Microbial fermentative processes, biomass, enzymes, amino acids

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Meaning of „fermentation“

- **Fermentation** is a *metabolic* process that converts *sugar* to acids, gases or *alcohol*. (anaerobic)
- It occurs in *yeast* and *bacteria*, and also in oxygen-starved muscle cells, as in the case of *lactic acid fermentation*.
- **Fermentation is also used more broadly to refer to the bulk growth of** *microorganisms* on a *growth medium*, often with the goal of producing a specific chemical product. (aerobic conditions)
Definition of fermentation

- Production of **alcohol**: grains and fruits are fermented to produce beer and wine. Preservation methods for food via **microorganisms** (general use).
- **Any process that produces alcoholic beverages** or acidic dairy products (general use).
- **Any large-scale microbial process** occurring with or without air (common definition used in industry).
- **Any energy-releasing metabolic process** that takes place only under **anaerobic conditions** (becoming more scientific).
- **Any metabolic process** that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (most scientific).
Fermenter design

• What should be the **basic points** of consideration while designing a fermenter?
• Productivity and yield
• Fermenter operability and reliability
• Product purification
• Water management
• Energy requirements
• Waste treatment
Fermenter - bioreactor

• **STIRRED TANK FERMENTER** - tank reactor having the following functions: **homogenization**, suspension of solids, **dispersion** of gas-liquid mixtures, **aeration** of liquid and **heat exchange**.

• The stirred tank reactor is provided with a baffle and a rotating stirrer is attached either at the top or at the bottom of the bioreactor.

• The typical decision variables are: **type**, **size**, location and the number of impellers; sparger size and location. These determine the **hydrodynamic pattern** in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc.
A bioreactor

Fermentor
AIR-LIFT FERMENTER - Airlift fermenter (ALF) is generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing. The turbulence caused by the fluid flow ensures adequate mixing of the liquid. The draft tube is provided in the central section of the reactor. The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor. The air/liquid velocities will be low and hence the energy consumption is also low. ALFs can be used for both free and immobilized cells. There are very few reports on ALFs for metabolite production.
Fermenter - bioreactor

**FLUIDISED BED BIOREACTOR** - (FBB) have received increased attention in the recent years due to their advantages over other types of reactors. Most of the FBBs developed for biological systems involving cells as biocatalysts are **three phase systems** (solid, liquid & gas). The fundamentals of three phase fluidization phenomena have been comprehensively covered in chemical engineering literature. The FBBs are generally operated in co-current upflow with **liquid as continuous phase** and other more unusual configurations like the inverse three phase fluidized bed or gas solid fluidized bed are not of much importance. Usually fluidization is obtained either by external liquid re-circulation or by gas fed to the reactor.
Gas-lift / fluidized bed reactors
Fermenter - bioreactor

PACKED BED BIOREACTOR - Packed bed or fixed bed bioreactors are commonly used with attached biofilms especially in wastewater engineering. The use of packed bed reactors gained importance after the potential of whole cell immobilization technique has been shown. The immobilized biocatalyst is packed in the column and fed with nutrients either from top or from bottom. One of the disadvantages of packed beds is the changed flow characteristic due to alterations in the bed porosity during operation. While working with soft gels like alginites, carragenan etc the bed compaction which generally occurs during fermentation results in high pressure drop across the bed. In
Special demands on fermentors

• **In citric acid** fermentation where pH may be 1 to 2, it will be necessary to use a stainless steel with 3-4% molybdenum (AISI grade 317) to prevent leaching of heavy metals from the steel which would interfere with the fermentation. AISI grade 304, which contains 18.5% chromium and 10% nickel, is used extensively for brewing equipment.

• With **plant** and **animal cell tissue culture**, a low-carbon version (type 316L) is often used.
Main types of products

- Microbial cells or **biomass** as the product, e.g. **single cell protein**, bakers yeast, lactobacillus, *E. coli*, etc.

- **Microbial enzymes**: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, etc.

