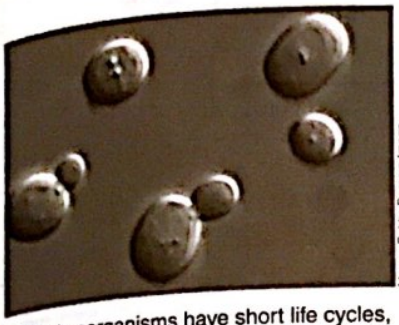
192 The Use of Microbes in Food Technology

Key Idea: Bacteria and fungi are used extensively in many aspects of food technology. They provide many advantages over other food technology techniques.

Bacteria and fungi have been used for thousands of years to preserve and produce a wide variety of foods (e.g. alcoholic beverages and bread). The microbes can play an important role in the food technology process (e.g. cheese production), or they may be the final food (e.g. fungal mycoprotein). Features of microbial biology make microorganisms well suited to producing a range of products on an industrial scale. The industrial-scale culture of microbes is given the general name fermentation because it occurs in fermentation tanks (bioreactors). It can be aerobic or anaerobic and is not a reference to the metabolism of the organisms themselves.

Advantages of using microorganisms



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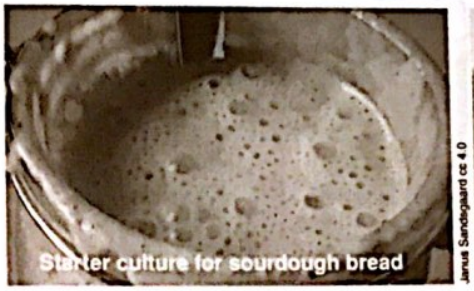
Most microorganisms have short life cycles, and can reproduce rapidly to increase their numbers. Baker's yeast (*Saccharomyces cerevisiae*, above) is used in brewing and baking. Under optimal conditions, it can double its population every 100 minutes.



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Microorganisms can be cost effective to produce as they can grow on a wide range of raw materials or even on waste products. Genetic modification of microorganisms can enhance the production of a naturally occurring substance or produce a novel desirable trait.

Jan Arvall cc3.0

Sometimes the microbe itself is the food. Mycoprotein is a fungal protein and is an alternative to animal protein. The fungi are grown in reactors, which occupy very little space so, unlike livestock farming, mycoprotein can be produced anywhere, independent of climate.

Food production using microbes

Genetic engineering provides alternative sources for products that were once available only through expensive or wasteful means. The enzyme rennin is used in cheese making and was traditionally obtained from the stomachs of calves. It is now produced by GM microbes.

Sauerkraut production involves the fermentation of cabbage using lactic acid bacteria. Not only does this process change the characteristic of the cabbage, but it helps to preserve it. The low pH prevents food spoilage bacteria growing.

In the production of **soy sauce**, filamentous fungi (*Aspergillus soyae* and *A.oryzeae*) digest soy proteins. The culture is fermented in the presence of lactic acid bacteria (*Lactobacillus* spp.) and acid tolerant yeast to develop the characteristic soy flavours.

1. Discuss the advantages of using microorganisms in food technology: _____

2. Describe one example of how genetic engineering has assisted a traditional biotechnology: _____

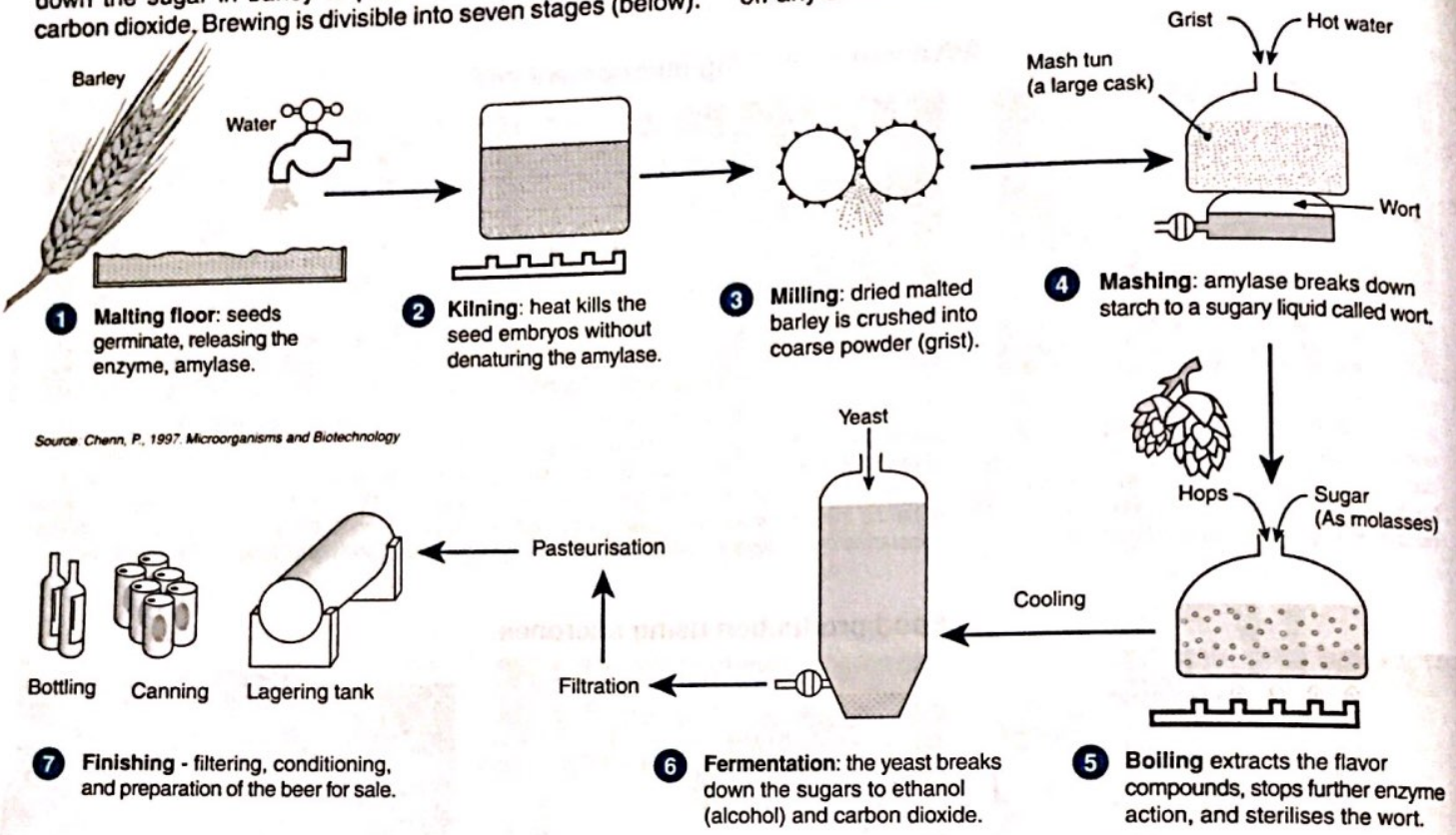
3. How can fermentation help to increase the shelf-life of sauerkraut? _____

