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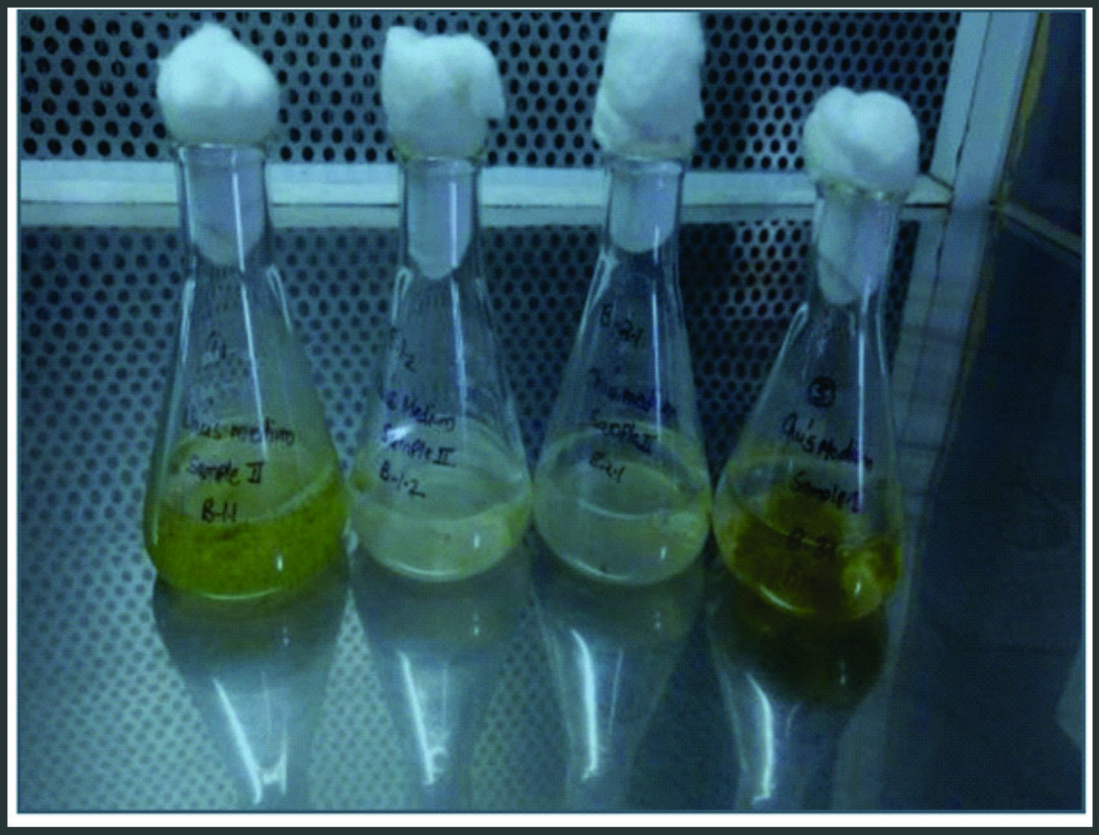
Basics of Industrial Biotechnology

Volume 2



JV'n Dr. Ritu Singh Rajput

JAYOTI VIDYAPEETH WOMEN'S UNIVERSITY, JAIPUR

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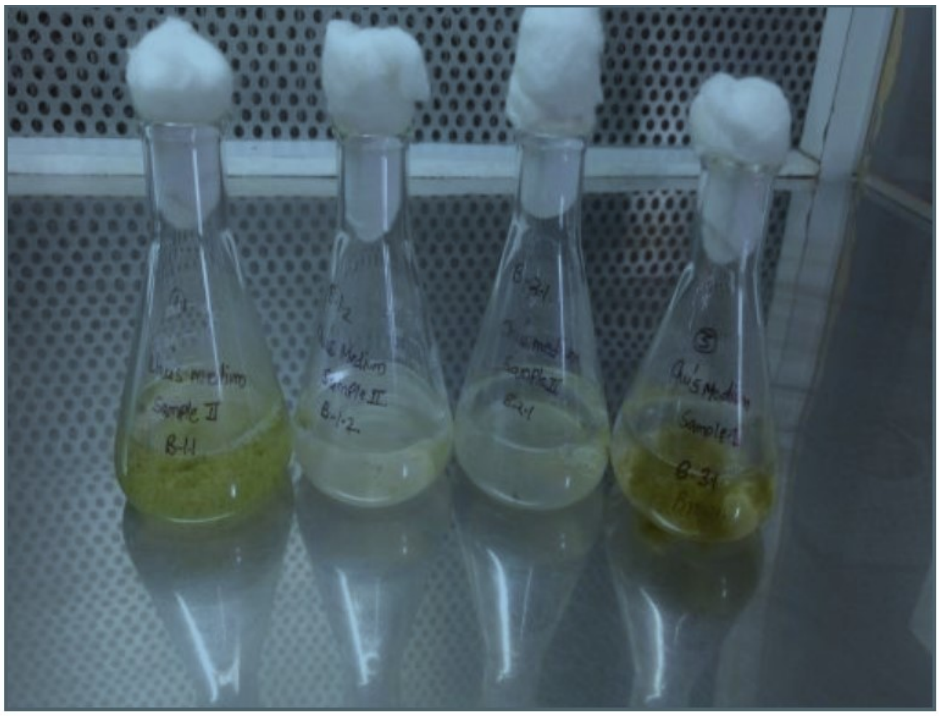

Author: Dr. Ritu Singh Rajput

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Basics of
Industrial Biotechnology
Volume 2
By
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Chapter 1:

Citric Acid Production

Introduction

The most important commercial commodity that is present in almost all plant and animal tissues is citric acid. C6H8O7, 2-hydroxy-1, 2, 3-propane tricarboxylic acid is the molecular formula of citric acid. In the field of food (60 percent) and pharmaceuticals (10 percent), organic acid is commonly used. First obtained from lemon juice in 1784 by W.SCHEELE as calcium citrate, which was treated with sulphuric acid, in the liquid process gave citric acid.

