



**AVIT**  
AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY



VINAYAKA MISSION'S  
RESEARCH FOUNDATION  
(Deemed to be University under section 3 of the UGC Act 1956)



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## **DEPARTMENT OF BIOTECHNOLOGY**

### **BIOPROCESSING ENGINEERING LABORATORY**

**Lab Code: 17BTCC91**

### **STANDARD OPERATING PROCEDURE**

**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****Digital Incubator**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Digital Incubator
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Digital Incubator in the Bioprocess engineering lab , Vinayaka Mission's Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology, Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR DIGITAL INCUBATOR**

- Ensure that the incubator is properly connected to the power supply and Switch on the main
- Turn on the red colour power knob towards 0-1.
- To set the incubator at 22°C , set the lower temperature 21°C by pressing the 'SET POINT -1' and simultaneously adjust the temperature with the help of screw of SET and RST by screw driver.
- Set the incubator temperature to 22°C. Wait till the set temperature is reached.
- Take a calibrated thermometer and dip it in a 500 ml beaker filled to 3/4 of the volume with Glycerol AR grade.
- Keep the beaker inside, at the center of the incubator. Close the incubator door. Allow the temperature to equilibrate for 30 minutes.
- By following the same procedure as above carry out calibration by setting the incubator temperature to 37°C, 44°C and 55°C
- Record any discrepancy observed during operation or during temperature monitoring to Quality Control Executive and notify the defect to technical assistant for rectification. Affix "BREAK DOWN" label on the instrument

**PRECAUTIONS TO BE FOLLOWED**

- Ensure that the power supply to the incubator is switched 'OFF'.



- Dedust the incubator daily externally with a clean dry cloth.
- Once a week remove adhered dust by wet mopping using detergent solution. Afterwards wipe the surface with a clean dry cloth to remove traces of detergent.
- Once in a month clean the interior surfaces with 2.5 % savlon solution using a clean cloth

*A. H.*

**HOD**

**STANDARD OPERATING PROCEDURE****HOT AIR OVEN**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Hot Air Oven
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Hot Air Oven in the Bioprocess engineering lab, Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology, Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR HOT AIR OVEN**

- Ensure the cleanliness of the instrument
- Open the ventilation knob provided on top of the oven
- Switch "ON" the power supply
- Electronic temperature controller displays the chamber temperature
- Set the required temperature by pushing the "PUSH" switch and first potentiometer knob clockwise or anticlockwise until the temperature comes to set one
- Set the temperature with the help of second potentiometer knob
- Release the "PUSH" switch
- Indicator Bulb glows indicates that the power to the heater is "ON"
- Use rotary switch for precise control of temperature

**PRECAUTIONS TO BE FOLLOWED**

- Wipe the surface, walls, top, bottom and trays of the oven with dry lint free cloth on the daily basis so that there will be no dust particles in the oven
- Wipe all the parts and the outer surface of the oven with the wet lint free cloth soaked in purified water, on weekly basis

**HOD**

## **STANDARD OPERATING PROCEDURE**

### **Laminar Airflow horizontal**

Name of the Lab./facility	Bioprocess engineering Lab
Purpose	To describe the procedure for the operation and maintenance of the Laminar Airflow
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Laminar Airflow in the Bioprocess engineering lab Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology, Lab Technicians, students

### **STANDARD OPERATING PROCEDURE FOR LAMINAR AIRFLOW**

- Switch "ON" the mains. Switch "OFF" U.V light
- Switch "ON" laminar air flow and light
- Check and ensure manometer reading "0" mm of water gauge before switching "ON". Check and ensure the manometer reading between 10 to 15 mm water gauge after switching "ON" the LAF and keep the record of reading
- In case the manometer reading is found out of limit, inform maintenance department for corrective action
- Clean the LAF bench with 70% IPA before use and after completion of work

### **PRECAUTIONS TO BE FOLLOWED**

- Validate the LAF twice a year by the third party for DOP test/smoke Test for air velocity and for nonviable particle count. Maintain U.V light burning record

**HOD****DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****UV-Vis Spectrophotometer**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the UV-Vis Spectrophotometer
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the UV-Vis Spectrophotometer in the Bioprocess engineering lab, Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR UV-VIS SPECTROPHOTOMETER**

