MICROBIAL BIOTECHNOLOGY

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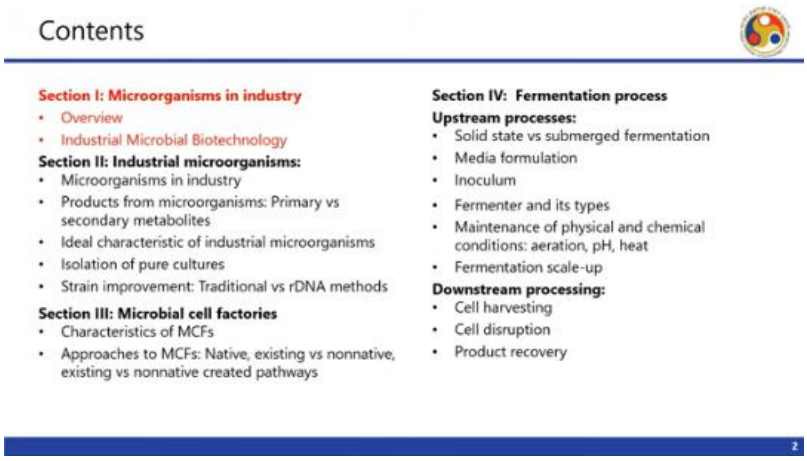
Lecture-19
Lec 19: Microorganisms in industry

Hello everyone, welcome to my course on microbial biotechnology. Today we will start Module 6, which will deal with industrial and pharmaceutical applications of microorganisms. In the first lecture, we are going to discuss microorganisms in industry. So, this is briefly the overview of the various things we are going to discuss, divided into three sections. In the first section, we will discuss microorganisms in industry and have an overview.



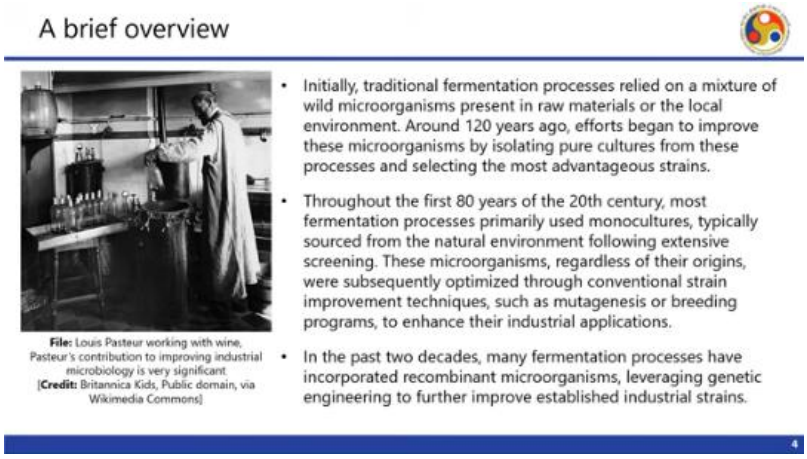
And then we'll go for the industrial microorganisms. Then we will discuss microbial cell factories. And finally, the fermentation process, which comprises upstream and downstream processes. Microorganisms play a vital role in industrial production of a wide variety of goods and services.

They are exceptionally valuable due to their ease of mass production, rapid growth, utilization of economic substrates—often waste materials—which can be converted into valuable products, and vast potential in generating diverse products. Furthermore, their capacity for genetic manipulation has opened boundless opportunities for innovation within fermentation industries. Briefly, industrial microbiology is the application of microorganisms to produce valuable products or perform processes on an industrial scale,

including the production of antibiotics, enzymes, biofuels, fermented foods, and waste treatment. This field encompasses a wide range of microorganisms found naturally and also laboratory-derived mutants.

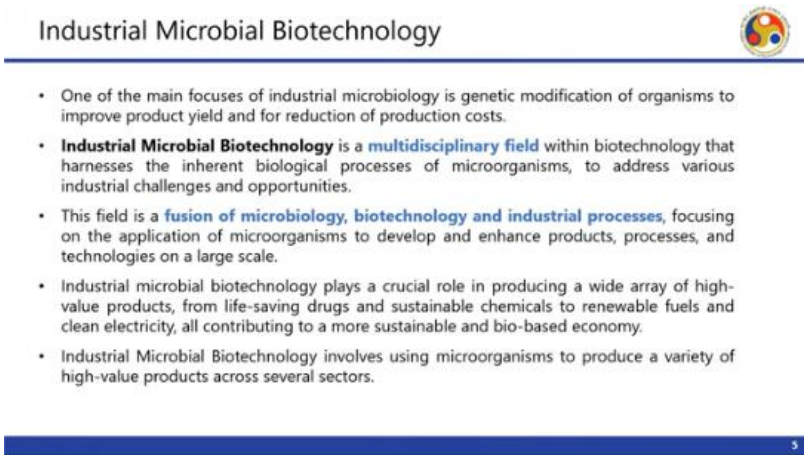
Initially, traditional fermentation processes relied on a mixture of wild microorganisms. These were present in raw materials or the local environment. Around 120 years ago, efforts began to improve these microorganisms by isolating pure cultures from these processes and selecting the most advantageous strains. In this picture, you can see Louis Pasteur working with wine. His significant contribution to improving industrial microbiology is very important.

For the first 80 years of the 20th century, most fermentation processes primarily used monocultures, which are cultures containing only one type of strain or species. These were typically sourced from the natural environment following extensive screening. These microorganisms, regardless of their origin, were subsequently optimized through conventional strain improvement techniques such as mutagenesis or breeding programs to enhance their industrial applications. In the past two decades, many fermentation processes have incorporated recombinant microorganisms, leveraging genetic engineering to further improve established industrial strains.



One of the main focuses of industrial microbiology is the genetic modification of organisms to improve product yield and reduce production costs. Industrial microbial biotechnology is a multidisciplinary field within biotechnology that harnesses the inherent biological processes of microorganisms to address various industrial challenges and opportunities. This field is a fusion of microbiology, biotechnology, and industrial processes, focusing on the application of microorganisms to develop and enhance products, processes, and technologies on a large scale.

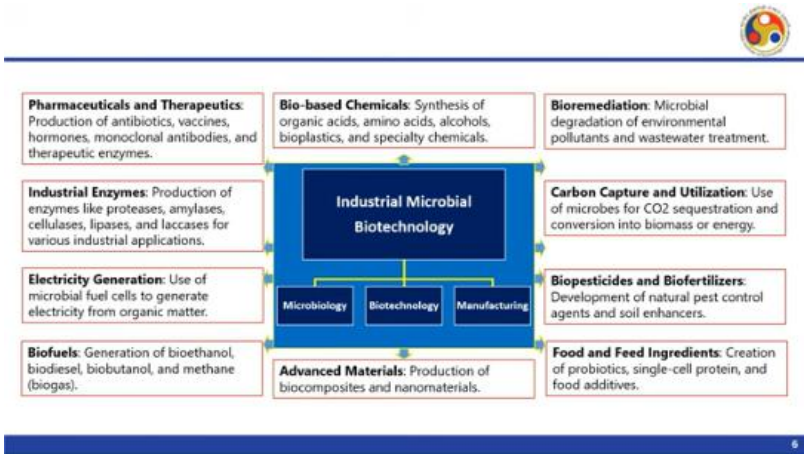
Industrial microbiotechnology plays a crucial role in producing a wide array of high-value products, from life-saving drugs and sustainable chemicals to renewable fuels and clean electricity, all contributing to a more sustainable and bio-based economy. Industrial microbiotechnology involves using microorganisms to produce a variety of high-value products across several sectors. So, if you look into the domain of industrial biotechnology, it is basically multidisciplinary, deriving from the fields of microbiology, biotechnology, and industrial processes or manufacturing. The outcome is bio-based chemicals, which include the synthesis of organic acids, amino acids, alcohols, bioplastics, and specialty chemicals. Or it gives pharmaceutical and therapeutics, where we produce antibiotics, vaccines, hormones, monoclonal antibodies, and therapeutic enzymes.



It also produces many industrial enzymes like proteases, amylases, cellulases, lipases, and lecases for various industrial applications. And then we can use these techniques for microbial fuel cells to generate electricity from organic matter. It also contributes to the generation of bioethanol, biodiesel, biobutanol, and methane, which is biogas. As well as advanced materials, including the production of biocomposites and, of late, nanomaterials. Industrial microbial biotechnology also contributes to manufacturing food and feed ingredients, such as the creation of probiotics, single-cell protein, and food additives. It also results in the production of biopesticides and biophagylases, which are natural pest control agents, as well as soil enhancers.

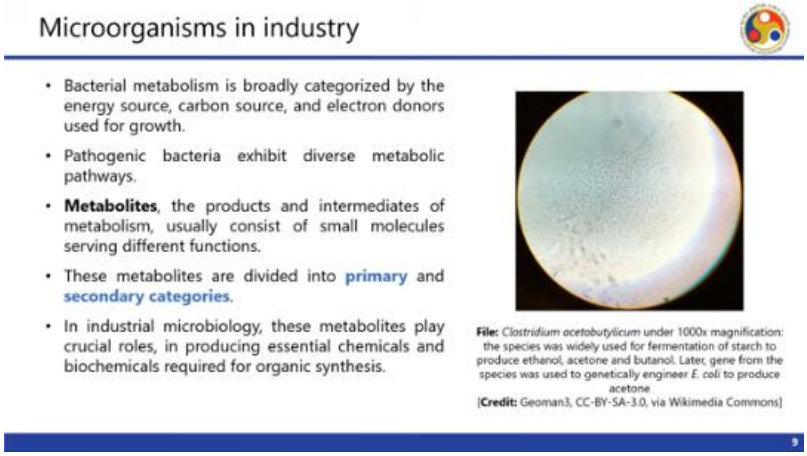
It also contributes to carbon capture and utilization, using microbes for carbon dioxide sequestration and conversion into biomass energy. And finally, it also contributes to bioremediation, whereby microbial degradation of environmental pollutants and wastewater treatment is undertaken. So, it's a very large domain, and we will be discussing many of these applications under various modules and lectures. So let us look into some of

the industrial microorganisms. Here you can see the picture of Penicillium, which is one of the most important industrial microorganisms and is essential for the manufacture of antibiotics.