193 Beer Brewing

Key Idea: Beer is an alcoholic drink produced by the fermentation of the sugars in barley by yeast. Ethanol and carbon dioxide are produced.

Using yeast to make foods and drinks is probably the oldest form of biotechnology. During beer production, yeast breaks down the sugar in barley to produce alcohol (ethanol) and carbon dioxide. Brewing is divisible into seven stages (below).

During the finishing stage, bacterial proteases are added to break down the yeast and prevent cloudiness. Amylase is added to break down sugars in the production of low calorie beers. Traditional beers are stored in barrels to develop their characteristic qualities. Beer is pasteurised, and standardised for colour and flavour before bottling. Pasteurisation also kills off any undesirable microbes.



Source: Chenn, P., 1997. Microorganisms and Biotechnology

1. **Malting floor**: seeds germinate, releasing the enzyme, amylase.
2. **Kilning**: heat kills the seed embryos without denaturing the amylase.
3. **Milling**: dried malted barley is crushed into coarse powder (grist).
4. **Mashing**: amylase breaks down starch to a sugary liquid called wort.
5. **Boiling** extracts the flavor compounds, stops further enzyme action, and sterilises the wort.
6. **Fermentation**: the yeast breaks down the sugars to ethanol (alcohol) and carbon dioxide.
7. **Finishing** - filtering, conditioning, and preparation of the beer for sale.

1. Write the word equation for the reaction taking place in the brewing process: _____

2. Investigate the production of beer brewing. Discuss the key processes involved in each of the seven steps illustrated:

(a) **Malting**: _____

(b) **Kilning**: _____

(c) **Milling**: _____

(d) **Mashing**: _____

(e) **Boiling**: _____

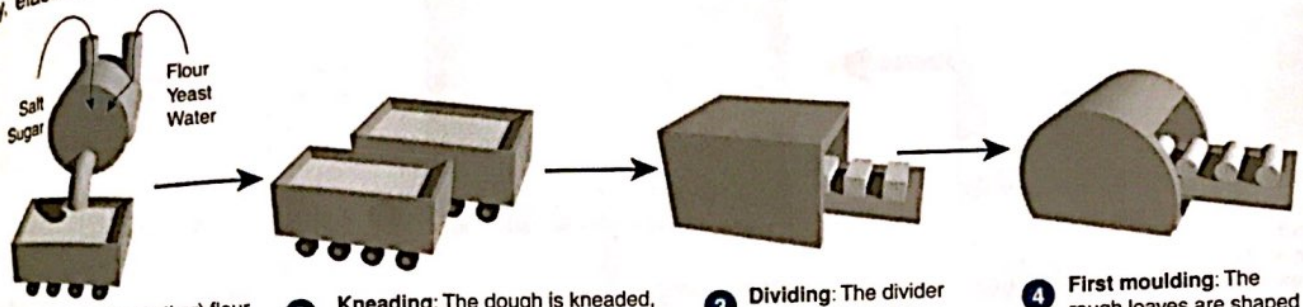
(f) **Fermentation**: _____

(g) **Finishing**: _____

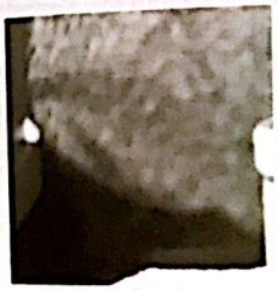
194 Bread Making

Key Idea: Bread is produced during a fermentation process using the yeast *Saccharomyces*. The carbon dioxide produced causes the bread to rise.

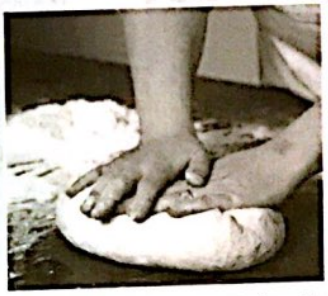
Leavened (risen) bread is produced using the yeast *Saccharomyces*. When the raw ingredients are mixed, the gluten (flour proteins) are hydrated and coalesce to form a sticky, elastic dough. Enzymes, having survived the milling process when grains are made into flour, act on the starch in the dough to make a mixture of sugars. Yeast uses the sugars (anaerobically) and produces ethanol and carbon dioxide gas which causes the bread to rise. *Lactobacilli* may grow during the early stages of proving, producing lactic acid which contributes to the final flavour and inhibits growth of other organisms. The commercial process is outlined below.



