homogenized" sample 25 times vigorously. Using a sterile wide bore pipette aseptically transfer 11 mL of well mixed "homogenized" sample into 99 mL of peptone dilution water, shake a minimum of 25 times. This is dilution "A". One mL of dilution "A" equals 10^{-2} mL of the original biosolid sample.

14.3.3 Using a sterile pipette (wide bore if necessary) aseptically transfer 11 mL of Dilution "A" into 99 mL of peptone dilution water, shake a minimum of 25 times. This is dilution "B". One mL of dilution "B" equals 10^{-3} mL of the original biosolid sample.

14.3.4 Using a sterile pipette (wide bore if necessary) aseptically transfer 11 mL of Dilution "B" into 99 mL of peptone dilution water, shake a minimum of 25 times. This is dilution "C". One mL of dilution "C" equals 10^{-4} mL of the original biosolid sample.

14.3.5 Spiked Class A samples require at least two additional dilutions. Use a sterile pipette to aseptically transfer 11 mL of Dilution "C" into 99 mL of peptone dilution water, shake a minimum of 25 times. This is dilution "D". One mL of dilution "D" equals 10^{-5} mL of the original sample. Then aseptically transfer 11 mL of Dilution "D" into 99 mL of peptone dilution water, shake a minimum of 25 times. This is dilution "E". One mL of dilution "E" equals 10^{-6} mL of the original biosolid sample.

Table 3. Sample Dilutions

Dilution ID	Amount of Original Sample	Grams of Original Biosolid Sample
Homogenized Sample	10^{-1}	1.0 g
Dilution "A"	10^{-2}	0.1 g
Dilution "B"	10^{-3}	0.01 g
Dilution "C"	10^{-4}	0.001 g
Dilution "D"	10^{-5}	0.0001 g
Dilution "E"	10^{-6}	0.00001 g

14.4 Inoculation of Class A Biosolids solid samples:

14.4.1 For unspiked samples use four series of five tubes for analysis with 1.0, 10^{-1}, 10^{-2}, 10^{-3} gram of the original sample. The first series of tubes must contain 2X LTB broth. For spike samples use 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} gram of original biosolid sample.

14.4.2 Use a sterile pipette to inoculate each of the first series of five tubes with 10 mL of the "homogenized" sample (unspiked samples only). This series of tubes must contain 10 mL of 2X LTB broth. This is 1.0 g of the original biosolid sample.

14.4.3 Use a sterile pipette to inoculate each of the second series of five tubes. The series of tubes can contain 10 mL of 2X LTB broth and 10 mL of the dilution "A" or 10 mL of 1X LTB broth and 1 mL of the "homogenized" sample. The amount of sample inoculated will depend on the consistency of the sample after homogenization. This is 10^{-1} (0.1) g of the original biosolid sample.

14.4.4 Use a sterile pipette to inoculate each of the third series of five tubes (10 mL of 1X LTB) with 1 mL of dilution "A". Use for spiked and unspiked samples. This is 10^{-2} (0.01) g of the original biosolid sample.

14.4.5 Use a sterile pipette to inoculate each of the fourth series of five tubes (10 mL of 1X LTB) with 1 mL of dilution "B". Use for spiked and unspiked samples. This is 10^{-3} (0.001) g of the original biosolid sample.

14.4.6 Use a sterile pipette to inoculate each of the fifth series of five tubes (10 mL of 1X LTB) with 1 mL of dilution "C". Use for spiked samples. This is 10^{-4} (0.0001) g of the original biosolid sample.

14.4.7 Use a sterile pipette to inoculate each of the sixth series of five tubes (10 mL of 1X LTB) with 1 mL of dilution "D". Use for spiked samples. This is 10^{-5} (0.00001) g of the original biosolid sample.

14.5 Class B biosolids-dilutions:

14.5.1 Follow steps for Class A biosolid sample dilutions "A" through "E".

14.6 Inoculation of Class B Biosolids:

14.6.1 Inoculate at least four rows of 5 tubes each, with the inoculation scheme dependent on the anticipated level of fecal coliform in the sample. If little is known regarding the fecal coliform content of the sample, setting up 5 rows (or more) is recommended. For most Class B biosolids, setting up 4 rows, with the first row of tubes all containing 0.01 g of original biosolids sample, and each successive row containing a serial dilution, is appropriate.

14.7 LTB/EC/BGB Procedure:

14.7.1 Place LTB tubes into the incubator for 24 ± 2 hours.

14.7.2 Score LTB tubes as positive or negative after 24 ± 2 hours of incubation. Tubes containing both growth and any amount of gas in the inverted tube are considered a positive result. Place LTB tubes that are negative at 24 hours ± 2 hours back into the incubator for an additional 24 hours, for a total of 48 ± 3 hours. Record results at 24 ± 2 hours and 48 ± 3 hours on the bench sheets.

14.7.3 Use a heat sterilized inoculation loop to transfer growth from the positive LTB tubes into tubes containing EC broth. No more than 30 minutes may elapse from the time the first transfer into EC occurs, until the tubes are placed into the water bath.

14.7.4 Score EC tubes from the 24-hour positive LTB tubes as positive or negative after 24 ± 2 hours incubation. Any amount of gas in the inverted tube is considered a positive result.

14.7.5 Score EC tubes from the 48-hour positive LTB tubes as positive or negative after 24 ± 2 hours incubation. Any amount of gas in the inverted tube is considered a positive result.

14.7.6 If total coliform analysis is occurring, use a heat sterilized inoculation loop to transfer from positive LTB tubes into BGB tubes at the same time as the transfer to EC broth. Transfer into EC broth first, then transfer into BGB broth. No more than 30 minutes may elapse from the time the first transfer into EC and BGB occurs and the start of incubation.

14.7.7 Incubate BGB tubes for 24 ± 2 hours. Place negative BGB tubes at 24 hours back into the incubator an additional 24 hours, for a total of 48 ± 3 hours. Record results observed at 24 ± 2 hours and 48 ± 3 hours on the bench sheets.

14.8 Spiking Suspension Dilution: Refer to Reagents and Standards section for the preparation of the undiluted spiking suspension.

14.8.1 Shake the undiluted spiking suspension vigorously 25 times at a minimum. Transfer 1mL of undiluted spiking solution into 99 mL of peptone dilution water to create a 10^{-2} dilution. This is solution "A". Shake the bottle vigorously 25 times at a minimum.

14.8.2 Transfer 11mL of Solution A into 99 mL of peptone dilution water to create a 10^{-3} dilution. This is solution "B". Shake the bottle vigorously 25 times at a minimum.

14.8.3 Transfer 11mL of Solution B into 99 mL of peptone dilution water to create a 10^{-4} dilution. This is solution "C". Shake the bottle vigorously 25 times at a minimum.

14.8.4 Transfer 11mL of Solution C into 99 mL of peptone dilution water to create a 10^{-5} dilution. This is solution "D". Shake the bottle vigorously 25 times at a minimum.

Table 4. Spiking Suspension Dilutions

DILUTION	PREPARATION INSTRUCTIONS	SOLUTION ID
10^{-2}	1 mL Undiluted spiking suspension + 99 mL peptone dilution water	Solution A
10^{-3}	11 mL Solution A + 99 mL peptone dilution H_2O	Solution B
10^{-4}	11 mL Solution B + 99 mL peptone dilution H_2O	Solution C
10^{-5}	11 mL Solution C + 99 mL peptone dilution H_2O	Solution D
10^{-6}	11 mL Solution D + 99 mL peptone dilution H_2O	Solution E
10^{-7}	11 mL Solution E + 99 mL peptone dilution H_2O	Solution F
10^{-8}	11 mL Solution F + 99 mL peptone dilution H_2O	Solution G

14.9 Spiking solution enumeration:

14.9.1 Prepare HIA plates several days before analysis and store inverted at room temperature until use. Ensure that agar surface is dry prior to use.

14.9.2 Pipette the following volumes onto HIA plates in triplicate for the evaluation of 3-9 plates including the appropriate QA blank plate.

14.9.2.1 0.1 mL of dilution C (10^{-4}) -----[10^{-5} mL (0.00001 mL of spiking solution A)]

14.9.2.2 0.1 mL of dilution D (10^{-5}) -----[10^{-6} mL (0.000001 mL of spiking solution A)]

14.9.2.3 0.1 mL of dilution E (10^{-6}) -----[10^{-7} mL (0.0000001 mL of spiking solution A)]

NOTE: 0.1 mL of dilution D in triplicate will usually suffice when a culture is 23 to 24 hours old.

14.9.3 Using a sterile bacti cell spreader or sterile bent glass rod distribute inoculum over the surface of the media.

14.9.4 Place inverted plates into the incubator for 24 ± 2 hours.

14.9.5 Count and record number of colonies per plate and calculate the concentration of *E. coli* (CFU/mL) in the undiluted spiking solution according to the spike suspension enumeration instructions listed in the Data Analysis and Calculations (Section 15.0) section of this SOP.

14.10 Matrix spike and matrix spike duplicate preparation:

14.10.1 Spike a 30-g sample of Milorganite® with 3 mL of the *E. coli* spiking suspension dilution "B" (10^{-3} dilution). Homogenize with 270 mL of peptone dilution water.

14.10.2 At least four rows of 5 tubes each are inoculated, with the inoculation scheme dependent on the anticipated level of fecal coliforms in the spiked samples.

14.10.3 Proceed to process and analyze matrix spike and matrix spike duplicate according to the LTB/EC/BGB procedure.

14.10.4 The matrix spike and the matrix spike duplicate samples are two aliquots from the same preparation.

15.0 DATA ANALYSIS AND CALCULATIONS

15.1 The estimated density of fecal coliform or total coliform bacteria are based on confirmation tests using BGB or EC broth respectively. The density is calculated in terms of MPN/g TS (dry weight basis). The results are calculated in three steps:

15.1.1 Selection of significant dilutions

15.1.2 Calculation of MPN/mL (wet weight)

15.1.3 Conversion to MPN/g TS (dry weight)

15.2 Select Significant Dilutions: The sample dilution refers to the grams (solid sample) of original sample that was inoculated into each series of tubes. Only three of the four dilution series are used to estimate the MPN. Significant dilutions only include those that were transferred to confirmative media: EC for fecal coliform and BGB for total coliform.

