BT503 FULL BOOK PPT VU BIOLOGY ZONE

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

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.

END

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

END

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

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

END

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.

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

Diverse Degradative Abilities

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

Particular interest to environmental biotechnologists.

END

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

END

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.

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).

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

END

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

END

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.

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

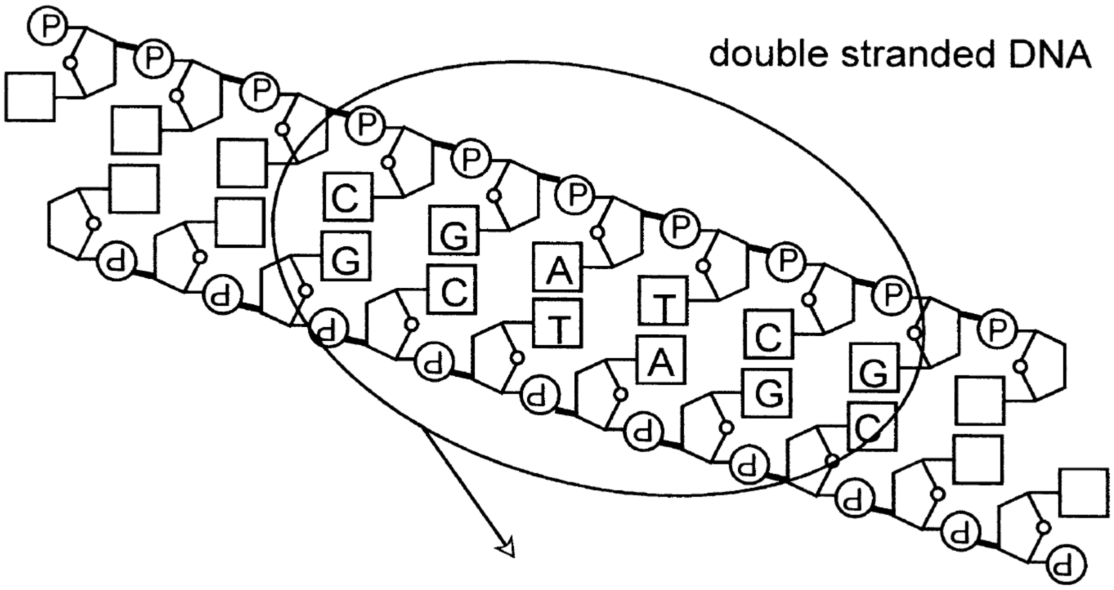
Next stage is to insert the DNA into the vector…

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



DNA sequence	-----CGATCG---- -----GCTAGC----	
digestion with *Sau* 3A	-----C GATCG---- -----GCTAG C----	sticky end (5' overhang)
digestion with Pvu I	-----CGAT CG---- -----GC TAGC----	sticky end (3' overhang)
digestion with *Dpn* I (methylated DNA)	-----CGA TCG---- -----GCT AGC----	flush end

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.

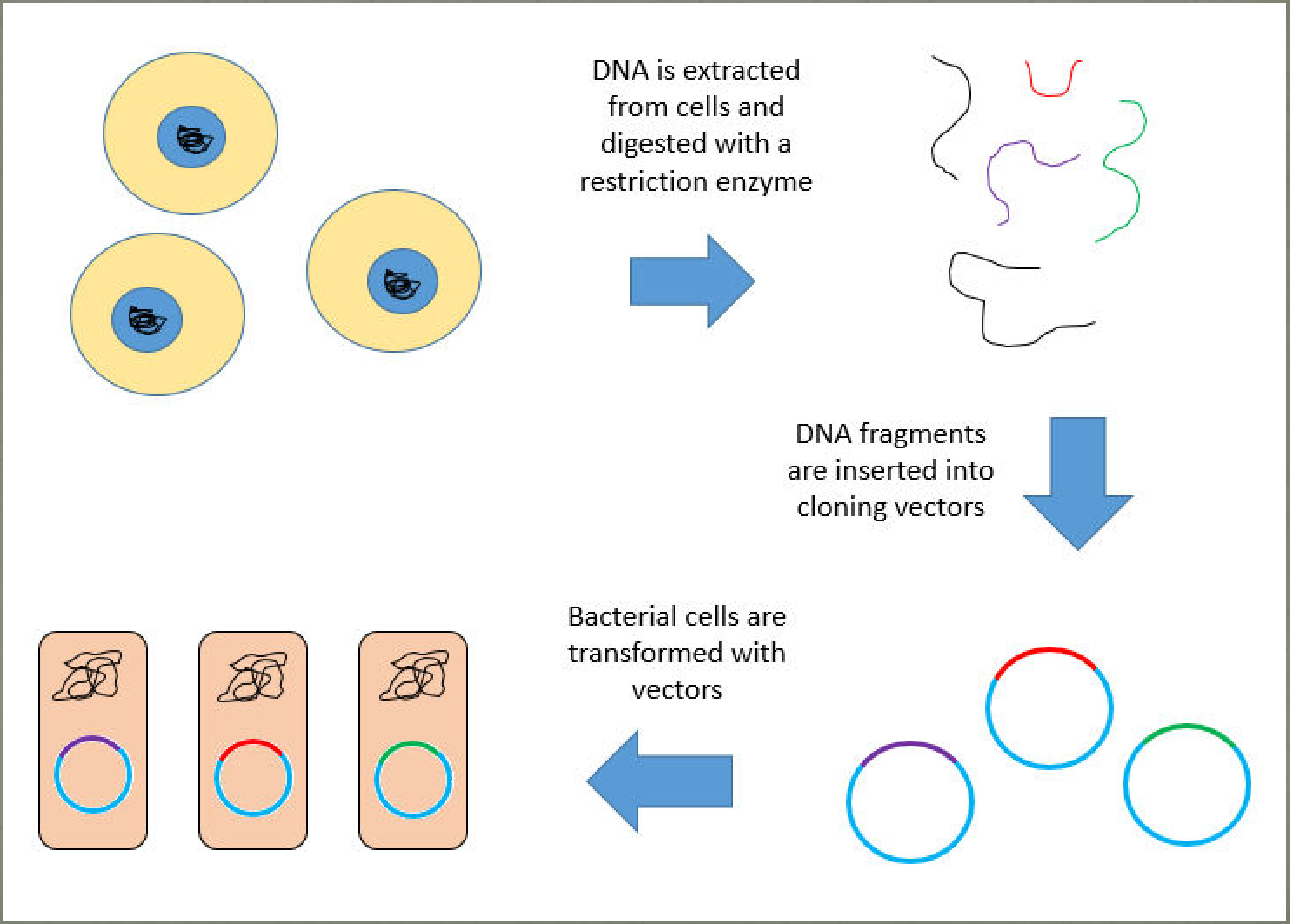
Basic Principles of Genetic Engineering: DNA for transfer

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



DNA is extracted from cells and digested with a restriction enzyme

DNA fragments are inserted into cloning vectors

Bacterial cells are transformed with vectors

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

cDNA libraries

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.

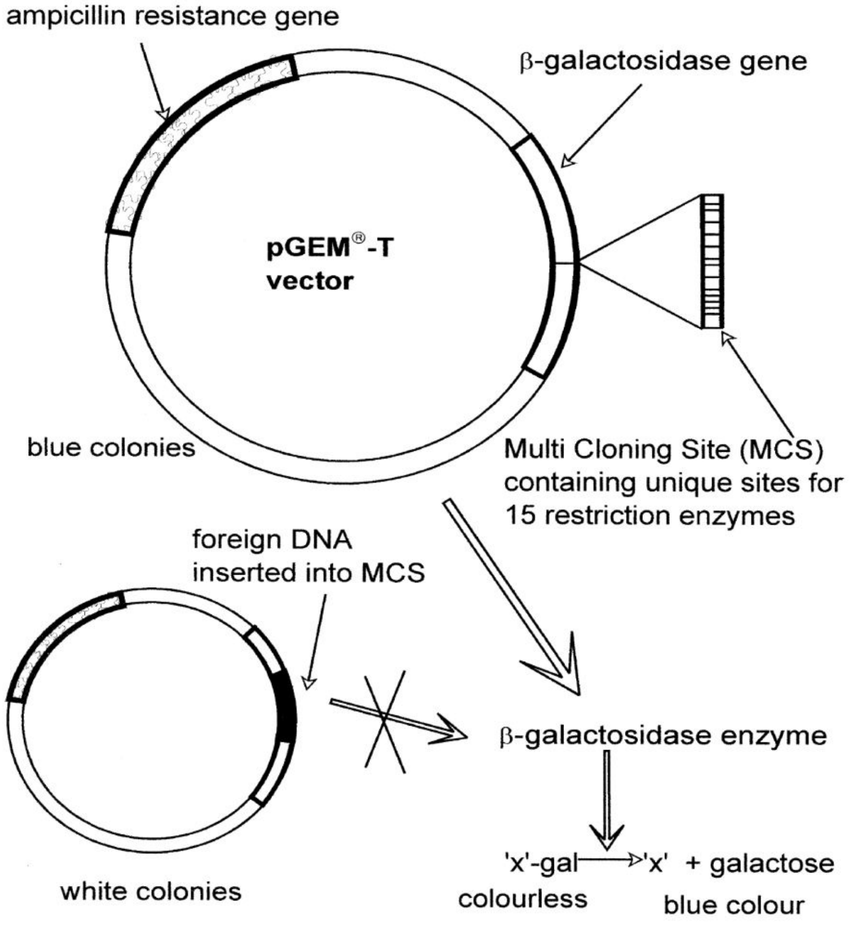
Basic Principles of Genetic Engineering: Cloning Vectors

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

example- pGEM®-T



pGEM®-T is a registered tradename (Promega)

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.

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

END

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

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)

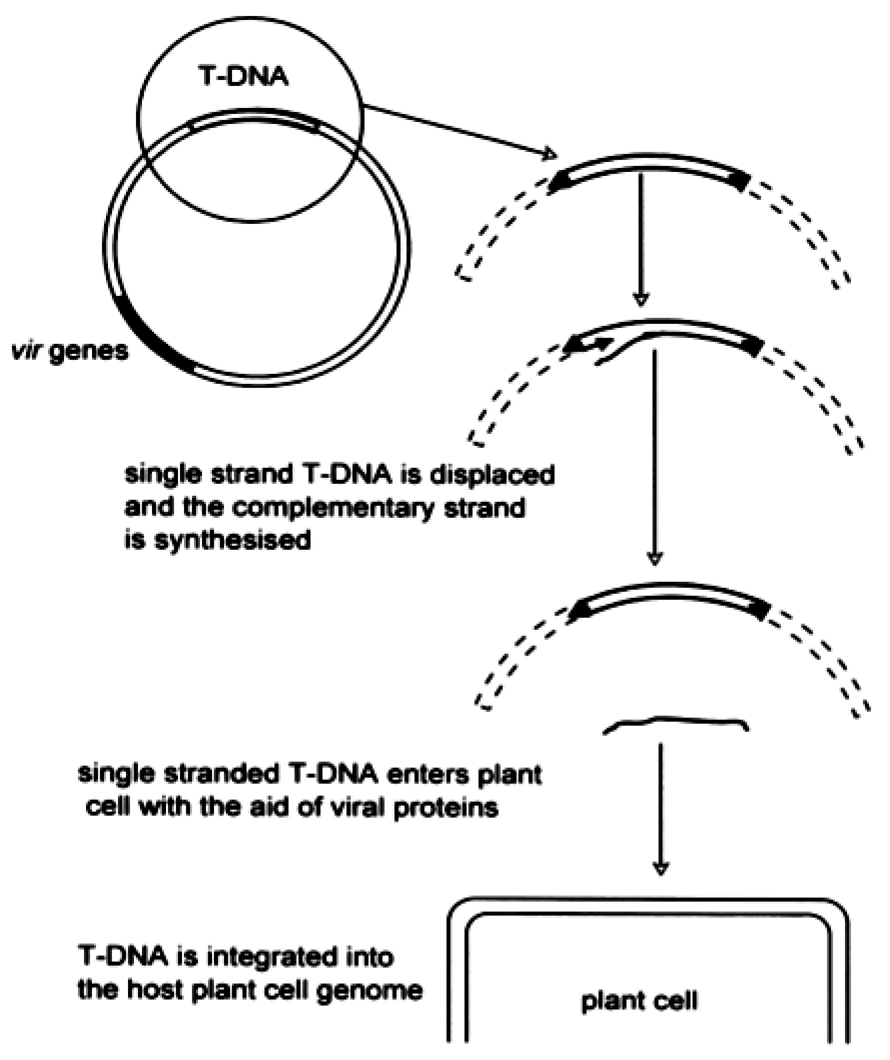
Transformation of plants

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



single strand T-DNA is displaced and the complementary strand is synthesised

single stranded T-DNA enters plant cell with the aid of viral proteins

T-DNA is integrated into the host plant cell genome

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

END

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.

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

Improved resistance to pests

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.

END

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

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

END

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.

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.

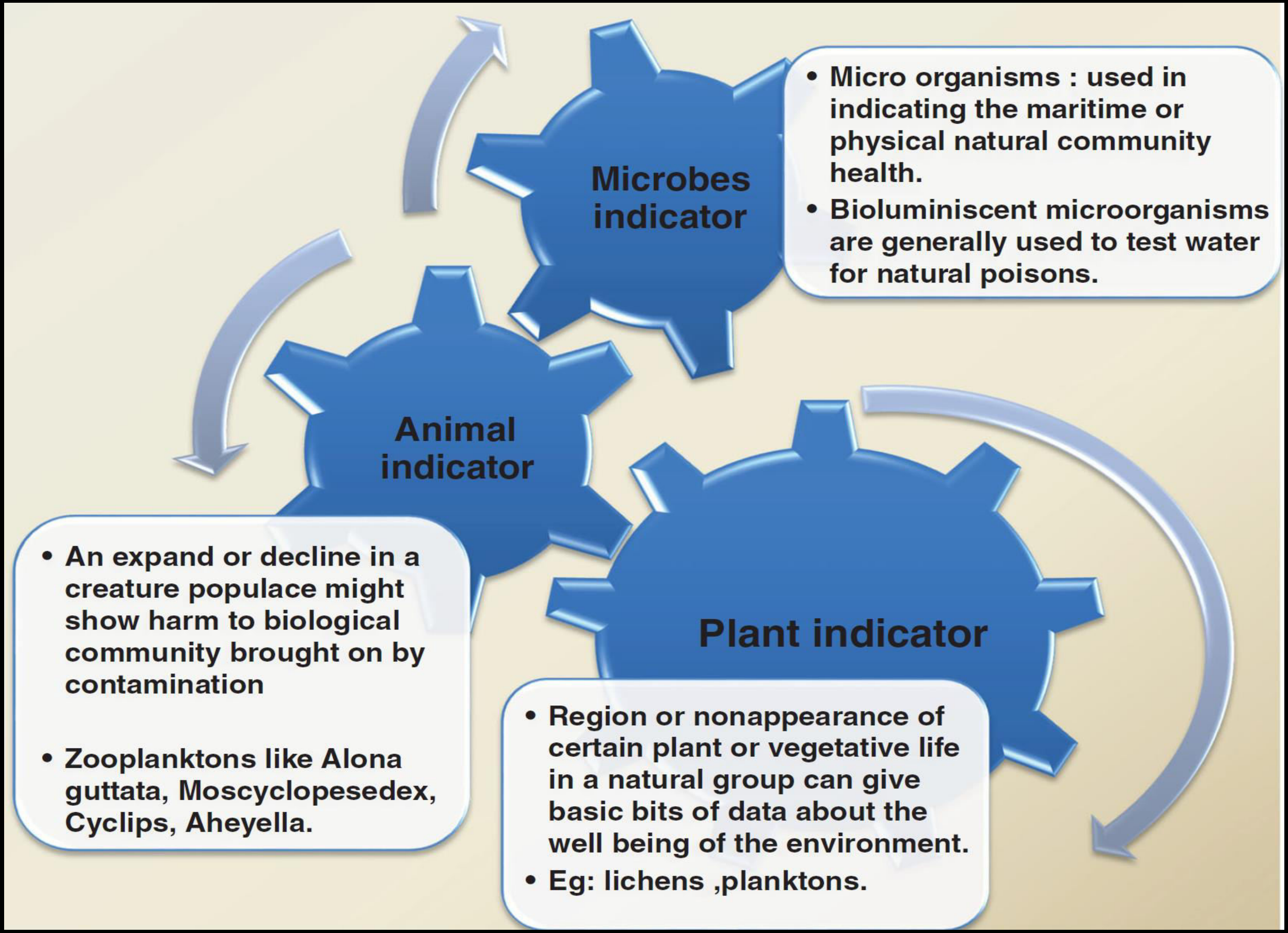
END

Environmental Biotechnology

Types of bioindicators:

Plant Indicators

Types of bioindicators : *Plant Indicators*



Microbes indicator

- Micro organisms : used in indicating the maritime or physical natural community health.
- Bioluminiscent microorganisms are generally used to test water for natural poisons.

Animal indicator

- An expand or decline in a creature populace might show harm to biological community brought on by contamination

- Zooplanktons like Alona guttata, Moscyclopesedex, Cyclips, Aheyella.

Plant indicator

- Region or nonappearance of certain plant or vegetative life in a natural group can give basic bits of data about the well being of the environment.
- Eg: lichens ,planktons.

http://dx.doi.org/10.1080/21553769.2016.1162753

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.

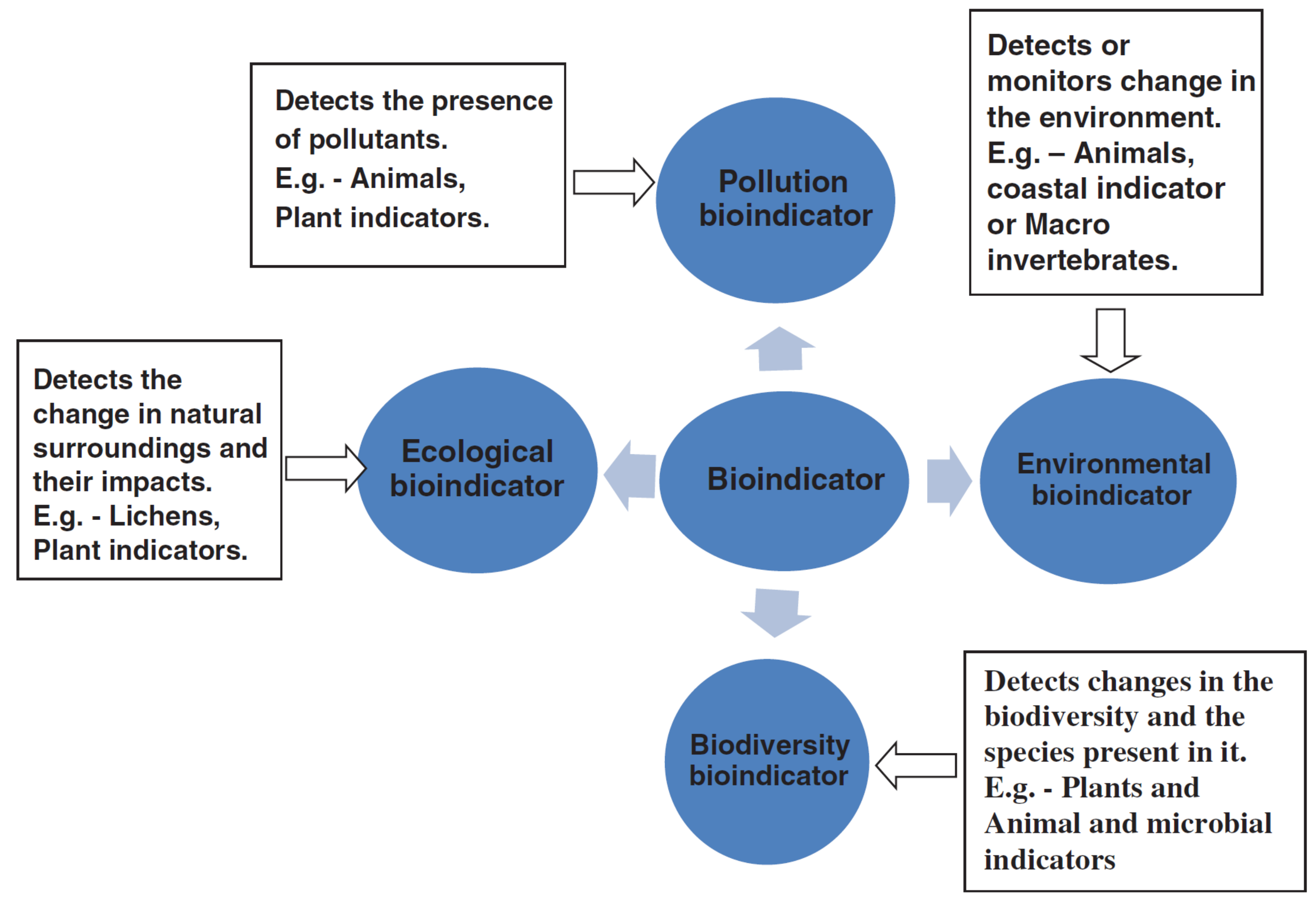
Types of bioindicators: *Microbial Indicators*

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*



Detects the presence of pollutants.
E.g. - Animals, Plant indicators.

Pollution bioindicator

Detects or monitors change in the environment.
E.g. – Animals, coastal indicator or Macro invertebrates.

Detects the change in natural surroundings and their impacts.
E.g. - Lichens, Plant indicators.

Ecological bioindicator

Bioindicator

Environmental bioindicator

Biodiversity bioindicator

Detects changes in the biodiversity and the species present in it.
E.g. - Plants and Animal and microbial indicators

http://dx.doi.org/10.1080/21553769.2016.1162753

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

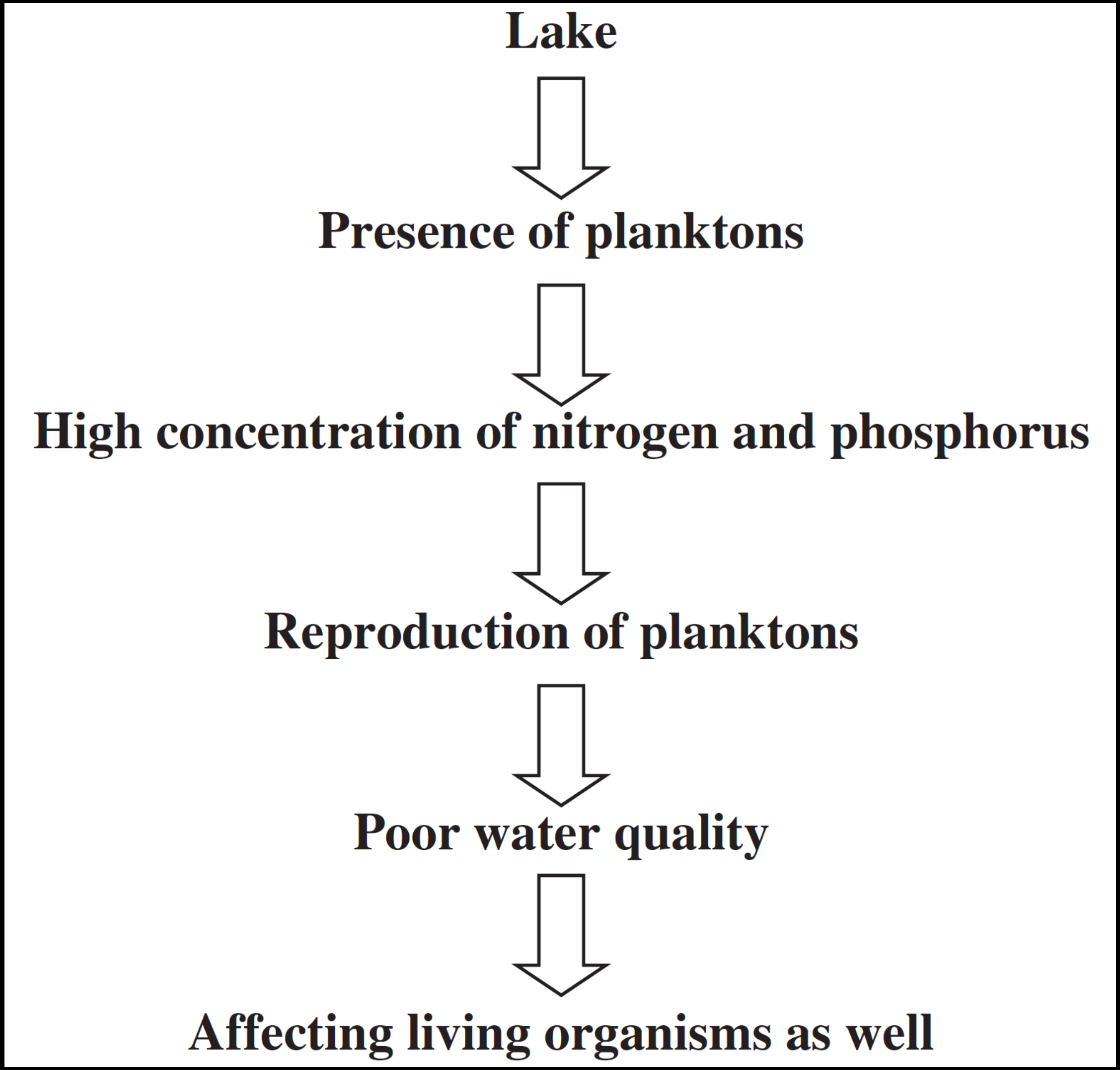
Planktons as an indicator of water pollution

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



Lake

↓

Presence of planktons

↓

High concentration of nitrogen and phosphorus

↓

Reproduction of planktons

↓

Poor water quality

↓

Affecting living organisms as well

http://dx.doi.org/10.1080/21553769.2016.1162753

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

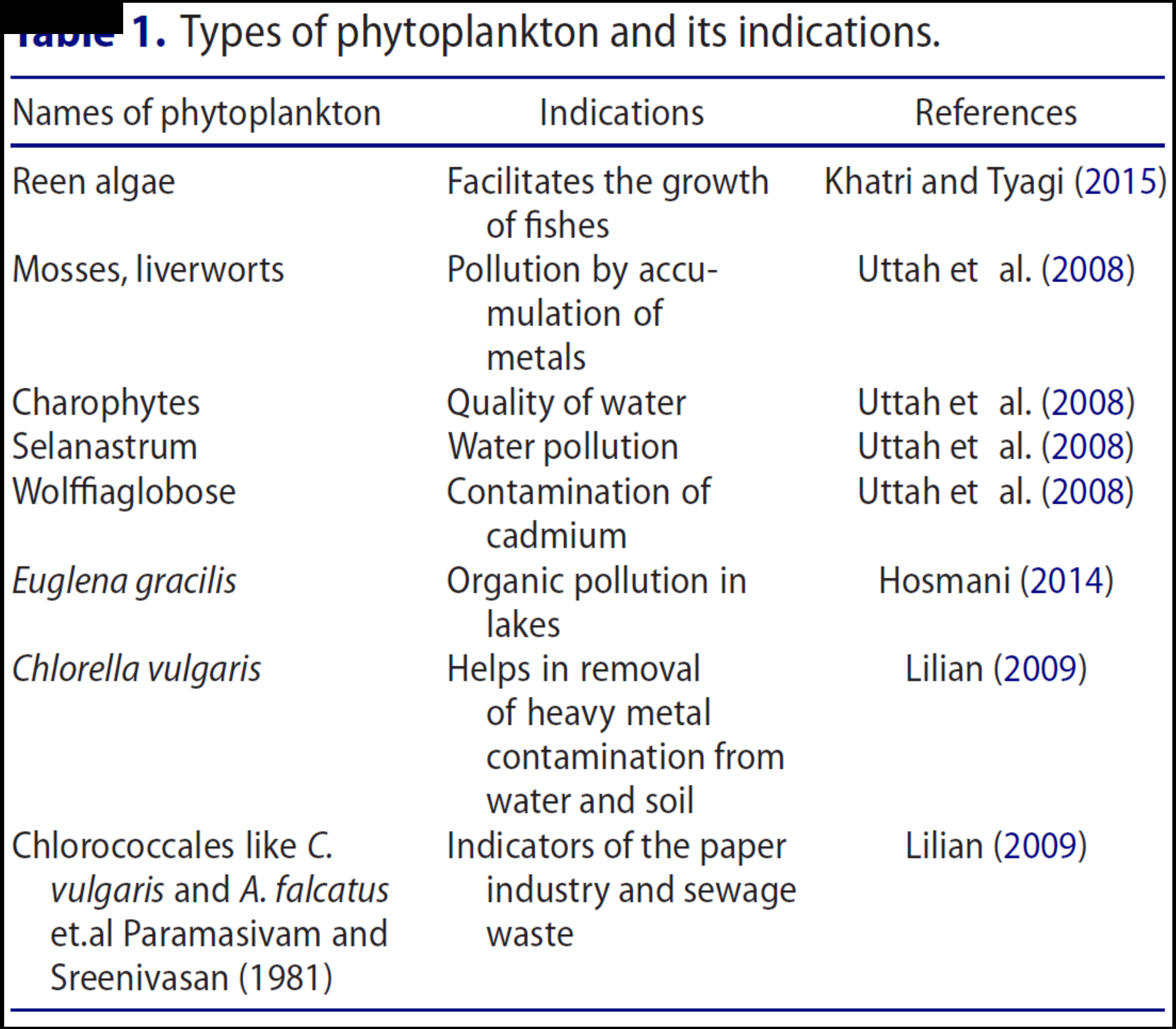
Phytoplankton

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



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)

http://dx.doi.org/10.1080/21553769.2016.1162753

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

END

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.

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

END

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)

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

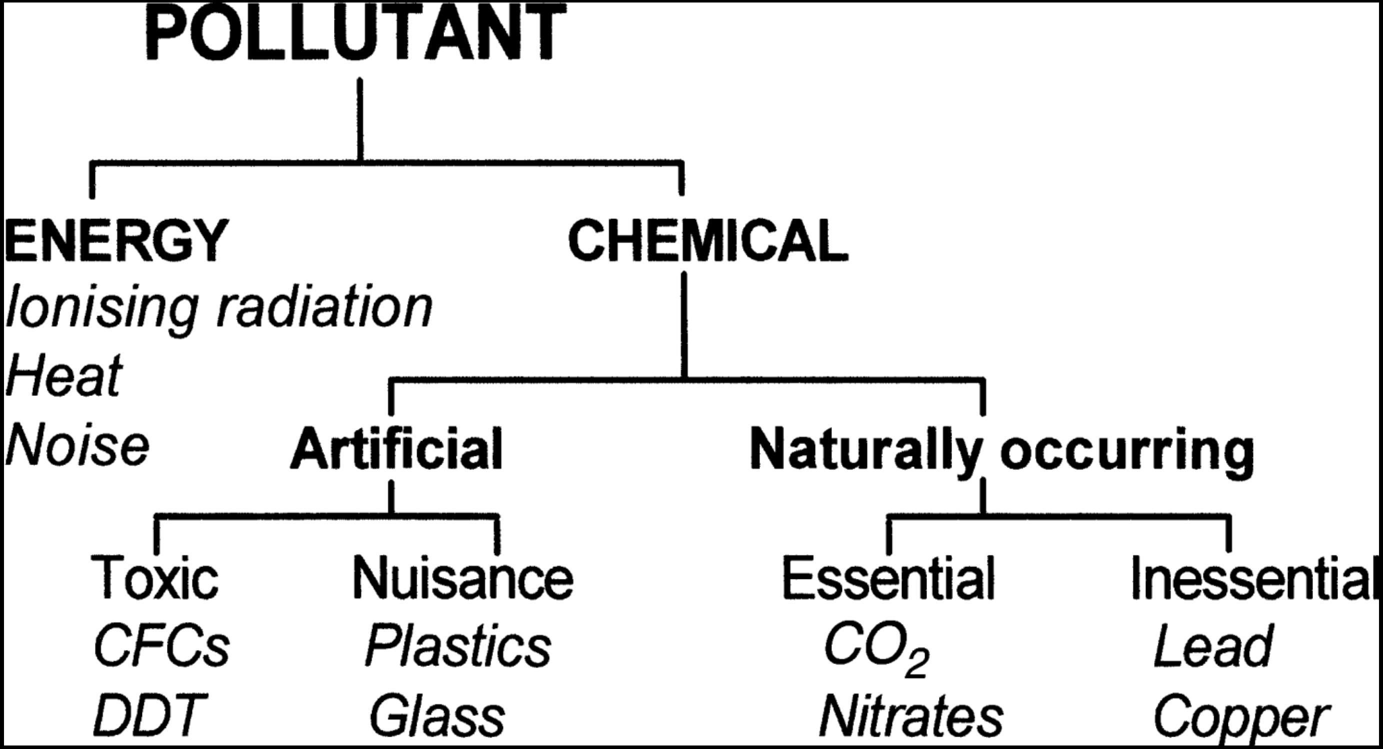
The diverse nature of potential pollutants makes their systematisation difficult in absolute terms. Possible to produce functional classifications on the basis of various characteristics. However, all such classification is essentially artificial and subjective.

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



POLLUTANT

- **ENERGY**
  - *Ionising radiation*
  - *Heat*
  - *Noise*
- **CHEMICAL**
  - **Artificial**
    - Toxic
      - *CFCs*
      - *DDT*
    - Nuisance
      - *Plastics*
      - *Glass*
  - **Naturally occurring**
    - Essential
      - CO_2
      - *Nitrates*
    - Inessential
      - *Lead*
      - *Copper*

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

END

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.

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

<u>Mobility</u>

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

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.

END

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

Bioaccumulation & Biomagnification

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

END

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

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

END

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

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

END

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.

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.

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

END

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

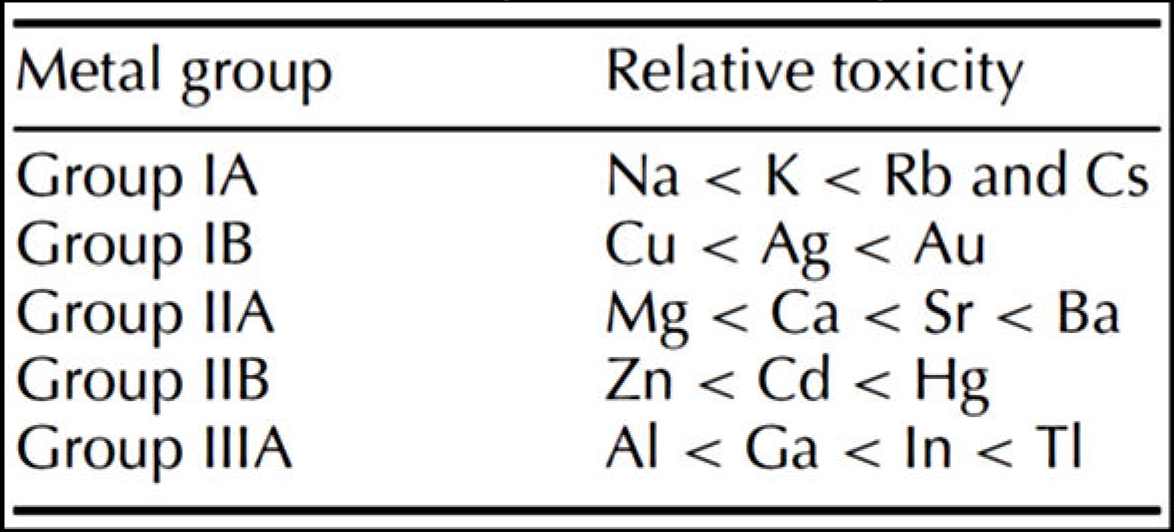
Practical Toxicity Issues (Part II)

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.

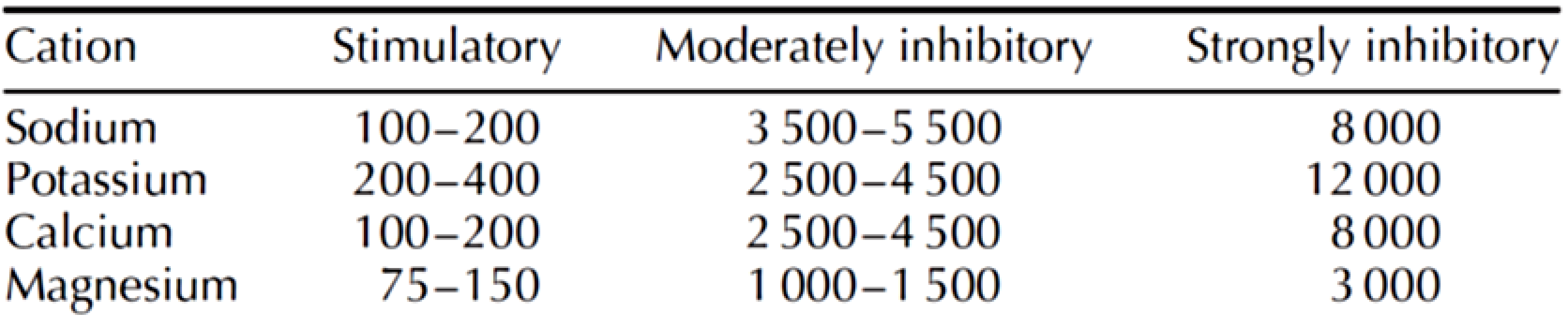
Practical Toxicity Issues (Part II)

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)

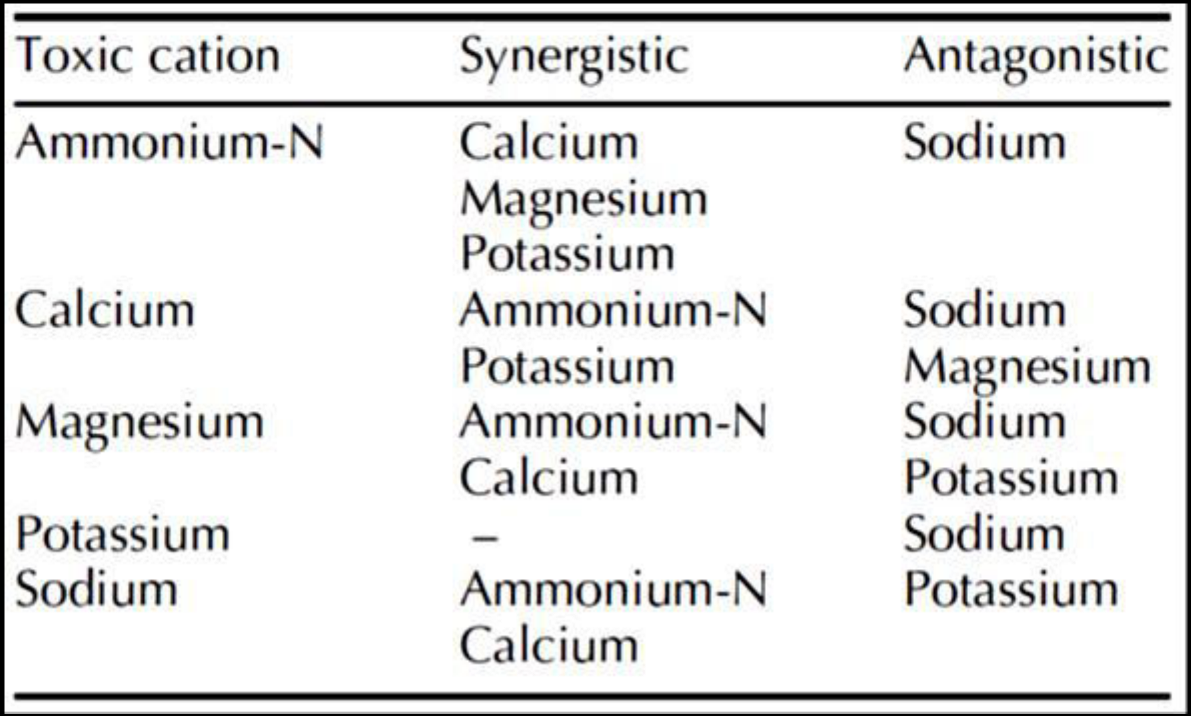
e.g., effects of metal cations within anaerobic bioreactors



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)

END

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.

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

END

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.

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

Environmental Biotechnology

Biofilters

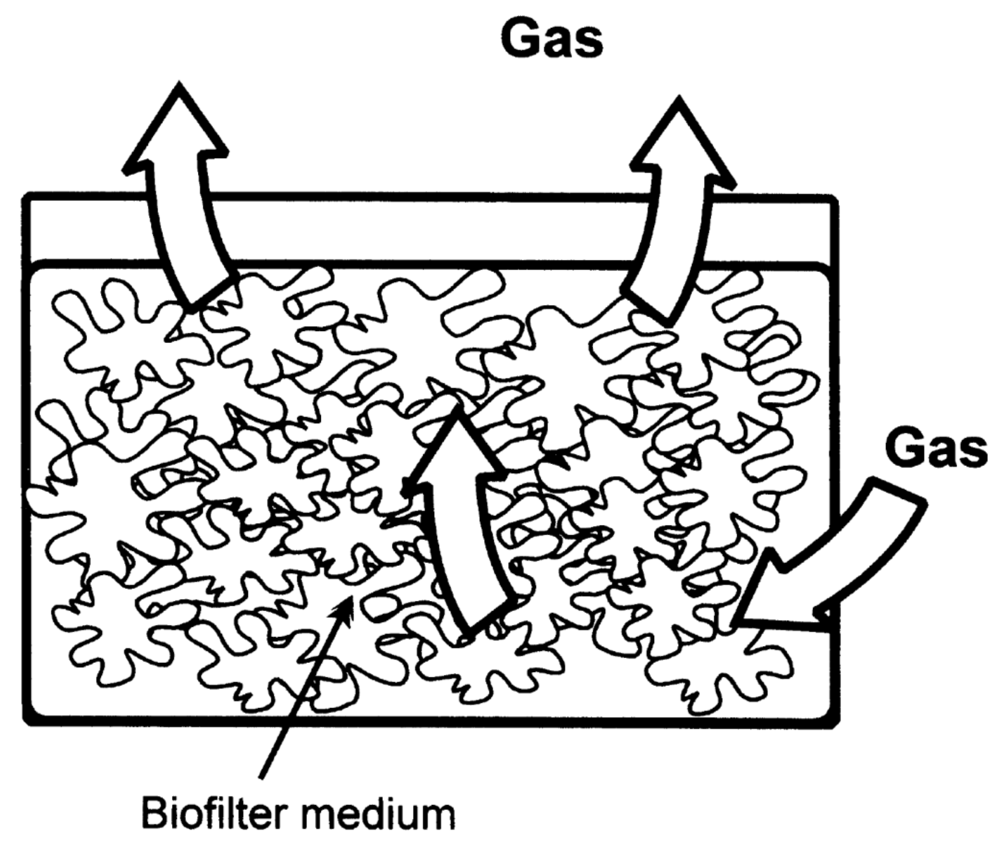
Biofilters

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.

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

END

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

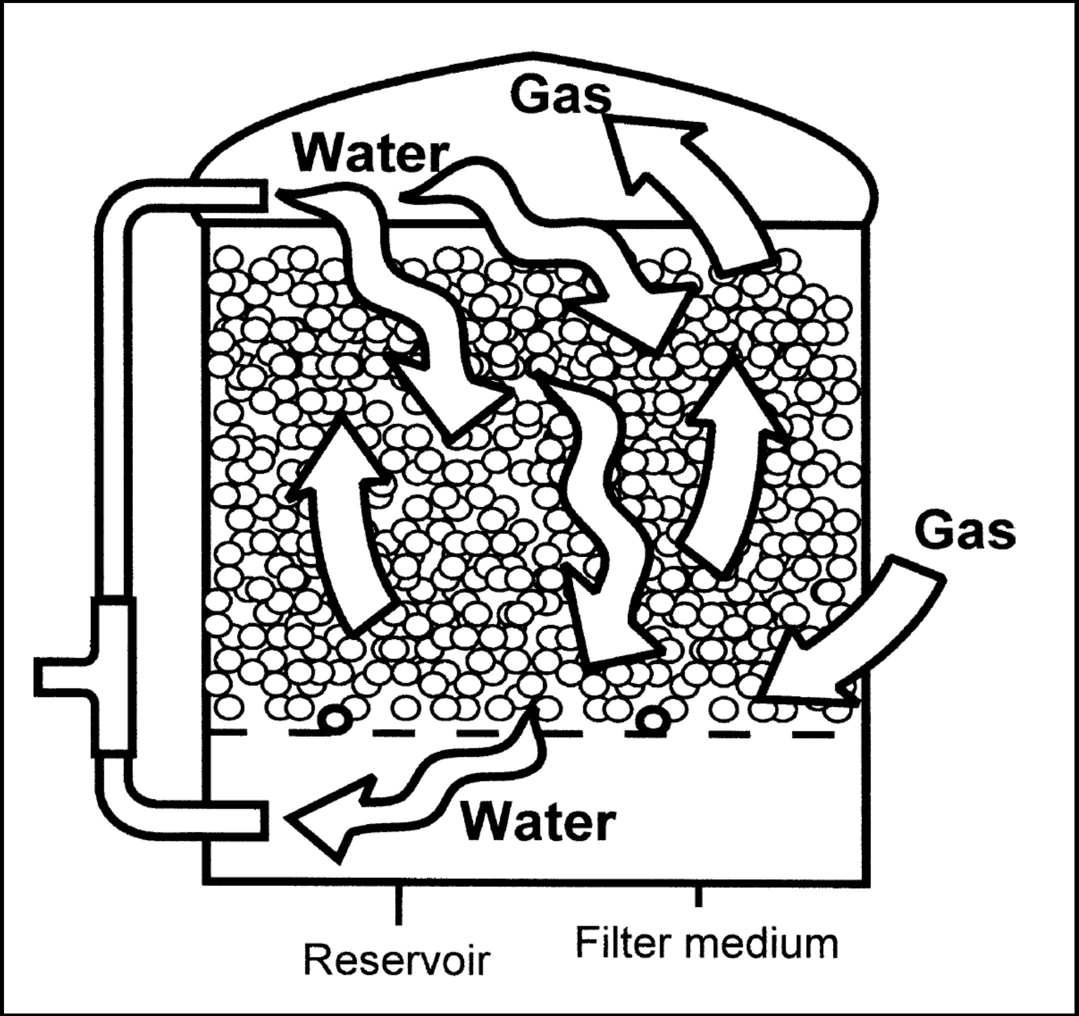
Being highly resistant to compaction, this also provides a large number of void spaces between particles and a high surface area relative to the overall volume of the filter.

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

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.

END

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.

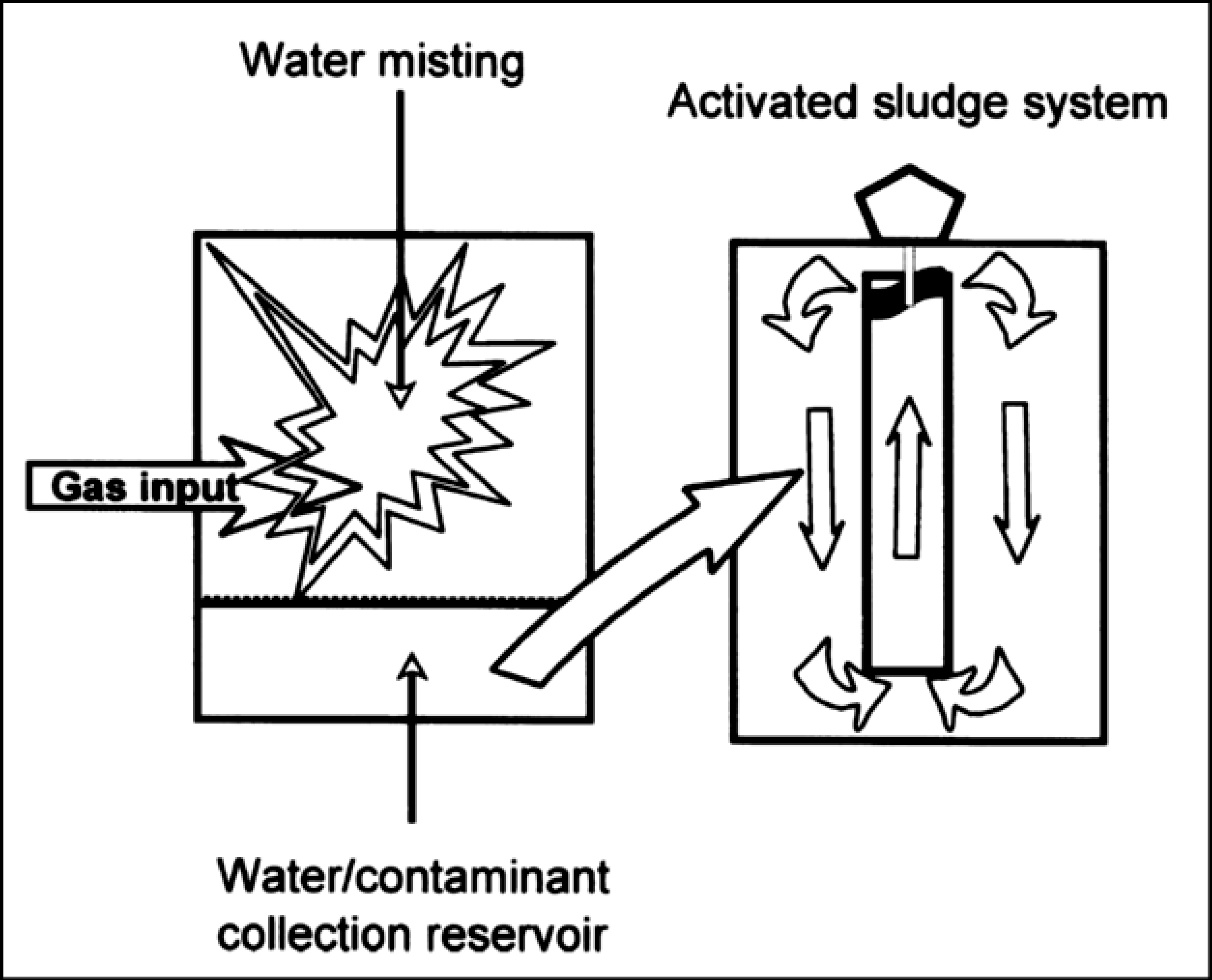
Most appropriate for hydrophilic compounds like acetone or methanol.

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

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

END

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

END

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

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

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.

END

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

END

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…

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

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.

END

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

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.

<u>Biological methods</u>: 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

END

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.

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

END

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.

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

END

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*

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

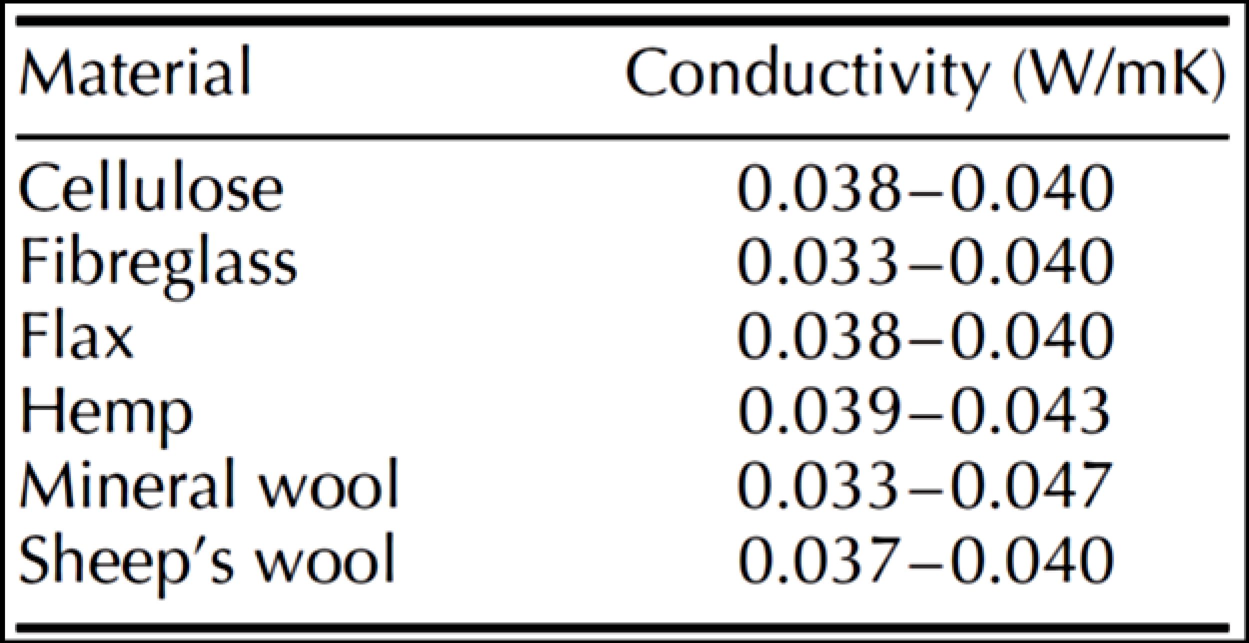
Issues of acoustic insulation often go hand-in-hand with the need for thermal insulation…

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:

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

where:

E = removal efficiency (%)
C_o = influent concentration of the pollutant (mg/L)
C_e = effluent concentration of the pollutant (mg/L)

END

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…

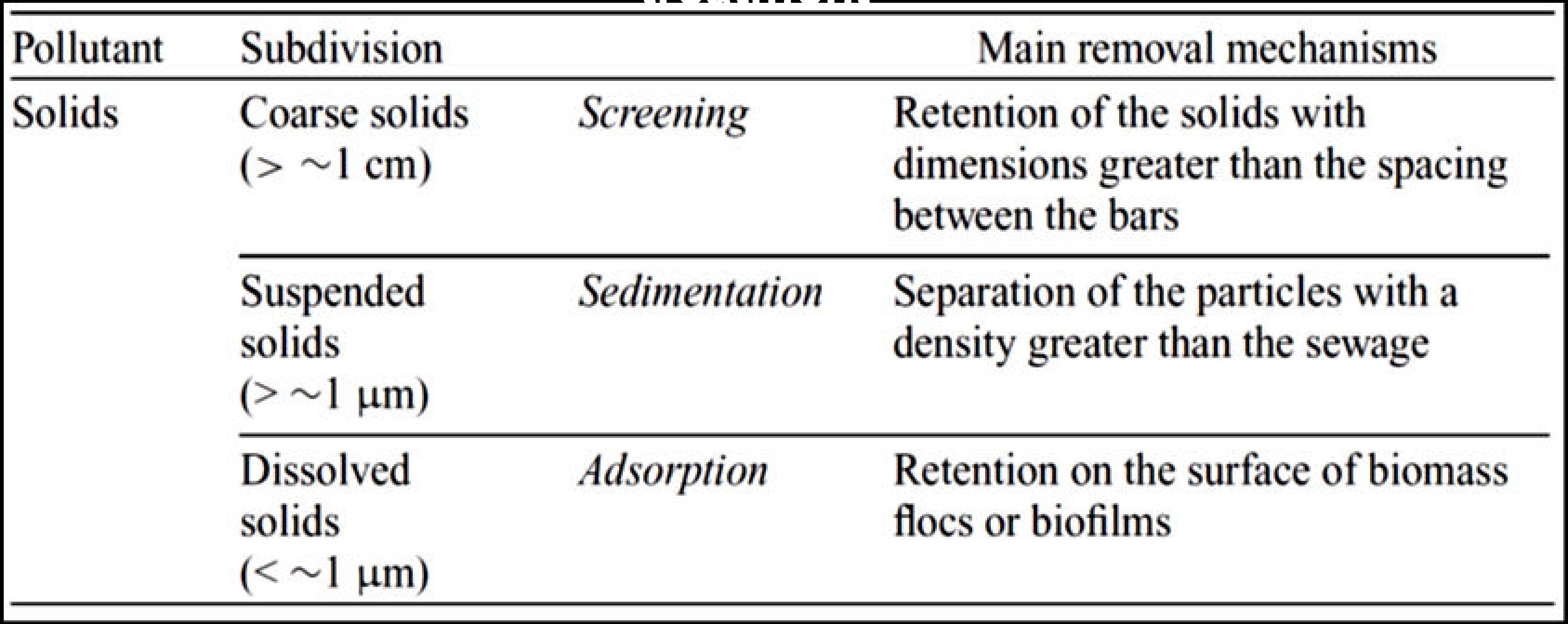
END

Environmental Biotechnology

Mechanisms for removal of pollutants in wastewater

Removal of Pollutants in Wastewater

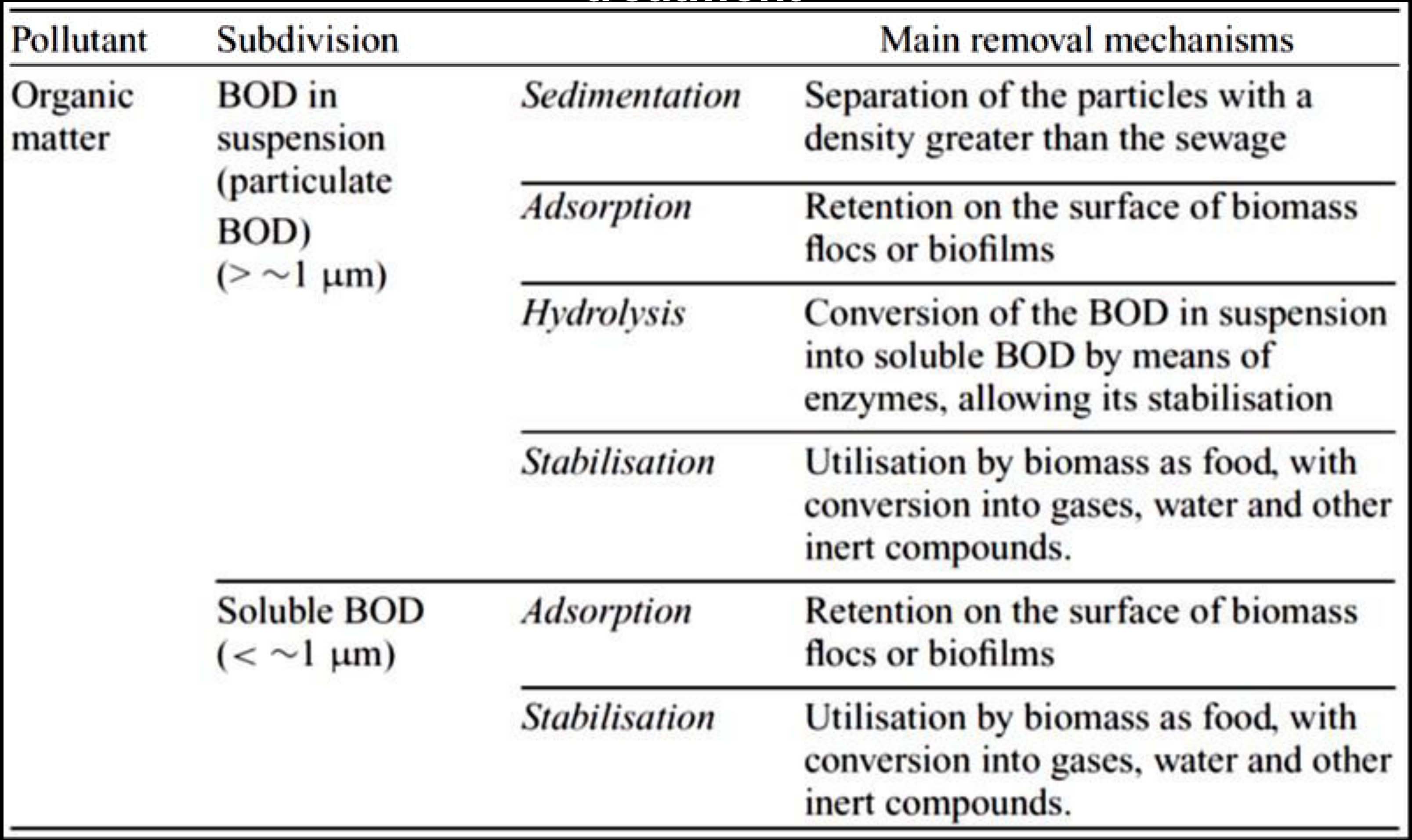
Main mechanisms for the removal of pollutants in wastewater treatment



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

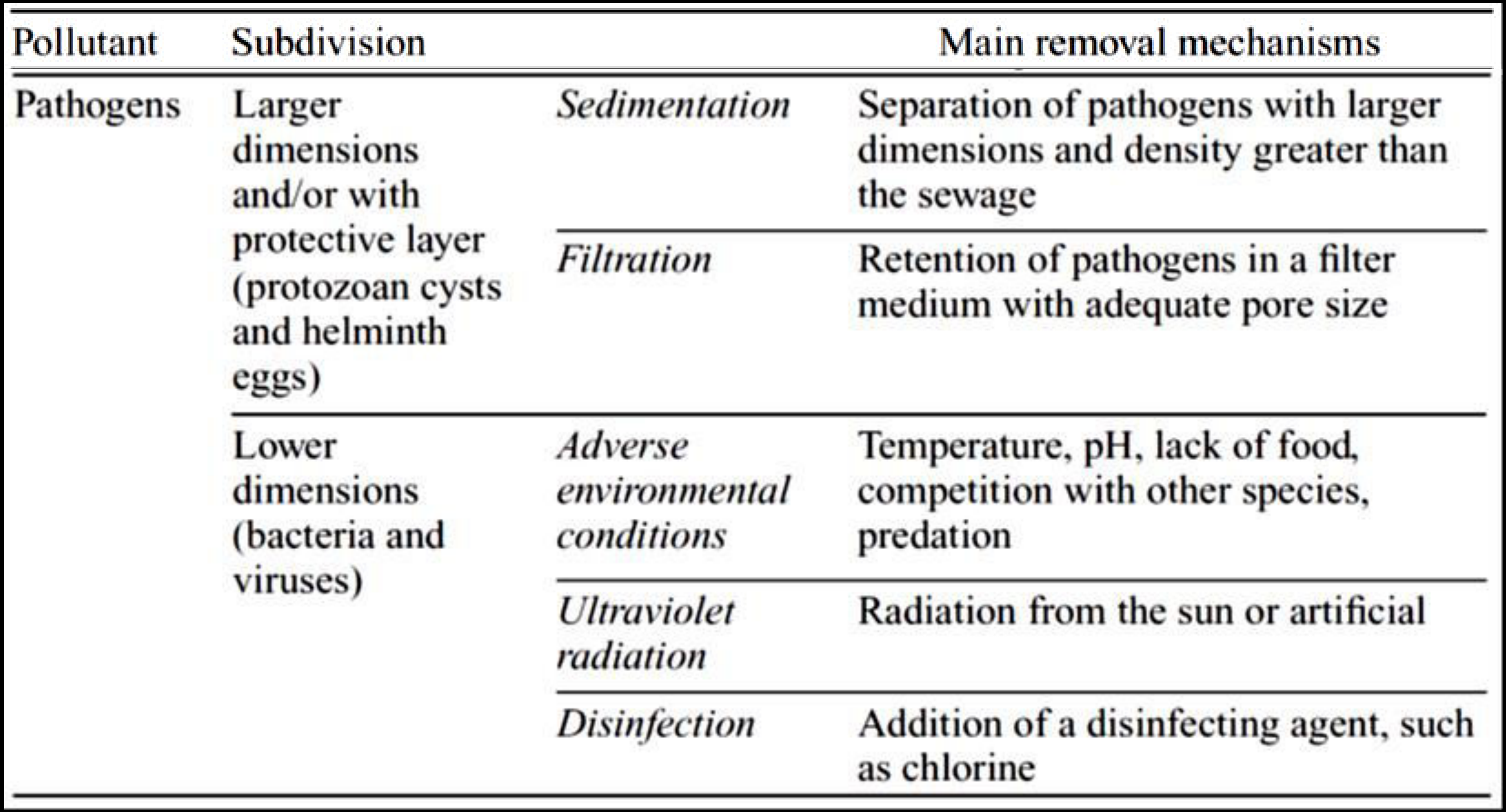
Main mechanisms for the removal of pollutants in wastewater treatment



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

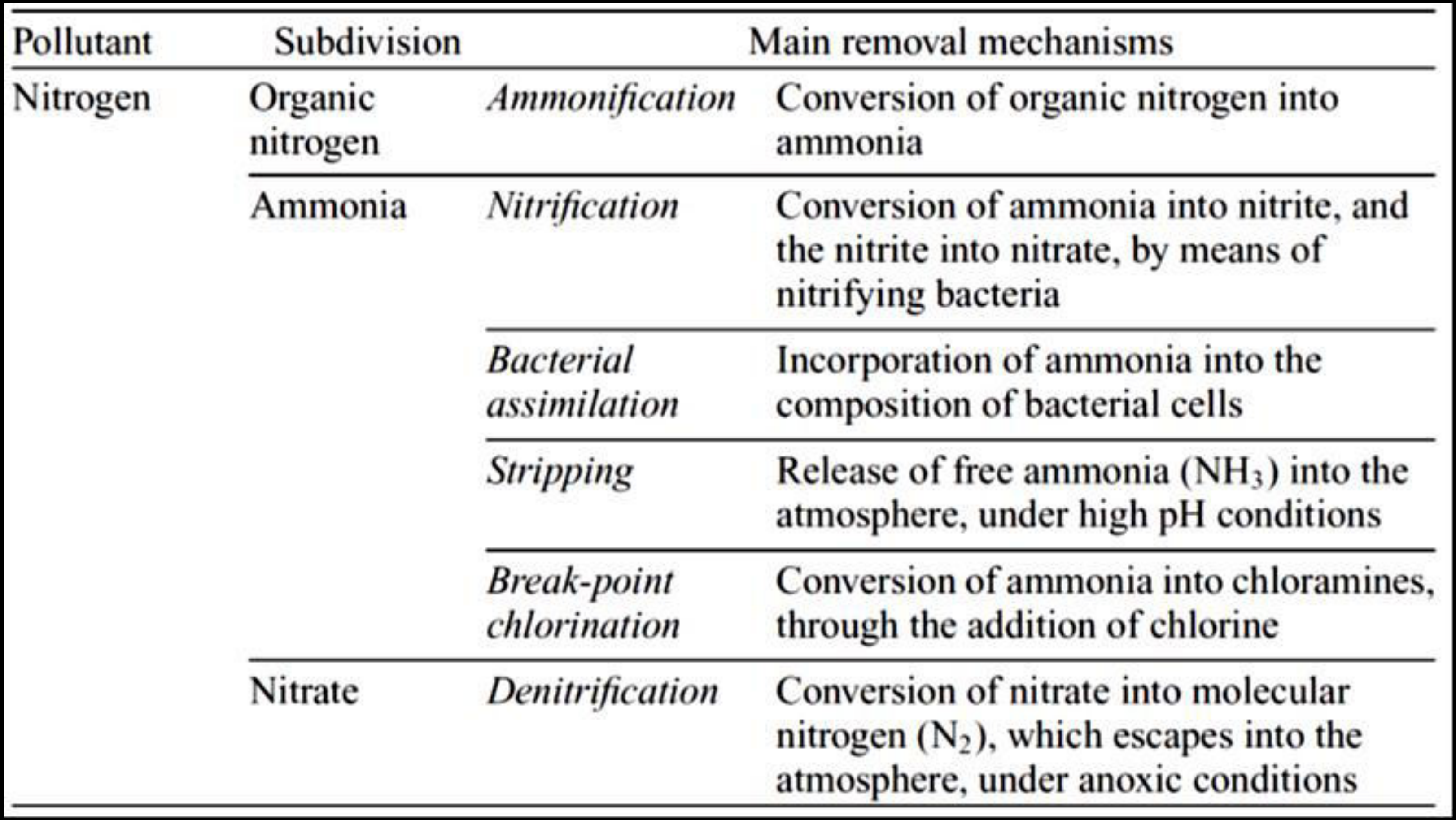
Main mechanisms for the removal of pollutants in wastewater treatment



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

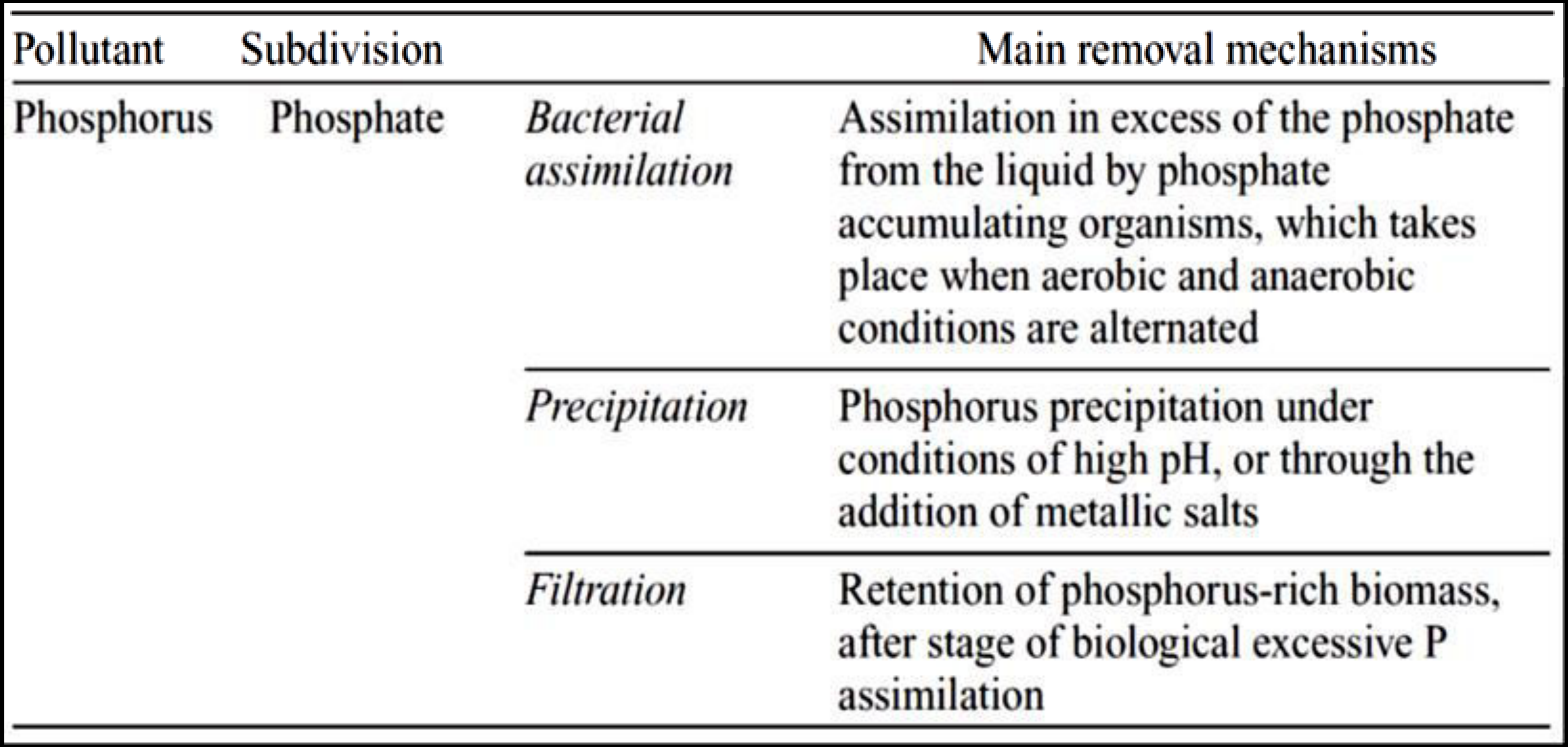
Main mechanisms for the removal of pollutants in wastewater treatment



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



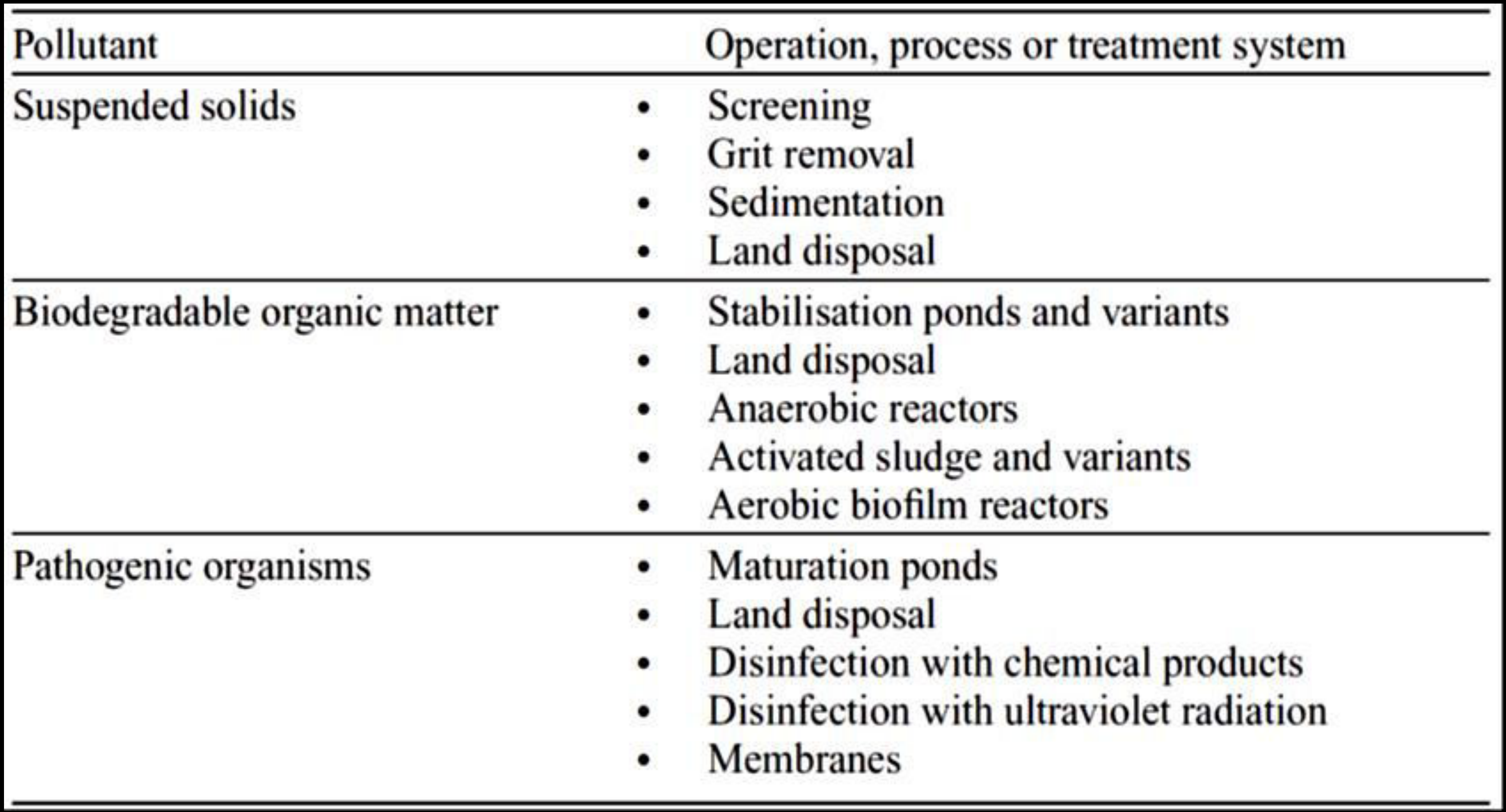
Pollutant	Subdivision	Main removal mechanisms	
Phosphorus	Phosphate	*Bacterial assimilation*	Assimilation in excess of the phosphate from the liquid by phosphate accumulating organisms, which takes place when aerobic and anaerobic conditions are alternated
		Precipitation	Phosphorus precipitation under 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

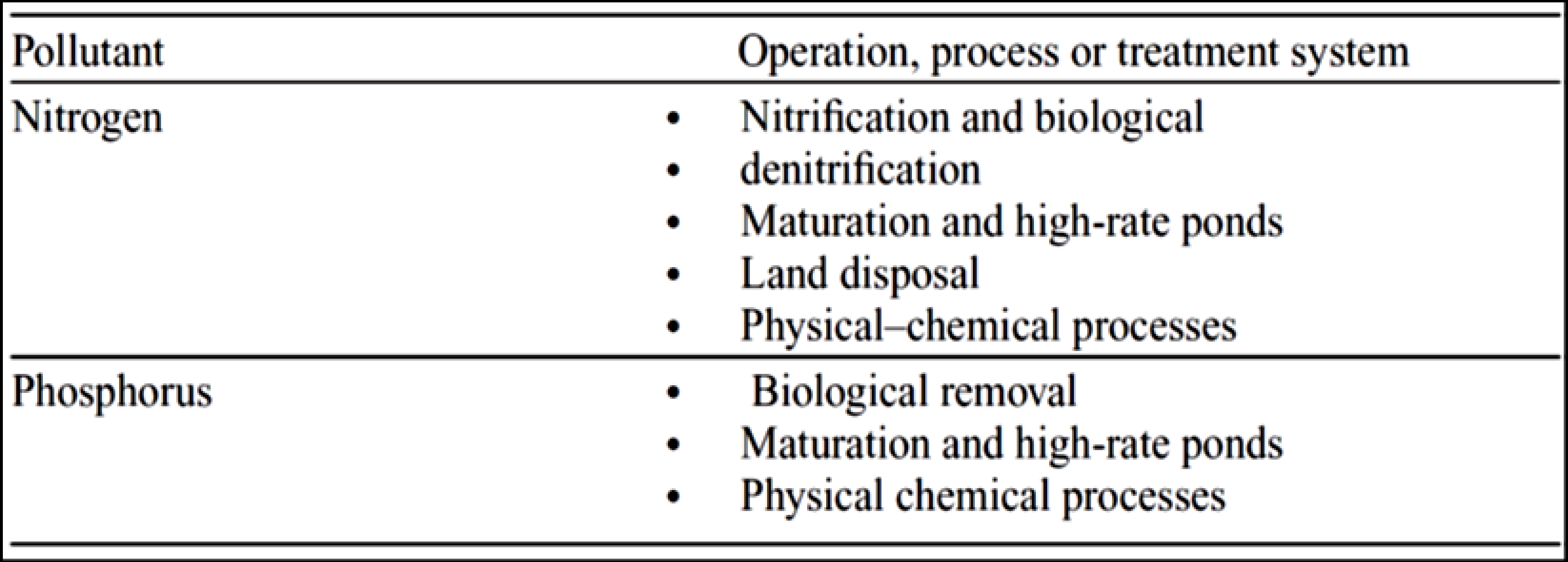
Treatment operations, processes and systems frequently used for the removal of pollutants from domestic sewage



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

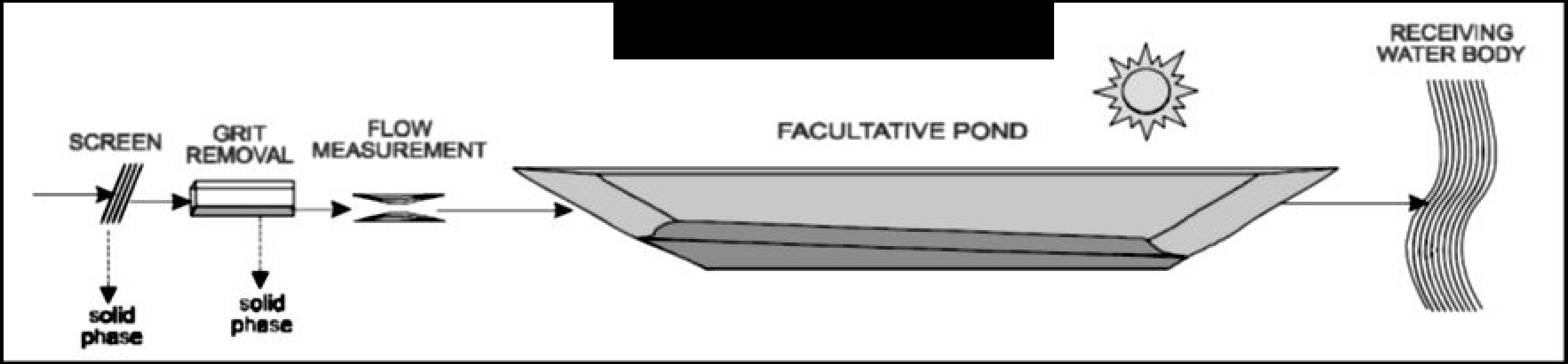
Facultative pond:

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

The land requirements are high.

