TECHNOLOGY AND ENGINEERING BIOPROCESSES: INDUSTRIAL AND FOOD PRODUCTIONS

created by Morenko A.
Full PhD
Lesya Ukrainka Eastern European National University

Main topics

- Industrial Production of GM-Products
- Biotechnology and Food Production

Industrial Production of GM-Products

Certain aspects regarding large scale production have to be taken into consideration:

- How to maintain production.
- Scale of production.

- Setup and auxiliary (lab, etc.) facilities for industrial application.
- Practical application - e.g. industrial production of antibiotics.

Safety Consideration in Biotechnology

A biotechnological application on a large scale production

Profound knowledge of system processes in order to avoid financial losses.

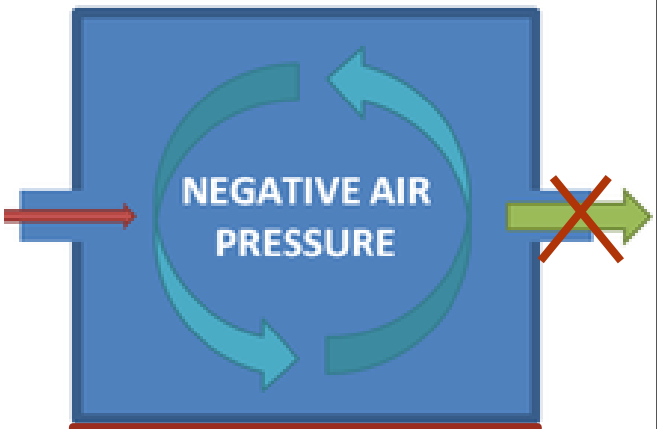
Strict guidelines are implemented

The processes have to run strictly according to the designed pathway.

Some of the safety considerations :

Any smooth surface must be kept aseptic at all times

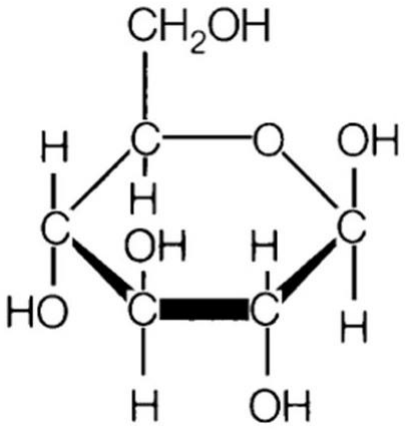
Full protective clothing



Preventing particles to vent out into the environment.

Preventing any escape or contamination of cultured media by or to the staff.

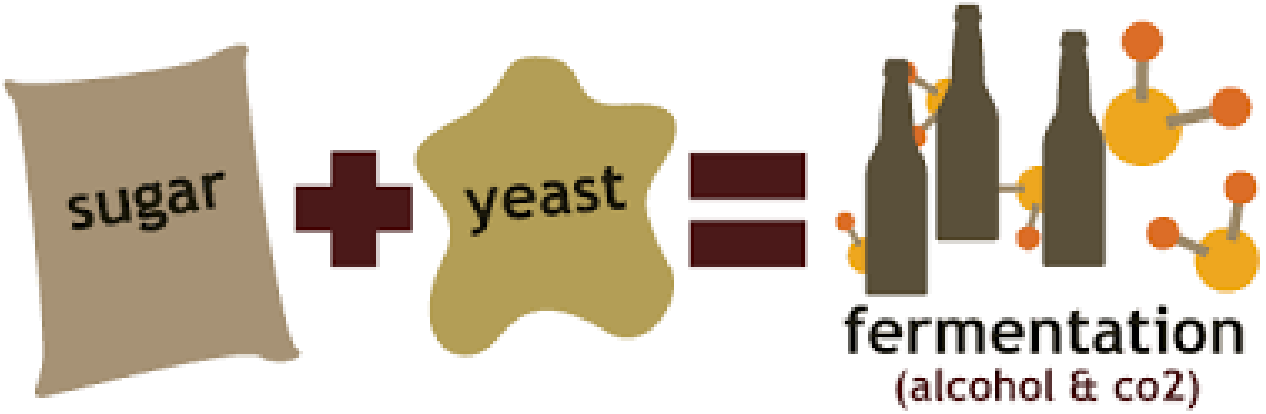
Fermentation is a chemical act or process



Carbohydrate (CH_2O)

Fermenting agents

Alcohol, acids, ets



Yeast converts the sugar in grape juice into alcohol, producing wine.

Fermentation involves the breakdown of carbohydrates **in the absence of oxygen**

Glucose

Pyruvate

Bacteria produce *lactic acid*, which modifies milk proteins to generate yogurts and cheeses

Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide.
In baking CO_2 evaporates.

Aspergillus	Lactobacillus	Saccharomyces		
Lactic acid	Lactic acid	Ethanol + CO_2	Ethanol	Carbon dioxide
				
Soy Sauce	Cheese, Yogurt	Beer	Wine	Bread

THE *FERMENTOR*

Beer fermenter tanks

The *fermentor* is a tightly sealed reaction chamber in which a controlled reaction can take place.

It keeps any contaminants from disturbing or even spoiling the enclosed reaction mechanism.

Key Aspects of a Fermentation Process

Double mechanical seals

Filters on exhaust pipes

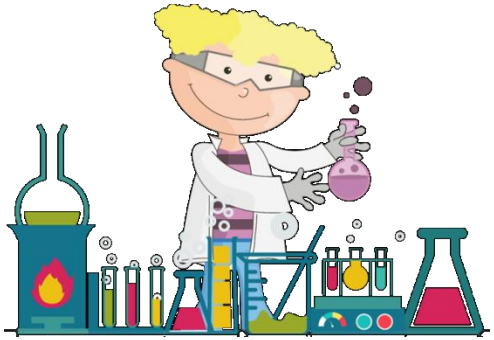
Safety precautions at sampling outlets

Autlets are considered the weakest point in the system - spills are very common

Grain Beer Brewing System

Sterility is the key word in biotechnology

Keeping out unwanted microorganisms that might interfere



One of the Key Aspects of a Fermentation Process is Aerosol Prevention

AEROSOLS

- Aerosols are perfect carriers of contaminants

O2 stimulates aerosol growth

- Aerosols can transport contaminants over large distances

- Off site contamination of people may be happened

So far has not yet occurred

Effluents

Maintaining aseptic conditions at all stages

A multiple cleaning process

Chemicals are partly used

Heat exchangers

Generating a substantial amount of heat

Maintaining optimal temperature conditions is necessary to keep the system process going

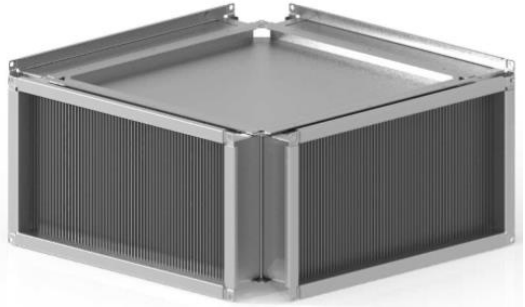
An excessive down-cooling mechanisms may cause the fermentor to burst (freezing).

Other Design Features of a Fermentation Process

In order to avoid any escape of GM-organisms into the environment

Pipes and tubes must be leak proof.

In centrifuges air-tight seals must be used.



Vents require microbiological filters

Usually a super heated steam system is used to flush and heat-sterilize the pipes, tubes, and the fermentor for the entire factory.

Running a Facility – Aseptic Operations Before

Successful fermentation means zero contamination.

Practically this implies that:

