Culture media for fermentation

The medium composition is as critical to product yields as high producing strains of microorganisms.

The medium not only provides the nutrients needed for microbial growth but also for the metabolite production.

The microorganisms vary greatly in their nutrient requirements from autotrophs, which produce all the biochemicals required from simple inorganic nutrients deriving their energy from oxidation of some inorganic component of the medium to the difficult organisms like lactic acid bacteria, which require many organic compounds for their growth.

According to the composition of culture media there are three types:-

1-Natural media.

2-Synthetic media.

3-Semi synthetic media. (1+2)

The general basis includes :-

A) Nutrients

Carbon source

Nitrogen source

Mineral source (depending on the microorganisms used and the type of the product)

Growth factors

B) Physical conditions

PH

Temperature

Oxygen (for aerobic processes)

Light for some processes.

The Sterilization of the fermenter and its Accessories

The fermenter itself, unless sterilized, is a source of contamination. Of the various methods, steam is the most practical for fermenter sterilization. Steam is used to sterilize the medium in situ in the fermenter but sometimes the medium may be sterilized separately in autoclave and subsequently transferred aseptically to a fermenter.

In order to avoid microbial growth within the fermenter when not in use, crevices and rough edges are avoided in the construction of fermenters, because these provide pockets of media in which undesirable microorganisms can grow. These crevices and rough edges may also protect any such organisms from the lethal effects of sterilization.

For the reasons discussed earlier, saturated steam should be used and should remain in contact with all parts of the fermenter for at least half an hour. Pipes which lead into the fermenter should be steam-sealed using saturated steam. The various probes used for monitoring fermenter activities, namely probes for dissolved oxygen, CO_2, pH, foam, etc., should also be sterilized.

Batch Sterilization

The medium can be sterilized after the fermenting tank has been filled by passing steam under pressure through a metal jacket that surrounds the outside of the fermenter vessel.

Continous Sterilization

The medium can be sterilized by a process called continuous sterilization The medium is pumped through a heater where it quickly rises to a temperature sufficiently hot so that it is sterilized in a few seconds and it is then pumped into the empty but previously sterilized fermenter.

Additives to culture media

In addition to sources of carbon , energy , nitrogen, minerals and growth factors , it is necessary to add some substances in which each one of them has a specific function.

1-Buffers

These are added in order to keep in the PH value against any change during the fermentation processes. This can be done through many ways :-

a- By controlling the ratio of carbon source to nitrogen source since protein ,peptides and amino acids have a high buffer capacity .

b- By adding substances which modify the PH (such as acid and base)

c- By adding substances which are considered as buffer and one of the constituents at the same time (Potassium dihydrogen monophosphate).

2.Induced substances

This is necessary in enzyme production since most of the produced enzymes are of the induced type.
Such as the addition of starch in amylase production.

3- Inhibitors

Sometimes it is necessary to add inhibitors to the culture media , these inhibitors are of different types and they have different purposes :-

A-Antibiotics are added to prevent contamination especially when the organisms used has a slow growth rate such as the animal and plant cell , while its usage are low in the case of microorganisms cells which they have a high growth rate.

B-Some inhibitors are added to inhibit certain pathways. Such as in the addition of bisulphate in order to produce glycerol instead of ethanol by yeasts .

C-Some inhibitors are added in order to prevent secondary metabolites processes so it enhance the primary metabolites processes.

D-Some inhibitors could change the permeability of the cell wall and cell membrane in which it lead to the leakage of some primary metabolites . Such as in the addition of penicillin in the glutamic acid production.

E-Some inhibitors are added for special purposes such as in the addition of Bromide to prevent the formation or integration of the Chlore in the tetracycline molecule, so another antibiotic (oxytetracycline) is produced.

4- Precursors

Sometimes the productivity is increased if precursors are added.

Such as in the addition of phenyl acetic acid and similar substances in the penicillin production . They are added in small concentration or at the end of the growth stage.

5- Chelating agents

They are added in order to regulate the concentration of heavy minerals to the physiological levels required by microorganisms through linking the minerals with the linkage group in the chelating agents.

6- Antifoams & Defoams

Antifoams are added before foam production .
Defoams are added after foam production .
Sometimes mechanical methods are used.

Starter (inoculum)

Definition of starter (inoculum)

Is a pure culture of microorganisms cell consist of one or many strains or mixed cultures which act singly or together .

Inoculum Development –

The preparation of a population of microorganisms from a dormant stock culture to an active state of growth that is suitable for inoculation in the final production stage is called inoculums development.

As a first step in inoculums development, inoculum is taken from a working stock culture to initiate growth in a suitable liquid medium.

Bacterial vegetative cells and spores are suspended, usually, in sterile tap water, which is then added to the broth.

In case of nonsporulating fungi and actinomycetes the hyphae are fragmented and then transferred to the broth.

Stock Culture

Is a culture of microorganisms maintained solely to keep it viable for subculture into fresh medium .

Working culture

a microorganisms preparation derived from a reference stock culture used as a control.

Inoculum development is generally done using flask cultures; flasks of 50 ml to 12 litres may be used and their number can be increased as per need. Where needed, small fermenters may be used.

Inoculum development is usually done in a stepwise sequence to increase the volume to the desired level. At each step, inoculum is used at 0.5-5% of the medium volume; this allows a 20-200-fold increase in inoculum volume at each step. Typically, the inoculum used for production stage is about 5% of the medium volume.

Inoculums preparation media are quite different from production media. These media are designed for rapid microbial growth, and little or no product accumulation will normally occur.

Many production processes depend on inducible enzymes.
In all such cases, the appropriate inducers must be included either in all the stages or at least in the final stages of inoculums development.
This will ensure the presence of the concerned inducible enzymes at high levels for the production to start immediately after inoculation .

The factors which effect on the starter efficiency & productivity.

1-Inoculums size or quantity.

Depend on two factors :-
a- Kind of microorganisms
Bacteria 0.1%- 3 - 5%
Fungi & actinomycetes 5 - 10%
Spore suspension 1 - 2 X 10^5 spores/l

b- Type of product
In the case of the production of secondary metabolites, the size of inoculums should increased to about 60% and sometimes to about 100%

2-Inoculums age & the physiological state.

The inoculum should be active .
In the case of production of primary metabolites the inoculums should be at the medium stage of the logarithmic phase and in the case of the production of secondary metabolites the inoculums should be at the stationary phase.

3-Effect of culture media

The culture media which are used for preparing the inoculums may be different from the culture media which are used for the production. Therefore adaptation of the inoculums is necessary by culturing them in a media at the final stage of preparing the inoculums which is similar to the culture media used for production so the inoculums processes is increased and the repression is inhibited .
This will lead that the lag phase is shortened or terminate.