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BT503

Environment Biotechnology

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BT503 – MIDTERM MERGED PPT
(LECTURE 01 TO 105)
Environmental Biotechnology

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Note:

Students must follow the course announcement for midterm syllabus & any up-gradation in the course.

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Introduction to Environmental Biotechnology

Environmental Biotechnology

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Introduction to Environmental Biotechnology

Biotechnology:

"The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services" (OECD, 2002).

Introduction to Environmental Biotechnology

There was a time when the biotechnology sector was seen as largely medical or pharmaceutical in nature, particularly amongst the general public…

Introduction to Environmental Biotechnology

The agricultural, industrial and environmental applications of biotechnology are potentially enormous

Introduction to Environmental Biotechnology

END

Environmental biotechnology is the branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass production, by exploiting biological processes (nature.com).

Environmental Biotechnology

Role & Scope

Role & Scope

As compared to other branches of Biotechnology….

Environmental Biotechnology deals with apparently less dramatic topics, though their importance may be every bit as great, and thus their direct relevance is far less appreciated by bulk of the population.

Role & Scope

The goals of Environmental Biotechnology are in everybody's interest… But for most it is simply addressing a problem that could have avoided already.

Role & Scope

The goals of Environmental biotechnology can be achieved in two ways:

- enhance or optimise conditions for existing biological systems to make their activities happen faster or more efficiently, OR
- Resort to some form of alteration to bring about the desired outcome.

Role & Scope

END

Scope for Use:

Three key points for environmental biotechnology interventions:

- manufacturing process,
- waste management,
- pollution control

Environmental Biotechnology

The Global Environmental Market

The Global Environmental Market

Massive growth in global environmental market.
A prediction: the market will have grown to 7400 billion US dollars by 2025.

The Global Environmental Market

Driving Factors:

- greater general awareness,
- widespread adoption of sustainable best practice by industry,
- geo-political changes,
- clean manufacturing applications, etc.

The Global Environmental Market

Major areas so far:

- energy production,
- waste management,
- land remediation,
- water treatment.

Water treatment accounts for 25% of the total global environmental market.

The Global Environmental Market

END

Unbreakable cycle of industrial growth at the cost of environmental damage.

The OECD (2001) concluded that the industrial use of biotechnology commonly leads to increasingly environmentally harmonious processes and additionally results in lowered operating and/or capital costs.

Environmental Biotechnology

Modalities and Local Influences

Modalities and Local Influences

Effect of local circumstances…

Contextual sensitivity…

Location, modalities, economics, legislation and customs…

Biotech intervention in one region or country, may be totally unsuited to use in another…

Modalities and Local Influences

These contexts can change…

Economic Pressure…

Legislation…

Integrated Approach

The controlled world of research translates imperfectly into the harsh realities of commercial implementation…

Often a dichotomy between theory and application…

Environmental biotechnology,
relies on existing natural cycles, often directly and in an entirely unmodified form…

Modalities and Local Influences

Potential for different biological approaches to be combine…

In some spheres, traditional biology has become rather unfashionable…

END

The fundamentals of living systems are the stuff of this branch of science…

Environmental Biotechnology

Using Biological Systems

Using Biological Systems

The manipulation of natural cycles lies at the heart of much environmental biotechnology…

Typically centres on adapting existing organisms and their inherent abilities…

Using Biological Systems

Optimization of the activities of particular organisms, or even whole biological communities…

Factors affecting the use of biological systems relate to the nature of the substances needing to be treated and to the localized environmental conditions…

Using Biological Systems

The intended target of the bio-processing must generally be both susceptible and available to biological attack, in aqueous solution, or at least in contact with water, and within a low to medium toxicity range.

Using Biological Systems

For land based applications, the soil types best suited to biotechnological interventions
are sands and gravels.

END

Moreover, nutrient availability, oxygenation and the presence of other contaminants can all play a role too.

Environmental Biotechnology

Extremophiles

Extremophiles

In general the use of biotechnology for environmental management relies on mesophilic micro-organisms.

Some microbial species tolerate extreme conditions, like high salinity, pressures and temperatures,

- useful for biotech applications requiring tolerance to these conditions

Extremophiles

The Archaea

- extreme thermophiles, extreme halophiles, methanogens, species that tolerate high levels of ionising radiation, pH, or high pressures…

- Extremophiles could provide a way of developing alternative routes to many conventional chemicals or materials…

Extremophiles

Much of the interest centres on the extremophile enzymes, the so-called extremozymes.

The promise of extremozymes lies in their ability to remain functional when other enzymes cannot.

Extremophiles

The widespread uptake and integration of biocatalytic systems as industrial production processes in their own right is not without obstacles which need to be overcome.

END

Environmental Biotechnology

Thermophiles

Thermophiles

Thrive at temperatures above 45°C.
Hyperthermophiles…
~85°C.

Thermus aquaticus…

Hot-springs, deep sea-vents, geothermal fluids, etc.

Thermophiles

Potential for the industrial exploitation…

Temperatures where other organisms do not survive.

Thermophiles

A good understanding of the way in which extremophile molecules are able to function at high temperatures is essential for any future attempt at harnessing the extremozymes for industrial purposes.

Thermophiles

In PCR:
DNA Polymerase of *Thermus aquaticus* (Taq Polymerase), *Pyrococcus furiosus* (Pfu Polymerase).

END

Clean energy:
bioethanol production, hydrogen production fuel cells, etc.

Environmental Biotechnology

Other Extremophiles

Other Extremophiles

In addition to Thermophiles, many other species survive under equally challenging environmental conditions.

May also have some potential for future methods of reduced pollution.

Other Extremophiles

Cold environments are more common on earth than hot ones…

Average oceanic temperature is around 1–3°C and vast areas of the global land mass are permanently or near-permanently frozen.

Psychrophiles can tolerate these circumstances.

Other Extremophiles

Halophiles... survive intensely saline environments, such as exist in natural salt lakes or salt evaporation ponds.

Other Extremophiles

Normal cells lose water and dehydrate in hypertonic saline environments.

Halophiles deal with this problem by ensuring that their cytoplasm contains a higher solute concentration than is present in their surroundings.

Other Extremophiles

Two distinct mechanisms: either manufacture or concentrate large quantities of solutes for themselves.

E.g., a number of halophile species accumulate KCl , and thus their extremozymes work only in the presence of KCl.

Other Extremophiles

Acidophiles thrive in the conditions of low pH, typically below 5.

Protective molecules in their cell walls, membranes or outer cell coatings to exclude acids.

Extremozymes capable of functioning below pH 1 have been isolated…

Other Extremophiles

Alkaliphiles flourish in environments at pH 9 or more such as soda lakes and heavily alkaline soils.

Rely on protective molecules in their surfaces
or in their secretions to ensure the external environment is held at bay.

Other Extremophiles

END

Diverse Degradative Abilities

Toluene degradation,
Phenol degradation,
Chloromethane degradation,
Cellulose degradation,
etc.
Novel biopolymers.

Particular interest to environmental biotechnologists.

Environmental Biotechnology

Xenobiotics and Other Problematic Chemicals

Xenobiotics and Other Problematic Chemicals

Greek word ‘xenos’ meaning foreign.
Xenobiotics are compounds which are man-made and not produced by a biological procedure and for which no equivalent exists in nature.

Subject to bioaccumulation especially if they are fat soluble:

- gets stored in the body fat of organisms providing an obvious route into the food chain

Xenobiotics and Other Problematic Chemicals

Xenobiotics can be degraded by micro-organisms.

Gratuitous degradation: a xenobiot resembles a natural compound so that it is recognised by the organism's enzymes and may be used as food, OR *Cometabolism:* a xenobiot is degraded by the organism's enzymes but in this case its catabolism does not provide energy and so cannot be the sole carbon source.

Xenobiotics and Other Problematic Chemicals

The ability of a single compound to be degraded can be affected by the presence of other contaminants.

This being the case, model studies predicting the rate of contaminant degradation may be skewed in the field.

Soil microorganisms are very versatile and can adapt to a food source due to exchange of plasmids.

Xenobiotics and Other Problematic Chemicals

In bacteria, the genes coding for degradative enzymes are often arranged in operons, which usually are carried on a plasmid.

Fast transfer from one bacterium to another.

END

Latent pathways in many Archaea.

Genetic modifications in laboratories.

Environmental Biotechnology

Endocrine Disruptors

Endocrine Disruptors

There are chemicals which resist degradation. Partly due to lack of organisms with degradative abilities, or worse due to microbial activities that change them to more toxic form.

E.g., synthetic oestrogens (such as 17α-ethinyloestradiol forming active ingredient of birth control pills) and natural oestrogens.

Endocrine Disruptors

Natural oestrogens are deactivated in humans by glucuronidation which is conjugation of the hormone with UDP-glucuronate, making the compound more polar and easily cleared from the blood by the kidneys.

However, many bacteria have the enzyme β-glucuronidase which removes this modification thus reactivating the hormone.

Endocrine Disruptors

Glucuronidation is also used to detoxify a number of drugs, toxins and carcinogens in the liver.

Raised levels of reactivated oestrogen in the waterways leading to disturbances of the endocrine, or hormonal, system in fauna.

E.g., feminisation of male fish in many species including minnows, trout and flounders.

Endocrine Disruptors

Many other chemicals, including PAHs, dichlorodiphenyltrichloroethane (DDT), alkyl phenols and some detergents may also mimic the activity of oestrogen.

END

Oestrogen is heat labile and susceptible to UV exposure, the effects of which are augmented by titanium dioxide.

Environmental Biotechnology

Ongoing discoveries

Ongoing Discoveries

Novel bacteria with capacity to degrade toxic, carcinogenic or teratogenic compounds.

Bacteria against PAHs (polycyclic aromatic hydrocarbons, e.g., naphthalene and phenanthrene).
Bacteria isolated from the same environments may vary in their abilities to degrade PAHs

- indicative of diverse catabolic pathways

Ongoing Discoveries

Polychlorinated biphenyls (PCBs) are xenobiotics having high level of halogenation.
PCBs are substrates for very few pathways normally occurring in nature.

A strain of *Pseudomonas putida* known to degrade PCBs (isolated from waste water outflow from a refinery).

Ongoing Discoveries

Two pathways encoded by two separate operons; the *tod* pathway (employed in toluene degradation), and the *cmt* pathway (responsible for the catabolism of p-cumate).

The mutation which allowed this strain to utilise the *cmt* pathway was found to be a single base change to the promoter-operator sequence.

Ongoing Discoveries

Phthalates are substituted single ring phenols (e.g., terephthalic acid and its isomers). These are the major chemicals used in the manufacture of polyester fibres, films, adhesives, coatings and plastic bottles.

END

Methanogenic consortium of over 100 bacterial clones with the capability to digest terephthalate.

Environmental Biotechnology

Mobility of DNA

Mobility of DNA

Movement of genes within and between organisms.

Genetic transfer through physical contact between bacteria (conjugation).
Genetic transfer through bacteriophages (Transduction).
Taking up of foreign DNA by bacteria (Transformation).

Called extra-chromosomal elements (viruses and plasmids)

Mobility of DNA

Rearrangement of genomic material within an organism stimulated by the presence of transposons.

Move from one point and reinsert into a second site. Insertion may be into specific sites or random.

Evolutionary process occurs principally by insertions and deletions of genomes caused by such activities.

Mobility of DNA

Such activities can also lead to rearrangement of existing structural genes into a different region of the genome. These genes then, therefore, operate under different parameters thus affecting the gene regulation.

Mobility of DNA

e.g., Viruses which infect a wide host range, such as some retroviruses, the alfalfa mosaic virus and the Ti plasmid of *Agrobacterium tumefaciens*.

Retroviruses have RNA as their genetic material. They replicate through double stranded DNA intermediate and thus can integrate into the host cell genome.

Mobility of DNA

RNA viruses tend to be more susceptible to mutation than DNA viruses presumably due to the less chemically stable nature of the macromolecule

A blurred distinction between chromosomal and extra-chromosomal elements (ECE).

END

No organism lives in true genetic isolation as long as it is susceptible to at least one of ECE.

Environmental Biotechnology

Genetic Manipulation

Genetic Manipulation

If selective breeding is to be considered as manipulation, then genes have been manipulated by man for a very long time.

Gregor Mendel.

Exchange of genetic information between organisms in nature is considerably more common than is generally imagined.

Genetic Manipulation

In bacteria, the most likely candidates for genetic transfer are plasmids and bacteriophages.

For eukaryotes, their most plausible vectors are eukaryotic viruses.

Exchange involving a vector requires compatibility between the organism donating the genetic material, the vector involved and the recipient organism.

Genetic Manipulation

Microorganisms involved in remediation do so in their ‘natural’ state largely because they are indigenous at the site of contamination.

In case of a sudden contamination, microbes are not able to adapt quickly. They may be ‘trained’ by artificial expansion of pre-existing pathways.

Or they can be genetically engineered.

Genetic Manipulation

Wild type and mutant.

Artificial mutations or deliberate reconstruction of the genome, often by the introduction of a novel gene.

Basis of genetic engineering and has several advantages over traditional breeding techniques.

The process is specific i.e. only selected gene(s) is transferred.

END

Environmental Biotechnology

The Manipulation of Bacteria Without Genetic Engineering

The Manipulation of Bacteria Without Genetic Engineering

A general procedure is to take a sample of bacteria from, at, or near, the site of contamination from which a pure culture is obtained in the laboratory and identified, using standard microbiology techniques.

The Manipulation of Bacteria Without Genetic Engineering

Improve the bacterium's tolerance to the pollutant or to increase the capabilities of its pathways.

Tolerance may be increased by culturing in growth medium containing increasing concentrations of the pollutant.

The Manipulation of Bacteria Without Genetic Engineering

Catabolic expansion:
Improving microbes ability to degrade a contaminant by culturing the bacteria in growth medium in which the contaminant is supplied as an essential part of the nutrition.
Only bacteria which have undergone a mutation enabling them to utilise this food source will be able to survive.

The Manipulation of Bacteria Without Genetic Engineering

An increased rate
of error may be forced upon
the organism, speeding up
the rate of mutation,
by including a mutagen in
the growth medium.

The Manipulation of Bacteria Without Genetic Engineering

END

Reintroduction of these “trained” bacteria to the polluted sites should give them an advantage over the indigenous bacteria as they would be better suited to survive and remediate the contamination.

Environmental Biotechnology

The Manipulation of Bacteria by Genetic Engineering

The Manipulation of Bacteria by Genetic Engineering

Genetic manipulation by the deliberate introduction of defined genes into a specified organism is a very powerful, established technique in constant development, some times at phenomenal rates of progress.

The Manipulation of Bacteria by Genetic Engineering

The techniques have produced some exciting hybrids in all areas of research, both microscopic; bacteria and fungi, usually described as recombinants, and macroscopic; principally higher plants and animals, commonly described as transgenics.

Deliberate transfer of a gene from one organism to another… ‘foreign gene’.

The Manipulation of Bacteria by Genetic Engineering

In practice, a very tiny proportion of all endeavour in environmental biotechnology has a direct reliance for its effectiveness on the type of recombinants and transgenics currently being developed.
Not because of the limits of GE, which in principal are almost boundless, but because of cost.

The Manipulation of Bacteria by Genetic Engineering

May be sustainable by pharmaceutical companies and perhaps to a lesser extent, agribiotechnology companies possibly able to command a high return on sales of the transgenic product.
However, it is rarely sustainable in applications of environmental technology.

The Manipulation of Bacteria by Genetic Engineering

Many problems in utilizing GMOs.
E.g., when a recombinant microbe is applied in bio augmentation it comes in competition with indigenous species which could outgrow it.

The recombinant bacterium may also lose its new capability through normal transfer of genes between bacteria.

A highly controlled and contained environment such as a bioreactor may circumvent some of these objections.

The Manipulation of Bacteria by Genetic Engineering

END

In reality, there is rarely any need to use recombinants or transgenics…

The required metabolic capability is mostly provided by indigenous organisms.

Environmental Biotechnology

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

Basic Principles of Genetic Engineering

Fundamental requirements of all cloning procedures:
the enzymes, solutions and equipment necessary to perform the procedures;
the desired piece of DNA to be transferred;
a cloning vector;
and the recipient cell.
It is also essential to have some means of determining whether or not the transfer has been successful. This is achieved by the use of marker genes.

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

Isolation of DNA…

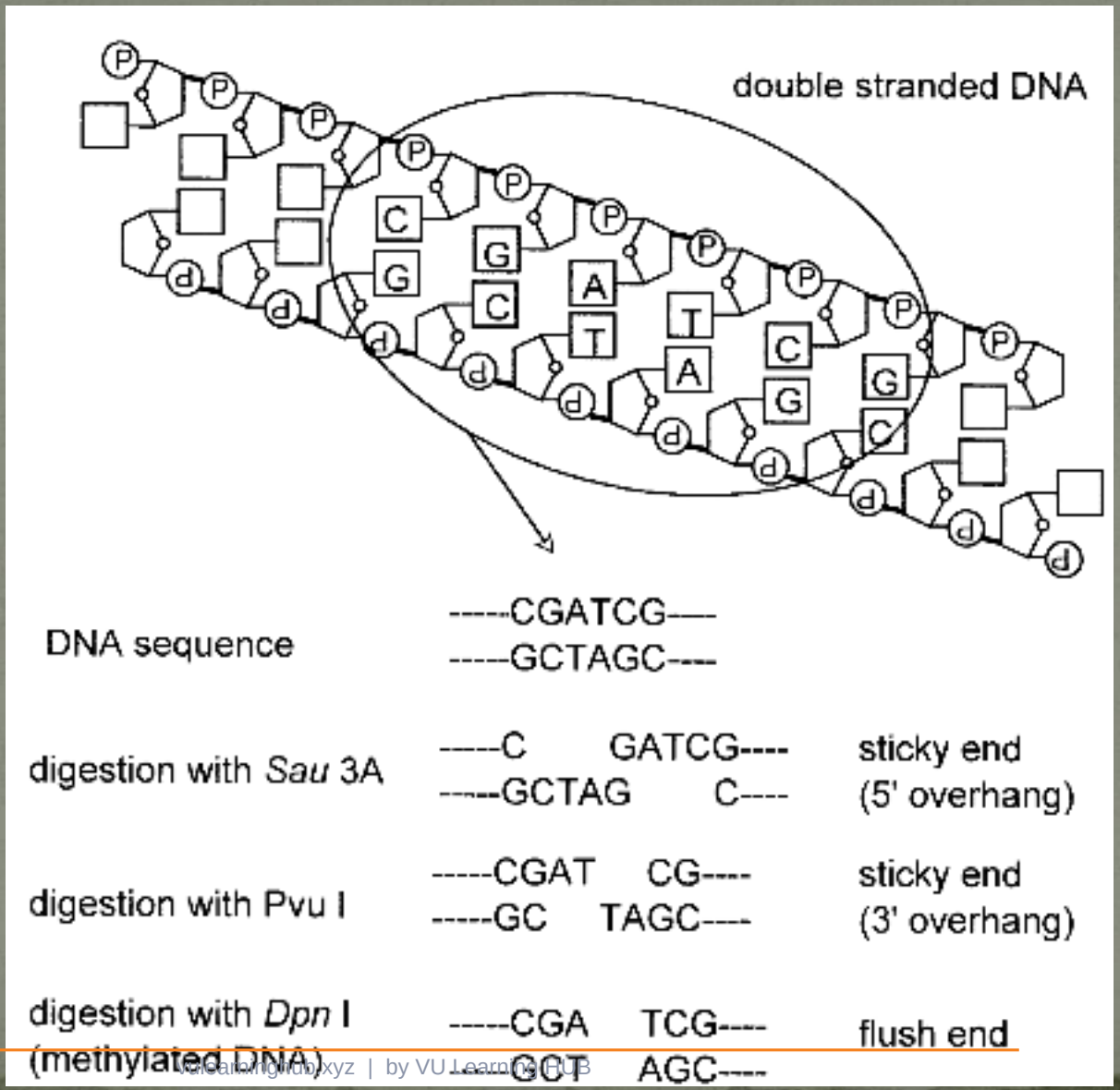
Once DNA has been isolated from an organism, it is purified from contaminating material such as protein and is precipitated out of aqueous solution by the addition of alcohol.
The DNA appears as a white, semi-transparent material, coiling out of solution on addition of the alcohol.
DNA may be collected by centrifugation and dried down.

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

Next stage is to insert the DNA into the vector…

Restriction Digestion or PCR, or restriction digestion of PCR products.
Restriction endonucleases recognise specific sequences within the DNA and cut at that site, either producing a flush/blunt or staggered end.
Other restriction nucleases recognise a site in DNA but cut at some distance from it (rarely of any value in cloning procedures).

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment



Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

Preparation of the vector is dictated by the type of end prepared for the insert DNA; flush or ‘sticky’.
If it is flush, it does not much matter how that was achieved so long as the vector receiving it is also flush,
But if it is sticky, the appropriate sticky end must be prepared on the vector by a suitable restriction endonuclease.

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

The next step is to stick the pieces together.
The prepared insert, or 'foreign' DNA is incubated with the prepared vector in an aqueous solution containing various salts and ligase Enzyme.

This recombinant DNA molecule may be transferred into a cell where it undergoes replication in the usual way.

Basic Principles of Genetic Engineering: Enzymes, solutions and equipment

END

If the DNA is not viral, introduction will be by direct entry through the cell membrane achieved by any one of a number of standard techniques all based on making the membrane permeable to the DNA molecule i.e. competent cells; cells able to undergo transformation.
However, if the 'foreign' DNA is part of a recombinant virus, it has to be packaged into particles, and then transferred into cells by infection.

Environmental Biotechnology

Basic Principles of Genetic Engineering

DNA for transfer:

Genomic Libraries

Basic Principles of Genetic Engineering: DNA for transfer

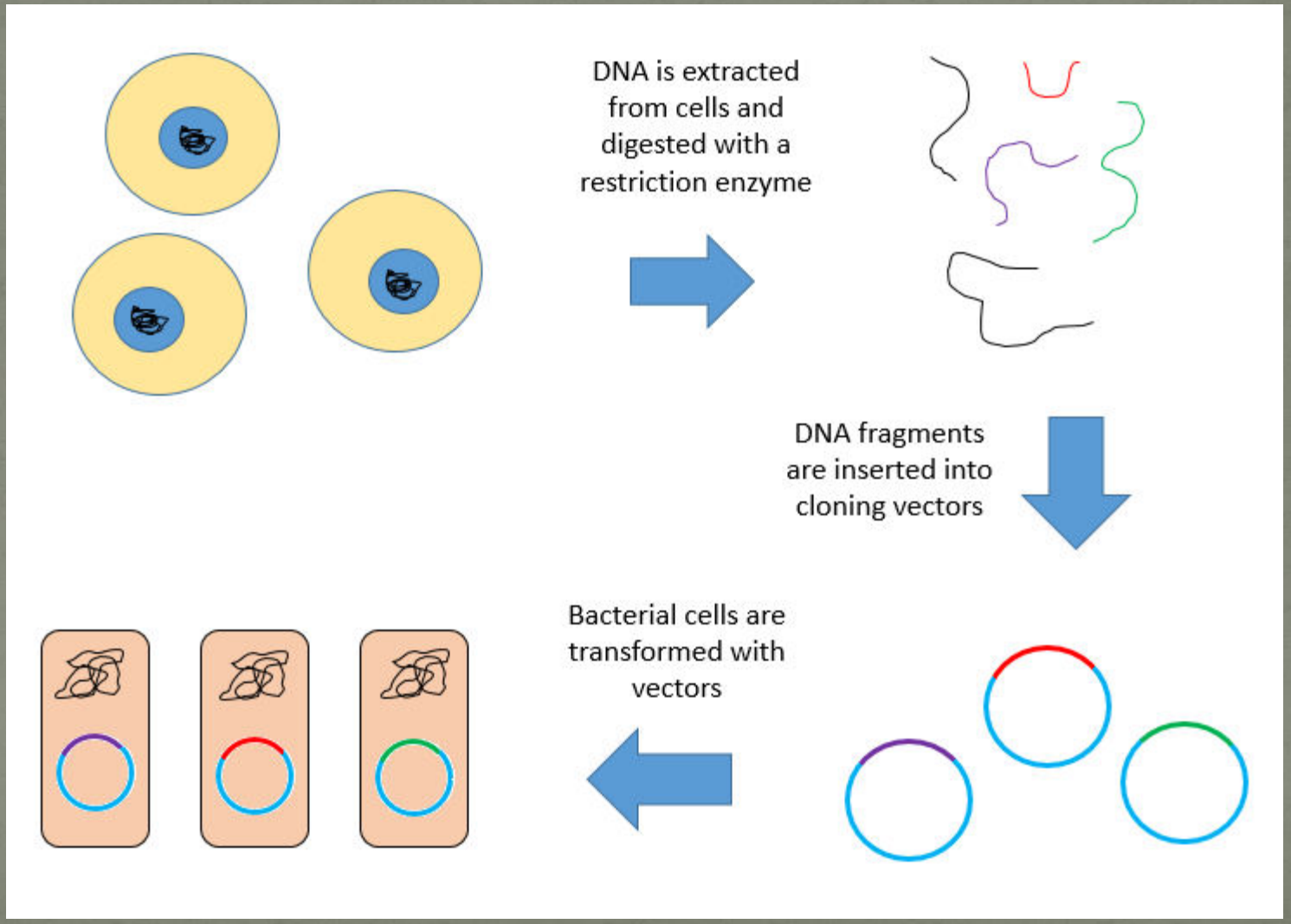
Most commonly, this is a piece of DNA which contains the coding sequence for a gene. It can be obtained from a number of sources, e.g., genomic DNA, a complementary DNA (cDNA) library, a product of a polymerase chain reaction (PCR) or a piece of DNA chemically produced on a DNA synthesiser machine. Another source is from a DNA copy of an RNA virus as in the replicative form of RNA viruses.

Basic Principles of Genetic Engineering: DNA for transfer

Genomic libraries

Collection of total Genomic DNA from an organisms.
The genomic DNA is isolated, purified and cut up into pieces of a size suitable to be inserted into a cloning vector, following by ligation and transformation into a host cell.

Basic Principles of Genetic Engineering: DNA for transfer



https://en.wikipedia.org/wiki/File:Genomic_Library_Construction.png

Basic Principles of Genetic Engineering: DNA for transfer

Genomic libraries

These DNA pieces may either be ligated as a total mixture, into a suitable vector to produce a genomic library, or a specific piece may be isolated and prepared as described ago.

Basic Principles of Genetic Engineering: DNA for transfer

END

Genomic libraries

Genomic libraries are very useful, as they may be amplified, and accessed almost limitlessly, to look for a specific DNA Sequence.

If the genomic library is of a eukaryotic origin the genes will contain non-coding regions, called introns. (Prokaryotes do not contain introns).
This is a problem if the gene is to be expressed.
This problem can be avoided by using cDNA.

Environmental Biotechnology

Basic Principles of Genetic Engineering

DNA for transfer:

cDNA libraries, & Polymerase chain reaction

Basic Principles of Genetic Engineering: DNA for transfer

cDNA libraries

In eukaryotes, the first product of transcription from DNA is not messenger RNA (mRNA) but heterogeneous nuclear RNA (hnRNA). This is mRNA prior to the removal of all the non-coding sections, or introns, which are discarded during the processing to produce the mature mRNA.

Basic Principles of Genetic Engineering: DNA for transfer

<u>cDNA libraries</u>

cDNA is DNA which has been artificially made using the mature mRNA as a template (through Reverse Transcriptase enzyme).

It is then used as the template for the second strand.

Thus the synthetic DNA product is simply a DNA version of the mRNA and so should overcome the problems of expression mentioned ago.

Basic Principles of Genetic Engineering: DNA for transfer

Polymerase chain reaction (PCR)

A powerful technique which amplifies a piece of DNA of which only a very few copies are available.

The piece must be flanked by DNA of somewhat known sequence.

This allows a short sequence of DNA to be synthesised to bind specifically to the end of the sequence and act as a primer for the DNA Polymerase. A second primer is used for the other end to allow the second strand to be synthesised.

Basic Principles of Genetic Engineering: DNA for transfer

Polymerase chain reaction (PCR)

The process is repeated by a constant cycling of denaturation of double stranded DNA at elevated temperature to approximately 95 ◦C, followed by cooling to approximately 55-60 ◦C to allow annealing of the primers, followed by extension of the 3’ end of the primer by Polymerase usually at 72 ◦C.

Basic Principles of Genetic Engineering: DNA for transfer

END

Polymerase chain reaction (PCR)

PCR requires the use of DNA polymerases able to withstand such treatment.
Thermus aquaticus is a thermophilic microorganism from which polymerase has been isolated and used for this purpose (Taq-polymerase).

DNA polymerases from other thermophiles are also being used.

Environmental Biotechnology

Basic Principles of Genetic Engineering

Cloning Vectors

Basic Principles of Genetic Engineering: Cloning Vectors

A cloning vector is frequently a plasmid or a bacteriophage (bacterial virus)

Must be fairly small and fully sequenced, able to replicate itself when reintroduced into a host cell, thus producing large amounts of the recombinant DNA for further manipulation.

Also it must carry on it ‘selector marker’ genes, which are indicators of genomic integrity and activity.

