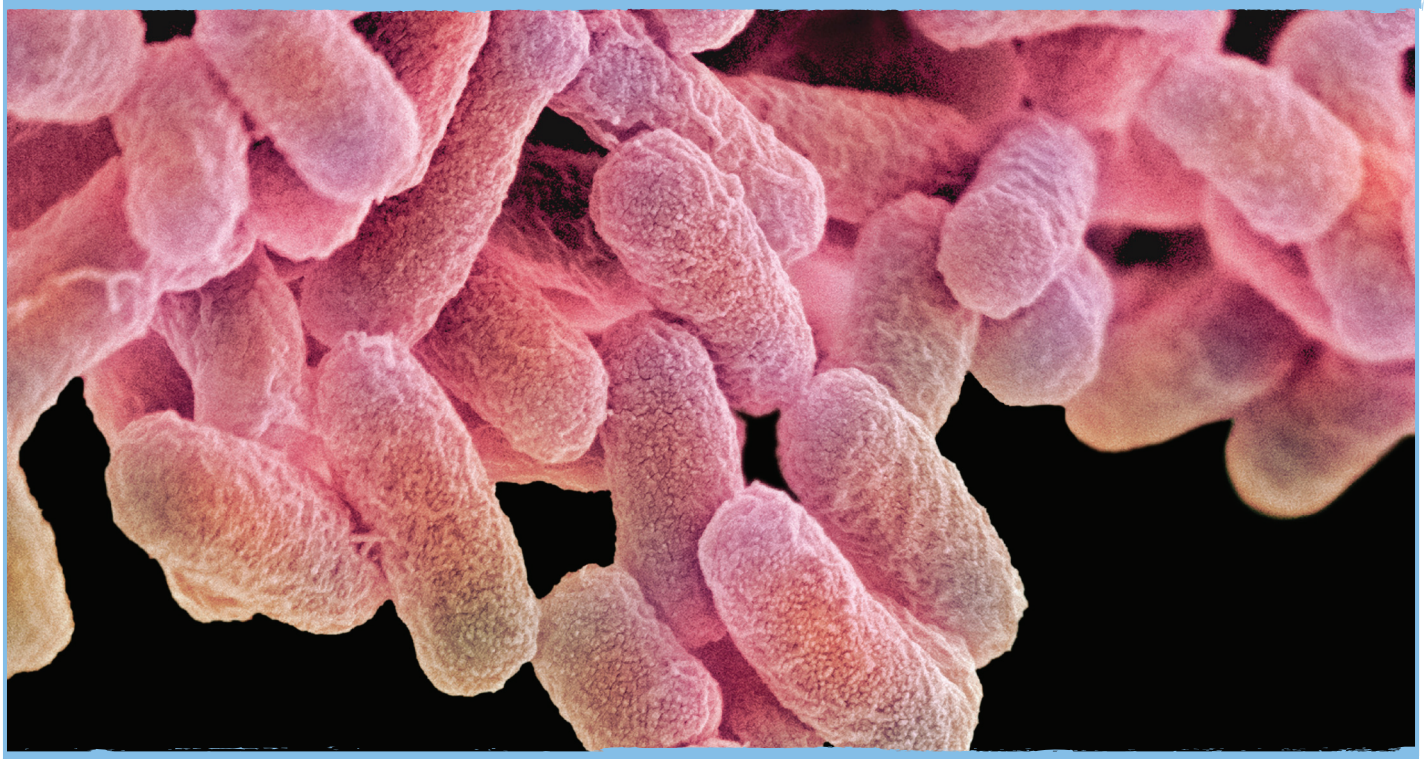


2.2 Commercial and industrial applications of microorganisms

cogent | Skills for Science Based Industries

Think of all the ways that we and other living organisms on Earth depend on microorganisms.

Humans have unknowingly made use of microorganisms for centuries to carry out fermentation processes to make wine, beer, yoghurt, cheese and bread. Since the 1860s we have known about the existence of bacteria and since the 1970s we have been able to genetically modify them, increasing the scope of biotechnology. Microbes are also used to process waste, such as sewage.

On successful completion of this topic you will:

- understand commercial and industrial applications of microorganisms (LO2).

