



Fermentation Technology Abstract



2005

An Abstract on Industrial Fermentation Technology

1. INTRODUCTION

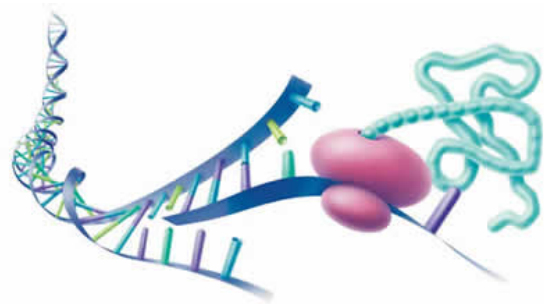
'**Biotechnology**', the short form of **Biological technology**, defies precise definition.

The term biotechnology came into general use in the mid 1970s, gradually superseding the more ambiguous 'bioengineering', which was variously used, to describe chemical engineering processes using organisms and/or their products, particularly fermenter design, control, product recovery and purification. Most scientists agree that all processes that utilize biological organisms constitute biotechnology, but what is disputed is which processes do not (Crafts-Lighty, 1983). Definitions of technology too vary from the simple 'applied science' to 'the scientific study of the practical or industrial arts' (Crafts-Lighty, 1983).

In consequence of the disagreements, there are at least ten different definitions of biotechnology, each qualified according to the context of use. The following are among the more widely used definitions of biotechnology:

a) "The application of biological organisms, systems or processes to manufacturing and service industry" (HMSO, 1980).

b) "The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services" (Bull et al., 1982). Agents include a wide range of biological substances, such as enzymes, as well as whole cells or multicellular organisms. 'Goods and services' covers processes such as waste and water treatment.



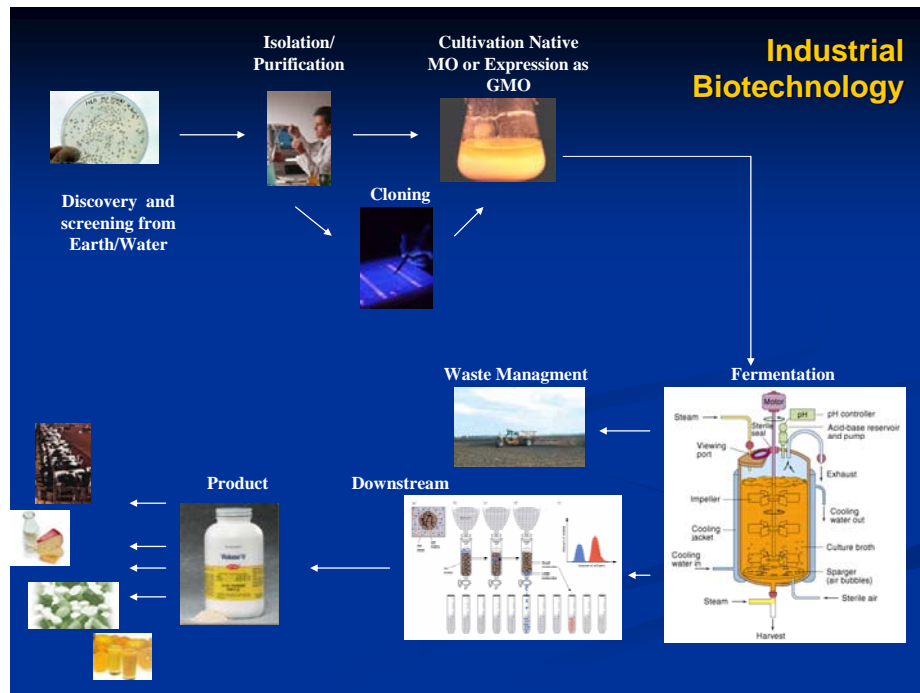
c) "The integrated use of biochemistry, microbiology and chemical engineering to exploit plant materials and genetic resources for the production of specific products and services" (Mantell, 1989).

Many definitions are rather vague about the nature of the organism or agents involved in biotechnology. It is argued that systematic farming of plants and animals for food and fuel also falls within these definitions.