Basic Principles of Genetic Engineering: Cloning Vectors

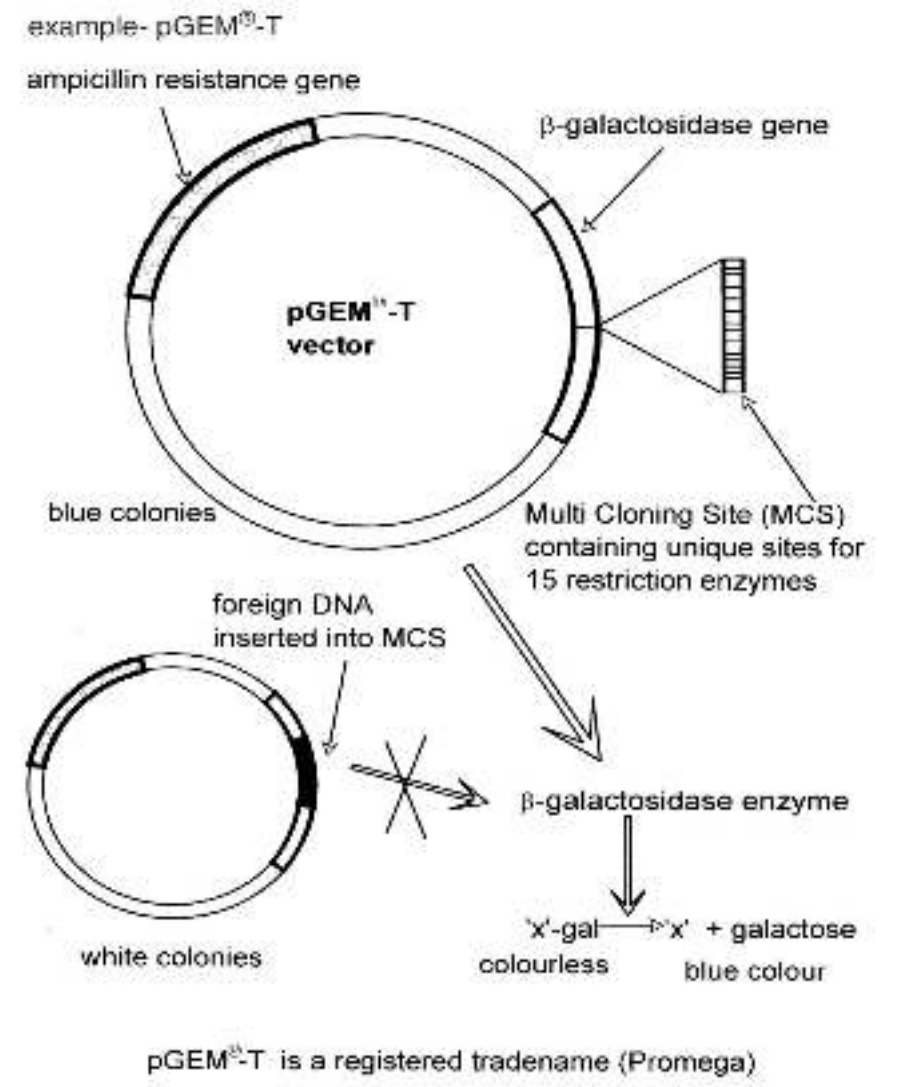
A common design of a cloning vector is one which carries two genes coding for antibiotic resistance.
The ‘foreign’ gene is inserted within one of the genes so that it is no longer functional therefore it is possible to discriminate by standard microbiology techniques, which bacteria are carrying plasmids containing recombinant DNA and which are not.

Basic Principles of Genetic Engineering: Cloning Vectors

To make the manipulations easier, selector marker genes normally contain a multi cloning site (MCS) which is a cluster of sites for restriction enzymes.

Insertion of foreign DNA in vector at MSC site causes disruption of these marker genes resulting in loss of the function of that gene.
For example, if that gene codes for antibiotic resistance, it will no longer be functional and will not protect the bacterium from that antibiotic.

Basic Principles of Genetic Engineering: Cloning Vectors



Basic Principles of Genetic Engineering: Cloning Vectors

Viruses may be used as vectors i.e., the DNA of viruses can be used to carry foreign DNA.

Most such recombinant viral DNA vectors need phage particles to perform proper gene transfer i.e. transduction.

END

Transfer of naked viral recombinant vector to host cells is called transfection, and tends to have lower uptake rates as compared to transduction.

Environmental Biotechnology

Basic Principles of Genetic Engineering

Expression Vectors and Reporter Genes

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Expression Vectors

These are similar to the vectors described above but in addition have the required regulatory regions located before and after the ‘foreign’ gene which direct the host cell to translate the product of transcription into a protein.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Expression Vectors

It is sometimes a difficult, expensive or time consuming procedure to analyze for product from the ‘foreign’ gene and so, in addition to the selector genes described above, there are frequently reporter genes to indicate whether or not the signals are ‘switched on’ allowing the ‘foreign’ DNA to be expressed.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Expression Vectors

In expression cloning, there are many circumstances which can be difficult to predict.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Reporter Genes

There are many such genes in common use and these usually code for a trait or an enzyme.
e.g., antibiotic resistance gene, or β-galactosidase, etc.
Blue/White screening.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Reporter Genes

Other reporter genes produce enzymes which can cause the emission of light such as the luciferase isolated from fireflies, or whose activity is easy and quick to assay like the bacterial β-glucuronidase (GUS) which is probably the most frequently used reporter genes in transgenic plants.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

Reporter Genes

Reporter genes can only be a guide to the process of transcription and translation process.

Basic Principles of Genetic Engineering:
Expression Vectors and Reporter Genes

END

Reporter Genes

As with selector genes, the reporter genes serve no useful purpose once the cloning procedure has been successfully accomplished to produce the finished product.

Environmental Biotechnology

Basic Principles of Genetic Engineering

Analysis of Recombinants

Basic Principles of Genetic Engineering:
Analysis of Recombinants

The design of the vector (plasmid) is so that the insertion of 'foreign' DNA allows for a colour test, or causes a change in antibiotic sensitivity.
This constitutes the first step in screening.
The second stage is usually to probe for the desired gene using molecules which will recognise it and to which is attached some sort of tag.

Basic Principles of Genetic Engineering:

Analysis of Recombinants

The next stage is normally to analyze the DNA isolated from possible recombinants, firstly by checking the size of the molecule or pieces thereof, or by sequencing the DNA.

However, if a large number of samples are to be analyzed it is usually quicker and cheaper to scan them by a procedure described as a Southern blot.

Basic Principles of Genetic Engineering:
Analysis of Recombinants

In Southern blotting, DNA fragments are resolved by electrophoresis on a gel.
These resolved fragments are then shifted to a membrane (nylon or nitrocellulose).
The membrane is then probed by a piece of DNA complementary to the sequence of interest.
If a band shows up on autoradiography then the probe has found a mate and the required sequences are present.
DNA sequencing is then required to confirm the results.

Basic Principles of Genetic Engineering:
Analysis of Recombinants

Northern blotting: RNA

Western blotting: Proteins (e.g., antibodies antigens interaction)

END

Environmental Biotechnology

Basic Principles of Genetic Engineering

Recombinant Bacteria & Yeast

Recombinant Bacteria & Yeast

GE of microorganisms for use in environmental biotechnology: expansion of metabolic pathways either to modify the existent metabolic capability, or to introduce new pathways.

Recombinant Bacteria & Yeast

Various applications, from the improved degradation of contaminants, to the production of enzymes for industry, thus making the process less damaging to the environment.

Recombinant Bacteria & Yeast

Many examples…
A strain of *Escherichia coli* into which was engineered some 15 genes originating from *Pseudomonas*.
These were introduced to construct a pathway able to produce indigo for the dyeing of denim (Bialy, 1997).
The traditional method requires the use of toxic chemicals.

Recombinant Bacteria & Yeast

Whether or not such routes will ultimately be taken up by industry still remains to be seen.

Recombinant Bacteria & Yeast

Yeast, being unicellular eukaryote, has become popular for cloning and expressing eukaryotic genes.
It is fairly simple to propagate, some species being amenable to culture in much the same way as bacteria.

Recombinant Bacteria & Yeast

Yeast cells are surrounded by a thick cell wall which must be removed to permit entry of DNA into the cell.

Several types of plasmid vectors available for GE, some of which have been constructed to allow replication in both bacteria and Yeast.

Recombinant Bacteria & Yeast

END

Such vectors have a region which permits integration into the host yeast genome by recombination.
Two crossover events take place between homologous regions on vectors and yeast genome which effectively swap over a piece of host DNA with the plasmid DNA.

Environmental Biotechnology

Basic Principles of Genetic Engineering

Recombinant Viruses

Recombinant Viruses

Viruses based vectors have their own advantages.

Many success stories.

e.g., The insect virus, *Baculovirus*, has been shown to be the method of choice for the over expression of genes in many applications of molecular biology.

Recombinant Viruses

Recombination in *Baculovirus* DNA occurs by much the same way as in yeast.

e.g., Replacement of p10 proteins of *Baculovirus* by the gene for a scorpion neurotoxin, with a view to improving the insecticidal qualities of the virus.

END

Environmental Biotechnology

Transgenic Plants

Transgenic Plants

Currently, GE in agro biotechnology is focusing on genetic modifications to improve crop plants with respect to quality, nutritional value and resistance to damage by pests and diseases.

Transgenic Plants

Other avenues under investigation aim to increase tolerance to extreme environmental conditions, to make plants better suited for their role in pollutant assimilation, degradation or dispersion by phytoremediation, or to modify plants to produce materials which lead to the reduction of environmental pollution.

Transgenic Plants

Crop quality improvements: Control of fruit ripening, production of cereals with improved nutritional value. These although are of great interest to the food industry, they are of more peripheral relevance to environmental biotechnology.

Transgenic Plants

END

Many of the transgenic plants have been produced using the Ti plasmid transfer system of *Agrobacterium tumefaciens* and are often used together with the 35S CaMV promoter.

Environmental Biotechnology

Transformation of plants (Part I)

Transformation of plants

Efforts by: AARISH ALI

There are two practical problems associated with GE of plants which make them more difficult to manipulate than bacteria. Firstly they have rigid cell walls and secondly they lack the plasmids which simplify so much of GE in prokaryotes.

Transformation of plants

The first problem is overcome by the use of specialised techniques for transformation, and the second by performing all the manipulations in bacteria and then transferring the final product into the plant.

Transformation of plants

The DNA construct contains regions of DNA which are complementary to the plant DNA to enable the inserted piece to recombine into the plant genome.

Transformation of plants

The most popular method of transforming plants is by the Ti plasmid, but there are at least two other methods also in use.

Transformation of plants

The first is a direct method where DNA is affixed to microscopic bullets which are fired directly into plant tissue.
e.g., introduction into sugarcane of genes able to inactivate toxins produced by the bacterium, *Xanthomonas albilineans*, causing leaf scald disease

Transformation of plants

This method of biolistic bombardment may increase in popularity in line with improvements to plastid transformation. It is now possible to produce fertile transgenics expressing foreign proteins in their edible fruit.

Transformation of plants

The second is by protoplast fusion which is a process whereby the plant cell wall is removed leaving the cell surrounded only by the much more fragile membrane. This is made permeable to small fragments of DNA and then the cells allowed to recover and grow into plants.

Transformation of plants

END

These methods can be unsuccessful due to difficulties in recovery of the cells from the rather traumatic treatments.

Environmental Biotechnology

Transformation of plants (Part II)

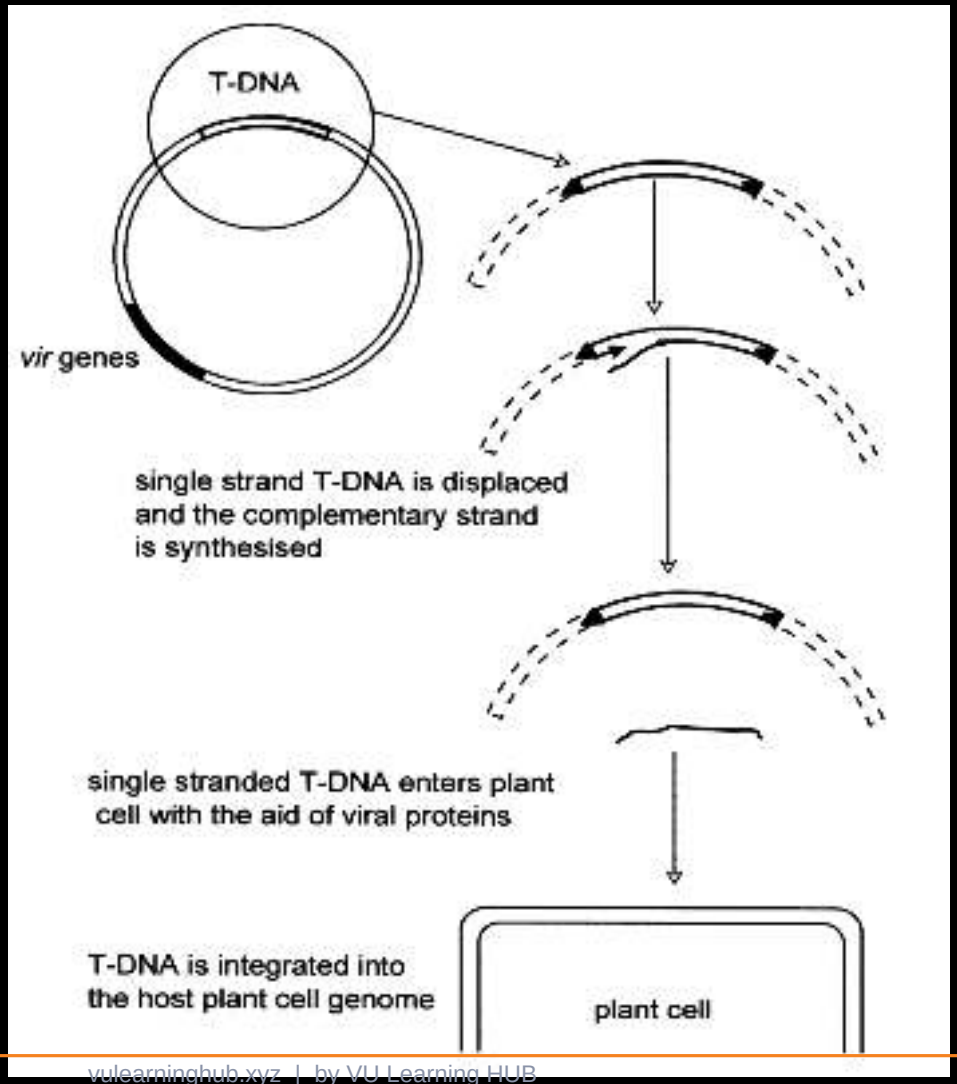
Transformation of plants

Transformation by the Ti plasmid of *Agrobacterium tumefaciens* suffers from few disadvantages other than the limitation that it does not readily infect some cereal crops: A problem been addressed by attempting to increase its host range.

Transformation of plants

Wild type plasmid contains genes which causes the transfer of a piece of DNA, ‘T-DNA’, into a plant cell, causing crown gall disease. This piece is flanked by 24 bp direct repeats. These genes may be cut out and replaced by DNA containing the gene of choice to be introduced into the plant.

Transformation of plants



Transformation of plants

In case of plants, recombination is not 100% efficient, and so a method of selection is required such that only plants containing the novel DNA grow. This is frequently a gene coding for weed-killer or antibiotic resistance.

Transformation of plants

END

Genes usually introduced more successfully on a second Ti plasmid during a co-infection with *Agrobacterium* carrying the plasmid containing the gene of interest. Other selector genes are introduced into the plasmids to ensure that growth is only possible if all the desired elements are present in the plant cell.

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:

Resistance against herbicides

Resistance against herbicides

So many different purposes to do GE in plants:
to reduce the amount of herbicide and pesticides, etc,
to improve tolerance of harsh conditions,
or to protect the plants from attacks, etc.

Resistance against herbicides

A general strategy to protect plants from various agents has been proposed using tobacco plants as a model.

The transgenics express the iron-binding protein, ferritin, in their cells which appears to afford them far ranging protection (De´ak et al., 1999).

Resistance against herbicides

‘Glyphosate’ (herbicide) is an analogue of phosphoenol pyruvate and inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase.
The gene coding for this enzyme has been identified, isolated and inserted into a number of plants e.g., Petunias.
The gene was expressed behind a CaMV promoter and introduced using *A. tumefaciens*.

Resistance against herbicides

The gene was expressed behind a CaMV promoter and introduced using *A. tumefaciens*.

As a consequence, the recombinant plants showed significant resistance to the effects of glyphosate (Shah et al., 1986).

Resistance against herbicides

Another example using *A. tumefaciens*: transfer of genes for mammalian cytochrome P450 monooxygenases, known to be involved in the detoxification of many xenobiotics including pesticides, into tobacco plants.

END

These transgenics displayed resistance to chlortoluron and chlorsulphuron (Yordanova, Gorinova and Atanassov, 2001).

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:

Resistance against pests

Resistance against pests

<u>Improved resistance to pests</u>

Plants have an inbuilt defense mechanism protecting them from attack by insects but the damage caused by the pests may still be sufficient to reduce the commercial potential of the crop. Attack by insects not only causes damage to the plant but also provides a route for bacterial or fungal infection.

Resistance against pests

Improved resistance to pests

With a view to increasing resistance to sustained attack, the genes coding for the δ-endotoxin of *Bacillus thuringiensis* have been transferred into plants. Examples are of a synthetic *B. thuringiensis* δ-endotoxin gene transferred by *A. tumefaciens* into Chinese cabbage (Cho et al., 2001).

Resistance against pests

Improved resistance to Viruses

Introduction of the genes expressing antibodies to the coat protein of Tobacco Mosaic Virus (TMV) by *A. tumefaciens*. Expression of these in the plant lead to complete immunity against TMV.

Resistance against pests

Ever growing research…

Insects are able to develop resistance to Bt products…

Different codon preference for bacteria (*Bacillus thuringiensis*) and eukaryotes (Plants)…

modification of the DNA sequence to compensate for these differences.

High expression and stability of the Bt proteins whose genes have been introduced into chloroplasts

END

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:

Improved resistance to disease

Improved resistance to disease

Bacteria communicate with each other by way of small diffusible molecules such as the N-acylhomoserine lactones (AHLs)…'quorum sensing'. These responses are diverse and include the exchange of plasmids and production of antibiotics and other biologically active molecules.
Many plant pathogens also cause pathogenicity through quorum sensing.

Improved resistance to disease

Plants are susceptible to bacterial pathogens such as *Erwinia carotovora*, which produces enzymes capable of degrading its cell walls.
The synthesis of these enzymes is under the control of AHLs and so they are made only once the appropriate threshold level of this chemical has been reached.

Improved resistance to disease

The rationale behind using AHLs for plant protection is to make transgenic plants, tobacco in this case, which express this signal themselves. The consequent high level of AHL presented to the pathogenic bacteria, wrongly indicates a very high number of similar organisms in the vicinity, and triggers the bacteria into responding.

Improved resistance to disease

END

As a consequence, they produce enzymes able to degrade the plant cell walls and continue infection.
The plant will mount its normal response to invasion but on a far greater scale than necessary to destroy the few bacteria actually causing the infection, thus improving the plant's resistance to the disease.

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:

Improved tolerance

Improved Tolerance

Pseudomonas syringae which colonises the surface of leaves.
This example is of bacterial rather than plant modification but impinges on interaction between the two.
Pseudomonas syringae produces a protein which promotes the formation of ice crystals just below 0 ◦C thus increasing the risk of frost damage.

Improved Tolerance

Scientists transferred this gene to *Escherichia coli* to simplify the genetic manipulations.
They deleted sufficient regions so that a non-functional, ice mediating protein was expressed.
The mutated gene was reintroduced into *Pseudomonas syringae* and selected for *ice*$^{-}$ mutants.
Protected the susceptible crop against frost damage.

Improved Tolerance

Salt tolerance in tomatoes has been established by introducing genes involved in Na+/H+ antiport, the transport of sodium and hydrogen ions in opposite directions across a membrane.

Improved Tolerance

The quality of the fruit was maintained by virtue of the fact that the sodium accumulation caused by the antiport occurred in leaves only and not in the fruit.

Improved Tolerance

Improved tolerance to drought, salt and freezing in Arabidopsis has been achieved by overexpressing a protein which induces the stress response genes.
However, if too much of this factor is produced, which was the case when the 35S CaMV was employed, severe growth retardation was observed.
No such problem existed when the expression was under the control of a promoter which switched on only when stressful conditions existed.

END

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:

Improved plants for phytoremediation

Improved plants for phytoremediation

Phytoremediation.

Phytovolatilisation.

Improved plants for phytoremediation

Genetic modification of a poplar to enable mercury to be removed from the soil and converted to a form able to be released to the atmosphere.

mer A gene gene is one of a cluster of genes involved in bacterial detoxification of mercury, and is the one coding for the enzyme, mercuric ion reductase, which converts mercury from an ionic to a volatile form.

Improved plants for phytoremediation

Initially the gene was transferred to *Arabidopsis thaliana*.

The gene was transferred by gene guns to poplar tree (*Liriodendron tulipifera*) embryogenic material.

The resulting poplar plantlets were found to exhibit tolerance to mercury and to volatalise it at 10 times the rate observed in untransformed yellow poplar plantlets.

Improved plants for phytoremediation

This study demonstrated the possibility that trees can be modified to become useful tools in the detoxification of soil contaminated with mercury.

Later it was found (in *Arabidopsis Thaliana*) that successful remediation also required the *mer B* genes coding for a lyase.

Improved plants for phytoremediation

A bacterial gene encoding pentaerythritol tetranitrate reductase, an enzyme involved in the degradation of explosives, have been transferred into tobacco plants.
The transgenics have been shown to express the correct enzyme and trials undertaken to determine the extent of their ability to degrade TNT.

END

Environmental Biotechnology

Selected examples of developments in plant Genetic Engineering:
New products from plants

New products from plants

Arabidopsis thaliana has become a popular choice for the production of recombinant species. One such recombinant is a plant where the fatty acid composition in the seed has been modified to produce triacylglycerols containing elevated levels of trierucinic acid suitable for use in the polymer industry.

New products from plants

Polyhydroxybutyrate suitable for the production of biodegradable plastics.

Synthesis of the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Arabidopsis* through *Agrobacterium tumefaciens* technology and the use of the 35S promoter from CaMV.

New products from plants

END

This copolymer can be produced by bacterial fermentation, but due to cost considerations, it is normally synthesised chemically.

Environmental Biotechnology

Introduction to Bioindicators

Introduction to Bioindicators

Bioindicators are living organisms such as plants, planktons, animals, and microbes, which are utilized to screen the health of the natural ecosystem in the environment.

The expression ‘Bioindicator’ is used as an aggregate term referring to all sources of biotic and abiotic reactions to ecological changes.

Introduction to Bioindicators

They are used for assessing environmental health and biogeographic changes taking place in the environment e.g., a plankton responding rapidly to changes taking place in the surrounding

Introduction to Bioindicators

Even the health of aquatic flora is best reflected by plankton, which acts as an early warning signal.

There are a certain factors which govern the presence of Bioindicators in environment such as transmission of light, water, temperature, and suspended solids.

Introduction to Bioindicators

Through the application of Bioindicators we can predict the natural state of a certain region or the level/degree of contamination.

Introduction to Bioindicators

END

Advantages of Bioindicators

- Biological impacts can be determined.
- To monitor synergetic and antagonistic impacts of various pollutants on a creature.
- Early stage diagnosis as well as harmful effects of toxins to plants, as well as human beings, can be monitored.
- Can be easily counted, due to their prevalence.
- Economically viable alternative when compared with other specialized measuring systems.

Environmental Biotechnology

Utilization of Bioindicators

Utilization of Bioindicators

Instead of simply working as gauges of natural change, taxa are utilized to show the impacts of natural surrounding changes, or environmental change.

They can also detect changes in the environment due to the presence of pollutants which can affect the biodiversity of the environment, as well as species present in it.

Utilization of Bioindicators

Hasselbach et al. utilized the moss i.e. *Hylocomium splendens* as a natural indicator of heavy metals in the remote tundra environment of northwestern Alaska.

In this study, lichens were utilized as biomonitors by utilizing the quantitative estimation of metal concentrations inside individual lichen.

Utilization of Bioindicators

Here, the ore of mineral is mined from Red Dog Mine, the world's largest creator of zinc (Zn), and is carried to a singular street to storage spaces on the Chukchi Sea

Utilization of Bioindicators

Hasselbach and her partners inspected whether this overland transport was influencing the encompassing physical biota.

Contents of heavy metals inside the moss tissue were analyzed at different distances from the street. The concentrations of metals in moss were most prominently adjacent to the haul street and reduced with distance.

Utilization of Bioindicators

Natural, biological, and biodiversity markers can be found in various organisms occupying different types of environments.

Lichens and Bryophytes are frequently used to monitor air contamination.

High surface region region to volume ratio…. to capture contaminates from the air.

Utilization of Bioindicators

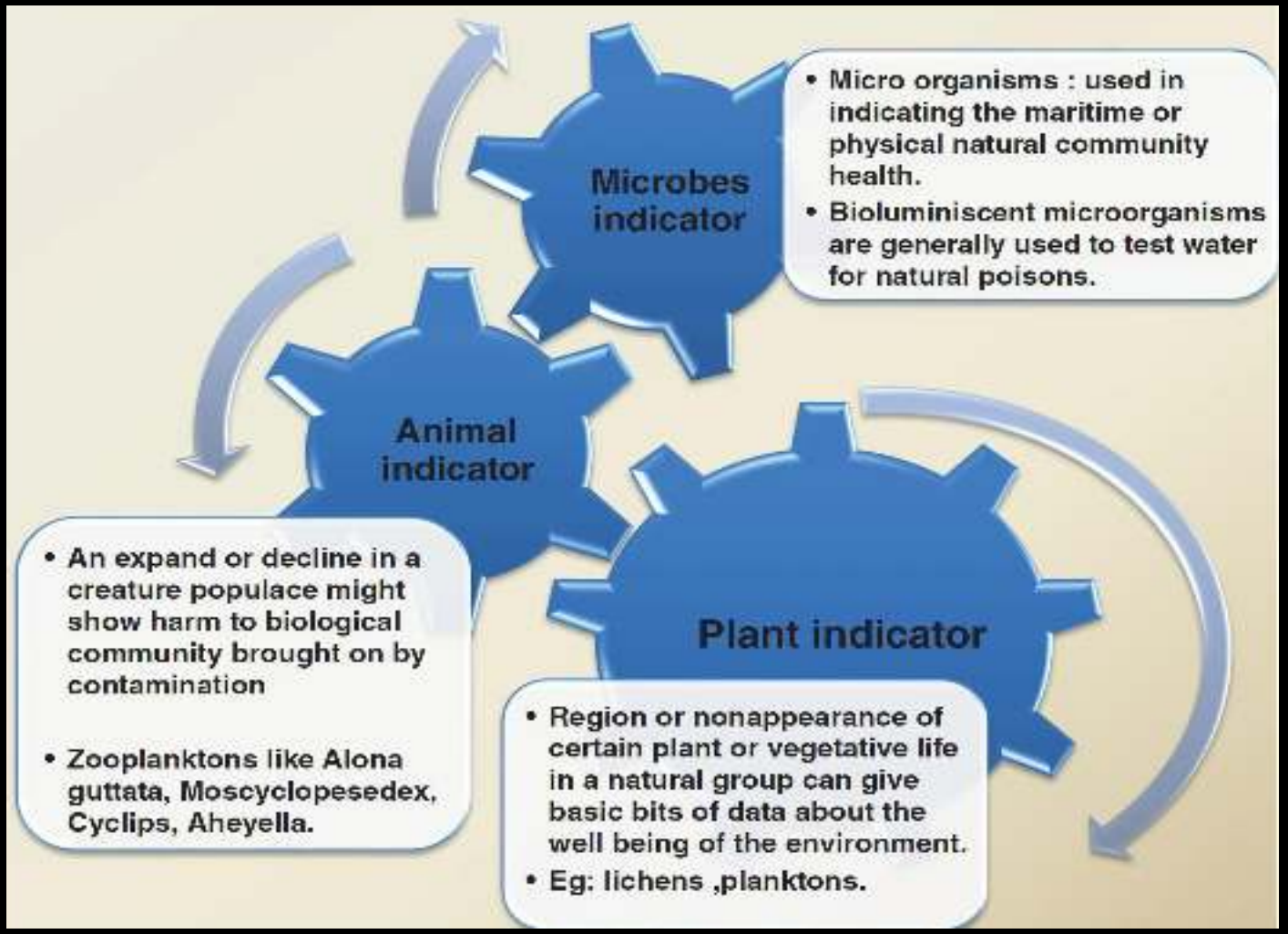
Cynophyta, a type of phytoplankton, is one particularly powerful bioindicator which is known to indicate rapid eutrophication of water bodies such as reservoirs, lakes, etc. via the creation of bloom formations.

END

Environmental Biotechnology

Types of bioindicators:
Plant Indicators

Types of bioindicators : *Plant Indicators*



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Types of bioindicators : *Plant Indicators*

Plants are used as very sensitive tools for prediction and recognition of environmental stresses.

The presence or absence of some specific plants or other vegetation provides ample information about environmental health.

Types of bioindicators : *Plant Indicators*

e.g., Lichens generally found on the trunks of trees and rocks, react to ecological changes in forests, including changes in the structure of the forest, air quality, and climate (e.g., increased level of SO_2, N_2, etc.)

Types of bioindicators : *Plant Indicators*

e.g., Wolffia globosa is an important tool for showing cadmium sensitivity and contamination

Types of bioindicators : *Plant Indicators*

Marine plants provide valuable information to predict the status of oceanic environment, as they are immobile and rapidly obtain equilibrium with their natural surrounding.

Types of bioindicators : *Plant Indicators*

END

Changes in the diversity of species of phytoplankton, including *Euglena clastica*, *Phacus tortus*, and *Trachelon anas*, indicate the pollution of marine ecosystems

Environmental Biotechnology

Types of bioindicators:

Microbial Indicators

Types of bioindicators: *Microbial Indicators*

Microorganisms are often used as health indicators of aquatic and terrestrial ecosystems. Due to their abundance, they are easy to test and readily available.

e.g., Some microorganisms when exposed to cadmium and benzene contaminants develops new proteins known as stress proteins which can be used as early warning signs.