To achieve a Pass in this unit you will need to show that you can:

- review commercially important products from microbial fermentation processes (2.1)
- review the species commonly used in the fermentation processes for the production of products (2.2)
- discuss the principles of biotechnology that underpin the manufacture of products (2.3)
- discuss the use of bioreactors in manufacturing (2.4)
- analyse the routine practices underlying water management in terms of sewage treatment and water purification (2.5).

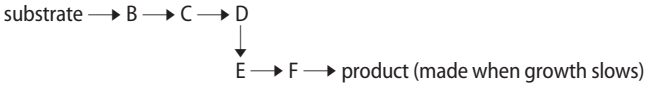
1 Fermentation processes

Besides classifying fermentation processes as batch, fed-batch or continuous (see *Topic guide 2.1*) they can be classified according to how dependent the product formation is upon energy consumption.

Type I: The product is a **primary metabolite**, a catabolic product, produced during the log phase and used by the organism as a source of energy or as biomass.

substrate ⟶ product

Type II: The product is a **secondary metabolite** and is produced from the substrate via a secondary metabolic pathway during the stationary phase, separate from the pathway where the substrate is utilised as an energy source.



Type III: The primary metabolism and the product formation occur at completely different times. The product is produced during an **amphibolic** pathway. The organism grows and consumes substrate and then uses intermediate metabolic reactions to produce the product.

Not all fermentations fit neatly into one of these three categories. The mycelium-forming actinobacteria, *Streptomyces rimosus,* grows and then fragments. It produces the antibiotic, oxytetracycline, during the growth of a secondary mycelium, mostly in the last part of the log phase and slowly during the stationary phase.

Key terms

Amphibolic: Pathway that involves both anabolic and catabolic reactions.

Primary metabolite: Useful substance produced by a microorganism during its log phase.

Secondary metabolite: Useful substance produced by a microorganism during its stationary phase.

Case study

Francesca Diaz works at a laboratory where the main products are organic acids such as citric acid made using mainly fungal microorganisms although *Corynebacterium* sp. can also be used. The citric acid produced is used mainly in the food and drinks industry but 10% is used by the pharmaceutical industry as a preservative for stored blood.

One of her tasks is to evaluate productivity and cost-effectiveness of each product made. Productivity is measured in $gdm^{-3}hr^{-1}$ and is found by dividing product concentration by fermentation time. She also has to consider the total production time, which includes time to set up and clean the fermenter; sterilisation time; length of the lag phase of the microorganism being used; operation costs including energy use and labour; and capacity of the vessels.

When considering energy costs she calculates the energy used by the motor for the stirrer, heat produced by that motor and by the respiration of the microbes, heat lost from the vessel surface by radiation and by conduction from the joints, and the effect of the cooling jacket. She has to ensure that the vessel is kept at the optimum temperature for the growth of the organism and subsequent production of the metabolite. For short-term fermentations of 8–70 hours' duration, the set-up time and length of lag phase are significant, whereas for long-term fermentations of 3 or more days, they are not significant and can be ignored.

She is also responsible for care of the master strains of microorganisms. Many strains used produce citric acid as a primary metabolite but mutants of *Aspergillus niger* are used commercially because these yield more citric acid per unit time than do strains of *Penicillium*. It is also easier to suppress the secretion of unwanted side-products, such as oxalic acid, gluconic acid and isocitric acid in these strains.

As the working strains are damaged during fermentation, or high-yielding mutants may undergo reverse mutation, a master strain of each microorganism is kept frozen or freeze-dried. A few days before a particular fermentation culture is to be set up, Francesca has to revive some of the preserved master strain and subculture from it into several small flasks. She then makes precultures so that the inoculum for each large fermenter is of an optimum size, otherwise growth of the microorganism in the fermenters will be delayed and this affects production time and costs. She also oversees the production of suitable nutrient media for each fermentation and checks all other parameters, such as optimum temperature, pH, oxygen requirements, stirrers, sensors and computers for sampling and monitoring during the process.

Why is the length of set-up time and lag phase significant for short-duration fermentations but not for longer duration fermentation processes?

Activity: Total and maximal productivity

Some fermentation processes are halted at the time of maximum productivity and some are left to run for longer, depending on the operating costs.

The graph in **Figure 2.2.1** shows data for two fermentation products.

1 For each one calculate the maximal productivity (slope of the graph) and total productivity.
2 Explain which would be halted at maximum activity and which would be run for longer.