Ancient fermented food processes, such as making bread, wine, cheese, curds, *idli*, *dosa*, etc., some of which are some 6,000 yr old, and developed long before man had any knowledge of the existence of the micro-organisms involved, also genuinely constitute biotechnology. However, for the sake of convenience, many people exclude these traditional processes from the realm of biotechnology. Conventional agriculture is a well-developed industry in its own right, but in practice, this is not included in biotechnology. Aspects of 'modern biotechnology' may have significant effects on 'traditional biotechnology'. Genetic manipulation to improve brewing and baking yeasts or to introduce new characteristics in crops, biological control of plant pests, and new methods of diagnosing and preventing plant, human and animal disease, are all now realisable. Whatever the definition, experimental

production of new varieties of organisms, is one of the important objectives of biotechnology.

In simpler words, biotechnology means the industry-scale use of organisms and/or their products. Now biotechnology virtually includes the scientific, technological and commercial aspects of almost every area of human welfare from agricultural production to pollution control.



2. FERMENTATION

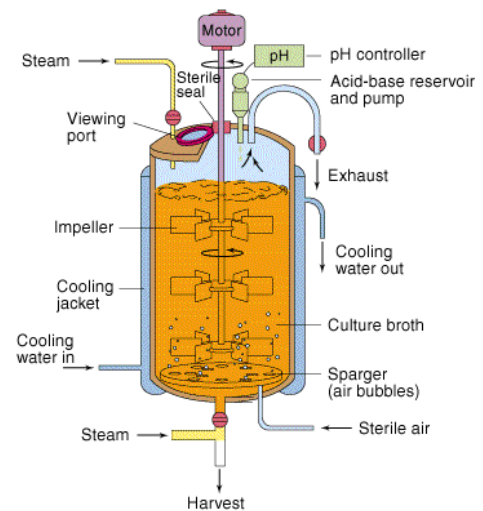
Fermentation technology is the oldest of all biotechnological processes. The term is derived from the Latin verb *fevere*, to boil--the appearance of fruit extracts or malted grain acted upon by yeast, during the production of alcohol.

Fermentation is a process of chemical change caused by organisms or their products, usually producing effervescence and heat.

Microbiologists consider fermentation as '**any process for the production of a product by means of mass culture of micro-organisms**'.

Biochemists consider fermentation as '**an energy-generating process in which organic compounds act both as electron donors and acceptors**'; hence fermentation is '**an anaerobic process where energy is produced without the participation of oxygen or other inorganic electron acceptors**'.

In biotechnology, the microbiological concept is widely used.



3. MICRO-ORGANISMS

Several species belonging to the following categories of micro-organisms are used in fermentation processes:

PROKARYOTIC	Unicellular: bacteria, cyanobacteria
	Multicellular: cyanobacteria
EUKARYOTIC	Unicellular: yeasts, algae
	Multicellular: fungi, algae

Unicellular and micro-fauna are rarely a part of fermentation processes, while isolated cells of multicellular animals are frequently cultured.

4. MICROBIAL GROWTH

A. REQUIREMENTS FOR ARTIFICIAL CULTURE

The growth of organisms involves complex energy based processes. The rate of growth of micro-organisms is dependent upon several **culture conditions**, which should provide for the energy required for various chemical reactions. The production of a specific compound requires very precise cultural conditions at a particular growth rate. Many systems now operate under computer control.

The rate of growth of micro-organisms and hence the synthesis of various chemical compounds under artificial culture, requires organism specific chemical compounds as the

growth (nutrient) medium. The kinds and relative concentrations of the ingredients of the medium, the pH, temperature, purity of the cultured organism, etc., influence microbial growth and hence the production of **biomass** (the total mass of cells or the organism being cultured), and the synthesis of various compounds. The nutrient sources for industrial fermentation are given in Table 1.



