STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Trace Elements Storage

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APPROVALS: Process Change Committee **DATE: April 16th, 2013**

A. Scope

This SOP describes the procedure to prepare, store and transfer the Trace Metals and Magnesium Sulfate with Sodium Metabisulfite solutions.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment listed in section D below before starting any process work.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the laboratory, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves

C. Related Documents and SOPs

1. MSDS Binder
2. Scale Manual
3. Sampling SOP-0511
4. Media Preparation SOP-2115
5. UV Water System SOP-9555

D. Preparation/Materials/Equipment

1. Sodium Metabisulfite
2. Hydrochloric Acid
3. Magnesium Sulfate Heptahydrate ($MgSO_4 \cdot 7H_2O$)
4. Sodium Metabifulfate ($Na_2S_2O_5$)
5. Ferric Chloride Hexahydrate ($FeCl_3 \cdot 6H_2O$)
6. Cobalt(II) Chloride Hexahydrate ($CoCl_2 \cdot 6H_2O$)
7. Copper(II) Chloride Dyhydrate ($CuCl_2 \cdot 2H_2O$)
8. Zinc Chloride ($ZnCl_2$)
9. Sodium Molybdate Dyhydate ($Na_2MoO_4 \cdot 2H_2O$)
10. Boric Acid (H_3BO_3)

11. Manganese (II) Chloride Tetrahydrate ($MnCl_2 \cdot 4H_2O$)
12. 36.5%(w/w) Hydrochloric Acid (HCl)

E. Detailed Procedure

E.1 Trace Metals Solution

E.1.1 Trace Metals Solution Preparation

1. Ensure Prep Tank is clean and empty according to Media Preparation SOP-2155.
2. Prepare the Trace Metal solution (1000X) in the Prep Tank according to the Media Preparation SOP-2155 using the following formula:
    a. Final volume of UV Water: 416 lbs (50 gallons).
    b. Add the trace metal components according to the table below:

Components	Amount (g)
36.5% HCl	2240.5
$FeCl_3 \cdot 6H_2O$	455
$CoCl_2 \cdot 6H_2O$	57
$CuCl_2 \cdot 2H_2O$	28
$ZnCl_2$	57
$Na_2MoO_4 \cdot 2H_2O$	57
H_3BO_3	14
$MnCl_2 \cdot 6H_2O_2$	156

Caution: Wear respirator when deal with 36.5% hydrochloric acid. Weigh the acid in fume hood.

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E.1.2 Trace Metal Solution Storage

1. Ensure valves are configured according to the initial valve table:

Table 2 Initial Valve Configuration

Trace Metal Solution Storage				
	Application	**Valve**	**Position**	**Check**
60 Gal Trace Metal Tank	Transfer from Prep Tank	3208-V-05	Open	
	Transfer to Magnesium Sulfate Tank	3208-V-06	Closed	
	Bottom drain	3208-V-07	Closed	
	To floor drain	3208-V-08	Closed	

2 Gal Trace Metal Tank 1	Transfer from 60 Gal Trace Metal Tank	3211-V-02	Open
	Bottom drain	3211-V-06	Closed
	Transfer to Propagator 3A	3211-V-27	Closed
	Transfer to Propagator 3B	3211-V-28	Closed
	Spare vales	3211-V-01	Closed
		3211-V-25	Closed
24 Gal Trace Metal Tank 2	Transfer from 60 Gal Trace Metal Tank	3211-V-04	Open
	Bottom drain	3211-V-08	Closed
	Transfer to Fermenter A	3211-V-16	Closed
	Transfer to Fermenter B	3211-V-18	Closed
	Transfer to Fermenter C	3211-V-19	Closed
	Spare valve	3211-V-17	Closed

2. Transfer 416 lbs of trace metal solution from Prep Tank to 60 Gal Trace Metals Tank (TS-3206) according to Media Preparation SOP-2115.
3. At HMI, turn on Trace Metals Tank Agitator (AG-3206).
4. Slowly open valve 3208-V-07 to transfer trace metal solution to 2 Gal Trace Metal Tank 1 and 24 Gal Trace Metal Tank 2 by gravity feed.
5. When Trace Metal Tank 1 is filled, close valve 3211-V-02.
6. When Trace Metals Tank 2 is filled, close valve 3208-V-07 and open valve 3211-V-02.
7. At HMI, turn off Trace Metals Tank Agitator.
8. When 60 Gal Trace Metals Tank contains less than 2 gallons of solution, repeat step E.1.1.1 through E.1.2.2 to refill the tank.

