STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Growth medium

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A. Scope

This procedure describes how to prepare growth media used during normal operations in the Lab and to determine the number of viable bacteria in samples taken from the process.

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material 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 Glasses
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. Autoclave Operation SOP-0504
2. ESCO Airstream Horizontal Laminar Flow Clean Bench Manual 2008
3. Laboratory Balance Manual
4. MSDS Binder

D. Preparation/Materials/Equipment

1. Autoclave
2. Autoclave tape
3. Laminar flow clean bench
4. Laboratory balance
5. Analytical balance

6. Nitrile gloves
7. Autoclave gloves
8. Erlenmeyer flasks (1 L)
9. Aluminum foil
10. Sterile pipettes (10 mL)
11. Sterile petri dishes
12. Stir plate
13. Stir bar
14. Sterile Filter Systems
15. Vaccum Pump
16. DI water
17. 70% Ethanol (v/v)
18. Agar
19. Hydrochloric acid
20. Weight boats
21. Sodium chloride
22. Tryptone
23. Yeast extract
24. Glucose
25. Ammonium phosphate dibasic ($(NH_4)_2HPO_4$).
26. Ammonium phosphate monobasic ($NH_4H_2PO_4$).
27. Magnesium sulfate heptahydrate ($MgSO_4 * 7H_2O$).
28. Potassium chloride (KCl).
29. Betaine.
30. Iron (III) chloride hexahydrate ($FeCl_3*6H_2O$).
31. Cobalt (II) chloride hexahydrate ($CoCl_2*6H_2O$).
32. Copper (II) chloride dehydrate ($CuCl_2*2H_2O$).
33. Zinc choride ($ZnCl_2$).
34. Sodium molybdate dyhydrate ($NaMoO_4*2H_2O$).
35. Manganese (II) chloride ($MnCl_2$).
36. Boric acid (H_3BO_3).

E. Detailed Procedure

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I. Liquid Medium

1. Make sure the Autoclave is ready according to SOP-0504.
2. Prepare a 500 mL stock solution (50% w/v) of glucose and sterilize it by filtration using a sterile filter system. This stock solution will be used along with the media at a final concentration of 2% (w/v).
3. Check the corresponding medium recipe and weigh all the components in the laboratory balance according to the desired medium (Luria Bertani or AM1).
   a. Prepare Luria Bertani media (LB) by:
      i. Weigh 10 g of Tryptone, 10 g of Sodium Chloride (NaCl), and 5 g of Yeast Extract. Dissolve all the components in 1 L of DI water using a stir bar and stir plate.
      ii. Transfer 500 mL of LB media to the Erlenmeyer flasks, tape the flasks with aluminum foil and sterilize the LB media by autoclaving at 250 °F (121 °C) and 30 min in the liquid cycle according to Autoclave Operation SOP-0504.
      iii. Turn on the laminar flow clean bench and wipe the work surface with a paper towel and 70% (v/v) ethanol.
      iv. After the autoclave cycle is complete, open the autoclave and take out the flasks using the autoclave gloves and put them inside the laminar flow clean bench.
      v. Add to each flask with LB media 20 mL of the glucose stock solution (50 % w/v) using a sterile pipette (final glucose concentration of 2 % w/v).
   b. Prepare AM1 media by:
      i. Weigh 131.5 g of ammonium phosphate dibasic ($(NH_4)_2HPO_4$) and 43.5 g of ammonium phosphate monobasic ($NH_4H_2PO_4$), and dissolve both salts in 1 L of DI water (50X stock solution). Sterilize the ammonium stock solution by filtration using a sterile filter system.
      ii. Weigh 246.3 g of magnesium sulfate heptahydrate ($MgSO_4 * 7H_2O$) and dissolve it in 1 L of DI water (1 M stock solution). Sterilize the magnesium stock solution by filtration using a sterile filter system.
      iii. Weigh 149.2 g of potassium chloride (KCl) and dissolve it in 1 L of DI water (2 M stock solution). Sterilize the potassium stock solution by filtration using a sterile filter system.
      iv. Weigh 117.15 g of betaine and dissolve it in 1 L of DI water (1 M stock solution). Sterilize the betaine stock solution by filtration using a sterile filter system.

