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REVIEW ARTICLE

Trends in Fermentation Process, Purification and Recovering of Biomolecules: A Review

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ABSTRACT

The word fermentation is derived from a Latin verb fervere which means to boil. However, events of boiling came into existence from the fact that during alcoholic fermentation, the bubbles of gas (CO_2) burst at the surface of a boiling liquid often give rise to Warty appearance. The conventional meaning of fermentation is the breakdown (Metabolism) of larger molecules, for example; carbohydrates, into simple ones under the influence of micro-organism for enzymes. In Micro-biological way, fermentation is defined as any process for the production of useful products through mass culture of microorganisms, whereas, in a biochemical sense, this word means the numerous oxides reduction reactions in which organic compounds, used as source of carbon and energy, act as acceptors or donors of hydrogen ions. The organic compounds used as substrate give rise to various products of fermentation which accumulate in growth medium. Although fermenting in brewing and wine production was done many hundred years ago. Fermentation can also be looked at as the process of increasing yields in volume without losing process yields. Microorganisms isolated from different sources (soil, water, air or genetic manipulation are cultivated in growth media, supplemented with sources of carbon, Nitrogen, phosphorous, amino acids, trace elements, sterilized and inoculated with specific micro-organism for the specific products. Fermenter or Bio-reactor is a vessel designed to carry out fermentation process that is biological reactions under control conditions.

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INTRODUCTION

The term 'fermentation' is derived from the Latin verb, fervere, to boil, thus describing the appearance of the action of yeast on extracts of fruit or malted grain. The boiling appearance is due to the production of carbon-dioxide bubbles caused by anaerobic catabolism of the sugars present in the extract. However, fermentation has come to have different meanings to biochemist and to industrial microbiologist or biochemical engineers. Its biochemical meaning relates to the generation of energy by the catabolism of organic compounds, whereas its meaning in industrial microbiology or biochemical engineering tends to be much broader (Sahoo, 2004).

The major groups of commercially important fermentation processes may be summarized as those that produce microbial cells (or cell biomass) as the product, those that produce microbial metabolites or enzymes and those that modify a compound which is added to the fermentation broth – the bio-transformation processes. The development of fermentation industry prior to 1900 was confined to products such as portable alcohol and vinegar. Between the years 1900 and 1940, the main new products were yeast biomass, glycerol, citric acid, lactic acid and acetone and butanol. Probably the most important advances during this period were the development in baker's yeast and organic solvent fermentations.

The development of acetone-butanol fermentation during the First World War by pioneering efforts of Weisman led to the establishment of the first truly aseptic fermentation. All the fermentation processes used previously could be conducted with relatively minimal contamination provided that good inoculum was used and reasonable standard hygiene employed. However the anaerobic acetone-butanol fermentation was susceptible to contamination in early stages by acid producing anaerobic ones, once anaerobic condition has been establish in the later stages of the process. The fermenters used were vertical cylinder with hemispherical tops and bottom and they could be steam sterilized under pressure and were constructed to minimize the possibility of contamination.

The next stage in the development of the fermentation industry arose as the result of the war time need to

produce the Penicillin in submerged culture under aseptic condition. The production of penicillin is an aerobic process which is very vulnerable to contamination. Thus, although the knowledge came from solvent fermentation, it was exceptionally valuable; the problem of sparging the culture with large volume of sterile air and mixing a highly viscous broth had to overcome. Also, unlike the solvent fermentation, the amount of penicillin produced in the fermentation broth was small and hence, it resulted in the establishment of strain improvement programme. Process development was also aided by introduction of pilot plant facilities and development of large-scale extraction process for recovery of Penicillin which was another major advancement of the time. This was probably the stage when the most significant charges in the establishment of many new processes over the period, including other antibiotics, vitamins, gibberellins, amino acids enzymes and steroid transformations. In the early 1960's the decision of the number of multinational companies to investigate the production of microbial biomass as a source of feed protein led to a number of developments.

The most recent stages of the progress of the industry have been initiated by the development in the in *vitro* genetic manipulation of organisms, commonly known as genetic engineering. Since the discovery of structure of DNA by Watson and Crick in 1953, many advances have been made in understanding DNA structure, organization and expression. One important technology developed in the field of molecular biology was in *vitro* construction of recombinant DNA molecule. This led to the development of independent replicating extra chromosomal plasmid DNA that can be used as vehicle for insertion into and amplification of any DNA segment in a suitable host cell. The technique, not only allow the transfer of genes between unrelated organisms but also enable the extremely precise alterations of genome of an organism.

