

# Microorganisms and Processes Involved in the Production of Single Cell Protein and Industrial Alcohol





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**Alcohols  
(ethanol)**

**Amino acids  
(lysine, threonine,  
phenylalanine,  
and tryptophan)**

**Organic acids  
(acetic, propionic,  
succinic, fumaric,  
and lactic)**

**Polyols (glycerol,  
mannitol,  
erythritol, and  
xylitol)**

**Polysaccharides  
(xanthan and  
gelan)**

**Sugars (fructose,  
ribose, and  
sorbose)**

**Vitamins  
(riboflavin,  
cyanocobalamin,  
and biotin)**

**Microbial primary metabolites used in the  
food and feed industries**



**Single-cell proteins or microbial proteins refer to edible unicellular microorganisms. The biomass or protein extract from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as an ingredient or a substitute for protein-rich foods, and is suitable for human consumption or as animal feeds.**





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# Why Single Cell Protein?????

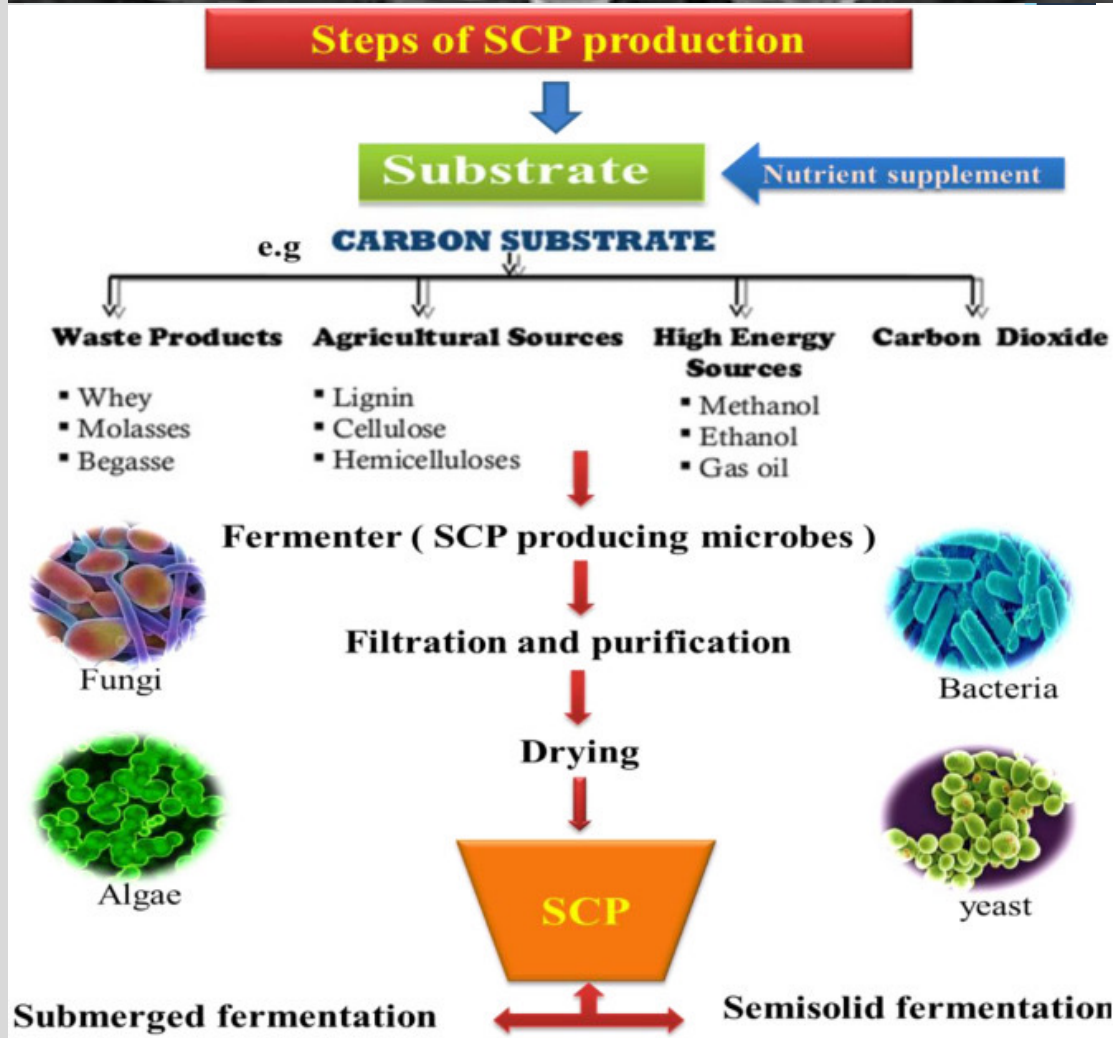
- **Microorganisms would help meet this world protein deficiency.**