Fermentor, auxiliary equipment plus medium must be sterile

Regular checks for mechanical micro-fracture

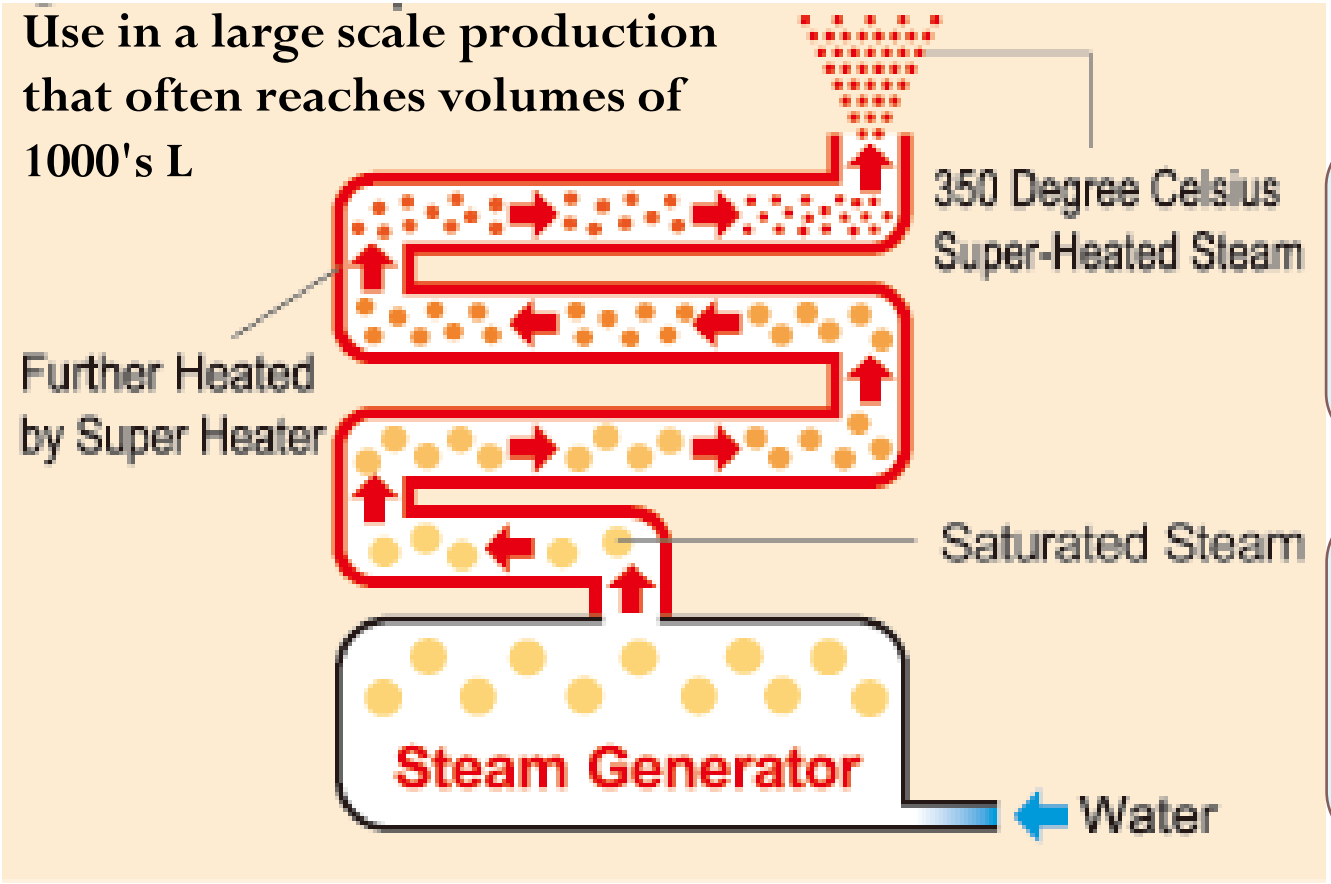
Medium culture sterilization with super-heated steam

Foam Formation must be steriled

Sterilization of Air-Supply

Antifoaming additives are necessary to avoid excessive foam formation

Sterilization with Super-Heated Steam



Medium passes through coils that are exposed to super-heated steam

Exposure time is crucial to avoid decomposition of the involved medium

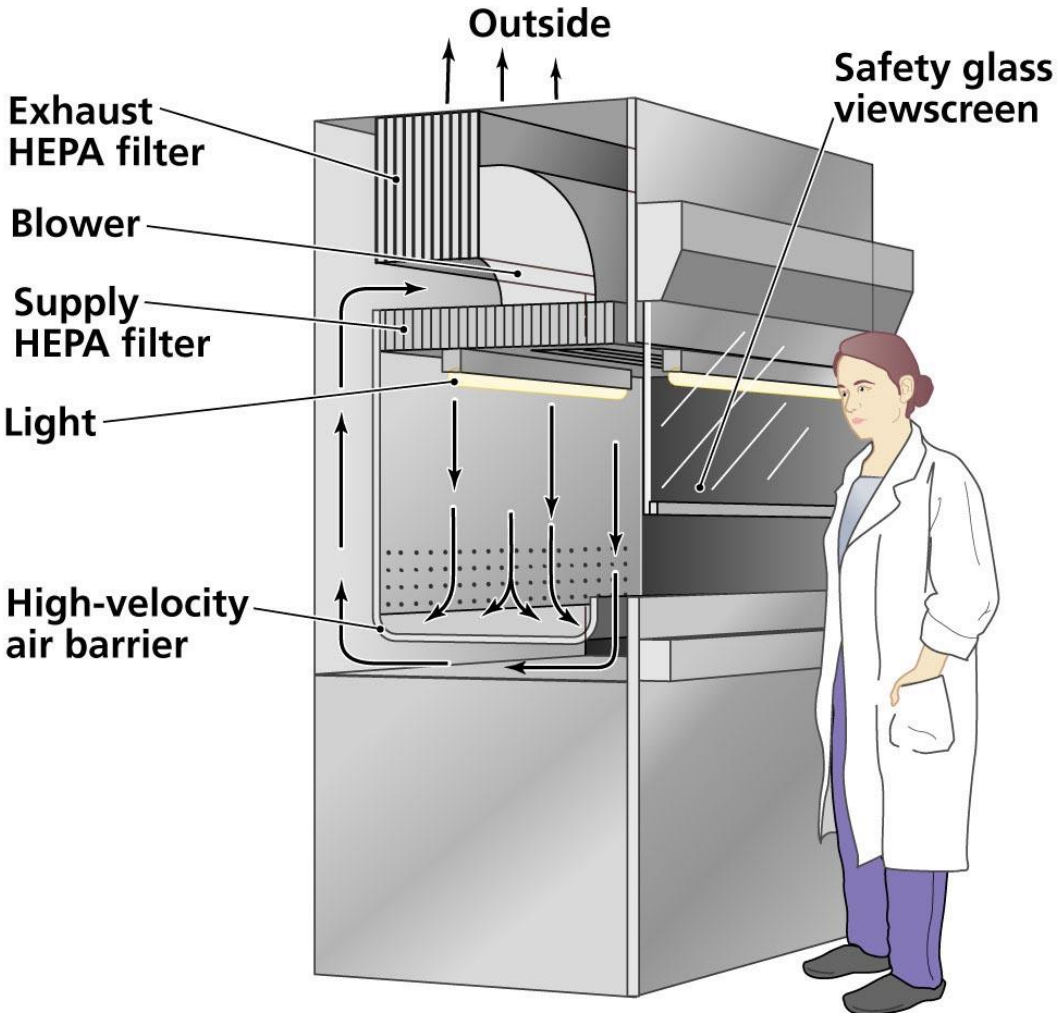
Such complex sterilization often requires doubling of costs through an extension of the production plant.

Sterilization of Air-Supply (HEPA Filters)

Bacteriophags cannot pass such narrow filters

Heating and subsequently cooling down the air before it passes the filter

The plant should not be located at a site where persistent winds redirect exhaust gases towards densely populated areas.