Microorganism used for Citric Acid Production

The processing of citric acid has been employed by a large number of micro-organisms. That involves bacteria, yeast, and fungi. *A.Niger*, however, and *saccharomycopsis s*p. Since it has many benefits, they are working for commercial production.

Advantages of using this micro-organism are:

- Its ease of handling.
- Its ability to ferment a variety of cheap raw materials.
- It provides high yields.

Micro-Organisms Used For Citric Acid Production

Yeasts

- *Candida tropicalis*
- *C.oleophila*
- *C.guilliermondii*
- *C.Citroformans*
- *Hansenula anamola*
- *Yarrowia lipolytica*

Bacteria:

- *Bacillus licheniformis*
- *Arthrobacter paraffinens*
- *Corynebacterium species*

Fungi:

- *Aspergillus nagger*
- *A.aculeatus*
- *A.awamori*
- *A. carbonarius*

Raw Material

Raw materials used for the manufacture of citric acid are split into two classes:

Group 1:

Substances with low ash content

e.g. cane or beet sugar, dextrose syrups, and crystallized dextrose

Group 2:

Materials which have high ash content.

e.g. cane and beet molasses, crude unfiltered starch hydro-lysates.

Citric Acid Cycle

Steps of Citric Acid Cycle

The citric acid cycle is also called the tricarboxylic acid cycle (TCA).

The steps include in the citric acid cycle are:

1. Formation of Citrate
2. Formation of Isocitrate via cis-Aconitate
3. Oxidation of Isocitrate to a-Ketoglutarate and CO2
4. Oxidation of a-Ketoglutarate to Succinyl-CoA and CO2
5. Oxidation of Succinate to Fumarate
6. Hydration of Fumarate to Malate
7. Oxidation of Malate to Oxaloacetate

Accumulation of citric acid

By mutation:

- Giving rise to a mutated organism that can accumulate an incomplete cycle of citric acid.
- By inhibition of enzymes:
- By changing environmental factors (pH, Temperature)
- Treat the medium with versions of ferrocyanide or ion exchange to inhibit the enzymes involved in the TCA cycle, except citrate synthase.

Citric acid production techniques

The industrial citric acid production can be carried in three different ways:

- Surface fermentation
- Submerged fermentation
- Solid-state fermentation

Surface Fermentation Process

- Molasses (15-20 percent sucrose, additional nutrients) substrate acidified with phosphoric acid to pH 6.0-6.5 and heated for 15 to 45 minutes at T 110c.
- In the hot substrate, potassium hexacyanoferrate is added to precipitate or complex trace metals (Fe, Mn, and Zn) and to function in abundance as a metabolic inhibitor that restricts development and facilitates the production of acid.
- Inoculation is carried out in two ways, either as a conidium suspension applied to the cooling medium or as a dried conidium combined with clean air and distributed over the trays as an aerosol.
- During fermentation, the temperature is kept steady at 30 degrees by means of the air present.
- The germinating spores begin to form a 2-3 cm blanket of mycelium floating on the surface of the substrate within 24 hours after inoculation. The pH of the substrate decreases to 2.0. as a result of the absorption of ammonium ions.
- As a dense white coating on the nutrient solution, the completely formed mycelium floats. After 8-14 days, the fermentation process ends.

- Mycelium recovery for citric acid extraction.
- This is all steps taken to generate citric acid during surface fermentation.

Solid State Fermentation

- Up to 65-70% of the water content of the firm substratum is soaked with water. The mass undergoes a steaming process after the elimination of the waste vapour.
- Sterile starch paste is inoculated by spraying Aspergillus niger conidia on the substrate surface in the shape of an aerosol or as a suspension of liquid conidia.
- The pH of the substrate is roughly 5-5.5, and by adding Alpha-amylase, incubation T 28-30C.growth can be accelerated. And with its own alpha-amylase, the fungus can hydrolyze starch. PH decreased to below 2 during the development of citric acid.
- The solid-state surface method takes 5 to 8 days, at the end of which hot water is used to remove the whole. In other times, to extract more citric acid from the cells, mechanical passes are often used.
- These are all steps taken to create citric acid during solid-state fermentation.

Submerged Fermentation

- Nutritional salts such as ammonium nitrate or potassium dihydrogen phosphate are added to the beet molasses substrate (12-15 percent, lowering sugar content), the substrate pH is kept at 5.5 to 5.9.
- Using suspensions of hydrophilic spores or germinated conidia from the propagator stage, the process will normally run in one or two steps.
- The growth of the hyphae and the aggregation normally involves a time of 9 to 25 hours at T 32c.
- Mycelia aggregation and spherical pellets, after 24 hours of inoculation, will detect the productive type.
- The pH change in this phase is from 5.5 to 3.5 for the substrate of beet molasses and 2.2 for the substrate of sucrose.
- The fermentation lasts up to 6-8 days and mycelium is later purified from citric acid.
- These are all steps taken to create citric acid during submerged fermentation.

Product Recovery: The biomass is separated by filtration. Then the liquid broth is transferred to the recovery process.

Purification

Purification is a simple way of getting pure citric acid followed by two simple techniques.