15.2.1 The significant dilutions are selected as follows:

15.2.1.1 Choose the highest dilution (the most dilute, with the least amount of sample) giving positive results in all five tubes inoculated and the two succeeding higher (more dilute) dilutions.

15.2.1.2 If the lowest dilution (least dilute) tested has less than five tubes with positive results, select it and the two next succeeding higher dilutions.

15.2.1.3 When a positive result occurs in a dilution higher (more dilute) than the three significant dilutions selected according to the rules above, change the selection to the lowest dilution (least dilute) that has less than five positive results and the next two higher dilutions (more dilute).

15.2.1.4 When the selection rules above have left unselected any higher dilutions (more dilute) with positive results, add those higher-dilution positive results to the results for the highest selected dilution.

15.2.1.5 If there were not enough higher dilutions tested to select three dilutions, then select the next lower dilution.

Table 5. Examples of Significant Dilution Selection and Calculation of MPN/mL [a]

Example (liquid or solid)	10^{-3} mL or g	10^{-4} mL or g	10^{-5} mL or g	10^{-6} mL or g	**Step 1:** Significant Dilutions	**Step 2:** (MPN / largest sig. dilution) =MPN / mL wet weight
A	5/5	5/5	3/5	0/5	5-3-0	(7.92 / 10^{-4}) = 79,200 MPN / mL 79,000 MPN / mL
B	4/5	5/5	1/5	0/5	4-5-1	(4.83 / 10^{-3}) = 4830 MPN / mL 4800 MPN / mL
C	0/5	1/5	0/5	0/5	0-1-0	(0.18 / 10^{-3}) = 180 MPN / mL
D	5/5	3/5	1/5	1/5	3-1-1	(1.37 / 10^{-4}) = 13,700 MPN / mL 14,000 MPN / mL
E	4/5	4/5	0/5	1/5	4-4-1	(3.98 / 10^{-3}) = 3980 MPN / mL 4000 MPN / mL
F	5/5	5/5	5/5	2/5	5-5-2	(54.22 / 10^{-4}) = 542,200 MPN / mL 540,000 MPN / mL
G	5/5	5/5	5/5	0/0	5-5-0	(23.98 / 10^{-4}) = 239400 MPN / mL 240,000 MPN / mL
H	5/5	5/5	5/5	5/5	5-5-5	(>160.9 / 10^{-4}) = >1609000 MPN / mL >1,600,000 MPN / mL
I	5/5	5/5	5/5	N/A [b]	5-5-5	(160.9 / 10^{-3}) = 160900 MPN / mL 160,000 MPN / mL [b]

[a] Significant dilutions are underlined and largest significant dilutions highlighted.

[b] If the most dilute tubes did not go positive in the presumptive LTB media, were not transferred to EC or BGB, and the significant dilutions were 5-5-5, then the MPN does not include the ">".

15.3 Calculate the MPN/mL (wet weight) for fecal coliform or total coliform: Obtain the MPN Index value from the MPN tables found in the appendix. The initial MPN result must be divided by the dilution volume to account for dilution of the sample during analysis to obtain the MPN / mL value:

$$\text{MPN/mL} = \frac{\text{MPN index}}{\text{Largest volume tested in the dilution series used for MPN determination}}$$

15.4 Calculation of MPN/g TS (dry weight) (calculation method for total coliform and fecal coliform is identical):

$$\text{MPN/g (dry weight)} = \frac{\text{MPN/mL}}{\text{Percent total solids (expressed as a decimal)}}$$

15.4.1 Example calculation: Dilutions containing 0.001, 0.0001, and 0.00001 mL of a Class B sludge yield a pattern of 5/5, 2/5, and 1/5 positive tubes in EC broth. The significant dilutions of 5-2-1 yield a value of 7.00 from the MPN Index Table. The percent total solids of the sample is 4.5%. The initial MPN value must be divided by 0.045 to express the result in terms of MPN/g TS. As shown below:

(7.00/0.001)/0.045 = 155,555.6 MPN/g TS (or 160,000 when rounded)

15.5 Calculation of geometric mean:

15.5.1 For each sample in a set of samples, calculate the base 10 logarithm.

15.5.2 Determine the mean of the logarithms.

15.5.3 Expression of the antilog of the mean of the logarithms yields the geometric mean (GM) – see the hypothetical data set below for Class B Biosolids:

Sample #1	1,600,000 FC/g TS	$\log_{10}$ = 6.204
Sample #2	1,100,000 FC/g TS	$\log_{10}$ = 6.041
Sample #3	46,000 FC/g TS	$\log_{10}$ = 4.663
Sample #4	1,200,000 FC/g TS	$\log_{10}$ = 6.079
Sample #5	870,000 FC/g TS	$\log_{10}$ = 5.940
Sample #6	1,600,000 FC/g TS	$\log_{10}$ = 6.204
Sample #7	420,000 FC/g TS	$\log_{10}$ = 5.623

Mean of Logarithms = 5.822

$10^{5.822}$ = 663,817, rounded to 660,000 FC/g TS as a Geometric Mean

15.6 Spike suspension enumeration:

15.6.1 Calculate concentration of *E. coli* (CFU/mL) in Undiluted Spiking Suspension:

$$EC_{undiluted\ spike} = \frac{(CFU_1 + CFU_2 + CFU_3)}{(V_1 + V_2 + V_3)}$$

Where:
$EC_{undiluted\ spike}$ = *E. coli* CFU/mL in undiluted spiking suspension
CFU = plate count between 30-300 CFU/mL
V = volume of sample

15.6.2 Calculate spiked *E. coli* [CFU/mL or g (wet weight)]:

$$Spiked\ EC_{wet\ weight} = (EC_{undiluted\ spike}) \times (V_{spiked\ per\ unit\ biosolids})$$

Where:
$V_{spiked\ per\ unit\ biosolids}$ = $1x10^{-4}$ mL per g of biosolids (wet weight)

15.6.3 Convert to "true" spiked *E. coli* CFU/g TS (dry weight):

$$T = \frac{Spiked\ EC_{wet\ weight}}{Percent\ total\ solids\ (expressed\ as\ a\ decimal)}$$

Where:
T = "true" spike of *E. coli* CFU/g TS (dry weight)

15.6.4 Calculate Percent Recovery:

$$R = 100 \times \frac{Ns - Nu}{T}$$

Where:
R = % recovery
N_s = spiked sample
N_u = unspiked sample
$R = [100 \times (N_s - N_u)/T]$

15.6.5 Calculate precision (RPD) between the matrix spike and matrix spike duplicate:

$$\%\ RPD = (|MS - MSD|) / \frac{|MS| + |MSD|}{2}$$

15.6.6 Acceptance criteria

Performance Test	Acceptance Criteria
Class A Biosolids MS/MSD Percent Recovery	30 – 424%
Class B Biosolids MS/MSD Percent Recovery	8 – 709%
Class A Biosolids Precision (RPD)	150%
Class B Biosolids Precision (RPD)	125%

16.0 METHOD PERFORMANCE

16.1 Details on the interlaboratory validation of this method can be found in EPA method 1680.

17.0 POLLUTION PREVENTION

17.1 The solutions and reagents used in this method pose little threat to the environment when recycled and managed properly.

17.2 Solutions and reagents should be prepared in volumes consistent with laboratory use to minimize the volume of expired materials to be disposed.

18.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QC MEASURES

18.1 All analytical data shall be reviewed by the analyst in accordance with the method and the standard operating procedure (SOP). Deviation from established criteria must be documented by the analyst.

18.2 Consult the QC Requirements Summary Sheets Manual for laboratory quality specifications by analytical method. This manual outlines the quality samples run by a specific method including frequency, flagging protocol, and corrective action for out of control data. Control limits are continuously calculated and maintained by the microbiology staff according to Standard Methods guidelines.

18.3 All analytical results will be verified by a peer before they are released. When a peer is unavailable, a team supervisor will perform the peer review duties, in addition to any supervisory data review. Review criteria is established in standard operating procedure SOP LPROS-041, Peer Review of Laboratory Data.

18.4 The MMSD Central Laboratory maintains various SOPs that apply to many areas in the lab and that provide guidance to procedures that affect most areas. These SOPs

are identified as process SOPs and have identification LPROS-XXX. These are maintained on SharePoint and may be printed as an uncontrolled document. All active SOPs are in SharePoint in the Laboratory Quality Assurance area. Analysts are responsible for keeping uncontrolled documents up to date in their areas if printed.

19.0 CORRECTIVE ACTION FOR OUT-OF-CONTROL DATA

19.1 Corrective action and root cause analysis procedures are documented in standard operating procedure SOP LPROS-020, Corrective Action and Root Cause Analysis.

19.2 Corrective action for analytical quality control samples is outlined for each method in the QC Requirements Summary Sheets Manual. Analysts may print this uncontrolled document and are responsible for keeping a current copy if printed.

19.3 Refer to SOP LPROS-041, Peer Review of Laboratory Data. for the MMSD Central Laboratory process of data review completed before entry to the LIMS system.

20.0 CONTINGENCIES FOR HANDLING OUT OF CONTROL DATA

20.1 If situations occur in which there are no written procedures for how to handle erroneous or out of control data consult a Team Supervisor, Quality Assurance Specialist, or Lab Manager.

20.2 Whenever an unexpected scenario occurs all steps that are taken to resolve the situation must be documented in the Corrective Action Logbook for that area. Refer to SOP LPROS-024, Logbook Maintenance Utilization and Review, for guidance.

21.0 WASTE MANAGEMENT

21.1 All hazardous waste must be handled in accordance with state and federal regulations.

21.1.1 Pipets, dilution bottles, and media must be autoclaved prior to disposal or transferred to the dish washing area.

21.2 The MMSD Central Laboratory follows pollution prevention procedures to eliminate the amount of hazardous waste produced.

22.0 REFERENCES

22.1 Standard Methods for the Examination of Water and Wastewater, 22nd ed. 2012, APHA, AWWA, WEF.

22.2 Microbiological Methods for Monitoring the Environment: Water and Wastes (EPA-600/8-78-017), 1978, US EPA.

22.3 Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC Medium, April 2010, US EPA, EPA-821-R-10-003.

22.4 US EPA Region 5 Central Regional Laboratory Standard Operating Procedure for the Analysis of Fecal Coliform Bacteria by Most Probable Number (MPN), CRL SOP MICR006, 1999, Chicago.

