Media Sterilization

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Media Formulation

- Formulation of medium is an vital phase in pilot-scale expansion, laboratory experiments and developmental processes.
- Few are some measures important to consider while manipulating a medium for the purpose of huge range of production:
  - It should produce maximum product.
  - It should give minimum yield of undesired product.
  - It should be cheap.
  - It should cause minimal problems in aeration, medium formation, sterilization, agitation, extraction, purification and waste treatment.
  - It should produce maximum concentration of biomass and must be available throughout the year.
  - Media must satisfy all nutritional requirements of the organism and fulfil the objectives of the process(Rodgers, 1986).

Components of Media

- Fermentation medium consists of macronutrients, micronutrients, trace elements, dissolved oxygen, vitamins, enzymes, other dissolved gases, and inhibitors.
- The components of the medium should accomplish the elemental necessities for metabolite construction and biomass production with sufficient provision of energy for biosynthesis (Springham & Moses, 1999).

Carbon + Nitrogen + Oxygen + Other requirements -- -----›
Biomass + Product + Carbon dioxide + Water + Heat

Water and Energy Sources

- Chief constituent of every fermentation medium is water and it is required in rinsing, cooling, and heating.
- It is significant to consider the dissolved salts, contamination, effluents, and pH, while evaluating the water supply.
- Water's mineral contents play an important function in brewing and are critical in squashing.
- Light or medium components of oxidation are main source of energy which is required for growth.
- As industrial microbes are chemoorganotrophs, so their source of energy is carbon source in form of lipids, proteins, and carbohydrates.
- While, in some cases, methanol or hydrocarbons may be used by some microorganisms as a source of energy or carbon (Bauchop and Elsden, 1960).

Carbon Source

- Carbon is considered as a main product of a fermentation process.
- If 60-70% of production cost is raw materials during single-cell protein or ethanol production, then the product's selling price will be indicated by expense of the carbon supply.

Carbohydrates

- Starch obtained from cereals, potatoes, and maize, is easily available as a source of carbohydrates and are extensively used in fermentation of alcohol.
- Grains (maize etc) are used in the form of powder and also as a source of carbohydrates (Atkinson and Mavituna, 1991).
- Cheapest source of carbohydrates is molasses, and used in organic acid, amino acid, single-cell protein, and alcohol fermentations (Bawa et al., 2010).

Fats and Oils

- Oils were firstly used as antifoaming agents in antibiotic processes (Solomons, 1969).
- Oils provide maximum energy per weight than sugars.
- Oils posses anti-foaming qualities but are used as additives.
- These may also be used for their high content of the fatty acids.

Nitrogen Source

- Industrially used microorganisms have ability to use organic as well as inorganic means of nitrogen.
- Inorganic source of nitrogen is supplied as ammonium salts, ammonia gas and nitrates (Hutner, 1972).
- Inorganic substrates which can be used as a source of nitrogen includes urea, ammonium salts, and ammonia.
- Ammonia is used to control pH during fermentation process.

Minerals

- Essential minerals which are used in all media formulation include potassium, sulphur, chlorine, phosphorous, magnesium, and calcium (Dahodet al., 2010).
- We require a minute amounts other minerals such as cobalt, manganese, zinc, iron, copper and molybdenum and they exist as impurities.
- The specific concentration of these all elements depends on the micro-organism.

Chelators

- Metal precipitation is avoided by addition of chelating agents.
- In large scale fermentation chelating agents are not necessary.
- Some other ingredients (yeast extract) will play role of formation of metal ion complexes.
- As EDTA is capable of forming bonds with magnesium and calcium ions thus they are widely used in soaps and detergents.

Growth Factors

- Few of the microbes cannot produce complete complement of components of the cell and consequently requisite some of the preformed components known as growth factors.
- Growth factors includes amino acids, vitamins, sterols and fatty acids.
- Some natural sources such as nitrogen and carbon are used in growth medium formulations having required growth factors.
- Cautious mixing of materials can be used to eliminate the vitamin deficiency.

