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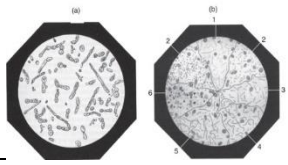
FACULTY OF SCIENCE

DEPARTMENT OF NATURAL AND LIFE SCIENCES

Course handout

Industrial Microbiology



Intended for 3rd year
Microbiology students.

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Preface

This industrial microbiology handout is intended for 3rd-year Microbiology degree students and is divided into two parts. The first part enables students to acquire a basic knowledge of the subject, through the study of the three main players in industrial microbiology: industrial microorganisms, industrial fermenters and industrial culture media. After acquiring the basic notions, the second part of the subject will discuss the various products of industrial fermentation, which are (i): microbial biomass: generally used as a source of proteins of unicellular origin, (ii): the industrial production of certain primary metabolites represented by amino acids, organic acids and biogases, and (iii) the industrial production of certain secondary metabolites represented by antibiotics, polysaccharides and vitamins. The teaching method used in this course is based on the use of simple and basic language, supported by examples. In addition, and in order to make the content more accessible to students, many illustrations in the form of simplified diagrams, photos and summary tables have been used. As a result, it is always encouraging and motivating to receive corrections, advice and recommendations from our teaching and research colleagues.

1 Historical Overview and Future Perspective

The field of industrial microbiology traces its roots to ancient times when humans first began understanding food spoilage, preservation, and storage. Early civilizations, through trial and error, developed methods to refine and preserve food based on experience. Over time, they unknowingly relied on the actions of microorganisms, which caused chemical changes in food. These techniques evolved gradually and were applied on a larger scale. Initially, knowledge of these processes was passed on orally and, later, through written records. Although artisans used various fermentation methods, they lacked awareness of the microorganisms involved and the biochemical processes behind them. It wasn't until around 1850 that scientists were able to identify these microorganisms and understand the chemical reactions they induced. Historical records up to this point highlight significant advancements in both food production techniques and microbiological discoveries. In ancient Egypt, by 2500 BC, beer was being produced from bread dough, a beverage known as "henket." It remains uncertain whether the Egyptians learned the brewing process from the Sumerians or developed it independently. In 1990, archaeologists excavated a 3,300-year-old brewery belonging to King Echnaton (1351–1334 BC), uncovering well-preserved brewing tools, clay vessels, and ingredients like malt, grain, and dates. These findings revealed that Egyptians had mastered essential brewing techniques such as malt preparation (germination and enzyme formation) and mashing (the enzymatic conversion of starch into sugar), which were crucial to beer production.

In Europe, during the late Middle Ages (around 1300), the production of saltpeter, or potassium nitrate (KNO_3), became prominent due to its essential role in making gunpowder. This compound was produced through a process involving the nitrification of nitrogen from organic materials in the soil, facilitated by nitrifying bacteria. Initially, surfaces covered in human and animal waste were used as raw materials for saltpeter production. Over time, more direct nitrogen sources, such as urine and blood, were introduced in specialized saltpeter huts, where the nitrification process was maintained in well-ventilated beds. This method of microbial production persisted until the discovery of vast natural deposits of saltpeter in Chile during the 19th century, which eventually led to the abandonment of microbial production techniques.

With the dawn of the modern era (around 1500) and the rise of natural sciences in the 17th century, scientists began studying natural phenomena systematically through observation, experimentation, and measurement. During this period, academic institutions were established to provide platforms for presenting and discussing the results of scientific inquiry, which were also published in scientific journals. The development of modern physics and chemistry

provided the necessary tools, along with several technical innovations like improved lens-making techniques, to scientifically analyze the production processes of bread, wine, beer, and vinegar during the 18th and 19th centuries.

The work of Antonie van Leeuwenhoek (1632–1723) is considered the foundation of microbiology. Using a simple microscope with a single lens, he was the first to observe microorganisms, including bacteria, which he meticulously described as "animalcules." While the fermentation process had long been in use, it was not yet understood that microorganisms played a key role in this transformation. Leeuwenhoek's discoveries laid the groundwork for the later understanding of microbial fermentation and its importance in food production and other processes.

Between 1850 and 1940, significant advancements in microbiology, spearheaded by scientists like Louis Pasteur and Robert Koch, laid the foundation for industrial microbiology. Pasteur demonstrated that fermentation processes were driven by specific microorganisms and their physiological abilities. His work from 1856 to 1875 focused on yeast life cycles and bacterial fermentation (lactic and butyric acid), revealing how failed fermentations were caused by contamination from unwanted microorganisms. Pasteur’s development of sterilization techniques, such as pasteurization, was pivotal for cultivating pure microbial cultures.

Industrial microbiology, a field that uses microorganisms for modifying and producing substances on an industrial scale, emerged from these breakthroughs. The contributions of Pasteur, along with Robert Koch’s discoveries on bacterial pathogens causing diseases like anthrax and cholera, were crucial to the rise of modern microbiology.

By the late 19th century, numerous research institutes across Europe focused on fermentation processes, food production, quality control, and hygiene. This growing understanding of microbes led to a boom in technical innovations, transforming food production from artisanal practices to industrial processes. This shift resulted in increased production, especially in beer, wine, and alcohol industries. For instance, beer production in Germany almost doubled between 1873 and 1890, and European wine production reached 11 billion liters by the century's end. Alcohol production, mainly from molasses, fruit, grains, and potatoes, saw significant growth, with two-thirds of it processed into high-proof beverages.

These developments not only boosted economic output but also led to improved food safety and a better understanding of microbial involvement in industrial processes.

2 Key Historical Developments in Industrial Microbiology (Late 19th Century to 1940s):

1. **Baker's Yeast and Lactic Acid Production:**
    - The *Vienna Process* was crucial for producing baker's yeast, utilizing bacteriologically pure cultures in sterile liquid environments.
    - Industrial-scale lactic acid production began in 1881, with advancements in sterile production methods and the use of pure microbial cultures.
2. **Butanol and Acetone Fermentation (Early 20th Century):**
    - Chaim Weizmann isolated *Clostridium acetobutylicum*, leading to the large-scale fermentation of butanol and acetone. These chemicals were crucial for synthetic rubber production and military explosives.
    - Glycerol production using yeast also became critical for making nitroglycerin during World War I.
3. **Citric and Gluconic Acid Production:**
    - Citric acid was produced on a large scale using *Aspergillus niger* starting in 1920. The transition to submersion cultures in 1940 greatly improved yields.
4. **Wastewater Treatment:**
    - Microbial wastewater treatment processes emerged in the late 19th century to reduce organic waste and prevent disease in growing cities. Techniques like the activated sludge process (1914) and anaerobic digestion advanced treatment methods.
5. **Enzyme Discoveries and Biochemical Research:**
    - Major discoveries in enzyme activity (e.g., *zymase* and glycolysis) occurred between 1880 and 1900, laying the foundation for enzyme technology. By the early 20th century, this research significantly advanced microbial metabolism understanding, aiding industrial production.

3 Introduction of Genetic Engineering (circa 1980):

Genetic engineering revolutionized industrial microbiology by enabling precise alterations to microbial genetic material. It allows for the targeted manipulation of enzymes and proteins, improving industrial processes without the unspecific side effects caused by traditional mutagenesis methods (e.g., radiation or chemical mutagens).

1. **Enhanced Microbial Strains:**

Genetically engineered strains surpass those developed through classical methods by producing higher yields, reducing unwanted by-products, and enabling the synthesis of proteins, including those from higher organisms. However, limitations remain in areas like post-translational modifications.

Foundations in Molecular Biology (1940s onward): Genetic engineering emerged from molecular biology research, with key discoveries such as:

- **1944:** Oswald Avery demonstrated that nucleic acids (DNA) carry genetic information, discrediting earlier protein theories.
- **1953:** James Watson and Francis Crick identified the double-helix structure of DNA.
- **1960s:** Decoding of the genetic code by Heinrich Matthaei and Marshall Nirenberg, followed by the operon model of gene regulation by François Jacob and Jacques Monod.

2. **Restriction Enzymes:** These enzymes, discovered between 1962 and 1968, became vital for genetic engineering by enabling precise cutting and splicing of DNA, facilitating recombination techniques.

3.1 Genetic Engineering Methods:

- Important milestones in microbiology research led to the development of critical genetic engineering tools, such as:
    - In vitro oligonucleotide synthesis (1964),
    - DNA sequencing methods by Maxam & Gilbert and Sanger (1977),
    - Polymerase chain reaction (PCR, 1987), and Cloning of synthetic genes (1988).

3.1.1 Advances in Genome Sequencing:

- Automated DNA sequencing decoded the first bacterial genome in 1995 and the yeast genome in 1997.
- Pyrosequencing (2001) and Illumina technology (2005) enabled high-throughput genome sequencing, facilitating the sequencing of over 4,000 prokaryotic and 450 eukaryotic organisms.

3.1.2 Emergence of Omics Technologies:

- DNA microarray technology (1990s) enabled genome-wide expression analysis.
- Proteomics and metabolomics emerged to identify proteins and metabolites in cells.
- The advent of bioinformatics helped process vast amounts of data from these methods, fostering **systems biology** (2000) to model entire metabolic networks in microorganisms.
    - **Metabolic Engineering:**

- Genetic engineering techniques are used to modify metabolic pathways in industrial microorganisms, improving their capacity to produce desired substances. Examples include optimizing carbon flow, expanding substrate range, and blocking unwanted by-products.

4 Applications in Industry:

- Human insulin, produced by genetically modified microorganisms (*E. coli*), was the first industrial product made using genetic engineering (1978).
- By 1986, genetically modified strains were used to manufacture various products such as alcohols, organic acids, amino acids, vitamins, and antibiotics.
- GM microorganisms are widely employed for enzyme production in industries like textiles, paper, and detergents, as well as for research and diagnostics.

These advancements have revolutionized industrial microbiology, enabling the large-scale production of proteins, enzymes, and chemicals through genetically engineered microorganisms.

5 Synthetic Microbiology

1. **Synthetic Biology Overview:**
    - Synthetic biology is a new subfield of **metabolic/genetic engineering** that involves engineering microorganisms with new, non-natural characteristics.
    - This includes designing microorganisms with minimal genomes that only retain pathways for producing a desired substance.
2. **Craig Venter's Milestone (2010):**
    - Craig Venter and his team made significant progress in synthetic biology by replacing the natural genome of *Mycoplasma capricolum* with a synthetically produced genome.
3. **Semisynthetic Production of Artemisinin:**
    - A major example of synthetic biology in industrial microbiology is the **semisynthetic production of artemisinin**, an important antimalarial drug.
    - Due to the fluctuating supply of artemisinin from the plant *Artemisia annua*, a semisynthetic process was developed where a precursor is produced via fermentation, followed by chemical conversion to artemisinin.
4. **Artemisinin Synthesis Pathway:**
    - Artemisinin is derived from **farnesyl diphosphate (FPP)** in plants. FPP is

converted into artemisinic acid via amorphadiene in yeast.

- The key enzyme responsible for converting amorphadiene to artemisinic acid was identified as **cytochrome P450 monooxygenase**.
- Yeast (*Saccharomyces cerevisiae*) was selected as the production organism since it expresses P450 enzymes efficiently.

5. **Yeast Engineering for Production:**
   - To increase FPP production in yeast, the genes in the **mevalonate pathway** (also known as the ERG pathway) were overexpressed, ensuring high concentrations of amorphadiene, the precursor to artemisinic acid.
   - The focus was on engineering yeast to make the semisynthetic process of artemisinin economically viable.

Synthetic biology represents a powerful approach to creating microorganisms that can efficiently produce essential substances, with significant applications in pharmaceuticals and other industries.

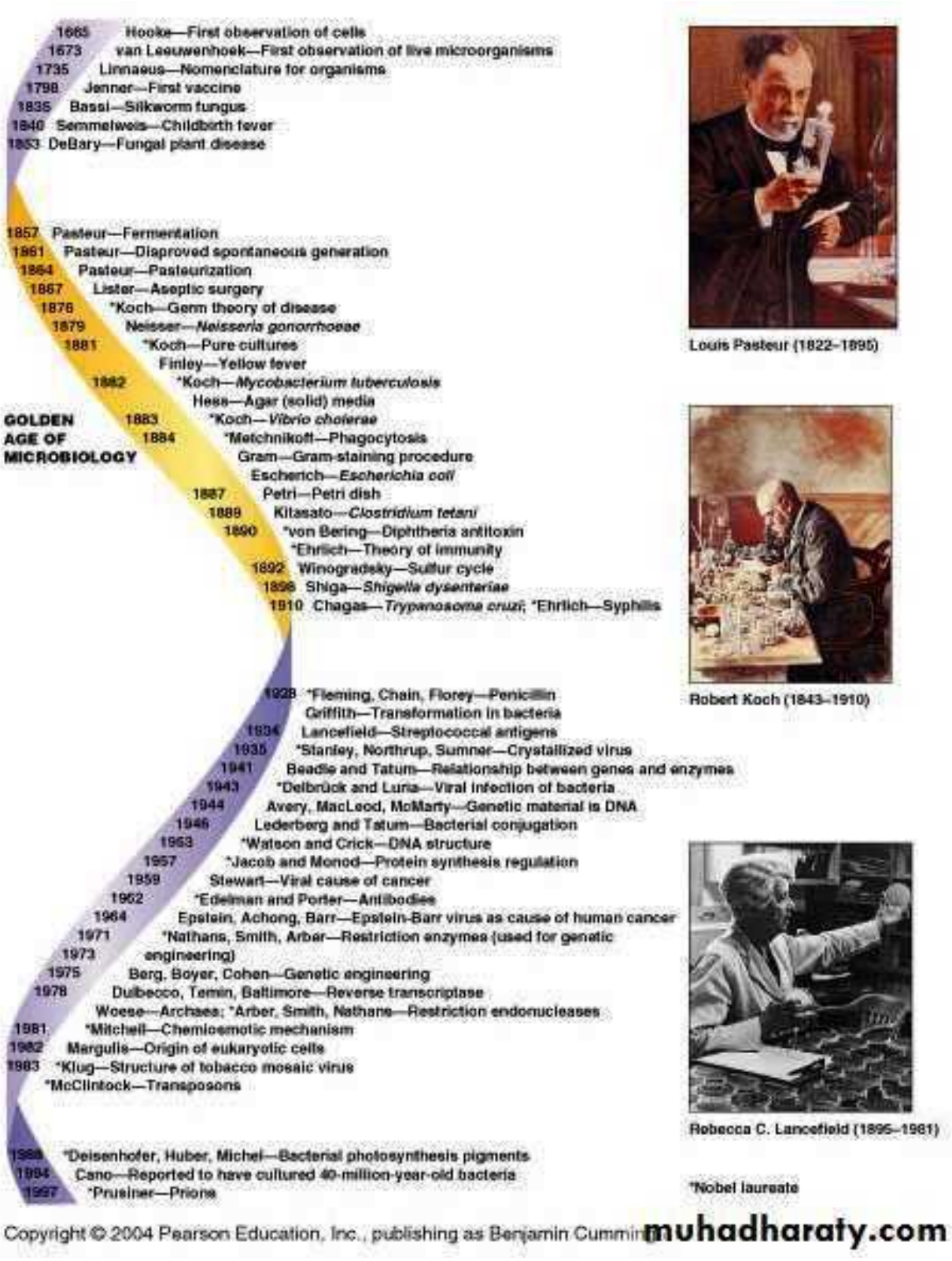

Figure 1. Timeline of industrial Microbiology (https://www.researchgate.net/publication/242049422_Biological_aspects_of_slow_sand_filtration_Past_present_and_future).

References

Cohen, S.N., Chang, A.C.Y., Boyer, H.W., and Helling, R.B. (1973). Construction of biologically functional bacterial plasmids *In Vitro*. *Proc. Natl. Acad. Sci. USA.* 70: 3240–3244. https://doi.org/10.1073/pnas.70.11.3240.