Types of bioindicators: *Microbial Indicators*

Microorganisms have a rapid rate of growth, and react to even low levels of contaminants and other physicochemical and biological changes.

From a research perspective they give important signs of environmental change

Types of bioindicators: *Microbial Indicators*

Microbial indicators can be used in a variety of ways to detect environmental pollutants in water including the use of bioluminescent bacteria. The presence of toxins in waters can be easily monitored by disturbance of microbial metabolism
e.g., changes in the amount of light emitted by the bacteria.

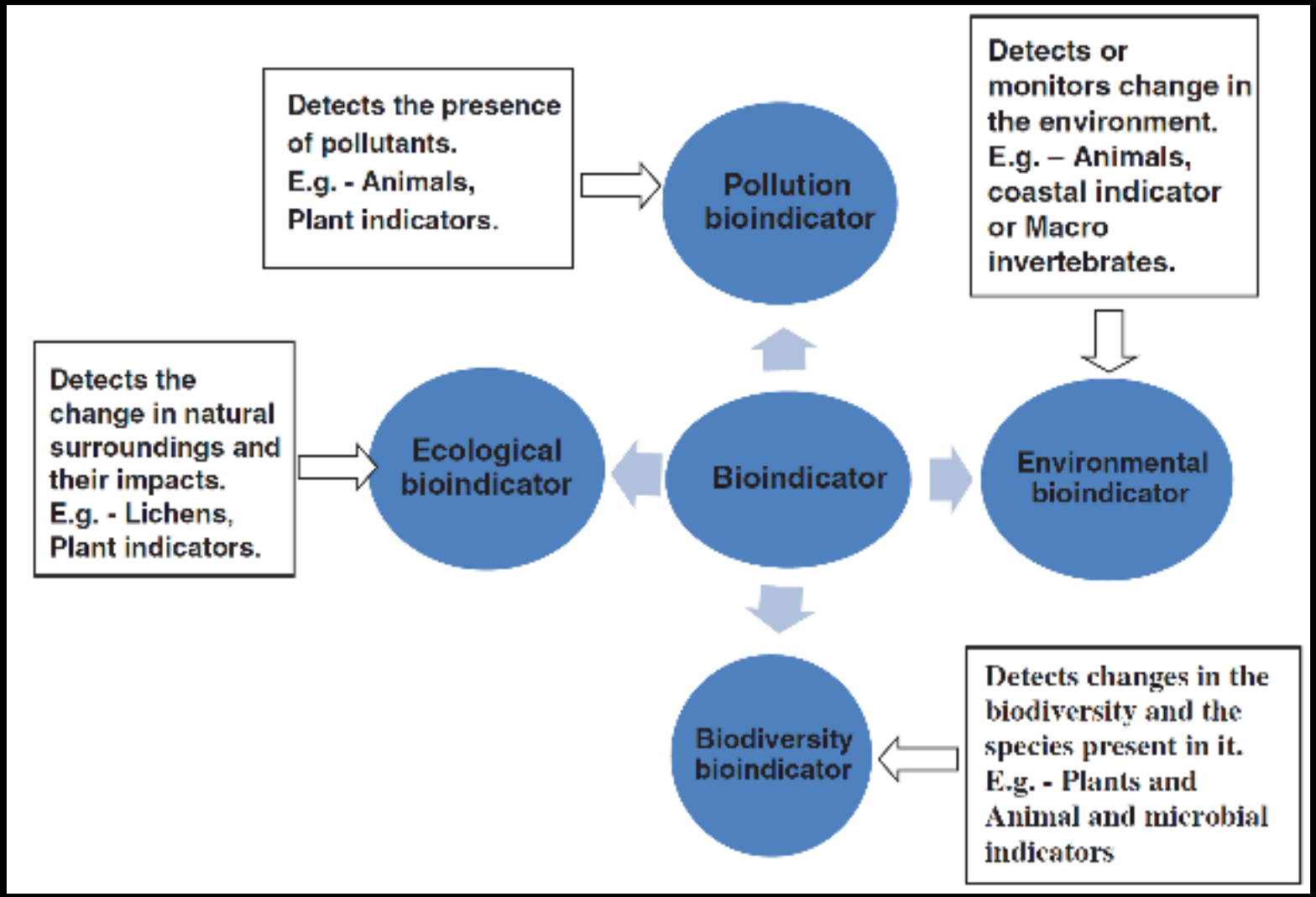
Types of bioindicators: *Microbial Indicators*

In comparison to other available traditional tests, these tests are very quick to monitor.

Types of bioindicators: *Microbial Indicators*

e.g., *Vogesella indigofera* which reacts to heavy metals quantitatively. In the absence of metal pollution, this bacterium produces blue pigmentation. Alternatively, under the vicinity of hexavalent chromium, the production of the pigment is blocked.

Types of bioindicators: *Microbial Indicators*



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Environmental Biotechnology

Biomonitoring

Biomonitoring

Bio-organisms are basically used to define the characteristics of a biosphere.
These organisms are known as Bioindicators or biomonitors, both of which may vary considerably.

Bioindicators indicate the quality of changes taking place in the environment while biomonitors are used to get quantitative information on the quality of the environment.

Biomonitoring

Monitoring can be done for various biological processes or systems with the objective of observing the temporal and spatial changes in health status.

Helps in assessing the impacts of specific environment or anthropogenic stressors and in assessing the viability of anthropogenic measures (e.g. reclamation, remediation, and reintroduction)

Biomonitoring

The species diversity is used as a prime aspect in biological monitoring.

Biomonitoring is one of the essential components for assessing the quality of water and has become an integral element of conducting studies on water pollution.

Biomonitoring

Biomonitors are freely available all around the world.
They fundamentally mirror the natural impact over creatures and can be used and understood with minimum preparation and training.

Biomonitoring

END

Though all natural species can be considered biomonitors to some degree, the above focal points apply well to planktons and similar species type, when water pollution is considered.

Environmental Biotechnology

Planktons

Planktons

In many water bodies, such as, seas, lakes, streams, and swamps, significant biological production is carried out by plankton.

Planktons

Planktons are composed of organisms with chlorophyll (i.e. phytoplankton and animals such as zooplanktons).

These planktons consist of communities that float along currents and tides, yet they fuse and cycle important quantities of energy that is then passed on to higher trophic levels.

Planktons

Indian lentic ecosystems were researched for planktons amid the mid-twentieth century. These studies demonstrated that the predominant planktons and their regularity are exceptionally variable in diverse water bodies relying upon their supplement status, age, morphometry, and other location variables … indicators of trophic state of the lakes

Planktons

Planktons react rapidly to ecological changes and are viewed as excellent indicators of water quality and trophic conditions due to their short time and rapid rate of reproduction.

Planktons

The occurrence of planktonic organisms is identified with the resistance range in relation to abiotic ecological components (Temperature, Oxygen fixation, and pH) as well as the biotic connections among organisms.

END

Changes within the communities of planktons provide the platform to determine the trophic state of water bodies.

Environmental Biotechnology

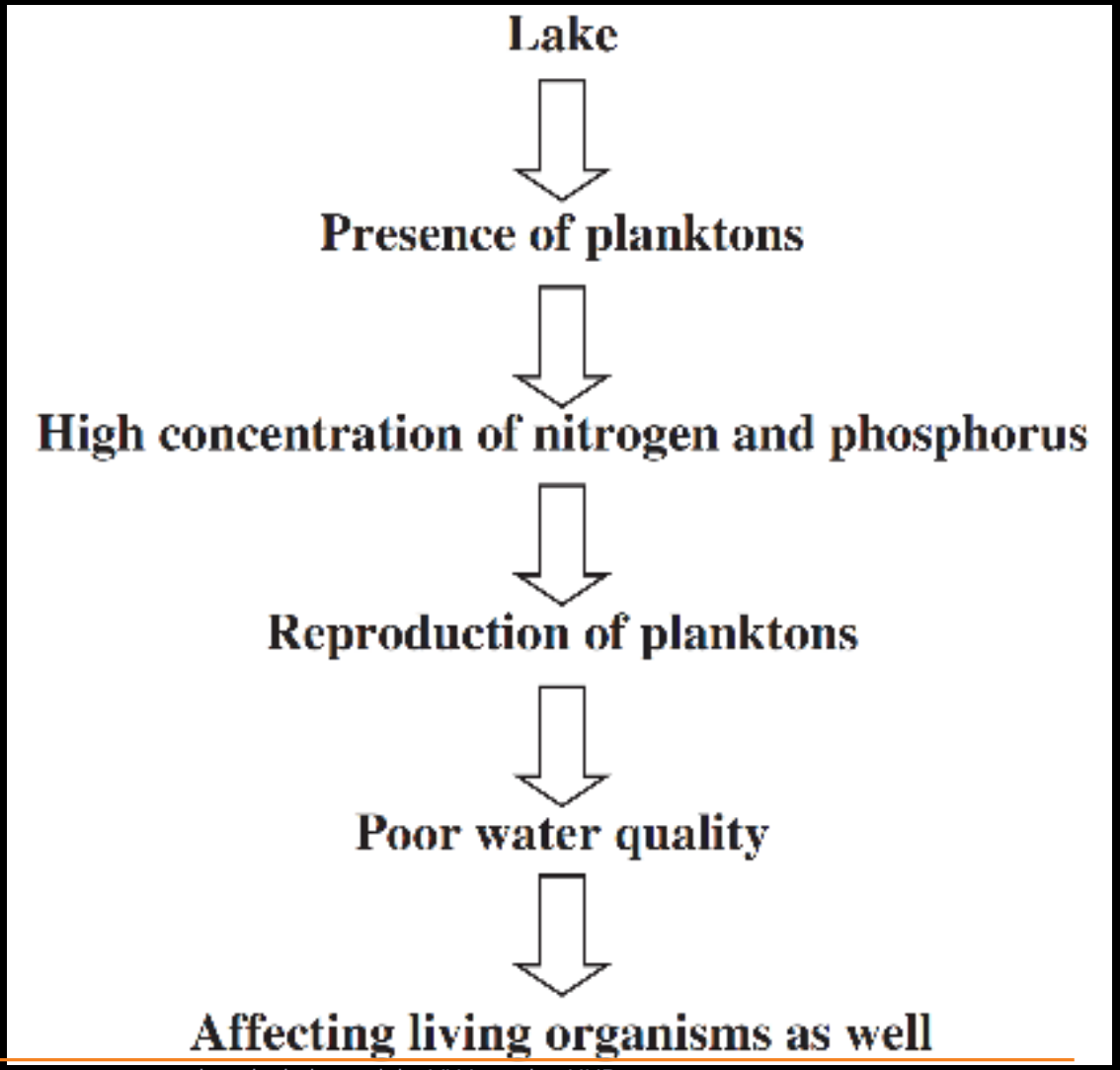
Planktons as an indicator of water pollution

Planktons as an indicator of water pollution

Planktons are profoundly sensitive to natural change and thus are best markers of water quality, especially lakes.

Many planktons reproducing at an increased rate in lakes when there are high centralizations of phosphorus and nitrogen.

Planktons as an indicator of water pollution



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Planktons as an indicator of water pollution

In addition to being a health indicator, planktons are also the fundamental sustenance for many larger organisms in the lake.

Planktons as an indicator of water pollution

Plankton also plays an important role in biological deterioration organic matter.
However, if plankton populations are too large this creates other problems in managing the water body.
Here, fish play an important role by grazing the planktons.

Planktons as an indicator of water pollution

END

Additionally, certain planktons such as cyanobacteria produce toxins which are harmful for fish growth. Thus planktons can be termed as useful or harmful, with respect to wastewater fed production of fishes.

Environmental Biotechnology

Phytoplankton

Phytoplankton

Phytoplanktons, also known as microalgae, are similar to terrestrial plants in that they contain chlorophyll and require daylight to live and develop.
Most are light and swim in the upper portion of the sea, where light infiltrates the water.

Phytoplankton

Algae are quite sensitive to contamination, and this may be reflected in their population levels and/or rates or photosynthesis.

Phytoplankton

Table 1. Types of phytoplankton and its indications.

Names of phytoplankton	Indications	References
Reen algae	Facilitates the growth of fishes	Khatri and Tyagi (2015)
Mosses, liverworts	Pollution by accumulation of metals	Uttah et al. (2008)
Charophytes	Quality of water	Uttah et al. (2008)
Selanastrum	Water pollution	Uttah et al. (2008)
Wolffiaglobose	Contamination of cadmium	Uttah et al. (2008)
Euglena gracilis	Organic pollution in lakes	Hosmani (2014)
Chlorella vulgaris	Helps in removal of heavy metal contamination from water and soil	Lilian (2009)
Chlorococcales like *C. vulgaris* and *A. falcatus* et.al Paramasivam and Sreenivasan (1981)	Indicators of the paper industry and sewage waste	Lilian (2009)

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Phytoplankton

END

When there is change in the diversity of phytoplankton species, or in the metabolites associated with it, it may indicate pollution of the marine ecosystem.

Environmental Biotechnology

Evidences pertaining to phytoplankton

Evidences pertaining to phytoplankton

Phytoplanktons have been used for successful observation of water contamination and are a useful indicator of water quality.

Relationship between the growth rate of an algal population, photosynthesis, and nutrient concentration in the water body.

Evidences pertaining to phytoplankton

Contaminations can influence the connection between rate of growth and each of these variables.
For example, if there is an industrial effluent which is colored or contains suspended solids, light may be filtered or absorbed causing a reduction in rate of growth.

Evidences pertaining to phytoplankton

Overnell et al. demonstrated that light prompted oxygen evolution from the freshwater species *Chlamydomonas reinhardtii* was sensitive to cadmium, methyl mercury, and lead. Moore et al. discovered that organo-chlorine compounds decrease use of bicarbonate by estuarine phytoplankton. Whitacre et al. also produced significant research on the effect of numerous chlorinated hydrocarbons on fixation of carbon by phytoplankton.

Evidences pertaining to phytoplankton

Phytoplanktons are also an important source of pollutant transfer from water to upper tropic levels and even to humans. Algae are unable to decompose the pesticides and are thus a link of transfer to herbivores when fed upon.

Evidences pertaining to phytoplankton

Substances gathering and intake plays an important role in pollution dynamics of phytoplankton.

If light is obstructed, it hampers the intake of ammonia and nitrate by aquatic phytoplankton as indicated by Mac Isaac and Dugdale.

END

Environmental Biotechnology

Zooplanktons

Zooplanktons

Zooplanktons are microscopic animals living near to the surface of the water body.
They are poor swimmers, instead relying on tides and currents as a transport mechanism.
They feed upon phytoplanktons, bacterioplanktons, or detritus (i.e. marine snow).

Zooplanktons

Zooplanktons constitute a vital food source for fish.
They also play an important role as Bioindicators and help to evaluate the level of water pollution.
They are assumed to be a vital part in indicating water quality, eutrophication, and production of a freshwater body.

Zooplanktons

In order to determine the status of a freshwater body it is necessary to measure seasonal variations and presence of zooplanktons.

Zooplankton development and conveyance are subject to abiotic (e.g. temperature, saltiness, stratification, and pollutants) and biotic parameters (e.g. limitation of food, predation, and competition)

END

Environmental Biotechnology

Evidences pertaining to zooplanktons

Evidences pertaining to zooplanktons

Lake Mirik, in Darjeeling, Himalayas, on was polluted due to toxins let into the lake resulting in a decreased pH and an increased acidity level. Brought on a reduction in the quantity of species and changes in species strength.
In this condition, cladocerans (Bosmina, Moina, and Daphnia) and copepods (Phyllodiaptomus and cylops) were found.

Evidences pertaining to zooplanktons

Trichopria tetrat could be utilized as contamination indicators as they have been seen in lakes rich in phosphorus and other heavy metals. This species was obtained in the past in sewage-contaminated tanks (Zannatul & Muktadir 2009).

Evidences pertaining to zooplanktons

Variation in the population of copepods, seasonally in various water bodies; density was highest in the rainy season, while it reduced in summers due to high temperatures.

Evidences pertaining to zooplanktons

END

Though Zooplankton may be present in an extensive variety of ecological conditions, yet disintegrated oxygen, temperature, salinity, pH, and other physicochemical parameters are restricting elements.

Environmental Biotechnology

Aquatic insects as environmental indicators

Aquatic insects as environmental indicators

https://www.youtube.com/watch?v=b4Gbv6-dktw

Environmental Biotechnology

Pollution and Pollution Control

Pollution and Pollution Control

Pollution, the most frequently talked about of all environmental Problems, yet in many respects, it can often remain one of the least understood.

Pollution and Pollution Control

The diverse nature of potentially polluting substances can lead to some confusion.

Not all pollutants are manufactured or synthetic, and under certain circumstances, many substances may contribute to pollution.

This inevitably leads to some difficulty in any attempt at classifying pollutants.

Pollution and Pollution Control

" 'Pollution of the environment' means pollution of the environment due to the release (into any environmental medium) from any process of substances which are capable of causing harm to man or any other living organisms supported by the environment." *(EPA 1990).*

Pollution and Pollution Control

END

"…the escape of any substance capable of causing harm to man or any other living organism supported by the environment." *(EPA 1990).*

Environmental Biotechnology

Classifying Pollution

Classifying Pollution

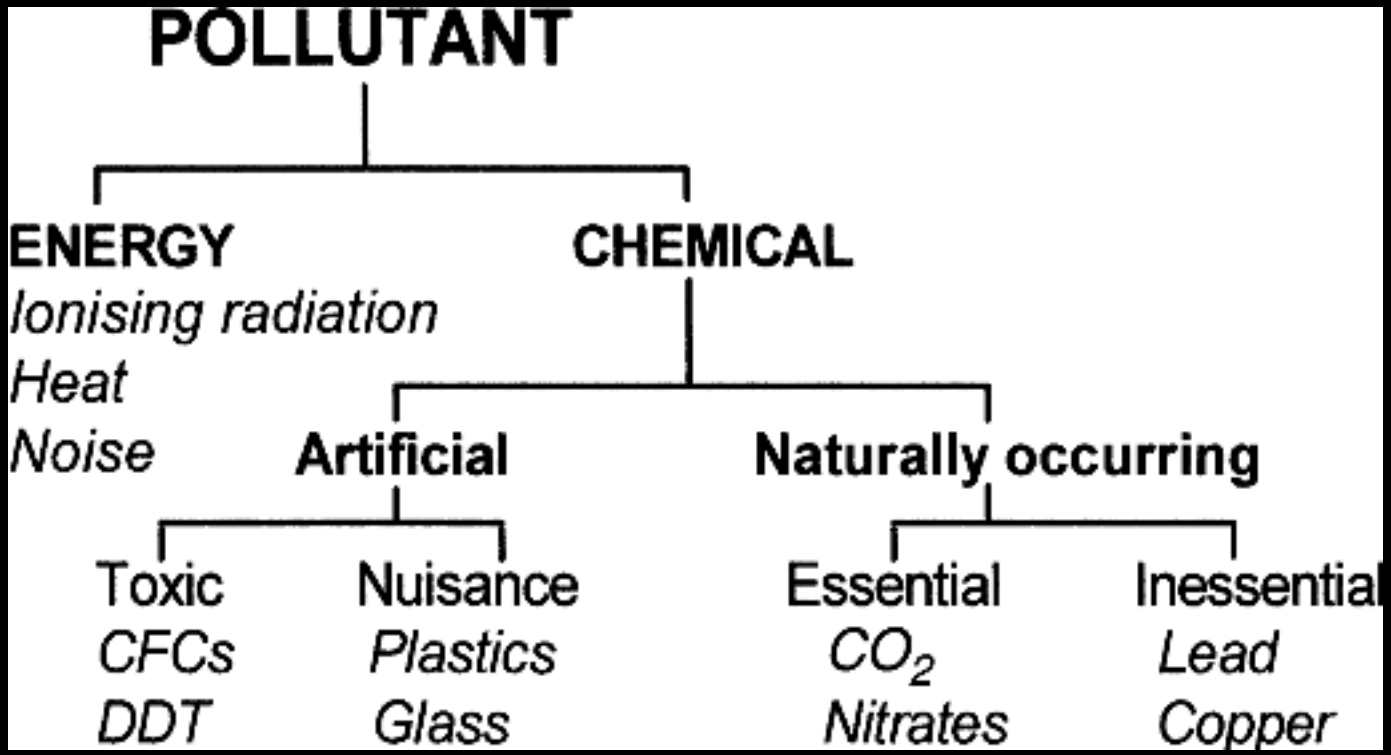
The diverse nature of potential pollutants makes their systematisation difficult in absolute terms. Possible to produce functional classifications on the basis of various characteristics. However, all such classification is essentially artificial and subjective.

Classifying Pollution

Classification may, for example be made on the basis of

- the chemical nature of the substance,
- physical nature of the substance,
- its source,
- the environmental pathway used,
- the target organism affected, or
- simply its gross effect.

Classifying Pollution



Classifying Pollution

END

The consideration of a pollutant's properties is a particularly valuable approach when examining real-life pollution effects.
E.g.,

- toxicity;
- persistence;
- mobility;
- ease of control;
- bioaccumulation;
- chemistry.

Environmental Biotechnology

Toxicity

Toxicity

Toxicity represents the potential damage to life and can be both short and long term.

It is related to

- type of the pollutant,
- concentration, and
- the time of exposure.

Toxicity

Intrinsically highly toxic substances can kill in a short time, while less toxic ones require a longer period of exposure to do damage.

In the case of low concentration exposure, some pollutants may also have an effect on an organism's behaviour or its susceptibility to environmental stress over its lifetime.

Toxicity

Availability also features as an important influence, both in a gross, physical sense and also in terms of its biological availability to the individual organism, together with issues of its age and general state of health.

END

Environmental Biotechnology

Persistence & Mobility

Persistence & Mobility

Persistence

How persistent a pollutant is.

Environmental persistence is a particularly important factor in pollution and is often linked to mobility and bioaccumulation.

Persistence & Mobility

Persistence

Highly toxic chemicals which are environmentally unstable and breakdown rapidly are less harmful than persistent substances, even though these may be intrinsically less toxic.

Persistence & Mobility

Mobility

The tendency of a pollutant to disperse or dilute is a very important factor in its overall effect, since this affects concentration, as well as exposure to the living organisms.

Persistence & Mobility

Mobility

Some pollutants are not readily mobile and tend to remain in ‘hot-spots’ near to their point of origin. Others spread readily and can cause widespread contamination.

Persistence & Mobility

END

Mobility

Whether the pollution is continuous or a single event, and if it has arisen from a single point or multiple sources, form important considerations.

Environmental Biotechnology

Ease of control, & Bioaccumulation

Ease of control, & Bioaccumulation

Ease of control

Many factors contribute to the overall ease with which any given pollutant can be controlled, including

- the mobility of the pollutant,
- the nature,
- extent or duration of the pollution event, and
- local site-specific considerations.

Ease of control, & Bioaccumulation

Ease of control

Clearly, control at source is the most effective method, since it removes the problem at its origin.
However, this is not always possible.
In such cases containment may be the solution, though this can itself lead to the formation of highly concentrated hot-spots.

Ease of control, & Bioaccumulation

Ease of control

For some substances, the dilute and disperse approach may be more appropriate, though the persistence of the polluting substances must obviously be taken into account when making this decision.

Ease of control, & Bioaccumulation

Bioaccumulation

Some pollutants, even when present in very small amounts within the environment, can be taken up by living organisms and become concentrated in their tissues over time.

Ease of control, & Bioaccumulation

Bioaccumulation

This is a major consideration, since even relatively low background levels of contamination may accumulate up the food chain.

Ease of control, & Bioaccumulation

Bioaccumulation & Biomagnification

Bioaccumulation refers to how pollutants enter a food chain, Biomagnification refers to the tendency of pollutants to concentrate as they move from one trophic level to the next.

Ease of control, & Bioaccumulation

END

<u>Bioaccumulation & Biomagnification</u>

In order for biomagnification to occur, the pollutant must be:

- long-lived,
- mobile,
- soluble in fats,
- biologically active.

Environmental Biotechnology

Chemistry of pollutants

Chemistry of pollutants

Pollution effects are not always entirely defined by the initial nature of the contamination.

The reaction or breakdown products of a given pollutant can sometimes be more dangerous than the original substance.

Chemistry of pollutants

The principle underlying much of practical bioremediation in general involves the break down of pollutants to form less harmful products.

Chemistry of pollutants

Other substances present and the geology of the site may also influence the outcome.

END

Both synergism and antagonism are possible.

Environmental Biotechnology

The Pollution Environment

The Pollution Environment

There is sometimes a tendency for contamination to be considered somewhat in isolation from its context.

It is important to remember that pollution cannot properly be assessed without a linked examination of the environment.

The Pollution Environment

The nature of the soil or water which harbours the pollution can have a major effect on the actual expressed end-result.

The Pollution Environment

In case of soil particularly, properties such as

- depth of soil,
- texture,
- type,
- porosity,
- humus content,
- moisture,
- microbial complement, and
- biological activity

can all have a effect on outcome of a pollutant.

The Pollution Environment

The more stable and robust the environmental system affected, the less damage a given pollution event will inflict.

Post pollution survival of a given environment depends on the maintenance of its natural cycles.

The Pollution Environment

Equally obviously, artificial substances which mimic biological molecules can often be major pollutants since they can modify or interrupt these processes and pollution conversion can spread or alter the effect

END

Environmental Biotechnology

Pollution Control Strategies:

Dilution and dispersal

Dilution and dispersal

Dilute and disperse

The attenuation of pollutants by permitting them to become physically spread out, thereby reducing their effective point concentration.

Dilution and dispersal

Dilute and disperse

The attenuation of pollutants by permitting them to become physically spread out, thereby reducing their effective point concentration.

Dilution and dispersal

It depends on the nature of the pollutant, and the characteristics of the specific pathway/ mechanism used to achieve this.

Dilution and dispersal thus has varying degrees of effectiveness in air, water or soil.

END

Environmental Biotechnology

Pollution Control Strategies:

Dilution and dispersal

Air, Water, & Soil

Dilution and dispersal: *Air, Water, & Soil*

Air:

Air movement gives good dispersal and dilution of gaseous emissions.

Lighter particles spread easily.

Heavier particulates tend to fall out near the source.

Dilution and dispersal: *Air, Water, & Soil*

Water:

Good dispersal and dilution potential in large bodies of water.

Smaller watercourses clearly have a correspondingly lower capacity.

Moving bodies of water disperse pollutants more rapidly than still ones.

Dilution and dispersal: *Air, Water, & Soil*

END

Soil:
Movement through the soil represents another opportunity for the dilute and disperse approach, often with soil water playing a significant part, except where excessive ecohydrologic separation becomes a limiting factor.

Typically aided by the activities of resident flora and fauna.

Environmental Biotechnology

Concentration and Containment

Concentration and Containment

The principle is completely opposed to the previous approach of Dilution and Dispersal.

It is an attempt to gather together the offending substance and prevent its escape into the surrounding environment.

Concentration and Containment

Concentration and Containment vs Dilution and Dispersal.

It is fair to say that there is a place for both, dependent on individual circumstances.

END

The idea of a ‘best’ method, at least in absolute terms, is of little value.

Environmental Biotechnology

Practical Toxicity Issues (Part I)

Practical Toxicity Issues (Part I)

There are two main mechanisms by which the toxic action of pollutants arises, often labelled ‘direct’ and ‘indirect.’

Practical Toxicity Issues (Part I)

In the ‘direct’ mechanism, the effect arises by the contaminant combining with cellular constituents or enzymes and thus preventing their proper function.
In the ‘indirect’ mechanism, the damage is done by secondary action resulting from their presence, typified by histamine reactions in allergic responses.

Practical Toxicity Issues (Part I)

The significance of natural cycles...
In many respects the functional toxicity of a pollution event is often no more than the obverse aspect of this same coin, just an overburdening of existing innate systems.

Inability to deal with the contaminant by normal routes, rather than the simple presence of the substance Itself.

Practical Toxicity Issues (Part I)

The case of metals is a good example.
Under normal circumstances, processes of weathering, erosion and volcanic activity lead to their continuous release into the environment.

Corresponding natural mechanisms exist to remove them from circulation, at a broadly equivalent rate.

Practical Toxicity Issues (Part I)

END

However, human activities, particularly after the advent of industrialization, have seriously disrupted these cycles

Environmental Biotechnology

Practical Toxicity Issues (Part II)

Practical Toxicity Issues (Part II)

The toxicity of metals is related to their place in the periodic table, and reflects their affinity for amino and sulphydral groups (associated with active sites on enzymes).

Practical Toxicity Issues (Part II)

Metal periodicity and toxicity

Metal group	Relative toxicity
Group IA	Na < K < Rb and Cs
Group IB	Cu < Ag < Au
Group IIA	Mg < Ca < Sr < Ba
Group IIB	Zn < Cd < Hg
Group IIIA	Al < Ga < In < Tl

Practical Toxicity Issues (Part II)

In broad terms, type-A metals are less toxic than type-B, but this is only a generalization and a number of other factors exert an influence in real-life situations.

Practical Toxicity Issues (Part II)

e.g., Passive uptake by plants: an initial binding onto the cell wall followed by diffusion into the cell itself, along a concentration gradient.

As a result, those cations which readily associate with particulates are accumulated more easily than those which do not.

Practical Toxicity Issues (Part II)

In addition, the presence of chelating ligands may affect the bio-availability and thus, the resultant toxicity of metals.

Whereas some metal–organic complexes (Cu–EDTA, for example) can detoxify certain metals, lipophilic organometallic complexes can increase uptake.