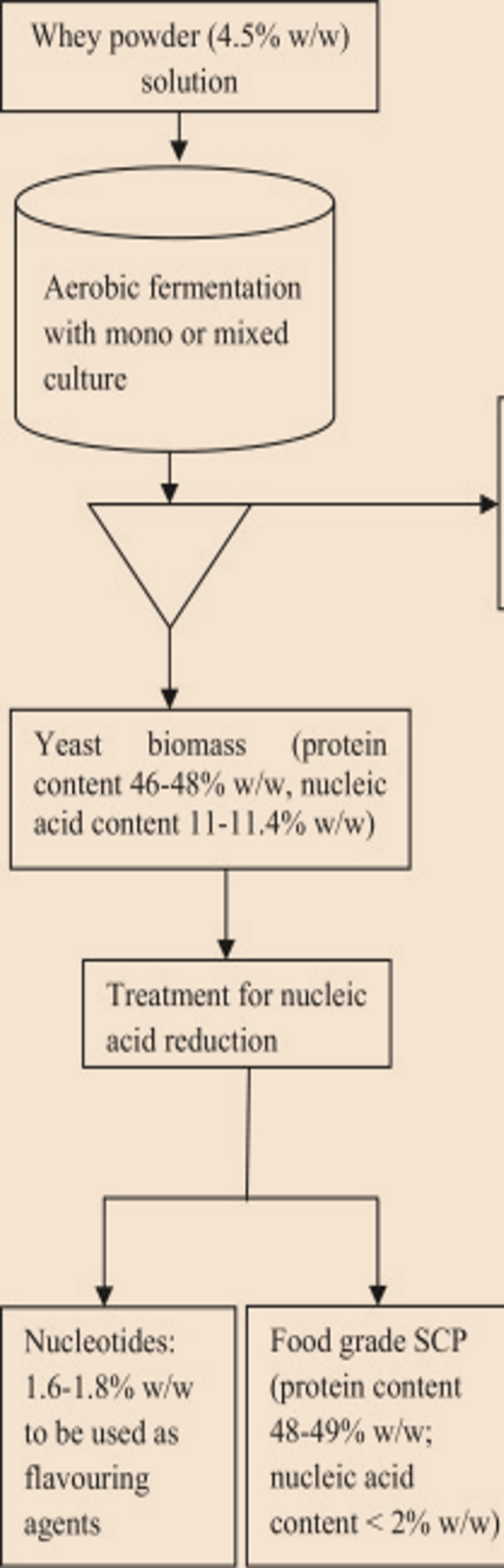
**Limitations of conventional sources of proteins include: (a) possible crop failure due to unfavorable climatic conditions in the case of plants; (b) need to allow a time lapse for replenishment of stock (fish) (c) limited land available for farming (plant production).**

- **Production of Single cell protein (SCP) has number of attractive features: (a) it was not subject to vicissitudes of the weather and can be produced every minute of year (b) Microorganisms have much more rapid growth than plants or animals.**

# Disadvantages of SCP:

- In developing countries, where protein malnutrition actually exists, lack of expertise and/or the financial resources to develop the highly capital intensive fermentation industries involved. This short-coming can be bridged by use of improvised fermentors and recovery methods which do not require sophisticated equipments.
- Microorganisms contain high levels of RNA and that its consumption could lead to uric acid accumulation, kidney stone formation and gout.





