Quadrant II – Transcript and Related Materials

Programme: Bachelor of Science (Third Year)

Subject: Microbiology (HONS.)

Paper Code : MIC106

Paper Title : Industrial Microbiology

Unit: Types of fermentation processes, bioreactors and measurement of fermentation parameters

Module Name: Monitoring and control of fermentation parameters: pH

Module No: 22

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Notes

Basic principles of pH measurement and control

So why do we need to monitor and control fermentation parameters.

You must have witnessed even in your own classroom if you do not have a monitor to control the class, there is chaos, similarly monitoring and control of parameters in a fermentation is required to obtain the desired product and to maximize yield.

We need to remember that the mos used in the fermentation were obtained from the natural environment which is very different from a laboratory setup. So whatever property of the microorganism we are exploiting for eg enzyme production or antibiotic production, that property must have evolved as a result of environmental factors. So if we want the organisms to do the same thing in the laboratory environment, we need to simulate the natural environment in the laboratory i.e control all the parameters such as pH, temperature, dissolved oxygen etc , so as to provide optimum conditions for growth and colonization , so that the mo is able to perform at its full potential.

The measurement and control of pH is one of the most critical process controls during cell culture.

Microorganisms have an optimum pH for growth. Bacteria prefer a neutral pH , fungi prefer an acidic pH. In general most organisms , be it bacteria or fungi, favour the range of 5-8. Some exceptions like acetic acid bacteria grow at very low pH and urea decomposing bacteria grow at very high pH.

In general, If the pH is too high or too low it will affect the mo and in turn the fermentation process and the product.

Reasons for pH change and its effects on microorganisms

pH changes due to metabolic activity of microorganisms. If you remember your biochemical pathways you will recall that when glucose is broken down via the EMP pathway we get pyruvic acid, or further lactic acid which will make the media acidic .

Similarly proteins may breakdown to urea and ammonia making the media alkaline respectively.

What will be the effect on the mo then? Eventually pH changes will affect proteins , be it cellular or enzymatic . Usually metabolism is halted, because the catalytic properties of the enzymes are lost .

Effect of pH change on fermentation

So pH change significantly affects microbial metabolism and in turn the cell growth, as a result you may not get the desired product or the yield will not be as expected.

Measure pH during fermentation

Therefore it is imp to control and monitor the pH,
pH is measured by a pH electrode or sensor (called pH probe) .
Two methods of placing the pH detecting elements:-

a) Element it can be immersed directly in the vessel or
b) It may be a system where there is continuous flow of media in a loop circuit through a chamber housing the sensing element .

Adv of the first one is that the response time is minimized but the disadv. is that if electrode fails, fermentation has to be stopped, to replace the electrode. Whereas you need not stop the fermentation with the second type

But now a new type of electrode is available for eg the Ingold enlectrode. It has many advantages -

1) It can be withstand repeated steam sterilization, temperature, pressure and mechanical stress.
2) It is a combined electrode with both H+ sensor and reference sensor as a single unit, therefore requires only one penetration in the fermenter.
3) Design of the sensor makes it possible to sterilize it within the fermenter itself or you can even remove it even under pressure, sterilize it in an autoclave and replace it without stopping the fermentation process.

pH measurement and control

1) In lab fermenters addition of acid and alkali is done with peristaltic pumps, using silicone tubing
2) Automatic pH control: In batch and continuous culture, pH is controlled by the controller which consists of a pH electrode (probe) to measure pH changes and provide a feedback signal to the controller, which activates the supply of acid or alkali (autotitrator) to bring the pH back to the set point.

   A set-point value as well as an upper and lower limit is fed into the controller to provide a "dead band" range. Acid or alkali will be added as required only if the pH crosses the upper or lower limit of the dead band range.

   To calculate dead band, For eg if Dead band is 5% and the set point is pH 7, then (7x5)÷100=35/100=0.35.
   So upper limit will be 7+.35=7.35~ 7.4 and lower limit is 7-0.35=6.65~6.7.
   This band is normally within ±0.5 pH units of the desired value.

2 control systems for autotitrator are used-

i) Two position control i.e. either on/off position;used in small lab fermenters and

ii) Proportional control where the control unit assumes any intermediate position; used in large scale fermentation.

pH control by buffer action :

This is achieved by natural buffers such as proteins, peptides and amino acids as in corn steep liquor present in the medium;

phosphates and calcium carbonate may also be used in the media for this purpose. The buffering effect of these compounds is however usually temporary and poor .

Acetic acid and organic acids also serve as buffers.

Criteria of buffers suitable for use-

1) Buffering capacity
2) Pure
3) Water soluble
4) Non volatile
5) Enzymatically and hydrolytically stable
6) Non toxic
7) Not affected by temperature and pH
8) Non metabolizable
9) Non inhibitory
10) Economical

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