UNIT 1
APPLICATIONS OF BIOLOGY

INTRODUCTION

- The applications of biology in our daily to day life are numerous.
- It helps us to understand every living organism, from the smallest bacteria to the blue whales.
  - Biologists often focus on a different subset of living organisms, such as birds, plants, or bacteria, to determine their character.
- Biological science is very useful science to determine where some diseases and pests come from (such as infections, animal pathologies, and damage to plants).
- Biology covers the study of:
  - the functions of living organisms
  - the evolution of species
  - the factors that produce diseases, as well as

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  - the discovery of new drugs

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- Biology allows human to explore fields such as genetic engineering, research applications with mother cells, and global warming.
- Biology helps to understand:
  - How living things evolve
  - Nature and how humans, animals and plants interact in life.
  - The rates of species extinction and
  - How a species depends on and affects the habitat where they lives improves the effectiveness of conservation efforts
- A practical application of biology in which most people are familiar is hand washing, traditional fermentation and domestication.
  - Regular soap washing removes pathogens from the skin and helps to control the spread of infectious diseases.
- Another application of biology is the set of instructions given to take all the pills of antibiotics in a recipe.

1.1 Applications in Conservation of Natural Resources

- **Conservation** is the careful maintenance and wise use of natural resources to prevent them from disappearing.
- **Natural resources** are physically supplies that exist in nature and include soil, water, air, plants, animals and energy.
- **Conservation biology** is a mission-oriented science that focuses on how to protect and restore biodiversity on Earth.
- Natural resources can be classifies as renewable and non-renewable.

Renewable resources:

- Are mainly living things and their products.
- The main sources of renewable resources are sun, wind, water, geothermal and biomass.
- When managed carefully, they can be used, reused and replaced.
- Examples include: crop plants, trees, cattle and chickens.

Non-renewable resources:

- Are natural substances that are not replaced at the same speed as that are used up.
- They are finite resources.
- Once used, they cannot be easily made or replaced
- Examples include: metals such as gold and iron and fossil fuels such as oil, natural gas and coal.

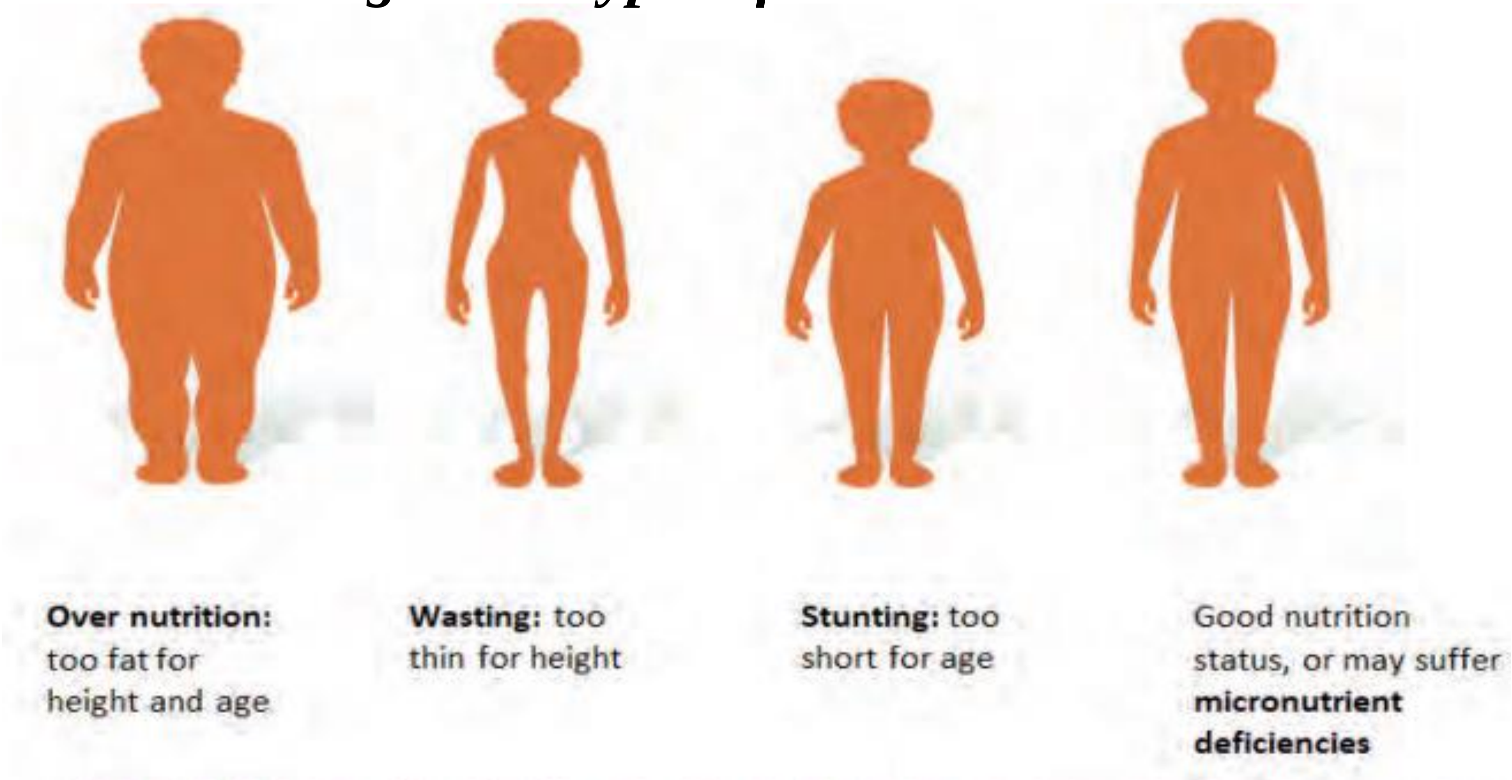
- Renewable resources can be lost (extinct), if we do not manage them carefully.
- Therefore, biologists takes a position in conserving species and saving them from extinction through:
  - The preservation of animals and plants in terms of zoos and seed banks and
  - By stopping the destruction of their natural habitats so the populations are able to thrive.

- To protect our natural resources, people have become more aware of the need for conservation of natural resources.
- Conservation is the protecting and preserving our natural environment so that:
  - Non-renewable resources are used sparingly, and
  - Renewable resources should be appropriately managed. Otherwise, they can last for an extended period of time in the future.

1.2 Food and Nutrition Security

- **Food security** is the state in which all people have physical, social and economic access to sufficient, safe and nutritious food.
- **Food Security**, as defined by the United Nations Committee on World Food Security (UNCWFS),
  - Is a state in which when all the people have physical, social and economic access to sufficient, safe and nutritious food that meets their food preferences and dietary needs for an active and healthy life at all time.
- However, **Food Insecurity** is often rooted in poverty and has long-term impacts on the potential of families, communities and countries to develop and prosper.
- Prolonged undernourishment, stunts growth, slows cognitive development and increases susceptibility to illness.

Figure 1: Types of Malnutrition



- **Nutrition security** is a situation whereby individual have:
  - Access to sufficient, safe and nutritious foods
  - Safe water and adequate sanitation
  - The ability to access health care services, and knowledge of household and community practices in child care
  - Food storage, preparation and hygiene.