23.0 TABLES, DIAGRAMS, FLOWCHARTS, AND DATA VALIDATION

23.1 EPA Most Probable Number Index Table for Five Tube – Three Dilution Series (next page).

EPA MPN Index Table for Combinations of Positive Results When Five Tubes are Used per Dilution [a]

Combination of Positives	MPN Index mL	95% Confidence Limits		Combination of Positives	MPN Index mL	95% Confidence Limits	
		Lower	Upper			Lower	Upper
0-0-0	<0.1803			1-3-0	0.83	0.12	1.96
0-0-1	0.18	0.03	0.63	1-3-1	1.04	0.20	2.43
0-0-2	0.36	0.03	1.01	1-3-2	1.25	0.29	2.96
0-0-3	0.54	0.03	1.37	1-3-3	1.47	0.38	3.64
0-0-4	0.72	0.08	1.74	1-3-4	1.69	0.48	4.60
0-0-5	0.91	0.15	2.12	1-3-5	1.91	0.57	5.66
0-1-0	0.18	0.03	0.63	1-4-0	1.05	0.21	2.45
0-1-1	0.36	0.03	1.01	1-4-1	1.27	0.30	3.00
0-1-2	0.55	0.03	1.38	1-4-2	1.48	0.39	3.70
0-1-3	0.73	0.08	1.75	1-4-3	1.70	0.48	4.68
0-1-4	0.91	0.15	2.14	1-4-4	1.93	0.58	5.75
0-1-5	1.10	0.23	2.56	1-4-5	2.15	0.67	6.57
0-2-0	0.37	0.03	1.02	1-5-0	1.28	0.30	3.03
0-2-1	0.55	0.03	1.39	1-5-1	1.50	0.40	3.75
0-2-2	0.74	0.08	1.76	1-5-2	1.72	0.49	4.77
0-2-3	0.92	0.15	2.15	1-5-3	1.95	0.58	5.83
0-2-4	1.11	0.23	2.58	1-5-4	2.17	0.68	6.64
0-2-5	1.29	0.31	3.07	1-5-5	2.40	0.77	7.31
0-3-0	0.56	0.03	1.40	2-0-0	0.45	0.03	1.19
0-3-1	0.74	0.09	1.77	2-0-1	0.68	0.06	1.64
0-3-2	0.93	0.16	2.17	2-0-2	0.91	0.15	2.13
0-3-3	1.12	0.23	2.60	2-0-3	1.15	0.25	2.69
0-3-4	1.30	0.31	3.10	2-0-4	1.39	0.35	3.38
0-3-5	1.49	0.39	3.72	2-0-5	1.64	0.46	4.37
0-4-0	0.75	0.09	1.79	2-1-0	0.68	0.06	1.66
0-4-1	0.94	0.16	2.19	2-1-1	0.92	0.15	2.16
0-4-2	1.12	0.24	2.63	2-1-2	1.16	0.25	2.72
0-4-3	1.31	0.32	3.13	2-1-3	1.41	0.36	3.43
0-4-4	1.50	0.40	3.77	2-1-4	1.66	0.46	4.47
0-4-5	1.69	0.48	4.62	2-1-5	1.92	0.57	5.71
0-5-0	0.94	0.16	2.21	2-2-0	0.93	0.16	2.18
0-5-1	1.13	0.24	2.65	2-2-1	1.18	0.26	2.76
0-5-2	1.33	0.32	3.17	2-2-2	1.43	0.36	3.49
0-5-3	1.52	0.40	3.82	2-2-3	1.68	0.47	4.56
0-5-4	1.71	0.48	4.70	2-2-4	1.94	0.58	5.81
0-5-5	1.90	0.56	5.63	2-2-5	2.21	0.69	6.75
1-0-0	0.20	0.03	0.68	2-3-0	1.19	0.26	2.79
1-0-1	0.40	0.03	1.08	2-3-1	1.44	0.37	3.55
1-0-2	0.60	0.03	1.49	2-3-2	1.70	0.48	4.67
1-0-3	0.81	0.11	1.91	2-3-3	1.97	0.59	5.91
1-0-4	1.01	0.19	2.36	2-3-4	2.23	0.70	6.83
1-0-5	1.22	0.28	2.87	2-3-5	2.51	0.82	7.59
1-1-0	0.40	0.03	1.09	2-4-0	1.46	0.38	3.61
1-1-1	0.61	0.03	1.50	2-4-1	1.72	0.49	4.77
1-1-2	0.81	0.11	1.92	2-4-2	1.99	0.60	6.00
1-1-3	1.02	0.19	2.38	2-4-3	2.26	0.72	6.92
1-1-4	1.23	0.28	2.90	2-4-4	2.54	0.83	7.68
1-1-5	1.44	0.37	3.54	2-4-5	2.82	0.94	8.36
1-2-0	0.61	0.03	1.51	2-5-0	1.74	0.50	4.88
1-2-1	0.82	0.12	1.94	2-5-1	2.01	0.61	6.10
1-2-2	1.03	0.20	2.40	2-5-2	2.29	0.73	7.00
1-2-3	1.24	0.29	2.93	2-5-3	2.57	0.84	7.76
1-2-4	1.46	0.38	3.59	2-5-4	2.86	0.95	8.45
1-2-5	1.67	0.47	4.51	2-5-5	3.15	1.07	9.10

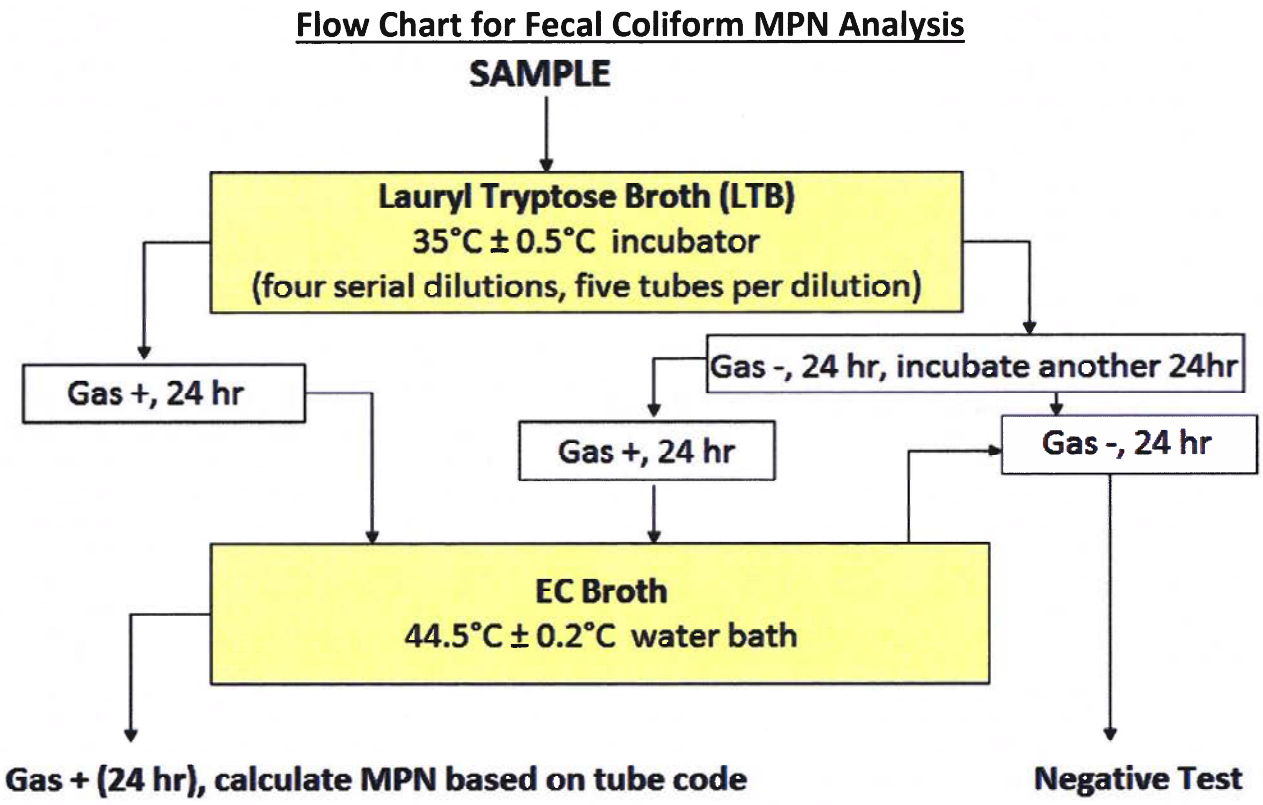
[a] Table was developed using the MPN calculator developed by Albert Klee

MPN Index Table (continued)