TABLE 1

Nutrient sources for industrial fermentation

Nutrient	Raw material
Carbon source	
Glucose	Corn sugar, Starch, Cellulose
Sucrose	Sugarcane, Sugar beet molasses
Lactose	Milk whey
Fats	Vegetable oils
Hydrocarbons	Petroleum fractions
Nitrogen source	
Protein	Soybean meal, Cornsteep liquor, Distillers' solubles
Ammonia	Pure ammonia or ammonium salts
Nitrate	Nitrate salts
Nitrogen	Air
Phosphorous source	Phosphate salts

B. PHASES OF MICROBIAL GROWTH

When a particular organism is introduced into a selected growth medium, the medium is **inoculated** with the particular organism. Growth of the inoculum does not occur immediately, but takes a little while. This is the period of adaptation, called the **lag phase**.

Following the lag phase, the rate of growth of the organism steadily increases, for a certain period--this period is the **log** or **exponential phase**.

After a certain time of exponential phase, the rate of growth slows down, due to the continuously falling concentrations of nutrients and/or a continuously increasing (accumulating) concentrations of toxic substances. This phase, where the increase of the rate of growth is checked, is the **deceleration phase**.



After the deceleration phase, growth ceases and the culture enters a **stationary phase** or a **steady state**. The **biomass** remains constant, except when certain accumulated chemicals in the culture **lyse** the cells (**chemolysis**). Unless other micro-organisms contaminate the culture, the chemical constitution remains unchanged. Mutation of the organism in the culture can also be a source of contamination, called **internal contamination**.

5. FERMENTERS AND BIOREACTORS

A fermenter is the set up to carry out the process of fermentation. The fermenters vary from laboratory experimental models of one or two litres capacity, to industrial models of several hundred litres capacity, which refers to the volume of the main fermenting vessel.

A **bioreactor** differs from a fermenter in that the former is used for the mass culture of plant or animal cells, instead of micro-organisms. The chemical compounds synthesised by these cultured cells, such as therapeutic agents, can be extracted easily from the cell biomass.



The design engineering and operational parameters of both fermenters and bioreactors are identical. With the involvement of micro-organisms as elicitors in some situations, the distinction between the two concepts is being gradually obliterated.

6. DESIGN OF INDUSTRIAL FERMENTATION PROCESS

The fermentation process requires the following:

- a) A pure **culture** of the chosen organism, in sufficient quantity and in the correct physiological state;
- b) **Sterilised**, carefully composed **medium for growth** of the organism;

c) A **seed fermenter**, a mini-model of production fermenter to develop an inoculum to initiate the process in the **main fermenter**;

d) A **production fermenter**, the functional large model; and

e) Equipment for i) drawing the culture medium in steady state, ii) cell separation, iii) collection of cell free supernatant, iv) product purification, and v) effluent treatment.

Items a) to c) above constitute the **upstream** and e) constitutes the **downstream**, of the fermentation process,

Fermenters/bioreactors are equipped with an aerator to supply oxygen in aerobic processes, a stirrer to keep the concentration of the medium uniform, and a thermostat to regulate temperature, a pH detector and similar control devices.

7. TYPES OF CULTURE SYSTEMS

A. BATCH PROCESSING OR CULTURE

At about the onset of the stationary phase, the culture is disbanded for the recovery of its biomass (cells, organism) or the compounds that accumulated in the medium (alcohol, amino acids), and a new batch is set up. This is **batch processing** or **batch culture**.



The best advantage of batch processing is the optimum levels of product recovery. The disadvantages are the wastage of unused nutrients, the peaked input of labour and the time lost between batches.

B. CONTINUOUS PROCESSING OR CULTURE

The culture medium may be designed such that growth is limited by the availability of one or two components of the medium. When the initial quantity of this component is exhausted, growth ceases and a steady state is reached, but growth is renewed by the addition of the limiting component. A certain amount of the whole culture medium (**aliquot**) can also be added periodically, at the time when steady state sets in. The addition of nutrients will increase the volume of the medium in the fermentation vessel. It is so arranged that the increased volume will drain off as an overflow, which is collected and used for recovery of products. At each step of addition of the medium, the medium becomes dilute both in terms of the concentration of the biomass and the products. New growth, stimulated by the added medium, will increase the biomass and the products, till another steady state sets in; and another aliquot of medium will reverse the process.