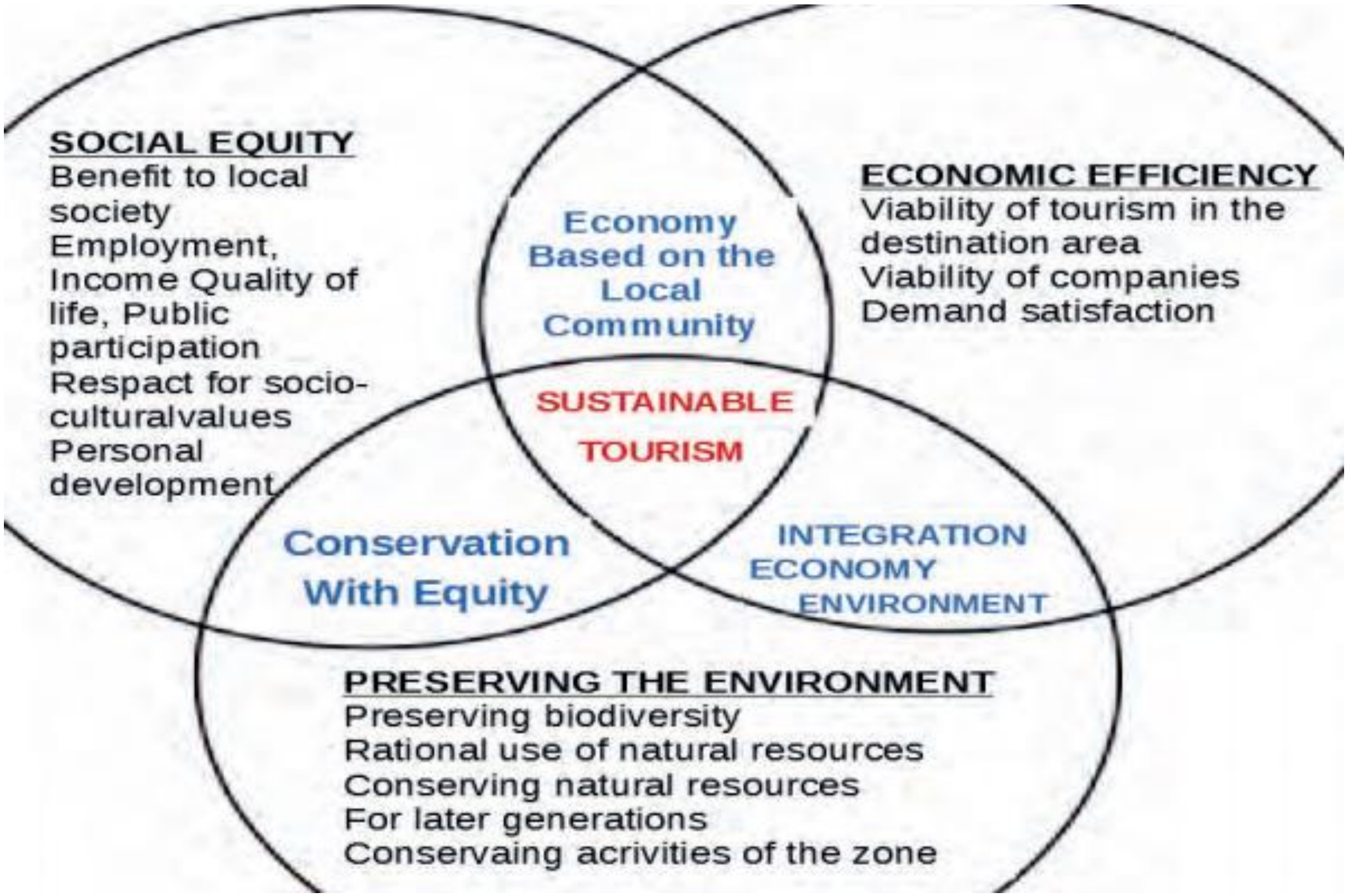
- Therefore, food security is ensured only if:
  - Enough food is available for all in a country;
  - When all individuals have the capacity to buy acceptable quality food, and
  - When there is no barrier to access food.
- Therefore, Biology plays a key role in:
  - Producing high-nutrient staple crops
  - Developing new products that can combat malnutrition, and thereby improving food utilization.
  - Designing the manufacturing processes and machinery used to produce food and drink in large quantities.
- Biologists work to ensure food security, through these innovations.

1.3 Creating Conscious Citizens and Ensuring Sustainable Development

- **A conscious citizen:**
  - Is one who places value on being fully human while connecting with a higher purpose
  - One who values human life and the relationship with all living things and
  - One who takes the responsibility for transforming skills into action through ethical decision making, to ultimately improve life and living on the planet.
- Biology has a vital role in creating a conscious citizen by expanding awareness of the social, global and environmental conditions.

- Biology empowers conscious citizens’ to assume personal responsibility by engaging in and being committed to initiating positive impact.
- The interplay of biotechnology is vital to facilitating sustainable development initiatives and conscious citizens will use biotechnology to improve life and living on the planet.
- Conscious citizen biologists:
  - Develop innovative and cost effective bio-based technologies which consume fewer resources, incorporate recycling, reuse components and reduce production of wastes, and
  - Use strategies for sustaining a greener earth and improving food production.

Figure 2: a model for sustainable development



- According to the World Conservation Union, 2006, the three dimensions of sustainability (economic, social and environmental) are represented either as pillars, embedded circles or in the popular Venn diagram of three overlapping circles
- The conscious global citizens (biologists) in Ethiopia involved in sustainable development through protecting, managing and monitoring the existing resources of our land including:
  - Analyzing soil, water and air for chemical pollution
  - Finding ways to clean up pollution
  - Identifying, recording and monitoring the plants and animals that share the land we use

1.4 Applications in Biotechnology

- **Biotechnology** is the application of technologies that involve the use of living organisms, or products from living organisms, for the development of products that benefit humans.
- **Genetically modified organisms (GMOs)** have received genetic material via recombinant DNA technology.
  - If an organism has received genetic material from a different species, it is called **a transgenic organism**.
  - A gene from one species that is introduced into another species is called **a transgene**.
- Crops can be modified to increase yields & to obtain novel products.
- Biotechnology can be used in:
  - The prevention and mitigation of contamination from industrial, agricultural and municipal wastes.
  - The diagnosis and treatment of diseases

I. Applications of Biology for Food Processing and Production

- This method involves the increasing of food productivity using microorganisms.
- ***A technology that shows some promise in increasing world food productivity. For example,***

➔ **Single Cell Protein (SCP)** is produced from waste materials such as molasses from sugar refining, petroleum by-products, and agricultural wastes.

➔ In developed countries, an animal feed called **Pruteen** is produced by mass culture of the bacterium *Methylophilus methylotrophus.*

➔ **Mycoprotein** is made from the fungus *Fusarium venenatum*. Its filamentous texture makes it a meat substitute product for human consumption.

- Health food stores carry bottles of dark green pellets or powder that is a culture of a spiral-shaped **cyanobacterium** called ***Spirulina***.
  - ***Spirulina*** is harvested from the surface of lakes and ponds.
  - ***Spirulina*** has become a viable alternative to plants as a primary nutrient source in parts of Africa, Asia, and Mexico. It can be eaten in its natural form or added to other food stuffs.
- ***Vitamins are also produced using biotechnology.***
- **Vitamin C** was the first vitamin to be produced during a fermentation process by using bacteria.
- Previously, **Vitamin B12** (**Cyanocobalamin**) and **B2** (**Riboflavin**) were obtained from animal liver extract.
- Nowadays the production of **vitamin-B12** involved fermentation by **propionic bacteria**.
  - In nature vitamin B2 is found in cereals, vegetables and yeast but the yield of B2 can be enhanced hundred to three hundred fold by using microbes.

A. Dairy Products

- ***Microorganisms are used in making a wide variety of dairy products.***

- **Cultured buttermilk** is made by adding ***Streptococcus cremoris*** to pasteurized skim milk and allowing fermentation to occur until the desired consistency, flavor, and acidity are reached.
  - Other organisms; *Streptococcus lactis*, *S. diacetylactis* and *Leuconostoc citrovorum*, *L. cremoris*, or *L. dextranicum* make buttermilk with different flavors because of variations in fermentation products.
- **Sour cream** is made by adding one of these organisms to cream.
- **Yogurt** is made by adding ***Streptococcus thermophilus*** and ***Lactobacillus bulgaricus*** to milk.
  - These organisms release other products, and so yogurt has a different texture and flavor.

- **Fermented milk** have been made for centuries in various countries, especially Africa, Asia, and Eastern European.
  - The products vary in acidity and alcohol content.
- **Acidophilus milk** is made by adding ***Lactobacillus acidophilus*** to sterile milk.
  - Sterilization prevents uncontrolled fermentation by organisms that might already be present in non-sterilized milk.
- **Bulgarian milk** is made by ***Lactobacillus bulgaricus***.
  - It is similar to buttermilk except that it is more acidic and lacks the flavor imparted by the **leuconostocs**.

B. Fermented Meats

- Microbes such as ***Lactobacillus plantarum*** and ***Pediococcus cerevisiae*** add flavor by fermenting meats such as salami, summer sausage, and Lebanon bologna.
- The **heterolactic acid** fermentation preserve the meat and gives it a tangy flavor.
- Fungi such as **Penicillium** and **Aspergillus**, growing naturally on the surfaces of country hams, help to produce their distinctive flavor.

C. Production of Beer, Wine, and Spirits

- **Beer** and **wine** are made by fermenting sugary juices.
- **Spirits** (such as whiskey, gin, and rum) are made by fermenting juices and distilling the fermented product.
  - **Distillation** separates alcohol and other volatile substances from solid and nonvolatile substances.
- Strains of **Saccharomyces** are the fermenters for all alcoholic beverages.