Combination of Positives	MPN Index mL	95% Confidence Limits		Combination of Positives	MPN Index mL	95% Confidence Limits	
		Lower	Upper			Lower	Upper
3-0-0	0.79	0.10	1.88	4-3-0	2.71	0.90	8.09
3-0-1	1.06	0.21	2.46	4-3-1	3.26	1.11	9.34
3-0-2	1.35	0.33	3.23	4-3-2	3.86	1.32	10.60
3-0-3	1.65	0.46	4.40	4-3-3	4.51	1.54	11.92
3-0-4	1.96	0.59	5.89	4-3-4	5.21	1.76	13.31
3-0-5	2.29	0.73	6.99	4-3-5	5.93	1.96	14.77
3-1-0	1.07	0.22	2.50	4-4-0	3.35	1.14	9.53
3-1-1	1.37	0.34	3.29	4-4-1	3.98	1.37	10.84
3-1-2	1.67	0.47	4.52	4-4-2	4.66	1.59	12.23
3-1-3	1.99	0.60	6.01	4-4-3	5.39	1.81	13.68
3-1-4	2.32	0.74	7.10	4-4-4	6.15	2.02	15.21
3-1-5	2.67	0.88	8.00	4-4-5	6.93	2.23	16.81
3-2-0	1.38	0.35	3.35	4-5-0	4.11	1.41	11.11
3-2-1	1.70	0.48	4.64	4-5-1	4.83	1.64	12.56
3-2-2	2.02	0.62	6.13	4-5-2	5.59	1.87	14.09
3-2-3	2.36	0.76	7.20	4-5-3	6.39	2.09	15.70
3-2-4	2.71	0.90	8.10	4-5-4	7.22	2.30	17.39
3-2-5	3.08	1.04	8.94	4-5-5	8.06	2.50	19.16
3-3-0	1.72	0.49	4.77	5-0-0	2.40	0.76	7.63
3-3-1	2.05	0.63	6.24	5-0-1	3.14	1.06	9.08
3-3-2	2.40	0.77	7.31	5-0-2	4.27	1.46	11.42
3-3-3	2.76	0.92	8.21	5-0-3	5.78	1.92	14.46
3-3-4	3.13	1.06	9.06	5-0-4	7.59	2.39	18.16
3-3-5	3.52	1.20	9.89	5-0-5	9.53	1.65	22.34
3-4-0	2.09	0.64	6.36	5-1-0	3.29	1.12	9.40
3-4-1	2.44	0.79	7.42	5-1-1	4.56	1.56	12.02
3-4-2	2.81	0.93	8.33	5-1-2	6.31	2.07	15.53
3-4-3	3.19	1.08	9.18	5-1-3	8.39	2.57	19.85
3-4-4	3.58	1.23	10.02	5-1-4	10.62	3.04	24.85
3-4-5	3.99	1.37	10.86	5-1-5	12.93	3.04	30.90
3-5-0	2.48	0.80	7.53	5-2-0	4.93	1.67	12.76
3-5-1	2.86	0.95	8.44	5-2-1	7.00	2.24	16.94
3-5-2	3.25	1.10	9.31	5-2-2	9.44	2.80	22.13
3-5-3	3.65	1.25	10.17	5-2-3	12.05	3.31	28.43
3-5-4	4.07	1.40	11.03	5-2-4	14.79	3.81	37.14
3-5-5	4.50	1.54	11.89	5-2-5	17.67	5.03	52.30
4-0-0	1.30	0.31	3.11	5-3-0	7.92	2.47	18.86
4-0-1	1.66	0.46	4.45	5-3-1	10.86	3.08	25.44
4-0-2	2.07	0.64	6.31	5-3-2	14.06	3.68	34.45
4-0-3	2.53	0.82	7.64	5-3-3	17.50	4.34	51.31
4-0-4	3.02	1.02	8.81	5-3-4	21.22	5.29	67.98
4-0-5	3.55	1.21	9.96	5-3-5	25.27	8.14	79.71
4-1-0	1.69	0.48	4.60	5-4-0	12.99	3.48	31.08
4-1-1	2.12	0.66	6.46	5-4-1	17.24	4.29	49.75
4-1-2	2.58	0.85	7.79	5-4-2	22.12	5.63	70.87
4-1-3	3.10	1.05	8.98	5-4-3	27.81	8.82	86.00
4-1-4	3.65	1.25	10.16	5-4-4	34.54	11.59	101.10
4-1-5	4.25	1.45	11.38	5-4-5	42.56	14.37	118.00
4-2-0	2.16	0.67	6.61	5-5-0	23.98	7.62	76.29
4-2-1	2.64	0.87	7.94	5-5-1	34.77	11.72	101.60
4-2-2	3.17	1.08	9.15	5-5-2	54.22	17.91	141.90
4-2-3	3.75	1.29	10.37	5-5-3	91.78	26.72	220.10
4-2-4	4.38	1.50	11.64	5-5-4	160.90	38.37	410.30
4-2-5	5.04	1.71	12.97	5-5-5	>160.90		

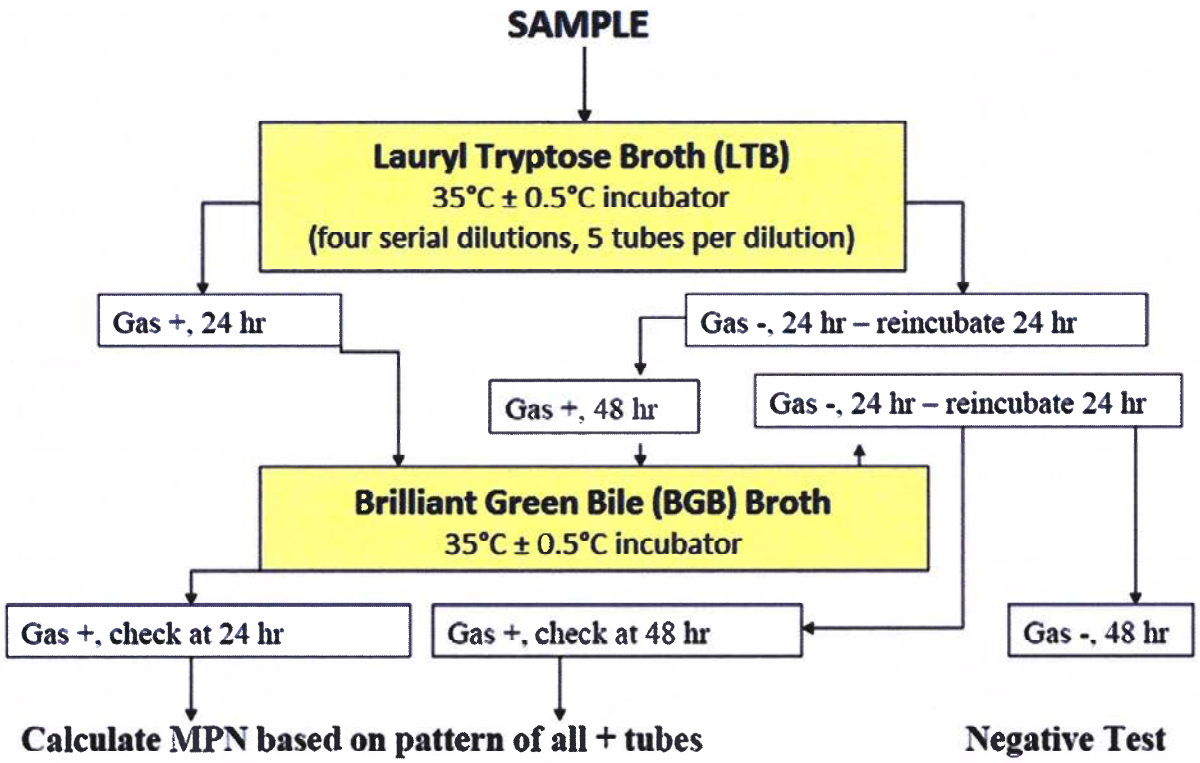
[a] Table was developed using the MPN calculator developed by Albert Klee

23.2 Flow Charts:

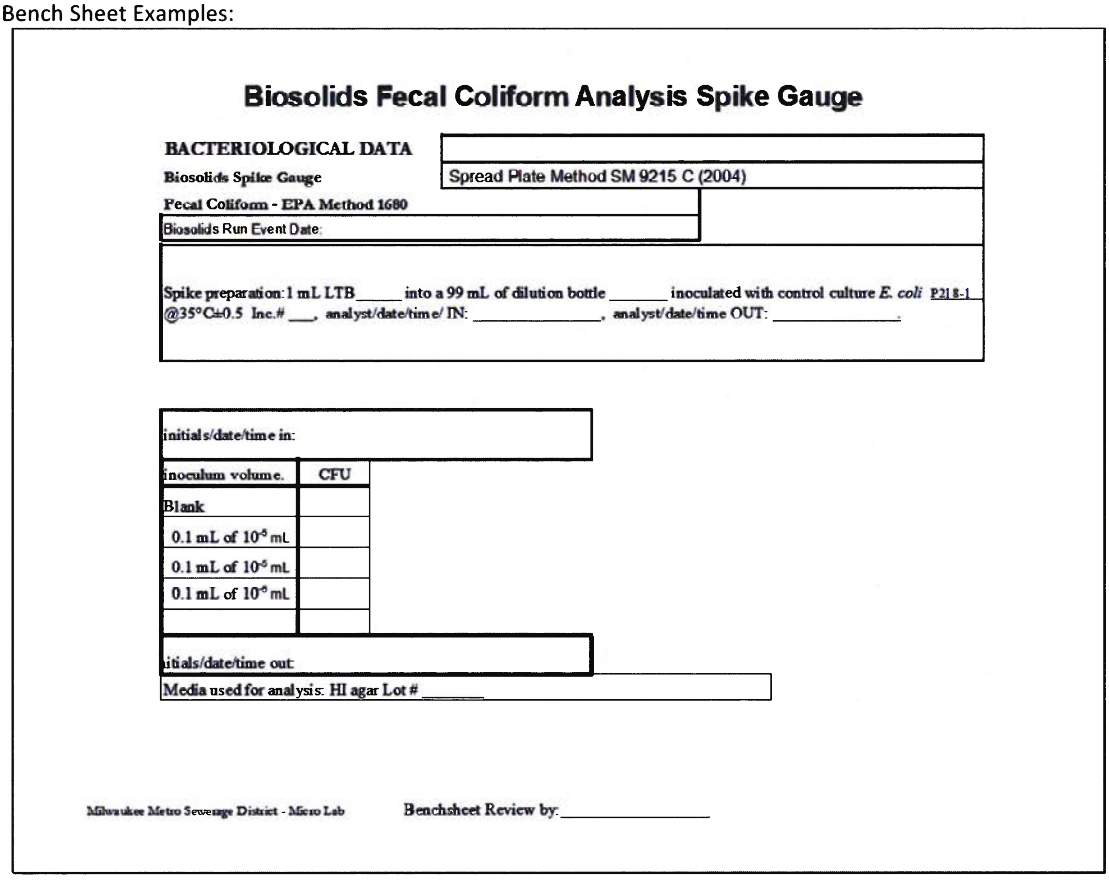


Flow Charts (continued):

Flow Chart for Total Coliform MPN Analysis



23.3 Bench Sheet Examples:



Biosolids Fecal Coliform Analysis Spike Gauge

BACTERIOLOGICAL DATA

Biosolids Spike Gauge | Spread Plate Method SM 9215 C (2004)

Fecal Coliform - EPA Method 1680

Biosolids Run Event Date:

Spike preparation: 1 mL LTB_____ into a 99 mL of dilution bottle _____ inoculated with control culture *E. coli* P218-1 @35°C±0.5 Inc.# ___, analyst/date/time/ IN: _____, analyst/date/time OUT: _____.