Figure 2.2.1: *Graph showing data for two fermentation products.*

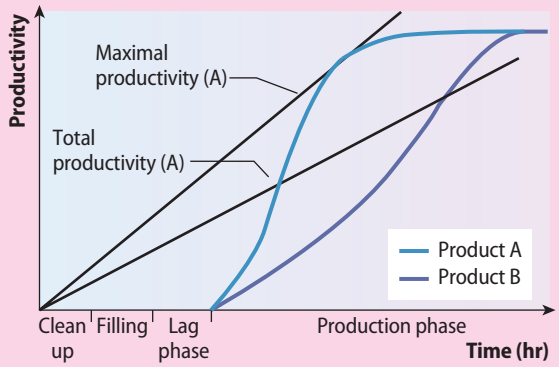


Table 2.2.1: *Some examples of fermentation products.*

Table 2.2.1 shows some examples of fermentation products.

Product	Information
beer	Anaerobic type I fermentation. Uses strains of yeast such as *Saccharomyces cerevisiae* and maltose obtained from malted barley as the substrate. Metabolites other than ethanol impart distinct flavours to the brew.
wine	Anaerobic type I fermentation. Uses strains of yeast that may occur naturally on the grapes or be added to give better consistency of quality. A second, slow fermentation takes place during the ageing process. Other metabolites besides ethanol give distinct aromas and flavours.
lactic acid • yoghurt (milk) • sauerkraut (cabbage) • kimchi (vegetables) • salami (meat) • silage	*Lactobacillus, Streptococcus* and *Leuconostoc* bacteria metabolise sugars in the substrate and produce lactic acid. This lowers the pH and inhibits the growth of other bacteria so preserves the food. It also alters the consistency, texture and flavour by coagulating proteins.
antibiotics	There are many thousands of antibiotically-active materials but about 150 are produced by fermentation and another 50 or so made by the action of enzymes (many derived from microorganisms) on some of these to make semi-synthetic antibiotics. Type III fermentation, usually under aerobic conditions. Antibiotics include bactericides and fungicides and can be used medicinally in humans, in agriculture to treat diseases of animals, and in horticulture to treat diseases of plants. Some antibiotics that are toxic to eukaryotic cells are used to treat tumours.
hormones	Insulin, human growth hormone and human gonadotrophin are some hormones made by genetically modified microorganisms.
amino acids	Many are used in the food and beverage industries to enhance flavours (glutamic acid is used to make sodium glutamate); as antioxidants (cysteine, L-tryptophan and L-histidine) and as sweeteners (aspartame made from aspartic acid and phenylalanine). Proline is used in cosmetics. Various types of bacteria, including *E. coli, Lactobacillus, Klebsiella, Corynebacterium* and *Bacillus* genera are used.

Continued on next page

Product	Information
vitamins	Many vitamins can be obtained from microorganisms but the most commercially cost-productive are vitamin B_{12}, thiamine, riboflavin and ascorbic acid. Bacteria of genera *Bacillus, Streptomyces, Pseudomonas* and *Propioni* are grown on substrates such as glucose or methanol, the latter two bacteria under anaerobic conditions.
enzymes	Microbial rennin has replaced calf rennin for cheese making since the mid-1960s. Lipases and proteases are used in washing powders, glucose isomerise in the production of fructose, pectinases in fruit juice extraction and many enzymes are used in the paper, textiles and animal feed production industries. Streptokinase is a clot buster and glucose oxidase is used by diabetics in biosensors to measure blood glucose levels.
citric acid	This is a primary metabolite used in the food and drinks industry as a flavour enhancer for marmalade, fruit juice and ice cream. In the pharmaceutical industry it is used to preserve stored blood, tablets and ointments. It is also used in cosmetics, as an anti-foaming agent and to soften textiles.
food	Some bacteria produce single cell protein (SCP), from methanol, methane or diesel oil, which can be added to animal feed. Marmite is made from hydrolysing spent yeast; *Spirulina* (a type of cyanobacteria) is eaten in parts of Africa and quorn is a mycoprotein made from *Fusarium venenatum*. This is made during aerobic fermentation and is high in both protein and dietary fibre while low in saturated fat. However, it is low in iron and may be high in purines (derived from nucleic acids), thought by some to increase risk of gout.

Sake (Japanese rice wine) is made from rice grains of high starch content being fermented by the mould fungus *Asperguillus oryzae*. The same species of fungus is used to ferment soy bean curd into paste and soy sauce.

