fermentation technology

(1) The word fermentation is Derived from latin & word fervere which means to Boil.

(2) But the Conventional Definition of fermentation is to Break down of larger Molecule into Smaller and Simple molecule using micro-organisms.

(3) In Biotechnology, fermentation mean any Process by which micro-organisms are grown in large quantities to Produce any type of useful material.

⟹ Culturing the Micro-organism / method -

After isolation of micro-organism they are grown in Culture medium. Different type of microbial Culture are used for Different Purposes. Some of the Common types of Culture are -

1) Batch Culture
2) Continuous Culture
3) fed - Batch Culture.

Batch Culture –

① It is the simplest method of culturing the micro-organism in which the micro-organism are grown on a limited amount of medium Until Essential nutrients are exhausted of toxic By products inhibit the growth.

(2) In a Batch Culture, the microbes pass through a number of Stage During their growth.

A. Leg phase –

① The growth of Micro-organism will not occur immediately After inoculation. They take Some time to Adjust or Adapt to the medium.

(2) This time is called leg phase.

(3) The leg phase Can be reduced by using relatively large Amount of Exponentially growing inoculum which is grown in a medium having Similar Composition as that used in the fermentation.

(B) Exponential or log phase—

In this phase, the microbes grow in an exponential manner consuming the nutrient present in the medium.

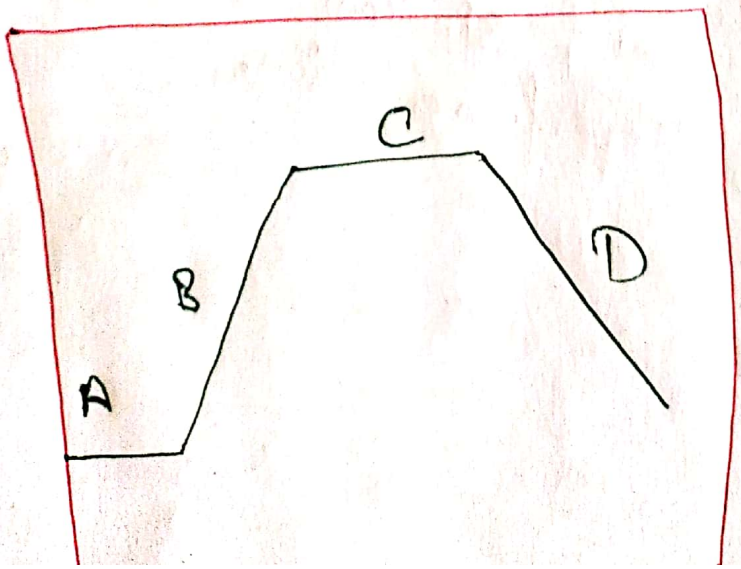
(C) Stationary or Deceleration Phase—

As soon as the level of Nutrient is restricted or exhausted in the medium, the growth of culture gradually slows down.

This may also occur due to Accumulation of toxic metabolites which inhibits the growth. During this phase, the micro-organism can not grown and hence their biomass can not increase.

(D) Death or Decline phase—

In this phase the nutrients in the medium exhaust completely and there will be Accumulation of toxic material which leads to death of microbial cell.



Advantages:-
1) Require less Space
2) Can be Easily handled, and
3) Less Chance of Contamination

② Disadvantage-
① Time Consuming
② Requires more time for cleaning, Sterilization, and Cooling
③ Product yield is low.

★ Continuous Culture-
1) if the Culture medium is Designed Such that the Cessation of growth is due to Depletion of nutrients rather than by Accumulation of toxins, the Exponential growth in the Batch Can be Prolonged by the Addition of fresh medium to the Culture vessel.
2) if the Addition of medium Displaces an Equal Amount of Culture, then Continous Production of cells Can be Achieved.
3) if the medium is Added Continously to such a System at a Suitable rate, the Displacement of Culture Can be Balanced by the Production of New Biomass and a steady State Can be Achieved.