Practical Toxicity Issues (Part II)

e.g., effects of metal cations within anaerobic bioreactors

Cation	Stimulatory	Moderately inhibitory	Strongly inhibitory
Sodium	100–200	3 500–5 500	8 000
Potassium	200–400	2 500–4 500	12 000
Calcium	100–200	2 500–4 500	8 000
Magnesium	75–150	1 000–1 500	3 000

Practical Toxicity Issues (Part II)

Interactions between cations under anaerobic conditions may lead to increased or decreased effective toxicity in line with the series of synergistic/antagonistic relationships shown

Toxic cation	Synergistic	Antagonistic
Ammonium-N	Calcium Magnesium Potassium	Sodium
Calcium	Ammonium-N Potassium	Sodium Magnesium
Magnesium	Ammonium-N Calcium	Sodium Potassium
Potassium	–	Sodium
Sodium	Ammonium-N Calcium	Potassium

Practical Toxicity Issues (Part II)

Toxicity is often dependent on the form in which the substance occurs and substances forming analogues which closely mimic the properties of essential chemicals are typically readily taken up and/or accumulated.

e.g., Arsenic and Phosphorus, Selenium and Sulphur.

END

Environmental Biotechnology

Industrial Pollution in Karachi

Industrial Pollution in Karachi

https://www.youtube.com/watch?v=No7lwt6DPzU

Environmental Biotechnology

Practical Applications to Pollution Control

Practical Applications to Pollution Control

Bacteria normally live in an aqueous environment which clearly presents a problem for air remediation. Frequently the resolution is to dissolve the contaminant in water, which is then subjected to bioremediation by bacteria.

Practical Applications to Pollution Control

However, there is scope for future development of a complementary solution utilising the fact that many species of yeast produce aerial hyphae which may be able to metabolise material directly from the air.

Practical Applications to Pollution Control

A variety of substances can be treated, including volatile organic carbon containing compounds (VOCs) like alcohols, ketones or aldehydes and odorous substances like ammonia and hydrogen sulphide (H_2S).

Practical Applications to Pollution Control

The removal of H_2S by biological means was first discussed as long ago as 1920 and the first patent for a truly biotech-based method of odor control was applied for in 1934.
In 1960s the real modern upsurge began, with the use of mineral soil filter media and the first true biofilters were developed in the succeeding decade.

Practical Applications to Pollution Control

The latest state-of-the-art developments have seen the advent of the utilisation of mixed microbial cultures to degrade xenobiotics, including chlorinated hydrocarbons like dichloromethane and chlorobenzene.

Practical Applications to Pollution Control

A number of general features of strategies applied to air contamination.

Typically an operational temperature within a range of 15–30 ◦C, abundant moisture, pH between 6 and 9, high oxygen, and nutrient availability. Most of the substances treated by these systems are water soluble.

Practical Applications to Pollution Control

The available technologies fall naturally into three main types: biofilters, biotrickling filters and bios rubbers.

All three can treat a wide range of flow rates, ranging from 1000 to 100 000m^3/h.

END

Selection based on the concentration of the contaminant, its solubility, the ease of process control and the land requirement.

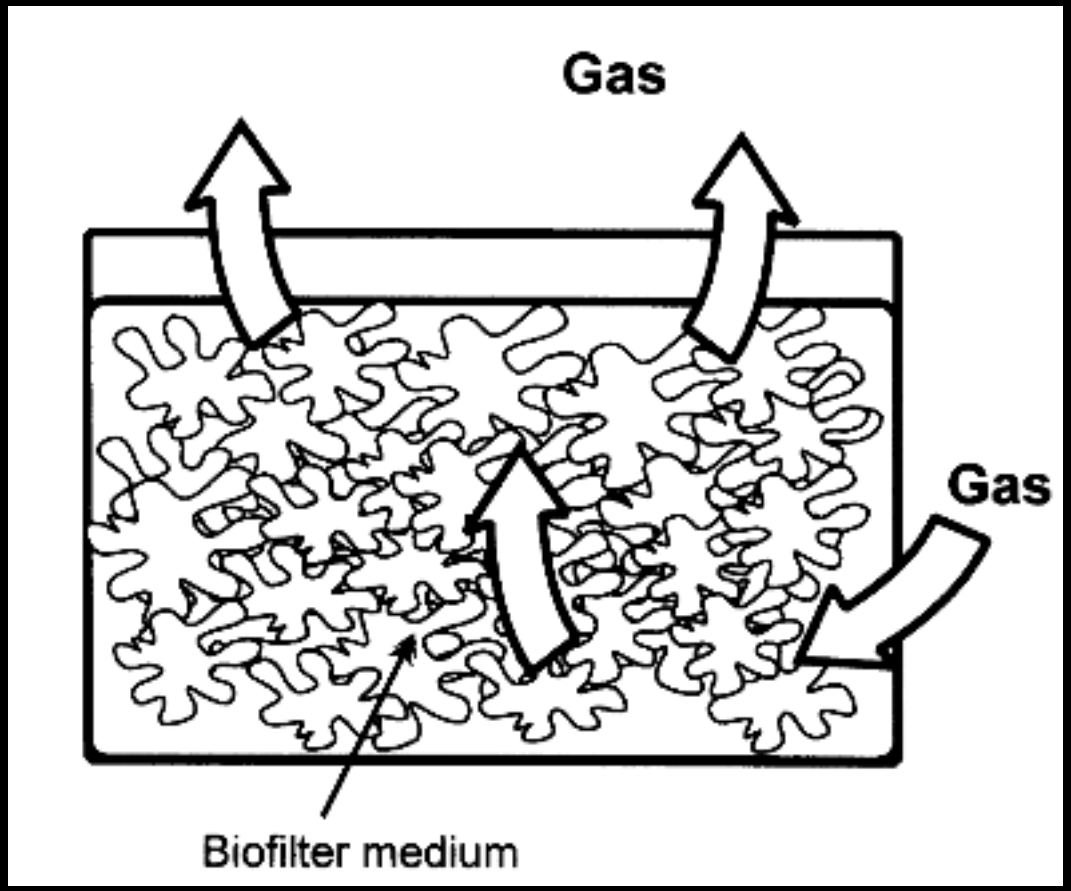
Environmental Biotechnology

Biofilters

Biofilters

The first methods to be developed.
Consists of a relatively large vessel or container, typically made of cast concrete, metal or durable plastic, which holds a filter medium of organic material such as peat, heather, bark chips and the like.
The gas to be treated is forced, or drawn, through the filter.

Biofilters



Biofilters

The medium offers good water holding capacity and soluble chemicals within the waste gas dissolve into the film of moisture around the matrix.

Bacteria, and other micro-organisms present, degrade components of the resultant solution, thereby bringing about the desired effect.

Biofilters

The medium itself provides physical support for microbial growth, with a large surface area to volume ratio, high in internal void spaces and rich in nutrients to stimulate and sustain bacterial activity.

Biofilters

Biofilters need to be watered sufficiently to maintain optimum internal conditions, but waterlogging is to be avoided as this leads to compaction, and hence, reduced efficiency.

END

Properly maintained, biofilters can reduce odour release by 95% or more.

Environmental Biotechnology

Biotrickling filters

Biotrickling filters

In many respects these represent an intermediate technology between biofilters and bioscrubbers, sharing certain features of each.

An engineered vessel holds a quantity of filter medium, but in this case, it is an inert material, often clinker or slag.

Biotrickling filters

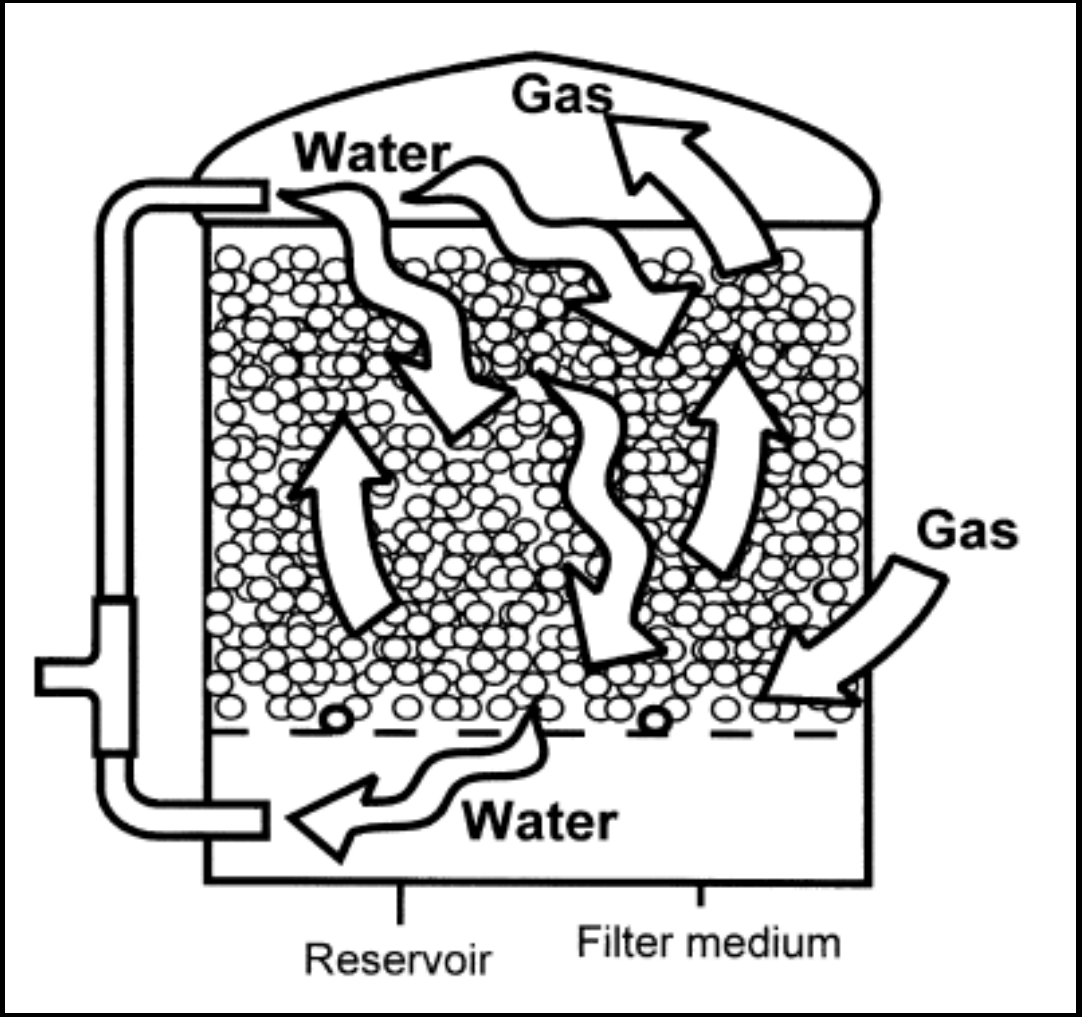
Being highly resistant to compaction, this also provides a large number of void spaces between particles and a high surface area relative to the overall volume of the filter.

The microbes form an attached growth biofilm on the surfaces of the medium.

Biotrickling filters

The odourous air is again forced through the filter, while water simultaneously recirculates through it, trickling down from the top, hence the name.

Biotrickling filters



Biotrickling filters

Thus a counter-current flow is established between the rising gas and the falling water, as shown in the diagram, which improves the efficiency of dissolution.

The biofilm communities feed on substances in the solution passing over them, biodegrading the constituents of the smell.

Biotrickling filters

Process monitoring can be achieved relatively simply by directly sampling the water recirculating within the filter vessel.

Process control is similarly straightforward.

Biotrickling filters

Though the efficiency of the biotrickling filter is broadly similar to the previous method, it can deal with higher concentrations of contaminant and has a significantly smaller foot-print than a biofilter of the same throughput capacity.

Biotrickling filters

END

However, as with almost all aspects of environmental biotechnology, these advantages are obtained by means of additional engineering, the corollary of which is, inevitably, higher capital and running costs.

Environmental Biotechnology

Bioscrubbers

Bioscrubbers

The bioscrubber is not itself truly a biological treatment system, but rather a highly efficient method of removing odour components by dissolving them.

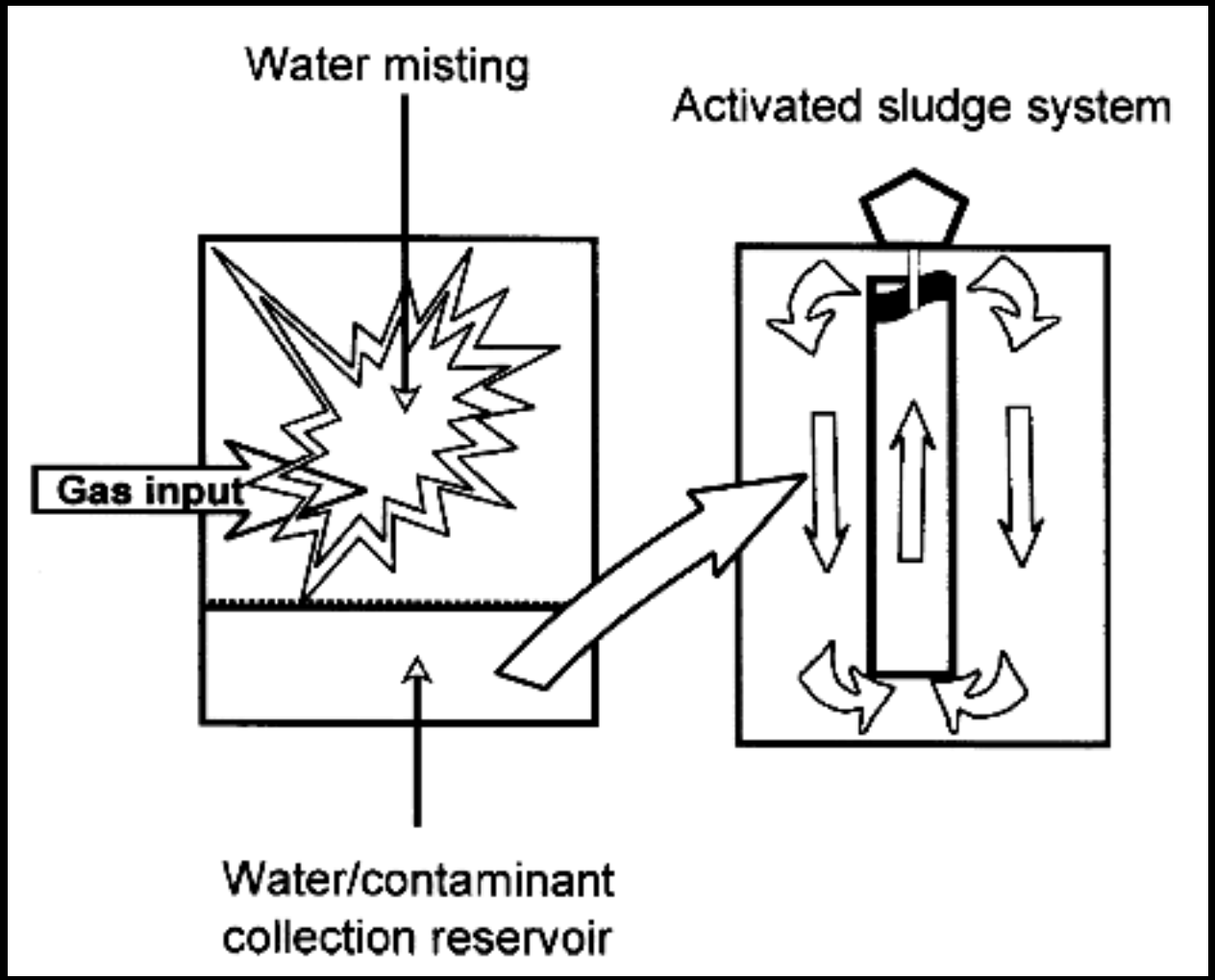
Most appropriate for hydrophilic compounds like acetone or methanol.

Bioscrubbers

The gas to be treated passes through a fine water spray generated as a mist or curtain within the body of the bioscrubber vessel.

The contaminant is absorbed into the water, which subsequently pools to form a reservoir at the bottom.

Bioscrubbers



Bioscrubbers

The contaminant solution is then removed to a secondary bioreactor where the actual process of biodegradation takes place.
In practice, activated sludge systems are often used in this role.

Bioscrubbers

END

As in the preceding case, process control can be achieved by monitoring the water phase and adding nutrients, buffers or fresh water as appropriate.

Environmental Biotechnology

Other options (for treating gases)

Other options (for treating gases)

Biotechnology is not the only answer to controlling air pollution.

Other options (for treating gases)

A number of alternative approaches exist.

END

Absorption, Adsorption, Incineration, Ozonation, etc.

Environmental Biotechnology

Absorption, Adsorption, Incineration, & Ozonation

Absorption

Absorbing the compound in a suitable liquid; this may oxidize or neutralize it in the process.

Adsorption

Activated carbon preferentially adsorbs organic molecules; this can be tailored to give contaminant-specific optimum performance.

Incineration

High temperature oxidation; effective against most contaminants, but costly.

Ozonation

Use of ozone to oxidize some contaminants, like hydrogen sulphide; effective but can be costly.

END

Environmental Biotechnology

Main advantages of biotechnological approaches

Advantages of biotechnological approaches

Main advantages of biotechnological approaches to the issue of air contamination…

- competitive capital costs;
- low running costs;
- low maintenance costs;
- low noise;
- no carbon monoxide production;
- avoids high temperature requirement or explosion risk;
- safe processes with highly ‘green’ profile;
- robust and tolerant of fluctuation.

Advantages of biotechnological approaches

Much of the focus of environmental biotechnology centers on the remediation of pollution or the treatment of waste products.

In many respects, this tends to form the natural constituency of the science and is, certainly, where the bulk of practical applications have generally occurred.

END

Environmental Biotechnology

‘Clean’ Technology

‘Clean’ Technology

The mechanisms by which pollution or waste may be reduced at source are varied.

They may involve changes in technology or processes, alteration in the raw materials used or a complete restructuring of procedures.

‘Clean’ Technology

Biotechnological interventions are principally limited to the technology or processes, though they may also prove instrumental in permitting procedural change

‘Clean’ Technology

The main areas in which biological means may be relevant fall into three broad categories:

- process changes;
- biological control;
- bio-substitutions.

‘Clean’ Technology

END

The field is a fast evolving one and many more types of biotechnological interventions are likely in the future, especially where commercial pressures derive a competitive advantage.

Environmental Biotechnology

Process Changes

Process Changes

Replacement of existing chemical methods of production with those based on microbial or enzyme action is an important potential area of primary pollution prevention and is one role in which the use of genetically modified organisms could give rise to significant environmental benefit.

Process Changes

Biological synthesis, either by whole organism or by isolated enzymes, tends to operate at a lower temperature and, as a result of high enzymatic specificity, gives a much purer yield with fewer by-products, thus saving the additional cost of further purification.

Process Changes

Many examples…
e.g., In the cosmetics sector, high demand for isopropyl myristate used in moisturizing creams. The conventional method for its manufacture runs at high temperature and pressure and the product needs further refinement.
An alternative approach, enzyme-based esterification…

END

Environmental Biotechnology

Textile industry

Textile industry

Long tradition of the use of biological treatment methods in the clothing and textile industry… Amylase enzymes from malt extract to degrade starch-based sizes for cheap and effective reduction of fabric stiffness and improvement to its drape.

Textile industry

Currently, novel enyzmatic methods provide a fast and inexpensive alternative to traditional flax extraction by breaking down the woody material in flax straw, reducing the process time from 7 to 10 days, down to a matter of hours.

Textile industry

The enzyme-based retting processes available for use on hemp and flax produce finer, cleaner fibres, and, consequently, novel processing techniques are being developed to take advantage of this.

Textile industry

Interest is growing in the production of new, biodegradable polymeric fibers which can be synthesized using modified soil bacteria, avoiding the current persistence of these materials in landfills, long after garments made from them are worn out.

Textile industry

In natural fiber production enzymes are useful to remove the lubricants which are introduced to prevent snagging and reduce thread breakage during spinning, and to clean the natural sticky secretions present on silk.

Textile industry

Bio-scouring for wool and cotton uses enzymes to remove dirt, wax, fats, etc.

Bio-bleaching uses enzymes to fade materials, avoiding both the use of caustic agents and the concomitant effluent treatment problems such conventional methods entail.

Textile industry

Biological catalysts have also proved effective in shrink-proofing wool, improving quality while ameliorating the wastewater produced and reducing its treatment costs.

Textile industry

Bio-polishing involves enzymes in shearing off cotton microfibers to improve the material's softness and the drape resistance to pilling of the eventual garments produced.

Textile industry

Bio-stoning has been widely adopted to produce ‘stone-washed’ denim, with enzymes being used to fade the fabric rather than the original pumice stone method, which had a higher water consumption and caused abrasion to the denim.

Textile industry

END

Incorporation of absorbers and microbes within a geotextile produced for use in land management around railways.
Soaking up and subsequently biodegrading diesel and grease, the textile directly reduces ground pollution, provides safer working conditions and reduces risk of fire.

Environmental Biotechnology

Leather industry

Leather industry

The leather industry has a lengthy history of using enzymes.
In the bating process, residual hair and epidermis, together with non-structural proteins and carbohydrates, are removed from the skins, leaving the hide clean, smooth and soft.
Traditionally, pancreatic enzymes were employed.

Leather industry

Around 60% of the input raw materials in leather manufacturing ultimately ends up being discarded and enzyme additions have long been used to help manage this waste.

Leather industry

Efforts by: AARISH ALI

Upsurge in the use of microbially-derived biological catalysts, cheaper and easier to produce, with the possibility of converting waste products into saleable commodities in the latter.

Leather industry

Chemical methods for unhairing hides dissolve the hairs....

Combining chemical and biological catalysts significantly lessens the process time and quantities of reagents used.

The enzymes also make intact hair recovery a possibility, opening up the prospect of additional income from a current waste.

Leather industry

It has been estimated that, in the UK, for a yearly throughput of 400 000 hides, enzymatic unhairing offers a reduction of around 2% of the total annual running costs (BioWise, 2001).

Leather industry

END

Degreasing procedures... Conventional treatments produce both airborne VOCs and surfactants. The use of enzymes in this role not only gives better results, consistent quality, better final color and superior dye uptake, but also considerably reduces VOC and surfactant levels.

Environmental Biotechnology

Desulphurization of coal and oil

Desulphurization of coal and oil

Lots of importance of microbial desulphurization of coal and oil.

The sulphur content of fossil fuels is of environmental concern, e.g., implicated in the production of acid rain, since it produces sulphur dioxide (SO_2) on combustion.

Desulphurization of coal and oil

Most of the work done to date has tended to focus on coal…

High-sulphur oils…

The sulphurous component of coal typically constitutes between 1 and 5%; the content for oil is much more variable.

Desulphurization of coal and oil

There are two main ways to reduce SO_2 emissions.

The first is to lessen the sulphur content of the fuel in the first place, while the second involves removing it from the flue gas.

Desulphurization of coal and oil

A number of conventional methods to remove Sulphur.

Methods for achieving a sulphur content reduction include washing pulverized coal and the use of fluidized bed technology.

To remove sulphur from gases, the most commonly encountered being wet scrubbing.

Desulphurization of coal and oil

Both organic and inorganic sulphur is present in coal.

Biological methods: Aerobic, acidophilic chemolithotrophs like *Thiobacillus* species, for desulphurization of the inorganic sulphur in coal.

Long been known to oxidize sulphur during the leaching of metals from low grade sulphide ores.

Desulphurization of coal and oil

One possible application which has been suggested would be the use of a heap-leaching approach to microbial desulphurization at the mine itself, which is a technique commonly employed for metals…

…Though cheap and simple solution, but difficult to maintain optimum conditions.

Desulphurization of coal and oil

Mesophiles are mostly used but rapid temperature increases coupled with the lengthy period of contact time is required.

Use of extreme thermophile microbes, like *Sulfolobus* sp. may offer the way ahead, though demanding more sophisticated and engineered environment of a bioreactor.

Desulphurization of coal and oil

Removal of organic sulphur from coal has been investigated by using dibenzothiophene (DBT).

A number of organisms have been shown to be able to remove organic sulphur, including heterotrophs like *Pseudomonas*, *Rhizobium* and the fungi *Paecilomyces* and chemolithotrophs like *Sulfolobus*, etc.

Desulfovibrio.

Desulphurization of coal and oil

Successful experiments using *Trametes versicolor* ATCC 200801 and *Phanerochaete chrysosporium* ME 446 to desulphurise Tuncbilek lignite, which has a typically high average sulphur content of around 2.59%

Desulphurization of coal and oil

A range of putative bioreactor designs for desulphurization have been put forward…

However, the state of the art is little advanced beyond the laboratory bench and so the benefit of large scale commercial applications remains to be seen.

END

Environmental Biotechnology

Biological Control

Biological Control

The use of insecticides and herbicides in agriculture…

Many of these chemicals are highly persistent…

One of the areas where biotechnological applications may have significant environmental impact…

Biological Control

Disastrous outcome of Australia's attempts to use the Cane Toad (*Bufo marinus*) to control the cane beetle…

However, in principle, the idea remains sound and considerable research effort…

Biological Control

Essence of the specifically environmental contribution of this type of biointervention lies in its ability to obviate the need for the use of polluting chemicals.

One of the major limitations on the effective use of bio-controls is that these measures tend to act more slowly than direct chemical attacks…

Biological Control

Biotechnology *per se* is not a central, or even necessary, requirement for all of biological control, as many methods rely on whole organism predators…

Biological Control

Biological control methods can provide an effective way to mitigate pesticide use and thus the risk represented to the environment and to public health.

Biological Control

Moreover, bio-controls are often highly target-specific, reducing the danger to other non-pest species.

Biological measures typically demand much more intensive management and careful planning than the simple application of chemical agents.

Biological Control

Success is much more dependent on a thorough understanding of the life-cycles of the organisms involved and can often be much more of a long-term project.

Biological Control

A large number of insects pose a threat to crops & thus represent an economic concern (global insecticide market estimated ~$10 billion (US) / year).

END

Accordingly, much of the biological control currently in practice relates to this group of animals.

Environmental Biotechnology

Whole organism approaches (Part-I)

Whole organism approaches

Three main ways:

Classical biological control requires the importation of natural predators and is principally of use when the pest in question is newly arrived in an area, often from another region or country, having left these normal biological checks behind.

Whole organism approaches

Another form of control involves conservation measures aimed at bolstering the predatory species, which may be a valuable approach when natural enemies already exist within the pest's range.

Whole organism approaches

The third method, augmentation, refers to means designed to bring about the increase in effectiveness of natural enemies to a given pest.

This may consist simply of artificially rearing them in large numbers or may extend to more intensive and sophisticated measures like the modification, either by selective breeding or genetic manipulation, of the predator…

END

Environmental Biotechnology

Whole organism approaches (Part-II)

Whole organism approaches

E.g., augmentation of entomopathogenic parasitic nematodes.

Juvenile stages of the nematodes enter soil insects and many carry pathogenic bacteria.

Once ingested, these bacteria pass out of the nematode and multiply inside the insect, typically causing death within a few days.

Whole organism approaches

Five species of nematode were originally made available on the US agricultural market, namely, *Steinernema carpocapsae*, *S. riobravis*, *S. feltiae*, *Heterorhabditis bacteriophora* and *H. megidis*, each being effective against different groups of insects. Later, *S. glaseri* and *H. marelatus*.

Whole organism approaches

Despite much research and development effort, the initial results were largely unpredictable.

Clearly, farmers and commercial growers will not adopt a biological control which does not reliably offer at least comparable efficacy with traditional insecticides.

Whole organism approaches

The gap between biological control and conventional agro-chemicals has been significantly narrowed… Due to technological improvements in organism production, dosage, quality control and product delivery, coupled with increased understanding of optimal timing and greater target application specificity

Whole organism approaches

Phasmarhabditis hermaphrodita has proven itself to be a highly effective against garden slug.

Augmentation is, obviously, a highly interventionist approach and relies on a regime of continual management to ensure its effectiveness.

Whole organism approaches

There is also a role for the engineered application of biologically derived chemicals in this sector. E.g., *Azadirachta indica*, the neem.

The compound azadirachtin has been identified and isolated from the plant and it has been shown to have broad spectrum insecticidal properties.
Also seems to repel many leaf-eating species.

END

Environmental Biotechnology

Semiochemical agents (Part-I)

Semiochemical agents

Semiochemicals are natural messenger substances which influence growth, development or behaviour in numerous plant and animal species

E.g., pheromones.

Semiochemical agents

END

This has been successfully applied to control various forms of insect pests, either directly to divert them from crops and trap them, or indirectly to trap their natural enemies in large numbers for introduction into the fields for defence.

Environmental Biotechnology

Semiochemical agents (Part-II)

Semiochemical agents

Crops worldwide suffer severe damage as a result of a number of pentatomid insects, amongst which are several of the common brown bugs of North America (*Euschistus* spp.).
They arrive late in the growing season and often cause major harm before detection.

Semiochemical agents

A major part of bio-control involves: a thorough understanding of their migration patterns, a pheromone methyl 2E,4Z-decadienoate…

Scope in three main directions.
Firstly, to capture and eliminate the pest.
Secondly, to harvest predatory bugs for bio-augmentation.
Thirdly, to identify more pheromones…

Semiochemical agents

The Siberian moth *Dendrolimus superans* is a vigorous defoliating pest of northern Asian coniferous forests.

In an attempt to provide a first line of defence against this potential threat in North America, it has been suggested that a blend of Z5,E7-dodecadienal and Z5,E7-dodecadienol, be deployed at US ports of entry.

Semiochemical agents

However, in the case of another pentatomid, *Nezara viridula*, the use of this approach to biological control failed.

Alternative method control: genetic engineering of its gut symbionts to produce a reduced tolerance of environmental stress.
A gram-negative bacterium *Yokenella* sp.

END

Environmental Biotechnology

Bio-Substitutions

Bio-Substitutions

The bio-substitution of many of today's polluting substances or materials is a major potential avenue for the environmental biotechnology.

Bio-Substitutions

END

Biofuels,
Polymers,
Biodegradable
alternatives…

Environmental Biotechnology

Barriers to uptake

Barriers to uptake

The pollution of many inland and coastal waters around the world is a well appreciated environmental problem and wider use of non-toxic, readily biodegradable alternative products could make a huge difference.

