# Medium formulation

Media

Medium formulation is an essential stage in the design of fermentation process. Most fermentation media require liquid media, although some solid-substrate fermentations are also operated. Fermentation media must satisfy all the nutritional requirements of the microorganisms and fulfill the technical objectives of the process. There are several stages where media are required in a fermentation process; inoculum (starterculture), propagation steps, pilot-scale fermentations and the main production fermentations.

According to Cruger W and Cruger A (1990); on a large scale, the sources of nutrients should be selected to create a medium which should meet as many as many possible of the following criteria:

- It should produce the maximum yield of product or biomass per gm of substrate used.
- It should produce maximum concentration of product or biomass.
- It should permit the maximum rate of product formation.
- There should be the minimum yield of undesired products.
- It should be of a consistent quality and be readily available throughout the year.
- It should cause minimal problems during media preparation and sterilization.
- It should cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction, purification and waste treatment.

The initial step in media for media formulation is the examination of the overall process on the stoichiometery for growth and product formation. The optimization of a medium should be carried out such that it meets as many as possible of the seven criteria. Different combinations and sequences of process conditions have to be investigated to determine growth conditions (Stanbury P. F and Whitaker A; 1995). Medium optimization can be carried by the classical method, in which one independent variable is changed while keeping all others at a certain level. An aerobic fermentation process may be represented as: Carbon and energy source + Nitrogen source + O2 + other requirements Biomass + products + CO2 + H2O + heat This primarily involves consideration of the input of the carbon and nitrogen sources, minerals and oxygen and their conversion to cell biomass, metabolic products.

Based on this information, it should be possible to calculate the minimum quantities of each element required to produce a certain quantity of biomass and metabolite According to Prasanthi V et al (2008); Chlorella vulgaris is a green, spherical, single celled fresh water microalga belongs to the phylum Chlorophyta. As per the study conducted so far it is found that green algae are the highest source of chlorophyll in the plant world and particularly, Chlorella one of the members of green algae is the richest source of chlorophyll which is widely used as ahealthfoodand feed supplement.

The aim of this work is to design different medium types to evaluate optimization combinations for maximum growth, morphology and pigment content of C. vulgaris. Effect of glucose Three different volumes of glucose from apple juice while other variables are kept constant. The volumes that were used are 5g/l, 15g/l and 30g/l. The highest chlorophyll production (12%) was obtained with a glucose concentration of 15g/l. Glucose is used as a carbon source which is required for all biosynthesis leading to reproduction, product formation and cell maintenance. It also serves as the energy source.

Carbon requirements may be determined from the biomass yield coefficient (Y), an index of the efficiency of conversion of a substrate into the cellular material: Ycarbon (g/g) = biomass produced  Glucose substrate utilized (g) An increase in glucose concentration of 30g/l resulted in the production of chlorophyll being at a constant this is because all the active sites of the microorganism are occupied and active carrying out biochemical reactions. At low glucose concentration of 5g/l very little biomass (chlorophyll) is obtained and also there is low growth rate.

Thus, glucose concentration significantly influences chlorophyll production and microbial growth of the microorganism. Constraints that can be generated include the fact that apple juice not only contains one type of sugar, glucose but also contains other sugars (fructose and sucrose) which the microorganism can either utilise for growth resulting in us not obtaining accurate optimization results and also the other sugars can inhibit the growth of the microorganism. Apple juice also contains soluble pectin these can be difficult to digest hence a reduction in biomass.

Effect of nitrogen from defatted soya Nitrogen being important constituent of the cell protein was needed for algal growth, either in combined or in molecular form. It is also a component of proteins nucleic acids some co-enzymes. Industrially important microorganisms can utilize both inorganic and organic nitrogen sources. Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia; these can be used in place of defatted soya. Ammonia can also be used to adjust the pH of the fermentation.

As nitrogen deficiency develops the amount of chlorophyll in the cells decreases faster than the nitrogen content in C. vulgaris. Nitrogen is a limiting factor if continually increased it can inhibit the production of chlorophyll. Varying concentrations of nitrogen were used i. e 0. 3g/l, 0, 6g/l and 2. 0g/l. At 0. 3g/l little chlorophyll is obtained this is due to the fact that nitrogen being a macronutrient it is required in high concentration. At 0. 6g/l high yields of chlorophyll are obtained and at 2. 0g/l nitrogen turns to be a limiting factor and can lead to culture toxicity.

Constraints can be generated when using Ammonia as a substitute for defatted soya this is due to the fact that ammonia leads to high pH which results in a precipitate formation in the medium but lower pH of the medium prevent the precipitation. Foaming in a microbiological process is due to media proteins that become attached to the air-broth interface where they denature to form stable foam. Non-treatment of foam may block air filters, resulting in loss of aseptic conditions. The foam production can be controlled by addition of chemical antifoam. Natural antifoams include plant oils (e. g. Soya, sunflower and rapeseed), hence defatted soya is used as a nitrogen source rather than ammonia. Also high concentrations of ammonium ions can be toxic to cells of the microbe. Effect of $Mg^{2+}$ $MgSO_4$ can be used as the source of magnesium. It promotes the maximum growth of the present alga and it is also incorporated as an enzyme co-factor component of chlorophyll. Three salt concentrations were used 0. 1g/l; 0. 5g/l and 1g/l. At low salt concentration of 0. 1g/l it results in a magnesium deficiency which interrupted cell division in Chlorella which results in abnormally large cell formation. Increase in salt concentration of 0. g/l and 1g/l of magnesium alone in the medium resulted in higher cell number, although increase in nitrogen alone did not make much difference that means cells need magnesium to synthesize chlorophyll. The process of multiplication requires a larger concentration of magnesium in the medium than does the production of cell material. Iron uptake is strictly required to optimize the process.

## References


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