# Organisms used in SCP production should have following properties:

**Absence of pathogenicity and toxicity:** Large-scale cultivation of organisms which are pathogenic to animals or plants could pose a great threat to health and so avoided. Organisms should also not contain or produce toxic or carcinogenic materials.

**Protein quality and content:** Amount of protein in the organisms should not only be high but should contain as much as possible of amino acids required by man.

**Digestibility and organoleptic qualities:**

Organism should be digestible and acceptable taste and aroma.

**Growth rate:** Grow rapidly in cheap, easily available medium.

**Adaptability to unusual environmental conditions**



<i>Gaseous hydrocarbons</i>	<i>Bacteria</i>	<i>Fungi</i>
i) Methane	<i>Methanomonas</i> sp. <i>Methylococcus capsulatus</i> <i>Pseudomonas</i> sp. <i>Hyphomicrobium</i> sp. Mixed <i>Acinetobacter</i> sp.	
ii) Propane	<i>Flavobacterium</i> sp. <i>Arthrobacter simplex</i>	
iii) Butane	<i>Nocardia paraffinica</i> <i>Nocardia paraffinica</i>	
<i>Liquid Hydrocarbons:</i>		
i) n-Alkanes (C <sub>10</sub> -C <sub>30</sub> )	<i>Mycobacterium phlei</i> <i>Nocardia</i> sp.	<i>Candida guilliermondii</i> <i>Candida lipolytica</i>
carbon length		
ii) n-Alkanes (unspecified)		<i>Candida Kofuensis</i> <i>Candida tropicalis</i> <i>Candida lipolytica</i> <i>Candida rigida</i>
iii) Liquefied petroleum gas		<i>Candida tropicalis</i> <i>Candida lipolytica</i>
iv) Gas oil	<i>Acinetobacter</i> <i>Pseudomonas</i>	
v) Diesel oil	<i>Acromobacter delcavate</i>	
<i>Alcohols</i>	<i>Methylomonas Methanolica</i>	<i>Torulopsis methansoba</i>
<i>Methanol</i>	<i>Methylphilus (Pseudomonas) methylotrophus</i>	<i>Torulopsis methanolove</i> <i>Candida boidini</i>
<i>Ethanol</i>	<i>Methylomonas clara</i>	<i>Hansenula polymorpha</i> <i>Candida ethanomorpha</i> <i>Candida tropicalis</i>
<i>Plant/Wood Wastes</i>		
Sulphite liquor	<i>Thermomonospora fusca</i>	<i>Paecilomyces variotii</i>
Cellulose pulping fines	<i>Brevibacterium</i> sp.	<i>Candida utilis</i>
Mesquite wood		
Wheat bran	<i>Rhodospseudomonas glutinosa</i>	
Wastes from carb, Palms, papaya, etc.		<i>Fusarium</i> sp <i>Aspergillus</i> sp
<i>Starch Wastes</i>		
Potato hydrolysate		<i>Rhodotomila rubra</i> <i>Candida tropicalis</i>
Tapioca (Cassava) starch		<i>Endomycopsis</i> <i>Libuligera</i>
<i>Diary Wastes</i>		<i>Kluyveromyces fragilis</i> <i>Trichosporon cutaneum</i>
Whey		
<i>Sugar Wastes</i>		
Molasses		<i>Candida utilis</i>
<i>Chemical Industries Wastes:</i>		
Oxanone Waste Water		<i>Trichosporon cutaneum</i> <i>Candida pseudotropicalis</i>
Waste polyethylene		

Strains which grow at low pH conditions or at high temperature are often chosen. Heterotrophic microorganisms used are bacteria, actinomycetes and fungi (molds and yeasts); protozoa have not been used in SCP production. Substrates currently in use, gaseous hydrocarbons (methane, propane, butane) are almost exclusively attacked by bacteria. Liquid hydrocarbons (n-paraffins, gas oil, diesel oil) and alcohols are utilized by both bacteria and yeasts. Fungi have the advantage that they are lower in RNA content and are easily harvested.