Barriers to uptake

In case of bio-lubricants, cost is a major issue.

Though, inevitably, users need to be convinced of the deliverable commercial benefits, the potential market is enormous.

Barriers to uptake

The petrochemical industry has sought to meet the growing demand for more environmentally friendly products by developing biodegradable lubricants based on crude oil.

Barriers to uptake

However, with the agricultural sector, particularly throughout Europe, being encouraged to grow non-food crops commercially, there is a clear opportunity for a sizeable vegetable oil industry to develop.

Barriers to uptake

No denying the burgeoning interest in bio-lubricants, the actual machinery to be lubricated is extremely expensive.

Only a few equipment operators are willing to risk trying these new, substitute oils, they are seldom willing to guarantee their performance.

END

Environmental Biotechnology

Biomimetics

Biomimetics

Biomimicry and biomimetics…

Biomimetics

Biomimicry and biomimetics…

"…field of science in which inspirations are elicited from nature to design practical materials and systems that can imitate structure and function of native biological systems."(Science direct)

Biomimetics

"Biomimetics is an interdisciplinary field in which principles from engineering, chemistry and biology are applied to the synthesis of materials, synthetic systems or machines that have functions that mimic biological processes." (Nature.com)

Biomimetics

Best of these is to be found in attempts to defeat aquatic, and especially marine, biofouling.

Biomimetics

The unwanted accumulation of various forms of life, including algae, microorganisms and sessile animals on submerged structures is a serious economic and practical nuisance to a number of industries.

Biomimetics

According to US Naval sources, micro-fouling by surface-adhering biofilms can increase drag on a warship by up to 20%, while the presence of macro-fouling by barnacles and other larger organisms can add more than 60% overall. Leads to 40% increase in fuel consumption and up to 10% reduction in speed.

Biomimetics

Conventional anti-fouling treatments rely on the biocidal action of various agents, but they are essentially indiscriminate and tend to both leach and accumulate in the ecosystem…

Biomimetics

A common example, tributyltin (TBT), for instance, has been shown to lead to genital abnormalities at concentrations as low as 1 ng/l in the dog whelk Nucella lapillus and at 20 ng/l, normal shell formation in the Japanese oyster *Crassostrea gigas* is disrupted.

Biomimetics

Unlike other marines species, sharks are characteristically untroubled by biofouling and studies of their skin have established that its unique texture coupled with its inherent antimicrobial properties help keep them clean of fouling organisms.

Biomimetics

Modelling of shark skin and the subsequent development of a novel biomimetic coating technology – known as Sharklet™ – which features biomimetic topography replicated in polydimethylsiloxane elastomer has yielded a system which has proven remarkably effective at inhibiting bio-fouling

Biomimetics

It has shown a zoospore settlement rate less than 85% that of a conventional smooth surface.

Also inhibits a range of health threatening microorganisms out of the water, including *Pseudomonas aeruginosa*, Vancomycin-Resistant *Enterococcus*, *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus*

END

Environmental Biotechnology

Simple bio-substitutions

Simple bio-substitutions

Not all bio-substitutions need be the result of lengthy chemical or biochemical synthesis.

Far simpler forms of biological production may provide major environmental benefits.

Simple bio-substitutions

e.g., The production of biomass fuels for direct combustion under short rotation coppicing management…

e.g., The use of ‘eco-building materials’ formed from hemp, hay, straw and flax, as an ecological alternative to conventional materials in the construction industry.

Simple bio-substitutions

Adequate soundproofing requirement at home or work settings…

Difficult or costly to achieve for many standard materials.

Walls made from eco-materials have been found to be particularly effective at sound suppression…

Simple bio-substitutions

Issues of acoustic insulation often go hand-in-hand with the need for thermal insulation…

Products such as Thermafleece™, a high density product manufactured from British sheep's wool…

…Also exhibit exceptional moisture absorption…

Simple bio-substitutions

Thermal conductivity table

Material	Conductivity (W/mK)
Cellulose	0.038–0.040
Fibreglass	0.033–0.040
Flax	0.038–0.040
Hemp	0.039–0.043
Mineral wool	0.033–0.047
Sheep's wool	0.037–0.040

Simple bio-substitutions

Conventional construction and demolition waste poses a considerable disposal problem.

Obvious advantage in relatively inexpensive, lightweight and sustainable materials which are truly biodegradable.
…Still in the quite early stages of their commercial development

END

Environmental Biotechnology

Wastewater Treatment levels

Wastewater Treatment levels

In planning studies for the implementation of the wastewater treatment, the following points must be clearly addressed:

- environmental impact studies on the receiving body
- treatment objectives
- treatment level and removal efficiencies

Wastewater Treatment levels

Wastewater treatment is usually classified according to the following levels:

- Preliminary
- Primary
- Secondary
- Tertiary

Wastewater Treatment levels

Preliminary treatment: the removal of coarse solids only.

Primary treatment: removal of settleable solids and part of the organic matter. (Physical pollutant removal mechanisms are predominant in both levels)

Wastewater Treatment levels

Secondary treatment:
The removal of organic matter and possibly nutrients (nitrogen and phosphorus) by predominantly biological mechanisms.

Tertiary treatment:
The removal of specific pollutants (usually toxic or non-biodegradable compounds) that were not sufficiently removed in the secondary treatment.

Wastewater Treatment levels

The removal efficiency of a pollutant in the treatment or in a treatment stage is given by the formula:

$$E = \frac{C_o - C_e}{C_o} .100$$

END

where:

E = removal efficiency (%)

C_o = influent concentration of the pollutant (mg/L)

C_e = effluent concentration of the pollutant (mg/L)

Environmental Biotechnology

WASTEWATER TREATMENT (WWT) OPERATIONS, PROCESSES AND SYSTEMS

WWT Operations, Processes And Systems

The treatment methods are composed by unit operations and processes, and their integration makes up the treatment systems.

WWT Operations, Processes And Systems

Physical unit operations:
Treatment methods in which physical forces are predominant (e.g. screening, mixing, flocculation, sedimentation, flotation, filtration).

WWT Operations, Processes And Systems

Chemical unit processes:
Treatment methods in which the removal or the conversion of the contaminants occurs by the addition of chemical products or due to chemical reactions (e.g. precipitation, adsorption, disinfection).

WWT Operations, Processes And Systems

Biological unit processes:
Treatment methods in which the removal of the contaminants occurs by means of biological activity (e.g. carbonaceous organic matter removal, nitrification, denitrification).

WWT Operations, Processes And Systems

Liquid phase…

Solid phase…

END

Environmental Biotechnology

Mechanisms for removal of pollutants in wastewater

Removal of Pollutants in Wastewater

Main mechanisms for the removal of pollutants in wastewater treatment

Pollutant	Subdivision		Main removal mechanisms
Solids	Coarse solids (> ~1 cm)	*Screening*	Retention of the solids with dimensions greater than the spacing between the bars
	Suspended solids (> ~1 μm)	*Sedimentation*	Separation of the particles with a density greater than the sewage
	Dissolved solids (< ~1 μm)	*Adsorption*	Retention on the surface of biomass flocs or biofilms

Removal of Pollutants in Wastewater

Main mechanisms for the removal of pollutants in wastewater treatment

Pollutant	Subdivision	Main removal mechanisms	
Organic matter	BOD in suspension (particulate BOD) (> ~1 µm)	*Sedimentation*	Separation of the particles with a density greater than the sewage
		Adsorption	Retention on the surface of biomass flocs or biofilms
		Hydrolysis	Conversion of the BOD in suspension into soluble BOD by means of enzymes, allowing its stabilisation
		Stabilisation	Utilisation by biomass as food, with conversion into gases, water and other inert compounds.
	Soluble BOD (< ~1 µm)	*Adsorption*	Retention on the surface of biomass flocs or biofilms
		Stabilisation	Utilisation by biomass as food, with conversion into gases, water and other inert compounds.

Removal of Pollutants in Wastewater

Main mechanisms for the removal of pollutants in wastewater treatment

Pollutant	Subdivision		Main removal mechanisms
Pathogens	Larger dimensions and/or with protective layer (protozoan cysts and helminth eggs)	*Sedimentation*	Separation of pathogens with larger dimensions and density greater than the sewage
		Filtration	Retention of pathogens in a filter medium with adequate pore size
	Lower dimensions (bacteria and viruses)	*Adverse environmental conditions*	Temperature, pH, lack of food, competition with other species, predation
		Ultraviolet radiation	Radiation from the sun or artificial
		Disinfection	Addition of a disinfecting agent, such as chlorine

Removal of Pollutants in Wastewater

Main mechanisms for the removal of pollutants in wastewater treatment

Pollutant	Subdivision	Main removal mechanisms	
Nitrogen	Organic nitrogen	*Ammonification*	Conversion of organic nitrogen into ammonia
	Ammonia	*Nitrification*	Conversion of ammonia into nitrite, and the nitrite into nitrate, by means of nitrifying bacteria
		Bacterial assimilation	Incorporation of ammonia into the composition of bacterial cells
		Stripping	Release of free ammonia (NH_3) into the atmosphere, under high pH conditions
		Break-point chlorination	Conversion of ammonia into chloramines, through the addition of chlorine
	Nitrate	*Denitrification*	Conversion of nitrate into molecular nitrogen (N_2), which escapes into the atmosphere, under anoxic conditions

Removal of Pollutants in Wastewater

Main mechanisms for the removal of pollutants in wastewater treatment

Pollutant	Subdivision		Main removal mechanisms
Phosphorus	Phosphate	*Bacterial assimilation*	Assimilation in excess of the phosphate from the liquid by phosphate accumulating organisms, which takes place when aerobic and anaerobic conditions are alternated
		Precipitation	Phosphorus precipitation under conditions of high pH, or through the addition of metallic salts
		Filtration	Retention of phosphorus-rich biomass, after stage of biological excessive P assimilation

Environmental Biotechnology

Mechanisms for removal of pollutants from domestic sewage

Removal of Pollutants from Domestic Sewage

Treatment operations, processes and systems frequently used for the removal of pollutants from domestic sewage

Pollutant	Operation, process or treatment system
Suspended solids	• Screening • Grit removal • Sedimentation • Land disposal
Biodegradable organic matter	• Stabilisation ponds and variants • Land disposal • Anaerobic reactors • Activated sludge and variants • Aerobic biofilm reactors
Pathogenic organisms	• Maturation ponds • Land disposal • Disinfection with chemical products • Disinfection with ultraviolet radiation • Membranes

Removal of Pollutants from Domestic Sewage

Treatment operations, processes and systems frequently used for the removal of pollutants from domestic sewage

Pollutant	Operation, process or treatment system
Nitrogen	• Nitrification and biological • denitrification • Maturation and high-rate ponds • Land disposal • Physical–chemical processes
Phosphorus	• Biological removal • Maturation and high-rate ponds • Physical chemical processes

Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Stabilization Ponds

Stabilization Ponds

Facultative pond:
Wastewater flows through a pond and remains therefore many days.
Soluble BOD is aerobically stabilised by planktonic bacteria.

BOD in suspension tends to settle, and is stabillised by anaerobic bacteria at the bottom of the pond.

Stabilization Ponds

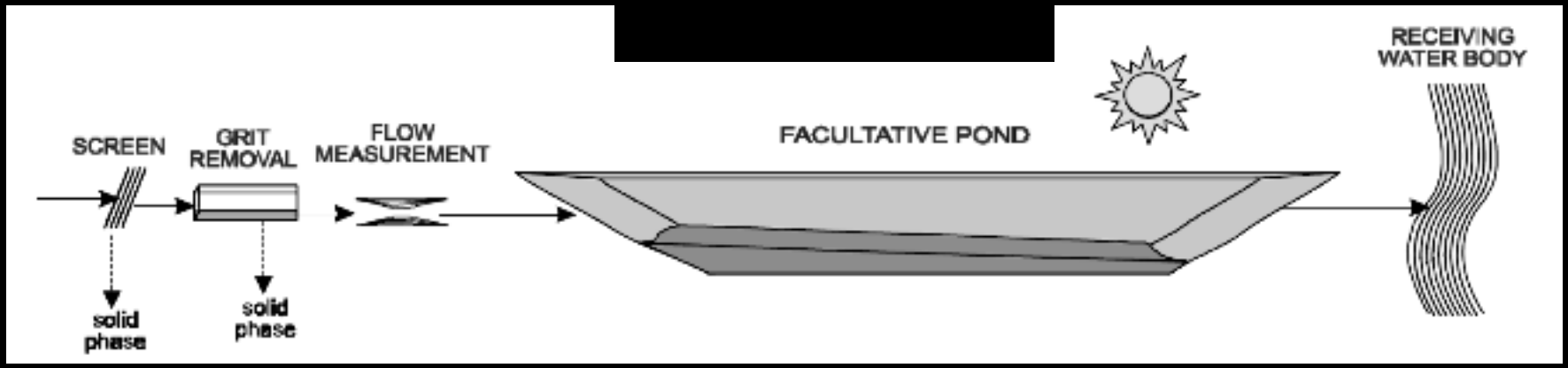
Facultative pond:

The oxygen required by the aerobic bacteria is supplied by algae through photosynthesis.

The land requirements are high.

Stabilization Ponds

Facultative Pond



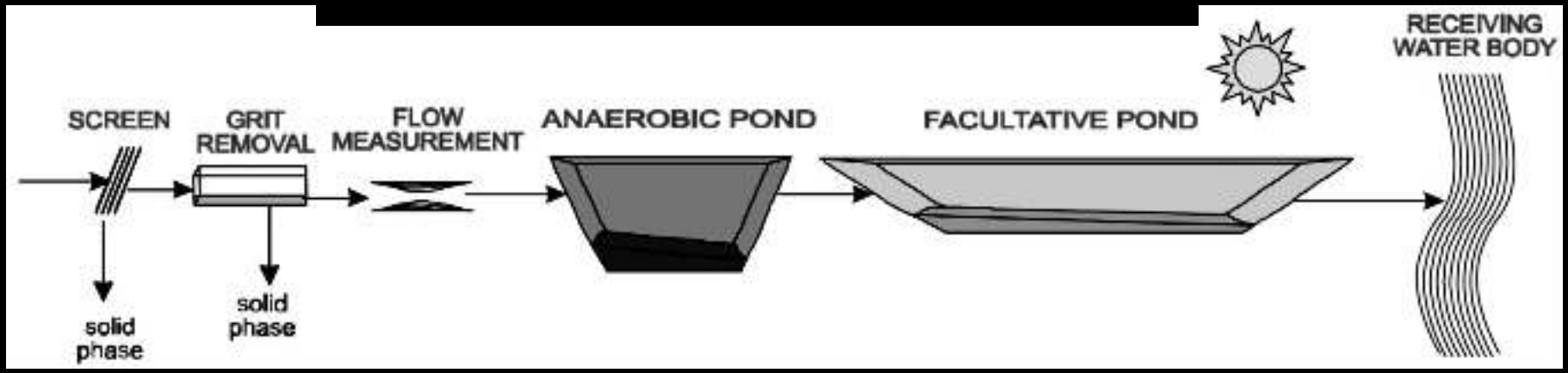
Stabilization Ponds

Anaerobic pond – facultative pond:

~50 to 65% of the BOD is converted in the anaerobic pond, while the remaining BOD is removed in the facultative pond.

Stabilization Ponds

Anaerobic pond – facultative pond



Stabilization Ponds

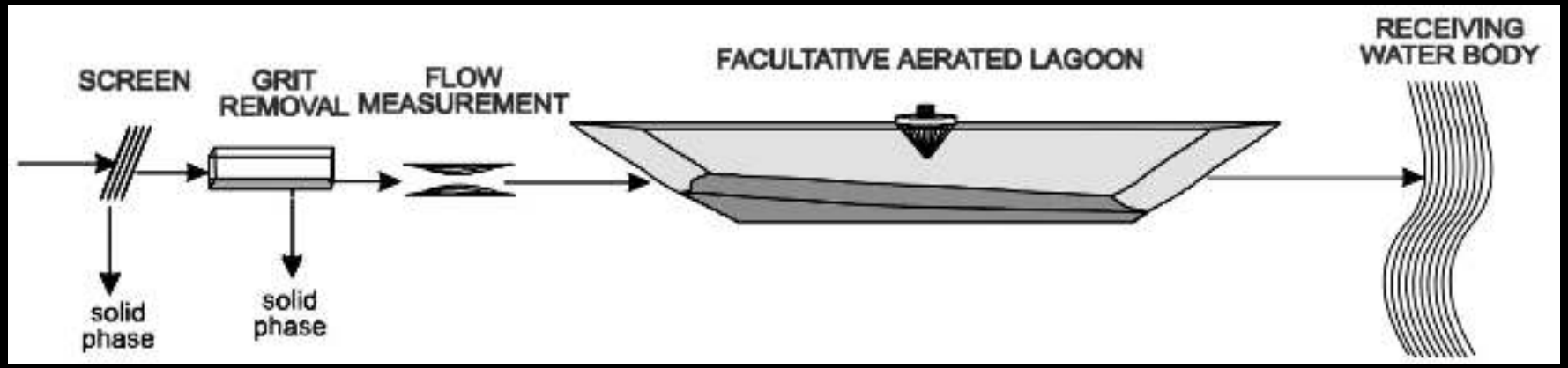
Facultative aerated lagoon:

Similar to facultative Pond, but oxygen is supplied by mechanical aerators.

Still a large part of the sewage solids and biomass settles, being decomposed anaerobically at the bottom.

Stabilization Ponds

Facultative aerated lagoon



Stabilization Ponds

Completely mixed aerated lagoon – sedimentation pond:

Complete mixing is done to disperse the solids (biomass) in the liquid medium.

This increases the BOD removal efficiency.

Smaller volume than a facultative aerated lagoon.

Stabilization Ponds

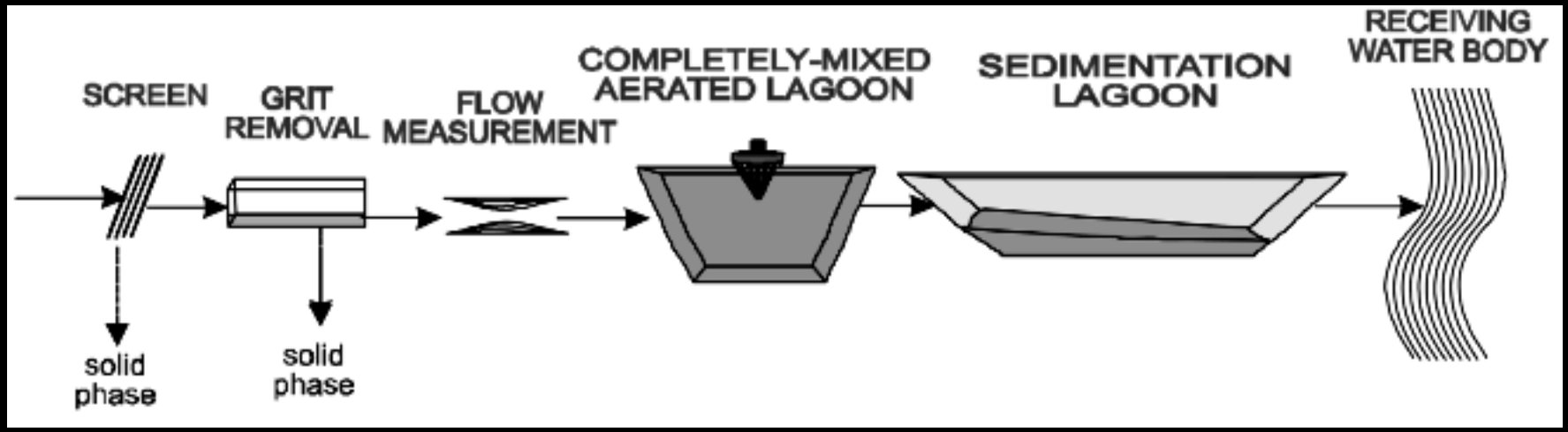
Completely mixed aerated lagoon – sedimentation pond:

However, the effluent contains high levels of solids (bacteria).

The sedimentation pond downstream provides conditions for this removal.

Stabilization Ponds

Completely mixed aerated lagoon – sedimentation pond



Stabilization Ponds

High rate ponds:

Totally aerobic environment to maximise algal production.

To accomplish this, lower depths are employed, allowing light penetration throughout the liquid mass.

Stabilization Ponds

High rate ponds:

Photosynthetic activity is high, leading to high dissolved oxygen concentrations and pH levels.

Increase of the pathogens die-off and to the removal of nutrients.

Stabilization Ponds

High rate ponds:

High rate ponds usually receive a high organic load per unit surface area.

Usually a moderate agitation in the liquid is introduced, caused by a low-power mechanical equipment.

Stabilization Ponds

Maturation ponds:

The main objective is the removal of pathogenic organisms.

Adverse environmental conditions, such as UV radiation, high pH, high DO, lower temperature, lack of nutrients, etc.

Stabilization Ponds

END

Maturation ponds:

Maturation ponds are a post-treatment stage for BOD-removal processes.

Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Land Disposal

Land Disposal

Slow rate system:

Objectives:
(a) wastewater treatment or (b) water reuse through crop production.

Land Disposal

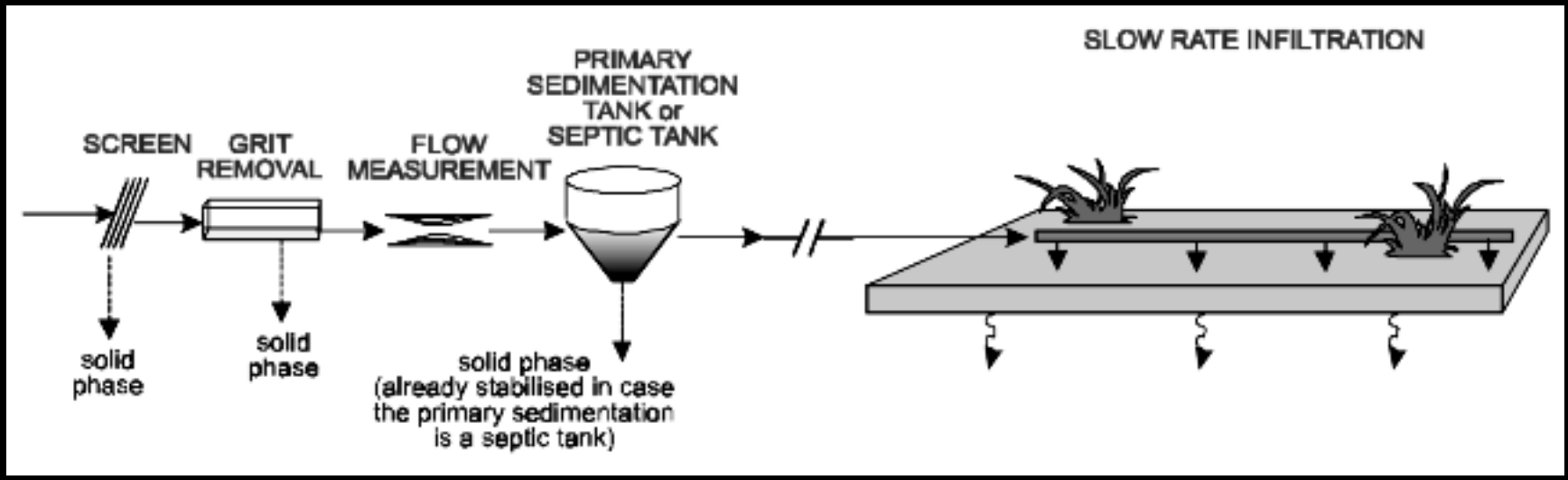
Slow rate system:

Wastewater is applied to the soil, supplying water and nutrients necessary for plant growth.

Some liquid evaporates, some percolates into the soil, and the largest fraction is absorbed by the plants…

Land Disposal

Slow rate system



Land Disposal

Rapid infiltration:

Wastewater is applied in shallow basins.

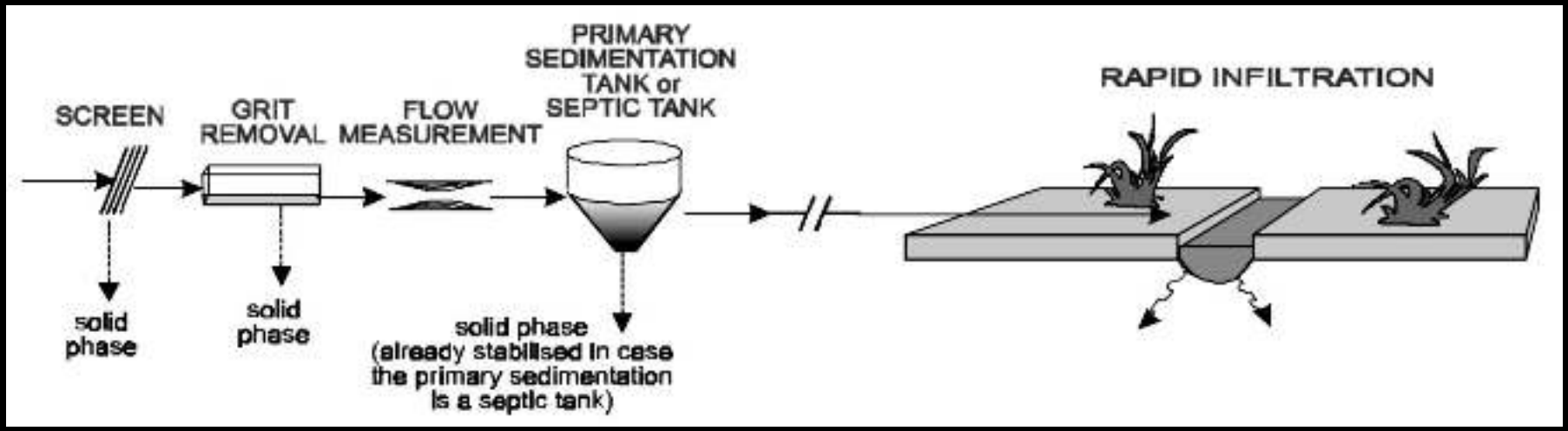
The liquid passes through the porous bottom and percolates into the soil.

Low evaporation loss, and vegetation may or may not be used.

Intermittent application…

Land Disposal

Rapid infiltration



Land Disposal

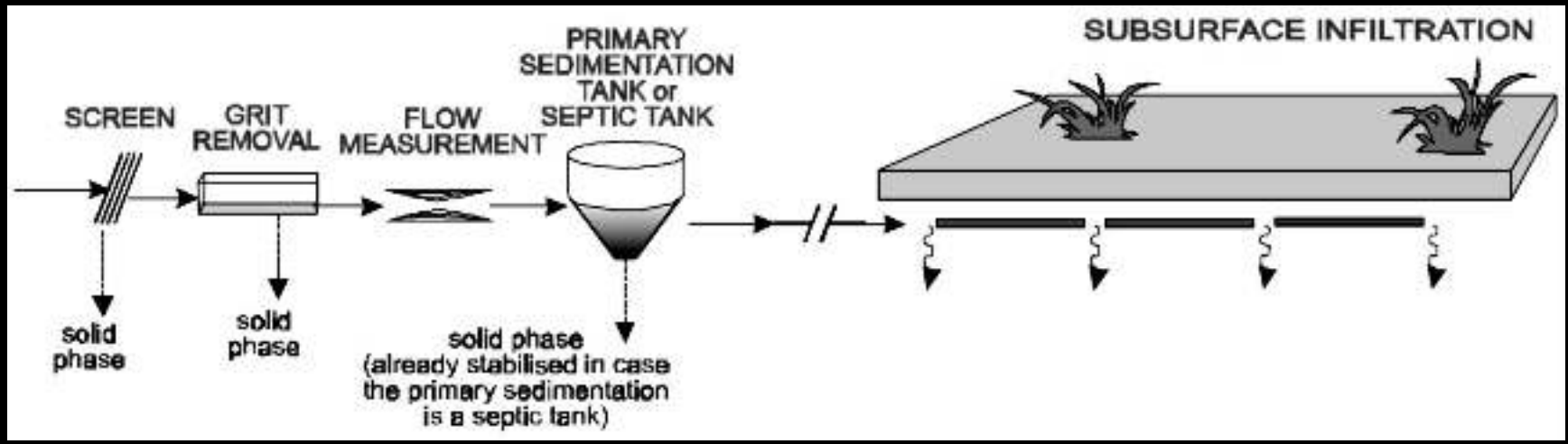
Subsurface infiltration:

Pre-settled sewage is applied below the soil surface.

The infiltration trenches or chambers are filled with a porous medium, which provides transportation, storage and partial treatment, followed by the infiltration itself.