E.1.3 Trace Metal Solution Transfer

1. Ensure 2 Gal Trace Metal Tank 1 and 24 Gal Trace Metal Tank 2 contain sufficient solution according to the Experimental Plan.
2. According to the Experiment Plan, transfer XX gal of trace metal solution from 2 Gal Trace Metals Tank 1 to Propagator 3A by:
   a. Ensure Propagator 3A is ready to receive trace metal solution according to Secondary Propagator 3A SOP 3220.
   b. Open valves 3211-V-06, -26.
   c. Start transfer according to Propagator 3A SOP-3220.
   d. Close valves 3211-V-06, -26.
   e. Refill 2 Gal Trace Metals Tank 1 according to section E.1.2 and repeat step E.1.3.2 if needed.

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3. According to the Experiment Plan, transfer XX gal of trace metal solution from 2 Gal Trace Metals Tank 1 to Propagator 3B by:
   a. Ensure Propagator 3B is ready to receive trace metal solution according to Secondary Propagator 3B SOP 3225.
   b. Open valves 3211-V-06, -27.
   c. Start transfer according to Propagator 3B SOP-3225.
   d. Close valves 3211-V-06, -27.
   e. Refill 2 Gal Trace Metals Tank 1 according to section E.1.2 and repeat step E.1.3.3 if needed.
4. According to the Experiment Plan, transfer XX gal of trace metal solution from 24 Gal Trace Metals Tank 2 to Fermenter A by:
   a. Ensure Fermenter A is ready to receive trace metal solution according to Fermentation Tank A SOP-3230.
   b. Open valves 3211-V-08, -16.
   c. Start transfer according to Fermenter A SOP-3230.
   d. At HMI, turn on Trace Metals Pump 1 (PT-3202).
   e. At HMI, monitor pressure in PI-3202-04.
   f. At HMI, turn off Trace Metals Pump 1 when transfer is completed.
   g. Close valves 3211-V-08, -16.
   h. Refill 24 Gal Trace Metals Tank 2 according to section E.1.2 and repeat step E.1.3.4 if needed.
5. According to the Experiment Plan, transfer XX gal of trace metal solution from 24 Gal Trace Metals Tank 2 to Fermenter B by:
   a. Ensure Fermenter B is ready to receive trace metal solution according to Fermentation Tank B SOP-3235.
   b. Open valves 3211-V-08, -18.
   c. Start transfer according to Fermenter B SOP-3235.
   d. Close valves 3211-V-08, -18.
   e. Refill 24 Gal Trace Metals Tank 2 according to section E.1.2 and repeat step E.1.3.5 if needed.
6. According to the Experiment Plan, transfer XX gal of trace metal solution from 24 Gal Trace Metals Tank 2 to Fermenter C by:
   a. Ensure Fermenter C is ready to receive trace metal solution according to Fermentation Tank A SOP-3240.
   b. Open valves 3211-V-08, -19.
   c. Start transfer according to Fermenter C SOP-3240.
   d. Close valves 3211-V-08, -19.

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e. Refill 24 Gal Trace Metals Tank 2 according to section E.1.2 and repeat step E.1.3.6 if needed.

E.2 Magnesium Sulfate Solution

E.2.1 Magnesium Sulfate Solution Preparation

1. Ensure Prep Tank is ready to receive trace metal solution according to Media Preparation SOP-2155.
2. Ensure UV Water System is operational according to the UV Water System SOP-9555.
3. Prepare the Trace Metal solution in the Prep Tank by:
   a. Ensure the Prep Tank Scale reads zero.
   b. At HMI, turn on the UV Water Pump (PC-9503) to transfer 416 lbs (50 gallons) of UV water to the Prep Tank.
   c. Add 34.07 kg of $MgSO_4 \cdot 7H_2O$ crystals to Prep Tank to give the concentration of 1.5 M.
   d. Add 53.94 kg of Sodium Metabisulfite to Prep Tank to give the concentration of 1.5 M.
   e. At HMI, turn on Prep Tank Agitator (AG-2109).
   f. Visually verify that crystals are dissolved.
   g. At HMI, turn off Prep Tank Agitator.