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## Nucleic Acids and their Removal from SCP

Another area of concern in SCP feeding is the consumption of high levels of nucleic acid. Man has lost the enzyme uricase which oxidizes uric acid to the soluble and excretable allantoin. When nucleic acid is eaten by man, it is broken up by nucleases present in the pancreatic juice, and converted into nucleosides by intestinal juices before absorption. Guanine and adenine are converted to uric acid, which cannot be converted to the soluble and excretable allantoin. As a result when foods rich in nucleic acid are consumed in large amounts, an unusually high level of uric acid occurs in the blood plasma. Owing to low solubility of uric acid, uricates may be deposited in various tissues, kidneys and joints where kidney stones and gout may result.





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- **Various ways have been devised for removal of nucleic acids from SCP:**
- **Growth and cell physiology method :** RNA content of cell is dependent on growth rate: Higher the growth rate the higher the RNA content. The growth rate is therefore reduced as a means of reducing nucleic acid. High growth is one of requirements of reducing costs in SCP, hence the method may have only limited usefulness.
- **Extraction with chemicals :** Dilute bases such as NaOH or KOH will hydrolyze RNA easily. Hot 10% sodium chloride may also be used to extract RNA. Cells usually disrupted before using these methods. In some cases the protein may then be extracted, purified and concentrated.
- **Use of pancreatic juice:** RNAase from bovine pancreatic juice, which is heat-stable, used to hydrolyze yeast RNA at 80°C at which temperature the cells are more permeable.
- **Activation of endogenous RNA:** RNAase of the organism itself may be activated by heat-shock or by chemicals. RNA content of yeasts have been reduced in this way.



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## **Fermentation:**

- 1. Fermentation process requires a pure culture of the chosen organism that is in the correct physiological state, sterilization of growth medium which is used for the organism, a production fermenter which is equipment used for drawing culture medium in the steady state, cell separation, collection of cell free supernatant, product purification and effluent treatment.**
- 2. Fermenters can vary in size from laboratory experimental models: most commonly used principle has been the chemostat: a perfectly mixed suspension of biomass into which medium is fed at a constant rate and culture is harvested at same rate so that the culture volume remains constant.**



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3. Among the designs, air-lift has enjoyed greatest success as configuration of choice for continuous SCP production.
4. The control of key process variables is a critical element of SCP production, from oxygen transfer, substrate and product concentration, to the appearance of minimal amounts of toxic compounds through undesired metabolic processes, which may compromise quality of final product.
5. Biomass from yeast fermentation processes is harvested normally by continuous centrifugation.
6. Filamentous fungi are harvested by filtration.
7. Biomass is then treated for RNA reduction and dried in steam drums or spray driers.
8. Drying is expensive, but results in stabilized product with shelf lives of years.





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9. Generally, under combined conditions of low water activity and presence of intractable solid substrate, fungi show luxuriant growth. Hence, proper growth of fungi in solid state fermentation gives much higher concentration of biomass and higher yield when compared to submerged fermentation .
10. Advantage in SSF process is the unique possibility of efficient utilization of waste as substrate to produce commercially viable products. Process does not need elaborate prearrangements for media preparation. Process of SSF initially concentrated on enzyme production.



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# Industrial Alcohol

1. Ethyl alcohol,  $\text{CH}_3\text{CH}_2\text{OH}$  (synonyms: ethanol, methyl carbinol, grain alcohol, molasses alcohol, grain neutral spirits, cologne spirit, wine spirit), is a colorless, neutral, mobile flammable liquid.
2. Rarely found in nature, found only in unripe seeds of *Heracleum giganteun* and *H. spondylium*.

