Understanding Temperature Control in Bioreactor Systems

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Key Words:

- **Bioreactor**
- **Reaction vessel**
- **Sub**
- **Single use bioreactor**
- **Jacketed reactor**
- **Reactor temperature control**

Abstract

Microbiologists, biochemists, and chemical engineers need to cultivate organisms in a highly controlled manner. As such, bioreactors have become integral to the pharmaceutical, food, and chemical industries. In order to successfully fulfill experimental requirements, suitable conditions and parameters must be provided, which include accurate temperature control, i.e., heating and cooling. In order to meet this need, Thermo Fisher Scientific manufactures a wide range of heated and cooled chillers and bath circulators; including the Thermo Scientific ThermoFlex series of re-circulating chillers and the new Thermo Scientific refrigerated and heated bath circulators.

Introduction

The environmental conditions of a bioreactor, including gas flow rates, temperature, pH, dissolved oxygen levels, and agitation rate need to be closely monitored and controlled in order to provide optimal growth conditions. As one of the key variables, temperature must be tightly regulated to ensure reliable and consistent data is obtained across the many different processes that use bioreactors. This is because a single batch can require both heating and cooling, with tight temperature control and rapid ramping across a broad temperature range. Even single temperature batches can require both heating (endothermic) and cooling (exothermic) reactions to maintain a stable and accurate temperature.

The Bioreactor

As the most commonly used type of reactor, batch bioreactors are typically made from glass or stainless steel, which must be sterilized before use. Newer single-use bioreactors are also becoming increasingly popular, which are manufactured from a pre-sterilized polymeric material. Sterile culture medium is subsequently added to the bioreactor and temperatures are stabilized before inoculation with cultured cells. Depending on the culture requirements, there may be provisions to agitate the medium using a stirrer and/or by providing a flow of gas (oxygen, nitrogen) and waste products, such as CO_2 are removed. This stirring also facilitates fluid mixing for an even distribution of temperature for increased uniformity.

Methods

Selecting the optimal temperature control equipment for applications using bioreactors has many considerations. Initially, the required temperature or temperature range needs to be determined, along with whether the reaction will be endothermic, exothermic or static. For example:

• When using a small vessel (typically 10 L or less) in combination with a temperature range of 13°C above ambient (33°C set-point in a 20°C room) and it is either endothermic or static, then a bath circulator should sufficiently manage the heat load.

• When using a larger vessel that still requires heating, temperature control measures can be instigated to provide the initial heat requirement, as well as provide the necessary chilling capacity.

50 L Water Jacket S.U.B.

• If the lowest temperature point is near or below ambient and the reaction is exothermic, or a controlled temperature change from high to low is required, then a bath circulator with refrigeration (for small vessels) or a larger chiller will be required.

In order to calculate the required heating or cooling capacity to reach the desired temperature, the heat load comprised of the specific internal energy must be determined. This is the energy associated with a change in temperature of a given mass, where no electrical, mechanical or chemical energy is being added to the system. Users must ensure that the final calculations include everything that is being cooled or heated, including the culture medium, the bioreactor, the fluid in the jacket, plumbing lines and temperature control unit.

The heat load calculation
$Q = MC\Delta T$
Q = heat load (Kilocalories/Kcal)
M = Mass of the substance that is changing temperature (kg)
C = specific heat of the substance that is changing temperature (calories/gram, /°Kelvin)
ΔT = change in temperature (°C)

Once the desired temperature or temperature range and heat load have been determined, and manipulated to compensate for the desired time frame, you will need to estimate the additional cooling or heating needed to poor heat transfer and heat gained from, or lost to the external environment.

Bioreactors often require a remote sensor, located within the culture medium, to control temperature. The chiller can therefore sense and control the internal environment and accurately maintain optimal conditions within the bioreactor. Utilizing a remote sensor also allows for a lower jacket temperature which will increase the rate of temperature change. For example, if a chiller without a remote sensor was set to 10°C, with a starting point of 20°C, the cooling rate will be significantly reduced as the batch approaches

jacket temperature. This is due to the system approaching thermal equilibrium and is explained by;

$Q = U \times A \times LMTD$
Q = exchanged heat in watts
U = heat transfer coefficient
A = area of heat exchange and
$LMTD$ = log mean temperature differential (average temperature differential across the heat exchange area)

Without this additional temperature differential created with the use of a remote sensor, the batch may never actually reach 10°C due to the insufficient flow of energy between the batch and the recirculating fluid.