Historically, the main tool for a fermentation laboratory was shake flasks or flatbed bottle. Science has made great strides since then, but the shake flasks or flatbed bottle still have an important role to play in modern fermentation laboratories. The next stage was the introduction of glass vessels with the stirrer, and this was followed by stainless steel vessel in various sizes and forms. The shake flasks and bottle can vary in size and forms. The shake flasks and bottle can vary in size and form and in some instances have been designed and developed for specialist application. Different plugs, made of cotton wool, glass wool, polyurethane foam or synthetic fibrous materials is used in flasks or bottle to prevent air borne microorganisms getting in to the medium while at the same time allowing the free flow of air in to the flasks.

The fermenters can be of different types such as stirred tank, air lift or bubble column. The stirred tank fermenter consists of a cylindrical tube with top driven or bottom driven agitator. For a smaller mini fermenter, borosllicate glasses used as cylindrical tank and a top plate of stainless steel clamped on. The vessel, medium and probes are usually sterilized together, minimizing the number of aseptic operation required. For safety reason, glass vessels are normally sterilized in an autoclave although some can be sterilized i*n situ* if the necessary safety precautions are taken. The stainless steel fermenter consists of hollow steel cylinder with either top or bottom drive and can be cleaned and sterilized *in situ.* The vessel will have a specific impeller design, baffle, and an air spager, sample port and pots for probes and other devices. In air lift fermenter there is no mechanical agitation system and it utilizes the air circulation with the fermenter to bring about mixing medium. The rather gentle system of mixing is ideal for cell culture such as animal cell culture. Other fermenter configurations include fixed bed, fluidized bed and rotating disc fermenter.

Regardless of the type of fermentation (with possible exception of some transformation process) or type of fermenter used, a fermentation process may be divided in to medium formulation, sterilization of fermenter with medium and accessories, production an active, pure culture in sufficient quantity to inoculate production vessels, the growth of an organisms in the production fermenter under optimum condition for product formation, the downstream processing for recovery, purification and formulation of the product and treatment and disposal of effluents produced by the process.

Fermenter: is a vessel designed to carry out fermentation process i.e. biological reactions under the control conditions. Fermenter is also called Bio-reactor (James, 2007).

Criteria for designing a fermenter

1. Long term operation is aseptic condition
2. Adequate aeration and agitation
3. PH control system functional.
4. Sampling facility
5. Minimum labour in operation harvesting, cleansing and maintenance.
6. Temperature control system.
7. Minimum evaporation losses from fermenter.
8. Suitable for variety of processes.

CULTURE PRODUCTION

Batch culture: This is the simplest type of culture in which microorganisms grow in a vessel, known as fermenter or bio reactor or fermentor. In batch culture, growth phase of microorganism passes through many stages. A microbe grows in the medium until the nutrients are exhausted or toxic metabolites secreted by the organism reach to inability level. After inoculation, the microbe takes some time to adjust in the new environment according to the size of the fermenter and hence does not grow in the medium. Thus the time taken for adaptation before it to come to its active growth is known as 'log phase'. Therefore, nutrients dependent logarithmic or exponential active growth and thereby increase in biomass is known as 'log' or exponential phase. As soon as the level of nutrients decreases, growth of culture is gradually slowed down. The stage of retarding the growth to reach to stationary phase is known as acceleration phase (Dep). However, during stationary phase, microorganisms do not grow and thus, fail to increase their bio mass. Ultimately the number of microbial cells declines due to accumulation of toxic metabolite. This stage is known as (death phase) (DP) (Kleman and Strohl, 1994).

BU Lock *et al* (1965) proposed different terminology for these growth stages. They used trophophase for the log phase and Idio phase - stationary phase of batch culture.
The growth rate and the concentration of the rate limiting substrate by relationship by the following formula:

$$\mu = \frac{\mu Max\ [S]}{Ks + [S]}$$ where μ = Growth rate constant

S = Concentration of limitary substrate
KS = Saturation constant value of limiting sub at which the growth rates half of the maximum growth rate (μ max).

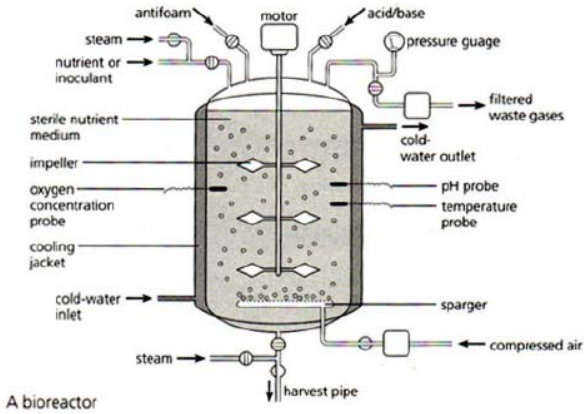
Shake flasks: 50-1000cm^3
The standard vessels are Erlenmeyer flasks with baffles filled with 50-500ml liquid. Oxygen saturation is assured by shaking the flask in a thermo stated reciprocal shaker.

