

- I. Industrial Production of Vinegar and Streptomycin
- II. Microbial sources and uses of
Enzyme- Amylase, Protease
Amino acid- Glutamic acid, Lysine
Polysaccharides Dextran
- III. Use of microbes as Biofertilizer and Biopesticide

Vinegar production

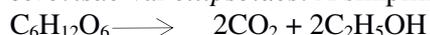
Vinegar is defined as a condiment made from sugary or starchy materials by an alcoholic fermentation followed by an acetous one. It must contain at least 4 gms of acetic acid per 100 ml to be a legal vinegar. Vinegars may be classified on the basis of materials from which they have been made

1. Those from the juices of fruits e.g. apples, grapes, oranges, berries, pears etc.
2. Those from starchy vegetables e.g. potatoes and sweet potatoes whose starch must first be hydrolysed to sugar.
3. Those from malted cereals such as barley, rye, wheat and corn.
4. Those from sugar such as syrup, molasses, honey etc.
5. Those from spirits and alcohols e.g. from waste alcoholic liquor, from yeast manufacturers or from oil, denatured ethyl alcohol etc. Anything in fact that contains sugar or alcohol and is in no way objectionable as food may be used as vinegar.

The manufacture of vinegar from saccharin materials involves 2 steps-

1. The fermentation of sugar to ethyl alcohol
2. The oxidation of alcohol to acetic acid

The first step is an anaerobic process carried out by yeast either naturally present in the raw materials or preferably added cultures of high alcohol producing strains of *Saccharomyces cerevisiae* var *ellipsoides*. A simplified equation for the process is:



Actually, a series of intermediate reactions takes place and small amount of other final products are produced such as glycerol and acetic acid. Also, there are small amounts of other substances produced from compounds other than sugar, including succinic acid and amyl alcohol.

The second step, oxidation of alcohol to acetic acid, is an aerobic reaction carried out by the acetic acid bacteria.



Acetaldehyde is an intermediate compound in this reaction. Among the final products are small amounts of aldehydes, esters, acetone etc. There are several species of *Acetobacter* that are acetic acid bacteria. The most common is *A. aceti*. The oxidation reaction in which *Acetobacter* converts ethanol to acetic acid is depicted below:



The genus *Gluconobacter* also contains species capable of oxidising ethanol to acetic acid.

Streptomycin production

Sources- *Streptomyces griseus*, *S. reticule*, *S. ranens*

Uses-

It is an aminoglycoside antibiotic which inhibits protein synthesis by combining irreversibly with 30S subunit of r-RNA

1. Broad spectrum antibiotic for treatment of diseases caused by Gram negative organisms like *E. coli*, *Haemophilus influenzae*
2. Affects Gram positive organisms like *Bacillus subtilis*, *Staphylococcus aureus*
3. Used for treatment of urinary infection, bacteraemia, meningitis, tuberculosis, plague
4. Used for treatment of plant diseases caused by bacteria
5. Inactivates bacteriophage
6. Inhibits nucleic acid and protein synthesis

Production

Applied Bacteriology

The medium for production of streptomycin is composed of soya bean meal (nitrogen source), glucose (carbon source) and NaCl. The medium is inoculated with bacterial cells. The culture is maintained for 10 days with high agitation and aeration at 28°C, pH 7.6-8.0.

First phase: There is rapid growth of microbe along with release of ammonia from the medium thereby increasing the pH of the medium. Small amount of streptomycin is produced in the medium.

Second phase: This phase is characterised by low microbial growth, accumulation of streptomycin (a bacterial secondary metabolite), consumption of glucose and ammonia from the medium.

Third phase: In this phase, carbohydrate level is depleted, streptomycin production stops and biomass declines due to autolysis. The PH level is about 9.0 due to release of ammonia from the lysed mycelium, and the process comes to an end.

Recovery of streptomycin:

After completion of the process, mycelia are separated by filtration. The streptomycin is then absorbed onto activated charcoal and then eluted with acid alcohol. It is then precipitated with acetone and purified by column chromatography.

Amylase

Sources- *Bacillus subtilis*, *B. diastaticus*, *Aspergillus oryzae*, *A. niger*

Uses-

1. Catalyses hydrolysis of α -1-4 glucosidic linkages of polysaccharides such as starch and glycogen
2. Used in textile, paper and brewing industry for removing starch and also for removing heavy starch paste when making corn and chocolate syrups
3. Used for making of bread
4. As animal feed
5. Used in detergents for removing food spots
6. Used for sewage treatment

Protease

Sources- *Bacillus subtilis*, *Bacillus* sp GX6644 (recombinant strain), *Pseudomonas* sp, *Clostridium* sp, *Proteus* sp

Uses-

1. Breaks peptide bonds and may be either endopeptidase or exopeptidase
2. Used in detergents for removing spots of milk, blood, egg etc
3. Recombinant strain of bacterium used to make kerazyme which dissolves hair
4. Improve quality of bread in baking industry
5. Used in leather industry and in textile industry for removing protein from silk
6. Makes meat soft
7. For removing gelatine from film to recover silver

Lysine

Sources- *Corynebacterium glutamicum*, *Enterobacter aerogens*, *E. coli* are used for production of lysine

Uses-

1. Used as food supplement for children to promote bone growth and collagen synthesis as proteins from vegetables and cereals are poor in lysine
2. Added to animal feed to increase growth rate in poultry and pigs

Glutamic acid

Sources – Glutamic acid is present nearly in all proteins, specially seed. L- glutamic acid and monosodium glutamate (MSG) can be produced by direct fermentation using *Brevibacterium*, *Arthrobacter*, *Corynebacterium* and *Micrococcus* sp. *Brevibacterium flavum* are widely used for large scale production of MSG.