- Check inside the UV-Vis chamber to assure that the appropriate sample holder (i.e., the liquid or solid sample holder) is in place. If it is not switch it out, the correct sample holder will be in the cabinet above the equipment.
- Turn the UV-Vis on by pressing the button in the front of the unit. The unit is not operational until the blinking light on the button goes to a solid green color
- To take a background, fill 2 cuvettes with the same solution, place them in the reference slot (R) and sample slot (S), and click on Baseline - The program will ask for range of wavelength. Type in 700 to 200nm, then click ok
- Replace the cuvette in the sample slot with your actual sample. Be sure that the cuvette is 2/3 full.
- Click Start
- After the scan is done, select where to save the data
- To view data points, click on the icon that looks like a paper with writing on it. (to the right of the icon with an M in a circle)
- To organize data, copy and paste data onto your own excel sheet



- To save a single scan, go to file>save as

### **PRECAUTIONS TO BE FOLLOWED**

- Click Disconnect
- Flip switch on UV-VIS off
- Always turn the system off when you do not plan to use it soon to conserve the lamp life

*A. H.*

**HOD**

**STANDARD OPERATING PROCEDURE****WATER BATH INCUBATOR SHAKER**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Water bath Incubator Shaker
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Water bath Incubator Shaker in the Bioprocess engineering lab, Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HoD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR WATER BATH INCUBATOR SHAKER**

- Fill and check the water level, if required fill purified water to the acceptance level. The minimum water level is indicated by a black line on the water level indicator on left
- Switch "ON" the ring both by pressing "ON/OFF" switch
- The digital temperature controller cum indicator will indicate the actual temperature of water
- Set the desired temperature by pressing the PRESS to SET switch and adjusting the SET pot

Approximately 1°C before the set temperature, the heater will start going on and off Heater action is indicated by the LED on the DTC. Allow a few minute for the temperature to stabilize

**PRECAUTIONS TO BE FOLLOWED** Do not operate without water. Switch OFF when the instrument is not in use

  
**HOD**



**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****MAGNETIC STIRRER**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Magnetic Stirrer
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Magnetic Stirrer in the Bioprocess engineering lab Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR MAGNETIC STIRRER**

- Place the magnetic stirrer on a stable well-levelled surface.
- Place the stir bar at the bottom of a glass container.
- Fill the glass container with the liquid to be stirred.
- Plug the mains cable into a suitably earthed socket.
- Check that the speed control knob is completely turned anticlockwise.
- Place the glass container on the centre of the magnetic stirrer.
- Press the On/Off switch to turn the magnetic stirrer On. The switch will light green.
- Adjust the speed control knob to a low stirring rate.
- Continue to adjust the speed control knob until the desired stirring speed is achieved.
- Wait until the liquid is properly mixed.
- Completely turn the speed control knob anticlockwise.
- Press the On/Off switch to turn the magnetic stirrer Off
- Manipulate another stir bar from the outside of the glass container to remove the immersed stir bar

**PRECAUTIONS TO BE FOLLOWED**

- Thoroughly wash the stir bar with distilled water after each application.



- Store stir bars in pairs to maintain their magnetic strength and increase their life span.

**RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

*A. J. H.*

**HOD**

**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****COOLING CENTRIFUGE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the cooling centrifuge
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the cooling centrifuge in Bioprocess engineering lab, Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR COOLING CENTRIFUGE**

- Open the upper lid by releasing the lock and lifting it up.
- Place the centrifuge tubes in the compartment provided for it.
- Switch on the main button.
- Set the desired time can be selected by pressing "SET TIME" by push button having range 0-60 minutes.
- Set the desired temperature can be set by adjusting knob.
- Increase the RPM of the centrifuge with the help of the adjustment knob.
- Gradually increase the rpm. Maximum 15000 rpm can be selected.
- When the desired rpm attained, now selected the time for centrifugation with the help of set time push button.
- After completion of the centrifugation time, a buzzer will beep, which indicates the completion of the cycle.
- After the beep, the motor will automatically off and rpm will come down to 0.
- Switch off the mains and remove the samples from the centrifuge.
- Clean the in wall of the centrifuge with dry lint free cloth.

**PRECAUTIONS TO BE FOLLOWED**

- Proper handling of the instrument.

- Ensure level and stability.
- Balance centrifuge tubes equally.
- Ensure use of rubber cushions for glass tubes.
- Bring speed knob to off and increase speed gradually.
- Do not open the lid in between the centrifugation cycle.

**RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

**HOD**

**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****CENTRIFUGE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Centrifuge
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the Centrifuge in the Bioprocess engineering lab, Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR CENTRIFUGE**

- Press the start/stop button and slowly increase the rpm to the desired speed using the dial
- Once a run is complete, make sure the rotor has COMPLETELY STOPPED before opening the centrifuge lid by depressing the red stop/start button.
- Remove sample vials.
- Remember to return the rpm dial back to zero after finishing.