Stabilization Ponds

Facultative Pond



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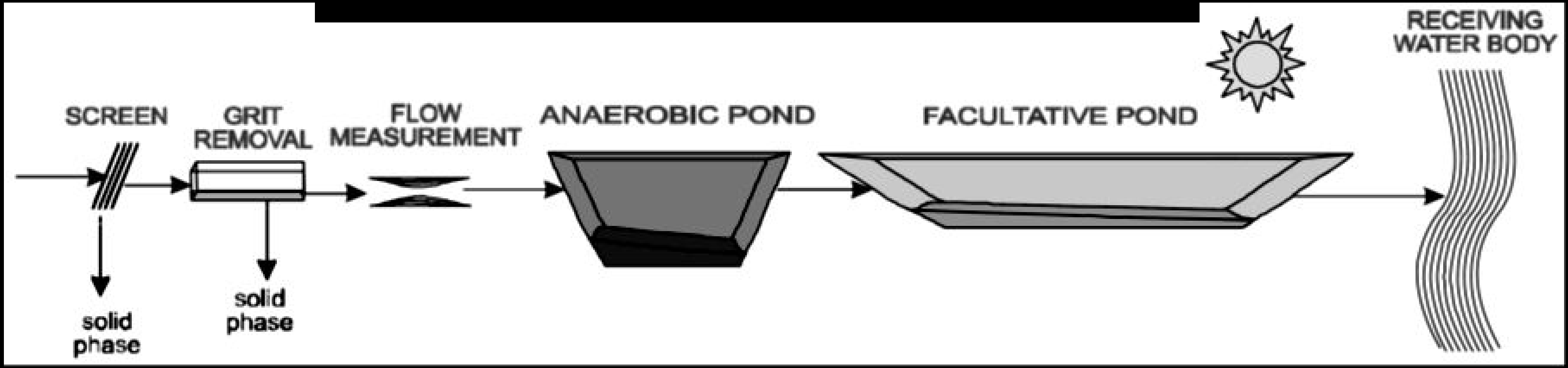
Stabilization Ponds

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



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Stabilization Ponds

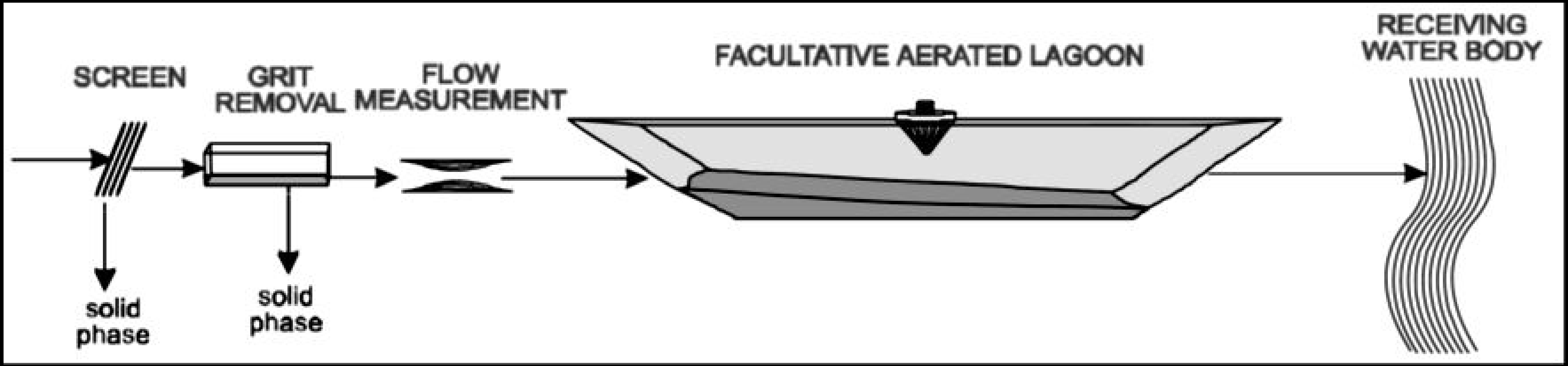
Facultative aerated lagoon:

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



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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

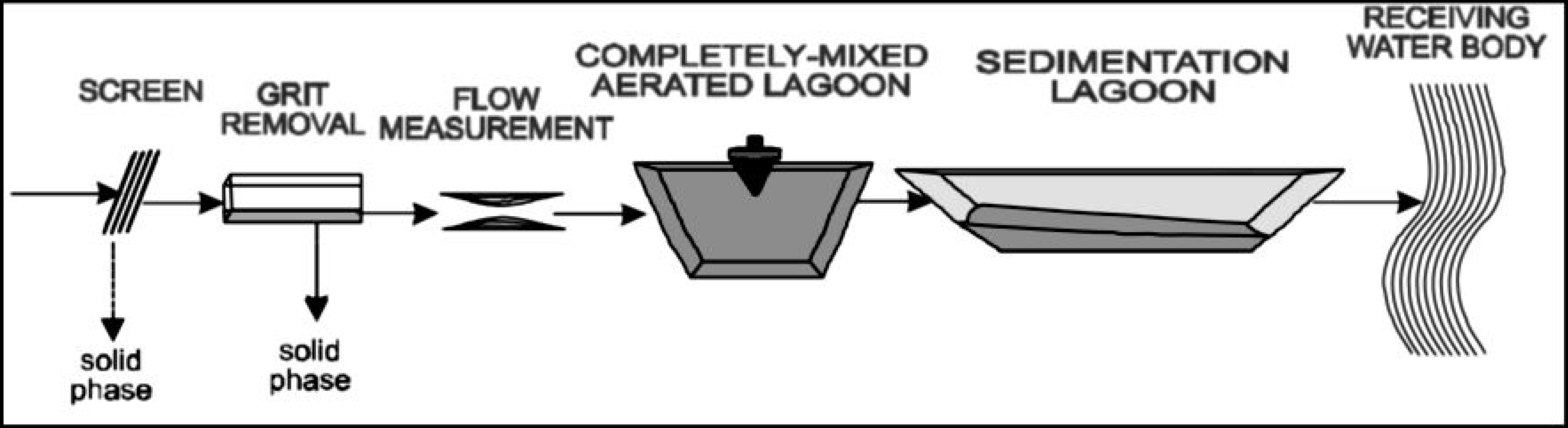
Completely mixed aerated lagoon – sedimentation pond:

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



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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

Maturation ponds:

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

END

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

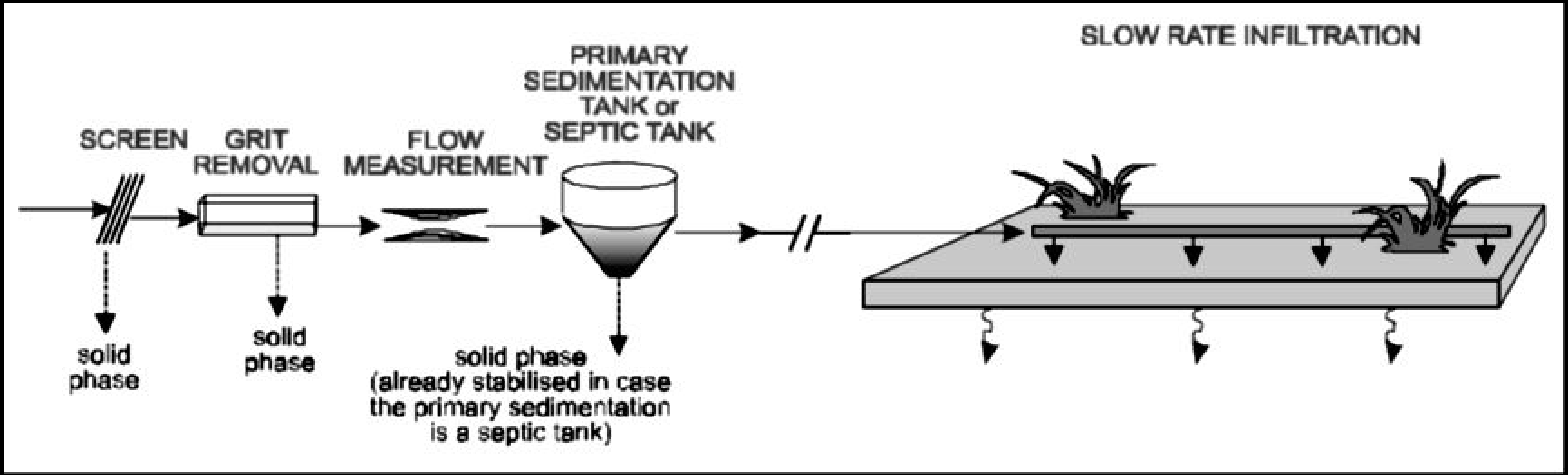
Slow rate system:

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



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Land Disposal

Rapid infiltration:

Wastewater is applied in shallow basins.

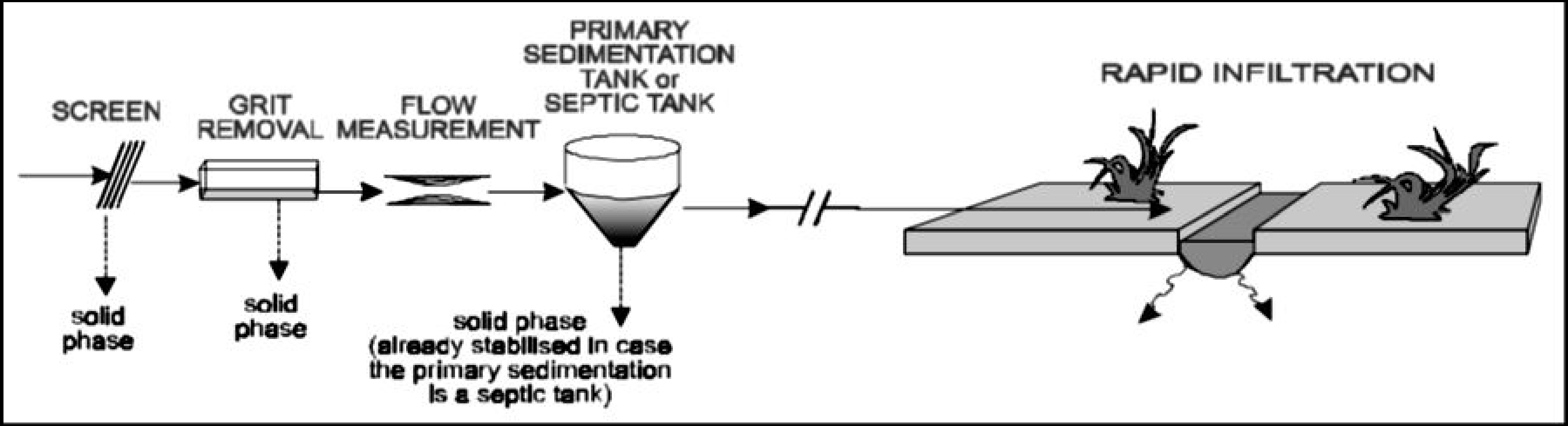
The liquid passes through the porous bottom and percolates into the soil.

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

Intermittent application…

Land Disposal

Rapid infiltration



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Land Disposal

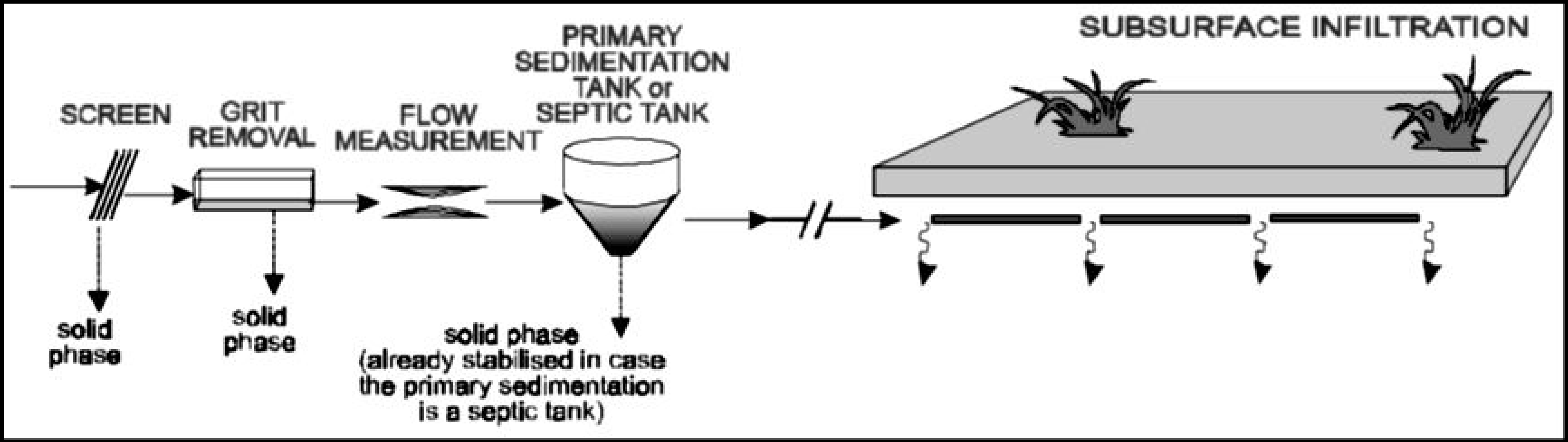
Subsurface infiltration:

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



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Land Disposal

Overland flow:

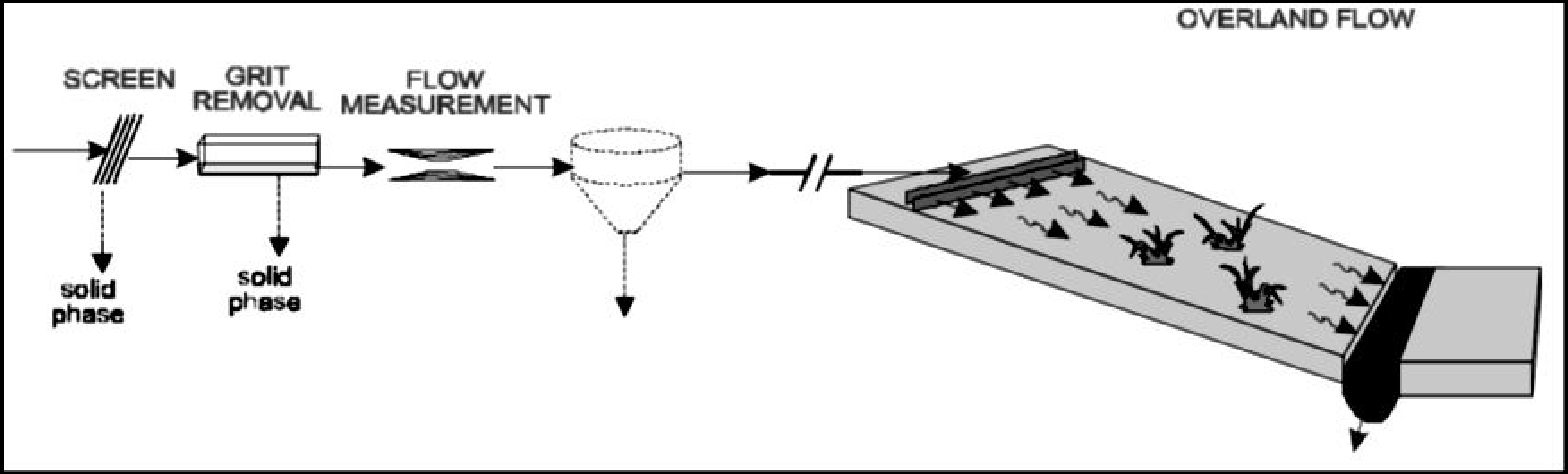
Wastewater is distributed in the upper part of vegetated slopes, flows over the slopes and is collected by ditches at the lower part.

Treatment occurs in the root-soil system.

The application is intermittent.

Land Disposal

Overland flow



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Land Disposal

Constructed wetlands:

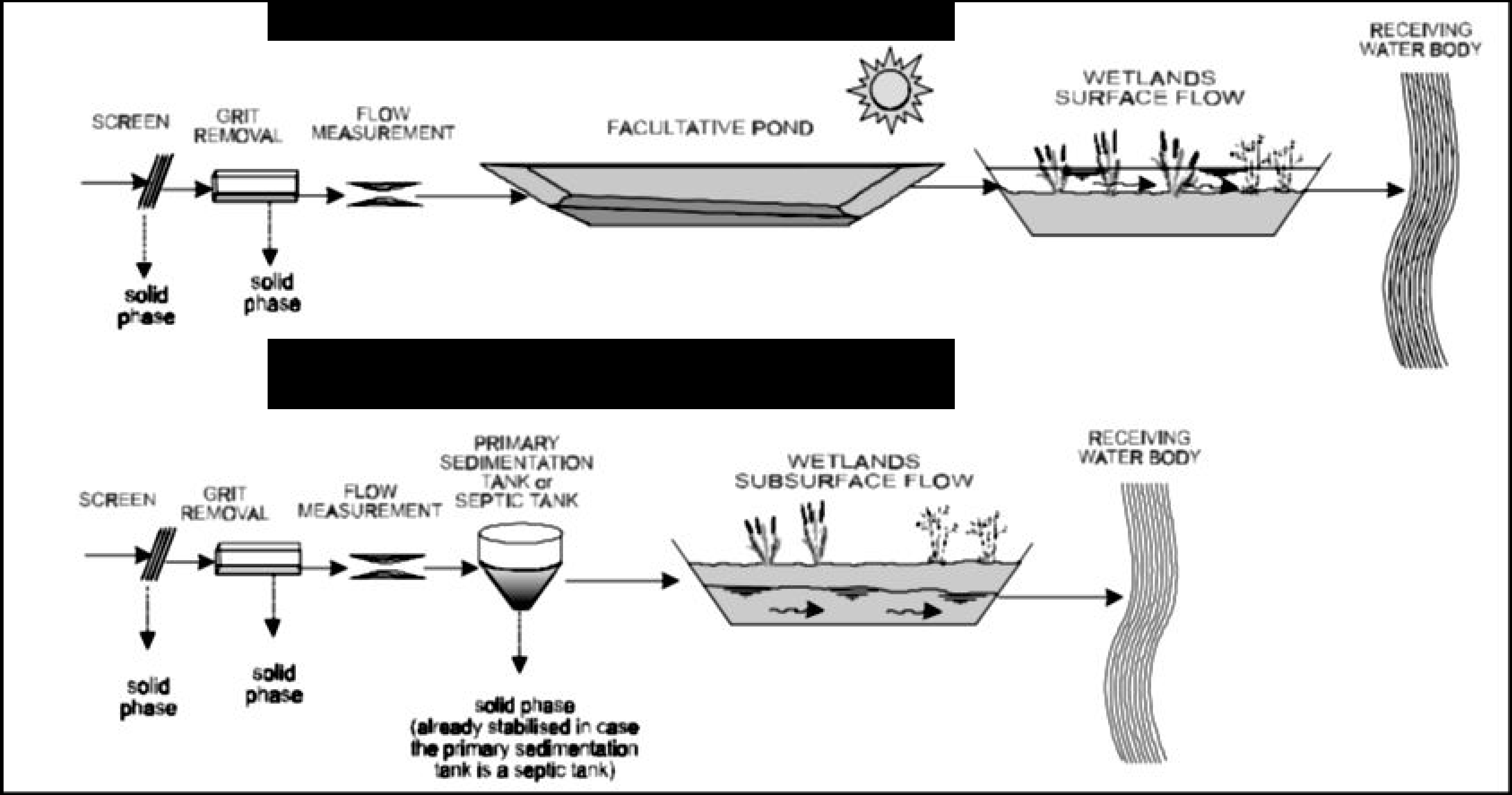
Aquatic-based systems, composed of shallow basins or channels in which aquatic plants grow.

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

Mechanisms operate on the root–soil system.

Land Disposal

Constructed wetlands



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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

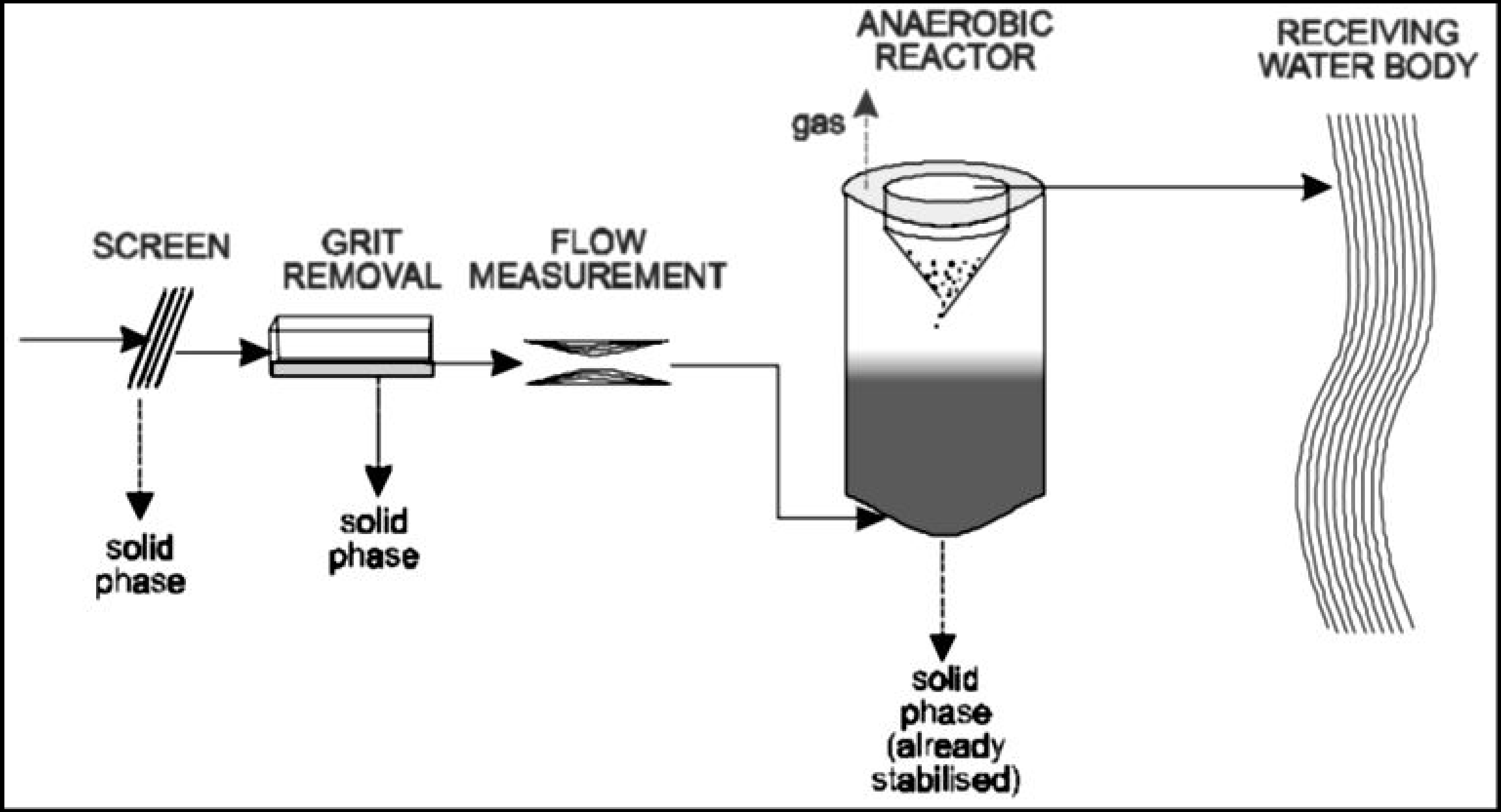
UASB:

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)



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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:

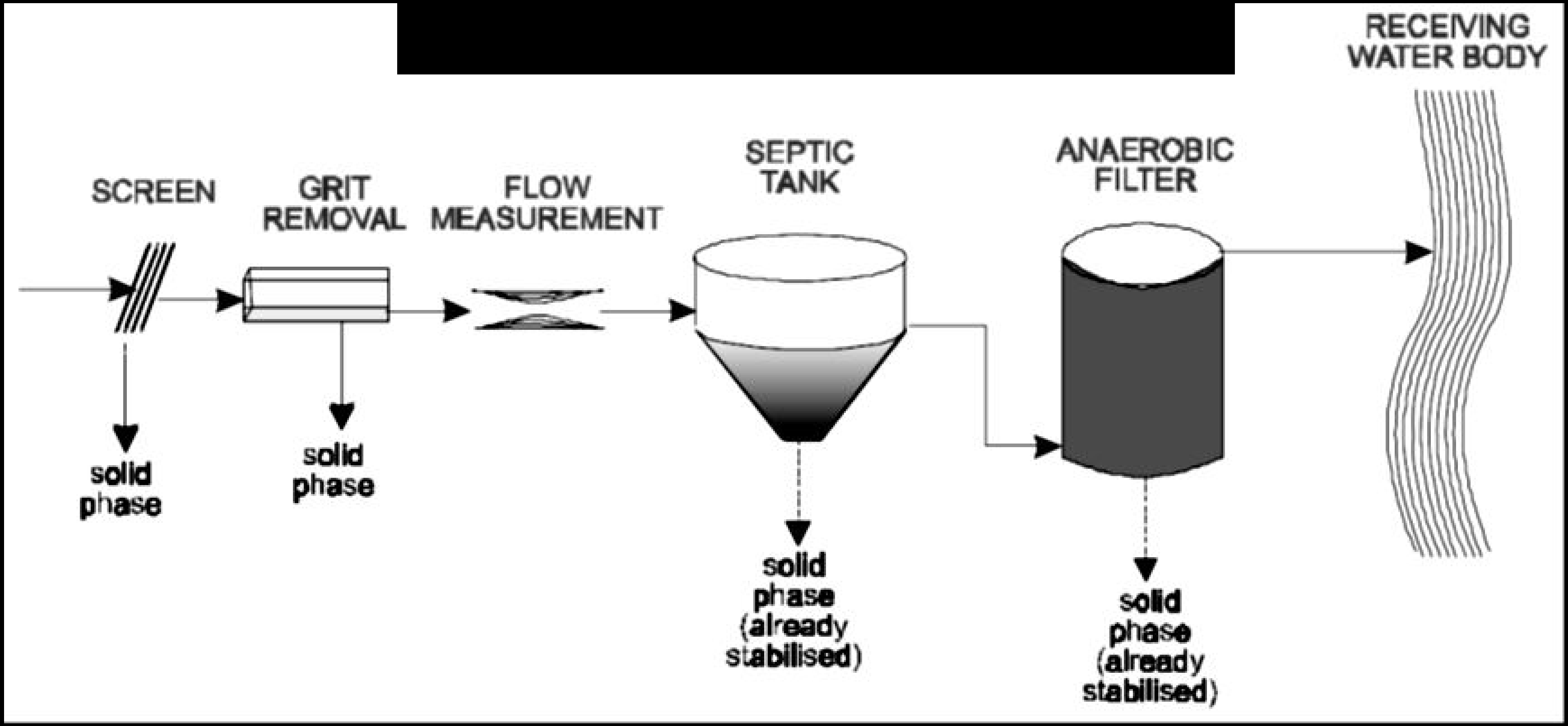
The tank works submerged and the flow is upwards.

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



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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).

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

END

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

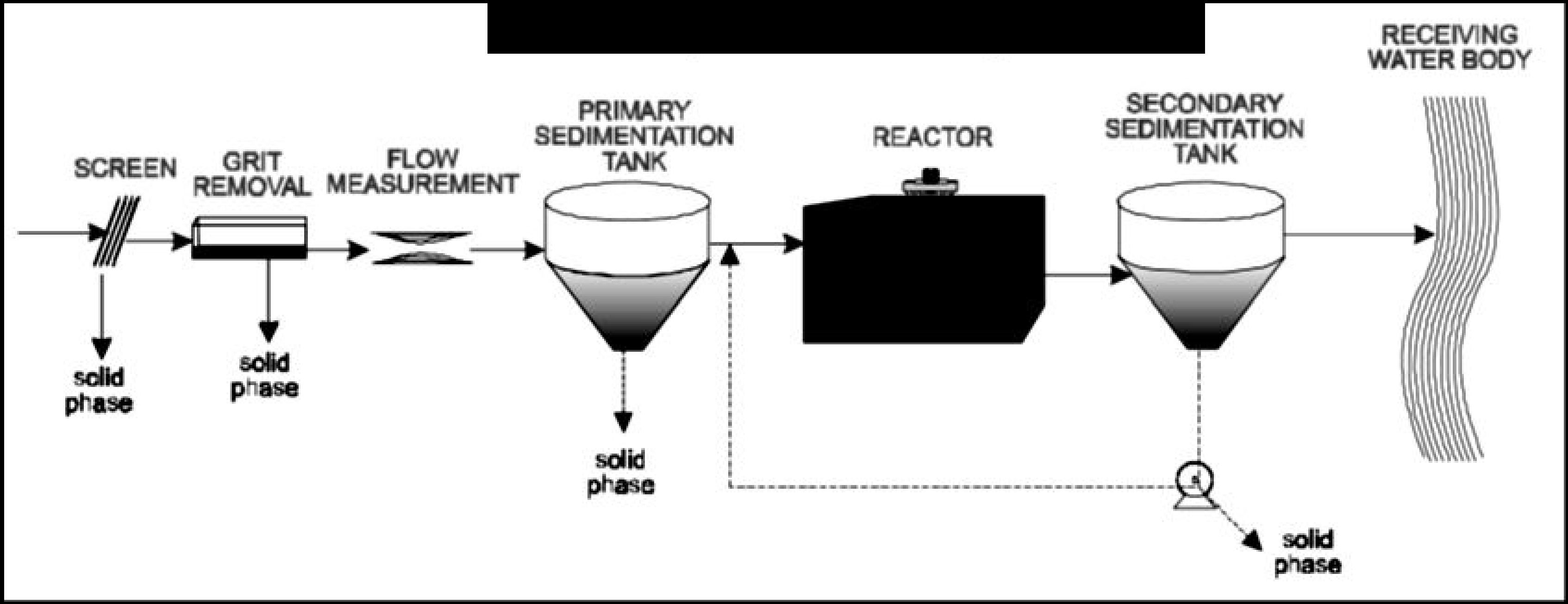
Conventional activated sludge:

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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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):

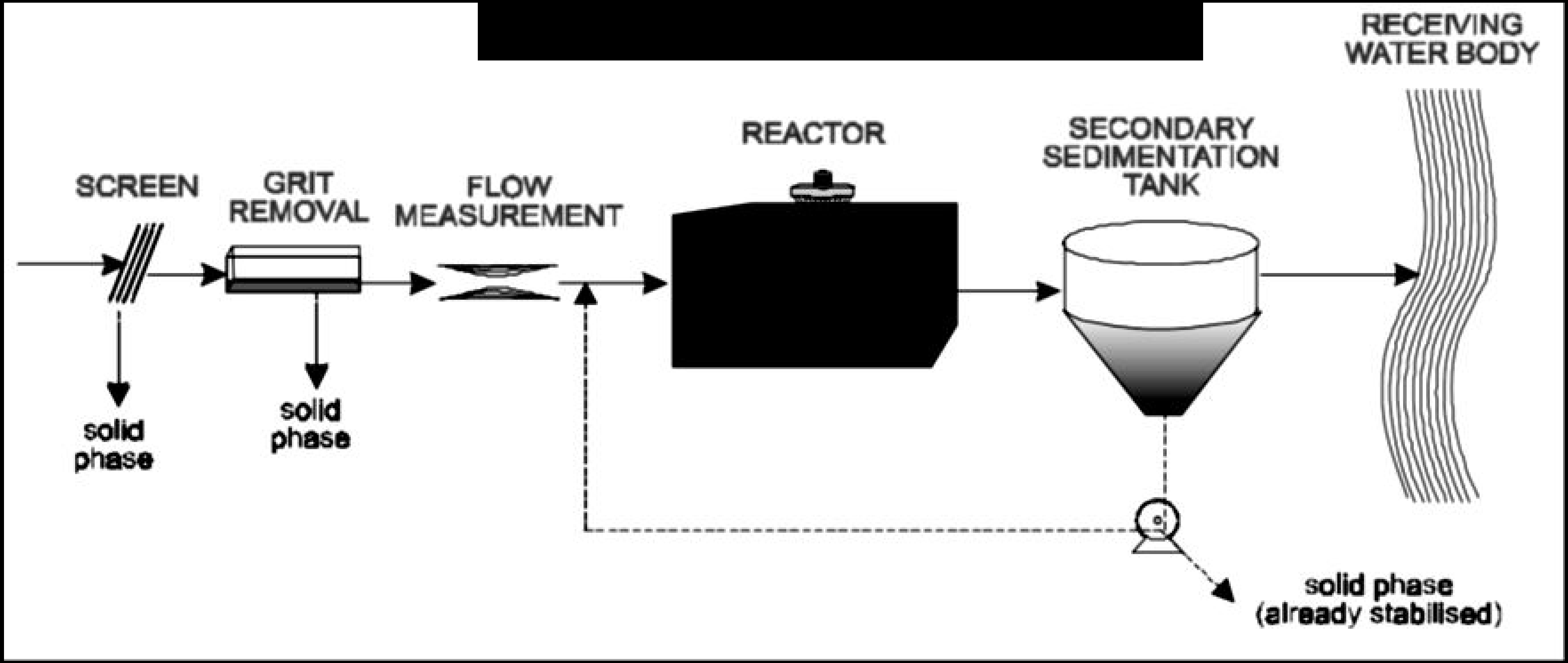
Less substrate available for the bacteria, which makes them use their own cellular material as organic matter.

Consequently, the removed excess sludge is already stabilised.

Primary sedimentation tank usually not included.

Activated sludge

Activated sludge
(extended aeration)



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Activated sludge

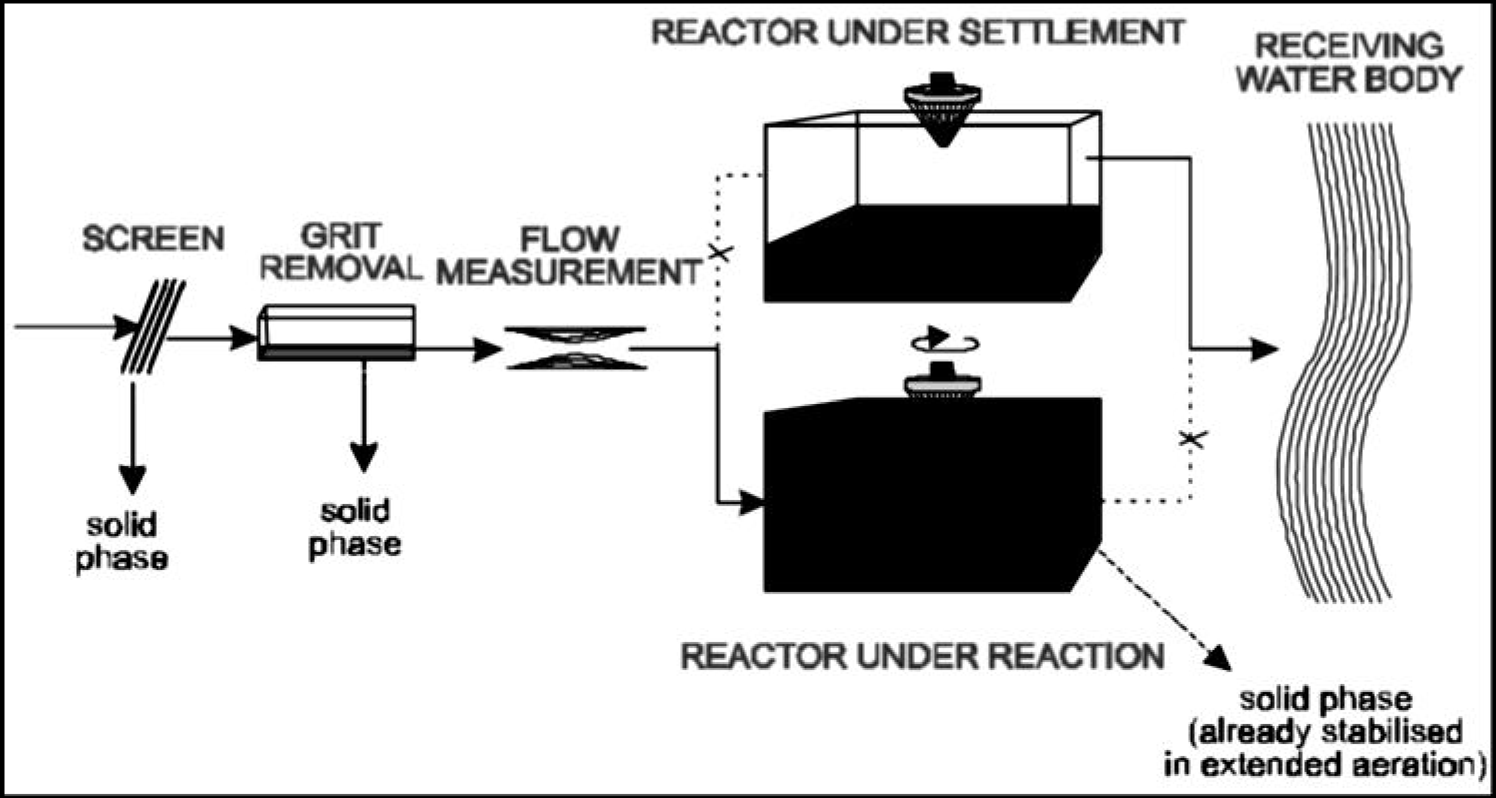
Intermittently operated activated sludge (sequencing batch reactors):

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)



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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.

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

END

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

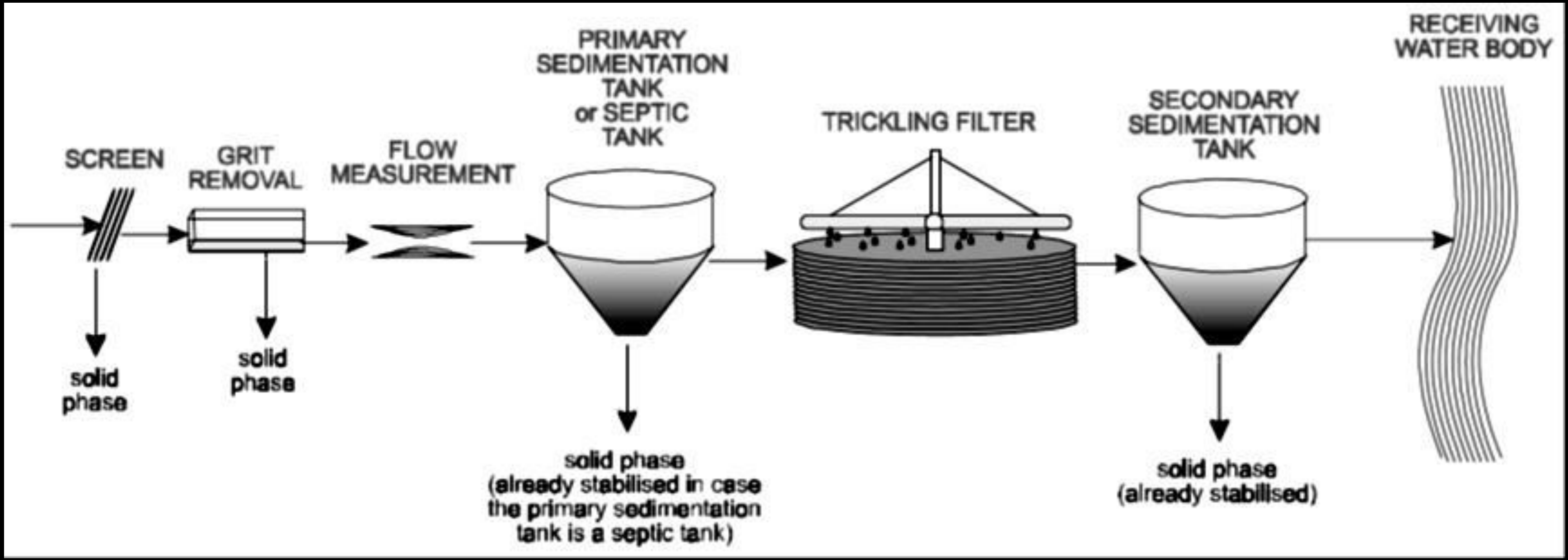
Low rate trickling filter:

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



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Aerobic Biofilm Reactors

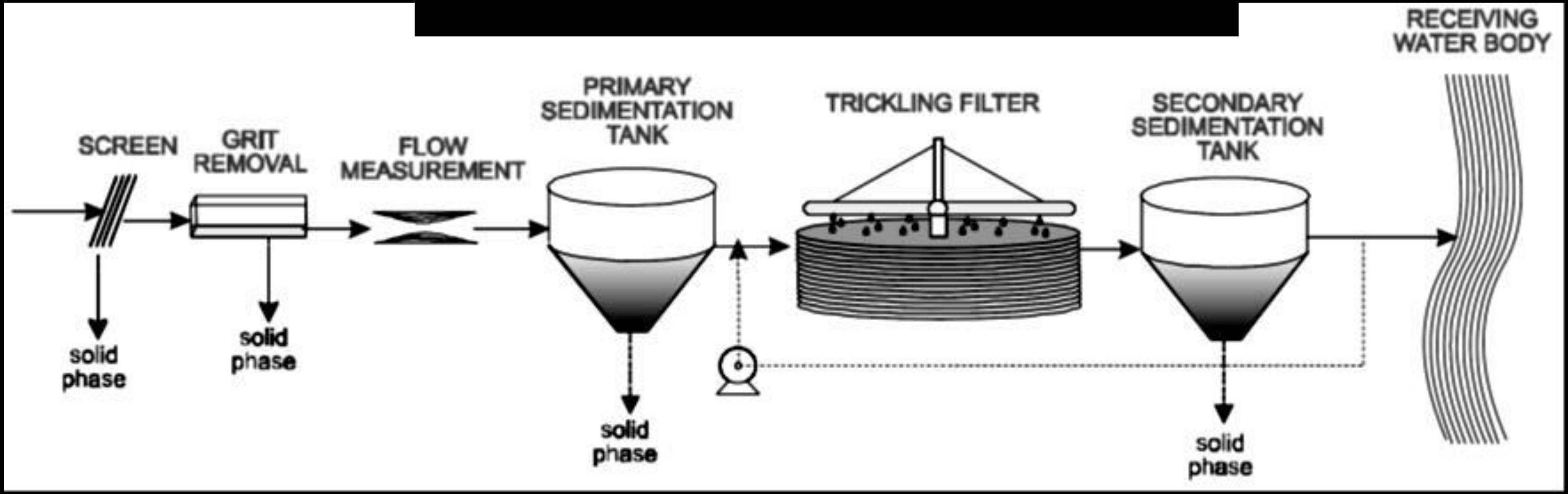
High rate trickling filter:

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



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Aerobic Biofilm Reactors

Submerged aerated biofilter:
Composed of a tank filled with a porous material (usually submerged), through which sewage and air flow permanently.

The biofilters with granular material undertake the removal of soluble organic compounds and particulate matter.

Aerobic Biofilm Reactors

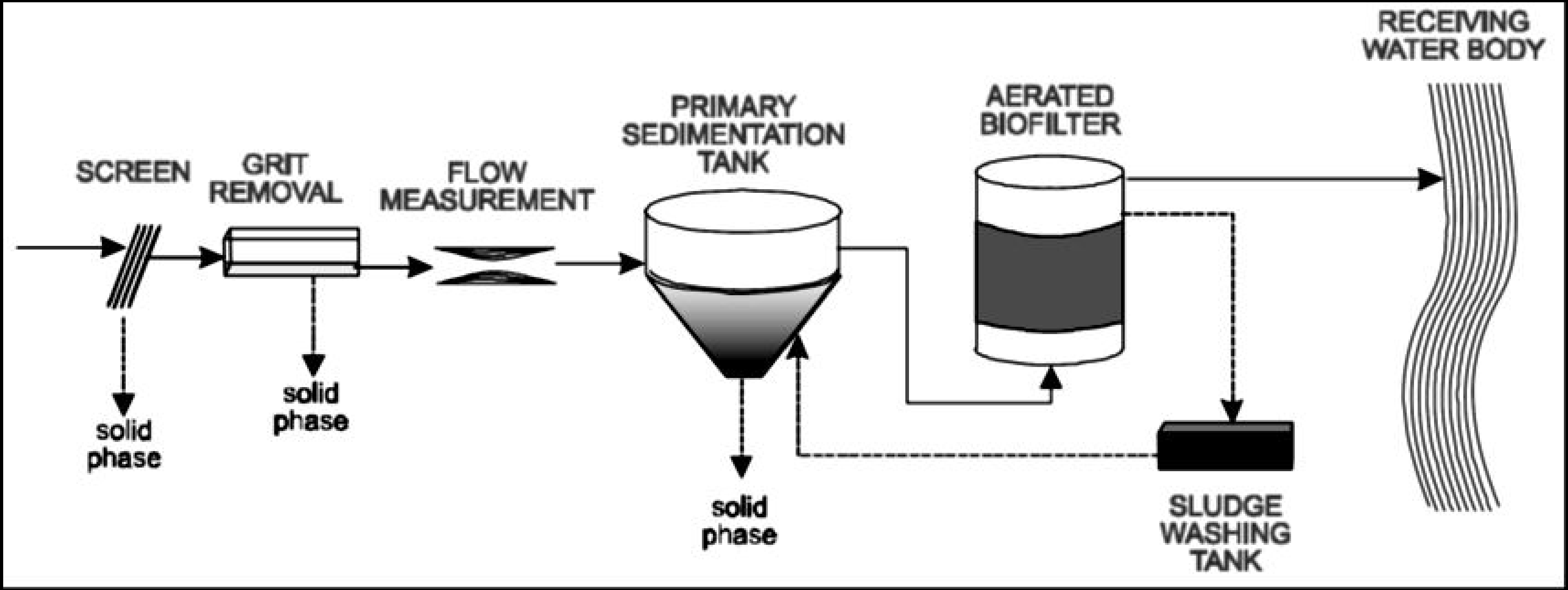
Submerged aerated biofilter:

Besides being a support medium for biomass growth, the granular material acts also as a filter medium.

Periodic washings are necessary to eliminate the excess biomass accumulated.

Aerobic Biofilm Reactors

Submerged aerated biofilter



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Aerobic Biofilm Reactors

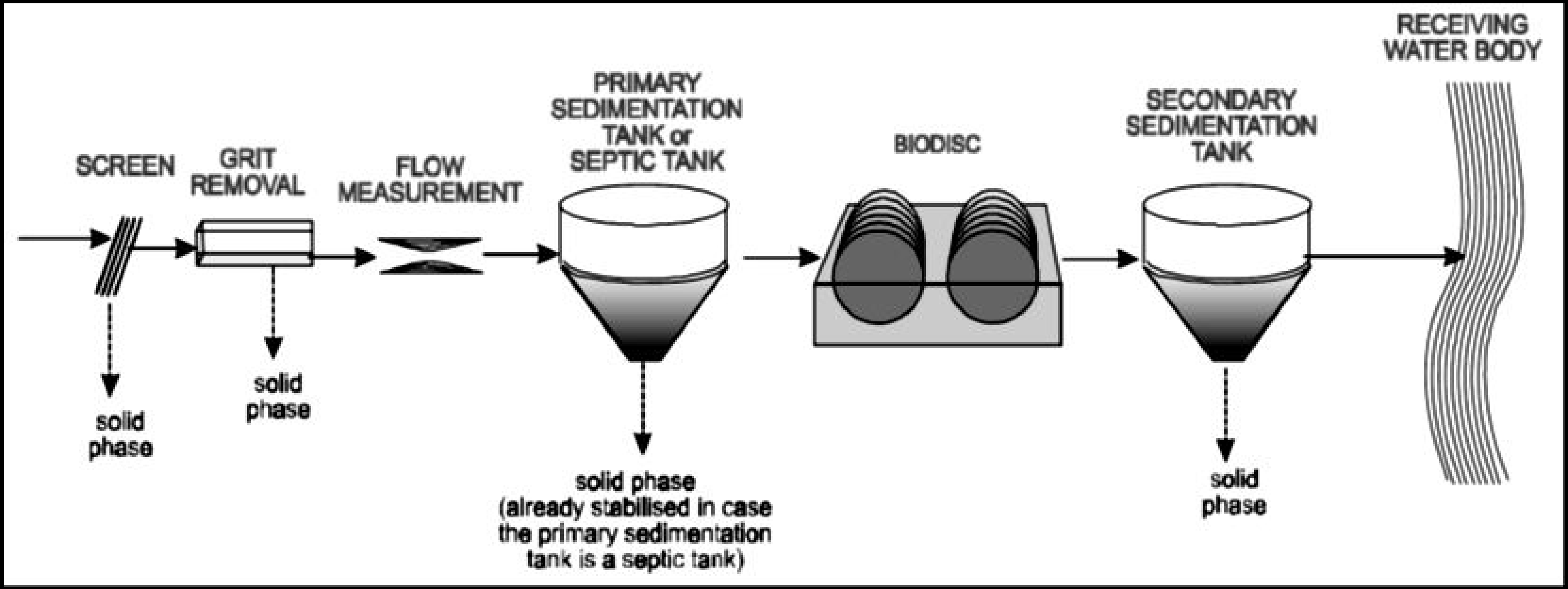
Rotating biological contactor (biodisc):

The biomass grows adhered to a support medium, which is usually composed by a series of discs.

The discs, partially immersed in the liquid, rotate, exposing their surface alternately to liquid and air.

Aerobic Biofilm Reactors

Rotating biological contactor (biodisc):



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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

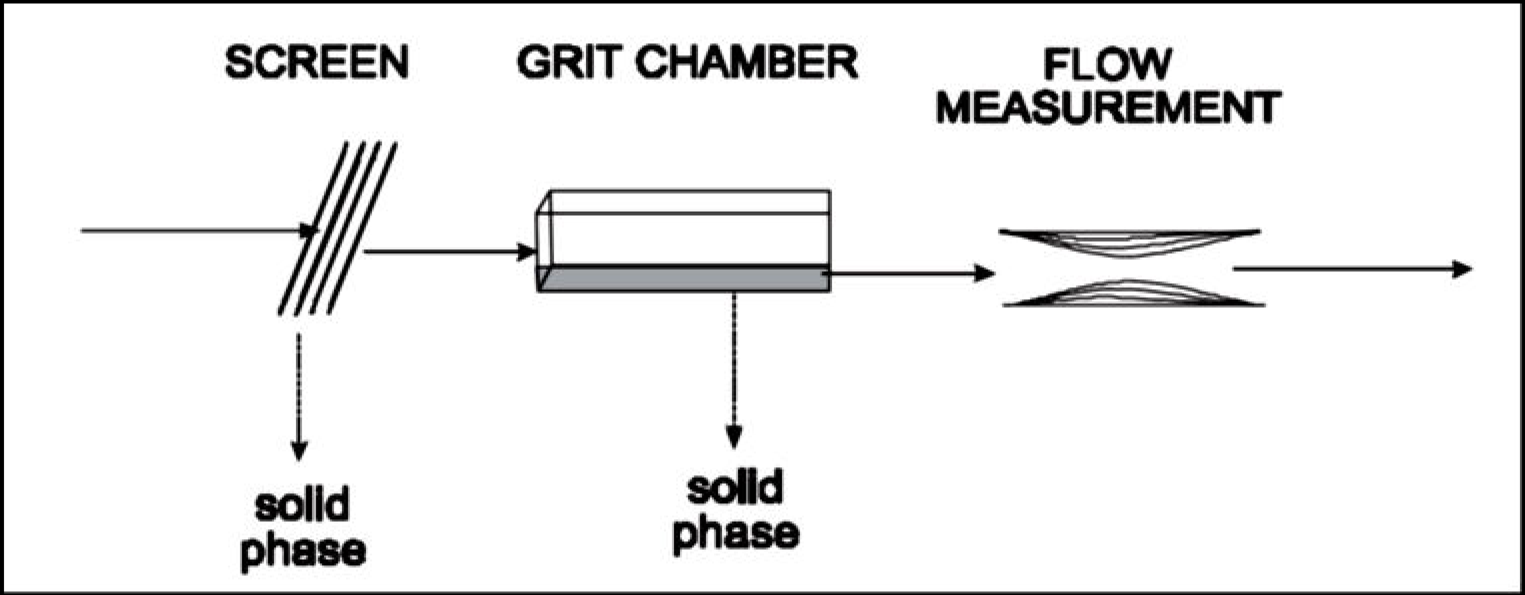
The removal of coarse solids is frequently done by screens or racks.

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



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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

END

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.

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

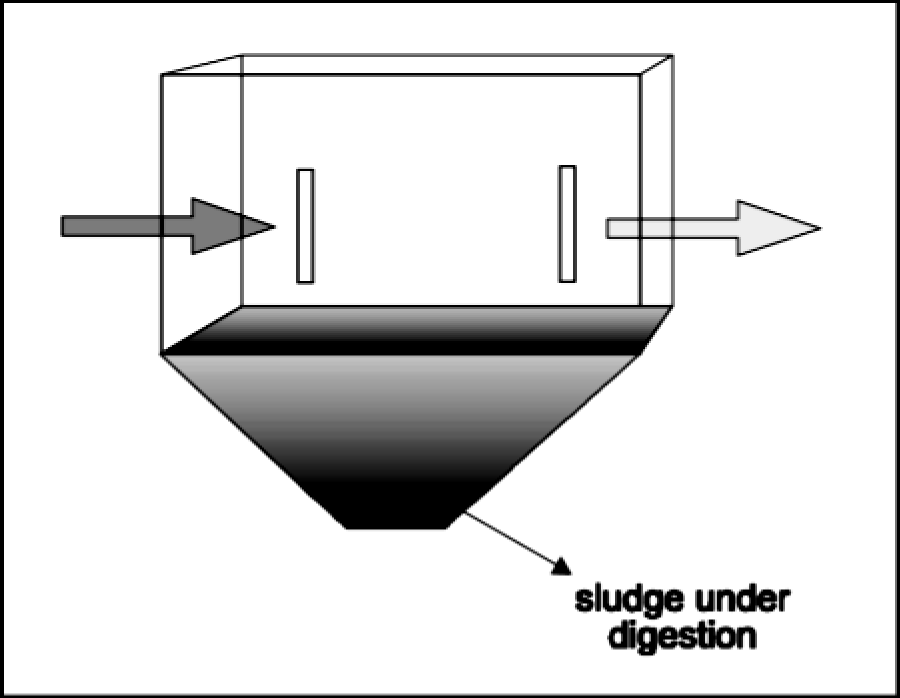
Septic tanks are also a form of primary treatment.

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



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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.

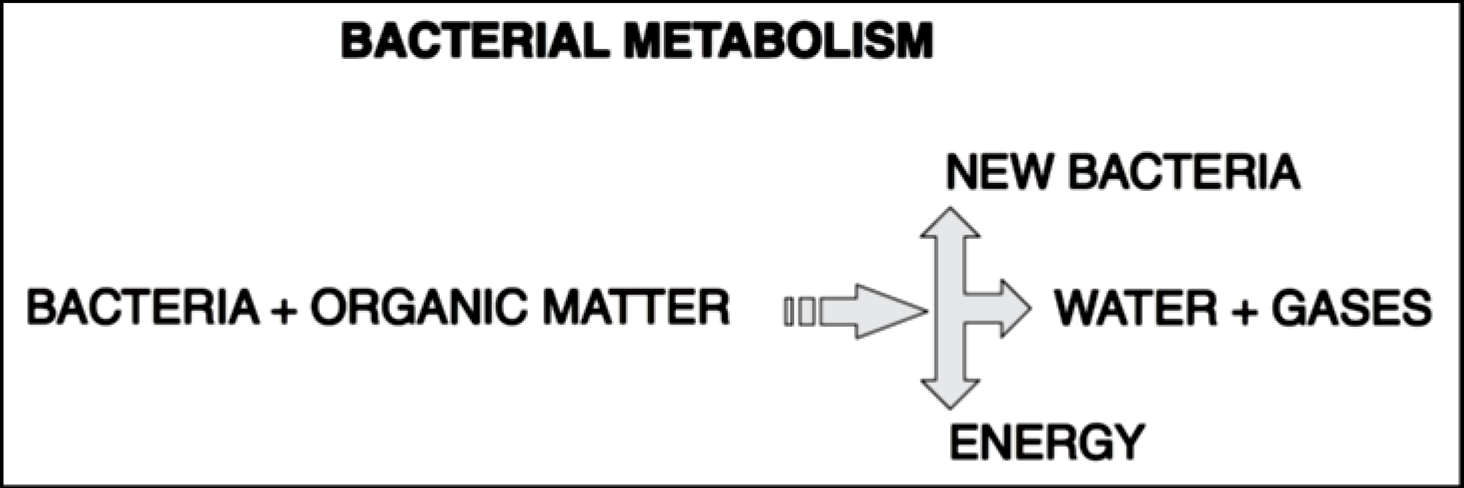
Secondary Treatment

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



BACTERIAL METABOLISM

BACTERIA + ORGANIC MATTER

NEW BACTERIA

WATER + GASES

ENERGY

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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.

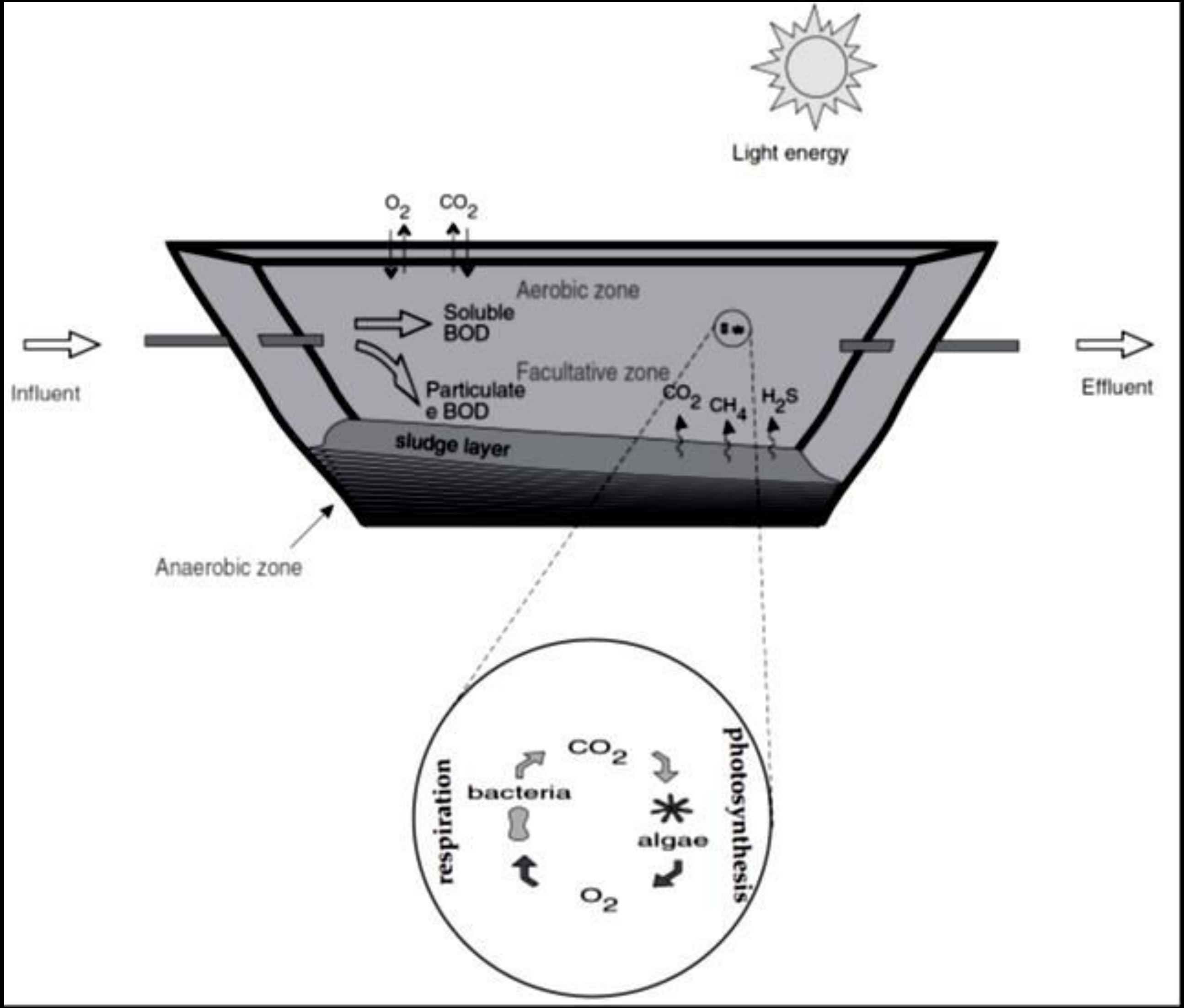
Hence the designation of facultative, which also defines the name of the pond.

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



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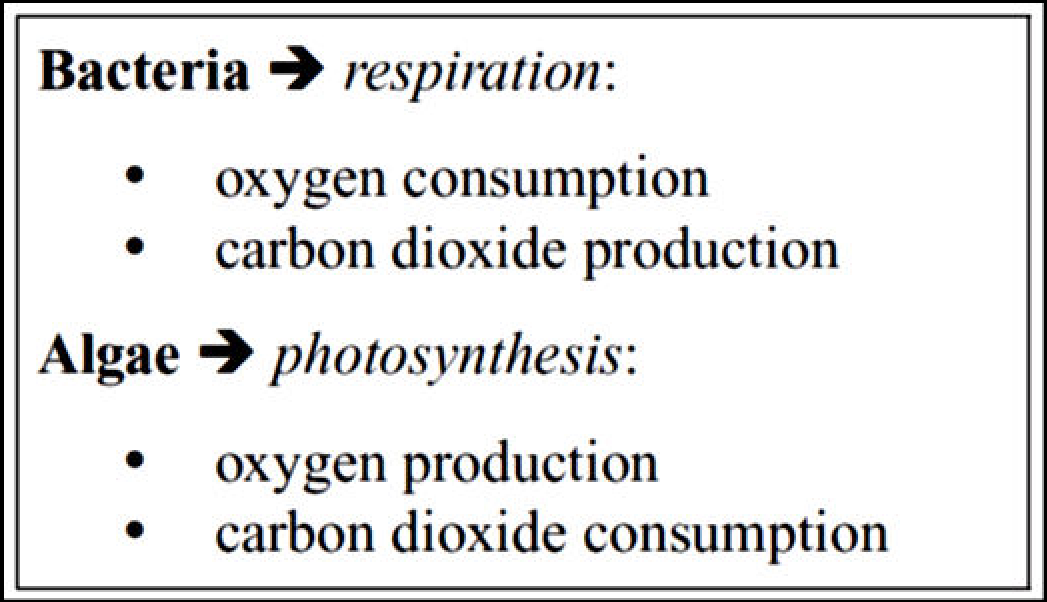
Stabilisation ponds: Facultative Ponds



Bacteria ➔ *respiration*:

- oxygen consumption
- carbon dioxide production

Algae ➔ *photosynthesis*:

- oxygen production
- carbon dioxide consumption

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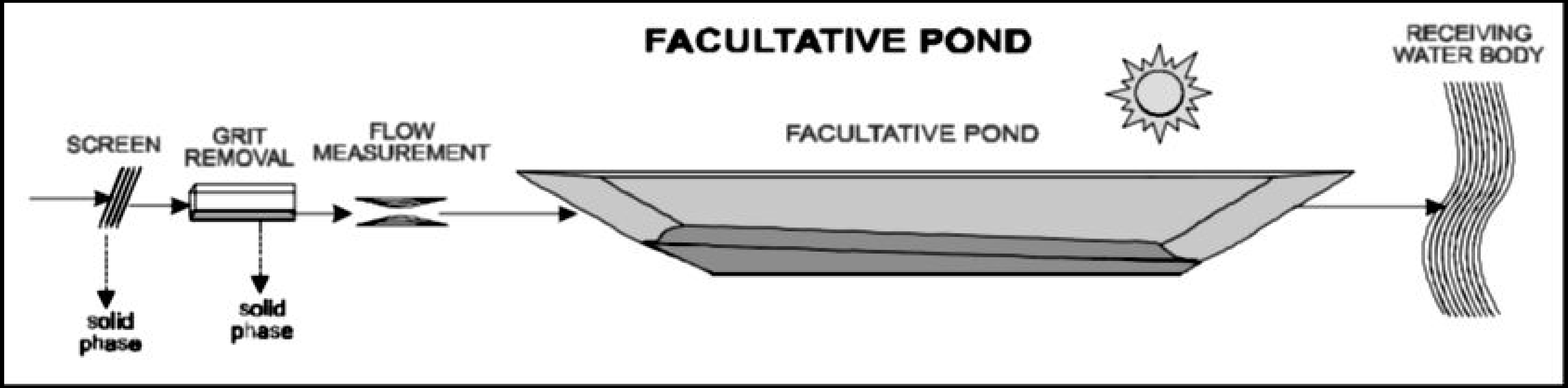
Stabilisation ponds: Facultative Ponds



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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

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.

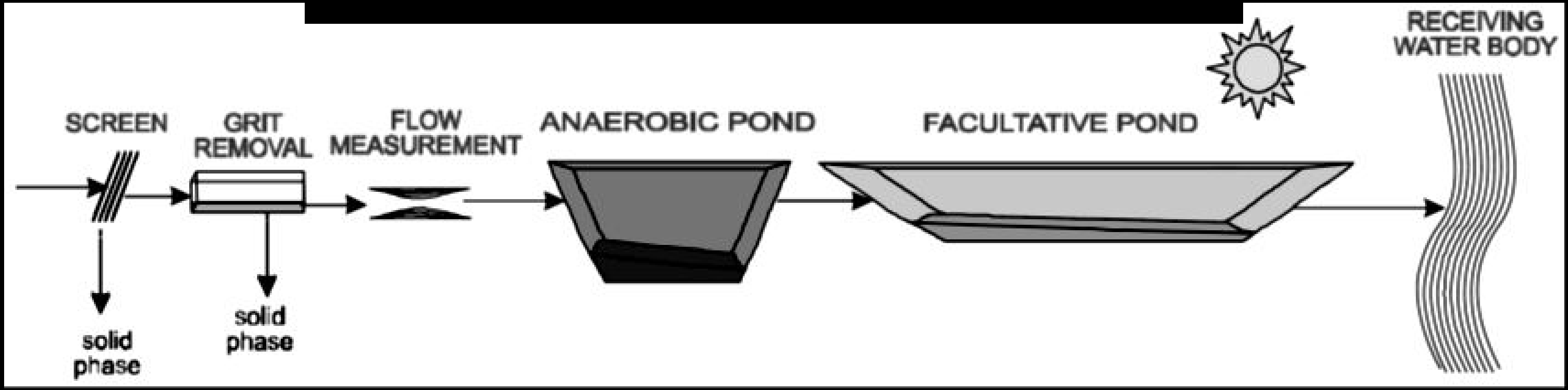
Anaerobic pond – facultative ponds systems

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



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Anaerobic pond – facultative ponds systems

END

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,

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.

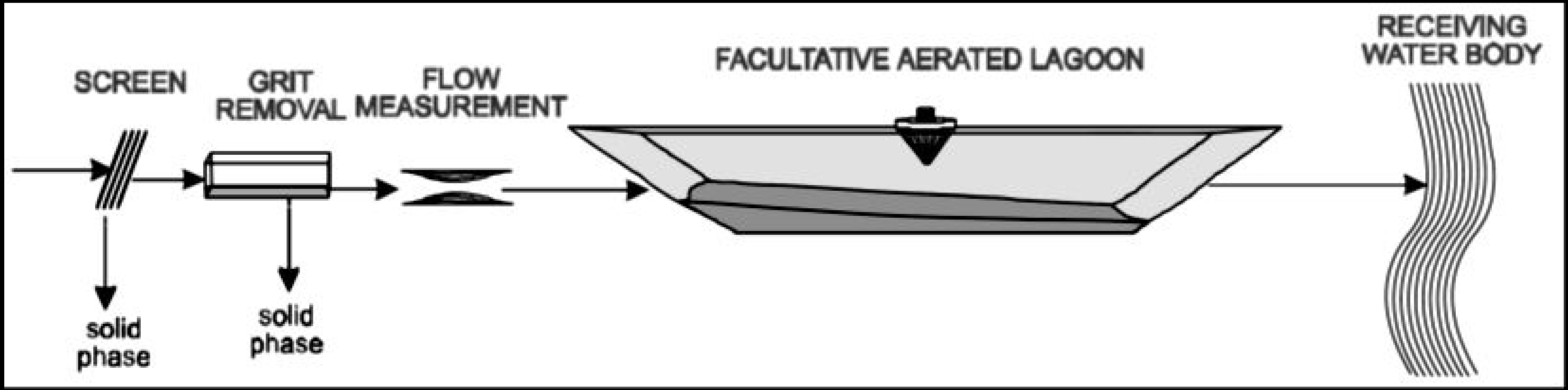
Facultative aerated lagoon

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



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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

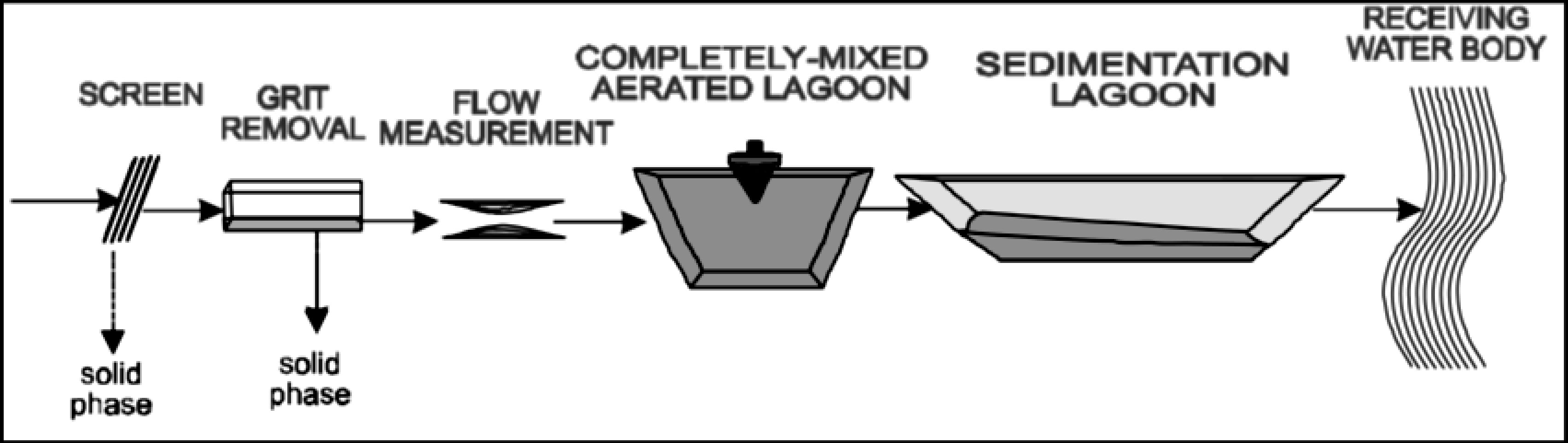
Sedimentation pond have short detention times, around 2 days.

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



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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

END

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.

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...