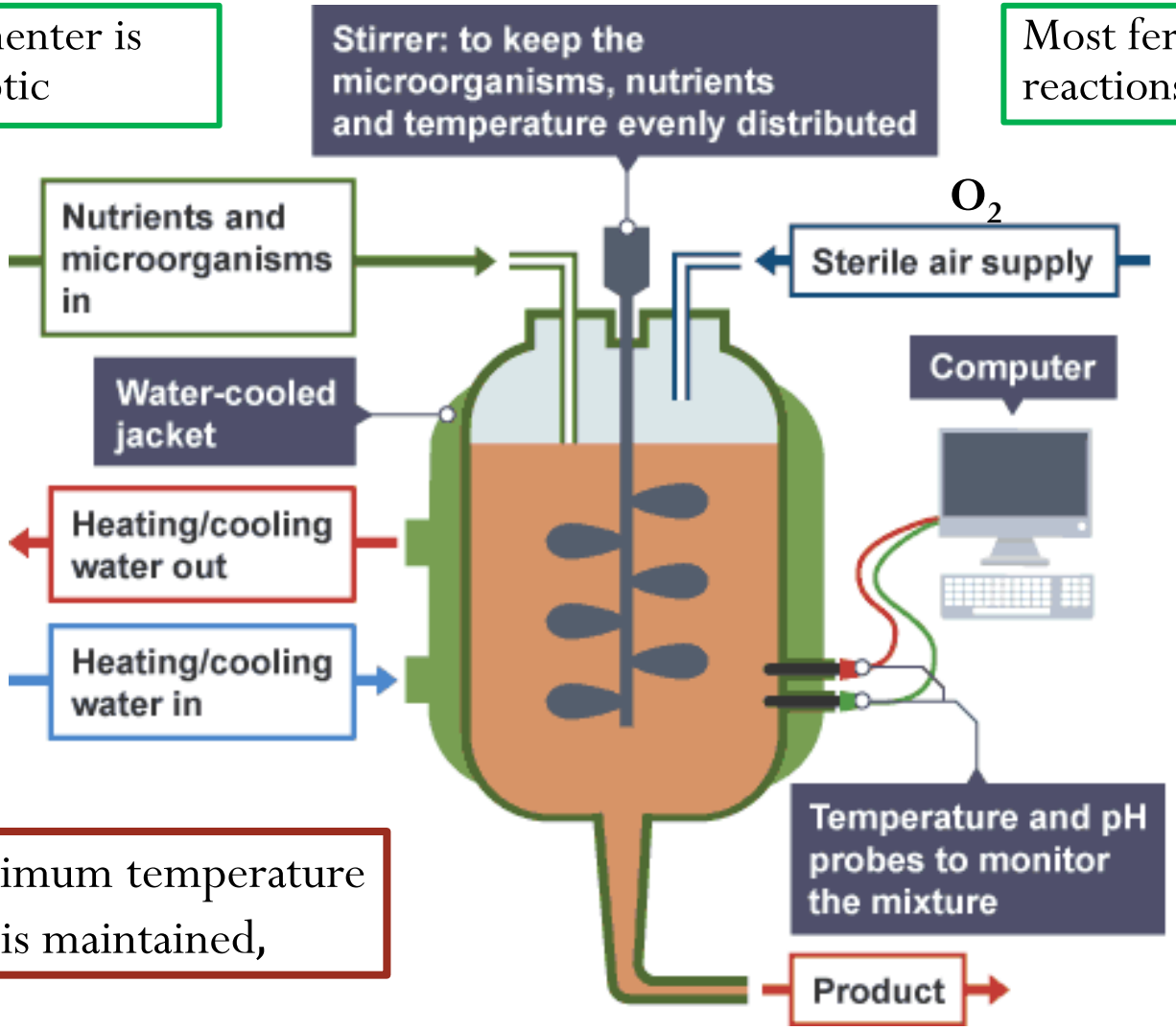


HEPA Filters Used in Pharmaceuticals Manufacturing

Fermentation process and conditions

The fermenter is kept aseptic

Most fermentation reactions are aerobic



The optimum temperature and pH is maintained,

Fermentor

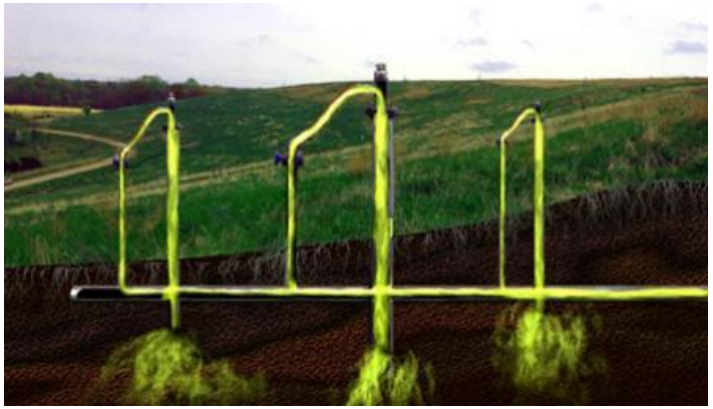
Fermentation Can Occur in Various Methods:

- **Solid substrate** is one of the most common.
- **Aqueous substrate:** fermentation that involves a liquid solution (mostly water).

Bread and cheese making is done with a solid substrate

Wine Fermentation on a liquid solution

Mushrooms are grown on a solid substrate.

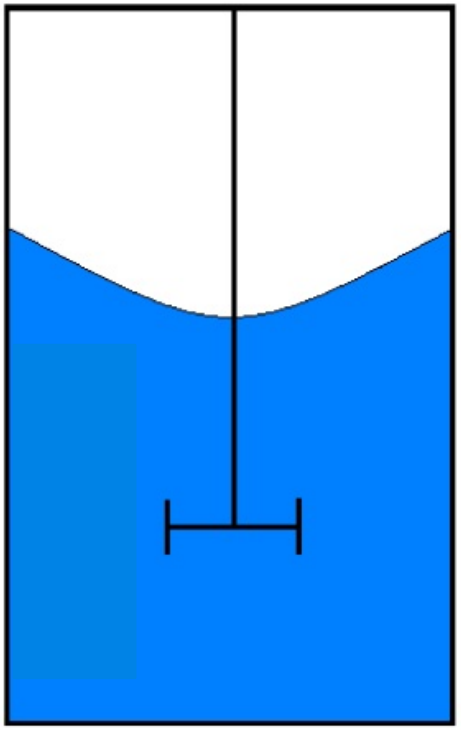


Microbial-mediated methane production from landfills

Fermentation systems

Batch

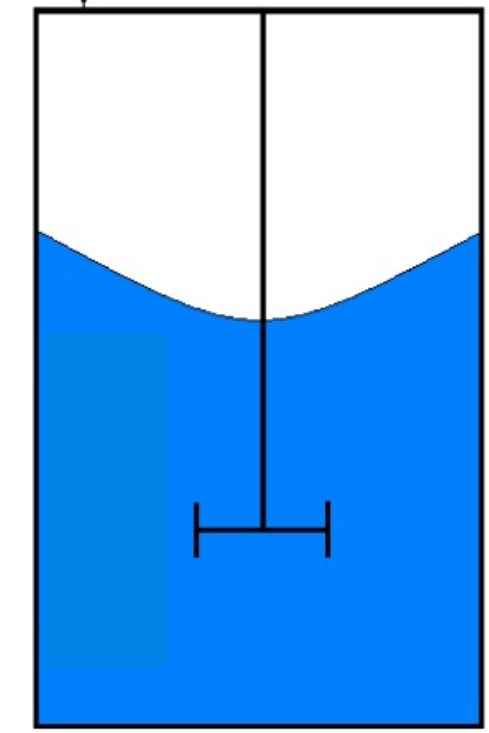
Closed FS



All nutrients are added at the beginning of fermentation.

Fed batch

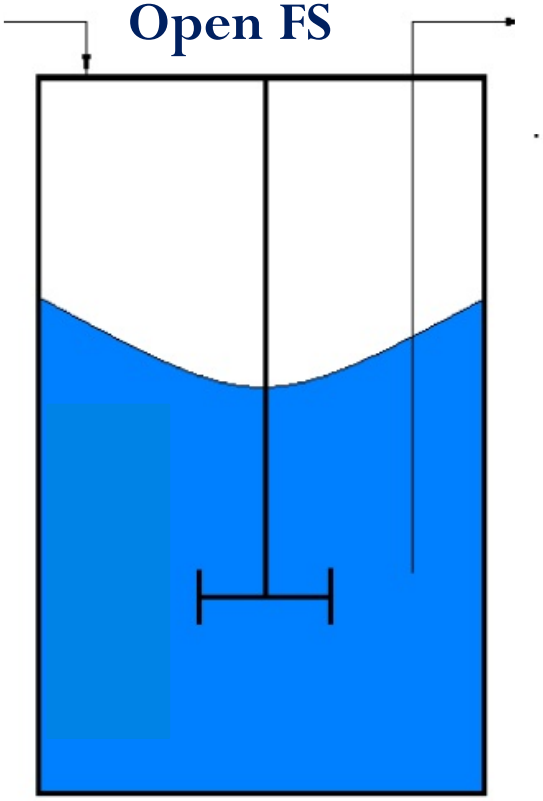
Closed FS



Nutrients are added at defined timing intervals.

Continuous

Open FS

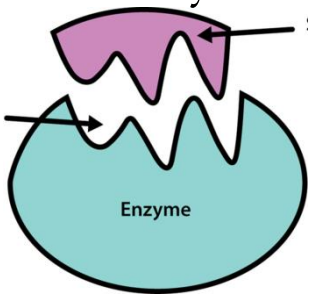


Nutrients are added at a constant rate.

Batch Procedure

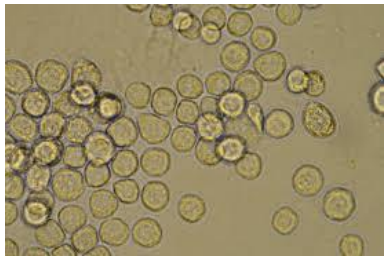
The preparation of beer

Production of antibiotics and enzymes



Once optimized conditions provides maximum accumulation of the desired product.

Fed Batch Procedure



Wine production

- This guarantees a continuos production until the fermentor's holding capacity is reached.
- They do not have constant outflow of content.
- The concentrations of limiting nutrients in the culture can be manipulated to remain at a constant level.

Continuous Procedure:

Continuous fermentation can only work if the reaction temperature, pH, O_2-concentration, ets. are carefully monitored.

Advantages of this fermentation is the continuous production of end-material.

Disadvantages:

If one parameter breaks down, the whole production collapses, generating considerable amount of waste;

Cells tends to clump and clog in inlet/outlet pipes;

The other complication is a foaming.

Fuel ethanol production

e.g. from corn

Continuous (Open) System

Setting up an Industrial Process:

Implementing a New Production Line Requires Three Elementary Steps:

Basic steps

Working with lab-flasks, petri dishes, screening techniques, determination of optimal growth parameters, harvest.

The average volumetric sample circles around 200 cm^3 , the energetic demands fluctuate around 1 kW.

Pilot plant

Finding out how upscaling affects reaction conditions compared to lab conditions

The construction of small scale fermentors (50-300 L)

Large Scale Production Facility

Conditions

- volumes of up to 10^3 L have to be handled properly,
- energetic demands,
- an appropriate cooling system,
- aseptic conditions for the entire factory,
- a constant flow of large volumes.

Processes

- Fermentation process,
- harvesting and filtering,
- drying,
- distilling,
- extracting,
- Byproducts.