So let us look into microorganisms in industry. Bacterial metabolism is broadly categorized by the energy source, carbon source, and electron donors used for growth. Pathogenic bacteria exhibit diverse metabolic pathways. Metabolites, which are the products and intermediates of metabolism, usually consist of small molecules serving different functions. These metabolites are divided into primary and secondary metabolite categories.

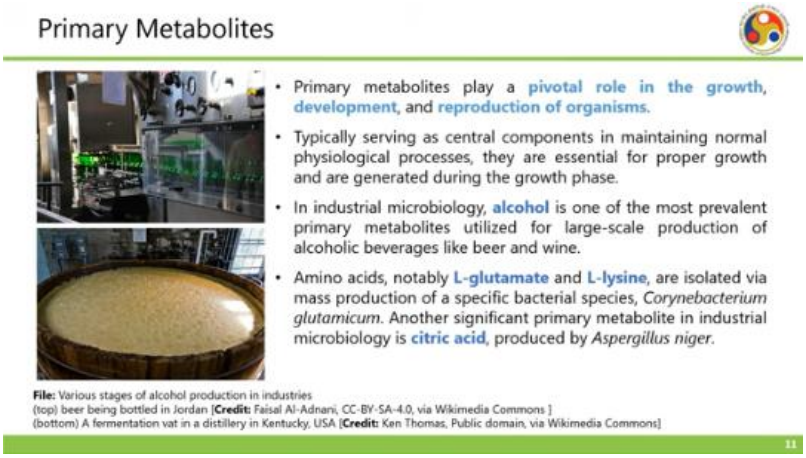
In industrial microbiology, these metabolites play crucial roles in producing essential chemicals and biochemicals required for organic synthesis. So, here you can see Clostridium acetobutylicum under 1000x magnification. This species is widely used for the fermentation of starches to produce ethanol, acetone, and butanol. A later gene from the species was used to genetically engineer Escherichia coli to produce acetone. What are the products from microorganisms?



Molecular products derived from microorganisms encompass a broad spectrum of substances suitable for applications in pharmaceuticals, food and beverage production, agriculture, and various other industries. These can range from antibiotics and enzymes to various organic acids, alcohols, and bioactive compounds, as well as many essential biomolecules like vitamins, amino acids, and polysaccharides. So, some of these include antibiotics, enzymes, biofuels, organic acids, vitamins, bioplastics, and bioactive compounds. We'll have a discussion on these various products and compounds in this lecture. What are primary metabolites?

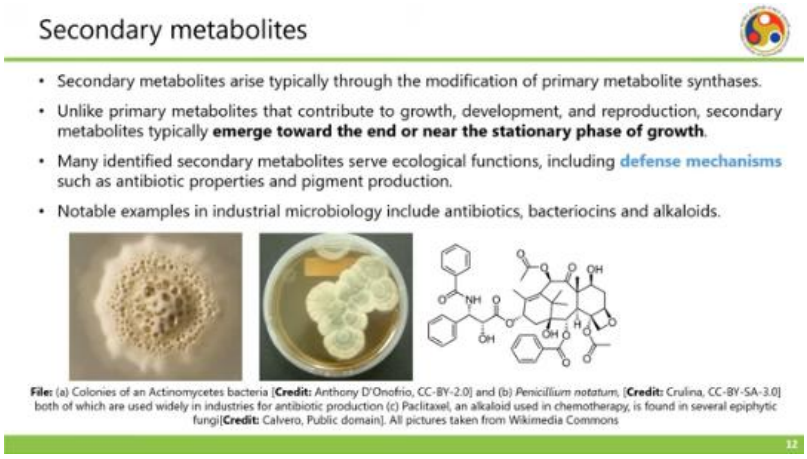
What role do they play? The primary metabolites play a pivotal role in the growth, development, and reproduction of organisms. Typically, they serve as central components in maintaining normal physiological processes. They are essential for proper growth and are generated during the growth phase. In industrial microbiology, alcohol is one of the most prevalent primary metabolites utilized for large-scale production of alcoholic beverages like beer and wine.

Amino acids, notably L-glutamate and L-lysine, are isolated by mass production of a specific bacterial species, Corynebacterium glutamicum. Another significant primary metabolite in industrial microbiology is citric acid, produced by Aspergillus niger. So, here in these photographs, you can see various stages of alcohol production in industries. In the top figure, you can see beer being bottled, and then in the figure below, you can see fermentation in a distillery in the USA. The secondary metabolites arise typically through the modification of primary metabolites.

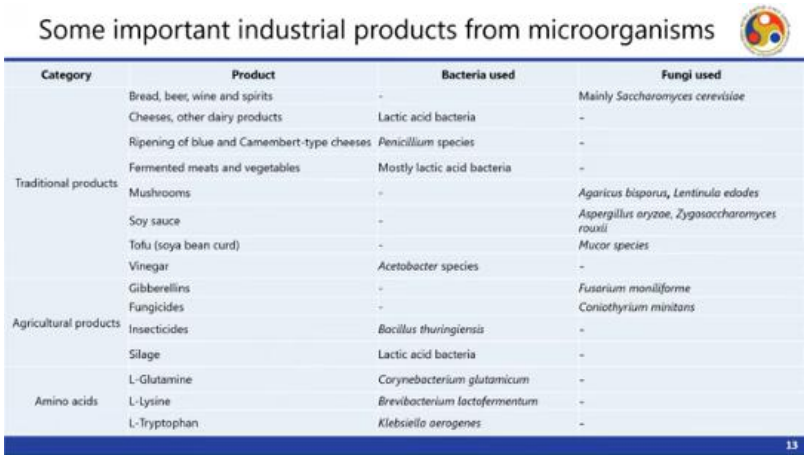


Unlike primary metabolites that contribute to growth, development, and reproduction, secondary metabolites typically emerge toward the end or near the stationary phase of growth. Many identified secondary metabolites serve ecological functions, including

defense mechanisms such as antibiotic properties and pigment production. Some of the notable examples include antibiotics, bacteriocins, and alkaloids. So, here in figure A, we can see colonies of actinomycetes bacteria. And then in B, we can see Penicillium notatum bacteria.



Both of which are used widely in industries for antibiotic production. C is the figure peclitexil, which is an alkaloid used in chemotherapy, is found in several epiphytic fungi. So, some of the important industrial products obtained from microorganisms or with the help of microorganisms are traditional products which have been utilized by mankind for thousands of years, like bread, beer, wine, spirits, and then seeds and other dairy products. Then you have the ripening of blue and Camembert-type seeds, fermented meats and vegetables, mushrooms, soy sauce, tofu, and vinegar. Various bacteria are used; for example, we use lactic acid bacteria for producing dairy products.



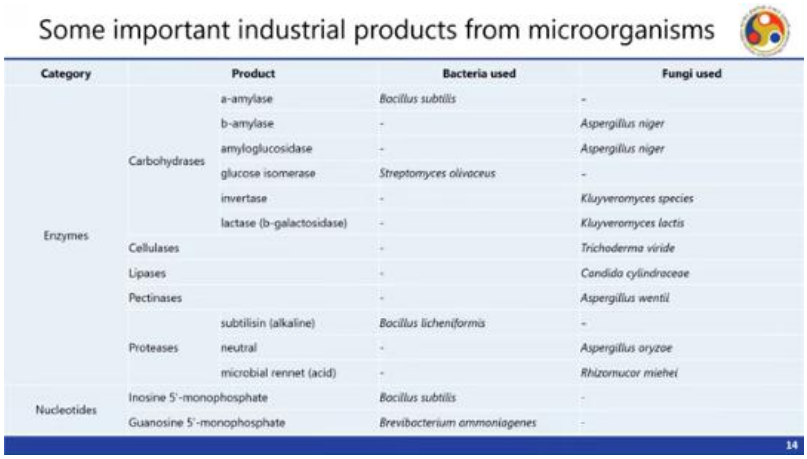
Category	Product	Bacteria used	Fungi used
Traditional products	Bread, beer, wine and spirits	-	Mainly *Saccharomyces cerevisiae*
	Cheeses, other dairy products	Lactic acid bacteria	-
	Ripening of blue and Camembert-type cheeses	*Penicillium* species	-
	Fermented meats and vegetables	Mostly lactic acid bacteria	-
	Mushrooms	-	*Agaricus bisporus*, *Lentinula edodes*
	Soy sauce	-	*Aspergillus oryzae*, *Zygosaccharomyces rouxii*
	Tofu (soya bean curd)	-	*Mucor species*
	Vinegar	*Acetobacter* species	-
Agricultural products	Gibberellins	-	*Fusarium moniliforme*
	Fungicides	-	*Coniothyrium minitans*
	Insecticides	*Bacillus thuringiensis*	-
	Silage	Lactic acid bacteria	-
Amino acids	L-Glutamine	*Corynebacterium glutamicum*	-
	L-Lysine	*Brevibacterium lactofermentum*	-
	L-Tryptophan	*Klebsiella aerogenes*	-

Then we use Penicillium species for the ripening of blue and Camembert-type seeds. For bread, wine, and spirits, we use fungi, Saccharomyces cerevisiae. Then there are certain

fungi, Agaricus, then Lentinula, which give us the mushrooms. And then for soy sauce, we use Aspergillus. Then for tofu, we use Mucor species.

