People's Democratic Republic of Algeria

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Ministry of Higher Education and Scientific Research

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Ibn Khaldoun University - Tiaret

Faculty of Natural and Life Sciences

Department of Environment, Ecology and Biotechnology

Handout of the Course

Bioprocesses

For the Benefits of Students:

1ère Year « Master Microbial Biotechnology»

Presented by :

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Academic year 2023-2024

I. General Informations :

Branch/Major : SNV/ Biotechnology
Target Public : 1 ère Year « Master Microbial biotechnology»
Subject : Bioprocesses
Nature of class : Course (UE Methodologie)
Semester = 2

Duration : 15 Weeks
Semestrele Volume of hours = 45 h
Weekly V.H = 3 h
Credit: 05
Coefficient: 2

2. Presentation:

This introductory course provides a comprehensive overview of the fundamental principles, technologies, and applications in the field of bioprocessing. Through a combination of theoretical learning, students will gain insight into the fascinating processes involved in harnessing biological systems for various industrial applications.

3. Objectives:

Bioprocesses play a crucial role in a wide range of industries, including pharmaceuticals, biotechnology, food and beverage, agriculture, environmental management, and more. This course serves as a foundation for understanding the underlying concepts and methodologies essential for the design, optimization, and operation of bioprocesses.
Key Topics Covered:

- Introduction to Bioprocessing: Understanding the scope, significance, and applications of bioprocess technology in different industries.
- Microbial Biotechnology: Exploring the diversity of microorganisms and their roles in bioprocesses, including fermentation, enzyme production, and biofuel synthesis.
- Bioreactor Design and Operation: Learning the principles of bioreactor design, selection, and operation for optimal cultivation of microorganisms and production of desired bioproducts.
- Downstream Processing: Understanding the techniques and methods for separating, purifying, and recovering target products from bioprocess streams.
- Upstream Processing: Exploring the upstream processes involved in cell culture, media preparation, and inoculum development for bioproduction.
- Bioprocess Monitoring and Control: Introduction to monitoring techniques, process analytics, and control strategies for ensuring product quality, consistency, and efficiency.
- Scale-Up and Scale-Down: Understanding the challenges and considerations in scaling bioprocesses from laboratory to industrial scale or vice versa.

4. Outline :

Introduction

Introduction :

The production of food through the processing of raw materials derived from plants or animals has been happening for over two million years. This practice achieved a significant breakthrough with the discovery of fire. One of the earliest forms of biotechnology, dating back to ancient times, involved the production of fermented foods like wine, bread, and cheese. These processes often involved heating the food as well.

In this era, bioprocesses are projected to supplant chemical industrial processes due to their environmental friendliness, as they rely on natural activities performed by living organisms. This shift towards bioprocesses ensures sustainability in the industrial sector. Moreover, a bio-based economy is gradually replacing the previous oil-based economy.

Bioprocess techniques are of paramount importance to the development and production of pharmaceuticals, microbial biomasses, and renewable energy, to name a few areas of application. A comprehensive discussion of bioprocess techniques is beyond the scope of one chapter. However, we intend to demonstrate the influence of engineering principles and quantitative analysis in such areas as bioreaction stoichiometry and kinetics, bioreactor design, bioreactor scale-up, and bioproduct engineering (separation and purification) on the development and production of products of biotechnology. Engineering has always been an integral part of chemical and biochemical reaction research and development, be it for the design and testing of new drugs, development of new chemical products, environmental applications, or various other areas. Many products of biotechnology are derived from microorganisms, and bioprocess techniques are tailored toward the development of cost-effective processes for the production of such products.

For a long time, micro-organisms have played a major role in the production of food and beverages. Traditional fermented foods include alcoholic beverages, particularly beers, wines and spirits, which are derived from sugars and starches; dairy products, especially cheeses, yogurt, sour cream and kefir; fish and meat products, such as fish sauce and fermented sausages; and plant products, including grain-based breads and fermented rice products; as well as fermented fruits, vegetables and legumes, including canned olives and pickles, sauerkraut, soy sauce, tofu, fermented cassava, cocoa and coffee beans. Some of these products originally evolved as a means of preserving food.

The role of microorganisms in food manufacturing is not limited to the production of fermented foods and beverages. Many products of microbial fermentations are also incorporated into foods as additives and supplements. They include antioxidants, flavors, colors, preservatives, sweeteners and vitamins; as well as amino acids, organic acids and polysaccharides, many of which also have non-food uses. The annual global food additives market is very important and growing at over 4% per year. Synthetic food additives produced by chemical processes are perceived as less desirable than natural compounds. Therefore, there is great interest and incentive for the development and use of natural food additives derived from microorganisms. There are more than 30 categories of functional food ingredients. Many of these additives are now called neutraceuticals, which are considered to provide medical or health benefits, including the prevention and treatment of disease.

Chapter 1

Bioprocess Techniques

1. Bioprocess Techniques:

1.1. Definition of Bioprocess Techniques

It can be defined as a method used to culture a pure microbial product, like enzymes or antibiotics. It includes aseptic transfer and subculturing to obtain a pure culture, preservation of culture, and inoculum preparation for large-scale fermentation. Then the main fermentation involves aseptically growing microorganisms on a large scale in a controlled media in a fermenter to obtain the maximum yield of the desired product. This is followed by downstream processing to isolate the product, and waste products are treated to conserve the environment. Nowadays, bioprocess has become synonymous with white biotechnology and biotechnological engineering. White biotechnology manipulates the interconnection between living organisms and their environment, especially through the materials processing industries. On the other hand, biotechnological engineering is defined as the integration of biological principles and process engineering. Both of these have led to the development of diverse bioprocess techniques.

A bioprocess is a technique developed through the united knowledge of biology and process engineering in order to manipulate cellular structure and function to produce the desired product. It is a technique that tells about the technique employed in its implementation. It is a multifaceted manipulation at the cellular level, which encompasses a wide range of biological and engineering knowledge. It involves the design of a biologically based process for the purpose of producing a product or series of products. It involves the use of living cells or their

components such as enzymes for the synthesis and release of a product and the use of organic substances by microorganisms for growth.

1.2. Importance of Bioprocess Techniques

- The development of tissue engineering has a greater scope for future medical treatments due to different kinds of tissue cell cultures and the production of artificial tissues. This can solve many medical problems like organ failures and bio-artificial organs.

- Large-scale production of monoclonal antibodies and specific gene products has created new avenues for medical treatments and diagnostics. Cell cultures have a greater role in these products, and these products have a huge market due to high specificity and fewer side effects.

- The industrial importance of plant and mammalian cell cultures has increased by multifold due to unlimited applications of their products. For example, enzymes and protein production from recombinant DNA technology find their applications in the production of detergents, food processing, medicinal use, and molecular biology. Similarly, plant cell cultures have vast applications from the production of secondary metabolites and antibodies to the production of transgenic products.

- Various chemical and physical engineering sectors have shifted their attention to bio-processing. For example, the production of antibiotics and other pharmaceutical products, production of beer and wine, waste treatment, production of bio-fuels, and the production of recombinant DNA products. The prime reason for this switchover is the simplicity of

downstream processing, environmental issues, no hazardous by-products, resource material, and ease for sterile operations.

1.3. Scope of Bioprocess Techniques

Bioprocess techniques have played a significant role in the large-scale production of new products and in the creation of new industries. Bioprocess techniques are used in the large-scale production of biological products from renewable raw materials, which include microorganisms, plant and animal cells, or tissue. It involves a chain of events where the raw material is subjected to change by various processing steps, leading to the manufacture of a product. The product of bioprocess may be a specific cell in the culture, an enzyme, or the product after the metabolism of a cell. So, the scope of bioprocess is very wide-ranging, from the very complex to an extremely simple product. To approach the topic, let us see the importance and scope of bioprocess techniques in brief. A waste product or secondary metabolite for one industry may be a valuable product for another. It always depends on which product to manufacture and to what extent it will be useful.

1.4. The range of bioprocesses

Five major groups of commercially significant fermentations include:

1. Those that produce **microbial cells** (or biomass) as the product.
2. Those that produce microbial **enzymes**.
3. Those that produce **microbial metabolites**.
4. Those that produce **recombinant** products.

5. Those that modify a compound which is added to the fermentation the **transformation** process.

1.4. 1. Microbial Biomass

The commercial production of microbial biomass may be divided into two major processes: the production of yeast to be used in the baking industry and the production of microbial cells to be used as human food or animal feed (single-cell protein).