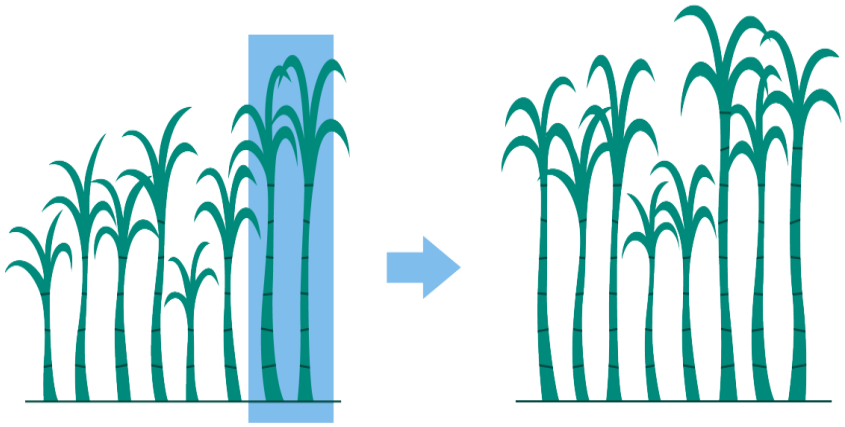
A single error can result in the total loss of both culture and medium which will force the plant out of operation for days, weeks or even months.

Biotechnology and Food Production

Topics:

- how Biotech is used in agriculture,
- pest-resistance in crops,
- BST and the turbo-cow,
- new foods obtain via microorganisms,
- byproducts of biotechnology as new food sources,
- biotechnology of making wine and fruit-juices.

- **Biotech and Agriculture**: promised improvements of crops yields, resistance against diseases, ets.
- **In the Animal sector, the biotech industry** tried to increase productivity via the use of modified hormones.

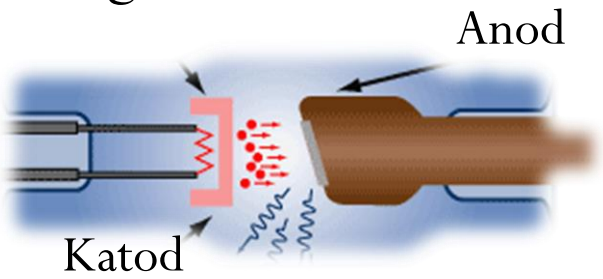


Selective cross breeding was common before the advent of biotechnology. Crossing for example one weed with another - the result of the hybrid was unpredictable.

Means of biotechnology

Treating weeds with x-rays or aggressive chemicals

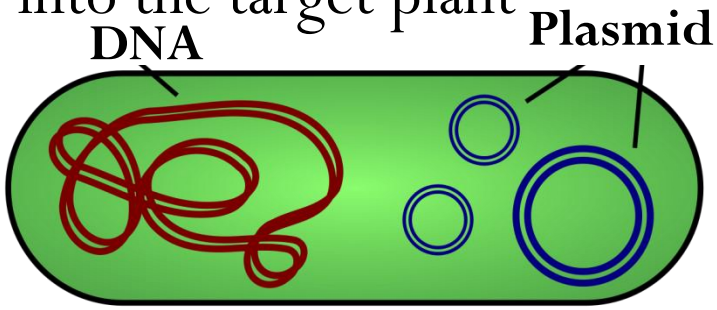
- generally induced uncontrollable mutations,
- resulted in a wide range of diversification in one's offspring,
- such treatment is a rather wasteful process that takes a long time.



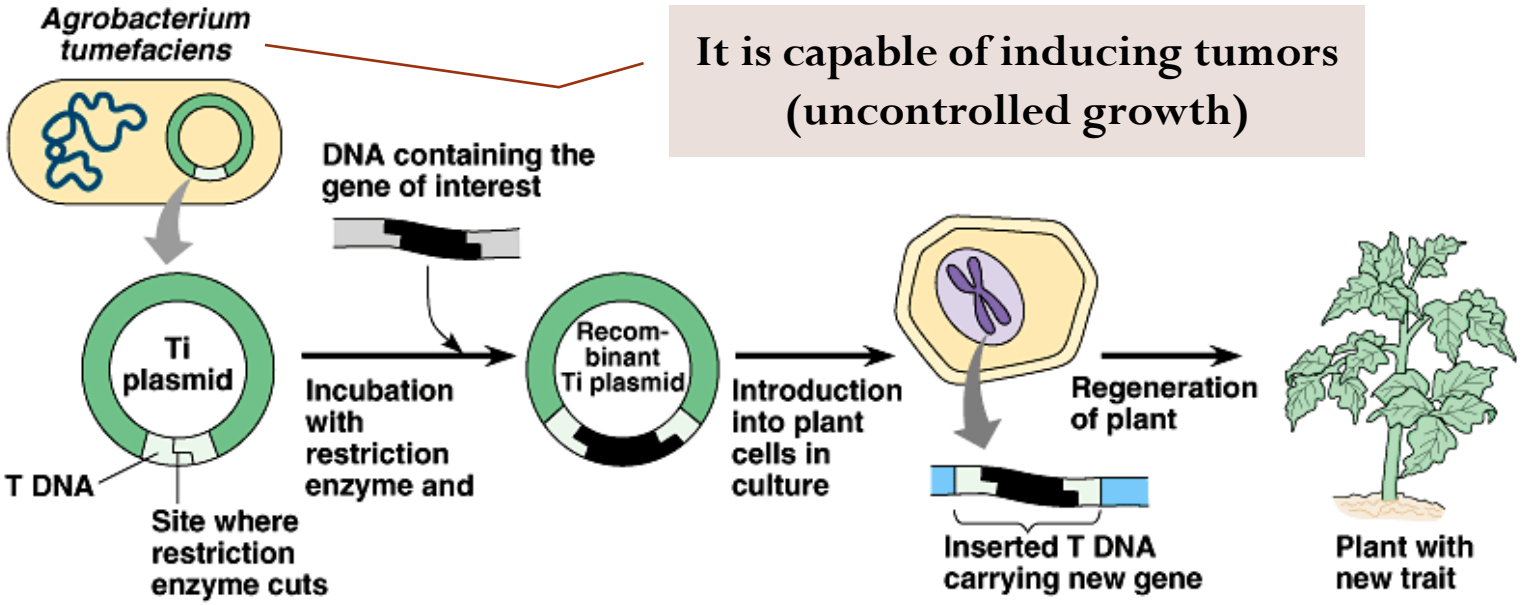
X-ray tube

Genetic engineering

- allows related species to be mixed,
- a process that is more efficient and less time consuming,
- By inserting the extracted gene into bacterial plasmids (vector), the genetic sequence is shipped into the target plant



Application of Genetic Engineering in the Agriculture Sector



1

The Ti plasmid normally integrates a segment of its DNA into its host plant and induces tumors.

2

Foreign genes can be inserted into the Ti plasmid (a version that does not cause disease) using recombinant DNA techniques.

3

The recombinant plasmid can be put back into Agrobacterium, which then infects plant cells, or introduced directly into plant cells.

Sky-Blue Glow Effect

A marine jellyfish

A bio-luminescent gene of a marine jellyfish (the *Aequinon)* is extracted.

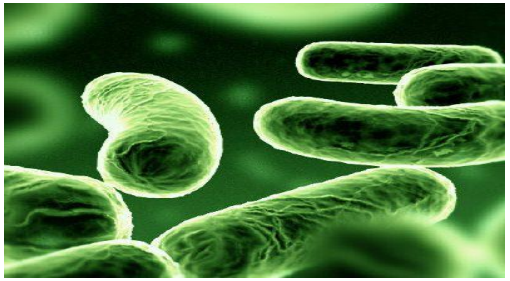
The plant starts to emit a bluish light.

In this way it indicates stress, drought, cold, nutrient insufficiency, etc. The stronger a factor – the more light is emitted.

Plants under stress contained higher contents of calcium and the *Aequinon* is used to detect this higher calcium concentration.

Ecosyl is Successfully Applied Modified Product

Lactobacillus plantarum



With wild strains of bacteria over the winter

Grass to feed the cows

Ecobale inhibits the formation of molds while grass is stored in bales on the field.

Ecosyle with the modified *Lactobacillus plantarum*

Ecobale as improved version

The bacteria utilize the grass' sugar content and converted it into acid

A randomly controlled process, resulting in huge variations from one silage to the next.

Converts the sugar more efficiently, keeps the grass' pH at a pretty constant level.

Makes silage more palatable for cows.
Grows very fast and competes very well.

Silage

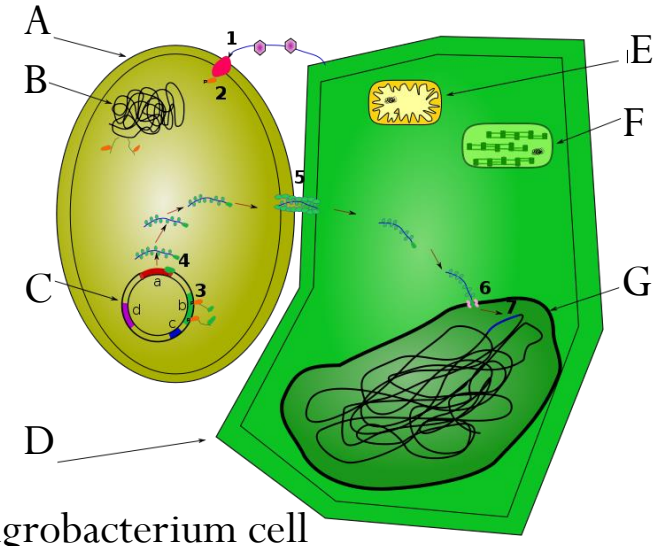
Breeding Disease Resistant Plants

Tobacco Mosaic Virus on tomatoes

Fungus disease on tobaccos

GENE TRANSFERRING METHOD

Agrobacterium is used to make plants resistant to virus, fungi, etc.