initials/date/time in:

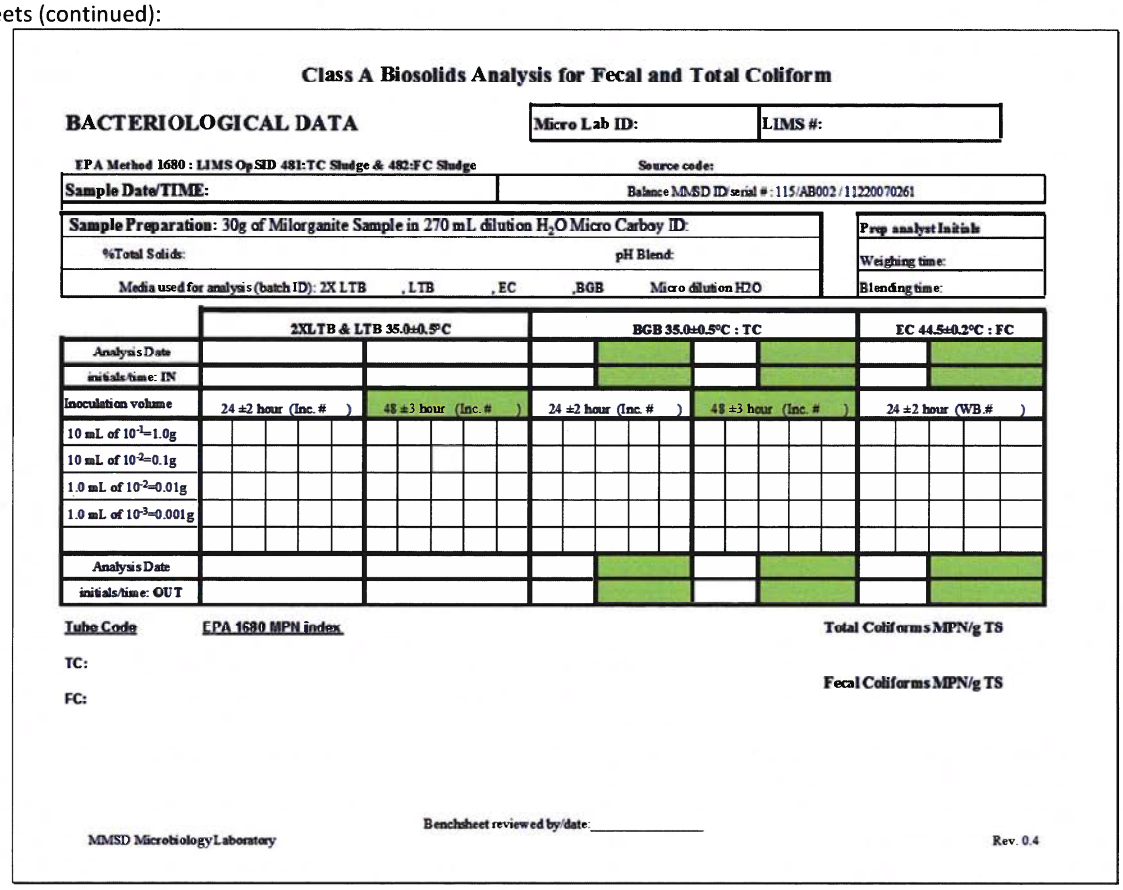
inoculum volume.	CFU
Blank	
0.1 mL of 10^{-5} mL	
0.1 mL of 10^{-6} mL	
0.1 mL of 10^{-7} mL	

initials/date/time out:

Media used for analysis: HI agar Lot # _____

Milwaukee Metro Sewerage District - Micro Lab Benchsheet Review by:_____

Bench Sheets (continued):



Class A Biosolids Analysis for Fecal and Total Coliform

BACTERIOLOGICAL DATA

Micro Lab ID:	LIMS #:

EPA Method 1680 : LIMS OpSID 481:TC Sludge & 482:FC Sludge — Source code:

Sample Date/TIME:	Balance MMSD ID/serial # : 115/AB002 / 11220070261

Sample Preparation: 30g of Milorganite Sample in 270 mL dilution H_2O Micro Carboy ID:	Prep analyst Initials
%Total Solids: pH Blend:	Weighing time:
Media used for analysis (batch ID): 2X LTB , LTB , EC , BGB Micro dilution H2O	Blending time:

	2XLTB & LTB 35.0±0.5°C				BGB 35.0±0.5°C : TC				EC 44.5±0.2°C : FC	
Analysis Date										
initials/time: IN										
Inoculation volume	24 ±2 hour (Inc. #)				24 ±2 hour (Inc. #)				24 ±2 hour (WB.#)	
10 mL of 10^{-1}=1.0g										
10 mL of 10^{-2}=0.1g										
1.0 mL of 10^{-2}=0.01g										
1.0 mL of 10^{-3}=0.001g										
Analysis Date										
initials/time: OUT										

Tube Code	EPA 1680 MPN index	Total Coliforms MPN/g TS
TC:		
FC:		Fecal Coliforms MPN/g TS

Benchsheet reviewed by/date:_____

MMSD Microbiology Laboratory — Rev. 0.4

Bench Sheets (continued):

Biosolids Fecal Coliform analysis

BACTERIOLOGICAL DATA

Micro Lab ID:	LIMS #:

EPA Method 1680 : LIMS OpSID 482:FC Sludge **LIMS OpSID 483:FC MPN index** **Source code: 978**

Sample Date/Time:	Balance MMSD ID/serial # : 115/AB002 / 11220070261 Blender serial # : 160517

Sample Preparation: 30g of Sample in 270 mL Micro Carboy ID:	**Prep analyst Initials**
%Total Solids: pH Blend:	Weighing time:
Media used for analysis (batch ID): 2X LTB: LTB: EC: dilution H2O:	Blending time:

	2XLTB & LTB 35.0±0.5°C										EC 44.5±0.2°C : FC				
Set-up Date															
initials/time: **IN**															
Inoculation volume	24 ±2 hour (Inc. #)					48 ±3 hour (Inc #)					24 ±2 hour (WB.#)				
10 mL of 10^{-1}=1 0g															
10 mL of 10^{-2}=0.1g															
1.0 mL of 10^{-2}=0.01g															
1.0 mL of 10^{-3}=0.001g															
Analysis Date															
initials/time: **OUT**															

Inoculation volume Key

10^{-1}=Straight Blend

10^{-2}=11 mL Straight Blend + 99 mL H_2O

10^{-3}=11 mL 10^{-2} + 99 mL H_2O

Dilution factor Multiplier Key

row 1 = 1

row 2 = 10

row 3 = 100

row 4 = 1000

Tube Code **Value from EPA MPN Index**

Fecal Coliforms MPN/g TS:

Benchsheet reviewed by/date:_____

MMSD Microbiology Laboratory

Uncontrolled Document

24.4 Spiking Solution Instructions

Spiking Solution Guidance

Preparation of *E. coli* undiluted spiking suspension:
SOP LAB-031, sec. 10.8 and EPA Method 1680 sec. 15.2.3

1. Prepare a 1% LTB solution. Add 1 mL of sterile single-strength LTB to 99 mL of peptone dilution water. Shake to mix.
2. Using the lab's reference stock culture of *E. coli*, inoculate the 1% of LTB solution using a flame sterilized loop.
3. Shake the bottle vigorously a minimum of 25 times.
4. Incubate at 35°C ± 3°C for 20 ± 4 hours.
5. Record the incubation time and corresponding lot #s to bench sheets for all materials used.

24.5 SOP Editorial Changes and Revisions

SOP Revision Updates/ Editorial Changes	
Date:	1/4/2021
Revision ID:	0.4
Reason for change:	Biannual SOP review.
Change:	Minor editorial changes throughout. Added single strength LTB, HI agar, and peptone dilution water instructions to section 10. Removed media sterility check information from section 12. This is detailed in LPROS-013. Added "Spiking Solution Instructions" to section 24.
Signatures	Alfredo Salomaya Jr. Laboratory Manager signature Kim M Walden QA Specialist signature

Milorganite Fecal Coliform

VWM–MMSD Sample Chain of Custody

Sample Location: Jones Island Milorganite Production, Shipping, and Bagging

Sample Date: _____

# of Bottles	Source Code	Sample ID	Analyses Requested: Fecal Coliform via EPA 1680	Analyses Requested: % Total Solids	Analyses Requested: LIMS Placeholder	Analyses Requested	Analyses Requested	Analyses Requested	Analyses Requested	Analyses Requested	Analyses Requested	Type (grab, comp)	Preservative	Sample Time(s): Start Time	Sample Time(s): (comp. only) End Time	Sample Time(s): Preserved Time	For Laboratory Use Only: LIMS #	For Laboratory Use Only: pH verified
	978/1	*Milorganite Production	X	X								Grab	None					
	978/1	*Milorganite Production			X							Grab	None					
	978/5	*Milorganite Shipping	X	X								Grab	None					
	978/5	*Milorganite Shipping			X							Grab	None					
	978/4	*Miloragnite Bagging	X	X								Grab	None					
	978/4	*Milorganite Bagging			X							Grab	None					

Microbiological samples must be collected after 4am and received by the lab no later than 9am

Remarks (any unusual conditions must be recorded here and supervisor informed):

VWM Sample Custody

Relinquished by:	Date	Time	Received by:	Date	Time

Sample Courier Custody ***Microbiological samples must be refrigerated upon collection and transported on ice***

Received by:	Date	Time	Relinquished by:	Date	Time	On Ice?
						Y / N

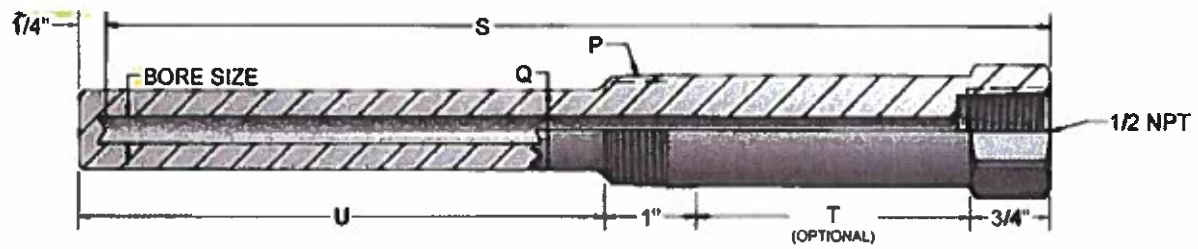
MMSD Lab Sample Custody

Received by:	Date	Time	On Ice?	Relinquished by:	Date	Time
			Y / N			

If not received on ice, sample temperature on receipt in lab: _____°C

Straight-Shank, Threaded Thermowells

Straight-Shank, Threaded Thermowells are available in a variety of materials, process connection sizes, lengths, and optional lagging extensions. Thermowell specifications should be determined based on process conditions which include strength, temperature, pressure and corrosion-resistance requirements. They are designed with a standard 0.260" or 0.385" bore diameter to accommodate sensing elements with either a 0.252" or 0.377" maximum diameter, respectively. These wells are available as separate components or as part of complete sensor assemblies.