And for vinegar, we use bacteria, Acetobacter species for production. And then for agricultural applications, we have gibberellins, then fungicides, and then insecticides, and silage. Gibberellin is produced by the fungi Fusarium moniliforme. And then fungicides are also produced by various microbes. For example, we can have Coniothyrium, and then insecticides like organisms such as Bacillus thuringiensis, which produces Bt toxin.

And the production of silage, which is assisted or mediated by lactic acid bacteria. Then there are certain amino acids like L-glutamine, L-lysine, and L-tryptophan produced by various bacteria like Corynebacterium, Bacillus, and Klebsiella, respectively. Then we have various kinds of products like enzymes such as cellulases, lipases, pectinases, proteases, and a broad class like carbohydrates, including amylase, alpha and beta amylase, invertase, glucose isomerase, lactase, etc. And these are produced by various bacteria and fungi, as can be seen in this table. And then we also have nucleotides, which are produced by Bacillus and Brevibacterium species.



Some important industrial products from microorganisms

Category		Product	Bacteria used	Fungi used
Enzymes	Carbohydrases	a-amylase	*Bacillus subtilis*	-
		b-amylase	-	*Aspergillus niger*
		amyloglucosidase	-	*Aspergillus niger*
		glucose isomerase	*Streptomyces olivaceus*	-
		invertase	-	*Kluyveromyces species*
		lactase (b-galactosidase)	-	*Kluyveromyces lactis*
	Cellulases		-	*Trichoderma viride*
	Lipases		-	*Candida cylindraceae*
	Pectinases		-	*Aspergillus wentil*
	Proteases	subtilisin (alkaline)	*Bacillus licheniformis*	-
		neutral	-	*Aspergillus oryzae*
		microbial rennet (acid)	-	*Rhizomucor miehei*
Nucleotides	Inosine 5'-monophosphate		*Bacillus subtilis*	-
	Guanosine 5'-monophosphate		*Brevibacterium ammoniagenes*	-

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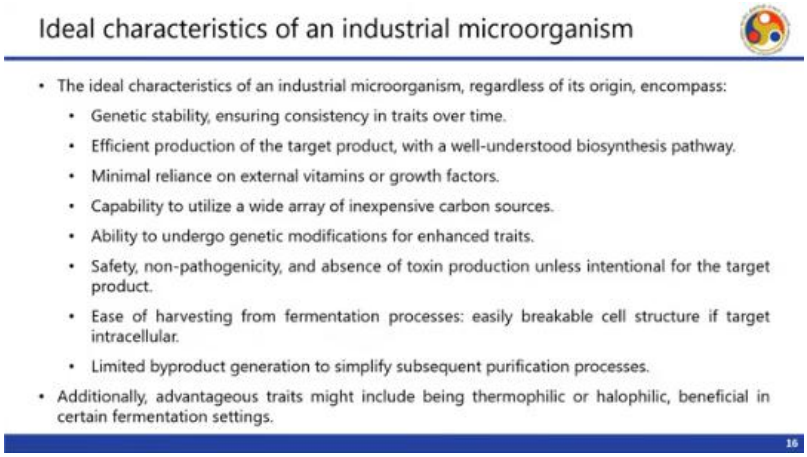
Then certain other industrial products like fuel and chemical feedstocks, such as acetone, butanol, ethanol, glycerol, and methane, are produced by Clostridium, Gymnomonas, Methanogenic species, or even fungi like Saccharomyces cerevisiae. And then we have organic acid producers like Acetobacter, which produces acetic acid; Aspergillus niger, which produces citric acid; and Rhizopus, which produces fumaric acid. And then gluconic acid, which is produced by Acetobacter suboxidans. Then itaconic acid by Aspergillus itaconicus. And then kojic acid by Aspergillus flavus.

And then lactic acid produced by Lactobacillus delbrueckii. So, what are the ideal characteristics of an industrial microorganism? The ideal characteristics of an industrial microorganism, regardless of its origin, are as follows. It should have genetic stability and ensure consistency in traits over time. Efficient production of the target product.

So, we saw so many different kinds of products produced by either bacteria or fungi, as discussed in the various tables just now. The production of those products should be highly efficient, and the biosynthetic pathway by which these products are made should be well understood. Minimal reliance on external vitamins or growth factors is one of the important features. They should have the capability to utilize a wide range of inexpensive carbon sources so that the production cost is lowered. They should have the ability to undergo genetic modifications for enhanced traits. Then, safety, non-pathogenicity, and absence of toxin production—unless intentional for the target product—are important features.

But, supposing our product itself is a toxin, then we would desire that the toxin production is highly efficient in that case. One important thing is the ease of harvesting from fermentation processes and easily breakable cell structure, because most of the time the product may be inside the microbial cell. If the target or the product is intracellular, limited byproduct generation simplifies the subsequent purification process. And then, if we have more of the product compared to the byproducts, the yield efficiency will also increase.

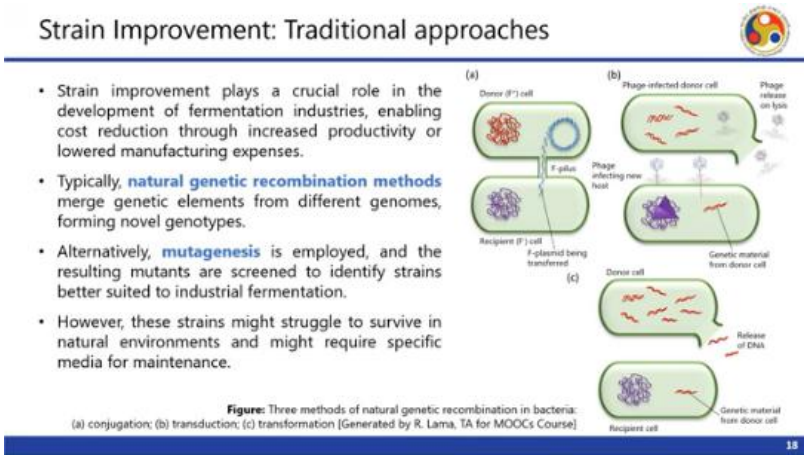
Additionally, advantageous traits like being thermophilic or halophilic will be beneficial in certain fermentation settings. Let us now discuss a pure culture because nowadays all industrial processes are carried out with pure culture or monoculture. So, we begin with collecting samples. After collecting the sample, the key challenge is determining appropriate growth media and conditions to isolate the target microorganisms.



The process begins by suppressing common organisms and promoting rare ones, often through enrichment cultures in batch or continuous systems to encourage organisms with desired traits. This approach works best when the target trait provides a competitive advantage and acts like a selection marker. Bio-cultures are then isolated on selective solid media under tailored conditions. Once isolated, each culture is screened for desired properties, such as enzyme production or inhibitory compounds, with strain development expected to improve performance later. Selected isolates are also evaluated for stability and other features, like non-toxicity.

So, let us discuss the traditional approach for strain improvement. There are three methods of natural genetic recombination in bacteria. Number A shows the method of conjugation, B shows transduction, and C shows transformation. Strain improvement plays a crucial role in the development of fermentation industries, enabling cost reduction through increased productivity or lowered manufacturing expenses. Typically, natural genetic recombination methods merge genetic elements from different genomes, forming novel genotypes.

Alternatively, mutagenesis is employed, and the resulting mutants are screened to identify strains better suited to industrial fermentation. However, these strains might struggle to survive in natural environments and might require specific media for maintenance. Although bacteria lack any form of sexual reproduction, they are able to exchange genetic material via the processes of conjugation, transduction, and transformation, as shown in these figures. Conjugation involves direct contact between a donor and recipient via a filamentous protein, a sex pilus, pulling them together.



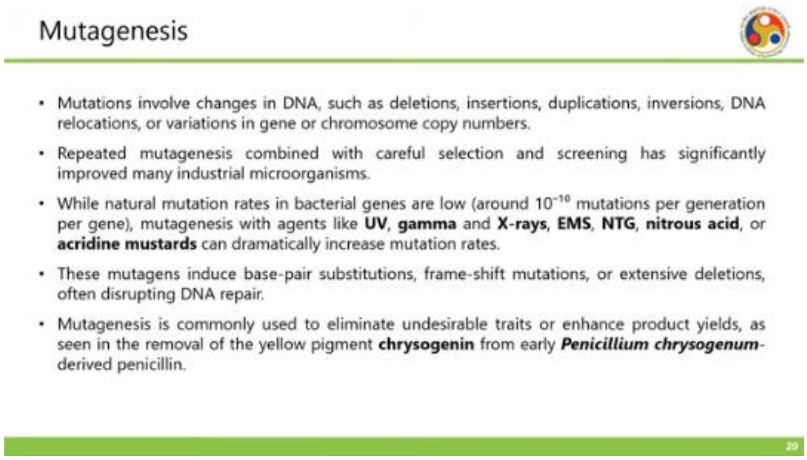
The donor transfers all or part of its plasmid through the pilus to the recipient as can be seen over here. Transaction involves a bacteriophage vector delivering genes between

bacteria. When the FAS attaches to a bacterial cell, its DNA gets incorporated into the host chromosome during its replication. The FAS may acquire bits of host DNA and carry them to new hosts. Bacteriophages can also acquire transposons, DNA pieces capable of relocating from one DNA to another and transfer them to the new bacterial cells.