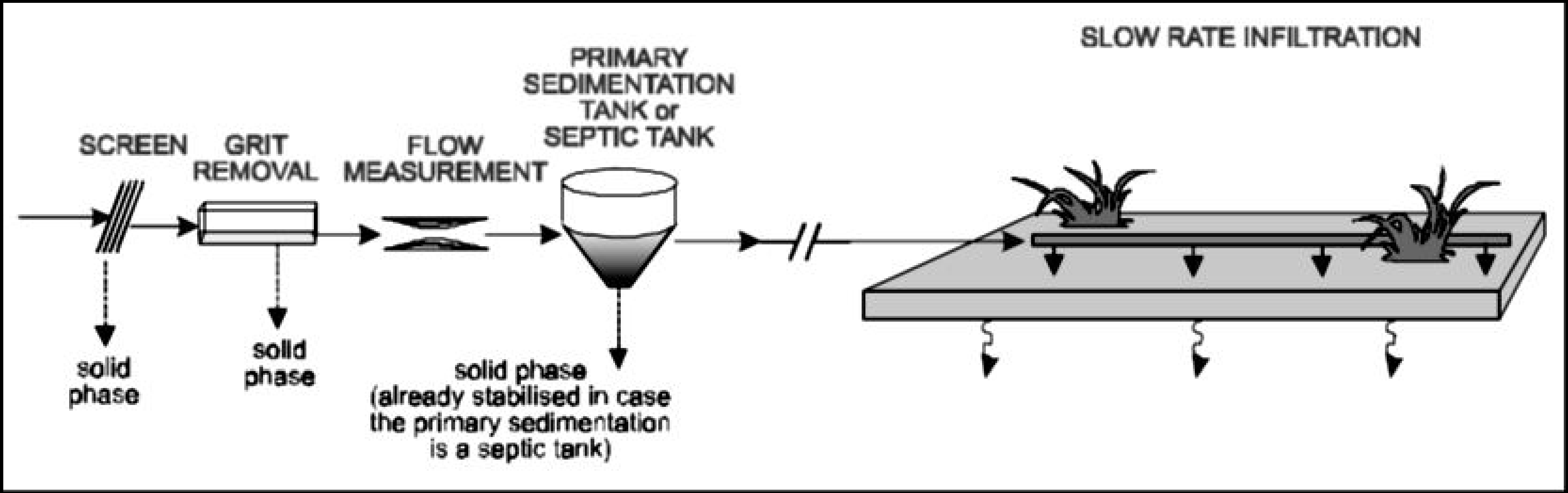
The systems are designed to apply sufficient wastewater to meet crop irrigation requirements.

Loading rates are based on the crop irrigation requirement.

Nitrogen loading must be checked to avoid excess nitrogen.

Slow-rate systems

Slow rate system



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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.

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

END

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

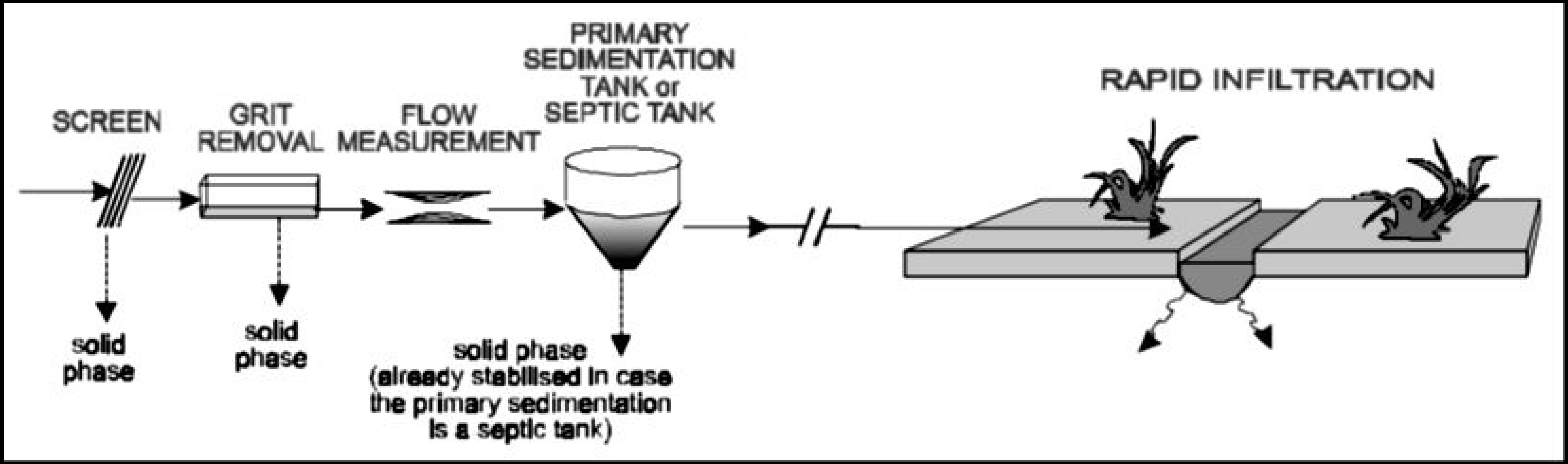
Wastewater is applied in shallow infiltration basins, from which wastewater percolates through the soil.

The application is intermittent.

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

Rapid infiltration

Rapid infiltration



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Rapid infiltration

END

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

Vegetation growth may or may not occur.

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.

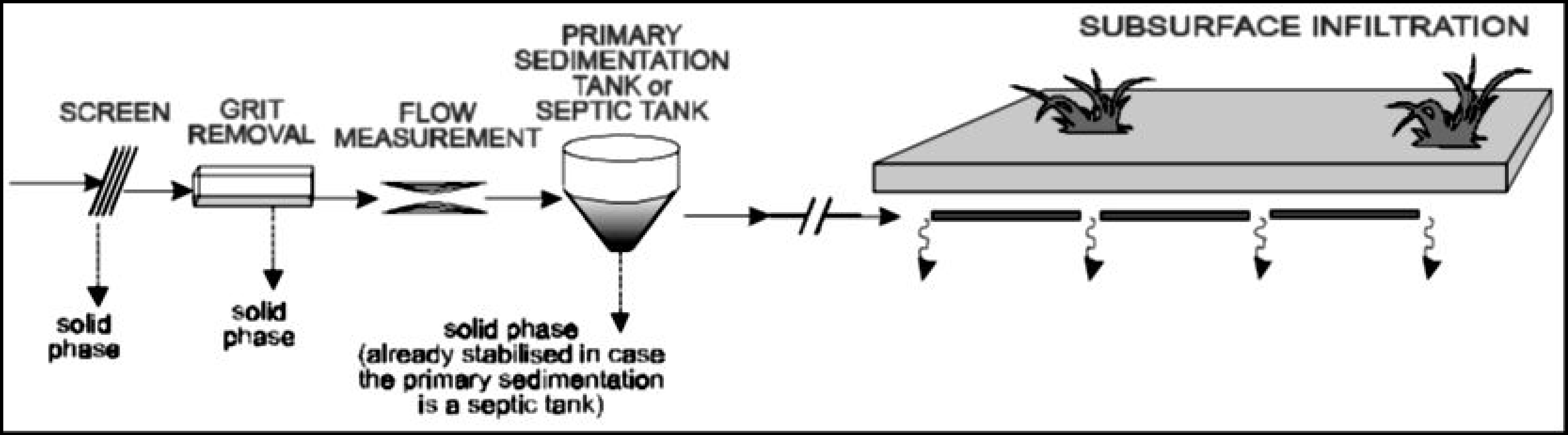
Subsurface infiltration

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



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Subsurface infiltration

END

These systems are normally used following septic tanks, etc.

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.

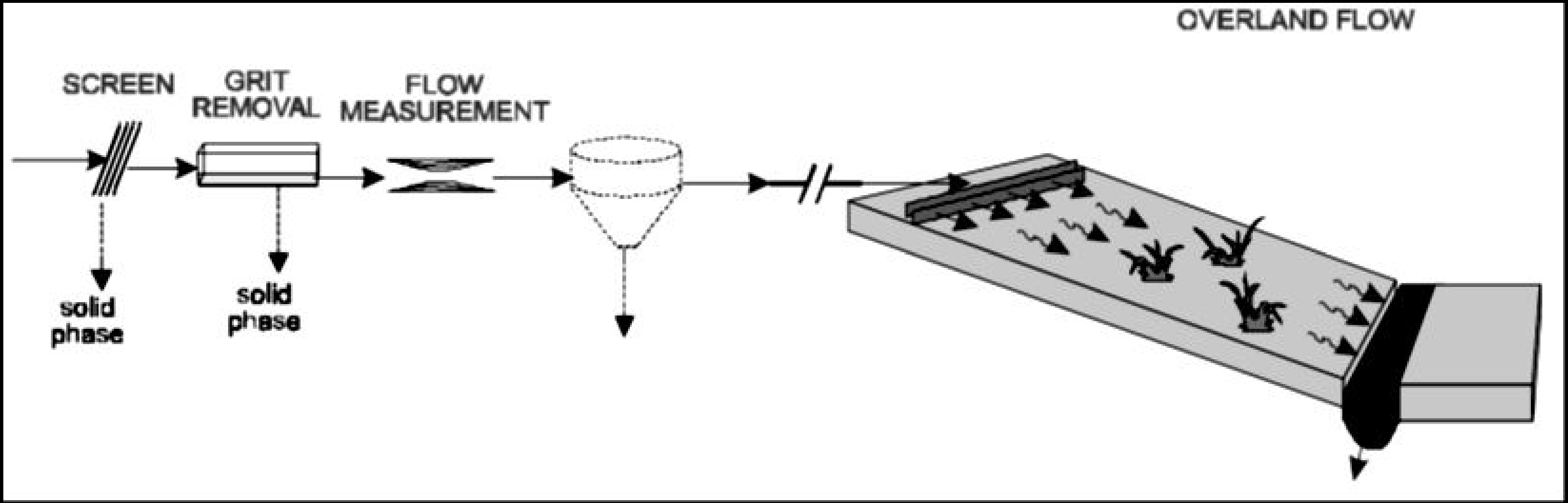
Wastewater flows gently downwards, having contact with the root-soil system, in which biochemical reactions take place.

Overland flow

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

Application is intermittent.

Overland flow



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Overland flow

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

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

Overland flow

END

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.

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.

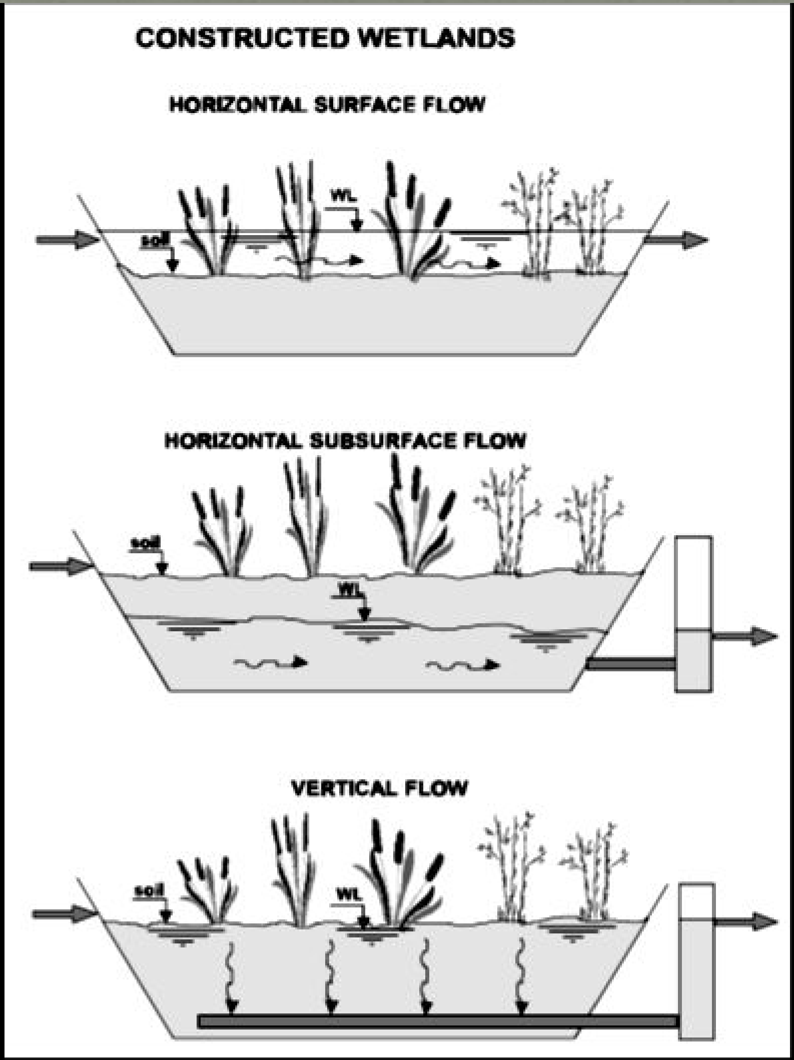
Constructed wetlands (Horizontal flow)

Horizontal flow:

The most classical conception of constructed wetlands.

May be with surface or subsurface flow.

Constructed wetlands



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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

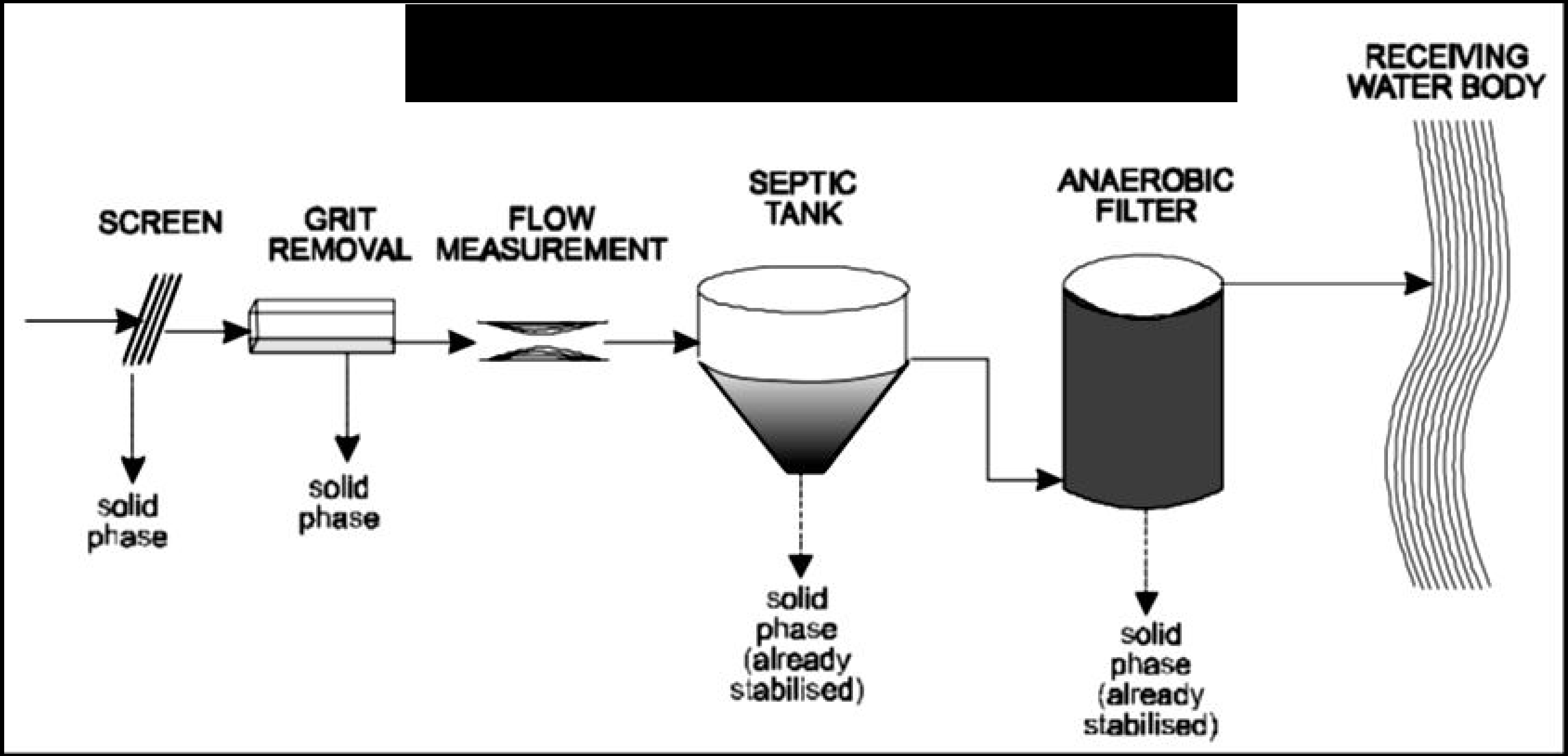
As septic tanks are sedimentation tanks (no biochemical reactions in the liquid phase), BOD removal is limited.

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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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…

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

END

Environmental Biotechnology

Secondary Treatment: Anaerobic reactors; Upflow anaerobic sludge blanket (UASB) reactors (I)

Upflow anaerobic sludge blanket (UASB) reactors

In UASB reactors, the biomass grows dispersed in the liquid (not attached to a support medium).

When biomass grows it can form small granules, which serve as a support medium for other organisms.

Concentration of biomass in the reactor is very high, justifying the name, and thus the volume required is greatly reduced...

Upflow anaerobic sludge blanket (UASB) reactors

Liquid enters at the bottom, where it meets the sludge blanket, leading to the adsorption of the organic matter by the biomass.

Due to anaerobic activity, gases are formed….

Upward flow.

Upflow anaerobic sludge blanket (UASB) reactors

The upper part of the anaerobic sludge blanket reactor presents a structure, whose functions are the separation and accumulation of the gas and the separation and return of the solids (biomass).

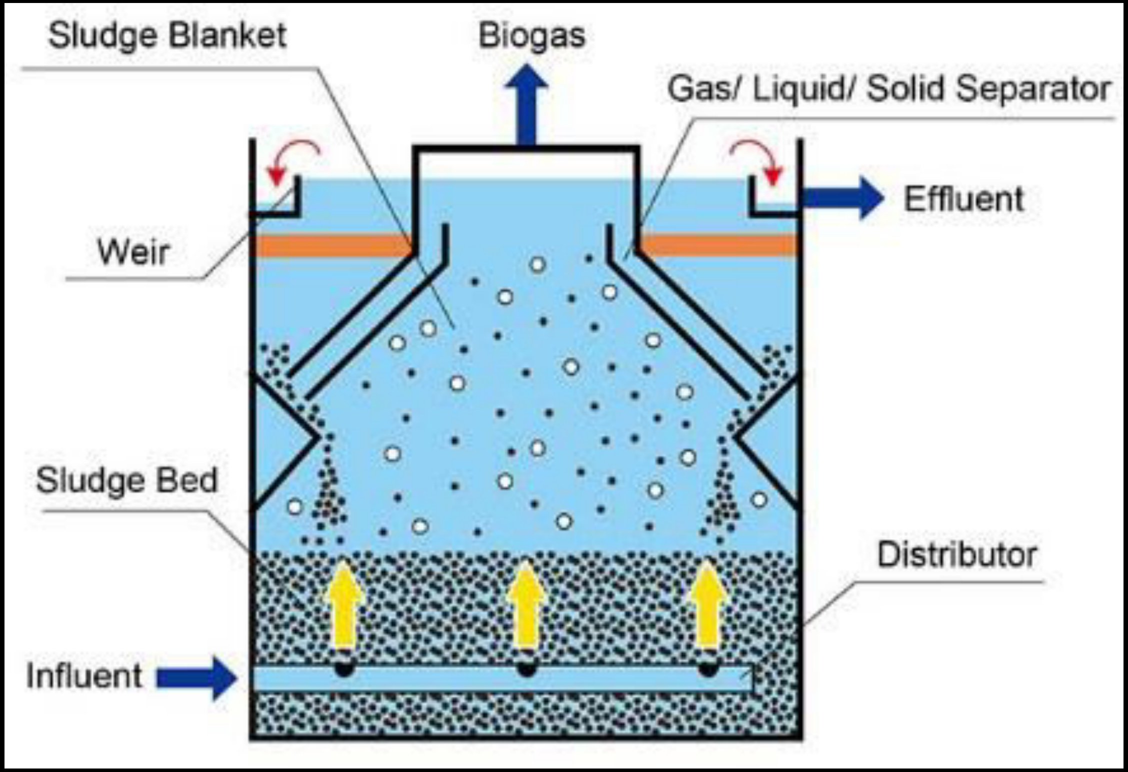
Upflow anaerobic sludge blanket (UASB) reactors

In this way, the biomass is kept in the system (leading to high concentrations in the reactor), and only a minor fraction leaves with the effluent.

This structure is called a three-phase separator, as it separates the liquid, solids, and gases.

The form of the separator is frequently that of an inverted cone or pyramid.

Upflow anaerobic sludge blanket (UASB) reactors



http://www.itriwater.org.tw/Eng/technology/More?id=96

Upflow anaerobic sludge blanket (UASB) reactors

The gas is collected in the upper part of the separator, in the gas compartment, from where it is removed. The solids settle in the upper part of the separator, in the settling compartment, and drain down the steeply inclined walls until they return to the reactor body.
In this way, a large part of the biomass is retained by the system by simple gravitational return…

END

Environmental Biotechnology

Secondary Treatment: Anaerobic reactors; Upflow anaerobic sludge blanket (UASB) reactors (II)

Upflow anaerobic sludge blanket (UASB) reactors

Owing to the high solids retention, the hydraulic detention time can be low (in the order of 6 to 10 h). Because the gas bubbles do not penetrate the settling zone, the separation of the solids-liquid is not impaired.

The effluent is relatively clarified when it leaves the settling compartment, and the concentration of the biomass in the reactor is maintained at a high level.

Upflow anaerobic sludge blanket (UASB) reactors

The sludge production is very low.

The sludge wasted from the reactor is already digested and thickened, and may be simply dewatered.

Upflow anaerobic sludge blanket (UASB) reactors

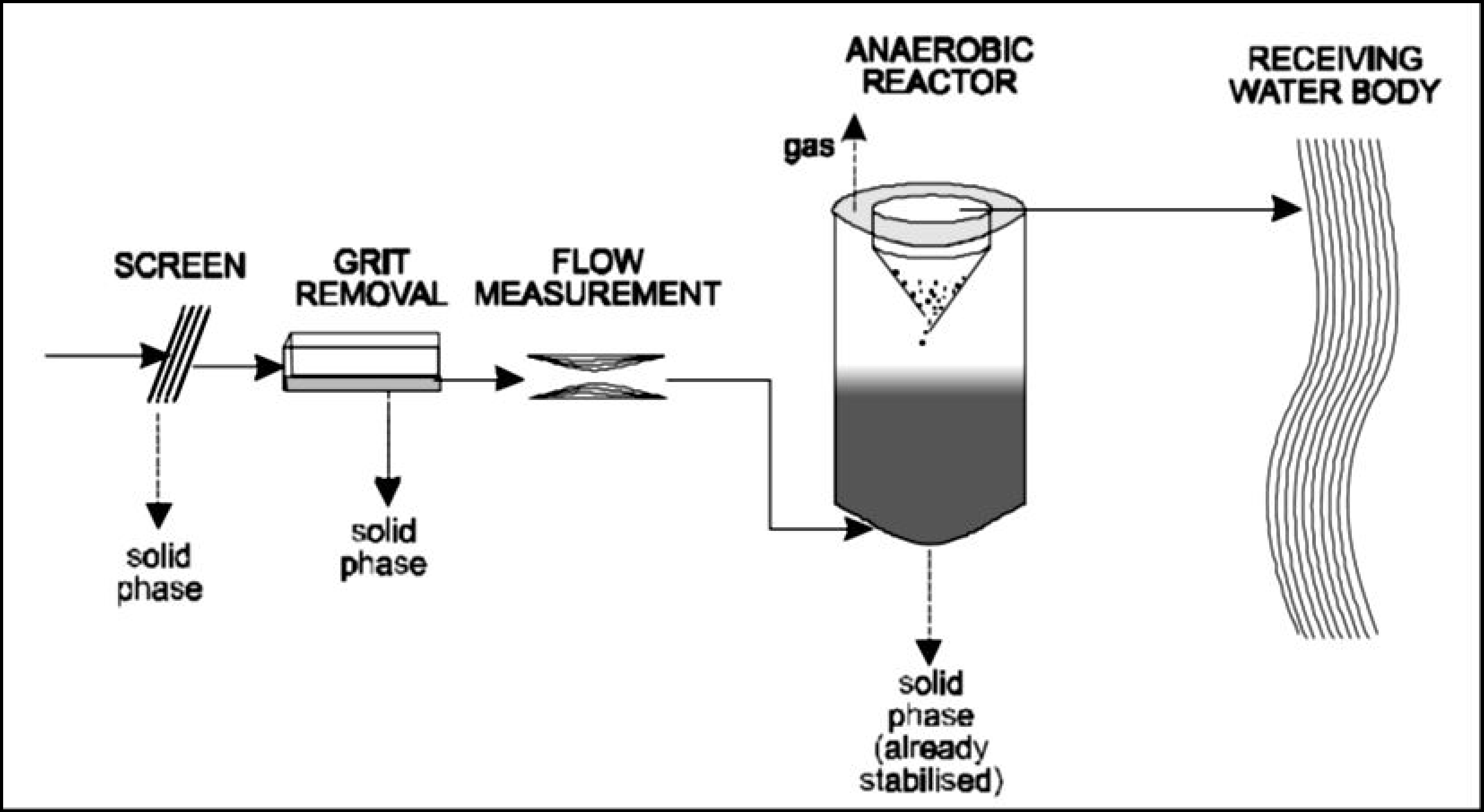
BOD removal efficiency is around 70% (lower than in most of the other systems).

To reach desired efficiency, some form of post-treatment must follow the UASB reactors.

The post-treatment process can be any of the secondary processes (aerobic or anaerobic)…

Upflow anaerobic sludge blanket (UASB) reactors

Upflow anaerobic sludge blanket (UASB) reactors



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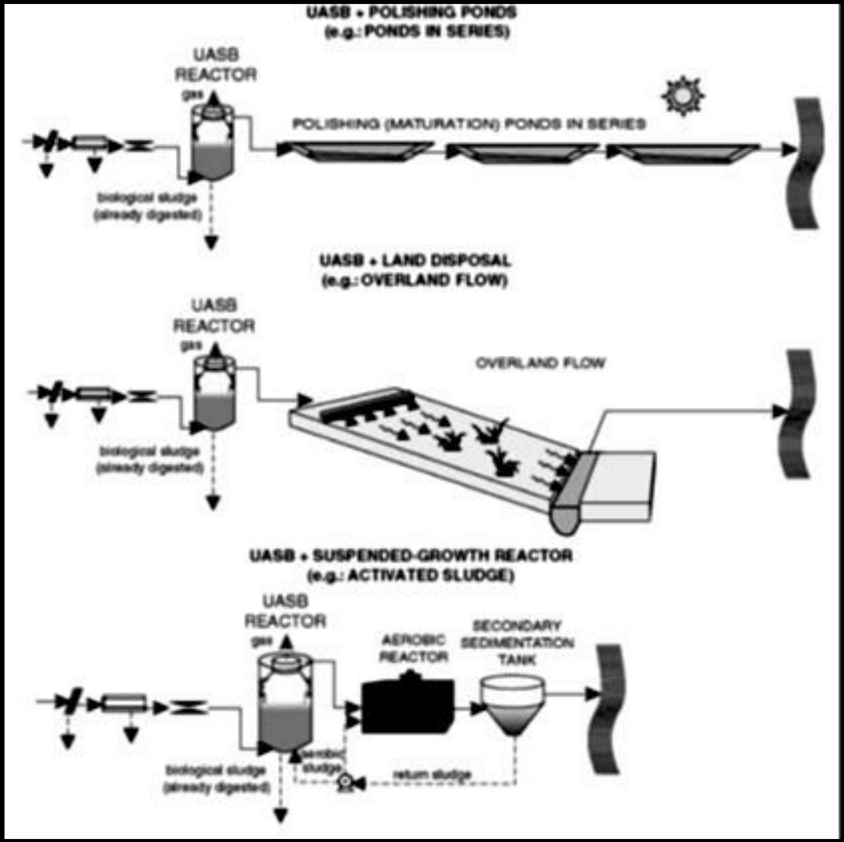
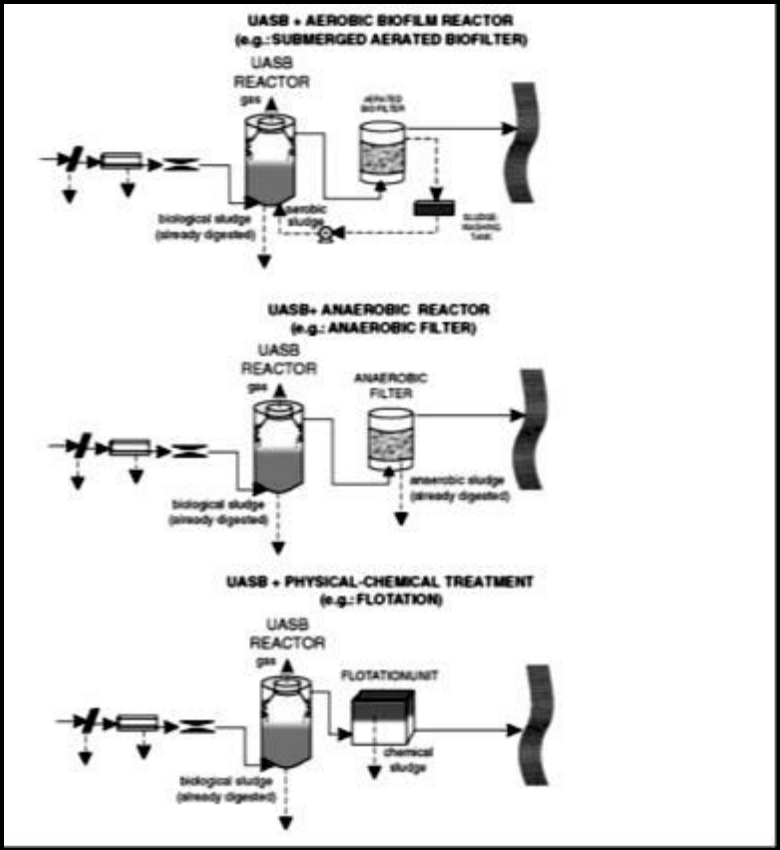
Upflow anaerobic sludge blanket (UASB) reactors

UASB reactor + Post-Treatment





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Environmental Biotechnology

Secondary Treatment: Activated Sludge System (I)

Activated Sludge System

Activated sludge systems may be classified…

- According to the sludge age:
  - *Conventional activated sludge*
  - *Extended aeration*
- According to flow:
  - *Continuous flow*
  - *Intermittent flow*
- Regarding the treatment objectives:
  - *Removal of carbon (BOD)*
  - *Removal of carbon and nutrients (N and/or phosphorus).*

END

Environmental Biotechnology

Secondary Treatment: Activated Sludge System (II)

Activated Sludge System

Main variants of the activated sludge process:

- conventional activated sludge (continuous flow)
- extended aeration (continuous flow)
- sequencing batch reactors (intermittent operation)
- activated sludge with biological nitrogen removal
- activated sludge with biological nitrogen and phosphorus removal

Activated Sludge System

END

All these systems may be used as post-treatment of the effluent from anaerobic (UASB) reactors.

In this case, primary sedimentation tanks (if existing) are substituted by the anaerobic reactor, and the excess sludge from the aerobic stage, if not yet stabilised, is pumped back to the anaerobic reactor.

Environmental Biotechnology

Secondary Treatment
Conventional Activated Sludge (I)

Conventional Activated Sludge

Evident that a reduction of the volume required could be reached by increasing the biomass concentration in suspension in the liquid.

The more bacteria there are in suspension, the greater the food consumption is going to be.

Conventional Activated Sludge

In previous systems, a storage of bacteria is still active in the settling unit.

If these could be returned to the aeration unit, the concentration of the bacteria in this unit will be increased.

This is the basic principle of the activated sludge system.

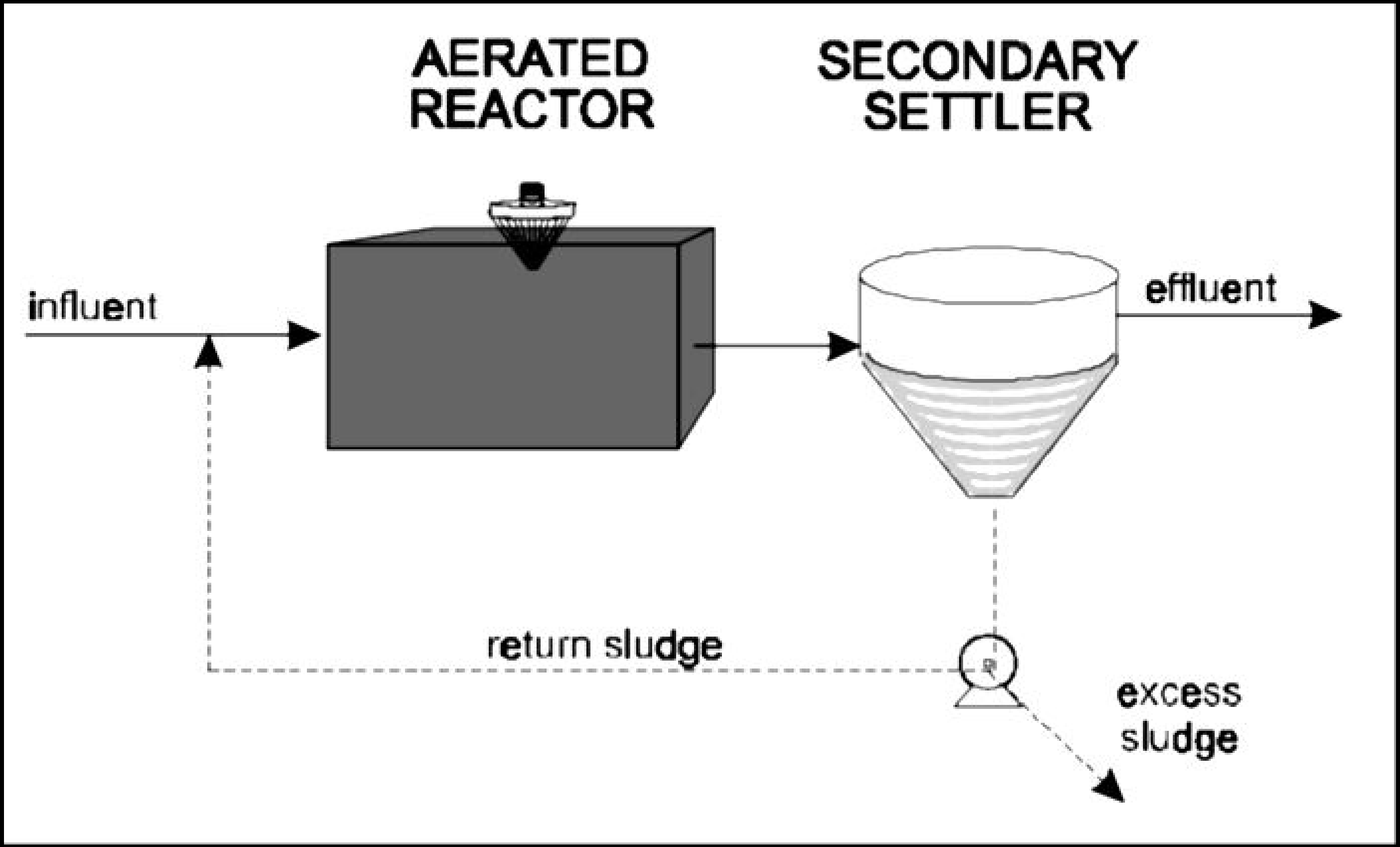
Conventional Activated Sludge

The following items are therefore essential in the activated sludge system :

- aeration tank (reactor)
- settling tank (secondary sedimentation tank, also called final clarifiers)
- pumps for the sludge recirculation
- removal of the biological excess sludge.

Conventional Activated Sludge

Schematics of the units of the biological stage of the activated sludge system



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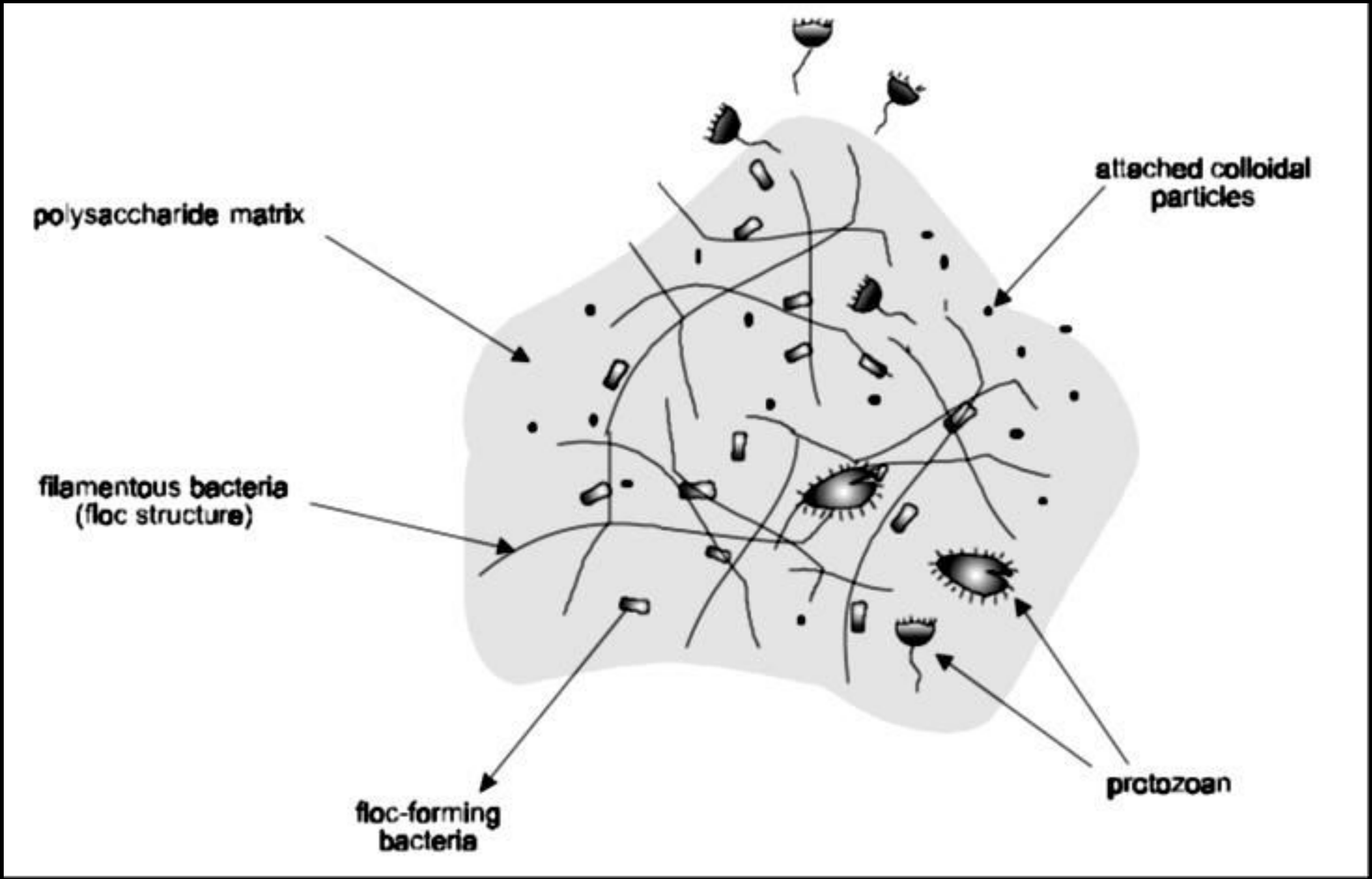
Conventional Activated Sludge

Schematics of bacteria and other microorganisms forming an activated sludge floc



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Conventional Activated Sludge

The biomass can be separated in the secondary sedimentation tank because of its property of flocculating.

This is due to the fact that many bacteria have a gelatinous matrix that permits their agglutination.

The floc has larger dimensions, which facilitates settling.

Conventional Activated Sludge

The concentration of the suspended solids in the aeration tank of the activated sludge system is more than 10 times greater than in a complete-mix aerated pond.

The detention time of the liquid is very low, of the order of 6 to 8 hours in the conventional activated sludge system.

Conventional Activated Sludge

Due to sludge recirculation, the solids (biomass) stay in the system for a longer time.

The retention time of the solids in the system is called sludge age or solids retention time, which is of the order of 4 to 10 days in this system.

The longer retention of the solids guarantees the high efficiency of the activated sludge.

END

Environmental Biotechnology

Secondary Treatment: Conventional Activated Sludge (II)

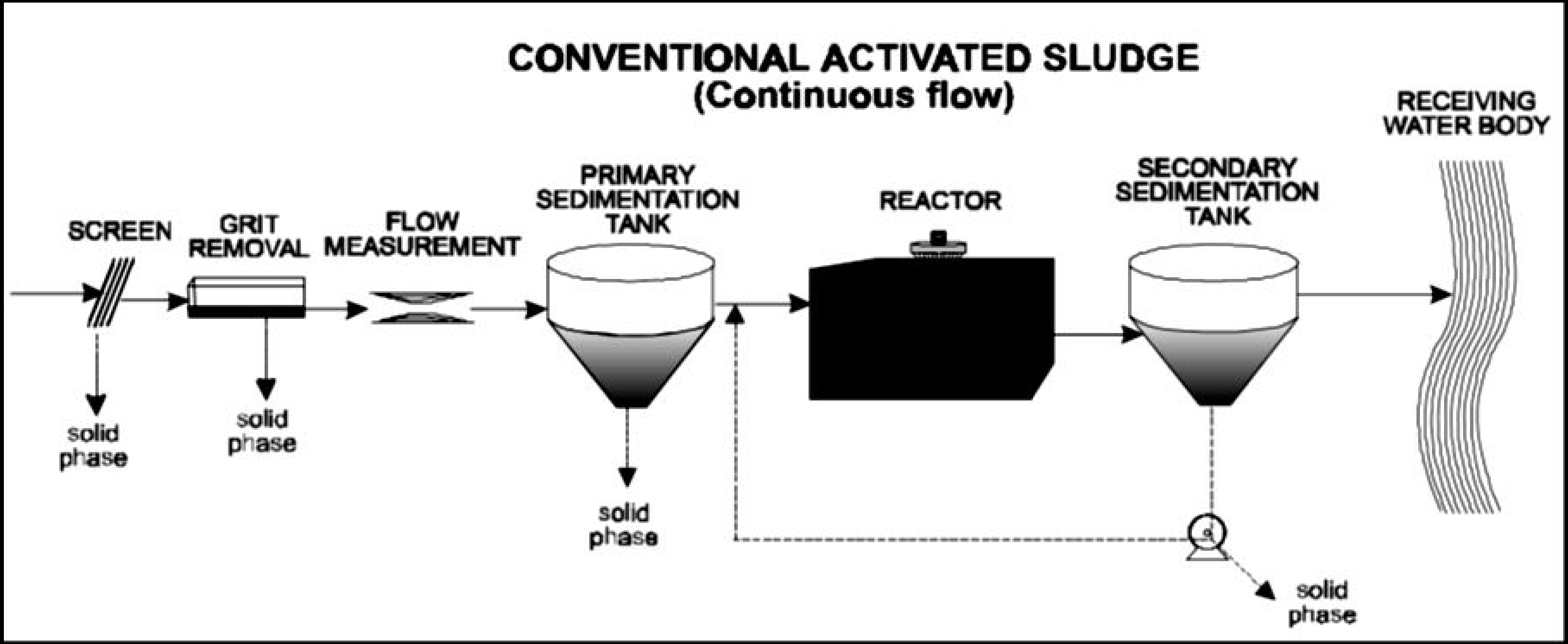
Conventional Activated Sludge

Part of the organic matter (in suspension, settleable) of the sewage is removed before the aeration tank, in the primary sedimentation tank.

Therefore, the conventional activated sludge systems have as an integral part also the primary treatment.

Conventional Activated Sludge

Typical flowsheet of the conventional activated sludge system



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Conventional Activated Sludge

Due to continuous arrival of food in the form of BOD to the aeration tank, bacteria grow and reproduce continuously.

If an indefinite population growth were allowed…

Conventional Activated Sludge

The secondary sedimentation tank would become overloaded, the solids would not settle well and they would start to leave with the final effluent…

Conventional Activated Sludge

To maintain the system in equilibrium, it is necessary to draw approximately the same quantity of biomass that has increased by reproduction.

The biological excess sludge can be wasted directly from the reactor, and must undergo additional treatment.

Conventional Activated Sludge

END

The conventional activated sludge system has low land requirements and good removal efficiencies.

However, the flowsheet of the system is more complex than in most other treatment systems.

Energy costs for aeration are higher than for aerated ponds.

Environmental Biotechnology

Secondary Treatment: Activated Sludge System; Extended Aeration

Activated Sludge System: Extended Aeration

In the conventional activated sludge system, high biomass containing sludge (due to long retention time of 4-10 days) requires a stabilisation stage in the sludge treatment…

Activated Sludge System: Extended Aeration

If the retention time is increased to 18-30 days (thus the name extended aeration), receiving the same BOD load as the conventional system, there is a lower food availability for the bacteria…

Activated Sludge System: Extended Aeration

The bacteria start to use the organic matter from their cellular material.

This cellular organic matter is converted into carbon dioxide and water through respiration.

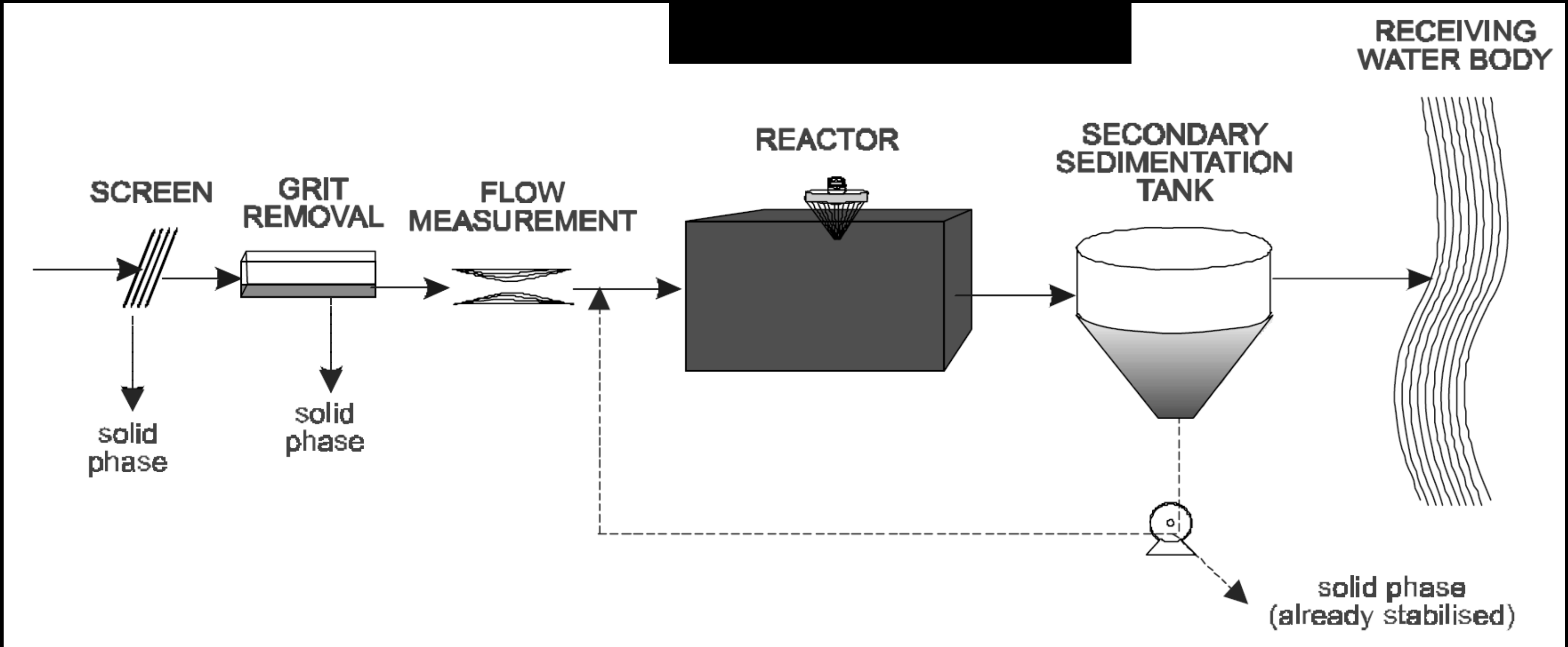
This corresponds to a stabilisation of the biomass, taking place in the aeration tank.

Activated Sludge System: Extended Aeration

Consequently, extended aeration systems do not usually have primary sedimentation tanks and no sludge digestion units.

Activated Sludge System: Extended Aeration

Flowsheet of the extended aeration system



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Activated Sludge System: Extended Aeration

END

The consequence of this simplification in the system is the energy expenditure for aeration, which is due, not only to the removal of the incoming BOD, but also for the aerobic digestion of the sludge in the reactor.

Environmental Biotechnology

Secondary Treatment: Activated Sludge System; Intermittent operation

Activated Sludge System; Intermittent operation

Intermittent flow of the liquid/sewage and operation, also called a sequencing batch reactor (SBR).

Incorporation of all the units, processes and operations normally associated to the conventional activated sludge within a single tank.

Activated Sludge System; Intermittent operation

Using only a single tank, these processes and operations become sequences in time and not separated units…

Activated Sludge System; Intermittent operation

This process can be used in the conventional or in the extended aeration sludge ages.

In the extended aeration mode, the tank also incorporates the role of the sludge digestion (aerobic) unit.

Activated Sludge System; Intermittent operation

The process consists of a complete-mix reactor where all the treatment stages occur.

This is accomplished by the establishment of operating cycles with defined duration.

Activated Sludge System; Intermittent operation

The biological mass stays in the reactor, eliminating the need for separate sedimentation and sludge pumping.

The retention of biomass occurs because it is not withdrawn with the supernatant.

Activated Sludge System; Intermittent operation

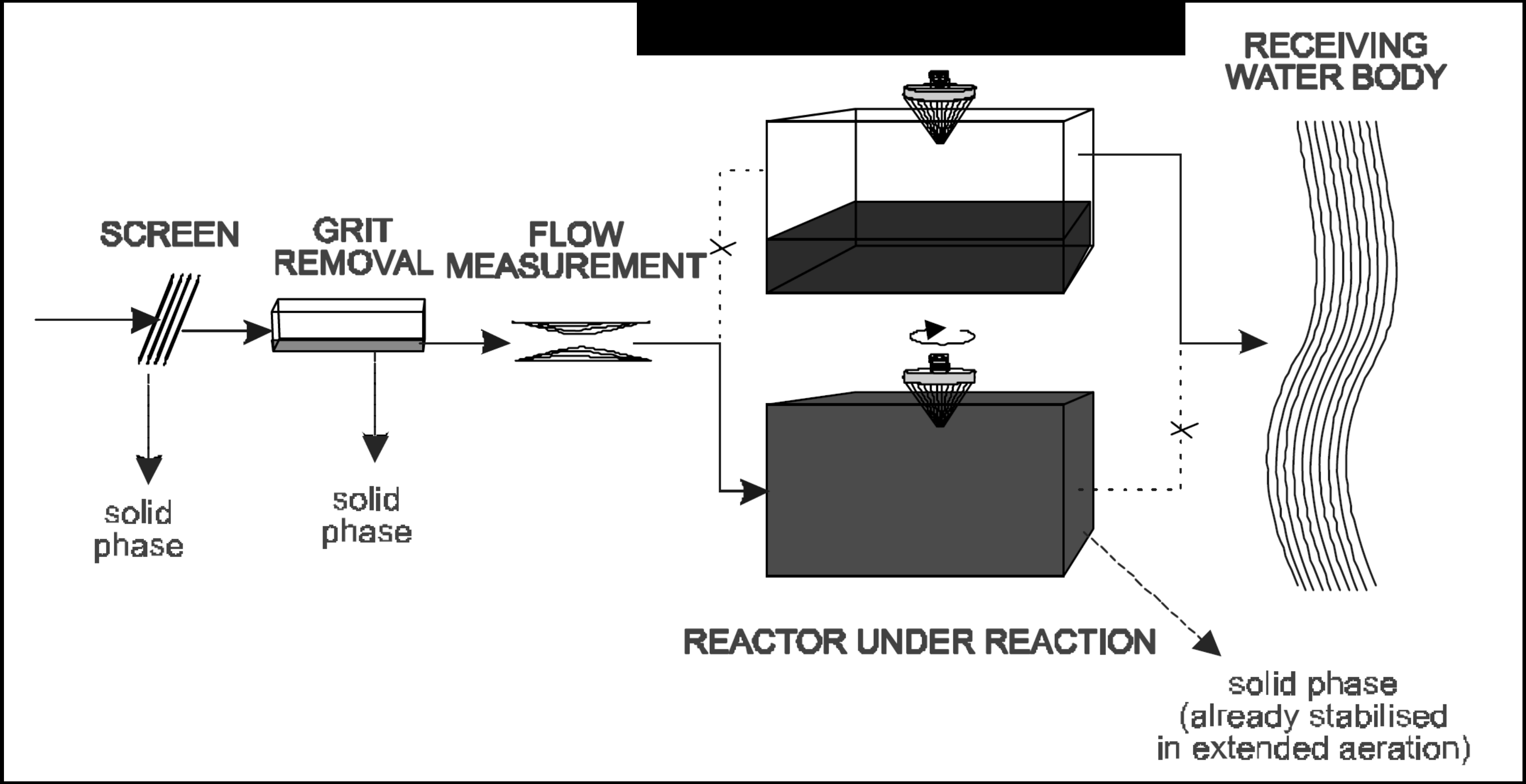
The normal treatment cycle is composed of the following stages:

- Fill
- React
- Settle
- Draw
- Idle…

The flowsheet of the process is greatly simplified...

Activated Sludge System; Intermittent operation

Flowsheet of a sequencing batch reactor system in the extended aeration



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Activated Sludge System; Intermittent operation

END

There are some variants of the sequencing batch reactor systems related to its operation (continuous feeding and discontinuous supernatant withdrawal) as well as in the sequence and duration of the stages within each cycle.

Environmental Biotechnology

Secondary Treatment: Activated sludge with biological nitrogen removal

Activated sludge with biological nitrogen removal

The activated sludge system is capable of producing a satisfactory conversion of ammonia to nitrate (nitrification).

In this case, only ammonia and not nitrogen is removed.

Activated sludge with biological nitrogen removal

Biological nitrogen removal is achieved in the absence of dissolved oxygen, but presence of nitrates.

A group of bacteria uses the nitrates in their respiration process, converting them to nitrogen gas…

A process called denitrification.

Activated sludge with biological nitrogen removal

To achieve denitrification in the activated sludge, process modifications are necessary, including the creation of anoxic zones and possible internal recycles.

Activated sludge with biological nitrogen removal

In activated sludge systems where nitrification occurs, it is interesting to induce denitrification due to following reasons: *Savings in oxygen* (energy economy in the aeration), as under anoxic conditions, facultative anaerobic bacteria remove BOD by using the nitrate in their respiratory processes.

Activated sludge with biological nitrogen removal

Savings in alkalinity (preservation of the buffering capacity).

During nitrification, H^+ ions are generated which can lead to a decrease in the pH.

Conversely, denitrification consumes H^+ and generates alkalinity, partially compensating the pH reduction.

Activated sludge with biological nitrogen removal

Operation of the secondary sedimentation tank (to avoid rising sludge).

If denitrification occurs in the secondary sedimentation tanks, there will be production of nitrogen gas bubbles, which can tend to adhere to the settling flocs, dragging them to the surface and causing a loss of biomass and deterioration in the final effluent quality.

Activated sludge with biological nitrogen removal

END

Nutrient control (eutrophication). The reduction of the nitrogen levels is important when the effluent is discharged into sensitive water bodies that are subjected to eutrophication.

Environmental Biotechnology

Secondary Treatment: Activated sludge with biological nitrogen and phosphorus removal

Activated sludge with biological N and P removal

It is essential to have anaerobic and aerobic zones in the treatment line for the biological removal of phosphorus.

The anaerobic zone gives good conditions for the development or selection of a large population of phosphorus accumulating organisms.

Activated sludge with biological N and P removal

When the biological excess sludge is wasted from the system, phosphorus is removed…

Activated sludge with biological N and P removal

END

For higher efficiencies of phosphorus removal, effluent polishing methods can adopted:

- *addition of coagulants* (metallic ions): phosphorus precipitation
- *effluent filtration*: removal of the phosphorus present in the suspended solids in the effluent
- *combination of the addition of coagulants and filtration*

Environmental Biotechnology

Secondary Treatment: Aerobic biofilm reactors: Low rate trickling filter

Low Rate Trickling Filter

Aerobic units are biofilm reactors, in which the biomass grows attached to a support medium.

Many variants:

- Low rate trickling filter
- High rate trickling filters
- Submerged aerated biofilters
- Rotating biological contactors

Low Rate Trickling Filter

These systems may be used as post-treatment of the effluent from anaerobic reactors.

In this case, primary sedimentation tanks are substituted by the anaerobic reactor, and the excess sludge from the aerobic stage, if not yet stabilised, is pumped back to the anaerobic reactor.

Low Rate Trickling Filter

Low rate trickling filter:

Consists of a coarse material bed, such as stones, gravel, etc, over which the wastewater is applied.

After application, the wastewater percolates in the direction of the drains at the bottom.

Low Rate Trickling Filter

This percolation allows the bacterial biofilm formation on the surface of the support medium.

With the passage of the wastewater, there is a contact between the microorganisms and the organic matter…

Air circulates between the empty spaces between the stones…

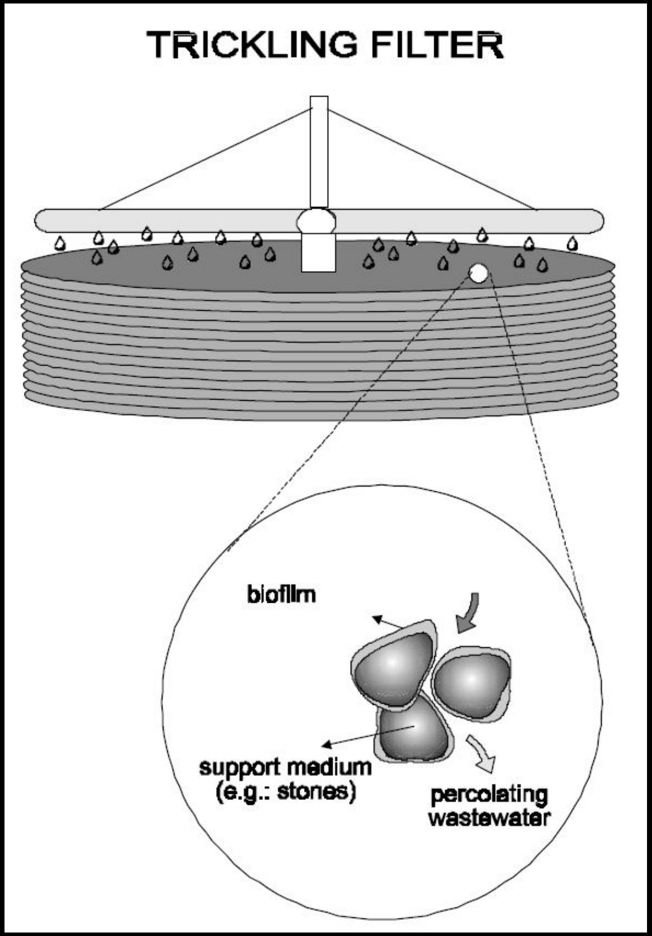
Low Rate Trickling Filter

Wastewater is usually applied through rotating distributor, moved by the hydraulic head of the wastewater.

The liquid percolates rapidly but organic matter so adsorbed by the microbial film, and is eventually stabilized.

Low Rate Trickling Filter

Schematics of a trickling filter



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Low Rate Trickling Filter

Contrary to what the name suggests, the primary function of the filter is not to filter.

The diameters of the stones are of the order of a few centimeters, which allows a large void space...

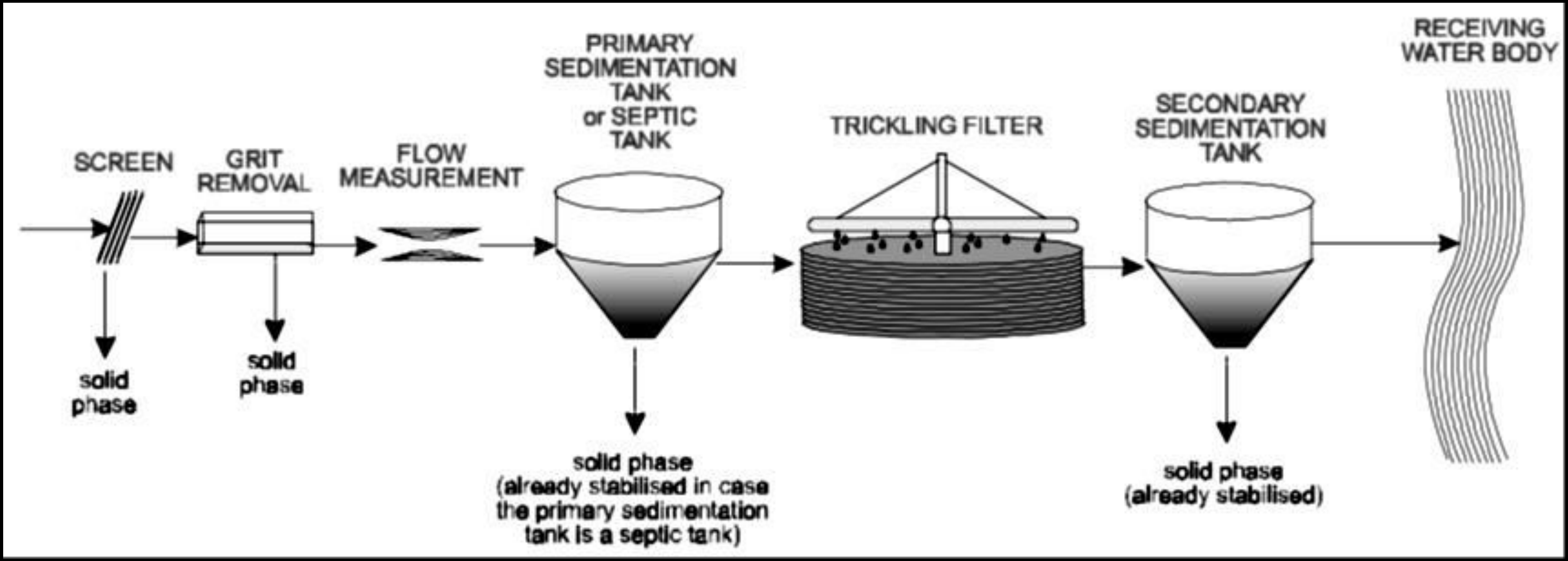
The function is only to supply support for the formation of the microbial film.

Low Rate Trickling Filter

With increase in biofilm mass, the empty spaces decrease, increasing the liquid velocity through the pores.
This causes a shearing stress that dislodges part of the attached biomass.
This is a natural form of controlling the microbial population in the medium.
The dislodged sludge must be removed by the secondary sedimentation tank.

Low Rate Trickling Filter

Typical flowsheet of a low rate trickling filter



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Low Rate Trickling Filter

The applied BOD load per unit area and volume is lower, therefore, food availability is lower. This results in a partial self digestion of the sludge and a higher BOD removal efficiency in the system.

High area requirement.

Low Rate Trickling Filter

END

The efficiency of the system is comparable with the conventional activated sludge system, the operation is simpler, although less flexible.

Environmental Biotechnology

Secondary Treatment: Aerobic biofilm reactors: High rate trickling filter

High Rate Trickling Filter

Conceptually similar to the low rate filters but receive a higher BOD load per unit volume.

Main differences from Low rate filters:
(a) the area requirements are lower;
(b) there is a slight reduction in the organic matter removal efficiency;
(c) Sludge is not digested in the filter.

High Rate Trickling Filter

Another difference is recirculation of the final effluent.

Main objectives of recirculation:

(a) maintaining approximately a uniform flow…;

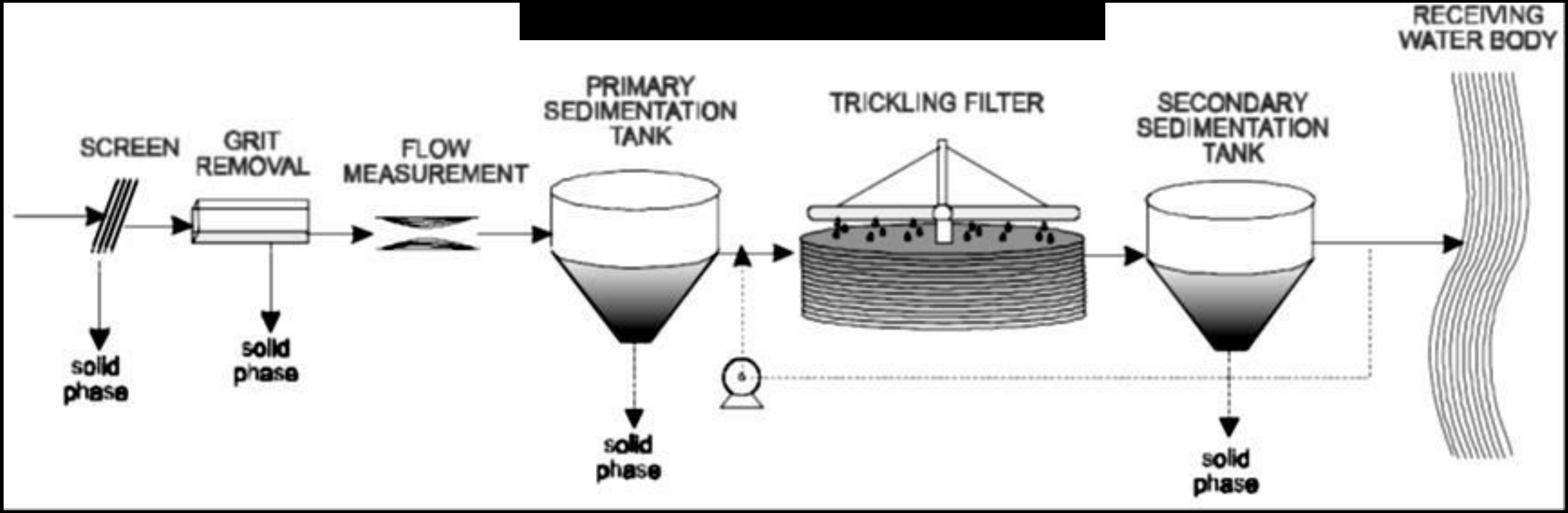
(b) balancing the influent load;

(c) Increasing contact between effluent organic matter and the biomass;

(d) Bringing dissolved oxygen…

High Rate Trickling Filter

Typical flowsheet of a high rate trickling filter



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High Rate Trickling Filter

Another way of improving the efficiency is by using two filters in series.
This is called a two-stage trickling filter system.

Some of the limitations of stone-bed trickling filters when operating with high organic loads refer to clogging of the void spaces…

High Rate Trickling Filter

END

Other materials such as corrugated plastic modules, plastic rings, etc., can also be used.

These offer larger surface areas, significant increase in the empty spaces, are much lighter and hence can be installed much higher e.g., filters with stones are ~3 meters in height, while filters with synthetic media can be more than 6 meters high, substantially reducing the land requirement.

Environmental Biotechnology

Secondary Treatment: Aerobic biofilm reactors: Submerged aerated biofilters

Submerged Aerated Biofilters

It consists of a tank filled with a porous material, through which wastewater and air permanently flow.

The porous medium is maintained under total immersion.

Submerged Aerated Biofilters

This biofilter is a three-phase reactor composed of:

Solid phase: consists of a support medium and biofilms,

Liquid phase: consists of the liquid in permanent flow through the porous medium,

Gas phase: formed by artificial aeration and by the gaseous by-products of the biological activity.

Submerged Aerated Biofilters

The airflow in the submerged aerated biofilter is always upflow, while the liquid flow can be upflow or downflow.

Submerged Aerated Biofilters

Biofilters with granular media remove, in the same reactor, soluble organic compounds and suspended solids. Besides serving as support medium, the granular material also works as an effective filter.

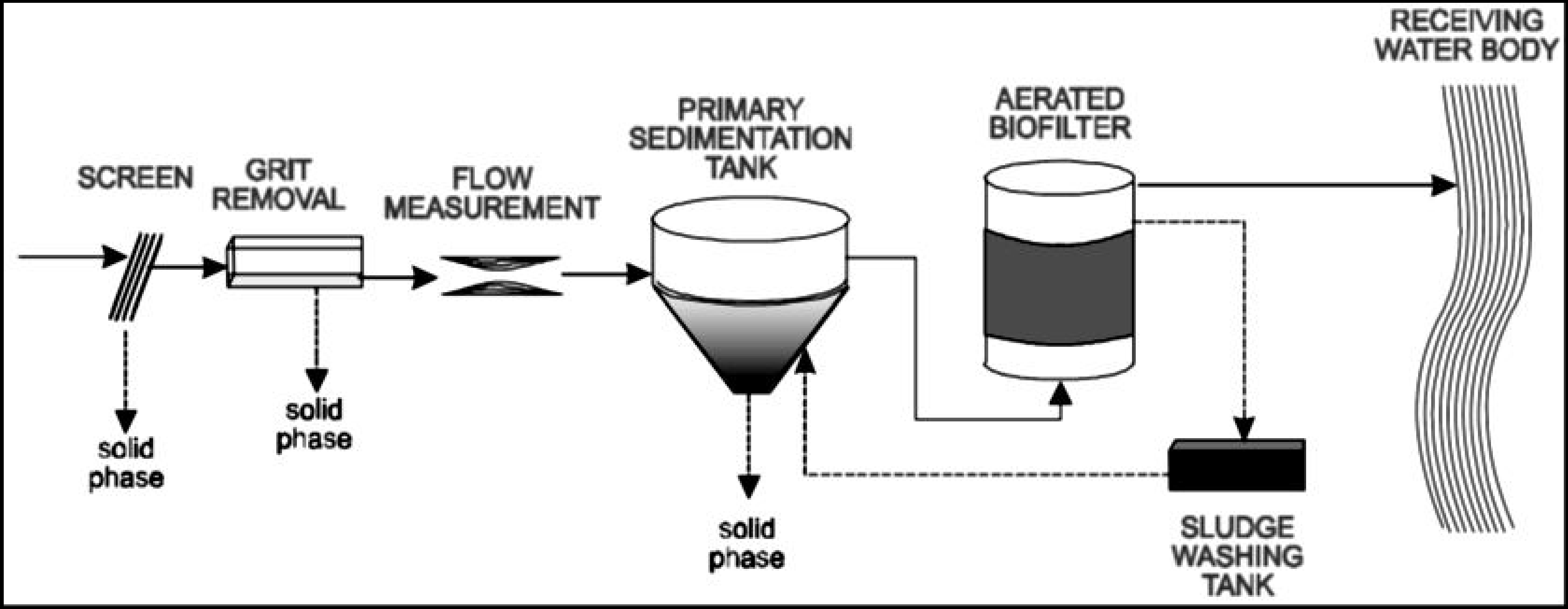
Submerged Aerated Biofilters

In this type of biofilter, periodic washing is necessary to eliminate the accumulated biomass.

There are two sources of sludge generation: the primary sedimentation tanks and the washing of the biofilter.

Submerged Aerated Biofilters

Typical flowsheet of a conventional submerged aerated biofilter system



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Submerged Aerated Biofilters

END

Submerged aerated biofilters achieve good nitrification efficiencies and can be modified for the biological removal of nitrogen, through the incorporation of an anoxic zone in the reactor.

Environmental Biotechnology

Secondary Treatment: Aerobic biofilm reactors: Rotating biological contactors

Rotating Biological Contactors

The most widely used are the biodiscs, a process that consists of a series of spaced discs, mounted on a horizontal axis.
The discs rotate slowly and around half the surface immersed in the sewage and the other half exposed to the air.
Biomass grows attached to the discs, forming a biofilm.

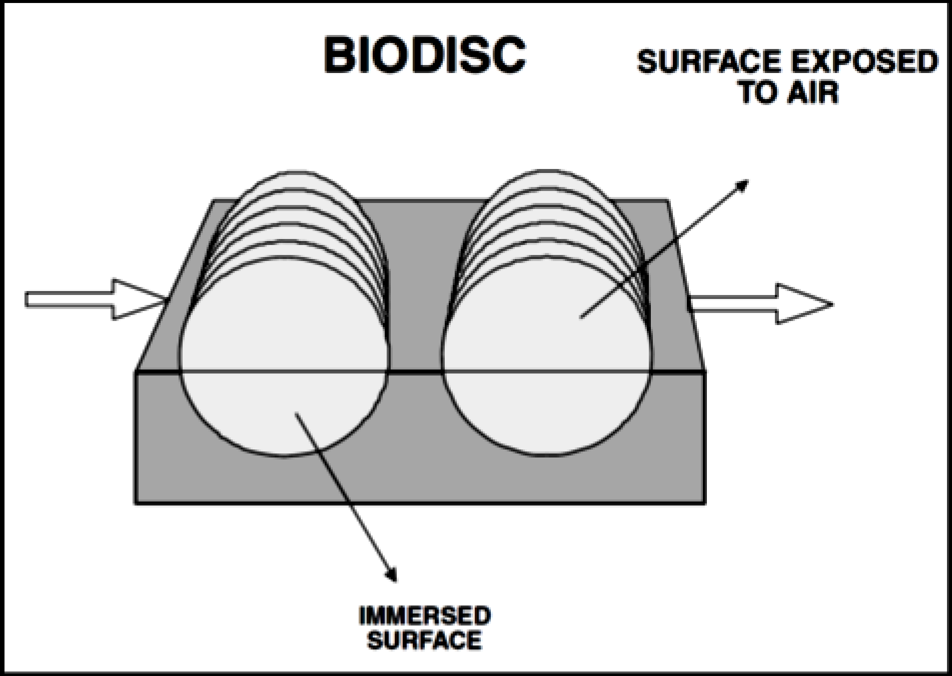
Rotating Biological Contactors

The discs usually are less than 3.6 metres in diameter and are generally constructed of low weight plastic.

When the system is put into operation and the discs rotate…
Biofilm formation,
Air mixing…
More food in the form of organic matter…

Rotating Biological Contactors

Schematics of a tank with biodiscs



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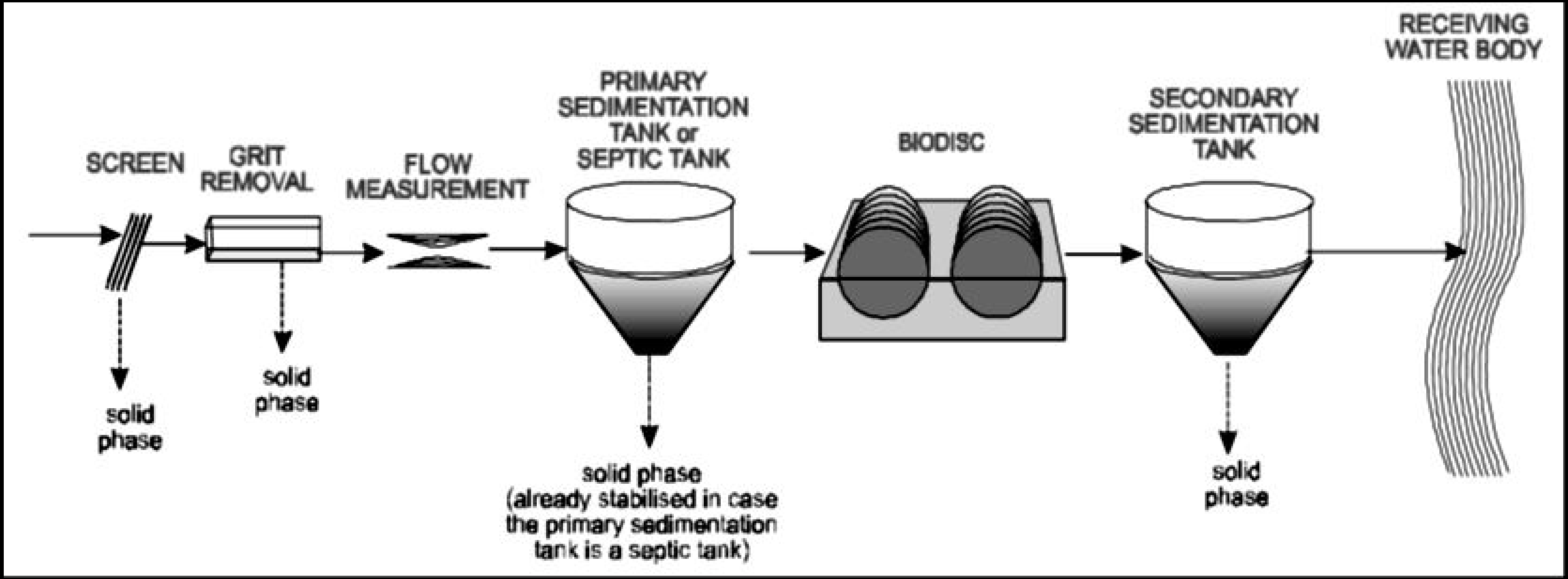
Rotating Biological Contactors

When the biological layer reaches an excessive thickness, it detaches from the discs.