This is **continuous culture** or **processing**. Since the growth of the organism is controlled by the availability of growth limiting chemical component of the medium, this system is called a chemostat. The rate at which aliquots are added is the **dilution rate** that is in effect the factor that dictates the rate of growth.

The events in a continuous culture are:

- a) The growth rate of cells will be less than the dilution rate and they will be washed out of the vessel at a rate greater than they are being produced, resulting in a decrease of biomass concentration both within the vessel and in the overflow;
- b) The substrate concentration in the vessel will rise because fewer cells are left in the vessel to consume it;
- c) The increased substrate concentration in the vessel will result in the cells growing at a rate greater than the dilution rate and biomass concentration will increase; and
- d) The steady state will be re-established.

Hence, a **chemostat is a nutrient limited self-balancing culture system, which may be maintained in a steady state over a wide range of sub-maximum specific growth rates.**

The continuous processing offers the most control over the growth of cells.

Commercial adaptation of continuous processing is confined to biomass production, and to a limited extent to the production of potable and industrial alcohol.

The steady state of continuous processing is advantageous as the system is far easier to control. During batch processing, heat output, acid or alkali production, and oxygen consumption will range from very low rates at the start to very high rates during the late exponential phase. The control of the environmental factors of the system becomes difficult. In the continuous processing, the rates of consumption of nutrients and those of the output chemicals are maintainable at optimal levels. Besides, the labour demand is also more uniform.



Continuous processing may suffer from contamination, both from within and outside. The fermenter design, along with strict operational control, should actually take care of this problem.

The production of growth associated products like ethanol is more efficient in continuous processing, particularly for industrial use.

Continuous culturing is highly selective and favours the propagation of the best-adapted organism in culture.

A commercial organism is highly mutated such that it will produce very high amounts of the desired product. But physiologically such strains are inefficient and give way in culture to inferior producers--a kind of contamination from within.

C. FED-BATCH CULTURE OR PROCESSING

In the **fed-batch system**, a fresh aliquot of the medium is continuously or periodically added, without the removal of the culture fluid. The fermenter is designed to accommodate the increasing volumes. The system is always at a **quasi-steady state**.

Fed-batch achieved some appreciable degree of process and product control.

A low but constantly replenished medium has the following advantages:

- a) Maintaining conditions in the culture within the aeration capacity of the fermenter;
- b) Removing the repressive effects of medium components such as rapidly used carbon and nitrogen sources and phosphate;
- c) Avoiding the toxic effects of a medium component; and
- d) Providing limiting level of a required nutrient for an **auxotrophic** strain.

Production of baker's yeast is mostly by fed-batch culture, where biomass is the desired product. Diluting the culture with a batch of fresh medium prevents the production of ethanol, at the expense of biomass; the moment traces of ethanol were detected in the exhaust gas.

The production of penicillin, a secondary metabolite, is also by fed-batch method. Penicillin process has two stages: an initial growth phase followed by the production phase called the '**idiophase**'.

The culture is maintained at low levels of biomass and phenyl acetic acid, the precursor of penicillin, is fed into the fermenter continuously, but at a low rate, as the precursor is toxic to the organism at higher concentrations.



8. PRODUCTS OF FERMENTATION PROCESSES

The growth of micro-organisms or other cells results in a wide range of products. Each culture operation has one or few set objectives. The process has to be monitored carefully and continuously, to maintain the precise conditions needed and recover optimum levels of products. Accordingly, fermentation processes aim at one or more of the following:

- a) Production of cells (biomass) such as yeasts;
- b) Extraction of metabolic products such amino acids, proteins (including enzymes), vitamins, alcohol, etc., for human and/or animal consumption or industrial use such as fertiliser production;
- c) Modification of compounds (through the mediation of **elicitors** or through **biotransformation**); and
- d) Production of **recombinant** products.