Activity: Making sauerkraut

Shredded cabbage is treated with salt, which plasmolyses the cells causing juice to leave the cabbage tissue. The brine solution allows growth of lactic-acid producing microorganisms, first cocci such as *Leuconostoc mesenteroides* and, when this has produced acid, *Lactobacillus plantarum, Lactobacillus brevis* and *Streptococcus faecalis*.

You will need per group: two cabbages, two bowls, aluminium foil or cling film, wooden boards to fit into the bowl, heavy weights, muslin, pH probe and meter, top pan balance, microscope slides and coverslips, Bunsen burner, inoculating loop, 10 ml disposable pipettes, knife, table salt (uniodised), 0.1M NaOH, methylene blue

1. Remove outer leaves from cabbage heads. Halve, core and wash the heads.
2. Shred the cabbage and weigh it.
3. Weigh out table salt at 3% of the mass of the cabbage.
4. Divide the cabbage into two equal batches.
5. Place layers of cabbage, sprinkling salt between layers, in each bowl.
6. Place the wooden board on top of the cabbage and put the weights onto the board.
7. Cover the bowl with muslin and foil or cling film.
8. Incubate at 30 °C for 14 days.
9. Leave one batch untouched for the whole time.
10. Examine the contents of the other bowl at 3, 7 and 10 days. Examine the contents of both bowls after 14 days.

When you make the examinations, observe and record the colour, aroma, appearance and texture of the fermented cabbage. Make prepared slides of the brine solution, heat fixed and stained with methylene blue and observe using oil immersion to find the shapes and sizes of bacteria present. Use the pH probe to find the pH of the brine solution.

Tabulate all your observations.

- Why do you think the salt should not contain any iodine?

Take it further: Lambic beer

Lambic beer is beer, traditionally brewed in Belgium, where instead of adding carefully selected strains of yeast, the wort (liquid obtained from heating barley malt and wheat) is cooled and exposed to the air so that wild yeasts of the area inoculate it and cause spontaneous fermentation. More than 80 species of yeasts and bacteria are involved in the fermentations to make this beer and most live in the brewery buildings or equipment. Hops are added to inhibit the growth of unwanted bacteria. The beer undergoes secondary fermentation in old wine, port or sherry wooden casks and fruit may be added to counteract the sourness of the beer.

1 Find out the names of some of the species of microorganisms that are used in brewing lambic beer.
2 How do you think consistency of flavours of the beers made in a particular brewery is maintained?

Professional profile

Karen Chapman, Technical brewer

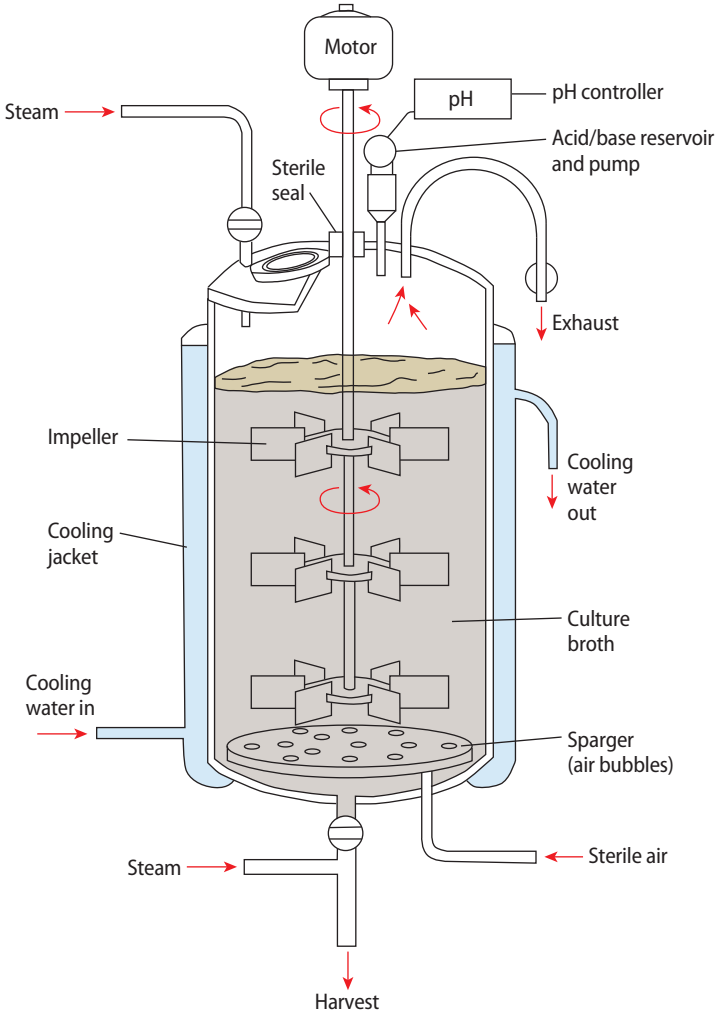
I have a first degree in Microbiology and, after working in a small brewery in Wiltshire under supervision, I studied for and obtained a Master's degree in Brewing Science. I still work in a small brewery – I am responsible for many more areas of the work so the job is more varied, which, at the moment, I personally find more interesting and rewarding.