1.4. 2. Microbial Enzymes

Enzymes have been produced commercially from plant, animal, and microbial sources. However, microbial enzymes have the enormous advantage of being able to be produced in large quantities by established fermentation techniques. Also, it is infinitely easier to improve the productivity of a microbial system compared with a plant or an animal one. Furthermore, the advent of recombinant DNA technology has enabled enzymes of animal origin to be synthesized by microorganisms. The uses to which microbial enzymes have been put are summarized in Table 1.1, from which it may be seen that the majority of applications are in the food and related industries.

Table 1: Some examples about commercial applications of Enzymes

Industry	Application	Enzyme	Source
Baking and milling	Reduction of dough viscosity, acceleration of fermentation, increase in loaf volume, Improvement of crumb softness, and maintenance of freshness Amylase Fungal Improvement of dough texture, reduction of mixing time, increase in loaf volume	Amylase Protease	Fungal Fungal/bacterial
Brewing	Mashing	Amylase	Fungal/bacterial
	Chill proofing	Protease	Fungal/bacterial
	Improvement of fine filtration	β-Glucanase	Fungal/bacterial
Cereals	Precooked baby foods, breakfast foods	Amylase	Fungal
Chocolate and cocoa	Manufacture of syrups	Amylase	Fungal/bacterial
Coffee	Coffee bean fermentation	Pectinase	Fungal
	Preparation of coffee concentrates	Pectinase, hemicellulase	Fungal
Confectionery	Manufacture of soft center candies	Invertase, pectinase	Fungal/bacterial
Cotton	Low temperature processing	Pectate lyase	Fungal

Chapter 2

Bioreactors

2. Bioreactors:

2.1. Definition and working principle

A bioreactor is a vessel in which living cells or organisms are grown under controlled conditions to produce a particular product. The bioreactor working principle is relatively simple, but the technology behind it is incredibly complex.

Bioreactors are devices that support a biologically active environment. This environment can be created in a number of ways, ranging from simple aeration of the organism's environment to agitation of the liquid, to a more complex setup such as an airlift fermentor. One of the key defining characteristics of a bioreactor is the mechanical agitation of the cultivation. In agitation culture, the bioculture is provided with oxygen by circulating an airflow or, in the case of a fermentor, by spinning a propeller located at the bottom of the vessel. This provides a well-mixed environment and allows for a more consistent product, as the organism is always exposed to the same conditions. In contrast, static culture methods hold the organism in place and allow it to grow on the vessel wall or a support material, with no movement of the liquid. Although this is suitable for some animal and plant cultures, it is not commonly used for microorganisms. Static methods have the disadvantage of a mass transfer that is lower than for agitated cultures and a higher likelihood of contamination due to the lack of protection from outside environments. Static culture can be either surface culture or submerged culture, just as with agitated cultures. Submerged static cultures are commonly done in small vessels.

2.2. Types of Bioreactors

The two most common types of bioreactor are anaerobic and aerobic. Anaerobic bioreactors are used to create an environment for microorganisms that do not require oxygen. These bioreactors are simpler in design compared to aerobic bioreactors and are often used for processes such as the production of methane and other gases. One of the advantages of anaerobic bioreactors is they can be relatively cheap to operate because energy does not have to be used to provide an oxygen source.

Aerobic bioreactors provide an environment in which microorganisms that require oxygen can thrive and do their work. The microbial degradation of organic matter in wastewater is one example in which an aerobic bioreactor is used. These bioreactors are designed to provide a large amount of oxygen to the microorganisms, often by using compressed air and aeration stones. Aerobic bioreactors are widely used in industry and are available in many different sizes, from huge tanks used to process large quantities of water or sewage to simple set-ups using bottles and tubes.

The bioreactor is one of the most important tools in bioprocess technology. In these vessels, a solution of microorganisms or animal cells utilizes their environment to produce a desired product. The design of a bioreactor is very important. A good design can lead to a successful experiment and obtain the required product, while a bad design can result in failure and possibly damage the bioreactor. The first bioreactors were designed for batch fermentation and were little more than a tank with a drain. Today, bioreactors are designed for many different types of operation and vary widely in size and complexity.

2.2.1. Stirred-Tank Bioreactors

These types of bioreactors have mechanically moving agitators or impellers within a baffled cylindrical vessel (Fig. 1). Baffles are usually flat vertical plates whose width is about one-tenth of the vessel diameter. Normally, 4–6 baffle plates are fitted to the inside vessel walls to aid mixing and mass transfer by increasing turbulence, preventing vortex formation and eliminating 'dead spaces'.

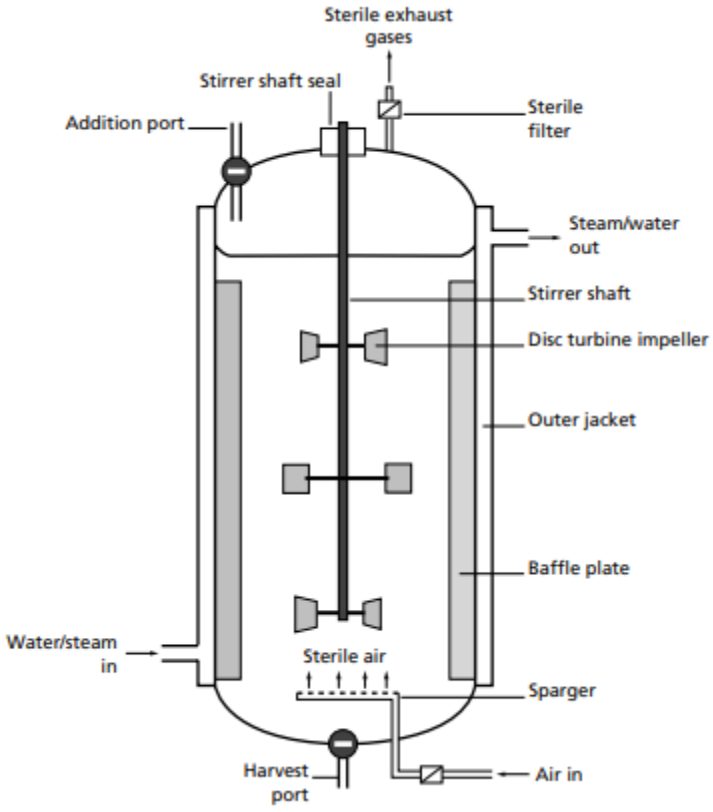

Figure 1: Diagram of a stirred tank reactor

2.2.2. Packed-Bed Bioreactors

A packed bed bioreactor draws its name from the nature of the growth support on which microorganisms are immobilized. The support particles are packed to a certain depth within a column through which the liquid culture medium containing the substrate flows. The substrate becomes attached to the surface of the particle and diffusion of substrate occurs within the particle to the site of utilization by the microorganisms. In order to obtain high substrate removal efficiency, a high microorganism culture density and expansion is required. This is achievable using a packed bed with high particle density and a small interstitial void volume. Flow through the reactor can be in the upward or downward direction. Packed bed bioreactors are particularly suited to the degradation of toxic substrate and low substrate concentration from which only small amounts of biomass are able to be produced. This is due to the fact that the toxicity of a substrate is inversely proportional to substrate concentration uptake rate, and the high biomass concentration within the floc will release large amounts of extracellular polymers and enzymes. Substrate and toxicity removal from the effluent of a waste treatment system is seen as essential. High microorganism culture density and particle growth mean that the risk of reactor contamination is reduced. It has been shown from the work of Liu et al. (2002) that the simultaneous biodegradation of high molecular weight and low molecular weight substances can be achieved in a single packed bed reactor. His work involved the degradation of phenol (a model for a low molecular weight substrate) in the presence of pentachlorophenol, cellulose, and various lignins (all high molecular weight). This is highly significant because each type of substance typically requires different types of microorganisms to effectively degrade it.

2.2.3. Membrane Bioreactors

It is basically the same as a conventional activated sludge system, however the separation of the biomass is what differentiates the two. The operation of an Membrane bioreactors (MBR) is the same as an activated sludge plant, however MBRs use a state-of-the-art aeration system to optimize biological treatment. The cross flow filtration method is what generally separates the biological system from the filter system. With continuous suction, a low fouling and higher porosity membrane, block/plate and frame or hollow fibre module can provide a very clear filtrate without being interrupted for an intensive backwash and solid separation.

Because the technology is so new and rapidly growing, it is difficult to provide an exact number, but what is known is the market is growing extremely rapidly due to its increasing acceptance and requirement for higher quality effluent throughout the world.

MBR combine a separation process like microfiltration or ultrafiltration with a biological wastewater treatment process. It has been used for treating wastewater. The main advantage of an MBR system over conventional wastewater treatment is the ability to separate the biological treatment unit from the solid-liquid separation unit. This means, one can obtain high quality effluent with lesser space.