So, here we have these FAS which is infecting the donor cell. And thereby genetic material is getting introduced from the donor cell into the fuzz infecting the new host. Then transformation involves the uptake of naked DNA from the environment. Here the fuzz is not there, neither there is a sex pylos. From the environment into the cell where it gets integrated, this process occurs randomly in natural settings as the DNA fragments originate from live cells.

However, only competent cells, which are cells which are ready to accept DNA in a specific physiological state permit DNA uptake and can incorporate these fragments. So, here you have a donor cell which is releasing DNA and this is the recipient cell which is competent and receives the genetic material from the donor cell. So, another method is mutagenesis which involves changes in DNA such as deletions, insertions, duplications, inversions, DNA relocations or variations in gene or chromosome copy numbers. Repeated mutagenesis combined with careful selection and screening has significantly improved many industrial microorganisms.

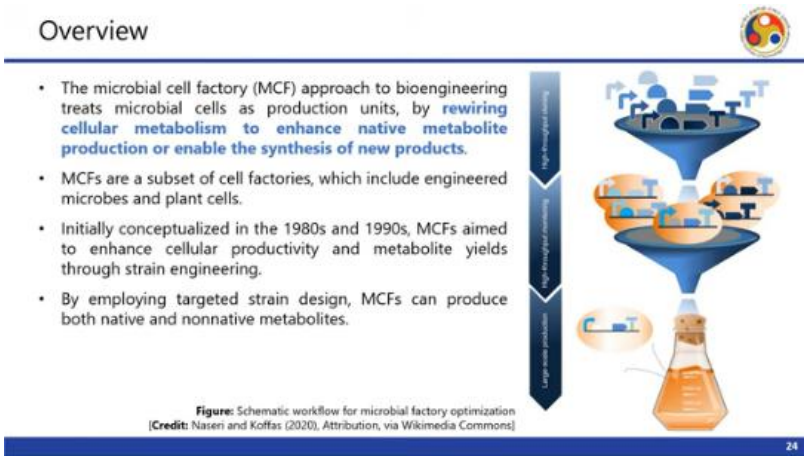
While natural mutation rates in bacterial genes are low, around 10 to the power of minus 10 mutations per generation per gene, mutagenesis with agents like UV, gamma and X-rays, or chemicals like ethyl methyl sulfonate, NTG, nitrosyl acid, or acridine mustards can dramatically increase mutation rates. These mutagens induce base pair substitutions, frameshift mutations, or extensive deletions, often disrupting DNA repair. Mutagenesis is commonly used to eliminate undesirable traits or enhance product yield, as seen in the removal of the yellow pigment chrysogenin from early Penicillium chrysogenum-derived penicillin. Now, another approach for strain improvement is using rDNA technology or recombinant DNA technology. Here, cell fusion techniques such as hybridoma formation for monoclonal antibody production have profoundly influenced industrial microbiology.



Unlike natural recombination, modern recombinant DNA technology provides precise control methods to create new gene combinations using genetic materials from nearly any organism, living or extinct. This allows specific gene sequences to be transferred between organisms, integrating new techniques into strain enhancement protocols to improve product yields by eliminating metabolic bottlenecks and modifying or amplifying metabolic steps. Microbes can be engineered to produce and secrete a wider range of enzymes, facilitating the synthesis of novel compounds and the use of more cost-effective substrates. With no constraints on gene origins, microorganisms now produce proteins from plants and animals, including valuable products like human growth hormone, insulin, and interferons. Let us now discuss microbial cell factories.

Here, we will discuss the characteristics of microbial cell factories. Then, a process to microbial cell factories. So, here in this picture, you can see the manufacturing of seeds. Whey from seed factories is often used in fermentation media. Briefly, the microbial cell factory approach to bioengineering creates microbial cells as production units by rewiring cellular metabolism.

To enhance native metabolite production or enable the synthesis of new products. Microwell cell factories are a subset of cell factories, which include engineered microbes and plant cells. Initially conceptualized in the 1980s and 1990s, these MCFs aim to enhance cellular productivity, and metabolite yields through strain engineering. By employing targeted strain design, MCFs can produce both native and non-native metabolites.



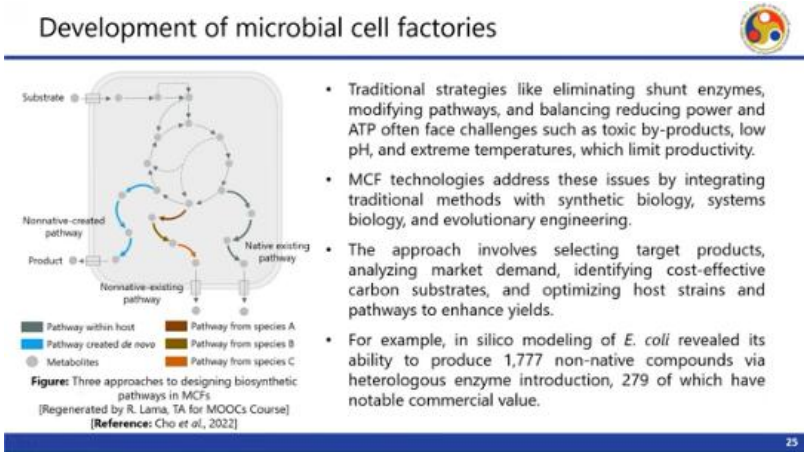
Moreover, they streamline synthesis cycles and simplify product separation processes. Traditional strategies like eliminating shunt enzymes, modifying pathways, and balancing reducing power and ATP often face challenges such as toxic byproducts, low pH, and extreme temperatures, which limit productivity. MCF technologies address these issues by integrating traditional methods with synthetic biology, systems biology, and evolutionary engineering. The approach involves selecting target products, analyzing market demand, identifying cost-effective carbon substrates, and optimizing host strains and pathways to enhance yield.

Let us look into the various approaches to designing biosynthetic pathways in microbial cell factories. Here, you can see the existing pathway within the host. We call them the native existing pathways. Then, we have these pathways created de novo. This is the de novo blue-colored pathway, which is non-native.

Created pathway. And then we have a pathway from, say, species A. So we clone the genes from that species and put them into here. Then we may have another pathway from species B and another pathway from species C. So, basically, we may have three approaches: one is utilizing the native existing pathway, or we may create a non-native pathway de novo—entirely new—or we may borrow from various organisms to build up a network of pathways, like taking from species A, B, and C as in the center. And then, the ultimate result is that we have a substrate which enters the cell, and this substrate is acted upon by any of these pathways—the native, non-native, de novo, or the non-native transferred existing pathway—and then transformed into products, all of which have economic value.

So, this cell is considered a factory on its own because you put in raw material and then you get a product, and that raw material is converted into products by numerous pathways, as discussed here. Now, in silico modeling of E. coli reveals its ability to produce 1,777

non-native compounds via heterologous enzyme introduction. If we introduce heterologous enzymes into the E. coli system, we can produce 1,777 non-native compounds. Then, there are 279 of which have notable commercial value.



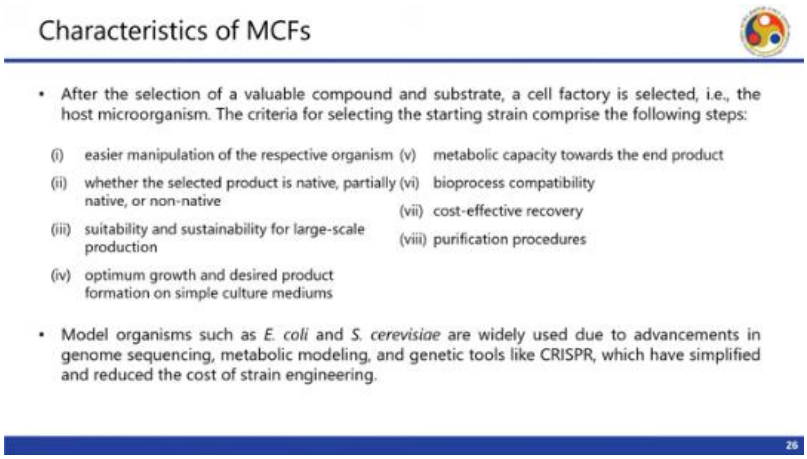
So, imagine one single cell, by the introduction of heterologous enzyme systems, suddenly gains the capability of producing roughly around 2,000 different chemicals, and then out of which roughly around 300 have high commercial value. So, that is the potential of microbial cell factories. So, what are the characteristics of microbial cell factories after the selection of a valuable compound and substrate? A cell factory selected is basically the host microorganism.

For example, in this case, E. coli can be a cell factory for these 279 products with commercial value. The criteria for selecting the starting strain comprise the following steps. Number one, the strain should be easier to manipulate of the respective organism. It should be easy to manipulate.

Whether the selected product is native, partially native, or non-native, we should have that knowledge. Suitability and sustainability for large-scale production. Because we are speaking about industrial-scale production, the strain should be suitable for that large-scale production. It should show optimum growth and desired product formation on a simple culture medium. The culture medium we are going to use should not be very costly.