I manage the production team and I'm ultimately responsible for working with them to maintain or improve the quality of our products. I liaise with suppliers and check the quality of raw materials we buy in. I have to make sure that the plant runs safely and effectively and need to be aware of all current relevant legislation and be computer literate. I have to accurately record sources of all raw materials, production stage timings and the results of quality checks. Sometimes we develop new speciality beers and I may be involved with designing their labels. All staff work shifts and have to work some weekends. I am often on call to solve any problems that may arise. My long-term goal is to obtain a PhD in Food Science and Brewing Technology and become a yeast fermentation specialist.

2 Bioreactors

Use of batch, fed-batch and continuous fermentation has been outlined in *Topic guide 2.1*. For industrial use the most versatile bioreactor is the simple stirred aerated fermenter as shown in **Figure 2.2.2**. There are many different variations on this design but for sizes up to 20 dm^3 they are made of glass and, if larger, are made of stainless steel. As reactors become larger their geometry changes and ratio of height to width varies from 2:1 to 6:1. See the *Case study* for more information.

Figure 2.2.2: *An industrial fermenter, illustrating the construction and facilities for aeration and process control.*



Case study

Ewan Matthews is an engineer who specialises in designing fermenters. The glass or stainless steel must be very smooth so as not to harbour microorganisms and possibly contaminate one batch with a previously used microorganism. The glass must also be strong enough to be sterilised with steam. There must be double mechanical seals on the mixer shafts and effective filters on the exhaust gas outlets to prevent potential pathogens escaping from the fermenter and to prevent entry of contaminants. Phage viruses must be kept out as they will infect and lyse bacterial cells, rendering a whole batch useless. The sampling portals must be designed to avoid spills and to not form aerosols as these provide oxygen-rich environments for microorganisms to thrive, can contaminate large areas of the laboratory and be inhaled by lab personnel. Centrifuges used in downstream processing must also have an airtight seal and their vents must be fitted with filters to prevent microorganisms passing through. All effluent from the fermenters must pass through a steriliser or killing tank containing disinfectants. During downstream processing operators must be aware that they should treat heat exchangers carefully. If the culture fluid freezes it will expand and crack the exchanger. High-quality piping that can be sterilised with steam has to be used and all joints must be securely welded.

Research and industrial laboratories have strict guidelines. Floors, walls and working surfaces must be smooth – this means they do not harbour bacteria and are easily cleaned. There may be two doors at the entrance to provide an

airlock so the lab is never directly open to the outside environment. The air pressure inside the lab is lower than that outside so air, which could carry harmful microorganisms, does not flow out of the lab. Workers may shower before and after each shift and during the shift they may wear protective clothing that is disposed of or sterilised afterwards. Employees may also be asked not to wear make-up or jewellery. Laminar flow cabinets may be used to provide a sterile working area as their airflow hoods filter all air entering and leaving the cabinet.

3 Biotechnology

Genetically modified bacteria can be made using recombinant DNA technology. Bacteria have a circular piece of naked DNA, called a plasmid, as well as their chromosome. Plasmids can act as **vectors** and carry a new gene into a bacterial cell. Genes for antibiotic resistance are present on the plasmids. Restriction endonuclease enzymes obtained from bacteria (they are part of the defences of bacteria to protect them from invasion by phage virus nucleic acids) cut DNA at specific base pair sequences.

Key term

Vector: Carrier. In genetic engineering refers to the structure, such as a plasmid, organism or virus, used to carry a gene into a cell.