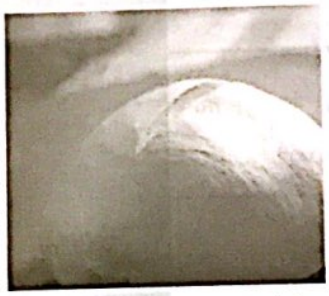
1. **Mixing**: Wheat (and other) flour, yeast, sugar, salt, ascorbic acid and water (or milk) are combined to form a **dough**.
2. **Kneading**: The dough is kneaded, left to ferment (rise) at 27-35°C for 10 minutes in a humid environment, and then remixed.
3. **Dividing**: The divider cuts the dough into rough loaf-sized pieces ready to be shaped.
4. **First moulding**: The rough loaves are shaped by a rounding machine.
5. **First proving**: The loaves are left to ferment for about 10 minutes.
6. **Second moulding**: The molder shapes the dough into cylinders
7. **Second proving:** A further period of fermentation at 40°C for 45 mins.
8. **Baking**: At 235°C for 25-40 mins. The bread is then cooled, sliced (optional) and wrapped ready for distribution.



Bread making is one of the oldest and simplest of biotechnologies, involving mixing wheat flour, water, and yeast to form a dough, which can be baked.



Kneading results in physical and chemical changes in the gluten, which give the dough its elastic and resilient texture and help it to rise.



During proving, the dough is left to ferment. The yeast metabolises sugars, producing ethanol and carbon dioxide. The carbon dioxide causes the dough to rise.

Baking kills the yeast, evaporates the ethanol, and cooks the flour. Vitamin C, whiteners, raising agents, stabilisers, and flavours may be added.

1. Explain the role of each of the following in the bread-making process:

   (a) Sugar: _____

   (b) Yeast: _____

   (c) Water (or milk): _____

2. (a) What happens to the dough during the fermentation (or proving) stages? _____

   _____

   (b) Why will bread not rise if it is baked too soon after adding the yeast? _____

   _____

3. Suggest why gluten free bread is flat and dense: _____

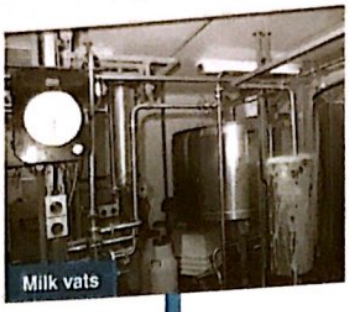
   _____

195 Cheese Making

Key Idea: The coagulation of the milk protein casein by acid and/or rennin forms the basis for cheese production.

Cheese is produced when the milk protein casein is coagulated (curdled) to form an insoluble curd. The process varies depending on the type of cheese made. Some cheese (e.g. cottage cheese) is produced by acid coagulation only. The acid is produced by the bacterial starter culture which is added to the milk during the process. Ripened cheese is produced using a combination of acid and the enzyme rennin to form the curd.

Milk is delivered under refrigeration. Most cheese is made from cow's milk, but goat and sheep milk is used for some cheese varieties (such as feta).



Milk vats

1 The milk is often pasteurised (heated to 72°C for 15 seconds). Pasteurisation kills off any undesirable microorganisms that could alter the characteristics of the cheese and, most importantly, kills off dangerous microbes that could cause harm if eaten. The milk is pumped into large, temperature controlled vats and kept cool between 20-30°C.

Stirring rennet and starter

2 A **starter culture** of specially selected bacteria is added to the cooled milk. Lactic acid producing bacteria, which metabolise the milk sugar to produce lactic acid are often used. The pH of the milk will begin to drop as lactic acid is produced and the casein proteins in the milk will begin to coagulate. In most cheeses, **rennet** (a mix of milk coagulating enzymes) is also added. The coagulation step is a function of the chemistry of milk. The casein proteins in milk are associated with other molecules, including calcium, to form stable structures called micelles. Acidity and hydrolysis by the proteases in rennet cause the micelles to destabilise, and the casein proteins precipitate out to form a gel.

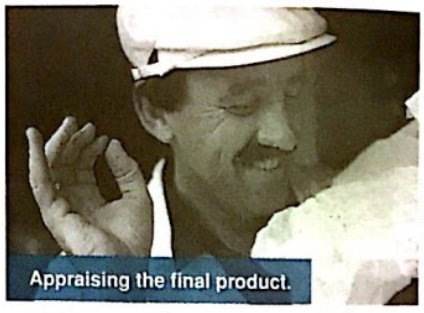
Ricotta - a low fat cheese resulting from processing of the drained whey

P S C

Cooking and draining remaining whey

Cutting the curd

3 Cutting the gel causes it to separate into **curds** (the solid portion consisting mainly of casein proteins) and **whey** (mainly water but does contain some whey proteins). The whey is removed from the curd by a combination of stirring, cooking, draining, salting, and pressing. The vigour with which the whey is removed has a profound effect on the final cheese product.



Appraising the final product.

