Large scale cultures of microorganisms Products production and purification

Schedule

- Important products and methodology of industrial microbiology
- Upstream and downstream processes
- Selected products of industrial microbiology
- Packaging – standardization of products

Important products

Product	Cell	Applications
Ethanol	*Saccharomyces cerevisiae*	Industrial solvent, beverages
Glycerol	*Saccharomyces cerevisiae*	Explosives
Lactic acid	*Lactobacillus bulgaricus*	Foods and pharmaceuticals
Aceton - Butanol	*Clostridium acetobutylicum*	Solvents
α-amylase	*Bacillus subtilis*	Starch hydrolysis

Important products of industrial microbiology

- Foods and beverages industries using fermentation processes (cheese, vinegar, yogurt, all beverages)
- Industries producing enzymes, ethanol and other solvents, citric acid, vitamins, single-cell protein, antibiotics and other drugs
- Industries producing energy by wastes recycling (ethanol or/and biogas from lignocellulosic wastes)
- Wastes treatment (industrial and municipal)
- Oil extraction and nitrogen fixation industries

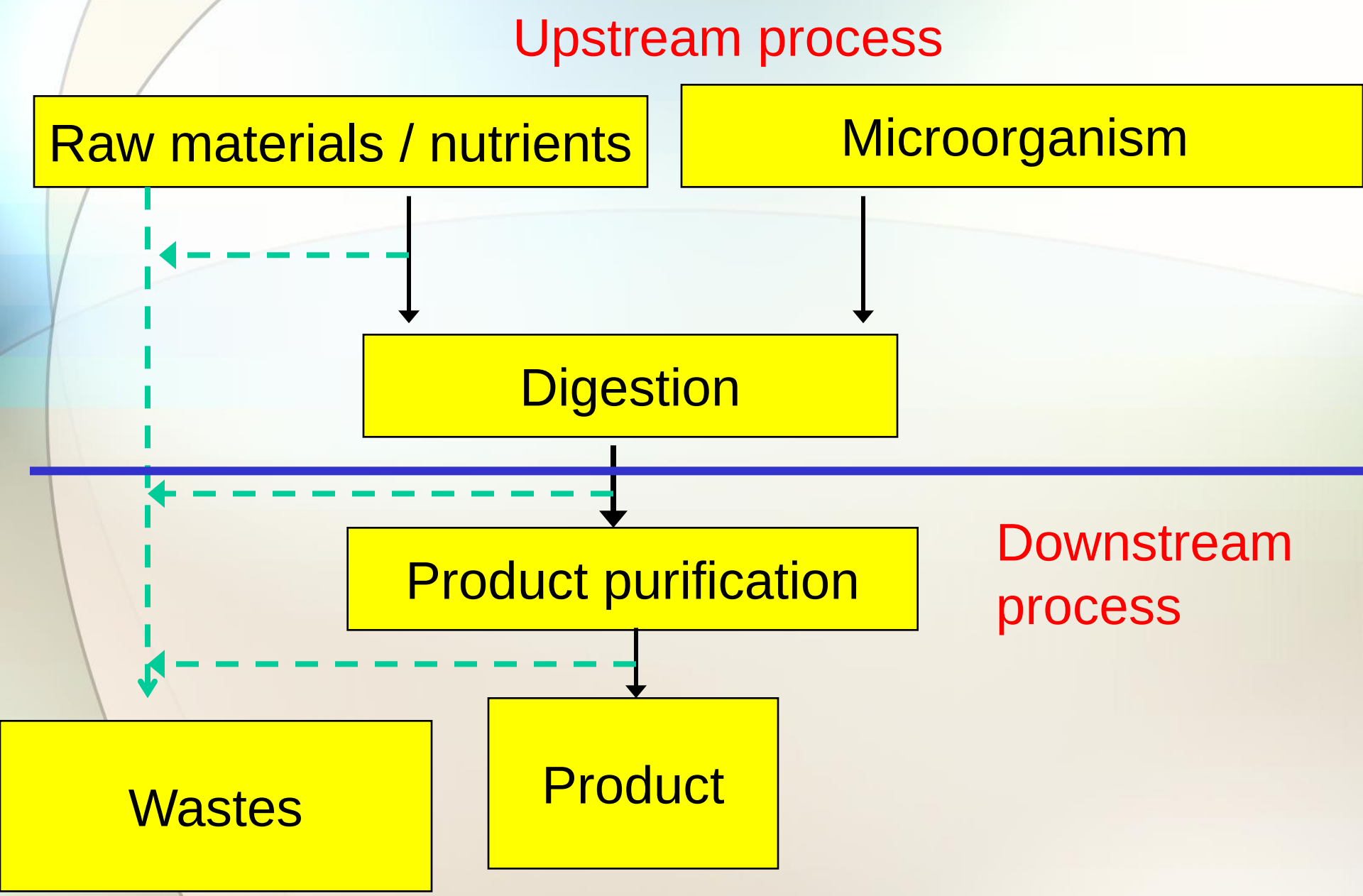
MAJOR ACHIEVEMENTS

- Ancient years up today: microbiological food production (wine, vinegar, cheese, bread, and later bier and yogurt
- Early 20th century: use industrial microbiology to produce **organic acids**, **solvents** and **biomass** under non aseptic conditions
- About 1940: using **sterilized** processes a lot of various products are produced, antibiotics (penicillin, streptomycin, tetracycline, etc), vitamin B12, alkaloids, gibberellins, cortisone, 5'-nucleotides, dextran, enzymes, etc
- Last years: two new achievements:
  - **Immobilized cell** technology
  - Recombinant DNA technology (**genetic engineering**)