Buffers

- pH has great influence on microbial growth.
- pH of the growth media can be maintained by addition of buffers that would resist pH changes.
- Many microorganisms have optimum pH range 7.0.
- Some of the examples of buffers that are commonly used include; ammonia sodium hydroxide and calcium carbonate.

Media Sterilization

- In industrial fermentations, components such as vessels, pipework, media, inlet air, and exhaust gases are frequently sterilized by a combination of wet-heat and filtration methods.
- Wet-heat methods are less expensive and more effective than dry-heat methods, and thus are employed commonly in fermentation industries to destroy unwanted microorganisms.
- The wet-heat sterilization conditions typically used to kill all microorganisms, including bacterial spores, are listed in Table 1.

Temperature (°C)	Time (min)	Pressure (kPa)
121	15	103.4
126	10	137.8
134	3	206.7
140	0.67	261.8

Physical Methods

- The physical methods such as filtration, centrifugation, and adsorption (to ion-exchangers or activated carbon) are in use.
- Among these, filtration is most widely used.
- Certain constituents (vitamins, blood components, antibiotics) of culture media are heat labile and therefore, are destroyed by heat sterilization.
- Such components of the medium are completely dissolved and then subjected to filter sterilization.
- **There are a couple of limitations of filtration technique:**
  - Application of high pressure in filtration is unsuitable for industries.
  - Some of the media components may be lost form the media during filtration.
  - Sometimes, a combination of filtration and heat sterilization are applied.
  - For instance, the water used for media preparation is filtered while concentrated nutrient solution is subjected to heat sterilization.
  - The filtered water is now added for appropriate dilution of the media.
- The chemical methods (by using disinfectants) and radiation procedures (by using UV rays, y rays, X-rays) are not commonly used for media sterilization.

Batch Sterilization

- The culture media are subjected to sterilization at 121°C in batch volumes, in the bioreactor.
- Batch sterilization can be done by injecting the steam into the medium (direct method) or injecting the steam into interior coils (indirect method).
- For the direct batch sterilization, the steam should be pure, and free from all chemical additives (that usually come from steam manufacturing process).

There are two disadvantages of batch sterilization:

1. Damage to culture media:

- Alteration in nutrients, change in pH and discolouration of the culture media are common.

