

OVERVIEW

Zenith BioFERM bioreactor is an all-purpose bioprocessing system designed to maximize yields in both fermentation and cell culture. Uses the newest advances in microprocessor control with successful stirred-tank technology, **BioFERM** blends multi-loop process control with the latest innovations in cell culture. Various accessories are available, including a selection of interchangeable bioreactor vessels. Controls pH, DO, temperature, air flow, stirring speed using microprocessor based control system.

The **BioFERM** bioreactor is controlled by micro processor based digital PID control algorithm. The temperature control range is from room temperature to 70°C. Using optional cooling system, temperature can be controlled from 10°C to 70°C. It has a stirrer agitation speed range from 50 to 1000 rpm and it can be used very usefully for anaerobic microorganism or aerobic microorganism fermentation process.

- Easy operation. LCD display. Space saving installation.
- Various size of vessels are interchangeable.
- An air pump is installed to supply air into vessel.
- The pH and DO sensors are attachable when required. Microprocessor based digital PID control.

Volume of sample run is 50% to 70% of vessel size.

MODULAR DESIGN

BioFERM offers a real alternative to our competitors with a truly modular system approach. Each module is totally independent, using its own power supply, pumps etc. Although individual, the modules can stack together to create an extremely neat, combined unit with inter-linking mains cable.

The controllers are easy to understand and simple to use, ideal for projects where equipment familiarisation time may be limited. Because the modules are totally independent, they can also easily be used to up-grade older equipment.

Basic unit has systems for

- Agitation
- Aeration
- Temperature control
- pH and DO display

The **BioFERM** range offers a combination of ease of use with robust and sophisticated instrumentation at competitive prices. A comprehensive, fully microprocessor controlled fermentation system which can have :

Temperature

With full PID control of heating and cooling using heater, chiller and RTD sensor, range 10-70°C.

pH

With fully isolated circuit with peristaltic pump for acid and base additions. Each pump has its own run timer, range 2-11pH.

Oxygen

Using galvanic electrodes with full control of oxygen addition. A rotameter with air control valve is also supplied.

Stirrer

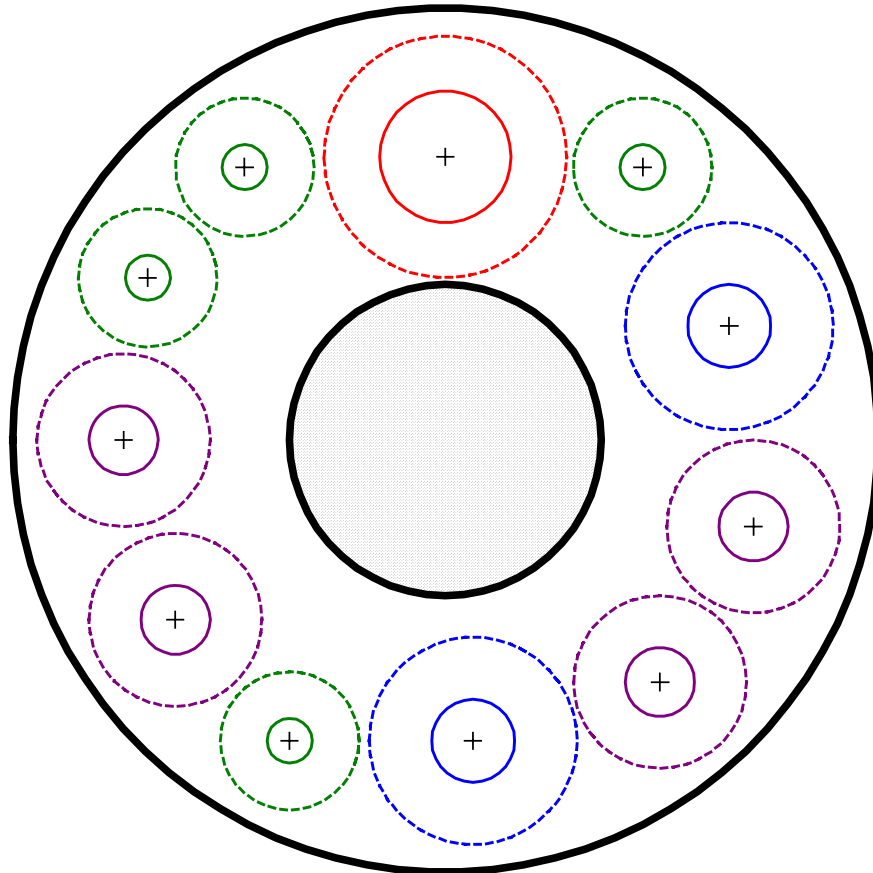
Overhead stirrer with powerful motor and reduction drive to increase torque. There is a display of speed and power used. The stirrer can be remotely controlled from the electronics. Motor power 80W. Speed range 70-1000rpm.