1. Precipitation
2. Filtration

The most widely used method is precipitation. It is carried out to form the slightly soluble tri-calcium citrate tetrahydrate by adding calcium oxide hydrate (milk of lime). By filtration, the precipitated tri-calcium citrate is removed and cleaned with water several times. It is then treated with calcium sulfate-forming sulphuric acid, which is washed out. Citric acid-containing mother liquor is treated with activated carbon and transferred through cation and anion exchangers. There are some anion-exchange resins available commercially.

Further purification

After purification, it can be produced in two forms

1. Monohydrate
2. Anhydrous.

Mono hydrate

- Each citric acid molecule contains one water molecule.
- Require repeated crystallization until the water content is approx. 7.5- 8.

Anhydrous

- Processed to remove all water from the end product
- Prepared by dehydrating the monohydrate citric acid product at a temperature above 36.6°C.

Factors Affecting Citric Acid Production

- Carbon source
- Nitrogen Source
- Phosphorus Source

- Trace Element
- the pH of fermentation medium
- Aeration

Uses of citric acid

- It is used in the detergent industry as a phosphate substitute.
- Used as a preservative and flavoring agent.
- Emulsifying agent in ice cream.
- Also used as an antioxidant.

Competitive questions from today topic (2 questions Minimum)-

Which of the following statements is NOT true regarding the closer affinity of Archaea to Eukarya than to Bacteria?

A. Both Archaea and Eukarya lack peptideglycan in their cell walls.

B. The initiator amino acid for protein synthesis is methionine in both Archaea and Eukarya.

C. Histones associated with DNA are absent in both Archaea and Eukarya.

D. In both Archaea and Eukarya the RNA polymerase is of several kinds.

Exam Name CSIR JUNE 2016

Which of the following is NOT a cell adhesion protein?

A. Cadherin B. Selectin

C. Immunoglobulin (Ig) superfamily D. Laminin

Exam Name CSIR NET DEC 2015

Questions to check understanding level of students-

Explain the citric production?

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Chapter 2

Cheese production

Cheese is a fermented food which is obtained from the milk of different mammals. Since humans started domesticating animals that produce milk around 10,000 B.C. The tendency of milk to split into curds and whey was known to them. When milk sours, a watery, grey substance containing lactose, nutrients, vitamins, and traces of fat breaks down into curds, lumps of phosphoprotein, and whey. It is the curds that are used to produce cheese, and almost every civilization on Earth has produced its own techniques, with China and the ancient Americas being the only significant exceptions.

The first cheeses were "fresh," not fermented, that is. These consisted entirely of salted white curds drained from whey, comparable to the cottage cheese of today. The next move was to develop methods to speed up the normal phase of separation. By adding rennet to the milk, this was done. Rennet is an enzyme from young ruminants' stomachs-a ruminant is an animal that very deeply chews its food and has a complicated digestive tract of three to four stomach chambers; cows are the best known creatures of this type in the United States. The most common method of "starting" cheese remains Rennet, although other starting agents are also used, such as lactic acid and various plant extracts.

Via A.D. In different countries, 100 cheese makers learned how to press, mature, and cure fresh cheeses, thereby making a food that could be kept for long periods. Different types of cheese, representing local ingredients and circumstances, were produced by each country or region. There is a staggering number of cheeses thus produced. Popular for the consistency and variety of its cheeses, France is home to around 400 cheeses that are commercially available.

In the 1860s, after Louis Pasteur invented the method that bears his name, the next crucial step in influencing the production of cheese took place. Without altering the underlying chemical composition, pasteurisation requires heating milk to partly sterilise it.

Since the procedure kills harmful micro-organisms, pasteurised milk is considered better, and today most cheese is made from pasteurised milk. The first and fastest way to increase the duration of cheese without spoiling was to actually age it. From the beginning, aged cheese was popular because it remained fine for domestic use. In the 1300s, to protect its freshness,

the Dutch began sealing cheese meant for sale in hard rinds, and the Swiss were the first to process cheese in the early 1800s.

They invented a method of grinding old cheese, applying filler additives, and heating the mixture to create a sterile, standardized, long-lasting product, disappointed by the pace at which their cheese went bad in the days before refrigeration. Another bonus of cheese production was that the producers were allowed to recycle edible, second-grade cheeses in a palatable shape.

Most people regarded cheese a specialty food prior to the twentieth century, manufactured in individual homes and consumed seldom. Since the introduction of mass manufacturing, however, both the availability of cheese and the market for it have risen. 13 per cent of milk was made into cheese in 1955. This figure had risen to 31 percent by 1984, and it continues to grow. Interestingly, while refined cheese is now readily available, only one-third of the cheese manufactured today accounts for it. While most cheeses are processed in big factories, the remainder is still manufactured using natural processes. In reality, in recent years, small, "farmhouse" cheese making has made a comeback. Many Americans now own their own small cheese-making companies, and, especially among connoisseurs, their products have become very popular.

Raw Materials

The milk comes from species as varied as cows, dogs, goats, horses, camels, water buffalo, and reindeer. Cheese is made from milk. The curdling method with rennet, lactic acid, or plant extracts, such as the vegetable rennet made from wild artichokes, fig leaves, safflower, or melon, is accelerated by most cheese producers.