- **Microbial metabolites**:
  - **Primary metabolites** – ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides etc.
  - **Secondary metabolites** – all **antibiotics** fermentation

- **Recombinant products**: insulin, hepatitis B vaccine, interferon, granulocyte colony-stimulating factor, streptokinase

- **Biotransformations**: phenylacetylcarbinol, steroids biotransformation, etc.
Biomass production
Single cell proteins

- **Single-cell protein (SCP)** refers to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for **protein-rich foods**, and is suitable for human consumption or as animal feeds.

- **SCP** is commonly grown on **agricultural waste products**, and as such inherits the ecological footprint and water footprint of industrial agriculture.

- SCP may also be produced entirely independent of agricultural waste products through autotrophic growth. Thanks to the high diversity of microbial metabolism, autotrophic SCP provides several different modes of growth, versatile options of nutrients recycling, and a substantially increased efficiency compared to crops.

- **The term SCP was coined in 1966 by Carroll L. Wilson of MIT.**
 SCP

• Research on Single Cell Protein Technology started a century ago when Max Delbrück and his colleagues found out the high value of surplus brewer’s yeast as a feeding supplement for animals.

• During World War I and World War II, yeast-SCP was employed on a large scale in Germany to counteract food shortages during the war. Inventions for SCP production often represented milestones for biotechnology in general: for example, in 1919, Sak in Denmark and Hayduck in Germany invented a method named, “Zulaufverfahren”, (fed-batch). Sugar solution was fed continuously to an aerated suspension of yeast instead of adding yeast to diluted sugar solution once (batch).

• In post war period, the Food and Agriculture Organization of the United Nations (FAO) emphasized on hunger and malnutrition problems of the world in 1960 and introduced the concept of protein gap, showing that 25% of the world population had a deficiency of protein intake in their diet.
In the 1960s, researchers at British Petroleum developed what they called "proteins-from-oil process": a technology for producing single-cell protein by yeast fed by waxy n-paraffins, a byproduct of oil refineries. Initial research work was done by A. Champagnat at BP's Lavera Oil Refinery in France; a small pilot plant there started operations in March in 1963, and the same construction of the second pilot plant, at Oil Refinery in BP "food from oil" idea became quite popular by the 1970s, with Champagnat being awarded the UNESCO Science Prize in 1976, and paraffin-fed yeast facilities being built in a number of countries. The primary use of the product was as poultry and cattle feed.
Ability of microalgae to mitigate CO₂ emission and produce oil with a high productivity show that it has the potential for applications of producing the third-generation of biofuels. For microalgae biofuel production there is a need of identification of preferable culture conditions for high oil productivity, development of effective and economical microalgae cultivation systems, as well as separation and harvesting of microalgal biomass and oil.

Chisti in 2007 proposed that under suitable culture conditions, some microalgal species are able to accumulate up to 50–70% of oil/lipid per dry weight.

Chisti also proposed the major reason of using microalgal oil for biodiesel production which is the tremendous oil production capacity by microalgae, as per hectare, they could produce up to 58,700 L oil, which is one or two magnitudes higher than that of any other energy crop.
Algae cultivation

- **Autotrophic microalgae** are cultivated on land in large ponds, or in enclosed so-called **photobioreactors**, using enriched CO$_2$. The CO$_2$ can come in the form of flue gases from power plants or be obtained from other fossil fuel combustion and biological processes. They thus can help recycle this specific greenhouse gas, and can help reduce greenhouse gas emissions overall when the algal biomass is converted into biofuels.

- **Heterotrophic microalgae** are grown in large fermenters using **sugar** or **starch**, similar to the corn ethanol fermentation already providing almost 10 percent of our liquid transportation fuels.
Microalgae cultivation
Microbial enzymes

In contrast to submerged (liquid state) fermentation, **Solid State Fermentation (SSF)** is the cultivation of microorganisms under controlled conditions in the absence of free water.

Examples of products of **Solid State Fermentation** include **industrial enzymes**, fuels and nutrient enriched animal feeds. The application of modern biotechnical knowledge and process control technologies can lead to significant productivity increases from this ancient process.
Global enzyme markets by application sectors, through 2009 ($ millions).