Vessel

A wide range of vessel sizes and types. Standard vessels are 2, 5 and 7 litres, flat bottomed with a stainless steel top plate.

ASSEMBLY

A figure of the head plate is shown:



Clockwise from top:

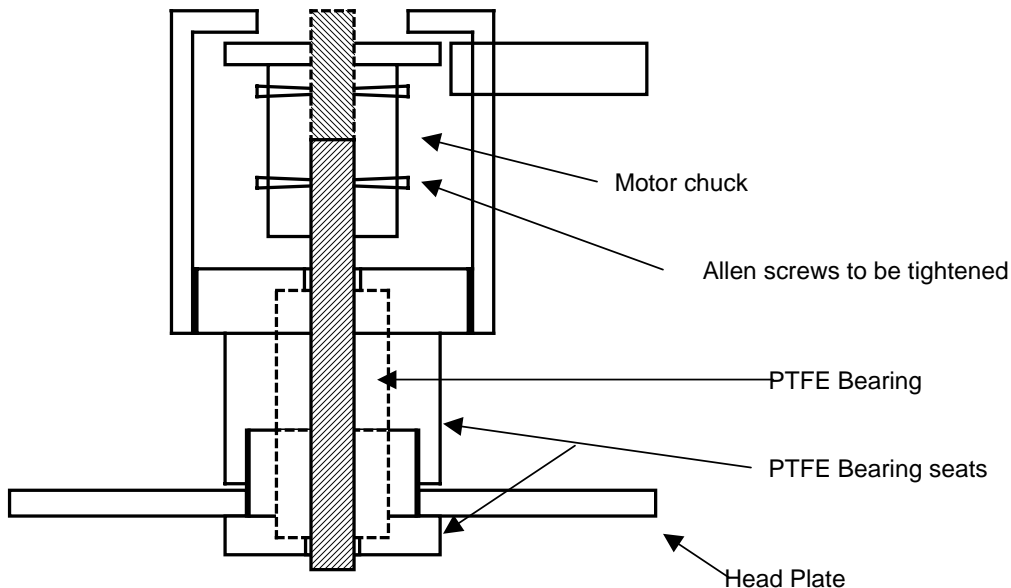
- | | |
|-----------------|-------------------------|
| 1. Heater | 7. Acid Addition |
| 2. Harvest | 8. DO Probe |
| 3. Exhaust Port | 9. Temperature Probe |
| 4. pH Probe | 10. Sparger |
| 5. Feed | 11. Base Addition |
| 6. Spare 10mm | 12. Motor (Centre port) |

ASSEMBLING THE BIOREACTOR

1. After the glass vessel is autoclaved it is ready to be placed in the cooler.
2. Place the large silicone gasket on the circular stainless steel stand in the cooler.
3. Now place the stainless steel ring which has the four threaded holes over the silicone gasket. Align the holes with holes of the stand on which it has been placed.
4. Place another of the silicone gaskets over this ring. The gasket has to be aligned inside the holes of this ring.
5. Carefully lift the glass vessel and place it over the gasket. Ensure the gasket is below the rim of the glass vessel.
6. Next place the third silicone gasket over the rim of the glass vessel.
7. Add amounts of silicone grease to it to ensure sealing.
8. In the head plate from below screw the stainless steel PTFE bearing holder. Tighten this as far as possible.
9. Take the sparger tube and pass it through its hole in the head plate. Which hole is which can be seen from the head plate diagram above.
10. Take the stirring rod and pass it through the center hole which now has the stainless steel bearing holder mentioned in (8) above.
11. Now carefully place the head plate and align its four holes to the holes in the stand.
12. Using the provided 1" long stainless steel bolts tighten the head plate to the stand so that the glass vessel is also firmly against the head plate.