E.2.2 Magnesium Sulfate Solution Storage

1. Ensure valves are configured according to the initial valve table.

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Table 3 Initial Valve Configuration

Magnesium Sulfate Solution Storage				
	Application	**Valve**	**Position**	**Check**
60 Gal $MgSO_4$ Tank	Transfer from Prep Tank	3208-V-06	Open	
		3208-V-05	Closed	
	Bottom drain	3208-V-10	Closed	
	To floor drain	3208-V-11	Closed	
2 Gal $MgSO_4$ Tank 2	Transfer from 60 Gal $MgSO_4$ Tank	3211-V-03	Open	
		3211-V-28	Open	
	Bottom drain	3211-V-07	Closed	
	Transfer to Propagator 3A	3211-V-22	Closed	
	Transfer to Propagator 3B	3211-V-23	Closed	

	Spare valve	3211-V-21	Closed
24 Gal $MgSO_4$ Tank 1	Transfer from 60 Gal $MgSO_4$ Tank	3211-V-05	Open
		3211-V-28	Open
	Bottom drain	3205-V-09	Closed
	Transfer to Fermenter A	3211-V-12	Closed
	Transfer to Fermenter B	3211-V-13	Closed
	Transfer to Fermenter C	3211-V-14	Closed
	Spare valve	3211-V-11	Closed

2. Transfer 416 lbs of $MgSO_4$ solution from Prep Tank to 60 Gal $MgSO_4$ Tank (TS-3207) according to Media Preparation SOP-2115.
3. At HMI, turn on Magnesium Sulfate Tank Agitator (AG-3207).
4. Slowly open valve 3211-V-10 to transfer trace metal solution to 24 Gal $MgSO_4$ Tank 1 and 2 Gal Trace Metal Tank 2 by gravity feed.
5. When 2 Gal Trace Metal Tank 2 is filled close valve 3211-V-03.
6. When 24 Gal Trace Metal Tank 1 is filled, close valve 3208-V-10 and open valve 3211-V-03.
7. At HMI, turn off 60 Gal $MgSO_4$ Storage Tank Agitator.
8. When 60 Gal $MgSO_4$ Tank contains less than 2 gallons of solution, repeat step E.2.1.1 through E.2.2.2 to refill the tank.

E.2.3 Magnesium Sulfate Solution Transfer

1. Ensure 24 Gal $MgSO_4$ Tank 1 and 2 Gal $MgSO_4$ Tank 2 contain sufficient solution according to the Experimental Plan.
2. According to the Experiment Plan, transfer XX gal of $MgSO_4$ solution from 2 Gal $MgSO_4$ Tank 2 to Propagator 3A by:
   a. Ensure Propagator 3A is ready to receive trace metal solution according to Secondary Propagator 3A SOP 3220.
   b. Open valves 3211-V-07, -22.
   c. Start transfer according to Propagator 3A SOP-3220.
   d. Close valves 3211-V-07, -22.
   e. Refill 2 Gal Trace Metals Tank 2 according to section ***E.2.2*** and repeat step E.2.3.2 when needed
3. According to the Experiment Plan, transfer XX gal of $MgSO_4$ solution from 2 Gal $MgSO_4$ Tank 2 to Propagator 3B by:
   a. Ensure Propagator 3A is ready to receive trace metal solution according to Secondary Propagator 3A SOP 3225.

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b. Open valves 3211-V-07, -23.
c. Start transfer according to Propagator 3B SOP-3225.
d. Close valves 3211-V-07, -23.
e. Refill 2 Gal Trace Metals Tank 2 according to section ***E.2.2*** and repeat step E.2.3.3 when needed.

4. According to the Experiment Plan, transfer XX gal of $MgSO_4$ solution from 24 Gal $MgSO_4$ Tank 1 to Fermenter A by:
   a. Ensure Fermenter A is ready to receive trace metal solution according to Fermentation Tank A SOP-3230.
   b. Open valves 3211-V-08, -12.
   c. Start transfer according to Fermenter A SOP-3230.
   d. Close valves 3211-V-08, -12.
   e. Refill 24 Gal $MgSO_4$ Tank 1 according to section ***E.2.2*** and repeat step E.2.3.4 when needed.
5. According to the Experiment Plan, transfer XX gal of $MgSO_4$ solution from 24 Gal $MgSO_4$ Tank 1 to Fermenter B by:
   a. Ensure Fermenter B is ready to receive trace metal solution according to Fermentation Tank B SOP-3235.
   b. Open valves 3211-V-08, -13.
   c. Start transfer according to Fermenter B SOP-3235.
   d. Close valves 3211-V-08, -13.
   e. Refill 2 Gal $MgSO_4$ Tank 1 according to section ***E.2.2*** and repeat step E.2.3.5 when needed.
6. According to the Experiment Plan, transfer XX gal of $MgSO_4$ solution from 24 Gal $MgSO_4$ Tank 1 to Fermenter C by:
   a. Ensure Fermenter C is ready to receive trace metal solution according to Fermentation Tank C SOP-3240.
   b. Open valves 3211-V-08, -14.
   c. Start transfer according to Fermenter C SOP-3240.
   d. Close valves 3211-V-08, -14.
   e. Refill 2 Gal $MgSO_4$ Tank 1 according to section ***E.2.2*** and repeat step E.2.3.6 when needed.

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