The processes

- processes to remove nutrients (e.g., biological treatment)
- processes to produce metabolic products (e.g., alcoholic fermentation)
- processes to produce biomass (algae, single-cell protein)
- bioconversion processes, i.e., use microorganisms as catalysts (e.g., hydroxylation of aromatics)

Fermentation process



Upstream process

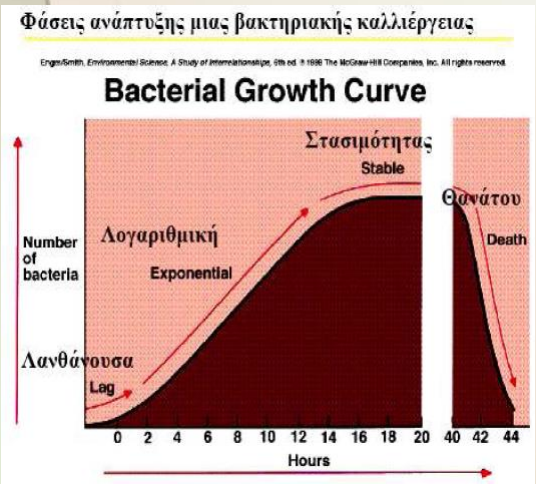
- Three key points:
  - Microorganism
    - Critical points
      - Selection/Isolation of the appropriate microorganism
      - Improving the strain to increase productivity and performance
      - Preparation of the appropriate material for inoculation
  - Culture medium
  - Fermentation process

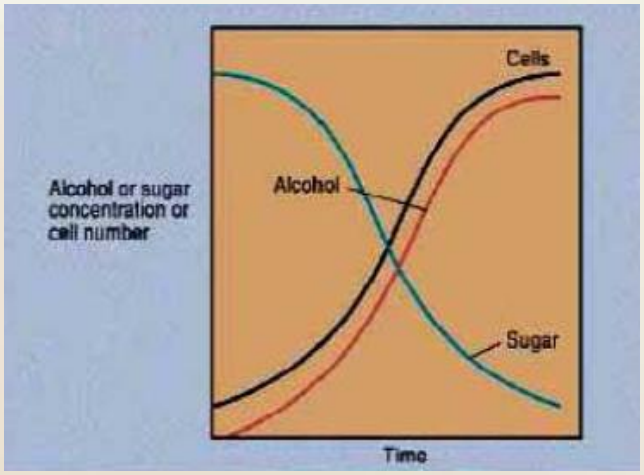
Examples of the applicability of microorganisms

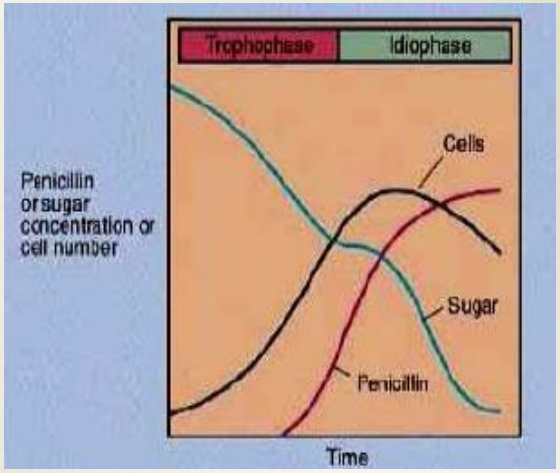
- *Bacillus thuringiensis*
  - Microbial insecticides
- *Lactobacillus sp.*
  - Starter cultures for the fermentation of dairy products
- *Streptococcus cremoris*
  - Industrialization of dairy products
- *Penicillium roqueforti*
  - Inoculations for cheese production
- *Rhizobium sp.*
  - Inoculations that promote nitrogen fixation
- *Pseudomonas syrigae*
  - Artificial snow production
- Mutant strains to protect plants from frost

Type of products

- Primary metabolites that produced during growth phase (logarithmetic phase)
- Secondary metabolites that produced during stable (stationary) phase







Secondary metabolites

- These are molecules synthesized by microorganisms during their last phase of development (stationary phase)
- They are not required for development
- Their actual function is not fully known
- The best known are antibiotics that inhibit the growth of other microorganisms, giving an ecological advantage to the producers
- Today more than 2500 antibiotic substances are produced by bacteria known as *Actinomycetes*