While flow rates through the jacket are not critical, higher flow rates are preferred to maintain efficient heat transfer. Some bioreactors, such as those made from glass, may have pressure limits below the capabilities of the chiller. In these cases, pressure limiting devices such as External Pressure Reducers (EPR) or Pressure Reduction Valves (PRV) are required.

Results

To calculate the specific internal energy of a 50 L stainless steel reactor, weighing 100 kg, with 5 L in the jacket, 7 L in the chiller and 3 L in the hoses, with a 10°C ΔT:

Use the following method:

For the culture medium (aqueous so we will use the same values as water):

• 50 L = 50 kg
• 50kg x 1.0 cal/g °K x 10°C ΔT = 500 Kcal

For the water in the jacket, hoses and chiller:

• 15 L = 15 kg
• 15 kg x 1.0 cal/g °K x 10 °C ΔT = 150 Kcal

For the bioreactor:

• 100 kg x 0.11 cal/g °K x 10 °C ΔT = 110 kcal

The total specific internal energy to be removed is therefore 760 Kcal.

Since the cooling capacity of chillers is traditionally given in watt hours:

• 760 Kcal x 1.16 watt/kcal = 881.6 watts per hour

As a result, a chiller with an average cooling capacity of 882 watts is required to achieve a 10°C temperature decrease in one hour. The average cooling capacity can be estimated using cooling capacity curves, for example, by adding the cooling capacity at 20°C, 15°C and 10°C and dividing by 3.

To calculate the specific internal energy for different time periods, the one hour watts value is simply divided by the new time period.

For two hours:

• 882 W / 2hrs = 441 W

For ½ hour:

• 882 W / 0.5hrs = 1764 W

However, these calculations do not compensate for poor heat transfer, or heat gained/lost from the external environment. Poor heat transfer is attributed to slow or non-existent stirring, as well as the use of materials such as glass, that possess poor heat transfer properties. Heat gained or lost is often a result of poor or non-existent insulation when the desired temperature is not close to the ambient temperature.

Additional temperature differential (ΔT) can facilitate the movement of energy from high to low between the jacket and the culture medium on bioreactors with poor heat transfer. Differentials of 5 – 10 °C are common, especially for glassware, but this ΔT may also be restricted by limits of the cellular activity. Since it would be undesirable to freeze, or destroy the cells with heat, jacket temperatures often need to be limited to prevent a limiting temperature, regardless of the desired temperature. These restrictions may result in a longer experimental time-frame, even if the required cooling or heating capacity is present. As such, the ThermoFlex re-circulating chiller with remote temperature sensors can be equipped to provide jacket temperature limits, ensuring that

the cultured cells are not adversely affected by extreme temperature alterations.

Heat gained from or lost to the external environment can be difficult to calculate due to a large number of variables, including surface area, surface orientation, air temperature and air flow within the room. When possible, it is highly advisable that optimal insulation measures are employed. However, if this is not possible, only data from the manufacturer will provide a reliable indication of the additional heating/cooling capacity required. Without insulation, it is possible that an otherwise properly sized chiller may not be able to reach the desired temperature. In all cases, flow rate should be kept as high as possible, within the pressure limits of the vessel and the flow capabilities of the pump.

Conclusion

Bioreactors are crucial for the development of many new processes that will replace traditional chemical based products. Since bioreactors are highly dependent on temperature control, it is vital that the correct temperature control equipment is selected based on the specific requirements of each application. However, in order for researchers to select the most appropriate temperature control equipment, they must first calculate the heat load (specific internal energy). This provides an indication of the volume of heat that needs to be dissipated in order to maintain the optimal environment.

References

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