Demain, A.L. (1971). Overproduction of microbial metabolites and enzymes due to alteration of regulation. *Advances in Biochemical Engineering/Biotechnology* 1: 113–142.

Erker L. (1574) Probirbuch, Library of Leipzig University.

Gillam, S. and Smith, M. (1979). Site-specific mutagenesis using synthetic oligodeoxyribonucleotide primers: I. Optimum conditions and minimum ologodeoxyribonucleotide length. *Gene* 8: 81–97.

Kornberg, A., Bertsch, L.L., Jackson, J.F., and Khorana, H.G. (1964). Enzymatic synthesis of desoxyribonucleic acid, XVI. Oligonucleotides as templates and the mechanism of their repication. *Proc. Natl. Acad. Sci. USA.* 51: 315–323. ttps://doi.org/10.1073/pnas.51.2.315.

Maxam, A.M. and Gilbert, W. (1977). A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA.* 74: 560–564. https://doi.org/10.1073/pnas.74.2.560.

Mullis, K.B. and Faloona, F.A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* 155: 335–350.

Pasteur, L. (1876). *Etudes sur la biere. Avec une theorie Nouvelle de la Fementation*. Paris: Gauthier-Villars.

Saiki, R.K., Gelfand, D.H., Stoffel, S. et al. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239 (4839): 487–491.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74: 5463–5467. https://doi.org/10.1073/pnas.74.12.5463.

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Introduction.

The use of microorganisms for human well-being is a very ancient practice dating back to 7000 BC, with the Sumerians as well as the Egyptians. Microorganisms were used during this period in an empirical manner, for the preservation of foodstuffs, for the preparation of bread, for the manufacture of vinegar and alcoholic beverages, as well as for the manufacture of cheese.**(Baltz et al, 2010).**

Following the discovery of microorganisms, as well as the knowledge and experiences accumulated over time, a new field emerged called industrial microbiology, which is a branch of applied microbiology, in which microorganisms of interest are exploited, for commercial interest, to carry out a large-scale biosynthesis, biotransformation or degradation process. Indeed, industrial microorganisms can be used to produce biomass rich in proteins, or for the production of molecules useful to humans, resulting either from primary metabolism, such as amino acids, organic acids and biogas, or secondary, such as antibiotics, vitamins or polysaccharides, as well as to carry out certain biotransformation reactions of toxic organic molecules, to render them harmless **(Okafor et al, 2020; Harwood et al, 2018).**

A large part of the success of the use of microorganisms in industry is related to their easy cultivation as well as their high growth speed, which reduces the cost and time of production. This makes it possible to overcome many problems encountered when using plants, for example. Indeed, the plants used as a source of commercial molecules of interest require a very large cultivation field, a longer time, as well as adequate climatological conditions (rainfall, temperature, humidity level, etc.), which increases the cost of production **(Wilson et al 2019).**

Many microorganisms are used in industrial microbiology; these include bacteria, archaea, yeasts, molds and microalgae. These microorganisms can be used either in their natural state, or are mutants selected in the laboratory or even genetically modified microorganisms (MOGM).**(Zhang et al, 2014).**

1. The fields of activity of industrial microbiology and the interest in the use of microorganisms.

The fermentation industry is an umbrella term applied to business processes that rely on the ability of microorganisms to produce useful molecules, on a large scale, in the presence or absence of air. Thus, contrary to the biochemical sense, the term industrial fermentation does not refer to the metabolism of the microorganism **(Humphrey et al, 1992).**

Fermentation takes place in fermenters or tanks, under rigorously controlled physicochemical conditions (temperature, pH, aeration, carbon source, nitrogen source, etc.)**(Walker et al, 2014).**

2. Interest in using microorganisms in industry:

The use of microorganisms in industry provides a wide range of products and services. They have proven particularly useful due to **(Demain et al, 2008):**

- Their easy cultivation;
- Their very short generation time;
- Their high growth rate.
- Their ability to be grown on cheap substrates, which in many cases are waste from the food industry. ;
- Are easy to manipulate genetically, which allows strains to be improved.

2.1. The objective sought in industrial fermentation.

- Collect the microorganisms themselves (baker's yeast, for example **(Ritala et al, 2017).**

- Collect a byproduct of the reaction (alcohol, antibiotics, etc.) **(Keller et al, 2019).**

- Bioconversion of molecules, transforming complex products into simple elements (wastewater treatment, recovery of industrial waste) **(Huang et al, 2017).**

In the food industry, the benefit of fermenting a food product is to improve its stability, for two reasons:

- Consumption of fermentable substrate avoids post-fermentation bacterial degradation of the food;

- The production of alcohol or acid limits bacterial contamination.

This vital conservation role generated by fermentation is accompanied by positive effects sought in terms of the texture and aromatization of the products produced.

3. Fields of activity in industrial microbiology.

Industrial microbiology can have different applications, namely:

- Agri-food sector: several food products come from industrial fermentation, this is the case, for example, in the manufacture of yogurt produced by the action of two bacteria, Lactobacillus bulgaricus and Streptococcus thermophilus. The lactic acid bacteria, Lactococcus lactis, is used for the manufacture of different types of fresh cheeses, due to its ability to acidify the environment following the fermentation of lactose into lactic acid, which thus facilitates the formation of curds.
- Several organic acids are produced industrially by microbial means, such as acetic acid and citric acid, produced by the species of the genus Acetobacter and the fungus Aspergillus niger, respectively.
- Pharmaceutical field: among the major sources of drugs that exist on the market are microorganisms, in particular bacteria members of the Actinobacteria phylum, which are the source of 80% of antibiotics that exist on the market.
- Field of bioremediation and environmental depollution: Thanks to its ability to biotransform toxic pollutants, microorganisms can be used in the decontamination of polluted environments, this process is called bioremediation.
- Renewable energy field: microorganisms play a vital role in the renewable energy field, in fact, through the intervention of several microorganisms, organic waste can be converted into biogas (methane) or bioethanol, which are then used as fuels to replace fossil energy.Other applications are described in **Table 1**.

Table 1: Some examples of the application of microorganisms in industry.

Microorganism	The product of fermentation	The app
Saccharomyces cerevisiae	Ethanol	**Fine chemistry**
Pseudomonas	2 keto-gluconic acid	**An intermediate in the production of ascorbic acid (vitamin C); precursor of acid synthesis isoascorbic.**
Aspergillus niger	Pectinase, protease	**Clarification of fruit juices**
Bacillus subtilis	Amylase	**Preparation of modified starch; To use during gluing of paper.**
Bacillus subtilis	Protease	**Protein hydrolysis**
Micrococcus glutamicus	Lysine	**Food additive**
Leuconostoc mesenteroides	Dextran	**Food stabilizer**
Gluconobacter suboxydans	Sorbose	**Ascorbic acid production**
Streptomyces olivaceus	Cobalamin (Vitamin B12)	**Dietary supplement**
Recombinant of E. coli	Insulin	**Human medicine**
Streptococcus thermophilus, Lactobacillus bulgaricus	Yogurt	**Starter in the dairy industry**
Candida utilis ***Fusarium*** **graminearum**	Proteins of unicellular origin (P.U.O)	**Human and animal nutrition**
Cephalosporium acremonium ***Saccharopolyspora erythrea***	Cephalosprin Erythromycin	**Antibiotics for treating infections**

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Chapter 01: Industrial Microorganisms and Product Formation

1. Industrial microorganisms:

1.1. Definition :

Microorganisms, also called microbes, are living things too small to be seen with the naked eye. The group includes bacteria, fungi (yeasts and molds), protozoa and microscopic algae. It also includes viruses, which are non-cellular entities located at the boundary of the living and non-living world.

1.2. Microorganisms used in industry:

Microorganisms used in industry are selected, most often empirically, for their particular metabolic abilities for transforming a substrate or biosynthesising a product.

These properties are originally present in wild microbial strains from the natural environment. These strains are isolated then improved in the laboratory by mutation and then selection of the most efficient mutants.

1.2.1. Bacteria :

Bacteria are organisms invisible to the naked eye. They are prokaryotes i.e. lacking a true nucleus, their genetic material is immersed in the cytoplasm. Depending on the composition of their wall, two large groups are distinguished: Gram negative and Gram positive. They exist in different shapes (cocci, sticks, etc.) and methods of grouping (chain, cluster, etc.).

1.2.1.1. Lactic bacteria:

These bacteria all have the fundamental property of transforming carbohydrates into lactic acid through the process of lactic fermentation.

It is this property which is exploited in the preparation of fermented dairy products (cheeses, yogurts). In fact, the metabolization of lactose (milk sugar) into lactic acid and the accumulation of the latter in the medium gradually lowers the pH of the milk by causing its spontaneous ripening (coagulation of proteins leading to the formation of curds). These bacteria are also used to make other fermented foods (sauerkraut, olives, fermented meats,

etc.)

They are present naturally in the animal microbiota, on plants (grapes and cereals) and in milk. The genera most used in industry are Lactobacillus, Lactococcus, Pediococcus, Streptococcus, Bifidobacterium and Leuconostoc.

1.2.1.2. Acetic bacteria:

These are strict aerobic bacteria that oxidize ethanol to acetic acid during vinegar production. Wine and cider can serve as a substrate for these bacteria, which therefore cause spoilage of these drinks.

They are naturally present on fruits and in the air. The best-known genera are Gluconobacter and Acetobacter.

1.2.1.3. Butter bacteria:

They are, for the most part, strictly anaerobic bacteria which belong to the genus Clostridium. The Clostridium acetobutylicum species is particularly used in the industrial production of butanol.

These bacteria are also used to ret flax and hemp. They attack pectin, a compound found in the middle lamella separating the cell walls of plant cells and which gives rigidity to the fibers. This process facilitates the release of textile fibers cemented by pectin.

1.2.1.4. Bacillus:

These are aerobic bacteria. They have a marked industrial interest since they produce toxins which are fatal for other bacterial species or for other types of living beings. Thus, the species Bacillus thuringiensis produces a protein which is toxic to the larvae of several insects: mosquitoes, black flies, spruce budworms, butterflies.

The bacilli cultivated in large capacity bioreactors are harvested, then the microbial suspension is incorporated into an emulsion which will act as a bioinsecticide.

1.2.1.5. Glutamic bacteria:

These bacteria of the Corynebacterium and Brevibacterium genus are used for the industrial production of amino acids (glutamic acid, lysine, tryptophan) as food additives.

1.2.1.6. Enterobacteria:

They are one of the largest families of bacteria and are grouped into many genera, such as Escherichia, Salmonella, Shigella, Proteus and Yersinia. These microbial species are often encountered in infectious pathology, but they also have several uses in bio-industries: production of 2,3-butanediol and organic acids, development of toxicity bioassays, manufacture of antiviral agents, etc. The Escherichia coli species is the most used in bioprocesses. Thanks to recombinant DNA technology, several strains of this common bacterium of the intestinal flora of mammals are exploited to produce recombinant proteins and plasmids.

1.2.2. Fungi:

They are unicellular or multicellular eukaryotic organisms, they are ubiquitous, i.e. are found wherever there is a food source. They are subdivided into two large groups: Yeasts (single-celled organisms) and molds (multi-cellular organisms)

1.2.2.1. Yeasts:

Thanks to their very important biochemical activities, yeasts are abundantly involved in the manufacture of leavened bread, alcoholic beverages and even in the production of recombinant proteins from foreign genes, including insulin, interferon and several classes of enzymes. The most used species is certainly the brewer's yeast Saccharomyces cerevisiae, the genetics of which are very well known, but the yeasts Pichia pastoris and Kluyveromyces lactis can also be used for the production of proteins.

1.2.2.2. Molds :

The use of molds is very common in the cheese industry (production of Camembert and Roquefort). But they are also used in the chemical and pharmaceutical industries, for the production of metabolites of major economic or medical interest: antibiotics (penicillin, cephalosporins), vitamins (B12), enzymes (amylases, proteases), organic acids (citric acid) and proteins.

1.2.3. Microalgae:

These are microscopic unicellular or multicellular species, eukaryotes, capable of ensuring their nutrition by photosynthesis thanks to the chlorophyll they contain. They also have pigments such as carotenoids, xanthophylls and phycocyanins which provide their coloring and play an important biochemical role. These microorganisms are widely distributed in fresh or saline waters and in soil. Microalgae are used in several fields (pharmaceuticals, cosmetics, food, bioenergy production).

Microalgae should not be confused with cyanobacteria, incorrectly called "blue-green algae", because they are two very different organisms on a biological level: the first are eukaryotic microscopic algae and the second constitute a branch of bacteria, therefore prokaryotes. . However, from a biochemical point of view, these two microorganisms are photosynthetic and are therefore often associated when it comes to their industrial valorization.

1.3. Obtaining industrial microorganisms:

1.3.1. The choice of strains:

The selection of a microbial species for carrying out a bioprocess is not based solely on the condition that it can synthesize a potentially useful compound or carry out a particular metabolic pathway. Indeed, the imperatives of profitability force manufacturers to look for strains which can meet other equally important criteria which will make it possible to optimize a biological process. The main criteria sought are:

1- Rapid growth on inexpensive organic substrates (e.g. molasses, corn liquor, whey).

2- Easy and abundant cultivation.

3- Development of products that are easy to extract and separate and in abundant quantity.

4- Obtaining the desired transformations in a simple and rapid way, with high yield and minimum energy.

5- Conservation of biochemical properties of strains over time (genetic stability)

6- Non-pathogenic

1.3.2. Improvement of strains:

1.3.2.1. The constraints of wild strains:

It is rare for natural strains to meet all of these criteria. They often have limited performance that must be amplified to reach the industrial profitability threshold. In addition, they sometimes have undesirable secondary characteristics that must be corrected before their use in bioprocesses can be considered.

- **Limited performance:**

Generally speaking, the productivity of a wild strain freshly isolated from the natural environment is quite low. It can be significantly increased by modifying the genome of the microorganism. Indeed, microorganisms are always equipped with metabolic regulation mechanisms, often of the negative feedback type, allowing them to produce, in nature, only the quantity of enzymes and metabolites they need to survive in a competitive environment. . By bypassing these mechanisms through genetic modifications, we can generate more productive microorganisms.

- **Undesirable characteristics:**

The wild strain may present certain undesirable characteristics which may also be modified, such as sensitivity to a bacteriophage, the propensity to generate a lot of foam in a liquid medium or the synthesis of a by-product which is difficult to eliminate during product purification. .

1.3.2.2. Techniques for improving wild strains:

The selection of more efficient strains requires the implementation of a genetic improvement program; induced mutation or DNA recombination techniques are frequently used.

- **The mutation :**

Mutation is a spontaneous process that occurs naturally and quite frequently in microorganisms, due to the rapid rate at which they divide. The principle is this: each time a cell divides, there is a small probability that a DNA replication error will occur and generate a cell carrying modified DNA. Thus, by simply allowing a microorganism to divide in a selective medium which promotes the growth of cells possessing the character we are looking for, we can hope to have a more efficient natural variant which we can then isolate.

However, it is more effective to increase the rate of mutation by inducing it using physical mutagens, such as UV rays, or chemical mutagens, such as nucleotide analogues. We first expose a microbial population to the mutagenic agent, then we inoculate it on a selective medium making it possible to isolate the most efficient mutants. This technique relies largely on chance, it involves quite exhaustive work in which the procedures for selecting and screening mutants are decisive.

- **Recombinant DNA:**

The recombinant DNA technique is more recent and also more efficient for the improvement of microbial strains. Indeed, it makes it possible to directly modify a targeted gene and therefore act precisely on a metabolic pathway.

However, this technology is expensive and involves complicated work, since it is necessary to know in detail the metabolism and genome of a strain to be able to modify it so precisely.

Despite this, it is booming in the industry, because it allows the expression of foreign genes in microorganisms, such as the bacterium Escherichia coli and the yeast Saccharomyces cerevisiae, whose genetics are perfectly known.