**PRECAUTIONS TO BE FOLLOWED**

- Proper handling of the instrument
- Ensure level and stability
- Balance centrifuge tubes equally
- Ensure use of rubber cushion for glass tubes
- Bring speed Knob to off and increase the speed gradually.
- Do not open the lid in between the centrifugation cycle

**RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

  
**HOD**

**DEPARTMENT BIOTECHNOLOGY**  
**STANDARD OPERATING PROCEDURE**

**ELECTRONIC WEIGH BALANCE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Electronic Weigh balance
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the weigh balance in the Bioprocess engineering lab , Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR ELECTRONIC WEIGH BALANCE**

- Switch ON the Power button
- Keep the butter sheet or aluminum foil, Press TARE to equivalence the weight
- Add the chemicals on to the butter sheet and weigh it accurately
- Wear clean cotton gloves (supplied with reference weights) or use forceps while handling reference weights. To avoid depositing oil and dirt onto the surface of the weight, do not touch weights with bare hands.
- Store reference weights in cases provided by the manufacturer.
- For optimal performance, place balance on a stable, even, horizontal surface with minimal vibration. Avoid areas with excessive heat and moisture, direct sunlight, aggressive chemical vapors, and drafts.
- If a balance is transferred to a different location, perform the accuracy check prior to use in the new location.
- Switch OFF the power button

**PRECAUTIONS TO BE FOLLOWED**

- Short circuit of the battery terminals or any source terminals has to be avoided.

- Avoid spilling of chemicals
- Clean the spilled chemicals/powders immediately to avoid deposition.
- Avoid over weighing, above the limit
- As it is air sensitive, handle with care
- Perform annual calibration of weigh balances at approximately the same time each year

**RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

**HOD**

**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****HEATING MANTLE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the Heating mantle
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the heating mantle in the Bioprocess engineering lab, Vinayaka Missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR HEATING MANTLE**

- Switch ON the Power button
- Set up flask and condenser as required.
- Connect hose to tap and turn on to give a gentle flow of water
- Switch on heating mantle and set to required temperature setting. Monitor temperature. Do not use a mercury thermometer.
- Place HOT warning sign near the heating mantle.
- Monitor system during heating procedure.
- When procedure complete, carefully remove glassware, using heat proof gloves.

Switch off heating mantle and leave HOT warning sign in place until everything is cool.

**PRECAUTIONS TO BE FOLLOWED**

- Know where the nearest firefighting equipment
- Know the emergency phone number 33#
- Refer to the SDS for any chemicals being used
- Place a HOT warning sign at the heating mantle
- Read and understand the procedure
- Check that the equipment is electrically compliant

**HOD**



**DEPARTMENT BIOTECHNOLOGY****STANDARD OPERATING PROCEDURE****HOT PLATE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the HOT PLATE
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the hot plate in the Bioprocess engineering lab, Vinayaka Missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology Lab technicians, students

**STANDARD OPERATING PROCEDURE FOR HOT PLATE**

- Plug in power cable and switch on the Hot Plate
- Keep the Glassware on the hot plate and set the temperature using knob
- Red light glow indicates the Hot plate is ON
- Once the appropriate time is over, turn the knob to zero
- Switch off the power switch, after use.

**PRECAUTIONS TO BE FOLLOWED**

- Short circuit of the battery terminals or any source terminals has to be avoided.
- Avoid spilling of chemicals
- Clean the spilled chemicals/powders after the usage to avoid deposition.
- As the plate is hot avoid touching with bare hands
- Always wear gloves and lab coats.

**RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

**HOD**

## **STANDARD OPERATING PROCEDURE**

### **TOP PAN BALANCE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the TOP PAN balance
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the weigh balance in the Bioprocess engineering lab , Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology, Lab Technicians

### **STANDARD OPERATING PROCEDURE FOR TOP PAN BALANCE**

- Switch ON the Power button
- Keep the butter sheet or aluminum foil, Press TARE to equivalence the weight
- Add the chemicals on to the butter sheet and weigh it accurately
- Wear clean cotton gloves (supplied with reference weights) or use forceps while handling reference weights. To avoid depositing oil and dirt onto the surface of the weight, do not touch weights with bare hands.
- Store reference weights in cases provided by the manufacturer.
- For optimal performance, place balance on a stable, even, horizontal surface with minimal vibration. Avoid areas with excessive heat and moisture, direct sunlight, aggressive chemical vapors, and drafts.
- If a balance is transferred to a different location, perform the accuracy check prior to use in the new location.
- Switch OFF the power button

### **SPECIFIC**

#### **Frequency**

Switch off/on the instrument mains. Open the sliding doors for the balance and remove the pan

inside the balance. Remove the dust or powder with a help of soft brush.