Antibiotics

- *Penicillium notatum*: penicillin
- *Bacillus licheniformis*: bacitracin
- *Streptomyces griseus*: streptomycin
- *Streptomyces erythraeus*: erythromycin
- *Streptomyces nodosus*: amphotericin B

Vitamins

- Vitamin B12
  - *Propionobacterium*, *Streptomyces griseus*
- Carotene (Vitamin A)
  - *Phycomyces blakesleeanus*
- Vitamin B2
  - *Eremothecium ashbyii*, *Ashbya gossipyii*

Flavoring agents

- Volatile compounds found in foods and perfumes giving the specific odor
- They are present in several natural products
- Today more than 100 flavoring agents are produced using microorganisms
- Vanillin:
  - Natural, from the vanilla fruit (~ $4,000/kg)
  - Synthetic, by fermentation of natural products (stilbene, eugenol) (~ $1,000/kg)
  - Similar to the natural but produced by microorganisms (~ $12/kg)

Flavorings and cheese production

- During the ripening of cheeses, pure cultures of bacteria are used, e.g., *Brevibacterium*, *Microbacterium*, with a multitude of odor components, such as fatty acids, alcohols, methyl ketones and cyclic compounds
- The appearance of color is often associated with the action of enzymes such as lipases and proteases
- The concentrations of the degradation products of these enzymes determine the aroma of each variety of cheese, e.g., *Penicillium roqueforti*, which imparts the characteristic aroma of Roquefort-type cheese

Citric acid production

- The most important organic acid
  - Huge amounts are produced (>1.000.000 tn/y)
  - Its use increases every year by 4%
  - It is used mainly as food (60%) and pharmaceutical (10%) additive
- It is produced by submerged fermentation of sugars (molasses, starch soluble products) using *Aspergillus niger* or *Candida sp*.
- Many other types of process are proposed, e.g., solid-state fermentation, as well as other sugar sources, e.g., agro-industrial waste

Citric acid production

- First isolated by Karls Scheels in 1874, England, from lemon juice, after precipitation with $Ca(OH)_2$
- Italian lemon producers had a monopoly on its production for almost 100 years, and its price was quite high
- Because of this, many attempts have been made for alternative production methods, chemical and microbial
- In 1923, Wehmer observed the presence of citric acid as a by-product of oxalate production by *Penicillium glaucum* and other researchers isolated two strains of fungi of the genus *Citromyces* (namely *Penicillium*)
- Unfortunately, industrial efforts have not been efficient, due to contaminations and high duration of fermentations

Citric acid production

- The industrial process was started by Currie, in 1917, who discovered that *Aspergillus niger* could accumulate citric acid during fermentation of sugars
- Currie showed that the production of citric acid is favored by high concentrations of sugars, where its growth is inhibited
- In the 1930s industrial units were developed in England, the Soviet Union and Germany to produce the
- The whole matter was fully clarified from a biochemical point of view in the 1950s, with the discovery of the glycolysis pathway and the tricarboxylic acid cycle
- Subsequently, the improved process of production through submerged fermentation was developed in the USA, which is followed today

Citric acid production

- There are many good methods of chemical synthesis of citric acid
- More successful methods are considered those through microbial fermentations
  - due to cleaner waste
  - due to lower energy expenditure
- Studies continue at the level
  - improvement of the strains
  - maintaining their productivity

Citric acid applications

- Mainly in food products as additive due to
  - Its pleasant sour taste
  - its high solubility in water
- It belongs to substances characterized as "GRAS" (generally recognized as safe)

Industry	Applications
Beverages	Fruit taste and odor. It increases microbicide effect. pH regulation.
Jellies, jams and spoon sweets	Taste. pH regulation.
Candy	Taste. It reduces the inversion of sucrose. It provides the dark color to hard candies. pH regulation.
Frozen fruits	It decreases pH to inactivate oxidizing enzymes. It protects ascorbic acid from bivalent metals.
Dairy Products	As emulsifier in ice-creams and soft cheese. As an acidifying agent and antioxidant in cheeses.
Fats and oils	Binds free radicals - antioxidant.
Pharmaceuticals	As an effervescent in powders and pills in combination with bicarbonate. Facilitates rapid dissolution of active ingredients. As an acidifying agent in mild astringent preparations. Anticoagulant.

Industry	Applications
Cosmetics and care products	As a component of buffer solutions and for pH adjustment. Antioxidant. As a metal ion chelator.
General industrial applications	Metal ion chelator. Neutralizer. Ingredient of buffer solutions.
Metal cleaning	Removes metal oxides from the surfaces of ferrous and non-ferrous metallic materials. Purification (for production or use) of copper and iron oxides.
Other	Electroplating (copper, etc.). Metal cleaning. Tanning of leather fabrics. Printing inks. Bottle cleaning materials. Floor cement. Fabrics. Photographic reagents. Concrete plaster. Refractory materials and moulds. Adhesives. Paper. Polymers. Tobacco – tobacco products. Waste treatment.