Part of these detached microorganisms is maintained in suspension in the liquid due to the movement of the disk, which increases the efficiency of the system.

Rotating Biological Contactors

Typical flowsheet of a biodisc system



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Rotating Biological Contactors

The main purposes of the discs are:

- to serve as the surface for microbial film growth;
- to promote the contact between the microbial film and the sewage;
- to maintain the biomass that detached from the discs in suspension in the liquid;
- to promote the aeration of the sewage that is adhered to the disc and the sewage immersed in the liquid.

Rotating Biological Contactors

The growth of the biofilm is similar in concept to the trickling filter, with the difference that the microorganisms pass through the sewage, instead of the sewage passing through the microorganisms.

END

Like the trickling filter process, secondary sedimentation tanks are also necessary, with the objective of removing the suspended solids.

Environmental Biotechnology

Removal of Pathogenic Organisms

Removal of Pathogenic Organisms

Types of Processes:

Natural:
Maturation Ponds,
Land treatment.

Artificial:
Chlorination,
Ozonisation,
UV radiation,
Membranes.

Removal of Pathogenic Organisms

Maturation ponds:
Shallow ponds, where the penetration of solar UV radiation and unfavourable environmental conditions causes a high mortality of the pathogens.

The maturation ponds do not need chemical products or energy, but require large areas.

They are highly recommended systems...

Removal of Pathogenic Organisms

Land treatment (infiltration in soil):

The unfavourable environmental conditions in the soil favour the mortality of the pathogens.

Chemical products are not needed.

Requires large areas.

Removal of Pathogenic Organisms

Chlorination:

Kills pathogenic microorganisms…

High dosages are necessary, which may increase operational costs.

There is a concern regarding the generation of toxic by-products.

The toxicity caused by the residual chlorine in the water bodies is also of concern…Dechlorination.

Removal of Pathogenic Organisms

Ozonisation:

Ozone is a very effective agent for the removal of pathogens.

Ozonisation is usually expensive.

There is less experience with ozonisation in most developing countries.

Removal of Pathogenic Organisms

Ultraviolet radiation:

UV radiation affects the reproduction of the pathogenic agents.

Toxic by-products are not generated.

Ideally, the effluent must be well clarified for the radiation to penetrate well in the liquid mass.

Removal of Pathogenic Organisms

END

Membranes:

The passage of treated sewage through membranes of minute dimensions (e.g. ultrafiltration, nanofiltration)…

The process does not introduce chemical products into the liquid.

The costs are still high.

Environmental Biotechnology

Analysis and Selection of the Wastewater Treatment Process, Criteria for Analysis

Criteria for Analysis

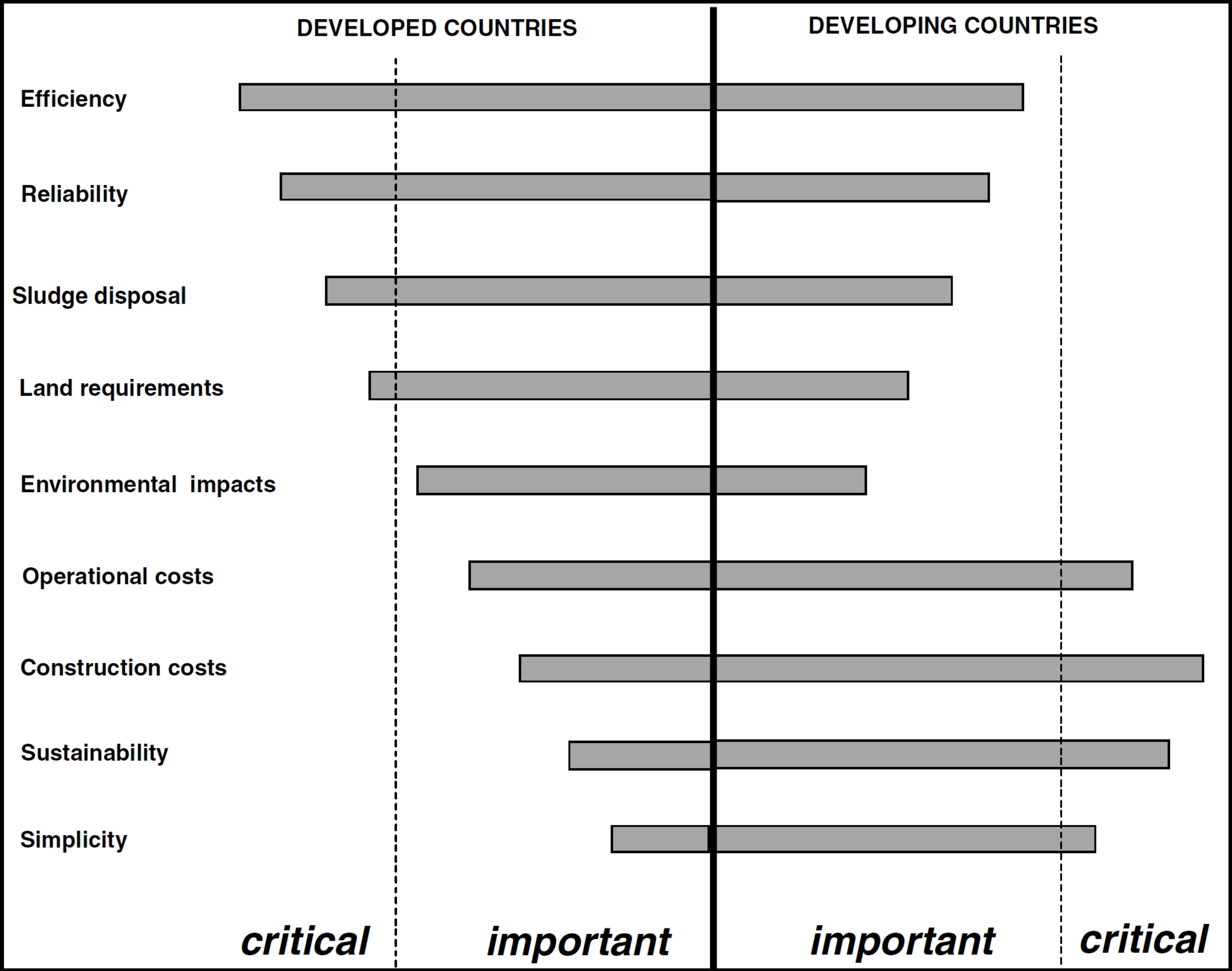
The decision regarding the wastewater treatment process to be adopted should be derived from a balance between technical and economical criteria, taking into account quantitative and qualitative aspects of each alternative.

Criteria for Analysis

Criteria or weightings can be attributed to the various aspects connected essentially with the reality in focus.

There are no such generalized formulae for this, and common sense and experience when attributing the relative importance of each technical aspect are essential.

Rotating Biological Contactors



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Criteria for Analysis

Important factors to be considered when evaluating and selecting unit operations and processes:

Process applicability,
Applicable flow,
Influent characteristics,
Inhibiting or refractory compounds,
Climatic aspects,
Performance,
Treatment residuals,
Sludge processing,
Chemical requirements,…

Criteria for Analysis

Important factors:

Energy requirements,
Personnel requirements,
Operating and maintenance requirements,
Complexity,
Compatibility,
Area availability.

Criteria for Analysis

END

Environmental impacts to be considered:

Odours,
Vector attraction,
Noise,
Sludge transportation,
Sanitary risks,
Air contamination,
Soil contamination,
Inconvenience to the nearby population…

Environmental Biotechnology

Comparison between the wastewater treatment systems

Comparison between the wastewater treatment systems

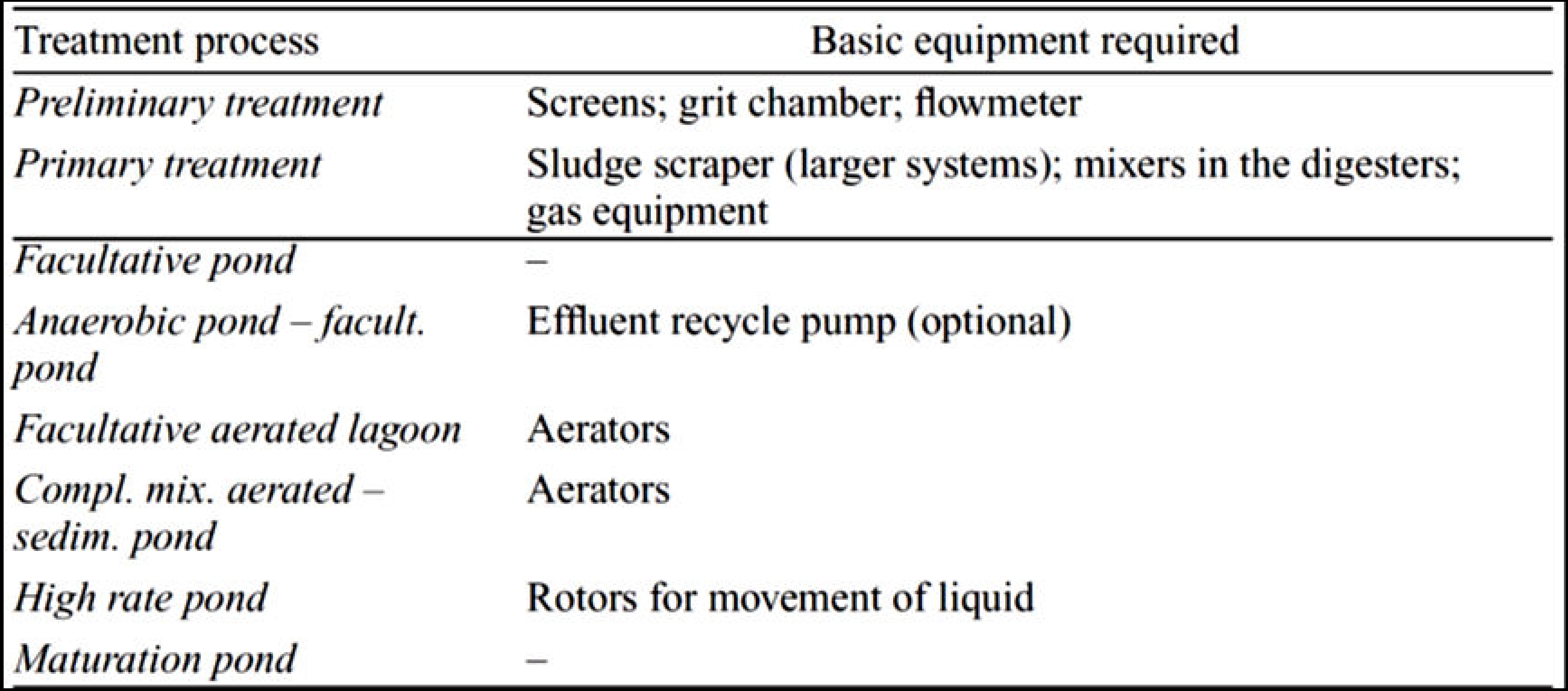
Comparison of average effluent concentrations and typical removal efficiencies of the main pollutants…

Comparison of typical characteristics of the main sewage treatment systems, expressed in per-capita values...

Comparison of the aspects of efficiency, economy, process and environmental problems…

Comparison between the wastewater treatment systems

Minimum equipment necessary for the main wastewater treatment processes



Treatment process	Basic equipment required
Preliminary treatment	Screens; grit chamber; flowmeter
Primary treatment	Sludge scraper (larger systems); mixers in the digesters; gas equipment
Facultative pond	–
Anaerobic pond – facult. pond	Effluent recycle pump (optional)
Facultative aerated lagoon	Aerators
Compl. mix. aerated – sedim. pond	Aerators
High rate pond	Rotors for movement of liquid
Maturation pond	–

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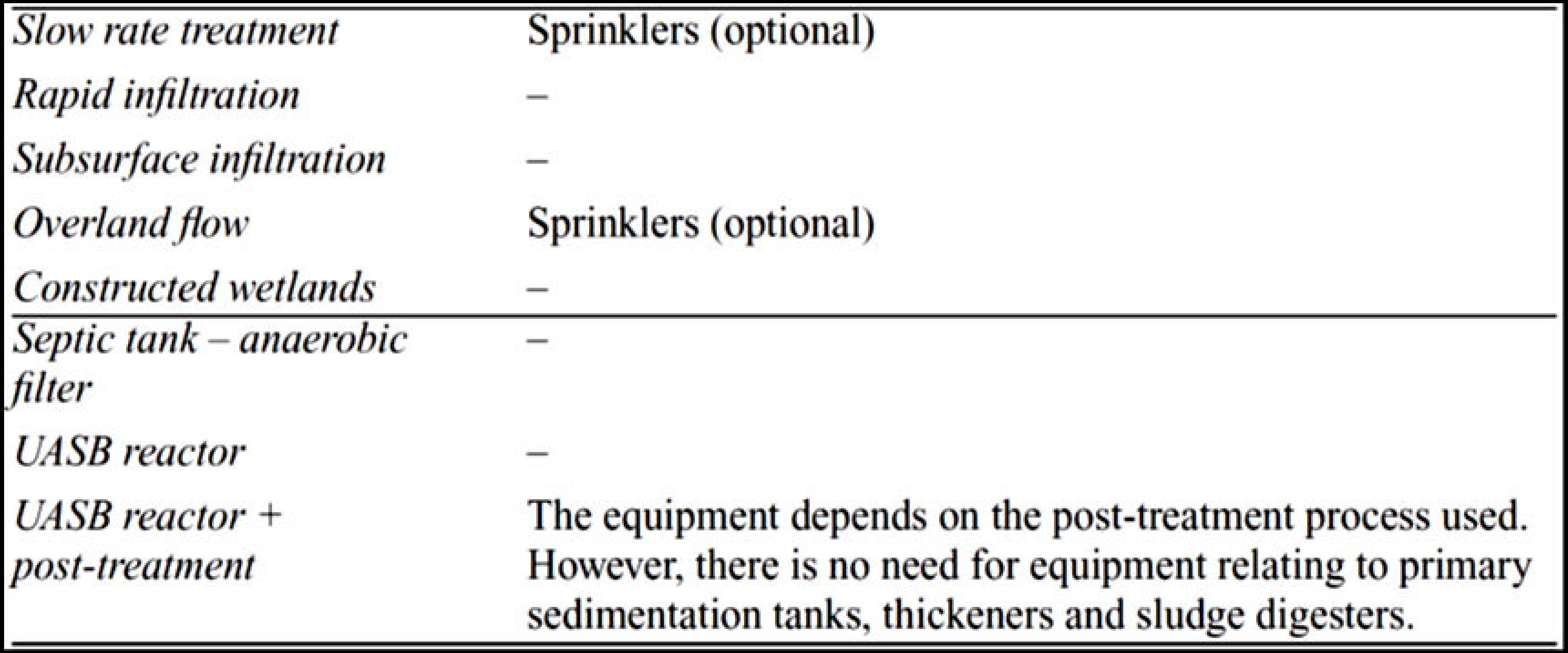
Comparison between the wastewater treatment systems

Minimum equipment necessary for the main wastewater treatment processes



Slow rate treatment	Sprinklers (optional)
Rapid infiltration	–
Subsurface infiltration	–
Overland flow	Sprinklers (optional)
Constructed wetlands	–
Septic tank – anaerobic filter	–
UASB reactor	–
UASB reactor + post-treatment	The equipment depends on the post-treatment process used. However, there is no need for equipment relating to primary sedimentation tanks, thickeners and sludge digesters.

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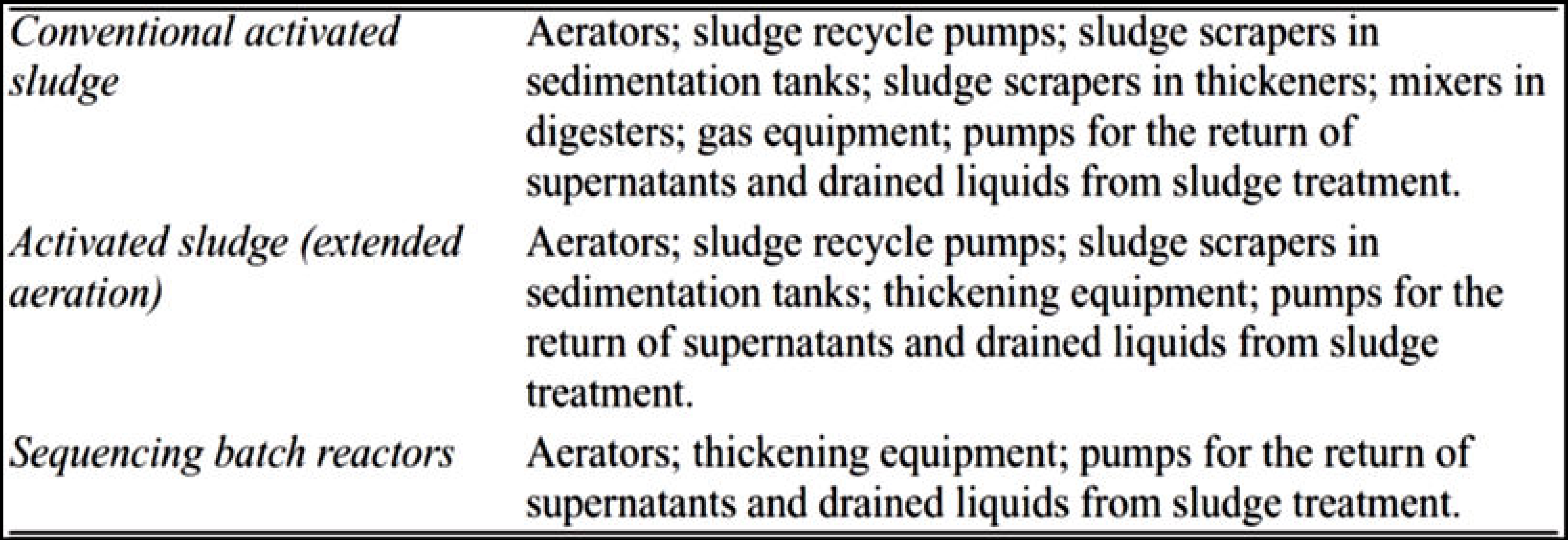
Comparison between the wastewater treatment systems

Minimum equipment necessary for the main wastewater treatment processes



Conventional activated sludge	Aerators; sludge recycle pumps; sludge scrapers in sedimentation tanks; sludge scrapers in thickeners; mixers in digesters; gas equipment; pumps for the return of supernatants and drained liquids from sludge treatment.
Activated sludge (extended aeration)	Aerators; sludge recycle pumps; sludge scrapers in sedimentation tanks; thickening equipment; pumps for the return of supernatants and drained liquids from sludge treatment.
Sequencing batch reactors	Aerators; thickening equipment; pumps for the return of supernatants and drained liquids from sludge treatment.

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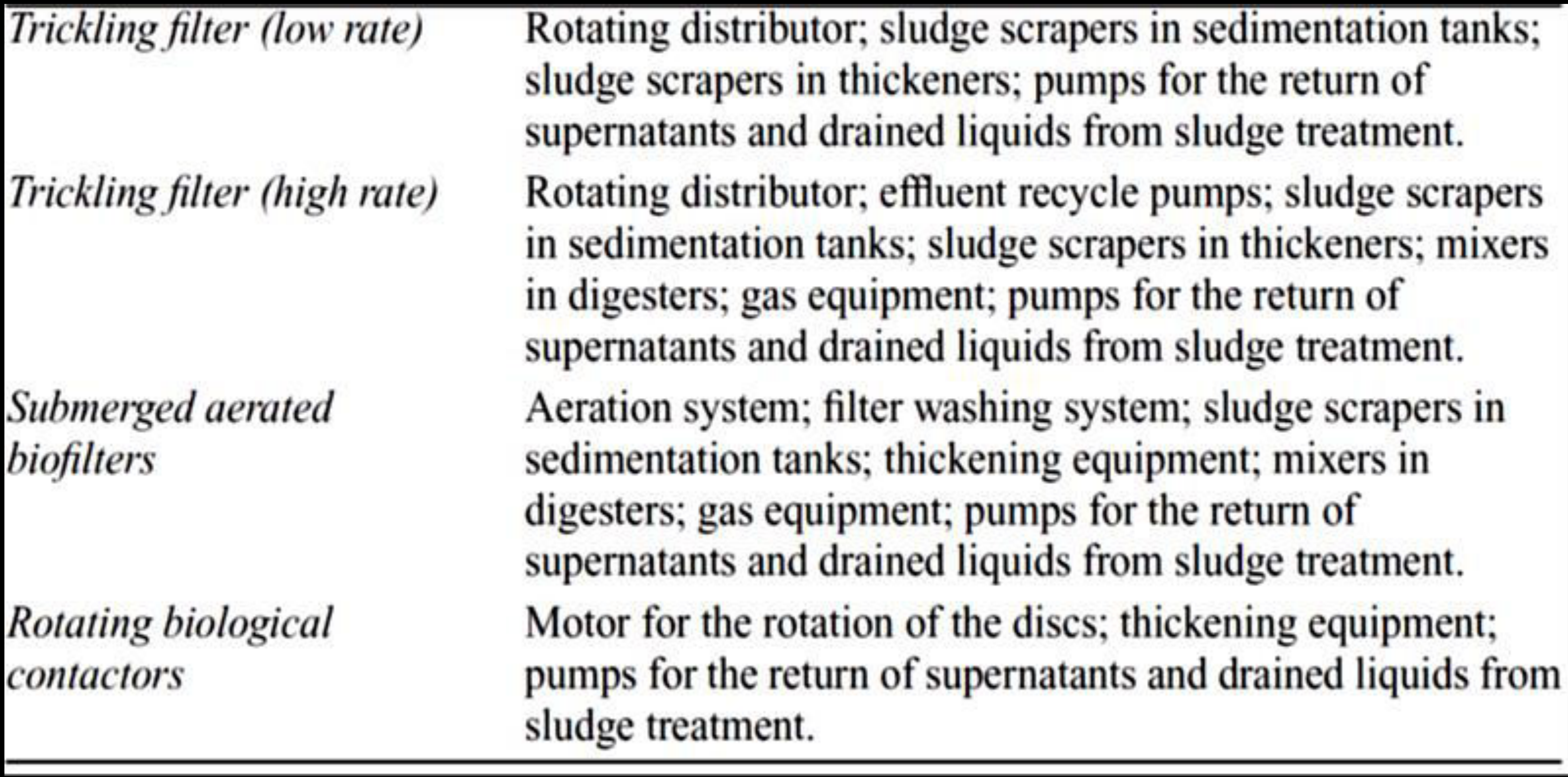
Comparison between the wastewater treatment systems

Minimum equipment necessary for the main wastewater treatment processes



Trickling filter (low rate)	Rotating distributor; sludge scrapers in sedimentation tanks; sludge scrapers in thickeners; pumps for the return of supernatants and drained liquids from sludge treatment.
Trickling filter (high rate)	Rotating distributor; effluent recycle pumps; sludge scrapers in sedimentation tanks; sludge scrapers in thickeners; mixers in digesters; gas equipment; pumps for the return of supernatants and drained liquids from sludge treatment.
Submerged aerated biofilters	Aeration system; filter washing system; sludge scrapers in sedimentation tanks; thickening equipment; mixers in digesters; gas equipment; pumps for the return of supernatants and drained liquids from sludge treatment.
Rotating biological contactors	Motor for the rotation of the discs; thickening equipment; pumps for the return of supernatants and drained liquids from sludge treatment.

Wastewater Characteristics, Treatment and Disposal, Vol 1, By Marcos

Comparison between the wastewater treatment systems

END

Comparative analysis of the main wastewater treatment systems; advantages and disadvantages.
(Table 4.21. Wastewater Characteristics, Treatment and Disposal, Vol 1, By Marcos).

Environmental Biotechnology

Sludge treatment & disposal (Introduction)

Sludge treatment & disposal

The main solid by-products produced in wastewater treatment are:

- screened material
- grit
- scum
- primary sludge
- secondary sludge
- chemical sludge (if a physical–chemical stage is included)

Sludge treatment & disposal

Even though the sludge, in most of its handling stages, is constituted by more than 95% water, it is only by convention that it is called a solid phase, with the aim at distinguishing it from the wastewater, or the liquid flow being treated (liquid phase).

Sludge treatment & disposal

Aspects needed to be taken into consideration when planning sludge management:

- production of the sludge in the liquid phase,
- wastage of the sludge from the liquid phase (removal to the sludge processing line)
- wastage of the sludge from the solid phase (removal from the wastewater treatment plant to the sludge disposal or reuse site)

Sludge treatment & disposal

The sludge production is a function of the wastewater treatment system used for the liquid phase.

In principle, all the biological treatment processes generate sludge.

Sludge treatment & disposal

The processes that receive raw wastewater in primary settling tanks generate the **primary sludge**, which is composed of the settleable solids of the raw wastewater.

In the biological treatment stage, there is the so-called biological sludge or **secondary sludge**.
This sludge is the biomass that grows at the expense of the food supplied by the incoming sewage.

Sludge treatment & disposal

Depending on the treatment system, the primary sludge can be sent for treatment together with the secondary sludge.

In this case, the resultant sludge of the mixture is called **mixed sludge**.

In treatment systems that incorporate a physical–chemical stage for improving the performance of primary or secondary settling tanks, a **chemical sludge** is produced.

Sludge treatment & disposal

Some treatment systems can store the sludge for all the operating horizon of the works (e.g. facultative ponds), others require only an occasional withdrawal (e.g. anaerobic reactors) and others need the continuous or very frequent removal (e.g. activated sludge).

END

Environmental Biotechnology

Relationships in sludge:
Relation between solid levels and water content

Relation between solid levels and water content

The relation between the level of dry solids and the water content in the sludge is given by:

$$\text{Water content (\%)} = 100 - \text{Dry solids level (\%)}$$

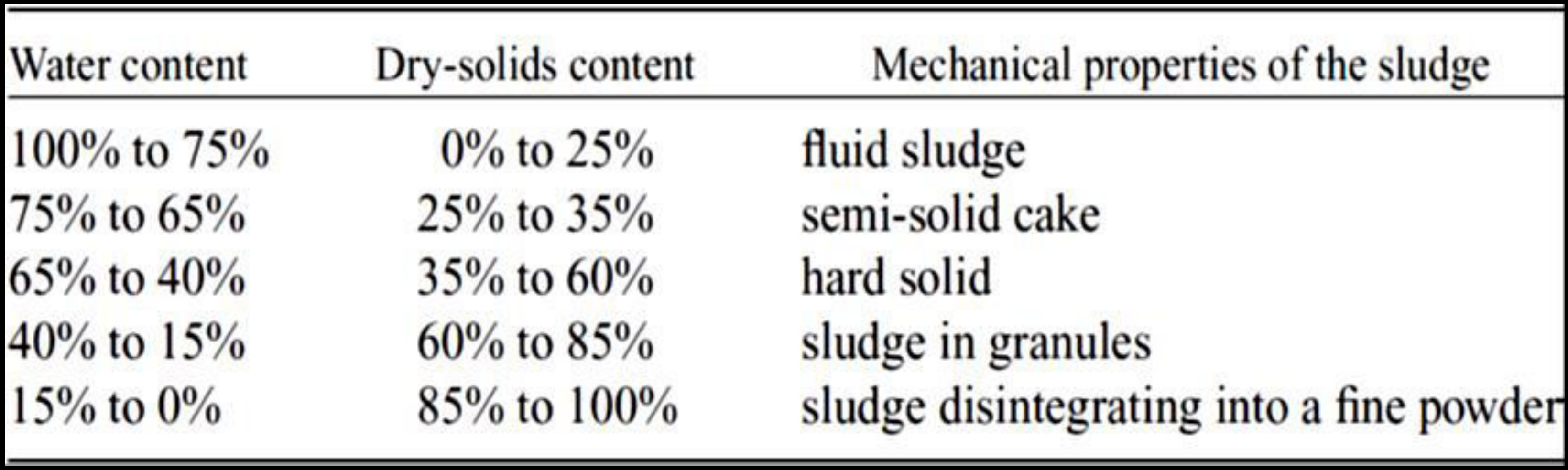
A sludge with a level of dry solids of 2% has a water content of 98%. Therefore, in every 100 kg of sludge, 98 kg are water and 2 kg are solids.

Relation between solid levels and water content

The water content influences the mechanical properties of the sludge and these influence the handling processes and the final disposal of the sludge.

Relation between solid levels and water content

Relation between the water content and the mechanical properties in most forms of sludges



Water content	Dry-solids content	Mechanical properties of the sludge
100% to 75%	0% to 25%	fluid sludge
75% to 65%	25% to 35%	semi-solid cake
65% to 40%	35% to 60%	hard solid
40% to 15%	60% to 85%	sludge in granules
15% to 0%	85% to 100%	sludge disintegrating into a fine powder

In the present context, dry solids (d.s.) are equivalent to total solids (TS), which, in the case of sludges, are very similar to total suspended solids (TSS or simply SS). These variables may be used interchangeably.

Wastewater Characteristics, Treatment and Disposal, Vol 1, By Marcos

Relation between solid levels and water content

Water in the sludge can be divided into four distinct classes.

- *Free water,*
- *Adsorbed water*,
- *Capillary water*,
- *Cellular water*.

Relation between solid levels and water content

- *Free water*: Can be removed by gravity (thickening, flotation).

- *Adsorbed water*: Can be removed by mechanical forces or by the use of a flocculating agent.

Relation between solid levels and water content

END

- *Capillary water*: Maintains itself adsorbed in the solid phase by capillary forces, and is distinguished from the adsorbed water by the need of a greater separation force.
- *Cellular water*: Is part of the solid phase and can only be removed by the change of the water aggregation state, that is, through freezing or evaporation.

Environmental Biotechnology

Relationships in sludge:
Sludge density
&
Expression of the concentration of dry solids

Sludge density

The density of the sludge during most of its processing is very close to water.

Usual values are between 1.02 and 1.03 (1020 to 1030 kg/m^3) for the liquid sludge during its treatment, and between 1.05 and 1.08 (1050 to 1080 kg/m^3) for the dewatered sludge going to final disposal.

Expression of the concentration of dry solids

The concentration of solids in the sludge is expressed in the form of dry solids, that is, excluding the water content of the sludge. The concentration can be in mg/L or in % (the latter being more frequent for sludge processing).

Expression of the concentration of dry solids

The concentrations in mg/L or in % are related by:

$$\text{Concentration (\%)} = \frac{\text{Concentration (mg/L)} \times 100}{1 \times 10^{6}\text{(mg/kg)} \times \text{Density (kg/L)}}$$

Since in most of the sludge processing stages the specific gravity is very close to 1.0 (except for the dewatered sludge), the above equation can be simplified to the following

$$\text{Concentration (\%)} \approx \frac{\text{Concentration (mg/L)}}{10{,}000}$$

For instance, a sludge with a concentration of 20,000 mg/L could have this same concentration expressed as 20,000/10,000 = 2.0% of dry solids. Thus, each 100 kg (or 100 litres) of sludge has 2 kg of dry solids (and 98 kg of water).

Environmental Biotechnology

Relationships in sludge:
Relation between flow, concentration and load

Relation between flow, concentration and load

The design of the sludge treatment and final disposal stages is based on the sludge flow (volume per unit time) or in many cases, the dry solids load (mass per unit time).

Relation between flow, concentration and load

The sludge flow is related to the SS load and concentration by:
Flow = Load / Concentration

$$\boxed{\text{Sludge flow (m}^3\text{/d)} = \frac{\text{SS load (kgSS/d)}}{\frac{\text{Dry solids (\%)}}{100} \times \text{Sludge density (kg sludge/m}^3\text{sludge)}}}$$

Considering that the density of the sludge in practically all of its processing stages is very close to 1000 kg/m^3, can be simplified to:

$$\boxed{\text{Sludge flow (m}^3\text{/d)} = \frac{\text{SS load (kgSS/d)}}{\text{Dry solids (\%)} \times 10}}$$

A sludge with a solids load of 120 kgSS/d and a solids concentration of 2.0 % (20,000 mg/L) will have a flow of 120/(2.0 × 10) = 120/20 = 6.0 m^3/d.

Relation between flow, concentration and load

To estimate the SS load from the sludge flow and SS concentration, the rearrangement of the previous equations can be used:

Load = Flow × Concentration

$$\text{Load (kgSS/d)} = \frac{\text{Flow } (\text{m}^3/\text{d}) \times \text{Concentration } (\text{g/m}^3)}{1000 \text{ (g/kg)}}$$

The conversion of the units is based on the fact that mg/L is the same as g/m^3 (as seen above). A sludge with 20,000 mg/L is the same as a with 20,000 g/m^3.

If the flow is 6 m^3/d, the solids load will be 6 × 20,000/1000 = 120 kg of dry solids per day (or 120 kgSS/d or 120 kgTS/d).

Environmental Biotechnology

Quantity of Sludge Generated in the Wastewater treatment Processes

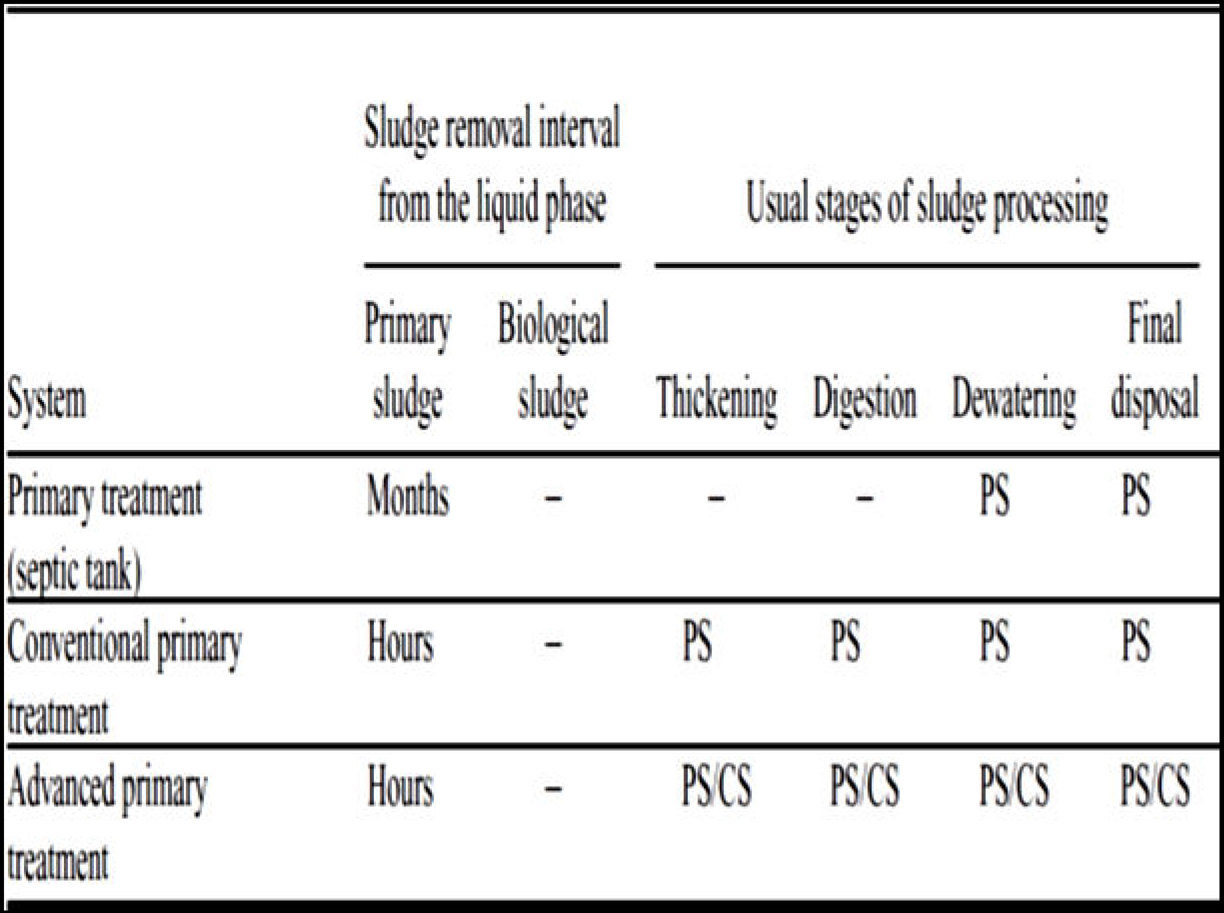
Quantity of Sludge Generated

The storage period has a large influence on the sludge characteristics and, as a result, on its treatment.

Sludges removed in intervals of weeks, months, years or decades are usually thicker and already digested.

Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



System	Sludge removal interval from the liquid phase		Usual stages of sludge processing			
	Primary sludge	Biological sludge	Thickening	Digestion	Dewatering	Final disposal
Primary treatment (septic tank)	Months	–	–	–	PS	PS
Conventional primary treatment	Hours	–	PS	PS	PS	PS
Advanced primary treatment	Hours	–	PS/CS	PS/CS	PS/CS	PS/CS

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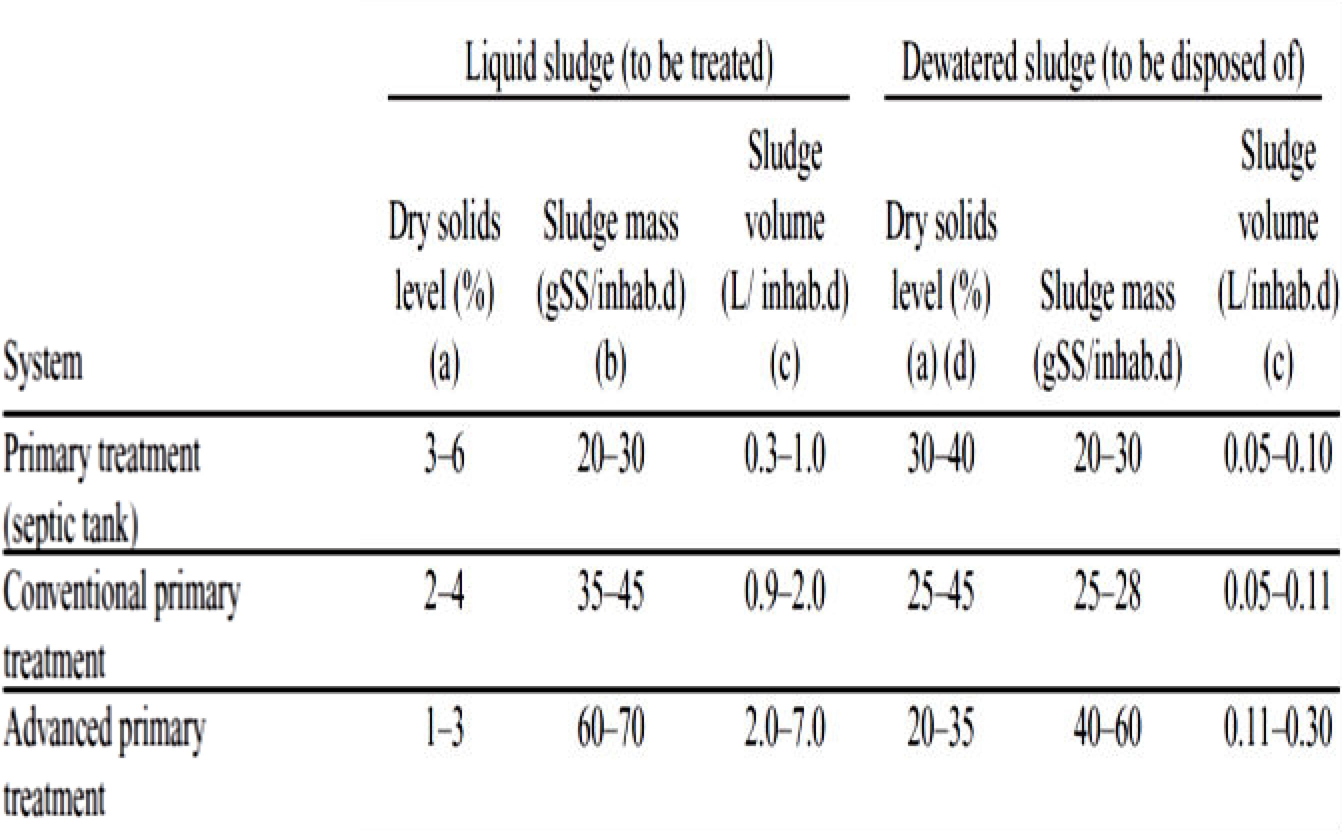
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Liquid sludge (to be treated)			Dewatered sludge (to be disposed of)		
System	Dry solids level (%) (a)	Sludge mass (gSS/inhab.d) (b)	Sludge volume (L/ inhab.d) (c)	Dry solids level (%) (a) (d)	Sludge mass (gSS/inhab.d)	Sludge volume (L/inhab.d) (c)
Primary treatment (septic tank)	3–6	20–30	0.3–1.0	30–40	20–30	0.05–0.10
Conventional primary treatment	2–4	35–45	0.9–2.0	25–45	25–28	0.05–0.11
Advanced primary treatment	1–3	60–70	2.0–7.0	20–35	40–60	0.11–0.30

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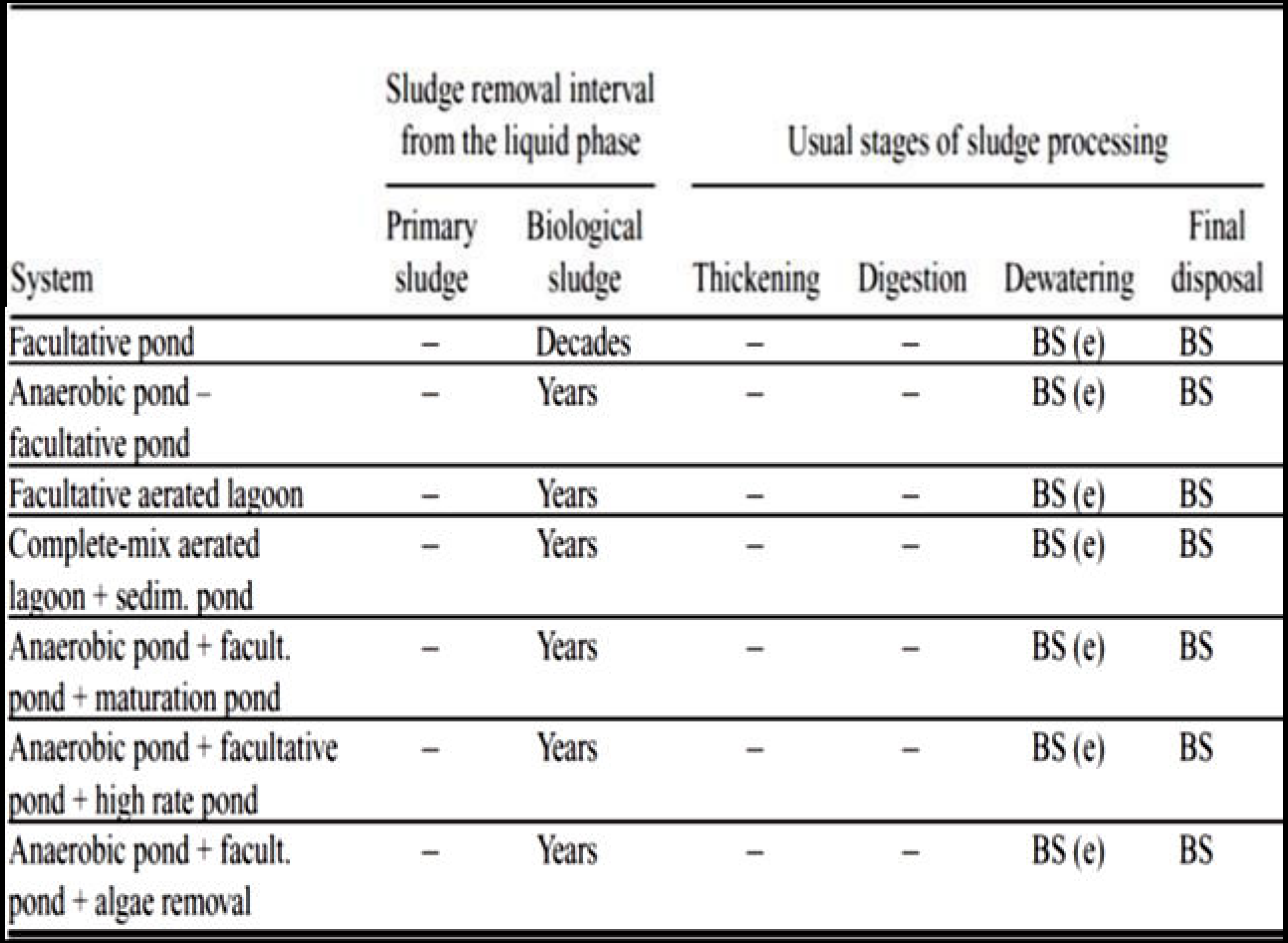
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



System	Sludge removal interval from the liquid phase		Usual stages of sludge processing			
	Primary sludge	Biological sludge	Thickening	Digestion	Dewatering	Final disposal
Facultative pond	–	Decades	–	–	BS (e)	BS
Anaerobic pond – facultative pond	–	Years	–	–	BS (e)	BS
Facultative aerated lagoon	–	Years	–	–	BS (e)	BS
Complete-mix aerated lagoon + sedim. pond	–	Years	–	–	BS (e)	BS
Anaerobic pond + facult. pond + maturation pond	–	Years	–	–	BS (e)	BS
Anaerobic pond + facultative pond + high rate pond	–	Years	–	–	BS (e)	BS
Anaerobic pond + facult. pond + algae removal	–	Years	–	–	BS (e)	BS

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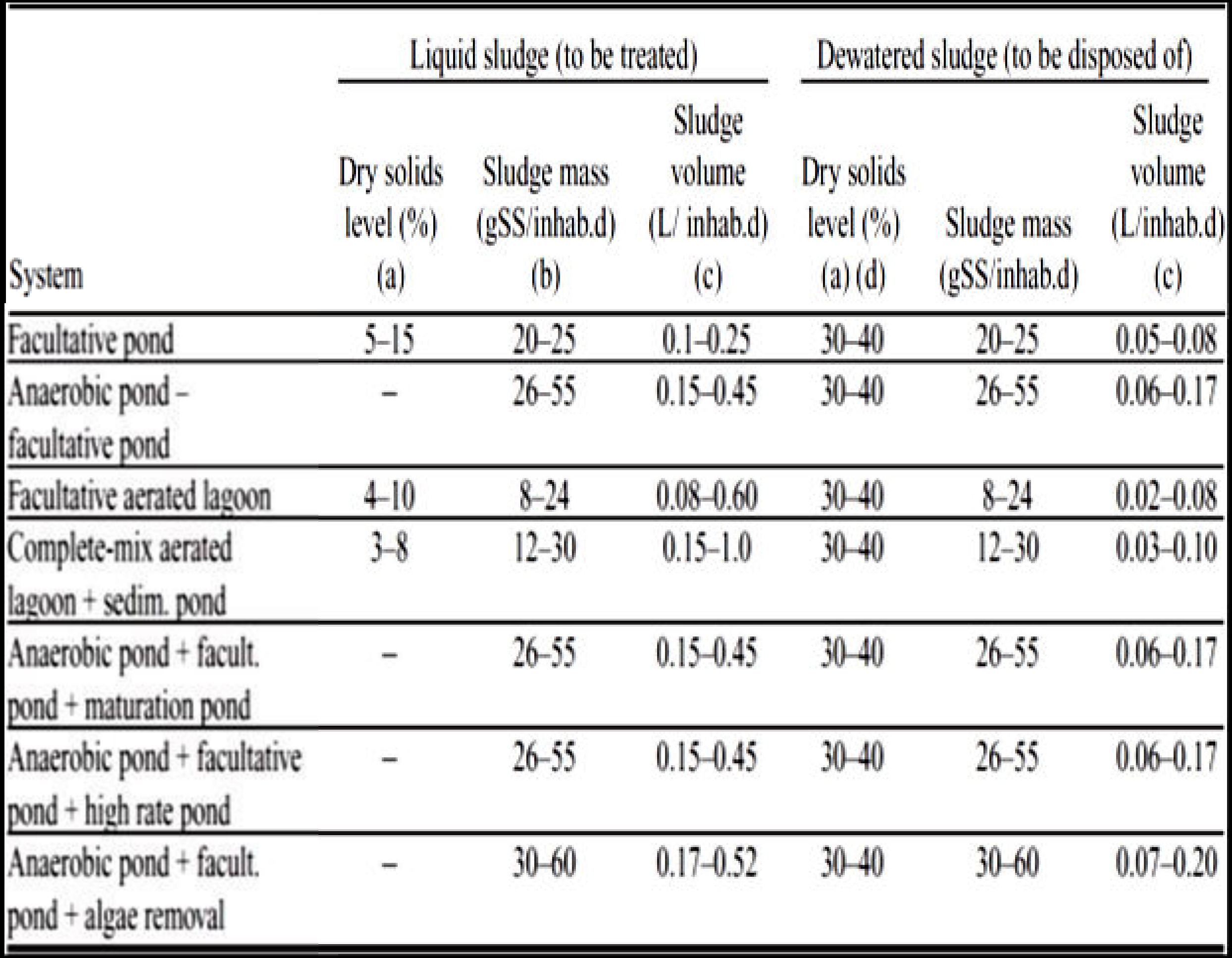
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Liquid sludge (to be treated)			Dewatered sludge (to be disposed of)		
System	Dry solids level (%) (a)	Sludge mass (gSS/inhab.d) (b)	Sludge volume (L/ inhab.d) (c)	Dry solids level (%) (a) (d)	Sludge mass (gSS/inhab.d)	Sludge volume (L/inhab.d) (c)
Facultative pond	5–15	20–25	0.1–0.25	30–40	20–25	0.05–0.08
Anaerobic pond – facultative pond	–	26–55	0.15–0.45	30–40	26–55	0.06–0.17
Facultative aerated lagoon	4–10	8–24	0.08–0.60	30–40	8–24	0.02–0.08
Complete-mix aerated lagoon + sedim. pond	3–8	12–30	0.15–1.0	30–40	12–30	0.03–0.10
Anaerobic pond + facult. pond + maturation pond	–	26–55	0.15–0.45	30–40	26–55	0.06–0.17
Anaerobic pond + facultative pond + high rate pond	–	26–55	0.15–0.45	30–40	26–55	0.06–0.17
Anaerobic pond + facult. pond + algae removal	–	30–60	0.17–0.52	30–40	30–60	0.07–0.20

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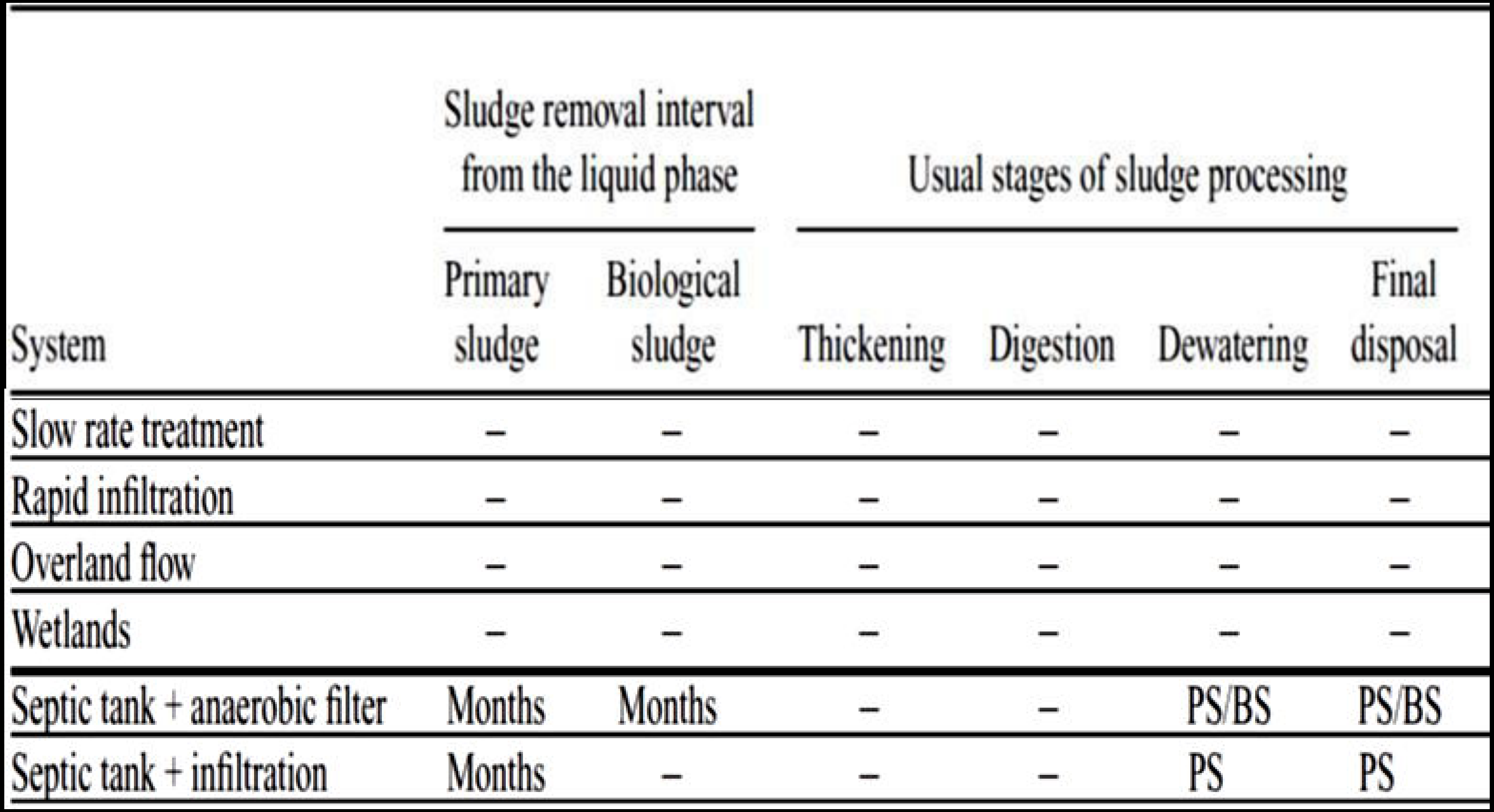
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Sludge removal interval from the liquid phase		Usual stages of sludge processing			
System	Primary sludge	Biological sludge	Thickening	Digestion	Dewatering	Final disposal
Slow rate treatment	–	–	–	–	–	–
Rapid infiltration	–	–	–	–	–	–
Overland flow	–	–	–	–	–	–
Wetlands	–	–	–	–	–	–
Septic tank + anaerobic filter	Months	Months	–	–	PS/BS	PS/BS
Septic tank + infiltration	Months	–	–	–	PS	PS

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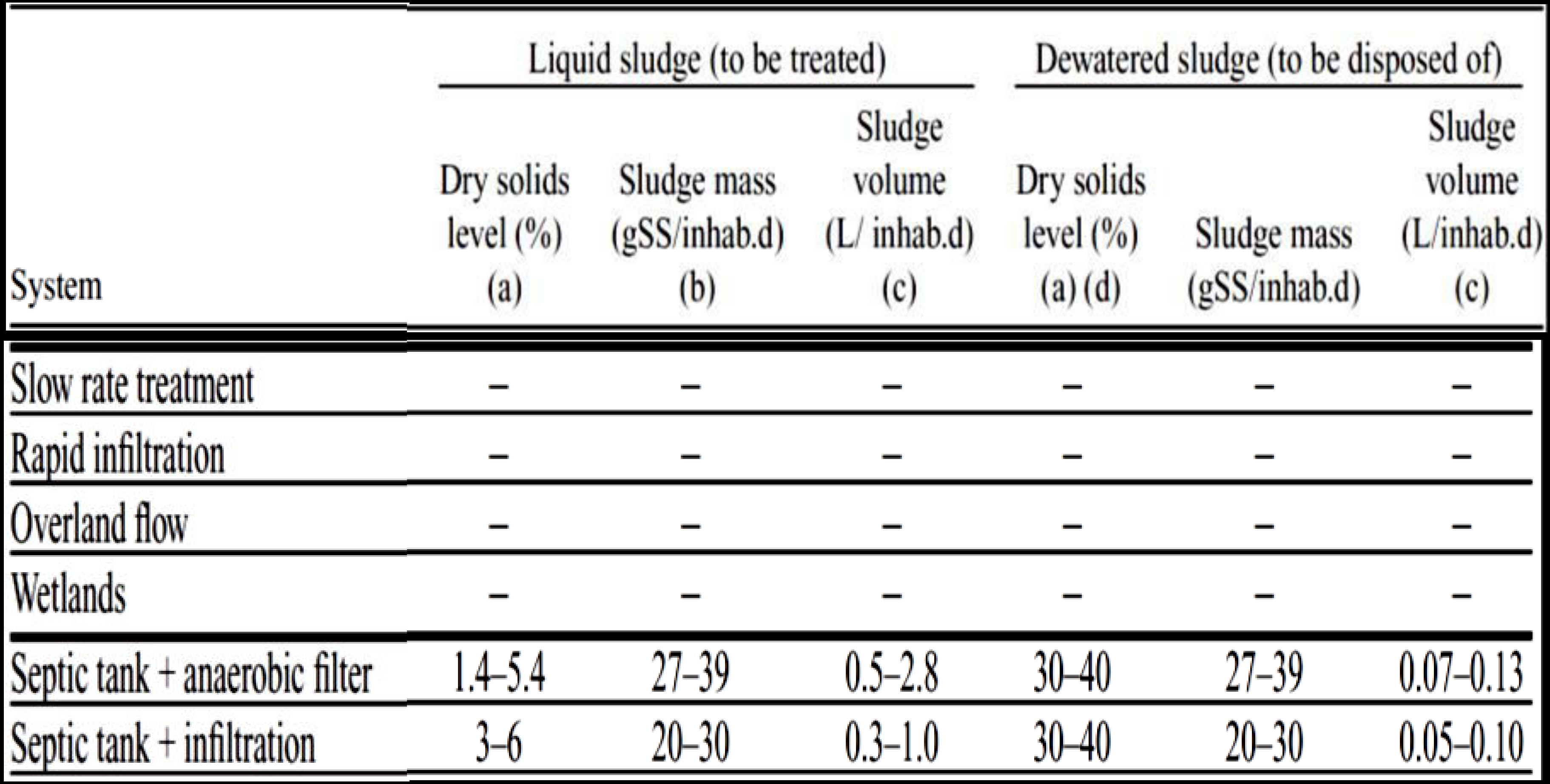
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



System	Liquid sludge (to be treated)			Dewatered sludge (to be disposed of)		
	Dry solids level (%) (a)	Sludge mass (gSS/inhab.d) (b)	Sludge volume (L/ inhab.d) (c)	Dry solids level (%) (a) (d)	Sludge mass (gSS/inhab.d)	Sludge volume (L/inhab.d) (c)
Slow rate treatment	–	–	–	–	–	–
Rapid infiltration	–	–	–	–	–	–
Overland flow	–	–	–	–	–	–
Wetlands	–	–	–	–	–	–
Septic tank + anaerobic filter	1.4–5.4	27–39	0.5–2.8	30–40	27–39	0.07–0.13
Septic tank + infiltration	3–6	20–30	0.3–1.0	30–40	20–30	0.05–0.10

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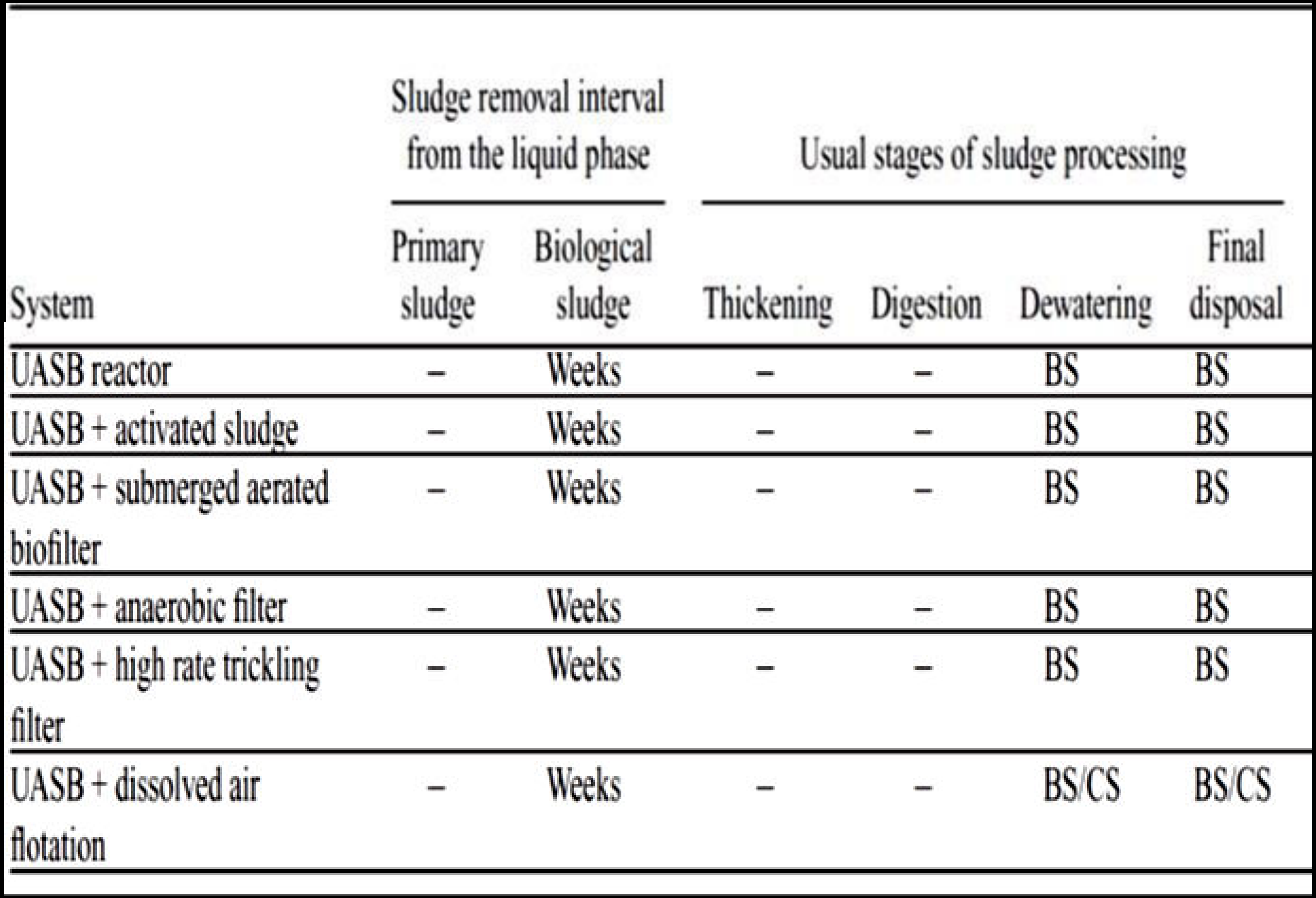
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Sludge removal interval from the liquid phase		Usual stages of sludge processing			
System	Primary sludge	Biological sludge	Thickening	Digestion	Dewatering	Final disposal
UASB reactor	–	Weeks	–	–	BS	BS
UASB + activated sludge	–	Weeks	–	–	BS	BS
UASB + submerged aerated biofilter	–	Weeks	–	–	BS	BS
UASB + anaerobic filter	–	Weeks	–	–	BS	BS
UASB + high rate trickling filter	–	Weeks	–	–	BS	BS
UASB + dissolved air flotation	–	Weeks	–	–	BS/CS	BS/CS

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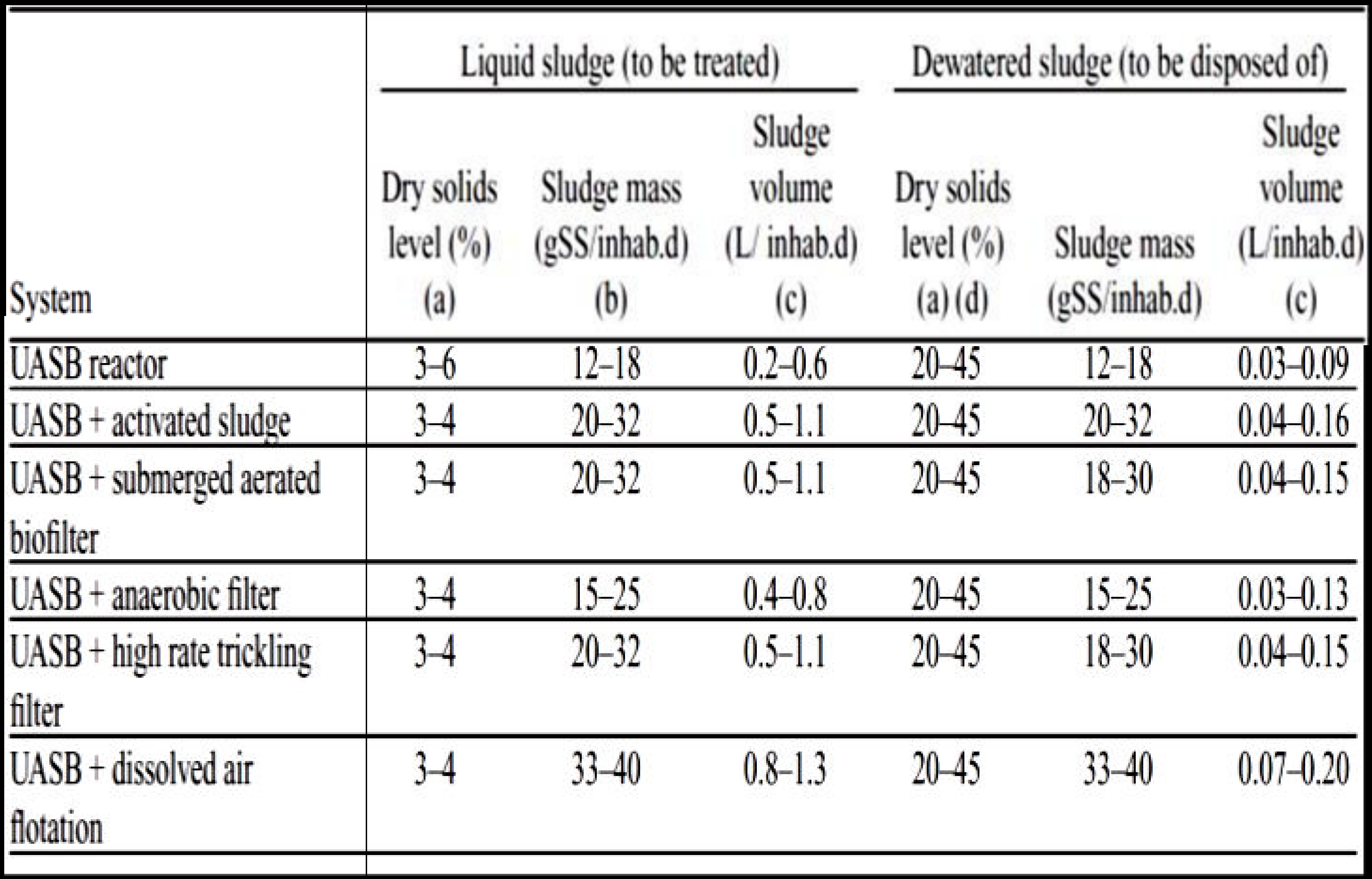
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Liquid sludge (to be treated)			Dewatered sludge (to be disposed of)		
System	Dry solids level (%) (a)	Sludge mass (gSS/inhab.d) (b)	Sludge volume (L/ inhab.d) (c)	Dry solids level (%) (a) (d)	Sludge mass (gSS/inhab.d)	Sludge volume (L/inhab.d) (c)
UASB reactor	3–6	12–18	0.2–0.6	20–45	12–18	0.03–0.09
UASB + activated sludge	3–4	20–32	0.5–1.1	20–45	20–32	0.04–0.16
UASB + submerged aerated biofilter	3–4	20–32	0.5–1.1	20–45	18–30	0.04–0.15
UASB + anaerobic filter	3–4	15–25	0.4–0.8	20–45	15–25	0.03–0.13
UASB + high rate trickling filter	3–4	20–32	0.5–1.1	20–45	18–30	0.04–0.15
UASB + dissolved air flotation	3–4	33–40	0.8–1.3	20–45	33–40	0.07–0.20

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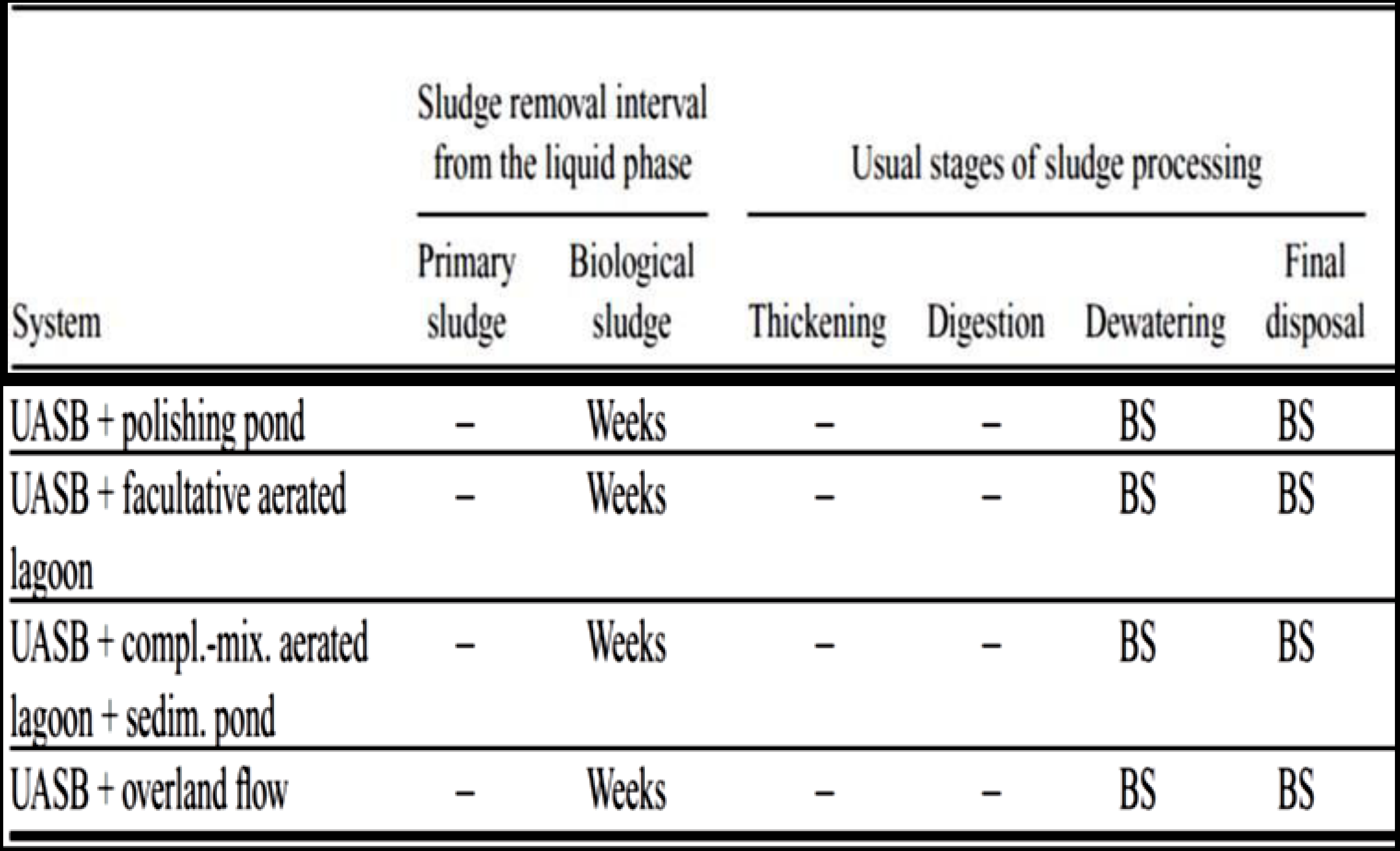
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



System	Sludge removal interval from the liquid phase		Usual stages of sludge processing			
	Primary sludge	Biological sludge	Thickening	Digestion	Dewatering	Final disposal
UASB + polishing pond	–	Weeks	–	–	BS	BS
UASB + facultative aerated lagoon	–	Weeks	–	–	BS	BS
UASB + compl.-mix. aerated lagoon + sedim. pond	–	Weeks	–	–	BS	BS
UASB + overland flow	–	Weeks	–	–	BS	BS

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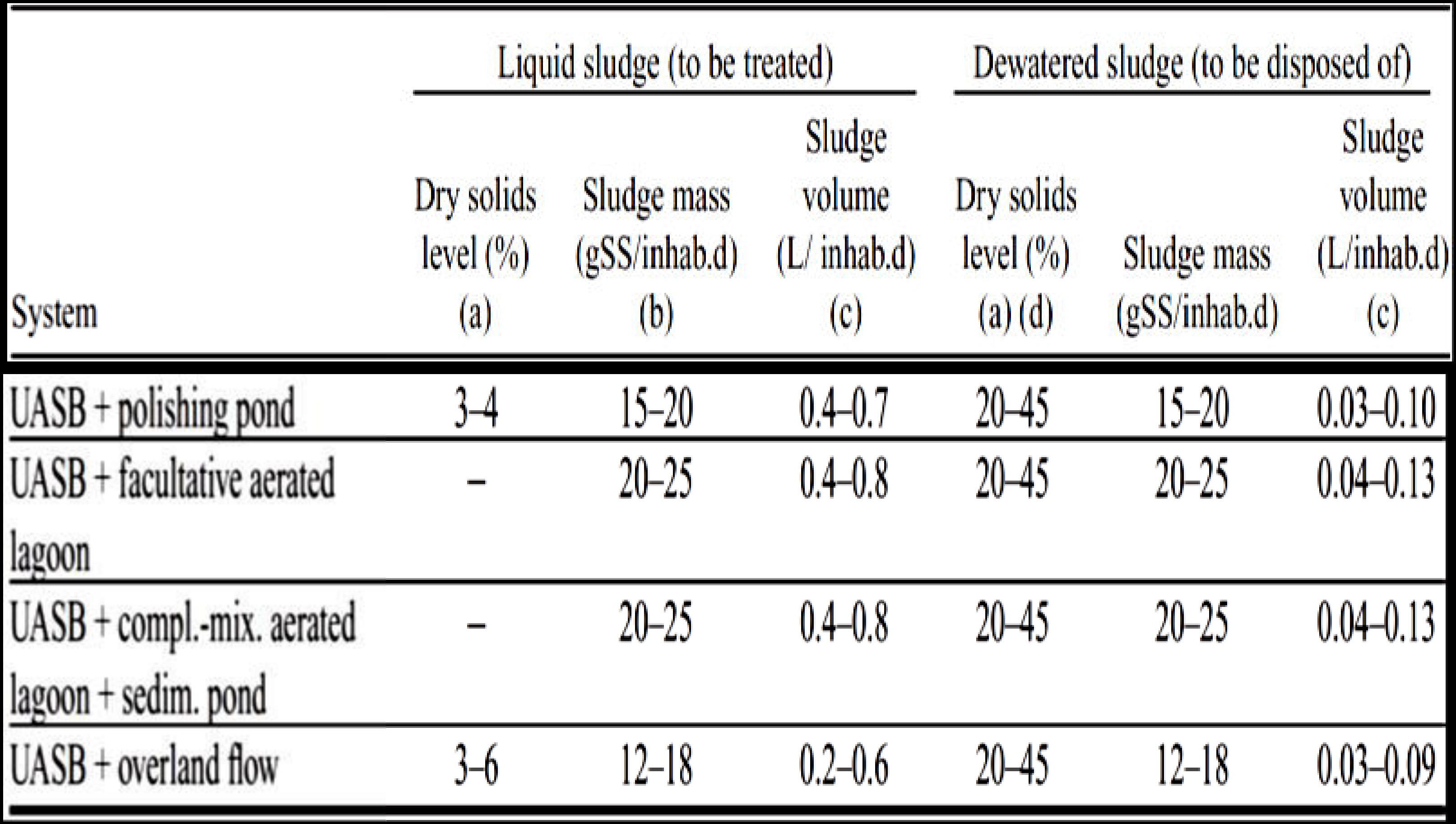
Quantity of Sludge Generated

Frequency of removal, treatment stages and characteristics of the sludge generated according to various sewage treatment processes



	Liquid sludge (to be treated)			Dewatered sludge (to be disposed of)		
System	Dry solids level (%) (a)	Sludge mass (gSS/inhab.d) (b)	Sludge volume (L/ inhab.d) (c)	Dry solids level (%) (a) (d)	Sludge mass (gSS/inhab.d)	Sludge volume (L/inhab.d) (c)
UASB + polishing pond	3–4	15–20	0.4–0.7	20–45	15–20	0.03–0.10
UASB + facultative aerated lagoon	–	20–25	0.4–0.8	20–45	20–25	0.04–0.13
UASB + compl.-mix. aerated lagoon + sedim. pond	–	20–25	0.4–0.8	20–45	20–25	0.04–0.13
UASB + overland flow	3–6	12–18	0.2–0.6	20–45	12–18	0.03–0.09

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Environmental Biotechnology

Sludge treatment stages

Sludge Treatment Stages

The main stages in sludge management:

- **Thickening**: removal of water (volume reduction)
- **Stabilisation**: removal of organic matter (mass reduction)
- **Conditioning**: preparation for dewatering
- **Dewatering**: removal of water (volume reduction)
- **Disinfection**: removal of pathogenic organisms
- **Final disposal**: final destination

Sludge Treatment Stages

Thickening:

It is a physical process of concentrating the sludge, with the aim of reducing its water content and, as a result, its volume, facilitating the subsequent sludge treatment stages.