Cheeses can contain various ingredients added to boost flavour and colour, in addition to milk and curdling agents. The world's great cheeses will gain their flavour from the unique bacterial moulds in which they have been inoculated, such as the popular *Penicillium roqueforti* used to

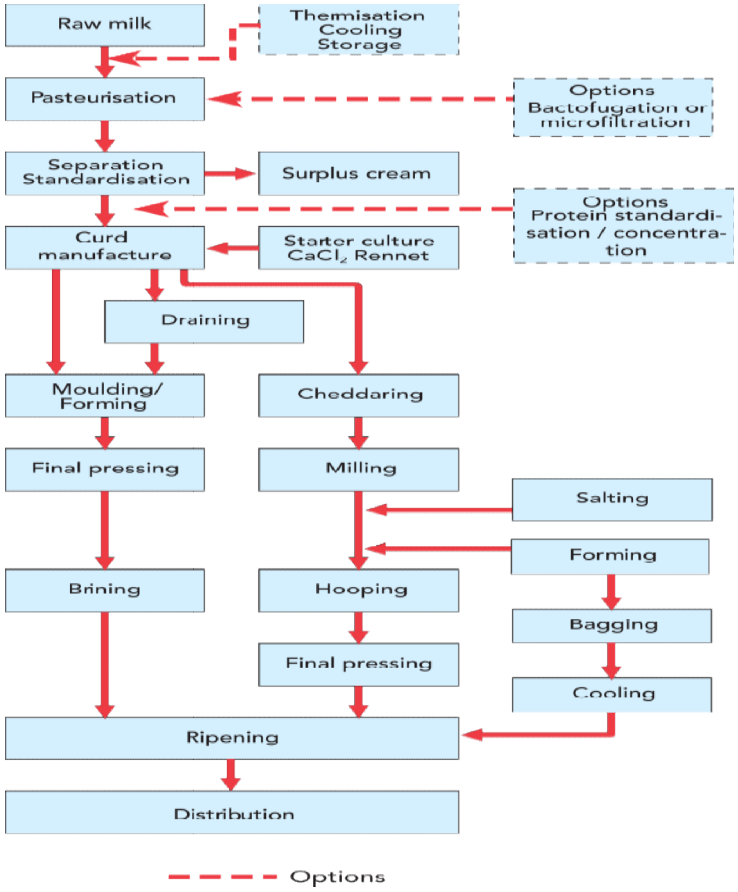

Diagram of Cheese Production make Roquefort of France and Stilton of England. It is also possible to salt or stain cheeses, usually with annatto, an orange dye made from the pulp of a tropical tree, or carrot juice.

They could be brine-washed or coated in ashes. The bacterial growth needed for curdling by a variety of odd methods can be promoted by cheese makers who wish to avoid rennet. These bacteria are present in some cheeses since they are made from unpasteurized milk. However, some cheeses are supposedly made from milk in which dung or old leather has been dunked; some also obtain their bacteria by being covered in dirt.

By mixing many forms of natural cheese and adding salt, milk-fat, cream, whey, water, vegetable oil, and other fillers, the unusual texture and taste of refined cheese is obtained. Preservatives, emulsifiers, gums, jelly, thickeners, and sweeteners can also be used in refined cheese as additives. Ingredients like paprika, pepper, chives, onions, cumin, car-away seeds, jalapeño peppers, hazelnuts, raisins, mushrooms, sage, and bacon are seasoned with most

refined cheese and some natural cheeses. In order to protect it and lend it a distinctive taste, cheese may even be burned.

The Manufacturing

Process

While cheese making is a linear process, several variables are involved. There are several types of cheese because various cheeses can be made at various points by finishing the basic preparation process, as ingredients or techniques can differ. For a long time, cheese making was considered a delicate process. It is understood that efforts to replicate the output of an old cheese factory have failed when situations at a new factory do not favour the development of the right bacteria.

Preparing the milk

Morning milk (which is richer), evening milk, or both, are approved by small cheese factories. This milk produces the bacteria required to create lactic acid, one of the agents that causes curdling, so it is normally bought from tiny dairies that do not refrigerate. The cheese makers let the milk sit until it has produced enough lactic acid to start manufacturing the unique type of cheese they are making. The cheese makers can then heat the ripening milk depending on the type of cheese being made. In large cheese factories, which buy pasteurized milk, this method varies marginally and must also incorporate a culture of bacteria to produce lactic acid.

Separating the curds from the whey

The next move is to add the milk to the animal or vegetable rennet, further separating it into curds and whey. The curds, once created, are cut with knives both vertically and horizontally. Big vats of curdled milk are sliced vertically in vast factories using sharp, multi-bladed, wire knives reminiscent of oven racks. Then the same unit agitates the curds and horizontally cuts them. Using a big, two-handled knife, the curds are sliced both ways if the cutting is performed manually. Soft cheeses are sliced into huge chunks, while small chunks are cut into hard cheeses. (The distance between the knives is about one-twentieth of an inch for cheddar, for example.) After cutting, the gap between the knives is about one-twentieth of an inch.

In a typical cheese-making operation, the first step is preparing the milk. Although smaller factories purchase unpasteurized milk that already has the bacteria present to produce lactic acid (necessary for curdling), larger factories purchase pasteurized milk and must add bacteria culture to produce the lactic acid. Next, the curds must be separated from the whey. Animal or vegetable rennet is added, and then the curds are agitated and cut using large knives. As the whey separates, it is drained. The curds are then pressed into molds, if necessary, to facilitate further moisture drainage, and aged for the proper amount of time. Some cheeses are aged for a month, others for several years.

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Moisture must then be removed from the curds, although the amount removed depends on the type of cheese. For some types with high moisture contents, the whey-draining process removes sufficient moisture. Other types require the curds to be cut, heated, and/or filtered to get rid of excess moisture. To make cheddar cheese, for example, cheese makers cheddar, or finely chop the curd. To make hard, dry cheeses such as parmesan, cheese makers first cheddar and then cook the curd. Regardless, if the curds are to be aged, they are then put into molds. Here, they are pressed to give the proper shape and size. Soft cheeses such as cottage cheese are not aged.