Advantage of CF.

1) Product forms continuously.
2) Product yield is good.
3) Inoculation of culture is done only once, and
4) Save time and labour.

Disadvantage of CF.

1) Complex and difficult to operate
2) More chance of contamination
3) operators with brief knowledge on fermentation and microbial behaviour and growth are required.

Fed-Batch Culture-

(1) It is also the Batch Culture which is fed Continuously with fresh medium with fresh medium without the removal of orginal Culture from the the fermentor.. The volume of medium in the fermenter increases Continously.

⇒ General requirement-

(1) Water - it forms the leading Component of the media in micro-organism.

(a) The water quality is highly Significant as it Affect microbial growth and Production of BioProduct.

(c) In media Production, there is quality Control of raw material. in many Biotechnological Proces, and it Supply and we Should be Carefully monitored and Controlled.

(2) Source of Energy- Carbon, a Nitrogen Source, inorganic Element, and Specific growth (for Some cell type) are the Basic nutritional requirment of micro-organism.

Carbohydrate Sources	Form	Nitrogen Sources (% Nitrogen by wt)
Glucose	Pure gluco, Monohydrate and hydo-lyed Starch	Barley (1.5-2.0) Beet Molasses (1.5-2.0)
Lactose	Pure Lactose whey Powder	Corn Steep Steep (4.5)
Starch	Barley, groundnut meal, oat flour, rye flour, and Soybean meal	Ground meal 8.0 Oat flour (1.5-2.0) Plasma media (8.0)

③ Sterili Sterilization Practices for Biotechnological media should Dabey maximum Contaminating micro-organism at minimum temperature and causing minimal Damage to the medium Component.

④ Preparation of the media in an Appropriate manner is the Basis of the Entire fermentation Process. Poor media Design lead to low Efficiency of growth and Poor Product formation.

★ Study of Media –

(i) Media is a mixture of various nutrient prepared Artificially to Support the growth and multiplication of micro-organism.

(2) Media are Continously being Developed and/or revised to be used for isolating and identifying Desired Bacteria in various field (like food, water, and Clinical Microbiology).

(3) Culture media are required for growing organism from infected material So that the Causative Agent Can be identified.

⇒ Constituent of Culture Media –

(i.) Water.
(ii) Electrolyte.
(iii) Peptone
(iv) Meat Extract
(v) Blood or Serum
(vi) Agar.

Type of Media –

(1.) Based on Physical State –

(a) liquid Media,
(b) Semi-Solid Media,
(c) Solid Media

(2.) Based on the Presence of Molecular oxygen and Reducing Substance in the Media

(a) Aerobic Media.
(b) Anaerobic media.

(3) Based on Nutritional factors –

(a) Simple media.
(b) Complex media
(c) Synthetic Media.
(d) Special media.
(i) Enriched media
(ii) Selective media
(iii) Enrichment media.
(iv) Differential media.
(v) indicator Media
(vi) Transport media.
(vii) Sugar Media

⇒ Culture Media for Lactobacilli and E. Coli –

(1) Autotrophs Can be Cultivated using Chemically Defined Media, which are also used for Defining the Nutritional requirement of Heterotrophs.

(2) However, Chemically Defined media is not Employed and instead Some Complex Organic materials. Eg – Peptones, meat Extract and

Yeast Extract) are used for the routine Cultivation of heterotrophs.

(3) The resulting media support the growth of a wide variety of heterotrophic bacteria.

(4) To obtain a solid medium, agar is added which serve as a non-nutritive solidifying Agent.

⇒ Culture media for viruses –

1) Primary Culture – This Culture is prepared by Dispersing cells, obtained from freshly removed host tissues, with trypsin, Generally, they Cannot grow for more than a few Passage in Culture.

2) Secondary Culture – This Culture included Diploid cell lines that have undergone a change Allowing limited Culture (up to 50 Passages).