It should be simple, abundant, and low-cost. It should have metabolic capacity toward the end product. Then, bioprocess compatibility is very important, important. Then, the recovery should be cost-effective, and the purification procedures should be, to the maximum extent, economical and simple. Model organisms like E. coli and

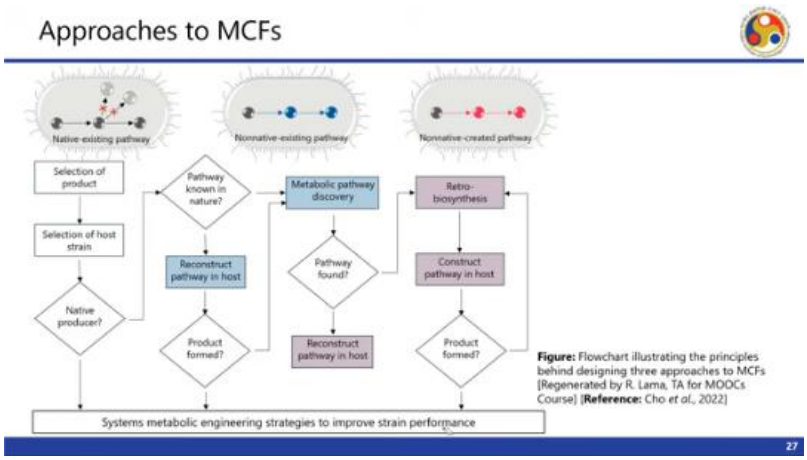
Saccharomyces cerevisiae are widely used due to advancements in genome sequencing because we know the entire genomes of these.



Organisms, and then we can understand whether the pathway is native or non-native. So, we can do metabolic modeling and use genetic tools like CRISPR-Cas9, which have simplified and reduced the cost of strain engineering. These tools are very well established in both of these two systems. Let us now discuss the various approaches to microbial cell factories. So, here you can see the different pathways we have just discussed: the native existing pathway, the non-native existing pathways, and the non-native created pathways. So, how are we going to make decisions at various levels?

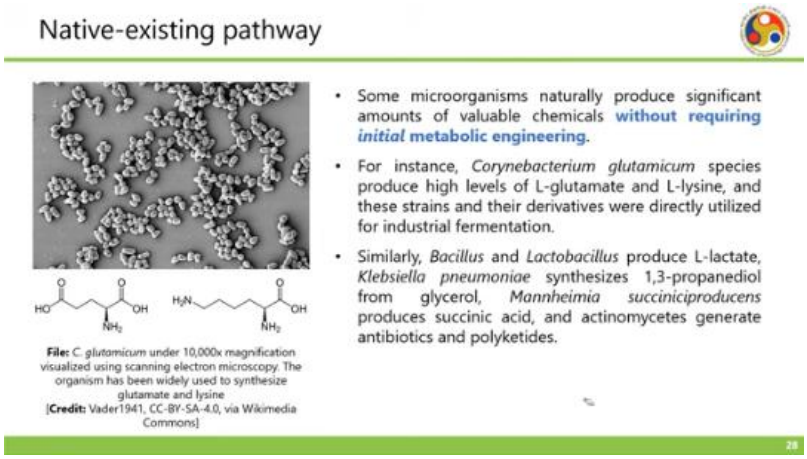
First, we need to consider the target product in our mind, and then for producing that particular product, we need to select the host strain. Once we have the strain in hand, we will try to examine whether this particular strain is a native producer of this particular product. If the answer is yes, we can proceed with production. If the answer is no, we will try to find out whether the pathway that produces this particular product is known in nature. Then we may take two approaches.

One is to reconstruct the pathway in the host, which will help us in the formation of the product. Or, if it is not known, we go for metabolic pathway discovery. Then we try to find the pathway, reconstruct it in the host, and proceed with retrobiosynthesis. Finally, we construct the pathway in the host, and the product will be formed. These are the three approaches by which we proceed with microbial cell factory and strain improvement production. Some microorganisms naturally produce significant amounts of valuable chemicals without requiring initial metabolic engineering. Here, they have the native existing pathway.



So here you can see glutamicum under 10,000x magnification visualized using scanning electron microscopy. This organism has been widely used to synthesize glutamate and lysine. So, this is the glutamicum species which produces glutamate and lysine. Similarly, there are other species like Bacillus and Lactobacillus, which produce L-lactate. Klebsiella pneumoniae synthesizes 1,3-propanediol from glycerol.

Then we have Menhemia, which produces succinic acid, and actinomycetes generate antibiotics and polyketides. These are all native existing pathways within these microorganisms, and we can directly use them for the production of these particular targeted products. So, we only have to go for optimal host selection and further engineering to improve production efficiency if it is low. The second approach is the non-native existing pathway. So, some target chemicals cannot be synthesized by microbial hosts due to missing biosynthetic genes because those genes are absent.



So, we can resolve this by incorporating enzymes and pathways from other organisms or metagenomes. So, we have databases like CAG, MetaPsych, and Brenda, which provide

detailed information on metabolic pathways, enzymes, and genes, enabling pathway reconstruction by integrating external genes. For products with incomplete pathway data or poorly functioning enzymes, novel enzymes can be identified through genome and transcriptome analysis of natural biosynthetic gene clusters. Then we have the non-native created pathway. Metabolic engineering has enabled the production of many chemicals using existing biosynthetic pathways.

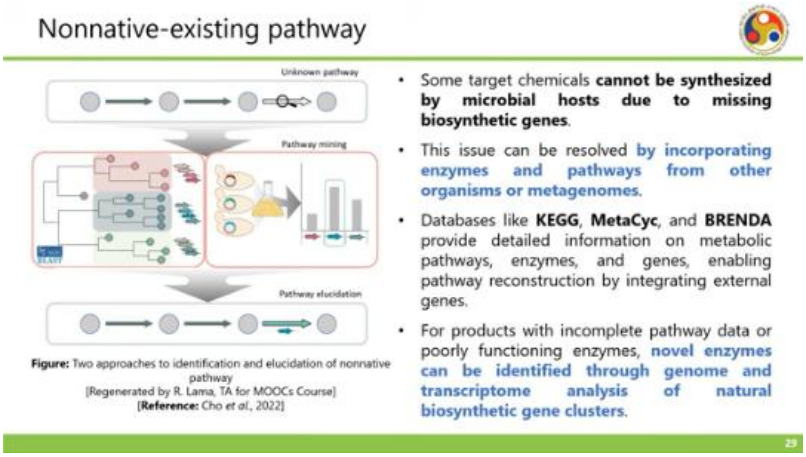
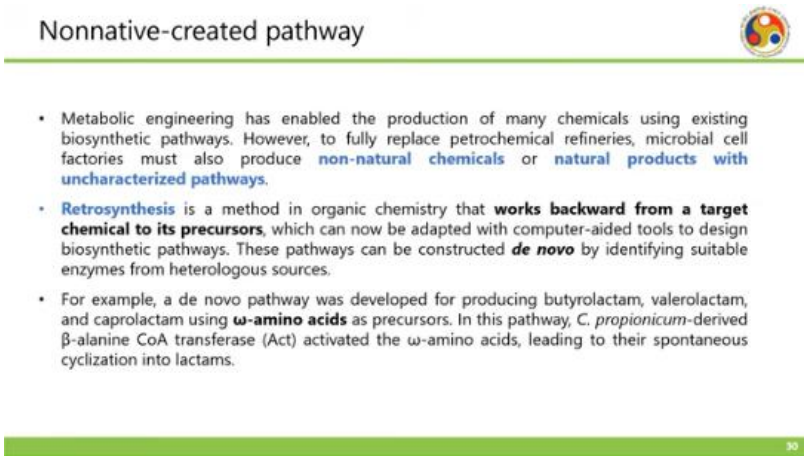


Figure: Two approaches to identification and elucidation of nonnative pathway
[Regenerated by R. Lama, TA for MOOCs Course]
[**Reference:** Cho *et al.*, 2022]

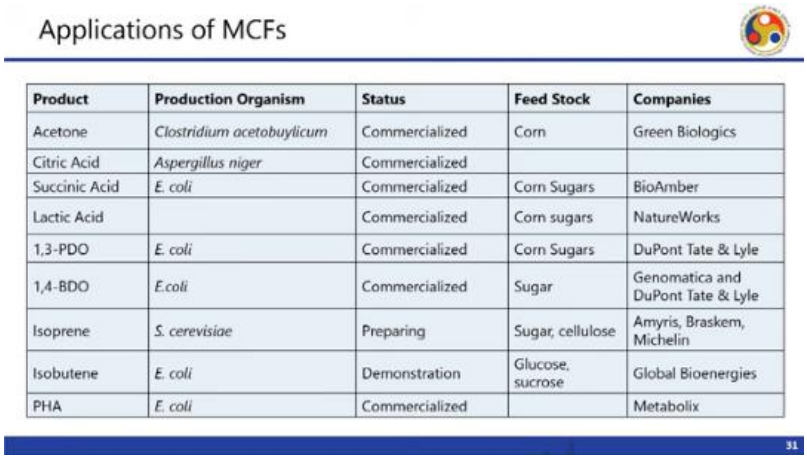
However, to fully replace petrochemical refineries, microbial cell factories must also produce non-natural chemicals or natural products with uncharacterized pathways. Retrosynthesis is a method in organic chemistry that works backward from a target chemical to its precursors. This can now be adapted with computer-aided tools to design biosynthetic pathways. These pathways can be constructed de novo by identifying suitable enzymes from heterologous sources. For example, a de novo pathway was developed for producing beta-lactam.