Continuous culture: To prevent culture retards due to depletion of nutrients. This is prevented by the addition of fresh medium to the fermentor and removal of spent medium and microbial bio mass this prolonged exponential phase. There is no gas exchange with the environment. There are 3 varieties of continuous fermentation modes.

Chemostats: where nutrient levels are held constant.

Turbidostats: where cell mass is held constant. It is the plug-flow reactor, in which the culture medium flows without back mixing through a tubular reactor (Klein, 1996).

Fed-Batch Culture: Basically it is the batch culture which is fed continuously with fresh medium without removal of the original medium from the fermenter. It results in continuous increase in volume of medium in the fermentor.

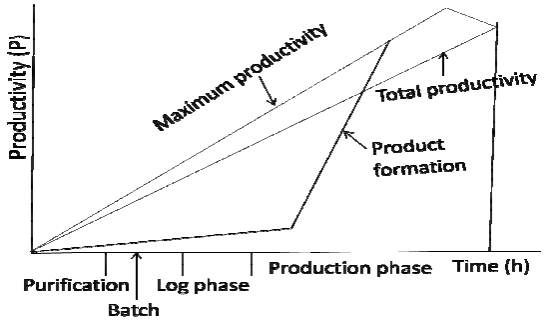


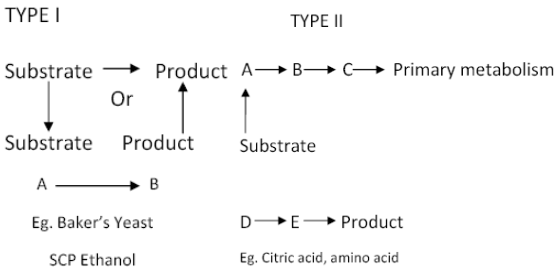
A bioreactor

Mixing: In a bio reaction is achieved by stirrers or pumps, resulting in a turbulent current.

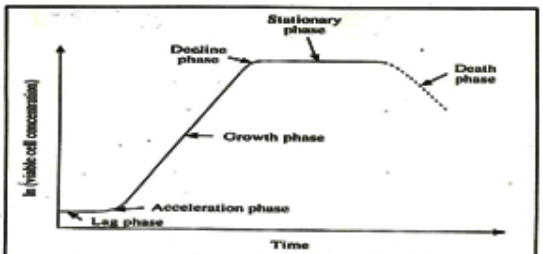
Materials:
Automatic pH control
Temperature profiling
Aeration, agitation: Distribution of air or O_2
Oxygen Transfer Monitor
Dissolved CO_2
Foaming control: use antifoaming agent. Such as erucic acid silicons is to be used.

Production formation

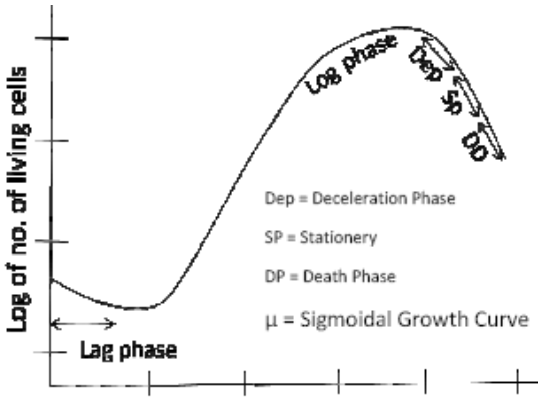




Typical batch growth curve



Phase	*Description*	*Specific growth rate*
Lag	Cells adapt to the new environment: no or very little growth	$\mu = 0$
Acceleration	Growth starts	$\mu < \mu_{max}$
Growth	Growth achieves its maximum rate	$\mu = \mu_{max}$
Decline	Growth slows due to nutrient exhaustion or build-up of inhibitory products	$\mu < \mu_{max}$
Stationary	Growth ceases	$\mu = 0$
Death	Cells lose viability and lyse	$\mu < 0$



Conclusion

The increasing importance of industrial operations based on biochemical engineering has been long recognized fermentation as biochemical processes, the

important industrial microbial process are aerobic organisms. An understanding of how oxygen is transferred to cells in a rector is crucial for successful design and analysis oxygen transfer plays an important role in the scale-up and economics of aerobic processes. For bacteria and yeast the critical oxygen concentration is about 10% to 50% of the saturated dissolve concentration (DO), which for optimum growth, it is important to maintain DO above this critical level, by sparing the fermenter with air or pure oxygen.

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