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 ( ) analytical methods can be paired in two groups:

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

The (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 (Analytical quality control) for following the 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

These together ensure that the necessary and relevant [ ] is performed so that [ ] 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 [ ] safety:

- sterility
- mycoplasma
- purity
- adventitious viral agents

and assessments of <u>other</u> product [ ] including:

- identity
- viability
- potency

At-line IPC methods

Quantitative analytical [ ] 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 [ ] 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 [ ] **kinetics**. Each cell produces a certain amount of [ ] product, termed the [ ] 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 [ ] is (stability) from one culture to the next.

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

We need to know when to add [ ] inducers. We need to know when to [ ] the product.

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

Cell counting techniques:

In-line / on-line

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

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

Indirect methods measure a [ ] 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 _____ is necessary (i.e. the need

to break the sterile boundary).

- _____ methods involve measuring cells as solid objects (e.g. cell dry weight; microscope counting; Vi-Cell®; turbidity / absorbance; dielectric spectroscopy; NIR; MIR)
- _____ 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
- time consuming
- _____ intensive
- person-to-person variation

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

Aufgabe Lückentext:

Folgende Wörter bitte in den Lückentext einfüllen.
Jedes Wort kommt einmal vor.
Bitte Gross- und Kleinbuchstaben beachten.

AQC, bioreactor, break, characteristics, cell, culture, Direct, former, given, growth, harvest, immobilized, Indirect, involve, IPC, labour, manufacturing, methods, need, off, on, perfusion, potential, present, products, protein, same, sampling, specific, techniques, testing