Table of content Chapter 02 Culture media and industrial microbiology

1 Introduction.

Industrial culture media must satisfy the nutritional requirements of the microorganism to allow optimal growth of cellular biomass, and at the same time, must provide the necessary nutrients for the biosynthesis of target metabolites.**(Okafor et al, 2020).**

Industrial fermentation is most often carried out on a liquid culture medium, although some fermentations are carried out on solid culture media.**(Tomorrow and 1981).**

2 Factors determining the choice of industrial cultivation medium.

The main factors that affect the choice of industrial growing media are as follows:**(El-Mansi et al, 2018):**

- Cheap (inexpensive) substrate.
- Constant physico-chemical quality even after sterilization.
- Availability throughout the year.
- Low transport and storage costs, especially in relation to temperature.
- Easy to handle in solid or liquid form.
- Easy to sterilize.
- A viscosity which does not interfere with agitation or aeration of industrial culture medium during the fermentation process.

3 The composition of industrial culture medium.

The composition of the culture medium must include a source of carbon which is most often the source of energy, in addition to a source of nitrogen, phosphorus and sulfur. Trace elements must also be present in the industrial culture medium. Certain demanding micro-organisms require the presence of certain growth factors, such as certain vitamins or amino acids **(Waites et al, 2009).**

3.1 Carbon sources.

The quantity as well as the quality of the carbon source used as an ingredient in the industrial culture medium can be determined from the biomass yield coefficient (Y_{carbon}), which is the ratio between the quantity of biomass produced and the quantity of carbonaceous substrate utilized (g of biomass / g of substrate)**(Waites et al, 2009).**

$$Y_{carbon}(g/g) = \frac{\text{biomass produced (g)}}{\text{carbon substrate utilized (g)}}$$

For cost reasons, pure carbon sources, such as carbohydrates, such as glucose or sucrose, are rarely used during fermentations on an industrial scale, however, other less expensive carbon sources can be used:

- **Molasses**: it is a by-product resulting from the refining of sugar extracted from sugar beet or sugar cane. It is a viscous, dark-colored syrup containing 50 to 60% (w/v) carbohydrates, mainly sucrose, as well as 2% (w/v) nitrogenous substrates, in addition to some vitamins and salts. minerals**(Koval et al, 2019).**
- **Malt extract:** Aqueous extracts of malted barley can be concentrated to form a syrup very rich in simple sugar and disaccharide, in addition to certain vitamins, peptides and amino acids. These substrates can be used for the cultivation of filamentous fungi, yeasts and actinomycetes**(Cvetković et al, 2002).**
- **Starch and dextrin:** Starch is obtained, most often, from corn, but it can also be obtained from other cereals. To be used as a source of carbon and energy, starch first undergoes hydrolysis into sugar syrup, containing mainly glucose and dextrin, by dilute acids or amylolytic enzymes.**(Laluce et al, 1988).**

- **Whey:** Also called whey, it is the result of milk coagulation. The

composition of whey varies depending on the method by which it was obtained, in fact, the whey obtained by rennet is very rich in serum proteins as well as lactose, while the whey obtained by lactic fermentation of milk, is rich in serum protein and calcium**(Pescuma et al, 2015).**

3.2 Source of nitrogen.

Most industrial microorganisms can use nitrogen sources in organic and inorganic forms. Inorganic nitrogen can be supplied in the form of ammonium salts, such as, ammonium sulfate (SO4-NH4+) and diammonium phosphate ($(NH_4)_2HPO_4$), while sources of organic nitrogen include amino acids, proteins and urea**(Kampen et al, 2014).**

The nitrogen source is often supplied in a raw form, which is primarily by-products from the food industry, such as corn liquor, yeast extracts, peptones and soy flour. Pure amino acids are only used in certain special cases**(Waites et al, 2009)**.

3.3 Mineral salts.

Mineral salts such as cobalt, copper, iron, manganese, molybdenum and zinc are present in sufficient quantities in water as well as other ingredients in the industrial culture environment. For example, corn steep liquor contains a satisfactory quantity of mineral salts to meet the fermentation requirements.**(Zhou et al, 2006).**

3.4 Vitamins and growth factors.

Many bacteria can synthesize all the necessary vitamins from basic elements. While other microorganisms require the addition of vitamins to the industrial culture medium. Other growth factors are needed, such as amino acids, fatty acids and sterols**(Chang 1999).**

3.5 O_2 intake.

According to the microorganism's O needs$_2$, as well as its respiratory type, oxygen is injected into the fermenter as air containing approximately 21% (v/v) oxygen, or it can be supplied as pure oxygen when microorganism requirements are particularly high. The air or oxygen injected into the fermenter must be sterilized by filtration **(Waites et al, 2009)**.

3.6 Adding antifoams.

Antifoams are necessary to reduce foam formation during fermentation, which is mainly due to proteins in the culture medium. If foam formation is not controlled, it can block air filters, resulting in loss of asepsis. There are three approaches to controlling foam formation: (i) modification of the composition of the culture medium; (ii) use of mechanical foam breakers;

(iii) the addition of chemical anti-foam products, which are surfactant molecules, such as for example vegetable oils (soybean oil, sunflower oil and rapeseed oil), fish oil**(Pelton et al, 2002).**

Chapter 3: Product Formation: Fermentation Technology

List of Figures

1 Fermenters or industrial bioreactors.

A bioreactor, also called a fermenter or propagator, is a device in which microorganisms (yeasts, bacteria, microscopic fungi, algae, animal and plant cells) are multiplied for the production of biomass, or for the production of a metabolite or again for the bioconversion of a target molecule **(Erickson, 2019)**. A bioreactor comprises **(Figure 1):**

1. A culture enclosure, made of glass or stainless steel, with a variable volume ranging from a few liters to several cubic meters in the case of industrial units. The tank is hermetically closed and does not allow air from the interior or the exterior environment to pass through.
2. An agitation system is used to ensure agitation and aeration of the culture, it is formed by an external motor, and one or more internal turbines (depending on the size of the fermenter).
3. A syringe to inject culture medium or nutrients.
4. Probes for checking temperature (thermometer), pH (pH meter), dissolved oxygen concentration (oximeter probe),
5. A control unit managed by a computer makes it possible to record and control all operating parameters.

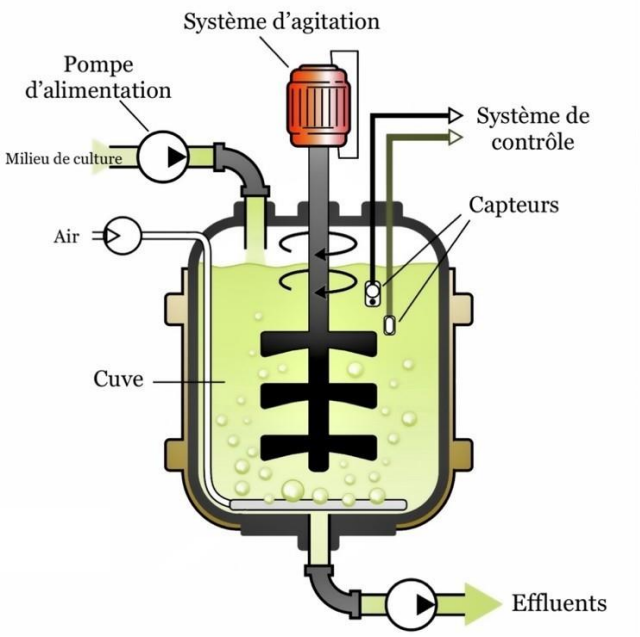


Figure1: Diagram of a bioreactor

2 Bioreactor design:

Bioreactors are classified according to their maximum volume:

1. Laboratory bioreactors that can be sterilized by autoclave up to 18 L
2. Laboratory bioreactors that can be sterilized in situ up to 30 L;
3. Pilot bioreactors up to 300 L;
4. Industrial bioreactors up to 500,000 L (500 m3).

2.1 Choice of installation location:

The bioreactors are located in premises, isolated and far from the laboratory. In order to choose the location installation of a bioreactor, two specific conditions must be taken into account:

- The preliminary examination of all risks of microbial pollution of air or water.
- The possibility of having a sufficient quantity of water to ensure cooling of the bioreactor.

2.1.1 Choice of bioreactor:

The choice of bioreactor as to him is governed by several criteria:

- Type of microorganisms used, we distinguish: aerobic or anaerobic bioreactors.
- Perfect contact between the liquid phase (the medium) and the solid phase (the biomass).
- Good transfer of material between the cell and the culture medium.
- Good oxygen transfer.
- Easy transfer of heat to the cells, then from the cells to the outside.
- Easy collection of products.

2.1.2 Preparation of the bioreactor:

Starting a bioreactor is controlled by the following parameters:

2.1.2.1 Sterilization:

Sterilization is done by heat treatment, using two methods:

✓ **In-situ sterilization:**

- Injection of water vapors at 121°C for 20 to 30 minutes
- Injection of superheated water (120°C to 140°C), duration varies depending on the size of the bioreactor
- Heating of the enclosure by resistors

✓ **Ex-situ sterilization:**

It concerns bioreactors in glass of small volume and is done by autoclaving.

2.1.2.2 Gas injection:

Some microorganisms require carbon dioxide (CO_2) or dinitrogen (N_2) for their growth. These gases are stored in pressurized bottles and are introduced into the bioreactor after passing through a sterilizing filter.

2.1.2.3 Temperature:

Cells generate energy in the form of heat during cultivation, causing temperature variations. Therefore, we use a probe which detects these variations to control the temperature suitable for growth.

Heating or cooling of the enclosure is ensured by an isolated water circuit (system cooling) which is not in contact with microorganisms.

2.1.2.4 Pressure:

Some productions require putting the bioreactor under pressure, for this manometer (pressure measuring device) are placed for permanent monitoring of the pressure level in all parts of the installation.

2.1.2.5 *The agitation:*

This parameter is essential because it allows the culture medium,

temperature and pH to be homogenized, which prevents cell aggregation.

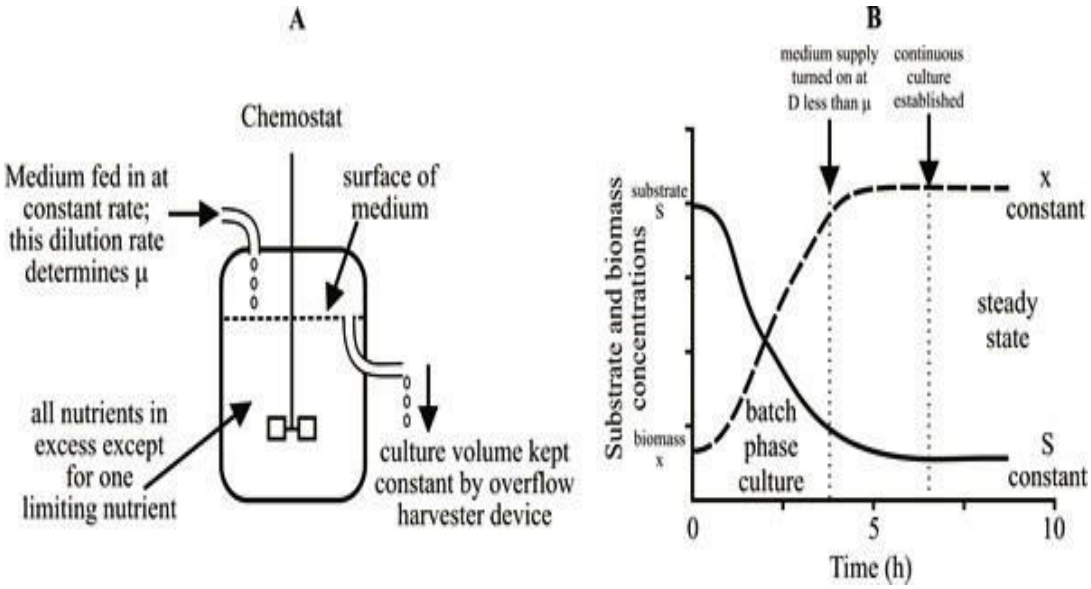
3 Cultivation methods in a bioreactor:

There are three types of fermenters:

3.1 Discontinuous (or batch) mode.

In this type of fermenter, the system is closed and maintains a constant volume. The tank is filled with the sterile culture medium, then it will be inoculated with the industrial strain. The fermentation takes place under stirring, and throughout the fermentation, the volume of the culture remains constant without additional introduction of culture medium. However, neutralization reagents, or even an anti-foam product, can be added **(Carmaux, 2008) (Figure 2).**

Figure2:Batch type fermenter (discontinuous) (Carmaux, 2008).



The biomass concentration increases according to the microbial growth curve. At the same time, the substrate is consumed by the microorganism and the concentration of desired products (P) increases. At the end of the culture, the fermenter is emptied and the desired product is extracted. **(Oosterhuis et al, 2011) (Figure.3).**

3.1.1 Advantages of batch fermentation.

- The desired product can be collected at any time.
- The risk of contamination is low.

3.1.2 The disadvantages of batch fermentation.

- The latency time is very long.
- The duration of the exponential phase is very short, so the biomass and the final product are produced in small quantities: limited yield.

3.2 Fed Batch (discontinuous and fed fermenter).

Figure3: Evolution of cell growth rate (X), relative to substrate conversion rates (S), metabolite production (P), during an industrial fermentation **(Oosterhuis et al, 2011).**

In this type of fermentation, in order to reduce the time of the lag phase and at the same time ensure a longer duration of the exponential growth phase, the culture begins with the use of a small volume of culture medium called a foot. tank, which will be inoculated with a microbial inoculum. When the microorganism reaches the exponential growth phase, the sterile culture medium is introduced into the tank. The feed rate is adjusted so that the substrate concentration is constant in the tank, and at the same time without exerting an inhibitory effect on biomass production (Figure 4). Fermentation is stopped as soon as the tank is filled with the culture medium. It should be noted that the risk of contamination in this type of fermenter is higher **(Evgenios et al, 2020).**

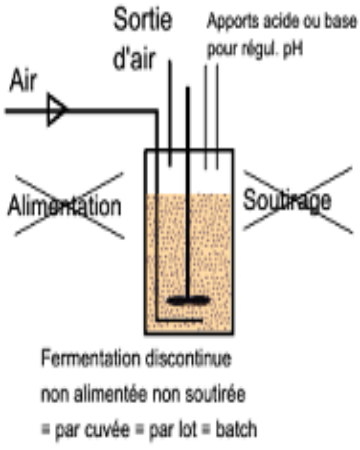


Figure 4: Fed Batch type fermenter (Evgenios et al, 2020).

2.1. Continuous fermentation.

In this type of fermentation, the exponential phase of microbial growth is permanently maintained, thanks to a regular addition of new culture medium by a constant flow rate, which allows a replenishment of nutrients and the maintenance of pH; and at the same time an equivalent quantity of added culture medium must be withdrawn, which helps avoid the accumulation of waste (Figure 5) (**Sarkar et al, 2003).**

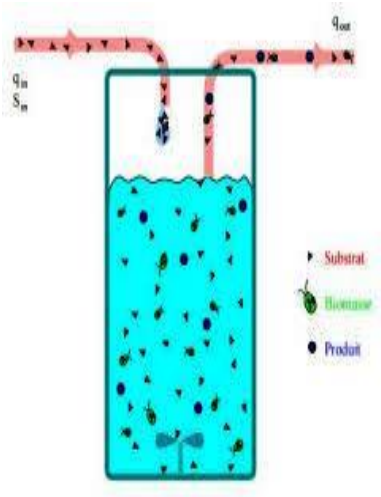


Figure 5: Fed Batch type fermenter ***(Sarkar et al, 2003)***.

Among the devices used, the turbidostat which is a culture fermenter in continuous mode (Figure 6). Thanks to turbidimetric control, the concentration of the culture medium is kept constant.

- If the microbial load tends to increase too much, new medium is added in order to dilute and return the microbial disorder to its initial value.
- If the microbial load decreases, there is a reduction in the supply of new medium until growth returns to its initial value.