3) Continuous cell Culture –
This Culture is is capable of more prolonged infefinite growth that has been Derived from Diploid cell lines or ~~form~~ from malignant tissue.
The type of cell Culture used for viral Cultivation Depend on the sensitivity of cells to a Particular virus.

Study of Equipment – (201) T.C.P.

⇒ Sterilization Method –

(1) Sterilization is an important operation which Differentiates biochemical and Chemical Process.

(2) The Process aims to Provide a Contamination-free Enviornment.

(3) fermentation Proceeds only with the involvement of

(i) A micro-organism (iii) A fermenter
(ii) A medium (iv) Nutrient / other Additives
(v) Air in the Case of Aerobic Process.

(4) A Contaminant may Affect the fermentation Process in the following ways.

(i) It may Contaminate the final Product
(ii) The medium would be Consumed to Support the growth of Contaminating organisms.
(iii) The Contaminated Product may be greater than the Desired Product.
(iv) Unteril Air in Aerobic fermentation may Spoil the fermentation Product.

⇒ Sterilization of fermenter 202
⇒ _____ Media 203
⇒ _____ Air 203

⇒ large Scale Production – fermenter Design – 20

large Scale Production – 20

⇒ Production of Penicilin—

① Antibiotics are Antimicrobial Agents Produced Naturally by other microbes (usually fungi or Bacteria.

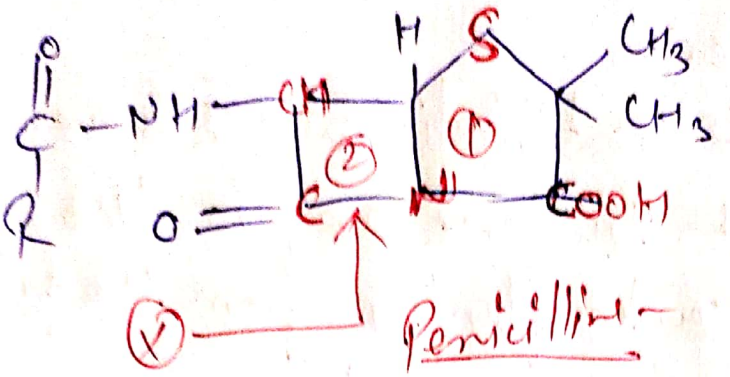
② The first Antibiotic was Discovered in 1896 by Ernest Duchene and in 1928, Rediscovered by Alexander fleming from the filamentous fungus Penicilium Notatum

③ The Antibiotic Substance, Named Penicilline, was not Purified Untill 1940, just in time to be used at the End of the Second world war

④ Penicilline was the first important Commercial Product Produced by an aerobic, Submerged fermentation.

⑤ Penicilline is Produce by the fungus Penicillium Chrysogenum— which require lactose, other Sugar, and a Source of nitrogen (in this Case Yeast Extract) in the medium to grow well.

⑥ Like all Antibiotics, Penicilline is a Secondary metabolite, so is only Produce in the Secondary Phase.



Properties-

1. Secondary Metabolite.
2. Against gram-tive Bacteria.
3. Soluble in water, very Soluble in Acetone, Ethyl Alcohol and Ether, less Soluble in Benzene, $CHCl_3$.
4. Aq. Penicillin are unstable & Store in refrigeration.
5. most Stable in the Ph range of 6.0 to 6.5 and reasonable Stable over Ph range 5.5 to 7.5

...rocess of Penicillin Production—

(1) The inoculum—

(i) inoculum is the micro-organism used in fermentation. generally, a high yielding Strain of (P. Chrylogenicum) is used.

(2) Spores (P. Chrylogenicum) from working Stock Cultured are Suspended in water or non-toxic ~~detergent~~ lauryl Sulphonate,

(3) Then Added to the flask Containing wheat Bran and Nutrient Soln.

(4) These flasks or bottle are ~~incu~~ incubated at 24°c for 5-7 days. to Support heavy Sporulation.

(5) This Prolem is repeated a number of times to have more Sporulation.

(2) The medium— Jakson in 1958 Prepared a media for Penicillin Production. the major Constituent of typical medium include.