2.3. Design Considerations for Bioreactors

2.3.1. Operating mode

The first decision in the design of a bioreactor is the choice of operating mode. The mode of operation can be batch, continuous or fed batch. Both continuous and fed batch modes require extra equipment to enable the separation of cells from the effluent and thus continuous stirred tank reactors designed for these modes have provisions for this. The decision on the operating mode is crucial to the design of the bioreactor and has economic implications throughout the lifetime of the plant.

The design of a bioreactor is largely determined by the process for which it is going to be used. However, there are a number of generic aspects in the design of a bioreactor that can be defined prior to the process taking place. Some of these aspects involve trade-offs and cost decisions. Although there are vast arrays of bioreactor designs, most of the engineering design considerations are similar. It is beyond the scope of this work to cover every bioreactor design, instead it will detail the considerations for the most common bioreactor: the continuous stirred tank reactor.

2.3.2. Oxygen Transfer Rate

In aerobic fermentation, oxygen transfer is one of the most important processes. Many fermentations are oxygen-limited because of the relatively low solubility of oxygen in water and the often high oxygen demand of microorganisms, cells, or enzymes. In some cases, oxygen may be toxic to the cells. Oxygen transfer has been a limiting factor in the development of fermentation technology. It has often been assumed to occur through the liquid phase by simple

diffusion to the cells. This has led to the use of shake flasks and simple stirred vessels at relatively large scales where it is assumed that oxygen transfer would be improved by increasing the rate of stirring. Unfortunately, these vessels often require the use of antifoam agents to control foam, and subsequent recovery of the product from these agents can be difficult. Even so, the shear forces involved in the use of these vessels may be detrimental to some cells. When adequate oxygen transfer is not achieved in such systems, it is common to see attempts to oxygenate the outlet gas from the vessel and return it to a sparger. Unfortunately, this can result in entrainment of the cells in the bubble phase and poor separation of cells from the product. This is often an unsatisfactory technology, and more research and development work is required to achieve efficient oxygen transfer to aerobic fermentations of all kinds.

2.3.3. Mixing and Agitation

It has been noted that "the inability to properly mix and maintain suspension of the cultured cells in the bioreactor can result in poor mass transfer and shear damage of the cells". Proper mixing is necessary in order to ensure a homogeneous environment in the bioreactor. When a predetermined set point of the Dissolved Oxygen (DO) is set, it is necessary that the oxygen level is the same throughout the whole bioreactor. Poor mixing can lead to concentration gradients of dissolved oxygen. This is because oxygen is consumed heterogeneously by the cells. Thus, regions of low oxygen concentration can result in cell damage or death. Cell death is likely when the oxygen level is too low to support respiration. This was the case in a study of the shear sensitivity of hybridoma cells. It was found that at low impeller speeds, the cells had decreased viability due to the low dissolved oxygen levels created by inefficient mixing. Mixing intensity

can be altered by changing the speed or type of impeller. This is possible to measure by the power number is influenced by the impeller type and speed. High power input can lead to higher shear stresses which may be detrimental to shear sensitive cells. Mixing patterns of the bioreactor are dependent on the design of the vessel and impeller. It was found that Rushton impellers in a draft tube sparged vessel provided an axial flow with good mixing and oxygen transfer but resulted in high shear damage of the cells. Particle image velocimetry and laser Doppler anemometry are both techniques to measure mixing and flow in bioreactors and are beyond the scope of this article.

2.3.4. Temperature Control

The basic principle of control involves a sensory system linked to a control system and feedback loop (Fig. 2). Sensors are used to measure and record the events within the bioreactor. In conjunction with process control, they maintain the difference between the measured and desired values at a minimum level. Overall control can be manual or automated; newer systems have integral and derivative control systems. Data recorded from the sensors and control decisions are downloaded to a computer where appropriate calculations can be performed to determine production of biomass and product, overall oxygen and carbon dioxide transfer rates, nutrient utilization, power usage, etc

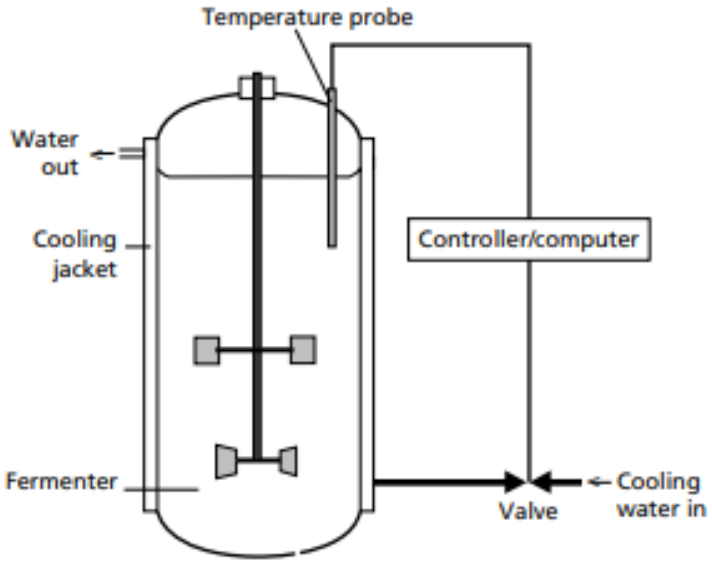

Figure 2: A scheme for controlling fermenter temperature

2.4. Sterilization and Aseptic Techniques

Aseptic techniques aim to prevent recontamination of sterilized components. This involves minimization of contact with non-sterile items and prevention of microbial contamination coming from the operator or the environment.

Steam sterilization in place is often used and many plastics can be sterilized by treatment with steam or hot air. However, these methods are slow and methods using gas such as ethylene oxide or vapor-phase peracetic acid are often used for plastic components. Filtration is a preferred method for sterilization of heat-sensitive liquids and gases and can be achieved by controlled pumping through a filter unit in a loop or by inline filtration of gases.

Sterilization: Sterilization is an absolute necessity in fermenter operation. The presence of microorganisms implies that the system is not sterile and therefore, by definition, any aseptic

technique is concerned with maintaining and/or regaining sterility in the system. Heat is an effective means of sterilization and many bioreactor components can be sterilized in an autoclave. However, heat sensitivity and the complexity of heat exchangers, probes, and transfer lines mean that alternative methods must often be sought.

Operational modes have major implications on bioreactor design and function, affecting mixing, heat transfer, and gas exchange. Batch bioreactors may be as simple as a glass bottle, but large fermenters are usually constructed from metal and can have complex internals.

Chapter 3

Fermentation Processes

3. Fermentation Processes:

In this context, a fermentation system refers to a complicated and dynamic collection of microorganisms or cells operating within a regulated setting to convert materials and energy into additional biomass and metabolic products. The microbial components of the fermentation system can exist as either a population, as commonly seen in most fermentation processes, or as a community, as observed in mixed culturing and traditional fermented foods. In the latter case, microbial cells exhibit varying degrees of growth and development, unless the culture is synchronized. Consequently, a fermentation system is intrinsically connected to a bioreactor, which creates the controlled environment necessary to fulfill specific biological and technological requirements.

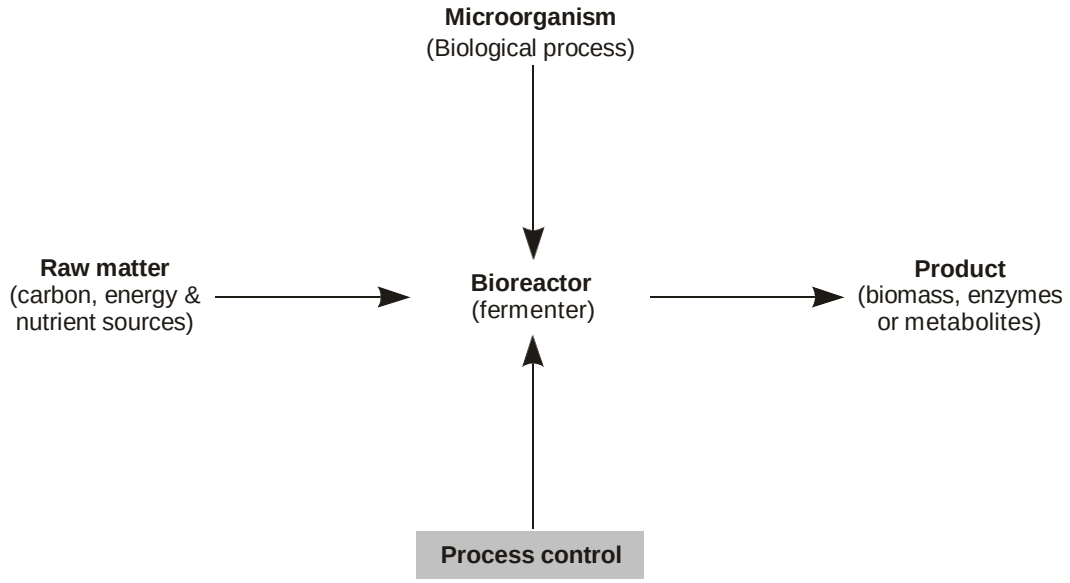

Figure 3: Diagram of fermentation process

Fermentation is a metabolic process which involves the conversion of complex organic compounds into simpler ones through the action of enzymes. In biotechnological processes, it is often desirable to produce a specific compound and for the organism to accumulate this. Depending on the microorganism and the specifics of the metabolic pathway, it may be best to perform the fermentation under specific conditions. This could be to maximize growth or the production of a specific compound. There are numerous factors which can affect the outcome of fermentation. This includes dissolved oxygen, pH, temperature, type of bioreactor to be used, and the specific fermentation process.