Using genetically modified *E. coli* to produce insulin and human growth hormone

- Plasmids obtained from a culture of *E. coli* bacteria are treated with a restriction endonuclease that makes a staggered cut, leaving exposed unpaired bases forming 'sticky ends'. The cut is in a gene for resistance to an antibiotic such as tetracycline but the plasmid will still contain an intact gene for resistance to another antibiotic, such as penicillin.
- The required human gene is obtained. It may be synthesised or made using mRNA obtained from cells producing the protein.
- This mRNA is treated with reverse transcriptase, obtained from retroviruses, to make complementary DNA. In this way the gene coding for the protein has been cloned.
- Nucleotides complementary to those on the sticky ends of the cut plasmids are added to the gene.
- Cut plasmids are mixed with copies of the cloned gene and ligase enzymes. The exposed complementary bases form hydrogen bonds and the DNA backbone joins to form a recombinant plasmid containing the human gene.
- Plasmids and *E. coli* bacteria are mixed. Calcium chloride ions are added to make the bacterial cell walls more permeable and the bacteria are subjected to heat shock treatment – a minute at 0 °C followed by a minute at 40 °C.
- Some bacteria will take up plasmids (these are transformed) and some will not. However, some will take up plasmids that have been cut but sealed up again without taking up the human gene.
- The culture is plated out onto agar plates that contain penicillin. The bacteria that grow have taken up a plasmid. Sterile velvet is pressed onto the agar plate to transfer bacteria from each colony onto another agar plate containing tetracycline. Colonies that grew on the first plate but not on the second are the ones that contain the transformed bacteria. These are used to inoculate sterile culture medium in a fermenter and the protein they secrete is obtained from the medium by downstream processing.

Activity: Flow diagram

Make a large annotated flow diagram to show the stages of producing genetically modified bacteria to produce insulin.

Insulin is used to treat patients with type I diabetes. Human growth hormone treats patients with pituitary dwarfism.

Blood clotting factors

These molecules are too large to be made in bacterial cells. Transgenic sheep have a copy of the human gene inserted into their embryonic cells, next to a promoter region for a gene coding for a milk protein. This means that the gene is only expressed in mammary tissue and the blood clotting factor (factors VIII and IX to treat haemophiliacs) can be extracted from the sheep milk.

Research is being carried out into producing factor VII (to treat a very rare form of haemophilia) in genetically modified *Tilapia* fish. The inserted gene is near a promoter region that acts as a switch so that the gene is only expressed in the fish liver and the protein passes into the fish blood. Genetically modified tobacco plants can also make this protein. The bacterium *Agrobacterium tumefaciens* has the human gene inserted and is the vector, carrying the gene into tobacco plants. The tobacco leaves can be ground up and the protein extracted. Research groups are investigating using genetically modified silk worm larvae or chicken eggs to make this blood clotting factor.

Professional profile

Katie Webb, Pharmaceutical Research and Development

I work at an R&D site in southeast England, researching ways to control oral bacteria that cause dental caries. I've tested many antibacterial products on the two main bacterial culprits – *Streptococcus mutans* and *Lactobacillus*. I've also exposed these bacteria to substances that do not kill them but prevent them from producing plaque acids. I'm now looking at ways of preventing plaque formation on the surfaces of dentures.

My supervisor always listens to my ideas on developing new ways to assess the antibacterial properties of the toothpastes and mouthwashes that the company makes. The company is also paying for me to study, on a part-time basis, and obtain a Master's degree in Microbiology.

Portfolio activity (2.3)

Using the kit provided, you can transform bacteria to take up the jellyfish gene so that the colonies will glow under UV light.

For your portfolio write a report of this protocol and record your observations.

4 Water management

Treatment of sewage effluent

Traditional sewage treatment involves screening out of large items of rubbish and then allowing sludge to settle out. This sludge is then anaerobically digested by bacteria and the methane generated in the process can be used for fuel. The liquid effluent is trickled over a percolating filter bed that supplies a large surface area for aerobic bacteria to digest organic matter. The treated effluent can then enter rivers.

