Basic Principles of Process Control Systems and Automation – Types of Analytical Methods, and Cell Counting

Your Objectives:

At the end of the lesson, you should be able to determine the importance of cell counting.

In-process control (IPC) analytical methods can be paired in two groups:

1) in-line or on-line
2) at-line or off-line

The former (1) will be discussed, and they are techniques used to follow the process in real time (e.g. temperature, pH, dissolved oxygen). The latter (2) are used in AQC (Analytical quality control) for following the same process and defining whether it falls into the acceptable limits.

Why the need for several analytical methods?

AQC makes up a part of **cGMP** (current Good Manufacturing Practices) which is concerned with:

- sampling
- specification
- testing
- documentation
- release procedures
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These together ensure that the necessary and relevant testing is performed so that products can be released for use, but only once the required quality are met.

Science-based assessments for biologics

The product testing standards in 21CFR 610/ICHQ6B encompass manufacturing safety:

- sterility
- mycoplasma
- purity
- adventitious viral agents

and assessments of other product characteristics including:

- identity
- viability
- potency

At-line IPC methods

Quantitative analytical methods are necessary for:

- cell counting
- metabolite analysis
- product quantification
- product quality
- contaminant determination

Cell counting methods

- microscope counting
- Vi-Cell®
- absorbance (e.g., at 600nm)
- dry cell weight (for microorganisms)
- colony-forming units (for microorganisms)

Why is cell counting necessary?

We need to know the total cell counts, viable cell counts, and viability, to determine **growth kinetics**. Each cell produces a certain amount of proteins, termed the specific productivity (g product/ number of cells/h). Therefore, the more cells are present, the more product will be formed.

We need to know how reproducible culture is (stability) from one culture to the next.

We need to know the health of the culture, i.e., how many cells are present and how many of these are actually viable.

We need to know when to add potential inducers. We need to know when to harvest the product.

Cell counting can be used to control the feed rate of a fresh medium to obtain a defined fed-batch, continuous or perfusion culture. It is relatively easy to measure cell counts in suspension cultures, but not so easy for immobilized cell cultures. Because there is a big chance of having large errors in cell counting techniques, we attempt to automate or standardize techniques, to avoid variability.

Cell counting techniques:

In-line / on-line

For methods used directly in the bioreactor, where no sampling is needed, these methods can provide continuous measurements suitable for monitoring and control (Process Analytical Technology, or PAT):

- Direct methods involve measuring cells as solid objects (e.g. turbidity / absorbance; dielectric spectroscopy; NIR spectroscopy; MIR spectroscopy)

Indirect methods measure a given component of a cell related to its cell count (e.g. fluorescence spectroscopy (measures NADH, or Nicotinamide adenine dinucleotide Hydrogen); glucose or other product determination; O_2 or CO_2 measurements).

The main weaknesses with such methods are:

- robustness
- instability over longer periods
- interferences from other cell components
- calibration of methods

Off-line

This method does not involve the bioreactor, and so sampling is necessary (i.e. the need to break the sterile boundary).

- Direct methods involve measuring cells as solid objects (e.g. cell dry weight; microscope counting; Vi-Cell®; turbidity / absorbance; dielectric spectroscopy; NIR; MIR)

- Indirect methods measure some component of a cell which is related to the cell number (e.g. fluorescence spectroscopy, which measures NADH; glucose or other product determination; O2 or CO2 measurements)

The pros and cons that come with the off-line method is that it is…:

- easy to calibrate
- low person-to-person variation
- time consuming
- labour intensive

The main weakness with such a method is the need to break the sterile boundary and to obtain a sample characteristic of **whole culture**.