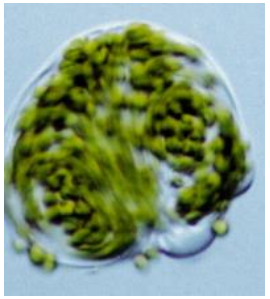


A. Agrobacterium cell
B. Agrobacterium DNA
C. Ti Plasmid
D. Plant cell
E. Plant mitochondria
F. Plant chloroplast
G. Plant nucleus

Once the protective gene sequence is inserted into a plant, it is able to block the pathway of the pathogen's metabolic synthesis.

Breeding Disease Resistant Plants

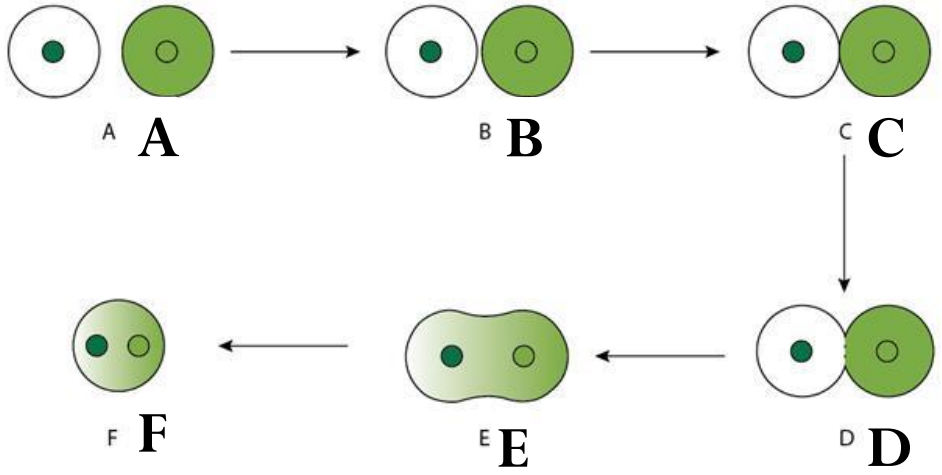
PROTOBLAST FUSION



Protoblast of *Bryopsis plumosa*.

A technique in which the plant's cell walls are dissolved under aseptic conditions and fused with another cell to generate a heterokaryonic cell.

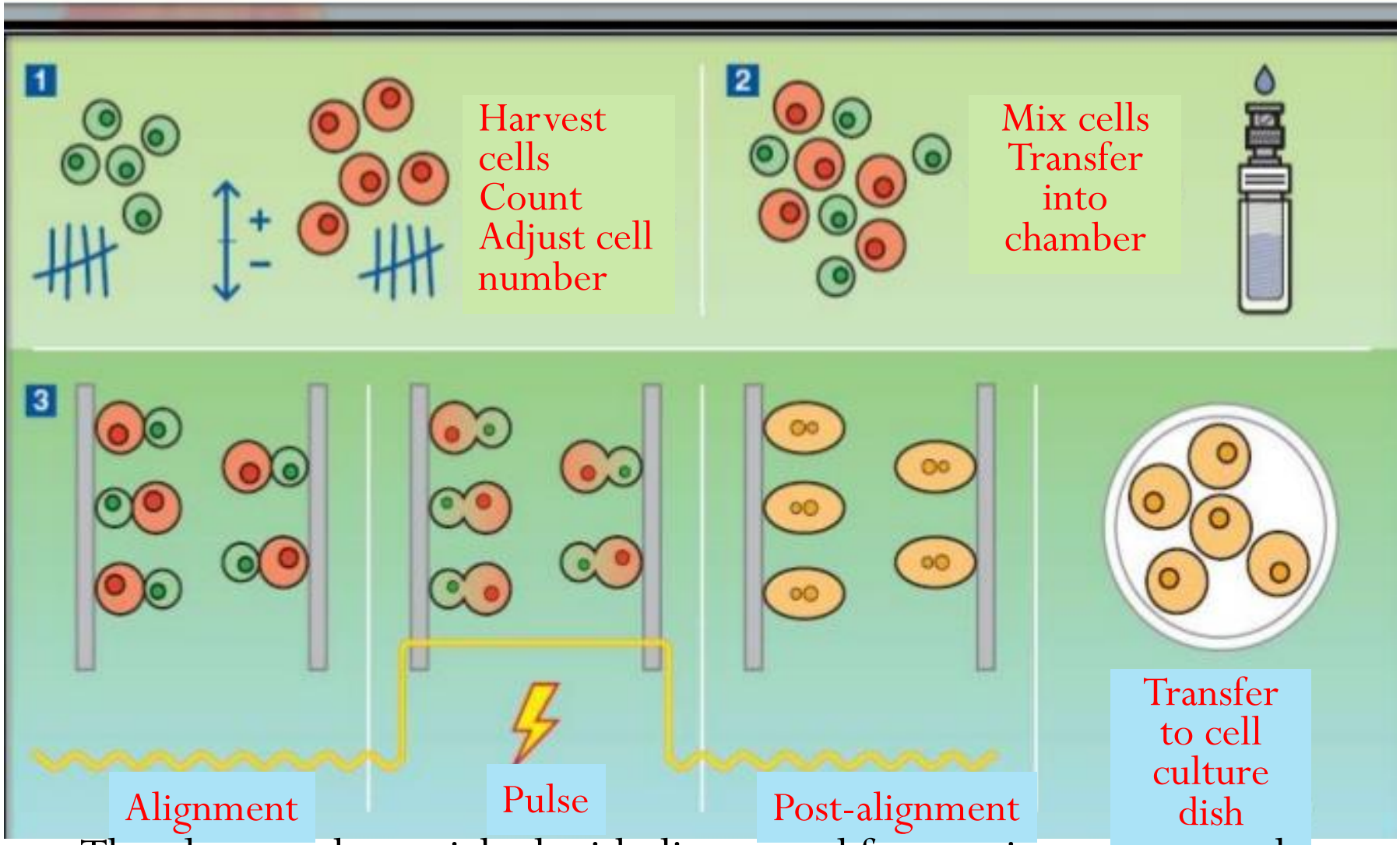
Protoblast – a cell with no cell wall; an embryonic cell.



Sequential stages in protoplast fusion.
(**A**) two separate protoplasts,
(**B**) agglutination of two protoplasts,
(**C** and **D**) Membrane fusion at localized site,
(**E** and **F**) development of spherical heterokaryon.

After vegetative reproduction, the lump of cell mass is then placed onto a cultivation dish (usually agar) to obtain the new organism.

Mechanism of protoplast fusion:



The plant can be enriched with disease and frost resistance, genes that encode for growth regulations, etc.

Growing Insect Resistant Plants

Broadband insecticides

DDT don't readily decompose, affect not only insects, but accumulate in plants, land, drinking water and ultimately in humans.

Narrow-band insecticides

Insecticides that only affects closely related insects or a particular species.

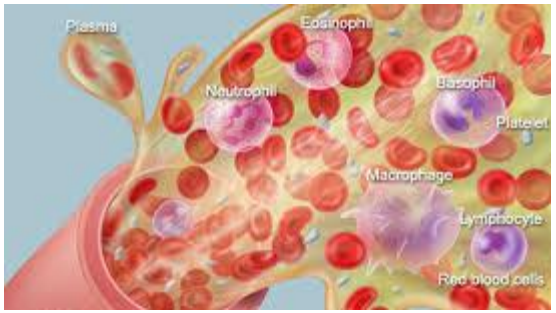
Selectively working insecticides

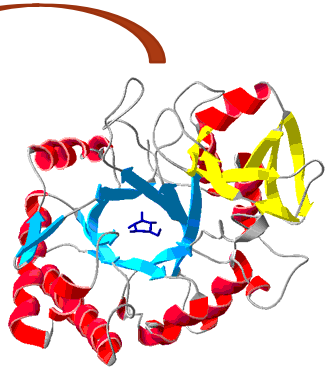
If a bacterium Bacillus Thuringiensis 's spore is ingested by an insect, it kills the insect – both the caterpillar and the adult insect are affected.

DDT has been banned by the WHO, but is still widely used in less developed countries.

Genetically Engineered Farm Animals

As the tick sucks blood



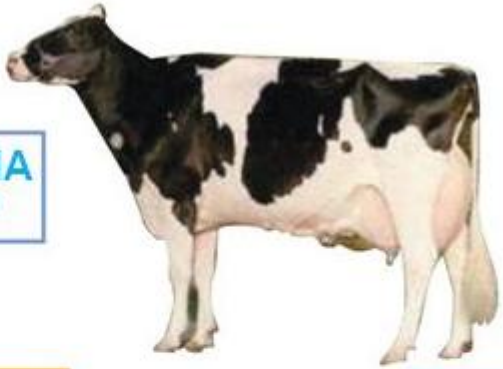


The modified blood plasma containing chitinase is able to break down the tick's chitin shell.

The tick resistant sheep

Somatropin is secreted by the pituitary gland.

rBST makes cows to produce 25 % more milk in which all fats from the body are deviated for the milk production.