(i) fermentable Carbohydrate — Corn Steep liquor (3.5%)
lactose (3.5%)
glucose (1%)

(ii) Potassium Di hydrogen phosphate → 0.4 %

(iii) Edible oil → 0.25%

(iv) Calcium Carbonate → 1.0 %

(v) pH After Sterilization → 5.5% to 6%

(vi) organic Nitrogen Source

(vii) phenyl Acidic Acid Precursor

③ Aeration – (Oxygen Supply)

① Supply of oxygen in a bioreactor is the limiting factor in Penicillin Biosynthesis. ~~Is the limiting factor.~~

② Aeration Speed is Between 3 to 1.5.

④ Temperature –

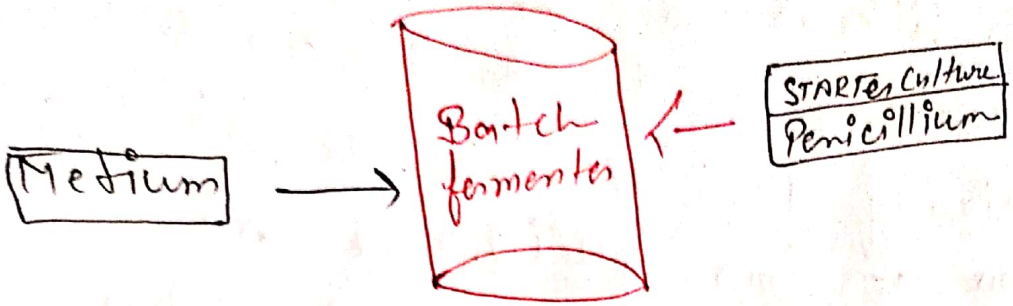
Temperature plays an important role in Penicillin Production it should be mentioned at 28°c.

⑤ Biomass Production –

Production of Penicillin Depend upon Biomass Production therefore, it is Desirable to have a high Biomass Conce in the vessel. It is achieved by increasing the Agitation rate and Power.

⑥ PH – It is maintained Early Neutrality by Calcium and magnesium Carbonated in the medium by phosphate Buffer. It is also Controlled by Adding Sodium hydroxide or Sulphuric Acid in the Medium.

Procedure of Penicillin —



↓

To time in 6 days
remove 30% Culture
add 30% fresh medium

↓

rotating filter.

→ fungal cell → Animal feed

↓ filtrate

↓ Dissolve in Butyl Acetate

↓ Potassium Ion Added to Precipitate Salt of Penicillin

↓ wash filter and Dry.

↓ 99.5% Pure Penicillin

↓ Chemical and Enzymatic modification to make new Antibiotic.

☆ Recovery & Purification of Penicillin -

① Harvested Culture Broth include Penicilline - G Along with a variety of other metabolites.

② Vaccum filter is used for Seperation of mycelium from the Broth on a rotary.

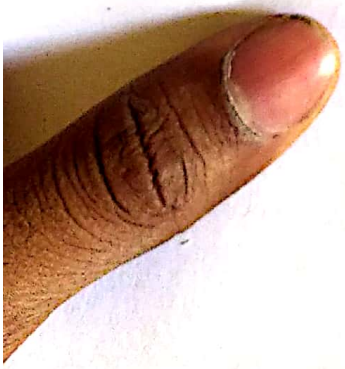
③ Conversion of Penicilline to the Anionic form occurs at low pH (2.2 to 2.5)

④ The lowering of pH is Done by Adding phosphoric Acid or Sulphuric Acid.

⑤ for removal of Pigment and other impurities from Solvent Containing Penicilline G it is treated with Active Charcoal.

⑥ The Product is back Extracted into water from Solvent by Adding Potassium or Sodium hydroxide to form G Salt.

⑦ The Product of Penicilline is then Crystallized into Sodium or Potassium Penicilline.