Sludge Treatment Stages

Stabilisation:

Stabilisation aims at attenuating the inconveniences associated with the generation of bad odours and disposing of the sludge.
This is accomplished through the removal of the biodegradable organic matter of the sludge.

Sludge Treatment Stages

Conditioning:

Conditioning is a sludge preparation process, based on the addition of chemical products (coagulants, polyelectrolytes) to increase its dewatering capability and to improve the capture of solids in the sludge dewatering systems.

Sludge Treatment Stages

Dewatering:

It can be done through natural or mechanical methods.

The objective of this phase is to remove water and reduce the volume even further, producing a sludge with a mechanical behaviour close to solids.

This has an important impact in its transport and final disposal costs.

Sludge Treatment Stages

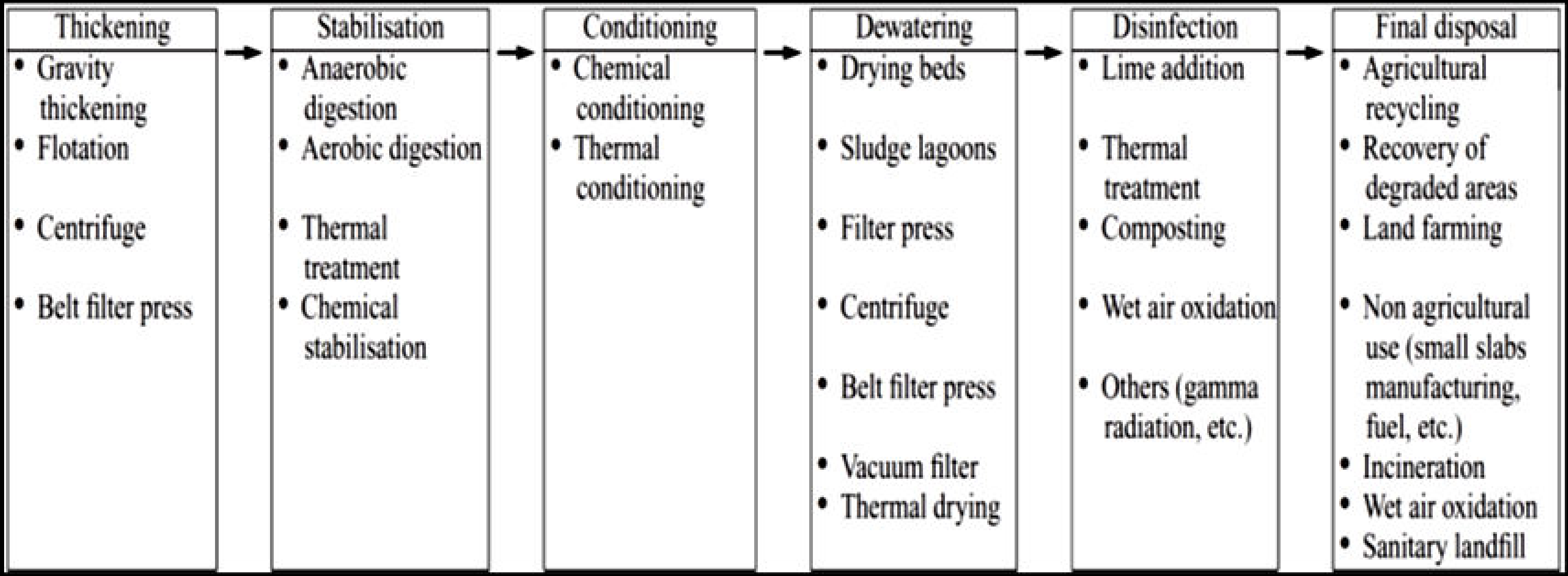
Disinfection:

This step is necessary, since the anaerobic or aerobic digestion processes usually employed do not reduce the pathogens content to acceptable levels.

Disinfection is not necessary if the sludge is to be incinerated or disposed of in landfills.

Sludge Treatment Stages

Sludge management stages and main processes used



Thickening	→	Stabilisation	→	Conditioning	→	Dewatering	→	Disinfection	→	Final disposal
• Gravity thickening • Flotation • Centrifuge • Belt filter press		• Anaerobic digestion • Aerobic digestion • Thermal treatment • Chemical stabilisation		• Chemical conditioning • Thermal conditioning		• Drying beds • Sludge lagoons • Filter press • Centrifuge • Belt filter press • Vacuum filter • Thermal drying		• Lime addition • Thermal treatment • Composting • Wet air oxidation • Others (gamma radiation, etc.)		• Agricultural recycling • Recovery of degraded areas • Land farming • Non agricultural use (small slabs manufacturing, fuel, etc.) • Incineration • Wet air oxidation • Sanitary landfill

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Environmental Biotechnology

Sludge Thickening and Sludge Stabilization

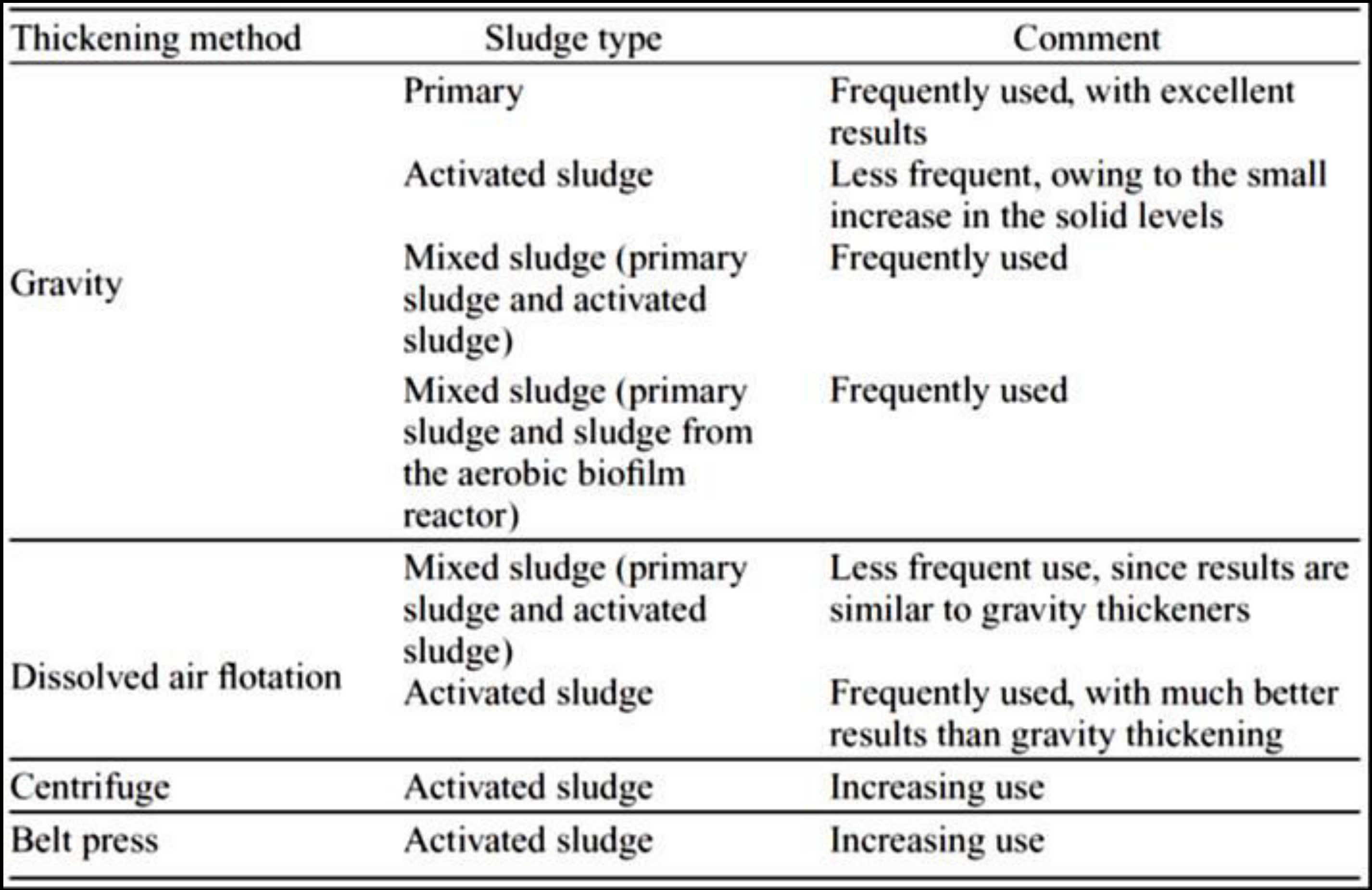
Sludge Thickening

The main processes used for sludge **thickening** are:

- gravity thickeners
- dissolved air flotation
- centrifuges
- belt presses

Sludge Thickening

Typical uses of the main sludge thickening methods



Thickening method	Sludge type	Comment
Gravity	Primary	Frequently used, with excellent results
	Activated sludge	Less frequent, owing to the small increase in the solid levels
	Mixed sludge (primary sludge and activated sludge)	Frequently used
	Mixed sludge (primary sludge and sludge from the aerobic biofilm reactor)	Frequently used
Dissolved air flotation	Mixed sludge (primary sludge and activated sludge)	Less frequent use, since results are similar to gravity thickeners
	Activated sludge	Frequently used, with much better results than gravity thickening
Centrifuge	Activated sludge	Increasing use
Belt press	Activated sludge	Increasing use

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Sludge Thickening

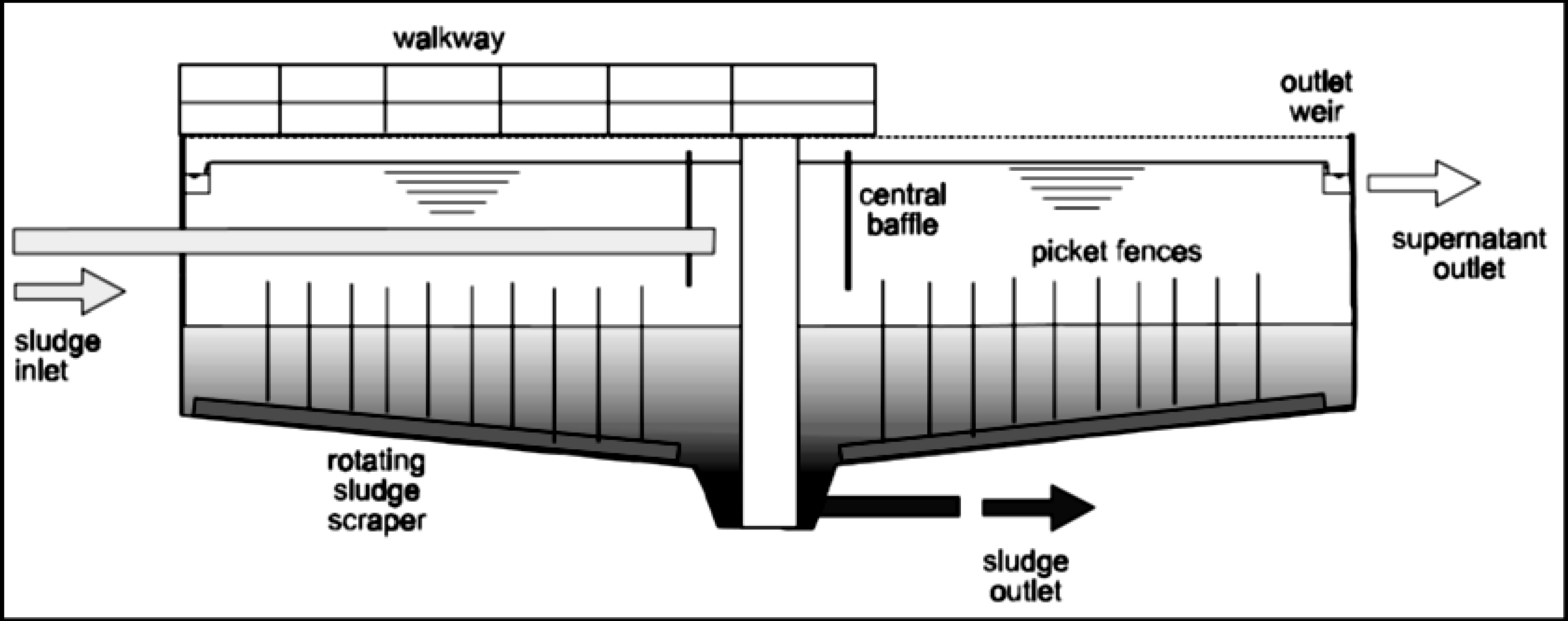
Gravity thickeners have a similar structure to settling tanks.

The format is usually circular with central feeding, a bottom sludge exit and a supernatant side exit.

The thickened sludge goes on to the next stage (normally digestion), while the supernatant is returned to the head of the works.

Sludge Thickening

Schematics of a gravity thickener



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Sludge Thickening

Dissolved air flotation:
In this process, air is dissolved in a solution maintained at high pressure.
When there is a depressurisation, the dissolved air is released in the form of small bubbles moving upward.
These bubbles tend to carry the sludge particles to the surface, from where they are removed.

Sludge Thickening

Thickening by flotation has a good applicability for activated sludge, which does not thicken well in gravity thickeners.

Sludge Stabilisation

The **stabilization** aims at stabilising (digesting) the biodegradable fraction of the organic matter present in the sludge, thus decreasing the risk of putrefaction, as well as reducing the concentration of pathogens.

Sludge Stabilisation

The stabilisation processes can be:

- biological stabilisation: use of specific bacteria to promote the stabilization of organic matter
- chemical stabilisation: by the chemical oxidation of the organic matter
- thermal stabilisation: obtained from the action of heat on the volatile fraction.

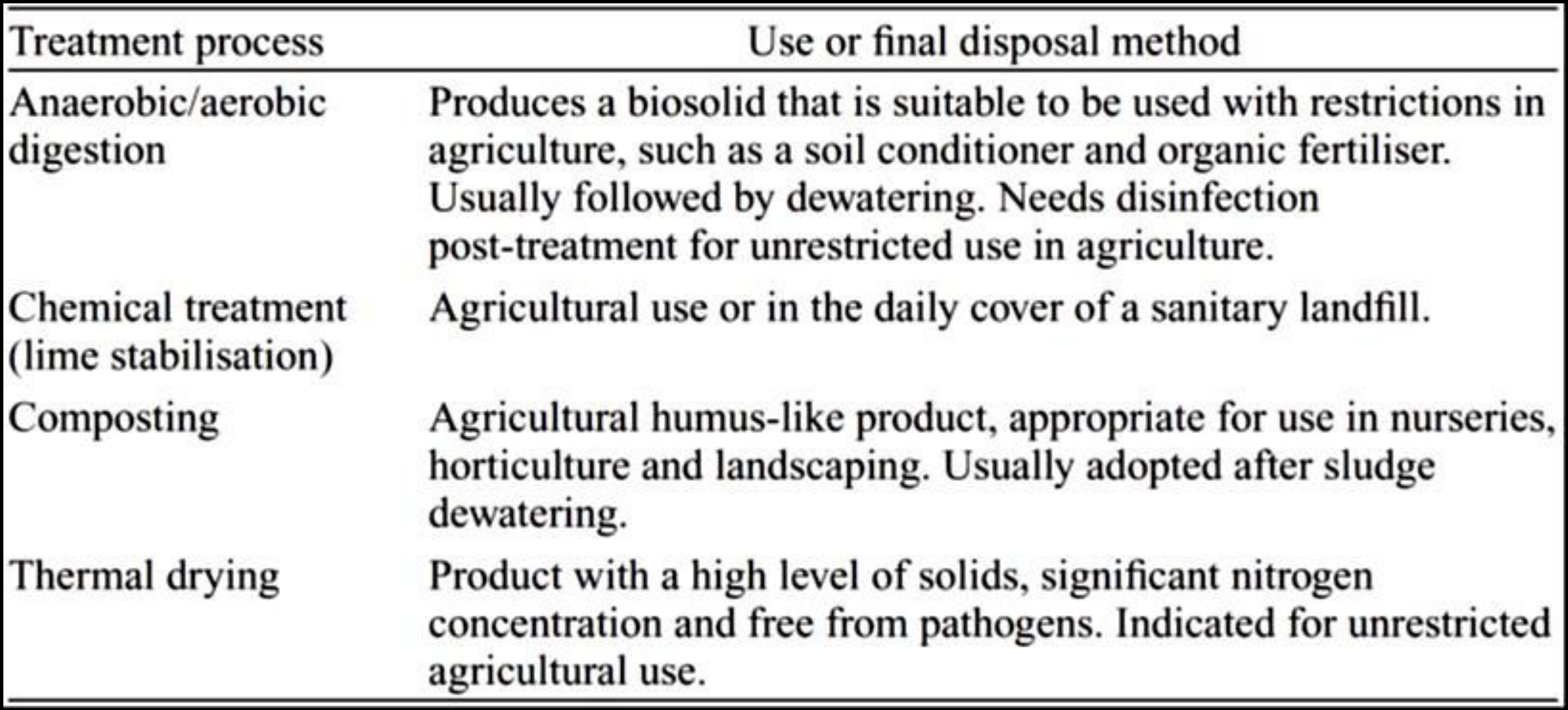
Sludge Stabilisation

Anaerobic digestion is the most frequently used sludge stabilisation process.

The stabilisation of the sludge facilitates its final disposal and opens alternatives for its reuse as an agricultural soil conditioner

Sludge Stabilisation

Sludge stabilisation technologies and final disposal methods



Treatment process	Use or final disposal method
Anaerobic/aerobic digestion	Produces a biosolid that is suitable to be used with restrictions in agriculture, such as a soil conditioner and organic fertiliser. Usually followed by dewatering. Needs disinfection post-treatment for unrestricted use in agriculture.
Chemical treatment (lime stabilisation)	Agricultural use or in the daily cover of a sanitary landfill.
Composting	Agricultural humus-like product, appropriate for use in nurseries, horticulture and landscaping. Usually adopted after sludge dewatering.
Thermal drying	Product with a high level of solids, significant nitrogen concentration and free from pathogens. Indicated for unrestricted agricultural use.

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Sludge Stabilisation

END

The anaerobic digesters are closed biological reactors constructed of concrete or steel.

Sludge is stabilised biologically under anaerobic conditions and converted into methane (CH_4) and carbon dioxide (CO_2)…

Environmental Biotechnology

Sludge Dewatering

Sludge Dewatering

The main objectives:

- reduction of the transportation costs;
- improvement of the handling conditions;
- increase of the calorific value of the sludge (for incineration);
- reduction in the volume for disposal;
- reduction in the production of leachate when the sludge is disposed of in landfills

Sludge Dewatering

Sludge dewatering can be done by natural or mechanised processes. Natural processes use evaporation and percolation as the main water removal mechanisms, thus requiring more time for dewatering.

Although simpler and cheaper to operate, they need larger areas and volumes for installation.

Sludge Dewatering

In contrast, the mechanized processes are based on mechanisms such as filtration, compaction, or centrifugation to accelerate dewatering, resulting in compact and sophisticated units, from an operational and maintenance point of view.

Sludge Dewatering

Natural sludge dewatering processes:

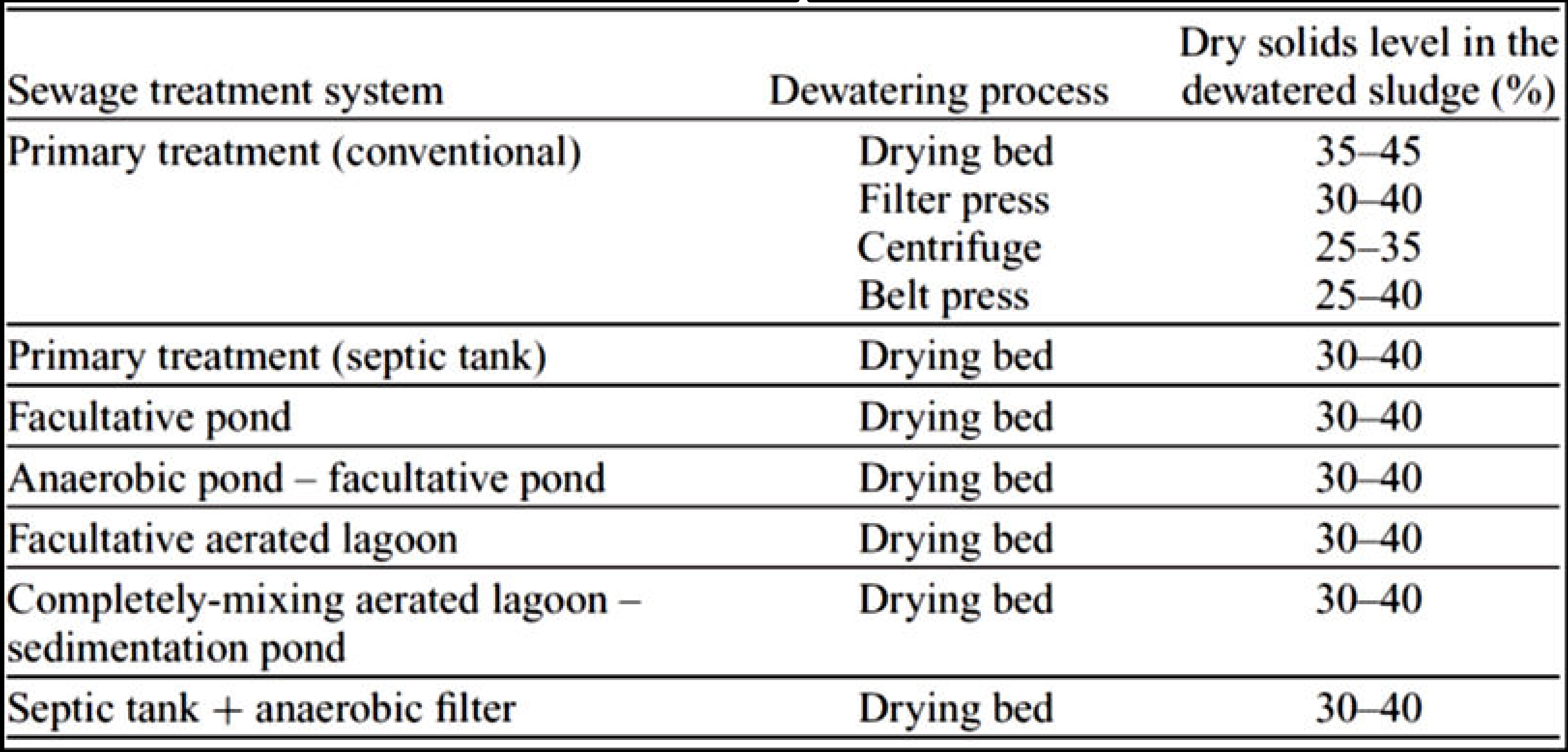
- Drying beds
- Sludge lagoons

Mechanised sludge dewatering processes:

- Centrifuges
- Vacuum filters
- Belt presses
- Filter presses

Sludge Dewatering

Typical dry solids levels in dewatered sludges from various wastewater treatment processes



Sewage treatment system	Dewatering process	Dry solids level in the dewatered sludge (%)
Primary treatment (conventional)	Drying bed Filter press Centrifuge Belt press	35–45 30–40 25–35 25–40
Primary treatment (septic tank)	Drying bed	30–40
Facultative pond	Drying bed	30–40
Anaerobic pond – facultative pond	Drying bed	30–40
Facultative aerated lagoon	Drying bed	30–40
Completely-mixing aerated lagoon – sedimentation pond	Drying bed	30–40
Septic tank + anaerobic filter	Drying bed	30–40

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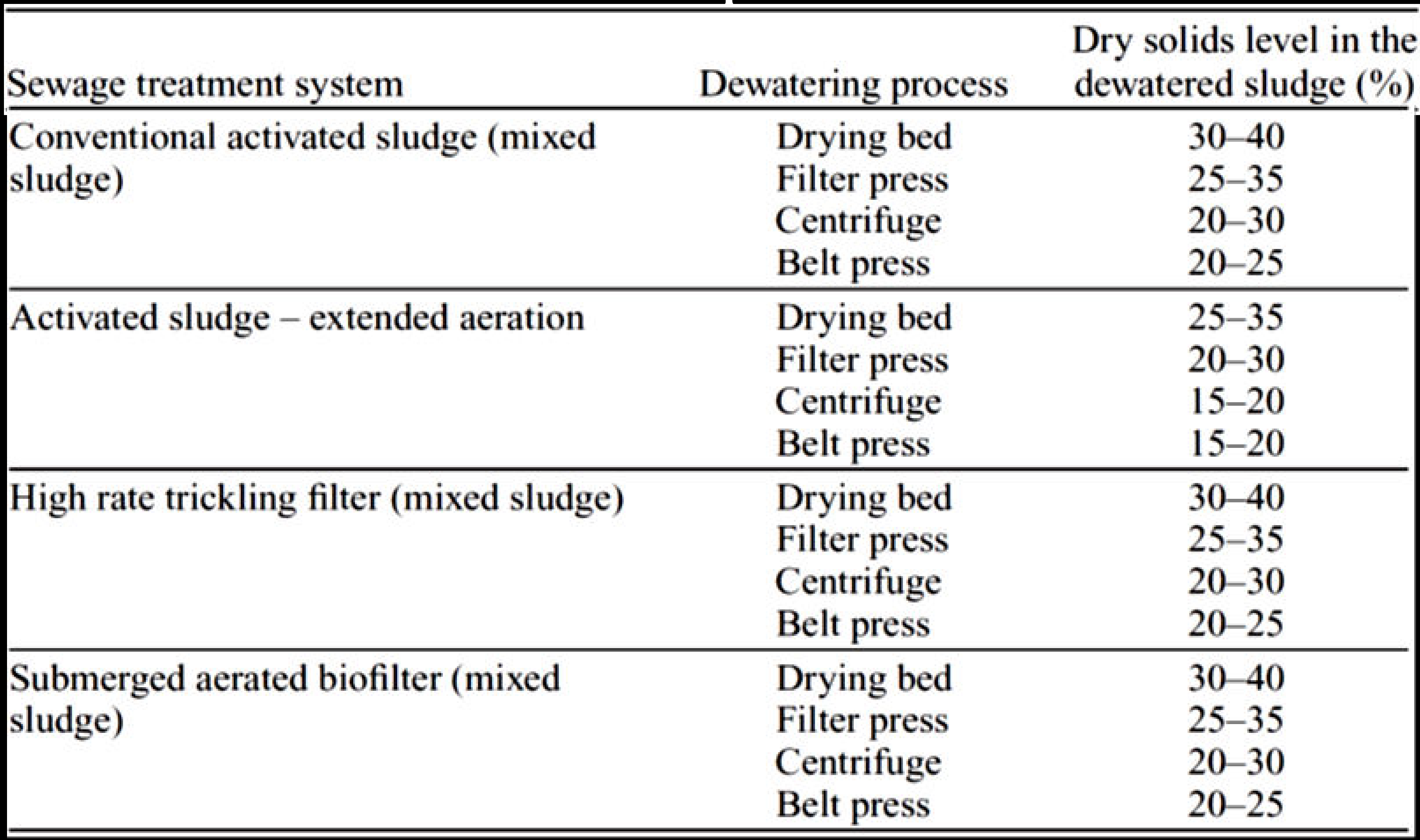
Sludge Dewatering

Typical dry solids levels in dewatered sludges from various wastewater treatment processes



Sewage treatment system	Dewatering process	Dry solids level in the dewatered sludge (%)
Conventional activated sludge (mixed sludge)	Drying bed	30–40
	Filter press	25–35
	Centrifuge	20–30
	Belt press	20–25
Activated sludge – extended aeration	Drying bed	25–35
	Filter press	20–30
	Centrifuge	15–20
	Belt press	15–20
High rate trickling filter (mixed sludge)	Drying bed	30–40
	Filter press	25–35
	Centrifuge	20–30
	Belt press	20–25
Submerged aerated biofilter (mixed sludge)	Drying bed	30–40
	Filter press	25–35
	Centrifuge	20–30
	Belt press	20–25

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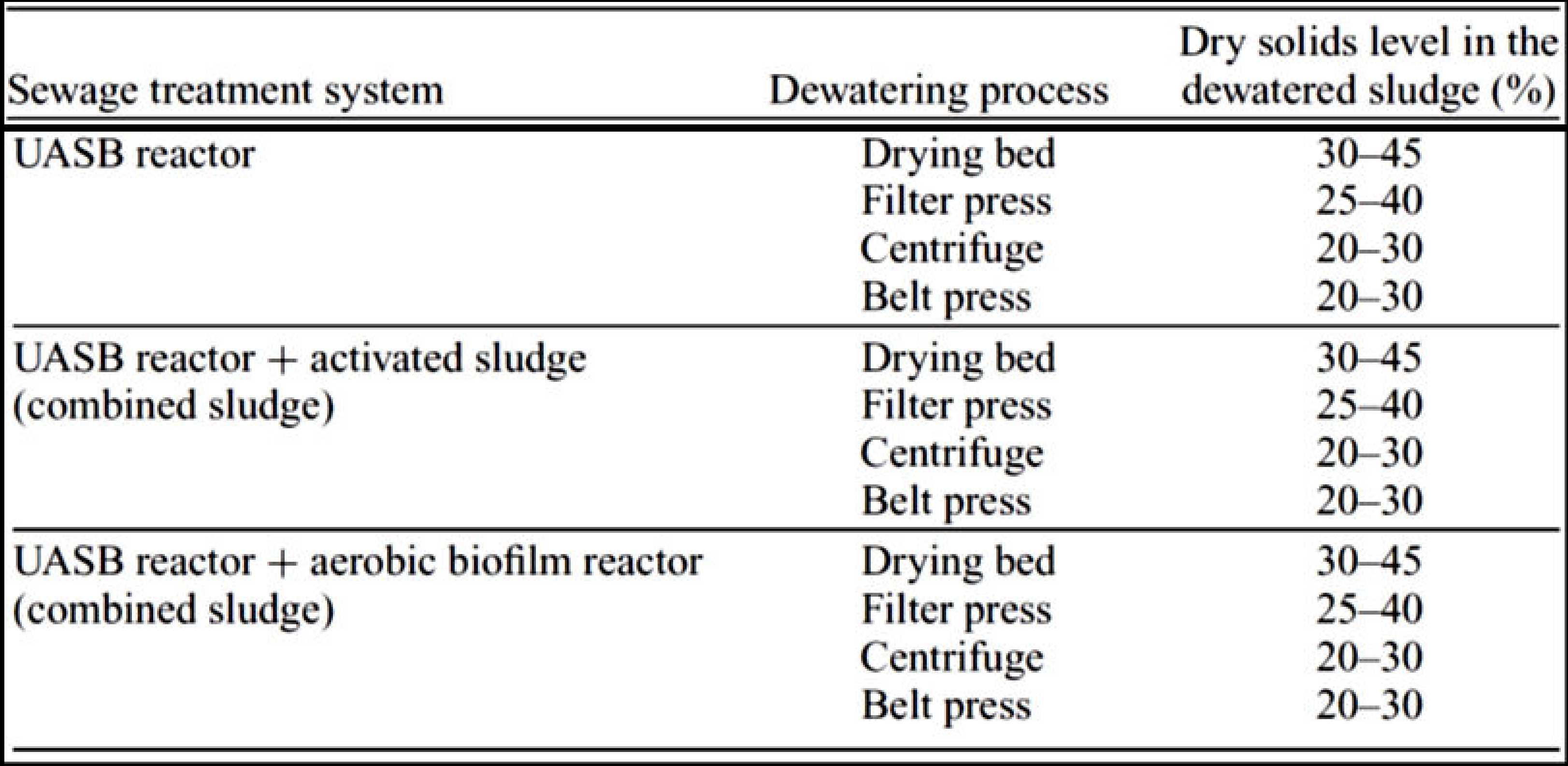
Sludge Dewatering

Typical dry solids levels in dewatered sludges from various wastewater treatment processes



Sewage treatment system	Dewatering process	Dry solids level in the dewatered sludge (%)
UASB reactor	Drying bed	30–45
	Filter press	25–40
	Centrifuge	20–30
	Belt press	20–30
UASB reactor + activated sludge (combined sludge)	Drying bed	30–45
	Filter press	25–40
	Centrifuge	20–30
	Belt press	20–30
UASB reactor + aerobic biofilm reactor (combined sludge)	Drying bed	30–45
	Filter press	25–40
	Centrifuge	20–30
	Belt press	20–30

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Sludge Dewatering

To increase the dewatering capability, the sludge can be submitted to a conditioning stage before dewatering.

The conditioning can be accomplished using chemical products or physical processes; heating or coagulants.

Sludge Dewatering

END

The main coagulants used are metallic salts and polyelectrolytes (polymers).

The most common metallic coagulants are:

- aluminium sulphate
- ferric chloride
- ferrous sulphate
- ferric sulphate
- quicklime/ hydrated lime

Environmental Biotechnology

Sludge Dewatering:
Sludge Drying Beds
& Lagoons

Sludge Drying Beds & Lagoons

Drying beds are one of the oldest techniques and very much used for solids-liquid separation in sludge. The construction costs are generally low in comparison with mechanical dewatering options.

The process generally has a rectangular tank with concrete walls and a concrete bottom.

Sludge Drying Beds & Lagoons

On the inside of the tank are the following devices to drain the water present in the sludge:

- support layer (bricks and coarse sand), on top of which the sludge is placed
- draining medium (fine to coarse sand followed by fine to coarse gravel)
- drainage system (open or perforated pipes)

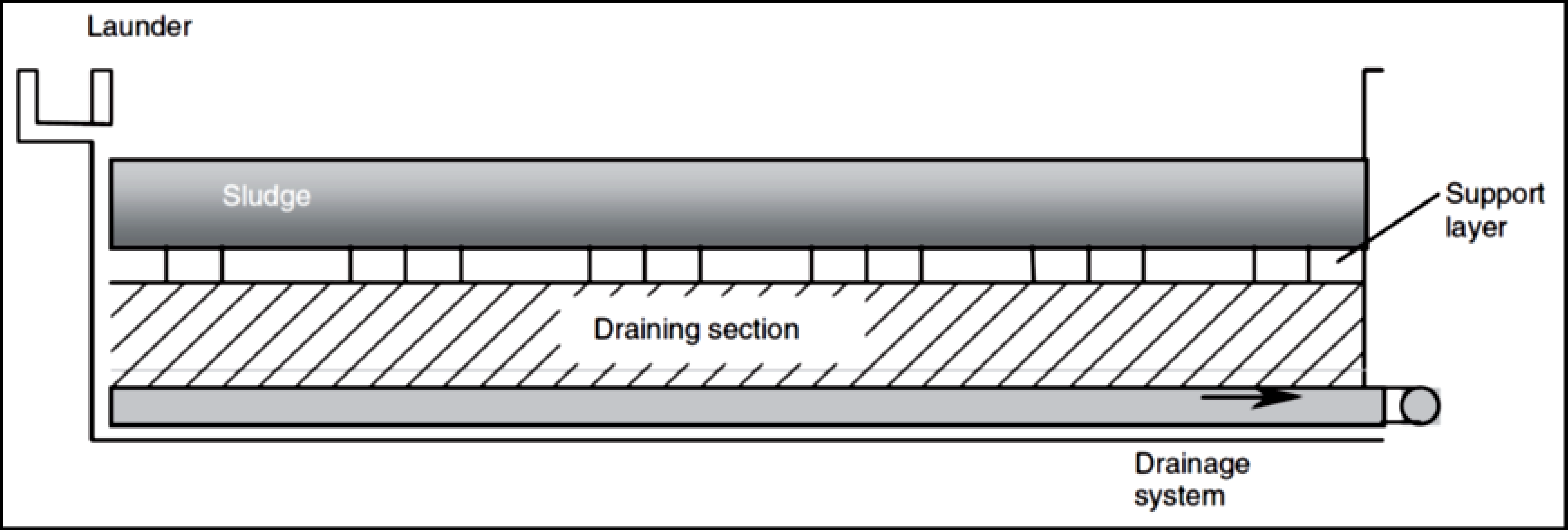
Sludge Drying Beds & Lagoons

Part of the liquid evaporates and part percolates through the sand and support layer.

The dewatered sludge stays in the layer above the sand.

Sludge Drying Beds & Lagoons

Schematics of a sludge drying bed (longitudinal section)



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Sludge Drying Beds & Lagoons

Sludge drying lagoons are used for thickening, complementary digestion, dewatering and even for the final disposal of sewage sludge.

Sludge Drying Beds & Lagoons

Drying lagoons are generally excavated in the soil, located in natural depressions in the land, where the discarded sludge from the WWTPs is accumulated for prolonged time periods (from 3 to 5 years).

Sludge Drying Beds & Lagoons

During this period, the sludge is thickened by the action of gravity, further digested by the microorganisms present in the sludge, and dewatered through drainage of the free water and evaporation.

Sludge Drying Beds & Lagoons

The process is only recommended for dewatering previously digested sludge by aerobic or anaerobic methods, and not for use in the dewatering of primary or mixed sludge.

Among the natural dewatering processes, the sludge lagoons are much less used than drying beds.

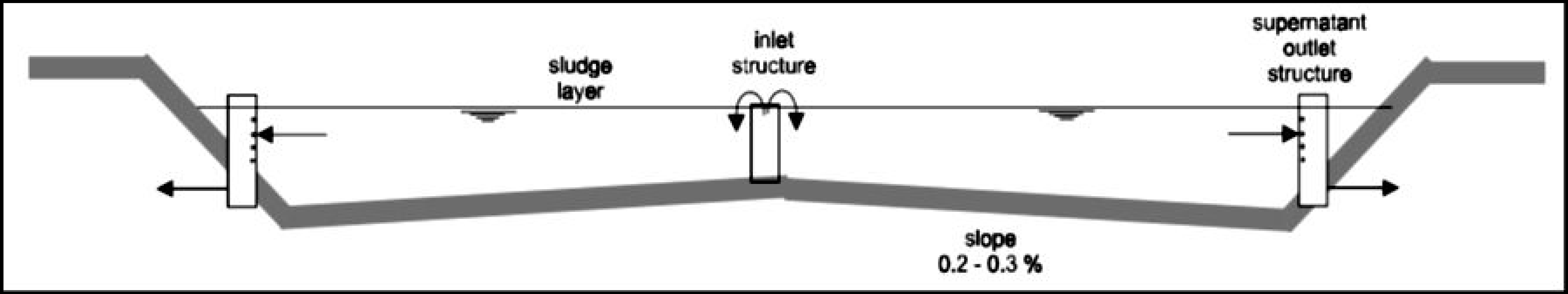
Sludge Drying Beds & Lagoons

The main difference between this process and the drying beds resides in the fact that evaporation is the principal mechanism of influence in the dewatering process. Percolation has a lesser effect than in the drying beds.

The use of drains at the bottom is not common practice in drying lagoons…

Sludge Drying Beds & Lagoons

Schematics of a sludge drying lagoon



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Sludge Drying Beds & Lagoons

When the lagoon is full, it can be put out of operation without the removal of the sludge, thus serving as a solution for final disposal.

Another possibility is the removal of the sludge from the full lagoon, allowing its reuse and utilisation as a continuous dewatering unit.

END

Environmental Biotechnology

Sludge Dewatering:
Centrifuge

Sludge Dewatering: Centrifuge

Centrifugation is a solids/liquid separation operation forced by the action of a centrifugal force.

Sludge Dewatering: Centrifuge

In first stage, the sludge particles settle at a velocity much higher than would occur under the action of gravity.
In second stage, compaction occurs when the sludge loses part of the capillary water under the prolonged action of centrifugation.

The cake is removed from the process.

Sludge Dewatering: Centrifuge

Centrifuges are equipment that may be used indistinctly for sludge thickening and dewatering.

The operating principle is the same…

It is possible to install the centrifuges in series, the first for the thickening of the sludge and the second for the dewatering.

Sludge Dewatering: Centrifuge

The main types:
vertical and horizontal-shaft centrifuges.

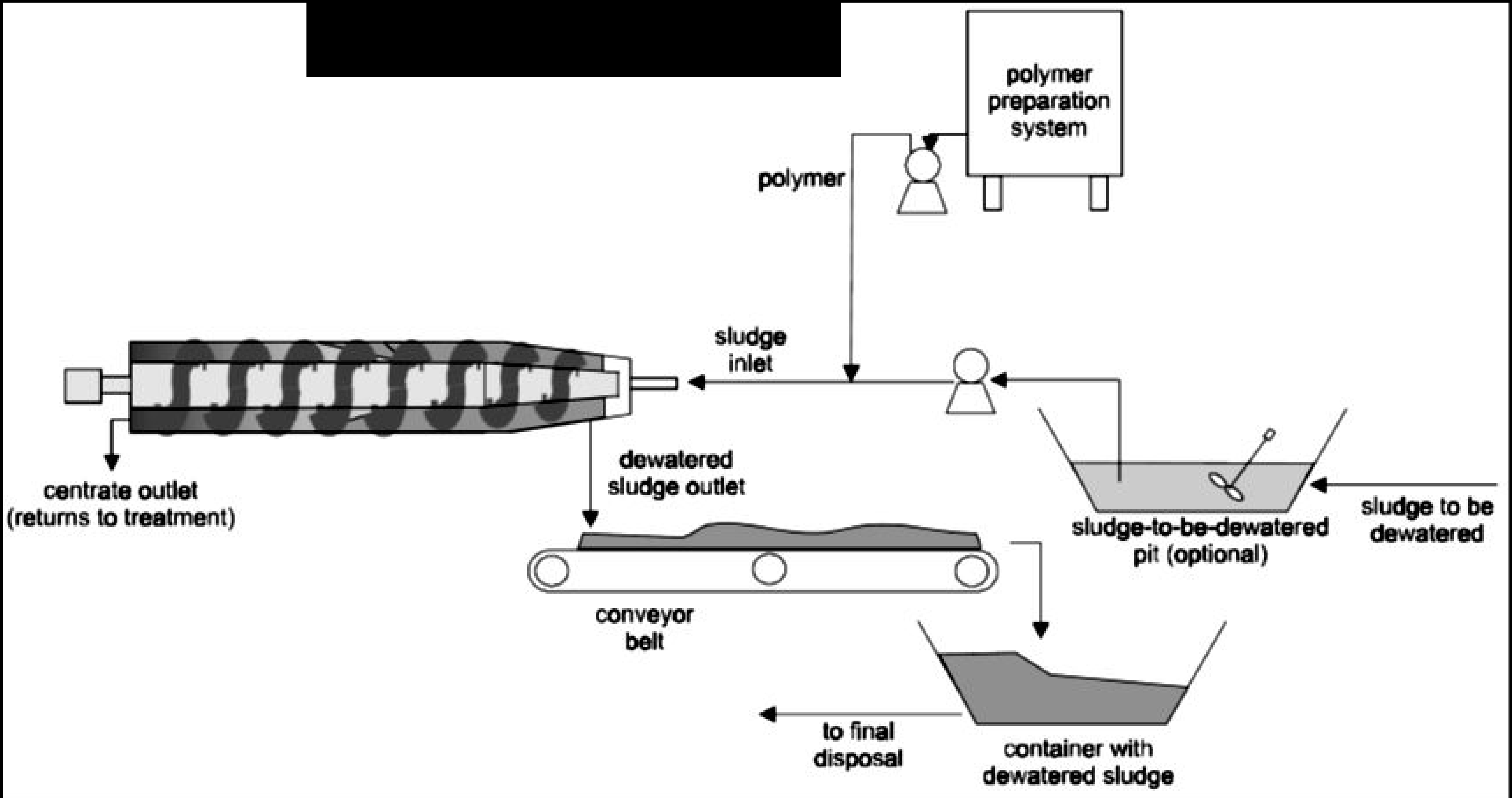
Main differences:

- type of feeding of the sludge,
- intensity of the centrifugal force, and the manner in which cake and liquid are removed.

Horizontal-shaft centrifuges are mostly used…

Sludge Dewatering: Centrifuge

Typical set up of a decanter-type centrifuge



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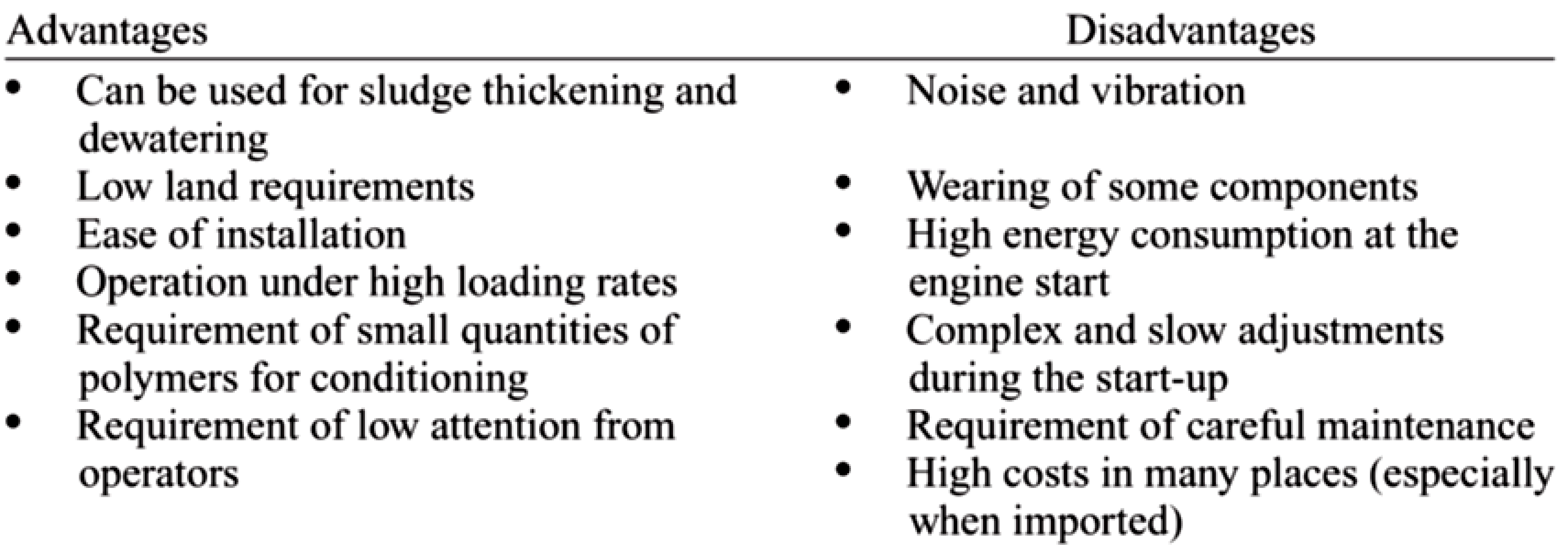
Sludge Dewatering: Centrifuge

Advantages and disadvantages of horizontal shaft centrifuges



Advantages	Disadvantages
• Can be used for sludge thickening and dewatering	• Noise and vibration
• Low land requirements	• Wearing of some components
• Ease of installation	• High energy consumption at the engine start
• Operation under high loading rates	• Complex and slow adjustments during the start-up
• Requirement of small quantities of polymers for conditioning	• Requirement of careful maintenance
• Requirement of low attention from operators	• High costs in many places (especially when imported)

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Environmental Biotechnology

Sludge Disinfection: Composting and Thermophilic aerobic digestion

Sludge Disinfection

The objective is to guarantee a low level of pathogens in the sludge...

However, the need to include a complementary sludge disinfection system will depend on the final disposal alternative to be adopted.

Sludge Disinfection

The application of sludge in public parks and gardens or its recycling in agriculture implies a higher sanitary level than other disposal alternatives, such as landfills.

Sludge Disinfection

Some stabilisation processes of the organic matter in the sludge also lead to a reduction of pathogenic microorganisms. Others reduce the pathogenic microorganisms to levels lower after the stabilisation of the organic matter, in a complementary sludge treatment stage.

Sludge Disinfection

The most important ones are:

a) Composting,
b) Thermophilic aerobic digestion
c) Lime stabilization,
d) Pasteurisation,
e) Thermal treatment.

Sludge Disinfection

Composting:

Composting is an aerobic organic matter decomposition process that is achieved through controlled conditions of temperature, water content, oxygen and nutrients.

The resultant product (compost) has a high agricultural value as a soil conditioner.

Sludge Disinfection

Composting:
The inactivation of the pathogenic microorganisms occurs mainly by the increase of temperature.

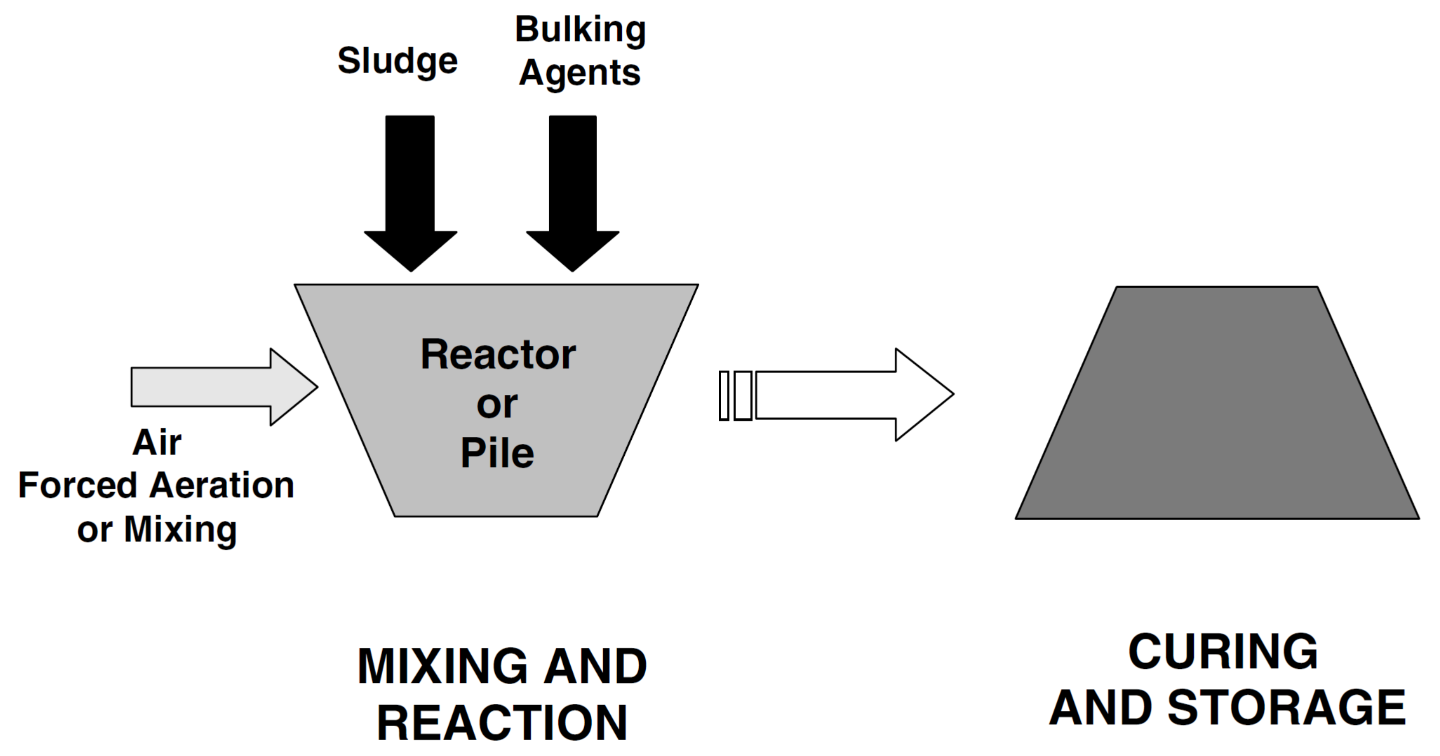
The temperature reaches values between 55 and 65 ◦C by means of biochemical reactions.

Sludge Disinfection

Composting:
Materials such as wood chips, leaves, green waste, rice straw, sawdust, or other structuring agents need to be added to the sludge to improve the water retention, increase the porosity, and balance the ratio between carbon and nitrogen.

Sludge Disinfection

Flowsheet of the composting process



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Sludge Disinfection

Composting can be carried out according to the three methods:

- Windrows: Periodical turning to allow aeration and mixture (50-90 days).
- Aerated static pile: Aeration by air blowers or exhausting systems (30-60 days).
- In-vessel biological reactors: Closed systems, with a greater control (14-21 days).

Sludge Disinfection

Thermophilic aerobic digestion (also called autothermal digestion)

Follows the same principles as the conventional aerobic digestion system.

The difference is that it operates in a thermophilic phase.

Sludge Disinfection

END

Thermophilic aerobic digestion

Two aerobic stages...

Since the reaction volume is small, the system is closed and the concentration of solids in the sludge is higher, the heat released from the aerobic reactions heats the sludge to temperatures higher than 50 ◦C in the first stage and 60 ◦C in the second.

Environmental Biotechnology

Sludge Disinfection: Lime stabilization, Pasteurisation and Thermal treatment

Sludge Disinfection

Lime stabilization is used to treat primary, secondary, or digested sludge.

Addition of lime increases the pH to 12, a reduction of microorganisms (including pathogens) as well as the potential occurrence of odours takes place.

Sludge Disinfection

Lime stabilization

Lime can be added to liquid or dewatered sludges.

Owing to the addition of lime, the quantity of sludge to be disposed of increases.

Sludge Disinfection

Pasteurisation involves heating of the sludge to 70 ◦C for 30 minutes, followed by a rapid cooling to 4 ◦C.

The sludge can be heated by heat exchangers or by hot steam injection.

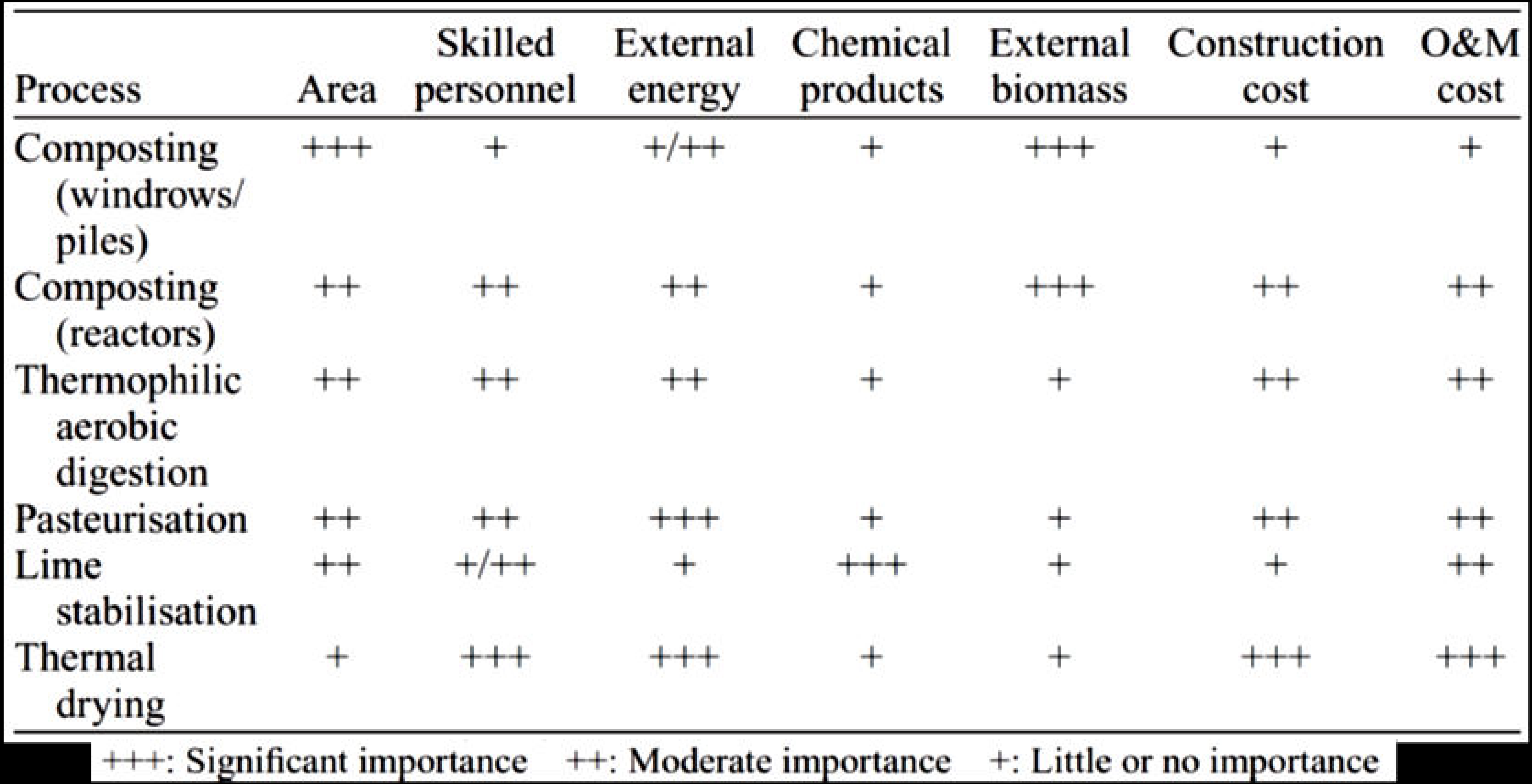
Sludge Disinfection

Thermal treatment consists of passing the sludge through a heat source that causes the evaporation of the existing moisture in the sludge and consequently the thermal inactivation of the microorganisms.

To be economically feasible, the sludge needs to be previously digested and dewatered.

Sludge Disinfection

Comparison between sludge disinfection technologies – Implementation



Process	Area	Skilled personnel	External energy	Chemical products	External biomass	Construction cost	O&M cost
Composting (windrows/ piles)	+++	+	+/++	+	+++	+	+
Composting (reactors)	++	++	++	+	+++	++	++
Thermophilic aerobic digestion	++	++	++	+	+	++	++
Pasteurisation	++	++	+++	+	+	++	++
Lime stabilisation	++	+/++	+	+++	+	+	++
Thermal drying	+	+++	+++	+	+	+++	+++

+++: Significant importance ++: Moderate importance +: Little or no importance

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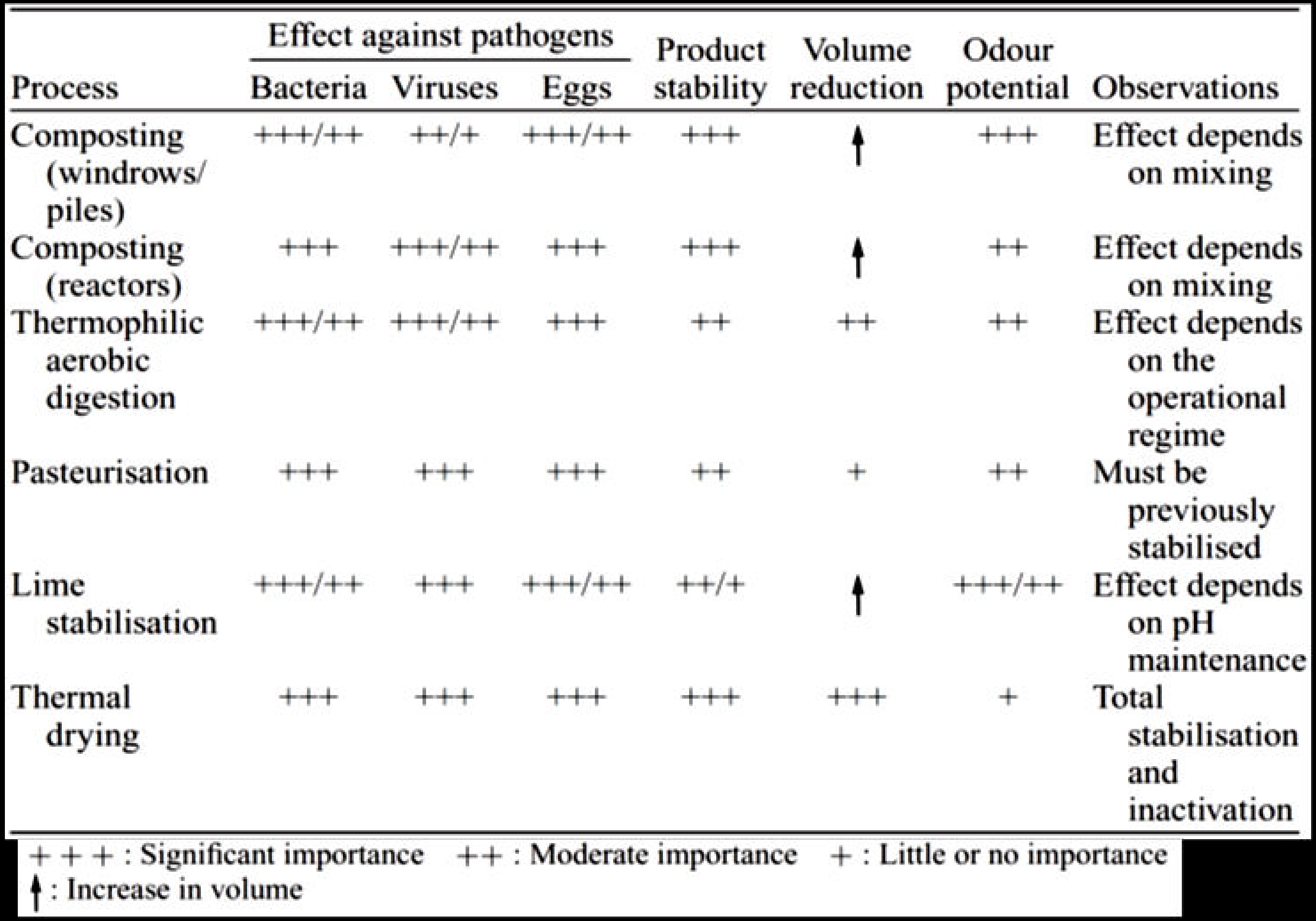
Sludge Disinfection

Comparison between sludge disinfection technologies – Operation



Process	Effect against pathogens			Product stability	Volume reduction	Odour potential	Observations
	Bacteria	Viruses	Eggs				
Composting (windrows/ piles)	+++/++	++/+	+++/++	+++	↑	+++	Effect depends on mixing
Composting (reactors)	+++	+++/++	+++	+++	↑	++	Effect depends on mixing
Thermophilic aerobic digestion	+++/++	+++/++	+++	++	++	++	Effect depends on the operational regime
Pasteurisation	+++	+++	+++	++	+	++	Must be previously stabilised
Lime stabilisation	+++/++	+++	+++/++	++/+	↑	+++/++	Effect depends on pH maintenance
Thermal drying	+++	+++	+++	+++	+++	+	Total stabilisation and inactivation

+ + + : Significant importance ++ : Moderate importance + : Little or no importance
↑ : Increase in volume

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Environmental Biotechnology

Final Disposal of the Sludge

Final Disposal of the Sludge

Main final disposal alternatives for the sludge:

- Ocean disposal,
- Incineration,
- Sanitary landfill,
- Landfarming,
- Land reclamation,
- Agricultural reuse.

Final Disposal of the Sludge

Ocean disposal:
After pre-conditioning, the sewage is disposed in the sea, through ocean outfalls or barges.

Disposal without beneficial uses.

Final Disposal of the Sludge

Ocean disposal:

Advantages:
- Low cost

Disadvantages:
- Ocean water, flora and fauna pollution

Final Disposal of the Sludge

Incineration:

Thermal decomposition process by oxidation, in which the volatile solids of the sludge are burnt in the presence of oxygen and are converted into carbon dioxide and water.
The fixed solids are transformed into ashes.

Disposal without beneficial uses.

Final Disposal of the Sludge

Incineration:

Advantages:

- Drastic volume reduction,
- Sterilisation.

Disadvantages:

- High costs,
- Ash disposal,
- Atmospheric pollution.

Final Disposal of the Sludge

Sanitary landfill:
Disposal of the sludges in ditches or trenches, with compaction and covering with soil, after which they are sealed. (can be co-disposed with urban solid wastes).

Disposal without beneficial uses.

Final Disposal of the Sludge

Sanitary landfill:

Advantages:

- Low cost

Disadvantages:

- Requirement of large areas,
- Problems with locations near urban centres,
- Requirement of special soil characteristics,
- Gas and leachate production,
- Difficulty in reintegrating the area after decommissioning.

Final Disposal of the Sludge

Landfarming:

Land disposal process, in which the organic substrate is biologically degraded in the upper layer of the soil and the inorganic fraction is transformed or fixed into this layer.

Disposal without beneficial uses.

Final Disposal of the Sludge

Landfarming:

Advantages:

- Low cost
- Disposal of large volumes per unit area

Disadvantages:

- Accumulation hardly decaying constituents…,
- Possible groundwater contamination,
- Odour release and vector attraction,
- Difficulty in reintegrating the area after decommissioning

Final Disposal of the Sludge

Land reclamation:

Disposal of sludge in areas that have been drastically altered, such as mining areas, where the soil does not offer conditions for development and fixation of vegetation, as a result of the lack of organic matter and nutrients.

Final Disposal of the Sludge

Land reclamation:

Advantages:

- High application rates,
- Positive results for the recovery of the soil and flora.

Disadvantages:

- Odours,
- Composition and use limitations,
- Contamination of the groundwater, fauna and flora.

Final Disposal of the Sludge

Agricultural reuse:

Disposal of the sludge in agricultural soils, in association with the development of crops. Beneficial use of the sludge (which, in this case, is named as a biosolid).

Final Disposal of the Sludge

Agricultural reuse:

Advantages:

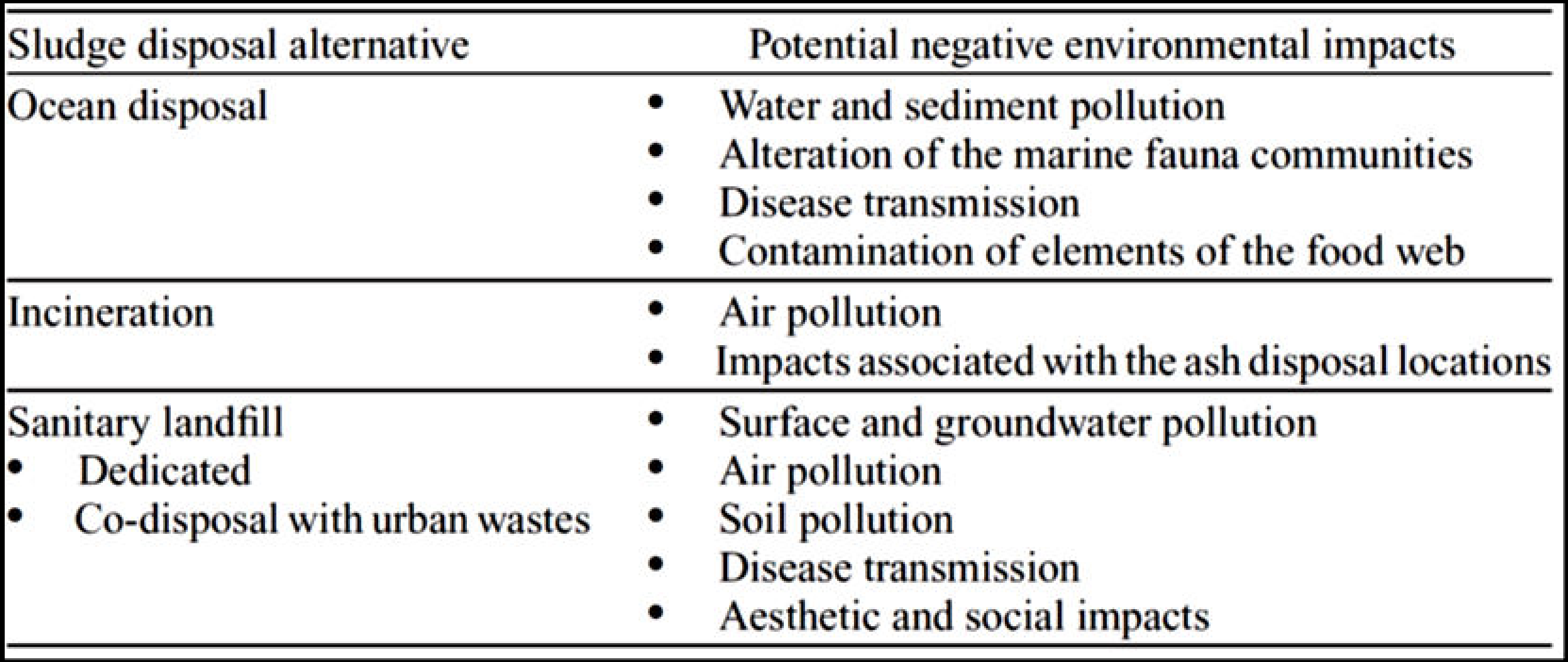
- Large area availability,
- Positive effects on soil,
- Long term solution,
- Potential as a fertilizer,
- Positive outcome for crops.

Disadvantages:

- Limitations regarding composition and application,
- Contamination of the soil by metals,
- Food contamination with toxic elements pathogens
- Odours.

Final Disposal of the Sludge

Environmental impacts related to the different sludge disposal alternatives



Sludge disposal alternative	Potential negative environmental impacts
Ocean disposal	• Water and sediment pollution • Alteration of the marine fauna communities • Disease transmission • Contamination of elements of the food web
Incineration	• Air pollution • Impacts associated with the ash disposal locations
Sanitary landfill • Dedicated • Co-disposal with urban wastes	• Surface and groundwater pollution • Air pollution • Soil pollution • Disease transmission • Aesthetic and social impacts

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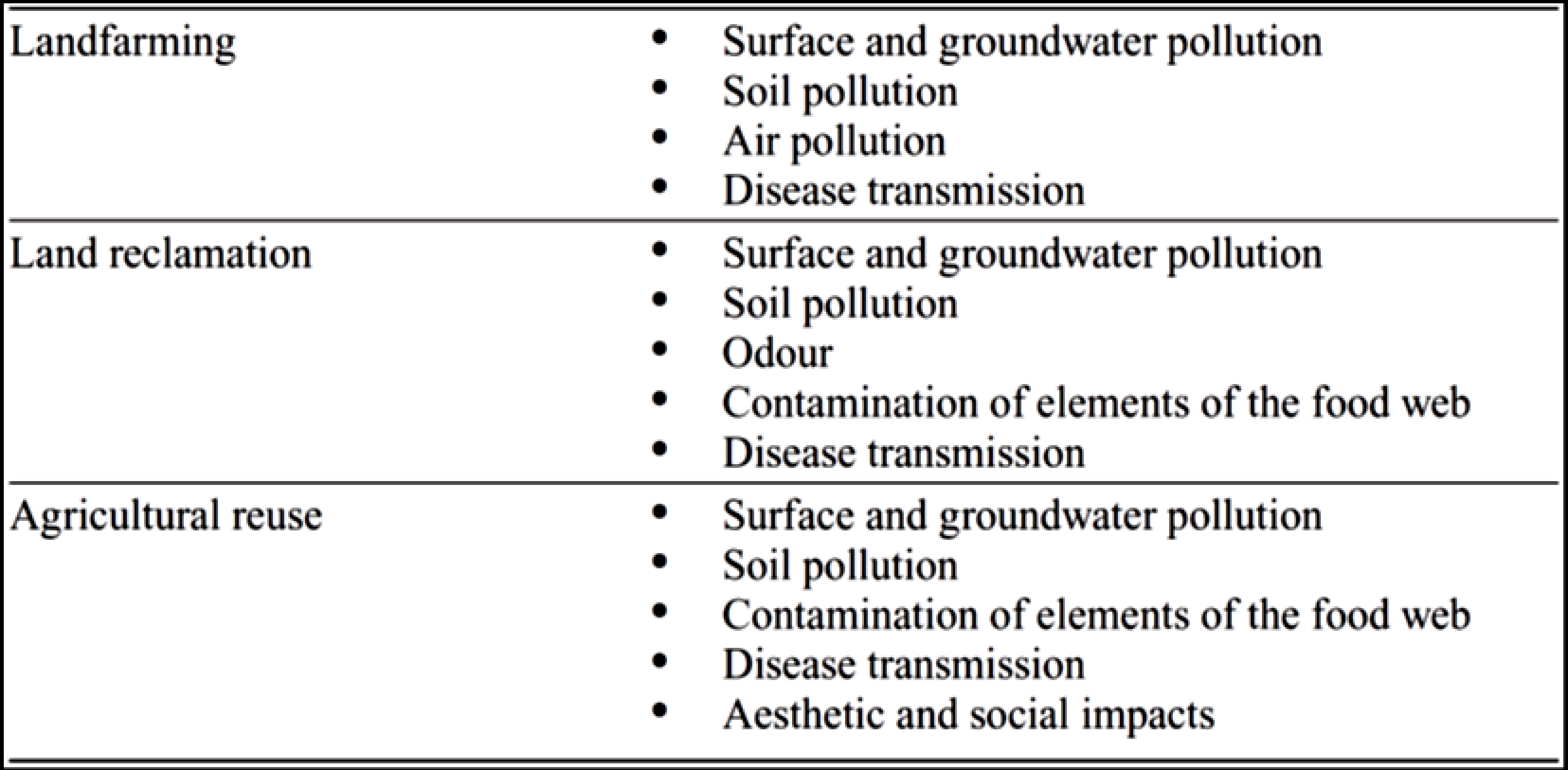
Final Disposal of the Sludge

Environmental impacts related to the different sludge disposal alternatives



Landfarming	• Surface and groundwater pollution • Soil pollution • Air pollution • Disease transmission
Land reclamation	• Surface and groundwater pollution • Soil pollution • Odour • Contamination of elements of the food web • Disease transmission
Agricultural reuse	• Surface and groundwater pollution • Soil pollution • Contamination of elements of the food web • Disease transmission • Aesthetic and social impacts

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Environmental Biotechnology

Contaminated Land
and
Bio-Remediation

Contaminated Land and Bio-Remediation

Contaminated land is another example of a widely appreciated, yet often poorly understood, environmental problem, in much the same way as pollution.

One is simply the manifestation of the other.