A. MICROBIAL BIOMASS

Microbial biomass is produced commercially as **single cell protein** (SCP) using such unicellular algae as species of *Chlorella* or *Spirulina* for human or animal consumption, or viable yeast cells needed for the baking industry, which was also used as human feed at one time. Bacterial biomass is used as animal feed. The biomass of *Fusarium graminearum* is also produced, for a similar use.

B. MICROBIAL METABOLITES

i) Primary metabolites:

During the log or exponential phase organisms produce a variety of substances that are essential for their growth, such as nucleotides, nucleic acids, amino acids, proteins, carbohydrates, lipids, etc., or by-products of energy yielding metabolism such as ethanol, acetone, butanol, etc. This phase is described as the **trophophase**, and the products are usually called **primary metabolites**. Commercial examples of such products are given in Table 2.

TABLE 2

Examples of commercially produced primary metabolites

Primary Metabolite	Organism	Significance
Ethanol	<i>Saccharomyces cerevisiae</i>	alcoholic beverages
	<i>Kluyveromyces fragilis</i>	Bio-Fuel
Citric acid	<i>Aspergillus niger</i>	food industry
Acetone and butanol	<i>Clostridium acetobutyricum</i>	solvents
Lysine	<i>Corynebacterium</i>	nutritional additive
Glutamic acid	<i>glutamaciun</i>	flavour enhancer
Riboflavin	<i>Ashbya gossipii</i>	nutritional
	<i>Eremothecium ashbyi</i>	
Vitamin B12	<i>Pseudomonas denitrificans</i>	nutritional
	<i>Propionibacterium shermanii</i>	
Dextran	<i>Leuconostoc mesenteroides</i>	industrial
Xanthan gum	<i>Xanthomonas campestris</i>	industrial

ii) Secondary metabolites:

Organisms produce a number of products, other than the primary metabolites. The phase, during which products that have no obvious role in metabolism of the culture organisms are produced, is called the **idiophase**, and the products are called **secondary metabolites**.

In reality, the distinction between the primary and secondary metabolites is not a straightjacket situation. Many secondary metabolites are produced from intermediates and

end products of secondary metabolism. Some like those of the Enterobacteriaceae do not undergo secondary metabolism. Examples of secondary metabolites are given in Table 3.

TABLE 3

Examples of commercially produced secondary metabolites

Metabolite	Species	Significance
Penicillin	<i>Penicillium chrysogenum</i>	antibiotic
Erythromycin	<i>Streptomyces erythreus</i>	antibiotic
Streptomycin	<i>Streptomyces griseus</i>	antibiotic
Cephalosporin	<i>Cephalosporium acrimonium</i>	antibiotic
Griseofulvin	<i>Penicillium griseofulvin</i>	antifungal antibiotic
Cyclosporin A	<i>Tolypocladium inflatum</i>	immunosuppressant
Gibberellin	<i>Gibberella fujikuroi</i>	plant growth regulator

Secondary metabolism may be **repressed** in certain cases. Glucose represses the production of actinomycin, penicillin, neomycin and streptomycin; phosphate represses streptomycin and tetracyclin production. Hence, the culture medium for secondary metabolite production should be carefully chosen.

C. PRODUCTION ENZYMES

Industrial production of enzymes is needed for the commercial production of food and beverages. Enzymes are also used in clinical or industrial analysis and now they are even added to washing powders (cellulase, protease and lipase). Enzymes may be produced by microbial, plant or animal cultures. Even plant and animal enzymes can be produced by microbial fermentation. While most enzymes are produced in the trophophase, some like the amylases (by *Bacillus stearothermophilus*) are produced in the idiophase, and hence are secondary metabolites. Examples of enzymes produced through fermentation processes are given in Table 4.