Ageing the cheese

At this stage the cheese may be inoculated with a flavoring mold, bathed in brine, or wrapped in cloth or hay before being deposited in a place of the proper temperature and humidity to age. Some cheeses are aged for a month, some for up to several years. Ageing sharpens the

flavor of the cheese; for example, cheddar aged more than two years is appropriately labeled extra sharp.

Wrapping natural cheese

Some cheeses may develop a rind naturally, as their surfaces dry. Other rinds may form from the growth of bacteria that has been sprayed on the surface of the cheese. Still other cheeses are washed, and this process encourages bacterial growth. In place of or in addition to rinds, cheeses can be sealed in cloth or wax. For local eating, this may be all the packaging that is necessary. However, large quantities of cheese are packaged for sale in distant countries. Such cheeses may be heavily salted for export (such as Roquefort) or sealed in impermeable plastic or foil.

Making and wrapping processed

Cheese

Edible yet inferior cheeses can be saved and made into processed cheese. Cheeses such as Emmental (commonly called Swiss), Gruyere (similar to Swiss), Colby, or cheddar are cut up and very finely ground. After this powder has been mixed with water to form a paste, other ingredients such as salt, fillers, emulsifiers, preservatives, and flavorings are added. The mixture is then heated under controlled conditions. While still warm and soft, the cheese paste is extruded into long ribbons that are sliced. The small sheets of cheese are then put onto a plastic or foil sheet and wrapped by a machine.

Quality Control

Cheese making has never been an easily regulated, scientific process. Quality cheese has always been the sign of an experienced, perhaps even lucky cheese maker insistent upon producing flavorful cheese. Subscribing to analytical tests of cheese characteristics may yield a good cheese, but cheese making has traditionally been a chancy endeavor. Developing a single set of standards for cheese is difficult because each variety of cheese has its own range of characteristics. A cheese that strays from this range will be bad-tasting and inferior. For example, good soft blue cheese will have high moisture and a high pH; cheddar will have neither.

One controversy in the cheese field centers on whether it is necessary to pasteurize the milk that goes into cheese. Pasteurization was promoted because of the persistence of

Mycobacterium tuberculosis, a pathogen or disease-causing bacteria that occurs in milk products. The United States allows cheeses that will be aged for over sixty days to be made from unpasteurized milk; however, it requires that many cheeses be made from pasteurized milk. Despite these regulations, it is possible to eat cheeses made from unpasteurized milk to no ill effect. In fact, cheese connoisseurs insist that pasteurizing destroys the natural bacteria necessary for quality cheese manufacture. They claim that modern cheese factories are so clean and sanitary that pasteurization is unnecessary. So far, the result of this controversy has merely been that connoisseurs avoid pasteurized milk cheeses.

Regulations exist so that the consumer can purchase authentic cheeses with ease. France, the preeminent maker of a variety of natural cheeses, began granting certain regions monopolies on the manufacture of certain cheeses. For example, a cheese labeled "Roquefort" is guaranteed to have been ripened in the Combalou caves, and such a guarantee has existed since 1411. Because cheese is made for human consumption, great care is taken to insure that the raw materials are of the highest quality, and cheese intended for export must meet particularly stringent quality control standards.

Because they possess such disparate characteristics, different types of cheese are required to meet different compositional standards. Based on its moisture and fat content, a cheese is labeled soft, semi-soft, hard, or very hard. Having been assigned a category, it must then fall within the range of characteristics considered acceptable for cheeses in that category. For example, cheddar, a hard cheese, can contain no more than 39 percent water and no less than 50 percent fat. In addition to meeting compositional standards, cheese must also meet standards for flavor, aroma, body, texture, color, appearance, and finish. To test a batch of cheese, inspectors core a representative wheel vertically in several places, catching the center, the sides, and in between. The inspector then examines the cheese to detect any inconsistencies in texture, rubs it to determine body (or consistency), smells it, and tastes it. Cheese is usually assigned points for each of these characteristics, with flavor and texture weighing more than color and appearance.

Processed cheese is also subject to legal restrictions and standards. Processed American cheese must contain at least 90 percent real cheese. Products labeled "cheese food" must be 51 percent cheese, and most are 65 percent. Products labeled "cheese spread" must also be 51 percent cheese, the difference being that such foods have more water and gums to make them spreadable. "Cheese product" usually refers to a diet cheese that has more water and less

cheese than American cheese, cheese food, or cheese spread, but the specific amount of cheese is not regulated. Similarly, "imitation cheese" is not required to contain a minimum amount of cheese, and cheese is usually not its main ingredient. In general, quality processed cheese should resemble cheese and possess some cheesy flavor, preferably with a "bite" such as sharp cheddar cheese has. The cheese should be smooth and evenly colored; it should also avoid rubberiness and melt in the mouth.

University Library Reference- Industrial Biotechnology by A. H Patel

Online Reference: Cheese Production

Ancient Indian Literature Reference –Competitive questions from today topic (2 questions Minimum) - Back at that period, cheeses were made from goat and sheep rennet which were the harbingers of modern-day cheese. Based on the Sanskrit text 'Charak Samhita', the earliest evidence of milk product is derived after the heat-acid coagulation of milk. Another reference comes from the Rig Veda 6.48.18 in which a kind of milk product is mentioned that sounds similar to cheese or paneer.