Contaminated Land and Bio-Remediation

The importance of land remediation lies in two spheres:
Firstly, environmental legislation is becoming increasingly stringent...
Secondly, as the pressure grows to redevelop old, unused or derelict so-called ‘brown-field’ sites, rather than develop previously untouched ‘greenfield’.

Contaminated Land and Bio-Remediation

A number of technologies are available to achieve such a clean-up, of which bio-remediation, in its many individual forms, is only one.

For some instances of contamination, non-biological methods of remediation may be the best practicable environmental option (BPEO).

Contaminated Land and Bio-Remediation

The idea of ‘contaminated land’ is the presence of substances which, when present in sufficient quantity or concentration, are likely to cause harm to the environment or human health and living beings.

Contaminated Land and Bio-Remediation

Many kinds of sites may give rise to possible contamination concerns, such as asbestos works, chemical works, garages and service stations, gas works, incinerators, iron and steel works, metal fabrication shops, paper mills, tanneries, textile plants, timber treatment plants, railway yards and waste disposal sites, etc.

Contaminated Land and Bio-Remediation

The motive force of land remediation is a largely commercial one.

This imposes its own set of conditions and constraints.

Remedies are normally sought only when and where there is unacceptable risk to human health, the environment and occasionally to other vulnerable targets.

Contaminated Land and Bio-Remediation

The actual remedies to be employed will be based on a realistic set of priorities and will be related to the risk posed.

This, of course will require adequate investigation and risk assessment to determine.

Contaminated Land and Bio-Remediation

Since the move to remediate is essentially commercial, only land for which remediation is either necessary or worthwhile will tend to be treated and then to a level which either makes it suitable for its intended use or brings it to a condition which no longer poses an unacceptable risk.

Contaminated Land and Bio-Remediation

In purely economic terms, remediation will only take place when one or more of the driving forces becomes sufficiently compelling to make it unavoidable. It will also tend towards the minimum acceptable standard necessary to achieve the required clean-up.

Contaminated Land and Bio-Remediation

The goal of treating land is to make it suitable for a particular purpose or so that it no longer poses unacceptable risk and once the relevant aim has been achieved, further treatment is typically not a good use of these resources.

Contaminated Land and Bio-Remediation

END

Sustainable Remediation Forum (SuRF) UK framework document identified six ‘key principles of sustainable remediation’, namely:

1. Protection of human health and the wider environment.
2. Safe working practices.
3. Consistent, clear and reproducible evidence-based decision making.
4. Record keeping and transparent reporting.
5. Good governance and stakeholder involvement.
6. Sound science.

Environmental Biotechnology

Remediation methods

Remediation methods

The currently available processes for soil remediation can be divided into five generalised categories:

- Biological,
- Chemical,
- Physical,
- Solidification / vitrification and
- Thermal.

Remediation methods

Biological:

This involve the transformation or mineralisation of contaminants to less toxic and more mobile, forms.

This can include fixation or accumulation in harvestable biomass crops.

Remediation methods

Biological:

Advantages:

- can destroy a wide range of organic compounds,
- can cause benefit to soil structure and fertility,
- are non-toxic.

Disadvantages:

- the process end-point can be uncertain and difficult to gauge,
- treatment can be slow
- not all contaminants are conducive to treatment by biological means.

Remediation methods

Chemical:
Toxic compounds are destroyed, fixed or neutralised by chemical reaction.

Remediation methods

Chemical:

Advantages:

- Destruction of biologically recalcitrant chemicals is possible
- toxic substances can be chemically converted to either more or less biologically available ones.

Disadvantages:

- Possible incomplete treatment,
- The reagents necessary may themselves cause damage to environment...

Remediation methods

Physical:

Physical removal of contaminated materials, often by concentration and excavation, for further treatment or disposal.

Not truly remediation.

Remediation methods

Physical:

Advantages:

- Purely physical approach with no reagent addition,
- Significantly reduces the risk of secondary contamination.

Disadvantages:

- Contaminants are not destroyed.
- The concentration achieved inevitably requires containment measures and further treatment.

Remediation methods

Solidification / vitrification:

It is the encapsulation of contaminants within a monolithic solid of high structural integrity, with or without associated chemical fixation.

Vitrification uses high temperatures to fuse contaminated materials.

Remediation methods

Solidification / vitrification:

Advantage:

Toxic elements and/or compounds which cannot be destroyed, are rendered unavailable to the environment.

Disadvantages:

- Contaminants are not actually destroyed.
- Significant amounts of reagents are required and not suitable for organic contaminants.

Remediation methods

Thermal: Contaminants are destroyed by heat treatment, using incineration, gasification, pyrolysis or volatisation processes.

Remediation methods

Thermal:

Advantages:

Contaminants are most effectively destroyed.

Disadvantages:

- Very high energy cost.
- Unsuitable for most toxic elements (generation of new pollutants).
- Soil organic matter, and, thus, at least some of the soil structure itself, is destroyed.

END

Environmental Biotechnology

In situ and *Ex situ* techniques

In situ and *Ex situ* techniques

A common way in which all forms of remediation are often characterized is as *in situ* or *ex situ* approaches.

These represent largely artificial classes, based on where the treatment takes place – on the site or off it.

In situ and *Ex situ* techniques

In situ:

The major benefit is the low site disturbance, which enables existing buildings and features to remain undisturbed.

They also avoid delays with methods requiring excavation and removal, while additionally reducing the risk of spreading contamination and the likelihood of exposing workers to volatiles.

In situ and *Ex situ* techniques

In situ:

These methods are suited to instances where the contamination is widespread throughout, and often at some depth within, a site, and of low to medium concentration.

Relatively slow to act, they are of most use when the available time for treatment is not restricted.

In situ and *Ex situ* techniques

In situ:

Disadvantages:

Stringent requirement for thorough site investigation and survey.

Since reaction conditions are not readily controlled, the supposed process ‘optimisation’ may, in practice, be less than optimum.

In situ and *Ex situ* techniques

Ex situ:

The main characteristic of *ex situ* methods is that the soil is removed from where it originally lay, for treatment.

In situ and *Ex situ* techniques

Ex situ:
These techniques are best suited to instances of relatively localized pollution within a site, typically in ‘hot-spots’ of medium to relatively high concentration which are fairly near to the surface.

In situ and *Ex situ* techniques

Ex situ:

Benefits:

- conditions are more readily optimized,
- process control is easier, monitoring is more accurate,
- simpler to achieve,
- introduction of specialist organisms is safer,
- faster than corresponding *in situ* techniques.

In situ **and** ***Ex situ*** **techniques**

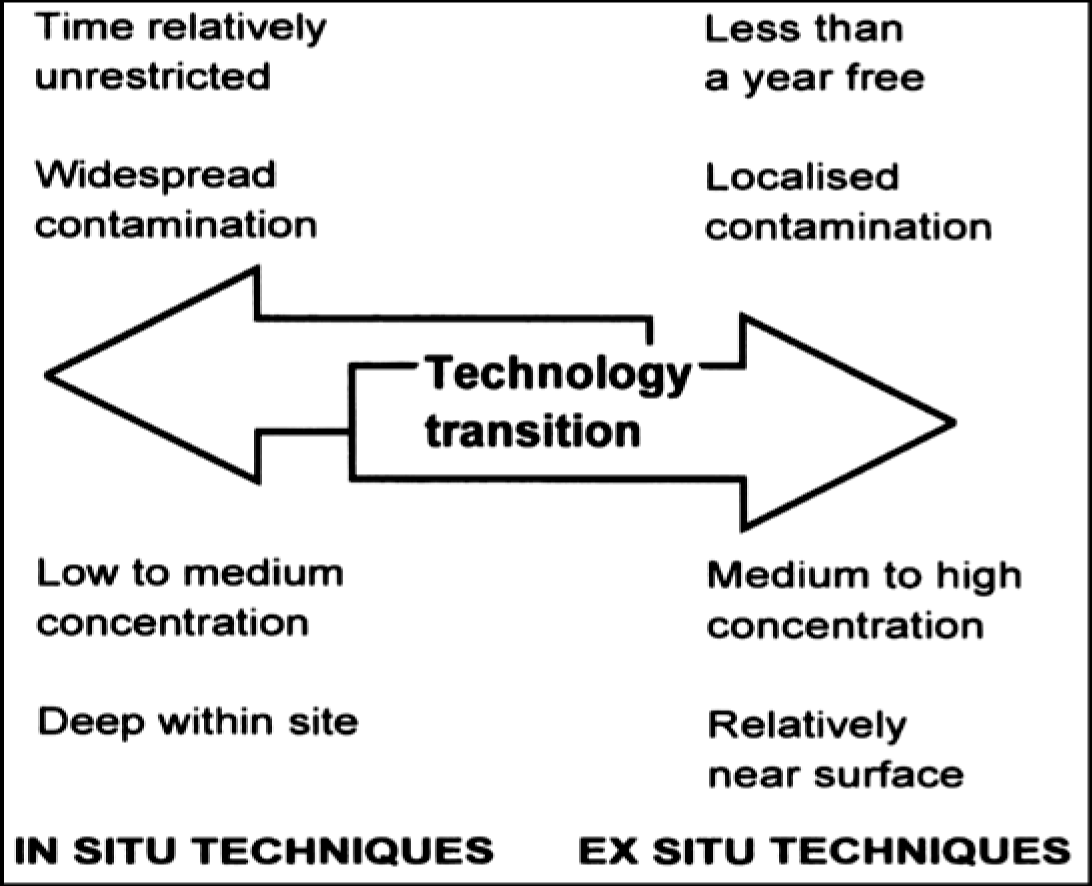
Ex situ:

Disadvantages:

- additional transport costs,
- increased likelihood of spillage,
- potential secondary pollution,
- require a supplementary area of land for treatment,
- more expensive than *in situ* techniques.

In situ and *Ex situ* techniques

Factors affecting technology suitability



Environmental Biotechnology Theory and application, 2nd Ed, By Gareth

Environmental Biotechnology

Intensive and Extensive Technologies

Intensive and Extensive Technologies

Intensive technologies can be characterized as fast acting, high intervention strategies, with a heavy demand for resources and high initiation, running and support costs.

Their key factors are a fast response and low treatment time, which makes them excellent for heavy contamination conditions.

E.g., Soil washing and thermal treatments.

Intensive and Extensive Technologies

Extensive methods are lower-level interventions, typically slower acting, based on simpler technology, with a smaller resource requirement and lower initiation, running and support costs.

These technologies have a slower response and a higher treatment time, but their lower costs make wider application possible, less damage to soil quality.

Intensive and Extensive Technologies

Extensive methods

Accordingly, they are well suited to large scale treatment where speed is not of the essence.
E.g., composting, promotion of biological activity *in situ* within the root-zone, precipitation of metal sulphides under anaerobic conditions and the cropping of heavy metal accumulator plants.

END

Environmental Biotechnology

Bioremediation
Documentary

Bioremediation Documentary

https://www.youtube.com/watch?v=fkVyBBl6aQo

Environmental Biotechnology

The Suitability of Bioremediation

The Suitability of Bioremediation

Bioremediation… a biotechnological intervention for cleaning up the residual effects of human activities.

Relies on the inherent abilities and characteristics of indigenous bacteria, fungi or plant species.

The Suitability of Bioremediation

The biological mechanisms underlying the relevant processes are:

- biosorption,
- demethylation,
- methylation,
- metal-organic complexation,
- chelation,
- ligand degradation,
- oxidation,
- reduction.

The Suitability of Bioremediation

Microbes capable of utilizing a variety of carbon sources and degrading a number of typical contaminants are commonly found in soils.

By enhancing and optimizing conditions for them, they can be utilized to do what they do naturally, but more efficiently.

The Suitability of Bioremediation

Three major routes of Bioremediation:

- Mineralization,
- Co-metabolism,
- Immobilization.

The Suitability of Bioremediation

Mineralization:

The contaminant is used as a food source by the microbes and thus is metabolized, thereby being removed and destroyed either completely or incompletely…

The Suitability of Bioremediation

Co-metabolism:
The contaminant is not used as food by the microbe, being metabolized fortuitously alongside the organism’s food into a less hazardous chemical.
Typically, there is no apparent benefit to the microorganisms involved.

The Suitability of Bioremediation

Immobilization:
The removal of contaminants, typically metals, by means of adsorption or bio-accumulation by various microbes or plant species.

The Suitability of Bioremediation

END

Bioremediation is most suited to organic chemicals…
(e.g., herbicides, hydrocarbons, etc.)

…but it can also be effective in the treatment of certain inorganic substances...
(e.g., metals, radionuclides, etc.)
Under certain circumstances their speciation can be changed…

Environmental Biotechnology

Factors Affecting the Use of Bioremediation

Factors Affecting the Use of Bioremediation

Possible to divide these factors into two broad groups;

- those which relate to the character of the contaminant itself, and
- those which depend on environmental conditions.

Factors Affecting the Use of Bioremediation

Character of the contaminant:

- chemical nature of the pollutant,
- physical state of the pollutant at the site,
- it must be susceptible to, and readily available for, biological decomposition…,
- it must also be dissolved, or at the very least, in contact with soil water, and
- typically of a low–medium toxicity range.

Factors Affecting the Use of Bioremediation

Environmental Factors:

Most significant are:

Temperature…

- 0 and 50 ◦C. Most efficient: 20–30 ◦C

pH…

- pH range of 5.0-9.0, however, 6.0-8.0 being most efficient.

Soil type…

- sands and gravels are the most suitable soil types for bioremediation…

Factors Affecting the Use of Bioremediation

Other factors:

- Nutrient availability,
- oxygenation,
- presence of other inhibitory contaminants
- whether the site is contained or if the groundwater runs off,
- what contaminants are present, what is their concentration and whether they are biodegradable, etc.

Factors Affecting the Use of Bioremediation

The whole issue may be viewed as hierarchical.

The primary influence consists of the contaminants themselves and actual origin

Factors Affecting the Use of Bioremediation

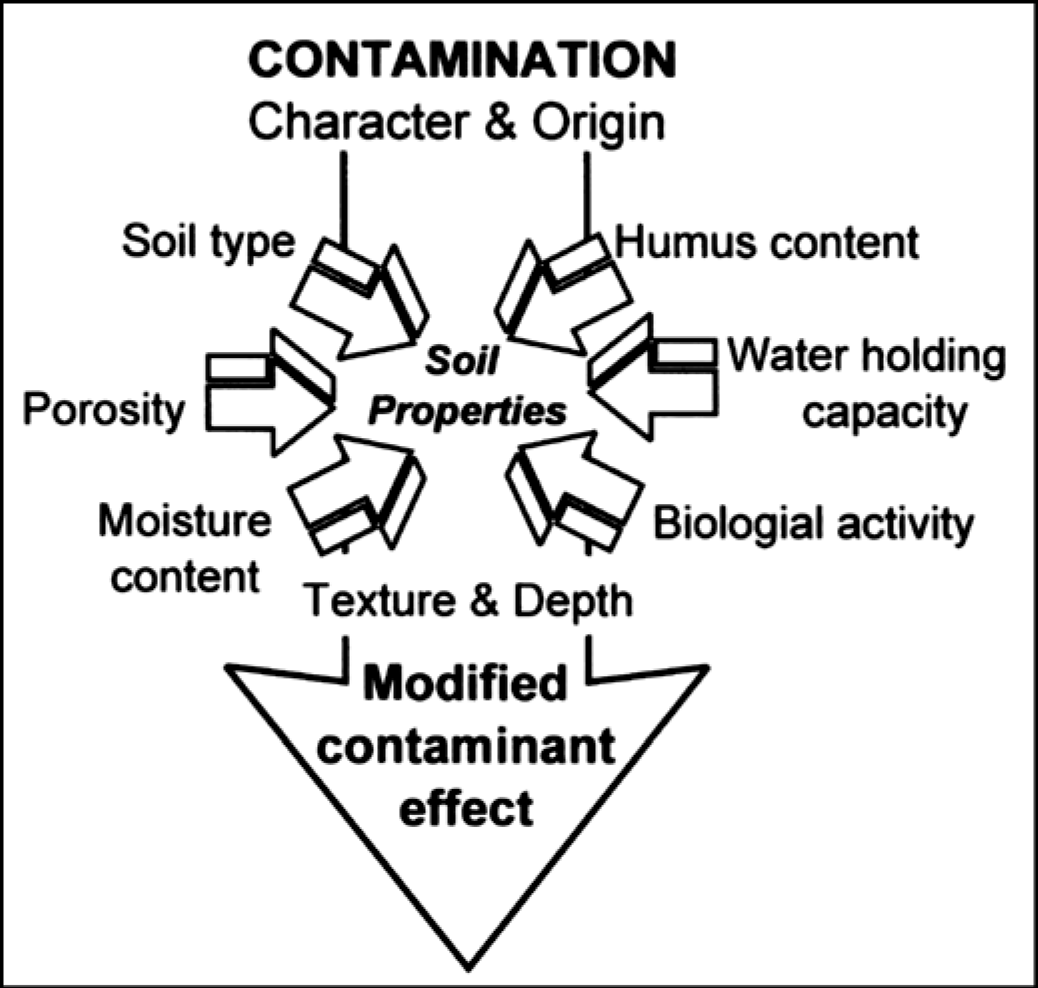
Edaphic factors such as

- soil type,
- depth,
- porosity,
- texture,
- moisture content,
- water-holding capacity,
- humus content, and biological activity

may all interact with the primary influences, and/or with each other.

Factors Affecting the Use of Bioremediation

Modification of effect by edaphic factors



Wastewater Characteristics, Treatment and Disposal, Vol 1, By Marcos

Factors Affecting the Use of Bioremediation

END

Which technique is the most appropriate?

This is a site-specific issue…

Decision is made on the basis of the edaphic matters, proper risk-assessment, characterization and quantification of resident contamination, etc.

Next is formulation of action plan…

Approval from the relevant regulatory bodies…

Implementation of the remediation work itself…

Environmental Biotechnology

Biotechnology
Selection

Biotechnology Selection

Effective biological treatments: 'natural attenuation', 'passive remediation', 'bioattenuation' or 'intrinsic remediation'…

The approach works with natural cycles and the preexisting indigenous microbial community to bring about the required treatment.

Biotechnology Selection

If natural attenuation is not appropriate, then some form of engineered response is required…

.

Biotechnology Selection

- Type and concentration of the contamination,
- its scale,
- level of risk,
- intended eventual site use,
- time available for remediation,
- available space and resources, and
- any site specific issues…

all influence this decision.

Biotechnology Selection

- All biotechnology treatments have certain central similarities.
- The majority of applications make use of indigenous microbes, though sometimes specialized organisms may be added.
- Thus, the functional biology may be described as a process of bioenhancement or bioaugmentation, or a mixture of both.

Biotechnology Selection

Bioenhancement concentrates solely on the existing micro-fauna, stimulating their activity by the manipulation of local environmental conditions.

Bioaugmentation requires the deliberate introduction of selected microbes to bring about the required clean-up.

Biotechnology Selection

Additions may be

- unmodified ‘wildtype’ organisms,
- a culture selectively acclimatized to the particular conditions to be encountered, or
- genetically engineered to suit the requirements.

Enzyme or other living system extracts may also be used…

Biotechnology Selection

In the final analysis, all biological approaches are expressly designed to optimize the activities of the various micro-organisms (either native to the particular soil or artificially introduced) to bring about the desired remediation.

Biotechnology Selection

END

This generally means letting them do what they would naturally do, but enhancing their performance to achieve it more rapidly and/or more efficiently…

Environmental Biotechnology

In situ techniques

In situ techniques

This involves introducing oxygen and nutrients to the contaminated area by various methods, all of which ultimately work by modifying conditions within the soil or groundwater.

In situ techniques

Three major techniques commonly employed, namely:

- Biosparging,
- Bioventing, and
- Injection Recovery.

In situ techniques

Biosparging:
a technique used to remediate contamination at, or below, the water table boundary.

The process involves super-aeration of the groundwater, thereby stimulating accelerated contaminant biodegradation.

In situ techniques

Biosparging:

- Air is introduced into the contaminated area and forms bubbles in the groundwater.
- This extra oxygen dissolves into the water, also increasing the aeration of the overlying soil.
- This leads to a speeding up of the natural ability of resident microbes to metabolize the pollutant.

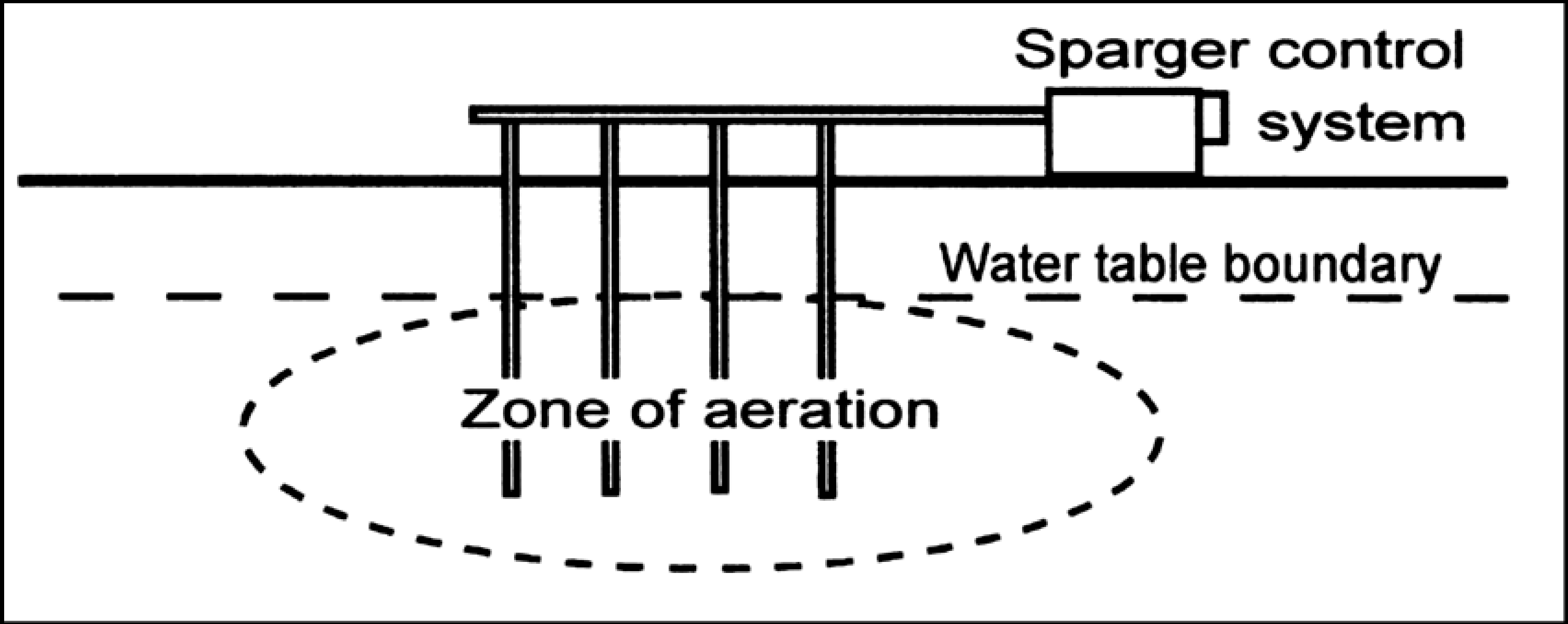
In situ techniques

Biosparging:

- Method of delivery can range from relatively simple to the more complicated.
- Typically the sparger control system consists of a pressure gauge and relief valve to vent excess air pressure.
- Sophisticated versions can also include data loggers, telemetry equipment and remote control systems.

In situ techniques

Biosparging



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In situ techniques

Bioventing:

Bioventing is a technique used to remediate contamination above the water table boundary.

This process also involves super-aeration, though this time it is taking place within the soil itself, instead of the groundwater.

In situ techniques

Bioventing:
The extra oxygen availability thus achieved, as in the previous approach described, stimulates the resident microbes, which then treat the polluting substances.

In situ techniques

Bioventing:

Volatile compounds are often mobilized during processing and thus more easily extracted. However, in many practical applications, the air extraction rate is adjusted to maximize decomposition underground.

In situ techniques

Bioventing:

As with the biosparger, control devices typically regulate the pressure, the flow rate is monitored in operation, with data loggers and telemetry systems again featuring in the more complex applications.

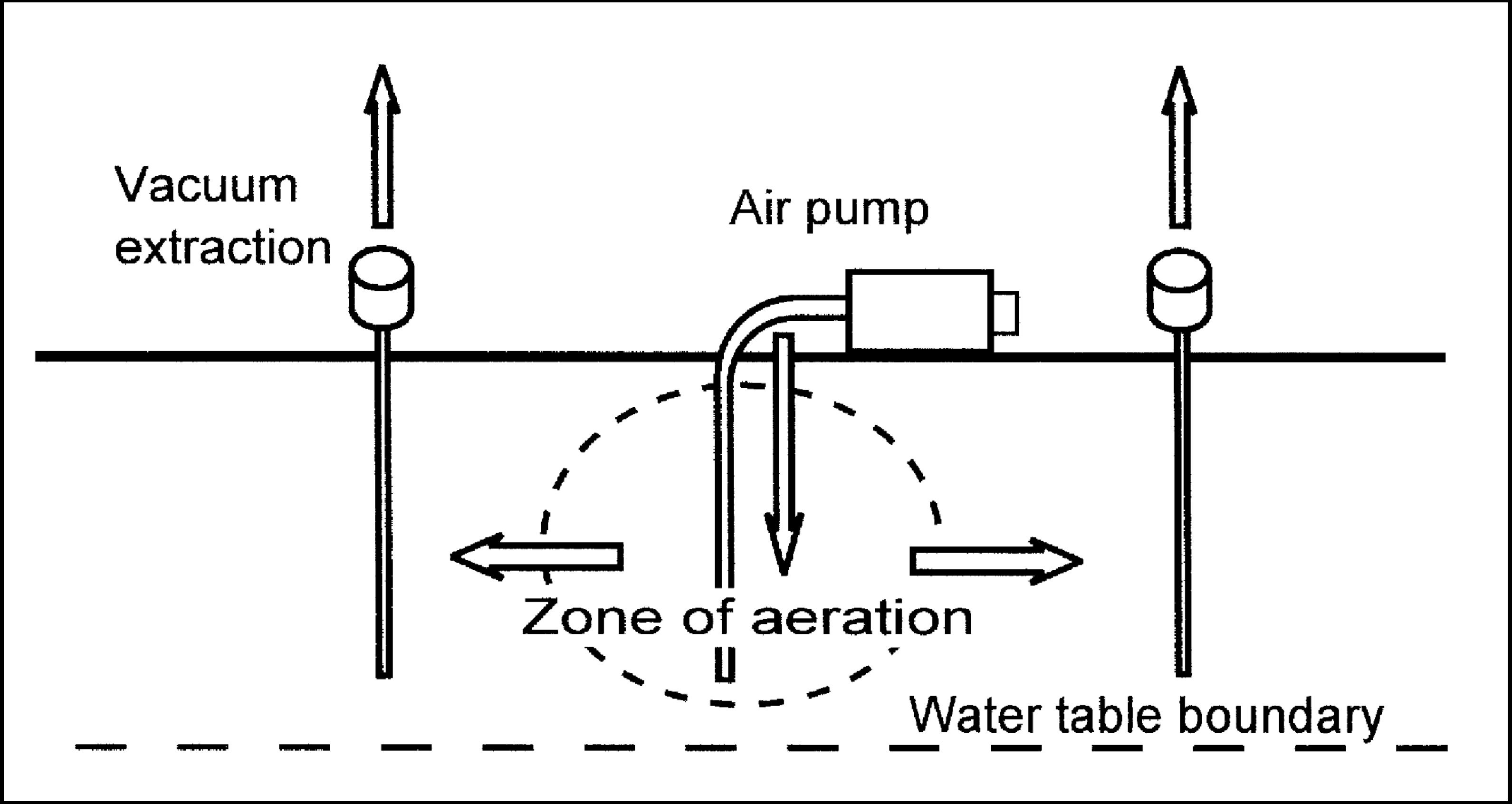
In situ techniques

Bioventing:

Bioventing is not generally suitable for remediating sites with a water table within 1.0m of the surface, nor for heavy or waterlogged soils, since air flow is compromised under these conditions.

In situ techniques

Bioventing



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In situ techniques

Injection recovery: This method makes use of the movement of ground water through the zone of contamination to assist the remediation process.

Shares many functional similarities with the preceding technologies, but is more sophisticated and refined.

In situ techniques

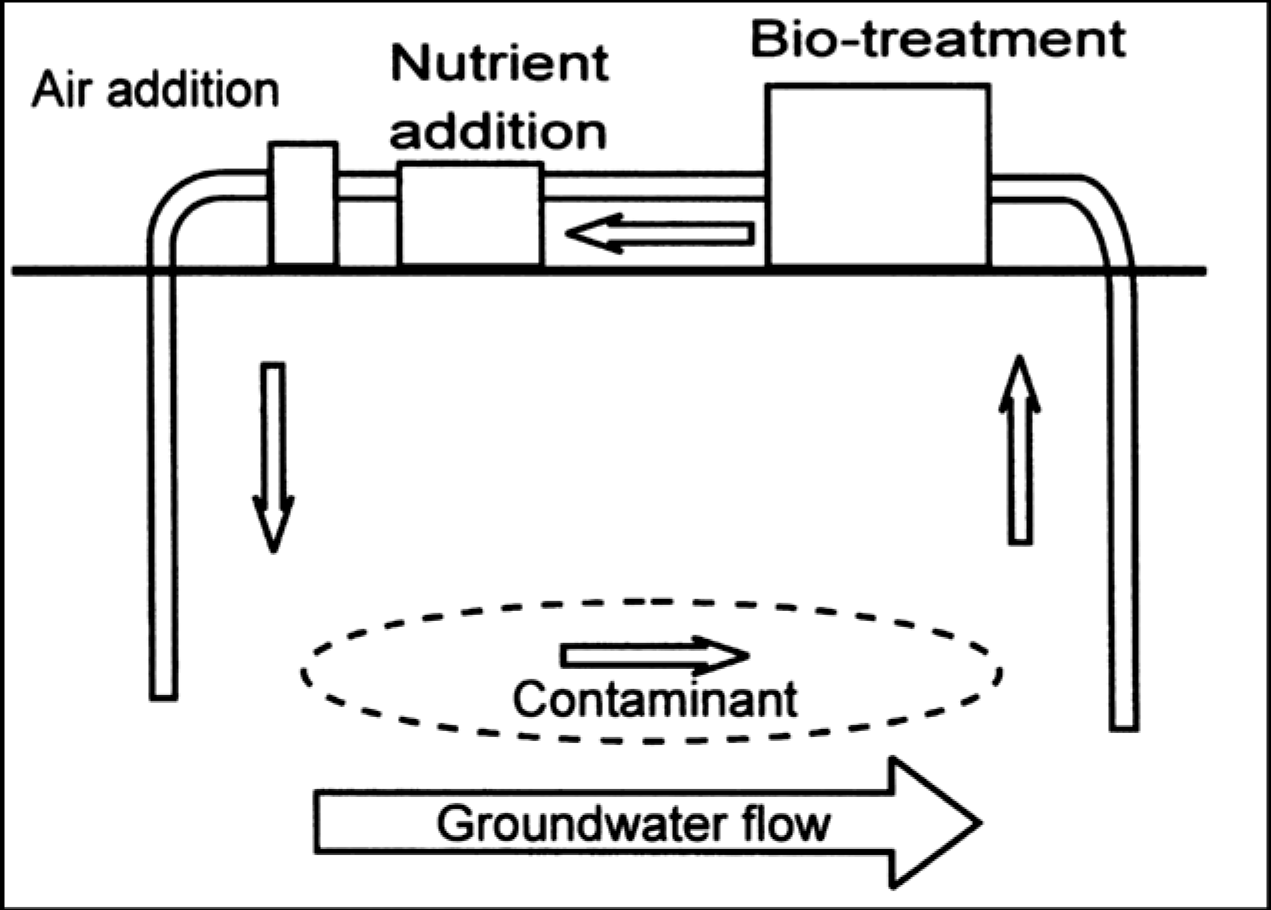
Injection recovery: There are two well system sunk into the ground, the ‘injection well’ and the ‘recovery well’, the former being located ‘upstream’ of the latter.

In situ techniques

Injection recovery: Nutrients and air are forced down the injection well, and as they flow through the contamination, they stimulate the growth and activity of the indigenous micro-organisms, which begin the process of remediation.

In situ techniques

Injection Recovery



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In situ techniques

END

Injection recovery: Groundwater, now rich in contaminant, is extracted via the 'recovery well'.

It then undergoes further biological treatment above ground in an associated bioreactor vessel, is replenished with air and nutrients, and is re-injected.

This cycle may be repeated many times.

Environmental Biotechnology

Site Monitoring for Biotechnological Applications

Site Monitoring for Biotechnological Applications

Environmental monitoring is well established as a separate science in its own right…

Plays an important role in the execution of bioremediation plans…

One particularly relevant aspect is the approach to sampling…

Site Monitoring for Biotechnological Applications

Sampling involves two distinct activities;

- the design of a sampling plan, and
- its practical execution.

Site Monitoring for Biotechnological Applications

The first steps are to define the objectives of the program and obtain agreement from all those concerned as to what will happen to the results produced and what decisions will be taken on the basis of the results obtained.

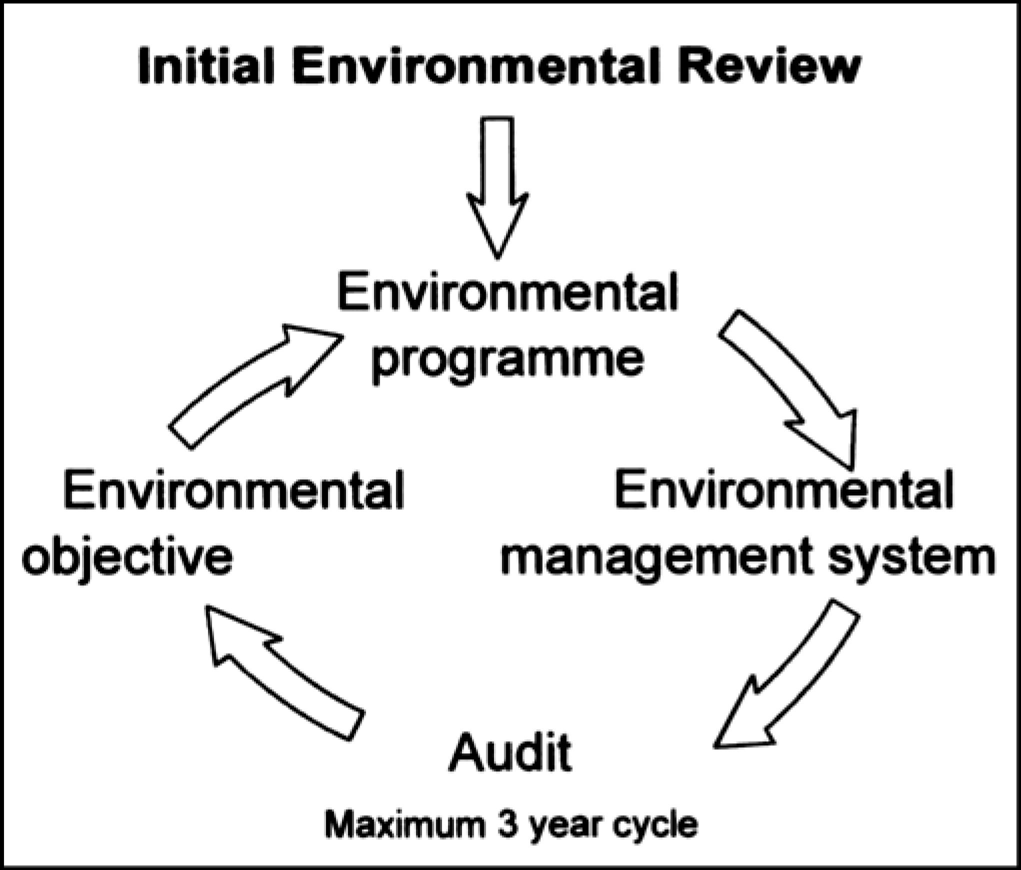
Site Monitoring for Biotechnological Applications

Actual sampling…

- Characterize the samples in terms of description, location, batch number, size, etc.,
- The manner in which they are to be taken,
- The total volume of the sample taken,
- The size of the final laboratory sample…,
- Transport, storage or preparation arrangements.

Site Monitoring for Biotechnological Applications

Illustrative long-term monitoring scheme



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Site Monitoring for Biotechnological Applications

END

Actual sampling…

- Safety aspects are both in terms of the site itself as well as protective clothing required, etc.

Environmental Biotechnology

Ex situ Techniques

Ex situ Techniques

Major benefits:

- greater ease of process optimization and control,
- relatively shorter treatment time, and
- safe introduction of specialized organisms.

Drawbacks:

- increased transport costs,
- additional land requirement,
- higher levels of engineering,
- more costly.

Ex situ Techniques

Three principal approaches:

- Land Farming,
- Soil Banking, and
- Soil Slurry Bioreactors.

Ex situ Techniques

Land Farming:

Accelerated natural attenuation…

Takes place off-site, within constructed earthwork banking to provide a low-tech bioreactor.

Pre-treatment stage involves excavation of soil, & screening for rocks and rubble.

Ex situ Techniques

Land Farming:

The process takes place in lined earthworks isolated from the surroundings by an impermeable clay or high density polyethylene (HDPE) liner.

Typically relies on the activities of indigenous microbes to bring about the remediation, though specialist microbes can be added.

Ex situ Techniques

Land Farming:

The soil to be treated is laid on a sand layer, which itself stands on a gravel bed, through which a series of drainage pipes have been laid.

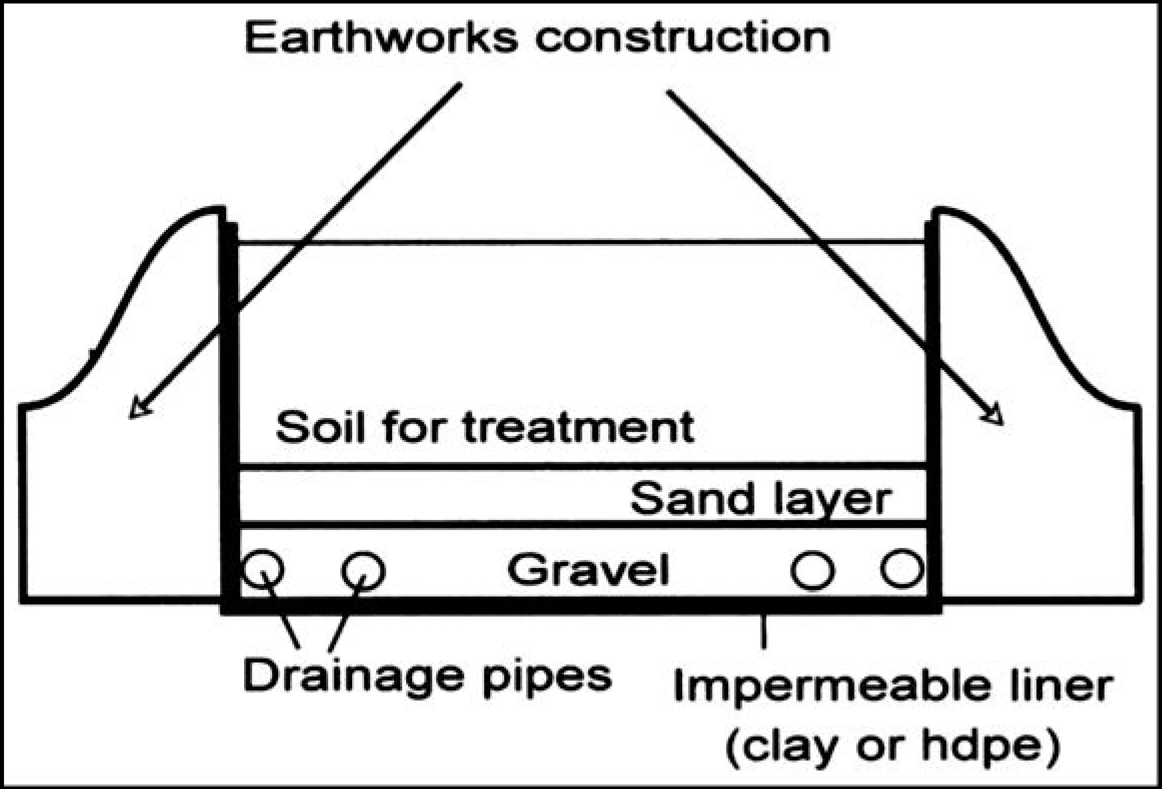
An impermeable clay or polymer lining isolates the whole system from direct contact with the underlying ground.

Ex situ Techniques

Land Farming: Water and nutrients are added to stimulate biological activity and soil aeration is maintained by means of turning or ploughing.

Ex situ Techniques

Schematic diagram of land farming



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Ex situ Techniques

Land Farming:
The inherent simplicity of the process, however, makes its effectiveness highly dependent on soil characteristics and climatic conditions.

Ex situ Techniques

Land Farming:

A process of sampling and monitoring helps to assess progress and compliance with required standards

At completion, the treated soil can be removed either for return to original site or use elsewhere.

Ex situ Techniques

Soil Banking:

Soil banking is an inverted version of the previous system, ranging from a long row of soil at its simplest, to a more engineered approach, with aeration pipes, a drainage layer, impermeable liner and a reservoir to collect leachate.

Ex situ Techniques

Soil Banking:
Soil is excavated and screened, and is formed into banks.

Wood chips, shredded organic matter, etc., can be added to increase aeration, water-holding capacity or organic matter content.

Also sometimes termed as ‘soil composting.

Ex situ Techniques

Soil Banking:
Faster than landfarming.

Indigenous microbes are again the principal agents of remediation, though specialized microbes can be introduced as required.

Nutrients are added to optimize and enhance their activities.

Ex situ Techniques

Soil Banking:

To boost efficiency, a more sophisticated version ‘engineered biopiling’, is used. Leachate is collected in a reservoir and recirculated... Contains a series of pipes that forces air through the biopile. The system is above an impermeable liner thus preventing leaching to underlying ground.

Ex situ Techniques

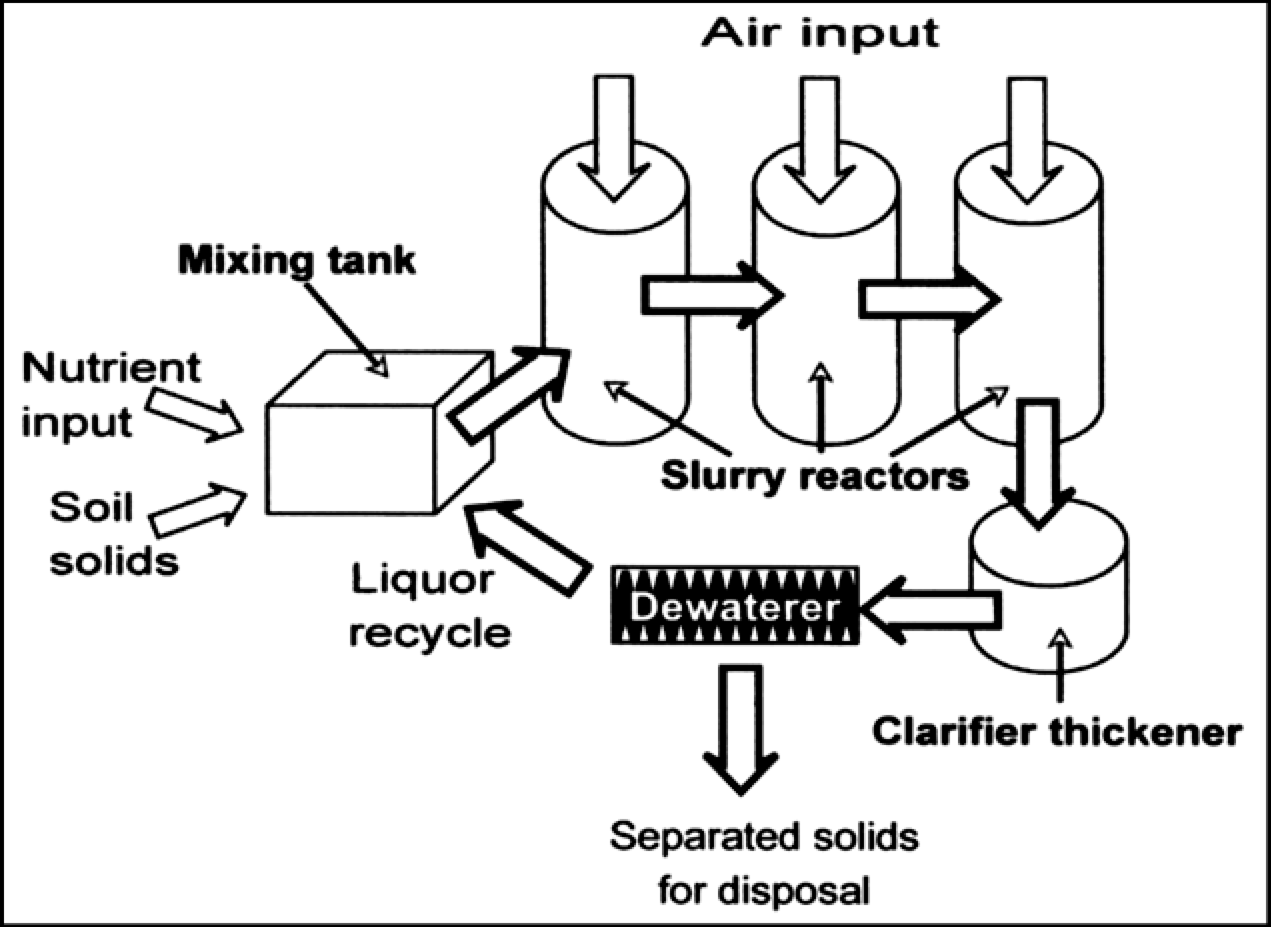
Soil slurry reactor: In most respects, this system shares essentially similar operating principles to the activated sludge system.

Ex situ Techniques

Soil slurry reactor:
Soil is introduced into a mixing tank, where a slurry is produced by mixing it with water. Nutrients are then added...
This suspension is transferred to a linked series of well-aerated slurry reactors and microbes treat
the contaminants progressively.
.

Ex situ Techniques

Schematic soil slurry bioreactor system



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Ex situ Techniques

END

Soil slurry reactor: Clarifiers and presses thicken the treated slurry and dewater it. Recovered liquid component is recirculated. Separated solids are removed for further drying followed by re-use or disposal.

Environmental Biotechnology

Process Selection and Integration

Process Selection and Integration

When complex mixtures of compounds are required to be treated, combining a series of different individual process stages within a series of inter-linked bioreactors, may often be a more appropriate and effective response.

Process Selection and Integration

Dependent on the specific type of contaminants …

Both aerobic and anaerobic procedures may be employed...,

Both biological and chemical steps may be employed...,

Each bioreactor may be so optimized to degrade particular contaminants.

Process Selection and Integration

Process of bioremediation employed depends on

- the site itself,
- the local area,
- economic instruments,
- reasons for remediation,
- the benefits and limitations of the technologies.

Process Selection and Integration

For any given contamination event, there may be more than one possible individual approach.

The potential will often exist for using integrated combinations of technologies to maximize the effectiveness.

Process Selection and Integration

END

The economic element remains a large factor, along with so many other factors.

However, global changes in attitude have seen the situation for bioremediation improve over the last decade.

Environmental Biotechnology

Case Studies

Case Studies: *Ex-Industrial Site Bioremediation (Utah, USA)*

Applying biotechnological solutions to some of the challenges has not always been successful.

Heavy hydrocarbons in the C15–C40 range, such as lubricating oils, drilling mud and crude oil, have at times proven slower or more recalcitrant to treatment.

Case Studies: *Ex-Industrial Site Bioremediation (Utah, USA)*

- Remediation of a former steel mill facility by Terra Nova Biosystems.
- C11–C21 diesel range organics (DROs) levels ~100000 mg/kg,
- along with naphthalene and PAH.

Case Studies: ***Ex-Industrial Site Bioremediation (Utah, USA)***

The site also contained dibenzofurans ~10000 mg/kg, along with lesser levels of benzene, toluene, ethyl benzene and xylenes.

Case Studies: *Ex-Industrial Site Bioremediation (Utah, USA)*

- Application of hydrocarbonoclastic bacteria (HCB) which have the innate ability to degrade hydrocarbon contaminants through metabolic action and biosurfactant production.
- Indigenous HCB were artificially augmented and additional oxygen and nutrients were supplied *in situ*.

Case Studies: ***Ex-Industrial Site Bioremediation (Utah, USA)***

The results far exceeded the US EPA/ DEQ standards for residential soils.

Final total petroleum hydrocarbon gasoline range organic (TPH GRO) concentration of <5 and ~1.0mg/kg for all other contaminants.

Case Studies: *Large-Scale Combined Bioremediation (Sweden)*

Large former industrial sites, those with a long and varied history, can often be host to more than one species of contaminants.

These poses an obvious challenge for remediation.

Solution lies in using a combination of appropriate bioremediation techniques.

Case Studies: *Large-Scale Combined Bioremediation (Sweden)*

K¨opmanholmen (Sweden) former industrial site homed chlorine, sulphur and cellulose factories as well as a timber yard, leading to contamination with oil, turpentine, mercury and PAHs.

Case Studies: *Large-Scale Combined Bioremediation (Sweden)*

MB Envirotech…

Demolition of some 3000 tonnes of concrete foundations

Ex-situ remediation of around 50000m^3 of contaminated soil.

A two-phase approach, (18–24 months), to first control the spread of contaminants and then remediate the soil and groundwater…

Case Studies: *Large-Scale Combined Bioremediation (Sweden)*

Biotechnologies involved bioventing, bioslurping – a combination process to remove free-phase contaminants and simultaneously bioremediate the soils of the vadose zone – and direct groundwater amelioration.

~15000m^3 of soil cleaned within the first six months...

END

Environmental Biotechnology

Oil Spill Bioremediation

Oil Spill Bioremediation

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

END

Environmental Biotechnology

Phytotechnology
and Photosynthesis

Phytotechnology and Photosynthesis

Phytotechnology is the use of plants in environmental biotechnology applications.

A wider topic…

Phytotechnology and Photosynthesis

Plants of one kind or another can be instrumental in the biological treatment of a large number of substances…

May be used to remediate industrial pollution, treat effluents and waste waters.

Phytotechnology and Photosynthesis

Phytoremediation:

“Direct *in situ* use of living green plants for the treatment of contaminated soil, sludges or ground water, by the removal, degradation, or containment of the pollutants present”.

Phytotechnology and Photosynthesis

Moreover, the role of phytotechnology is not limited solely to phytoremediation…

Phytotechnology and Photosynthesis

Phytoremediation:

- Removal and accumulation of unwanted substances within the plant tissues themselves,
- Their removal and subsequent volatilization to atmosphere, or
- The facilitation of in-soil treatment.

Phytotechnology and Photosynthesis

Phytoremediation:

- Makes use of natural cycles within the plant and its environment.
- To be effective, the right plant must be chosen.
- The plant must be appropriate for the climate.
- It must be able to survive in contact with the contamination.
- It may also have a need to be able to encourage localized microbial growth.

Phytotechnology and Photosynthesis

One of the major advantages is their almost universal approval and a big part of the appeal lies in the aesthetics…

Considerably cheaper than rival systems…

Phytotechnology and Photosynthesis

The processes of photosynthesis is fundamental in driving a solar-energy driven, passive and un-engineered system and hence may be said to contribute directly to the low cost of the approach.

Phytotechnology and Photosynthesis

END

Two general sections

- Terrestrial Phyto-Systems (TPS),
- Aquatic Phyto-Systems (APS),

Environmental Biotechnology

Terrestrial Phyto-Systems:
Metal Phytoremediation

Terrestrial Phyto-Systems: Metal Phytoremediation

A large range of plant species can be used, ranging from pteridophyte ferns, to angiosperms, and poplar trees…

These collectively employ a number of mechanisms to remove pollutants.

Terrestrial Phyto-Systems: Metal Phytoremediation

Over 400 different species can be used...

- Some hyper-accumulate contaminants within the plant biomass itself, which can subsequently be harvested,
- others act as pumps or siphons, removing contaminants from the soil before venting them into the atmosphere,
- while others enable the biodegradation of relatively large organic molecules…

Terrestrial Phyto-Systems: Metal Phytoremediation

Best suited to sites on which low to moderate levels of contamination are present fairly close to the surface and in a relatively shallow band

Terrestrial Phyto-Systems: Metal Phytoremediation

Metal Phytoremediation:

- Bioaccumulation,
- Phytoextraction,
- Rhizofiltration, or
- Phytostabilisation

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction

Uptake of metal contaminants from soil by the roots and their translocation into the above-ground regions of the plants.

Hyperaccumulators, have an innate ability to absorb exceptionally large amounts of metals typically 50–100 times more as compared to most ordinary plants.

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction
Wild forms often found in naturally metal-rich regions… …evolutionary selective advantage.

Copper, nickel and zinc, have been the best candidates for phytoextraction.

Nickel, cadmium, chromium, arsenic, etc…

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction

Two indica rice cultivars MORETSU and IR-8 for extracting cadmium (Ibaraki *et al*., 2009).

After 2 years, cadmium concentration decreased by 18%.

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction:
Hyperaccumulation

- *Pteris vittata*, accumulates 2.3% arsenic,
- Certain strains alpine pennycress (*Thlaspi caerulescens*) can bioaccumulate around 1.5% cadmium.

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction:
Hyperaccumulation

- Hyperaccumulation of metabolically required elements such as copper or zinc, can be viewed as the outcome of an over-efficient natural mechanism.
- However, uptake of a completely non-essential metal, particularly in such amounts, remains open to speculation…

Terrestrial Phyto-Systems: Metal Phytoremediation

Phytoextraction:

- Plants are chosen…
- Permitted to grow for a suitable length of time,
- Are harvested and the metal accumulated is permanently removed from the original site.
- Composting or incineration…

Terrestrial Phyto-Systems: Metal Phytoremediation

END

Phytoextraction:

- Dried plant biomass could be taken to processing works for recycling and for metals like gold…
- By contrast, low value materials, like lead, would not be a feasible prospect…
- Potential for biomining the metal out of former mines where further traditional methods are no longer practical

Environmental Biotechnology

Terrestrial Phyto-Systems: Rhizofiltration

Terrestrial Phyto-Systems: Rhizofiltration

Rhizofiltration

- Absorption,
- Adsorption, or
- Precipitation, onto plant roots of contaminants present in the soil water.

The principle difference between this and the previous approach: rhizofiltration is typically used to deal with contamination in the groundwater, rather than within the soil itself.

Terrestrial Phyto-Systems: Rhizofiltration

Rhizofiltration

The plants to be used are normally brought on hydroponically and gradually acclimatized to the specific character of the water which requires to be treated.

- Plantation on the site,
- Begin taking up the solution of pollutants,
- Harvesting,
- Final treatment…

Terrestrial Phyto-Systems: Phytostabilization

END

Phytostabilization

- Close similarities with both phytoextraction and rhizofiltration…
- Unlike the preceding regimes, harvesting the grown plants is not a feature of the process.
- In this sense, it does not remove the pollutants, but immobilizes them, or locks them up within the plant biomass…

Environmental Biotechnology

Terrestrial Phyto-Systems (TSP): Organic Phytoremediation –

Phytodegradation, Rhizodegradation, & Phytovolatilisation

TPS: Organic Phytoremediation

- Wide variety of organic pollutants such as pesticides, solvents and lubricants, etc.
- Tend to adhere closely to soil particles and are localized within 2m of the surface.
- Since they are effectively in direct contact with the rhizosphere, they are ideal candidates for phytoremediation.

TPS: Organic Phytoremediation

Typical mechanisms of organic phytoremediation are:

- Phytodegradation,
- Rhizodegradation and
- Phytovolatilisation.

TPS: Organic Phytoremediation - *Phytodegradation*

Phytodegradation

Biological breakdown of contaminants by plants. Either

- Internally, *having first been taken up by the plants*, or
- Externally, *using enzymes secreted by them*.

This leads to biodegradation into simpler substances and subsequent incorporation into the plant tissues…

TPS: Organic Phytoremediation - *Phytodegradation*

Phytodegradation

Important that the metabolites which accumulate are either non-toxic, or at least significantly less toxic than the original pollutant…

TPS: Organic Phytoremediation - *Rhizodegradation*

Rhizodegradation
Also described as phytostimulation or enhanced rhizospheric biodegradation.

Refers to the biodegradation of contaminants in the soil by edaphic microbes enhanced by the inherent character of the rhizosphere itself.

TPS: Organic Phytoremediation - *Rhizodegradation*

Rhizodegradation
Rhizosphere supports high microbial biomass.

Consequently increases the speed and efficiency of the biodegradation of organic substances within the rhizosphere as compared to other soil regions and microfloral communities.

TPS: Organic Phytoremediation - *Rhizodegradation*

Rhizodegradation
Plant roots increase the soil oxygenation in their vicinity and exude metabolites into the rhizosphere…

Denitrifying bacteria, *Pseudomonas* spp., and general heterotrophs are the principal beneficiaries.

Mycorrhizae fungi is also there...

TPS: Organic Phytoremediation - *Rhizodegradation*

Rhizodegradation
Unique enzymatic pathways that enable the biodegradation of organic substances that could not otherwise be transformed solely by bacterial action.

A much slower process than the previously described phytodegradation…

TPS: Organic Phytoremediation - *Phytovolatilization*

Phytovolatilization

Involves the uptake of the contaminants by plants and their release into the atmosphere, typically in a modified form.

Relies on the transpiration pull of fast growing trees, which accelerates the uptake of the pollutants in ground water solution, which are then transformed and released through the leaves.

TPS: Organic Phytoremediation - *Phytovolatilization*

Phytovolatilization

- E.g., genetically modified variety of the Yellow Poplar, *Liriodendron tulipifera* Engineered by the introduction of mercuric reductase gene (*mer A*).
- This confers the ability to tolerate higher mercury concentrations, and removal of the pollutant from the soil and volatize it.

TPS: Organic Phytoremediation - *Phytovolatilization*

Phytovolatilization
In a number of studies, poplars have been shown to be able to volatilize around 90% of the TCE (Trichloroethylene) they take up.

TPS: Organic Phytoremediation - *Phytovolatilization*

END

Phytovolatilization

- Is any danger from this kind of pollutant release into the atmosphere???
- Phytoremediation tends to be limited to sites where the pollutants are located fairly close to the surface.
- However, recent research has shown that deeply penetrating roots of trees allow deeper contamination to be treated.

Environmental Biotechnology

Terrestrial Phyto-Systems (TSP): Phytoremediation – *Plant Selection*

TPS: Phytoremediation - *Plant Selection*

Major criteria for plant selection are

- the particular requirements for the method to be employed, and
- the nature of the contaminants involved.

TPS: Phytoremediation - *Plant Selection*

For example, in the case of organic phytotransformation, species of vegetation which are

- hardy and fast growing,
- easy to maintain,
- have a high transpiration pull, and
- transform the pollutants present to non-toxic or less toxic products.

TPS: Phytoremediation - *Plant Selection*

On some sites, the planting of grass varieties in conjunction with trees, often in between rows of trees to stabilize and protect the soil, may be the best route…

TPS: Phytoremediation - *Plant Selection*

The selection of appropriate plant species is not limited solely to their direct ability to treat contaminants…

e.g., legumes can be of great benefit to naturally nitrogen deficient soils, since they have the ability, via symbiotic root nodule bacteria, to directly fix nitrogen from the atmosphere…

END

Environmental Biotechnology

Aquatic Phyto-Systems (APS) & Macrophyte Treatment System (MaTS) - I

Aquatic Phyto-Systems (APS)

Many aquatic plant species have the potential to be used in treatment systems.

The biological mechanisms by which some of the effects they achieve are largely familiar to the terrestrial systems.

Aquatic Phyto-Systems (APS)

There are a number of ways in which APS can be categorized.

Most useful categorization relates to the natural division between algae and macrophytes…

A macrophyte is an aquatic plant that grows in or near water and is either emergent, submergent, or floating.

APS: Macrophyte Treatment Systems (MaTS) - I

There has been a resurgence of interest in simpler, more natural methods for wastewater treatment…

Consequently, MaTS, in particular, have received much attention…

APS: Macrophyte Treatment Systems (MaTS) - I

These treatment systems are characterized by the input of effluent into a reservoir:

- either in the form of an artificial pond, or
- an expanse of highly saturated soil held within a containment layer,

within which the macrophytes have been established.

APS: Macrophyte Treatment Systems (MaTS) - I

- A gentle hydraulic flow is established.
- This causes the incoming wastewater to travel slowly through the system.
- The relatively long retention period allows adequate time for processes of settlement, contaminant uptake, biodegradation and phytotransformation...

APS: Macrophyte Treatment Systems (MaTS) - I

Mechanisms of pollutant removal are essentially the same, irrespective of the treatment system and whether the macrophytes are submerged, floating or emergent.

Treatment systems:

- natural wetland,
- a constructed monoculture
- or polyculture.

APS: Macrophyte Treatment Systems (MaTS) - I

- Both biotic and abiotic methods are involved.
- Main biological mechanisms are direct uptake and accumulation.
- The remainder is brought about by chemical and physical reactions, principally at the interfaces of :
  - water and sediment,
  - sediment and the root,
  - or the plant body and the water.

APS: Macrophyte Treatment Systems (MaTS) - I

Primary processes (by microbes and plants):

- Uptake and transformation,
- Subsequent biodegradation and biotransformation;
- Absorption,
- Adsorption,
- Ion exchange,
- Filtration,
- Chemical precipitation,
- Settlement of suspended solids,
- Chemical transformation.

APS: Macrophyte Treatment Systems (MaTS) - I

Planting densities in engineered systems are typically high.

The species involved tend to be included solely for their desired phytoremediation properties…

Much of the biological pollutant abatement potential of the system exists through the synergistic activity of the entire community…

APS: Macrophyte Treatment Systems (MaTS) - I

- Strong parallels between this and rhizospheric processes (exactly same mechanisms).
- In addition, particularly in the case of submerged vegetation, the surface of the plants themselves becomes a large extra substrate for the attached growth of closely associated bacteria and other microbial species.

APS: Macrophyte Treatment Systems (MaTS) - I

- The combined rhizo- and circum-phyllo-spheres support a large total microbial biomass.
- This exhibits a high level of bio-activity, relative to other microbial communities.
- Increased localized oxygenation and presence of significant quantities of plant metabolic exudates…

APS: Macrophyte Treatment Systems (MaTS) - I

In this way, the main role of the macrophytes clearly is more of an indirect one:

- bringing about local environmental enhancement and optimization for remediative microbes,
- rather than being directly implicated in activities of primary biodegradation.

In addition, physico-chemical mechanisms are also at work.

APS: Macrophyte Treatment Systems (MaTS) - I

The iron plaques which form on the plant roots trap certain metals, notably arsenic.

Direct adsorption and chemical/biochemical reactions also play a role in the removal of metals.

APS: Macrophyte Treatment Systems (MaTS) - I

- The ability of emergent macrophytes to transfer oxygen to their submerged portions is a well appreciated phenomenon.
- This enables them to cope with effective waterlogging and functional anoxia.
- As much as 60% of the oxygen transported to these parts of the plant can pass out into the rhizosphere.

APS: Macrophyte Treatment Systems (MaTS) - I

- This creates aerobic conditions for the microbial community associated with the submerged surfaces…

- This accounts for a significant increase in the dissolved oxygen level….

APS: Macrophyte Treatment Systems (MaTS) - I

- The aerobic breakdown of carbon sources is facilitated by this oxygen transfer.
- Have a major bearing on the rate of organic carbon biodegradation.
- This also makes possible the direct oxidation of hydrogen sulphide (H_2S) within the root zone...

END

Environmental Biotechnology

APS: Macrophyte Treatment System (MaTS) - II

APS: Macrophyte Treatment Systems (MaTS) - II

- Small, fast growing macrophytes have a high potential uptake rate.
- E.g., *Eichhornia* spp. can increase their biomass by 10 g/m^2/day under optimum conditions.
- This represents an enormous demand for nitrogen and carbon from their environment.

APS: Macrophyte Treatment Systems (MaTS) - II

The direct uptake of nitrogen from water by these plants can be up to 6000 kg/ha/year.

They are also effective in degrading phenols and in reducing copper, lead, mercury, nickel and zinc in effluents.

APS: Macrophyte Treatment Systems (MaTS) - II

Emergent macrophytes are also particularly efficient at removing and storing nitrogen in their roots, and some remove and accumulate phosphorus as well.

APS: Macrophyte Treatment Systems (MaTS) - II

Microbial nitrification and denitrification: the major nitrogen-affecting mechanisms.

- Anaerobic denitrification takes place in the sediment, causing loss to atmosphere,
- Aerobic nitrification facilitates nitrogenous incorporation within the vegetation.

APS: Macrophyte Treatment Systems (MaTS) - II

For the effective final removal of assimilated effluent components, accessibly harvestable material is essential, and above-water, standing biomass is ideal.

APS: Macrophyte Treatment Systems (MaTS) - II

One of the most important advantages of these systems is their potential to create habitats not just for ‘popular’ species, like waterfowl, but also for many less well known organisms, which can be instrumental in bolstering the ecological integrity of the area.

APS: Macrophyte Treatment Systems (MaTS) - II

At the same time, they can be ascetically pleasing, enhancing the landscape while performing their function.

These systems can have relatively low capital and running costs.

APS: Macrophyte Treatment Systems (MaTS) - II

Once properly set up, a well designed and constructed facility is almost entirely self-maintaining.

The system has low energy requirements:

- gravity drives the water flow, and
- all the remediating organisms are solar powered, either directly or indirectly…

APS: Macrophyte Treatment Systems (MaTS) - II

- The effluent treatment is as good or better than that from conventional systems.

- The system is also very tolerant of variance in organic loadings and effluent quality…

- These systems can often be very effective at odour reduction…

APS: Macrophyte Treatment Systems (MaTS) - II

One of the major constraints on the use of aquatic systems is an adequate supply of water throughout the year.

APS: Macrophyte Treatment Systems (MaTS) - II

If the effluent character or BOD is known, then it is a relatively simple procedure to calculate the necessary system size.

APS: Macrophyte Treatment Systems (MaTS) - II

Importance of substrate:
River sands, gravels, pulverized clinker, soils have been used.
The main factors are its hydraulic permeability and absorbance potential for nutrients and pollutants.

The substrate must also be able to provide an optimum growth medium for root development and penetration.

APS: Macrophyte Treatment Systems (MaTS) - II

Importance of substrate:

- Chemical nature of the chosen material may also affects system efficacy.
- Soils with low inherent mineral content tend to encourage direct nutrient uptake.
- While highly humeric soils have been shown to have the opposite effect in some studies.

APS: Macrophyte Treatment Systems (MaTS) - II

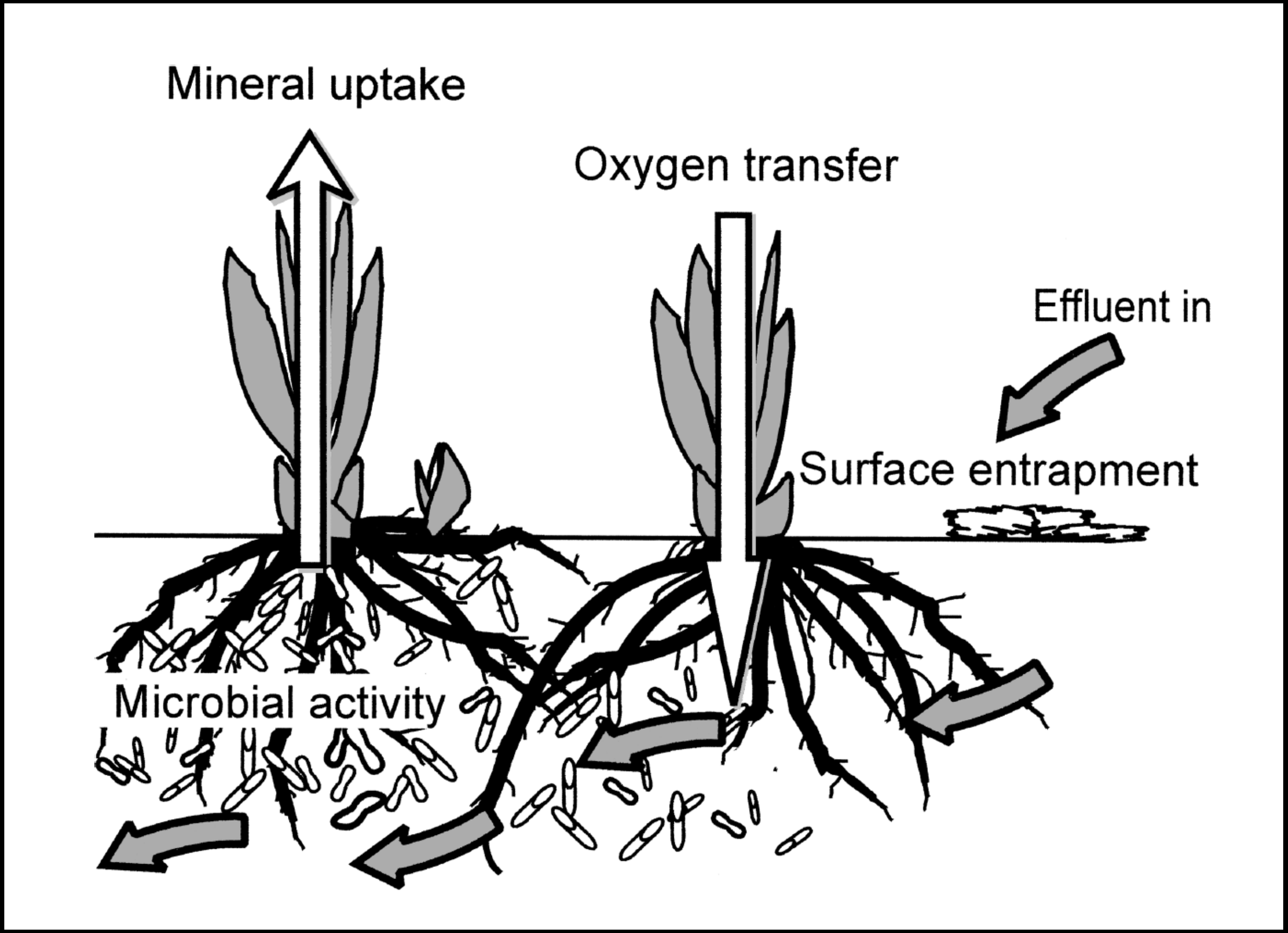
The hydraulic flow draws the effluent down through the rhizosphere, where the biodegradable components come into direct contact with the root zone's indigenous micro-organisms, which are stimulated to enhanced metabolic activity by the elevated aeration and greater nutrient availability.

APS: Macrophyte Treatment Systems (MaTS) - II

There is a net movement of oxygen down through the plant and a corresponding take up by the reeds of nitrates and minerals made accessible by the action of nitrifying and other bacteria.

APS: Macrophyte Treatment Systems (MaTS) - II

Diagrammatic root zone activity in MaTS



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APS: Macrophyte Treatment Systems (MaTS) - II

END

These systems are very efficient at contamination removal, typically achieving 95% or better remediation of a wide variety of pollutant substances.

Environmental Biotechnology

Nutrient Film Techniques (NFTs)
&
Algal Treatment Systems (ATS)

Nutrient Film Techniques (NFTs)

It involves growing plants on an impermeable containment layer, in a thin film of water.

The wastewater flows directly over the root mass…

Nutrient Film Techniques (NFTs)

Does not appear to have been developed further and little is known as to the conditions which govern its successful practical application.

Algal Treatment Systems (ATS)

Algae have principally been employed to remove nitrogen and phosphorus from wastewaters.

Some organic chemicals can also be treated.

Efficient carbon sequestration potential.

Algal Treatment Systems (ATS)

Algal effluent treatment systems work on the basis of eutrophication and rely on a dynamic equilibrium between the autotrophic algae themselves and the resident heterotrophic bacteria.

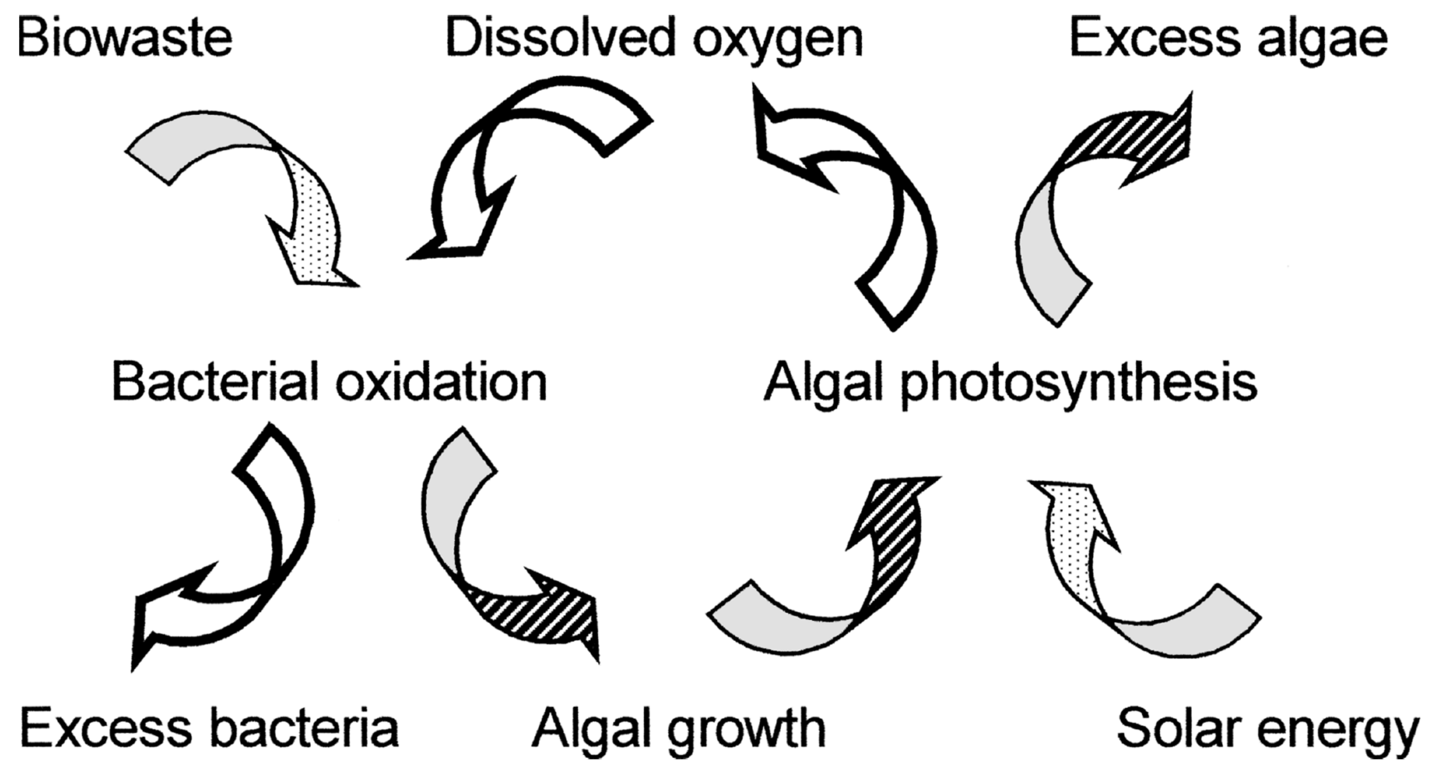
Algal Treatment Systems (ATS)

A two stage biodegradation/ assimilation process.

- Organic contaminants are biologically decomposed by the aerobic bacteria, which make use of oxygen provided by algal photosynthesis,
- while the algae grow using the nutrients produced by this bacterial breakdown, in a cyclic manner.

Algal Treatment Systems (ATS)

Algal and bacterial equilibrium



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Algal Treatment Systems (ATS)

END

Though the process is self-sustaining, it is also self-limiting and if left to proceed unchecked, will result in eutrophic stages.

The removal of excess algal and bacterial biomass is, therefore, an essential feature, vital to maintaining the system’s efficiency.

Environmental Biotechnology

Carbon sequestration by ATS

Carbon sequestration by ATS

A simpler process, only requiring the algae themselves.