Land Disposal

Subsurface infiltration



Land Disposal

Overland flow:

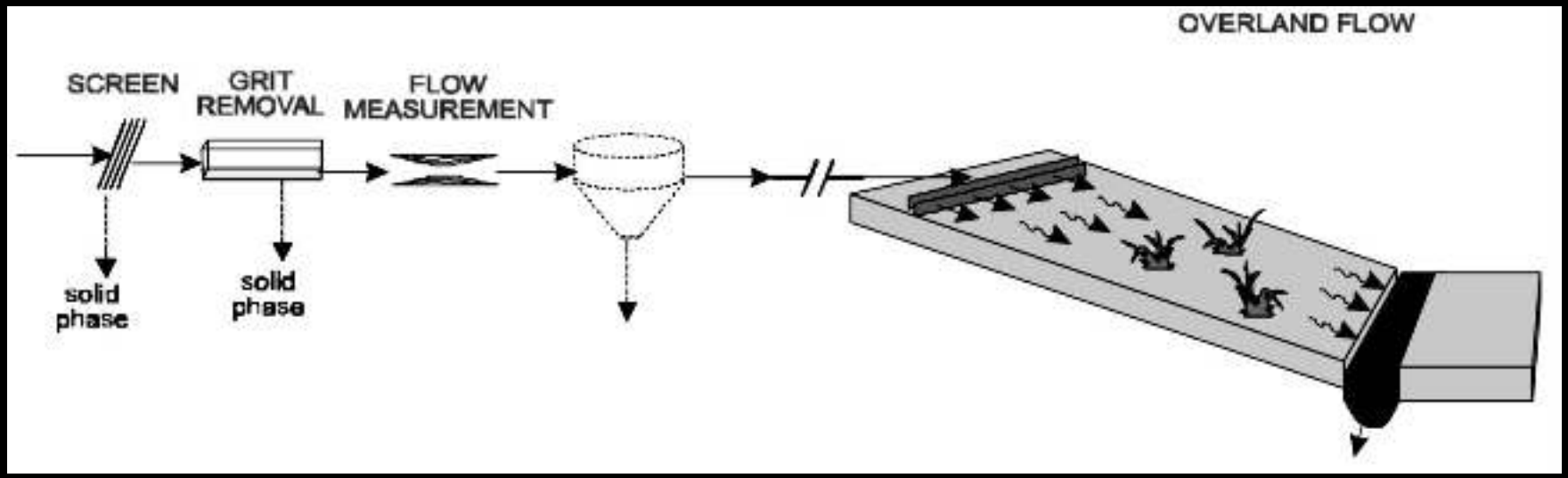
Wastewater is distributed in the upper part of vegetated slopes, flows over the slopes and is collected by ditches at the lower part.

Treatment occurs in the root-soil system.

The application is intermittent.

Land Disposal

Overland flow



Land Disposal

Constructed wetlands:

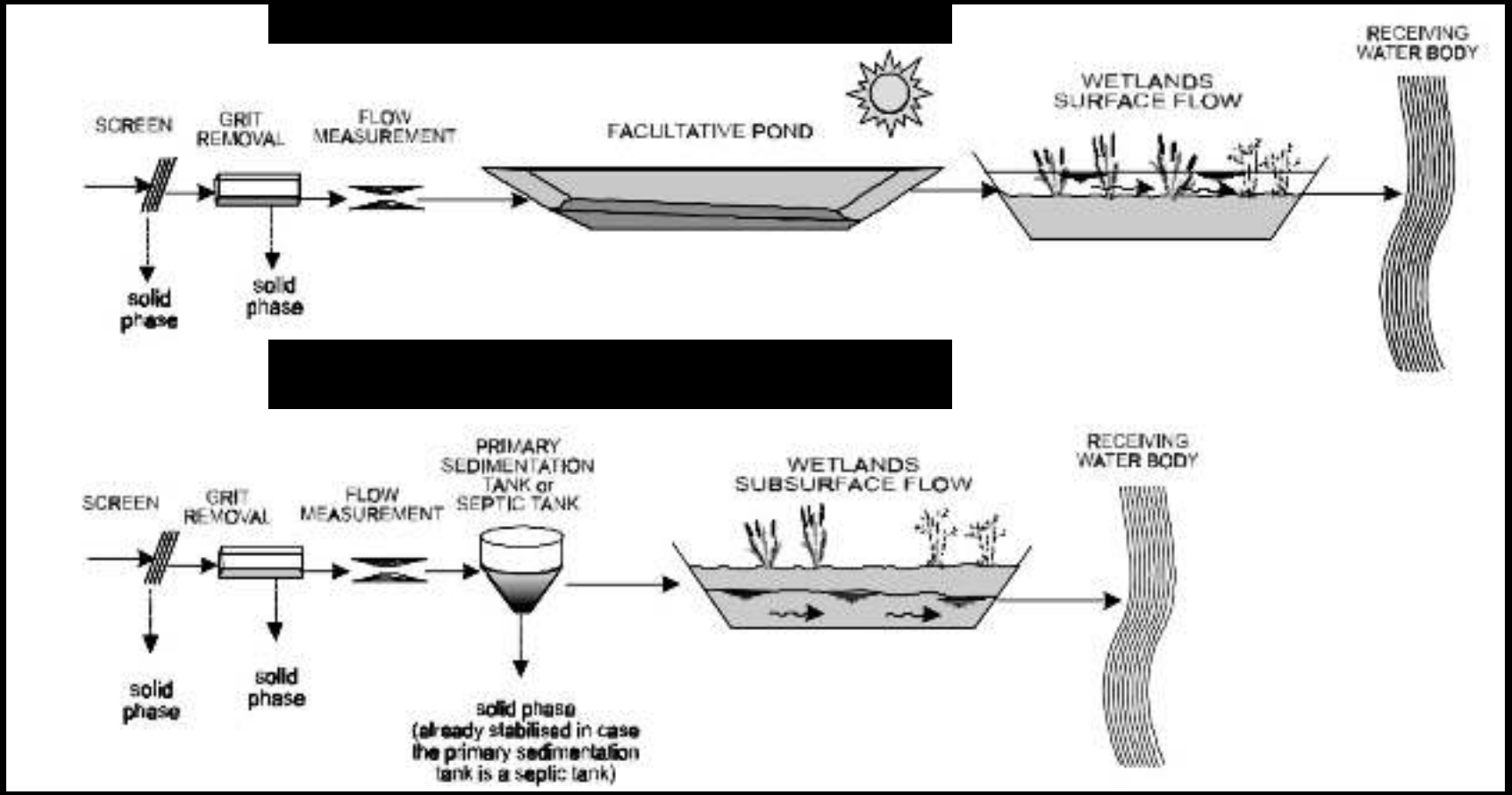
Aquatic-based systems, composed of shallow basins or channels in which aquatic plants grow.

The system can be of free-water surface or subsurface flow.

Mechanisms operate on the root–soil system.

Land Disposal

Constructed wetlands



Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Anaerobic System

Anaerobic System

Upflow anaerobic sludge blanket reactor (UASB):

BOD is converted anaerobically by bacteria dispersed in the reactor.

The liquid flow is upwards.

Anaerobic System

UASB:

The upper part of the reactor is divided into settling and gas collection zones.

The settling zone allows the exit of the clarified effluent.

Amongst the gases formed is methane.

Anaerobic System

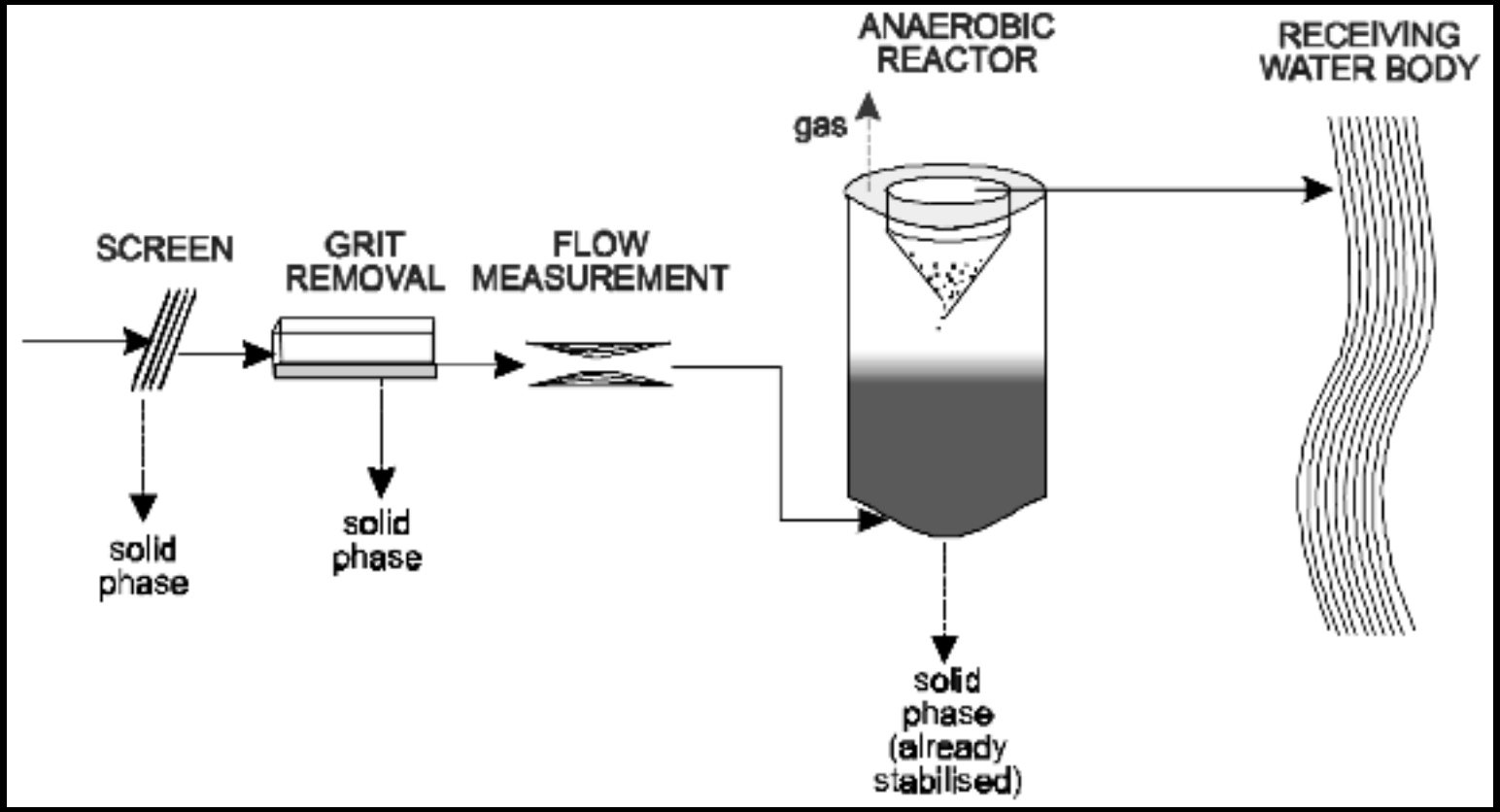
UASB:

No primary sedimentation tank.

The sludge production is low, and the excess sludge wasted is already thickened and stabilised.

Anaerobic System

Upflow anaerobic sludge blanket reactor (UASB)



Anaerobic System

Anaerobic filter:

BOD is converted anaerobically by bacteria that grow attached to a support medium (usually stones) in the reactor.

Anaerobic System

Anaerobic filter:

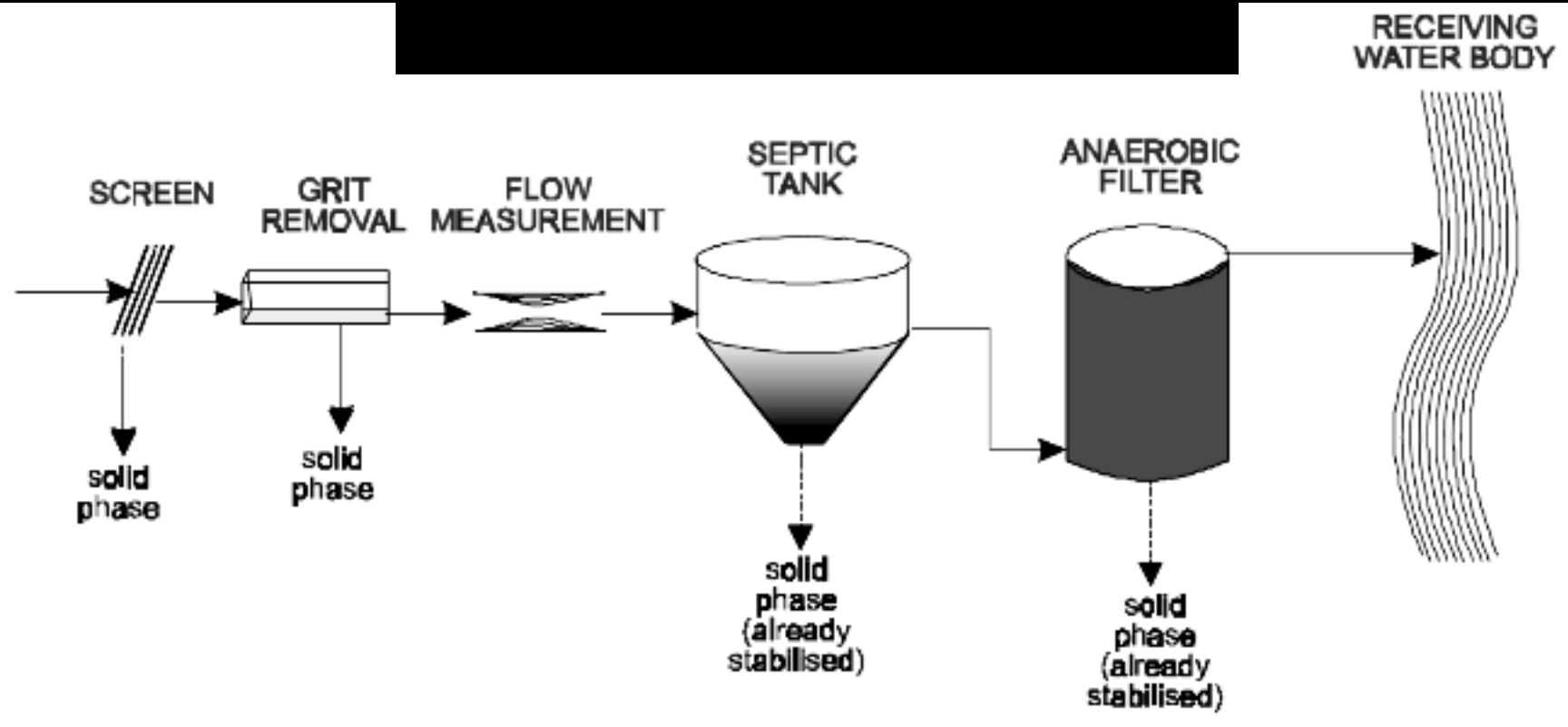
The tank works submerged and the flow is upwards.

The system requires a primary sedimentation tank (frequently septic tanks).

The sludge production is low and the excess sludge is already stabilised.

Anaerobic System

Anaerobic filter



Anaerobic System

Anaerobic reactor – post-treatment:

UASB reactors produce an effluent that has difficulty in complying with most existing discharge standards.

Therefore, some form of post treatment is frequently necessary.

Anaerobic System

Anaerobic reactor – post-treatment:

The post treatment may be biological (aerobic or anaerobic) or physical-chemical (with the addition of coagulants).

END

Practically all wastewater treatment processes may be used as a post treatment of the anaerobic reactors.

Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Activated sludge

Activated sludge

Conventional activated sludge:

The biological stage comprises two units: aeration tank (reactor) and secondary sedimentation tank.

Activated sludge

Conventional activated sludge:

High biomass concentration in the reactor due to the recirculation of the settled bacteria from the bottom of the secondary sedimentation tank.

Biomass remains in the system longer than the liquid, resulting in high BOD removal efficiency.

Activated sludge

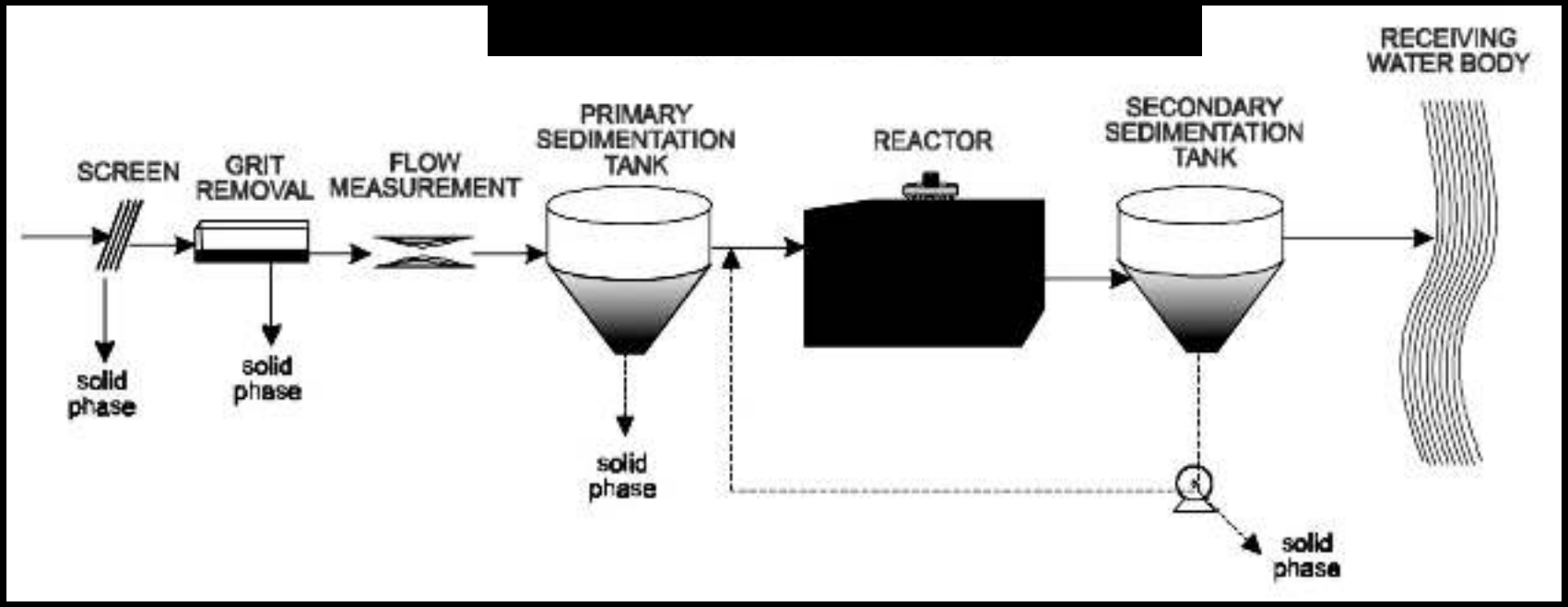
Conventional activated sludge:

It is necessary to remove a quantity of the sludge (biomass) that is equivalent to what is produced.

This excess sludge removed needs to be stabilised in the sludge treatment stage.

Activated sludge

Conventional activated sludge



Activated sludge

Activated sludge (extended aeration):

Similar to the previous system, but with the difference that the biomass stays longer in the system (the aeration tanks are bigger).

Activated sludge

Activated sludge (extended aeration):

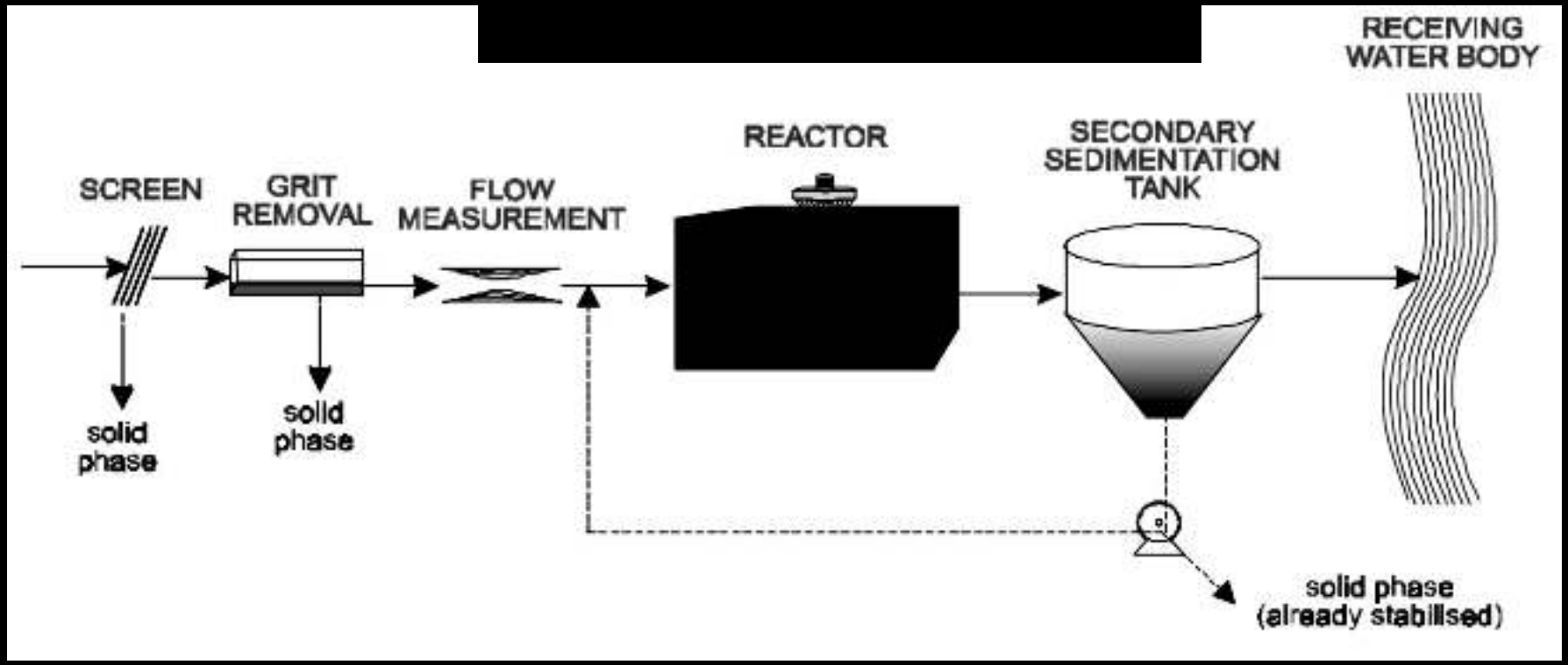
Less substrate available for the bacteria, which makes them use their own cellular material as organic matter.

Consequently, the removed excess sludge is already stabilised.

Primary sedimentation tank usually not included.

Activated sludge

Activated sludge (extended aeration)



Activated sludge

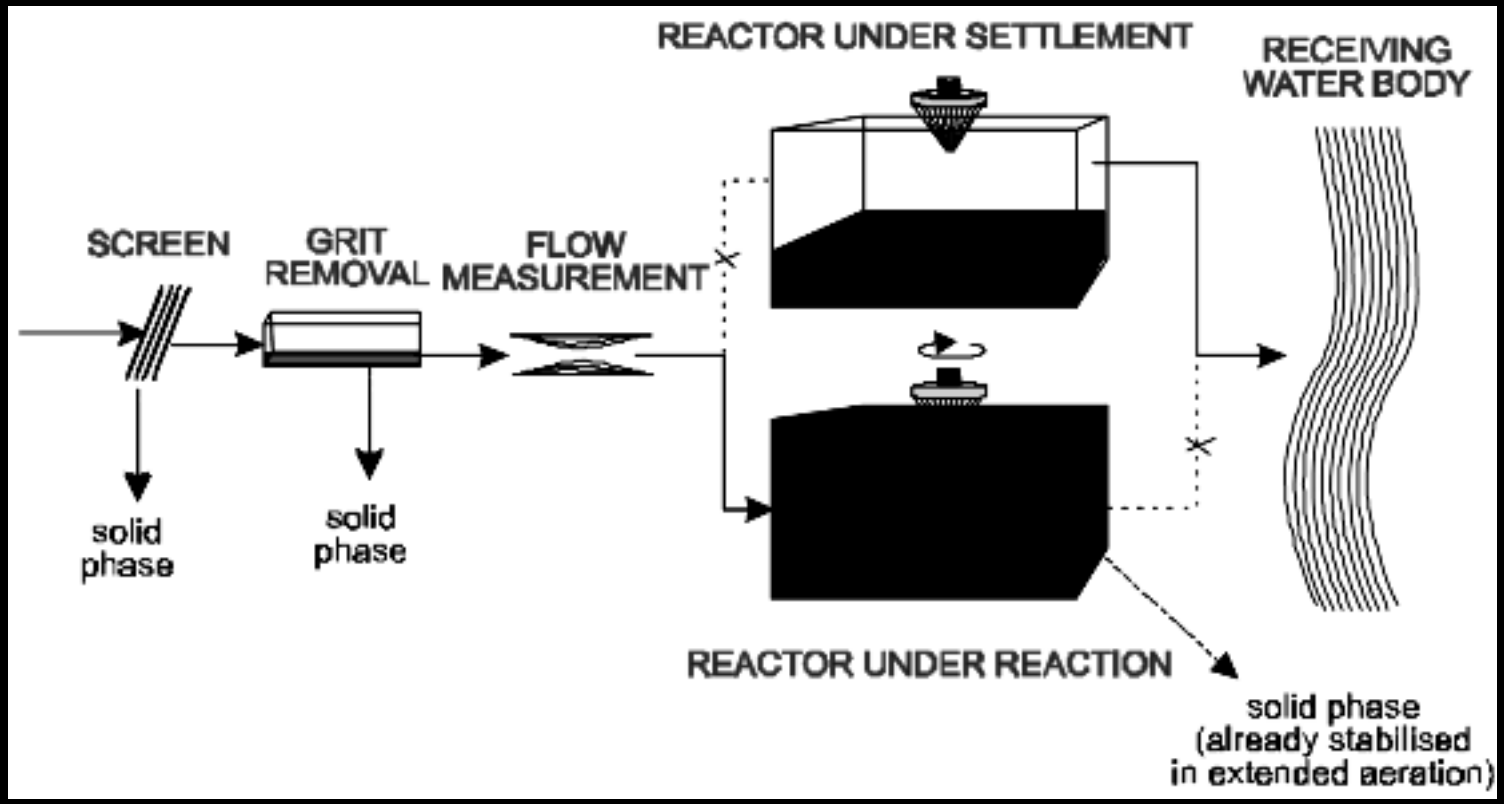
Intermittently operated activated sludge (sequencing batch reactors):

The operation of the system is intermittent.

In this way, the reaction (aerators on) and settling (aerators off) stages occur in different phases in the same tank.

Activated sludge

Intermittently operated activated sludge (sequencing batch reactors)



Activated sludge

Activated sludge with biological nitrogen removal:

The biological reactor incorporates an anoxic zone, either upstream or downstream of the aerated zone.

Activated sludge

Activated sludge with biological nitrogen removal:

The nitrates formed in the aerobic zone are used in the respiration of facultative microorganisms in the anoxic zone.

END

Nitrates are reduced to gaseous molecular nitrogen, which escapes to the atmosphere.

Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Aerobic Biofilm Reactors

Aerobic Biofilm Reactors

Low rate trickling filter:

BOD is stabilised aerobically by bacteria that grow attached to a support medium.

The sewage is applied on the surface. The liquid percolates and leaves from the bottom, while the organic matter is retained and further removed by the bacteria.

Aerobic Biofilm Reactors

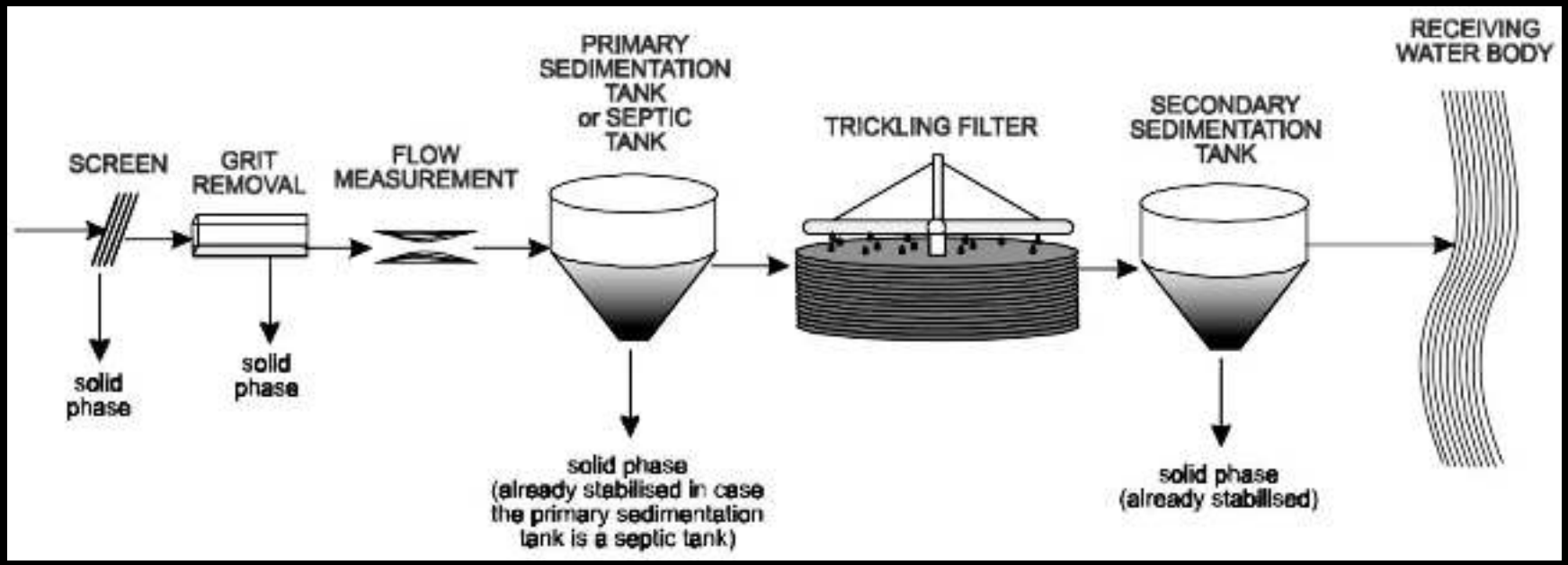
Low rate trickling filter:

Low availability of substrate (BOD) for the bacteria, which makes them undergo self-digestion and leave the system stabilised.

Sludge that is detached from the support medium is removed in the secondary sedimentation tank.