All photos kindly supplied by Kaath Cheeses Ltd

Key to finishing processes

P	Pressing	C	Cooking
R	Ripening	T	Turning
M	Internal mold	W	Washing curd
M	External mold	B	Brining**
CH	Cheddaring*	S	Salting

* Cheddaring involves the 'milling' (breaking up) of cooked curd and stirring

** Brining involves soaking in a salt solution

Ripened cheeses

Aeration allows the growth of internal molds

M P S

Blue vein
Stilton
Roquefort

W P B R

Surface coatings

Edam
Gouda

S P T M

C P B R

Gas producing bacteria

P B

C CH S P R

W S P R

Camembert
Brie

Swiss cheese
Gruyere

Feta

Cheddar
Cheshire

Mild, slightly sweet cheddar style

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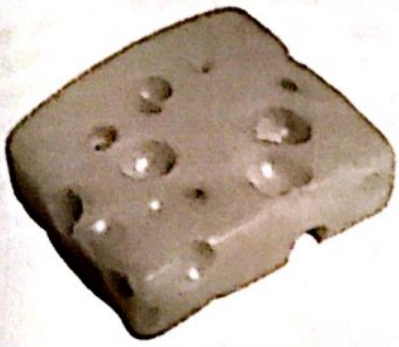
Microorganisms can alter cheese characteristics



Additional microorganisms are used to give specific characteristics to cheeses. *Propionibacterium freudenreichii* produces the carbon dioxide gas that produces the holes in swiss cheese (above) and creates the characteristic sweet nutty flavour.

Species of *Penicillium* produce the veining on blue cheese (above). The texture of the cheese is loose enough that oxygen can reach the aerobic moulds. Fungi in blue cheese use the lactic acids produced during the cheese-making process and release the odorous by-products associated with blue cheese.

Lactococcus lactis strains are used for most cheeses cultured at 30°C and 38°C. Thermophilic bacteria, which grow best at 42°C, are used for cheeses requiring higher cooking temperatures, such as Swiss and Parmesan (above). Unlike mesophilic bacteria, thermophilic strains will survive the cooking process.

1. Use an annotated diagram to summarise the general cheese making process:

2. Using examples from the flow opposite, explain how different actions during the ripening stage can influence the characteristics of a cheese:

3. (a) What could happen if milk was not pasteurised before the cheese-making process began?

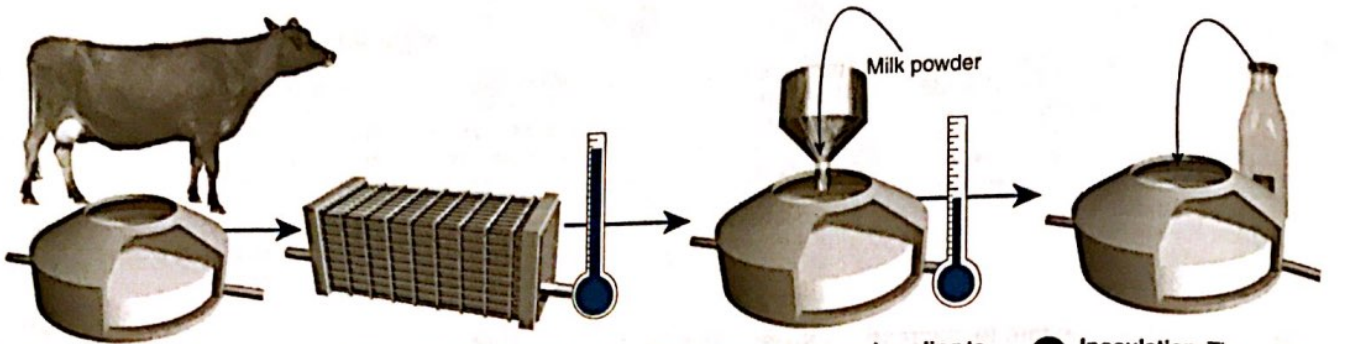
   (b) Why is the milk cooled to 30°C before the starter culture is added?

4. Explain what is happening when the casein protein coagulates to form the curd:

5. Why are thermophilic bacterial varieties used for producing cheeses that require higher cooking temperatures?

196 Yoghurt Making

Key Idea: Yoghurt is produced by the bacterial fermentation of milk. It is a way to preserve the nutritional qualities of milk. The biochemistry of yoghurt production is similar to that of cheese. Lactic acid bacteria are added into milk and the lactic acid they produce coagulates the milk proteins and thickens the yoghurt. The starter culture for yoghurt contains two symbiotic bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *L. bulgaricus* metabolises lactose in the milk anaerobically to produce the lactic acid responsible for the formation of the yoghurt. *L. bulgaricus* also produces peptidases, which break down the milk proteins into peptides and amino acids. These stimulate the growth of the *Streptococcus* in the culture. *S. thermophilus* produces carbon dioxide and methanoic acid, which together lower the pH and, in turn, stimulate the growth and metabolism of the *Lactobacillus*.



1. **Pre-treatment** of the raw milk which contains milk protein (casein) and milk sugar (lactose).
2. **Heating** to 85-95°C for 15-30 minutes to kill all the bacteria, drive out the air, and denature the milk proteins.
3. **Homogenising and cooling** to an incubation temperature of just below 45°C. Concentrated milk or milk powder is added to standardise the milk composition.
4. **Inoculation**: The addition of a bacterial starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.
5. **Incubation & fermentation**: The culture is left to incubate at 38-45°C for 3-6 hours
6. **Cooling**: the natural yoghurt is now at about pH 4, with 2% lactic acid and 30 ppm ethanal. It is cooled to 4.5°C and held at this temperature until packaging.
7. **Addition of fruit, nuts, colour, and flavouring** These are optional extras. Sterilisation of the product is also optional.
8. **Packaging, storage and distribution** in a chilled environment (2°C).