Production of Beer

- *To make beer, cereal (usually barley) are malted (partially germinated) to increase the concentration of starch-digesting enzymes that provide the sugar for fermentation.*
- *Malted grain is crushed and mixed with hot water (about 65°C), producing mash.*

- *After a few hours, a liquid extract called* ***wort*** *is separated from the mix.*
- *Hops (flower cones from the hop plant) are added to the wort for flavoring, and the mixture is boiled to stop enzyme action and precipitate proteins.*
- *A strain of Saccharomyces is added.*
- *Fermentation produces:*
  - *Ethyl alcohol,*
  - *Carbon dioxide, and other substances, including*
  - *Amyl and Isoamyl alcohols and*
  - *Acetic and Butyric acids, which add to the flavor of the beer.*
- *After fermentation, the yeast is removed, and the beer is filtered, pasteurized, and bottled.*

Production of Wine

- *Most **wine** is made from juice extracted from **grapes**, although it can be made from any fruit and even from nuts or dandelion blossoms.*
- *Juice is treated with **sulfur dioxide** to kill any wild yeasts that may already be present.*
- *Sugar and a strain of Saccharomyces are then added, and fermentation proceeds.*
- *Although **ethyl alcohol** is the main product of fermentation, other products similar to those in beer add to the flavor of the wine.*
- *In both beer and wine, the particular characteristics of the juice and the yeast strain determine the flavor of the final product.*
- *When fermentation is completed, liquid wine is siphoned to separate it from yeast sediment and, if necessary, cleared with agents such as charcoal to remove suspended particles.*
- *Finally, it is bottled and aged in a cool place.*

Production of Spirits

- ***Spirits*** *are made from the fermentation of a variety of foods, including: malted barley (Scotch whiskey), rye (Rye whiskey, gin), Corn (Bourbon), wine or fruit juice (Brandy), potatoes (Vodka), and molasses (Rum).*
- *After fermentation, distilling separates alcohol and other volatile substances that impart flavor from the solid and nonvolatile substances.*
- *Because of distillation, the alcohol content of spirits ranges from 40 to 50% much higher than the typical 12% for wine and 6% for beer.*

D. Bread Making

- Microorganisms accomplish three functions in bread making:
  1. Leavening the flour-based dough
  2. Imparting flavor and odor and
  3. Conditioning the dough to make it workable
- **Leavening** is achieved primarily through the release of gas to produce a porous and spongy product.
- Although various microbes and leavening agents can be used, the most common ones are various strains of the baker's yeast called ***Saccharomyces cerevisiae***.
- Other gas-forming microbes such as **coliform** bacteria, **Clostridium** species, **heterofermentative** lactic acid bacteria, and **wild yeasts** can be employed, depending on the type of bread desired.
- Yeast metabolism requires a fermentable sugar such as maltose or glucose.

- Because the yeast **respires aerobically** in bread dough, the chief products of maltose fermentation are CO_2 and H_2O.
- Other contributions to bread texture come from **kneading**, which incorporates air into the dough and from microbial enzymes, which break down flour proteins (**gluten**) and give the dough elasticity.
- Besides **CO_2** production, bread fermentation generates other **volatile organic acids** and **alcohols** that impart delicate flavors and aromas. These are especially well developed in home-baked bread, which is leavened more slowly than commercial bread.
- Yeasts and bacteria can also impart unique flavors, depending upon the culture mixture and baking techniques used.
  - The pungent flavor of **rye bread**, for example, comes in part from starter cultures of **lactic acid bacteria** such as *Lactobacillus plantarum*, *L. brevis*, *L. bulgaricus*, *Leuconostoc mesenteroides* and *Streptococcus thermophilus*.
- **Sourdough bread** gets its unique tang from ***Lactobacillus sanfrancisc***.

1.5 Genetic Engineering

- **Genetic engineering** is the process of transferring DNA from one organism into another that results in a genetic modification (production of a **transgenic organism**).
- Genetic engineering is being used in the production of:
  - Pharmaceuticals
  - Gene therapy
  - Development of transgenic plants and animals.

A. Animal Breeding, Transgenic Animals, Plants and Disease, and Pest Management

- Selecting for breeding animals with superior traits in growth rate, egg, meat, milk, wool production, or other desirable traits has revolutionized the livestock and plant production throughout the entire world.

- There are many potential applications of transgenic methodology in developing new and improved strains of livestock.
- Practical applications of transgenic technology in livestock production include:
  - Enhancing the prolificacy and reproductive performance
  - Increasing feed utilization and growth rate
  - Improving carcass composition
  - Improving milk production and/or composition
  - Modification of hair or fiber and
  - Increasing disease resistance in animals
- The development of transgenic farm animals allow flexibility in the direct genetic manipulation of livestock.
- Gene transfer is a relatively rapid way of altering the genome of domestic livestock

B. Tissue culture

- **Tissue culture**, a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function.
- The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ.
- By using tissue culture technique or technology:
  - Large quantity of plants can be propagated quickly. Plants produced by this technique include, palm trees, orchids, bananas, and carrots.
  - Large quantity of food with desired quality can be produced in reasonably little area

- Therefore, tissue culture is an important biotechnology for developing countries for:
  - The production of disease-free and high quality planting material
  - The rapid production of many uniform plants

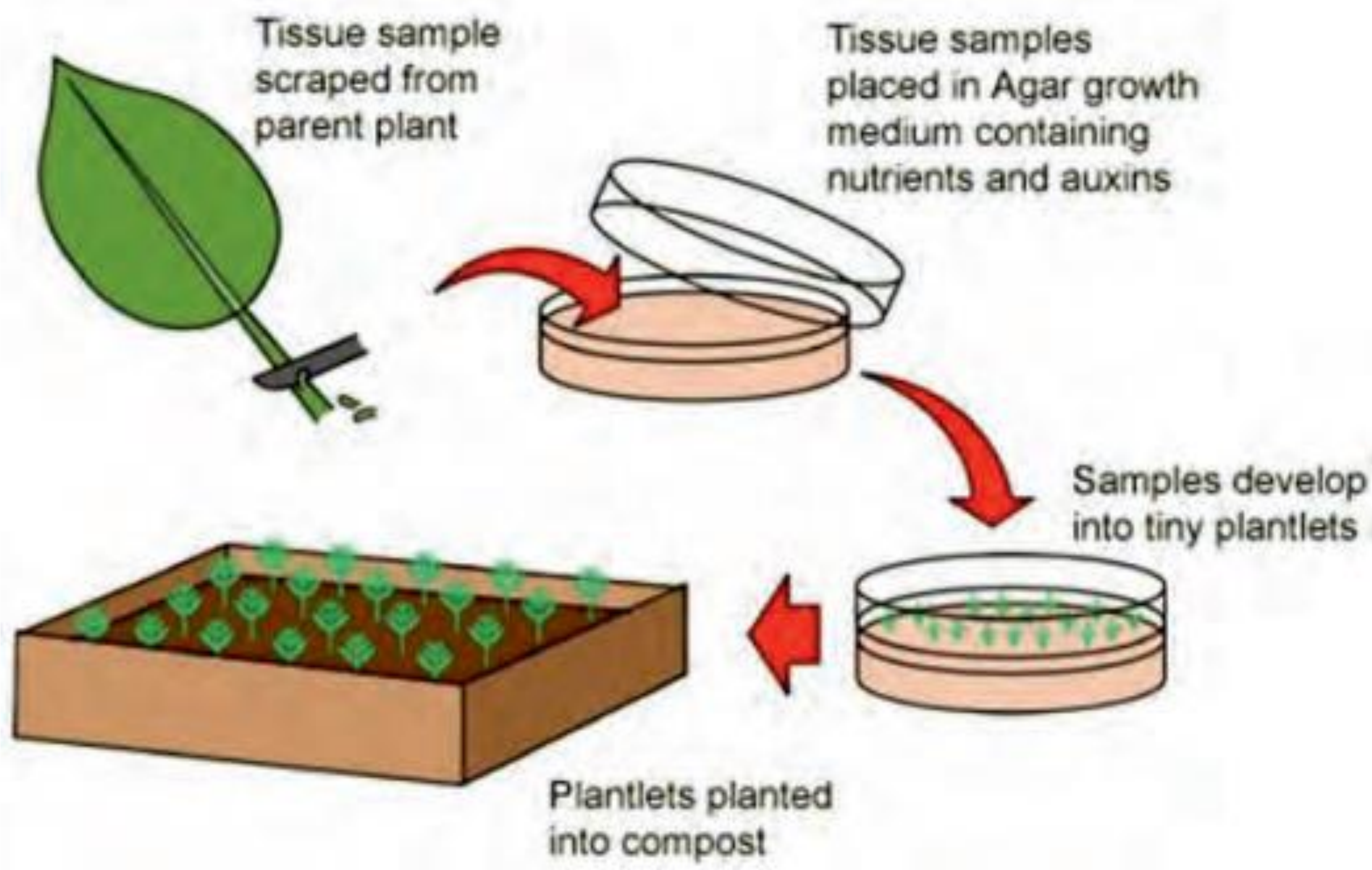


Figure 3: Techniques of tissue culture

1.6. Health and Wellbeings

- Human drugs such as:
  - **Insulin** for diabetics
  - **Growth hormone** for individuals with pituitary dwarfism, and
  - **Tissue Plasminogen Activator** for heart attack victims
- As well as animal drugs like:
  - **Growth hormones**, **Bovine** or **Porcine somatotropin**, are being produced by the fermentation of transgenic bacteria that have received the appropriate gene from human, cow, or pig.