Bacterial promoter and RBS

BST mRNA from cow

Bovine somatotropin mRNA

Expression vector

Convert BST mRNA to cDNA using reverse transcriptase

Bovine somatotropin cDNA

Inject rBST into cow to increase milk yield

Artificial BST lacks extra amino-acids.

rBST cows require extra food to cope with the increased milk production

rBST

Transform into cells of *Escherichia coli*

Commercial production

Side-effects of injected BST in cows

	Food	Increase of milk production	Ppb DST
No BST	34	28	2
BST	38	37	10

Prolonged use of BST lowers the cows immune system. rBST have a 78% higher chance of getting sick

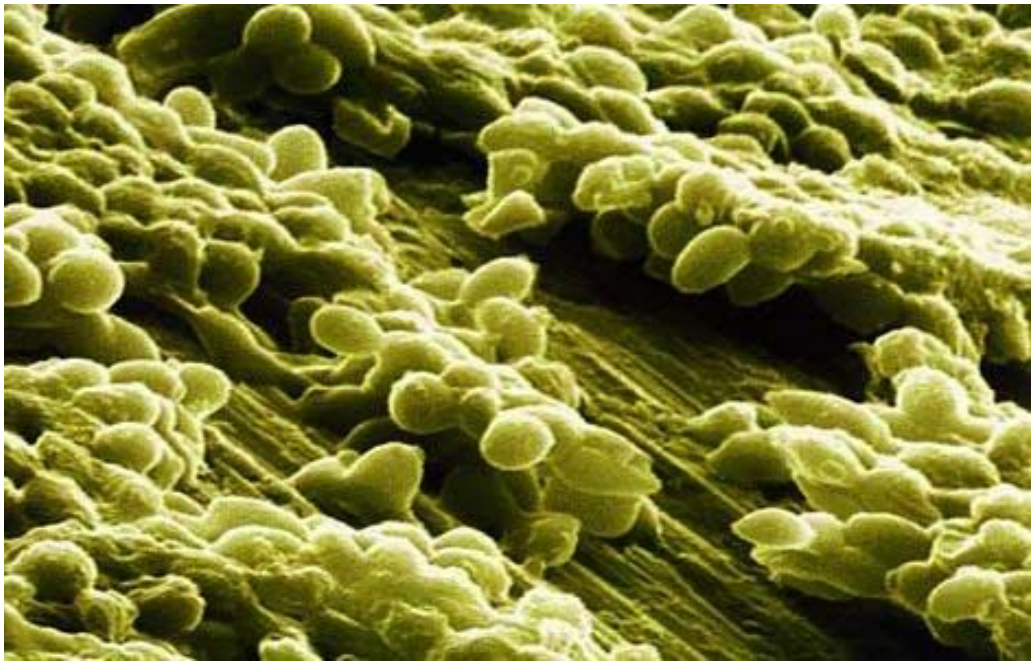
There might be side-effects like birth defects, or changes in behavior. The are not observed so far.

In 1993 the FDA (Food and Drug Administration) in USA approved it.

The EU has asked for more clinical studies, since they fear that BST may have effects on humans.

Single-Cell Protein (SCP)

- Proteins derived from microbial cells for use as food or food supplements;
- SCP's are biotech's real success story as these proteins are usually obtained from so called “waste” substrates, and are widely used in the UK and the USA.



Applications of Single-Cell Protein

GM-yeast remnant lactose proteins, vitamins

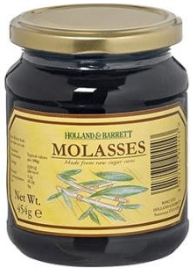
Food supplements for cattle

Earlier the huge amounts was dumped as sewage

Further processed by GM-bacteria

Cheese Industry

Molasses : the end product



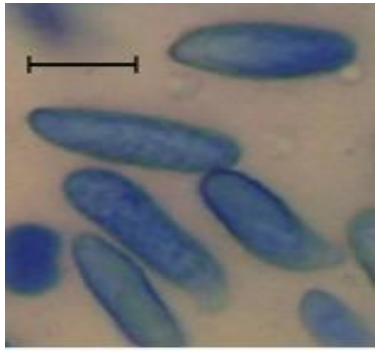
Sugar refinery

Sulfite liquor

Paper mills

Yields bio-degradable waste.

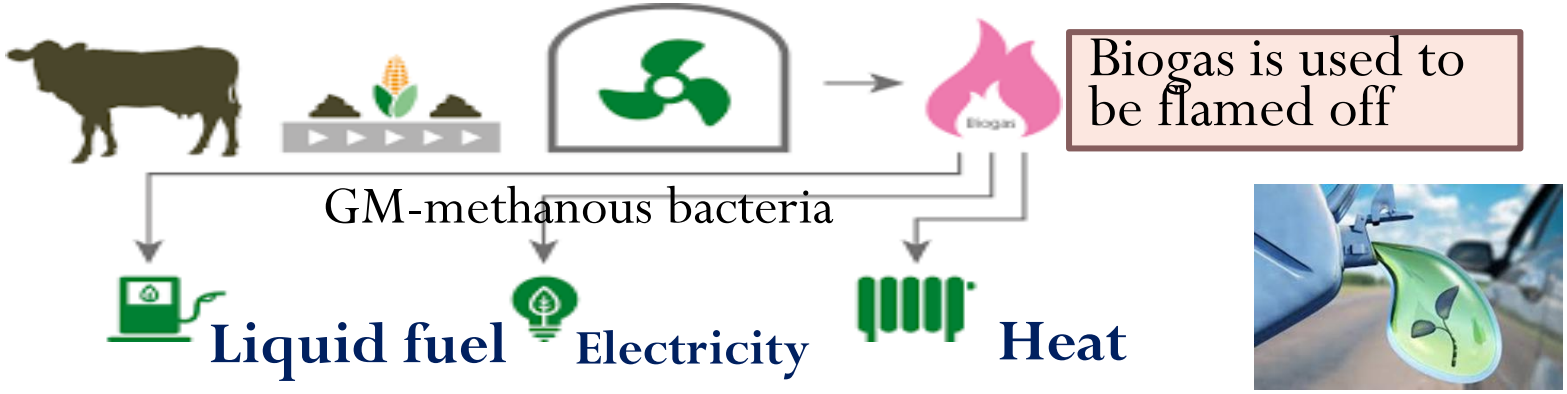
Paper waste product with low-level nutrient, sugar.



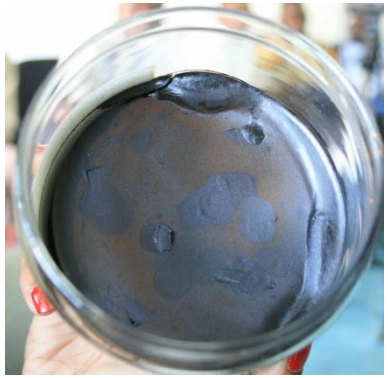
GM-fungi

Generating substantial amount of heat that can be further used.