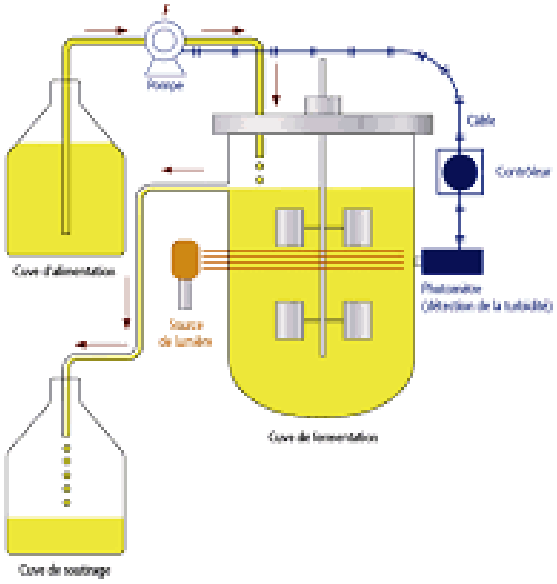


Figure6: A continuous bioreactor of the turbidostat type (Lee et al, 2011).

3.3 Scaling (or extrapolation) process: scale up.

Whatever the field of industrial microbiology applications, the transition from laboratory Erlenmeyer flasks to industrial bioreactors remains a challenge. Hence the idea of a scaling process, or scale up in English, which consists of transferring the microbial culture, prepared in Erlenmeyer flasks in the laboratory, to small volume laboratory bioreactors, then to pilot bioreactors, then the culture will be introduced into an industrial fermenter (Figure 10). To ensure the success of the extrapolation, the various physicochemical parameters are analyzed and then modified during each step of the scale-up, because the physicochemical and enzymatic reactions of the microbial cells, which occur inside the bioreactor vary. depending on the volume of the reactor used. So, we seek to obtain the same yield, despite the increase in the volume of the crop **(Levin, 2001; Schmidt et al, 2005; Xing et al, 2009).**

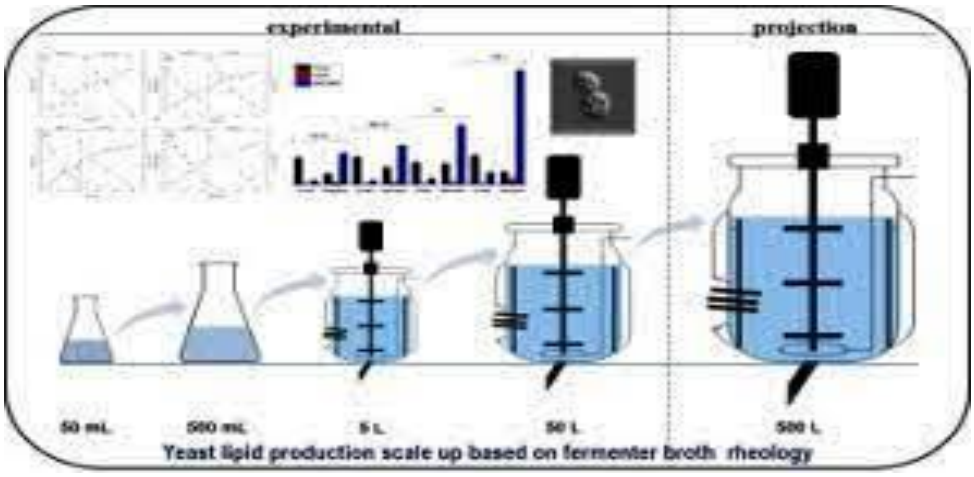


Figure7:Scaling up process (Levin, 2001).

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1 Secondary metabolites obtained by microbial fermentation "Pharmaceutical Products"

The role of microbiology in advancements in the pharmaceutical and medical industry has led to great discoveries. The biochemical properties of microorganisms are in fact exploited in numerous pharmaceutical processes such as the synthesis of antibiotics, vaccines and other molecules of medical interest.

1.1 Antibiotics

Antibiotics are natural or synthetic substances which have the property of inhibiting growth (bacteriostatic activity) or even destroying target bacteria (bactericidal activity). Antibiotics are naturally produced by microorganisms (bacteria and fungi) during their growth to survive in a competitive environment.

The immense therapeutic value of antibiotics has required the development of industrial techniques for the production of these molecules. Antibiotics are most often manufactured microbiologically, exploiting the metabolic activity of a few bacteria and fungi on an industrial scale.

1.1.1 Overall procedure for the industrial production of antibiotics:

The production and marketing of a new antibiotic must go through a series of studies at several levels:

1. Preliminary studies to isolate and conserve strains of interest.
2. Study of laboratory production by fermentation.
3. Analytical studies of physical and chemical properties.
4. Pharmacological studies of antimicrobial activities as well as toxicity and therapeutic effect on animals.
5. Study of industrial production for the implementation of manufacturing and control methods.
6. Economic and commercial study.

1.1.2 Qualities sought in a new antibiotic:

For suitable medical use, the new antibiotic must present a number of qualities:

- The absence of toxicity.
- Solubility in water at acceptable pH.
- Stability.

- The absence of pyrogens and histamine compounds.
- Good tolerance.
- The absence of side effects on the cell, serum, red blood cells and leukocytes.

1.2 Examples of antibiotic production:

1.2.1 Penicillin:

Despite its discovery by Fleming in 1929, penicillin was not manufactured industrially until 1941. Since that date, numerous researches have brought such improvements that this production currently constitutes a highly efficient microbiological process.

This antibiotic can be produced by many species of *Penicillium* and *Aspergillus*. However, the first strain Fleming isolated, *Penicillium notatum*, produced only a small amount of the antibiotic. In 1946, the NRRL (Northern Regional Research Laboratory, USA) isolated a more productive species, *Penicillium chrysogenum*, which was then improved by a series of mutations and selections. As a result, the variants used today produce 55 times more Penicillin than the strain isolated by NRRL, and thousands of times more than the one isolated by Fleming.

Penicillin is produced by fermentation in aerated and stirred vats. Penicillium are capable of producing 5 types of penicillin (penicillin G, X, F, K and dihydropenicillin F). However, penicillin G was favored due to the ease of its manufacture.

1.2.1.1 I.3.1. Tetracycline:

First discovered in 1948 by Duggar, tetracyclines constitute the most economically important group of antibiotics thanks to their broad spectrum of activity and low toxicity. Their synthesis has been mainly attributed to the species *Streptomyces aureofaciens*, whose production capacities have been considerably increased by selective isolations and induced mutations.

Besides Streptomyces aureofaciens, many other species can produce tetracycline: *Streptomyces mediolanum, Streptomyces psammoticus, Streptomyces antibioticus, Streptomyces viridifaciens*, etc.

Tetracycline is produced by the fermentation process in aerated and agitated stainless steel or aluminum fermenters. Manufacturing takes place in two stages: a growth stage and a production stage. Fermentation generally lasts two days.

2 Vitamins

Vitamins are active organic substances essential in small quantities for the metabolism of living organisms (growth factors). Man and higher animals cannot biosynthesize vitamins, so they must find them in their food. However, the absence or insufficiency of vitamins in the diet leads to the appearance of very serious deficiency diseases.

I.1. Production of vitamins by microorganisms:

Microorganisms are capable of synthesizing vitamins from several compounds widespread in nature, hence the interest in the industrial application of this property in the manufacture of some types of vitamins.

However, the production of vitamins by microorganisms is generally low and these factors do not accumulate in the culture medium. Therefore, the manufacture of vitamins by chemical processes proves to be more economically and practically advantageous.

However, we can see that yeasts (*Saccharomyces cerevisiae* and *Candida utilis*) constitute an important source of vitamins and produce a vitamin complex called "complex B" (B1, B2, B3, B5, B8, B9) which is used in industry. human and veterinary pharmaceuticals.

In addition to yeasts, other microorganisms also have the property of producing mixtures of various B vitamins such as Bacillus *polymyxa*, Bacillus *megatherium* and *Aspergillus aerogenes*. For most vitamins, microbiological production is associated with the addition of precursors to the culture medium. Table 01 presents some examples of vitamins produced by microorganisms.

Table 1: Examples of vitamins produced by microorganisms

Thiamine (vitamin B1)	***Ashbya gossypii; Torula utilis Sacharomyces cerevisae; Eremothecium ashbyii***
Riboflavin (vitamin B2)	***Clostridium acetobutylium; Ashbya gossypii; Eremothecium ashbyii ; Pichia miso***
Cobalamin (vitamin B12)	***Bacillus megaterium; Streptomyces olivaceus; Pseudomonas denitrificans; Propionibacterium shermanii***
Retinol (vitamin A)	***Blakeslea trispora; Rhodotorula gracilis; Dunaliella sp.***
Calciferol (vitamin D)	***Saccharomyces cerevisiae; Aspergillus niger***
Tocopherol (vitamin E)	***Euglena gracilis***

2.1 Examples of vitamin production:

2.1.1 Riboflavin (vitamin B2):

Riboflavin, also called vitamin B2, plays a fundamental role in the formation of ATP. It is found in microorganisms linked to certain nucleotides to constitute FMN (riboflavin mononucleotide) or FAD (riboflavin adenine dinucleotide).

Many microorganisms are capable of synthesizing this vitamin in very large quantities exceeding their requirements and which therefore accumulate in the culture medium. In fact, microorganisms produce excess riboflavin in the event of abnormal formation of toxic purines in the environment in order to eliminate this compound.

II.2.1. Cobalamin (vitamin B12):

Cobalamin, also called vitamin B12, is an essential vitamin for the normal functioning of the brain (it participates in the synthesis of neurotransmitters), the nervous system and the formation of blood. It is involved as a cofactor in cellular metabolism, more particularly in the synthesis of DNA and fatty acids as well as in energy production.

Vitamin B12 can only be produced by microorganisms, including bacteria and

Ascomycetes. Therefore, the industrial production of this vitamin by microbiological means has taken on considerable economic importance.

II. Amino acids

Amino acids are the main constituents of proteins. They are vital molecules and their contribution to the functioning of the body is crucial.

Man and higher animals are not capable of synthesizing amino acids and must therefore find them in their food. However, food products of plant origin contain only very low levels of essential amino acids such as lysine, methionine or tryptophan.

II.1. Production of amino acids by microorganisms:

A large number of microorganisms have the property of producing the amino acids necessary for the synthesis of their proteins and their growth from carbohydrates and mineral salts (Table 2).

Table 02: Examples of amino acids produced by microorganisms

Amino acids	Producing microorganisms
Aspartic acid	*Bacillus megaterium; Pseudomonas*
Alanine	*Pseudomonas; Corynebacterium; Brevibacterium*
Arginine	*Corynebacterium glutamicum; Brevibacterium flavum*
Glutamic acid	*Corynebacterium glutamicum; Brevibacterium; Micrococci*
Lysine	*Brevibacterium lactofermentum; Saccharomyces ; Candida*
Methionine	*Corynebacterium glutamicum; Rhodotorula; Candida*
Phenylalanine	*Brevibacterium lactofermentum; Flavobacterium spp; Micrococci*

The industrial production of amino acids by microbiological means is expanding and is currently of great social and economic importance. These methods have managed to compete with classical chemical synthesis techniques.

In addition, the microbiological route makes it possible to directly produce the L forms of amino acids, usable by mammals, unlike chemical methods which can only provide racemic compounds.

II.2. Example of glutamic acid production:

Glutamic acid (glutamate) is not an essential amino acid for humans or animals. However, it is widely used in the food industry as a condiment or as a flavor corrector. Glutamate is also one of the most active neurotransmitters in the brain. It can be extracted from natural sources, such as beet molasses, soy flour, or gluten, but in quantities insufficient to meet demands.

The industrial production of glutamic acid can be carried out according to two main processes:

2.1.1.1 The single-stage process:

In this process, the amino acid is produced directly by a single microorganism. The synthesis of the carbon chain of the amino acid is carried out from intermediate products of carbohydrate metabolism (glycolysis, pentose phosphate pathways and Krebs cycle): Glucose is exidated to form citrate which is then oxidized to acid α-ketoglutaric acid. Glutamic acid is subsequently produced from α-ketoglutarate using glutamate dehydrogenase.

The strains used in this process are *Miccus glutamicus, Corynebacterium glutamicum, Brevibacterium flavum, Brevibacterium lactofermentum and Brevibacterium divaricatum.*

2.1.1.2 The two-stage process:

This process consists of first preparing α-ketoglutaric acid microbiologically, which is then transformed into glutamic acid, either using a second microorganism or enzymatically.

This process is also of considerable industrial importance, due to the fact that the intermediate product, α-ketoglutaric acid, is easily obtained by well-studied microbiological techniques. It can be produced by several kinds of microorganisms from glucose, a source of mineral or organic nitrogen and mineral salts.

3 Vaccines:

3.1 Composition of vaccines:

The vaccine is an antigenic preparation, made from bacteria or complete viruses, their constituents (nucleic acids, polysaccharides, proteins) or their products (toxins), of which the capacity to produce the disease is reduced or removed while retaining that of inducing a protective immune response (immunogenicity).

In addition to the antigen (active ingredient), vaccines contain two classes of components which participate in the formulation of the final product:

- The adjuvant, which has the role of stimulating and directing the immune response.
- The stabilizing agent, responsible for maintaining the properties of the product over a long period of time under defined conditions.

3.2 Production of vaccines:

The production of vaccines on an industrial scale boomed during the second half of the 20th century. The manufacturing process of vaccines is longer and more complex than that of other drugs, since the raw materials are biological substances and living microorganisms that require delicate handling and strict control to ensure the quality of the final product.

Vaccine manufacturing includes two main stages: biological manufacturing and pharmaceutical manufacturing.

3.3 Organic manufacturing:

3.3.1 The germ bank:

The aim of this step is to constitute a “reserve” of the target bacteria or virus. The strains in this bank must be highly stable and free of mutations to guarantee the quality of the final product.

3.3.2 Culture :

The strain is cultured in a bioreactor. The culture of a viral strain is closely linked to cell culture, due to the inability of viruses to multiply outside living cells.

Fermentation takes place under rigorously controlled conditions (time, temperature, pH, agitation, aeration, oxygen content, etc.).

3.3.3 Harvesting the antigen:

After the growth time, the bacterial or viral antigen is harvested from the culture medium by centrifugation or filtration.

3.3.4 The concentration of the antigen:

This operation reduces the volume to be purified. It is based on the concentration of the antigen by fractionation with salts (calcium salts) or solvents (ethanol) and/or by ultrafiltration using filter membranes of well-determined molecular porosity.

3.3.5 Antigen purification:

This step involves removing all impurities from the manufacturing process. It makes it possible to isolate the antigen and separate it from the other components included in the crude extract. The purification technique is based on the distinct physicochemical characteristics of the antigens (chromatography, centrifugation, precipitation, solvent extraction, etc.).

3.3.6 Antigen inactivation:

This operation consists of removing the pathogenicity of microorganisms by physical (heat) and chemical (formalin) processes while preserving their immunogenic capacity, thus obtaining the active ingredient.

3.3.7 Pharmaceutical manufacturing:

The resulting antigen will be used as an active substance for the pharmaceutical production of the vaccine. It will undergo successive treatments (formulation, distribution, freeze-drying, crimping, candling, packaging, batch control and release) in order to produce the finished product.

4 Enzymes

Enzymes are biological catalysts of a complex protein nature. They are purified from various raw biological materials.

4.1 The interest in enzymes in bioprocesses:

Enzymes are widely used in bioprocesses and advantageously replace synthetic chemicals thanks to:

Their biodegradability.

- Their low energy consumption.
- Their high specificity.
- Reducing waste and the risk of pollution.

4.2 Production of enzymes by microorganisms:

Given the availability of microorganisms and their exponential growth, only microbial enzymes produced by fermentation have experienced significant expansion and are thus prepared industrially. In fact, more than half of the enzymes used industrially come from fungi or yeast and around a third are of bacterial origin.