Aerobic Biofilm Reactors

Low rate trickling filter



Aerobic Biofilm Reactors

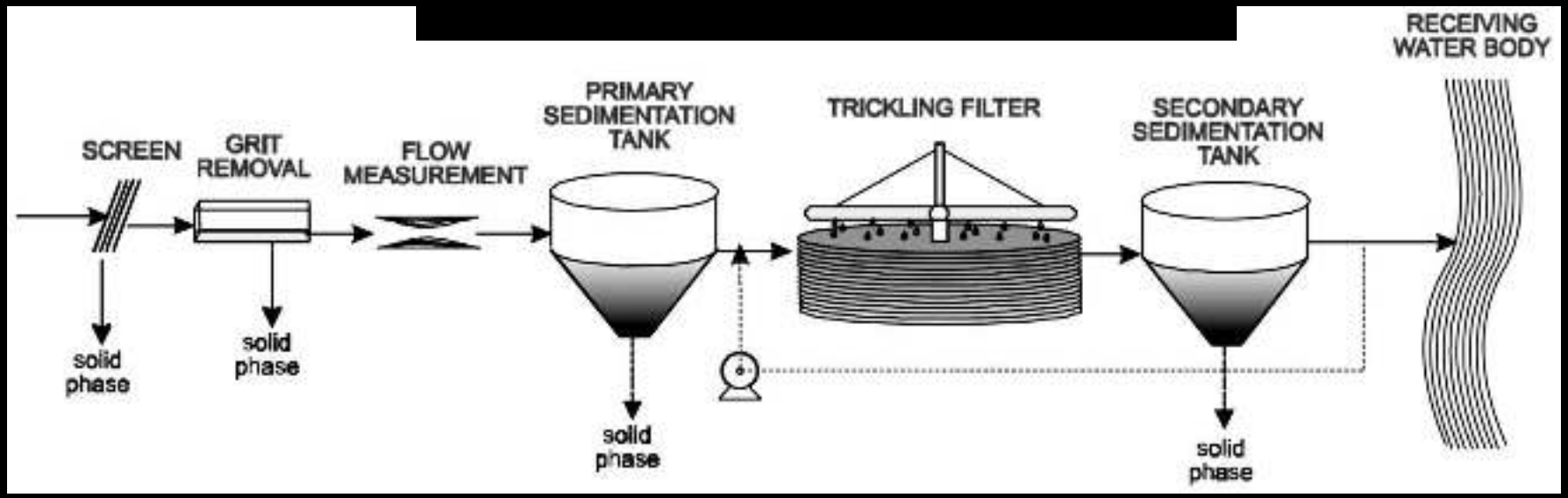
High rate trickling filter:

Similar to the previous system but with the difference that a higher BOD load is applied.

The effluent from the secondary sedimentation tank is recirculated to the filter in order to dilute the influent.

Aerobic Biofilm Reactors

High rate trickling filter



Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Preliminary Treatment

Preliminary Treatment

Mainly intended for the removal of:

- Coarse solids
- Grit

The basic removal mechanisms are of a physical order.

There is also a flow measurement unit.

Preliminary Treatment

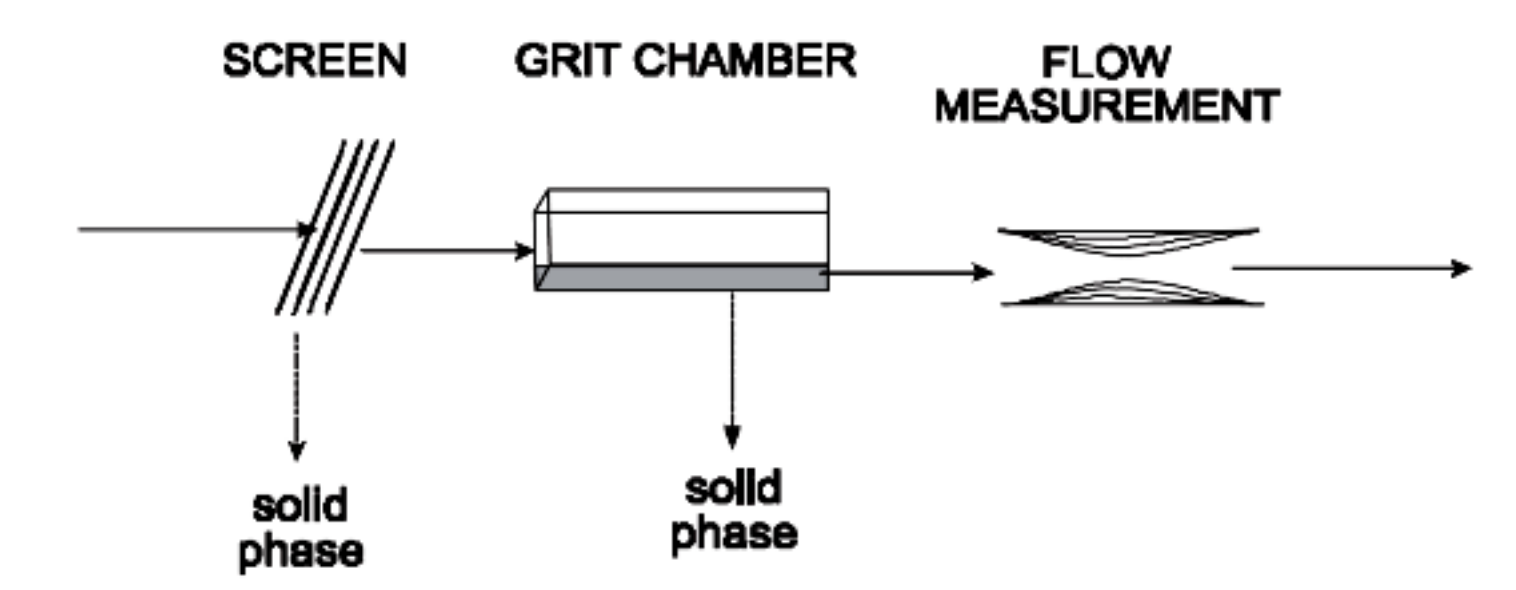
The removal of coarse solids is frequently done by screens or racks.

In the screening, material with dimensions larger than the spaces between the bars is removed.

There are coarse, medium, and fine screens.

Preliminary Treatment

Typical flowsheet of the preliminary treatment



Preliminary Treatment

The main objectives of the removal of coarse solids are:

- protection of the wastewater transport devices (pumps and piping)
- protection of the subsequent treatment units
- protection of the receiving bodies.

Preliminary Treatment

The removal of sand contained in the sewage is done through grit chambers.

The sand removal mechanism is simply by Sedimentation.

Organic matter stays in suspension and goes on to the downstream units.

Preliminary Treatment

The basic purposes of grit removal are:

- to avoid abrasion of the equipment and piping
- to eliminate or reduce the possibility of obstructions in piping, tanks, orifices, siphons, etc.
- to facilitate the transportation of the liquid, principally the transfer of the sludge in its various phases.

END

Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Primary Treatment

Primary Treatment

Primary treatment aims at the removal of:

- settleable suspended solids
- floating solids.

A significant part of these suspended solids is comprised of organic matter.

Its removal by simple processes such as sedimentation implies a reduction in the BOD load …

Primary Treatment

The sedimentation tanks can be circular or rectangular.

Sewage flows slowly through the sedimentation tanks, allowing the suspended solids with a greater density to slowly settle to the bottom.

Primary Treatment

The mass of solids accumulated in the bottom is called raw primary sludge.

This sludge is subsequently removed.

Floating material, such as grease and oil (having low density), rise to the where they are collected and removed.

Primary Treatment

The efficiency of primary treatment may be enhanced by the addition of coagulants.

This is called advanced primary treatment or chemically enhanced primary treatment (CEPT).

Primary Treatment

Coagulants may be aluminium sulphate, ferric chloride or other, aided or not by a polymer.

Phosphorus may be also removed by precipitation.

More sludge is formed…

Primary Treatment

The primary sludge may be digested by conventional digesters, but in some cases it may also be stabilised by lime…

Primary Treatment

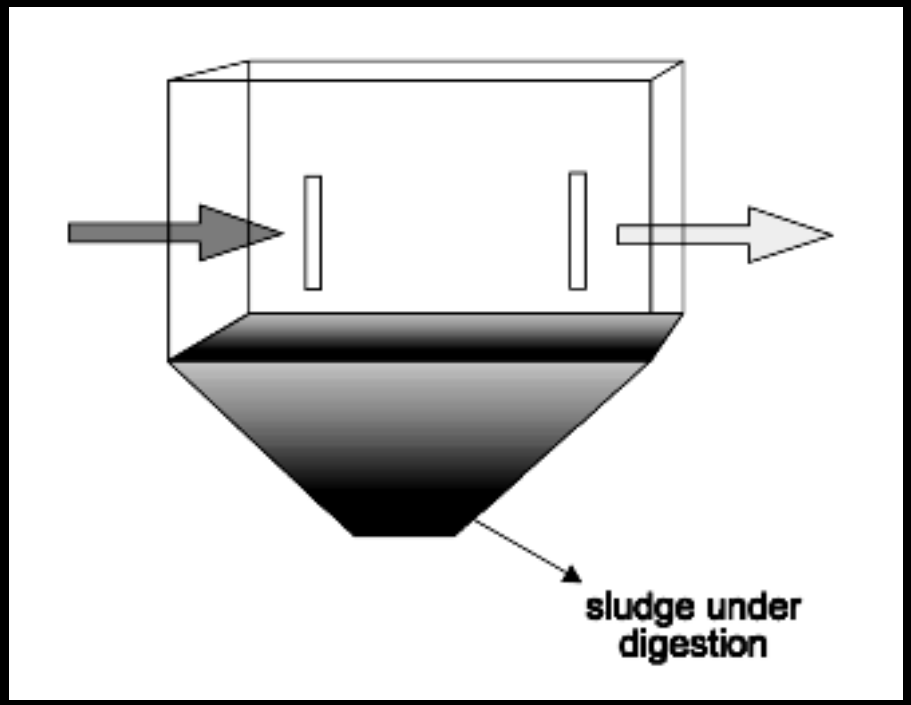
Septic tanks are also a form of primary treatment.

The septic tanks and their variants, such as Imhoff tanks, are basically sedimentation tanks…

These solids (sludge) remain at the bottom for a long period of time which is sufficient for their digestion.

Primary Treatment

Schematics of a single-chamber septic tank



Environmental Biotechnology

Main Biological Wastewater Treatment Systems: Secondary Treatment

Secondary Treatment

The main objective of secondary treatment is the removal of organic matter which is present in the following forms:

- Dissolved organic matter (soluble or filtered BOD) that is not removed by merely physical operations…;
- Organic matter in suspension (suspended or particulate BOD)…

Secondary Treatment

The secondary treatment processes are conceived in such a way as to accelerate the decomposition mechanisms that naturally occur in the receiving bodies…

The essence of secondary treatment of domestic sewage is the inclusion of a biological stage (as compared to previous treatments)…

Secondary Treatment

A great variety of microorganisms take part in the process: bacteria, protozoa, fungi and others.

The basis of the whole biological process is the effective contact between these organisms and the organic matter contained in the sewage.

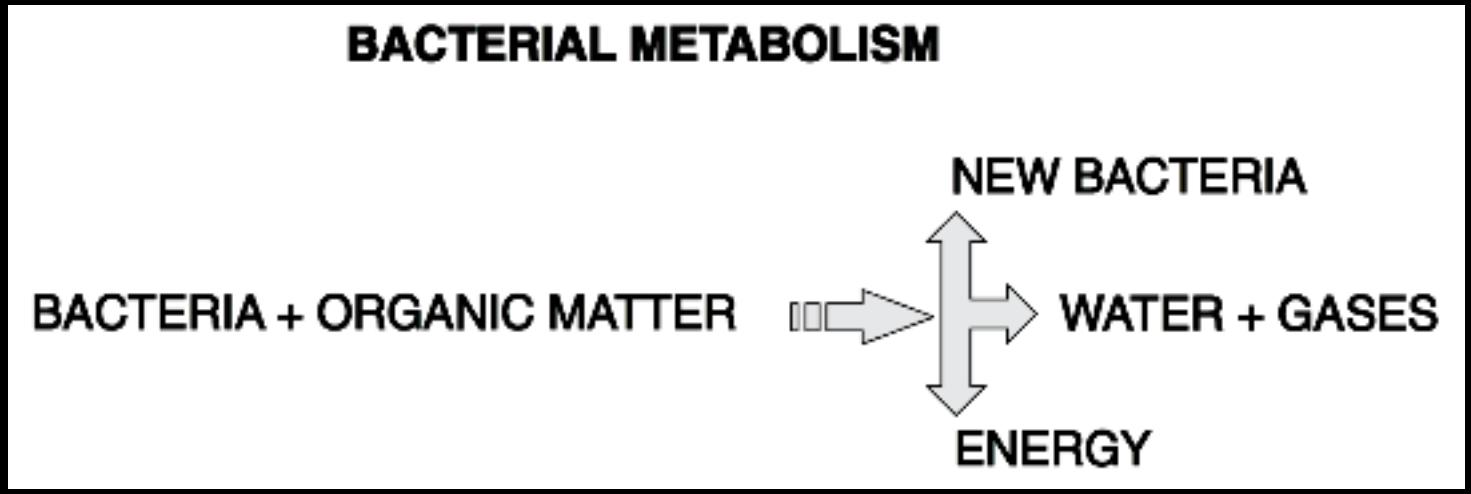
Secondary Treatment

The microorganisms convert the organic matter into carbon dioxide, water and cellular material...

This requires the presence of oxygen, as well as other favourable environmental conditions, such as temperature, pH, contact time, etc.

Secondary Treatment

Simplified diagram of bacterial metabolism



Secondary Treatment

There exists a large variety of secondary treatment processes, and the most common ones are:

- Stabilization ponds
- Land disposal systems
- Anaerobic reactors
- Activated sludge systems
- Aerobic biofilm reactors

END

Environmental Biotechnology

Secondary Treatment:
Stabilisation ponds:
Facultative Ponds

Stabilisation ponds: Facultative Ponds

When facultative ponds receive raw sewage, they are also called primary ponds.

The process of facultative ponds is the simplest, relying only on natural phenomenon.

The influent enters continuously in one end of the pond and leaves in the opposite end after many days.

Stabilisation ponds: Facultative Ponds

Suspended organic matter tends to settle, constituting the bottom sludge.

Anaerobic microorganisms convert it to carbon dioxide, methane and other compounds.

The inert fraction (non-biodegradable) stays in this bottom layer.

Stabilisation ponds: Facultative Ponds

The dissolved organic matter does not settle and stays dispersed in the liquid mass.

Its decomposition is through facultative anaerobic bacteria.

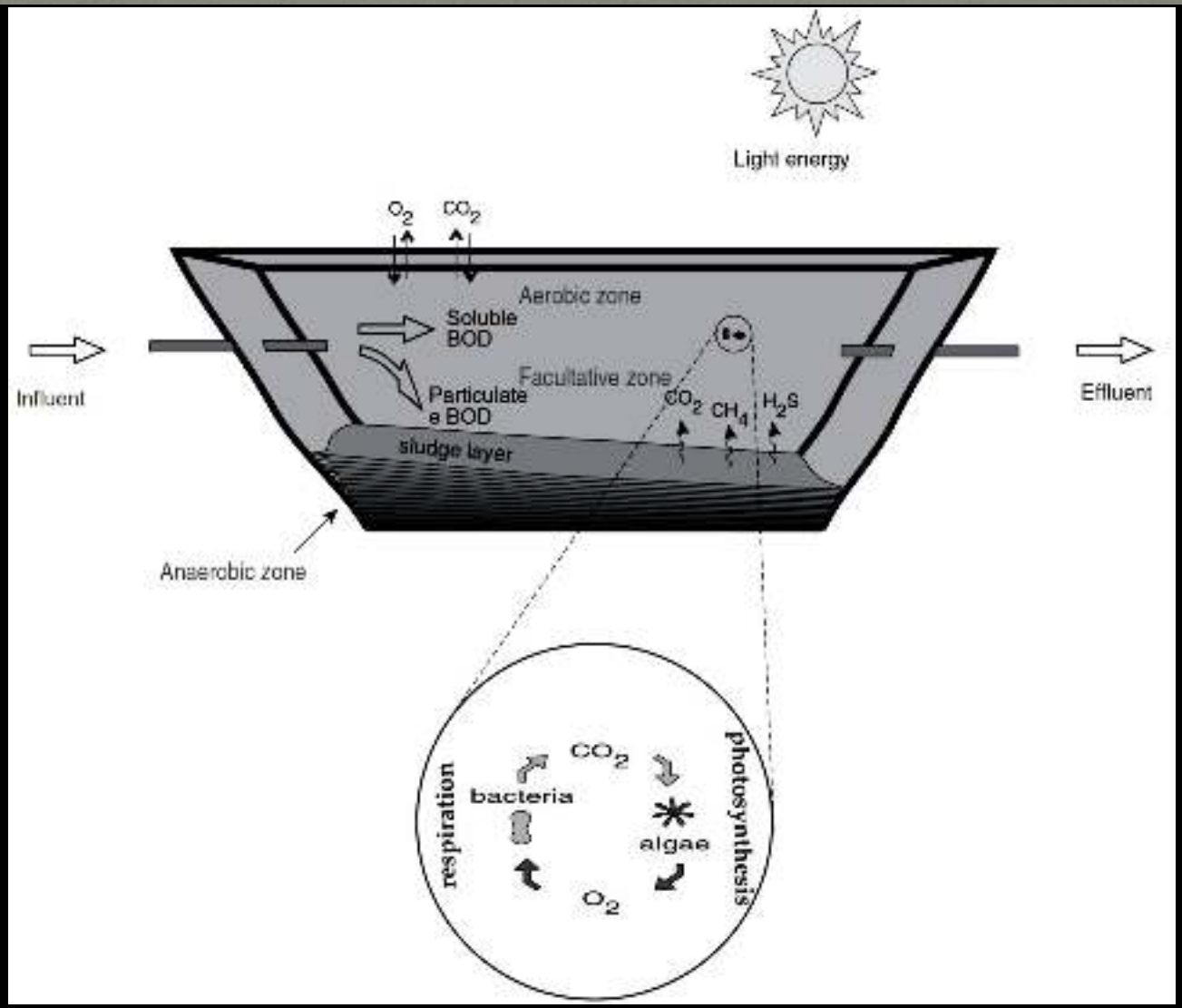
Hence the designation of facultative, which also defines the name of the pond.

Stabilisation ponds: Facultative Ponds

The presence of oxygen is necessary in aerobic respiration, and it is supplied to the medium by the photosynthesis carried out by the algae.

There is an equilibrium between consumption and the production of oxygen and carbon dioxide.

Stabilisation ponds: Facultative Ponds



Stabilisation ponds: Facultative Ponds

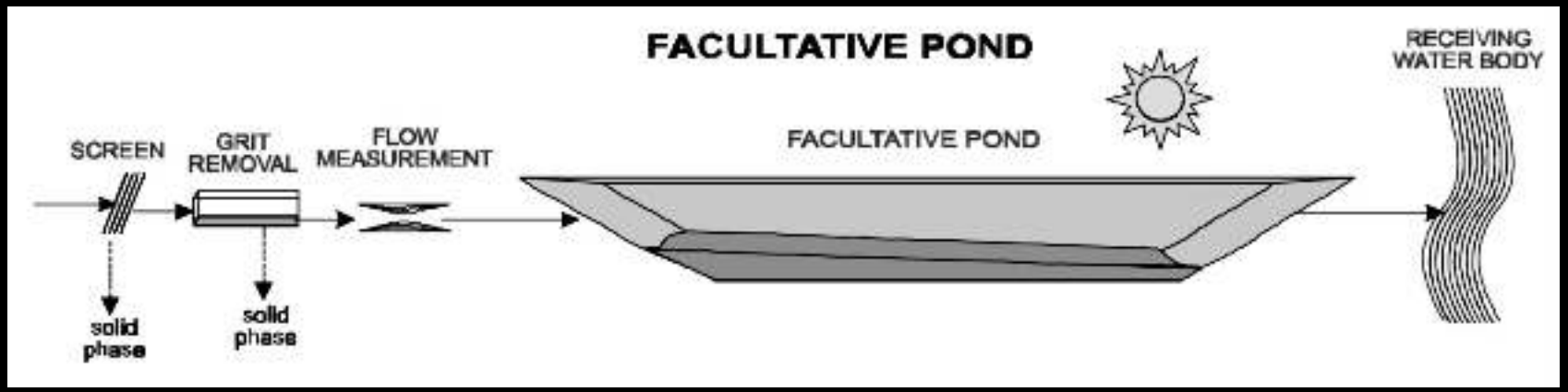
Bacteria ➔ *respiration*:

- oxygen consumption
- carbon dioxide production

Algae ➔ *photosynthesis*:

- oxygen production
- carbon dioxide consumption

Stabilisation ponds: Facultative Ponds



Stabilisation ponds: Facultative Ponds

Photosynthesis is higher near the water surface. In deep regions the light penetration is low, so oxygen consumption (respiration) is dominant over its production (photosynthesis).

So, it is essential that the main bacteria responsible for the stabilisation of the organic matter are facultative anaerobes.

Stabilisation ponds: Facultative Ponds

END

The process is natural, so does not require any equipment.
Slow rate of organic matter stabilization, so need more time (more than 20 days).
Effective photosynthesis needs a large exposure surface area, so area required is one of the largest.
Fundamental importance in developing countries.

Environmental Biotechnology

Secondary Treatment:

Stabilisation ponds:

Anaerobic pond – facultative ponds systems

Anaerobic pond – facultative ponds systems

Facultative ponds require a large area.

One of the solutions is the system of anaerobic ponds followed by facultative ponds.

In this case, the facultative pond is also called a secondary pond.

Anaerobic pond – facultative ponds systems

Efforts by: AARISH ALI

Raw sewage enters a pond that has smaller dimensions and is deeper.

Due to this, photosynthesis practically does not occur.

Oxygen consumption is much higher resulting in anaerobic conditions.

Anaerobic pond – facultative ponds systems

Anaerobic bacteria have a slower metabolic and reproduction.

In 2-5 days, there is only partial decomposition of the organic matter.

However, 50-70% BOD removal represents a large contribution.

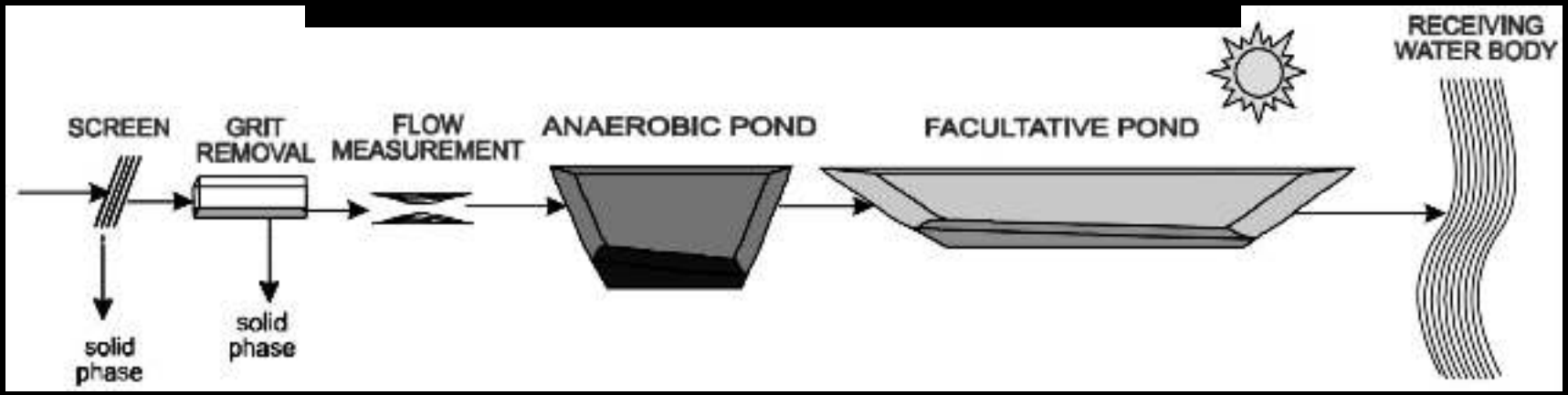
Anaerobic pond – facultative ponds systems

Facultative pond receives a load of only 30 to 50% of the raw sewage load, which therefore allows it to have smaller dimensions.

Land savings in the order of 1/3 are obtained, compared with just a single facultative pond.

Anaerobic pond – facultative ponds systems

Anaerobic pond – facultative pond



Anaerobic pond – facultative ponds systems

The efficiency of the system is similar or only slightly higher than that of a single facultative pond.

The existence of an anaerobic stage in an open unit can cause release of malodours, such as hydrogen sulphide,

END

Environmental Biotechnology

Secondary Treatment:

Stabilisation ponds:

Facultative aerated lagoon

Facultative aerated lagoon

If a predominantly aerobic system is desired with smaller dimensions, a facultative aerated lagoon can be used.

In facultative aerated lagoons the oxygen is supplied by mechanical equipment called aerators.

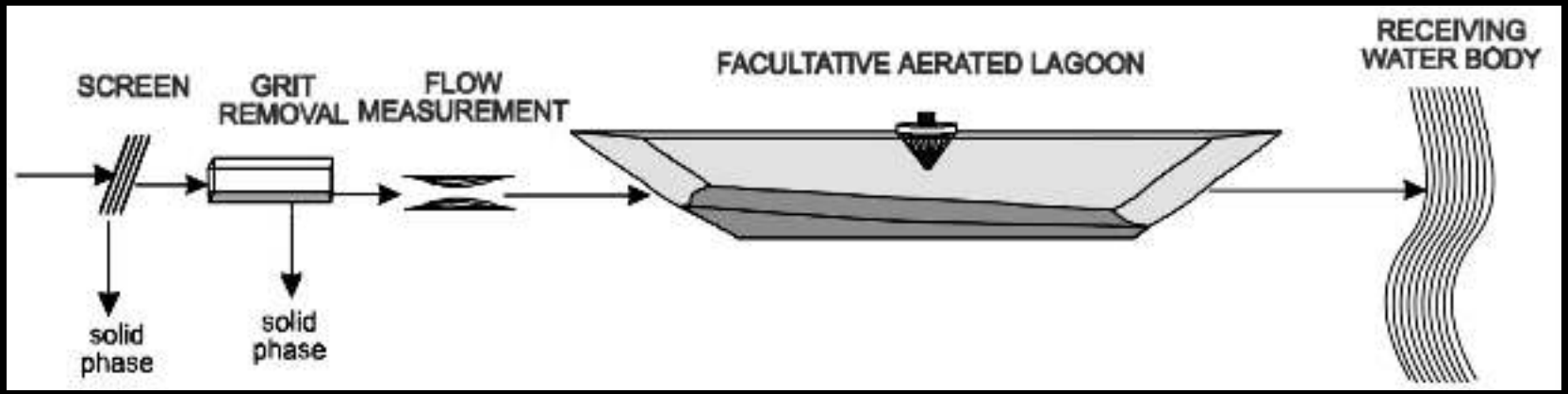
Facultative aerated lagoon

Most common mechanical aerators are having a vertical axis that rotates at a high speed.

A greater oxygen introduction is achieved, in comparison with the conventional facultative pond, resulting in less detention time, and smaller land requirements.

Facultative aerated lagoon

Facultative aerated lagoon



Facultative aerated lagoon

Called facultative because the level of aeration is only sufficient for the oxygenation, but not to maintain the solids in suspension in liquid mass.

END

Solids tend to settle and constitute a sludge layer at the bottom of the pond, to be decomposed anaerobically.

Environmental Biotechnology

Secondary Treatment:
Stabilisation ponds:
Complete-mix aerated lagoon – sedimentation pond systems

Complete-mix aerated lagoon – sedimentation pond systems

Volume of aerated pond can be reduced by increasing the aeration level per unit volume of the lagoon, thus creating a turbulence that, besides guaranteeing oxygenation, allows all the solids to be maintained in suspension in the liquid medium.

Complete-mix aerated lagoon – sedimentation pond systems

A larger concentration of bacteria in the liquid medium, leads to larger organic matter – biomass contact.

Increased efficiency and reduced volume.

Complete-mix aerated lagoon – sedimentation pond systems

The biomass stays in suspension in all the volume and thus can leave with the pond effluent...

Its discharged into the receiving body would deteriorate water quality.

Complete-mix aerated lagoon – sedimentation pond systems

Therefore, a unit called sedimentation pond is present downstream in which the suspended solids (predominantly the biomass) can settle and be separated from the liquid (final effluent).

Complete-mix aerated lagoon – sedimentation pond systems

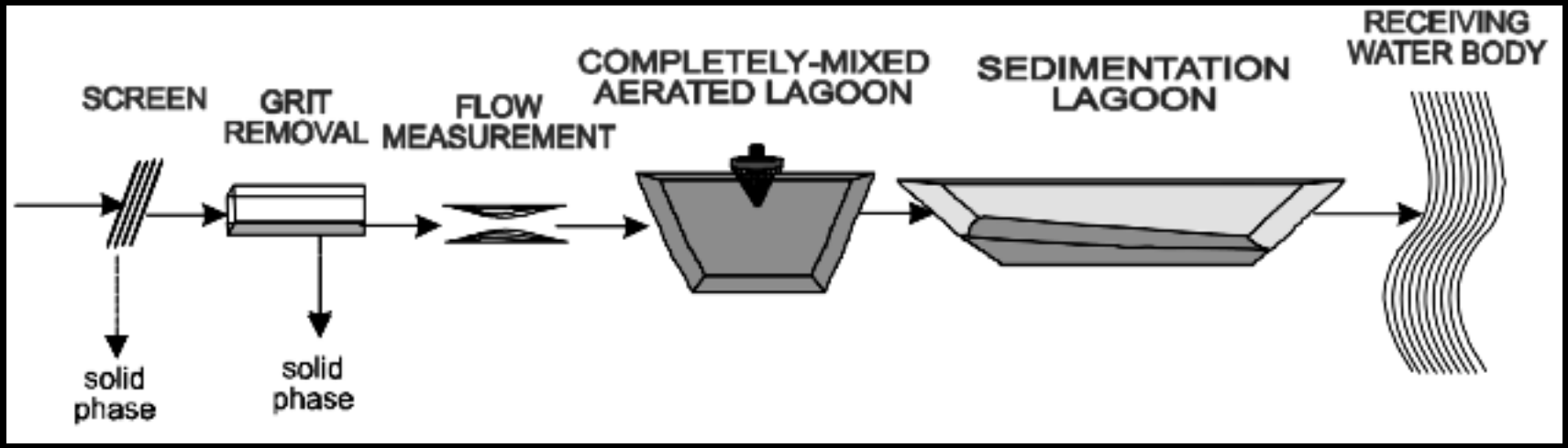
Sedimentation pond have short detention times, around 2 days.