1. Describe the mutualistic association between the two starter bacteria, *L. bulgaricus* and *S. thermophilus*: _____

2. Why does the pH fall during the incubation stage? _____

3. Why is it not necessary to kill the bacteria before eating the yoghurt? _____

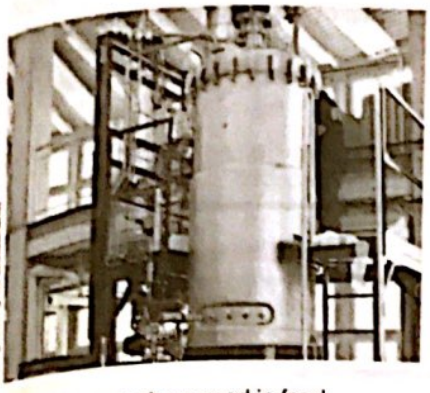
4. Many cows are given antibiotics, which can end up in their milk. How do you think antibiotics might affect the yoghurt making process?

197 Using Microbes To Make Food: Pros and Cons

Key Idea: There are many advantages to using microorganisms in food technology processes, but consumers have some concerns about their use.

Bacteria and fungi are used extensively in many food technology processes and also in the improvement of food crops. While traditional food safety issues (e.g. microbial contamination) are still valid, the use of genetically modified microorganisms has generated more debate about the safe use of microbes in food production. Some important aspects of this debate are described below.

The advantages of using microbes in food production

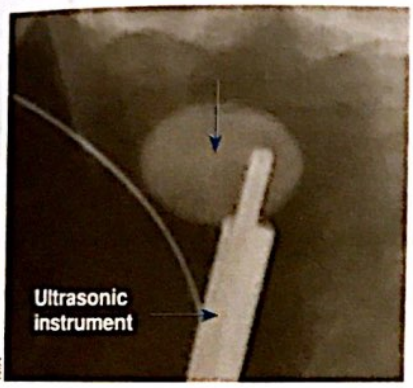


Many microorganisms used in food production are grown in bioreactors (above). The growth conditions (e.g. temperature and pH) can be easily adjusted to maintain maximum growth. This allows manufacturers to alter production to meet changes in demand. Often the microorganisms are grown on cheap waste products (e.g. whey) making their production cost efficient.

Microbes have been used to produce genetically modified crop plants. Genetic modification for pest resistance has improved crop yields in corn and rice, providing more food. Lactic acid producing bacteria have been genetically modified to improve the characteristics of the final product (e.g. nutritional value, flavour, or texture of cheese).

Many proteins are inaccessible to vegetarians because they are animal derived. However, single cell protein (SCP) is high quality protein derived from yeast, fungi,or bacteria. Quorn is an example of SCP. Historically, animal derived rennin was used to make cheese, but the use of recombinant rennin means many cheeses are now suitable for vegetarians.

The disadvantages of using microbes in food production



If bacteria are not removed from the finished food product, their high nucleic acid content can cause elevated uric acid levels in humans. This can result in a variety of painful diseases such as gout or kidney stones (above) which form when uric acid crystallises and precipitates out forming a "stone".

Many consumers have negative perceptions of food produced using microbes, especially those that have been genetically modified. The safety and ethics of GMO foods are often questioned and can sway consumer choice when it comes to purchasing food products. In the EU, GMO foods must be labelled.

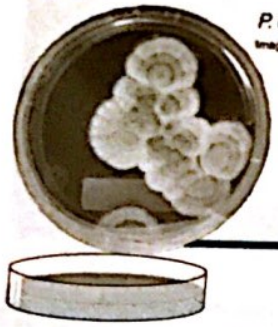
Microbial rennin (a component of rennet) used in cheese making can sometimes produce bitter cheeses. Rennin produced using genetic engineering does not contain all of the enzymes found in natural calf rennin, so can require additional enzymes to be added to achieve a more realistic flavour profile.

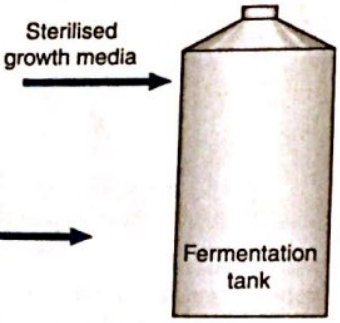
1. Discuss the advantages and disadvantages of using bacteria and fungi to make food for human consumption:

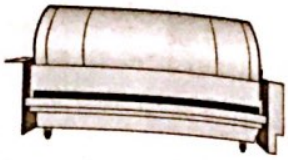
198 Penicillin Production

Key Idea: Penicillin is an antibiotic derived from *Penicillium* fungi. It is produced commercially by fermentation using a fed-batch culture technique.

Penicillin is an antibiotic derived from the fungal genus *Penicillium*. Penicillins are widely used against many common bacterial infections. Penicillin is a secondary metabolite, i.e. a compound that is not immediately essential to the microbe's survival or reproduction, and is only produced in the stationary phase of growth. Industrial production of penicillin occurs by fermentation under aerobic conditions in a fed-batch culture in which nutrients are added periodically, but the culture is not removed until the end of the process. Batch feeding optimises the conditions required for maximum penicillin production. If too many nutrients are supplied, the organism grows, but does not produce penicillin. The generalised process below describes the production of penicillin from *P. chrysogenum*, which yields high quantities of penicillin compared to other species of the genus.





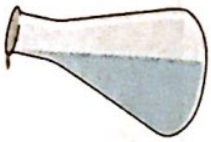


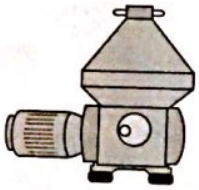
1 Isolation: *Penicillium chrysogenum* is grown in the laboratory.

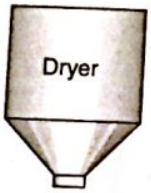
2 Inoculation: The *Penicillium* culture is added to a small tank of growth media for about 24 hours. Growth media contains a carbon and nitrogen source, amino acids, vitamins, minerals, and buffer to maintain the pH.