Generally speaking, an enzyme-producing microorganism must provide good enzyme production in a minimum of time and be able to grow on less expensive substrates. Extracellular enzymes are often preferable to endocellular ones (the extraction of which is difficult to achieve).

Each microorganism produces, generally in small quantities, numerous enzymes involved in cellular mechanisms (Table 3). However, some enzymes are produced in much larger quantities by certain microorganisms (for example, for the degradation of complex molecules such as cellulose, starch, or proteins).

Table 02:Examples of enzymes produced by microorganisms

Invertase	*Saccharomyces cerevisae; Kluyveromyces lactis; Aspergillus usamii*
Lipase	*Mucor javanicus; Lucor mihei; Rhizopus arrhizus; Aspergillus effusus*
Pectinesterase, Pectin lyase, Polygalacturonase	*Aspergillus usamii; Aspergillus wentii; Aspergillus niger; Penicillium funiculosum*
α-amylase	*Bacillus licheniformis; BacillusAspergillus niger subtilis; Aspergillus oryzae*
Glucose isomerase	*Streptomyces albus; Actinoplanes missouriensis; Bacillus coagulans*
Lactase	*Kluyveromyces fragilis; Kluyveromyces lactis*

Production media, whether synthetic or complex, must contain the nutrients necessary for microbial growth and production (energy, carbon, nitrogen, phosphorus, sulfur, vitamins, etc.)

The fermenters used can reach volumes of 100 to 200 m3. Depending on the enzymes and processes, fermentation lasts from 30 to 150 hours. It is done in a rich environment, where the physicochemical parameters are continuously regulated: oxygen, pH, temperature, addition of anti-foam. In addition, the measurement of enzymatic activity is carried out at regular intervals.

The enzyme produced can be excreted into the culture medium (extracellular enzymes) or present inside the cell (intracellular enzymes). Depending on the requirement, the commercial enzyme can be crude or purified. In addition, it can be in solid or liquid form.

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Chapter 03 bis industrial fermentation products

1 Products of industrial fermentation.

1.1 Proteins of Single Cell Origin (SCP).

We call “Proteins of Unicellular Origin” (PUO), or Single Cell Proteins (SCP), any microbial biomass rich in proteins intended for human or animal food. SCP are not pure proteins, but also contain carbohydrates, lipids, nucleic acids, mineral salts and vitamins **(Nasseri et al, 2011).**

2 The reason for the global need for proteins of single-cell origin.

Protein sources are mainly covered by animal proteins, represented mainly by meat of different types, however, due to its high price, the diet of many countries has a serious deficit in animal proteins, and the search for new Protein resources is one of the concerns of these countries. Therefore, the motivation for the use of SCP in human food is mainly aimed at overcoming undernutrition in these countries, and therefore makes it possible to satisfy their protein needs. In countries where there are no serious problems of undernutrition, the use of SCP in human food is very limited, and it is rather intended for animal feed. **(Ravindra et al, 2000)**.

3 Microorganisms used for the production of SCP

Generally, four types of microorganisms are used. These are microalgae, bacteria, yeasts and filamentous fungi. Below are some examples **(Gervasi et al, 2018):**

- The yeasts generally used as SCP are mainly: Saccharomyces cerevisiae: used mainly as a food additive.

- *Candida uses*: after inactivation by heating, it is used as a nutritious food, since it is rich in proteins and in free amino acids, and has a light meaty flavor.

- The mold, Fusarium venenatum, is used in the production of Quorn, which is a brand of mycoprotein-based meat substitute with a taste resembling chicken meat. **(Reihani et al, 2019).**

- Cyanobacteria can be used as food supplements, for example spirulina, produced mainly from the species, *Arthrospira platensis* Or *Arthrospira maxima*. Spirulina is rich in proteins (60% protein) and vitamins, as well as mineral salts and trace elements **(Avila-Leon, et al, 2012).**

4 Criterion for choosing a microorganism for SCP production

Before being used as a source of SCP, the microorganism must meet certain criteria, in fact, it must be **(Nangul et al, 2021)**:

- Non-pathogenic
- Possessing a high protein level.
- High growth rate;
- Ease of harvesting;
- Good resistance to variations under production conditions.

5 Industrial production of SCP

5.1 Cultivation conditions.

The ideal ratio for the different sources of carbon, nitrogen and phosphorus, entering into the composition of the culture medium for the production of SCP, must be equal to 100 / 5 / 1. The incubation temperature is generally between 30 and 35°C, depending on the microorganism, while the pH must be maintained between 4.0 and 5.5. A critical parameter is the dissolved oxygen concentration. For aerobic fermentations, the medium should be 40% saturated with oxygen. **(Waites et al, 2009)**.

5.2 Fermentation and purification of SCP

After sterilizing the culture medium, biomass production is carried out in a fermenter. The SCP are then recovered by multiple centrifugations, then stored in barrels, or are dried to obtain a powder free of any living cells. **(Ghanem, 1992).**

2. Advantages and disadvantages of SCPs.

Use of microorganism as a protein source of unicellular origin has certain advantages compared to protein sources of animal or plant origin **(Nasseri et al, 2011)** :

- Rapid growth rate, compared to livestock.
- High protein content (30–80% of dry weight);
- The possibility of using a wide range of cheap (inexpensive) substrates,

including organic waste, for their growth.

- Requires little space and little water, compared to breeding.
- Helps solve an environmental problem.
- SCP production is independent of climatic variations.

A few disadvantages that may accompany the use of SCP as a protein source **(Fabregas and Herrero, 1985) :**

- They can produce toxins or other harmful metabolites.
- The nucleic acid content of SCP limits their use in human food. Indeed, a large consumption of nucleic acids raises the concentration of uric acid in the blood plasma. There is then a risk of precipitation of urea in the tissues and joints, which results in symptoms similar to gout disease.

6 Types of metabolites:

- The metabolites produced by microorganisms are divided into two categories: primary metabolites and secondary metabolites, depending on the phase in which they are produced.

6.1 Primary metabolites:

- Primary metabolites are produced during the phase of exponential growth at the time of cell division. They are produced in abundant quantities by essential metabolic pathways that are common in many living organisms.

- Primary metabolites often arise from energy metabolism and are involved in cell growth and development, such as amino acids (protein synthesis), carbohydrates (energy source and cell wall synthesis), and lipids (energy source and synthesis of cell membranes)

- In industrial fermentation, the primary metabolites of interest may arise from anabolism or catabolism. The first are mainly amino acids and vitamins produced for food or pharmaceutical purposes, while the latter are mainly alcohols, solvents and organic acids used in the food or chemical industry **(Table 01).**

Table 1:Some examples of primary metabolites

Metabolite	Producer microorganism	Industrial interest
Organic acids		
Lactic acid	*Lactobacillus*	- Manufacturing fermented foods - Preservative
Acetic acid	*Acetobacter aceti*	- Manufacturing of vinegar - Preservative
Citric acid	*Aspergillus niger*	- Food additive - Pharmaceuticals and cosmetics
Alcohols		
Ethanol	*Saccharomyces cerevisiae*	- Fuel - Solvent industrial
Butanol	*Clostridium acetobutylicum*	-Industrial solvent
Amino acids		
Glutamic acid	*Corynebacterium glutamicum*	- Flavor enhancer
Lysine	*Brevibacteriumlactofermentum*	- Food supplement
Vitamins		
Cobalamin (B12)	*Propionibacteriumshermanii*	- Dietary supplement
Riboflavin(B 2)	*Eremothecium ashbyii*	- Dietary supplement

6.2 Secondary metabolites:

Secondary metabolites, for their part, are produced late during the stationary phase, and even sometimes during the decline phase. They are produced in small quantities and are not directly involved in the fundamental physiological processes of the body.

These metabolists are obtained by particular metabolic pathways exclusive to a few species and which usually give them a survival advantage in the natural environment, such as: antibiotics, growth factors and enzymatic inhibitors (table 02).

Table 2:Some examples of secondary metabolites

Metabolite	Producer microorganism	Industrial interest
Penicillin	*Penicillium chrysogenum*	- Antibiotic
Streptomycin	*Streptomyces griseus*	- Antibiotic
Benzaldehyde	*Trametes suaveolens*	- Almond aroma

6.3 Reminder: microbial growth:

During growth, there is, on the one hand, a depletion of the culture medium in nutrients and, on the other hand, an enrichment in metabolic by-products, as well as in growth waste.

Microbial growth includes 5 phases (figure 1):

1. **Latency phase:** the growth rate is zero (μ = 0). This is the time needed for the bacteria to adapt to the new substrate (synthesis of the enzymes necessary for growth).
2. **Exponential growth phase:** growth rate reaches peak(μ=max). The growth rate is constant and the bacteria doubling time is the shortest.
3. **Slowdown phase:** the growth rate declines. There is an exhaustion of the culture medium, an accumulation of waste and the beginning of autolysis of the bacteria.
4. **Stationary phase:** the growth rate becomes zero (μ = 0). Bacteria begin to synthesize metabolites to better resist damage.
5. **Decline phase:** the growth rate is negative (μ < 0). All nutritional resources are exhausted. There is accumulation of toxic metabolites. A reduction in viable organisms and cell lysis occurs under the action of endogenous proteolytic enzymes. However, growth persists through the release of metabolites during cell lysis.

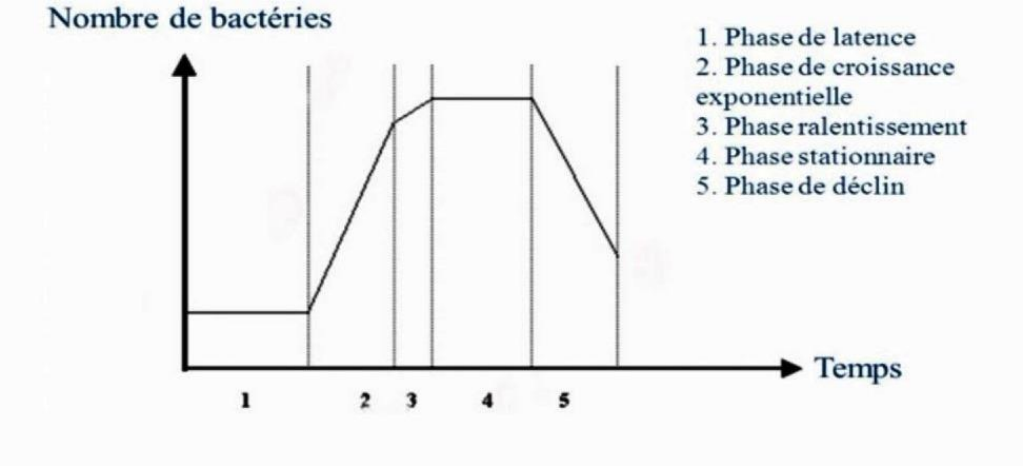

Figure1:The microbial growth curve

7 Primary metabolites obtained by microbial fermentation.

7.1 Amino acids.

Many microorganisms can synthesize amino acids from inorganic nitrogen compounds via industrial fermentation processes. These amino acids are mainly used as food supplements for human or animal nutrition. However, several amino acids are used as ingredients in pharmaceutical and cosmetic products, and in the chemical industry for the manufacture of polymers, for example glutamic acid, lysine, and tryptophan. **(Demain et al, 2008).**

In the rest of the course, we will discuss the example of the industrial production of glutamic acid.

7.2 Example of the industrial manufacture of glutamic acid.

Glutamic acid is a non-essential amino acid, produced industrially in the form of Monosodium Glutamate (MSG, E621). This molecule is used mainly as a food additive to enhance the taste of several food products, because it is responsible for the umami taste. **(Ikeda, 2003).**

1.1. The main microbial strains used.

Glutamic acid-producing bacteria include species that all belong to the Actinobacteria phylum, including Arthrobacter, Brevibacterium, Corynebacterium, Microbacterium and Micrococcus. They are Gram-positive bacteria, immobile, strictly aerobic, auxotrophic for biotin **(Tatsumi, 2012)**.

The industrial strains used for the production of glutamic acid are mutants of the species Corynebacterium glutamicum, Corynebacterium callunae, Brevibacterium flavum and Brevibacterium lactofermentum. In order to overcome the constraints encountered during the industrial production of glutamic acid, several steps are followed. **(Hermann et al, 2003; Ikeda, 2013) :**

- Genetic modification of industrial strains which mainly aims to block the metabolic pathways which lead to the biosynthesis of undesirable by-products.
- The inhibition of the regulatory mechanism of glutamic acid production by feedback inhibition, in fact, in wild strains of Corynebacterium glutamicum, when the final product accumulates and reaches a desirable final concentration, the cytoplasmic production of the glutamic acid is inhibited by the feedback inhibition mechanism,
- Decrease the activity of the enzyme complex, α-ketoglutarate dehydrogenase, also called, oxoglutarate dehydrogenase complex (OGDC), which are Krebs cycle enzymes, catalyze the conversion reaction ofα-ketoglutarateAtsuccinyl-CoA **(Figure 2)**.

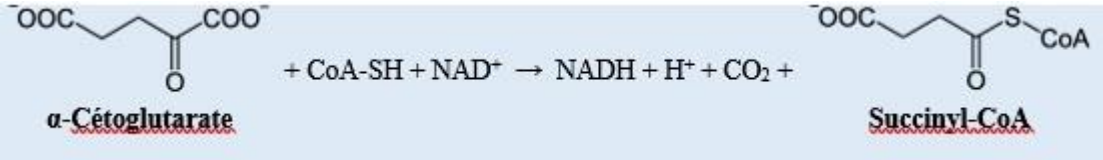

Figure2:Conversion reaction of α-ketoglutarate to succinyl-CoA (Ikeda, 2013).

Indeed, the production of glutamic acid is catalyzed by the enzyme glutamate dehydrogenase (Figure 3), which uses oxoglutarate as a substrate (α-ketoglutarate), which is a Krebs cycle intermediate **(Ertan, 1992).**

Figure3: Reaction of the formation of L-glutamic acid (Ertan, 1992).

In order to direct the metabolism of mutant strains towards an overproduction of glutamic acid, the activity of the enzyme, oxoglutarate dehydrogenase (OGDC), decreases under conditions limiting biotin, which allows an accumulation of α-ketoglutarate and therefore leads to an improvement in the production yield of glutamic acid. It is important to note that

the optimal concentration of biotin to produce glutamate is between 2 and 5 µg/L **(Schultz et al, 2007).**

1.2. The main treatments used to promote the secretion of glutamic acid.

As glutamic acid biosynthesis is cytoplasmic, and mutant strains do not secrete glutamate, a range of treatments are used to make cells more permeable and facilitate the release of the amino acid into the external environment. These treatments include:

- The limitation of biotin during glutamic fermentation is the cause of the decrease in the level of membrane phospholipid, which has the effect of increasing the ratio of saturated fatty acids/unsaturated fatty acids, which is the cause of glutamate excretion **(Gutmann, 1992).**
- The addition of penicillin appears to be used during the industrial production of glutamic acid. Indeed, penicillin inhibits peptidoglycan biosynthesis in producing strains, which causes an enlargement of cell size, and at the same time, causes an improvement in the yield of glutamate production. **(Kim et al, 2010).**
- Treatment with surfactants, especially with Tween 40, in fact, it was observed that final concentrations of 80 g/L in glutamate were obtained by the C. glutamicum strain, using Tween 40 **(Kim et al, 2009; Hoischen and Kramer, 1990).**

1.3. Cultivation conditions.

Fermentation is carried out in stainless steel fermenters with a maximum capacity of 450 m. The carbon sources used are generally glucose or sucrose. Sugar cane or beet molasses can also be used. The nitrogen source (ammonium salts, urea or ammonia) is fed slowly to avoid inhibition of L-glutamate production **(Hirasawa et al, 2016).**

The culture is kept shaking under aerobic conditions, at a temperature of 30–37 °C, depending on the microorganism used. To avoid decreasing the pH of the culture medium, as L-glutamate is excreted into the medium, the pH is maintained between 7 to 8. The duration of fermentation is normally between 35 to 40 h. and reached glutamic acid levels in broth of 80 g/L **(Delaunay et al, 1999).**

Purification of glutamic acid involves centrifugation to remove microbial biomass. The pH of the supernatant is gently lowered, by hydrochloric acid, to the isoelectric point of L-glutamic acid (pH = 3.2). The L-glutamic acid crystals are then recovered by centrifugation, then washed several times. MSG is prepared by adding sodium hydroxide solution to crystalline

l-glutamic acid followed by recrystallization **(Schultz et al, 2007).**

8 Organic acids.

Organic acids are organic molecules having acid groups (COOH) in their carbon skeleton. Most organic acids have an agri-food application, and are mainly used as preservatives, flavoring, acidulant and antioxidant. Among the main organic acids useful to humans, we find: citric acid, acetic acid, lactic acid and propionic acid **(Sauer et al, 2008).**

8.1 Definition and use of citric acid.

Citric acid, or citrate, is an organic acid, present in abundance in lemon, hence its name. It is widely used in the food industry, in the form of a food additive (number E330), as an acidulating and flavoring agent in drinks, confectionery, sour candies and other foods. Citric acid can have other non-food applications, especially medical and pharmaceutical **(Max et al, 2010).**

8.2 Industrial microorganisms producing citric acid.

Many microorganisms, can be used to produce citric acid, including:

- Bacteria: Bacillus licheniformis and Bacillus **subtilis (Xu et al, 2005).**
- Molds: Aspergillus niger, Aspergillus awamori, Aspergillus foetidus, and *Penicillium restrictum* **(Show et al, 2015).**
- Yeasts: Candida lipolytica, Candida intermedia, and Saccharomyces cerevisiae

(Yalcin et al, 2010).

