AN OVERVIEW OF BIOPROCESSING METHODS

Abstract

The development of recombinant DNA innovation has extended and expanded the capability of bioprocesses. Bioprocess technology is the modern use of natural cycles, including living cells and their components into substrates. The significant benefits of bioprocesses over conventional synthetic cycles are that they require gentle response conditions, are more explicit and proficient, and produce inexhaustible results (biomass).

Keywords: Recombinant, Bioprocess, Conventional, Inexhaustible.

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I. INTRODUCTION

A bioprocess is any interaction that uses living cells and their components as substrates. The capability of bioprocesses has been increased and enhanced by the development of recombinant DNA innovation. The fact that bioprocesses require mild response conditions, are more explicit and proficient, and yield infinitely renewable outcomes (biomass) are important advantages over standard synthetic cycles (Subramanian, 2018).

Upstream processing in biotechnology refers to operations at the very beginning of the ageing process. This covers all methods for growing microorganisms, such as cell culture, cell division, and cell collection. The cells or microorganisms are gathered and delivered to the downstream handling bioprocess, where they are further handled, once they have attained the desired thickness. The creation of supplements is a part of upstream management. Cells in a culture need the right nutrients to function and generate the desired value item, just like human beings do (Gronemeyer *et al.*, 2014). The majority of the supplements are made up of different ingredients, such as sugars (glucose), nitrogen (amino acids), fats (lipids), and small amounts of salt.

The fixes often have a powder composition and dissolve in water of high virtue. It is essential that the components are thoroughly incorporated. The mixed mixture is then transferred to the fermenter. The process of fermentation involves converting raw materials into desired products with the help of organic experts like microbes. It must be both monetarily feasible for an extensive scope and provide the optimal environment for the microbiological combination of the ideal item. They can be divided into procedures for the surface (emersion) and for submersion (Gronemeyer et al., 2014; Tripathi and Shrivastava, 2018; Gupta S. K. et al., 2019). Reactors that are run continuously or in a cluster may be the final option. The microorganisms grow on the surface of a liquid or sturdy substrate in surface techniques. These techniques are quite complicated and hardly used in business. The microorganisms fill up a liquid medium during the submersion procedures. In addition to the traditional ageing of beer and wine, the medium is kept in fermenters and blended to achieve a uniform distribution of cells and medium. The majority of cycles consume oxygen, hence the medium should be eagerly circulated air through them. Submersion processes finish enormously important current cycles (production of biomass and protein, antitoxins, chemicals, and sewage treatment).

The division of cells from the maturation stock, the purification and concentration of desired items, as well as the removal or recycling of waste, are all examples of downstream processing. The various stages of downstream processing, which come after the ageing system, incorporate reasonable recuperation, decontamination, and depiction methods for the optimum ageing item. Many other downstream processing methods, including chromatography, filtration, and centrifugation, can be used. These methods change in accordance with the final result's real composition and character, as well as its desired grade. The development of the cell comes next. When the environment is right, cells grow and develop. Unmistakably, this is constructed of growth supplements and cell culture chambers for regulating gases and temperature. Cell collection, or cell partition, is the most recent development. Centrifugation is usually used as a preliminary step. The cells in this place are separated from the way of life by the dregs and produced external powers. The principal

contaminants from the collect are removed during the subsequent filtration procedures. Finally, gastrointestinal filtration and sterile-grade filtration ensure that all impurities, no matter how tiny or microbiological, are removed (Peebo and Neubauer, 2018; Rahimi et al., 2019).

II. UPSTREAM PROCESSING

In order to get the energy they require to maintain their life processes, several organisms engage in fermentation. (Most organisms use aerobic respiration, which occurs in the presence of free oxygen, to produce the energy needed for these processes.) Yeasts, as well as some moulds and bacteria, and other microbes, get their energy from fermentation. Products from numerous fermentation processes are crucial in the realms of medicine, food preparation, and other industries (Fisher *et al.,* 2018).