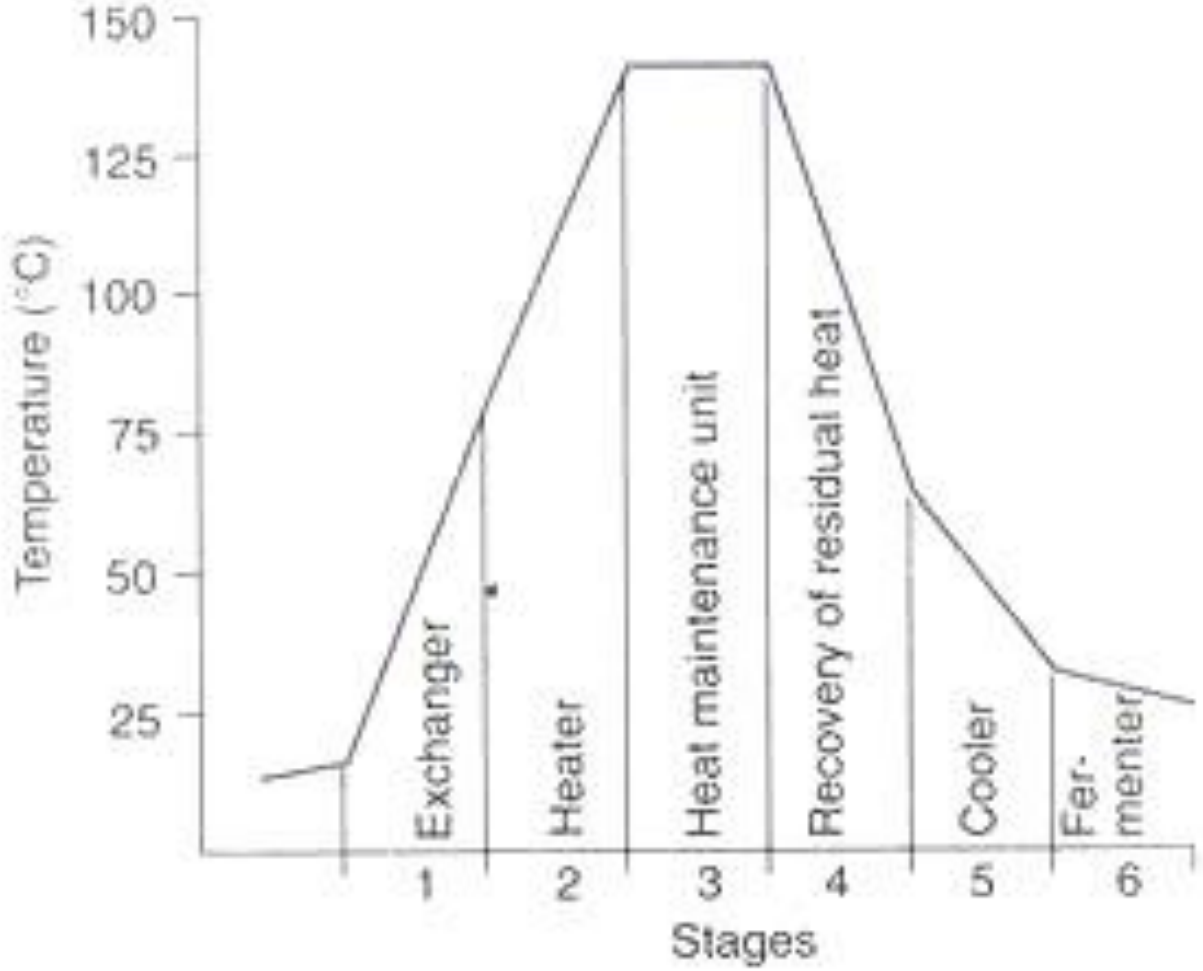
2. High energy consumption:

- It takes a few hours (2-4 hrs.) for the entire contents of the bioreactor to attain the requisite temperature (i.e. 120°C).
- Another 20-60 mins for the actual process of sterilization, followed by cooling for 1-2 h.
- This process involves wastage of energy, and therefore batch sterilization is quite costly.

Continuous Sterilization

- Continuous sterilization is carried out at 140°C for a very short period of time ranging from 30 to 120 seconds.
- Continuous sterilization is carried out by directly injecting the steam or by means of heat exchangers.
- In either case, the temperature is very quickly raised to 140°C, and maintained for 30- 120 seconds.
- The stages of continuous sterilization process and the corresponding temperatures are depicted in Fig. 1.
- The different stages are— exchanger, heater, heat maintenance unit, recovery of residual heat, cooling and fermenter.

Different stages in Continuous Sterilization Process in Relation to Temperature



… Continuous Sterilization

- In the continuous sterilization process, 3 types of heat exchangers are used.
  - The first heat exchanger raises temperature to 90-120°C within 20-30 seconds.
  - The second exchanger further raises temperature to 140°C and maintains for 30-120 seconds.
  - The third heat exchanger brings down the temperature by cooling in the next 20-30 seconds.
- The actual time required for sterilization depends on the size of the suspended particles. The bigger is the size, the more is the time required.
- The main advantage with continuous sterilization is that about 80-90% of the energy is conserved.
- The limitation however, is that certain compounds in the medium precipitate (e.g., calcium phosphate, calcium oxalate) due to very high temperature differences that occur in a very short time between sterilization and cooling.
- The starch-containing culture media becomes viscous in continuous sterilization and therefore is not used.