The choice between batch, continuous or fed-batch can have a great effect on the specifics of the fermentation and the outcome. Different fermentation processes can affect yield and productivity, time to product formation, and downstream processing of the product. Batch fermentation is the most basic type of fermentation, which is easy to set up and use. It is a closed culture system that means the conditions of the culture can be closely regulated. This fermentation process is carried out in an agitated tank, a defined amount of substrate is added, and the culture is left to ferment without any further nutrient addition. Batch fermentation is usually used for small-scale production and products that are heat sensitive. No new material is added or taken out of the culture during fermenting.

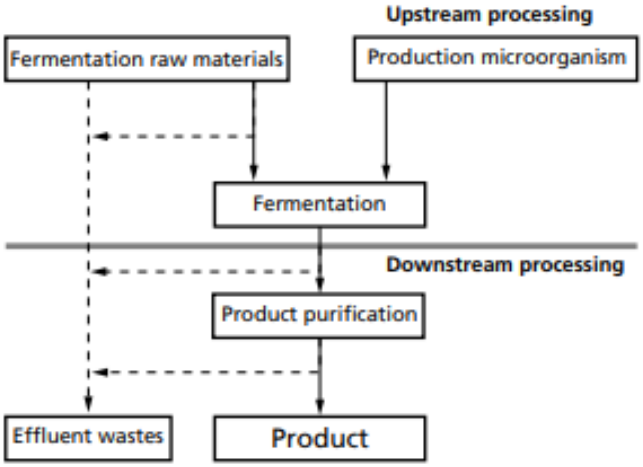

Figure 4: Outline of a fermentation process.

3.1. Batch Fermentation

In batch fermentation, a culture medium of fixed composition is agitated in a fermentation vessel using an inoculum of fixed viable cell concentration. Cells grow in agitated liquid culture, producing cell products and more cells until the substrate is depleted and/or toxic metabolites accumulate to growth limiting levels. The duration of the process is called fermentation time. Changes in cultivation conditions are not possible during such a process. For example, changing glucose concentration to grow a different *E. coli* strain would be classified as a different process. Batch fermentation is most suitable for the production of high cell concentration and/or products formed intracellularly. If contamination occurs, the batch process has the greatest probability of rescuing an uncontaminated culture. This is because a sample of uncontaminated culture can be taken out and stored at a low temperature for a long time. High cell density and/or product is advantageous in comparison to continuous process because the cost for

separation and purification are proportional to the volume of the culture medium or liquid product. Due to these reasons, batch fermentation methods are often used during research and process development in aiming to produce a large quantity of desired material using other methods.

Batch fermentation is a system in which all the components are added at the beginning of the process and the processing of the material takes place with no addition of any feed material. The output of the material will be after a fixed interval of time. Bread baking is an example of a batch process during which raw dough is converted to bread. The composition at the time of baking, i.e. conversion of dough to bread, changes during the process, but no addition of material is done to convert bread back to dough. This is a discontinuous process, since the material changes composition during processing but no further addition of any material is done. In food and fermentation industries, batch processes can be made discontinuous by pausing the incubation.

Batch culture is a closed culture system with an initial limited amount of nutrient. The inoculated culture passes through a number of phases, as illustrated in Figure 5.

The latency phase: corresponds to a period of metabolic adaptation of the microorganism to the environment. The latency phase will be reduced to a minimum in industrial fermentation.

The exponential growth phase: the specific growth rate is maximum. The amount of nutrients is in excess and the biomass increases most rapidly. The products formed during this phase are

the primary metabolites. As long as no growth-limiting factor appears, the exponential phase continues.

The stationary phase: After a slowdown phase, the stationary phase appears. It occurs following the consumption of nutrients and the presence of bacteriostatic and bactericidal microbial waste. Microorganisms can survive without cell multiplication in an environment unfavorable to their development; cell divisions can also occur but each gives rise to two cells, one of which is stillborn.

The death phase: The decline phase corresponds to the decrease in biomass linked to cell lysis.

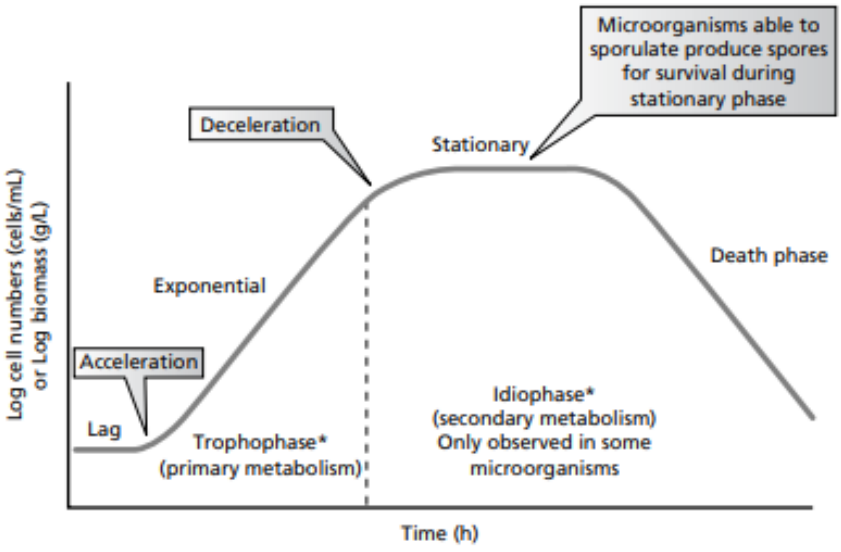

Figure 5: Growth of a microorganism in a batch culture.

3.2. Continuous Fermentation

Continuous fermentation is a process by which fresh media is continuously added to a fermenter and products are continuously removed (Figure 6). The fermenter size, culture duration, specific growth, and product formation rates are designed to maintain steady state. Continuous fermentation has several advantages over batch fermentation. This system was used to evaluate the effect of dissolved oxygen levels on the glycolytic and fermentative metabolism of *Lactococcus lactis subsp*. cremoris MG1363 during growth on a complex (YNB) and a defined (YNBD) media.

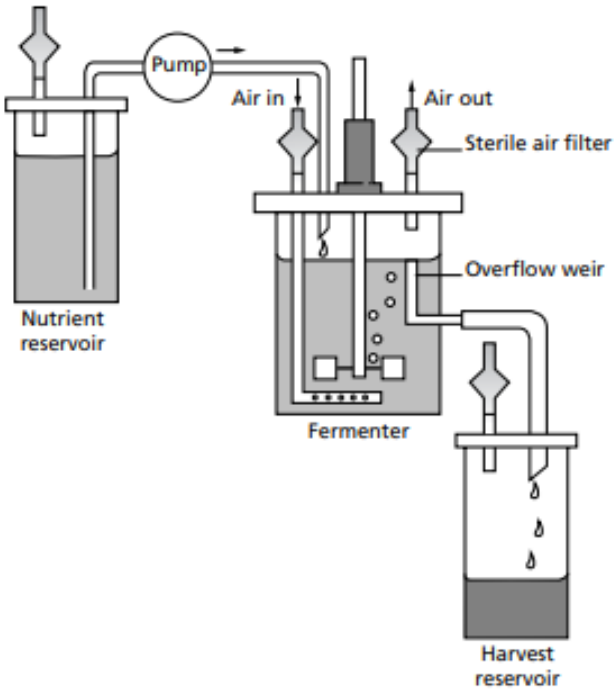

Figure 6: Continuous culture apparatus.