A. The manufacture of Antibiotics

- When micro-organisms are used for the production of antibiotics, it is not their fermentation products that are wanted, but complex organic compounds, called **antibiotics**, that they synthesize.

- Most of the antibiotics we use come from bacteria or fungi that live in the soil. The function of the antibiotics in this situation is not clear.
  - One theory suggests that the chemicals help to suppress competition for limited food resources, but the evidence does not support this theory.
- One of the most prolific sources of antibiotics is **Actinomycetes**.
  - Actinomycetes are filamentous bacteria, that resemble microscopic mould.
- The actinomycete ***Sireptomyces*** produces the antibiotic known as **Streptomycin**.
- Perhaps the best known antibiotic is **Penicillin**, which is produced by the mould fungus **Penicillium** and was discovered by Sir Alexander Fleming in 1928.

- **Penicillin** is still an important antibiotic but it is produced by mutant forms of a different species of **Penicillium** from that studied by Fleming.
  - The different mutant forms of the fungus pencillium produce different types of penicillin.
  - The penicillin types are chemically altered in the laboratory to make them more effective and to tailor them for use with different diseases.
  - Examples include: Ampicillin, Methicillin and Oxacillin.
- ***Antibiotics attack bacteria in a variety of ways:***
  - Some of them **disrupt the production of the cell wall** and so **prevent** the bacteria from **reproducing**, or even cause them to burst open
  - Some **interfere** with **protein synthesis** and thus arrest bacterial growth.

- Those antibiotics that:
  - Stop bacteria from reproducing are said to be **Bacteriostatic.**
  - Kill the bacteria are **Bacteriocidal**.
- Animal cells do not have cell walls, and the cell structures involved in protein production are different.
- Consequently, antibiotics do not damage human cells although they may produce some side-effects such as allergic reactions
- Some **vaccines** are also adaptable to mass production through fermentation.
  - Vaccines for Bordetella pertussis, Salmonella typhi, Vibrio cholerae, and Mycobacerium tuberculosis are produced in large batch cultures.
  - Corynebacterium diphtheriae and Clostridium tetani are propagated for the synthesis of their toxins, from which toxoids for the DT vaccines are prepared.

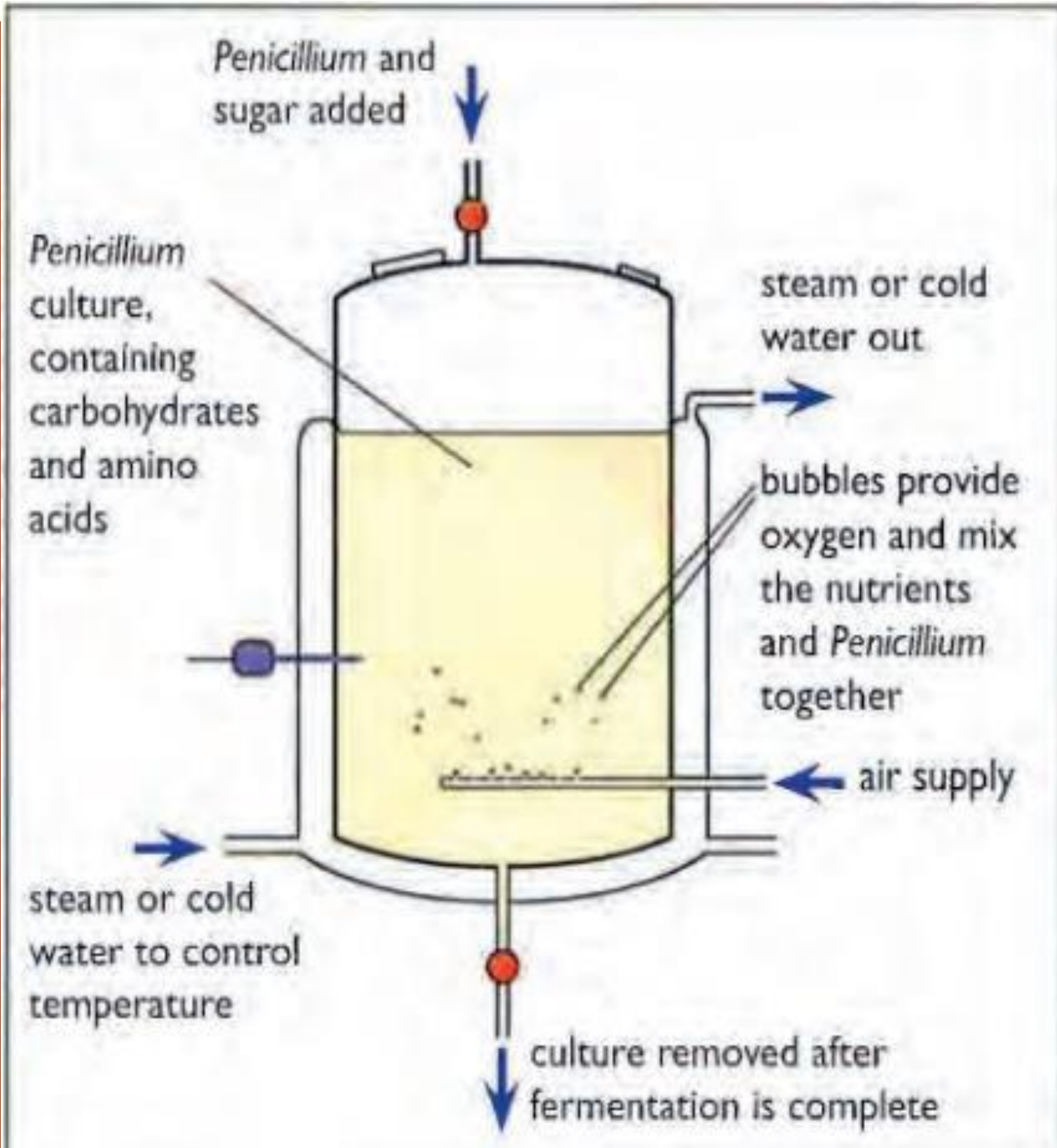


Figure 4: A fermenter used for producing penicillin

B. Biosensors

- A rapidly developing area of biotechnology, arousing intense international scientific interest, is that of biosensor production.
- In this field of bioelectronics, living microorganisms (or their enzymes or organelles) are linked with electrodes, and biological reactions are converted into electrical currents by these biosensors.
- Biosensors are being developed to:
  - Measure specific components in beer
  - Monitor pollutants and
  - Detect flavor compounds in food
  - Measure the concentration of substances from many different environments
- Applications of biosensor include the detection of glucose, acetic acid, glutamic acid, ethanol, and biochemical oxygen demand.

- The applications of biosensor include:
  - The detection of glucose, acetic acid, glutamic acid, ethanol, and biochemical oxygen demand.
  - To measure cephalosporin, nicotinic acid, and several B vitamins has been described.
- Recently biosensors have been developed using **immunochemical-based detection system**.
  - These new biosensors will detect pathogens, herbicides, toxins, proteins and DNA.
  - Many of these biosensors are based on the use of a **streptavidin-biotin recognition system**.
- One of the most interesting recent developments using these approaches is a **handheld aflatoxin detection system** for use in monitoring food quality.

- This automated unit, based on a new column-based **immunoaffinity fluorometric procedure**, can be used for 100 measurements before being recharged.
  - The unit can detect from 0.1 to 50 ppb of aflatoxins in a 1.0 ml sample in less than 2 min. Aflatoxins.
- Rapid advances are made in all areas of biosensor technology. These include major improvements in:
  - The **stability** and **durability** of these units, which are being made **more portable** and **sensitive**.
- Microorganisms and metabolites such as glucose can be measured, thus meeting critical needs in modern medicine.

C. Forensic Science

- Forensic biologists inspect crime scenes to examine potential sources of evidence using blood, saliva, and hair, and then they analyze the specimens in a laboratory, focusing on DNA analysis.
- Additionally, Fingerprints are important tools to **investigate crime** and determine the **paternity case** of a child
  - This is because each individual has unique fingerprints that do not change throughout life.
- After investigations, forensic biologists write up their findings in technical reports called **upon to testify in court**.
- Finally, this data is used to investigate the related transgression, and are put forward in the court that helps to castigate the criminal.
- These days **bioinformatics** is widely acceptable in forensic science because, with the help of computational tools, it has become easer and reliable to gather evidence regarding a particular crime scene.