3 Fermentation: The fermentation tank is stirred to distribute nutrients and fungus. Penicillin production occurs after 40 hours. The tanks are maintained at 23-27°C and pH 6.5. After 3-5 days, penicillin production stops.

4 Rotary filtration: This separates the solid (waste) material from the liquid phase containing the penicillin.







Filtrate

5 Penicillin extraction: Organic solvents are added to isolate and extract the penicillin.

6 Centrifugation and extraction: The crude filtrate undergoes several steps to extract and purify the penicillin.

7 Drying

8 Processing: The penicillin is processed into the desired form (e.g. liquid or tablet).

1. Why is the fermentation tank stirred during the process? _____

2. (a) Why is air added to the fermentation tank? _____

   (b) What could happen if unsterilised air was added? _____

Explain why there is not a continuous supply of nutrients added during the penicillin process: _____

The waste product from the process is sometimes used as animal feed. Suggest a possible problem with this: _____

LINK

199 Insulin Production

Key Idea: The microorganisms *E.coli* and *Saccharomyces* have been genetically modified to produce human insulin. This has solved the problems associated with traditional insulin sources such as high costs and allergic reactions.

Type 1 diabetes is a metabolic disease caused by a lack of insulin. Around 25 people in every 100 000 suffer from type 1 diabetes and currently the disease can only be treated with injections of insulin. In the past, insulin was obtained from the pancreatic tissue of cows and pigs and purified for human use. The method was expensive and some patients had severe allergic reactions to the foreign insulin or its contaminants. The insulin used to treat type 1 diabetes patients today is produced by recombinant DNA technology. The human insulin gene is inserted into a plasmid vector, which is taken up by the bacterium *Escherichia coli* or the yeast *Saccharomyces*. These organisms then produce the human insulin in culture conditions. At the end of production the insulin is harvested and purified. Human insulin produced using recombinant DNA technology has fewer side effects than insulin sourced from non-human mammals.

Insulin production using the bacterium *E. coli*

Concept 1

DNA can be cut at specific sites using **restriction enzymes** and joined together using **DNA ligase**. New genes can be inserted into self-replicating bacterial **plasmids** at the point where the cuts are made.

Concept 2

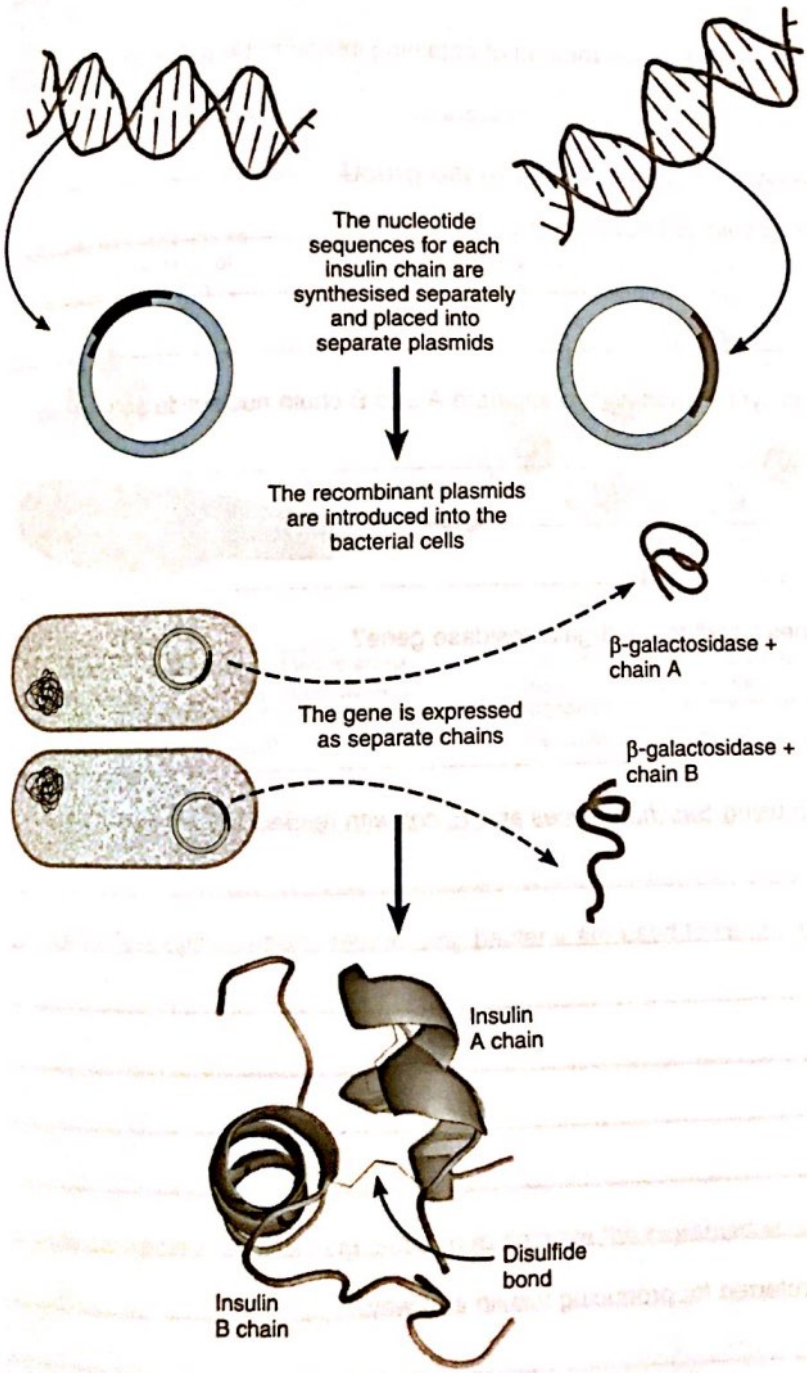
Plasmids are small, circular pieces of DNA found in some bacteria. They usually carry genes useful to the bacterium. *E. coli* plasmids can carry promoters required for the transcription of genes.