Enzyme and Microbial Technology, Volume 39, Issue 2, 2006, 235–251

http://dx.doi.org/10.1016/j.enzmictec.2005.10.016
Solid state fermentor

SSF (Maize crop)

AgSF (Agar media)
Solid state fermentor
Proteases

- **Commercially useful enzymes** (CUEs) are enzymes which have commercial uses.
- Microbial enzymes have well-known applications as biocatalysts in several areas of industry, such as biotechnology, agriculture, pharmaceuticals, etc.
- **Metagenomic data** provide a unique resource for discovering novel commercially useful enzymes (CUEs) from yet unidentified microbes belonging to complex microbial communities in diverse ecosystems.
- **Proteases** are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale.
- **The major uses of free proteases** occur in dry cleaning, detergents, meat processing, cheese making, and silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and the virulent wounds.
NEUTRAL PROTEASE

- Neutral protease is produced at industrial level using agro-industrial residues as substrate e.g. wheat bran, rice husk, rice bran, spent brewing grain, coconut oil cake, palm kernel cake, sesame oil cake, jackfruit seed powder and olive oil cake etc.
- For developing a production medium it is very important to monitor the cost-effectiveness of the medium so these agro-industrial residues mentioned above are very cheap and easily available.
- Seven fungal cultures, i.e. three strains of *Aspergillus oryzae* and four strains of *Penicillium* species e.g. *P. funiculosum*, *P. funiculosum*, *P. pinophilum*, *P. aculeatum* were evaluated using a plate assay for enzyme production, which showed a strain of *A. oryzae* NRRL 1808 as the most useful culture.
- Protease enzyme is produced in two fermentor systems, in solid-state fermentors (SSF) and sub-merged fermentors (SmF).
Amylases

• Completely replaced chemical hydrolysis of starch in starch processing industry. Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amyloliquifaciens* and *Aspergillus niger*.

• Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries.

• *Rhizopus oligosporous* 1HS13, *Aspergillus niger*, *Rhizopus oryzae*, and *Conidiobolus spp* have ability to produce proteases.
GLUCOAMYLASE

• *Aspergillus* sp. A3 is used for the production of glucoamylase under solid state fermentation. Different substrates like wheat bran, green gram bran, black gram bran, corn flour, barley flour, jowar flour, maize bran, rice bran and wheat rawa are the best substrate and give best results among all these. Wheat bran showed the highest enzyme activity. The maximum enzyme activity under optimum conditions was 247 U/g of wheat bran.

• The optimum conditions are fructose as additive 1% w/w, urea as additive 1% w/w, incubation time of 120 h, incubation temperature at 30 °C, 2:10 (v/w) ratio of salt solution to weight of wheat bran, inoculum level 10% v/v, moisture content of solid substrate 80%, 1:50 ratio of substrate weight to flask volume and pH 5.0.
Cellulases

- **Cellulase** is any of several enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze cellulolysis, the decomposition of cellulose of some related polysaccharides.
- The name is also used for any naturally occurring **mixture or complex of various such enzymes**, that act serially or synergistically to decompose cellulosic material.
- The specific reaction involved is the hydrolysis of the 1,4-beta-D-glycosidic linkages in cellulose, hemicellulose, lichenin, and cereal beta–D-glucans.
- Because **cellulose molecules bind strongly** to each other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides such as starch.
Synergy between endoglucanases, cellobiohydrolases and β-glucosidases

Hexagones - glucose

Reducing ends black

Crystalline region Amorphous region

↓ Adsorption of cellulases

Endoglucanases

Cellbiohydrolases

β-glucosidases
Cellulase types

Cellulose (crystal) → Endocellulase → Cellulose

Endocellulase → Cellulose

Exocellulase → Cellulobiase (β-glucosidase) → Glucose

Cellulobiase or Cellotetrose → Glucose
Cellullases

• Several different kinds of cellulases are known.
• Synonyms, derivatives, and specific enzymes associated with the name "cellulase" include
• endo-1,4-beta-D-glucanase (beta-1,4-glucanase, endoglucanase D, 1,4-(1,3,1,4)-beta-D-glucan 4-glucanohydrolase),
• carboxymethyl cellulase (CMCase), avicelase, celludextrinase, cellulase A, cellulosin AP, alkali cellulase, cellulase A 3, 9.5 cellulase, and pancellase SS.
• Endocellulases (EC 3.2.1.4) randomly cleave internal bonds at amorphous sites that create new chain ends.
Cellulases

- **Exocellulases** or celllobiohydrolases (EC 3.2.1.91) cleave two to four units **from the ends of the exposed chains** produced by endocellulase, resulting in tetrasaccharides or disaccharides, such as cellobiose.