The solids settle to the bottom where they will undergo digestion and be stored for a period of some years.

Some sedimentation ponds with continuous removal of the bottom sludge using pumps.

Complete-mix aerated lagoon – sedimentation pond systems

Completely mixed aerated lagoon – sedimentation pond



Complete-mix aerated lagoon – sedimentation pond systems

The land required for this pond system is the smallest within the pond systems.

The energy requirements are similar to or only slightly higher than those in the facultative aerated lagoons.

Complete-mix aerated lagoon – sedimentation pond systems

As storage period is smaller, the sludge needs to be removed periodically.

END

The removal of the sludge is a laborious and expensive task.

Environmental Biotechnology

Secondary Treatment:

Stabilisation ponds:

High rate ponds

High rate ponds

Conceived to maximise the production of algae in a totally aerobic environment.

The ponds are shallow (less than 1.0 m depth), thus guaranteeing the penetration of light.

High rate ponds

The photosynthetic activity is high, leading to high DO concentrations and an increase in pH.

These factors contribute to the increase in the death rate of the pathogenic microorganisms and the removal of nutrients

High rate ponds

Ammonia removal occurs by stripping of the free ammonia (NH_3), since in high pH conditions the ammonia equilibrium shifts in the direction of free ammonia (with a reduction in the concentration of the ammonium ion NH^{+4}).

The increase in the NH_3 concentration leads to its release to the atmosphere.

High rate ponds

Phosphorus removal also occurs as a result of the high pH, which causes the precipitation of the phosphates into the form of hydroxyapatite or struvite.

High rate ponds

The high rate ponds receive a high organic load per unit surface area.

There is usually the introduction of moderate agitation in the pond…

High rate ponds

END

The high rate ponds can come after facultative ponds, in which a large part of the BOD is removed, leaving the polishing in terms of pathogen and nutrient removal for the high rate ponds.

Environmental Biotechnology

Secondary Treatment:

Stabilisation ponds:

Maturation ponds

Maturation ponds

Maturation ponds aim at polishing the effluent from any stabilisation pond system previously described.

The main objective of maturation ponds is the removal of pathogenic organisms.

Economic alternative for the disinfection of the effluent.

Maturation ponds

The maturation pond is designed in such a way as to optimise various factors that contribute to the death of the pathogens such as temperature, solar radiation, pH, food shortage, predator organisms, competition, toxic compounds, etc.

Maturation ponds

Many of these mechanisms become more effective with lower pond depths, which justifies the fact that maturation ponds are shallower than the other types of ponds.

Maturation ponds

Can reach extremely high coliform removal efficiencies (Up to 99.99%).

To maximise efficiency, maturation ponds can be designed as three or four ponds in series or a single pond with baffles.

END

Environmental Biotechnology

Secondary Treatment: Land disposal

Land disposal

The land application of wastewater can be considered as a form of final disposal.

Land application of wastewater leads to groundwater recharge and/or to evapotranspiration.

Sewage supplies the plants with water and nutrients.

Land disposal

In the soil, a pollutant has basically four possible destinations:

- retention in the soil matrix
- retention by the plants
- appearance in the underground water
- collection by underdrains

Land disposal

Mechanisms in the soil for the removal of pollutants:

- physical (settling, filtration, radiation, volatilisation, dehydration)
- chemical (oxidation and chemical reactions, precipitation, adsorption, ion exchange, complexation, photochemical breakdown)
- biological (biodegradation and predation)

Land disposal

The capacity of the soil to absorb complex organic compounds depends on its properties and on climatic conditions.

Infiltration rates and types of vegetation are important factors in the use of soil as a medium for the degradation of organic compounds.

Land disposal

These reactions require good soil aeration, which is inversely related to the humidity of the soil.

Insufficient aeration leads to a lower assimilation capacity of the organic compounds by the soil.

Land disposal

The removal results from the filtering action of the soil, followed by the biological oxidation of the organic material.

Soils with clay (fine texture) or with some organic matter will also retain wastewater constituents through mechanisms of adsorption, precipitation and ion exchange.

Land disposal

The most common types of land application are:

Soil-based systems:

- slow-rate systems
- rapid infiltration
- subsurface infiltration
- overland flow

Aquatic-based systems:

- constructed wetlands

Land disposal

The selection of the treatment method depends on the required efficiency, climatic conditions, depth to ground water, soil permeability, slope etc.

The application of wastewater can be done by methods such as sprinklers, furrows, graded border, drip irrigation and others…

END

Environmental Biotechnology

Secondary Treatment:
Land disposal;
Slow-rate systems

Slow-rate systems

Classified according to two types:

a) Slow infiltration systems.
Main objective is wastewater treatment.
The amount of wastewater applied is not controlled by crop requirements.
The systems are designed to maximise the amount of wastewater applied per unit land area.

Slow-rate systems

Classified according to two types:

b) Crop irrigation systems.

Main objective is water reuse for crop production...

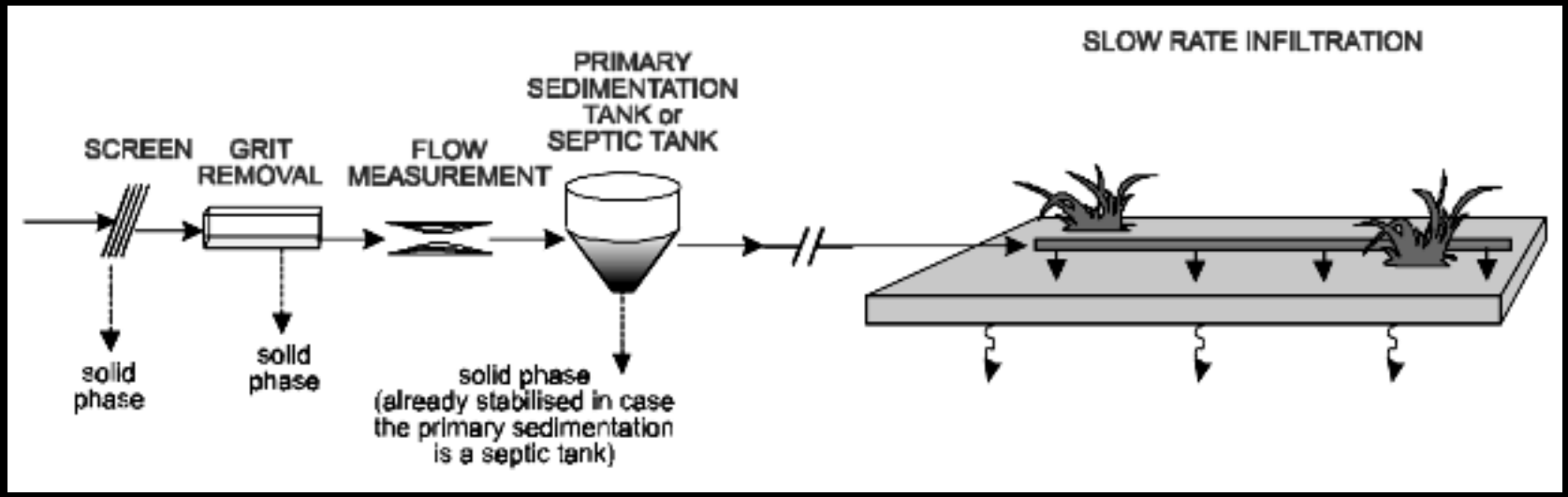
The systems are designed to apply sufficient wastewater to meet crop irrigation requirements.

Loading rates are based on the crop irrigation requirement.

Nitrogen loading must be checked to avoid excess nitrogen.

Slow-rate systems

Slow rate system



Slow-rate systems

In arid zones, wastewater can be used for irrigation throughout the year.
On the other hand, in wet areas, the application of wastewater in rainy periods can lead to anaerobic conditions and consequently odour and insect appearance problems.

Slow-rate systems

Irrigation is a treatment system that requires the largest surface area per unit of wastewater treated. It is natural system with the highest efficiency.

The plants are those mainly responsible for the removal of nutrients, such as P and N.

END

Microorganisms in the soil perform the removal of the organic substances.

Environmental Biotechnology

Secondary Treatment: Land disposal; Rapid infiltration

Rapid infiltration

In this system, soil is used as a filtering medium, characterised by the percolation of the wastewater.

Wastewater is purified by the filtering action of the porous medium.

The percolated wastewater may be used for groundwater recharge or be collected.

Requires the lowest area within all the land disposal processes.

Rapid infiltration

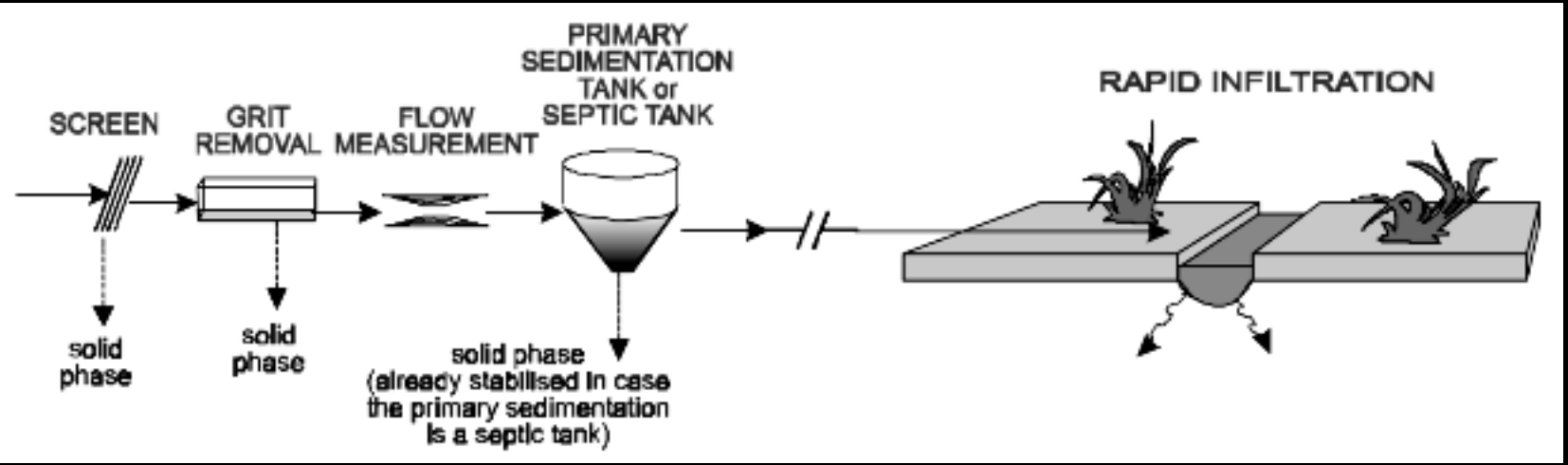
Wastewater is applied in shallow infiltration basins, from which wastewater percolates through the soil.

The application is intermittent.

Evaporation losses are small and most of the liquid percolates through the soil, undergoing treatment.

Rapid infiltration

Rapid infiltration



Rapid infiltration

The application can be done by direct discharge (furrows, channels, perforated pipes) or by high capacity sprinklers.

Vegetation growth may or may not occur.

END

Environmental Biotechnology

Secondary Treatment: Land disposal; Subsurface infiltration

Subsurface infiltration

In this systems, pre-settled or pre-treated sewage is applied below ground level.

The infiltration sites are prepared in buried excavations, and filled in with a porous medium.

Subsurface infiltration

The sewage percolates through unsaturated soil, where additional treatment occurs.

This treatment process is similar to rapid infiltration, the main difference residing in the application below ground level.

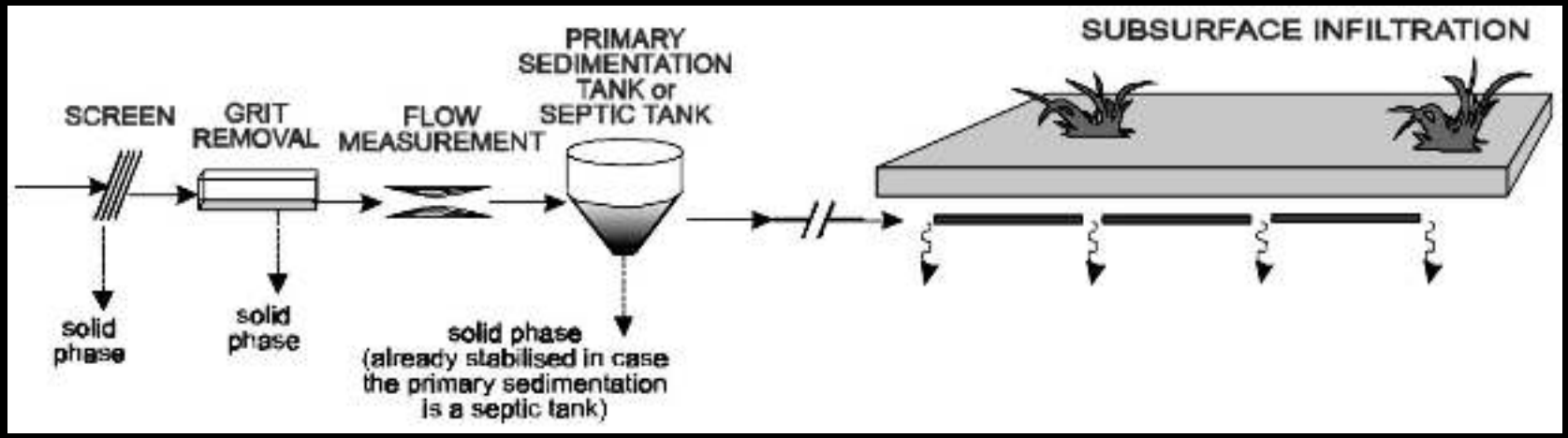
Subsurface infiltration

The subsurface infiltration systems have the following variants:

- infiltration trenches or pits (without final effluent: wastewater percolates to groundwater)
- filtration trenches (with final effluent: collection by underdrain system).

Subsurface infiltration

Subsurface infiltration



Subsurface infiltration

These systems are normally used following septic tanks, etc.

END

The applicability is usually for small residential areas or rural dwellings.

Environmental Biotechnology

Secondary Treatment: Land disposal; Overland flow

Overland flow

These systems consist of the application of wastewater in the upper part of sloped terraces, planted with water resistant grasses.

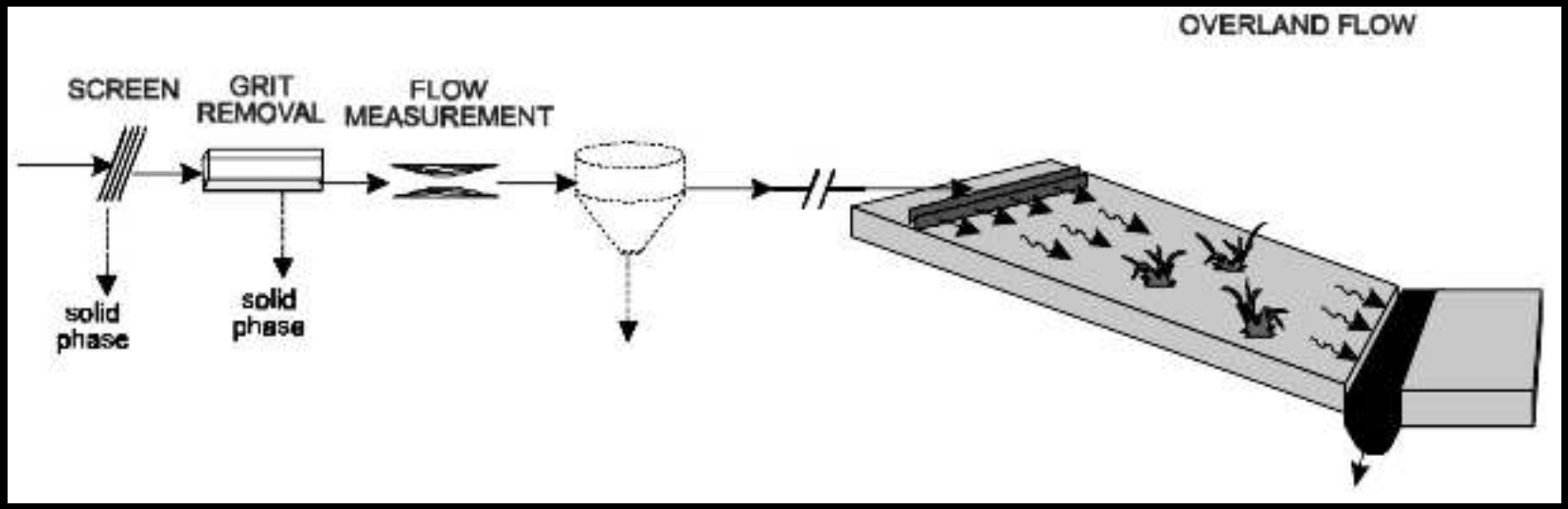
Wastewater flows gently downwards, having contact with the root-soil system, in which biochemical reactions take place.

Overland flow

The final effluent is collected at the lower end by drainage channels.

Application is intermittent.

Overland flow



Overland flow

The soils should have a low permeability (e.g. clay).

The slope should be moderate (between 2 and 8%).

Overland flow

The use of vegetation is essential to increase the absorption rate of the nutrients.

The vegetation represents a barrier to the free surface flow of the liquid in the soil, increasing the retention of suspended solids and avoiding erosion.

END

Environmental Biotechnology

Secondary Treatment: Land disposal; Constructed wetlands (Surface flow wetlands)

Constructed wetlands (Surface flow wetlands)

Natural wetlands are areas saturated by surface or groundwater that support a vegetation adapted to these conditions.

The natural wetlands include marshes, swamps and similar areas, that shelter diverse forms of aquatic life.

Constructed wetlands (Surface flow wetlands)

Constructed wetlands are purposely built wastewater treatment processes.

These consist of ponds, basins or shallow canals (a depth less than 1.0 m) that shelter aquatic plants, and use biological, chemical and physical mechanisms to treat the sewage.

Constructed wetlands (Surface flow wetlands)

These have an impermeable layer of clay or membrane, and structures to control the flow direction, hydraulic detention time and water level.

Depending on the system, they can contain an inert porous medium such as stones, gravel or sand.

Constructed wetlands (Surface flow wetlands)

Two types of constructed wetlands:

a). Surface flow (free water surface) wetlands.

b). Subsurface flow wetlands (vegetated submersed bed systems).

Constructed wetlands (Surface flow wetlands)

Surface flow Wetlands resemble natural wetlands in appearance, because they have plants which can be floating and/or rooted (emergent or submerged) in a soil layer at the bottom, and water flows freely between the leaves and the stems of these plants.

Constructed wetlands (Surface flow wetlands)

Plant genera in use include:
(a) emergent: *Typha*, *Phragmites*, *Scirpus*,
(b) submerged: *Potamogeon*, *Elodea*, etc.,
(c) floating: *Eichornia* (water hyacinth), *Lemna* (duckweed).

Native plants are preferred.

Constructed wetlands (Surface flow wetlands)

A very complex aquatic ecology.
May or may not have a lined bottom, depending on the environmental requirements.

Water depth is between 0.6 and 0.9 m for the vegetated zones (or less, in the case of certain emergent plants), and 1.2 to 1.5 m for free water zones.

END

Environmental Biotechnology

Secondary Treatment: Land disposal; Constructed wetlands (Sub-Surface, Vertical, Horizontal)

Constructed wetlands (Sub-Surface flow)

Subsurface flow wetlands are also called as vegetated submersed bed systems.

Constructed wetlands (Sub-Surface flow)

Do not resemble natural wetlands because there is no free water on the surface.

There is a bed composed of small stones, gravel, sand or soil that gives support to the growth of aquatic plants.

Constructed wetlands (Sub-Surface flow)

Water level stays below the surface of the bed, and sewage flows in contact with the roots of the plants (where a bacterial biofilm is developed).
The medium height is 0.5-0.6 m and water depth is 0.4-0.5 m.
Plant genera used are: *Typha*, *Scirpus*, *Carex* and *Phragmites*.

Constructed wetlands (Sub-Surface flow)

The gravel should have a size that allows the continuous flow of the sewage without clogging.

A large part of the subsurface zone is anaerobic, with aerobic sites immediately adjacent to the rhizomes and roots.

Constructed wetlands (Sub-Surface flow)

These wetlands are suited to receive effluents from septic tanks and anaerobic reactors.

Constructed wetlands (Vertical flow)

Vertical flow:

Typically, a filter of sand or gravel planted with vegetation.

At the bottom of the filter medium there is a series of underdrains that collect the treated sewage.

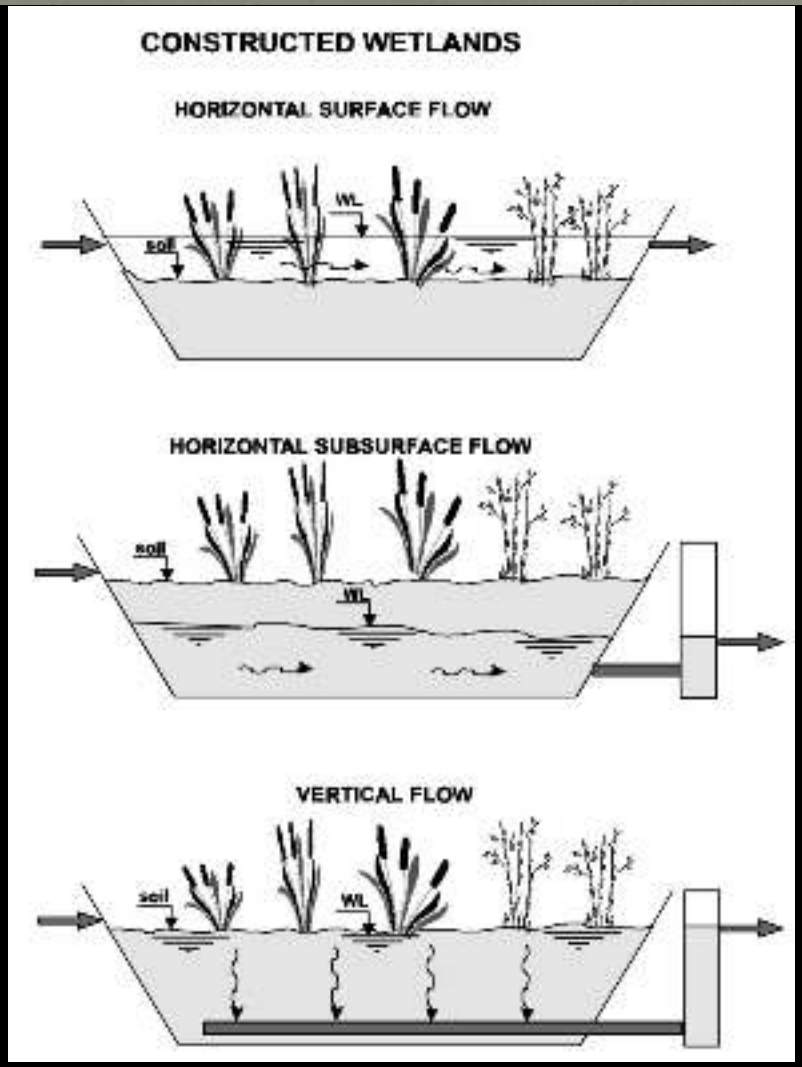
Constructed wetlands (Horizontal flow)

Horizontal flow:

The most classical conception of constructed wetlands.

May be with surface or subsurface flow.

Constructed wetlands



Environmental Biotechnology

Secondary Treatment: Anaerobic reactors; Septic Tank – anaerobic filter system

Septic Tank– anaerobic filter system

Widely used in rural areas and in small sized communities…

Septic tanks remove most of the suspended solids, which settle and undergo anaerobic digestion at the bottom of the tank.

Septic Tank– anaerobic filter system

The septic tank can be a single-chamber tank or a two-compartment tank (called an Imhoff tank).

In the single chamber tank, there is no physical separation between the regions of the raw sewage solids sedimentation and bottom sludge digestion.

Can be single or in series.

Septic Tank– anaerobic filter system

In the Imhoff tank, settling occurs in the upper compartment (settling compartment).

The settled solids pass through an opening at the bottom of the compartment and are directed to the bottom compartment (digestion compartment).

Septic Tank– anaerobic filter system

The accumulated sludge then undergoes anaerobic digestion.

The gases originating from the anaerobic digestion do not interfere with the settling process, as they cannot penetrate inside the sedimentation chamber.

Septic Tank– anaerobic filter system

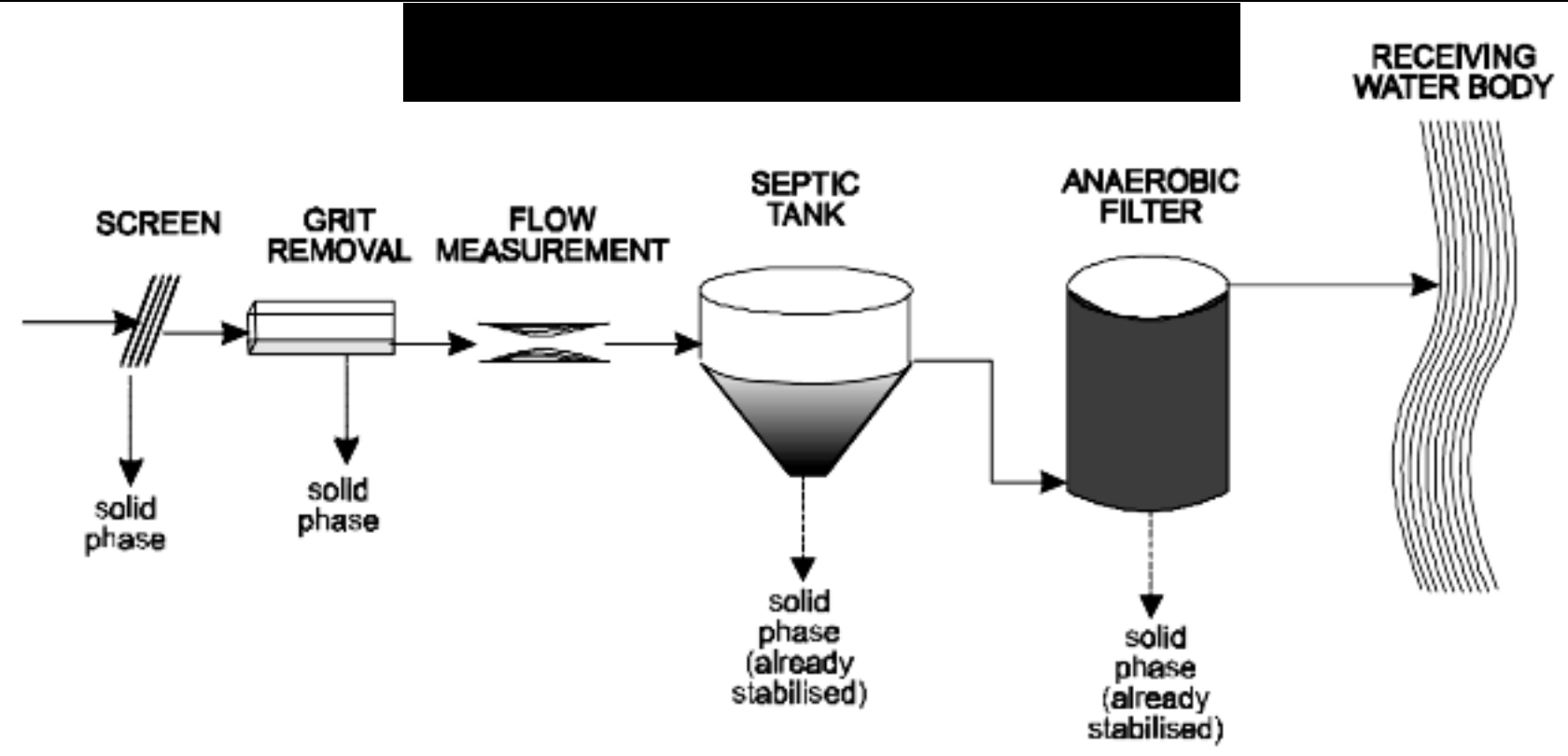
As septic tanks are sedimentation tanks (no biochemical reactions in the liquid phase), BOD removal is limited.

The effluent, still with high organic matter concentration, goes to the anaerobic filter...

The filter is a biofilm reactor…

Anaerobic pond – facultative ponds systems

Septic Tank– anaerobic filter system



Septic Tank– anaerobic filter system

Characteristic of anaerobic filters different from the trickling filters:

- the liquid flow is upwards…
- the anaerobic filter works submerged…
- the unit is closed…
- the BOD load applied per unit volume is very high, which guarantees anaerobic conditions…

Septic Tank– anaerobic filter system

The efficiency of a septic tank – anaerobic filter is usually less compared with fully aerobic systems…

Sludge production in anaerobic systems is very low…

END

The excess sludge is already digested and can go directly to dewatering…

Final Term Syllabus
Lecture 01 to 105
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Contact us to join our esteemed group
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Note:

Students must follow the course announcement for midterm syllabus & any up-gradation in the course.

REMEMBER IN PRAYERS

KEEP SMILING

SAY NO TO CURRUPTION

HELP TO OTHERS

Regards,

AARISH ALI & TEAM

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