***Use as a chemical feed stock:*** In chemical industry, ethanol is an intermediate in many chemical processes because of its great reactivity

***Solvent use:*** Ethanol is widely used in industry as a solvent for dyes, oils, waxes, explosives, cosmetics

***General utility:*** Alcohol is used as a disinfectant in hospitals, cleaning and lighting in home, and in laboratory.

***Fuel:*** Ethanol is mixed with petrol or gasoline up to 10% and known as gasohol and used in automobiles



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## **Manufacture of Ethanol:**

**Ethanol may be produced by either synthetic chemical method or by fermentation.**

**Due to the increase in price of crude petroleum, source of ethylene used for alcohol production.**

**Fermentation alcohol has the potential to replace two important needs currently satisfied by petroleum, namely the provision of fuel and that of feedstock in the chemical industry.**

**The production of gasohol (gasoline – alcohol blend) appears to have received more attention than alcohol use as a feed stock.**





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## Substrates

- Sugary Materials:** Sugarcane and its by products/wastes (molasses, bagasse) and sugar beet, tapioca, sweet potatoes, fruit juice, sweet sorghum, etc.
- **Starchy Materials :** Tapioca, maize, wheat, barley, oat, sorghum, rice and potatoes. Tapioca and corns are the two major substrates of the interest. 11.7 kg of corn starch can be converted into about 7 liters of ethanol.
- **Lignocellulosic Materials:** Agricultural wastes and wood. Yield of ethanol from lignocellulose is low because of lack of suitable technology and failure of conversion of pentoses into ethanol. 409 liters of ethanol can be produced from one tonne of lignocelluloses. Production of ethanol from lignocelluloses follows hydrolysis, fermentation, and recovery.



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## Fermentation

1. Alcohol-resistant yeasts, strains of *Saccharomyces cerevisiae* are used, and nutrients such as nitrogen and phosphate lacking in the broth are added.

## Distillation:

1. After fermentation, fermented liquor contains alcohol as well as low boiling point volatile compounds (acetaldehydes, esters and the higher boiling, fuel oils).
2. Alcohol is obtained by several operations. First, steam is passed through beer which is said to be steam-stripped. The result is a dilute alcohol solution which still contains part of the undesirable volatile compounds. Secondly, dilute alcohol solution is passed into the center of a multi-plate aldehyde column in which following fractions are separated: esters and aldehydes, water, and an ethanol solution containing about 25% ethanol. Thirdly, dilute alcohol solution is passed into a rectifying column where a constant boiling mixture, an azeotrope, distils off at 95.6% alcohol concentration.