- Exocellulases are further classified into **type I**, that work processively from the **reducing end** of the cellulose chain, and **type II**, that work processively from the **nonreducing end**.

- **Cellobiases** (EC 3.2.1.21) or beta-glucosidases hydrolyse the exocellulase product into individual monosaccharides.
Lipases

- Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are a class of enzymes which catalyse the hydrolysis of long chain triglycerides. Microbial lipases are currently receiving much attention with the rapid development of enzyme technology.
- The high-level production of microbial lipases requires not only the efficient overexpression of the corresponding genes but also a detailed understanding of the molecular mechanisms governing their folding and secretion. The optimization of industrially relevant lipase properties can be achieved by directed evolution.
- Numerous species of bacteria, yeasts and molds produce lipases. Taxonomically close strains may produce lipases of different types.
Microbial metabolites:
Primary products formation during exponential phase

- metabolites – ethanol,
- citric acid,
- glutamic acid
- lysine,
- vitamins,
- polysaccharides
Polysaccharide $\rightarrow$ Sugar $\rightarrow$ Pyruvate $\rightarrow$ Acetyl-CoA $\rightarrow$ Acetate, Butyrate $\rightarrow$ Propionate

Additional pathways:
- Methanogenic species (B) with $\rightarrow$ CH$_4$
- Lactate-fermenting species (C) with $\rightarrow$ Lactate, Acryllyl-CoA $\rightarrow$ Succinate $\rightarrow$ Oxalacetate

Metabolites and endproducts:
- $2H$, CO$_2$, H$_2$
- Intermediate metabolite
- Endproduct

Species categories:
- Carbohydrate fermenting species (A)
- Methanogenic species (B)
- Lactate-fermenting species (C)
Distribution of fermentations
Ethanol fermentation

1. Glucose is converted to 2 ATP through the process of glycolysis, releasing 2 ADP + 2 Pi.
2. The resulting 2 pyruvate molecules are converted to 2 acetaldehyde and 2 CO₂.
3. The 2 ethanol molecules are produced as the final product of the fermentation process.

NAD⁺ and NADH are involved in the redox reactions, with NAD⁺ being reduced to NADH and then reoxidized to NAD⁺.
Lactic acid fermentation

- **Homolactic fermentation** (producing only lactic acid) is the simplest type of fermentation.
- The pyruvate from glycolysis undergoes a simple redox reaction, forming lactic acid.
- It is unique because it is one of the only respiration processes to not produce a gas as a byproduct. Overall, one molecule of glucose (or any six-carbon sugar) is converted to two molecules of lactic acid:

\[
C_6H_{12}O_6 \rightarrow 2 \text{CH}_3\text{CHOHCOOH}
\]
Heterolactic fermentation

Bacteria of heterolactic fermentation do not have aldolase – direct oxidation of glucose by pentose cycle on pentosa-5-phosphate and CO$_2$.

In presence of anorganic phosphate it is split on acetylphosphate (CH$_3$-COO-PO$_3$H$_2$) and glyceraldehyde-3-phosphate

acetylfosfát → ETHANOL
glyceraldehyd → LAKTÁT
CO$_2$
The pentose phosphate pathway (also called the phosphogluconate pathway and the hexose monophosphate shunt) is a metabolic pathway parallel to glycolysis that generates NADPH and pentoses (5-carbon sugars) as well as ribose 5-phosphate, a precursor for the synthesis of nucleotides.