Figure 2.2.3: *Mersey Valley Processing Centre – sludge treatment and incineration plant, Widnes, Cheshire.*

In some sewage treatment plants, such as the one shown above, the liquid effluent is placed in large aerated tanks and some sludge, containing bacteria, is added. The bacteria digest organic matter. However, these large tanks take up a lot of space, are open, which means that they release odours, and are difficult in terms of process control. Increasingly the contaminants such as heavy metals and other compounds in sewage sludge are becoming a problem and research is being carried out into how to introduce specific cultures of bacteria to metabolise specific compounds from industrial wastes, as well as compounds from household chemicals that enter the sewage system. See the Case study for more information.

Tower reactors, tubular loop reactors and airlift fermenters, which are all types of activated sludge fermenters, take up less space, have lower energy costs and reduced investment costs and have improved oxygen transfer, making the digestion process more efficient.

Three groups of bacteria take part in the anaerobic process.

- The first group, with *Clostridia* (obligate anaerobes), *Streptococci* and enteric bacteria (facultative anaerobes), digest starches, lipids and proteins to organic acids.
- The second group convert the organic acids to acetic acid, hydrogen and carbon dioxide.
- The third group are strict anaerobic methanogens. They grow slowly and, using hydrogen as the energy source and carbon dioxide as the carbon source, produce methane.

Case study: Waste Water Treatment Works Operator

Damian Evans works for Severn Trent Services at a sewage works in East Anglia. He has five years' experience of sewage treatment, maintenance procedures and safe working practices. He has a good understanding of the processes involved in sewage treatment. He works various shifts including some weekends and bank holidays as he has to carry out the routine operating procedures at the plant and be able to solve any problems as they arise. He enjoys working outdoors although there is some clerical work reporting on maintenance, problems and their resolution.

Drinking water

Water used to produce drinking water is tested at every stage for pollutants such as heavy metals, organic toxins and microorganisms.

Water is pumped from source into holding tanks where it is screened to remove large debris. Flocculating agents, for example, aluminium sulfate, are added to clump suspended particles, such as clay, silt, algae, bacteria and viruses.

As the water passes into a sedimentation tank these clumped particles settle out. The settled sludge is removed and treated as the liquid effluent passes into another tank where air is bubbled through and attaches to any remaining floc causing it to float up to the surface for removal.

The water is then filtered, either through rapid or slow sand filters to remove suspended particles. It is filtered next through a membrane that removes any particles greater than 0.2 µm. Ions can be removed by ion exchange resin and then **disinfection** using chlorination, **chloramine**, hydrogen peroxide or UV light kills any remaining microorganisms.

Key terms

Chloramine: Derivative of ammonia where one or more hydrogen atoms is replaced with chlorine atoms.

Disinfection: The process of destroying vegetative pathogens but often not their endospores, or viruses. Disinfectants reduce microbial growth but do not sterilise. Disinfectants are used on non-living surfaces, whereas antiseptics are used on surfaces, for instance, skin, of living organisms.

Professional profile

Priya Gupta, Microbiology Department, Institute of Freshwater Ecology: Freshwater ecologist

I work in the microbiology department at the Institute of Freshwater Ecology. I began working there as a laboratory technician where I made up the media for investigations, washed equipment and carried out many microbiological techniques including sub-culturing. I have received in-house training in extraction, identification and manipulation of bacterial DNA. After being promoted, I am now responsible for maintaining equipment and laboratories and for ordering chemicals. I also maintain the strains of bacteria that are used for research and carry out some investigations, for which I have to keep meticulous records. I have been trained in the use of radioactive isotopes, so I have gained many transferable skills in molecular biology techniques. I am also studying part-time at the Open University for a degree in Environmental Science. I hope eventually to work abroad and I am investigating the possibility of working for the World Wide Fund for Nature (WWF) as a freshwater scientist.

Activity: Presentation

Choose one of the topics below and prepare a PowerPoint® or poster presentation for the rest of your class:

- sewage treatment
- water treatment
- production of antibiotics
- brewing beer
- making wine
- yoghurt and cheese making.

Checklist

In this topic you should now be familiar with the following ideas:

- ✓ microorganisms are grown in fermenters to make a variety of products including beer, wine, soy sauce, yoghurt, antibiotics, hormones, vitamins, enzymes and food.
- ✓ microorganisms can be genetically modified to produce human proteins for medicinal treatment
- ✓ transgenic mammals are used to produce large human proteins for medicinal treatments
- ✓ bacteria are used in the digestion of sewage
- ✓ water for drinking is treated to make sure there are no harmful microorganisms or pollutants present.

Further reading

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Acknowledgements

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