Soak the tissue paper in isopropyl alcohol and clean the balance to remove the oil substance or any spot inside..Finally clean it with tissue and dry in air

Check air bubble is in the centre of the circle

Do spirit level check, pan clean and adjustment , zero error check and calibrate properly before use

### **PRECAUTIONS TO BE FOLLOWED**

- Short circuit of the battery terminals or any source terminals has to be avoided.
- Avoid spilling of chemicals
- Clean the spilled chemicals/powders immediately to avoid deposition.
- Avoid over weighing, above the limit
- As it is air sensitive, handle with care
- Perform annual calibration of weigh balances at approximately the same time each year

### **RECORD TO BE MAINTAINED**

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

*A. J. H.*

**HOD**

## **STANDARD OPERATING PROCEDURE**

### **MICROPIPETTE**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the MICROPIPETTES
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the weigh balance in the Bioprocess engineering lab , Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology, Lab Technicians

#### **STANDARD OPERATING PROCEDURE FOR MICROPIPETTE**

##### **A. Choosing the Right Instrument**

1. Each micropipette has a range of volumes for which it is accurate and reliable. This range is stamped on the pipette below the display window and should never be exceeded.
2. The volumes of the micropipettes in this lab are 1-10 µl, 10- 100 µl and 100-1000 µl. Proper selection of the correct pipette is very crucial to accurate pipetting.

##### **B. Pipetting**

1. Set the pipette to the desired volume by depressing the adjustment button next to the display and rotating the plunger button on the end until the correct numbers appear in the display. CAUTION: Do not rotate the volume in the display beyond the minimum or maximum numbers stamped on the micropipette. Do not rotate the plunger button without depressing the adjustment button first.

##### **B. Fit an appropriate size tip on the tip holder by using a slight twisting motion when pressing the tip holder into a pipette tip to help form an airtight seal.**

CAUTION: If when aspirating a liquid, you see a drop appear at the end of the tip as you hold the plunger button down, then there is a tip leak and you should refit a new tip.

3. Pre-rinse the tip by aspirating the first volume of liquid and then dispensing it back into the sample container.

4. Aspirate by pressing the plunger button to the first stop slowly and smoothly.
5. While still holding the plunger button down, hold the pipette vertically and immerse the tip into the liquid and hold it at a constant depth below the surface of the liquid.
6. Slowly release the plunger button so that the pipette will aspirate the liquid in the sample. As the depth of liquid lowers, so must the tip of the pipette. Once the plunger button has completely been released, hold the pipette in the liquid for 1 additional second to complete aspiration.
7. Remove the pipette tip from the solution and continue to hold the pipette vertically.
8. To dispense the sample, place the tip against the inside wall of the tube that is receiving the sample at a slight angle. Press the plunger button slowly and smoothly to the first stop.
9. Wait at least one second as it dispenses and then press the plunger button further to the second stop to expel any residual liquid from the tip.
10. Keep the plunger button pushed down as you withdraw your sample from the tube so as not to re-aspirate any of the transferred sample.
11. Release the plunger button slowly and smoothly.

#### C. Tip Removal

1. The tip may be ejected from the tip holder by pressing the blue button at the base of the end button while holding the pipette tip over a waste container.
2. Tip changes are required only if aspirating a different liquid, sample or reagent or volume. If contamination of the tip is a concern, then a tip change is always appropriate.
3. Once done with dispensing samples, reset volume to maximum volume for proper storage

#### RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

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**HOD****STANDARD OPERATING PROCEDURE****BIOREACTOR**

Name of the Lab./facility	Bioprocess engineering lab
Purpose	To describe the procedure for the operation and maintenance of the BIOREACTOR
Scope	This Standard Operating Procedure (SOP) applies to the staff and students using the weigh balance in the Bioprocess engineering lab , Vinayaka missions' Research foundation to carry out research works and experimental purpose.
Responsibility	Faculty i/c of the facility, HOD/Biotechnology, Lab Technicians, students/

**STANDARD OPERATING PROCEDURE FOR BIOREACTOR**

Description and Uses of Fermentor can be used for batch culture with microprocessor control of pH, dissolved oxygen (DO), agitation, temperature, pump feed rate, antifoam, foam/level, and additional analog/digital inputs and outputs. It has a maximum volume of 5-L and a maximum operating temperature of 150°C.