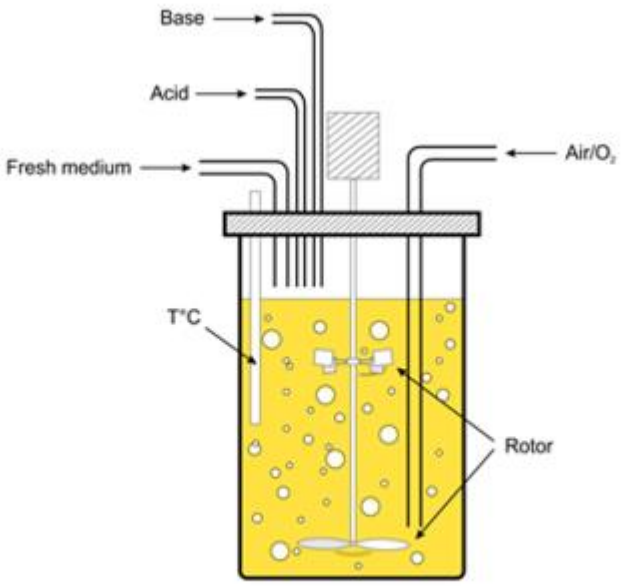
Microorganisms synthesizing citric acid

Μικροοργανισμοί	
Mycetes	*Aspergillus niger* *A. aculeatus* *A. awamori* *A. carbonarius* *A. wentii* *A. foetidus* *Penicillium janthinelum*
Yeasts	*Saccahromicopsis lipolytica* *Candida tropicalis* *C. oleophila* *C. guilliermondii* *C. parapsilosis* *C. citroformans* *Hansenula anamola*
Bacteria	*Bacillus licheniformis* *Arthrobacter paraffinens* *Corynebacterium sp.*

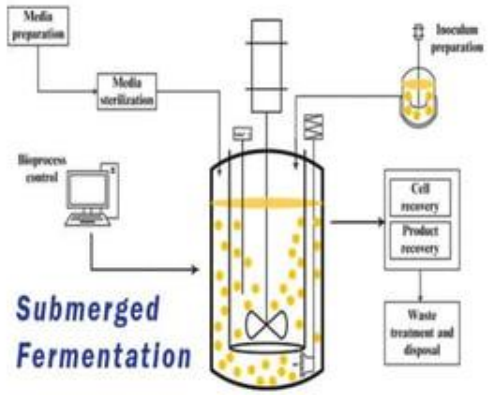
Liquid fermentation: Submerged fermentation

- This is the most used path
- About 80% of world production
- Many advantages, mainly
  - High performance
  - High productivity
  - Low labor costs
- Two types of fermentors
  - stirred fermentors
  - tower fermentors
    - preferred due to purchase price, size and mode of operation
- The digesters
  - are made of stainless steel
  - require good ventilation (rich oxygen supply)
  - they do not require resistance to high pressures
    - sterilization is done by passing hot steam
  - cooling is done by circulating cold water

Submerged fermentation



Submerged Fermentation



Liquid fermentation: Submerged fermentation

- The culture medium may contain simple carbohydrates or starch hydrolysis derivatives
- Molasses, and other wastes, require pre-treatment, addition of other ingredients and sterilization
- Inoculation is done by inserting
  - either seed suspension
    - detergent is added to help disperse them
  - or pre-cultured micelles
    - the inoculum is about 10% of the culture medium volume
- Submerged digestion is completed in 5-10 days depending on process conditions
- A batch reactor is usually used

Liquid fermentation: Submerged fermentation

Raw material	Strain	Citric acid	Yield, %
Brewery wastes	*A. niger* ATTC 9142	19 g/L	78.5
Beet molasses	*A.niger* ATTC 9142	109 g/L	-
	Yarrow lipolytica A101	54 g/L	68.7[a]
Cane molasses	*A. niger* T 55	-	65
Wood Hemicellulose	*A. niger* IMI- 41874	27 g/L	45[a]
	S. lipolytica IFO 1658	9 g/L	41
Date syrup	*A. niger* ATTC 9142	-	50
Corn starch	*A. niger* IM-155	-	62
Starch hydrolysate	*Y. lipolytica* DS-1	-	-
	Y. lipolytica A-101	-	75
Rapeseed oil	*Y. lipolytica* A-101	-	57
Soybean oil	*Y. lipolytica* A-101	-	63
Coconut oil	*C.lipolytica* N-5704	-	99.6[b]
Palm oil	*C.lipolytica* N-5704	-	155[b]
Olive oil	*C.lipolytica* N-5704	-	119[b]
Soybean oil	*C.lipolytica* N-5704	-	115[b]
Glycerol	*C.lipolytica* N-5704	-	58.8[b]
n-Paraffin	*C.lipolytica* N-5704	-	161[b]

