RICE UNIVERSITY
Institute of Biosciences and Bioengineering
NIH Biotechnology Research Training Program

Method Title: Bioreactors in Biochemical and Metabolic Engineering

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from: http://www.sysbio.org/technologies/mcdl.stm

CONTENTS:

FERMENTATION



from: http://www.zeri.org/expo/prj_beer.htm, http://www.vitacost.com/science/hn/Food_Guide/Tempeh.htm, http://www.vtonly.com/cheese.htm

The basic process of fermentation has been used for centuries for producing a variety of foods around the globe. Such examples include breads, beer, buttermilk, cheese, pickles, sauerkraut, yogurt, tempeh, just to name a few. Up until the work of Louis Pasteur during the mid to late 1800s, little was know about the process of fermentation or the involvement of microorganisms.

Fermentation is often defined as a process where cells produce energy anerobically, or without oxygen.

In general, fermentation involves the breaking down of complex organic substances into simpler ones. Microbial or animal cells obtain energy through glycolysis, splitting a sugar molecule, and removing electrons in the process. The electrons are then passed to an organic molecule such as pyruvic acid. This results in the formation of waste products that are excreted from the cell. Waste products formed in this way include ethyl alcohol, butyl alcohol, lactic acid, and acetone.

from: http://www.sd01.k12.id.us/schools/borah/academics/programs/bioproj/diseases00/anthrax/history.htm

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THE NEED FOR BIOREACTORS

The discoveries of Louis Pasteur soon lead to the development of bioreactors for the large scale culture of bacteria and yeasts.

Small cultures originally were performed in dishes which could not create appreciable quantities of fermentation products (such as acetone, ethyl alcohol). These products had value if they could be produced in large volumes. To achieve this goal, the development of bioreactors was required (beginning in the early 1900s) that could culture at least a few liters of cells and media. Eventually, bioreactors would be able to ferment thousands of liters at a time.

from: http://picturethis.pnl.gov/picturet.nsf/by+id/SMAA-3V9T37?opendocument

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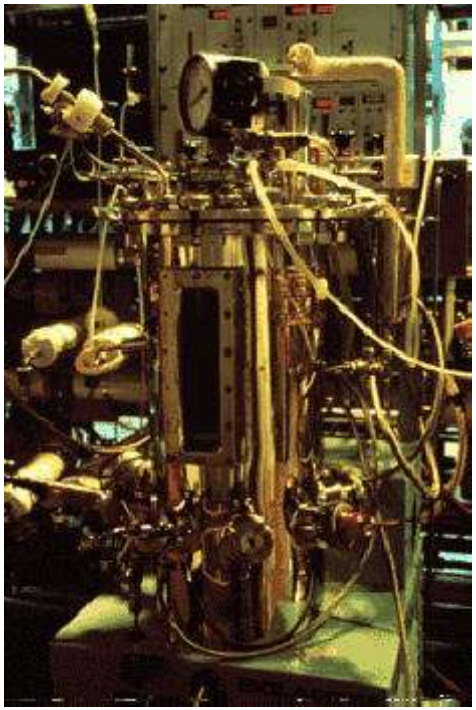
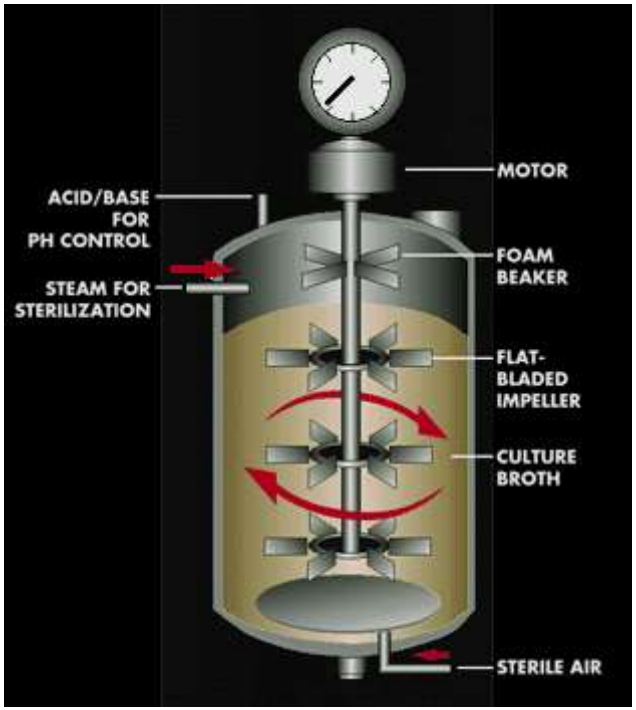
BIOREACTORS IN INDUSTRY

In the first half of the 20th century, ethyl alcohol and butyl alcohol were the most important industrial fermentations in the world. Such products were vital to the synthesis of synthetic rubber, especially during World War I. This required the development of bioreactors that could culture thousands of liters. By the 1960's, the fermentation of many simple organic products diminished as chemical synthesis (mostly from petroleum) provided a cheaper alternative.

