Media formulation

Literature, Russian Literature

MEDIA FORMULATION 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 (1). There are several stages where media are required in a fermentation process; inoculum (starter culture), propagation steps, pilot-scale fermentations and the main production fermentations (2). 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: i. It should produce the maximum yield of product or biomass per gm of substrate used. ii. It should produce maximum concentration of product or biomass. iii. It should permit the maximum rate of product formation. iv. There should be the minimum yield of undesired products. v. It should be of a consistent quality and be readily available throughout the year. vi. It should cause minimal problems during media preparation and sterilization. vii. 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 (3). An aerobic fermentation process may be represented as: Carbon and energy source + Nitrogen source + O_2 + other requirements Biomass + products + CO_2 + H_2O + 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. Once the nutritional requirements of a microorganism have been determined, then suitable nutrient sources can be incorporated into the media (3). The medium adopted also depends on the scale of the fermentation. For small scale laboratory fermentations pure chemicals are often used in well defined media. Industrial scale fermentation processes use cost effective complex substrates, where many carbon and nitrogen sources are almost indefinable (1). For example, for preparing a medium for culturing yeast for animal consumption vegetable waste matter can be used as a starting material. Although, such a waste matter is not suitable for direct feeding of animals. Corn-cob and/or stalks, reeds, sunflower stalks, fallen autumn leaves are preferred representatives (4). Carbon sources A carbon source 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 (g) __________________ Carbon substrate utilized (g) Carbohydrates are traditional carbon and energy sources for microbial fermentation, although other sources may be used, such as alcohols, alkanes and organic acids. In addition to main carbon source, animal fats and plant oils may be incorporated into some media as supplements (1, 3). Nitrogen sources 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. Ammonia can also be use to adjust the pH of the fermentation. Organic nitrogen sources include amino acids, proteins and urea. Growth is fastened with a supply of organic nitrogen, and a few microorganisms have an absolute requirement of amino acids. Nitrogen is often supplied in crude forms that are essentially byproducts of other industries, such as corn steep liquor, yeast extracts, peptones and soya meal. Purified amino acids act as precursors for specific products, are added as precursors for specific products, and are added only in special situations (1). Water Most fermentation, except solid substrate fermentation, require large quantities of water in which medium is formulated. It also provides trace mineral elements. It is not only a major component of all media, but is also used for ancillary equipment and cleaning. Prior to use, removal of suspended solids, colloids and microorganisms and removal of hardness is usually required. In order to minimize water costs, recycle/ reusage of water is practiced, which also reduces the volume requiring waste water treatment (3). Minerals Usually sufficient quantities of cobalt, copper, iron, manganese, molybdenum and zinc are also present in water supplies and as impurities in other media ingredients. For example, corn steep liquor satisfies the requirements of minor and trace mineral trace mineral needs. Specific salts of calcium, magnesium, phosphorus, potassium, sulphur and chloride ions have to fulfill the requirements (1, 3). Vitamins and growth factors Some bacteria, filamentous fungi and yeasts cannot synthesize all necessary vitamins from basic elements; they must be added as supplements to the fermentation medium. Most natural carbon and nitrogen sources are also contain at least some of the required vitamins as minor contaminants. Other necessary growth factors, amino acids, nucleotides, fatty acids and sterols are added either in pure form, or as plant and animal extracts (3). Precursors Specific precursors have to be added in some fermentation, notably for secondary metabolite production. They are often added in controlled quantities and in a relatively pure form. For example, D-threonine is used as a precursor in L-isoleucine production by Serratia marsescens (1). Inducers and elicitors Majority of enzymes which are of industrial use, being inducible, require a specific inducer or a structural analogue, which must be incorporated into the culture medium or added at some specific stage. Inducers are often necessary in fermentation of genetically modified microorganisms (GMMs) (2, 3). Inhibitors When certain inhibitors are added to fermentation, a specific product may be produced or a metabolic intermediate which is normally metabolized is accumulated. For example, sodium bisulphate is an inhibitor specifically employed to redirect metabolism, used in production of glycerol by Saccharomyces cervisiae (1). Buffers The optimal productivity in a fermentation process can be achieved by the control of pH. Many media are buffered at about pH 7. 0 by incorporation of calcium carbonate. The balanced use of carbon and nitrogen sources also aids in pH control, as buffering capacity can be provided by proteins, peptides and amino acids, such as corn steep liquor (3). Cell permeability modifiers These compounds increase cell permeability by modifying cell walls and/or membranes, promoting the release of intracellular products into the fermentation medium. For example, penicillins and surfactants are frequently added to amino acids fermentations, including processes for producing L-glutamic acid using the members of genera Corynebacterium and Brevibacterium(1, 3).

Oxygen Depending on the amount of oxygen required by the organism, it may be supplied in the form of air containing about 21%(v/v) oxygen, or occasionally as pure oxygen, when requirements are high (1). Antifoams 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 following any of these three approaches: modification of medium composition, use of mechanical foam breakers and addition of chemical antifoam. Natural antifoams include plant oils (e. g. Soya, sunflower and rapeseed), decolorized fish oil, mineral oils and tallow. The synthetic antifoams are mostly silicon oils, poly alcohols and alkylated glycols (1, 3). Medium optimization 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. Medium optimization can be carried by the classical method, in which one independent variable is changed while keeping all others at a certain level (3). In a research study Amla (Emblica officinalis Gaertn.) was used for wine production. The conditions for achieving the highest alcohol content and improving the sensory qualities have been standardized by evaluating the effect of addition of various exogenous nutrients, environmental conditions, fermentation technology and by maturing the wine. The supplementation of ammonium sulphate, potassium dihydrogen phosphate, proline and biotin to the hot water extract of amla proved to be best nutritional factors for highest alcohol production (12%) during the fermentation of the amla based medium with a new strain of S. cerevisiae in a batch fermentation (5). Other alternative strategies e. g. Plackett-Burman design for several variables may be followed for optimization. Response surface methodology (RSM) includes factorial design and regression analysis which helps in evaluating the effective factors, selection of the optimum conditions of variables for a desirable response and building models to study interactions (3) . An artificial neural network (ANN) is a superior and more accurate modeling technique when compared to the RSM method, as it represents the non linearities in a much better way (7). References 1. Waites MJ, Morgan NL. Publisher- Wiley-blackwell. Industrial microbiology: an introduction by Michael J. Waites. 2001. 2. Crueger W, Crueger A. A Textbook of Industrial Microbiology . Publisher: Panima Publishing Corporation. Biotechnology: 1990 3. Stansbury PF, Whitaker A . Pergamon Press. Principles of fermentation technology . 1995 4. Simon A, Lengyl Z. Process for the preparation of fermentation media suitable for culturing yeast for animal consumption and microspores and/or for the production of protein from vegetable waste matter. United States patent, Jan 6, 1981. Patent No.-4, 243, 685 5. Soni SK, Bansal , Soni R. Standardization of conditions for fermentation and maturation of wine from Amla (Emblica officinalis Gaertn.). Natural Product Radiance. 2009. Vol. 8(4), pp. 436-444 6. Jayati R D, Pranab K D, Rintu B. Optimization of culture parameters for extra cellular protease production from a newly isolated Pseudomonas Sp. under response surface and artificial neural network models. Process Biochemistry. 2004. 39: 2193-2198. 7. Prasanthi v, Yugandhar M, Nikku, Vuddaraju SP, Nalla KK, Raju CAI, Donthireddy SRR. Optimization of the fermentation media using statistical approach and artificial neural networks for the production of an alkaline protease from Bacillus subtilis. International Journal of Natural and Engineering Sciences. 2008. 2 (3): 51-56