However, global industrial production is ensured by the mold Aspergillus niger, due to its ease of handling, and its ability to ferment a variety of inexpensive substrates, as well as their high citric acid production yields **(Papagianni, 2007).**

8.3 Natural biosynthesis of citric acid.

Citric acid is a Krebs cycle metabolic intermediate, resulting from the condensation of the acetyl residue (2 carbons) of acetyl-CoA on oxaloacetate (four carbons) to form citrate (six carbons), with release of CoA, the reaction is catalyzed by the enzyme citrate synthase (Figure 4) **(Szczodrak, 1981):**

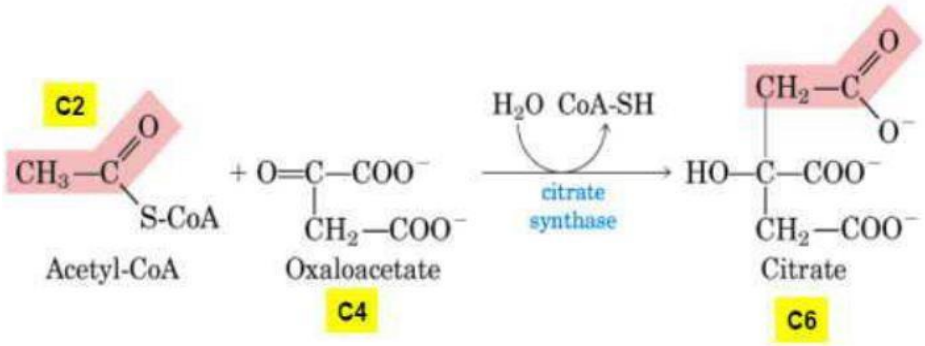

Figure4:Reaction of the formation of citric acid (Szczodrak, 1981).

In industry, in order to accumulate citrate, the reaction of conversion of citrate to cis-aconitate must be blocked. This is achieved by inhibiting aconitase, the enzyme that catalyzes this reaction. Inhibition of this enzyme can be done in several ways, including **(Ramakrishnan et al, 1955; Kubicek and Röhr, 1985):**

- The elimination of iron ions (Fe+2), in fact, the aconitase enzyme uses iron ions as a cofactor, so if we eliminate iron from the industrial culture medium, the enzyme will automatically be blocked. Iron ions can be eliminated by adding certain iron chelating agents such as: fluorocitrate.

- The genetic modification of Aspergillus niger strains, by causing mutations in the gene responsible for the biosynthesis of the aconitase enzyme, in order to have transgenic strains devoid of this enzyme.

8.4 The industrial culture medium used for the production of citric acid.

The process of industrial fermentation of citric acid involves the cultivation of the mold, A. niger, in aerobic conditions, in a culture medium which must contain:

- **A source of carbon.**

The carbon source could be starch, starch hydrolyzate, sugarcane juice, glucose, sucrose or molasses. In industry, the most used source of carbon is molasses. In order to have a good production yield of citric acid, the sugar concentration in the culture medium must be at least 140 g / L (14%) **(Ikram et al, 2004).**

- **A source of nitrogen.**

Are generally ammonium salts, especially, ammonium sulfate, ammonium nitrate…etc, which are generally supplied at concentrations of 0.1 to 0.4 g/L **(Papagianni et al, 2005).**

- **Mineral salts.**

Mineral salts, especially Fe+2, must be removed from the culture medium, because the latter inhibits the formation of citric acid beyond a critical concentration. The elimination of mineral salts is carried out by ion exchange chromatography **(Choudhary et al, 1966).**

- **The pH.**

In order to initiate the growth of Aspergillus niger, the initial pH of the culture medium is generally between 5 and 7. Then, it must be maintained below 2, this has the advantage of controlling contamination and inhibiting the formation of oxalic acid and gluconic acid (undesirable products) **(Papagianni et al, 2005)**.

8.5 The stages of industrial production of citric acid.

8.5.1 Fermentation.

The industrial production of citric acid can be done either by surface fermentation or by submerged fermentation:

8.5.2 The surface fermentation process:

This method involves placing the sterile culture medium, which is usually molasses, in shallow (5 to 20 cm deep) aluminum or stainless-steel trays, placed in a sterile enclosure **(Kılıç et al, 2002).**

8.5.3 The submerged fermentation process:

More than 80% of the world's citric acid production is produced by submerged fermentation in fermenters of 200 to 900 m³ volume. Fermenters are corrosion resistant, usually made of stainless steel. **(Darouneh et al, 2009).**Unlike surface fermentation, the sterile culture medium is seeded by vegetative cells, and no longer by spores of the A. niger mold. The culture is kept stirring at a temperature of 30°C and a pH which does not exceed pH=3.5. Fermentation lasts five to fourteen days and reaches a yield of approximately 18.0 kg/m³ per day **(Khan et al, 1990).**

8.5.4 Precipitation, extraction and purification of citric acid.

After fermentation is completed, citric acid purification begins with the separation of fungal mycelium, by filtration or centrifugation, from the culture medium. The solution obtained is heated then mixed with lime (CaO) to form a precipitate of calcium citrate (Ca3(C6H5O7)2). This is separated by filtration, then treated by dilute sulfuric acid to generate citric acid plus a precipitate of calcium sulfate (gypsum). The latter will be eliminated by filtration. The citric acid solution obtained will be decolorized by the activated carbon then evaporated to produce citric acid crystals. These crystals are recovered by centrifugation, then dried and packaged **(Vandenberghe et al, 1999).**

The stages of industrial citric acid fermentation are summarized in Figure 5.

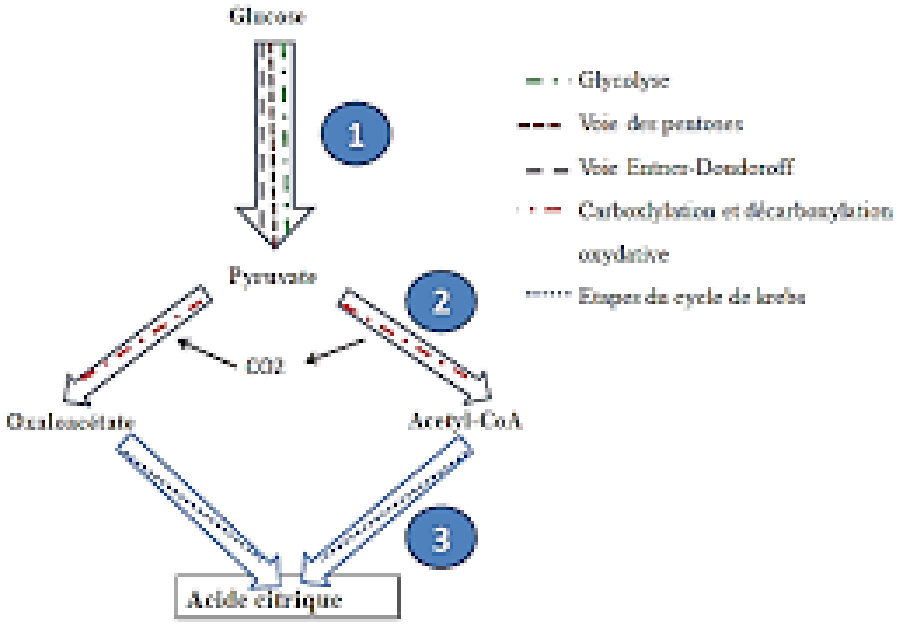

Figure5: Steps in the production of citric acid.

Chapter 04: Food Products

A large number of microorganisms are exploited in the food industry. These contribute to the preparation of foods, to the improvement of their organoleptic properties and even to their preservation, thanks to the particular metabolic abilities that they possess.

1 Fermented food

Fermentation has always been a common way of preserving and processing food. The range of products concerned is very wide. It includes many foods traditionally obtained by natural fermentation and, now, industrially processed.

Fermented foods are foods transformed by microorganisms aerobically or anaerobically. These foods make up 20-40% of our diet and have been around for millennia.

Depending on the type of fermentation, there are several categories of fermented food products:

1.1 Dairy products:

These are the most widely consumed fermented foods in the world. Milk is composed of various substances, including fats, which are stabilized by a protein, casein, and carbohydrates represented mainly by lactose.

Caseins make up the majority of milk proteins. They are present in the form of micelles composed by the association of different caseins held together by calcium phosphate. These micelles are suspended in the aqueous phase of the milk and their destabilization by acidification leads to its coagulation. This is called lactic technology.

The milk is then inoculated with lactic ferments (lactic acid bacteria) which

transform lactose into lactic acid, thus causing the acidification of the milk and its coagulation.

This coagulation will have various consequences: it modifies the texture, taste and quality of the milk. The pH is also reduced, which limits the growth of unwanted bacteria and therefore the conservation of milk. As for them, lactic acid bacteria multiply and produce compounds which are at the origin of the organoleptic properties of fermented dairy products.

1.2 Yogurt:

Yogurt results from the fermentation of milk by two lactic acid bacteria, Streptococcus thermophilus living in symbiosis with Lactobacillus bulgaricus.

The production of yogurt goes through several stages:

1. Pasteurization of milk (heating to 72°C for 15 seconds) to eliminate pathogenic germs.
2. Seeding with lactic ferments (Streptococcus thermophilus and Lactobacillus bulgaricus) after cooling to 43°C which is the optimal growth temperature for lactic acid bacteria.
3. Steaming, where the milk is potted for 3 hours, which allows the ferments to develop and transform the milk as follows:

- *S.thermophilus* grows first and causes an acidification of the environment up to 0.8% lactic acid. This acidity level will inhibit the growth of S. thermophilus and promote the growth of L. bulgaricus.
- The growth of L. bulgaricus will increase the acidity of the medium further up to 1.5-2% leading to coagulation of milk.

Notice: The lactic acid bacteria must still be alive when the yogurt is consumed, which allows for better digestion and better transit. The content of viable ferments upon marketing must be greater than 107 germs/g of product, with a ratio of 1/1 between the two bacterial species.

1.3 Cheeses:

Cheeses have been known for thousands of years and have more than 2000 varieties. This diversity is due to the variety of milks used, the nature of the ferments inoculated and the treatment applied to the curds.

In cheese making, milk coagulation results from the combination of the microbiological action of the ferments and the enzymatic action of rennet (coagulant made up of enzymes, notably chymosin). The manufacturing steps are as follows:

1. Pasteurization of milk.
2. Addition of lactic ferments and rennet.
3. Transfer of milk into molds for its steaming and thus its coagulation by the action of ferments and enzymes.
4. Draining the formed curds to reduce their water content (fresh and white cheeses are consumed directly after this step).
5. Unmolding of the cheese and salting either by surface sprinkling or by brine (immersion in a saturated sodium chloride solution).
6. Refining of cheeses, which corresponds to a maturation period allowing the physico-chemical evolution of the organoleptic characteristics (taste and flavor), color and particular texture.

1.4 Lacto-fermented vegetables:

Lactic fermentation is not only used to preserve dairy products, it also allows the preservation of mushrooms and vegetables of all kinds: cabbage, beets, carrots, beans, onions, etc. This technique consists of preserving vegetables by promoting the development of lactic acid bacteria which acidify the environment and thus inhibit the growth of other undesirable organisms.

For fermentation to take place, lactic acid bacteria must be provided with all the conditions necessary for their development:

- Vegetables must provide sugar, B vitamins and mineral salts.

- Fermentation takes place in an anaerobic environment, so oxygen must be driven out of the environment.
- Vegetables are most often covered in salt water (salt inhibits the bacteria responsible for decomposing vegetables).
- The temperature must be between 18 and 22°C at the start of fermentation.

Fermentation then takes place in 3 phases:

1. **Pre-fermentation:** lasting 2-3 days where many species of microorganisms develop, causing vegetables to decompose and soften (to make them less tough).
2. **Fermentation:** which begins when the level of lactic acid bacteria increases compared to other microorganisms.
3. **Storage:** when the pH drops below 4. Undesirable microorganisms are no longer able to develop and new aromas are revealed.

The vegetables can then be stored for at least a year even if the temperature rises above 10°C. This method of preservation is therefore not only economical since it does not require any energy input but also good for health because lactic acid bacteria produce many vitamins in parallel and lactic acid has many digestive properties.

1.5 Fermented meat:

Meat products of various origins (cattle, poultry, fish, etc.) are prepared in different ways and presented in several forms (hams, sausages, etc.) and despite their great diversity, they all undergo fermentation at a certain stage of their production.

The fermentation processes applied to meats are most often carried out by strains of Lactobacillus, the development of which contributes to the maturation of the product and its acidification which causes the progressive disappearance of contaminating microorganisms.

The microbial quality of finished products is also due to various specific manufacturing operations: cooking, acidification, drying, salting, etc.

1.6 Bread :

The leaven for making bread is made up of strains of the Saccharomyces cerevisiae yeast which is cultivated aerobically to promote the production of CO_2 which will cause the dough to swell through the formation of air bubbles. In addition, sourdough modifies the structure of gluten and produces the characteristic flavors of bread.

The activity of the yeast begins as soon as it is incorporated into the dough and stops 5 minutes after the start of cooking:

- During aerobic kneading, cells multiply rapidly. Then during proofing (1st fermentation), the yeast ferments and produces CO_2 but also a lot of alcohol, which evaporates but contributes to the development of aromas alongside a reduction in pH (acidification).
- After 1 hour of activity, the pre-existing simple sugars in the flour are consumed. It then continues its action thanks to the maltose coming from the hydrolysis of starch.
- During the preparation (2nd fermentation), the production of CO_2 is greater, it increases further during the first minutes of cooking up to 50°C where the yeast is inactivated.

Notice: Certain breads are produced by specific sourdoughs made up of mixed cultures of lactic acid bacteria and yeast.

Références bibliographiques.

Abbaszadeh, S., Sharifzadeh, A., Shokri, H., Khosravi, A. R., Abbaszadeh, A. (2014). Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. Journal de Mycologie Medicale, 24(2), e51-e56.

Al Farraj, D. A., Varghese, R., Vágvölgyi, C., Elshikh, M. S., Alokda, A. M., & Mahmoud, A. H. (2020). Antibiotics production in optimized culture condition using low cost substrates from Streptomyces sp. AS4 isolated from mangrove soil sediment. Journal of King Saud University-Science, 32(2), 1528-1535.