3.3. Fed-Batch Fermentation

Fed-batch fermentation is an adaption of batch culture designed to allow production of a target amount of biomass or product while maintaining a defined growth rate. This is achieved by feeding the culture with a medium containing a carbon source of limited availability. In this type of culture, there are two simultaneously occurring processes: cell growth which is represented by an exponential growth phase and production of the desired product. The exponential growth phase occurs during the batch phase of the culture. The batch phase is defined by the fact that no feed is added to the fermenter for a period of time. This is followed by the fed-batch phase where the carbon source is added at a predetermined rate. The carbon limited growth phase occurs by feeding just enough to prevent the culture from becoming carbon replete. At the conclusion of the fed-batch phase, the feed is stopped and the culture proceeds to a metabolic shift which may result in product formation. The product formation phase occurs at a time when there is no carbon source available. Fed-batch can be used to increase the yield of product formed, the productivity of product formation, and to alter the proportion of a mixed product formed by providing different feeds at different stages of the culture. This technique also provides an environment nearer to steady state which has advantages for metabolic studies and the simplification of large-scale fermentations.

3.4. Solid-State Fermentation

Solid-state fermentation is defined as "a fermentation process in which the growth of microorganisms is carried out on a solid surface in the absence of free water." Although it has been practiced for centuries for the production of fermented foods, only in the last few decades

has it been recognized as a very versatile process with great potential. Its unique characteristic is the solid substrate on which the microorganism grows. This substrate provides the microorganism with the necessary water and nutrients for growth, and acts as support to allow the formation of desired product. The substrate can range from natural products such as grains or leaves, to synthetic materials such as resins. Processes involving natural substrates are usually similar in nature to the original method of producing fermented foods and utilize the natural microflora of the substrate. Processes involving synthetic substrates are usually designed to produce a specific product and use a known culture of microorganisms. The types of products which can be produced by solid-state fermentation are extremely varied and include traditional fermented foods, enzymes, organic acids, aroma compounds and fungal tissue mass for use in human food, animal feed and various other products. Due to the large diversity of possible applications and products, solid-state fermentation is a very relevant process to many different industries.

Chapter 4

Downstream Processing

4. Downstream Processing :

Downstream processing is a key to the production of the desired product, and the purification methods depend on the specific molecule properties and the intended end use of the product. Downstream processing involves the recovery and purification of the biosynthetic product. The general scheme of downstream processing is shown in Fig 7. This section addresses the recovery and purification of the product. Recovery results in the desired product being unbound from the aqueous solution, while purification involves a number of additional steps, depending on the specific molecule being processed. In most instances, the recovery process is followed by purification in order to obtain a product of sufficient purity. In general, the cost of downstream processing is dependent on the quantity of product to be processed, and ranges of processing techniques have been employed with varying success. These include techniques traditional in chemical engineering (for example, precipitation and filtration) and also techniques which are relatively unique to bioprocessing (for example, chromatography). Precipitation involves the addition of a non-solvent which results in a decrease in solubility of the product, thus allowing its separation from the solution. The technique is most effective when the product has a low solubility and is often followed by centrifugation or filtration. Ultrafiltration involves the separation of lower molecular weight components from higher molecular weight solutes in a solution. This is achieved by the passage of the solution across a membrane, which is a filter having a specified pore size. High-value products have been purified using preparative ultrafiltration to capture the product in the high molecular weight retentate. This technique is often used in conjunction with diafiltration, which is used to change the

composition of the solution by adding or removing components to or from the permeate. Both ultrafiltration and diafiltration have become more common as they can provide a more efficient and mild method of purification than traditional solvent extraction. Flow-through centrifugation techniques can also provide an efficient method of continuous separation of components of different densities.

4.1. Cell Separation

There are various methods of cell separation; centrifugation is the first preferred one, but it is not effective. If the cell properties are such that centrifugation is not effective, other methods like filtration, floatation, membrane processes can be tried. But all these methods suffer common disadvantage that they are based on physical or chemical parameters for separations. Increasing interest in the recent times to separate biologically automated affinity and immunological methods are also not discussed as they are bench scale techniques. An ideal cell separation scheme should between recovery of desired material and a cell free discharge which can be followed affirmation in process or culture system at consistent at limit cost.

When a fermentation process is complete, the product has to be separated from the broth carrying it. There are various methods used for this process, but the relevant one to the type of fermentation carried out in this case is cell separation. Cell separation biologically implies the selective removal of a specific type of cells from a culture containing more than one type of cells without disturbing the viability of the separated cells. Thus it can be said to be isolation process, which selective release and recapture cells, immobilization or affinity of specific types of cells are in reality specific types of separations.

4.2. Purification Techniques

The simplest method of product recovery is through precipitation. Salting out involves the addition of a high concentration of salt or water-miscible organic solvent to give a condition of low solubility of the product. This is effective for proteins and nucleic acids. The most common precipitation technique is the addition of ammonium sulfate. Gradual addition of the salt in a saturated solution allows selective precipitation at different levels of saturation. The technique is effective in separating proteins of similar size and shape. Osmotic shock can be used to cause the release of products from cells without significant damage to the cells, which will allow subsequent cultivation. This may involve growing the cells in a hypotonic medium. High levels of sugar or other solutes, and pressure changes can also be used to break cells.

First, it is necessary to recover the product from the culture medium. If the product is intracellular, this step will involve disruption of the cells to release the product.

A variety of purification techniques are available for isolating the desired product from impurities. The choice of method will depend on the nature of the product and the degree of purity required.

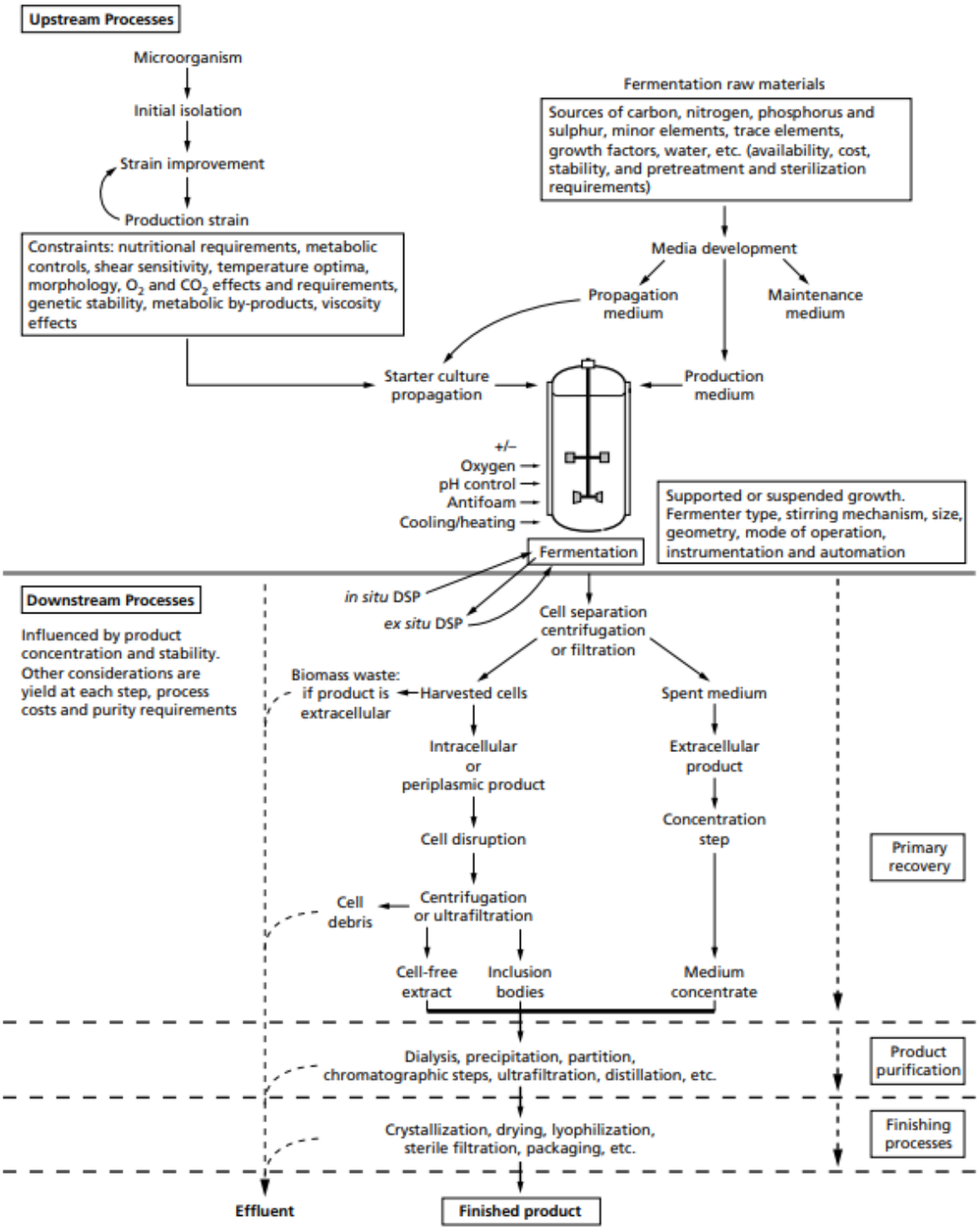

Figure 7: An outline of upstream and downstream processing operations.