Valerolactam and caprolactam were produced using omega amino acids as precursors in these pathways. C. propionicum-derived B-alanyl coenzyme A-transferase activated the omega amino acids, leading to their spontaneous cyclization into lactams. Now, let us have an overview of the applications of MCFs. We have various products here, such as acetone, citric acid, succinic acid, and so on. We also have the organisms that can produce them.



For example, acetone is produced by Clostridium acetobutylicum. Citric acid is produced by Aspergillus niger. E. coli produces succinic acid. Then, 1,3-BDO, 1,4-BDO, isoprene (produced by Saccharomyces cerevisiae), and isobutene (produced by E. coli). Many of these have already been commercialized.

Some are in various stages like preparation, demonstration, and some have already been commercialized again. Now, we use various kinds of feedstocks, and in one of the slides, we discussed that the substrate feedstocks should be very, very low cost. For example, we use corn, corn sugars, sugar, cellulose, glucose, and sucrose, and various companies have already taken the lead in producing these particular chemicals. Then we have ethanol production from sugarcane using Saccharomyces cerevisiae. Then we have farnesene, butanol, and isobutanol.



Product	Production Organism	Status	Feed Stock	Companies
Acetone	*Clostridium acetobuylicum*	Commercialized	Corn	Green Biologics
Citric Acid	*Aspergillus niger*	Commercialized		
Succinic Acid	*E. coli*	Commercialized	Corn Sugars	BioAmber
Lactic Acid		Commercialized	Corn sugars	NatureWorks
1,3-PDO	*E. coli*	Commercialized	Corn Sugars	DuPont Tate & Lyle
1,4-BDO	*E.coli*	Commercialized	Sugar	Genomatica and DuPont Tate & Lyle
Isoprene	*S. cerevisiae*	Preparing	Sugar, cellulose	Amyris, Braskem, Michelin
Isobutene	*E. coli*	Demonstration	Glucose, sucrose	Global Bioenergies
PHA	*E. coli*	Commercialized		Metabolix

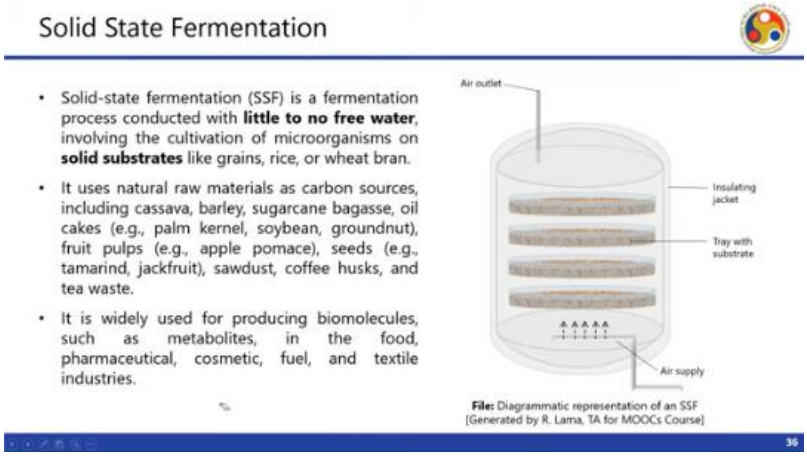
These have all already been commercialized. Let us now move to Section 4, where we will discuss the fermentation process overall, including the various upstream and downstream processes. So, here in this picture, you can see industrial bioreactor filtration and

sterilization equipment. Fermentation, in general, refers to the chemical transformation of organic substances into simple compounds by the action of enzymes produced by microorganisms such as yeast, molds, or bacteria. It can be classified based on the substrate used, like solid-state fermentation or submerged fermentation.

While solid-state fermentation is practiced, most fermentations employ liquid media, often termed as broth, under aerobic or anaerobic conditions. Most fermentation processes involve stirring, aeration, and adherence to aseptic protocols, with the exception of most alcoholic fermentations. Let us discuss solid-state fermentation first. So, here is a diagrammatic representation of a solid-state fermentation process.

So, here we have a fermenter with an insulating jacket. Then this is stacked with certain trees having the substrate, and then there is an air supply from below and an air outlet because the air supply will be continuous, and air has to be let out from the system. So, it uses natural raw materials as carbon sources. So, these are the natural raw materials placed in these trays, which may be cassava, barley, sugarcane bagasse, oil cakes, palm kernel, soybean, groundnut, fruit bulbs, seeds, tamarind, sawdust, coffee husks, and tea waste.

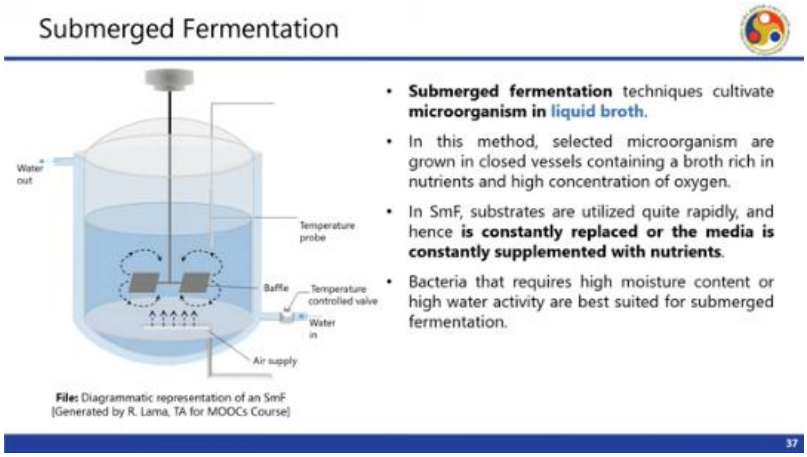
So, you can see from these that many of these are, in general, agro-wastes, for example, or they may be waste from the timber industry. It is widely used for producing biomolecules such as metabolites in the food, pharmaceutical, cosmetic, fuel, and textile industries. Now, in the summer's fermentation, the fermenter is different. So, here you can see a fermenter which has a water inlet. So, basically, this is a water jacket which keeps the temperature of the fermenter fixed.



So, you have these temperature control valves over here. Water is heated to a desired temperature, which will carry the heat and provide warmth to this fermenter. There is an

optimum temperature condition at which these microbes will do the fermentation. And of course, there is a continuous supply of air from below, and then you have certain probes over here: the temperature probe, which will keep track of the temperature of this media, and then you have a stirrer which will keep on mixing the media. So, submerged fermentation techniques cultivate microorganisms in liquid broth.

Selected microorganisms are grown in closed vessels containing a broth rich in nutrients and a high concentration of oxygen. Substrates are utilized quite rapidly and hence are constantly replaced or the media is constantly supplemented with nutrients. Bacteria that require high moisture content or high water activity are best suited for submerged fermentation. Submerged fermentation is primarily used in the extraction of secondary metabolites that need to be used in liquid form. So, now let us discuss the fermentation media in a little more detail.



Most fermentation processes primarily utilize liquid media, which is often termed as broth, although there are instances of solid substrate fermentations, as we have discussed just now. So, here on the left we can see algae fermentation media at lab scale, and in the other figure we can see molasses, which is one of the most widely used carbon sources in the industrial setting. The fermentation medium is crucial, needing to meet both the nutritional needs of the microorganism and fulfill the technical goals of the process. Thus, the nutrient composition should be carefully devised to stimulate the production of the desired output, be it cell biomass or a specific metabolite. In almost all fermentations except those involving solid substrate, substantial volumes of water are used in formulating the media.

Fermentation media

- Most fermentation processes primarily utilize liquid media, often termed broth, although there are instances of solid-substrate fermentations being employed.
- The fermentation medium is crucial, needing ***to meet both the nutritional needs of the microorganism and fulfill the technical goals of the process***.

File: (left) Algal fermentation media in lab-scale production of biofuels at Oak Ridge National Laboratory, USA
[**Credit:** Oak Ridge National Laboratory, CC-BY-2.0, via Wikimedia Commons]
(right) Molasses is one of the most widely used carbon source in industries
[**Credit:** Tractorboy60, Public domain, via Wikimedia Common]

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Apart from water, let us see the composition of the fermentation media. So, essential components of a typical medium include a carbon source, which serves as both an energy source and carbon unit for biosynthesis, along with nitrogen, phosphorus, and sulfur sources. Additionally, minor and trace elements must be provided, and some microorganisms may require supplementation with specific vitamins like biotin and riboflavin. Aerobic fermentations rely on a constant supply of molecular oxygen, while even some anaerobic fermentations necessitate initial aeration of the media.

Media typically include buffers or employ pH control through the addition of acids and alkalis, and antifoam agents are very, very essential. Certain processes may require the introduction of precursors, inducers, or inhibitor compounds at specific fermentation stages. So, what are these precursors and antifoams? In certain fermentations, specific precursors are added in controlled amounts and in relatively pure forms. Some examples are phenylacetic acid or phenylacetamide in penicillin production, D-threonine in L-isoleucine production by Serratia marcescens.

Composition of the fermentation media

- In almost all fermentations except those involving solid substrates, substantial volumes of water are used in formulating the medium.
- Essential components of a typical medium include a **carbon source**, which serves as both an energy source and carbon units for biosynthesis, along with **nitrogen**, **phosphorus**, and **sulfur** sources.
- Additionally, minor and trace elements must be provided, and some microorganisms may require supplementation with specific vitamins like **biotin** and **riboflavin**.
- Aerobic fermentations rely on a constant supply of **molecular oxygen**, while even some anaerobic fermentations necessitate initial aeration of the media.
- Media typically include buffers or employ pH control through the addition of acids and alkalis, and antifoam agents might be essential.
- Certain processes may require the introduction of precursor, inducer, or inhibitor compounds at specific fermentation stages.