Concept 3

Under certain conditions, Bacteria are able to lose or pick up plasmids from their environment. Bacteria can be readily grown in vat cultures at little expense.

Concept 4

The DNA sequences coding for the production of the two polypeptide chains (A and B) that form human insulin can be isolated from the human genome.



Techniques

The **gene** is **chemically synthesised** as two nucleotide sequences, one for the **insulin A chain** and one for the **insulin B chain**. The two sequences are small enough to be inserted into a plasmid.

Plasmids are extracted from the bacteria *Escherichia coli*. The gene for the bacterial enzyme β-galactosidase is located on the plasmid. To make the bacteria produce insulin, the insulin gene must be linked to the β-galactosidase gene, which carries a promoter for transcription.

Restriction enzymes are used to cut plasmids at the appropriate site and the A and B insulin sequences are inserted. The sequences are joined with the plasmid DNA using **DNA ligase**.

The **recombinant plasmids** are inserted back into the bacteria by placing them together in a culture that favours plasmid uptake by bacteria.

The bacteria are then grown and multiplied in vats under carefully controlled growth conditions.

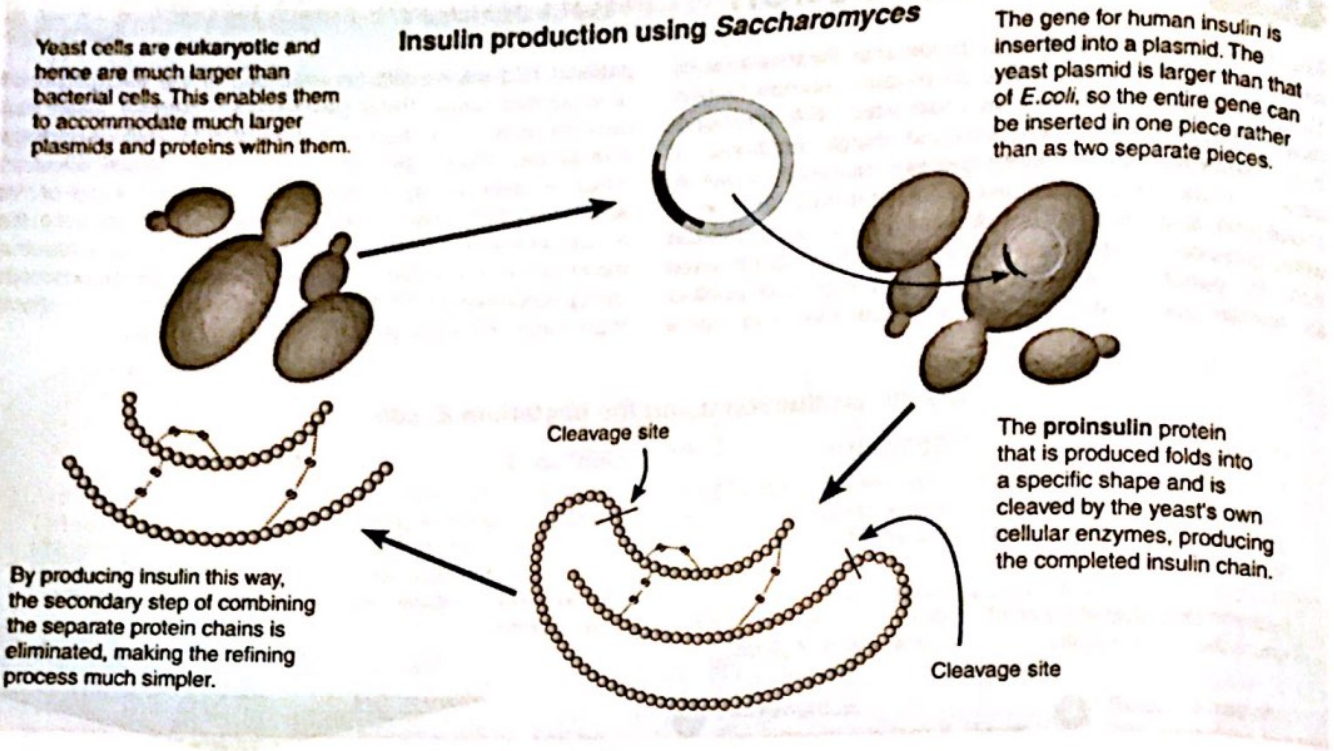
Outcomes

The product consists partly of β-galactosidase, joined with either the A or B chain of insulin. The chains are extracted, purified, and mixed together. The A and B insulin chains connect via **disulfide cross linkages** to form the functional insulin protein. The insulin can then be made ready for injection in various formulations.

Other Applications

The techniques involved in producing human insulin from genetically modified bacteria can be applied to a range of human proteins and hormones. Proteins currently being produced include human growth hormone, interferon, and factor VIII.

Insulin production using *Saccharomyces*



1. (a) Describe some of the problems associated with the traditional method of obtaining insulin to treat diabetes:

(b) What are the advantages of using recombinant insulin to treat diabetes?