**PRECAUTIONS TO BE FOLLOWED**

**Safety Precautions:****High Voltage/Possible Electric Shock (220-V)**

Make sure that the wall outlet receptacle is properly wired and grounded, and matches the instrument's power cord and plug. • Do not touch the power cord or plug if hands or feet are wet, or if standing on a wet/damp surface, as severe electrical shock or death may result.

**Excessive Weight/Possible Damage to Hands or Fingers when Moving or Repositioning Parts**

Certain parts of the fermentor, such as the head plate, condenser, etc. are very heavy. Use extreme caution when handling or moving these parts during fermentor assembly/disassembly. If available, wear shoes with steel caps when using the fermentor.

**Hot Surfaces/Possible Burns to Hands and/or Extremities**

The fermentor is made of stainless steel and has a "jacket" that is filled with steam under pressure during the sterilization-in-place (SIP) process. As a result, the outer surfaces of the fermentor become HOT enough to cause serious skin burns if the fermentor is touched. Use extreme caution when working near the fermentor during sterilization.

**Biohazard/Possible Infection from use of Pathogenic Microbes**

Normal operation involves the use of many types of microorganisms, all of which can be opportunistic pathogens. Occasional use may also involve working with genetically modified microbes. Use extreme caution and good sterile techniques when working with these organisms. After use, autoclave the fermentor to kill any remaining microbes.

**Required Personal Protective Equipment**

- Lab Coat
- Hair Net
- Safety Glasses or Goggles
- No Open-toed or Open-heeled Shoes
- Insulated Rubber Gloves
- Steel-toed Shoes are recommended

**Required Training**

- Machine & Site-Specific Training
- Fire Safety & Extinguisher Training



**Operation****Operation: Start-Up**

1. Be sure to complete all the required training listed above, including machine and site-specific training by the Fermentation Facility manager. It is highly recommended that the 5-L KEMI Fermentor be operated under the direct supervision of the Fermentation Facility manager.
2. The Operating Manual provides a more complete description of the KEMI Fermentor. Be sure to read the manual before operating the fermentor to become familiar with its correct operation and individual component parts.
3. Inspect the fermentor to see if it is clean. If not, clean the vessel with warm soapy water. Then, rinse with tap water followed by a distilled-water rinse. If necessary, clean the underside of the head plate, impeller shaft, and paddles.
4. Once clean, fill the fermentation vessel with deionized (DI) water to a depth sufficient enough to cover the pH probe so that the probe does not dry out.
5. Turn ON the fermentor controller using the key on the front of the Control Panel.
6. Open the "Instrument Air Valve" located above the central lab bench.
7. Open the "5-L Vessel Air Valve" to sparge the fermentor.
8. Close the "Cooling Water Valve" to the heat exchanger and close the Thermostat Pressure Valve first. It should read ~1.5 bar.
9. Calibration of the pH Probe is necessary prior to sterilization and filling the fermentor with media. To calibrate the probe, perform the following:
  - a. Drain the fermentor vessel. This is necessary because the pH probe must be disconnected from the vessel during calibration. If the vessel is full of water, it will spill out when the pH probe is removed.
  - b. Connect the pH probe cable to the fermentor controller.
  - c. Remove the pH probe from the fermentor vessel.
  - d. turn ON the fermentor pH meter using the flip switch.
  - e. Set the temperature knob to ambient (~20°C).



- f. Switch the pH meter to “Manual.”
- g. Rinse the pH probe w/deionized (DI) water. Then, immerse the probe in pH 7 buffer.
- h. Adjust the  $\Delta$  pH knob until the pH reading is 7.0.
- i. Rinse the pH probe with DI water. Then, immerse the probe in pH 4 buffer.
- j. Keep the probe immersed in either the pH 4 or pH 7 buffer until just prior to filling the fermentor vessel with media to prevent it from drying out.
10. Check the pH probe O-ring for possible damage. If necessary, replace the O-ring.
11. Apply a small amount of glycerol or silicone grease to the pH probe O-ring.
12. Insert and secure the pH probe inside the fermentor.
13. Rinse the fermentor vessel with deionized (DI) water prior to filling and preparing the media. When finished, close the vessel drain valve.
14. Fill the vessel with DI water and add medium ingredients.
15. Set the agitator at ~100 rpm to dissolve and mix media ingredients. Note: The level of media should be high enough to cover the probes.
16. Check that all connections (e.g., sample port, inoculation port, etc.) to the vessel are securely closed.