Chapter 5

Scale-up & Scale-Down

5. Scale-Up and Scale-Down:

Scale-up of a bioprocess is the process of increasing the capacity of the process. This may be dictated by the manufacturing of a new product, increase in the demand of a product already being manufactured, or the need to produce the product at a larger scale for a continuous amount of time. For modern biotechnological processes, it really should be thought of as 'scale-up/scale-down' since a large number of the processes are intensively research based and are therefore still in development. Attempting to 'scale-down' a process in order to carry out experiments and trials at the new smaller scale can often be more difficult than the scale-up. The most obvious case of this is within the pharmaceutical industry, where there has been much development in recent years in continuous small scale fermentations for the production of protein products. This is due to the large amount of these products that are produced using gene expression systems in transgenic animals. These are inherently batch processes, but continuous fermentations are a much cheaper process. These new pharmaceutical products are still being developed and changes in the production process are likely, thus the ability to carry out small scale trials will be essential. Also, the current economic climate has forced many companies to attempt to cut production costs by moving to a smaller scale. This may often involve the production of the same product at a new smaller scale and there are obviously many biotech companies operating at a small scale that would wish to outsource the production of a particular product.

5.1. Challenges in Scaling Up Bioprocesses

A bioprocess usually runs from grams to a few kilograms and then to tons. This increase in the quantity of the product is commonly referred to as scale-up. The scale-up of an entire process is a difficult task, especially in biotechnology, because of the complexity of living systems and the many interactions that occur in any biological process. An ideal scale-up method is one which is successful in achieving the desired objectives with an acceptable balance of risk, cost, time, and with minimal changes to the original process. It is usual to have to compromise over an ideal method, and in most cases, scale-up is an iterative process that involves repeated experimentation and learning from the effects of scaling individual components of the process. Despite the difficulties and risks of scale-up, most large organizations and investors are often not interested in the early stages of process development and will only consider an investment if the processes can be successfully scaled up to give reasonable production levels.

5.2. Techniques for Scale-Up

The method which may yield better results in terms of the process are simple changes of operating conditions, and this is what in which it is desired to be changed as little as possible in scaling up a process.

There are many methods that can be employed when scaling up a bioprocess, and there is no one true way that will fit all. It has been stated that scale-up is a black art, best understood by experience, and this is because of the complexity of the bioprocess due to its broths and cultures and the cells that are being processed with it. Speitel et al (1980) identified three categories in which scale-up methods can be classified, and these methods can be seen in varying degrees

throughout the industry. These methods are often used in combination and are dependent on the nature of the process to be scaled. The first method is known as the empirical method, in which it is a case of trial and error by using a series of experiments to try to duplicate the laboratory results. This method is perhaps the simplest scaling strategy, but in turn, it is probably the most costly and time-consuming method, and modern industrial practice will not tolerate using this method on its own. A modified version of this method is the adaptive approach, and this uses a systematic series of experiments in order to migrate the process to full scale. This method allows for a more structured and less trial and error approach and allows changes where necessary. The second method is known as the theoretical scale-up, and this method applies scale-up mathematical models to predict the large-scale behavior based on the understanding of the small-scale process. This method uses the knowledge of how and why a process works as it does and attempts to quantify it so that it can be duplicated on a larger scale. This method is probably the most common method employed in the industry as science and engineering can be applied without excessive increase in costs. The final method known as the all-in-one-go method, and this method is the most straightforward to the point.

Scale-up of a bioprocess is the process of increasing the process to produce in a larger scale. The need for such a process can be attributed to the success of the small-scale process, which in turn may be due to higher sales of the product, new processing regulations, the requirement of more clinical material for trials, and an increase in the demand for the product. Lund (2000) defined scale-up as a process that carries the results obtained in laboratory and pilot plant experiments without appreciable change in the process or in the types of equipment, to a more satisfactory

completion of the process in a larger production plant. This definition refers to the want of it all, which is to get as close to production without changing as possible. During the development process of a product, there will be many changes and improvements mainly due to the knowledge gained during each phase, and with scale-up, it is said to be necessary to have a defined point where it starts. This will prevent the changes made to improve the product from being incorporated into the methods, and thus changes to the process with intent to characterize later improvements may require the process to start again at that new level of understanding. The method which may yield better results in terms of the process are simple changes of operating conditions, and this is what in which it is desired to be changed as little as possible in scaling up a process.

Chapter 6

Process Monitoring and Control

6. Process Monitoring and Control:

Process monitoring and control technologies are essential for any scale of operation in order to maintain the reproducibility and stability of the product. In process monitoring, the critical variables which affect the process or product are continuously measured and the information is fed back to an operator. If the information is to be used to affect the variables in the same batch, the control system can be said to be 'closed loop'. In 'open loop' control, the information is used to affect the process at some time in the future, either in the next batch or by changing a continuous process. An example of closed loop control is the addition of acid to a fermentation in order to maintain the pH of the culture. Open loop control might involve a change in temperature setting on a freezer, at the time of next use. Control may be manual, using the operator to make decisions and change the settings of process equipment, or automatic, where the control system makes the decision - comparing the value of the measured variable to a pre-set value known as the set point - and changes the process settings. Control systems generally operate on the basis of a deviation, which is the difference between the measured variable and the set point. The more the control system knows about the process and the relationship between the process settings and the product, the more effective it can be in affecting the desired change. This is one reason that mathematical modeling procedures are now popular in the bioprocess industries. Control systems can be designed to control a single process variable, or several, using what is known as multi-variable control. Step changes in process settings and variables can be inefficient and affect the quality of the product. A more gradual and controlled change involves the use of optimization techniques and computer aided process engineering tools. In batch and continuous processes, it is important to know how the process is proceeding

and whether or not the process is under control. If it is known that a process variable is subject to change due to a disturbance, or at a specific point in the process, then control system can be designed to change the settings of another variable in order to counteract the change or to affect a desired change in the other variable. This type of control is known as feed forward control and it useful in preventing changes in the process from affecting the product. Control systems require information from the process and this is obtained using sensors. In the modern age of bioprocessing, biological and biochemical sensors are now crucial in obtaining information about process variables and cell culture conditions. Control systems can be as simple as a time switch turning a bioreactor stirrer on and off to prevent cell aggregation, or highly complex involving a computer and several algorithms coordinating a large number of variables in a continuous bioreactor.

6.1. Sensor Technologies

6.1.1. Introduction

The use of sensors is an integral part of process monitoring and control, which is becoming of increased importance with the growth of bioprocessing. During the monitoring of a process, a sensor is used to measure the state of the system, and from this information, an assessment of the process's performance can be made. For a sensor to be of value, it should have high sensitivity and selectivity to the variable being measured. It should have a rapid response time and be robust enough to survive in the conditions in which it will measure the variable. An example of a typical variable for a bioprocess to measure is the concentration of a key compound in the system broth. A biosensor designed specifically to measure this will have great

significance for process monitoring and control. This is just one example of a vast number of potential applications for a sensor, and the importance of sensors is significant and broad.

6.1.2. Sensor classification

There are many categories into which sensors can be classified; however, the most relevant to bioprocessing are direct/indirect and on-line/off-line. A direct sensor measures a variable on which it is acting, whereas an indirect sensor will measure a variable by determining the effect it has on another variable. So if the variable to be measured is the concentration of a particular compound in a solution, a direct sensor will measure the concentration of the compound itself, and an indirect sensor may measure the solution's absorbance of a specific light spectrum. Direct sensors tend to be preferred to indirect sensors as the measurement made on another variable will not always have a simple and constant relation with the variable to be measured. On-line sensors are those for which the measurement obtained can be immediately used to make a control decision, and off-line sensors are those that measure a variable in such a manner that a control decision cannot be made based on the measurement at the time it is made. An example of this is the difference between measuring a solution's pH using a pH probe (on-line) and taking a sample of the solution to be measured at a later time in the laboratory (off-line).

6.1.3. Temperature Sensors

As the temperature rises, the rate of chemical reactions increases. This rate doubles for every 10 °C rise in temperature. Thus, cells should grow faster as the temperature is raised. However, there are maximum limits beyond which some temperature-sensitive macromolecules (proteins, nucleic acids and lipids) will become denatured, and hence, non-functional. There is also a

minimum temperature for growth, below which the lipid membrane is not fluid enough to function properly. All organisms have an optimum temperature for growth and different groups of microorganisms have evolved to grow over different temperature ranges. A typical microorganism, referred to as stenothermal, can grow over a temperature range of approximately 30 °C, and eurythermal organisms grow over even wider ranges.