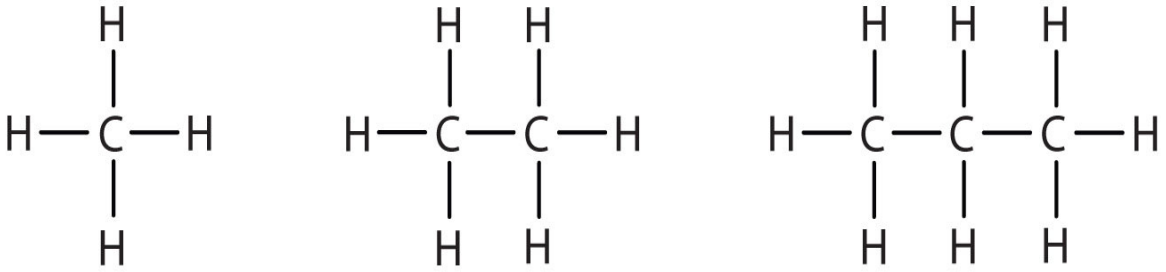
Liquid fuel



Alkanes



Waxy oil looks like



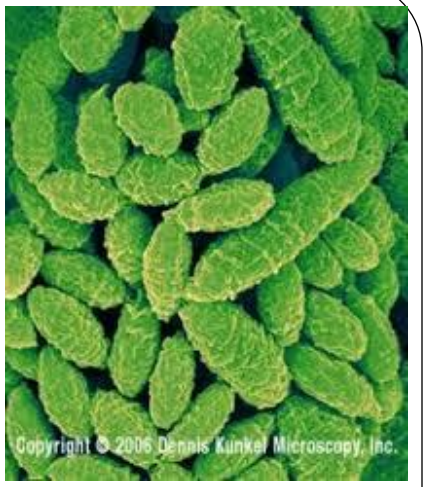
Methane Ethane Propane

Gene modified bacteria

Primary substrates for the synthetic protein production

Advantages of Single-Cell Protein

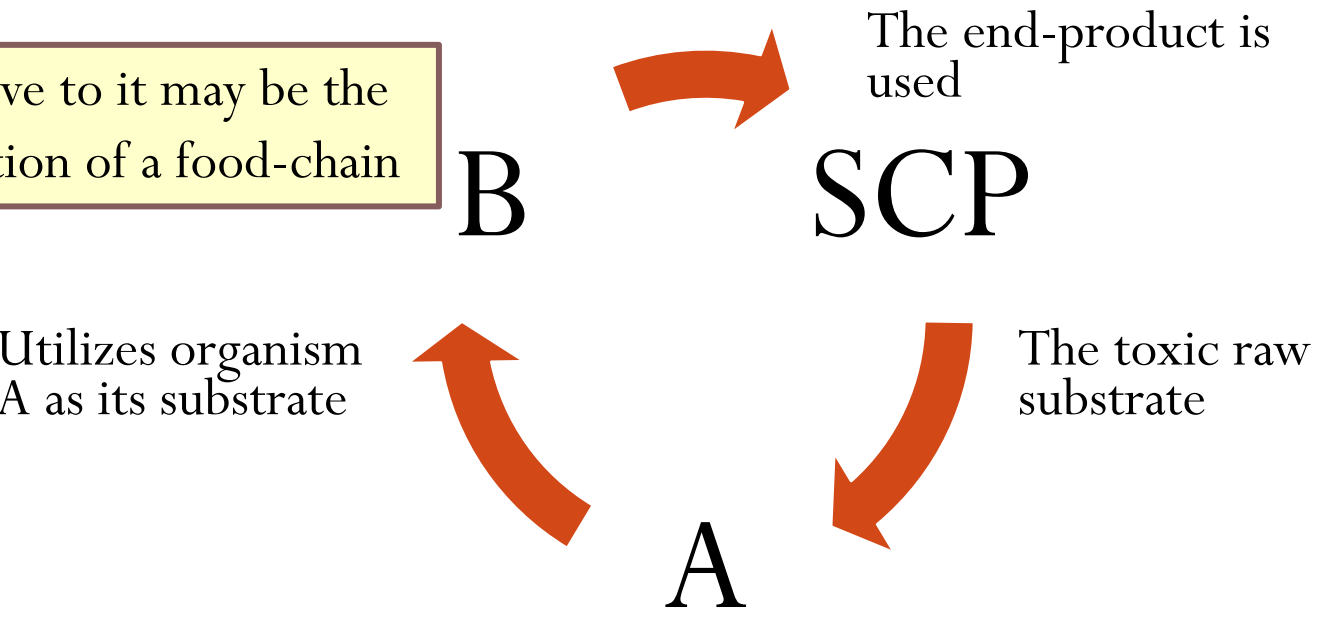
- microorganisms grow and multiply rapidly; some microbes are able to double their biomass up to three times an hour:
- microorganisms can exploit a great variety of (waste) substrates - represents a huge profit;
- the genotype of microorganisms can be manipulated very easily; screening of the manipulated organisms is facilitated by their rapid growth rates;
- microorganisms in relation to their size, are not only rich in proteins, but also in amino-acids;
- providing the warmth and ideal growth conditions, they can be easily accounted for in a fermentor;
- furthermore, fermentors do not require that much space as traditional techniques in agriculture.



Disadvantages of Single-Cell Protein

- The high percentage of contaminants that can be found in the extract (traces of sewage, excrements, waste, etc);
- SCP's are economically not yet profitable enough. Purification at this stage is rather expensive and no one can guarantee a 100% pure product.

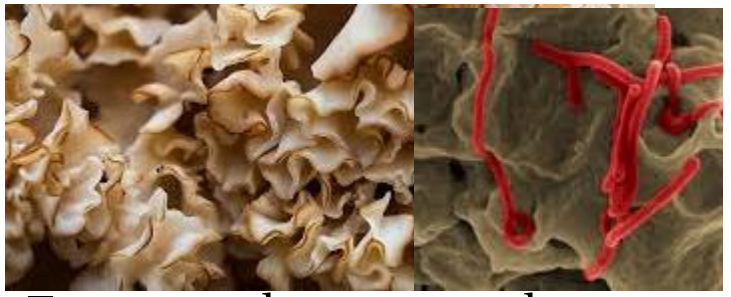
An alternative to it may be the implementation of a food-chain



Even though the final product is free of contaminants, there are some setbacks in this approach:

- Food-chains are usually less efficient (energy losses) - roughly 10-30% is obtained at the end;
- Food-chains have the low nutrient level and higher financial costs;
- The SCP's nucleic and amino-acid content is around 15%, while those in conventional food is only around 4%.
- So, SCP can cause serious health problems, like kidney stones, diarrhea, vomiting, etc.

Food supplements for humans and animals



Fungi can be seen in the introduction of eukaryotes , that contain far less of it.

Selected Items Made of SCP's:

Marlow food belons to the food products made of GM-fungi, that is very easy, fast, and convenient to cook.

The success story is that the fungi, *Fusarian gramineaum*, has lots of fibers that can be arranged to look like meat - a similar product is known under the brand name *Quorn*.

Quorn Foods

Health Care Products - the **GM-autotrophic blue-green algae** *Spirulina sp.*, is used as a food additive; it has a very high nutritional value and is most appreciated by health conscious individuals.

Prutein

A high protein food for cattle

In 1972 ***prutein*** was the largest investment of ICI

ICI were forced to completely shut down production of this GM-product all together.

Fish meal came onto the market

There are chances though, if fish stocks reduce that they may relaunch production again.

The wine making industry used to rely on traditional methods

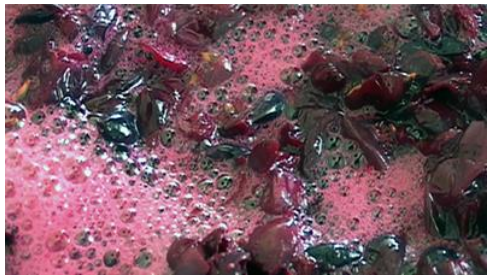
Grapes that contain different strains of wild yeast that can be found on the grape's skin

So, the flavor of a wine depends on which strain of yeast dominates the season's harvest and which geographical area the grapes are cultivated in.

Nowadays, vineyards tend to cultivate their yeast strains separately to guarantee that every harvest yields the same unique taste that consumers might look for.

The Basic Steps of Making Wine

1. To kill the naturally present strains of yeast (usually strains of *Saccharomyces cervisiae*).



The grapes of the first must

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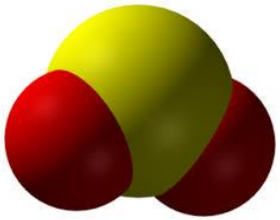


Sulfur dioxide

All reactions are carried out in huge stainless steel fermentation tanks.

Neutralization of the acid. Stop the beginning of fermentation process

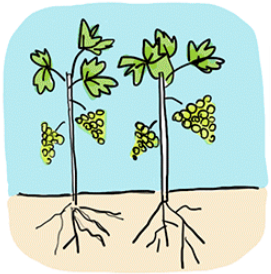
2. To start the actual fermentation, which ultimately, yields white wine.

+

GM-yeast

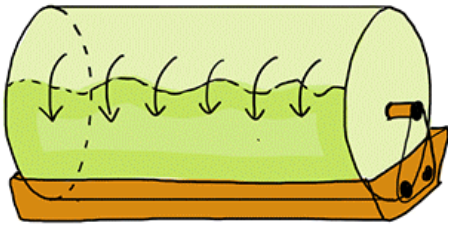
How white wine is made

Grow some grapes

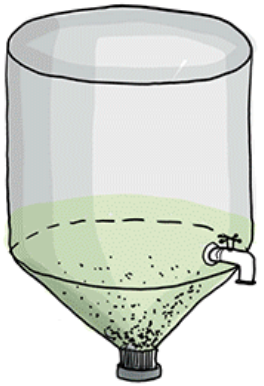


Harvest

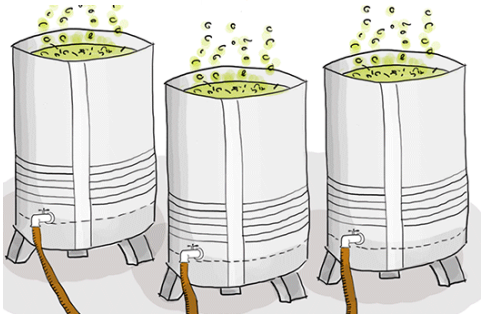
Press. Extract juice from the grapes



Settling. Particles form sediment at the bottom of the tank



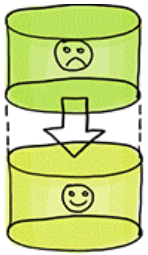
Inoaked



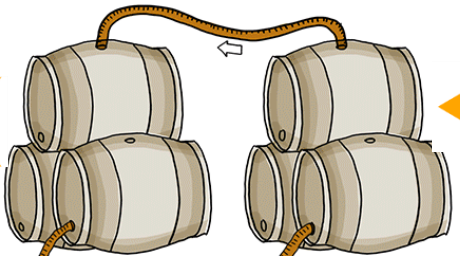
Alcochol Fermentation.
Sugar+Yeast=
Alcochol + CO_2

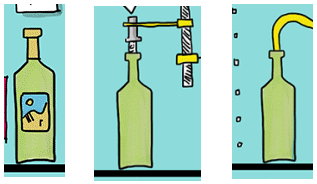
Oaked

Maloactic conversion.
Malic Acid –
into Lactic acid



Racking.
Removing the clear wine from the sediment.





Lable Corc Fill

Bottling.