The specific product that results from fermentation depends on the type of microbe driving the process and the substrate in which the fermentation takes place. For instance, while yeast in fruit juice ferments to produce wine, it ferments to produce beer in grain. Antibiotics, which are drugs used to treat infectious illnesses, are produced by both bacterial and mould fermentation. Enzymes are used to speed up chemical reactions in a number of commercial and medical processes through fermentation by a variety of microorganisms (Manahan et al., 2019). Bacterial fermentation results in the production of vinegar and cheese. Leavened bread is produced through yeast fermentation. The laboratory work is done at this point. The microorganisms chosen are those that are best suited for producing a specific chemical. Similar strains are created to increase both the yield and the quality. The media is prepared. The media is designed to be most suited for the best growth of the microorganisms utilised, depending on the biomass used.

The next step is sterilisation, which involves cleaning the equipment that will be used for fermentation and other processes of contaminants including fungi, bacteria, viruses, etc. As a result, the nutrients supplied in the medium are available only to the required microorganisms and, if present, not to any other unneeded microbes. An exact quantity of inoculum is made. That number of microorganisms will be employed to kick off the fermentation in the fermentor. The quantity must be adequate to start the fermantation.

III. FERMENTATION

A fermenter is required for successful production because it provides the following facilities for the process: a sterile environment, maintenance of a specific temperature, maintenance of agitation and aeration, pH control, monitoring of Dissolved Oxygen (DO), ports for feeding nutrients and reagents, ports for inoculation and sampling, fittings and geometry for scaling up, minimising liquid loss, and a growth facility for a variety of organisms.

Generally Recognised as Safe refers to some organisms. The known pathogenicity of the organism, its level of virulence, the quantity of organisms needed to start an infection, the routes of infection, the known incidence of infection, the presence of local vectors and microorganism reserves, the volume of organisms used in the process, cultivation and harvesting methods, prophylaxis measures, and treatment facilities are among the criteria for

determining whether an organism is hazardous. If an organism is determined to be pathogenic based on all the criteria, the fermentation process is kept under control. GILSP, or good industrial large scale practise, calls for the use of highly productive and secure organisms in the process (Stanberry, 2013).

IV. DOWNSTREAM PROCESSING

Some creatures are referred to as generally recognised as safe. Among the criteria for determining whether an organism is hazardous are its level of virulence, known pathogenicity, the number of organisms required to start an infection, the routes of infection, the known incidence of infection, the presence of local vectors and microorganism reserves, the volume of organisms used in the process, cultivation and harvesting techniques, prophylaxis measures, and treatment facilities. If an organism meets all the requirements for pathogenicity, the fermentation process is controlled. The utilisation of highly productive and secure organisms is required by GILSP, or good industrial large-scale practises (Stanberry, 2013).

Product isolation refers to the elimination of components whose characteristics significantly differ from those of the target product. Water is the main impurity for the majority of products, and isolation processes are designed to remove the majority of it, lowering the amount of material that needs to be handled and concentrating the result. Some of the unit processes include solvent extraction, adsorption, ultrafiltration, and precipitation. Product purification is carried out to separate impurities with qualities that closely mimic those of the product. As a result, these steps are expensive to complete and call for delicate and advanced machinery. A sizeable portion of the total cost of downstream processing is contributed by this stage. Affinity, size exclusion, reversed phase chromatography, crystallisation, and fractional precipitation are a few examples of procedures. Product polishing refers to the latter stages of processing that result in the packing of the product in a way that is reliable, practical, and transportable (Stanberry, 2013).

V. FERMENTATION PROCESSES IN A VARIATION

Commercially significant fermentations can be divided into five broad categories. Those who generate biomass (microbial cells) as a byproduct, those who generate microbial enzymes. Those that manufacture recombinant goods. Those who alter a substance that is added to the fermentation process, which is the transformation process. Those that produce microbial metabolites. The two main procedures for the commercial production of microbial biomass are the generation of yeast for the baking industry and the creation of microbial cells for use as food for humans or animals (single cell protein). Commercial production of enzymes from microbial, animal, and plant sources has been done (Fisher *et al.*, 2018).