Competitive questions from today topic (2 questions Minimum)-

The bacteria that require specialized nutrients for growth, which can be difficult to meet in the laboratory, are called

A. facultative B. obligate

C. fastidious D. non-fermentor

Exam NameJAM-2015

Match the industrial products mentioned in Group I with their producer organisms in Group II Group I Group II P. Citric acid 1. Trichoderma viride Cellulase 2. Clostridium acetobutylicum R. Vitamin B12 Q3. Aspergillus niger S. Butanol 4. Propionibacterium freudenreichii

A. (A) P-4, Q-3, R-1, S-2B. (B) P-3, Q-1, R-2, S-4

C. (C) P-2, Q-1, R-4, S-3D. (D) P-3, Q-1, R-4, S-2

Exam Name BT 2018

Questions to check understanding level of students-

Explain the Cheese Production?

Reference

Soetaert, W. and Vandamme, E., 2006. The impact of industrial biotechnology. *Biotechnology Journal: Healthcare Nutrition Technology*, *1*(7-8), pp.756-769.

Chapter 3 :

Antibiotics production

Antibiotics are medicines that help stop infections caused by bacteria. They do this by killing the bacteria or by keeping them from copying themselves or reproducing. The word antibiotic means “against life.” Any drug that kills germs in your body is technically an antibiotic. But most people use the term when they’re talking about medicine that is meant to kill bacteria. Before scientists first discovered antibiotics in the 1920s, many people died from minor bacterial infections, like strep throat. Surgery was riskier, too. But after antibiotics became available in the 1940s, life expectancy increased, surgeries got safer, and people could survive what used to be deadly infections. As more and more bacteria continue to develop resistance to currently produced antibiotics, research and development of new antibiotics continues to be important. In addition to research and development into the production of new antibiotics, repackaging delivery systems is important to improving efficacy of the antibiotics that are currently produced. Improvements to this field have seen the ability to add antibiotics directly into implanted devices, aerosolization of antibiotics for direct delivery, and combination of antibiotics with non antibiotics to improve outcomes. The increase of antibiotic resistant strains of pathogenic bacteria has led to an increased urgency for the funding of research and development of antibiotics and a desire for production of new and better acting antibiotics.

An agar plate streaked with microorganisms

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over that already in use, or have no other practical applications.

Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified.

A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture. Research into antibiotic identification has shown the opportunity exists to move away from lawn spotting methodology, a methodology which increases the chances of cross contamination. This new methodology involves using Lactobacillus species and shows a clear zone of inhibition as well as allowing for a determination of minimum inhibitory concentration.

Industrial production techniques

Fermentation

Industrial microbiology can be used to produce antibiotics via the process of fermentation, where the source microorganism is grown in large containers (100,000–150,000 liters or more) containing a liquid growth medium. Oxygen concentration, temperature, pH and nutrient are closely controlled. As antibiotics are secondary metabolites, the population size must be controlled very carefully to ensure that maximum yield is obtained before the cells die. Once the process is complete, the antibiotic must be extracted and purified to a crystalline product. This is easier to achieve if the antibiotic is soluble in organic solvent. Otherwise it must first be removed by ion exchange, adsorption or chemical precipitation.

Semi-synthetic

A common form of antibiotic production in modern times is semi-synthetic. Semi-synthetic production of antibiotics is a combination of natural fermentation and laboratory work to maximize the antibiotic. Maximization can occur through efficacy of the drug itself, amount of antibiotics produced, and potency of the antibiotic being produced. Depending on the drug being produced and the ultimate usage of said antibiotic determines what one is attempting to produce. An example of semi-synthetic production involves the drug ampicillin. A beta lactam antibiotic just like penicillin, ampicillin was developed by adding an addition amino group (NH2) to the R group of penicillin. This additional amino group gives ampicillin a broader spectrum of use than penicillin. Methicillin is another derivative of penicillin and was discovered in the late 1950s,the key difference between penicillin and methicillin being the addition of two methoxy groups to the phenyl group.These methoxy groups allow methicillin

to be used against penicillinase producing bacteria that would otherwise be resistant to penicillin.

Synthetic

Not all antibiotics are produced by bacteria; some are made completely synthetically in the lab. These include the quinolone class, of which nalidixic acid is often credited as the first to be discovered. Like other antibiotics before it the discovery of nalidixic acid has been chalked up to an accident, discovered when George Lesher was attempting to synthesize chloroquine. However a recent investigation into the origin of quinolones have discovered that a description for quinolones happened in 1949 and that patents were filed concerning quinolones some 5 years before Lesher's discovery.

Strains used for the production

In the earliest years of antibiotic discovery the antibiotics being discovered were naturally produced antibiotics and were either produced by fungi, such as the antibiotic penicillin, or by soil bacteria, which can produce antibiotics including streptomycin and tetracycline. Microorganisms used in fermentation are rarely identical to the wild type. This is because species are often genetically modified to yield the maximum amounts of antibiotics. Mutation is often used, and is encouraged by introducing mutagens such as ultraviolet radiation, x-rays or certain chemicals. Selection and further reproduction of the higher yielding strains over many generations can raise yields by 20-fold or more. Another technique used to increase yields is gene amplification, where copies of genes coding for enzymes involved in the antibiotic production can be inserted back into a cell, via vectors such as plasmids. This process must be closely linked with retesting of antibiotic production.

Advancements

Penicillin was the first of the antibiotics to be discovered. After the discovery there was the issue of taking the raw naturally produced penicillin and developing a method so that wide-scale production of a clinically significant antibiotic could occur. Over the course of many years a team led by Florey and Chain and based in Oxford was able to successfully purify, concentrate, and produce the antibiotic. Advances in scientific technology have not always led to better conditions for the production of antibiotics. Since 1987 there have been no new classes of antibiotics discovered for industrial production and widespread usage. However new developments in genomic sequencing and technology have led to improvements and

discovery in the field of antibiotic production. Genomic engineering of antibiotic gene clusters has already been shown to lead to an increase in production of different antibiotics.