TABLE 4

Examples of commercially produced enzymes

Organism	Enzyme
<i>Aspergillus oryzae</i>	Amylases
<i>Aspergillus niger</i>	Glucamylase
<i>Trichoderma reesii</i>	Cellulase
<i>Saccharomyces cerevisiea</i>	Invertase
<i>Kluyveromyces fragilis</i>	Lactase
<i>Saccharomycopsis lipolytica</i>	Lipase
<i>Aspergillus</i> species	Pectinases and proteases
<i>Bacillus</i> species	Proteases
<i>Mucor pusillus</i>	Microbial rennet
<i>Mucor meihei</i>	Microbial rennet



D. FOOD INDUSTRY PRODUCTS

A very wide range of innumerable products of the food industry, such as sour cream, yoghurt, cheeses, fermented meats, bread and other bakery products, alcoholic beverages, vinegar, fermented vegetables and pickles, etc., are produced through microbial fermentation processes. The efficiency of the strains of the organisms used, and the processes are being continuously improved to market quality products at more reasonable costs.

E. RECOMBINANT PRODUCTS

Recombinant DNA technology has made it possible to introduce genes from any organism into micro-organisms and *vice versa*, resulting in **transgenic** organisms and the latter are made to produce the gene product. Genetically manipulated *Escherichia coli*, *Saccharomyces cerevisiae*, other yeasts and even filamentous fungi are now being used to produce interferon, insulin, human serum albumin, and several other products.



F. BIOTRANSFORMATION

Production of a structurally similar compound from a particular one, during the fermentation process is transformation, or **biotransformation**, or **bioconversion**. The oldest instance of this process is the production of acetic acid from ethanol.

Immobilised plant cells may be used for biotransformation. Using alginate as the immobilising polymer, digitoxin from *Digitalis lanata* was converted into digoxin, which is a therapeutic agent in great demand. Similarly, codeinone was converted into codeine and tyrosine from *Mucuna pruriens* was converted into DOPA.

G. ELICITORS

It is possible to induce production or enhance production of a compound in cultures by using **elicitors**, which may be micro-organisms. For example, *Saccharomyces cerevisiae* was an efficient elicitor in the production of glyceollin (*Glycine max*) and berberine (*Thalictrum rugosum*). *Rhizopus arrhizus* trebled diosgenin production by *Dioscorea deltoidea*. The production of morphine and codeine by *Papaver somniferum* was increased 18 times by *Verticillium dahliae*.

9. GENETIC IMPROVEMENT OF FERMENTATION PROCESSES

The **genome** of the organism ultimately controls its metabolism. Although improved fermenter engineering design and optimal cultural conditions can quantitatively enhance the microbial products, this will only be up to a limit. Genetic improvement of the organism is fundamental to the success of fermentation technology. Mutation and recombination are the two ways to meet this end.

A. MUTATION

A certain amount of mutational change in the genome occurs as a natural process, though the probability is small. Exposing a culture of a micro-organism to UV light, ionising radiation or certain chemicals, enhances the rate of occurrence of mutations. But it is a tremendous task for the industrial geneticist to screen the very large number of randomly produced mutants and to select the ones with the desired qualities.

The synthesis of a number of products of cell metabolism is controlled by a '**feed-back inhibition**'. When a compound reaches a particular level of accumulation, its synthesis is stopped. Synthesis starts again when the level of the compound falls below the specific level. If a mutant is produced, in which the feedback signalling is suppressed, the product is synthesised continuously. By such a manipulation, a high producing strain of *Corynebacterium glutamacium* was developed to recover very high quantities of lysine. Such strains that do not produce controlling end products are called **auxotrophs**.

B. RECOMBINATION

Recombination is defined as any process that brings together genes from different sources.

A strain of *Brevibacterium flavum* is a high producer of lysine, but is limited by its poor capacity to absorb glucose. Another strain of the bacterium, which is an efficient absorber of glucose but which does not produce lysine, was used to develop a recombinant strain, through **protoplast fusion**. The new strain utilises high levels of glucose and yields higher levels of lysine.



A gene for the synthesis of phenylalanine was transferred to a chosen strain of *Escherichia coli*, which was a non-producer, but a good experimental and production tool.

Transformation of a high cephalosporin producing strain of *Cephalosporium acremonium* with a **plasmid** containing the gene REXH has significantly increased the titre.