However, even as a functional algal monoculture, without external intervention to limit the standing burden of biomass within the bioreactor, reduced efficiency and, ultimately, system collapse is inevitable.

Carbon sequestration by ATS

Huge amounts of many elements are held in global reservoirs, regulated by biogeochemical cycles. For carbon, a considerable mass is held in organic and inorganic oceanic stores, with the seas themselves being dynamic and important parts of the planetary carbon cycle.

Carbon sequestration by ATS

Marine phytoplankton utilize carbon dissolved in the water during photosynthesis, incorporating it into biomass and simultaneously increasing the inflow gradient from the atmosphere.

Carbon sequestration by ATS

When these organisms die, they sink, locking up this transient carbon and taking it out of the upper oceanic ‘fast’ cycle into the ‘slow’ cycle, which is bounded by long-term activities within the deep ocean sediments.

Carbon sequestration by ATS

The system may be likened to a biological sequestration pump, effectively removing atmospheric CO_2 from circulation within the biosphere.

Carbon sequestration by ATS

Increased quantities of CO_2 as well as greenhouse gases these days…

Application of phytotechnology stands as one very promising means by which to reduce this level.

Several attempts to commercialize the benefits of algae as carbon sinks.

Carbon sequestration by ATS

BioCoil: To remove CO_2 from generator emissions.

Use of unicellular algal species in a narrow, water containing, spiral tube made of translucent polymer, through which the exhaust gases from the generator were passed.

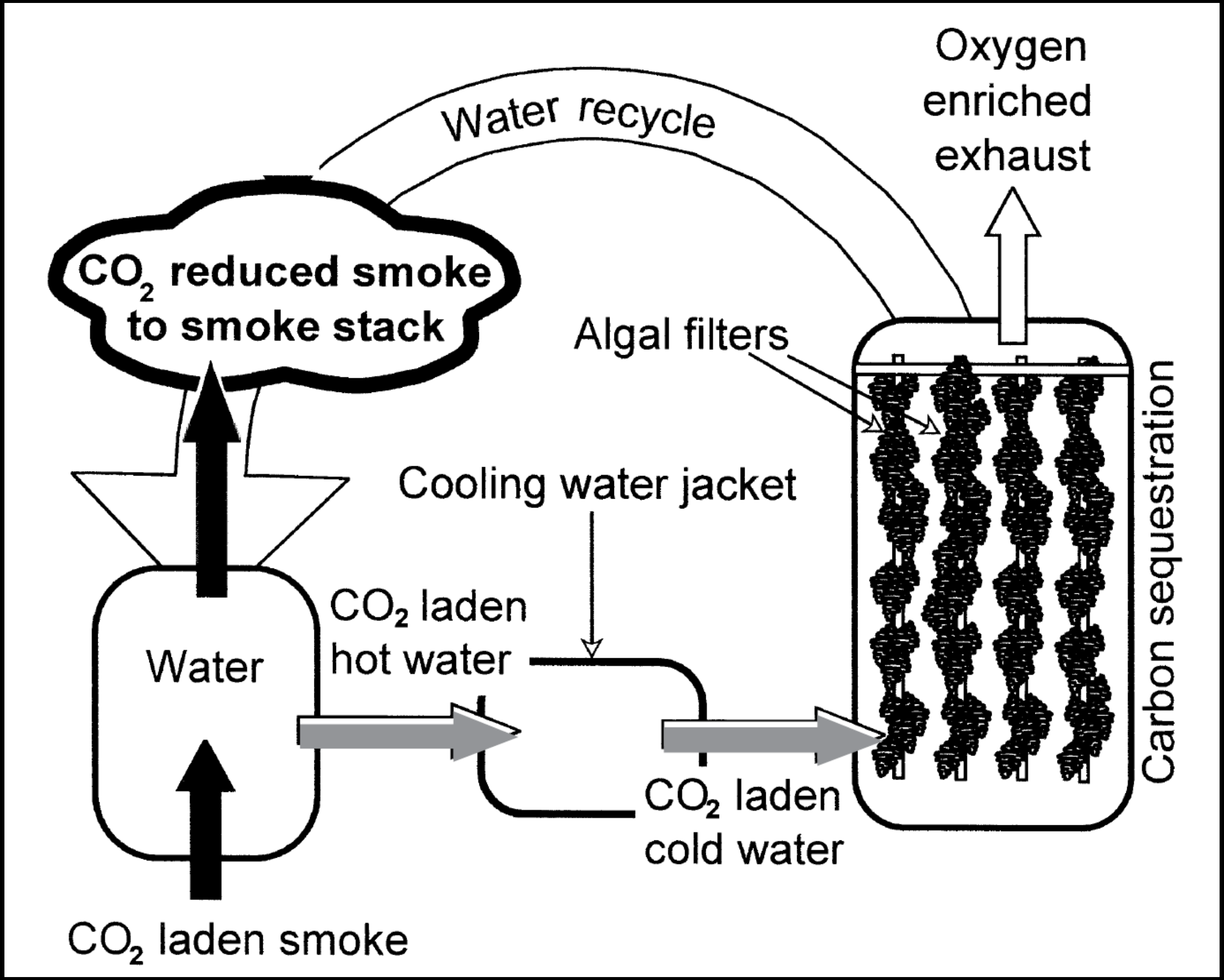
The CO_2 rich waters provided conditions for photosynthesis that was further enhanced by the use of additional artificial light...

Carbon sequestration by ATS

ACSACS (Algal cultivation system and carbon sink), Used filamentous algae, growing as attached biofilter elements on a polymeric lattice support. CO_2 rich exhaust gas was passed into the bottom of a bioreactor vessel, containing the plastic filter elements in water, and allowed to bubble up to the surface through the algal strands…

Carbon sequestration by ATS

Schematic ACSACS



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Environmental Biotechnology

Pollution Detection

Pollution Detection

Possible use of plants in a variety of industrial sectors as pollution detectors.

The aim is to provide information about the toxic components of contamination from a wide variety of sources.

Pollution Detection

Unlike biosensors, the plants are used as entire biological test systems in this approach.

The varieties used have been selected for their abilities to identify contaminants by reacting to the specific effects these substances have on the plant's vital functions.

Pollution Detection

- Functional within a broad range of pH and under varied climatic conditions.
- Responsive to both long-term pollution or incidental spillages.
- Can be applied to either laboratory or on-site investigations to monitor air, soil or water.

END

Environmental Biotechnology

Case studies

Case studies

Algal Biofuel Production (USA):

Algae have an obvious attraction when it comes to biofuel production. Soy, canola and palm yield around 470, 1500 and 5700 L /Ha oil respectively…
Some kinds of algae can provide approaching 20000 L /Ha…

Case studies

Algal Biofuel Production (USA):

Problems in achieving high yield:

Demands for resources and land.

In the case of enclosed bioreactors, the cost of construction and operation too.

Case studies

Algal Biofuel Production (USA):

NASA came up with the idea of huge plastic osmotic containers, filled with sewage and floating at sea, in which to grow algae.

Semi-permeable 'forward-osmosis membranes'

Case studies

Algal Biofuel Production (USA): Offshore membrane enclosures for growing algae (OMEGA), additional internal fresh water can safely escape, without the danger of ingress into the system of the external salt water.

Case studies

Algal Biofuel Production (USA): The nature of the material also allows for the necessary gaseous exchange, while temperature control is provided by the natural buffer effect of the sea's specific heat capacity. Wave action will help keep the system mixed.

Case studies

Algal Biofuel Production (USA): the OMEGA system also promises sustainable wastewater treatment, fertilizer production and carbon sequestration.

Case studies

Sustainable Engineered Phytoremediation (Dhulikhel, Nepal)
United Nations Human Settlements Program (UN-HABITAT), the Environment and Public Health Organization (ENPHO), the Dhulikhel municipality combine venture.

Case studies

END

Dhulikhel, Nepal

A community-based wastewater treatment plant…

Utilizing constructed wetlands to return cleaned water to the Punyamata River.

Six 175m^3 horizontal reed beds…

Two 75m^3 biogas reactors…

The sludge solids produced are used as agricultural fertilizer.

Environmental Biotechnology

Solid Waste Management

Solid Waste Management

- Solid waste refers here to all non-liquid wastes.
- Can create significant health problems and a very unpleasant living environment…
- This may provide breeding sites for insect-vectors, pests, snakes and vermin (rats) that increase the likelihood of disease transmission.
- It may also pollute water sources and the environment.

Solid Waste Management

Associated risks:
Disease transmission

Decomposing organic waste attracts animals, vermin and flies.

Flies may play a major role in the transmission of faecal-oral diseases.

Rodents may increase the transmission of diseases such as Leptospirosis.

Solid Waste Management

Associated risks:
Disease transmission

Solid waste may also provide breeding sites for mosquitoes…

Aedes genus… Dengue fever,
Anopheles genus… Malaria…

Solid Waste Management

Associated risks:
Disease transmission

In times of food scarcity, members of the affected population may be attracted to waste heaps to scavenge for food.

Likely to increase the risk of gastro-enteritis, dysentery and other illnesses.

Solid Waste Management

Associated risks:
Pollution

Poor management of the solid waste may lead to leachate pollution of surface water or groundwater.

Can cause significant problems if the waste contains toxic substances...

Solid Waste Management

Associated risks:
Pollution

Where large quantities of dry waste are stored in hot climates this may create a fire hazard. Related hazards include smoke pollution and fire threat to buildings and people.

Solid Waste Management

END

Associated risks: Effect on morale

The effect of living in an unhygienic and untidy environment may lead people to become demoralized and less motivated to improve conditions around them. Waste attracts more waste and leads to less hygienic behaviour in general.

Environmental Biotechnology

Sources and Types of Solid Waste

Sources and Types of Solid Waste

Sources of solid waste:

- Medical centers
- Food stores
- Feeding centers
- Food distribution points
- Slaughter areas
- Warehouses
- Markets
- Domestic areas

Sources and Types of Solid Waste

Sources of solid waste:

- Solid waste management strategies may vary for institutional, communal and domestic sources, and
- Depends on the types and volumes of the waste…

Sources and Types of Solid Waste

Type and Quantity of solid waste:

The main factors are:

- the geographical region (developed or less-developed country or region);
- socio-cultural practices and material levels among affected population;
- seasonal variations (affecting types of food available);

Sources and Types of Solid Waste

Different categories of solid waste include:

Organic waste: Waste from preparation of food, market places, etc.

Combustibles: Paper, wood, dried leaves, packaging, etc. (high organic and low moisture content)

Sources and Types of Solid Waste

Different categories of solid waste include:

Non-combustibles: Metal, tin cans, bottles, stones, etc.

Ashes/dust: Residue from fires used for cooking.

Bulky waste: Tree branches, tyres, etc.

Sources and Types of Solid Waste

END

Different categories of solid waste include:

Dead animals: Carcasses of domestic animals and livestock.

Hazardous waste: Oil, battery acid, medical waste.

Construction waste: Roofing, rubble, broken concrete, etc.

Environmental Biotechnology

Initial Steps in Solid Waste Management

Initial Steps in Solid Waste Management

1. Identify the types of waste.

2. Identify the sources of waste.

3. Determine the potential health hazards from waste.

4. Determine the volume of waste generated

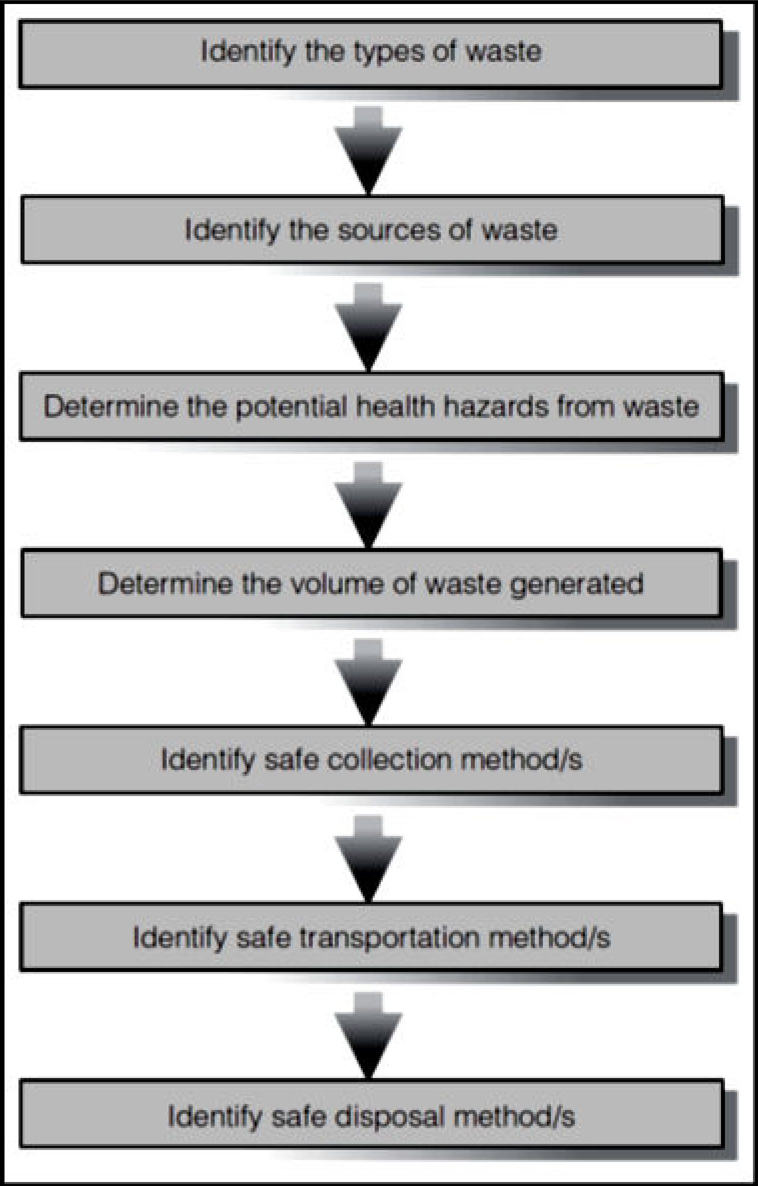
Initial Steps in Solid Waste Management

5. Identify safe collection method/s.

6. Identify safe transportation method/s.

7. Identify safe disposal method/s.

Initial Steps in Solid Waste Management



Identify the types of waste

Identify the sources of waste

Determine the potential health hazards from waste

Determine the volume of waste generated

Identify safe collection method/s

Identify safe transportation method/s

Identify safe disposal method/s

http://ec.europa.eu/echo/files/evaluation/watsan2005/annex_files/WEDC/es/ES07CD.pdf

Environmental Biotechnology

Key Components of Solid Waste Management

Key Components of Solid Waste Management

Five key components:

- Generation
- Storage
- Collection
- Transportation
- Disposal

Key Components of Solid Waste Management

Generation:

- At this stage the materials become valueless to the owner.
- Since they have no use for them and require them no longer, they wish to get rid of them.
- However, items which may be valueless to one individual may not necessarily be valueless to another.

Key Components of Solid Waste Management

Storage:

- Storage is a system for keeping materials after they have been discarded and prior to collection and final disposal.
- For on-site disposal systems are, storage may not be necessary.

Key Components of Solid Waste Management

Storage:

- In worst conditions, a population may discard domestic waste in heaps close to dwelling areas.
- In this case, improved disposal or storage facilities should be provided and should be located where people are able to use them easily.

Key Components of Solid Waste Management

Storage:

- ➢ Improved storage facilities include:
  - Small containers: household containers, plastic bins, etc.
  - Large containers: communal bins, oil drums, etc.
    - Shallow pits
  - Communal depots: walled or fenced-in areas.

Key Components of Solid Waste Management

Storage:

- The size, quantity and distribution of storage facilities depends on:
  - the number of users,
  - type of waste,
  - maximum walking distance, and
  - the frequency of emptying.

Key Components of Solid Waste Management

Collection:

- Simply refers to how waste is collected for transportation to the final disposal site.
- Should be carefully planned to ensure that storage facilities do not become overloaded.
- Collection intervals and volumes of collected waste must be estimated.

Key Components of Solid Waste Management

Transportation:

- This is the stage when solid waste is transported to the final disposal site.
- The method chosen depends upon local availability and the volume of waste to be transported.

Key Components of Solid Waste Management

Transportation:

Types of transportation can be divided into three categories:

- Human-powered: open hand-cart, hand-cart with bins, wheelbarrow, tricycle
- Animal-powered: donkey-drawn cart
- Motorised: tractor and trailer, standard truck, tipper-truck

Key Components of Solid Waste Management

END

Disposal:

The final stage is safe disposal.

There are four main methods:

- Land application: burial or landfilling
- Composting
- Burning or incineration
- Recycling (resource recovery)

The most common is the land application.

Environmental Biotechnology

On-Site Disposal Options-I

(For Solid Waste)

Communal Pits & Bins

On-Site Disposal Options - I

Communal Pit Disposal:

- Simplest solid waste management system is where consumers dispose of waste directly into a pit.
- The size depends on the number of people it serves.
- The pit should be fenced off…
- It should not be more than 100m from the dwellings to be served.
- Should be covered weekly with a thin layer of soil…

On-Site Disposal Options - I

Communal Pit Disposal:

Advantages:

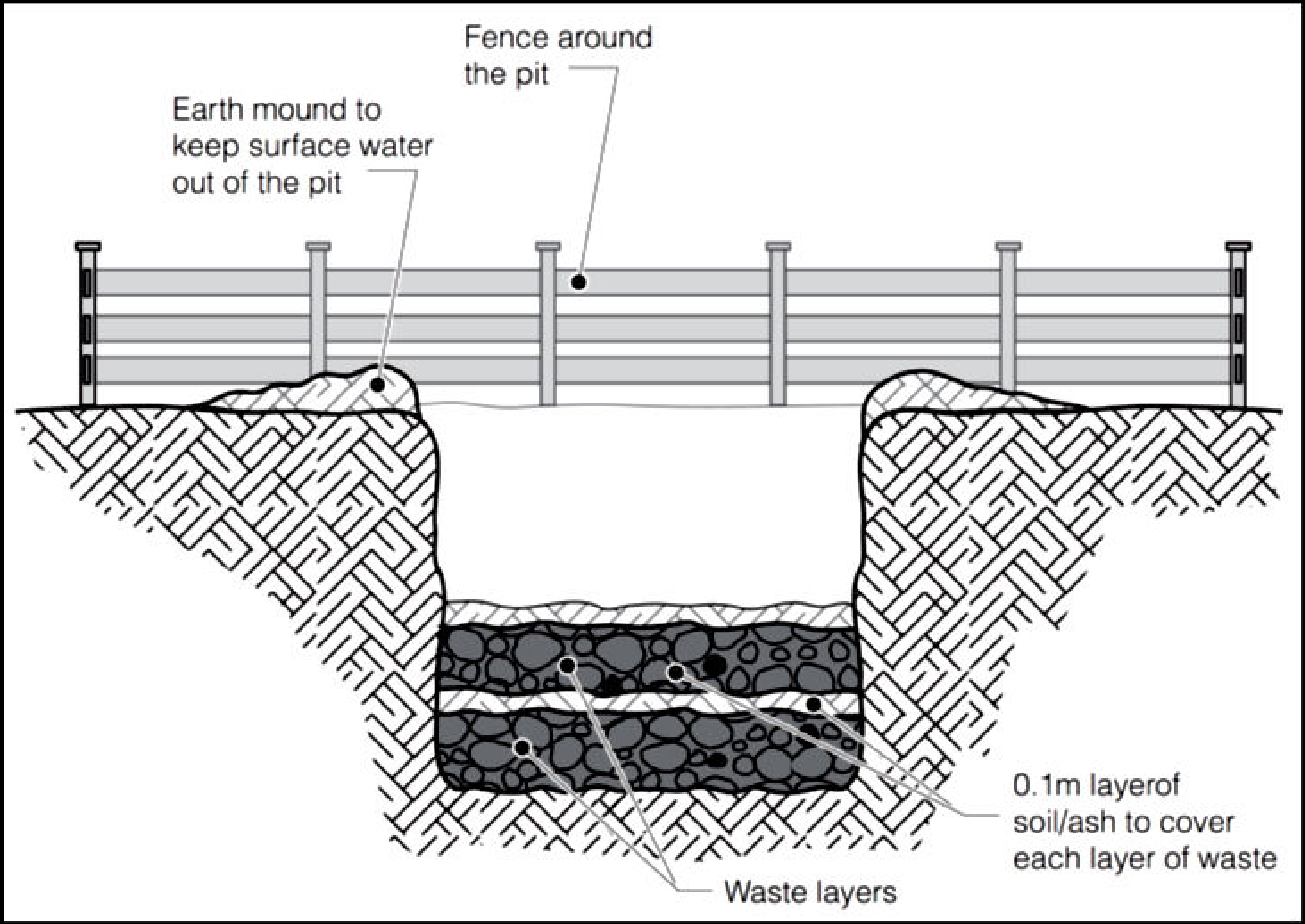
- Rapid to implement,
- Requires little operation and maintenance.

Constraints:

- Distance to the pit may cause indiscriminate disposal;
- Waste workers required to manage pits.

On-Site Disposal Options - I

Communal Solid Waste Pit



http://ec.europa.eu/echo/files/evaluation/watsan2005/annex_files/WEDC/es/ES07CD.pdf

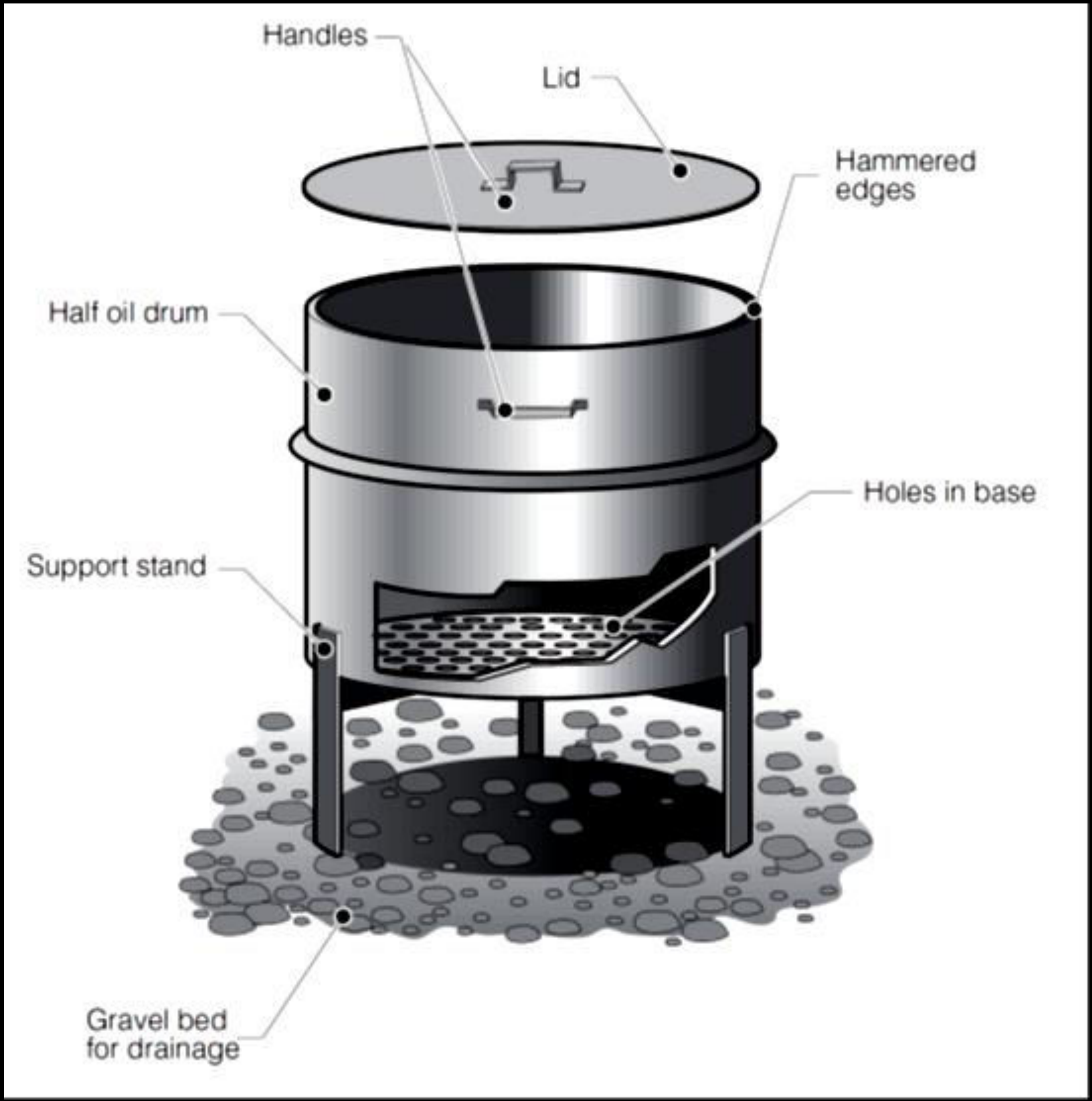
On-Site Disposal Options - I

Communal Bins:

- These containers are designed to collect waste, from where it can be easily removed.
- Plastic containers are generally not appropriate…
- A popular solution is to provide oil drums cut in half, with their bases perforated.
- A lid and handles can be provided if necessary.

On-Site Disposal Options - I

Communal Bin Made from an Old Oil Drum



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On-Site Disposal Options - I

END

Communal Bins:

Advantages:

- Bins are potentially a highly hygienic and sanitary management method; and
- Final disposal of waste is well away from dwelling areas.

Constraints:

- Collection, transportation and human resources are required;
- System takes time to implement; and
- Efficient management is essential.

Environmental Biotechnology

Transportation Options

(For Solid Waste)

Transportation Options

Where bins or collection containers require emptying, transportation to the final disposal point is required.

Three methods:

- Human-powered,
- Animal-powered or
- Motorized.

Transportation Options

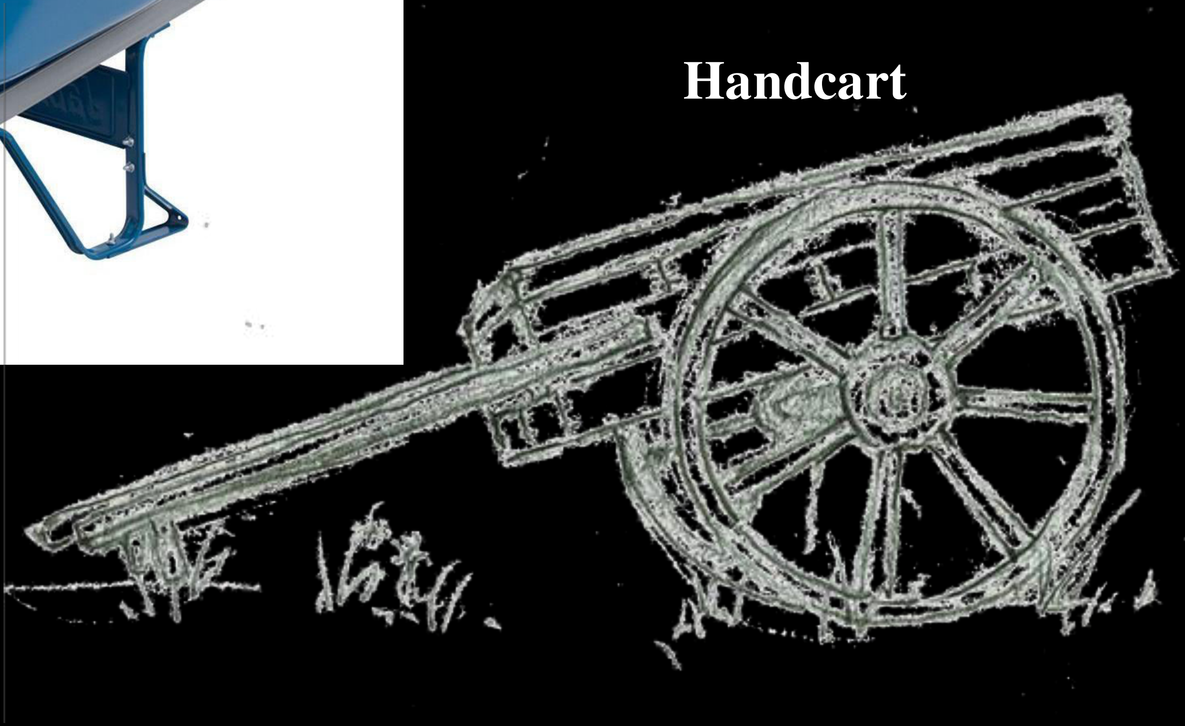
Human-powered:

- Wheelbarrows are ideal for the transportation of waste around small sites.
- Not appropriate where waste must be transported distant off-site.
  - Handcarts are better for longer distances since these can carry more waste and can be pushed by more than one person.
- Carts may be open or can be fitted with several containers or bins.

Transportation Options

Wheelbarrow

Handcart



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Transportation Options

Animal-powered:

- Horse or donkey with cart are likely to be appropriate where they are commonly used locally.
- This may be ideal for transportation to middle distance sites.
- Inappropriate for much longer distances.

Transportation Options

Motorized:

Motorized vehicle may be the ONLY appropriate option…

- where the distance to the final disposal site is great, or
- where the volume of waste to be transported is high,

➢ Options include tractor and trailer, a standard truck, or a tipper-truck,

➢ The choice depends largely on availability and speed of procurement.

Transportation Options

- For large volumes of waste it may sometimes be appropriate to have a two-stage transportation system requiring a transfer station.
- For example, waste is transported by handcart to a transfer station where it is loaded into a truck to be taken to an off-site disposal site several kilometers away.

END

Environmental Biotechnology

Off-Site Disposal Options

(For Solid Waste)

Off-site Disposal Options

Landfilling:

- Once solid waste is transported off-site it is normally taken to a landfill site.
- The waste is placed in a large excavation (pit or trench) in the ground, The pit is back-filled with excavated soil.
- Ideally, about 0.5m of soil should cover the deposited refuse…

Off-site Disposal Options

Landfilling:

- The location of landfill should be decided by consultation with the local authorities and the affected population.
- Sites should preferably be fenced, and
- Should be at least one kilometer downwind of the nearest dwellings.

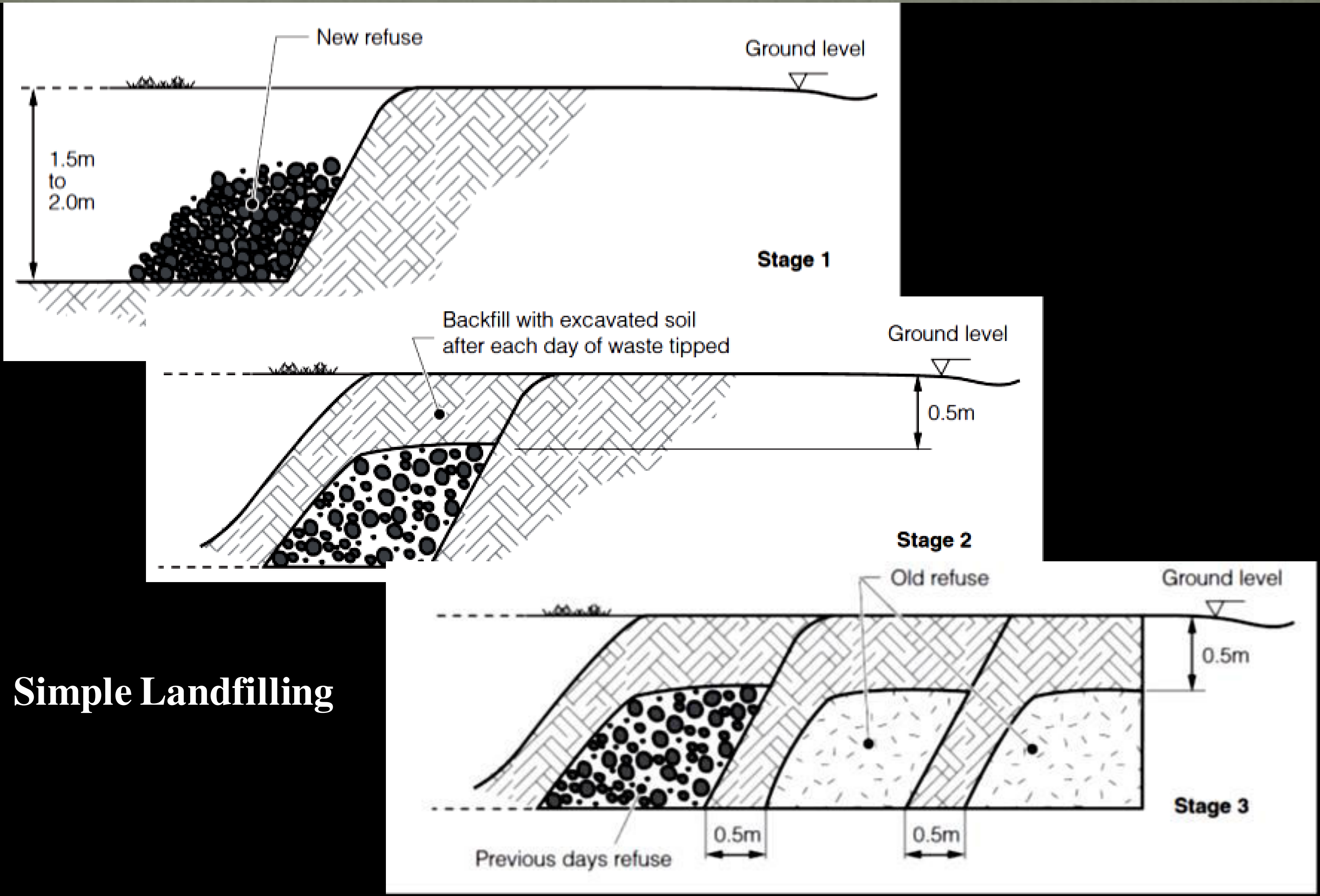
Off-site Disposal Options

Landfilling:

Advantages:
A sanitary disposal method if managed effectively.

Constraints:
A reasonably large area is required.

On-Site Disposal Options - I



Simple Landfilling

http://ec.europa.eu/echo/files/evaluation/watsan2005/annex_files/WEDC/es/ES07CD.pdf

Off-site Disposal Options

Incineration:

- This should generally only take place off-site or at a considerable distance downwind of dwellings.
- Burning refuse within dwelling areas may create a significant smoke or fire hazard, especially if several fires are lit simultaneously…

Off-site Disposal Options

Incineration:

- Can be used to reduce the volume of waste.
- Appropriate where there is limited space for burial or landfill.
- Waste should be ignited within pits and covered with soil once incinerated.

Off-site Disposal Options

Incineration :

Advantages:

- Burning reduces volume of combustible waste considerably; and
- It is appropriate in off-site pits.

Constraints:

- A reasonably large area is required.

Off-site Disposal Options

Composting:

- Where people have their own gardens or vegetable plots, organic waste can be dug into the soil to add humus and fibre.
- This makes the waste perfectly safe and also assists the growing process.

Off-site Disposal Options

Composting:

- Requires careful monitoring of decomposing waste to control moisture and chemical levels and promote microbial activity.
- This is designed to produce compost which is safe to handle and which acts as a good fertilizer.

Off-site Disposal Options

Composting:

Advantages:

- Composting is environmentally friendly; and beneficial for crops.

Constraints:

- Intensive management and experienced personnel are required for large-scale operations.

Off-site Disposal Options

Recycling:

- Complex recycling systems are unlikely to be appropriate but the recycling of some waste items may be possible on occasions.
- In most developing country contexts there exists a strong tradition of recycling leading to lower volumes of waste than in many more developed societies.

Off-site Disposal Options

Recycling:

Advantages:
- Recycling is environmentally friendly.

Constraints:
- Expensive to set up.

END

Environmental Biotechnology

Intervention Levels

(For Solid Waste)

Intervention Levels

Immediate action:

- Clearing of scattered waste
- Burning and burial of waste on site
- Temporary communal pits
- Temporary communal bins and off-site disposal
- Repairing or upgrading of existing facilities.

Intervention Levels

Short-term Measure:

- Communal pits
- Family pits
- Communal bins and off-site disposal

Intervention Levels

END

Long-term Measure:

- Communal pits
- Family pits
- Communal bins and off-site disposal
- Repairing or upgrading of existing facilities
- Recycling

Environmental Biotechnology

Protective Measures

(For Solid Waste Management)

Protective Measures

- In order to minimize disease transmission there are several protective measures that can be undertaken.
- These concern equipment for staff and the siting and management of disposal sites.

Protective Measures

Staff:

- Workers involved should be provided with appropriate clothing and equipment.
- Gloves, boots and overalls should be provided.
- Where waste is burned, or is very dusty, workers should have protective masks.
- Water and soap should be available for washing.

Protective Measures

Siting of disposal sites:

- The location of all disposal sites should be determined through consultation with key stakeholders.
- Appropriate siting should minimize the effects of odour, smoke, water pollution, insect vectors and animals.

Protective Measures

Siting of disposal sites:

On-site disposal is appropriate where

- waste volumes are small,
- plenty of space is available, and
- waste is largely organic or recyclable.

If the above conditions are not met, Off-site disposal is adopted.

Protective Measures

Siting of disposal sites:

For off-site disposal, the following measures should be taken:

- Locate sites at least 500m downwind of nearest settlement.
- Locate sites downhill from groundwater sources.
- Locate sites at least 50m from surface water sources.
- Provide a drainage ditch downhill of landfill site.
- Fence and secure access to site.

END

Environmental Biotechnology

Solid Waste Management

(A Video)

Solid Waste Management (A Video)

https://www.youtube.com/watch?v=9STFO5HjuP8

END

Environmental Biotechnology

Integrated Environmental Biotechnology

Integrated Environmental Biotechnology

- Essence of environmental biotechnology: *harnessing of pre-existing organisms and natural cycles to bring about a desired goal.*

- Sometimes achieved by relatively unsophisticated means.

- At other times requires engineering, adaptation or modification.

Integrated Environmental Biotechnology

- The exact form may differ, the underlying paradigm remains the same.
- Applying a naturalistic model leads to some inevitable conclusions with far-reaching implications.

Integrated Environmental Biotechnology

- Mutual interactions in nature...
- Natural cycles dovetail together at both the gross and the microscopic levels…
- Interplay exists between the organism and its environment as well as between the various central metabolic pathways.

Integrated Environmental Biotechnology

Since such integration exists already between bio-processes, and these are the very stuff upon which environmental biotechnology is based, the potential for integrated applications is clear.

Integrated Environmental Biotechnology

Three basic requirements:

- Gather material resources,
- deal rationally with the waste, and
- the requirement for adequate and affordable energy.

Integrated Environmental Biotechnology

The desire between compromising neither commercial success nor environmental stewardship is particularly important for the long-term future of the economy.

Integrated Environmental Biotechnology

Extremist environmentalist thought against industries is scarcely helpful:

- hardly likely to react constructively to criticism from its avowed detractors…
- industry in its widest sense is what has defined humanity from the outset…

Integrated Environmental Biotechnology

The move towards integration is inevitable…

Sustainable development inherently demands a cogent view of resource management, and this implicitly covers materials, waste and energy.

It becomes impossible to consider them in isolation.

END

Environmental Biotechnology

Bioenergy

Bioenergy

- Concept of obtaining energy from biomass…

- Methane and ethanol have been long established as fuels in many parts of the world…

- However, to most people, the most familiar forms of biofuel are far more directly utilized. E.g. burning of wood.

Bioenergy

- The energy of all biofuels derives ultimately from the sun…

- During biomass combustion, and in various metabolic processes, organic carbon reacts with oxygen, releasing energy...

- The residual matter feeds back into natural cycles for reuse…

Bioenergy

This relationship of energy and matter within the biospheric system is of fundamental importance to understanding the whole question of biomass and biofuels.

Bioenergy

END

- The crux of this particular debate ultimately centers on issues of greenhouse gases and global warming…
- Bio-energy simply releases the carbon it took up during its own growth.
- Thus, only ‘modern’ carbon is returned, avoiding any unwanted additional atmospheric contributions of ancient carbon dioxide.

Environmental Biotechnology

Derived Biofuels:
Methane biogas I

Derived Biofuels: Methane biogas I

- Biogas is a methane-rich gas resulting from the activities of anaerobic bacteria, responsible for the breakdown of complex organic molecules.

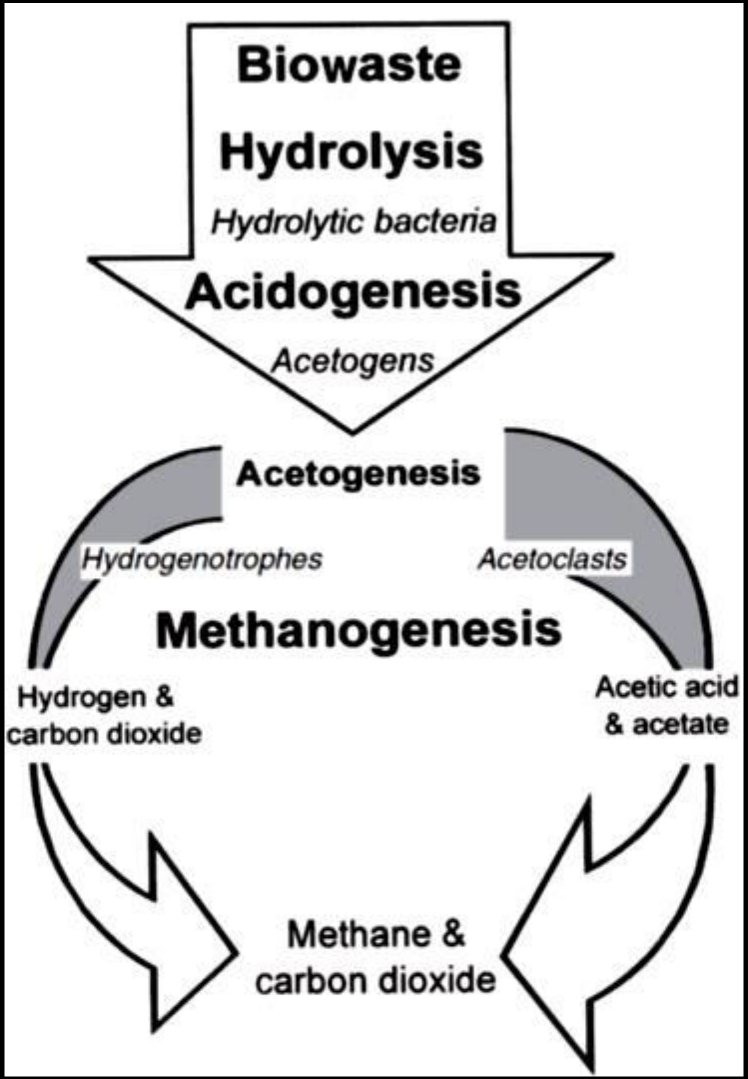
- It is combustible, with an energy value typically in the range of 21–28MJ/m^3.

Derived Biofuels: Methane biogas I

- The main route for methane production involves acetic acid/acetate and accounts for around 75% of gas produced.

- The remainder is made up via methanol or carbon dioxide and hydrogen.

Derived Biofuels: Methane biogas I



The methanization of biowaste

Environmental Biotechnology Theory and application, 2nd Ed by Gareth.

Derived Biofuels: Methane biogas I

Cellulose decomposition and gas evolution can be broadly characterized as:

Stage I: Peak biowaste cellulose loadings; dissolved oxygen levels fall to zero; nitrogen, and carbon dioxide tend to atmospheric levels.

Derived Biofuels: Methane biogas I

Stage II: Carbon dioxide, hydrogen and free fatty acids levels peak; nitrogen levels fall to around 10%; cellulose begins to be broken down.

Derived Biofuels: Methane biogas I

Stage III: Carbon dioxide decreases and plateaus to hold at around 40%; methane production commences and achieves steady state at around 60%; free fatty acids decrease to minimum levels; cellulose breakdown continues at a linear rate with respect to time; nitrogen levels fall to near zero.

Derived Biofuels: Methane biogas I

END

Stage IV: Carbon dioxide and methane continue in steady state at circa 40 and 60% respectively; cellulose component reduces steadily.

Stage V: Cellulose becomes fully decomposed, ultimately leading to zero methane and carbon dioxide production; oxygen and nitrogen revert to atmospheric levels.

Environmental Biotechnology

Derived Biofuels:
Methane biogas II

Derived Biofuels: Methane biogas II

Anaerobic digestion for methane production, notably in

- the waste management,
- sewage treatment,
- agricultural, and food processing industries.

The process has also been successfully used on a relatively small scale, commonly with animal manures as its feedstock.

Derived Biofuels: Methane biogas II

- With a theoretical yield of $400m^3$ of biogas per wet cellulosic tonne, the prospect of high energy production simultaneous with waste treatment has clear appeal.
- However, it is not feasible to optimize conditions to that extent…
- Therefore, only around a quarter of the potential biogas yield is actually achieved.

Derived Biofuels: Methane biogas II

Biogas is much cleaner and far less contaminated as compared to LFG.

LFG may contain 1,2 dichloroethene, alkylbenzene, butylcyclohexane, carbon disulphide, propylcyclohexan, methanethiol, decane, dichlorobenzene, undecane, ethylbenzene, dodecane, trimethylbenzene, tridecane, toluene, dimethyl disulphide, nonane and sulphur dioxide.

Derived Biofuels: Methane biogas II

- Biogas, is relatively pure by comparison.
- Bulk of the inorganic matter and many pollutants are excluded, either by source or mechanical separation, as part of the waste preparation process.

Derived Biofuels: Methane biogas II

- The main concern is hydrogen sulphide (H_2S), which is a metabolic by-product of sulphur-reducing bacteria.
- Depends largely on the relative abundance of sulphur containing compounds in the original biowaste.

Derived Biofuels: Methane biogas II

H_2S is acidic and poses a major corrosion risk.

- scrubbing of hydrogen sulphide out of biogas, Or
- to use a high-alkalinity lubricant oil which is changed often.

Derived Biofuels: Methane biogas II

- Biogas utilization involves burning it, with some of the energy being transformed to electrical.
- Different types of engines available…

END

Environmental Biotechnology

Derived Biofuels:
Ethanol Fermentation

Derived Biofuels: Ethanol Fermentation

Fermentation produces a solution of ethanol in water.

Can be further treated to produce fuel-grade ethanol by subsequent

- simple distillation, to 95% ethanol, or
- to the anhydrous form by azeotropic co-distillation using a solvent.

Derived Biofuels: Ethanol Fermentation

- Liquid fuels are of considerable importance due to the relative ease of transport and handing, and inherent controllability of combustion.

- Ethanol is a prime example in this respect, since it can be used either as a direct replacement for petrol, or as a co-constituent in a mix.

Derived Biofuels: Ethanol Fermentation

There are thriving ethanol industries in many countries of the world, generally using specifically energy-farmed biomass in the form of primary crop plants, like corn in the US and sugarcane in Brazil.

Derived Biofuels: Ethanol Fermentation

Commercial viability of the operation depends on:

- Indigenous economy,
- Employment,
- Transport costs,
- Government policy,
- Taxation, and
- Fiscal incentives.

Derived Biofuels: Ethanol Fermentation

- Brazil, an Excellent example.
- Ethanol/petrol mixing has been routine since the 1970s.
- Although dates back to the 1930s, the real upsurge lay in an unusual combination of events, partly driven by the energy-crisis of the mid-1970s.

Derived Biofuels: Ethanol Fermentation

- Rising oil prices…
- Came at the same time as a fall in sugar revenue...

- The Brazilian sugarcane industry, which had shortly before invested heavily, faced collapse.

- Production of fuel from the newly available biomass crop became a sound commercial move…

Derived Biofuels: Ethanol Fermentation

- The situation which exists today with biowaste is almost the same …

- This application potentially provides a major solution to two of the largest environmental issues, energy and waste.

Derived Biofuels: Ethanol Fermentation

- The distillation gives rise to relatively large volumes of potentially polluting by-products in the form of ‘stillage’.

- High in BOD and COD, between 6 and 16 liters are produced for every liter of ethanol distilled out.

Derived Biofuels: Ethanol Fermentation

END

- Developments in anaerobic treatments have begun to offer a better approaches for its treatment.
- The most integrated approach would be
- biowaste fermentation for ethanol distillation,
- biogas production from the stillage, and
- a final aerobic stabilization phase

Environmental Biotechnology

Short Rotation Coppicing I

Short Rotation Coppicing I

- Coppice: an area of woodland in which the trees or shrubs are periodically cut back to ground level to stimulate growth and provide firewood or timber.

- Coppicing: cut back (a tree or shrub) to ground level periodically to stimulate growth.

Short Rotation Coppicing I

Traditional method of woodland management which exploits the capacity of many species of trees to put out new shoots from their stump or roots if cut down.

Short Rotation Coppicing I

- Typically uses fast growing varieties.
- Involves establishing plantations which are then harvested to provide a long-term source of biomass material for combustion.

Short Rotation Coppicing I

- Substantial land requirement associated with SRC.
- Two to four year lead-in period.
- A yield of 8-20 dry tonnes per ha per year can be expected, with a calorific value of around 15 000 MJ/tonne.

Short Rotation Coppicing I

- Utilization is by burning, usually in the form of chips or short lengths.

- In addition, the potential for producing electricity is becoming increasingly important.

Short Rotation Coppicing I

- It is possible to characterize any given fuel in terms of its calorific value per unit mass, which is referred to as its energy density (ED).

- Wood, however, is a relatively low ED fuel and hauling it to a centralized facility, thus, becomes costly.

Short Rotation Coppicing I

There is a clear advantage, then, in maximizing the final yield of energy cropped trees and integrated biotechnology can assist in this regard.

Short Rotation Coppicing I

END

Major factors that govern biomass energy to land area ratio:

- The climate of the growing location,
- the irrigation needs of the particular trees being grown,
- the available nutrients in the soil, and
- the management regime…

Environmental Biotechnology

Short Rotation
Coppicing II

Short Rotation Coppicing II

Potential for nutrient and humus recycling from biowaste back into the soil, via composted, digested or otherwise biologically treated material.

Have water holding applications and form another example of the natural potential for environmental biotechnologies to self-integrate.

Short Rotation Coppicing II

It has been shown that at an application rate of around 250 tonnes of composted material per hectare, the land is able to hold between 1000 and 2500 tonnes of rainwater (Butterworth, 1999).

Short Rotation Coppicing II

Trials of large-scale compost treatment in East Anglia (Butterworth, 1999), showed that the compost would allow SRC crops to be grown without any further watering.

Short Rotation Coppicing II

- It also showed that the additional irrigation required is greatly reduced.
- Relatively immature composts are more effective as they can absorb and retain between 2-10 times their own weight of water.

Short Rotation Coppicing II

The chief end uses of biowaste-derived compost is as a horticultural amendment and fertilizer replacement.

There is no clear consensus between those working in the field as to how much nutrient is removed from the system when SRC wood is harvested, both N and P.

Short Rotation Coppicing II

In the case of soils with naturally low fertility, or those which have been used for coppice cropping for some years, supplementary mineral input may well be required.

Short Rotation Coppicing II

- If biowaste-derived material is used for its water-holding properties, the concomitant humus and mineral donation would represent what might be described as a gratuitous benefit.
- Process integration in this fashion brings evident economic advantages to any commercial coppicing operation.

Short Rotation Coppicing II

- Compost brings with it a ready-made microbial community which can significantly augment the compliment already present in naturally impoverished soils.
- With better physical structure, aeration is improved and root growth facilitated.

Short Rotation Coppicing II

Lead to a reduction in chemical fertilizers use.

Reduces nitrogenous inputs, reduces the farm’s pollution potential.

Example of cleaner production, since by biocycling nutrients back into the chain of biomass utility…

END

Environmental Biotechnology

Biodiesel

Biodiesel

- Biodiesel is derived mostly from vegetable oils…
- Transesterification…
- It can be used directly, in unmodified engines, with the additional bonus that it can perform as a single, pure fuel, or as part of a mix with its traditional counterpart.

Biodiesel

- Good evidence that particulate emissions are significantly reduced.
- Biodiesel exhaust is generally less harmful to both human health and the planet.
- Contains significantly lower levels of polycyclic aromatic hydrocarbons (PAHs) and nitrited polycyclic aromatic hydrocarbons (nPAHs).

END

Environmental Biotechnology

The Great Biofuel Conundrum

The Great Biofuel Conundrum

- Enormous energy demand particularly in industrialized countries…
- The production of biofuels has accordingly burgeoned in recent years.
- Driven by the twin pressures of environmental and economic necessity.

The Great Biofuel Conundrum

The rush towards biofuels could have larger social and environmental consequences.

UN reported growing evidence that food prices were being driven up in poorer nations of the world as oil-rich crops were grown for fuel.

The Great Biofuel Conundrum

Worldwatch Institute warned that current production methods could be exerting too heavy a price on land and water resources.

Some forms of biomass derived energy, such as SRC and waste-to-biogas, for example are outside of the ‘food or fuel’ discussion *ab initio*.

The Great Biofuel Conundrum

"*Biofuels are neither a panacea nor a pariah but like all technologies they represent both opportunities and challenges. Therefore a more sophisticated debate is urgently needed*".

Achim Steiner, UN Under-Secretary General and Executive Director of the UN Environment Program.

The Great Biofuel Conundrum

END

Using biomass in a balanced way, has certain clear advantages over the sequestration-only option.

Energy farmed biomass crops can support jobs, both directly and indirectly within the region.

Environmental Biotechnology

Integrated Agricultural Applications & Plant Disease Suppression I

Plant Disease Suppression I

There is a natural fit between agricultural and environmental biotechnologies and hence, a significant potential for integration both between and within them.

Plant Disease Suppression I

Another application is the suppression of plant diseases.

Expensive losses result from plant disease infection.

Previously crop rotation, use of animal manures, and then chemical fumigation methods were used… e.g., Methyl bromide… ozone depletion…

Plant Disease Suppression I

Alternative options are being explored:

- soil pasturisation
- using steam,
- ultraviolet treatment,
- the development of resistant cultivars.

Plant Disease Suppression I

The use of compost extracts – so-called 'compost teas'…

Twofold action:

- protection against foliar diseases, and
- an inoculant to restore or enhance soil microbial communities.

Plant Disease Suppression I

- Direct competition with the relevant pathogen
- Disease suppression,
- Induced disease resistance, and
- Inhibited germination of spores.

Plant Disease Suppression I

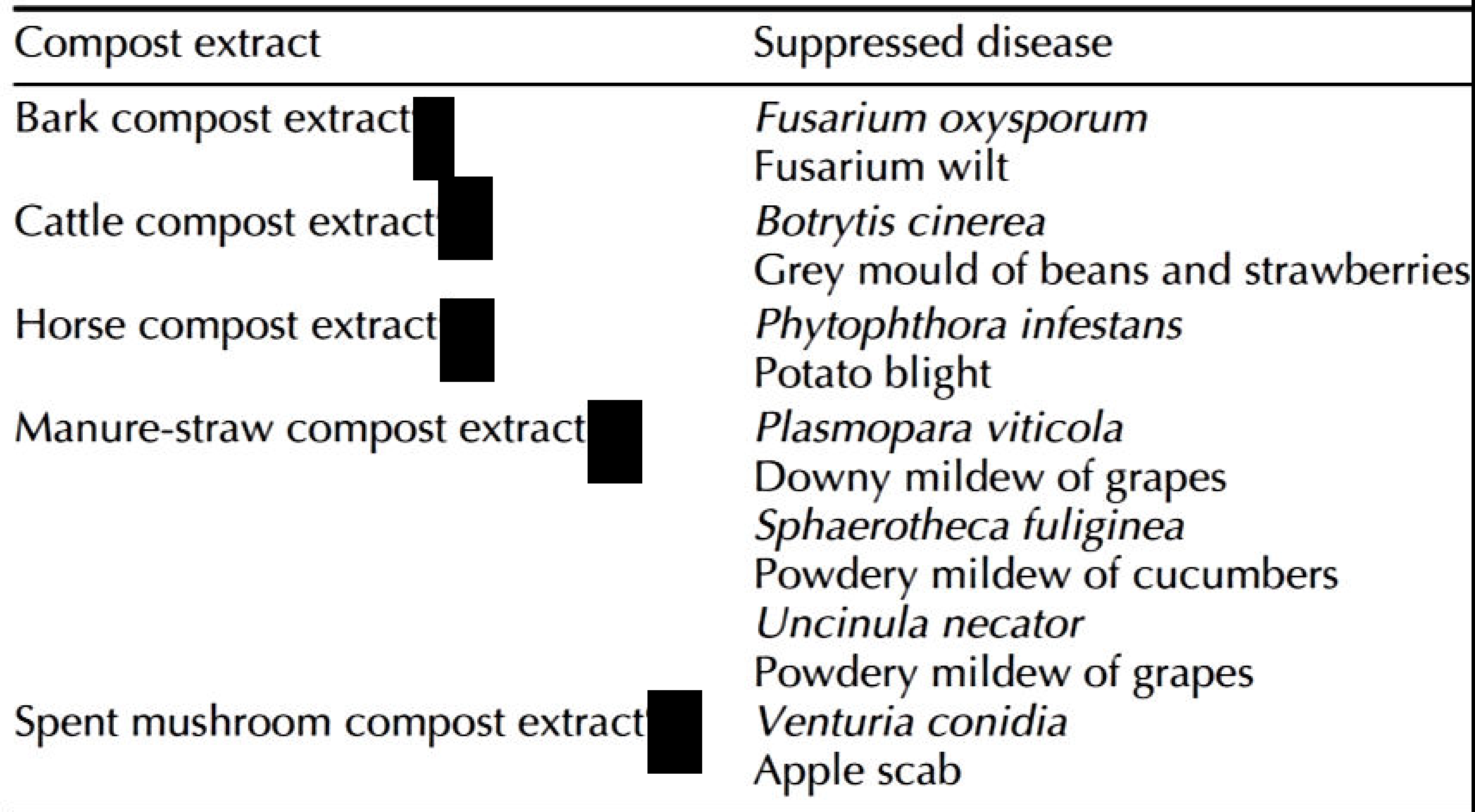
- Brought about by extract’s action on the surface of the leaves and stimulatory effect on the associated microorganisms.
- Microbes present in the extracts have been shown to be active agents.
- Evidence also points to a number of organic chemicals, including phenols and various amino acids.

Plant Disease Suppression I

The nature of the extracts seems to be principally biological, since fine filtration and sterilization by heat treatment significantly reduce the extract's effectiveness.

Plant Disease Suppression I

Plant disease suppression using selected compost extracts



Compost extract	Suppressed disease
Bark compost extract [redacted]	*Fusarium oxysporum* Fusarium wilt
Cattle compost extract [redacted]	*Botrytis cinerea* Grey mould of beans and strawberries
Horse compost extract [redacted]	*Phytophthora infestans* Potato blight
Manure-straw compost extract [redacted]	*Plasmopara viticola* Downy mildew of grapes
	Sphaerotheca fuliginea Powdery mildew of cucumbers
	Uncinula necator Powdery mildew of grapes
Spent mushroom compost extract [redacted]	*Venturia conidia* Apple scab

Environmental Biotechnology Theory and application, 2nd Ed by Gareth.

Environmental Biotechnology

Integrated Agricultural Applications & Plant Disease Suppression II

Plant Disease Suppression II

The abilities of properly prepared biowaste composts to suppress and control soil-borne plant diseases have been well established.

Plant Disease Suppression II

Particularly against

- *Phythium*,
- *Phytophthora,*
- *Fusarium* as well as
- *Rhizoctonia solani*.

Plant Disease Suppression II

- ➢ Public acceptability and quality assurance issues are major concerns. E.g.,
- Bovine spongiform encephalopathy (BSE),
- the concerns raised by genetically modified (GM) crops, or
- animals reared on GM crops…
- ➢ Safety code for the treatment and application of sewage sludge to agricultural land.

Plant Disease Suppression II

- Depend on factors which are more societal and economic than scientific or technical.
- In any novel application, cost is a major issue.
- Although the potential market may be enormous, the commercial benefits must be clear.

END

Environmental Biotechnology

Microbial Pesticides I

Microbial Pesticides I

- Chemical pesticides are notoriously recalcitrant.
- Their use may lead to a build up of chemicals damaging to the environment.
- Insects develop resistance to pesticides and so new and perhaps more poisonous chemicals might be needed.

Microbial Pesticides I

- Pesticides are rarely targeted to specific problematic species…

- Balanced natural environments have an equilibrium between assailant and victim…

Microbial Pesticides I

- Many bacteria produce toxins that kill certain insects.
- May not be sufficient to satisfy commercial crop production.

Microbial Pesticides I

Sufficient time may elapse between ingestion of the toxin by the larva and its ensuing death, for it to have caused considerable damage by feeding on the crop.

Microbial Pesticides I

- The best studied pesticidal bacterial toxin is the δ-endotoxin from *Bacillus thuringiensis*.
- Frequently abbreviated to ‘Bt toxin’, is active against members of the Lepidoptera (butterflies and moths), Diptera (flies, midges and mosquitoes) and Coleoptera (beetles) families.

Microbial Pesticides I

- Several strains of the bacterium each one producing a toxin active against a limited number of insect species.

- A leading candidate for development into an even more effective biopesticide.

Microbial Pesticides I

END

Limitations of Bt-Toxin:

- limited range of susceptible insects,
- require dosing with multiple toxins,
- insufficient ingestion by insects…,
- stability issues of the toxin when sprayed, and
- development of resistance by the insects.

Environmental Biotechnology

Microbial Pesticides
II

Microbial Pesticides II

Nematodes especially *Steinernema* sp., are demonstrating great potential as biological control agents applied as sprays.

It is anticipated that they will complement *Bacillus thuringiensis*.

Microbial Pesticides II

- *Bacillus sphaericus* produces a toxin more potent but more specific than Bt.
  Specific against mosquito larvae.
- *Bacillus popillae* kills its host by weight of bacterial numbers.
  Active against the Japanese beetle.

Microbial Pesticides II

Several Baculoviruses as insecticides against

- Maize bollworm (*Heliothis zea* SNPV),
- Gypsy moth (*Lymantira dispar* MNPV),
- Douglas fir tussock moth (*Orgyia pseudotsugata* MNPV) and in the UK against
- Pine sawfly (*Neodiprion sertifer* MNPV) and
- Pine beauty moth (*Panolis flammea* MNPV).

Microbial Pesticides II

Members of Baculoviruses (Nuclear Polyhedrosis Virus) cycle:

- Several virus particles are bound together in a large crystal of proteins.
- Present on leaves, ingested by an insects.
- Enzymes in the gut digest this protein releasing the viruses.
- Virus nucleocapsids enter the insect’s cells, make their way into the cell nucleus, and start replication.

Microbial Pesticides II

- In 12 hours, virus particles are released and spread to other cells.
- By 24 hours, the protective polyhedrin protein is being produced in sufficient quantities to start assembling the crystal structures again.
- By this time all the insect tissues are severely damaged leading to its death.

END

Environmental Biotechnology

Plant/Microbe Interactions

Plant/Microbe Interactions

All plant forms have interactions with microbes.

Most important biotechnologically is the interaction of microbes with higher plants.

Plant/Microbe Interactions

Two Categories:

Microbes external to the plants (epiphytes)
e.g. soil bacteria and soil fungi.

Microbes internal to the plants (endophytes)
e.g., nitrogen fixing bacteria, internal fungi.

Pathogens…

Plant/Microbe Interactions

The associations may involve bacteria, fungi or viruses.

In some cases, some quite complex interactions involving three or four different organisms

Often bring great benefit to the plant.

Plant/Microbe Interactions

Positive interaction

Mutualism
Syntrophism
Protocooperation
Commensalism

Negative interaction

Amensalism
Competition
Parasitism
Predation

END

Environmental Biotechnology

Microbes External to the Plants

Microbes External to the Plants

Two distinct areas:

- Above ground (on surface of leaves, stems, seeds and flowers), and
- Below ground in zones of increasing distance away from the root mass.

Microbes External to the Plants

These rhizospheres, or zones around the roots, a continuous gradient of nutrients…

Mostly due to plant metabolic activity...

Microbes External to the Plants

Colonization of the rhizosphere by bacteria is stimulated by exudate from the plant.

- The first phase is attraction to the plant roots,
- The second is a ‘settlement’ phase during which bacteria grow and
- The third is a ‘residence’ phase when a balance is established between root mass and bacterial numbers.

Microbes External to the Plants

Microbes in the rhizosphere are dependent on the plant for a supply of many useable organic substances.

Consequently, plants affect the composition of the microbial community.

Some plants may also produce inhibitors.

Microbes External to the Plants

The microbes also have an effect on the plant.

Some release plant growth factors such as gibberellins and cytokinins…

Microbes External to the Plants

Mycorrhizae:
Associations of fungi with roots of vascular plants

Quite common and in some cases be very beneficial to the plant.

They may be
- external, ectomycorrhizal, or
- internal, endomycorrhizal.

Microbes External to the Plants

Aid the growth of the plants as a result of their mycelia reaching far out into the surrounding soil, thus assisting the plant in nutrient uptake.

This quality has received commercial attention…

Microbes External to the Plants

- *Pseudomonas syringae* produces a protein known to act as a point of nucleation of ice crystals.
- Plants which harbour this bacterium run an increased risk of frost damage…
- *P. syringae* has been subjected to genetic engineering which successfully reduced the problem.

Microbes External to the Plants

END

- Plant–microbe interactions can play role in reducing ‘sick buildings’ effect…
- Bacteria resident in the soil of potted plants are able to degrade many of the volatiles, which include phenolics, formaldehyde and trichloroethylene.
- The plants also contribute by producing oxygen during photosynthesis…

Environmental Biotechnology

Microbes Internal to the Plants

Microbes Internal to the Plants

Two categories fulfil this description:

- The internal fungi or endomycorrhizae, and
- endophytic bacteria.

Plant pathogens, which may be bacterial or viral in form..

The term “endophytes” is usually used to denote non-pathogenic microorganisms.

Microbes Internal to the Plants

Commensals:
Neither benefit nor harm the plant, but there are also those which are beneficial to plant growth.

Symbiotes:
Achieve this status either by promoting plant growth or by protection against plant pathogens.

Microbes Internal to the Plants

END

Several roles played by endophytes…

- PGRP production,
- Antimicrobial compounds production,
- Nitrogen fixation, Etc.

Environmental Biotechnology

Symbiotic Nitrogen Fixation

Symbiotic Nitrogen Fixation

- Classic example: Nitrogen fixation by *Rhizobium* bacteria within plant root tissue.
- Limited number of organisms able to fix nitrogen, all of which are prokaryotes.
- All living organisms are dependent ultimately on such organisms, due to a universal and essential requirement for nitrogen.

Symbiotic Nitrogen Fixation

- Nitrogen reduced to ammonia either by free living organisms or by plant symbiots.
- Essential to have an oxygen free environment…
- Some prokaryotes achieve this naturally (e.g., *Clostridium*)
- Others have to create such an environment (e.g., *Cyanobacteria* and *Azobacter*).

Symbiotic Nitrogen Fixation

Although these free-living organisms have a vital role to play in their particular niches, approximately 10 times more nitrogen is fixed by plant symbiots.

Symbiotic Nitrogen Fixation

- Most probably because the plant provides the necessary levels of ATP...
- Plant supplies the endophytes with dicarboxylic acids, such as malate and succinate and
- Other nutrients, like iron, sulphur and molybdenum which is a component of the nitrogen fixing enzymes.

Symbiotic Nitrogen Fixation

Plant also provides its nitrogen-fixing symbiots with an oxygen free environment.

Symbiotic Nitrogen Fixation

- The analogous chemical process has an enormous energy requirement to achieve ~500 ◦C, and a pressure of ~200 atm…
- Moreover, leaching of surplus fertilizer into waterways, causing algal blooms.
- Therefore, drive to engineer plants both to increase nitrogen fixation (80% more efficient), and to extend the range of varieties which have this capability.

Symbiotic Nitrogen Fixation

Nitrogen fixation by bacteria occurs only in response to local need and so is very unlikely to be a source of pollution.

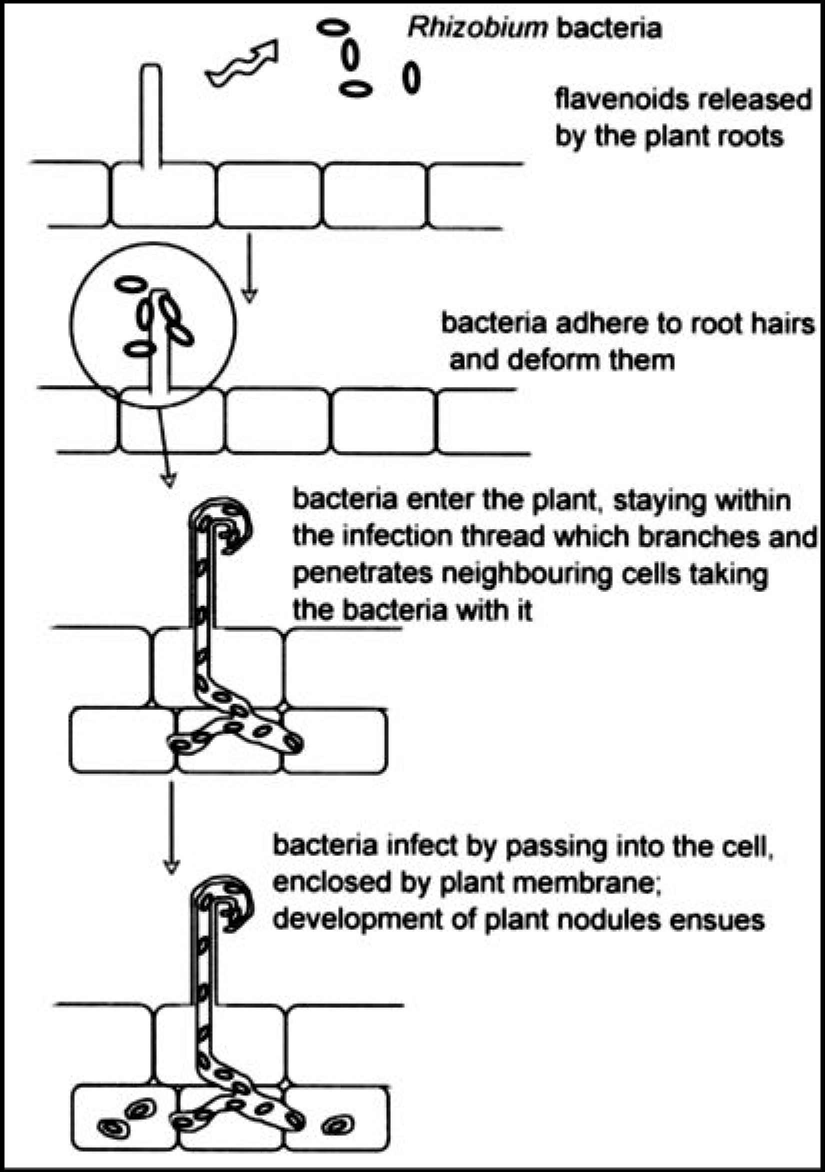
Symbiotic Nitrogen Fixation

In case of *Rhizobium*,

- Plant is invaded by a member of the *Rhizobium* family.
- Specific relationship between plant and bacterium...
- *nod* genes are coded for by the bacterium, activated by a mixture of flavonoids released by the plant.

Symbiotic Nitrogen Fixation

Root Nodule Formation



flavenoids released by the plant roots

bacteria adhere to root hairs and deform them

bacteria enter the plant, staying within the infection thread which branches and penetrates neighbouring cells taking the bacteria with it

bacteria infect by passing into the cell, enclosed by plant membrane; development of plant nodules ensues

Environmental Biotechnology Theory and application, 2nd Ed by Gareth.

Symbiotic Nitrogen Fixation

- Bacteria invade root hairs, and reach the cells of the inner root cortex.
- Drawn into the cell by endocytosis.
- This structure then develops into nodules.
- Followed by several changes to the plant including synthesis of different proteins e.g., leghaemoglobin, which may reach levels of up to 30% of the total nodule protein.

Symbiotic Nitrogen Fixation

Enzymes for fixation are coded by *nif* genes.

END

Environmental Biotechnology

Endomycorrhizae and Plant Pathogens

Endomycorrhizae and Plant Pathogens

In addition to bacteria, some fungi may also live within plants.

Found in various grasses and a wide range of commercial crops including tomatoes, apples, beans, wheat and corn.

Endomycorrhizae and Plant Pathogens

- Fungal hyphae penetrate plant cells where a variety of structures may develop such as swellings or the development of coils or small branches.
- Vesicles and arbuscules which are branched structures reminiscent of a tree, are common features of this invasion.

Endomycorrhizae and Plant Pathogens

- A beneficial association for the plant.
- In exchange for energy derived from the plant, the fungus may enhance the supply of available nutrients to the plant.
- In dry conditions may help the plant in the uptake of water.

Endomycorrhizae and Plant Pathogens

Some fungi have also been found to protect plant from insects by producing alkaloids.

Endomycorrhizae and Plant Pathogens

Pathogens:

Many bacteria, fungi and viruses can infect a plant and cause disease.

Most microorganisms will not be pathogens for a particular plant...

Endomycorrhizae and Plant Pathogens

Pathogens:

- Infection elicits numerous responses some of which may be quite complicated, and so has been the center of some fairly intensive research.
- Identification and isolation of plant genes involved in resistance to pathogens and pathogen virulence genes.

Endomycorrhizae and Plant Pathogens

Agrobacterium tumefaciens:

A tumour-like growth, seen as a crown gall in plants.

Consequence of injection by the bacterium of a small piece of DNA (on Ti plasmid)

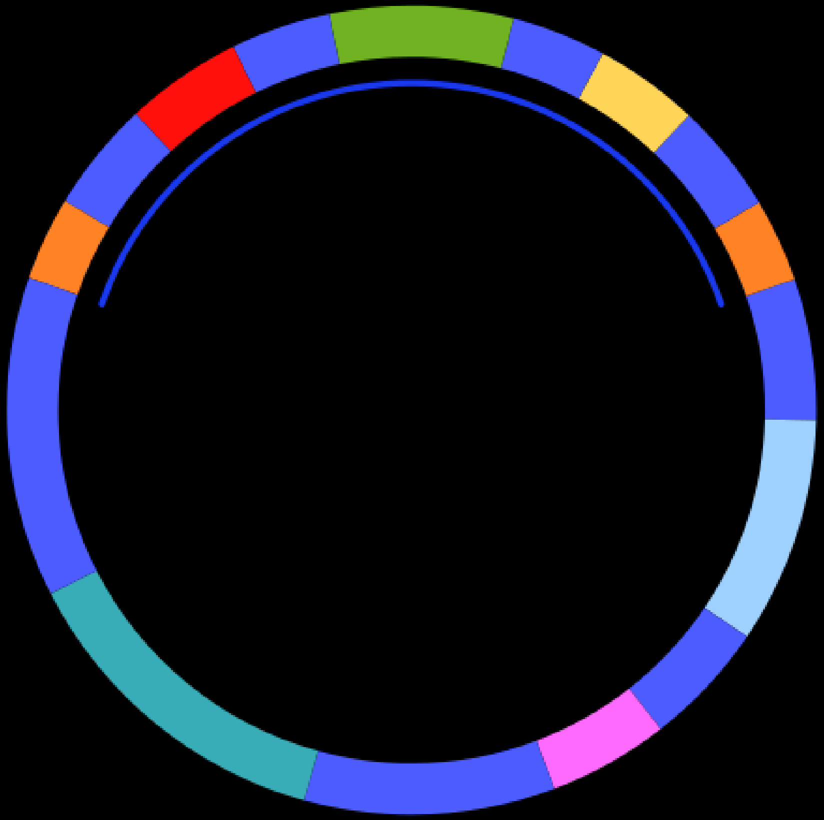
Endomycorrhizae and Plant Pathogens

Agrobacterium tumefaciens:

The transferred DNA carries genes for

- opines which encourage further invasion of the plant by the bacterium, and
- plant growth hormones including auxin to stimulate plant growth thus produces the characteristic tumour.