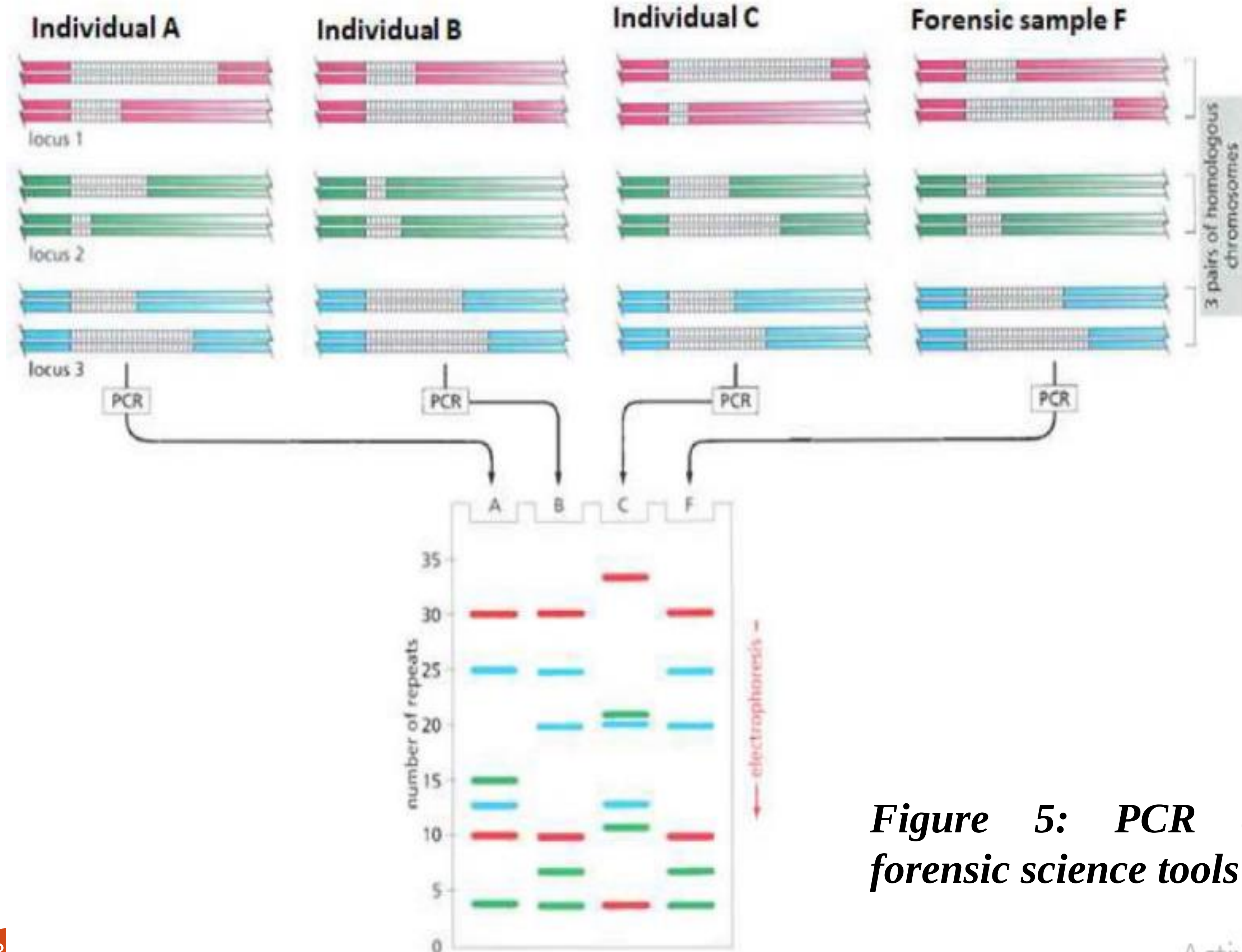


Figure 5: PCR as forensic science tools

- When examining the variability at 5-10 different Variable Number of Tandem Repeat (VNTR) loci, the odds that two random individuals would share the same genetic pattern by chance can be approximatel1y 1 in 10 billion.
- In the case shown in the figure 5,
  - Individuals A and C can be eliminated from further enquiries
  - Whereas individual B remains a clear suspect for committing the crime.

- A similar approach is now routinely used for paternity testing.
- As shown in the figure 6 below, the gel electrophoresis results of two-suspected paternity tests.
  - Therefore the suspected person (DNA of suspected 2) is the father of the baby (victim).

Figure 6: PCR in forensic application

Applications in Biomining

1. Microbiological Mining

- As the availability of mineral-rich ores decreases, methods are needed to extract minerals from less concentrated sources. This need spawned the new discipline known as **Biohydrometallurgy**.
    - ***Biohydrometallurgy is the use of microbes to extract metals from ores.***
- Copper and other metals originally leached from the wastes of ore crushing as a result of inorganic chemicals reaction such as those reactions used to extract metals from ores.
    - This leaching was due to the action of ***Thiobacillus ferrooxidans***.
    - This **chemolithotrophic acidophilic** bacterium lives by oxidizing the sulfur that binds copper, zinc, lead, and uranium into their respective sulfide minerals, with a release of the pure metal.

- **Copper** in low-grade ores is often present as **copper sulfide**.
  - When acidic water is sprayed on copper sulfide ore, *T. ferrooxidans* obtains energy as it uses atmospheric oxygen to oxidize the **sulfur** atoms in sulfide ores to **sulfate**.
  - The bacterium doesn't use the copper; it merely converts it to a water-soluble form that can be retrieved and used by humans.
- **Iron** can be degraded by *T. ferrooxidans*, it releases iron from iron sulfide by the same process.
  - Combinations of *T. ferrooxidans* and *T. thiooxidans* degrade some **copper** and **iron ores** more rapidly than either one does alone.
  - Another combination of *Leptospirillum ferrooxidans* and *T. organoparus*, degrades pyrite (FeS2) and chalcopyrite (CuFeS2), although neither organism can degrade the minerals alone.
- Other bacteria can be used to mine uranium, and bacteria may eventually be used to remove arsenic, lead, zinc, cobalt, and gold.
- However, of late, fewer mining companies are actually using microbes in their processing.

Application in the Environment

A. Solid waste treatment: Composting and Landfill

- Most of it ends up in landfill sites- huge holes in the ground where refuse is deposited to prevent it being a hazard.
  - The **non-biodegradable** components (metals, plastics, rubble, etc.) remain there more or less indefinitely.
  - However, **biodegradable** material (food waste, textiles, paper, etc.) undergoes a decomposition process, over a period of time.
- The rate at which this happens is dependent on:
  - The nature of the waste and
  - The conditions of the landfill, but can take several decades.
- Aerobic processes give way to anaerobic ones and a significant result of the latter is the generation of **methane**.

- Modern landfill sites incorporate systems that remove methane to prevent it being a fire/explosion hazard, and may put it to good use as fuel source.
- Many householders separate organic wastes such as vegetable peelings and grass cuttings and use them to make **compost**.
- This practice, apart from providing a useful gardening supplement, also substantially reduces the volume of material that has to be disposed of by other means.
- Fungi and bacteria, particularly **actinomycetes**, break down the organic matter to produce CO2, water and humus (a relatively stable organic end product).
- Compost is not really a fertilizer, because, its nitrogen content is not high, but it provides nutrients to a soil and helps to improve its condition.
- **Composting** is carried out on a large scale by local authorities using the waste generated in municipal parks and gardens.

B. Wastewater Treatment

- The aim of wastewater treatment is the removal of undesirable substances and hazardous microorganisms in order that the water may safely enter a watercourse such as a river or stream.
- Wastewater treatment is fundamental to any society, because it reduces the incidence of **waterborne** diseases such as cholera.
- Wastewater may come from domestic or commercial sources; highly toxic industrial effluents may require pretreatment before entering a water treatment system.
- **Sewage** is the term used to describe liquid wastes that contain faecal matter (human or animal).
- The effectiveness of the treatment process is determined mainly by the reduction of the wastewater's **BOD (*biochemical oxygen demand)*.**
  - **BOD** is a measure of the amount of oxygen needed by microorganisms to oxidize its organic content.

