Department of Biochemical Engineering and Biotechnology
IIT Delhi
BEL850 (Advanced Biochemical Engineering Laboratory)
Experiment 4

OBJECTIVE:
Understanding the dissolved oxygen (DO) measurement system of a bioreactor and its calibration.

INTRODUCTION:
Dissolved oxygen concentration in fermentation broth is one of the most important chemical variables in the fermentation process as oxygen is required in the aerobic respiration by the cells. In case of facultative bacteria, low dissolved concentration (e.g., <20% of saturation value) may reduce cell yield to a great extent. Therefore, DO monitoring / control assumes great importance.

DESCRIPTION:
The DO monitoring system comprises of the following:

Sensing unit: It is a Clark type or amperometric sensor (PYE-In Gold Make) which can be *in situ*, sterilized by steam. The electrode measures partial pressure (pO_2) of dissolved oxygen and not the dissolved oxygen concentration can be obtained from pO_2 data using the Henry's law constant. The sensor comprises of a platinum cathode and an Ag/AgCl anode. Both the cathodic and anodic surfaces are in contact with each other through an electrolyte solution (saturated with AgCl) contained in a cartridge fitted with Teflon membrane, which is permeable to oxygen. Thus, the membrane separates the probe internals from the liquid medium.

Reactions at cathode:
Oxygen in liquid medium, diffusing through the membrane, gets reduced at cathode

$$\frac{1}{2}\,O_2 + H_2O + 2e^- \xrightarrow{Pt} 2OH^-$$

The above equation does not proceed on its own until electrons are available at the cathode. This is done by applying a constant negative bias potential of -0.64V across the cathode and anode.

Reaction at anode:

$$Ag + Cl^- \longrightarrow AgCl + e^-$$

Thus current flow in the circuit is measured, which in turn is correlated to oxygen flux reaching the cathodic surface. Drift caused by accumulation of hydroxyl ions on chloride depletion is a common draw back.

2) Monitoring unit: It is an amplifier (AMP) capable of accepting current signal (~nA) from the DO sensor. Two potentiometers (POTS) and a range selection switch are available on the front panel, namely.

ZERO POT, which is used to adjust the AMP for zero electrical signal.
SPAN POT which is used to adjust the AMP at 100% saturation value corresponding to maximum probe out from a medium saturated with air, and
RANGE switch for attenuation of input signal.

CALIBRATION

1. The DO sensor's tip, covered by Teflon membrane, is very delicate. Take precautions so that it does not touch any solid surface. Very carefully fit the DO sensor through a port on the top plate of the given bioreactor and connect the sensor with the amplifier.

2. Switch ON the power supply of the DO-AMP and wait for 15 minutes to let it stabilize and then set the range switch to 100 (signal attenuation is zero).

3. Sparge nitrogen through the air sparger tube and set agitation at about 500 rpm. Wait till the DO reading on the AMP display comes down to minimum value close to zero. Use the ZERO POT to the display reading at zero.

   If the range selector switch has a marking for zero, sparging nitrogen as explained above, may be avoided for setting the zero. The range selection switch at zero position shorts the electrical probe output. Now set the ZERO POT till the display reading shows zero. The current output of the DO probe is negligibly small corresponding to 0% DO and, therefore, setting the AMP's zero with range selection switch at ZERO position may be practically erroneous. However, if very small DO concentrations are to be measured, the zero must be set by using nitrogen gas. The calibration of AMP with ZERO is not required to be done frequently. However, it may be done occasionally.

4. Stop sparging nitrogen and start air supply the sparger. Wait for the DO reading to come to a maximum. Use SPAN-POT to set the meter reading at 100%. The probe is now calibrated. When doing the fermentation work, the 100% reading must be set after sterilization when the temperature has come down to desired value.

The output of the DO probe is proportional to partial pressure of oxygen (pO_2) in the liquid phase which in turn is proportional to the partial pressure of oxygen in the gas phase and, as such, the head space air pressure above the medium, the medium temperature and water vapour pressure must be taken into account in determining the pO_2.

The pO_2 in the liquid bulk is given by the equation:

$$PO_2 = (P_T - P_V + \frac{h}{33}) * 0.21$$

Where,

P_T is the headspace pressure, P_v is the water vapour pressure, and h is the hydrostatic head. If the hydrostatic head (h) is significant, as is the case with large industrial fermentors, we must take into account the hydrostatic head as well.

The saturation concentration of DO also depends upon pO_2 in the gas phase. Increasing the pO_2 by increasing the headspace pressure will increase the DO saturation level. The oxygen solubility of aerated distilled water at 20^{o}C and normal atmospheric pressure is 12 mg/l. However, for the fermentation media, this value goes down to as low as 8mg/l or 8ppm. Thus, a 100% reading in a well aerated and sterile medium will corresponds to 8ppm DO concentration. If required, the actual saturation DO concentration at operating conditions should be determined by Winkler's method.

MEASUREMENT

Estimate Response time of the DO probe for a step-up and step-down change.

DISCUSSION

Write a discussion on your observation.

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