While it does involve oxidation of glucose, its primary role is anabolic rather than catabolic.
Amino acids fermentation

- Market development has been particularly dynamic for the flavor-enhancer glutamate and the animal feed amino acids L-lysine, L-threonine, and L-tryptophan.
- They are produced by fermentation processes using highperformance strains of *Corynebacterium glutamicum* and *Escherichia coli* from sugar sources such as molasses, sucrose, or glucose.
- Four production methods for amino acids—extraction, synthesis, fermentation, and enzymatic catalysis—it is particularly the last two biotechnological processes, their economic and ecological advantages, that are responsible for this spectacular growth.
## Overview of amino acids production

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Strain/mutant</th>
<th>Titer (g/l)</th>
<th>Estimated yield (g/100 g scr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Lysine HCl</td>
<td><em>C. glutamicum</em> B-6</td>
<td>100</td>
<td>40–50</td>
</tr>
<tr>
<td>L-Threonine</td>
<td><em>E. coli</em> KY 10935</td>
<td>100</td>
<td>40–50</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td><em>C. glutamicum</em> KY</td>
<td>58</td>
<td>20–25</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td><em>E. coli</em></td>
<td>45</td>
<td>20–25</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td><em>E. coli</em> MW</td>
<td>51</td>
<td>20–25</td>
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<tr>
<td>L-Arginine</td>
<td><em>Brevibacterium flavum</em></td>
<td>36</td>
<td>30–40</td>
</tr>
<tr>
<td>L-Histidine</td>
<td><em>C. glutamicum</em> F81</td>
<td>23</td>
<td>15–20</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td><em>E. coli</em> H-8461</td>
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<td>20–30</td>
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<tr>
<td>L-Serine</td>
<td><em>Methylobacterium</em> sp.</td>
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<td>30–35</td>
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<tr>
<td>L-Valine</td>
<td><em>C. glutamicum</em> VR 3</td>
<td>99</td>
<td>30–40</td>
</tr>
</tbody>
</table>
Amino acids production

• All 20 amino acids are sold, albeit each in greatly different quantities. *Essential amino acids?*

• Amino acids are used as *animal feed additives* (lysine, methionine, threonine),

• *flavor enhancers* (monosodium glutamic, serine, aspartic acid)

• *nutrients* in the medical field.

  Glutamic acid, lysine and methionine account for the majority, by weight, of amino acids sold.

Glutamic acid and lysine are made by fermentation; methionine is made by chemical synthesis.

The major producers of amino acids are based in Japan, the US, South Korea, China and Europe.
How to increase production

- **Auxotrophic mutation**- lack of the formation of regulatory end product (i.e. repressor or regulatory effector). The intermediates of the metabolic pathways accumulate and get excreted.

- **Genetic recombination**- Protoplast fusion in certain bacteria is used for development of hybrids e.g. *Corynebacterium glutamicum* and *Bacillus flavum*.

- **Recombinant DNA technology**- E: coli and cloning vector pBR322 were used to increase the genes for the production of amino acids e.g. glutamic acid, lysine, phenylalanine, valine.
Secondary metabolites-- all antibiotics fermentation

• **Penicillin** - Nobel prize 1945 Sir A. Fleming

• The **production of antibiotics** has been widespread since the pioneering efforts of **Florey** and **Chain** in 1938. The importance of antibiotics to medicine has led to much research into their discovery and production.

• Useful antibiotics are often discovered using a **screening process**. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that **inhibit the growth of test organisms**.

• Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified.
Antibiotics production

• **Source microorganism** is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium.

• **Oxygen concentration**, temperature, pH and nutrient levels must be optimal, and are closely monitored and adjusted if necessary.

• As antibiotics are **secondary metabolites**, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die.

• Once the process is complete, the antibiotic must be **extracted** and **purified** to a crystalline product.

• This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.
Producers of antibiotics

• Microorganisms used in fermentation are rarely identical to the wild type.
• Species are often genetically modified to yield the maximum amounts of antibiotics.
• Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals.
• Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more.
• Another technique used to increase yields is gene amplification - copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production.
Typical producers

• **Actinomycetes** have provided important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances.

• **Streptomyces** is the largest genus of Actinobacteria and the type genus of the family *Streptomycetaceae*. Over 500 species of *Streptomyces* have been described.

• As with the other Actinobacteria, Streptomyces are **Gram-positive** and have genomes with high GC-content.