Production of Citric Acid —

★ Introduction —

① Citric Acid was first Discovered as a Constituent of lemon. Citric Acid at the current time is known as Intermediate of Krebs' Cycle and therefore is present in Every living organism.

② Previously, it was isolated from lemons (which Contain 7-9 % Citric Acid), and Now-a-days 99 % of it is obtained by Microbial fermentation.

★ Citric Acid Production —

① fermentation is the most Economical and widely used for Synthesis Citric Acid Production.

② Citric Acid Production Can be Carried in three Different ways.

(i) Surface fermentation
(ii) Submerged fermentation
(iii) Solid State fermentation.

(i) Surface fermentation.

1) Surface fermentation using Aspergillus niger may be done on rice bran as in the case in Japan, or in liquid Soln in flat Aluminium or Stainless steel Pans.

2) Special Strains of Aspergillus niger which can Produce Citric Acid Despite the higher Content of trace metals in rice bran are used.

(ii) Submerged fermentation—

1) In this case, the Strains are inoculated of About 15 cm Depth in fermentation tanks

2) The Culture is Enhanced by giving Aeration using Air Bubbles.

3) And it is Allowed to grow for About 5 to 14 Days at 27 to 33 Degree Celsius.

4) The Citric Acid Acid Produced in the fermentation tank and it is purified

(iii) Solid State fermentation—

1) It is Simplest method for citric Acid Production.

2) Solid State fermentation is also Known as Koji Process, was first Developed in Japan

3) Citric Acid Production reached a maximum (88g/kg Dry matter) when fermentation as Carried out with Guava having initial moisture of 62% at 26 Degree Celsius for 120 hours.

Citric Acid Production

CA → Calcium
PDA → Potato Dextrose Agar

A. Niger CA 16 and 19/20
↓
Growth PDA Agar Slant
↓
A. Niger Spore 7 Days old Culture

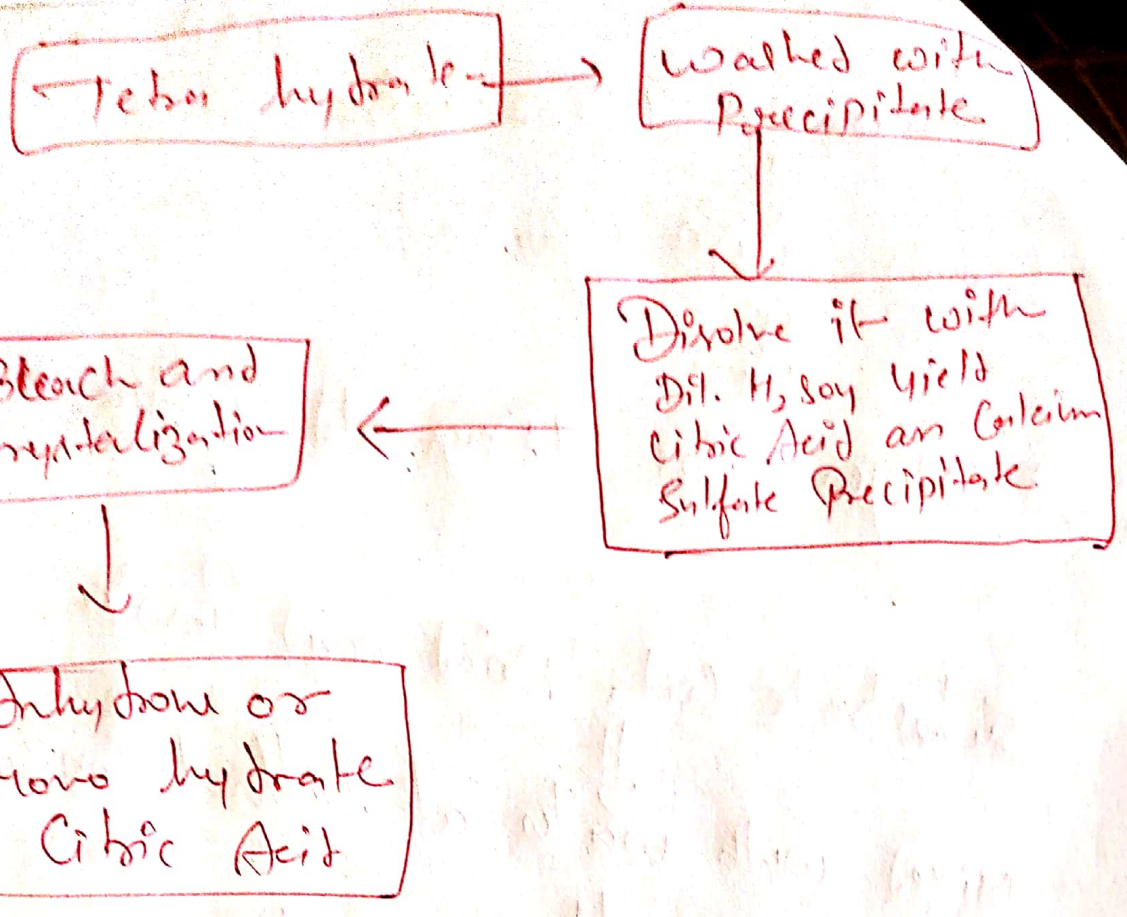
Substrate Cut, Dried and Powder
↓
Mixed with water at Different Concentration
↓
filteration ② Sterilization
↓
inoculation with 1X10 Spores / 25ml
↓
filteration ⟹ filtrate for citric Acid
↓
Cell BioMass.