v. For making a Trace Elements stock solution (1000 X): weigh 1.6 g of iron (III) chloride hexahydrate ($FeCl_3$*$6H_2O$); 0.2 g of cobalt (II) chloride hexahydrate ($CoCl_2$*$6H_2O$); 0.1 g of copper (II) chloride dehydrate ($CuCl_2$*$2H_2O$); 0.2 g of zinc choride ($ZnCl_2$); 0.2 g of sodium molybdate dyhydrate ($NaMoO_4$*$2H_2O$); 0.33 g of manganese (II) chloride ($MnCl_2$); and 0.05 g of boric acid (H_3BO_3), and dissolve all those salts in 1 L of 120 mM hydrochloric acid (weigh 4.38 g of HCl and bring it to 1 L with DI water). Sterilize the Trace Elements stock solution by filtration using a sterile filter system.

vi. Mix all the AM1 salts stock solutions (steps 3.b.i-v) in 1 L of sterile DI water (using a sterile screw cap bottle) in order to obtain a final concentration of 1X of ammonium stock solution (step 3.b.i; add 20 mL of this solution for 1 L of water); 1.5 mM of $MgSO_4$ * $7H_2O$ stock solution (step 3.b.ii; add 1.5 mL of this solution for 1 L of water); 1 mM of KCl stock solution (step 3.b.iii; add 0.5 mL of this solution for 1 L of water); 1mM of betaine stock solution (step 3.b.iv; add 1.0 mL of this solution for 1 L of water); and 1X of Trace Elements stock solution (step 3.b.v; add 1.0 mL of this solution for 1 L of water).

vii. Add 40 mL of glucose stock solution (50% w/v) for a 2% (w/v) final glucose concentration.

4. Use the sterile liquid medium the same day or keep it in the corresponding cabinet for 2 weeks.

II. Agar Media Plates

1. Make sure the autoclave is ready according to SOP-0504.
2. Prepare LB media plates by:
   a. Weigh all the compounds referred in the step E.I.3.a.i, and add 15 g of agar per liter of medium.
   b. Dissolve all the components in an Erlenmeyer flask (1 L) with DI water using a stir bar and stir plate for few minutes. Do not fill up the Erlenmeyer flask more than half full and cap the flask with aluminum foil and autoclave tape.
   c. Put all the Erlenmeyer flasks (1 L) with the LB agar medium into the autoclave and set the temperature and time at 250 °F (121 °C) and 20 minutes in the liquid cycle according to Autoclave Operation SOP-0504.

d. Turn on the laminar flow clean bench and wipe the work surface with a paper towel and 70% (v/v) ethanol.
e. After the autoclave cycle is complete, open the autoclave and take out all the sterile material using the autoclave gloves and put it inside the laminar flow clean bench.
f. Place the media in a 45 °C water bath for 20 min in order to cool the media without allowing it to solidify.
g. In the laminar clean bench add 20 mL of glucose stock solution (step I.2) with a sterile pipette for a final concentration of 2 % (w/v).
h. Pour 20-25 mL of agar solution into the Petri dishes and allow them to solidify (about 20 minutes).
i. Put the agar media plates into a Petri dish bag and store them in the refrigerator (4 °C) for no longer than a 1 month.
j. Turn off the laminar flow clean bench.

3. Prepare AM1 media plates by:
   a. Sterilize an Erlenmeyer Flask (500 mL work volume) with 15 g of agar in the autoclave at 250 °F (121 °C) for 20 minutes in the liquid cycle according to Autoclave Operation SOP-0504.
   b. Turn on the laminar flow clean bench and wipe the work surface with a paper towel and 70% (v/v) ethanol.
   c. After the autoclave cycle is complete, open the autoclave and take out all the sterile material using the autoclave gloves and Place the media in a 45 °C water bath for 20 min in order to cool the media without allowing it to solidify.
   d. In the laminar clean bench add all the AM1 salts as mentioned in the step E.I.3.b.vi to the Erlenmeyer flask with 20 mL of glucose (glucose stock solution 50 % w/v) and agar.
   e. Pour 20-25 mL of agar solution into the Petri dishes and allow them to solidify (about 20 minutes).
   f. Put the agar media plates into a Petri dish bag and store them in the refrigerator (4 °C) for no longer than a 1 month.
   g. Turn off the laminar flow clean bench.