Asif, A., Mohsin, H., Tanvir, R., & Rehman, Y. (2017). Revisiting the mechanisms involved in calcium chloride induced bacterial transformation. Frontiers in microbiology, 8, 2169.

Avila-Leon, I., Chuei Matsudo, M., Sato, S., & De Carvalho, J. C. M. (2012). Arthrospira platensis biomass with high protein content cultivated in continuous process using urea as nitrogen source. Journal of Applied Microbiology, 112(6), 1086-1094.

Awad, H. M., ELâ, K. Y., Aziz, R., Sarmidi, M. R., & Elâ, H. A. (2012). Antibiotics as microbial secondary metabolites: Production and application. Jurnal Teknologi, 59(1).

Baltz, R. H., Demain, A. L., & Davies, J. E. (Eds.). (2010). Manual of industrial microbiology and biotechnology. American Society for Microbiology Press.

Bayles, K. W. (2000). The bactericidal action of penicillin: new clues to an unsolved mystery. Trends in microbiology, 8(6), 274-278.

Becker, J., & Wittmann, C. (2017). Industrial microorganisms: Corynebacterium glutamicum. Industrial biotechnology: microorganisms, 1, 183-220.

Bergmans, HE, van Die, ,I.M., Hoekstra, WP. 1981. Transformation in Escherichia coli: stages in the process. J. Bacteriol. 146:564-570.

Bregni, C., Degrossi, J., Garcia, R., Lamas, M. C., Firenstein, R., & D'aquino, M. (2000). Alginate microspheres of Bacillus subtilis.

Brindha, J., Ramudu, K. N., & Balamurali, M. M. (2020). An efficient single pot DNA recombination method for protein library generation. International journal of biological macromolecules, 146, 661-667.

Cepeda-García, C., Domínguez-Santos, R., García-Rico, R. O., García-Estrada, C., Cajiao, A., Fierro, F., & Martín, J. F. (2014). Direct involvement of the CreA transcription factor in penicillin biosynthesis and expression of the pcbAB gene in Penicillium chrysogenum. Applied microbiology and biotechnology, 98(16), 7113-7124.

Chang DE, Jung HC, Rhee JS, Pan JG (1999) Homofermentative production of D()) or L(+)lactate in metabolically engineered Echerichia coli RR1. Appl. Environ. Microbiol. 65: 1384–1389.

Chang, A. Y., Chau, V. W., Landas, J. A., & Pang, Y. (2017). Preparation of calcium competent Escherichia coli and heat-shock transformation. JEMI methods, 1, 22-25.

Chawla, J., & Kvarnberg, D. (2014). Hydrosoluble vitamins. Handbook of clinical neurology, 120, 891-914.

Choudhary, A. Q., & Pirt, S. J. (1966). The influence of metal-complexing agents on citric acid production by Aspergillus niger. Microbiology, 43(1), 71-81.

Christy, P. M., Gopinath, L. R., & Divya, D. (2014). A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renewable andSustainable Energy Reviews, 34, 167-173.

Cirne DG, Lehtomaki A, Bjornsson L, Blackall LL. (2007). Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. J Appl Microbiol.103: 516–27.

Cvetković, D. D., & Markov, S. L. (2002). Cultivation of tea fungus on malt extract medium. Acta periodica technologica, (33), 117-124.

Darouneh, E., Alavi, A., Vosoughi, M., Arjm, M., Seifkordi, A., & Rajabi, R. (2009). Citric acid production: Surface culture versus submerged culture. African Journal of Microbiology Research, 3(9), 541-545.

Davies, J. (2006). Are antibiotics naturally antibiotics?. Journal of Industrial Microbiology and Biotechnology, 33(7), 496-499.

De La Jara, A., Ruano-Rodriguez, C., Polifrone, M., Assunçao, P., Brito-Casillas, Y., Wägner, A. M., & Serra-Majem, L. (2018). Impact of dietary Arthrospira (Spirulina) biomass consumption on human health: main health targets and systematic review. Journal of Applied Phycology, 30(4), 2403-2423.

Degu, A., Hatew, B., Nunes-Nesi, A., Shlizerman, L., Zur, N., Katz, E & Sadka, A. (2011). Inhibition of aconitase in citrus fruit callus results in a metabolic shift towards amino acid biosynthesis. Planta, 234(3), 501-513.

Delaunay, S., Gourdon, P., Lapujade, P., Mailly, E., Oriol, E., Engasser, J. M., ... & Goergen, J. L. (1999). An improved temperature-triggered process for glutamate production with Corynebacterium glutamicum. Enzyme and microbial technology, 25(8-9), 762-768.

Demain, A. L. (1981). Industrial microbiology. Science, 214(4524), 987-995.

Demain, A. L., & Adrio, J. L. (2008). Contributions of microorganisms to industrial biology. Molecular biotechnology, 38(1), 41.

Devi, P., D'Souza, L., Kamat, T., Rodrigues, C., & Naik, C. G. (2009). Batch culture fermentation of Penicillium chrysogenum and a report on the isolation, purification, identification and antibiotic activity of citrinin.

Diethard, M., Gasser, B., Egermeier, M., Marx, H., & Sauer, M. (2017). Industrial Microorganisms: Saccharomyces cerevisiae and other Yeasts. Industrial Biotechnology: Microorganisms, 2, 673-686.

Dumitriu, S. (Ed.). (2004). Polysaccharides: structural diversity and functional versatility. CRC press.

Dziegielewska-Gesiak, S., Fatyga, E., Kasiarz, G., Wilczynski, T., Muc-Wierzgon, M., & Kokot, T. (2019). Vitamins B12 and D deficiencies and macro-and microelement disturbances among diabetic elderly patients. Journal of biological regulators and homeostatic agents, 33(2), 477-483.

El Khoury, J. (2019). Étude de la résistance aux B-lactamines chez Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae et Pseudomonas aeruginosa par des approches omiques.

Elefsiniotis P, Oldham WK. 2004. Anaerobic acidogenesis of primary sludge: the role of solids retention time. Biotechnol Bioeng;44: 7–13.

El-Mansi, E. M. T., Nielsen, J., Mousdale, D., & Carlson, R. P. (Eds.). (2018). Fermentation microbiology and biotechnology. CRC press.

El-Nawwi, S. A., & Abd El-Kader, A. (1996). Production of single-cell protein and cellulase from sugarcane bagasse: effect of culture factors. Biomass and bioenergy, 11(4), 361-364.

Enzmann, F. Mayer, M. Rother, D (2018). Holtmann Methanogens: biochemical background and biotechnological applications AMB Express., 8, 1.

Erickson. L.E (2011). Bioreactors for Commodity Products. Editor(s): Murray Moo-Young, Comprehensive Biotechnology (Second Edition), Academic Press,. 653-658.

Erickson. L.E (2019). Bioreactors, Editor(s): Thomas M. Schmidt, Encyclopedia of Microbiology (Fourth Edition), Academic Press. 536-541.

Ertan, H. (1992). Some properties of glutamate dehydrogenase, glutamine synthetase and glutamate synthase from Corynebacterium callunae. Archives of microbiology, 158(1), 35-41.

Evgenios K, Christos C (2020). Model-based dynamic optimization of the fermentative production of polyhydroxyalkanoates (PHAs) in fed-batch and sequence of continuously operating bioreactors. Biochemical Engineering Journal.162 : 107702.

Fabregas, J., & Herrero, C. (1985). Marine microalgae as a potential source of single cell protein (SCP). Applied microbiology and biotechnology, 23(2), 110-113.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. Perspectives in medicinal chemistry, 6, PMC-S14459. Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. Perspectives in medicinal chemistry, 6, PMC-S14459.

Ferreira, A. F., Ribeiro, A. M., Kulaç, S., & Rodrigues, A. E. (2015). Methane purification by adsorptive processes on MIL-53 (Al). Chemical Engineering Science, 124, 79-95.

Francioso, O., Rodriguez-Estrada, M. T., Montecchio, D., Salomoni, C., Caputo, A., & Palenzona, D. (2010). Chemical characterization of municipal wastewater sludges produced by two-phase anaerobic digestion for biogas production. Journal of hazardous materials, 175(1-3), 740-746.

Franklin, M. J., Nivens, D. E., Weadge, J. T., & Howell, P. L. (2011). Biosynthesis of the Pseudomonas aeruginosa extracellular polysaccharides, alginate, Pel, and Psl. Frontiers in microbiology, 2, 167.

Garcıa-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. Biotechnology advances, 18(7), 549-579.

Gervasi, T., Pellizzeri, V., Calabrese, G., Di Bella, G., Cicero, N., & Dugo, G. (2018). Production of single cell protein (SCP) from food and agricultural waste by using Saccharomyces cerevisiae. Natural product research, 32(6), 648-653.

Ghanem. (1992). Single cell protein production from beet pulp by mixed culture.

Gumus, T., Demirci, A.S., Mirik, M., Arici, M., Aysan, Y., 2010. Xanthan gum production of Xanthomonas spp. isolated from different plants. Food Science Biotechnology 19 (1), 201–206.

Gutmann, M., Hoischen, C., & Krämer, R. (1992). Carrier-mediated glutamate secretion by Corynebacterium glutamicum under biotin limitation. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1112(1), 115-123.

Hamidi, M., Kennedy, J. F., Khodaiyan, F., Mousavi, Z., & Hosseini, S. S. (2019). Production optimization, characterization and gene expression of pullulan from a new strain of Aureobasidium pullulans. International journal of biological macromolecules, 138, 725-735.

Harwood, C. R., Park, S. H., & Sauer, M. (2018). Editorial for the thematic issue on “Industrial Microbiology”.

Hazra, A. B., Han, A. W., Mehta, A. P., Mok, K. C., Osadchiy, V., Begley, T. P., & Taga, M. E. (2015). Anaerobic biosynthesis of the lower ligand of vitamin B12. Proceedings of the National Academy of Sciences, 112(34), 10792-10797.

He, F., Yang, Y., Yang, G., & Yu, L. (2010). Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from Streptomyces virginia H03. Food Control, 21(9), 1257-1262.

Hermann, T. (2003). Industrial production of amino acids by coryneform bacteria. Journal of biotechnology, 104(1-3), 155-172.

Hirasawa, T., & Wachi, M. (2016). Glutamate fermentation-2: mechanism of L-glutamate overproduction in Corynebacterium glutamicum. Amino Acid Fermentation, 57-72.

Hoischen C, Kra¨mer R (1990) Membrane alteration is necessary but not sufficient for effective glutamate secretion in Corynebacterium glutamicum. J Bacteriol 172(6):3409–3416.

Huang, C., Luo, M. T., Chen, X. F., Xiong, L., Li, X. M., & Chen, X. D. (2017). Recent advances and industrial viewpoint for biological treatment of wastewaters by oleaginous microorganisms. Bioresource technology, 232, 398-407.

Huang, X., Liu, X., Chen, F., Wang, Y., Li, X., Wang, D., ... & Yang, Q. (2020). Clarithromycin affect methane production from anaerobic digestion of waste activated sludge. Journal of Cleaner Production, 255, 120321.

Humphrey, A. E., & Lee, S. E. (1992). Industrial fermentation: principles, processes, and products. In Riegel's handbook of industrial chemistry (pp. 916-986). Springer, Dordrecht.

Ikeda, M. (2003). Amino acid production processes. Microbial production of l-amino acids, 1-35.

Ikeda, M., & Takeno, S. (2013). Amino acid production by Corynebacterium glutamicum.In Corynebacterium glutamicum (pp. 107-147). Springer, Berlin, Heidelberg.

Ikram-Ul, H., Ali, S., Qadeer, M. A., & Iqbal, J. (2004). Citric acid production by selected mutants of Aspergillus niger from cane molasses. Bioresource Technology, 93(2), 125-130.

Jang, C, Magnuson, T. 2013. A Novel Selection Marker for Efficient DNA Cloning and Recombineering in E. coli. Plos One. 8:e57075.

Jiang, L. (2013). Effect of nitrogen source on curdlan production by Alcaligenes faecalis ATCC 31749. International journal of biological macromolecules, 52, 218-220.

Johansen, E. (2017). Future access and improvement of industrial lactic acid bacteria cultures. Microbial cell factories, 16(1), 1-5.

Kalai, S., Bensoussan, M., Dantigny, P. (2014). Lag time for germination of Penicillium chrysogenum conidia is induced by temperature shifts. Food Microbiology, 42, 149-153.

Kampen, W. H. (2014). Nutritional requirements in fermentation processes. In Fermentation and biochemical engineering handbook (pp. 37-57). William Andrew Publishing.

Keller, N. P. (2019). Fungal secondary metabolism: regulation, function and drug discovery. Nature Reviews Microbiology, 17(3), 167-180.

Khan KH, Shaukat SS (1990). Citric acid production with mixed strains of Aspergillus niger in submerged culture. Acta Microbiol. Hung 37: 9–13.

Kılıç, M., Bayraktar, E., Ateş, S., & Mehmetoglu, Ü. (2002). Investigation of extractive citric acid fermentation using response-surface methodology. Process Biochemistry, 37(7), 759-767.

Kim, J., Fukuda, H., Hirasawa, T., Nagahisa, K., Nagai, K., Wachi, M., & Shimizu, H. (2010). Requirement of de novo synthesis of the OdhI protein in penicillin-induced glutamate production by Corynebacterium glutamicum. Applied microbiology and biotechnology, 86(3), 911-920.

Kim, J., Hirasawa, T., Sato, Y., Nagahisa, K., Furusawa, C., & Shimizu, H. (2009). Effect of odhA overexpression and odhA antisense RNA expression on Tween-40-triggered glutamate production by Corynebacterium glutamicum. Applied microbiology and biotechnology, 81(6), 1097-1106.

Koval, O., & Oliinichuk, S. (2019). Influence of melasses non-sugars on efficiency of fermentation of mash from sacchariferous raw material.

Kräutler, B. (2005). Vitamin B12: chemistry and biochemistry. Biochemical Society Transactions, 33(4), 806-810.

Kriger, O. V., & Noskova, S. Y. (2018). Properties of lactic acid microorganisms: long-term preservation methods. Food Processing: Techniques and Technology, 51(4), 30-38.

Krishnakumar, V., Seshadri, S., & Muthunatasen, S. (2007). Analysis of vibrational spectra of 5, 6-dimethyl benzimidazole based on density functional theory calculations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 68(3), 811-816.

Kubicek, C. P., & Röhr, M. (1985). Aconitase and citric acid fermentation by Aspergillus niger. Applied and environmental microbiology, 50(5), 1336-1338.

Kushkevych, I., Kobzová, E., Vítězová, M., Vítěz, T., Dordević, D., & Bartoš, M. (2019). Acetogenic microorganisms in operating biogas plants depending on substrate combinations. Biologia, 74(9), 1229-1236.

Laluce, C., Bertolini, M. C., Ernandes, J. R., Martini, A. V., & Martini, A. (1988). New amylolytic yeast strains for starch and dextrin fermentation. Applied and environmental microbiology, 54(10), 2447-2451.

Lee, K. S., Boccazzi, P., Sinskey, A. J., & Ram, R. J. (2011). Microfluidic chemostat and turbidostat with flow rate, oxygen, and temperature control for dynamic continuous culture. Lab ona Chip, 11(10), 1730-1739.

Leela, J. K., & Sharma, G. (2000). Studies on xanthan production from Xanthomonascampestris. Bioprocess Engineering, 23(6), 687-689.

Lehtomäki, A. (2006). Biogas production from energy crops and crop residues (No. 163). University of Jyväskylä.

Lyu, Z., Shao, N., Akinyemi, T., & Whitman, W. B. (2018). Methanogenesis. Current Biology, 28(13), R727-R732.