. The ability to manufacture microbial enzymes in large quantities using recognised fermentation procedures is a huge benefit. Recombinant DNA technology has made it possible for humans to synthesise animal-derived enzymes like insulin.

It is possible to collect several microorganisms' metabolites. 2 types of microorganisms' metabolites are first-order metabolites, Subsequent metabolites. Fermentation is used to produce many key metabolic products, many of which are of

significant economic significance. Numerous secondary metabolites have antibacterial activity, whereas others serve as particular enzyme inhibitors, promoters, or pharmacologically significant promoters.

With the advancement of recombinant DNA technology, there are now a wider range of fermentation products that could be produced. Microbial cells may receive genes from higher organisms, allowing the recipients to generate proteins from organisms other than their own. A few examples of the many different microbial cells that have been used as hosts for these systems are E. coli, Saccharomyces cerevisiae, and filamentous fungi (Stanberry, 2013). Microorganisms have the ability to change a chemical into a more valuable but structurally similar molecule. To catalyse a single reaction, for example, in the biotransformation of steroids, a sizable amount of biomass must be produced throughout the transformation fermentation process.

VI. CURRENT UPSTREAM PROCESSING DEVELOPMENTS

Recombinant protein-based biopharmaceuticals are now produced quickly, affordably, and with a high yield thanks to recent innovation in upstream processing. High-throughput (HTP) technologies, single-use devices, statistical optimisation of media and environmental factors, QbD, PAT, and continuous upstream processing are some of the cutting-edge technologies employed for effective upstream process improvements.

High-throughput cultivation and screening techniques enable the miniaturised, cost-effective processing of several samples in parallel. However, for phototrophic species like microalgae or cyanobacteria, these techniques have not yet typically been established (Langer, 2015). Single-use technology (SUT) is currently crucial for bioprocessing research, scale-up, and commercial biomanufacturing. For instance, during the COVID-19 pandemic, the development and mass manufacture of vaccines and medications considerably increased the already rapidly expanding demand for single-use systems, sometimes referred to as disposable systems. The demand for adaptive next-generation cell culture facilities for the production of monoclonal antibodies (mAb), other recombinant proteins, and biosimilars is on the rise, as is the market for cell and gene therapy (Whitford, 2018).

VII. RECENTLY MADE DOWNSTREAM PROCESSING ADVANCES

Recent developments in downstream purification techniques include high-throughput equipment, single-use systems, QbD and PAT, modelling, continuous downstream processing, and integrated continuous downstream processing. PAT approaches are used in downstream processing to investigate protein concentration, purity, host cell proteins, host cell DNA, endotoxins, variances (misfolding), and process-related pollutants. Circular dichroism, HPLC, spectrometry, and other techniques are utilised to check critical quality characteristics in the chromatography processes. Next-generation sequencing could be used for virus screening even though it is very difficult. Additional investigation is necessary to guarantee the viral safety of therapeutic proteins (Fisher et al., 2018).

For automated sampling of a product stream eluting from a chromatography process column, one study used an on-line HPLC as a PAT tool (Tiwari *et al.,* 2018). The degree of PEGylation in chromatography was also estimated in-line, nearly real-time, using FTIR

spectroscopy as a PAT tool (Sanden et al., 2019). HEK293 cell-produced encapsulated VLPs containing the HIV-1 Gag protein linked to the Green Fluorescence protein were processed downstream using at-line multi-angle light scattering and fluorescence detectors (Aguilar *et al.,* 2019).

While expanding or gathering experimental data, modelling and simulations can greatly reduce the number of experiments required (Hanke and Ottens, 2014). Mechanistic models are based on physical qualities, whereas empirical models are based on a priori determined output data within a specified design space (Baumann and Hubbuch, 2017). Chromatography has benefited from the application of mechanistic modelling, a crucial tool for accelerating process development. Depending on the application, these models can provide a level of detail for the downstream unit functioning (Benner *et al.,* 2019).

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