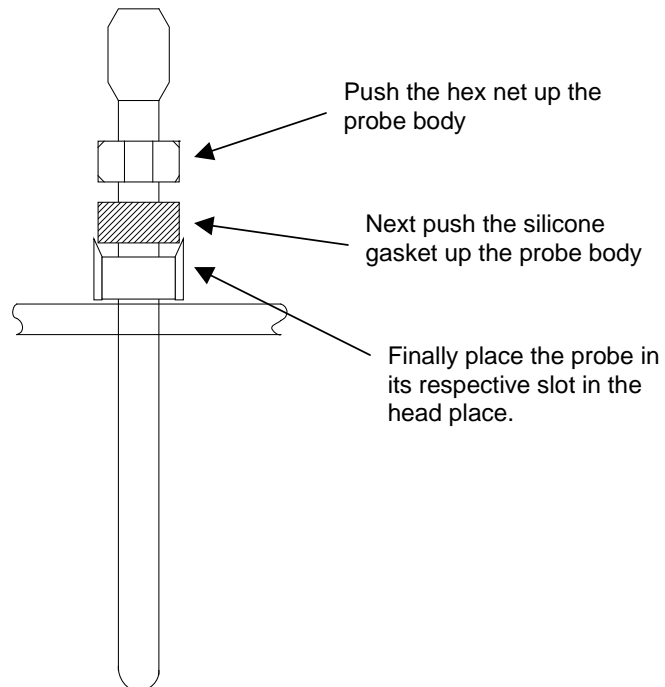
MOUNTING THE AGITATION MOTOR

1. In the center of the head plate there is a bearing seat for PTFE bearings provided. Apply silicone grease around the bearing and inside it as well. Push the PTFE bearing into this seat.
2. Rub some amount of silicone grease on the bearing inside and pass the stirrer rod through it from below the head plate.
3. Next screw the top half of the bearing seat and tighten it hard.
4. Push the stirrer rod upwards a little and then mount the motor assembly ensuring that the stirrer rod fits into the motor chuck.
5. From the side hole in the motor assembly tighten the chuck so that the stirrer rod is tightly held.
6. Finally screw the motor assembly into the bearing seat and tighten it hard.
7. Connect the motor supply wire (the big one) to its socket in the back of bioreactor control module.
8. Connect RPM sensor (the one with four terminals) to its socket in the back of bioreactor control module.



MOUNTING THE PROBES, SPARGER

1. Mount the different hex nuts into their respective probes, sparger, temperature probe etc.



2. After this mount the silicone gaskets on to the probe etc.
3. Carefully place the probe etc. into its respective holder.
4. Firmly tighten the hex nut so that the probe remains firm in its position and does not move.

CONNECTING SENSOR CABLES & POWER CORDS

1. In the back of the bioreactor module, there are sockets marked for various sensors.
2. All except DO and pH sensor sockets are unique and cannot be interchanged. These are BNC types with 2 terminals each.
3. The RPM sensor has 5 terminals.
4. The temperature sensor has 3 terminals.
5. Connect the sensors as per the labels over them.
6. The DO and pH sensors are marked and ensure their correctness.
7. The motor power supply is chord has 4 terminals.
8. Apart from these, there are two other power terminals of 220VAC types.
9. One is marked heater and other cooler (HTR & CLR). Connect the cooler chord from the base unit to the CLR socket and from the heating finger to the HTR socket.
10. There is another socket, which is unmarked on the side of the bioreactor. That is for field upgrades to the microprocessor inside.

1. Connect the probe wires to the respective probes at one end and the module at other end.
2. To recognize the pH and DO probe:



pH Probe has a small spherical bulb at the end



DO Probe has a cylindrical bulb at the end

3. The probes have to be calibrated before. See the calibration procedure to calibrate the probes.
4. The rotameter on the front of bioreactor controller has a pipe connector on top. Using the provided silicone tube connect it to the sparger pipe.
5. The temperature RTD sensor should be coated with the silicone grease and dropped into the thermowell in the head plate.
6. The acid, base, addition, harvest glass tubes should be fitted with the thin silicone tubes and attached to the peristaltic pumps.
7. The bioreactor is now ready for operation.