⑤ PH Adjustment

Application

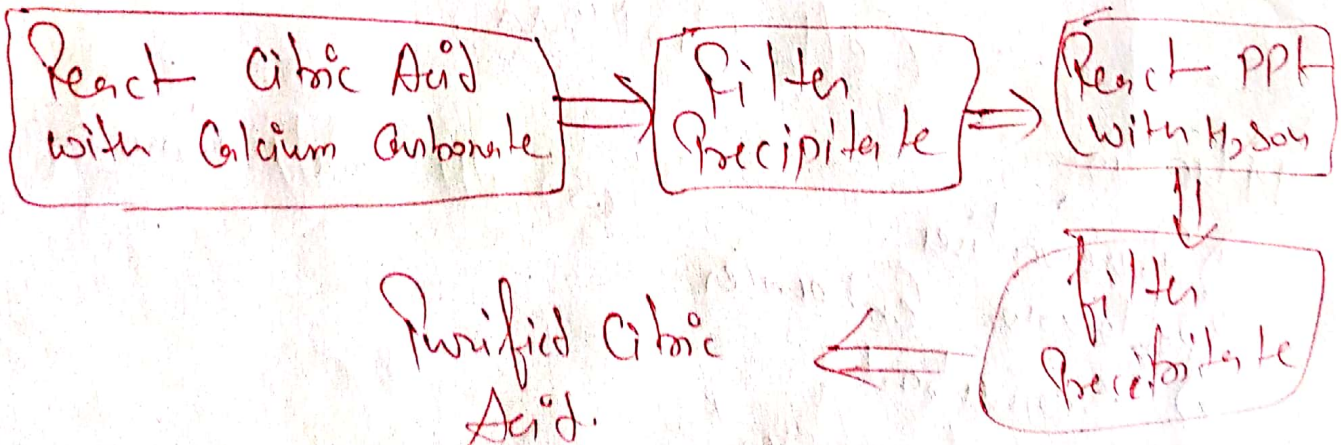
1) Preservatives
2) Flavour Enhancer
3) Sequestrant
4) Emulsifying Agent

Separation Process:-



Purification:-

1. Purification is a simple form of getting a pure Citric Acid followed by two simple technique.
2. Precipitation
3. filteration



Vitamin B_{12} –

1) Vitamin - B_{12} . also called Cobalmine , is a water Soluble vitamin that has a key role in the normal functioning of the Brain and Nervous System , and the formation of red Blood cells.