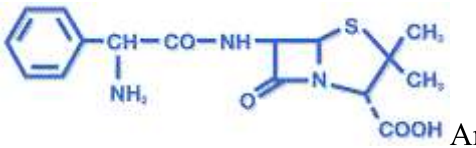




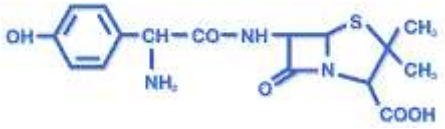
from: http://www.accessexcellence.org/LC/SS/ferm_graphics/reactor.html

Bioreactors and bacterial fermentation have played a vital role in the production of many medicinal products including the majority of antibiotics. The first large scale manufacture of antibiotics occurred during World War II with the production of penicillin. It was discovered early on that the most efficient production of penicillin occurred through fermentation processes rather than using direct chemical synthesis. Since this time many other antibiotics have been characterized and mass-produced using fermentation.

from: http://home.att.net/~steinert/wwii.htm



Ampicillin



Amoxicillin

from: http://www.antibioticos.it/default.htm

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PLANT CULTURE

Plant secondary metabolites are important for plant interactions with their environments. These metabolites give plants important properties such as antibiotic, antifungal, antiviral, UV protection, and pest deterrents. These chemicals have also been used by man for thousands of years as medicines. Today plants are responsible for the production of over 30,000 types of chemicals including pharmaceuticals, aromas, pigments, cosmetics, nutraceutics, and other fine chemicals.

Catharanthus roseus
from: http://www.nybg.org/bsci/belize/gallery.html

It was discovered early that metabolites could be accumulated in higher quantities using plant cell cultures as compared with whole plant cultivation. However, since plant cell cultures are low yield compared to their bacterial counterparts, they are primarily used only for high-value metabolites.

The biosynthetic routes and mechanisms describing the production of plant secondary metabolites are often very complex and can involve dozens of enzymes. Through the study of plant metabolism a new discipline called metabolic engineering has emerged. The goal of metabolic engineering is to improve cellular activity by manipulating enzymatic, transport and regulatory functions of a cell. This is accomplished through the use of recombinant DNA technology.

from: http://www.linnaeus.uu.se/online/fysik/mikrokosmos/fylevande.html

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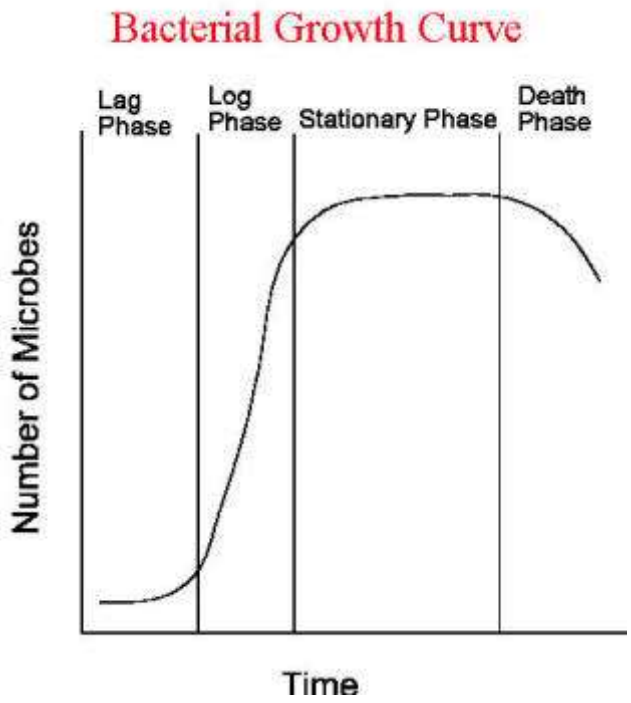
GROWTH PHASES AND SECONDARY METABOLITES

The growth of a bacterial culture can be represented by a curve that consists of four stages or phases:

- Lag phase - growth and reproduction are just beginning
- Log phase - reproduction is occurring at an exponential rate

- Stationary phase - environmental surroundings and food supply cannot support any more exponential growth
- Death phase - when all of the nutrients have been exhausted, the population dies off



from: http://www.eng.auburn.edu/~wfgale/usda_course/pasteur_page1.htm

The production of secondary metabolites (such as antibiotics) often occurs during the stationary growth phase (or idiophase) of a bacterial culture. Secondary metabolites are non-growth related and seem to play no obvious role in cell maintenance. The production of secondary metabolites largely concerns the first growth phases of a bacterial culture. This production is considered a two-stage process, with a trophophase (growth phase) and an idiophase (production phase), which usually occurs when culture growth has slowed or ceased. The major aim for mass production of secondary metabolites is to maximize the biomass in a short trophophase, while optimizing the conditions for high, sustained idiophase production.