[a] based on sugar consumed; [b] based on oils and fatty acids

Liquid fermentation: Surface fermentation

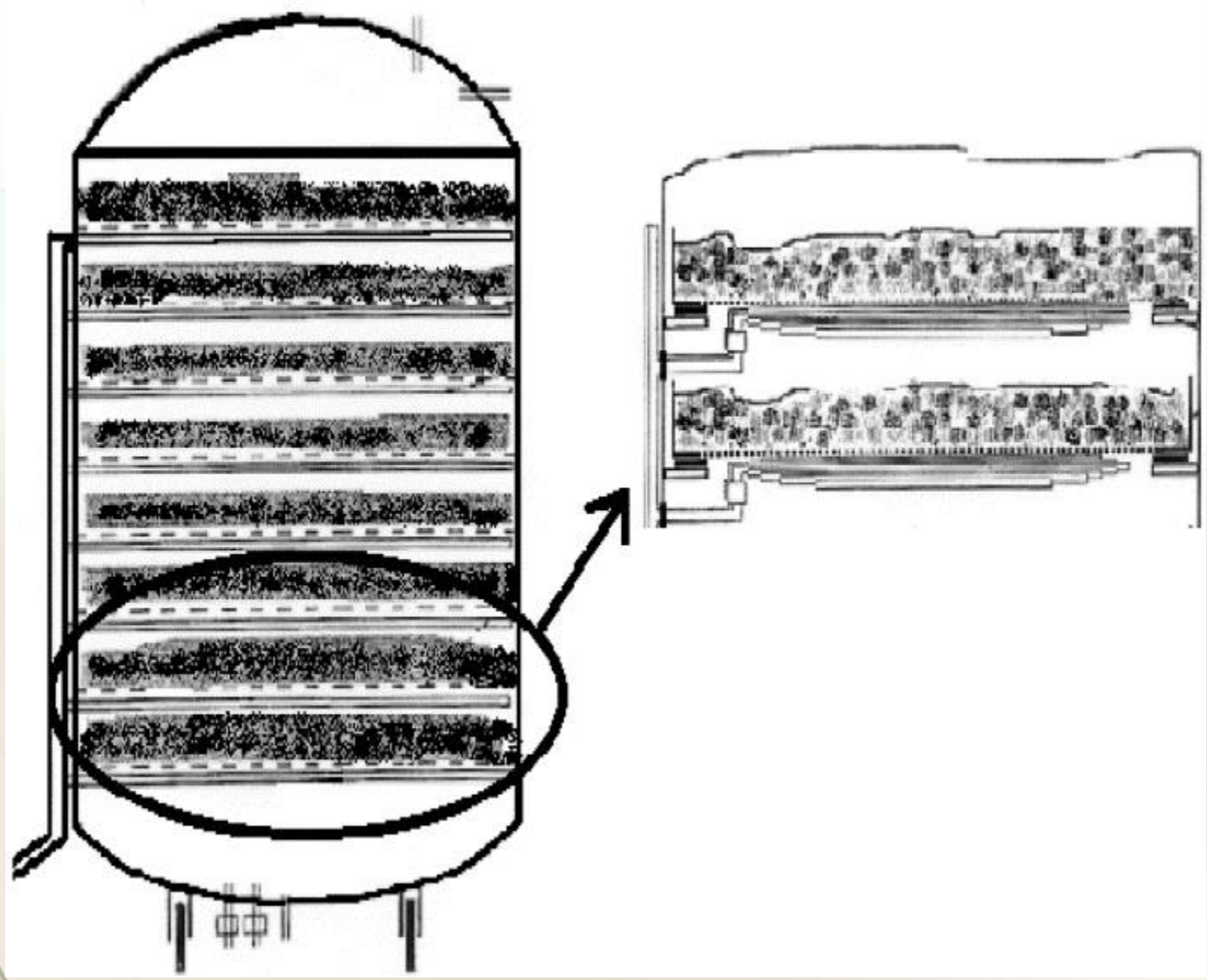
- The first process followed for the production of citric acid was wet surface culture (1919, Belgium and 1923, USA)
- The technique is relatively simple, easy to install and operate and with less energy expenditure
- The culture is maintained in shallow 50-100 L aluminum or stainless-steel trays and the fungus grows as a mycelial carpet on its surface
- The trays are placed as shelves in the reactor, which is regulated for temperature, air supply and humidity
- Pure or non-pure sucrose, corn syrup or molasses after oxidation with hexacyanoferric is used as carbon source
- Sterilization must be maintained for at least the first 2 days, until the spores germinate
- Common infections are from Penicilia, Aspergilli, yeasts and lactic acid bacteria