6.2. Control Strategies

The complexities of process and controller dynamics those are present in many modern bioprocesses, particularly those involving fed-batch and continuous culture, make PID control difficult to achieve. However, simulation and development of control strategies in these processes has been made easier with the aid of computer simulation techniques. In some situations, the development of a more complex controller may mean that we have actually made the control of the process variable fall outside of the processes reachable set. For a given system and desired range of process variable, there exists a set of all possible values of manipulated variable for which the process variable and manipulated variable could achieve. Any process variable can be steered to any desired value within its allowable range by suitable choice of manipulated variable. An attempt to change the process variable to a value which can't be achieved within the reachable set will lead to an unstable system with the process variable deviating from the desired value. An extension of this situation to multivariable processes can be seen as a scheme in which two processes are taking place during the same time frame. One process is an inverse of the other and can be used to cancel its effect on the process variable. This can be an unrealistic situation or the inverse process may only be valid within a limited

range. Control of modern bioprocesses may require sophisticated control strategy and many different manipulations of the same variable. This can potentially lead to a state of confusion for the process engineer who may try to implement a number of strategies, each with an adverse effect on the process variable. Simulation of the process and the control strategy can be useful as an aid to avoiding this situation.

When the controlled variable is a process variable, it is frequently necessary to store a portion of the signal from the sensor within an integrator. This can be visualized as a graphical function of error signal with respect to time. Given that the rate of a particular reaction or change in the process variable can be represented by a gradient of this function, it is possible to make the integral of the error equal to the cumulative deviation of the process variable from the set point. The strategy now would be to return the process variable back to its set point by using a change in the manipulated variable proportional to the error signal. This is known as proportional and integral control or simply PI control. An extension on this can be made to include differential control, with a portion of the signal from the sensor being differentiated and the rate of change in the process variable being calculated. Steps can be taken to change the process variable at a rate equal to but opposite to the rate present, causing the rate of change to the process variable to approach zero. This is known as proportional, integral, and differential control or PID control.

Modifications of process variables are caused by changes in the upstream variables, addition rate, or parameters associated with the kinetics or biochemistry of the production system. Given that the sources of change in the process variable are varied, this leads to the conclusion that control of bioprocess is frequently complex and at times can be difficult. Loop structure and

complexity will depend on the nature of the variable that is to be controlled and the corrective action that can be taken to a disturbance in this variable. Simple on/off control is frequently used, particularly when a change in the variable can be related to a change in the upstream variables. This means that the manipulated variable is switched between two states, returning to the original state each time the set point is deviated from. An example of this is the control of air flow rate to a fermenter in order to maintain dissolved oxygen concentration at a suitable level. High air or sparge flow rate can promote a change in the dissolved oxygen concentration. In order to correct this, the controller would increase the air flow rate set point, triggering an increase in manipulated air flow rate.

Chapter 7

Bioprocess Optimization

7. Bioprocess Optimization:

Although it seems common sense, this is often disregarded in bioprocess work and can result in much time wasted trying to discern the effects of multiple factors in what amounts to a confusing melee of results. An experiment widely used in bioprocess optimization is the Plackett-Burman design which is a type of fractional factorial design in which n variables can be assessed with only n experiments. This is particularly useful in the assessment of large numbers of factors in complex media.

Experimental Design is of the utmost importance to bioprocess optimization. It provides a method to the "madness" often associated with empirical methods in biotechnology. Due to the high degree of complexity in biological systems, bioprocess work can often become disjointed and uncoordinated with no clear goal. By careful planning and execution of bioprocess experiments facilitated by the methods of engineering science, it is possible to get the most information for the least expenditure of time and effort. This will help pinpoint specific areas for improvement in the process. The key to successful experimentation lies in the principle of changing only one factor at a time.

From an engineering perspective, bioprocess optimization aims to improve the synthesis of target products and minimize the consumption of resources in bioreactor systems. Due to the highly complex nature of cells and bioreaction systems, chances of selecting the "optimal" condition offhand are unlikely. Thus, a systematic approach to bioprocess optimization is required. With the utilization of modern methods of data acquisition and analysis, in complex

with new advances in genetic engineering, it is now likely to comprehend and regulate biological systems more accurately and closer to the desired result.

7.1. Experimental Design

A good experimental design shows a cause and effect relationship between process variables and the resulting output. It should make a clear distinction between the vital few and the trivial many. The vital few are the process variables that have the most effect on the output of the system. The trivial many are the variables that don't affect the output of the system. By identifying which process variables are important, a system can be optimized. This will lead to cost reductions and reduce the time the product takes to get to the market. There are many methods that can be used for experimental design, for example, the full factorial design, fractional factorial design, and response surface methodology. They vary in the amount of experimentation and the cost of each experiment. A full factorial design is an experiment where all the process variables are changed at once. This is good because it shows the effect of one process variable and the interaction between process variables. However, this can be very costly with a large number of process variables. A fractional factorial design is a more economical approach that allows the engineer to screen the important process variables at a fraction of the cost of a full factorial design. The engineer must then use the results of the fractional factorial design and the knowledge about the system to set up an optimization system using the fewest experiments necessary. Depending on the nature of the system, this could be using algorithms, trial and error, or DOE simulation, using the results of the DOE to guide the direction of the optimization.

7.2. Statistical Analysis

When an experimental strategy includes several modest changes with the hope of uplifting a complex biological response, it is essential to extract as much information as possible from a limited number of experimental runs. In this situation, it may be worth attempting an empirical model even though the scientific understanding of the process is inadequate. The quantitative information on the magnitude of the various effects is unobtainable with qualitative theoretical models. Statistical methods provide a system for making objective inferences, testing the reliability of the estimates, and revealing the strength and nature of the effects in the experimental system. Bioprocess experiments are becoming integrated with different informatics technologies. It is likely that the statistical methodology needs to be more flexible; the method will often depend on the type of model being used as well as its complexity. The analyses may also depend on the type and scale of the data being generated. It is important that the right statistical analysis is used, as many experimental studies may be overanalyzed with methods that provide results with no increased information gain relative to the analysis's complexity. An incorrect or overcomplicated analysis can consume a disproportionate amount of time in comparison to the experimental study itself.

Chapter 8

Industrial Applications

8. Industrial Applications:

During the coming years, there is likely to be a large demand for a more environmentally friendly alternative to petrol and diesel, and it is expected that second-generation biofuels such as biobutanol and biodiesel will become economically viable. This and the increased demand for ethanol will create a booming industry in biofuel production. All of these biofuels are produced or can be produced by fermentation. Ethanol is currently the only biofuel produced in large quantity in the world, more than 40 million m^3 per year, which is about 5% of the world production of all fuels. This is still a relatively small percentage of world fuel production and at present, most ethanol is used as a fuel additive to reduce exhaust emissions. However, this is likely to change in the very near future due to the Kyoto agreement, under which 155 countries including the European Union, have signed up to legally binding measures to reduce greenhouse gas emissions. The primary target is to reduce emissions of CO_2, the main method is thought to be the reduction of car use and the use of more efficient engines. However, as it is expected that the overall number of cars will actually increase, there will be a greater demand for the cleaner burning fuel, ethanol. Another factor that will increase the demand for ethanol is the expected banning of the current CFC air conditioning systems in cars, as it is likely that these will be replaced with ethanol-based absorption pumps.

Fermentation has widespread use in industrial applications. The most well known applications are the production of biofuels, organic acids, solvents, and alcoholic drinks. However, fermentation has a large number of other uses in a wide range of fields.

8.1. Biofuel Production

Microorganisms have the capacity to produce biofuels from biomass. For example, bioethanol and biodiesel are produced from crops, which are grown in great quantities. Use of first generation biofuels (produced directly from crops) has raised many issues because of the increase in cost of food and alcoholism. This has led to a movement to produce second generation biofuels from non-food crops such as grasses and wood. Use of these biofuels is even more environmentally friendly than current biofuels as non-food crops are grown on land which is unsuitable for food crop growth and so do not subtract from food available. Biomass can be converted to biofuel using a series of processes which ultimately turn long chain polysaccharides into usable monosaccharides. Cellulose, one of the most common polysaccharides, is converted in simple terms to glucose through acid hydrolysis, lignin and hemicellulose are broken down via heat and a series of enzymes into alcohols and other organic compounds. Agrofuels are a very interesting phenomenon in which microorganisms are used to fuel crop performance. An example of this is nitrogen fixing bacteria such as Rhizobium which can be used to enhance growth of legumes in a symbiotic process. The use of microorganisms as fertilizers is environmentally friendly and sustainable compared to western fertilizer and has a notable increase on crop yield which could be an immense benefit in some developing countries.