2) It is involved in the metabolism of Every Cell of the human Body, Especially Affecting DNA Synthesis , fatty Acid and Amino Acid metabolism

(3) It is Synthesized only by micro-organism and not by Animals (~~Animals~~ including Human) & Plant

(4) People with B_{12} Deficiency may Eventually Develop Pernicious Anemia.

(5) It can be Produced Industrially only through Bacterial Synthesis.

★ Production of vitamin B_{12} —

① Cyano-Cobalmin, is the industrially Produced Stable Cobalmin form which is not found in Nature.

② vit B_{12} is Entirely Produced on a Commercial Basis by the fermentation.

③ It is usually manufacture by Submerged Culture Process. Such a fermentation Procen is Completed in 3-5 Days.

④ Most of the B_{12} fermentation Procen use glycose as a Carbon Source.

⑤ The micro-organism that may be Employed in the Industrial Production Procen are —

(i) Steptomyces griseus
(ii) Steptomyces olivaceus
(iii) Bacillus Coagulans
(iv) Bacillus megaterium

★ Step Involved in Production —

1) formulation of the medium
2) Sterilization of the medium
3) Making Stenter Culture.
4) Anaerobic fermentation.
5) Recovery.

Streptomyces olivaceus —

① Production by Streptomyces olivaceus yield About 3.3 mg/L of vitamin B_{12}.

→ Process —

Ⓐ Preparation of inoculum —

Pure Slant Culture of S. olivaceus is inoculated in 100-250 ml of inoculum medium in flask

↓↓

Seeded flask is incubated on Platform of a mechanical Shaker to aerate the System

↓↓

flask Culture is then Subsequently used to inoculate larger inoculum tanks.

[2 or 3 Successive transfers are made to obtain required Amount of inoculum Cultures.].

② Media used in Preparation of inoculum in Bennetts agar.

Component	Amount (g/L)
Yeast	1.0
Beef Extract	1.0
Casein	2.0
Glucose	10.0
Agar	15.0
Dist water	1000 ml
PH	7.3

(B) Production medium –

1) Consist of Carbohydrate, Proteinaceous material, and Source of Cobalt and other Salts.
2) It is necessary to add Cobalt to the medium for maximum yield to Cobalmin.
3) Cyanide is added for Conversion of other Cobalamine to vitamin B12.

(C) Sterilization of the medium –

1) Sterilization can be done Batchwise or Continously.

- ~~Ste~~
- Batch → Medium Heated at 250°F for 1 hour.
- Continous → 330°F for 13 min by mixing with Steam.

(D) Temperature, PH, Aeration & Agitation –

1) Temperature :- A temperature of 80°F in Production tank is Satisfactory. During fermentation
2) PH :- At Starting of Process PH falls due to rapid Consumption of Sugar, then rises After 2 to 4 hr to lysis of mycelium, PH (5) is maintained with H_2SO_4 and reduce Agent Na_2SO_4.
3) Aeration & Agitation – optimum rate of aeration is volume Air / volume medium / min.

Anti foam Agent, Prevention of Contamination—

1) Antifoam Agent—

Defoaming Agent like Soyer Been oil, Corn oil, lard oil and Silicone Can be used.

2) Prevention of Contamination—

Essential to maintaine Sterility Contamination result in reduced yield, Equipment must be Sterile and all transfer are Carried out under specific Condition

(F) Recovery—

1) During fermentation, malt of Cobalmine is Subjected to further Process to obtain Crystalline B_{12}.

filtration of broth to remove mycelium

⇓

filtered broth is treated with Cyanide to bring Conversion of Cobalmine to CyanoCobalmine.

⇓

Adsorption of CyanoCobalmine from the Soln is done by Passing it thrugh Adsorbing agent packed in a Column.

⇓

CyanoCobalmine is the Eluted from the Adsorbent by the use of an aqueous Soln of organic Base or Soln of Na-Cyanide and thiocyanate.