Surface fermentation

Solid-state fermentation

- Solid-sate fermentation (SSF) is the main alternative process for citric acid production from agro-industrial wastes
- The production of citric acid via SSF (Koji process) developed in Japan and it is the simplest one
- SSF utilizes a great variety of wastes
- The substrate is mixed with water up to 70%
  - This depends to the adsorption capacity of the substrate
- Initial pH is 4.5-6.0 and incubation T between 28 and 30°C

Solid-state fermentation



Solid-state fermentation

Wastes used

Raw material	Strain		Citric acid
Apple pomace	*A.niger*	NRRL2001	766 g/kg[a]
		NRRL 2270	816 g/kg[a]
		NRRL 599	771 g/kg[a]
		NRRL 328	798 g/kg[a]
		NRRL 567	883 g/kg[a]
Grape pomace	*A.niger*	NRRL2001	413 g/kg[a]
		NRRL 2270	511 g/kg[a]
		NRRL 599	498 g/kg[a]
		NRRL 328	523 g/kg[a]
		NRRL 567	600 g/kg[a]
Kiwifruit peel	*A .niger*	NRRL 567	100 g/kg[a]
Cellulose hydrolysate and Sugar cane	*A. niger*		29 g/kg
Orange waste	*A. niger*		46 g/kg
Beet molasses (Ca-alginate gel)	*A.niger*	ATCC 9142	35 g/L
Saccharose (Sugar cane bagasse)	*A. niger*	CFTRI 30	174 g/kg[b]
Coffee husk	*A. niger*	CFTRI 30	150 g/kg[b]
Carrot waste	*A.niger*	NRRL 2270	29 g/kg[a]
Okara (soy residue)	*A. niger*		96 g/kg[a]
Pineapple waste	*A.niger*	ATCC 1015	132 g/kg[b]
	A.niger	ACM 4942	194 g/kg[b]
Glucose (Sugar cane bagasse)	*A.niger*	CBS733.88	21.24 g/L
Kumara (starch containing)	*A. niger*	Yang no 2	103 g/kg[b]
Mussel processing wastes (polyurethane foams)	*A. niger*		300 g/kg
Cassava bagasse	*A. niger*	LPB-21	347 g/kg[b]

[a] *based on sugar consumed;* [b] *based on dry matter*

Solid-state fermentation

- The most commonly used organism is *A. niger*
  - Certain yeasts are also used
- One of the advantages of SSF is that the presence of trace elements usually does not affect the production of citric acid, as it does in SmF
- Therefore, no pre-treatment of the substrate is required

Solid-state fermentation

- Different types of fermentors have been used
  - Simple conical flasks
  - Glass tubular reactors
  - Disc reactors
- Not related to the type of substrate
- Higher yields have been achieved in non-aerated flask reactors
  - with minimal spore development
- Similar yields have been achieved in tubular fermentors with minimal ventilation
- The production of citric acid from disk fermentors seems ultimately preferable

Factors affecting citric acid production

The medium and its constituents

Carbon source

- Citric acid accumulation is greatly affected by the nature of the carbon source
- The presence of easily metabolized carbohydrates is important for good production
- Sucrose>>glucose>>fructose>>galactose
  - Galactose contributes to a low growth of the fungus and does not favor the accumulation of citric acid
- Other carbon sources, sugar derivatives or not, e.g., ethanol, cellulose, mannitol, lactate, malate, α-ketoglutarate, allow limited growth and low production
  - Starch, pentoses (xylose and arabinose), sorbitol and pyruvate retard growth, thus minimizing production

Factors affecting citric acid production

The medium and its constituents

Carbon source

- The initial concentration of sugars is very critical to produce citrate and other organic acids
  - *A. Niger* needs an initial sugar concentration of 10-14% (optimal)
  - No citric acid is produced at a sugar concentration < 2.5%
  - Immobilized *A. niger* cells need a lower concentration of sucrose than simple culture to achieve high yields (200 g citrate/L culture, and 120 g/L immobilized cells)
- A lot of waste can be used
- Critical factors are cost and pretreatment
- A more critical factor is the presence of trace elements that can act as inhibitors as well as activators
  - therefore, their presence should be checked and removed

Factors affecting citric acid production

The medium and its constituents

Nitrogen source

- Citrate production is directly affected by the nitrogen source
- Ammonium salts are preferred
  - Urea, peptone, malt, etc. can also be used
- Nitrogen consumption leads to a decrease in pH, being very important in the fermentation process
- However, it is necessary to maintain a constant pH value on the first day of digestion, before the culture grows satisfactorily
  - Urea helps some how in pH stability
- The nitrogen source concentration required is 0.1 to 0.4 N
  - Higher nitrogen concentration increases cell growth and sugar consumption, but decreases citrate production

Factors affecting citric acid production

The medium and its constituents

Phosphorus source

- The presence of phosphorus in the culture medium has a very positive effect on the yield in citrate
  - $KH_2(PO_4)$ is considered the most suitable source of phosphorus
- Phosphorus is required at concentrations of 0.5 to 5.0 g/L in the culture medium for maximum citrate production
- Phosphate is essential for the growth and metabolism of *A. niger*
- Low phosphate levels favor citrate production
- High (excess) phosphate levels lead to
  - Biosynthesis of acid-derived carbohydrates
  - Reduction in CO_2 assimilation, and reduced growth
    - Phosphates act at the level of enzyme activity and not at the level of gene expression