• *Streptomyces* sp. are widely recognized as industrially important organisms for their ability to elaborate different kinds of **novel secondary metabolites**.
Phases during fermentation of streptomycin

**PHASE 1:** Rapid growth producing mycelial biomass. Little production of Streptomycin is obtained.

**PHASE 2:** Additional production of mycelium. Streptomycin accumulates in the medium.

**PHASE 3:** Process has completed. Finally, the mycelium is separated by filtration and antibiotic recovered.

Proteolytic activity of the microbe releases NH3 to the medium from the soybean meal, causing a rise in pH. The glucose and NH3 released are consumed during this phase. The pH remains fairly constant between 7.6 and 9.0.
Recombinant products

• insulin,
• hepatitis B vaccine,
• interferon,
• granulocyte colony-stimulating factor,
• streptokinase
Insulin

In the **1980s**, researchers used genetic engineering to manufacture a human insulin. In 1982, the **Eli Lilly Corporation** produced a human insulin that became the first approved genetically engineered pharmaceutical product. Without needing to depend on animals, researchers could produce genetically engineered insulin in unlimited supplies. It also did not contain any of the animal contaminants. According to the Eli Lilly Corporation, in **2001 95% of insulin** users in most parts of the world take **some form of human insulin**. Some companies have stopped producing animal insulin completely. Companies are focusing on synthesizing human insulin and insulin analogs, a modification of the insulin molecule in some way.
Insulin

The insulin gene is coding a protein consisting of two separate chains of amino acids, an A above a B chain, that are held together with bonds. Amino acids are the basic units that build all proteins. The insulin A chain consists of 21 amino acids and the B chain has 30.

Before becoming an active insulin protein, insulin is first produced as preproinsulin. This is one single long protein chain with the A and B chains not yet separated, a section in the middle linking the chains together and a signal sequence at one end telling the protein when to start secreting outside the cell.
Insulin

After **preproinsulin**, the chain evolves into **proinsulin**, still a **single chain** but without the signaling sequence. Then comes the active protein insulin, the protein without the section linking the A and B chains. At each step, the protein needs specific enzymes (proteins that carry out chemical reactions) to produce the next form of insulin.

One method of manufacturing insulin is to grow the **two insulin chains separately**. This will avoid manufacturing each of the specific enzymes needed.

Manufacturers **need the two mini-genes**: one that produces the A chain and one for the B chain. Since the exact DNA sequence of each chain is known, they synthesize each mini-gene's DNA in an amino acid sequencing machine.
interferon

- **Interferons (IFNs)** - a group of **signaling proteins** made and released by host cells in response to the presence of several **pathogens** (viruses, bacteria, parasites) and also **tumor** cells. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses. First described in **1957**.

- IFNs belong to the large class of proteins known as **cytokines**, molecules used for communication between cells to trigger the protective defenses of the **immune system** that help eradicate pathogens.

- Interferons are named for their ability to "interfere" with viral replication. IFNs also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating **antigen presentation** by virtue of increasing the expression of major histocompatibility complex (MHC) antigens.
Interferon production

- In 1978 – 1981, the purification of the type I interferons IFN-α and IFN-β.
- By the early 1980s, genes for these interferons had been cloned, adding further definitive proof that interferons were responsible for interfering with viral replication.
- Gene cloning also confirmed that IFN-α was encoded by a family of many related genes. The type II IFN (IFN-γ) gene was also isolated around this time.[1]
- Interferon was scarce and expensive until 1980, when the interferon gene was inserted into bacteria using recombinant DNA technology, mass cultivation and purification from bacterial cultures or derived from yeasts.
- Interferon can also be produced by recombinant mammalian cells.
Streptokinase

- **Streptokinase (SK)** is an enzyme secreted by several species of *streptococci* (*Streptococcus equisimilis*) that can bind and activate human plasminogen.
- SK is used as an effective and inexpensive thrombolysis medication in some cases of **myocardial infarction** (heart attack) and pulmonary embolism.
- It is on the WHO List of Essential Medicines, the most important medications needed in a basic health system.
- The wholesale cost is between 30.00 and 138.00 USD per dose as of 2014.
- It is no longer available in the United States. SK is used as thrombolysis for acute MI in about half of countries worldwide because it is affordable.
- “In countries where patients do not have rapid access to catheterisation, streptokinase is still important.”
Aspergillus - Penicillium