Wells are made from round bar with milled wrench hex. 1 1/4" NPT and 1 1/2" NPT wells are supplied as round bar with milled wrench flats.

("U" length for non-lagging wells) = "S" -1 1/2"
("U" length for lagging wells) = "S" -1 1/2" -"T"
(To solve for "T"), "T" = "S" -"U" -1 1/2" (When "U" and "S" are specified)

Thermowell Dimensions

"P"	"Q"
1/2" NPT	5/8" Dia.
3/4" NPT	3/4" Dia.
1" NPT	7/8" Dia.
1 1/4" NPT	1 1/4" Dia.
1 1/2" NPT	1 1/2" Dia.

ORDER CODES

Example Order Number: **ST 4 D 09 08 T2 C8S** (1-0 1-1 1-2 1-3 1-4 1-5 1-6) ST4D090BT1(1/2)

1-0 Well Type

CODE	DESCRIPTION
ST	Straight-shank threaded

1-1 Bore Size

CODE	DESCRIPTION
4	0.260 Dia. bore
6	0.385" Dia. bore

1-2 Pipe Size "P"

CODE	DESCRIPTION
C	1/2" NPT
D	3/4" NPT
E	1" NPT
F	1 1/4" NPT
G	1 1/2" NPT

1-6 Options

CODE	DESCRIPTION
C8	316 stainless steel well cap and chain
C22	Brass well cap and chain
S	Well stamped with customer-specified part number

1-5 Optional "T" Lag Dimension

CODE	DESCRIPTION
	Leave blank if no lag is required
T_	Specify "T" dimension in inches

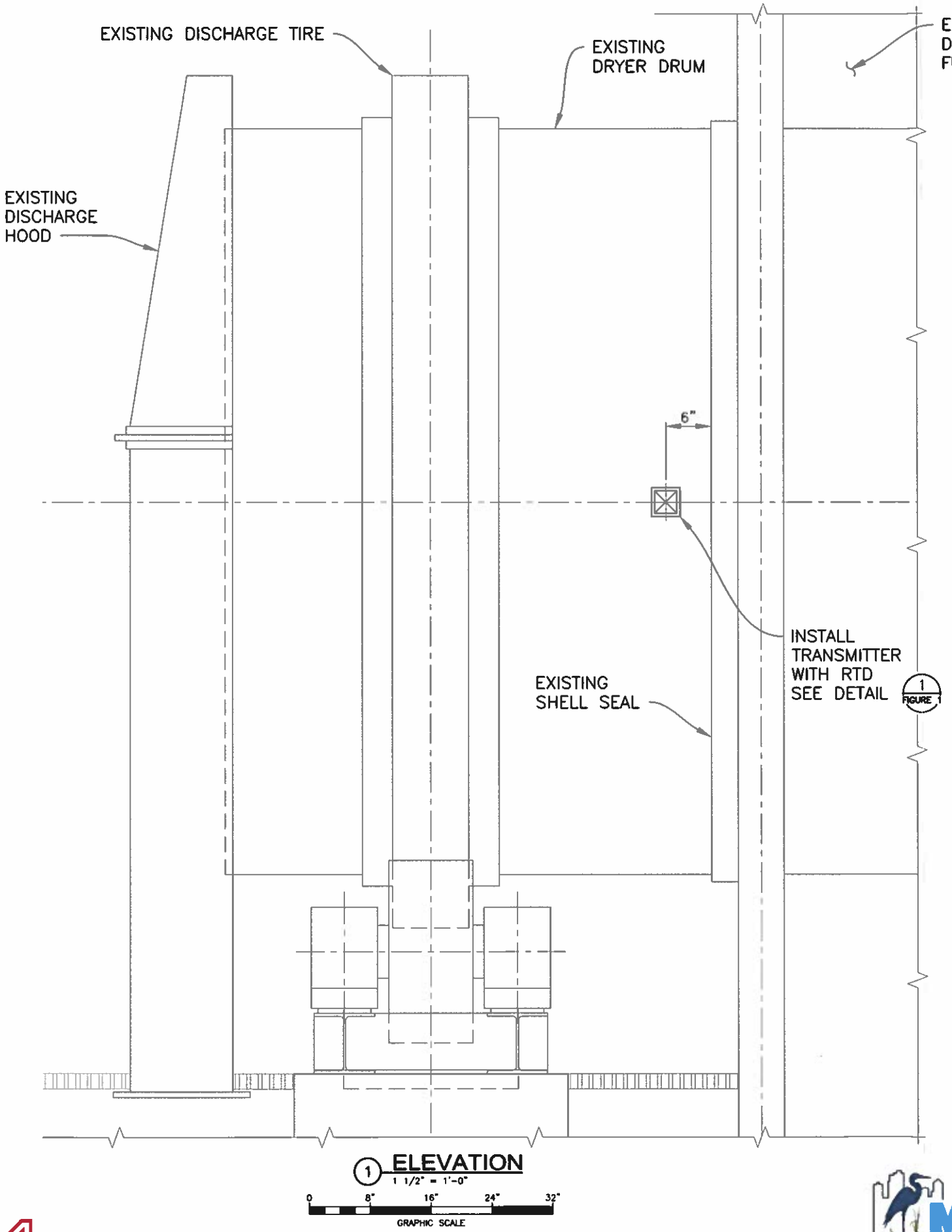
1-4 Material 316SS

CODE	DESCRIPTION
XX	Specify two digit material code as stated in the Thermowell Material Table located earlier in section

1-3 "S" Length

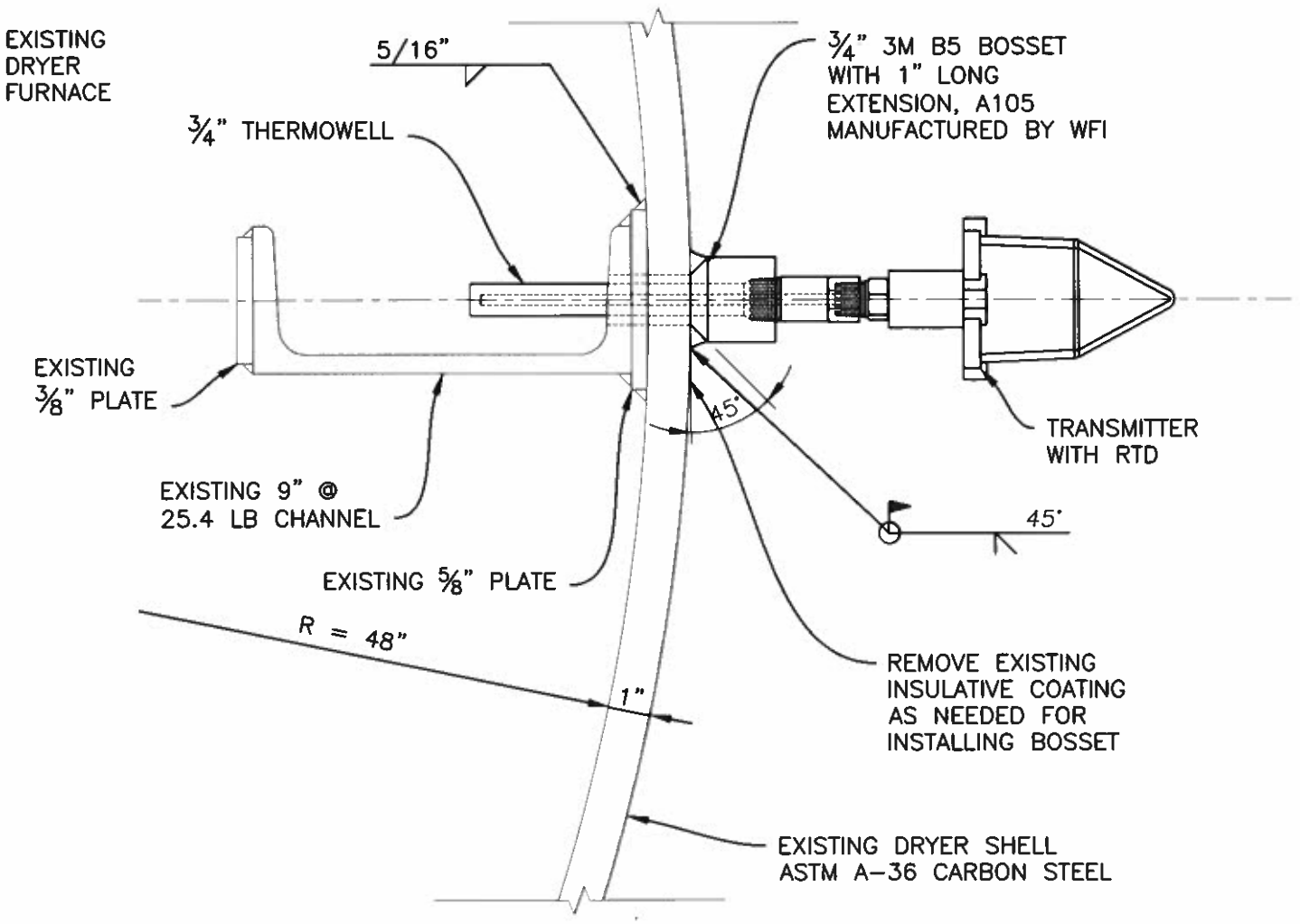
CODE	DESCRIPTION
XX	Specify length in inches using two digits plus fractional length

(1")



1 ELEVATION
1 1/2" = 1'-0"

0 8" 16" 24" 32"
GRAPHIC SCALE



1 DETAIL
FIGURE 1 6" = 1'-0"

0 2" 4" 6" 8"
GRAPHIC SCALE

NOTES:

1. BORE 1-7/32" HOLE IN DRYER SHELL AND WELD BOSSET IN PLACE.
2. WELDING SHALL COMPLY WITH AWS STANDARDS AND SHALL BE ACCOMPLISHED USING THE METALARC OR GAS-SHIELDED ARC METHOD DESCRIBED IN THE AMERICAN WELDING SOCIETY'S WELDING HANDBOOK. AS SUPPLEMENTED BY OTHER AWS STANDARDS QUALIFICATION OF WELDERS, ALL WELDING SHALL COMPLY WITH AWS AND ASME STANDARDS.
3. 100% X-RAY REQUIRED ON SHELL TO BOSSET WELD.