8.2. Pharmaceutical Industry

Simultaneously, with the development of processes, on-line monitoring, and intelligent control in fermentation, antibacterial products are expected to be widely used to support preventive

efforts and medical treatment for infectious diseases and their derivatives in a much safer and more effective way than in previous eras.

Many pharmaceutical products are traditionally obtained by extraction from plant or animal tissue, and fermented products are associated with prevention and treatment. Anti-microbial and antibiotic production is achieved through complex reactions of primary and secondary metabolism. The main product of fermentation is anti-microbial secondary metabolites, which are obtained from specific microbial cultures. The product can be obtained from fermentation residue or cell-free broth, and the method of extraction can vary depending on how to obtain the maximum yield of the product. Using specific extraction methods, such as fermentation of chitosan, chitin, and cell membrane to alkaline and solvent extraction, can lower costs and meet higher demands for domestic production. The obtained anti-microbial agents can be used as intermediate products to produce other kinds of products, such as anti-cancer and anti-diabetes medications. Antibiotics produced with specific strains of microbes have a higher possibility of yielding a compound, and current research and development in genetic engineering are focused on modifying microbes to obtain a high antibiotic yield. Antibiotic-resistant microbes have become prevalent in the past few decades, so anti-microbial and antibiotic alternatives are being explored.

Microbial fermentation has been used for millennia to produce useful compounds at the single-cell organizational level, fermenting aerobically and anaerobically. These microbes obtain energy for their various functions, such as maintenance, growth, synthesis, and cell division, through the anaerobic breakdown of complex molecules into simple molecules. This process

involves three types of fermentations: primary metabolic fermentation, secondary metabolic fermentation which ends with specific compound biosynthesis, and single-cell proteins fermentation.

8.3. Waste Treatment

The waste generated from the fermentation processes is high in organics. In most cases, it is also at a high level of carbon to nitrogen. This is a good food source for many microorganisms, and there is potential to reduce the waste using an aerobic biological treatment. Aerobic treatment systems are simple in design and operation. Waste is mixed with oxygen and the right bacteria, and the conditions are maintained until desired treatment levels are achieved. High energy waste could be utilized to create biogas in an anaerobic digester. This is a simple process mimicking the natural decomposition of organic matter. The biogas produced can be used to create energy, thus offsetting the energy costs for the fermentation process. The treatment of waste for fermentation processes has been estimated to be as high as 30% of the total operating costs. High treatment costs and strict environmental regulations have driven the development of new and cleaner treatment processes to prevent the pollution of waterways and destruction of soil ecosystems.

Chapter 9

Health Benefits of Fermented Foods

9. Health Benefits of Fermented Foods:

Fermented dairy products such as yogurt have been shown to aid in digestion of lactose, as the bacteria used in fermentation of these products can convert lactose into a more digestible form. This can be seen in a study where subjects with lactose maldigestion consumed 18 different dairy products over a 6-week period. Their findings showed that yogurts and cheeses with fermentation times of 24 and 48 hours caused less hydrogen excretion (accumulation of hydrogen is used to diagnose lactose maldigestion) in comparison with the same products that were not fermented. Another example of how fermented products are easier to digest is the processing of sauerkraut from cabbage. In this case, the fermentation process can increase the vitamin C content and digestive properties of the cabbage, making it more beneficial to health than when it was raw. This could be particularly important for people who have stomach ulcers or damage to the tissue in their upper GI tract due to a study being run to find ways of delivering more anti-inflammatory and antimicrobial treatments to these areas.

Dairy products, vegetables, and other types of food can be processed in a way which allows them to be preserved for a longer period of time. This process is known as fermentation. There are microorganisms all around us, and when they are given the right conditions they can easily be cultivated on food and can bring about desired changes. The most common example of fermentation is the processing of milk into cheese and yogurt. This essay will discuss the numerous ways in which fermentation is of benefit to us.

9.1. Probiotics and Gut Health

There is evidence that consuming probiotics can improve gastrointestinal health. Diarrhea is one of the most common side effects of taking antibiotics, this is because antibiotics do not discriminate between killing pathogenic bacteria and beneficial bacteria. The absence of beneficial bacteria can allow pathogenic bacteria to occupy the space left by the beneficial bacteria, this can often lead to infections. Consuming probiotics has been shown to reduce the incidence of antibiotic-associated diarrhea. A review published in the Journal of the American Medical Association found that consumption of probiotics reduced the risk of antibiotic-associated diarrhea by 42%. The strains that were most effective were *Lactobacillus rhamnosus* and *Saccharomyces boulardii*. Lactose intolerance is caused by a deficiency of lactase in the small intestine. Undigested lactose reaches the colon and is fermented by colonic bacteria, this produces gas and can often lead to diarrhea. In a small trial, it was found that lactose intolerant individuals who consumed kefir (a fermented milk drink) had reduced breath hydrogen levels compared to consuming regular milk. Breath hydrogen is a measure of lactose digestion, high levels indicate that lactose is reaching the colon undigested. The subjects also reported a reduction in abdominal pain and diarrhea when consuming kefir. This suggests that the microorganisms present in kefir were able to break down the lactose, negating its ability to be a fuel for colonic bacteria.

In recent years, consumers have become more aware of the connection between diet and health. As a result, people are choosing functional foods - often called nutraceuticals - that provide specific health benefits. There are many definitions for functional foods, but the general

consensus is that they are foods which provide a health benefit beyond basic nutrition. Probiotics are an example of a functional food which has gained attention for its potential to improve health. Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. Fermented foods are the most common vehicle used to deliver probiotics, however not all fermented foods contain live probiotics.

9.2. Enhanced Nutrient Absorption

In a system using Caco-2 cells, a model for the human small intestinal mucosa, results provided data showing increased iron absorption due to the presence of probiotics. The cells were loaded with iron and then exposed to a strain of Lactobacillus. Cells that had been exposed to Lactobacillus had an increased percentage of ferritin, a protein in cells that stores iron, suggesting an increase in iron absorption. High-performance liquid chromatography was used to identify the Lactobacillus reuteri gene involved in the production of a ferritin mRNA binding protein, which may explain the increase in iron absorption. Lactobacillus has also been frequently linked with an increase in lactose digestion due to the B-galactosidase activity. This is seen with an increase in lactose tolerance of lactose intolerant individuals who consume yogurt and other fermented milk products.

Through the process of fermentation in foods, organic acids and various strains of beneficial bacteria and yeast are developed. The organic acids present in the fermented foods facilitate the absorption of minerals and the production of B-vitamins. In addition, many of the strains of bacteria in fermented foods can produce enzymes, which assist in the breakdown of food and absorption of nutrients. For example, the enzyme phytase breaks down phytates, compounds

found in grains and legumes, which inhibit the absorption of minerals such as iron, zinc, and magnesium. The breakdown of phytates by phytase results in an increase in mineral absorption.

The consumption of fermented foods also has the added benefit of increasing nutrient absorption. This is primarily due to the live microorganisms present in fermented foods. According to a review article published in the journal Nutrition Reviews, gut microflora assists in the digestion and absorption of carbohydrates, fats, organic acids, and some minerals, and synthesize vitamins. Probiotics are suspected of facilitating absorption of certain nutrients and the uptake of minerals.

9.3. Potential Disease Prevention

These isothiocyanates prevent diseases through several biological activities. First, they detoxify and eliminate carcinogens in the body before they damage DNA, which is the initial step of cancer initiation. The isothiocyanates then cause cell cycle arrest, essentially stopping potentially cancerous cells from continuing to divide. They induce apoptosis, also known as cell suicide, in cells that are beyond repair. Isothiocyanates have anti-angiogenic effects, from preventing the enablement of a blood supply for tumor growth to obstructing the dissemination of cancer cells to other body parts. Phase 2 of the isothiocyanates is increased during metabolism and these are the compounds that are excreted in the urine. This explains why isothiocyanates have been mainly linked to prevention of bladder cancer, as a study of people in Singapore showed that increased cruciferous vegetable consumption resulted in decreased bladder cancer risks. Due to these biological activities, the consumption of isothiocyanate containing foods such as sauerkraut can aid in prevention of many types of cancer.

There have been numerous modern-day studies that have associated fermented foods with a decrease in the risks of certain cancers. As an example, a Swedish study found that the consumption of fermented milk products resulted in a decreased breast cancer rate in Norwegians. Kimchi, a Korean fermented vegetable dish, is also said to be linked to a lower rate of colon cancer in Korea. It was also found that the risk of bladder cancer in a group of people who consumed fermented garlic was reduced. What is it in these fermented foods that decrease cancer risks? The study of sauerkraut provides a reasonable answer to this question.

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