Martin, M. R., Fornero, J. J., Stark, R., Mets, L., & Angenent, L. T. (2013). A single-culture bioprocess of Methanothermobacter thermautotrophicus to upgrade digester biogas by CO2-to-CH4 conversion with H2. Archaea, 2013.

Mattanovich, D., Sauer, M., & Gasser, B. (2017). Industrial microorganisms: Pichia pastoris. Industrial Biotechnology: Microorganisms, 2, 687-714.

Max, B., Salgado, J. M., Rodríguez, N., Cortés, S., Converti, A., & Domínguez, J. M. (2010). Biotechnological production of citric acid. Brazilian journal of Microbiology, 41(4), 862-875.

Menzel, C., Olsson, E., Plivelic, T. S., Andersson, R., Johansson, C., Kuktaite, R. & Koch, K. (2013). Molecular structure of citric acid cross-linked starch films. Carbohydrate polymers, 96(1), 270-276.

Min, B. E., Hwang, H. G., Lim, H. G., & Jung, G. Y. (2017). Optimization of industrial microorganisms: recent advances in synthetic dynamic regulators. Journal of Industrial Microbiology and Biotechnology, 44(1), 89-98.

Naessens, M., Cerdobbel, A. N., Soetaert, W., & Vandamme, E. J. (2005). Leuconostoc dextransucrase and dextran: production, properties and applications. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology, 80(8), 845-860.

Nampoothiri, K. M., Singhania, R. R., Sabarinath, C., & Pandey, A. (2003). Fermentative production of gellan using Sphingomonas paucimobilis. Process Biochemistry, 38(11), 1513-1519.

Nangul, A., & Bhatia, R. (2021). Microorganisms: a marvelous source of single cell proteins. Journal of Microbiology, Biotechnology and Food Sciences, 2021, 15-18.

Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H., & Ghasemi, Y. (2011). Single cell protein: production and process. American Journal of food technology, 6(2), 103-116.

Nunez, C., Pena, C., Kloeckner, W., Hernández-Eligio, A., Bogachev, A.V., Moreno, S., Guzmán, J., Büchs, J., Espín, G., 2013. Alginate synthesis in Azotobacter vinelandii is increased by reducing the intracellular production of ubiquinone. Appl. Microbiol. Biotechnol. 97, 2503-2512.

Ohnishi, Y., Ishikawa, J., Hara, H., Suzuki, H., Ikenoya, M., Ikeda, H., & Horinouchi, S. (2008). Genome sequence of the streptomycin-producing microorganism Streptomyces griseus IFO 13350. Journal of bacteriology, 190(11), 4050.

Okafor, N., & Okeke, B. C. (2020). Modern industrial microbiology and biotechnology.

Oosterhuis, N.M.G. Hudson, T. D'Avino, A. Zijlstra, G.M. Amanullah, A (2011). Disposable Bioreactors, Editor(s): Murray Moo-Young, Comprehensive Biotechnology (Second Edition), Academic. 249-261.

Page, M. G. (2012). Beta-lactam antibiotics. In Antibiotic Discovery and Development (pp. 79- 117). Springer, Boston, MA.

Palaniraj, A., & Jayaraman, V. (2011). Production, recovery and applications of xanthan gum by Xanthomonas campestris. Journal of Food Engineering, 106(1), 1-12.

Pandey, N., & Cascella, M. (2020). Beta lactam antibiotics. StatPearls [Internet].

Papagianni, M. (2007). Advances in citric acid fermentation by Aspergillus niger: biochemical aspects, membrane transport and modeling. Biotechnology advances, 25(3), 244-263.

Papagianni, M., Wayman, F., & Mattey, M. (2005). Fate and role of ammonium ions during fermentation of citric acid by Aspergillus niger. Applied and environmental microbiology, 71(11), 7178-7186.

Parmar, A., Kumar, H., Marwaha, S. S., & Kennedy, J. F. (2000). Advances in enzymatic transformation of penicillins to 6-aminopenicillanic acid (6-APA). Biotechnology advances, 18(4), 289-301.

Paul, P. E. V., Sangeetha, V., & Deepika, R. G. (2019). Emerging trends in the industrial production of chemical products by microorganisms. In Recent developments in applied microbiology and biochemistry (pp. 107-125). Academic Press.

Pelton, R. (2002). A review of antifoam mechanisms in fermentation. Journal of industrial microbiology and biotechnology, 29(4), 149-154.

Pescuma, M., de Valdez, G. F., & Mozzi, F. (2015). Whey-derived valuable products obtained by microbial fermentation. Applied microbiology and biotechnology, 99(15), 6183-6196.

Piao, Y., Yamashita, M., Kawaraichi, N., Asegawa, R., Ono, H., & Murooka, Y. (2004). Production of vitamin B12 in genetically engineered Propionibacterium freudenreichii. Journal of bioscience and bioengineering, 98(3), 167-173.

Piwowarek, K., Lipińska, E., Hać-Szymańczuk, E., Kieliszek, M., & Ścibisz, I. (2018). Propionibacterium spp.—source of propionic acid, vitamin B12, and other metabolites important for the industry. Applied microbiology and biotechnology, 102(2), 515-538.

Porter, N. T., & Martens, E. C. (2017). The critical roles of polysaccharides in gut microbial ecology and physiology. Annual review of microbiology, 71, 349-369.

Purama, R. K., Goswami, P., Khan, A. T., & Goyal, A. (2009). Structural analysis and properties of dextran produced by Leuconostoc mesenteroides NRRL B-640. Carbohydrate Polymers, 76(1), 30-35.

Ramakrishnan, C. V., Steel, R., & Lentz, C. P. (1955). Mechanism of citric acid formation and accumulation in Aspergillus niger. Archives of biochemistry and biophysics, 55(1), 270-273.

Ravindra, P. (2000). Value-added food:: Single cell protein. Biotechnology advances, 18(6), 459-479.

Reihani, S. F. S., & Khosravi-Darani, K. (2019). Influencing factors on single-cell protein production by submerged fermentation: A review. Electronic Journal of Biotechnology, 37, 34-40.

Ritala, A., Häkkinen, S. T., Toivari, M., & Wiebe, M. G. (2017). Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. Frontiers in microbiology, 8, 2009.

Robyt, J. F., Yoon, S. H., & Mukerjea, R. (2008). Dextransucrase and the mechanism for dextran biosynthesis. Carbohydrate research, 343(18), 3039-3048.

Rodríguez-Sáiz, M., Díez, B., & Barredo, J. L. (2005). Why did the Fleming strain fail in penicillin industry?. Fungal genetics and Biology, 42(5), 464-470.

Rosalam, S., & England, R. (2006). Review of xanthan gum production from unmodified starches by Xanthomonas comprestris sp. Enzyme and Microbial Technology, 39(2), 197-207.

Sagagi, B., Garba, B., & Usman, N. (2009). Studies on biogas production from fruits and vegetable waste. Bayero Journal of Pure and Applied Sciences, 2(1), 115-118.

Salam, N., Jiao, J. Y., Zhang, X. T., & Li, W. J. (2020). Update on the classification of higher ranks in the phylum Actinobacteria. International journal of systematic and evolutionary microbiology, 70(2), 1331-1355.

Salehizadeh, H., Yan, N., & Farnood, R. (2018). Recent advances in polysaccharide bio-based flocculants. Biotechnology advances, 36(1), 92-119.

Sarkar, D., & Modak, J. M. (2003). Optimisation of fed-batch bioreactors using genetic algorithms. Chemical Engineering Science, 58(11), 2283-2296.

Schink, B (1997). Energetics of syntrophic cooperation in methanogenic degradation. Microb Mol Biol Rev 61:262–280.

Schultz, C., Niebisch, A., Gebel, L., & Bott, M. (2007). Glutamate production by Corynebacterium glutamicum: dependence on the oxoglutarate dehydrogenase inhibitor protein OdhI and protein kinase PknG. Applied microbiology and biotechnology, 76(3), 691-700.

Shao, W., Ma, K., Le, Y., Wang, H., & Sha, C. (2017). Development and use of a novel random mutagenesis method: in situ error-prone PCR (is-epPCR). In In Vitro Mutagenesis (pp. 497-506). Humana Press, New York, NY.

Shewale, J. G., & Sudhakaran, V. K. (1997). Penicillin V acylase: its potential in the production of 6-aminopenicillanic acid. Enzyme and microbial technology, 20(6), 402-410.

Show, P. L., Oladele, K. O., Siew, Q. Y., Aziz Zakry, F. A., Lan, J. C. W., & Ling, T. C. (2015). Overview of citric acid production from Aspergillus niger. Frontiers in life science, 8(3), 271-283.

Silva, M.F., Fornari, R.C.G., Mazutti, M.A., Oliveira, D., Padilha, F.F., Cichoski, A.J., Cansian, R.L., Luccio, M.D., Treichel, H., 2009. Production and characterization of xanthan gum by Xanthomonas campestris using cheese whey as sole carbon source. Journal of Food Engineering 90,119–123.

Singh, R. S., Saini, G. K., & Kennedy, J. F. (2008). Pullulan: microbial sources, production and applications. Carbohydrate polymers, 73(4), 515-531.

Sonenshein, A. L. (2001). The Krebs citric acid cycle. Bacillus subtilis and its Closest Relatives: from Genes to Cells, 151-162.

Steiger, M. G., Rassinger, A., Mattanovich, D., & Sauer, M. (2019). Engineering of the citrate exporter protein enables high citric acid production in Aspergillus niger. Metabolic engineering, 52, 224-231.

Suarez, C., & Gudiol, F. (2009). Beta-lactam antibiotics. Enfermedades infecciosas y microbiologia clinica, 27(2), 116-129.

Sworn, G. (2021). Xanthan gum. In Handbook of hydrocolloids (pp. 833-853). Woodhead Publishing.

Szczodrak, J. (1981). Biosynthesis of citric acid in relation to the activity of selected enzymes of the Krebs cycle in Aspergillus niger mycelium. European journal of applied microbiology and biotechnology, 13(2), 107-112.

Tatsumi, N., & Inui, M. (Eds.). (2012). Corynebacterium glutamicum: biology and biotechnology (Vol. 23). Springer Science & Business Media.

Tian, G., Zhang, W., Dong, M., Yang, B., Zhu, R., Yin, F., ... & Cui, X. (2017). Metabolic pathway analysis based on high-throughput sequencing in a batch biogas production process. Energy, 139, 571-579.

Tiwari, S., Jamal, S. B., de Carvalho, P. V. S. D., Hassan, S. S., Silva, A., & Azevedo, V. Industrial Microbiology & Biotechnology.

Vandenberghe, L. P., Soccol, C. R., Pandey, A., & Lebeault, J. M. (1999). Microbial production of citric acid. Brazilian Archives of Biology and Technology, 42(3), 263-276.

Waites, M. J., Morgan, N. L., Rockey, J. S., & Higton, G. (2009). Industrial microbiology: an introduction. John Wiley & Sons.

Walker, G. M. (2014). Fermentation (Industrial): media for industrial fermentations.In Encyclopedia of food microbiology (pp. 769-777). Academic Press.

Wilson, D. B., Sahm, H., Stahmann, K. P., & Koffas, M. (Eds.). (2019). Industrial Microbiology. John Wiley & Sons.

Xu, H., Jiang, M., Li, H., Lu, D., & Ouyang, P. (2005). Efficient production of poly (γ-glutamic acid) by newly isolated Bacillus subtilis NX-2. Process Biochemistry, 40(2), 519-523.

Yalcin, S. K., Bozdemir, M. T., & Ozbas, Z. Y. (2010). Citric acid production by yeasts: fermentation conditions, process optimization and strain improvement. Current research, technology and education topics in applied microbiology and microbial biotechnology, 9, 1374- 1382.

Yamauchi, R., Maguin, E., Horiuchi, H., Hosokawa, M., & Sasaki, Y. (2019). The critical role of urease in yogurt fermentation with various combinations of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus. Journal of dairy science, 102(2), 1033-1043.

Yang, H., Swartz, A. M., Park, H. J., Srivastava, P., Ellis-Guardiola, K., Upp, D. M & Lewis, J. C. (2018). Evolving artificial metalloenzymes via random mutagenesis. Nature chemistry, 10(3), 318.

Yang, M. J., Lee, H. W., & Kim, H. (2017). Enhancement of thermostability of Bacillus subtilis endoglucanase by error-prone PCR and DNA shuffling. Applied Biological Chemistry, 60(1), 73- 78.

Ye, B., Li, Y., Tao, Q., Yao, X., Cheng, M., & Yan, X. (2020). Random mutagenesis by insertion of error-prone PCR products to the chromosome of Bacillus subtilis. Frontiers in Microbiology, 11.

Yongsmith, B., Sonomoto, K., Tanaka, A., & Fukui, S. (1982). Production of vitamin B 12 by immobilized cells of a propionic acid bacterium. European journal of applied microbiology and biotechnology, 16(2), 70-74.

Zähner, H., Anke, H., & Anke, T. (2020). Evolution and secondary pathways. In Secondary metabolism and differentiation in fungi (pp. 153-171). CRC Press.

Zhang, B., Jiang, Y., Li, Z., Wang, F., & Wu, X. Y. (2020). Recent Progress on Chemical Production From Non-food Renewable Feedstocks Using Corynebacterium glutamicum. Frontiers in Bioengineering and Biotechnology, 8.

Zhang, W., & Nielsen, D. (2014). Synthetic biology applications in industrial microbiology. Frontiers in microbiology, 5, 451.

Zhou, S., Du, G., Kang, Z., Li, J., Chen, J., Li, H., & Zhou, J. (2017). The application of powerful promoters to enhance gene expression in industrial microorganisms. World Journal of Microbiology and Biotechnology, 33(2), 23.

Zhou, S., Shanmugam, K. T., Yomano, L. P., Grabar, T. B., & Ingram, L. O. (2006). Fermentation of 12%(w/v) glucose to 1.2 M lactate by Escherichia coli strain SZ194 using mineral salts medium. Biotechnology letters, 28(9), 663-670.

Semestre : 6

Unité d'enseignement Fondamentale 1 (UEF 3.2.1) : Microbiologie Appliquée

Matière 1: Microbiologie industrielle

Crédits : 5

Coefficient : 3

Objectifs de l'enseignement :

Cette matière permet l'étude :

- Du fonctionnement des fermenteurs et de la pratique industrielle des fermentations.
- Des potentialités des souches microbiennes en matière de biosynthèse de métabolites importants (vaccins, antibiotiques, enzymes, protéines, levures, P.O.U., fromages, arômes,...)
- Des optimisations et des améliorations de souches sauvages (facteurs et conditions du milieu, mutagénèse, recombinaison génétique en vue d'une production maximale de métabolites.

Des méthodes d'isolement, de purification et de l'obtention des métabolites.

Connaissances préalables recommandées :

Contenu de la matière :

1. Introduction: Les domaines d'activité d la microbiologie industrielle et intérêt de l'utilisation des microorganismes, cellule bactérienne : produit microbien d'intérêt industriel

2. Les Microorganismes utiles (Archaea, bactéries, Archaea, champignons, algues et Virus) : Rappel de Taxonomie, importance des microorganismes en industrie.

3. Les milieux de culture industriels.

4. Les fermentations industrielles :

-Le fermenteur

-Les protéines d'organismes unicellulaires : les P.O.U. ou SCP, les organismes utilisés et les substrats bon marché les plus adaptés

5. Les produits de fermentations industrielles :

5.1. Les métabolites primaires obtenus par fermentation microbienne:

-Les acides aminés

-Les acides organiques

-Les Biogaz (H2, CH4, ...)

-Les vaccins

5.2. Les métabolites secondaires :

-Les antibiotiques (pénicilline, streptomycine, tétracycline

-Les vitamines (B12)

-Les polysaccharides

5.3. Les enzymes.

Travaux pratiques :

N°1 : Initiation aux techniques de criblage d'antibiotiques

N°2 : Les techniques de conservation des souches microbiennes industrielles

N°3 : Production de P.O.U. la levure

N°4: Production d'une enzyme microbienne.