A number of human proteins, such as insulin, human growth hormone, bone growth factor, alpha, beta and gamma interferons, interleukin-2, tumour necrosis factor, tissue plasminogen activator, blood clotting factor VIII, epidermal growth factor, granulocyte colony stimulating factor, erythropoietin, etc., are being produced through recombinant micro-organisms.

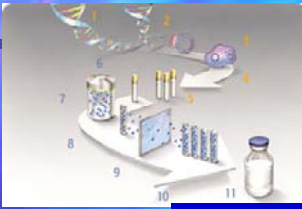
C. DNA MANIPULATION

In vitro DNA technology was used to increase the number of copies of a critical pathway gene (**operon**), as for example the production of threonine in *Escherichia coli*, at rates 40 to 50 times higher than usual.

10. CONCLUSION

Fermentation technology is a very vibrant and fast growing area of biotechnology, absorbing an ever increasing processes and products. With a longer history than any area of biological sciences, fermentation technology has a longer and brighter future, in the service of mankind, covering such important areas as food and medicine.

ensymm Strain Development Network for Industrial Application



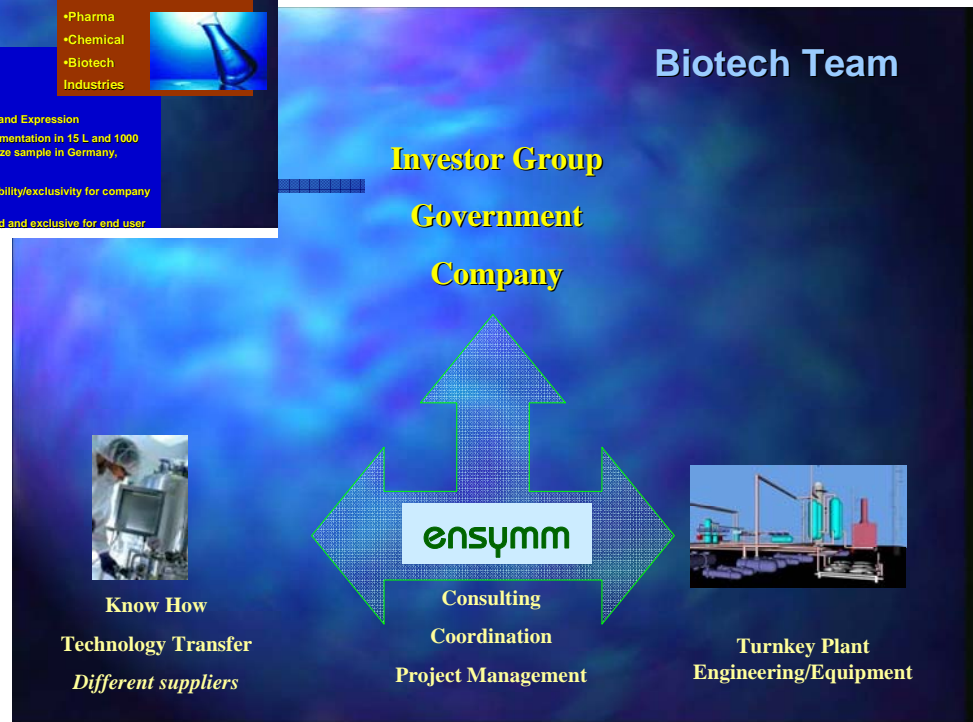
Optimized high performance strain useful for the production of enzymes for

- Food
- Pharma
- Chemical
- Biotech Industries

Scope of service:

- Proof of concept /Screening
- Strain development
- Gene Optimisation, Cloning and Expression
- Cultivation in shake flask/Fermentation in 15 L and 1000 L/Strain transfer, Reserve freeze sample in Germany,
- Documents /Transport
- Guarantees for yield and stability/exclusivity for company or territory
- License agreement: unlimited and exclusive for end user

in 11 steps from Earth to Product





We thank you for your attention



looks forward to a fruitful co-operation
between your company and our network

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