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Then we have anthranilic acid in L-tryptophan production by Hansenula anomala. Then, antifoams are also essential. Foam forms when media proteins denature at the air-broth interface, leading to issues like filter blockage, contamination, and microorganism release. Antifoams, being surface-active agents, reduce the surface tension that binds foam, thereby improving throughput. Natural antifoams encompass plant oils like soy, sunflower, and rapeseed oils, deodorized fish oil, mineral oils, and tallow.

Synthetic antifoams primarily consist of silicone oils, polyalcohols, and alkylated glycols. Then there are inducers and inhibitors, which are very, very essential in fermentations involving genetically modified microorganisms. Inducers are frequently essential for the expression of cloned genes in these genetically modified organisms. When turned on, they can impede their growth due to exceptionally high levels of transcription and translation. Consequently, these cloned genes incorporate inducible systems that initially maximize growth to establish high biomass density.

Subsequently, the cloned gene can be activated by introducing a specific chemical inducer. Inhibitors are employed to redirect metabolism toward the desired product and curtail the formation of other metabolic intermediates. Inhibitors halt a pathway at a particular stage, preventing further metabolism of the target product. For instance, in the production of glycerol by Saccharomyces cerevisiae, sodium bisulfite is used as an inhibitor to redirect metabolism.

One of the important points is inoculum. In industrial fermentation, the term inoculum refers to the initial culture of microorganisms—whether bacteria, yeast, or fungi—that is introduced into a fermentation process. So, in this picture, we can see the preparation of a wine yeast starter culture with yeast getting rehydrated.

This initial culture serves as the starting point for the production of desired products through fermentation. The inoculum contains a high concentration of the selected microorganisms and is used to inoculate a larger fermentation vessel, providing the microorganisms with the necessary conditions to grow, multiply, and carry out the fermentation process on a larger scale. The goal is to achieve optimal conditions for the production of various substances, including biofuels, enzymes, organic acids, or pharmaceuticals, depending on the specific industrial application. Let us now discuss one of the important apparatuses or equipment in this fermentation process: the fermenter.

Inoculum

- In industrial fermentation, the term "**inoculum**" refers to the initial culture of microorganisms (such as bacteria, yeast, or fungi) that is introduced into a fermentation process.
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File: Preparation of a wine yeast starter culture with rehydrated yeast [**Credit:** Agne27, CC-BY-3.0, via Wikimedia Commons]

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The primary role of a fermenter is to create an optimal environment where an organism can efficiently generate a desired product, whether it's cell biomass, a metabolite, or a bioconversion product. Most fermenters aim to maintain high concentrations of biomass, which is crucial for many fermentation processes. Control strategies vary based on the specific processes and their objectives. The performance of a fermenter hinges or relies on various factors, with key control parameters being agitation rate, oxygen transfer, pH, temperature, and foam production. So, in this picture on the left, you can see the fermenting and conditioning tanks over here.

Fermenters

- The primary role of a fermenter is to create an optimal environment where an organism can efficiently generate a desired product, whether it's cell biomass, a metabolite, or a bioconversion product.
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- Control strategies vary based on the specific process and its objectives.
- The performance of a fermenter hinges on various factors, with key control parameters being agitation rate, oxygen transfer, pH, temperature, and foam production.

File: (left) Fermenting & conditioning tanks in Sheffield, UK [**Credit:** Glyn Baker, CC-BY-SA-2.0, via Wikimedia Commons]
(right) Lab-scale fermentation tank with mammalian cells [**Credit:** Karel Schmiedberger, CC-BY-3.0, via Wikimedia Commons]

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And then in the other picture, we can see the lab-scale fermentation tank with mammalian cells. And this is just to give an idea that fermentation may also be carried out by non-microbial cells, like the mammalian cells in this case. There are two types of fermenters. One is the smaller ones, which are used in the lab, and the bigger ones, like these, which are used in industrial settings. So, laboratory-scale fermentations might use simple bottles or conical flasks that can be agitated to provide aeration when needed.

These vessels are typically sealed with cotton wool or a Styrofoam bung to prevent microbial contamination from outside. But this sealing method can lead to evaporation losses and limited gas exchange. Consequently, specifically designed vessels for fermentation are generally preferred even at the laboratory level. In industrial applications, fermenters with capacities reaching several hundred thousand liters are utilized. These are typically purpose-built and tailored for a particular process, although some flexibility might be required in certain cases.

Their design, construction quality, mode of operation, and level of sophistication are largely dictated by factors like the production organism. Optimal operating conditions for the target product, product value, and production scale. The primary considerations include reliability and the imperative to minimize both initial investment and ongoing operational expenses. Let us now discuss the control of physical and chemical conditions. Controlling the key parameters of a bioreactor, such as temperature, pH, pure oxygen, and pressure, is essential to maintain cells in a physical and chemical environment, optimizing their performance.

The majority of cells have an optimal temperature of operation. Temperatures higher than this optimum quickly have a dramatic effect on cell viability, while lower temperatures can result in slower cell metabolism. This optimum is maintained by a temperature sensor, a water jacket on the bioreactor, and a temperature control unit. Similarly, cells have an optimal operational pH maintained by buffers like bicarbonate buffer. Most bioreactors or fermenters employ a proper agitation system, which allows the media, the cells, and the gases to be maintained in an ideal mixing state.

Additionally, aerobic fermentations may require optimal concentrations of molecular oxygen, which is maintained by measuring the oxygen transfer rate, representing the rate at which oxygen can be delivered to the biological system. In lab-scale fermentation, after selecting a microorganism for a specific process, initial optimization is conducted in laboratory-scale conditions, typically using fermenters ranging from 1 to 10 liters. Here, you can see a lab-scale bio-fermenter. This involves analyzing media composition, exploring various feeding strategies—whether we opt for batch, fed-batch, or continuous fermentation—and determining the most suitable fermentation system types, such as stirred tank, airlift, packed bed, solid-state, hollow fiber, etc. Other critical considerations encompass reactor setup and the management of pH, dissolved oxygen, foam, and temperature control.

Control of physical and chemical conditions

- Controlling the key parameters of a bioreactor, such as temperature, pH, pure O_2, and pressure are essential to maintain cells in a physical and chemical environment, optimizing their performance.
- The majority of cells have an **optimal temperature of operation**: temperatures higher than this optimum can quickly have a dramatic effect on the cell viability, while lower temperature can result in a slower cell metabolism. This optimum is matained by a temperature sensor, a water jacket on the bioreactor, and a temperature control unit (TCU).
- Similarly, cells have optimal operation pH, maintained by buffers like **bicarbonate buffer**.
- Additionally, most bioreactors employ a proper agitation system, which allows the media, the cells, and the gases to be maintained in an ideal mixture state.
- Additionaly, aerobic fermentations may require optimal concentrations of molecular O_2, which is maintained by measuring the **oxygen transfer rate (OTR)**, representing ***the rate at which oxygen can be delivered to the biological system***.

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Once optimal product yield is achieved in the laboratory, the process undergoes scaling up, initially to pilot scale, ranging from 10 to 100 liters. So from 1 to 10 liters, we go to 100 liters and eventually to industrial scale, which will go up to 1,000 to 100,000 liters or more, depending on the specific process capacity of the plant. So, scaling up is very, very important. So, here you can see a small flask of around 500 ml. Then we move to 5 liters, then 50 liters, and then 500 liters.

Lab scale fermentation

File: A lab-scale biofermenter
(**Credit:** Sarathkumaran Ranganathan, CC-BY-SA-4.0, via Wikimedia Commons)

- After selecting a microorganism for a specific process, initial research is conducted in laboratory-scale conditions, typically using fermenters ranging from 1 to 10 liters.
- This phase involves analyzing media composition, exploring various feeding strategies (batch, fed-batch, continuous, etc.), and determining the most suitable fermentation system type (such as stirred tank, airlift, packed bed, solid-state, hollow fiber, etc.).
- Other critical considerations encompass reactor setup and the management of pH, dissolved oxygen, foam, and temperature.
- Once optimal product yield is achieved in the lab, the process undergoes scaling-up initially to pilot scale (ranging from 10 to 100 liters) and eventually to industrial scale (ranging from 1,000 to 100,000 liters or more, depending on the specific process).

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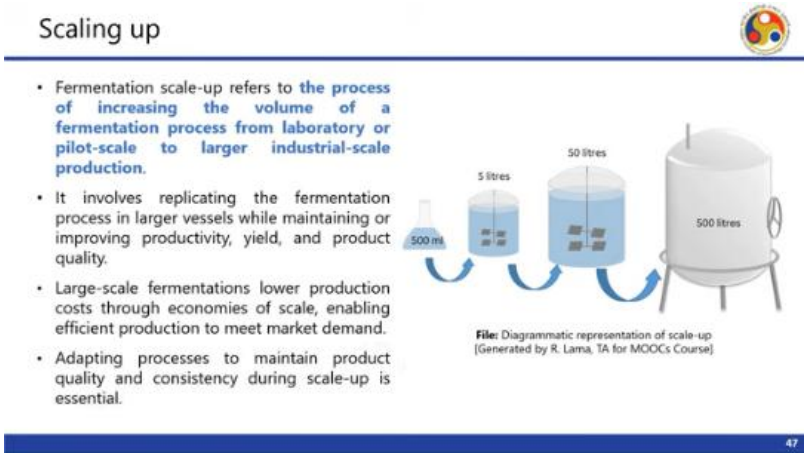
So, as we move from a small volume to a large volume, this process is called scaling up. Fermentation scale-up refers to the process of increasing the volume of a fermentation process from laboratory or pilot scale to a larger industrial-scale production. It involves replicating the fermentation process in larger vessels while maintaining or improving productivity, yield, and product quality. So, the yield and quality in these small volumes and the yield and quality in these large volumes should ideally be similar, if not the same. Large-scale fermentation lowers production costs through economies of scale, enabling efficient production to meet market demand.