3. To obtain red wine.

The prefermented must

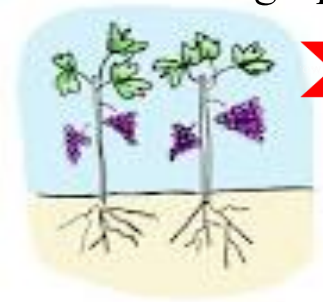
Tiny amounts of dark-colored grape-skin

4. To reduces the acidity of wine within the 1st year with a second fermentation (with *Malo-lactic acid*).

5. To soften the taste of the wine, a culture of *Pediococcus* bacteria is added to the must

How red wine is made

Grow some grapes



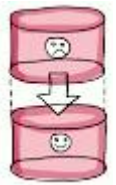
Harvest

Alcochol Fermentation.
Sugar+Yeast=
Alcochol + CO_2

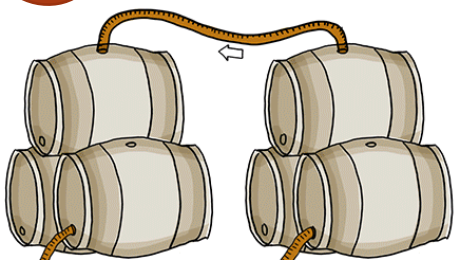
Grape-skin

Press

Ageing process.



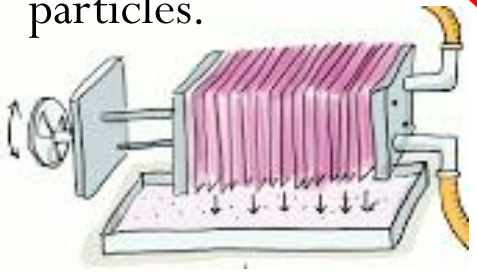
Maloactic conversion.
Malic Acid – into Lactic acid



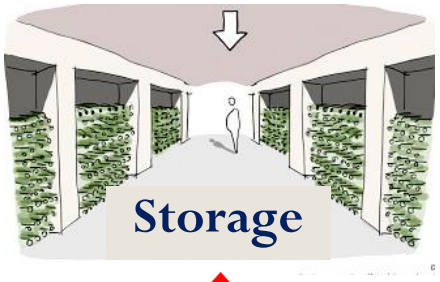
Racking.
Removing the clear wine from the sediment.

Fining

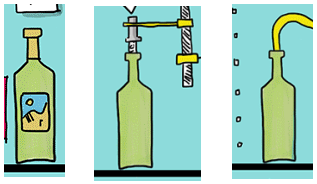
Removing sediment and particles.



Filtraion



Storage



Labled Corc Fill

Bottling

The Production of Sparkling Wine

Requires further alcohol fermentation.

Extra amounts of sugar

The tightly sealed fermentor

A fair amount of CO_2

CO2 becomes dissolved in the champagne

Champagne

To obtain different brands (flavors) of Champaign, the must is enriched with selected blends of red or white wine

Sparkling

Making Sherries

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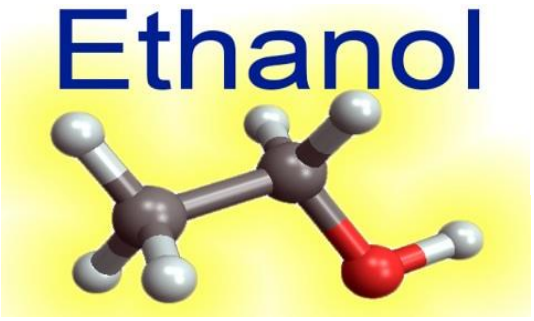
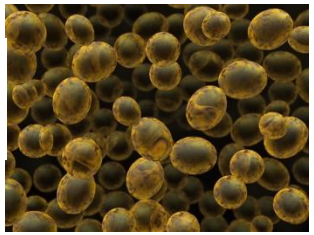


Increasing the alcohol content to 15 %

Fermentation process has to take place under atmospheric conditions.



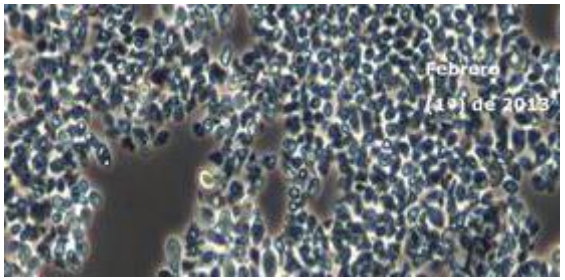
A thick film of yeast that floats on top

To maintain a typical Sherry's taste, most often producers expose their must to GM-modified yeast cultures.

The end product possesses the characteristic flavor of the yeast

Making Dessert Wines

Wines have to be extensively exposed to microbial treatment.



GM-fungi *Botrytis sinerae*

Dehydration of the grapes (loss of water)

Rendering the wine less acidic

Boosting the wines sugar content

Making Fruit Juices

Conventionally made fruit juices

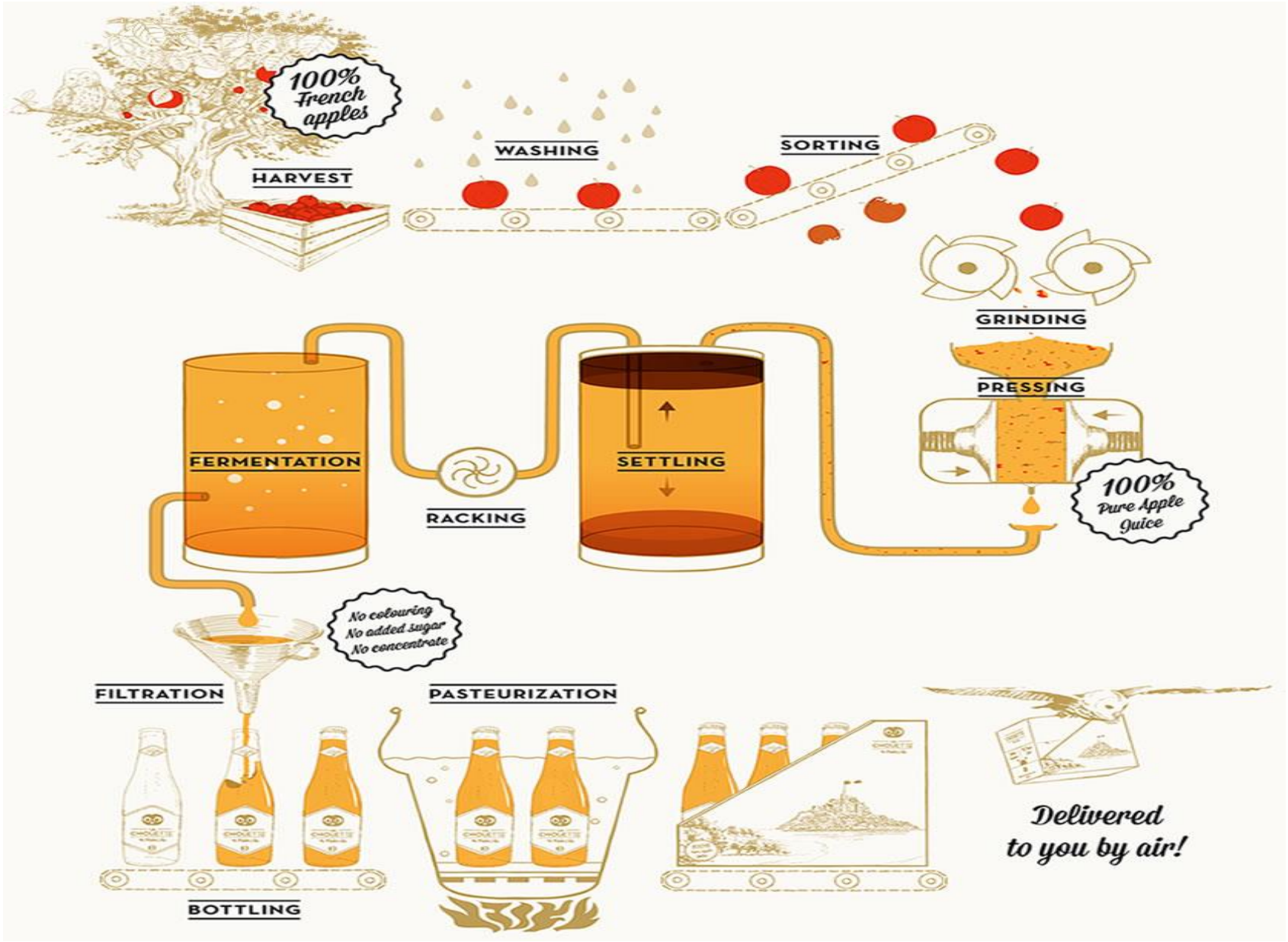
- The fruits are harvested, pressed and bottled.
- A profitable large scale production required year round supply of fruits.
- Fruits stored in a cool and CO_2-rich environment, tend to loose part of their sugar contents.

The industry is able to produce juices of fruits that have been unthinkable before

GM-making jouces

- To boost the levels of sugars extra GM-enzymes (e.g. pectinase) are adde to the pulp.
- A cocktail of tailored enzymes are used specifically for different kinds of juices.
- The production of juices require precisely adjusted technical equipment.
- Enlarging the different types of juices;

Fruit Juce Production line



Thank You For Attention