- A **high BOD** leads to the removal of oxygen from water, which is a certain indicator of pollution.
- ***Wastewater treatment usually occurs in different stages:***

First stage (Primary treatment):

- Is purely physical
- It involves the removal of floating objects followed by sedimentation
  - **Sedimentation** is a process that removes up to one-third of the BOD value

Second stage (Secondary treatment)

- Involves microbial oxidation, leading to a substantial further reduction in BOD.
- This may take one of two forms, both of which are aerobic;
  - The traditional trickling filter and
  - The more recent activated sludge process

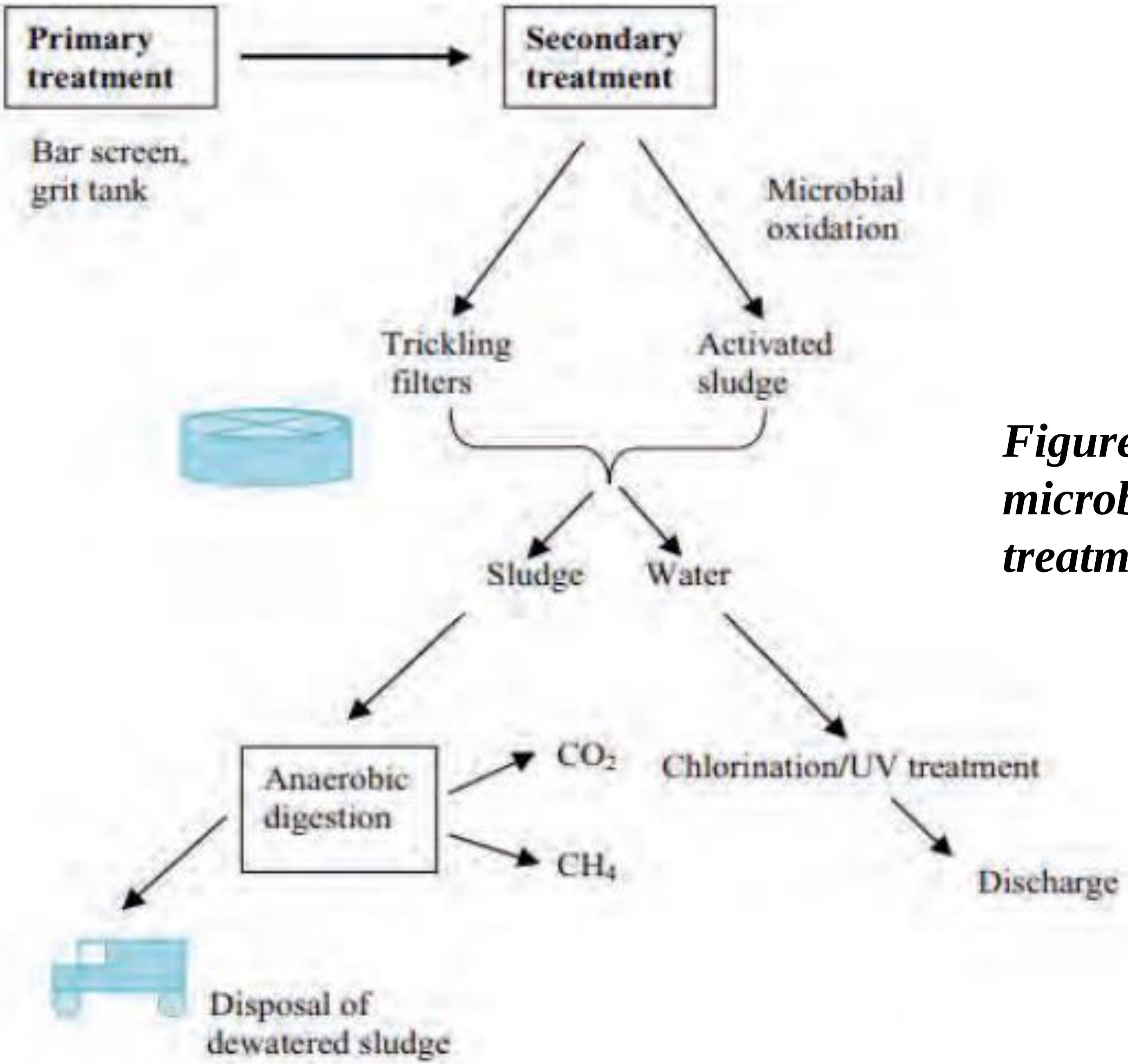


Figure 7: the role of microbes in wastewater treatment

- **In the former (Trickling filter):**
  - the wastewater is passed slowly over beds of stones or pieces of moulded plastic.
  - these develop a biofilm comprising bacteria, protozoans, fungi and algae
  - the resulting treated water has its BOD reduced by some 80- 85%.
- **In Activated sludge process:**
  - Activated sludge facilities achieve an even higher degree of BOD reduction.
  - Here the wastewater is aerated in tanks that have been seeded with a mixed microbial sludge.
  - The main component of activated sludge is the bacterium **Zoogloea**, which secretes slime, forming aggregates called **flocs**, around which other microorganisms such as protozoans attach.
  - Some of the water's organic content is not immediately oxidized, but becomes incorporated into the **flocs**.

- After a few hours residence in the tank, the sludge is allowed to settle out, and the treated water passes out of the system.
- Before being discharged to a watercourse, it is treated with chlorine to remove any pathogens that may remain.

- The principal operating problem encountered with activated sludge is that of **bulking**.
  - This is caused by filamentous bacteria such as ***Sphaerotilus natans***; it results in the sludge not settling properly and consequently passing out with the treated water.
- Both secondary treatment processes result in some surplus sludge, which undergoes anaerobic digestion, resulting in the production of methane and CO_2.
  - The methane can be used as a fuel to power the plant and
  - Any remaining sludge is dewatered and used as a soil conditioner.
- Care must be taken in this context, however, that the sludge does not contain toxic heavy metals.

C. Bioremediation

- **Bioremediation** the use of living organisms or their products for the **detoxification** and **degradation** of **environmental pollutants**.
- Today many pollutants are degraded with the help of saprophytic microbes; this process is also known as **biodegradation**.
- Genetically engineered bacteria metabolically breakdown toxic pollutants into harmless compounds. For example,
  - Mercury resistant bacteria process metallic mercury (which damages the nervous system) into a nontoxic compound.
- During bioremediation via microorganisms:
  - Enzymes produced by a microorganism modify a toxic pollutant by **altering** or **transforming** its structure. This process is called **biotransformation**.
- Biotransformation without biodegradation can also occur.
  - For example, toxic heavy metals can be rendered less toxic by oxidation/reduction reactions carried out by microorganisms.

D. Biofuels

- **Biofuels** (chiefly ethanol) is obtained by the fermentation of plant material, specifically crop residues.
  - **Corn** is currently the substrate of choice, the use of crop residues could significantly boost biofuel yields.
- Crop residues are the plant material that consists of:
  - Cellulose and
  - Hemicellulose
- These polysaccharides are polymers of five different hexoses and pentose sugars:
  - Glucose
  - Xylose
  - Mannose
  - Galactose and
  - Arabinose

- Even though, there isn't naturally occurring microorganism to ferments all five sugars:
  - ***Saccharomyces cerevisiae*** strain has been engineered to ferment **xylose** and
  - ***E. coli*** strain that expresses **Zymomonas mobilis genes** is able to ferment all these sugars.
- Another area of research focuses on degrading the cellulose and hemicellulose to release these monomers.
  - This is commonly done by **heating** the plant material and **treating it with acid**, which is both expensive and corrosive.
- Work to harvest **cellulase** and **hemicellululase** producing fungi as well as **bioprospecting** for enzymes from **thermoacidophiles** are ongoing in an effort to replace the **harsh thermochemical approach** with a **biological treatment**.

E. Biogas Production

- **Biogas** is a combustible mixture gas formed when **bacteria** and **archaeans** breakdown of organic matter (such as faeces, manure or animal waste, plant matter and household organic waste) in anaerobic condition in fermenters.
- Depending on the construction of the fermenter, biogas is mostly **methane** with some carbon dioxide and other gases may be present.
- ***Three different communities of anaerobic microbes are required.***
- **The first group**
  - Converts the raw organic waste into a mixture of organic acids, alcohol, hydrogen and carbon dioxide.
- **The second group**
  - Use the organic acids and alcohol from the first stage to produce acetate, carbon dioxide and hydrogen.
  - These first two communities are **Eubacteria**.

- **The last group** are **Archaea** called **methanogens**.
  - The methanogens produce methane by one of the following two reactions:

1. Reduction of carbon dioxide to methane

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_20$$

2. Splitting ethanoic acid to form methane and carbon dioxide

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

Advantages of Biogas

1. Biogas is a fuel used to cook food, and light lamps.
2. Slurry left after biogas production forms a soil conditioner (manure).
3. Biogas is much cheaper than Liquefied Petroleum Gas for home use.