FIGURE 1
DRYER TEMPERATURE ELEMENT
MMSD - JONES ISLAND WWTP

Appendix F

Vendor Equipment Information

Milorganite® 503 Compliance

Vendor and procurement printed information is contained in this appendix for the equipment listed below.

Equipment Installed for Compliance at Dryers

- Accutech RT10 – Wireless RTD transmitter. Two each installed in each dryer drum.

  (The new Accutech RT10 is much larger and heavier. MMSD will have an engineering analysis performed to determine if the new model RT10 will still be suitable for the dryer drum application. Banner makes a lighter weight wireless transmitter that uses the same Spread Spectrum technology, which could be an alternative if the new model RT10 cannot be used as a replacement.)

- Accutech BR21 Base Radio – One installed in each dryer control panel.
- Accutech 4AO-8SW Analog Output Module – One installed in each dryer control panel.
- Emmerson Sola-HD Power Supply – One installed in each dryer control panel.

Equipment Installed for Compliance at Recycle Bins

- Pyromation Spring-Loaded RTD with Hardwired Transmitter – Three installed in the removable access panels on the southside of each recycle bin.
- Pepperl+Fuchs Inductive Sensor – One installed at the closing end of each recycle slide gate discharging to Classification.

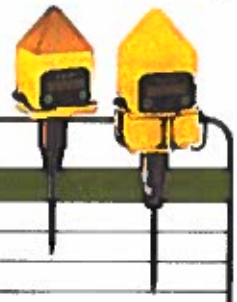
Product Data Sheet Accutech RT10
Model Code

TBUARTTJPN00S1B090

	TBUARTTJPN00B0N000 represents a typical part number.
Model	Type
TBUART	Wireless RTD Temperature Field Unit
Code	Select: RF Module Type
T	902MHz - 928MHz band (FCC / IC)
D	915MHz - 928MHz band (Australia)
N	915MHz - 921MHz band (New Zealand)
F	2.4GHz
Code	Select: Safety Certifications
J	Intrinsically Safe Protection CSA – see product data sheet for certification details
Q	ATEX & IECEx – see product data sheet for certification details
Code	Select: Housing & Battery Pack
P	NEMA4 Polycarbonate Housing with 1 Cell (Available with Intrinsically Safe Rating)
Code	Select: Future Option
N	None
Code	Select: Integral Antenna or Cable & Connector Interface
00	Integral Antenna with antenna cover. The 2.4GHz unit also comes with an external antenna connector.
	For 900MHz RF Module Systems Only:
01	External YAGI Antenna, 6db, attached to base of unit
10	10ft. (3.01m) cable with N-Male connector for remote antenna configurations
25	25ft. (8.72m) cable with N-Male connector for remote antenna configurations
Code	Select: Sensor Mounting
S	Integrated RTD (Requires selection of Type, Fitting and Probe length below)
B	Remotely mounted RTD - c/w NEMA4 Aluminum rear-entry junction box (RTD & Bracket not included)
D	Remotely mounted RTD - c/w NEMA4X Stainless Steel rear-entry junction box (RTD & Bracket not included)
Code	Select: RTD Type
0	No RTD (purchased separately)
1	4 Wire DIN curve 100 ohm platinum RTD
Code	Select: Fitting
N	No RTD (Purchased separately - junction box provided for field termination)
B	Spring-loaded fitting (Customer to install in thermowell)
D	Direct-insertion, welded
Code	Select: Probe Length – 0.5 inch increments only
000	No RTD (Purchased separately)
XXX 090	Enter Required Probe length XX . X inches as XXX (no decimal point) - contact factory for > 9 inches

Schneider Electric

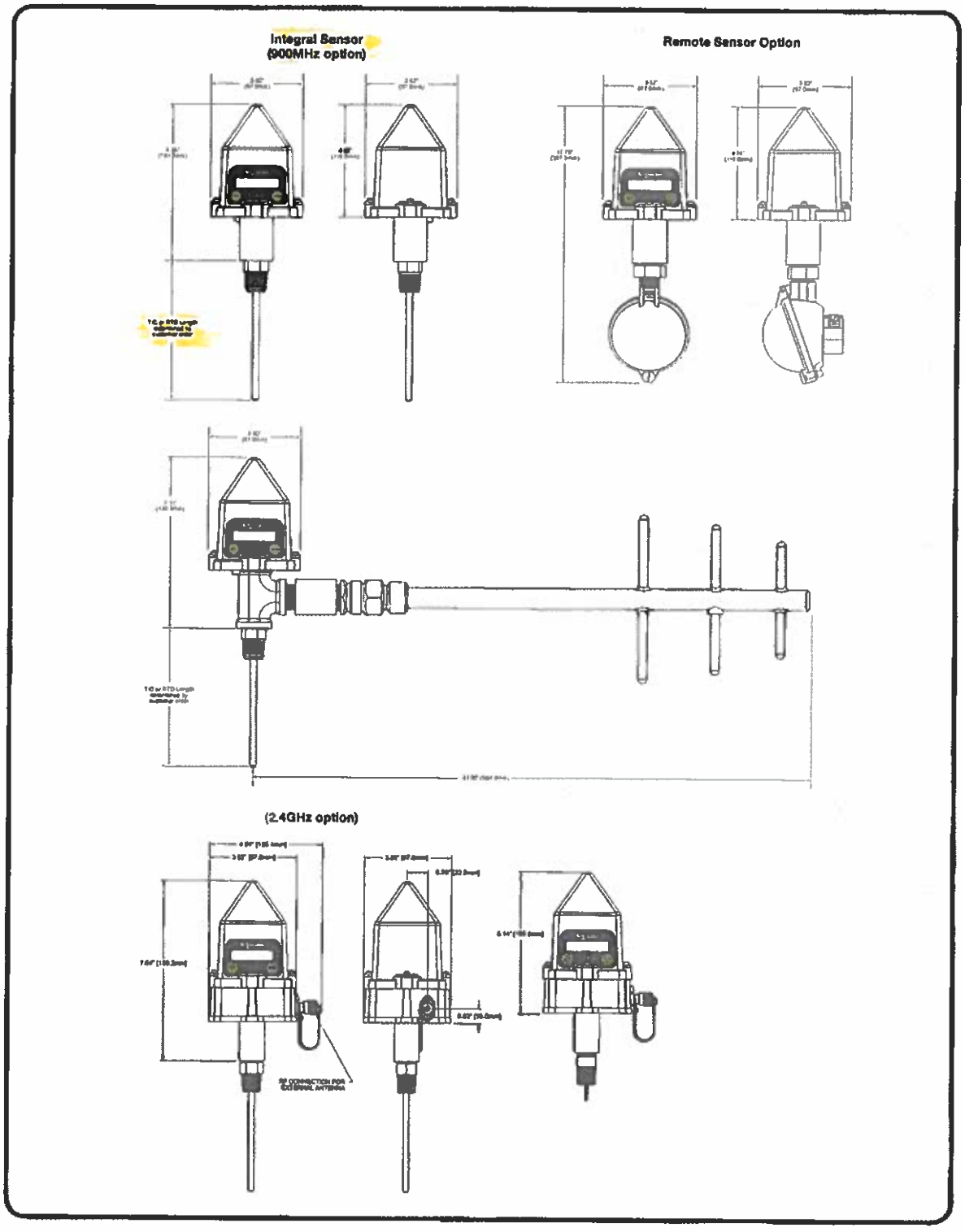
Product Data Sheet Accutech RT10
Specifications