Factors affecting citric acid production

The medium and its constituents

- It is worth noting that different strains require different concentrations of N and P in the culture medium
- In practice, N and P limitation is a very important factor in citrate production because there is an interaction between them
- The general picture shows that most strains produce larger amounts of citrate
  - **under conditions of low N and P levels in submerged culture**
  - **under conditions of high levels in solid state culture**
    - this is due to the low diffusion of nutrients in solid state culture
- In conclusion, the strains with high N and P requirements do not favor citrate production, due to the limitations placed on nutrients

Factors affecting citric acid production

Trace elements

- Trace element nutrition is the most important factor affecting citrate yield
- Many divalent metals such as Zn, Mn, Fe, Cu and Mg directly affect citrate production by *A. niger*
- The dependence of the action of trace elements on the culture medium is also important
  - **Zn favors citrate production when added in the presence of KH_2PO_4**
  - **The presence of Mn and high concentrations of Fe and Zn lead to a decrease in citrate yield only in phosphate-free medium**
- Few differences are found between SSF and SmF in the response of *A. niger* to trace elements
- SSF systems can overcome the undesirable side effects of high concentrations of trace elements in the culture medium
  - Thus, there is no need to add chelating agents (ferrocyanides) for their binding

Factors affecting citric acid production

Trace elements

- Cu complements the ability of Fe (at optimal concentration) to favor citrate biosynthesis
  - The optimal initial concentration of $CuSO_4.5H_2O$ is 78 mg/L
- The accumulation of citrate decreases with the increase of Fe, which also influences the growth of the microorganism
- Mn deficiency results in **suppression** of enzymes of glycolysis and tricarboxylic acid cycle, **except for citrate synthase**
  - This leads to an overaccumulation of citrate as an end product of glycolysis
- Low levels of Mn (at ppm level) can reduce citrate yield by 10%
- Mg is essential for both growth and citrate production
  - The optimal starting concentration of magnesium sulfate is between 0.02-0.025%

Factors affecting citric acid production

Other constituents: alcohols, esters, fats

- Addition of low C alcohols favors the production of citrate from glucose or other carbohydrate by-products
  - More suitable are: methanol, ethanol, isopropanol, but also methyl acetate
  - The optimal concentration of methanol or ethanol depends on the strain and the composition of the culture medium
    - It is usually between 1-3%
- Methanol or ethanol acts by increasing the activity of citrate synthase (100%) and aconitase (75%)
  - The other enzymes of the TCA cycle are slightly increased
- Coconut oil (3% w/w in medium containing sucrose) enhances citrate production
- In general, alcohols act mainly on the permeability of membranes, affecting their phospholipid composition (mainly of the cell membrane)
- At the same time, they affect the growth and sporulation of the microorganism, acting in addition on the spatial organization of the membranes and the lipid composition of the cell wall

Factors affecting citric acid production

Other constituents

- Certain substances (CaF_2, NaF and KF) that act by inhibiting metabolism, accelerate the production of citrate
- Conversely, others ($K_4Fe(CN)_6$) lead to a reduced yield
- Different substances act in different ways when favor the accumulation of citrate
- Some of them react contrary to the effect of metal ions and other toxic substances
- and favor the growth of the microorganism in the initial phase
- Some of these are: 4-Methylumbelliferone, 2-naphthoate, 3-hydroxy-2-naphthoate, benzoic acid, $Fe(CN)_2$, quaternary ammonium bases, oximes, starch, EDTA, vermiculite, etc.

Factors affecting citric acid production

Process parameters

- **pH:** the pH value in a culture can vary due to the metabolic activity of the microorganism
- Production of organic acids that lead to a decrease in pH
  - Citrate, acetate, lactate
- The change in pH kinetics is highly dependent on the microorganism
  - In *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., the pH decreases very rapidly to a value below 3.0.
  - In other fungi, *Trichoderma*, *Sporotrichum*, *Pleurotus* sp., the pH is more stable (between 4 and 5)
- The nature of the substrate affects pH kinetics
  - A pH value below 2.0 is required for optimal citrate production
- Low initial pH values are advantageous because
  - they do not allow contaminations
  - inhibit oxalate production
- A pH value of 2.2 is optimal for microorganism growth and citrate production
- When using molasses as a substrate, higher pH values (5.4 to 6.0-6.5) are required for optimal citrate production