C. Composting

- **Composting** is an aerobic microbial driven process that converts solid organic wastes into a stable, sanitary, humus-like material that has been considerably reduced in bulk and can be safely returned to the environment.
- To be totally effective, compositing should only use as substrates readily decomposable solid organic waste.
- In large-scale operations using domestic solid organic wastes,
  - The final product is mostly used for **soil improvement**.
- In more specialized operations using specific organic raw substrates (straw, animal manures, etc.),
  - The final product can become the substrate for the worldwide commercial production of the mushroom ***Agaricus bisporus***.
- A composting plant must function under environmentally safe conditions.

- The primary aim of composting operation is:
  - to obtain, in a limited time within limited compost, final compost with a desired product quality.
- Composting is carried out in a packed bed of solid organic particles in which the indigenous microbes will grow and reproduce.
- The basic **biological reaction** of the composting process is the **oxidation** of the mixed **organic substrates** with **oxygen** to produce **carbon dioxide**, **water** and other **organic by-products**.

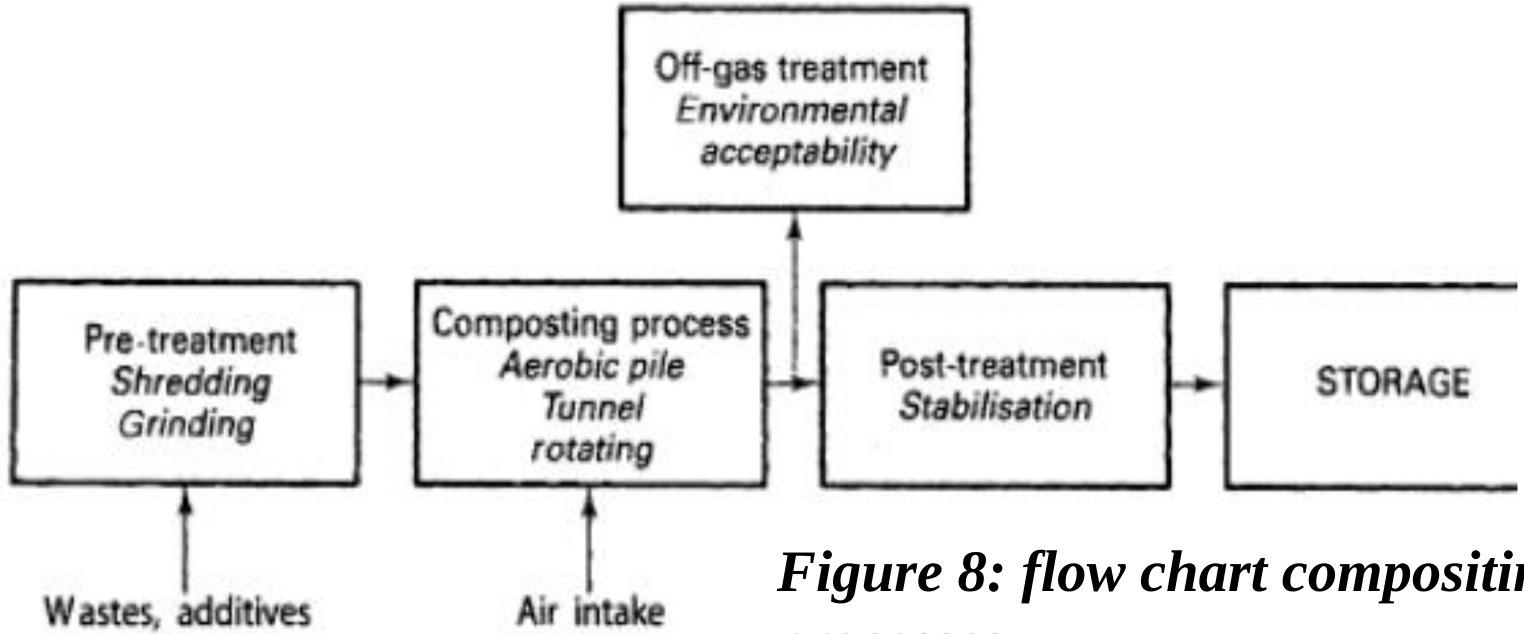


Figure 8: flow chart compositing plants processes

Application in Industry

A. Enzymes

- Enzymes can be produced by commercial fermentation using readily available feed stocks such as corn-steep liquor molasses.
- Fungi (e.g. **Aspergillus**) or bacteria (e.g. **Bacillus**) are two of the chosen and commonest organisms used to produce the enzymes.
  - This is because, they are **non-pathogenic** and **do not produce antibiotics**.
- The fermentation process is similar to that described for **penicillin**.
- If the enzymes are **extracellular:**
  - Then the liquid feedstock is filtered from the organism and the enzyme is extracted.
- If the enzymes are **intracellular**,
  - The micro-organisms have to be filtered from the feedstock.
  - They are then crushed and the enzymes extracted with water or other solvents.

Some commercial uses of enzymes are listed below:

- **Proteases**:
  - In washing powders for dissolving stains from, e.g. egg, milk and blood
  - Removing hair from animal hides
  - Cheese manufacture
  - Tenderizing meat
- **Lipases**:
  - In cheese for flavors enhancer
  - In washing powders for removal of fatty stains
- **Pectinases**:
  - Clarification of fruit juices
  - Maximizing juice extraction
- **Amylases**:
  - Production of glucose from starch

B. Biological Washing Powders

- The majority of commercial enzyme production involves protein-digesting enzymes (**proteases**) and fat-digesting enzymes (**lipases**) for use in the food and textile industries.
- When combined in washing powders, they are effective in removing stains in clothes caused by:
  - Proteins (such as blood, egg and gravy) and
  - Fats (such as grease)
- Protein and fat molecules tend to be large and insoluble.
  - When they have been digested the products are small and soluble molecules, which can pass out of the cloth.
- Biological washing powders save energy because they can be used to wash clothes at lower temperatures.
  - However, if they are put in boiled water, the enzymes become denatured and they lose their effectiveness.

Applications in Agriculture

A. Biopesticides

- There has been a long-term interest in the use of bacteria, fungi, and viruses as **Bio-insecticides** and **Bio-pesticides**.
  - These are known as **biological agents**, (such as bacteria, fungi, viruses, or their components), which can be used to **kill** a **susceptible insect**.

Bacteria:

- *Bacillus thuringiensis* and *B. popilliae* are the two major bacteria of interest, because they are considered harmless to humans.

➢ ***Bacillus thuringiensis*** is used on a wide variety of vegetables, field crops, fruits, shade trees, and ornamentals.

➢ ***Bacillus popilliae*** is used primarily against Japanese beetle larvae.

➢ ***Pseudomonas fluorescens***, (contains **toxin-producing gene** from *B. thuringiensis*), is used on maize to suppress **black cutworms**.

Viruses:

- **Three** major virus groups that do not appear to replicate in warm-blooded animals are used:
  - Nuclear polyhedrosis virus (NPV)
  - Granulosis virus (GV) and
  - Cytoplasmic polyhedrosis virus (CPV)
- These occluded viruses are protected in the environment.

Fungi:

- Over 500 different fungi are associated with insects. Infection and disease occur primarily through the insect cuticle. ***Four major genera of fungi have been used:***

- ***Beauveria bassiana:*** is used to control Colorado potato beetle
- ***Metarhizium anisopliae:*** is used to control froghopper in sugarcane plantations
- ***Verticillium lecanii*** and ***Entomophthora*** species have been associated with control of aphids in greenhouse and field environments.

B. The Use of Ti Plasmid as a Vector

- Use of **tumor-inducing (Ti) plasmid** of *Agrobacterium tumefaciens* to introduce **glyphosate resistance** into soybean crops.
- One way to introduce **transgenes** into plants is to use *Agrobacterium tumefaciens*.
  - This bacterium has a plasmid, called the **Ti plasmid**, that causes tumors in the plants it infects.
  - The **glyphosate resistance gene** is inserted into the Ti plasmid along with an **antibiotic resistance gene**.
  - The construct (recombinant DNA) is then re-inserted into *A. tumefaciens*.
  - Plant cells are then exposed to the **transgenic bacterium** and cultured on a plate containing **antibiotic**.
- *The only plant cells that grow are those that have taken up the plasmid. The others are killed by **antibiotic**.*

C. Insect-Resistant Crops

- Crop plants can be Genetically modified to protect themselves against attack by insect pests.
- **Maize** is protected against the **corn borer**, which eats the leaves of the plants and then burrows into the stalk, eating its way upwards until the plant cannot support the ear.
- **Cotton** is protected against pests such as the **boll weevil**.
- In both maize and cotton, the yields are improved.
- Insect-resistant **tobacco** plant is protected against the **tobacco bud worm**, but as yet it has not been grown commercially.
- The most likely detrimental effects on the environment of growing an insect-resistant crop are:
  - The evolution of resistance by the insect pests
  - A damaging erect on other species of insects
  - The transfer of the added gene to other species of plant

- However, less pesticide is used, reducing the risk of spray carrying to and erecting non-target species of insects in other areas.
- Remember that only insects that actually eat the crop are erected.