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9. Set sterilization time (STERILIZATION) on the Control Panel. This should be 20-25 min at 121°C for refined media and longer for media with a high suspended-solids content.
10. Turn ON the thermostat. Then, set the thermostat to the desired temperature to be used during the fermentation.
11. Hold the “Sterilization” switch up for a few seconds. The indicator will show the sterilization time in minutes. While the vessel heats up, the time will be flashing. When the vessel reaches sterilization temperature (121°C), the time on the indicator will be steady.
12. To cool down the fermentation vessel after sterilization, pressurize the vessel to avoid drawing a vacuum as follows
  - a. Close the “Exhaust Valve” by turning on
  - b. Close the “Inlet Air Condensate Valve.” This valve is located below the “Sterilizable Gas Filter.”
  - c. Open the “Inlet Air Valve”
  - d. Open the “Inlet Air Control Valve” a few turns.
  - e. Open the “Cooling Water Valve” to the heat exchanger slowly until it is fully open.
  - f. Turn on the cooling water to the condenser by fully opening the “Condenser Water Value.”
  - g. Wait until the fermentor temperature falls below 80°C.
  - h. Close the steam supply to the sample and drain ports. Then, close the “5-L Main Steam Valve.”
  - i. Turn the air on to 1 m<sup>3</sup>/h (positive air). Use the “Inlet Air Pressure Regulator” to decrease the air pressure to ~0.5 bar. Then, adjust the “Inlet Air Control Valve” to keep the pressure between 0.5 and 1.0 bar.

#### **Operation: Fermentation**

1. When the temperature in the vessel approaches the temperature to be used during the fermentation, reduce the flow of water to the heat exchanger by partially closing the “Cooling Water Valve.” This valve should remain open just a “crack.” Note: During the actual fermentation, keep the valve open slightly. This keeps cold water flowing to the heat exchanger. The exchanger performs a “balancing act” between the cold water and steam going to the fermentor to keep the fermentor temperature at the desired set point.
2. If the fermentation is to be performed aerobically, turn the “Sparge/Overlay Valve” fully clockwise to “Sparge.”

3. After the medium has cooled to the desired fermentation temperature, sparge the fermentor with either sterile air or oxygen, as required, to saturate the medium with oxygen.

4. Fermentor Inoculation: Depending on the inoculum volume (~50-1,000 mL), the inoculum can be added to the fermentor using a large sterile syringe and a sterile large-bore needle. For volumes of several hundred mL and larger it is better to add the inoculum from a sterile bottle using a peristaltic pump. In either case, the inoculum is added through a sterilizable, self-sealing septum on the lid of the fermentor.

a. By peristaltic pump: Insert an 8-10 inch long piece of stainless-steel tube in one end of the #25 tubing. Insert the stainless-steel tube through a rubber stopper on a screw-cap for a 0.5-1.0 L bottle. Wrap foil around the cap. Then, autoclave the tubing and bottle assembly. After sterilization, aseptically transfer the desired volume of inoculum into the sterile bottle. Sterilize the septum on the fermentor lid with several mL of alcohol. Uncover the needle and insert it through the septum. Turn ON the pump to deliver the inoculum to the fermentor. After the inoculum has been added, turn OFF the pump, withdraw the needle and wash the septum again with alcohol.

7. Foam Control: If the medium is foamy, add a few drops of sterile antifoam using a sterile needle/ syringe assembly. Add the anti-foam through one of the sterilizable, self-sealing septa on the fermentor lid.

8. Sampling the Fermentor:

a. To periodically collect samples during the fermentation, open the "Vessel Port Steam Valve." This will send steam through the sampling port to sterilize it and prevent introduction of contaminants during the sampling procedure.

b. After 1-2 minutes, remove the cylindrical metal "sleeve" from the sampling port.

c. Position a collection vessel (e.g. a beaker or flask) below the sampling port and turn the sampling valve clockwise until the medium drains from the vessel into the sampling container.

d. When a sufficient sample has been collected, turn off the sampling valve until the drainage stops.

e. Replace the metal sleeve over the sampling port and steam the valve for ~2 min (as stated above in Step 8a).

## RECORD TO BE MAINTAINED

- Laboratory Manual containing the experiments that can be performed with the equipment
- Maintenance Record

**HOD**



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