Antibiotic production and delivery method

Antibiotics do not render themselves fully functional and deliverable simply by being produced. Often modifications must be made to the antibiotics so that maximum efficiency is attained. Post-production modifications include making antibiotics aerosolized so as to bypass doing unnecessary damage to bacteria located in other parts of the body and instead going directly to the lungs. No socomial infections can lead to serious complications during and in the recovery following surgery or a hospital stay in general. By merging surgical implants with antibiotics, healthcare providers are able to strike at a specific high risk area of infection without having to use a body wide size dosage of antibiotics. Meropenem is an antibiotic that is delivered into the body via injection. When produced meropenem is a crystalline antibiotic, so it must be mixed in with solution before injection can occur. During this process meropenem is mixed with sodium carbonate, then diluted in water after which it can be injected.

Aerosolization of antibiotics is necessary because infections of the lung are especially troublesome, which is why direct targeting of the infection is needed. Broad spectrum antibiotics can have detrimental side effects when their action is also taken against necessary non-pathogenic bacteria residing in the human microbiome. Aerosolization is effective in bypassing the microbiome that exists in the gastrointestinal tract by directing the antibiotic directly to the lungs. This process is undertaken after the production of the antibiotic itself.

The rise of antibiotic-resistant bacteria has affected implantation of medical devices. In some cases it is no longer enough for devices to be sterile when they are implanted into an individual, now they must be proactive in fighting off bacterial infection. As such antibiotics are now being added into the surface of implanted devices as an added layer of defense against the threat of infection. One such infection is Osteomyelitis which can offer unique challenges in treatment efforts, one novel approach has been the creation of antibiotic cement nails which can be inserted into the infected bone. First described by Paley and Herzenberg antibiotic cement nails have dual purpose, both of stabilization of the bone being treated, and prevention against post-procedure infection. Antibiotic cement nails are inserted during surgery, and are produced around the time of procedure using materials available in the operating room setting. Antibiotics are mixed in with cement filling then molded around a

support anchor, often chest tubes are used to ensure proper molding. Chest tubes have the advantage of being cheap and ubiquitous and have been shown to have uniformity in the production of antibiotic cement nails. The antibiotics fill the voids within the cement matrix and upon drying and setting can be inserted into the bone. The antibiotic has a direct contact with the area of infection and retains its properties in acting upon the infection. In addition to cement nails, antibiotic cement spacers have been used to treat and prevent osteomyelitis, and for a longer period of time. In producing the antibiotic cement material it is necessary to choose antibiotics that will be effective in this hybrid form, it has been found that antibiotics in powder form that are broad spectrum are of best use. There are recommendations for the amount of antibiotic that is used when mixing in with the cement, but industry wide guidelines have not been established.

Challenges

Development of antibiotics is difficult, whereas many drug discoveries have been a result of concerted effort and intensive research and development; antibiotics have seemingly been discovered by chance. Since 1987 there have been no discoveries or development of a new class of antibiotics. This is partly due to the finicky nature of antibiotics. As most are produced biosynthetically they require an organism to produce. Historically this has meant that different species are grown and observed for any antimicrobial activity. Not only does this require a culturable species to start off with, but the conditions the species are grown in must be adequate for production of antibiotics as well as having the number of antibiotics produced reach a density threshold so that their function can be observed.

Another reason behind the lack of new antibiotic production is the diminishing amount of return on investment for antibiotics and thus the lack resources put into research and development by private pharmaceutical companies. The World Health Organization has recognized the danger of antibiotic resistance bacteria and has created a list of "priority pathogens" that are of the utmost concern. In doing so the hope is to stimulate R&D that can create a new generation of antibiotics. In the United States, the Biomedical Advanced Research and Development Authority (BARDA) aims to support the work of the industry to produce new antibiotics.

The buildup of inorganic phosphate can limit the biosynthetic production of certain antibiotics, researchers found that by using an inorganic phosphate trapping agent, the phosphate would be sequestered away and antibiotic production would return to normal

levels, thus allowing production to continue. Meropenem is mixed with sodium carbonate post-production before being injected into the body, subsequent analysis of this mixture using Nuclear Magnetic Resonance has shown that a second form of Meropenem is produced. This second form has an additional carbon dioxide on it, and exists alongside the pure form. In order to ensure that Meropenem stays in the correct form a four-step process was developed wherein the crude form is mixed together with a base in water, a proper pH is established, the product is treated with alkanols, and then the pure form is isolated.

University Library Reference- Industrial Biotechnology by A. H Patel

Online Reference: Production of Antibiotics

https://doi.org/10.1016%2Fj.ijantimicag.2015.05.011

Ancient Indian Literature Reference –Competitive questions from today topic (2 questions Minimum)- Relevant literature pertaining to challenge of antibiotic resistance and the beginning and end of the antibiotic era, were studied and incorporated in correlation to Ayurveda views. Many classical texts regarding Ayurveda drugas an antibiotic property as well as the herbal and mineral ingredients mentioned in some formulation are compiled from Ayurveda texts such as 'Ashtangsangraha" Charaksamhita' and 'Sushruta Samhita'

Competitive questions from today topic (2 questions Minimum)-

Identif, the mismatch

A. Peptone media - ComPlex media

B. Crystal violet media - Characteristic media

C. MacConkey media- Selective and differential media

D. Chocolate agar media- Enrichment media

Exam Name puducherry ENTRANCE 2016

The compound added to the medium which is directly incorporated into the product is called as

A. precursor B. inhibitor

C. inducer D. chelator

Exam NameJNU-MTB-2015

Questions to check understanding level of students-

Explain the Production of Antibiotics?