SETTING UP THE PERISTALTIC PUMPS

1. Open the front lids of the peristaltic pumps. The silicone tube is now placed next to the rollers.
2. Rotate the motors a bit. While the rollers are rotating insert the tubes between the rollers and the tube will be in place.
3. The tubes are then held in place by the spring clamps provided at the top and bottom of the pump opening.
4. To prime the pumps press the three way switch on top of the motor to ON and the pumps will start working. Prime the tubes and then switch OFF the pumps.
5. To let the pumps be operated by the controller, press the three way switch to the CTRL position. In this way the pumps will be switched on and off by the pH controller in the control system.

SETTING UP THE ACID/BASE BOTTLES

1. There are two opening on the caps of the bottles.
2. In the smaller opening, connect a suitable sized silicone tube about 2" long and at the other end connect a micron filter.
3. In the other opening place a glass tube that reaches to the bottom and connect the silicone tubes from the peristaltic pumps to the opening of the glass tube.

SETTING UP THE CONTROL

1. The front of the bioreactor has an on-off switch that powers up the unit.
2. There is a LCD with a 16 key keypad.
3. When the unit powers up, the main menu displays four options:
 - CALIBRATE**
 - SET**
 - START**
 - SHUTDOWN**
4. The **CALIBRATE** option is to calibrate the pH, DO and temperature sensors.
 - a. Press calibrate key to enter the calibration procedure.
 - b. There will be options for calibrating pH, DO and temperature. Press the appropriate key to enter respective calibrating procedure.
 - c. For calibrating the temperature sensor, two temperature calibration is needed at 0°C (using ice+water mixture) and at 100°C (boiling water). Prepare these solutions and dip the sensor in one of them. Press the appropriate key and wait for calibration to stop. Then dip in other solution, press key and wait for calibration to stop. If the calibrating is okay and you want the data saved, press TICK key (√). If you don't want the data saved or if the calibration was false, press CROSS key (X).
 - d. To calibrate the pH sensor, you will need 2 of 3 buffer solutions (4.0, 7.0 or 9.2). Prepare these solutions at 25°C or whatever the buffer tablets say. Dip the electrode in first buffer solution, wait for a minute, press appropriate key and wait for calibration to stop. Before dipping in other solution, clean electrode by washing with deionised water and wiping with soft paper or cloth. Now dip in other solution, wait for a minute, press appropriate key and wait for calibration to stop. If the calibrating is okay and you want the data saved, press TICK key (√). If you don't want the data saved or if the calibration was false, press CROSS key (X).

- e. Calibration of DO sensor is always done at the temperature the fermentation is to be run at. Dip the sensor in vessel where the fermentation is being run. Connect the silicone tube from the rotameter to the sparger tube. Open the rotameter valve to the fullest. Press the Calibrate at 100% key and let it finish. After this remove the sensor connector at the back of the control unit. Press the Calibrate at 0% key and wait for it to finish. If the calibrating is okay and you want the data saved, press TICK key (\checkmark). If you don't want the data saved or if the calibration was false, press CROSS key (X).
- f. Calibration can be done before autoclaving for pH and temperature sensors. For the DO sensor it should be done after autoclaving.
- g. To **SET** the control parameters, press the key marked SET on the keypad. Using the " > " key you can step one digit ahead while setting. To set the values, use the number keys. After setting if you want the values saved, press TICK key (\checkmark). If you don't want the data saved, press CROSS key (X).
- h. If after setting you press SET button once again, you will enter the DeadBand setting procedure. DeadBand is the hysteresis of control. It provides the region around the control values where no control takes place. For example, if the temperature set is 30°C and DB value for temperature is 0.5°C, then between 29.5°C and 30.5°C no control action (neither heating or cooling) will take place. Deadband is to prevent excessive control action and cycling around the set point. In the DB setting procedure the first value is hysteresis value below the setpoint and the second one is for above the setpoint. After setting if you want the values saved, press TICK key (\checkmark). If you don't want the data saved, press CROSS key (X). If you are unsure what to do, press CROSS key (X).
- i. After the calibration and setting procedures you can press the START key to begin the run.