Factors affecting citric acid production

Process parameters

- **Aeration:** Aeration plays a key role in the production of citrate through digestion
- Increased aeration leads to increased yields and shorter process time
- It is important to keep the oxygen concentration above 25% saturation
  - An interruption in the oxygen supply has harmful consequences
- The high oxygen requirements are achieved by the construction of suitable ventilation systems, to overcome the viscosity of the culture medium
- Aeration is carried out throughout fermentation with the same intensity and supply in the range of 0.5 – 1.5 vvm
  - For economy reasons, it is preferable to use low aeration (0.1 – 0.4 vvm) at the beginning of the process

Factors affecting citric acid production

Process parameters

- High air flow results in intense foaming, especially in the growth phase
- The addition of chemical defoamers and the construction of mechanical defoamers are necessary
- The way the microorganism grows affects the dissolution of oxygen
- During fermentation, the formation of microbial aggregates (pellets) is preferred over filaments (due to metal impurity), because dissolved oxygen is reduced to 50% of the original, even if microbial growth has not exceeded 5%

Citric acid recovery

- Citrate recovery from liquid fermentation proceeds via
  - precipitation (with calcium hydroxide)
    - tricalcium citrate tetrahydrate is obtained
  - extraction
    - the product is filtered, washed and treated with sulfuric acid
    - the calcium sulfate is removed by filtration
  - purification with an ion exchanger
    - the liquid is treated with activated carbon and subjected to anion- and cation-exchange treatment
  - crystallization under vacuum at 20-25°C in the form of citric acid monohydrate or at a higher temperature in the form of anhydrous citric acid
- The process is set by the FDA
- The resulting citrate is used as additive in food and medicine

Optimal growth conditions and optimal bioprocess conditions

e.g., *Aspergillus Niger*

- produces citric acid in significant quantities when the nutrient medium is deficient in manganese
- **The growth of the organism, however, requires manganese**

- A citric acid production process based on the use of this organism must include two phases:
- one to develop a sufficient number of cells
- second for citric acid production

Downstream process

- It is the process followed after fermentation:
  - Collection of cells
  - Cell lysis
  - Purification of the product from the cells extract

  - In cases the product is secreted from cells, the culture medium is used for its purification

Steps in downstream processing

	Step	Unit Operations
1.	Separation of insoluble material	filtration, extraction, precipitation, adsorption
2.	Product isolation	extraction, adsorption, ultrafiltration, sedimentation
3.	Purification	chromatography, crystallization, fractional sedimentation
4.	Finish	drying, crystallization

Substitutes

- Substances added to the final product for its stabilization

- Bovine serum albumin
  - Resistance to low pH or elevated temperature
  - It does not allow the product to stick to the walls of the packaging container
  - It stabilizes the natural conformation of proteins

Substitutes

- Amino acids
  - Glycine
    - It stabilizes proteins against temperature changes
- Alcohols (and other poly-ols)
    - They stabilize proteins in solution
- Detergents
    - They reduce the surface tension, preventing the aggregation of proteins and their denaturation

Final product

- Quality control (QC testing)
- Sterilization by passing through a 0.22 μm filter
- Aseptic filling of containers
  - In the case of liquids, use an automated dispensing system

Final product

- Lyophilization
  - The product is obtained as a powder
  - Possible biological or chemical deterioration of the product is reduced
  - Longer product life
  - Optimum way of storage of products taken parenterally

Final product

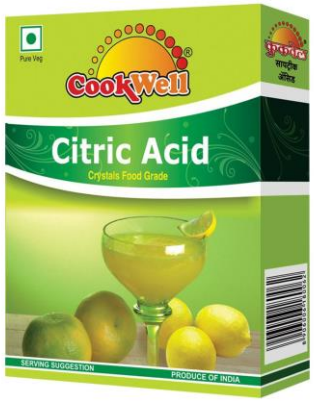
- Requirement for cryoprotectants addition
  - Glucose or sucrose
  - Bovine serum albumin
  - Amino acids
  - Poly-ols

Examples


HOME · KITCHEN · LAUNDRY · BATH
milliard
A HOME ESSENTIAL
CITRIC ACID
PURE · FINE GRAIN · ANHYDROUS
0%
NON GMO Project VERIFIED
NET WT. 5LB./2.3KG


Citric Acid
Monohydrate
BP/USP/FCC/E330
ENSIGN
GMO Free
Net Weight: 25kg
See the Seal


ultrap laboratories
Citric Acid B.P.
100g e
Store out of reach of children
Roche's Chemist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Since 1947
L-LYSINE
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SKIN & TISSUE PROTECTION*
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free-form
Easy Absorption
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Hyaluronic Acid 55mg
Healthy Cartilage and Joints
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HYALURONIC ACID
HYDRATING HAND CREAM
BATH & BODY WORKS
1 FL OZ / 29 ML


HYALURONIC ACID
100ml


Insulin


SCHERING
Interferon alfa-2b recombinant
Intron A
SCHERING
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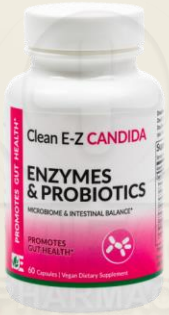
Intron A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