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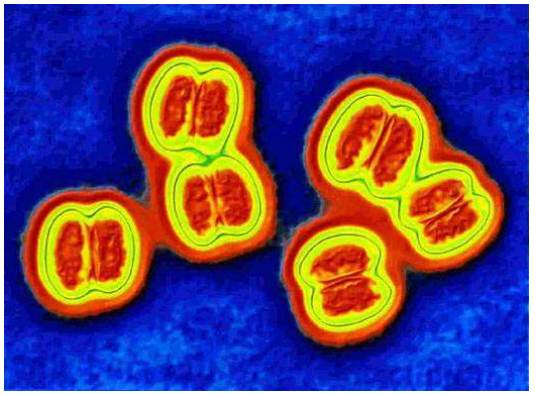
CULTURE CONDITIONS

A variety of parameters are important to influence the production of desired secondary metabolites in bioreactor cultures.

These include:

- Substrate
- Slow or fast utilization
- Nitrogen requirements
- Amino acid supplements
- Temperature
- pH
- Oxygen concentration
- Pressure



from: http://www.greeninstitute.org/GSP/gartips/worms/wormbin01.html

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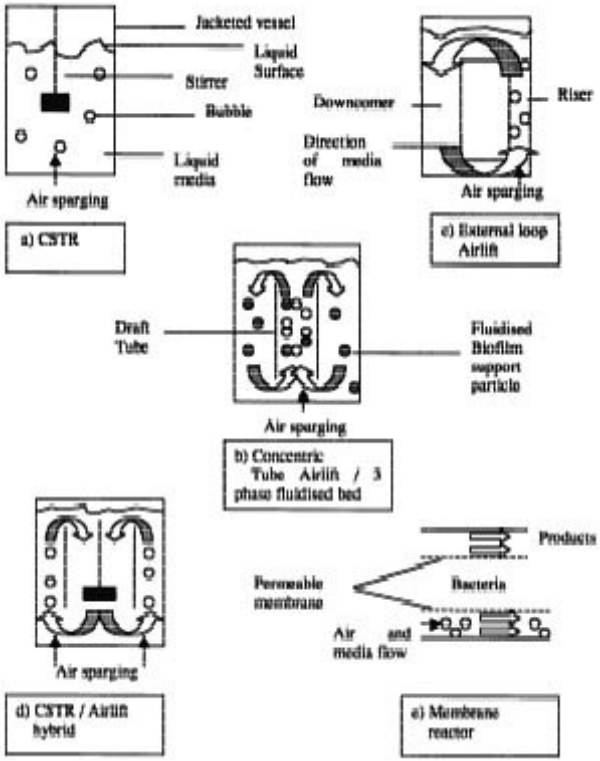
TYPES OF BIOREACTORS

Several types of bioreactors enjoy use in both research and industry. Here are some of the most common:

- CSTR - mechanical mixing and air-sparged liquid media
- Airlift - uses air sparging to pneumatically mix the media
- Combinations and variations of CSTR and Airlift reactors
- Membrane - two permeable membranes to deliver nutrients and export wastes and products from bacteria in a center tube



from: Marwick, J.D., P.C. Wright, and J.G. Burgess, Bioprocess Intensification for Production of Novel Marine Bacterial Antibiotics Through Bioreactor Operation and Design. 1999. 1(5): p. 495-0507

Bacterial and plant cultures can be grown in bioreactors using several modes of operation including: batch, semi-batch, or continuous.

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SCALE-UP CONSIDERATIONS

This process is the important step for transferring bench-top fermentations to mass production. Scale-up would be very simple if all parameters affecting bacteria remained the same. Numerous empirical and semi-empirical relationships are often used to correlate variables such as shear rates and oxygen mass transfer with physical parameters such as impeller speed and reactor dimensions. One of the most

important and often overlooked factors in scale-up is power consumption. When scaling-up a process, it is important to factor in the power costs of operating large fermenters.

from: http://www.av.fh-koeln.de/professoren/rieckmann/forschung/rieckmann_forschung.htm

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BIOREACTORS AT RICE

Several research groups in the departments of Bioengineering, Biochemistry, and Chemical Engineering utilize bioreactors for large scale culturing of microorganisms.

Dr. Ka Yiu San's lab utilizes the Bioflo 110 Fermentor from New Brunswick Scientific (link: http://www.nbsc.com/ferm_eq/bf110.asp). This bioreactor allows the precise control and monitoring of cultures of *E. coli* and similarly can be used for a variety of cell types. This model of bioreactor contains numerous ports for sampling and measurement of culture conditions.

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USEFUL LINKS

- Brock Biology of Microorganisms (http://www.prenhall.com/brock/)
- Biotechnology educational resources (http://www.nal.usda.gov/bic/Education_res/)
- Dr. San's web page with many links, (http://www.ruf.rice.edu/~ksan/)
- Bioreactors/fermentation equipment, (http://www.nbsc.com/ferm_eq/ferm.htm), (http://www.broadleyjames.com/bionet-overview.html), (http://www.bellcoglass.com/intl/bioreactors.shtml),(http://www.sartorius-bbi-systems.com/)
- Mammalian cell culture by NASA, (http://science.msfc.nasa.gov/newhome/br/bioreactor.htm)

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This Webpage was created by Nic Leipzig
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