⇓

Extraction is Carried out by Counter-Current Distribution b/w Cresol, Amylphenol, or Benzyl Alcohol and water or a Single Extraction into an organic Solvent (Eg. phenol) is Carried out.

⇓

Chromatography on Alumina and final Crystallization Complete the Procen.

Propionibacterium freudenreichii —

1) Production by Propionibacterium freudenreichii yield about 20mg/L of vit. B12.

A) Production Media —
- glycose, Corn-Steep, beteine & Cobalt.
- Cobalt — 5ug/ml [Excess Gme reduced Cobalmine]

B) PH — 7.5

C) Temperature — 30°C.

D) fermentation — It involve to cycles; Anaerobic fermentation Cycle of 70 hours and Aerobic fermentation Cycle of 50 hours.

(i) Anaerobic fermentation —
- → formation of ~~Cobalt~~ Cobinamide occurs.
- → The PH fall from 7.5 to 6.5 and then rise upto 8.5.
- → Necessary to Add 0.1% — of 5.6% - Dimethyl Benzimizazole to the Production medium.

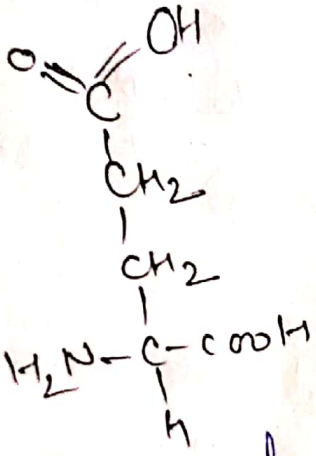
(ii) Aerobic fermentation —
- → Nucleotide formation take Place
- → This Nucleotide then link with Cobinamide to give Cobalmin

☆ Glutamic Acid –

① Glutamic Acid is an α- Amino Acid that wee in Bio-Synthesis of Proteins.

② Commercial Production of glutamic Acid by microbial fermentation offer 90 % of world's total Demands and the remaining 10 % is Provited by Chemical method.

③ for Actual fermentation, microbial Strains are grown in fermenters with volume of 500 m³.



glutamic Acid –

☆ Industrial Production of glutamic Acid –

1) The manufacturing Process of glutamic by fermentation. Comprises –

(a) fermentation (b) Crude isolation

(c) Purification Processes.

There are 3 types of fermentation are used -

(a) Batch fermentation
(b) feed - Batch fermentation
(c) Continous fermentation.

⇒ Medium -

1) Starch Sol^n, Cane molases are the Carbohydrate used as Carbon source.
2) The media Containg Cane Molasses with high Biotin Content is also Added with Penicillin During the Active growth of cell.
3) Ammonia, Ammonia gas, Urea etc are use as Nitrogen Source & other Ions included.
4) Biotin Should be Added in the fermentating media in Concentration below 5ug/L.
5) media Should be maintained at PH b/w 7-8 and temp b/w 30-35°C.
6) fermentation takes 2-4 days to Complete.

⇒ Industrial Production of glutamic Acid—

1) Natural Product such as Sugar Cane is used.
2) Then the Sugar Cane is ~~Sea~~ Squeezed to make molasses.
3) The Heat Sterilize raw material and other Nutrient are Put in the tank of the fermenter
4) The micro-organism [Corynebacterium glutamicum] Production glutamic Acid is Added to the fermentation broth.
5) The micro-organism react with Sugar to Produce glutamic Acid
6) Then, the fermentation broth is Acidified and the glutamic Acid is Crystallized.

✱ Separation & Purification—

1) The glutamic Acid Crystals is Added to the Sodium hydroxide Solⁿ and Converted into monosodium glutamate.
2) MSG is more Soluble in water, less likely Absorb moisture and has Strong Umami taste.
3) The MSG is Cleaned by using Active Carbon, which has many micro-holes on their Surface.

- The Clear MSG Soln is Cooled by heating and the monosodium glutamate Crystal is formed.

5) - The Crystal Produce are Dried with a Hot Air in the Closed System