Uses-

Glutamic acid is involved in the transport of K^+ in the brain and also detoxifies ammonia in the brain by forming glutamine, which can cross the blood brain barrier. In the CNS glutamic acid is decarboxylated to 4-aminobutyric acid by glutamate decarboxylase.

1. It is used as a condiment and flavour enhancing agent
2. As sodium salt (MSG) to impart a meat flavour to food
3. The hydrochloride is used to improve taste of beer
4. It has therapeutic properties and is used as an antiepileptic, Mg salt hydrobromide as anxiolytic and gastric ulcers are treated with glutamic acid.

Dextran

Sources- *Leuconostoc mesenteroides*, *L. dextranicum*

Uses-

1. Used to increase volume of blood plasma
2. Extracts are used to make dextran gels which act as molecular sieves. The polysaccharide cross linkages determines the size of the pores (more cross linking means smaller size of the pores). The cross linked dextran are sold by the trade name Sephadex.

Use of microbes as Biopesticides

Pesticides based on microorganisms and their products have proven to be highly effective, species specific and eco-friendly in nature, leading to their adoption in pest management strategies around the world. The microbial biopesticide market constitutes about 90% of total biopesticides and there is ample scope for further development in agriculture and public health, although there are challenges as well. In addition to the proteinaceous toxins, microorganisms are also known to produce anti-pest chemical compounds. The bacteria that are used as biopesticides can be divided into four categories: crystalliferous spore formers (such as *Bacillus thuringiensis*); obligate pathogens (such as *Bacillus popilliae*); potential pathogens (such as *Serratia marcescens*); and facultative pathogens (such as *Pseudomonas aeruginosa*). The viruses used for insect control are the DNA-containing baculoviruses (BVs), Nucleopolyhedrosis viruses (NPVs), granuloviruses (GVs), acoviruses, iridoviruses, parvoviruses, polydna viruses, and poxviruses and the RNA-containing reoviruses, cytoplasmic polyhedrosis viruses, nodaviruses, picorna-like viruses and tetraviruses. Some of the most widely used species include *Beauveria bassiana*, *Metarhizium anisopilae*, *Nomuraea rileyi*, *Paecilomyces farinosus* and *Verticillium lecani*.

Advantages

Microbial pesticides are non-toxic and non-pathogenic to non-target organisms and the safety offered is their greatest strength. Action of microbials is specific to a single group or species of pests, therefore, do not affect directly beneficial animals such as predators and parasitoids. Microbial pesticides can be used in many habitats where chemical pesticides have been prohibited. Such habitats include recreational and urban areas, lake and stream borders of watersheds, and near homes and schools in agricultural settings. Residues of microbial pesticides are non-hazardous and are safe all the time, even close to harvesting periods of the crops. They have a potential to control vectors. Some pathogenic microbes can establish in a pest population or its habitat and provide control during subsequent seasons or pest generations.

Disadvantages

Owing to the specificity of the action, microbes may control only a portion of the pests present in a field and may not control other type of pests present in treated areas, which can cause continuous damage. As heat, UV light and desiccation reduces the efficacy of microbial pesticides, the delivery systems become an important factor. Special formulations and storage procedures are necessary. Shelf life is a constraint, given their short shelf lives. Given their pest specificity, markets are limited. The

development, registration and production costs cannot be spread over a wide range of pest control sales; for example, insect viruses are not widely available.

Use of microbes in mineral processing.

Biomining, the use of micro-organisms to recover precious and base metals from mineral ores and concentrates, has developed into a successful and expanding area of biotechnology. Biomining processes employ microbial consortia that are dominated by acidophilic, autotrophic iron- or sulfur-oxidizing prokaryotes. Mineral biooxidation takes place in highly aerated, continuous-flow, stirred-tank reactors or in irrigated dump or heap reactors, both of which provide an open, non-sterile environment. The use of microbes in ore processing has some distinct advantages over the traditional physicochemical methods. Almost without exception, microbial extraction procedures are more environmentally friendly. They do not require the high amounts of energy used during roasting or smelting and do not produce sulfur dioxide or other environmentally harmful gaseous emissions.

Biomining entails the use of acidophilic microbes to facilitate the recovery process of metals from sulfide minerals in the processes of bioleaching and biooxidation. **Biooxidation** is the enrichment of metals, particularly gold, by mobilization and thus removal of interfering metal sulfides from ores bearing the precious metals. **Bioleaching** is the solubilization of metals of interest such as cobalt, copper, and nickel from sulfide minerals. The two processes are industrially well established and are commercially applied worldwide. The microbes found in these environments are (extreme) acidophiles growing at a pH of 3 or lower and span a wide range of different phyla. The majority belong to the bacterial and archaea domains; however, unicellular eukaryotes have also been reported.

The use of acidiphilic, chemolithotrophic iron- and sulfur-oxidizing microbes in processes to recover metals from certain types of copper, uranium, and gold-bearing minerals or mineral concentrates is now well established. Microorganisms important in commercial mineral mining are *Acidithiophilus* sp, *Leptospirillum* spp, *Acidiphillum* spp, *Acidimicrobium ferrooxidans*, *Sulfobacillus thermosulfidooxidans*, *Ferroplasma acidarmanus*, *Sulfolobus metallicus*.