Application Note

Fermentation protocol using BIOFERM bioreactor

Saccharomyces Cerevisiae - Aerobic Baker's Yeast Fermentation

1. Introduction

Yeast belongs to the class of Protoascomyceten and more specifically to the physiological class of *Saccaromyces cerevisiae*. *Saccaromyces cerevisiae* is crabtree positive yeast, which is sensitive to high substrate concentrations that results in the reduction of the oxygen uptake rate. As a result glucose is metabolised to ethanol. This metabolic pathway can be reduced by introduction of a regulated feed procedure adapted to the specific growth rate of the yeast. This is done using a fermentor.

2. Equipment and Materials Used

- BIOFERM 7L Bioreactor Assembly
- Balance
- pH Meter
- Photometer
- Magnetic stirrer plate
- Miscellaneous lab glassware like:
 - 1 graduated flask 5000 ml
 - 1 graduated flask 1000 ml
 - 1 graduated cylinder 250 ml
 - 1 graduated cylinder 100 ml
 - 1 beaker 500 ml
 - 1 beaker 250 ml
 - 1 beaker 100 ml
 - 2 graduated pipettes 10 ml
 - 1 graduated pipettes 1 ml
- Ethanol test kit
- Glucose analyzer or glucose test kit
- Drying chamber (Oven)
- Baker's yeast
- Autoclave

3. Setting up procedure

a.) Timetable

Step 1: Preparation main culture medium and bioreactor assembly
Step 2: Inoculation bioreactor / fermenter

b.) Bioreactor

- Calibration and installation of the pH-electrode
- Installation of the pO_2 probe
- Preparation and sterilization of base, acid manual filling of the tubes
- Sterilization of the culture vessel including the medium
- Calibration of the pO_2 probe at cultivation mixing speed
- Sterile connection of peripheral equipment

c.) Medium

3 litres of nutrient medium are prepared as follows:

$(NH_4)_2SO_4$	2.0 g/L
$K_2HPO_4 \cdot 3H_2O$	2.0 g/L
$MgSO_4 \cdot 7H_2O$	0.5 g/L
KCl	2.0 g/L
Yeast extract	0.1 g/L
Glucose in H_2O	11 g/L
H_2SO_4	5.0 mol/L

Add salts to a 5L flask and dissolve in 2 L distilled water.

Add 10 ml sulphuric acid (1 mol/L).

Adjust the pH value to pH = 4.5 with 1M NaOH, add 1 ml antifoam agent and adjust to 3.0 L with distilled water.

Transfer the salt solution into the prepared culture vessel and autoclave at 121°C for 20 minutes.

Dissolve 44 g glucose in 100 ml distilled water and autoclave in a separate flask.

Transfer the sterile glucose solution into the bioreactor vessel.

d.) Inoculum

For inoculation mix 15 g bakers yeast with 40 ml sterile nutrient medium.

- e.) **Corrective Agents**
Antifoam 1.0% (w/w)
Acid 0.1% (w/w) H₂SO₄
Base 1 mol/L NaOH
- f.) **Culture Conditions**
Culture volume 4 L
Temperature 30° C
pO₂ 40%
pH value 4,5 controlled
Stirrer 250 rpm

4. Analytical Procedure

: Measurement of Optical Density

Optical density (OD) is determined using a spectrophotometer at a wavelength of 600 nm. Samples should be diluted in such a way that the measured extinction is between 0.2 and 0.4. Measurements are made in cuvettes with a layer thickness of 1 cm.

OD is calculated according to the following formula:

$$\text{OD}_{600\text{nm}} = E * F [-]$$

With E = measured extinction

F = dilution factor

: Measurement of Biomass Production

There are different methods for biomass detection available:

- BM determination using a moisture analyzer
- BM determination in a drying chamber
- BM determination using a microwave

: Measurement of Glucose Concentration

Glucose measurements can be made using test kit for glucose (Roche Diagnostics) according to the respective manufacturer protocol.

: Measurement of Ethanol Concentration

Ethanol concentration can be determined using an ethanol kit. This photometric method of ethanol determination using enzyme alcohol dehydrogenase (ADH) is simple to use and characterised by high specificity and reproducibility.