D. Pest Resistant Crops

- Pest attack is one of the very common problems in a number of different crops all around the globe; these crops may include fodder crops or other crops for the purpose of getting food.
- One the example of such crops is **BT-Cotton**.
  - The **genes** of ***Bacillus thuringiensis*** **(Bt),** a very common, are inserted in cotton crop for development of certain **protein** in it.
  - The protein is very toxic to a number of different insects.
- With this aid of biotechnology, the developed BT-Cotton leads to a less pest attack ultimately leading to a significant more production.

E. Transgenic Animals

- Although several recombinant proteins used in medicine are successfully produced in bacteria, some proteins require a eukaryotic animal host for proper processing.
  - For this reason, the desired genes are cloned and expressed in animals, such as sheep, goats, chickens, and mice.
- Animals that have been modified to express recombinant DNA are called **transgenic animals**.
- Several human proteins are:
  - Expressed in the **milk** of **transgenic sheep** and **goats** and
  - Some are expressed in the **eggs** of **chickens**.
  - **Mice** have been used extensively for expressing and studying the effects of **recombinant genes** and **mutations**.

F. Transgenic Plants

- Manipulating the DNA of plants (creating GMOs) has helped to create desirable traits, such as:
  - Disease resistance
  - Herbicide and pesticide resistance
  - Better nutritional value and
  - Better shelf-life

❖ **Attention:** *Transgenic plants are being created to produce: Pharmaceuticals and Biologically active compounds*

❖ *Plants that have received recombinant DNA from other species arc called* ***transgenic plants****.*

❖ *Transgenic plants and other GMOs are not natural, they are closely monitored by government agencies to ensure that they are fit for human consumption and do not endanger other plant and animal life.*

❖ *Because foreign genes can spread to other species in the environment, extensive testing is required to ensure ecological stability.*

❖ *Staples like corn, potatoes, and tomatoes were the first crop plants to be genetically engineered.*

F. Pest Resistance

- The bacterium, *Bacillus thuringiensis*, produces a **toxin** that **kills** caterpillars and other insect larvae.
- The toxin has been in use for some years as an insecticide.
  - The **gene** for the **toxin** has been successfully introduced into some plant species using a **bacterial vector**.
  - As a result, the plants produce the toxin and show increased resistance to attack by insect larvae.

G. Herbicide Resistance

- Some of the safest and most effective herbicides are those, such as **glyphosate**, which **kill** any green plant but become **harmless** as soon as they **reach the soil**.
  - These herbicides cannot be used on crops because they kill the crop plants as well as the weeds.
  - A **gene** for an **enzyme** that breaks down **glyphosate** can be introduced into a plant cell culture. This lead to a reduced use of herbicides.

Cloning

- **Cloning** is a method of producing **identical copies** of **genes**, **cells**, or **organisms**.
- The products of cloning are called **a clone**.
  - **A clone** is a group of **genetically identical** organisms or cells produced either by **asexual reproduction** or **artificially** by cloning techniques.
- The main advantage of cloning is that, they can make large numbers of plants or animals, which are exact copies of a parent with desirable characteristics. Cloning is also used to produce skin or other tissues needed to treat a patient.

H. Animal Cloning

- Animals cannot be cloned in the same way as plants from parts of their bodies.
- If animal embryos are divided up at an early stage into several pieces, each piece can develop into a separate animal.

- However, it is hard to predict which embryos can develop into animals with desirable features and should therefore be cloned.
- The first successful reproductive cloning of an adult with known characteristics is **Dolly the sheep**.

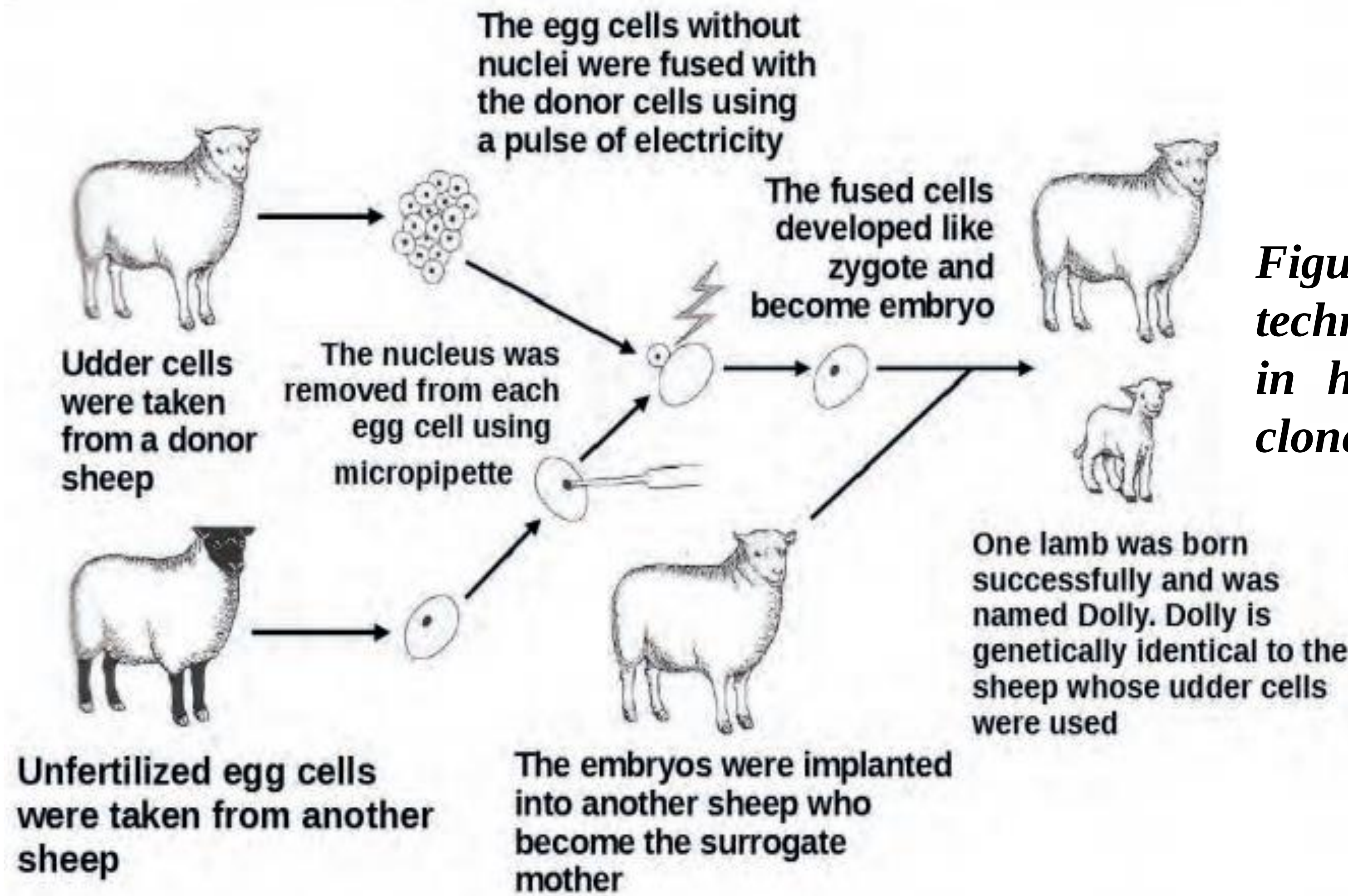


Figure 9: shows the techniques involved in how Dolly was cloned

Biological Warfare

- **Biological warfare (BW)** also known as **germ warfare** is the use of biological toxins or infectious agents such as bacteria, viruses, and fungi with the intent to kill or incapacitate humans, animals or plants as an act of war.
- **Biological weapons** include any microorganism (bacteria, viruses, or fungi) or toxin (poisonous compounds produced by microbes) found in nature that can be used to kill or injure people.
- The **act of bioterrorism** can range from a simple **hoax** to the actual use of these **biological weapons**, also referred to as **agents**.

- A number of nations have or are seeking to acquire biological warfare agents.
  - There are concerns that terrorist groups or individuals may acquire the technologies and expertise to use these destructive agents.
  - Biological agents may be used for an isolated assassination, to cause incapacitation or death to thousands.
  - If the environment is contaminated, a long-term threat to the population could be created.