>	Accutech RT10
Functional	
Sensor Type	RTD Temperature High accuracy, high temperature: -200° to 800°C (-330° to 1470°F)
Location	Field Unit
Frequency Range	900MHz and 2.4GHz license-free bands
Power	Integrated battery
Features	
Linearisation	RTD linearisation to ± .05°C (.09°F), custom linearisation with 22-point curve
Remote Configuration Interface	Accutech Manager, WindowsTM-based GUI software, providing network-wide fault and performance-management features and field unit configuration capabilities
Local Configuration Interface	• Integrated LCD with membrane-switch buttons • Display provides temperature reading and error messages, if applicable • Configure sampling and RF parameters locally using membrane-switch buttons.
Sensor	
Accuracy	Electronics accuracy: • ± 0.1% of full scale reading Ambient temperature effect: • ± 0.002% of reading per °C (1.8°F) ambient temperature difference from reference condition (20°C or 68°F). Stability: • Deviation per year is less than 0.025% RTD accuracy: • 100ohm platinum RTD: ± (0.15+0.002*|T|) for temperatures in the range -100°C < T < 450°C • For user-provided thermocouples see the manufacturer's data sheet.
Stability	Stability deviation per year is less than 0.025%
RF Characteristics	900MHz: • 902 to 928MHz Frequency Hopping Spread Spectrum (FHSS), FCC certified ISM license-free band • 915 to 928MHz (Australia) • 921 to 928MHz (New Zealand) • Data Rates: 4,800, 19,200 or 76,800bps • 0.4W maximum 2.4GHz: • 2400 to 2483.5MHz ISM license-free band Frequency Hopping Spread Spectrum (FHSS) Radio • Data Rates: 50/100kbps (FSK Modulation), 200kbps (GFSK Modulation) • Typical Electrical Transmit Power: +10.6dBm • Typical Receive Sensitivity (0.1% BER): - 102dBm @ 50kbps, - 99dBm @ 100kbps, - 99dBm @ 200kbps • Typical CW Receiver Blocking Rejection: 84dB for CW @ +/- 5MHz, 74dB for CW @ +/- 30MHz
Self-Diagnostics	• Low battery notification – indicates the need to replace the battery (approximately one month advance notification). • Contains software and hardware that continuously monitors operation. Any sensor or device parameter that is out of spec is identified and reported.
General	
Operating Ambient Environment	• –40 to 85°C (–40 to 185°F) electronics • –20 to 70°C (–4 to 158°F) display • –40 to –20°C (–40 to –4°F) display (extreme cold can reduce LCD visibility) • Humidity: 0 to 95%, non-condensing
Materials of Construction	• Type 316 stainless-steel base and RTD sheath • GE Lexan® cover. V-0 rating and UV resistant • Process Connection: 1/2" MNPT
Power	• Self-contained power • Standard Accutech field units include a single C-Cell (900MHz) or D-Cell (2.4GHz) lithium battery that offers battery life up to ten years of service, depending on data rates and battery options.
Operating Shock and Vibration	Tested per IEC 60068-2-6 (vibration) and 2-27 (shock)
Random Vibration Characteristics	Tested to withstand 6 g's, 15 minutes per axis from 9 – 500Hz
Electromagnetic Compatibility	• This equipment complies with the EU RTTE directive (1999/5/EC). • Australian C-Tick - registration number N15744
Certifications	North America HAZLOC: • cCSAus Intrinsically Safe: • Ex ia IIC. T3; Class I, Zone 0, AEx ia IIC. T3 • Class I, Div. 1, Groups A, B, C & D, T3 • Class II, Div. 1, Groups E, F and G, T3 • Class III, T3 • Class I, Div. 2, Groups A, B, C & D, T4 • Class II, Div. 2, Groups F and G, T4 • Class III, T4 ATEX/IECEx HAZLOC: • Intrinsically Safe • Ex ia IIC T3 • LCIE 10 ATEX 3109 X • IECEx LCI 10.0045X EMC & Radio: • North America: FCC , IC • Europe: CE Mark (R&TTE) • Australia/New Zealand: C-Tick
Disclaimer: Schneider Electric reserves the right to change product specifications. For more information visit www.schneider-electric.com.	

Schneider Electric

Product Data Sheet Accutech RT10
Dimensions



Schneider Electric

Sensor Wiring

The RT10 monitors one RTD input.

Wire switch inputs and outputs, where provided, using the instructions for Intrinsic Safety compliant installations.

⚠WARNING

UNEXPECTED EQUIPMENT OPERATION

Use wires of a diameter that conform to 0.326 mm² (AWG 22) or larger to avoid drops in voltage, overheating, and unexpected equipment operation.

Follow all local and national safety codes and standards.

Failure to follow these instructions can result in death, serious injury, or equipment damage.

Inspect contacts at regular intervals for corrosion. Clean corroded contacts and eliminate the source of the corrosion.

Additional Conditions for Intrinsic Safety Compliant Installations

The apparatus must only be powered by an approved battery. Refer to the label on the enclosure for the part number. For battery replacement instructions, see Maintaining Field Units.

The apparatus must be connected according to drawings included in the technical file. Refer to the Intrinsic Safety Drawings section for installation instructions. The apparatus must be wired to a simple device to result in an Intrinsically Safe compliant installation. A simple device is one that meets the conditions set forth in the Intrinsic Safety Control Drawings.

⚠WARNING

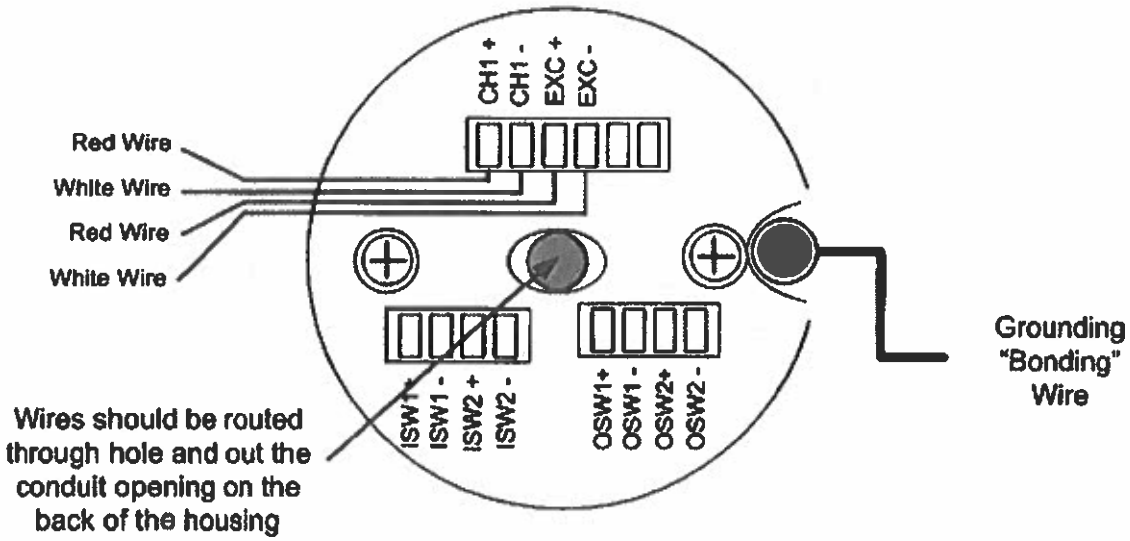
RISK OF EXPLOSION

Wire the device according to intrinsic safety practices and in accordance with the conditions set forth in the Intrinsic Safety Control Drawings.

Failure to follow these instructions can result in death, serious injury, or equipment damage.

RTD Wiring

Wire the 4-wire RTD input as shown in the figure below:



For 3-wire RTD operation, run the four wires as close a possible to the RTD and then connect the CH1+ and EXC+ together at one side of the RTD wiring.

RTD Flying Lead Wiring

The RT10 field unit is available without the junction box. A wiring harness, consisting of two twisted pair cables, is made available for wiring connection to user devices. The two twisted pair cables are labeled and the connections for each pair are:

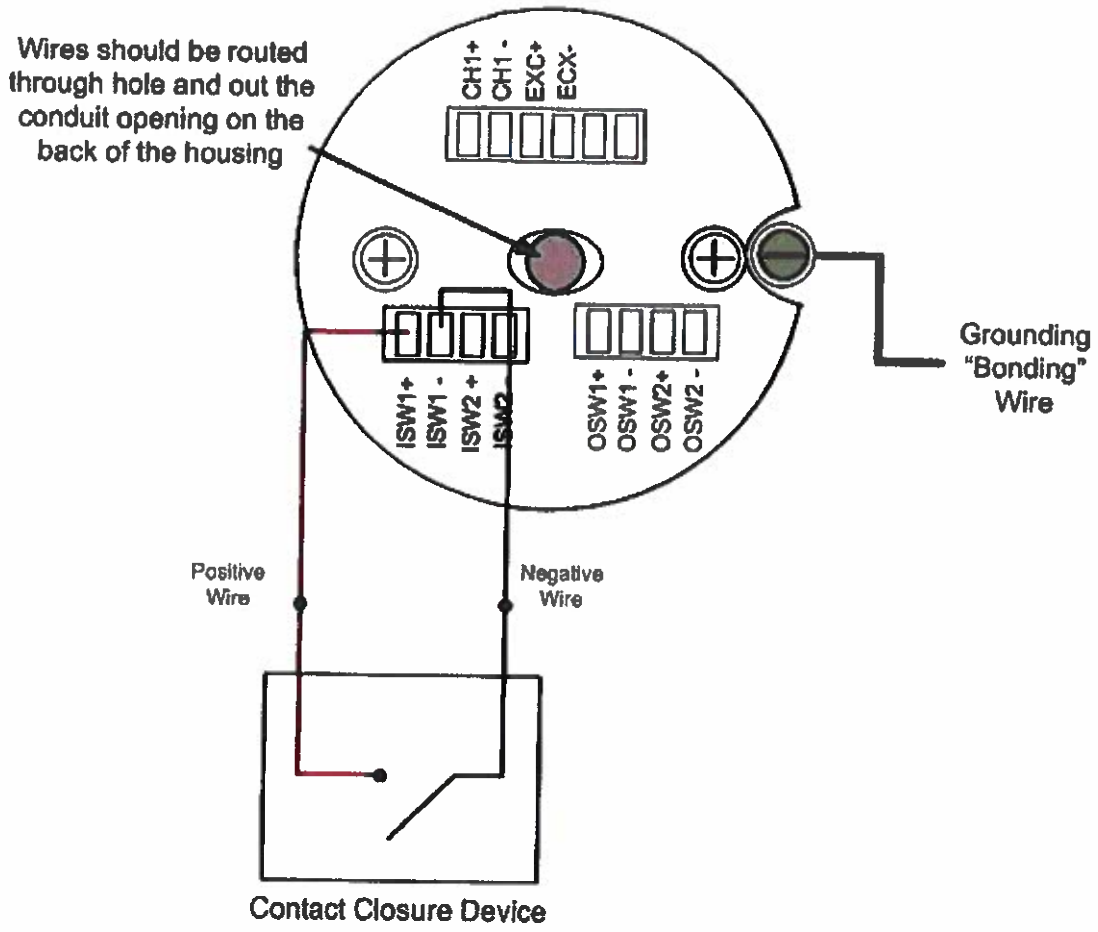
Twisted Pair 1 Red (CH1 +) and Black (CH1 –)

Twisted Pair 2 Red (EXC +) and Black (EXC –)

Switch Input Wiring

To wire a switch input device to the RT10 field unit, follow the wiring diagram shown below. Circuit power does NOT need to be supplied as the RT10 field unit supplies the monitoring power. The RT10 field unit has the capability of monitoring one input switch.

A common application for the switch inputs is to monitor a contact closure. However, the input switches can only be attached to simple devices. A simple device is one that meets the conditions set forth in the Intrinsic Safety Control Drawing.


Wires should be routed through hole and out the conduit opening on the back of the housing
CH1+
CH1 -
EXC+
ECX-
ISW1+
ISW1 -
ISW2 +
ISW2
OSW1+
OSW1 -
OSW2+
OSW2 -
Grounding "Bonding" Wire
Positive Wire
Negative Wire
Contact Closure Device