Introduction to USP – Critical Biotech Parameters

Your Objectives:

At the end of the lesson, you should be able to identify critical biotech parameters.

A bioreactor's functions

The bioreactor is equipped with any, or all, of the following:

- an (often controlled) [ ] which differs from any external environment
  (e.g.: temperature, pressure, redox potential*, ionic strength, etc.)
- [ ] conditions (where necessary)
- support (if necessary)
- Containment
- agitation/mixing (homogeneous [ ])
- aeration (if required)
- anærobic conditions (if required)

Provisions of a controlled growth environment

A controlled growth environment involves [ ] the following:

- suspension or adherent cell [ ], i.e., liquid (STR) or solid (fixed-bed)

- provision of oxygen if ærobic or, if anærobic, ærobic exclusion of [ ]

  (*Redox potential (E_h) is a measure of the degree of oxygenation of a medium)
- temperature
- pressure
- [ ] strength
- pH

Aeration (oxygenation)

The four types of bioprocesses are as follows: (mnemonic: "A.F.M.A.")

1. Ærobic
2. Facultative anærobic
3. Microærophilic
4. Anærobic

Ærobic cells

Most cell types, e.g. all animal cells, most yeasts and many bacteria are [ ].

Ærobic means that cells require [ ] to grow and [ ]. Obligate ærobes must have oxygen or else they will lose viability and die (e.g. animal cells).

Some cells require oxygen and grow ærobically. But if, for some reason, oxygen is not available, they may grow [ ]. These are called facultative anærobes.

Certain cells (animal cells), grow ærobically but if they are supplied with excessively high levels of sugars (glucose), they begin to ferment. This is called the **Crabtree effect**, **overflow metabolism,** or **catabolite repression**.

Facultative Anærobic cells

Many bacterial cells (E. coli) and yeast (e.g. ** Saccharomyces cerevisiae) can grow either ærobically or anærobically—also called facultative anærobes.

If these cells are growing without oxygen ( ) and then oxygen is suddenly given so that they will automatically switch to growth with the oxygen (respiration)—called the **Pasteur effect**.

Growth in the presence of oxygen is more common and efficient than in its . Both the growth rate and biomass yield are higher when grown ærobically.

Microærophilic cells

Some cells (e.g. Lactobacilli) and yeast (e.g. S. cerevisiae) cannot grow in the complete absence of oxygen.

Lactobacilli do not need oxygen to grow but can grow better (higher growth rate and yield) in its presence.

S. cerevisiae cannot completely anærobically; it must have trace amounts of oxygen so that it can produce the fatty acids that it needs for growth.

S. cerevisiae can grow anærobically provided it is supplied with certain fatty acids in the medium (e.g. oleic acid) together with ergosterol, for which the oxygen was originally needed to produce them (brewing)!

Anærobic cells

Generally speaking, anærobic organisms are exclusively bacteria, the most commonly recognised ones being Clostridia species. – e.g. Clostridium tetani (Cl. tetani); Clostridium

difficile (Cl. difficile); Clostridium botulinum (Cl. botulinum) - others include Bacillus anthracis; Methanobacteria, etc.

[ ] of these organisms require media from which all oxygen has been removed. Reducing agents are oftentimes added to the medium to ensure that every [ ] of oxygen is removed. Oxygen is poisonous (toxic) for such cells, and cells would die instantly. Reducing [ ] are especially important in the production of biogas and for certain [ ] products.

Aeration (oxygenation)

How is oxygen supplied to a [ ] ?

- Through the **headspace** above the culture medium
- Through **sparging** of the medium in the bioreactor with air or oxygen-enriched air, ether

pH levels

- Animal cells grow at pH 7.2 – 7.4
- Yeast cells generally at pH 4 – 6
- Bacteria at pH 2 – 8

Temperatures

- Psychrophiles (-5°C – 20°C)
- Mesophiles (15°C – 42°C)
- Thermophiles (38°C – 65°C)
- Extremophiles (<5°C or >65°C)

[ ] growth is based on several chemical [ ] ; as such, temperature effect chemical reactions. Namely, the reaction rate approximately doubles with a rise of 10-degree [ ] .

Pressure levels

There are two types of pressure in a [ ] :

- **Atmospheric pressure**
    - Acidophiles, Neutrophiles and Basophiles
- **Osmotic* pressure**
    - 330-360 mOsmole, Osmophiles

Mammalian cells are neutrophiles and require strict osmotic pressure limits.

* Osmosis: the tendency of a solvent to pass through a semipermeable membrane, as the wall of a living cell, into a solution of higher [ ] , so as to equalize concentrations on both sides of the membrane. osmotic pressure, thereby, prevents osmosis from occurring.

Sterile environment

Achieving sterility for a bioreactor requires:

- Thermal sterilization
- Chemical [ ]
    - liquids (e.g. 0.1-1N NaOH)
    - Gases (e.g. ethylene oxide)
- Irradiation
- Electronic beam irradiation (E beam)

NB[1]: When letters ‘ae’ are pronounced in a word as a **monothong** (single sound), they are written together as ‘æ’— (e.g. ærosol, æroplane, anærobic). When a word, however, is pronounced as a **diphthong** (two separate sounds), ‘ae’ is written separately — (e.g. aeration, cerevisiae).

NB[2]: ‘**i.e**.’ addresses one specific example or illustration, whereas ‘**e.g.**’ serves to give just one of several possible examples.

Aufgabe Lückentext:

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

aerobic, absence, agents, anærobically, bioreactor, Cultures, conditions, controlling, Cell, Celsius, culture, concentration, culture, cosmetic, environment, fermentation, grow, ionic, metabolise, microbial, molecule, oxygen, oxygen, reactions, sterile, sterilization