Reference

Tang, W.L. and Zhao, H., 2009. Industrial biotechnology: tools and applications. *Biotechnology Journal: Healthcare Nutrition Technology*, *4*(12), pp.1725-1739.

Chapter 4

Amino Acid Production

As a bulk biochemical, amino acids are used in many agricultural applications to manufacture a wide variety of products, such as animal feed additives, taste enhancers in human foods or as components in cosmetics and medicinal products. In addition to the essential role of amino acids and intermediates as building blocks of proteins, they are active in controlling critical metabolic pathways and processes that are critical to organisms' development and maintenance. In specific, they foster wellness by many steps, including optimizing the potency of food utilization, decreasing adiposity, regulating the synthesis of muscle protein and controlling the organism's development and immunity. it is well documented that an amino acids deficiency causes serious diseases both in humans and animals.

The interest in researching and designing new routes for processing them in a more cost-effective and sustainable manner has therefore increased considerably in recent years. Different methods, such as extraction from protein hydrolysates, chemical synthesis or enzymatic and fermentation pathways, may generate amino acids with the assistance of microorganisms. In particular, because of the modern genetic engineering techniques used to optimize the yield, specificity and efficiency of the target compounds, the fermentation process is now one of the most exciting methods for the industrial processing of amino acids.

Amino acid production : a short history

Over the years, interest in the development of amino acids has grown, resulting in a number of technologies being developed. In 1907, at the Imperial University of Tokyo, Kikunae Ikeda began his studies with the intention of defining and purifying the concept of flavour enhancement from the seaweed konbuu (Laminaria japonica). He found that the extract was made of MSG after a year of study. Soon after his discovery, Ajinomoto Co. started to remove MSG from and market it as a taste enhancer from acid-hydrolyzed wheat gluten or defatted soybean.

Kikunae Ikeda is considered the founder of MSG since he provided the basis for the manufacturing of amino acids. The emergence of new amino acid applications, such as pharmaceuticals, food additives, feed additives, cosmetics, polymer materials and agricultural chemicals, has contributed to a rapid rise in the production of amino acids. Indeed, the overall

demand for amino acids was valued at about USD 5.4 billion in 2008 and is projected to be worth more than USD 9.4 billion by 2018. However, it is also important to refine the manufacturing processes for processing amino acids.

For this reason, many companies and academic institutions started research in this field with the aim of finding more cost-effective and sustainable routes to produce amino acids.

The industrial production of amino acids is carried out by one or more of the following three processes:

Extraction:

In the structure of proteins, amino acids are the building blocks. The proteins can be subjected to hydrolysis and the necessary amino acids, such as cysteine, tyrosine and leucine, can be isolated.

Chemical synthesis:

A combination of D- and L-amino acids comes from chemical synthesis. The L-category contains the majority of the amino acids needed for commercial applications. Chemical processes are, however, used for the synthesis of glycine (optically inactive) and certain other amino acids which can be used for some applications in L- or D-form (D, L-alanine, D, L-methionine).

Microbiological production:

For the large- scale production of amino acids, microbiological methods are employed. There are three different approaches.

(a) Direct fermentation methods:

Microorganisms may generate amino acids using many sources of carbon, such as glucose, fructose, alkanes, ethanol, glycerol, propionate, etc. It is also possible to use such agricultural byproducts, such as molasses and starch hydrolysate. Methanol, a cheap source of carbon, is being sought, but with little results, for amino acid processing.

(b) Conversion of metabolic intermediates into amino acids:

In this approach, the microorganisms are used to carry out selected reactions for amino acid production e.g. conversion of glycine to serine.

(c) Direct use of microbial enzymes or immobilized cells:

For the processing of amino acids, resting cells, immobilised cells, crude cell extracts or enzyme-membrane reactors may often be used. Any explanations below are given. For the amination of alpha-keto acids, amino acid dehydrogenases from some bacteria (e.g. Bacillus megaterium) can be used to manufacture L-amino acids, e.g. alanine (from pyruvate), leucine (from alpha-ketoisocaproic acid) and phenylalanine (from phenyl pyruvate). It is possible to use immobilised cells or enzyme- membrane reactors. Enzymes or immobilised cells are also used to make a variety of other amino acids, such as tryptophan, tyrosine, lysine and valine.

Strain Development for Amino Acid Production:

The metabolic pathways for microorganisms to synthesise amino acids are closely regulated and work in an economical way. Natural overproduction of amino acids is, however, an unusual phenomenon. Some strains that excrete such amino acids, such as glutamic acid, alanine or valine, have been isolated.

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Online Reference: Amino Acid Production

Ancient Indian Literature Reference –Competitive questions from today topic (2 questions Minimum) –

Competitive questions from today topic (2 questions Minimum)-

Identify the amino acids containing nonpolar, aliphatic R groups.

A. Phenylalanine, tyrosine, and tryptophan B. Glycine, alanine, leucine

C. Lysine, arginine, histidine D.Serine, threonine, cysteine

Exam Name JAM-2015

Which among the following is a non-essential amino acid?

A. Serine

B. Threonine

C. Lysine

D. Histidine

Exam Name BT 2018

Questions to check understanding level of students-

Explain the Amino Acid Production?

References

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Garcia, N., Lopez Elias, J.A., Miranda, A., Martinez Porchas, M., Huerta, N., Garcia, A., 2012. Effect of salinity on growth and chemical composition of the diatom Thalassiosira weissflogii at three culture phases. Lat. Am. J. Aquat. Res. 40 (2), 435–440.

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