Adapting processes to maintain product quality and consistency during scale-up is essential. Several key factors influence yield during the scale-up process. Number one: differences in inoculum propagation procedures and the quality and quantity of inoculum used. Selection of the medium; cost limitations at industrial scales might involve cheaper nutrient sources. Industrial-scale sterilization methods might cause greater degradation of heat-sensitive compounds, affecting the medium quality.



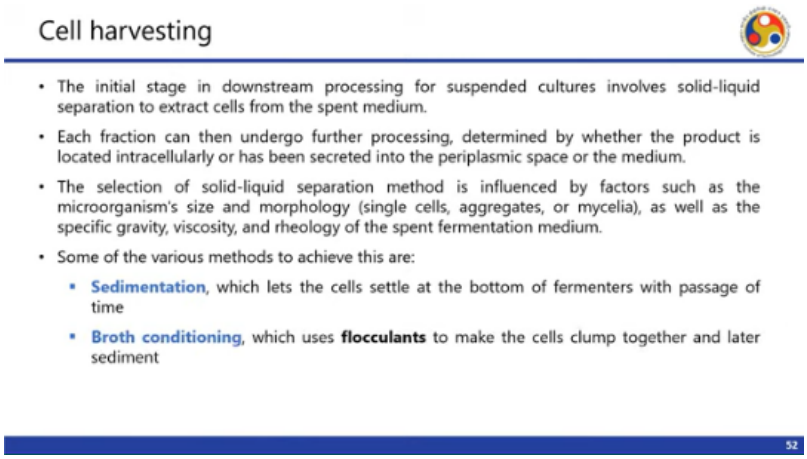
Development of gradients in larger fermenters including nutrient, temperature, pH and oxygen which were absent in smaller well-mixed systems. Alterations in form generation, shear forces and carbon dioxide removal rates. Variations in these factors can significantly affect operational conditions and subsequently influence productivity compared to laboratory-scale fermentations. Let us now have a discussion on the downstream processing where we will be discussing about cell harvesting disruption and the product recovery. So, here we see a industrial centrifuge used for multiple purposes in downstream processing and you can see the size of it which is very very big compared to the laboratory centrifuge.

So, downstream processing encompasses all post fermentation procedures after we take out the product out of the fermenter aimed at recovering the target product efficiently, consistently, and safely while maximizing yield and minimizing cost. The process depends on fermentation protocols and microorganisms properties such as morphology size and cell wall rigidity, which influence filterability, sedimentation and homogenization. Fermentation byproducts, media impurities and additives like antifoams can hinder downstream processing and product analysis.

Effective purification strategies must account for upstream and downstream factors including product properties, concentration and location as well as stability to prevent degradation. So, let us start with cell harvesting. The initial stage in downstream processing for suspended cultures involves solid-liquid separation to extract cell from the spent medium. Each fraction can then undergo further processing determined by whether the product is located intracellularly or has been secreted into the periplasmic space or the medium. The selection of solid-liquid separation method is influenced by factors such as the microorganism size and morphology , as well as the specific gravity, viscosity, and rheology of the spent fermentation medium.

Some of the various methods to achieve this are sedimentation, which lets the cells settle at the bottom of fermenters over time. Flocculation conditioning uses flocculants to make the cells clump together and later sediment; centrifugation employs a centrifugal field; and filtration uses porous materials like cloth, glass wool, or cellulose to retain the solids. The next important downstream operation is cell disruption. Disrupting microorganisms to release intracellular products like enzymes and recombinant proteins can be challenging due to the need to breach the cell wall and membrane. Mechanical methods for cell

disruption include liquid shear, which exposes cells to sudden pressure drops by discharging them through a narrow valve.

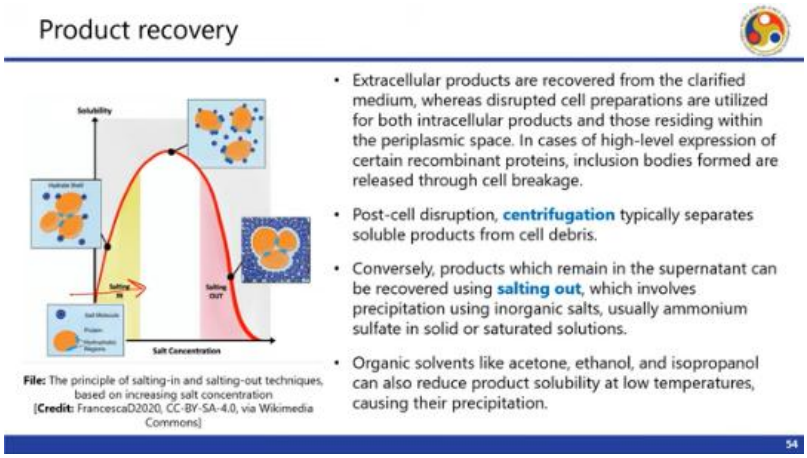


Manual grinding causes cell breakage due to shear forces, grinding, and collisions. Ultrasonic disruption uses cavitation. Alternative chemical techniques include autolysis, which uses the cell's own enzymes to break down its components. Osmotic shock involves treating cells with 20% sucrose and then rapidly suspending them in cold water. Organic solvents,

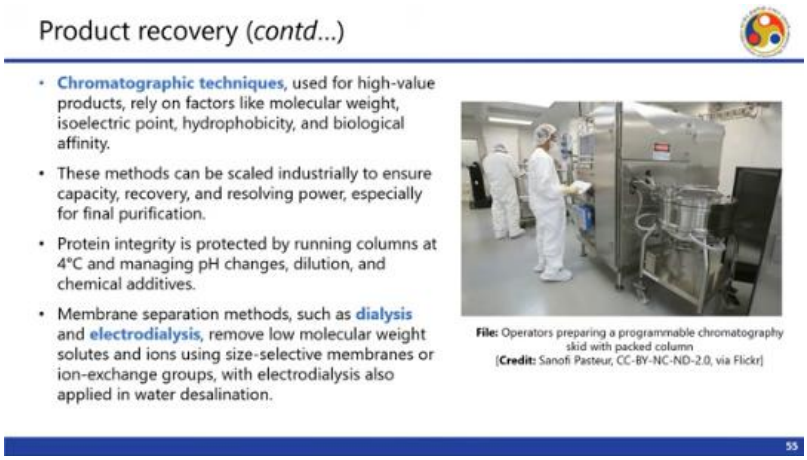
like acetone, butanol, chloroform, or methanol, or detergents like sodium lauryl sulfate or Triton X-100, disrupt membrane dynamics. Additionally, cell wall-degrading enzymes like lysozyme target bacterial peptidoglycan, while antibiotics like penicillin and cycloserine are also used. The next important operation is product recovery. Extracellular products are recovered from the clarified medium, whereas disrupted cell preparations are used for both intracellular products and those within the periplasmic space. In cases of high-level expression of certain recombinant proteins, inclusion bodies formed are released through cell breakage. This figure illustrates the principle of salting-in and salting-out techniques based on increasing salt concentration. Here, the salt concentration is increasing.

So, the salting in sets in, in the beginning. So, then the phase is reached where there is say maximum solubility. and then the solubility goes down after this salt concentration. So, this is the salting out phase where you can see that these salt molecules which were binding helping in the protein interactions particularly the hydrophobic regions So, as the salt concentration increases and the salts concentration will induce the hydrophobic regions of the proteins to come together and then finally the coagulation will start happening and they will become very heavy and then they will precipitate.

So, this is the salting out technique which is shown here diagrammatically. Post cell disruption, centrifugation typically separates soluble products from the cell debris. Conversely, products which remain in the supernatant can be recovered using salting out, which involves precipitation using inorganic salts, ammonium sulfate in solid or saturated solutions. Organic solvents like acetone, ethanol and isopropanol can also reduce product solubility at low temperatures, causing their precipitation. Then there are chromatographic techniques which are used for high value products, rely on factors like molecular weight, isoelectric point, hydrophobicity and biological affinity.



Here in this picture you can see operators preparing a programmable chromatography skid with packed column. These methods can be scaled industrially to ensure capacity recovery and resolving power especially for final purification. Protein integrity is protected by running columns at 4 degree centigrade and managing pH changes, dilution and chemical additives. Membrane separation methods such as dialysis and electrodialysis removes low molecular weight solutes and ions using size selective membranes or ion exchange groups with electrodialysis also applied in water desalination. Then another process is the crystallization.



Product crystallization encompasses evaporation, low-temperature treatments, or introducing a reactive chemical to the solute. Solubility reduction of the product can be achieved through various means, such as adding solvents, salts, polymers like non-ionic polyethylene glycol, polyelectrolytes, or adjusting the pH. Drying entails applying heat to the wet material and removing moisture as water vapor. This process typically aims to preserve the biological activity of the product. Factors influencing drying include the physical characteristics of the solid-liquid system, inherent properties of the solute, environmental conditions during drying, and heat transfer mechanisms involving direct contact, convection, or radiation.

So, with this, we come to the end of this lecture. Thank you for your present attention.