2. Explain why, when using *E. coli*, the insulin gene is synthesised as two separate A and B chain nucleotide sequences:

3. Why are the synthetic nucleotide sequences ('genes') 'tied' to the β-galactosidase gene?

4. Discuss the differences in the production of insulin using *Saccharomyces* and *E. coli* with respect to:

(a) Insertion of the gene into the plasmid:

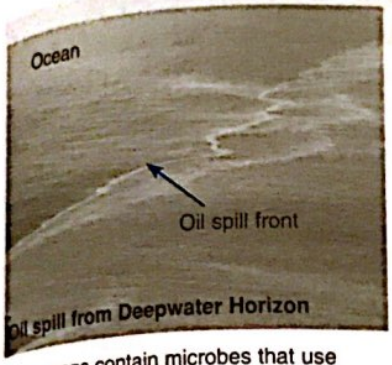
(b) Secretion and purification of the insulin:

(c) Which organism do you think would be most preferred for producing insulin and why?

200 Bioremediation

Key idea: Microorganisms can be used to remove or break down environmental contaminants such as oil and organic waste. This process is called bioremediation.

Bioremediation uses living organisms to remove or neutralise pollutants from a contaminated site. Microorganisms are often used in bioremediation processes where they break down the pollutant through a series of metabolic processes. Different microbes, but especially bacteria, naturally metabolise a range of different substrates, and this is one of the features which makes them useful in bioremediation. Some bacteria have been genetically modified to remove pollutants that no other organism naturally can (e.g. the bacterium *Deinococcus radiodurans* has been genetically engineered to digest ionic mercury produced from radioactive nuclear waste).



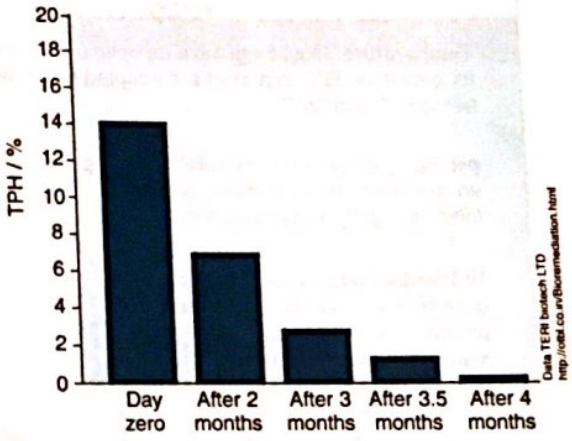
Oil spill from Deepwater Horizon

The oceans contain microbes that use the hydrocarbons found in oil as their energy source. The bacterium *Alcanivorax borkumensis* is one such organism. Its numbers quickly increase after an oil spill and it breaks down the oil into harmless compounds (H_2O and CO_2). Bioremediation was used in the *Deepwater Horizon* oil spill in the Gulf of Mexico in 2010.

In 2008, an oil spill occurred near Gujarat (Western India) due to a crude oil trunk line rupture. Crude oil contaminated a wide area of farm land. Oil soaked soil was excavated and transported off site for bioremediation. Oilzapper (a commercial product containing five different oil degrading bacteria) was applied to the soil.

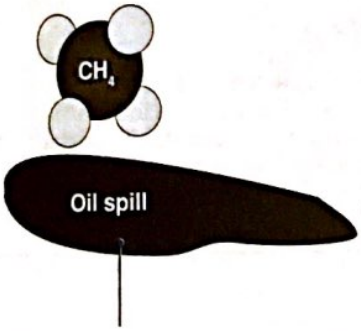
The results are shown right. This is an example of *ex-situ* bioremdiation (treatment that occurs away from the initial site of pollution). According to the Energy and Resources Institute of India, 5000 hectares of oil contaminated cropland has been reclaimed in India and more than 26 000 tonnes of oily sludge has been successfully treated with Oilzapper.

Total petroleum hydrocarbon (TPH) in contaminated soil after treatment with Oilzapper



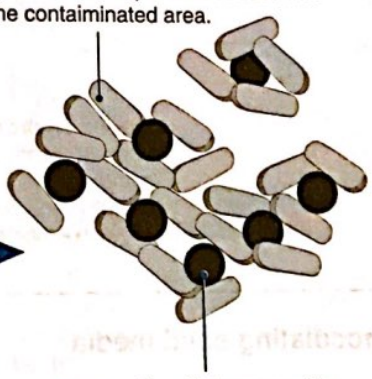
Using bacteria to metabolise hydrocarbons

Oil contains hydrocarbons (compounds made up of hydrogen and carbon).



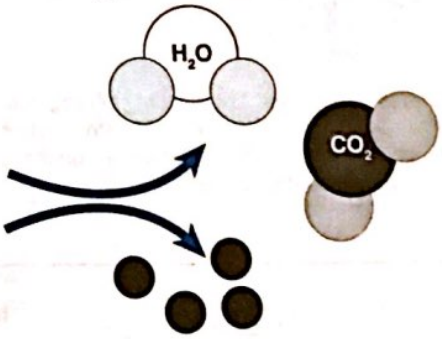
Chemical dispersants are added to an oil spill to break the oil up into smaller droplets. Nutrients are also added in the dispersant to encourage microbial growth.

Hydrocarbon digesting bacteria (e.g. *A. borkumensis*) are introduced into the contaiminated area.



The smaller oil drops provide more surface area for the bacteria to work on. As a result, the breakdown of the oil is faster.

The microbes metabolise the hydrocarbons. Hydrogen and carbon from the oil are added to oxygen to form water and carbon dioxide.



Not all the oil can be broken down by the microbes, but because there is less, it is more easily dispersed by ocean currents and the wind.

1. What is bioremediation? _____

2. Outline how hydrocarbon-metabolising bacteria are used to clean up an oil spill: _____

3. How can genetic engineering be used to improve the bioremediation process? _____