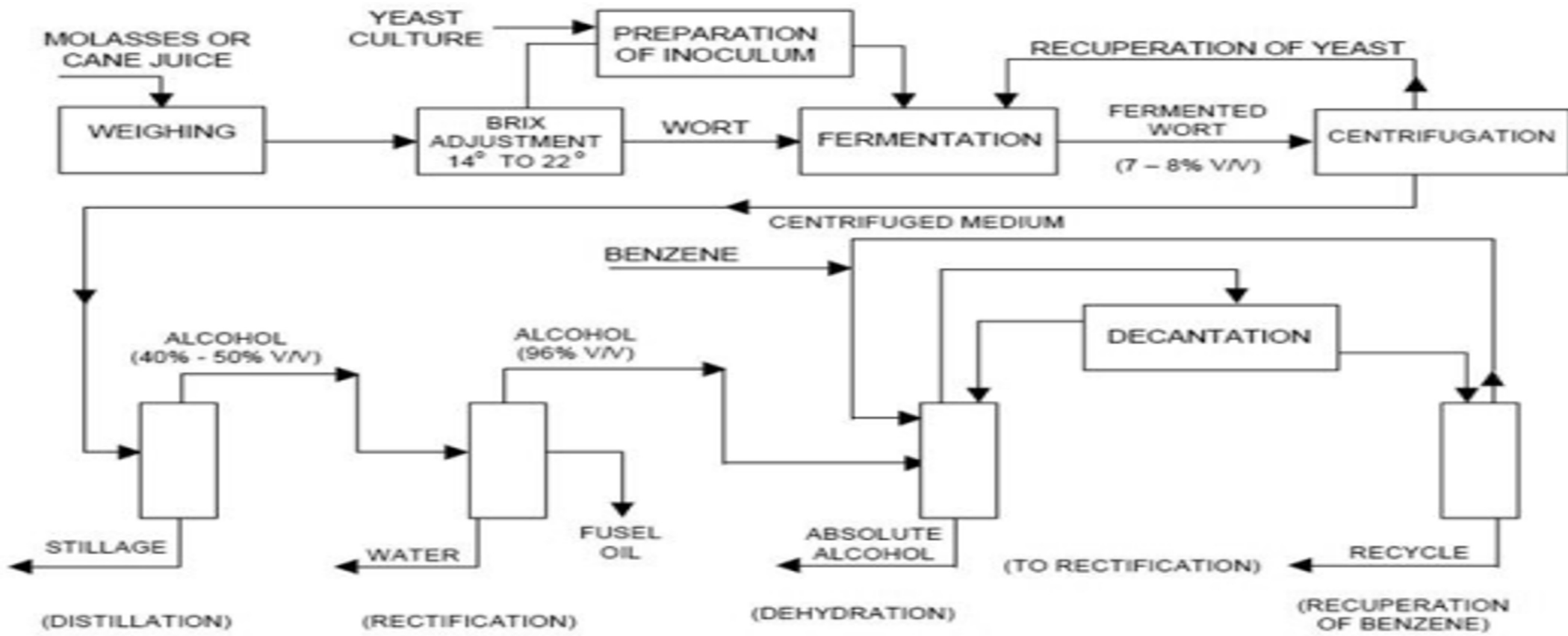
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- To obtain proof alcohol, such as used in gasohol blending, the 96.58% alcohol is obtained by azeotropic distillation. The principle of this method is to add an organic solvent which will form a ternary (three membered) azeotrope with most of the water, but with only a small proportion of the alcohol.
- Benzene, carbon tetrachloride, chloroform, and cyclohexane may be used, but in practice, benzene is used.
- Azeotropes usually have lower boiling point than their individual components and that of benzene-ethanol-water is 64.6°C.



- On condensation, it separates into two layers. Upper layer, which has about 84% of condensate, has percentage composition: benzene 85%, ethanol 18%, water 1%. The heavier, lower portion, constituting 16% of the condensate, has the following composition: benzene 11%, ethanol 53%, and water 36%.
- Condensate is not allowed to separate out, but arrangement of plates within the columns enable separation of the alcohol. Four columns are usually used. First and second columns remove aldehydes and fusel oils, respectively, while last two towers are for concentration of alcohol.





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# Developments in Alcohol Production

- Developments of new strains of yeast of *Saccharomyces uvarum* able to ferment sugar rapidly, to tolerate high alcohol concentrations, flocculate rapidly, and whose regulatory system permits it to produce alcohol during growth.
- Use of continuous fermentation with recycle using the rapidly flocculating yeasts.
- Continuous vacuum fermentation in which alcohol is continuously evaporated under low pressure from fermentation broth.
- Use of immobilized *Saccharomyces cerevisiae* in a packed column, instead of in a conventional stirred tank fermentor. Higher productivity consequent on a higher cell concentration was said to be the advantage.
- *Zymomonas mobilis*, a Gram-negative bacterium which is found in some tropical alcoholic beverages, rather than yeast is advocated.



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## Advantages claimed for the use of *Zymomonas* are the following:

- Higher specific rates of glucose uptake and ethanol production.
- Higher ethanol yields and lower biomass than with yeasts.
- Ethanol tolerance is at least as high or even higher than with yeast.
- Tolerates high glucose concentration.
- Grows anaerobically and does not require controlled addition of oxygen for viability at high cell concentrations used in cell recycle.
- Techniques for genetic engineering already worked out in bacteria can be easily applied to *Zymomonas* spp. for greater productivity.