Endomycorrhizae and Plant Pathogens



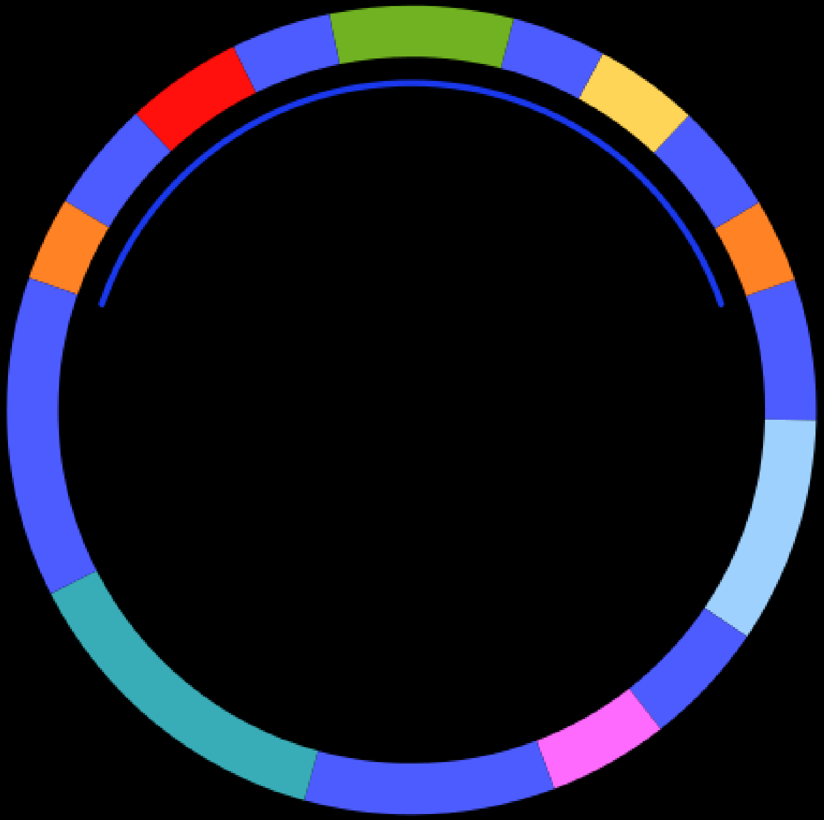
https://en.wikipedia.org/wiki/Ti_plasmid#/media/File:Ti_plasmid.svg

Endomycorrhizae and Plant Pathogens

Agrobacterium tumefaciens:

- The process of infection is stimulated by exudate from a plant which has been wounded by some means.
- The *vir* genes are activated leading to nicks being introduced at the borders of the T-DNA leading to the release of one of the strands…

Endomycorrhizae and Plant Pathogens



https://en.wikipedia.org/wiki/Ti_plasmid#/media/File:Ti_plasmid.svg

Endomycorrhizae and Plant Pathogens

Agrobacterium tumefaciens:

- The single stranded piece of T-DNA is transferred into the plant cell through a wound site and into the plant cell nucleus.
- The complementary strand is synthesized and the resulting double stranded DNA integrates into the plant genome.

Endomycorrhizae and Plant Pathogens

END

Agrobacterium tumefaciens:
Once the T-DNA is integrated into the host plant cell genome, these genes may be expressed leading to the establishment of crown gall disease.

A very effective natural delivery system for bacterial genes into a plant cell.

Environmental Biotechnology

Cauliflower Mosaic Virus

Cauliflower Mosaic Virus

- The study of plant viruses has lagged behind that of animal or bacterial viruses due to difficulties in their culture and isolation.

- Some viruses may be grown in isolated plant cultures, some may require the whole plant and some also require an insect intermediate either for transmission or for replication.

Cauliflower Mosaic Virus

Transfer of viruses between plants by insects is the most common means, especially by insects which penetrate and suck plants e.g., aphids.

Other routes also exist such as transfer by plant parts through infected seed, tubers or pollen.

Cauliflower Mosaic Virus

Genetic material of most plant viruses is RNA...

Caulimovirus (CaMV) is having a DNA genome.

This has proven very fortuitous for genetic engineering as it possesses two very strong constitutive promoters, the 35S and the 19S.

Cauliflower Mosaic Virus

Any DNA construct made with the intention of expressing the ‘foreign’ gene in a plant has a higher likelihood of the signals being recognized by the transcription machinery of the plant.

Cauliflower Mosaic Virus

These promoters have proved very successful and particularly the 35S, often designated in publications as ‘35S CaMV’.

END

Environmental Biotechnology

Case Study

Case Studies: ***Microbial Control and Cell Signaling***

- The prospect of being able to adjust the metabolic rate of bacteria, to either speed things up or slow them down, presents a powerful tool.
- Potential applications in a wide range of industries and activities.

Case Studies: *Microbial Control and Cell Signaling*

E.g., Effluent treatment.

- Sewage is rich with a wide variety of bacteria which accumulate in the sewerage pipes that convey effluent to treatment plants.
- Over time, biofilms are formed and give rise to both foul-smelling gases and corrosion.

Case Studies: ***Microbial Control and Cell Signaling***

- Australian company Biosol researched the cell signaling chemicals (quorum sensing),
- Developed commercial products capable of precisely influencing bacterial activity.
- Therefore, the dosed sewage travels inoffensively along sewer pipes, incapable of forming the biofilms.

Case Studies: *Microbial Control and Cell Signaling*

END

Once inside the treatment works, reversing the process with the dosing of ‘speed-up’ signals,

- to accelerate microbial breakdown
- to provide a faster processing,
- to achieve higher treatment throughput,
- a better quality final effluent,
- with lower residual biosolids load.

Environmental Biotechnology

Detoxification of Hazardous Chemicals I

Detoxification of Hazardous Chemicals I

A large number of synthetic organic chemicals…

Many are recalcitrant…

Many cause adverse effects on living beings and the environment…

Many have been banned…

Detoxification of Hazardous Chemicals I

Biodegradable organic chemicals generally do not have as many adverse effects as those that are highly resistant to biodegradation.

The latter tend to persist in the environment and spread throughout the world's ecosystems.

Detoxification of Hazardous Chemicals I

Biodegradation generally leads to detoxification of a chemical.

Thus, the biodegradation potential of a new compound is of great interest when judging its potential for environmental harm.

Detoxification of Hazardous Chemicals I

Among the first synthetic organic chemicals to create environmental problems were synthetic detergents.

Excellent cleaning properties.

However, it soon became apparent that it creates a problem of foaming…

Detoxification of Hazardous Chemicals I

- Alkyl benzene sulfonate (ABS) detergent not readily biodegradable.
- Primarily due to the quaternary carbon that attaches the aromatic structure to the alkyl part.
- Sulfonate group and the high degree of branching in the alkyl chain also contributes to resistance against biodegradation.

Detoxification of Hazardous Chemicals I

END

- The solution was to straighten the alkyl chain, which removed the branching and the quaternary carbon.
- The result was linear alkylbenzene sulfonate (LAS) which solved the foaming problem.

Environmental Biotechnology

Detoxification of Hazardous Chemicals
II

Detoxification of Hazardous Chemicals II

- Publication of *Silent Spring* by Rachel Carson (1962).
- Noted the growing incidences of problems to wildlife from pesticides.
- Concerns raised about chlorinated pesticides.
- One of the major forces that led to the environmental movement.

Detoxification of Hazardous Chemicals II

Other problematic organic chemicals are polychlorinated biphenyls (PCBs), halogenated solvents, and chlorofluorocarbons (CFCs).

Detoxification of Hazardous Chemicals II

Microbial infallibility:

All organic compounds (generally formed through biological activity) can and will be destroyed in the environment through biological activity.

Inaccurate as a general concept.

Ongoing Research…

Detoxification of Hazardous Chemicals II

Many naturally occurring compounds also are difficult to biodegrade.

E.g., aromatic components of gasoline, such as benzene, ethylbenzene, toluene, and xylenes (BETX); polycyclic aromatic hydrocarbons (PAHs); and dioxins, etc.

Detoxification of Hazardous Chemicals II

It was also thought that synthetic organic compounds are resistant to biodegradation…

But many of these organic compounds are biodegradable under some conditions.

Detoxification of Hazardous Chemicals II

- The fact that biodegradation can occur under some conditions is not sufficient to protect humans and the environment from a hazardous organic compound.
- The compound must be biodegradable under most natural conditions, including aerobic and anaerobic environments.

Detoxification of Hazardous Chemicals II

END

The ultimate goal is to mineralize organic compounds to inorganic elements, particularly carbon dioxide and water.

Environmental Biotechnology

Factors Causing Molecular Recalcitrance

Factors Causing Molecular Recalcitrance

Factors causing organic compounds to resist biodegradation:

- Structural characteristic of the molecule…
- The compound is inaccessible…
- Some factor essential for growth is absent…
- The environment is toxic.
- Requisite enzymes are inactivated.
- Some physiological inadequacy…

Factors Causing Molecular Recalcitrance

Molecular Structure:

- Most organic compounds are biodegradable by microbes...
- A portion of the organic material serves as electron and energy source....
- The other portion of the organic carbon is synthesized into cellular material…
- Such conversions mostly take place in aerobic environments…

Factors Causing Molecular Recalcitrance

Molecular Structure:

- Such conversions also occur in anaerobic environments…
- Nitrate, sulfate, carbon dioxide, other oxidized molecules serve as electron acceptors...

- Engineered systems can be designed and operated…

END

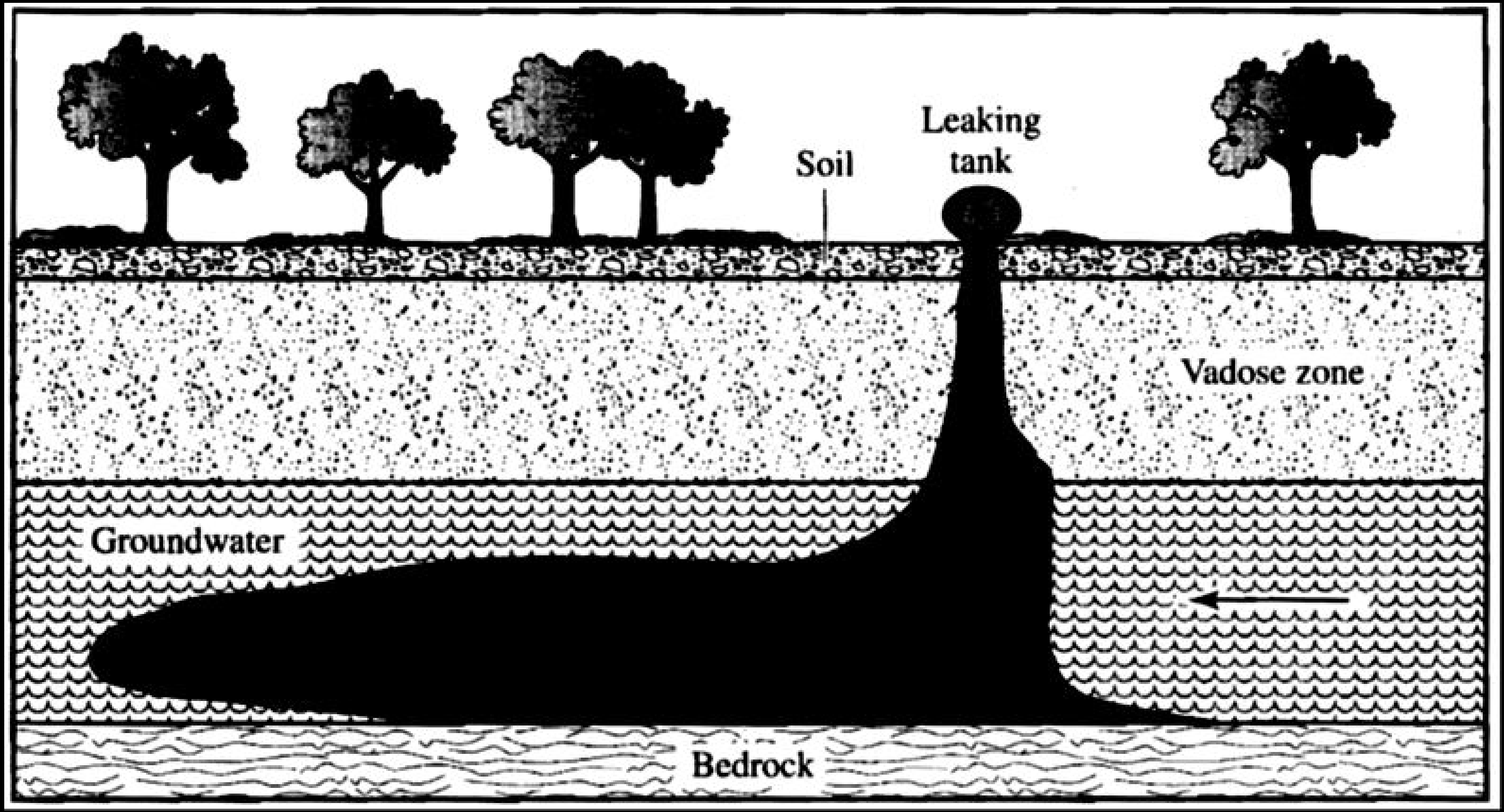
Environmental Biotechnology

Factors Causing Molecular Recalcitrance: Environmental Conditions

Environmental Conditions

- If a leakage of a storage tank happens…
- The liquid is pulled downward by gravity,
- Residuals left behind contaminate the surface soil, the unsaturated (vadose) zone, and finally the aquifer containing the groundwater itself…

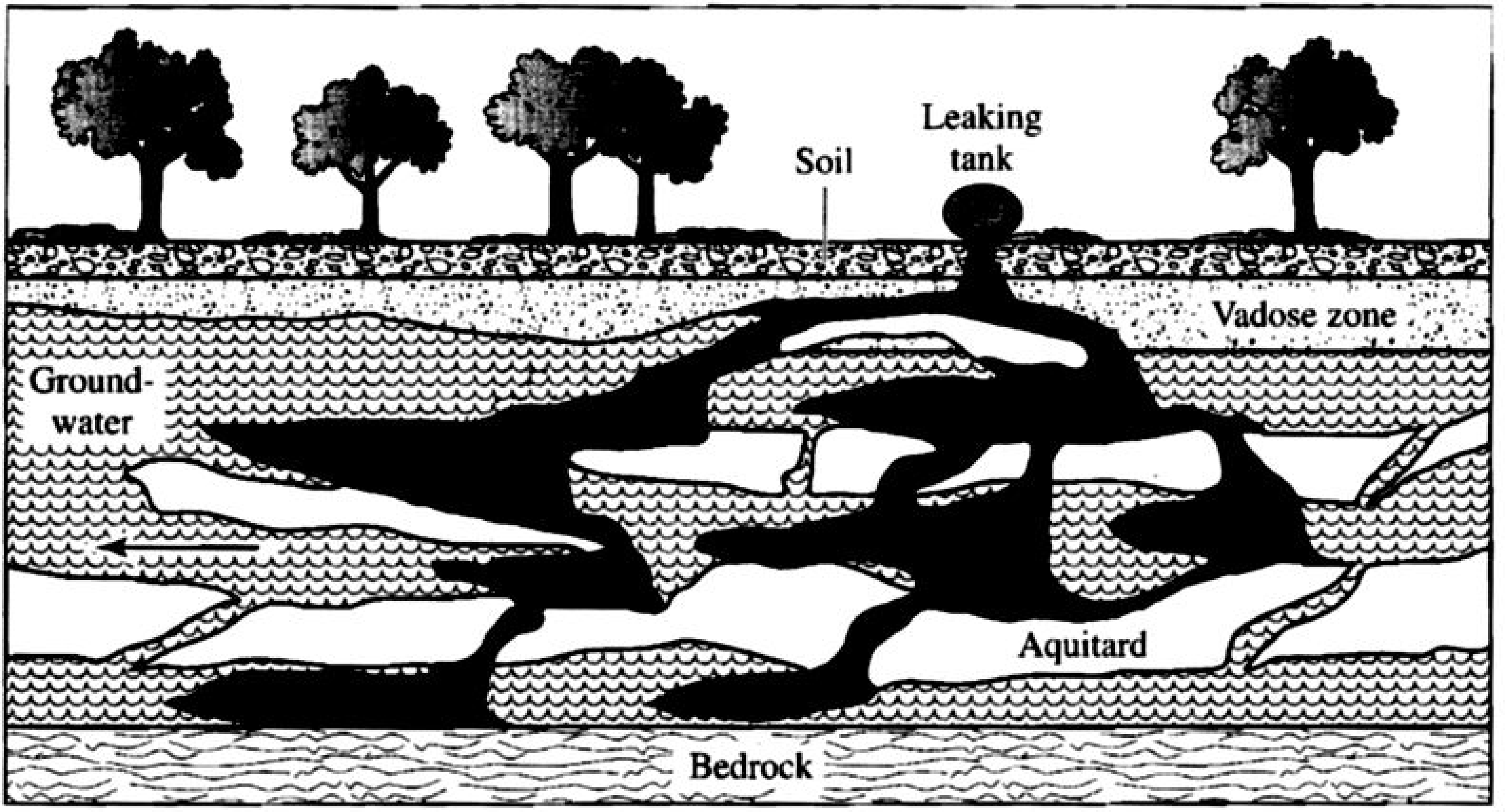
Environmental Conditions



Groundwater contamination resulting from a leaking tank

Environmental Biotechnology: Principles and Applications, by Bruce

Environmental Conditions



Groundwater contamination resulting from a leaking tank

Environmental Biotechnology: Principles and Applications, by Bruce

Environmental Conditions

After the leakage is stopped and the most highly contaminated soil around the tank is excavated, one must deal with a lower concentration residual in the soil, the vadose zone, and the groundwater.

Environmental Conditions

- Subsurface environments usually are much more complex…
- Layering of permeable (sands and gravels) and impermeable (silts, clays, and rock) strata are common.
- The mixture of gravel, sand, silt, clay, and organic matter can vary widely…

Environmental Conditions

Recalcitrance may result from

- The contaminant remaining in the nonaqueous phase liquid,
- Penetrating into fissures or small pores in minerals, and
- Strongly sorbing to particle surfaces.
- All of these render the contaminants unavailable to microorganisms.

Environmental Conditions

- The absence of suitable electron acceptors can be a key factor affecting biodegradability
- For aromatic hydrocarbons, degradation rates are generally enhanced through aerobic decomposition.

Environmental Conditions

- Recalcitrance also may result from insufficient nutrients, such as nitrogen and phosphorus, for bacterial growth.
- When these nutrients are below optimum levels, the amount of biomass is limited, and rates of biodegradation slow considerably

Environmental Conditions

END

- Where environmental conditions are suitable (and the proper microbes are present), complete mineralization of organic contaminants can occur.
- Where environmental conditions are not ideal, degradation of many organic chemicals still may take place at reduced rates.

Environmental Biotechnology

Factors Causing Molecular Recalcitrance: Microorganisms Presence

Microorganisms Presence

- Even though a contaminant is known to be readily biodegradable, the absence of a suitable microbial population may be a limiting factor.

Microorganisms Presence

- Several methods to determine the presence of microorganisms.

- Exposure of aseptically obtained soil or water to the contaminants...
  - If the microorganisms are naturally present, then degradation of the contaminant occurs.

Microorganisms Presence

- Isolation of species known to biodegrade the compounds of interest.
- Molecular techniques can identify the presence of specific microorganisms, nucleic acid sequences, or
- enzymes that are key to compound degradation.

Microorganisms Presence

- If appropriate organisms are absent, they may be introduced, a technique called *bioaugmentation*.

Microorganisms Presence

- Bioaugmented organisms may be natural, but not ubiquitous in nature.
  - *legally and politically acceptable*
- Genetically engineered microbes can be developed to degrade compounds that are inherently recalcitrant.
  - *legal and political concerns*
  - *questions about the fate of these organisms after their release*

Microorganisms Presence

END

An important question is, whether specialized organisms (natural or engineered) can survive in the new environment…

Environmental Biotechnology

Energy Metabolism
Versus Cometabolism

Energy Metabolism Versus Cometabolism

- Organic compounds that can be used by organisms to obtain energy for growth are the ones most susceptible to biodegradation.
- Microorganisms that obtain electrons and energy from the transformations carry out most degradation in biological wastewater treatment plants.

Energy Metabolism Versus Cometabolism

- Some compounds, are not used as a source of energy for microorganisms.

- Some such compounds may be transformed through cometabolism.

Energy Metabolism Versus Cometabolism

Cometabolism:

"A reaction in which microbes transform a contaminant even though the contaminant cannot serve as an energy source for the organisms.
To degrade the contaminant, the microbes require the presence of other compounds (primary substrates) that can support their growth."

https://www.igi-global.com/dictionary/hydrocarbon-biodegradation-using-agro-industrial-wastes-as-co-substrates/49449

Energy Metabolism Versus Cometabolism

- Most enzymes are quite specific, but some are not...
- Excellent examples of nonspecific enzymes are oxygenases that initiate the oxidation of hydrocarbons such as methane or toluene.

Energy Metabolism Versus Cometabolism

- These oxygenases often fortuitously oxidize a compound such as trichloroethene (TCE), forming an epoxide that is chemically unstable and degrades to simpler organic compounds that are readily biodegraded

Energy Metabolism Versus Cometabolism

- Methanotrophs have been demonstrated to produce methane monooxygenase, an oxidase that can degrade over 300 compounds.

Energy Metabolism Versus Cometabolism

END

In order to have cometabolic degradation of TCE, the normal electron donor for the organisms (methane or toluene) must also be present.

Peroxidases used by fungi to degrade lignin are also often not very specific and can bring about biodegradation of many different organic chemicals.

Environmental Biotechnology

Electron Donor Versus Electron Acceptors - I

Electron Donor Versus Electron Acceptors - I

- Organic compounds normally are electron donors in energy metabolism.
- However, that is not always the case.
- The most common exception is in fermentations, where one part of an organic compound is oxidized and another part is reduced.

Electron Donor Versus Electron Acceptors - I

E.g.,
$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$

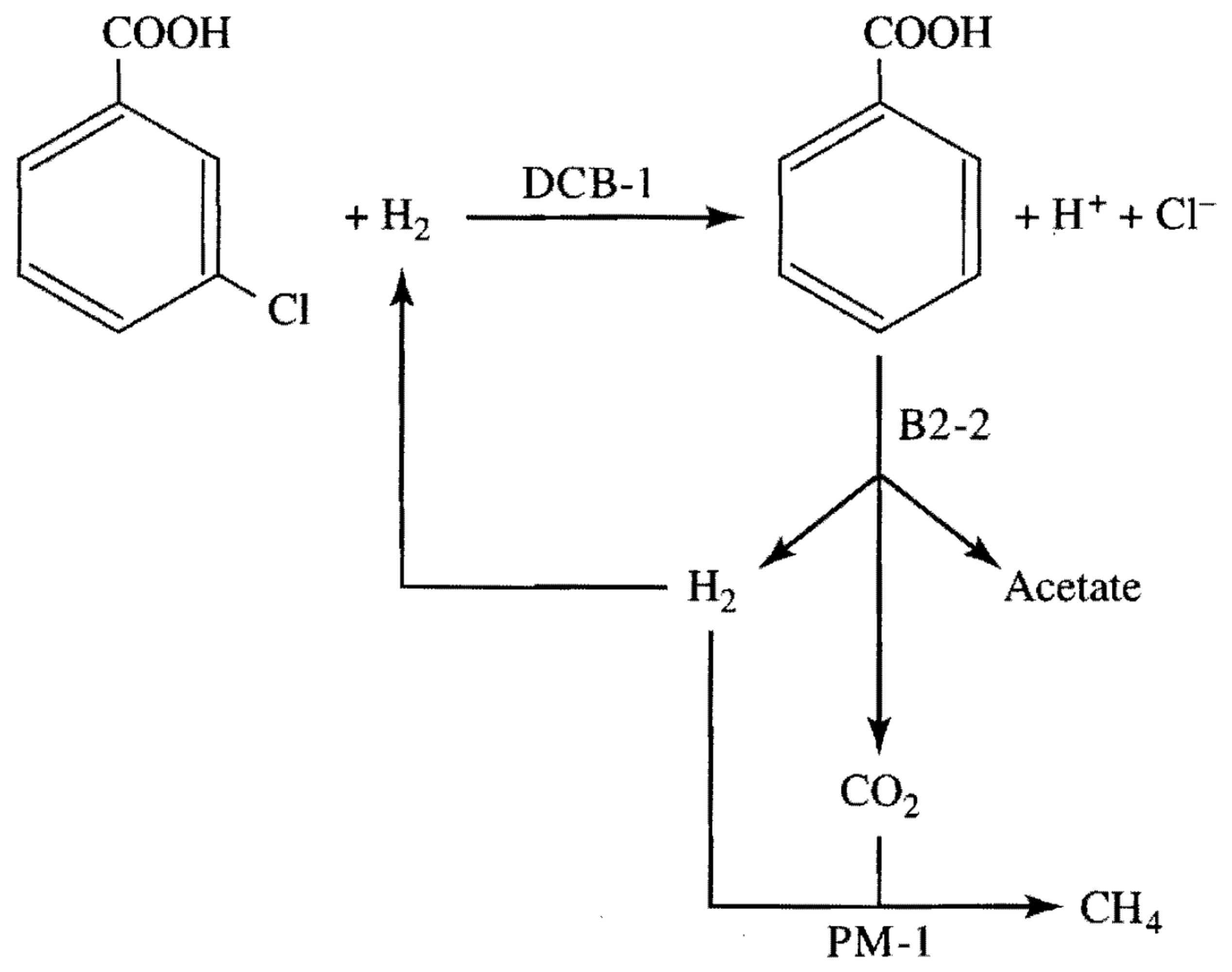
Four carbon atoms in glucose are partially reduced to ethanol, while two of the carbon atoms are oxidized to CO_2.

The part of the glucose that becomes ethanol is an electron acceptor, while the part becoming CO_2 is an electron donor.

Electron Donor Versus Electron Acceptors - I

- Halogenated organic compounds as electron acceptors
- Reductive dehalogenation of chlorobenzoate

Electron Donor Versus Electron Acceptors - I



Anaerobic degradation of m-chlorobenzoate

Environmental Biotechnology: Principles and Applications, by Bruce

Electron Donor Versus Electron Acceptors - I

- DCB-l obtains energy from hydrogen as an electron donor and chlorobenzoate as an electron acceptor.
- An example of dehalorespiration.
- Several other chlorinated organic chemicals serve as electron acceptors.

END

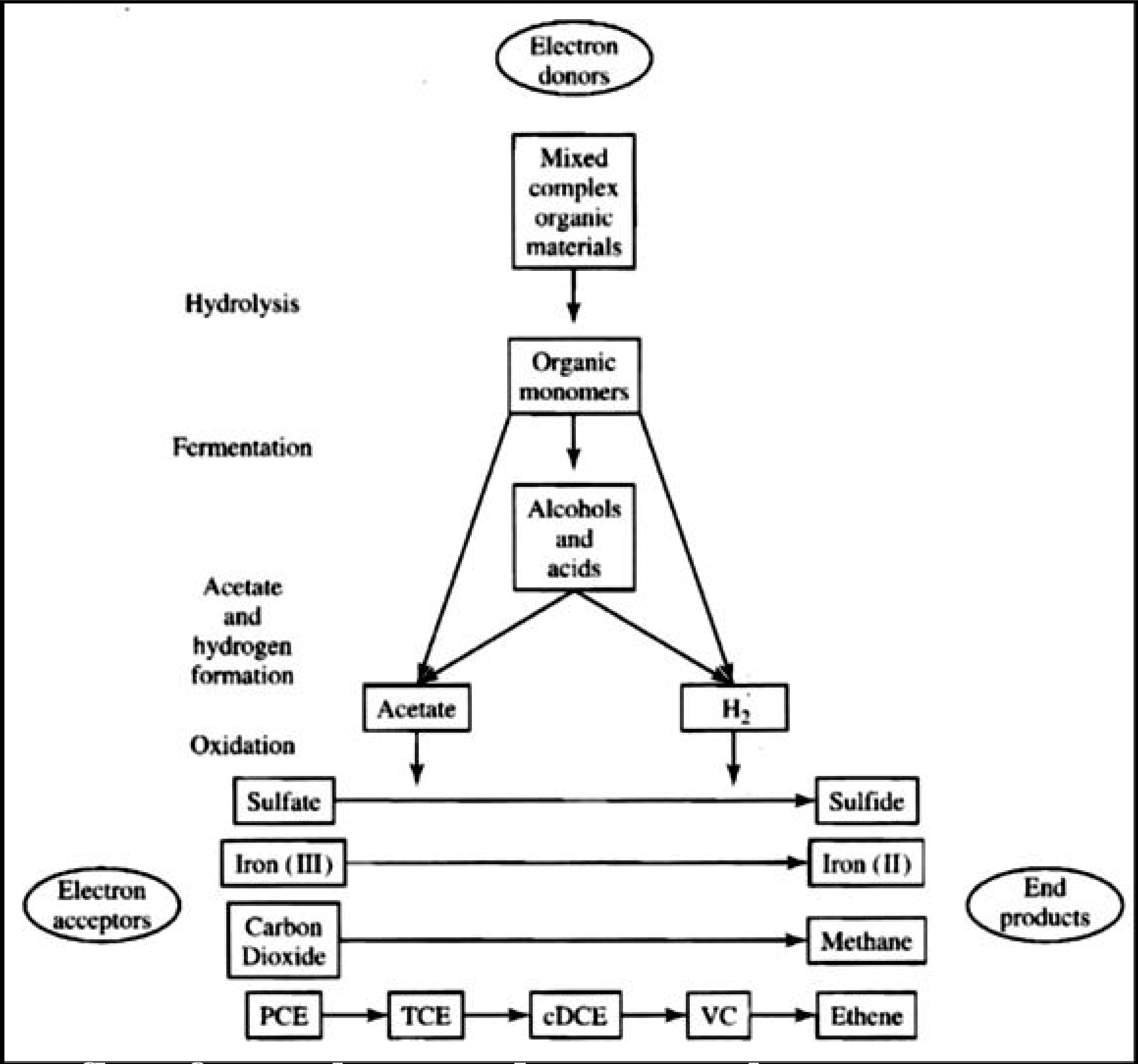
Environmental Biotechnology

Electron Donor Versus Electron Acceptors - II

Electron Donor Versus Electron Acceptors - II

- Dehalorespiration does not require that the dehalogenated compound also serve as the source of H_2.
- If a different organic compound is the initial electron donor, then competition for the available hydrogen can be quite keen.

Electron Donor Versus Electron Acceptors - II



Electron flow from electron donors to electron acceptors in the anaerobic oxidation of mixed and complex organic materials.

Environmental Biotechnology: Principles and Applications, by Bruce

Electron Donor Versus Electron Acceptors - II

This illustrates that, while reductive dehalogenation can be very important in the transformation of halogenated organics, it is not something that can be assumed to occur automatically.

Electron Donor Versus Electron Acceptors - II

END

- Organisms tend to be quite specific in transformation of compounds.
- Organisms that dehalogenate m-chlorobenzoate may be different from those that dehalogenate o-chlorobenzoate.
- Organisms that dehalogenate tetrachloroethene generally are different from those that dehalogenate dichloroethene.

Environmental Biotechnology

Minimum Substrate Concentration - I

Minimum Substrate Concentration - I

- Biodegradation of synthetic organic compounds is strongly affected by S_{min},
- *The lower threshold concentration below which reaction speed is insufficient to supply the organism with sufficient energy for net growth.*

Minimum Substrate Concentration - I

- Many hazardous organic compounds have drinking-water standards that are very low.
- Such concentrations are frequently less than S_{min}
- While a given compound may be easily biodegradable at higher concentrations, the question is whether or not it can be biodegraded at concentrations that meet regulatory and health standards.

Minimum Substrate Concentration - I

- Concentrations less than S_{min} can be obtained in two circumstances.
- The first is when the contaminant in used as a secondary substrate.
- Here, a microorganism obtains energy by utilizing more than one compound.
- The rates of electron and energy gain are the sum of the rates for all compounds.

Minimum Substrate Concentration - I

- E.g., Growth of one organism on acetate and dichloromethane.
- If acetate is present at a concentration higher than its S_{min}, it can be the main substrate supporting growth.
- The organism may use dichloromethane at the same time in order to obtain additional energy, even when dichloromethane is present below S_{min}.

END

Environmental Biotechnology

Minimum Substrate Concentration - II

Minimum Substrate Concentration - II

- Secondary utilization also works when many compounds present at concentrations less than S_{min} are utilized simultaneously.
- No one compound is the main primary substrate, but the primary substrate is an aggregate of many secondary substrates.
- E.g., a mixture of chlorinated benzenes (aerobic conditions) and halogenated methanes (anaerobic conditions).

Minimum Substrate Concentration - II

- The second method by which a contaminant can be biodegraded below S_{min} is through proper design of a treatment system.
- E.g., if the contaminant concentration in a wastewater is above its S_{min}, a plug-flow reactor (PFR) is theoretically capable of reducing the concentration to below S_{min}, while a continuous flow stirred-tank reactor (CSTR) is not.

Minimum Substrate Concentration - II

END

- The key is that the biomass must be retained in the plug-flow system by attachment as a biofilm or recycling of suspended growth.
- Then, the biomass at the effluent end of the process can remove the contaminant to a very low ($<< S_{min}$) concentration, because it was grown at the head end of the process, where the concentration was well above S_{min}.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Synthetic Detergents

Biodegradation of Synthetic Detergents

- Three basic types of synthetic detergents: anionic, cationic, and nonionic.
- The anionic detergents still are the major detergents used in laundry soaps.
- Cationic detergents are toxic to microorganisms.
- Used mostly when good disinfection, as well as cleaning action, is desired.

Biodegradation of Synthetic Detergents

- Nonionic detergents are not ionized and depend for solubility on polymers of ethylene oxide connected to a hydrophobic end that dissolves in grease.

- Alkyl-type of nonionic detergents tends to persist in natural waters.

Biodegradation of Synthetic Detergents

- The relative persistence of akylphenol polyethoxylates is related to the branched alkyl hydrophobic group connected to the aromatic ring.
- They generally contain 8 (octyl) or 9 (nonyl) carbons.
- As with the older ABS anionic detergents, the high degree of branching of these alkyl groups imparts resistance to biodegradation.

Biodegradation of Synthetic Detergents

- The general pathway for biodegradation begins with degradation of the ethylene oxide side chain.
- Cleavage of the side chain from the aromatic ring leaves an alkyl phenol residue.

Biodegradation of Synthetic Detergents

END

- Upon chlorination of water the alkyl group can become chlorinated or brominated, leading to halogenated organic compounds similar in structure to the 2,4-D pesticides and may have toxic properties.
- Even without halogenation, the residues of alkyl phenol polyethoxylates show toxicity to fish and are suspected of being endocrine disrupters.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Pesticides - I

Biodegradation of Pesticides - I

- Rachael Carson's 1962 book, *Silent Spring*, a major force behind the change in thinking of the public about the introduction of new chemicals into the environment.
- The main problem chemicals in 1962 were the chlorinated pesticides the first major one of great importance being DDT.

Biodegradation of Pesticides - I

- DDT was of great benefit to humans in their fight against malaria.
- Highly effective in controlling the *Anopheles* mosquito.
- While malaria was not a disease of significance in the United States, DDT found other uses for fighting crop insects.

Biodegradation of Pesticides - I

- Because of its great resistance to biodegradation and hydrophobic character, DDT's presence in the fatty tissues of birds and mammals, including humans, increased rapidly.
- DDT causes weakening of egg shells of birds, which led to dramatic decreases in the populations of many species.
- Leading to its ban.

Biodegradation of Pesticides - I

END

- Moreover, the experience with DDT was one of the first examples that target organisms develop a resistance to the toxic chemicals…
- Because DDT-resistant strains of the *Anopheles* mosquito risen, DDT's value for malaria control greatly diminished.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Pesticides - II

Biodegradation of Pesticides - II

- Two characteristics of chlorinated pesticides that led to great harm are
  - *their resistance to biodegradation and*
  - *their hydrophobicity.*
- Chlorinated pesticides are taken up by plankton and concentrated.
- Further concentrated in the fatty tissue of fish, birds and carnivores.
- This tendency to bioaccumulate magnifies their negative impacts.

Biodegradation of Pesticides - II

- The chemical structure of the chlorinated pesticides also makes them poorly susceptible to biotransformation reactions, especially under aerobic conditions.
- The chlorine substituents and the heavy branching block the normal sites for enzyme attack.

Biodegradation of Pesticides - II

- While chlorinated pesticides are highly resistant to transformation under aerobic conditions, anaerobic conditions are more favorable.
- Hill and McCarty (1967) found that lindane added to digested sludge from methanogenic fermentation disappeared rapidly, within days.

Biodegradation of Pesticides - II

- Reductive dehalogenation occurs with strongly reducing anaerobic conditions for essentially all chlorinated aromatic and aliphatic compounds.
- However, strong reducing conditions do not occur everywhere in the environment.
- So cannot be counted on to bring about detoxification of chlorinated compounds in general.

Biodegradation of Pesticides - II

END

- Moreover, reductive dehalogenation does not necessarily result in the complete destruction of the chemical.
- May create products that are hazardous to humans and the environment.
- The formation of DDD from DDT is an example.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Pesticides - III

Biodegradation of Pesticides - III

- Phosphorus-based pesticides…highly toxic, but upon exposure to water are enzymatically or chemically hydrolyzed.
- Hydrolysis occurs at the ester linkage in malathion, yielding ethanol and carboxylated pesticide residue, or between the carbon/oxygen bond in the carbon/ oxygen/ phosphorous linkages.

Biodegradation of Pesticides - III

- Many other transformations…
- Conversion of the sulfur atom connected to the phosphorus, forming a double bond to oxygen.
- E.g., Conversion of parathion to paraoxon in soil, a compound that is much more potent as a chlolinesterase inhibitor than the parent compound…

Biodegradation of Pesticides - III

- Carbamate pesticides transformed rapidly in the environment through hydrolysis.
- Atrazine is one of the most commonly found members of the s-triazines…most widely used herbicides.
- As triazines have relatively low octanol/ water partition coefficients, they do not sorb readily on soils, but become groundwater contaminants.

Biodegradation of Pesticides - III

- Atrazine biodegradation involves the sequential removal of the alkyl side chains followed by deamination, dehalogenation, and ring cleavage.
- Alkyl carbon can serve as a carbon and energy source...
- Ring carbon is fully oxidized and does not serve for catabolic or biosynthetic purposes.

END

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Hydrocarbons

Biodegradation of Hydrocarbons

- Organic compounds composed solely of carbon and hydrogen are termed hydrocarbons.
- Widely distributed natural products having many aliphatic and aromatic representatives.

Biodegradation of Hydrocarbons

- Can be rather simple compounds, such as methane or benzene, or
- Highly complex and large molecules, as represented by petroleum and coal.
- Many are saturated completely, while others contain unsaturated carbon bonds.

Biodegradation of Hydrocarbons

- Biodegradable aerobically.
- Rate of aerobic biodegradation depends on the complexity of the molecule.
- Large hydrocarbons with much branching or containing many aromatic rings are difficult to degrade.

Biodegradation of Hydrocarbons

- One aspect of their poor biodegradability is their low water solubility.
- The other is that the complex structure makes it difficult for microorganisms to find a location for an initial enzymatic attack.

Biodegradation of Hydrocarbons

The initial step in hydrocarbon degradation by bacteria:

- *the introduction of oxygen into the molecule with an oxygenase*,
- *energy investment in the form of NAD(P)H and molecular oxygen.*

Biodegradation of Hydrocarbons

- The hydrocarbon oxidized by two electrons for each -OH group added.
- The organisms do not recover the electrons as NADH.
- The products of the oxygenation reactions are more available…
- Attacked in subsequent reactions, which yield NADH.

Biodegradation of Hydrocarbons

- Many aromatic hydrocarbons are known to be biodegradable in the absence of oxygen.
- Organisms effecting the biodegradation of many aromatic hydrocarbons have been isolated, and biochemical pathways are being discovered.

Biodegradation of Hydrocarbons

END

- In general, the anaerobic reactions are quite slow compared with their aerobic counterparts.
- These are of importance to the fate of aromatic hydrocarbons.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: BTEX and MTBE I

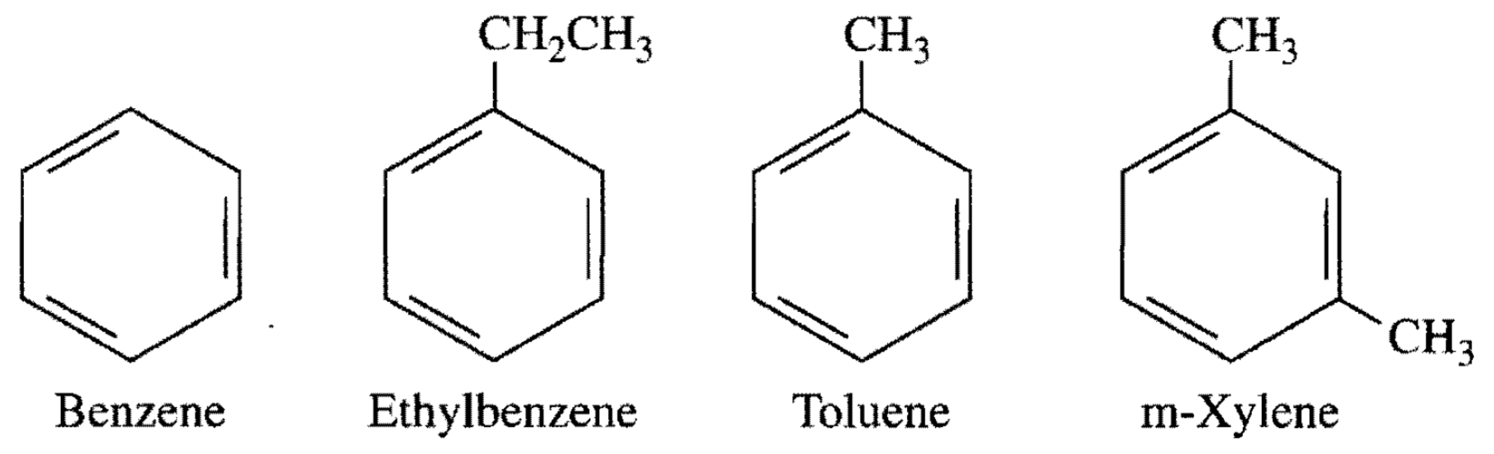
Biodegradation of BTEX and MTBE I

- Gasoline is a complex mixture of hundreds of different hydrocarbons.
- Most are saturated and contain 4 to 12 carbon atoms per molecule.
- From 22 to 54 % of gasoline is composed of aromatic hydrocarbons.

Biodegradation of BTEX and MTBE I

- Most common are benzene, toluene, ethylbenzene, and different isomers of xylene, which comprise the BTEX compounds.

Biodegradation of BTEX and MTBE I



The major aromatic hydrocarbon constituents of gasoline, generally referred to as the BTEX compounds

Environmental Biotechnology: Principles and Applications, by Bruce

Biodegradation of BTEX and MTBE I

- Gasoline may also include methylethyl benzenes and trimethyl benzenes.
- Toluene the most dominant, followed by the isomers of xylene, then ethylbenzene and benzene in about equal amounts.
- Jet fuels and lubrication oils have somewhat lower aromatic content.

Biodegradation of BTEX and MTBE I

- BTEX compounds are toxic and more water-soluble than the other hydrocarbons in gasoline.
- Benzene is a known human carcinogen.
- Individual BTEX compounds also are of commercial interest
- *used as solvents and in the production of other chemicals.*

Biodegradation of BTEX and MTBE I

- BTEX are natural products.
- The widespread human use of gasoline and other products containing BTEX has caused their wide environmental distribution.

Biodegradation of BTEX and MTBE I

END

- Being natural products, they are biodegraded under aerobic conditions.
- The biochemical pathways and enzymes involved in aerobic oxidation are well established.
- Numerous ubiquitous microbes can oxidize BTEX.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: BTEX and MTBE II

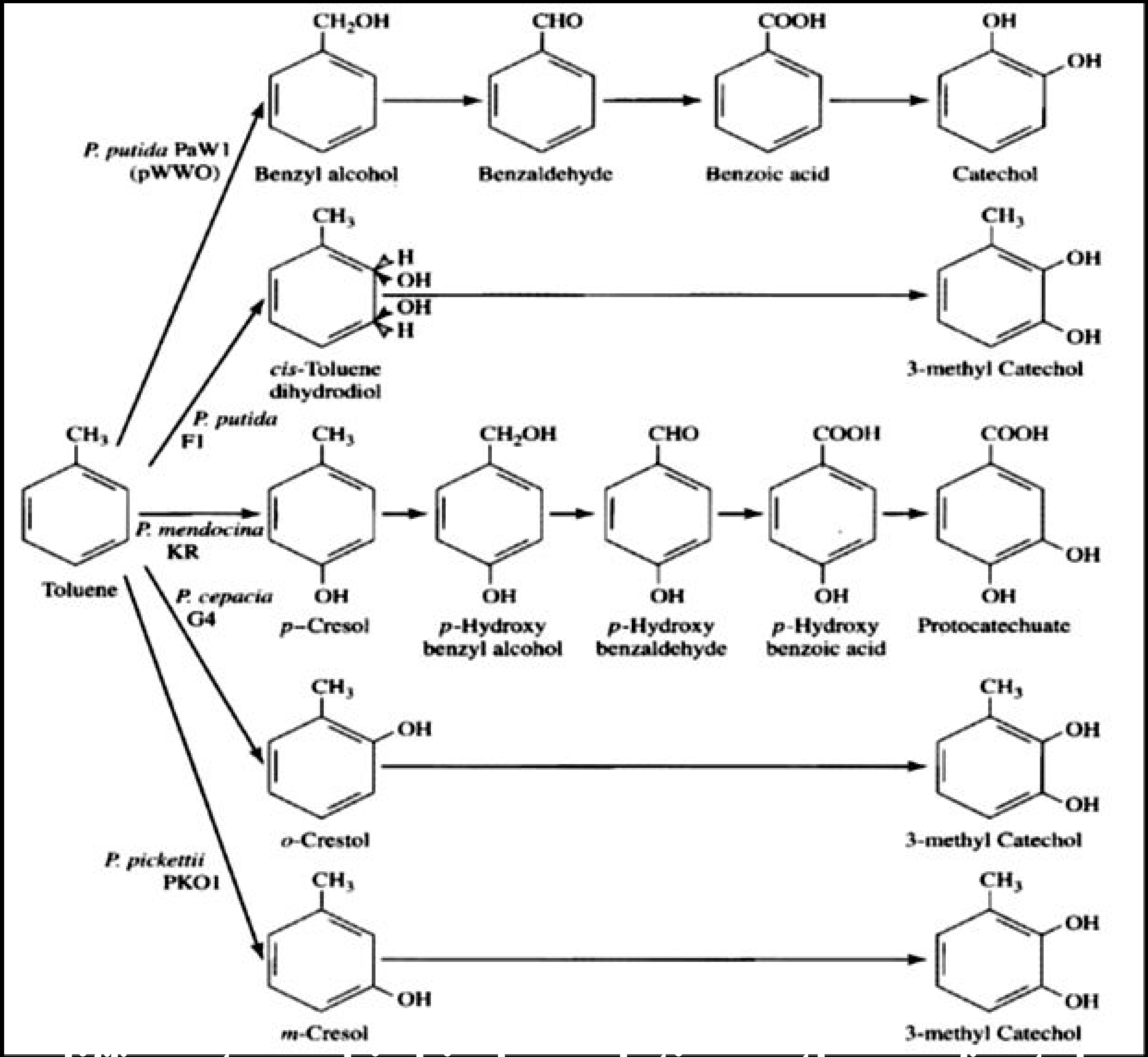
Biodegradation of BTEX and MTBE II

- The initial oxidation step is difficult
- Requires an oxidase, along with molecular oxygen and energy in the form of NADH.
- Many different oxidases can initiate BTEX biodegradation.
- Oxidation reactions occur up to the point where two hydroxyl groups are adjacent to each other on the ring.

Biodegradation of BTEX and MTBE II

- The ring is then cleaved by addition of more oxygen either
- *between the two carbons connected to the hydroxyl groups (ortho fission) or*
- *at one of the two adjacent double bonds (meta fission).*
- This results in a carboxylated aliphatic compound.
- This can be oxidized further by the usual dehydrogenation and hydroxylation reactions.

Biodegradation of BTEX and MTBE II



Five different aerobic biodegradation pathways for toluene

Environmental Biotechnology: Principles and Applications, by Bruce

Biodegradation of BTEX and MTBE II

- BTEX compounds can also be biodegraded under anoxic conditions.
- Numerous microbes capable of degrading BTEX compounds under anoxic conditions have been isolated, and the biochemical pathways determined.

Biodegradation of BTEX and MTBE II

- Many microorganisms can biodegrade toluene, ethylbenzene, and xylenes through denitrification.
- Isolates that can degrade BTEX compounds under more reducing conditions are more limited.

Biodegradation of BTEX and MTBE II

- Degradation of BTEX compounds under sulfate-reducing or methanogenic conditions in complex mixture of organisms has been observed frequently.
- Use of Fe(III) or Mn(IV) as electron acceptors is also possible.

Biodegradation of BTEX and MTBE II

- In anaerobic conditions, the rates of degradation tend to be slower than under aerobic conditions.
- The organisms that can carry out the reactions do not appear to be as prevalent.

Biodegradation of BTEX and MTBE II

- In California (in the past), Methyl tertiary butyl ether (MTBE) used to be added to gasoline in order to reduce smog-producing emissions.
- While the BTEX compounds in gasoline biodegrade, MTBE often does not…
- Led to a reversal of California's earlier decision…

END

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Polycyclic aromatic hydrocarbons (PAHs) I

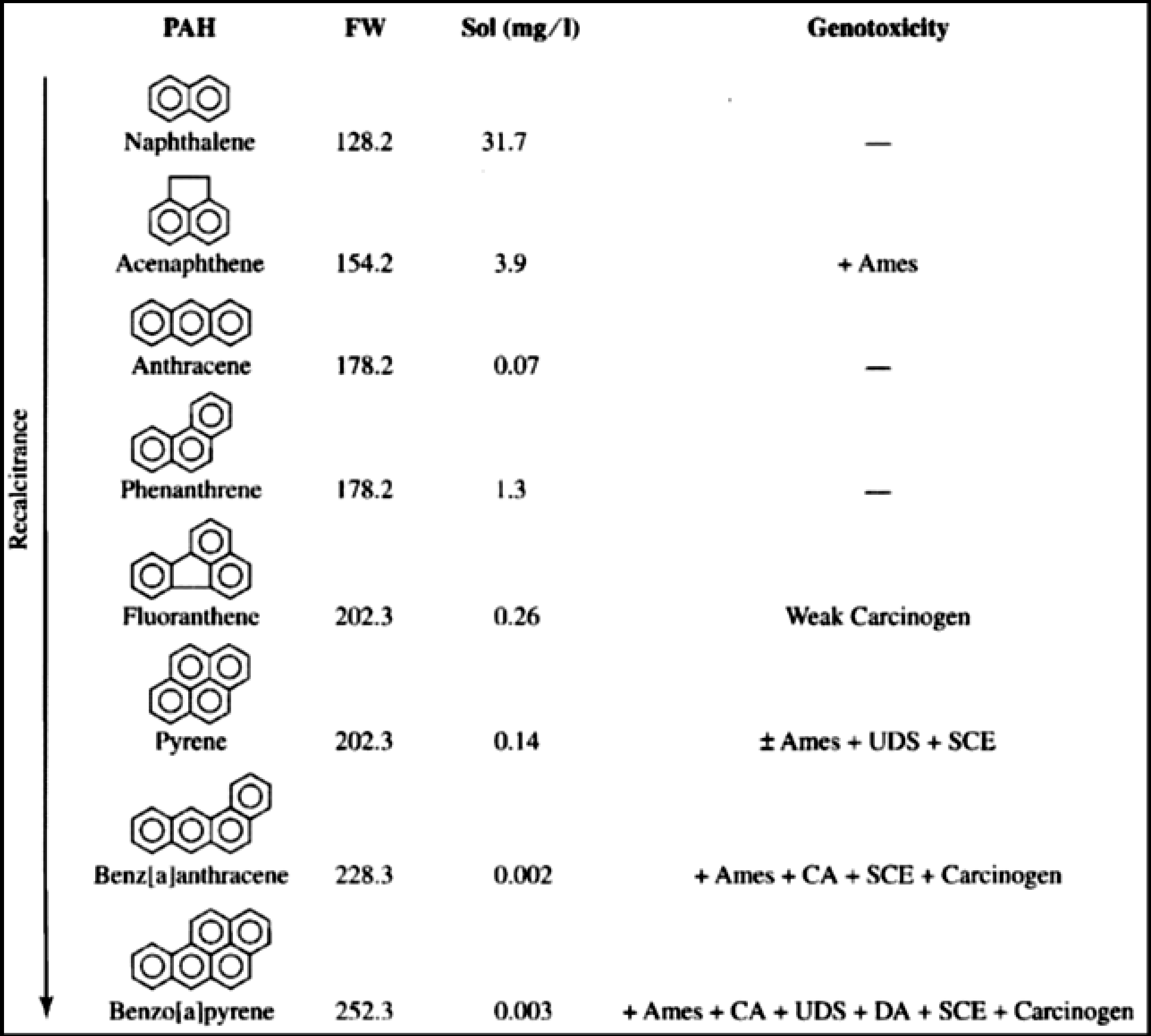
Biodegradation of PAHs - I

- Naturally formed aromatic hydrocarbons
- Of general concern because of toxicity.
- Potential for causing cancer.
- Formed from incomplete combustion of organic matter-naturally and through human activities-and thus are found widely in the environment.

Biodegradation of PAHs - I

- Major concerns today are the high concentrations of PAHs surrounding commercial processes that involve incomplete combustion of coal.
- The coal-tar residue, which contains PAHs, contaminates soils surrounding such plants.

Biodegradation of PAHs - I



Recalcitrance

PAH	FW	Sol (mg/l)	Genotoxicity
Naphthalene	128.2	31.7	—
Acenaphthene	154.2	3.9	+ Ames
Anthracene	178.2	0.07	—
Phenanthrene	178.2	1.3	—
Fluoranthene	202.3	0.26	Weak Carcinogen
Pyrene	202.3	0.14	± Ames + UDS + SCE
Benz[a]anthracene	228.3	0.002	+ Ames + CA + SCE + Carcinogen
Benzo[a]pyrene	252.3	0.003	+ Ames + CA + UDS + DA + SCE + Carcinogen

Various polycyclic aromatic hydrocarbons of importance.

Environmental Biotechnology: Principles and Applications, by Bruce

Biodegradation of PAHs - I

- Tend to remain in surface soils and do not migrate in groundwaters significantly.
- In general, PAHs are assumed to be primarily soil, rather than water, contaminants.
- However, they can concentrate in the sediments of rivers, lakes, and streams.

END

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Polycyclic aromatic hydrocarbons (PAHs)-II

Biodegradation of PAHs - II

- PAHs are biodegradable under aerobic conditions.
- Two- to four-ring compounds are quite readily biodegraded aerobically.

Biodegradation of PAHs - II

- Oxidation is initiated by oxygenases that require O_2.
- Higher ringed PAHs are quite resistant.
- Due to their structure complexity and very low solubility…
- Many-ringed PAHs can be biodegraded aerobically…
- *may be through cometabolism by organisms living on PAHs with a few rings.*

Biodegradation of PAHs - II

- Anaerobic degradation of PAHs has also been reported.
- E.g., naphthalene biodegradation through denitrification, biodegradation of phenanthrene, etc.
- Rates are generally quite low as compared with aerobic biodegradation.

Biodegradation of PAHs - II

END

- PAHs that are tightly bound to soils should be of environmental concern???
- If not bioavailable to microbes, are they bioavailable to cause harm??
- Alternatives to biodegradation are removal and burial in a secured landfill or incineration.
- Both of which can be very expensive.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Chlorinated Solvents and other Halogenated Aliphatic Hydrocarbons

- I

Biodegradation of Chlorinated Solvents - I

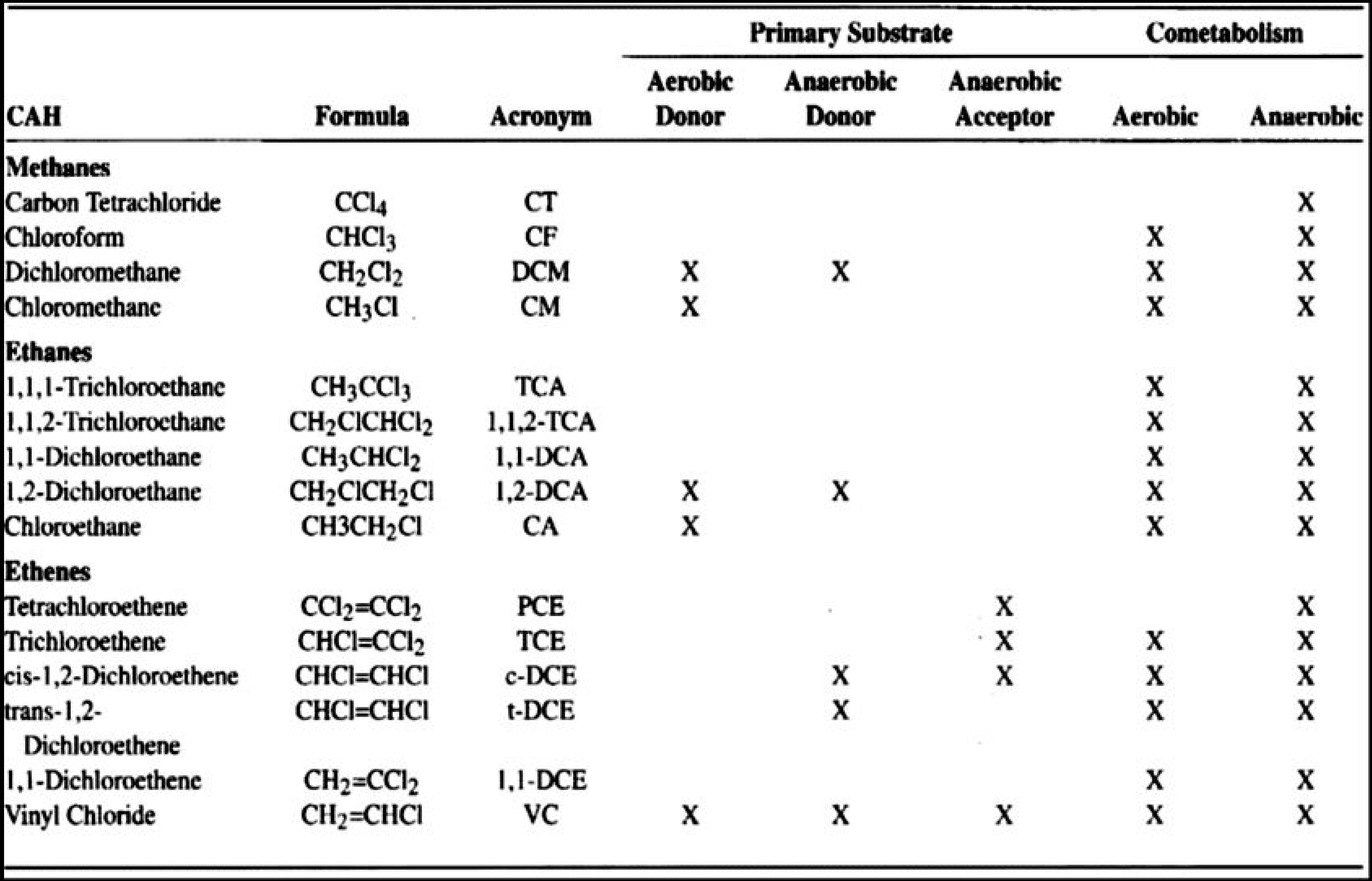
- Chlorinated solvents are among the most prevalent organic contaminants in groundwater.
- The chlorinated aliphatic hydrocarbons (CAHs) and related chlorinated compounds generally contain one or two carbon atoms and one to six chlorine atoms.

Biodegradation of Chlorinated Solvents - I

- CAHs with two carbon atoms may be saturated (ethanes) or unsaturated (ethenes).
- Chlorinated solvents are widely used for cleaning clothes, engines, electronic parts, and other items contaminated with grease.
- Superior to hydrocarbon alternatives being not explosive or readily combustible.

Biodegradation of Chlorinated Solvents - I

Major chlorinated aliphatic hydrocarbon (CAH) contaminants



			Primary Substrate			Cometabolism	
CAH	Formula	Acronym	Aerobic Donor	Anaerobic Donor	Anaerobic Acceptor	Aerobic	Anaerobic
Methanes							
Carbon Tetrachloride	CCl_4	CT					X
Chloroform	$CHCl_3$	CF				X	X
Dichloromethane	CH_2Cl_2	DCM	X	X		X	X
Chloromethane	CH_3Cl	CM	X			X	X
Ethanes							
1,1,1-Trichloroethane	CH_3CCl_3	TCA				X	X
1,1,2-Trichloroethane	$CH_2ClCHCl_2$	1,1,2-TCA				X	X
1,1-Dichloroethane	CH_3CHCl_2	1,1-DCA				X	X
1,2-Dichloroethane	CH_2ClCH_2Cl	1,2-DCA	X	X		X	X
Chloroethane	$CH3CH_2Cl$	CA	X			X	X
Ethenes							
Tetrachloroethene	$CCl_2{=}CCl_2$	PCE			X		X
Trichloroethene	$CHCl{=}CCl_2$	TCE			X	X	X
cis-1,2-Dichloroethene	$CHCl{=}CHCl$	c-DCE		X	X	X	X
trans-1,2-Dichloroethene	$CHCl{=}CHCl$	t-DCE		X		X	X
1,1-Dichloroethene	$CH_2{=}CCl_2$	1,1-DCE				X	X
Vinyl Chloride	$CH_2{=}CHCl$	VC	X	X	X	X	X

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Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Chlorinated Solvents and other Halogenated Aliphatic Hydrocarbons - II

Biodegradation of Chlorinated Solvents - II

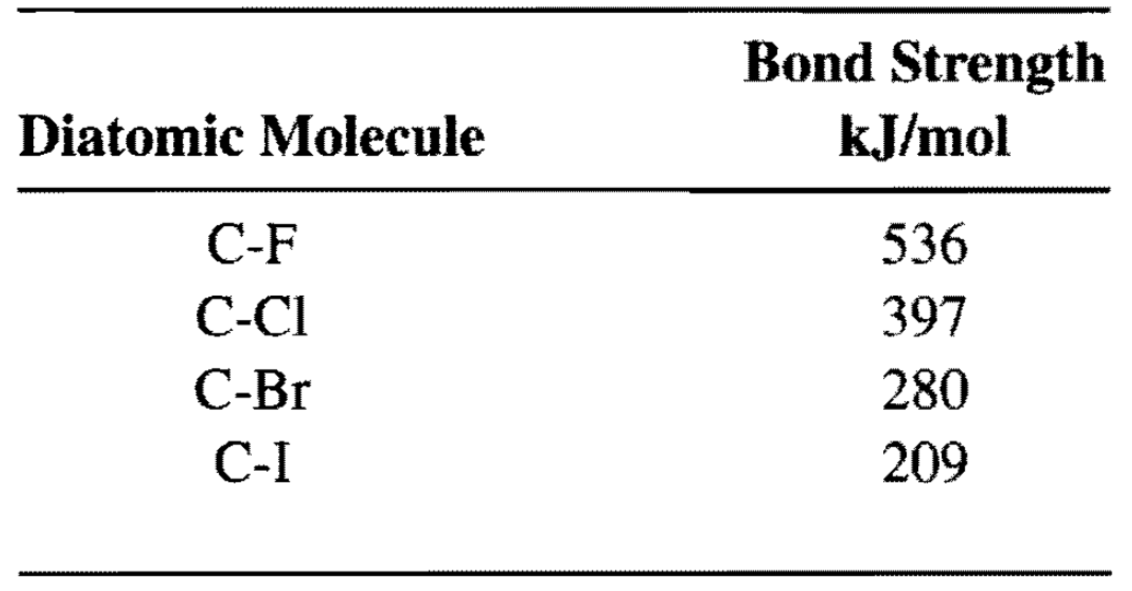
- Most CAHs are not natural compounds,
- Perhaps for this reason enzyme systems capable of their degradation have not evolved sufficiently.
- The order of their resistance is closely related to the halogen-carbon bond strength.

Biodegradation of Chlorinated Solvents - II

- The relative resistance to biodegradation of halogenated organics increases as one goes from bromide to chloride to fluoride.
- Many CAH can be transformed biologically under suitable conditions.

Biodegradation of Chlorinated Solvents - II

Strengths of carbon-halogen bonds



Diatomic Molecule	Bond Strength kJ/mol
C-F	536
C-Cl	397
C-Br	280
C-I	209

Handbook of Chemistry and Physics. Boca Raton, Fl: CRC Press

Environmental Biotechnology: Principles and Applications, by Bruce

Biodegradation of Chlorinated Solvents - II

- Some can be used as an electron donor for energy generation and growth…
- Others are biotransformed strictly through cometabolism.
- Some can be used as an electron acceptor, either for energy generation or through cometabolism.

Biodegradation of Chlorinated Solvents - II

- Chloromethane and dichloromethane can be used by many microbes for energy and growth under aerobic or anaerobic conditions.
- Dichloroethane and vinyl chloride also can be used aerobically for energy and growth.
- When conditions are suitable, these CAHs are readily biodegraded.

Biodegradation of Chlorinated Solvents - II

END

- Highly halogenated compounds-such as trichloroethene (TCE), dichloroethene (DCE), 1,1,1-trichloroethane (l,l,l-TCA), and chloroform (CF)-can be biodegraded
- *aerobically through cometabolism or*
- *anaerobically as electron acceptors, which may either represent cometabolism or dehalorespiration.*

Environmental Biotechnology

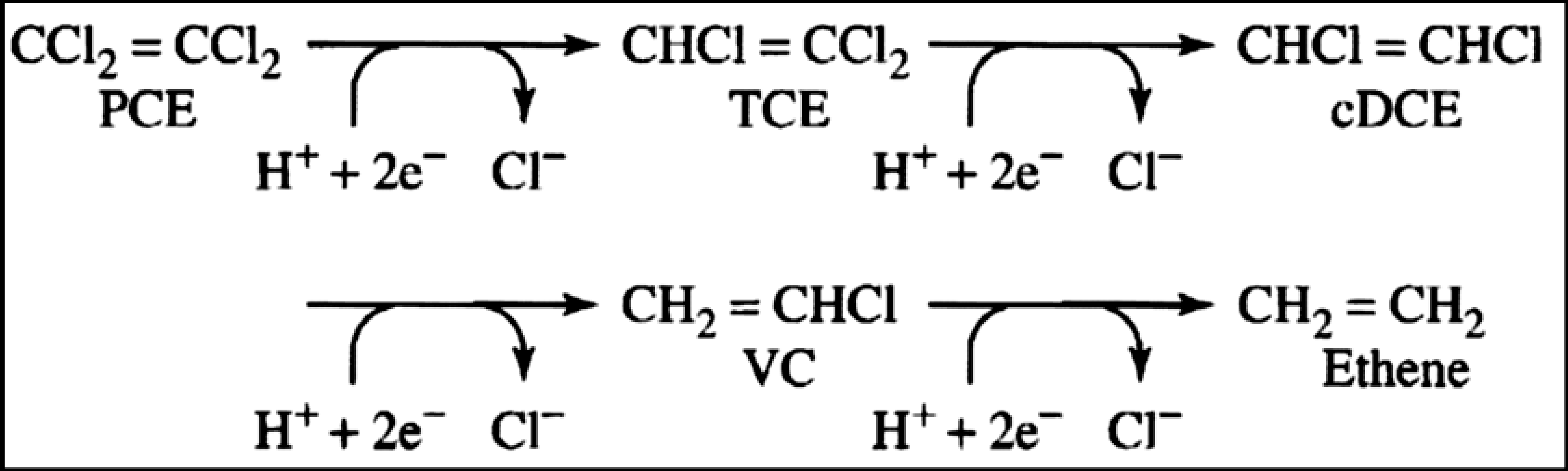
Biodegradation of Problem Environmental Contaminants: CAHs as Electron Acceptors - I

CAHs as Electron Acceptors - I

- Chlorinated organic compounds can be used as electron acceptors, a process termed reductive dehalogenation.

CAHs as Electron Acceptors - I

PCE conversion to ethene



Two electrons and a proton are accepted by perchloroethylene (PCE), which is converted to trichloroethene (TCE), releasing a chloride ion to solution.
TCE can then be an electron acceptor and is converted in a similar fashion to dichloroethene (DCE).
Of the three possible isomers of DCE, cis-1 ,2-dichloroethane (cDCE) is the product most commonly formed from biotransformation.
DCE then can be reduced to VC, which in turn can be reduced to ethene.
Even ethene can accept electrons and be converted to ethane (seldom observed).

CAHs as Electron Acceptors - I

- Reductive dehalogenation may be cometabolic (the organisms obtain no benefit from the process).
- Or, the CAH may be a terminal electron acceptor in energy metabolism through dehalorespiration.

CAHs as Electron Acceptors - I

- Ability to dehalogenate CAHs is widespread among microbes.
- Generally, strictly anaerobic microbes bring about reductive dehalogenation.
- Facultative microbes can bring about reduction of PCE to TCE and cDCE.

CAHs as Electron Acceptors - I

- Some methanogens can convert PCE to TCE through cometabolism.
- Many other organisms can convert PCE to cDCE as part of energy metabolism.
- Most of the CAH reducers use hydrogen as an electron donor, but some can use organic electron donors, such as acetate and pyruvate.

END

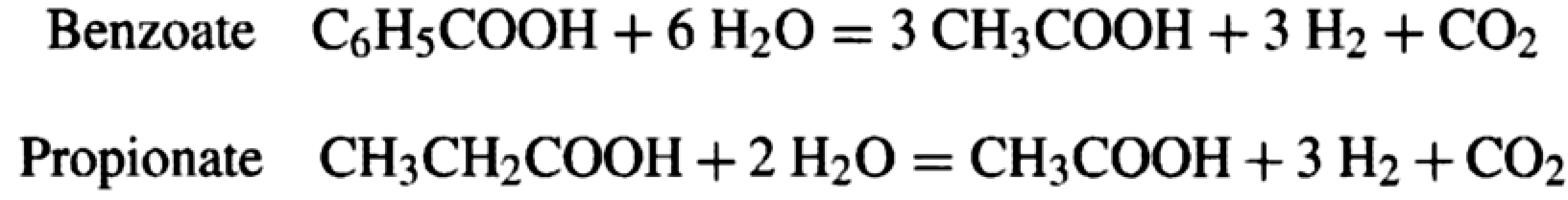
Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: CAHs as Electron Acceptors - II

CAHs as Electron Acceptors - II

- The conversion of cDCE to VC and VC to ethene tends to be slower than the steps from PCE to TCE and TCE to cDCE.
- Hydrogen that serves as electron donor normally is produced through fermentation of organic compounds in the absence of other readily used electron acceptors.

CAHs as Electron Acceptors - II



Benzoate $C_6H_5COOH + 6\ H_2O = 3\ CH_3COOH + 3\ H_2 + CO_2$

Propionate $CH_3CH_2COOH + 2\ H_2O = CH_3COOH + 3\ H_2 + CO_2$

Both compounds are fermented to 3 mol of H_2, but benzoate fermentation results in the production of two more moles of acetate (CH_3COOH), which ties up eight electrons per mole.
Thus, the percentage of electrons converted to hydrogen with propionate (43 percent) is much higher than with benzoate (20 percent).

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CAHs as Electron Acceptors - II

- H_2 released by organic fermentation can be used by different organisms, such as sulfate reducers, Fe(III) reducers, Mn(IV) reducers, methanogens.
- As with methanogens, there is a thermodynamic threshold below which hydrogen cannot be used because of energy limitations.

CAHs as Electron Acceptors - II

- The threshold for hydrogen-using methanogens occurs at a hydrogen partial pressure of about 10^{-6} atm.
- Translated to hydrogen solution concentration, this is 8 to 10 nM.
- The threshold for dehalogenation is lower, about 1 to 2 nM

CAHs as Electron Acceptors - II

- The thresholds for sulfate, iron, and manganese reducers are similar or a bit lower than that for dehalogenation.
- Thus, these electron acceptors compete with and may limit reductive dehalogenation.

CAHs as Electron Acceptors - II

- H_2 thresholds suggest that, in the absence of other electron acceptors, dehalogenating organisms should win over methanogens in a competition for H_2.
- This would be true if the H_2 concentration is below 8 to 10 nM.
- May be possible in a CSTR, but may not be in a plug-flow reactor.

CAHs as Electron Acceptors - II

END

- Rapid fermentation of organic matter leads to H_2 concentrations above methanogen threshold levels.
- The situation can be improved if slowly fermented organic donors are used.
- Slow release of H_2 helps to maintain H_2 near the lower H_2 threshold for dehalogenation.

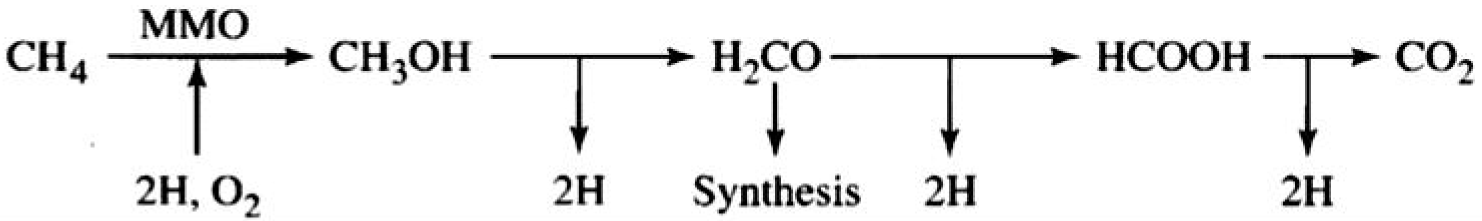
Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: *Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons I*

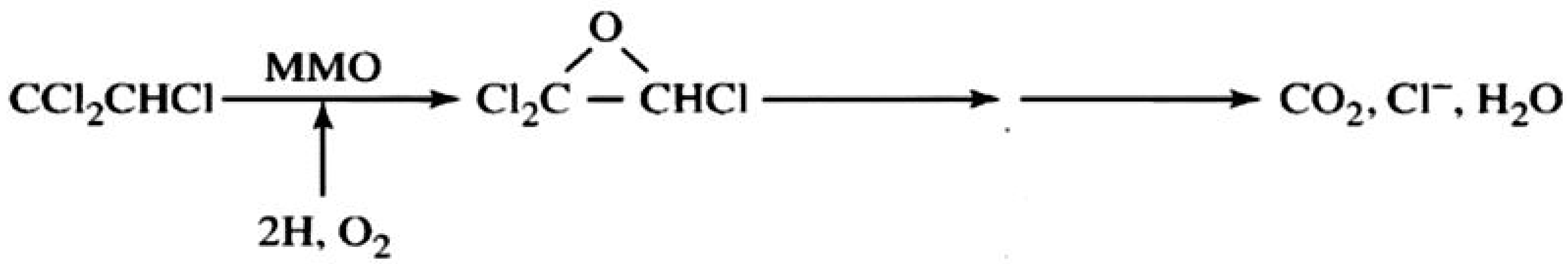
Aerobic Cometabolism of CAHs - I

Several of the CAHs can be transformed aerobically through cometabolism. Transformation of TCE by methanotrophic bacteria. The two steps are shown here:

Methane Oxidation (Normal Metabolism):



TCE Epoxidation (Cometabolic Dechlorination):



Methane monooxygenase (MMO) initiates the oxidation of methane by forming methanol.

Requires molecular oxygen and a supply of reducing power, noted as 2H.

MMO also fortuitously oxidizes TCE, converting it into TCE epoxide, an unstable compound that degrades to a variety of products…

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Aerobic Cometabolism of CAHs - I

- TCE cometabolism does not benefit the methanotrophs.
- Degradation products of TCE epoxide are toxic to methanotrophs.
- This led to the concept of transformation capacity and the related transformation yield.

Aerobic Cometabolism of CAHs - I

- Transformation capacity (Tc) is the quantity of TCE that a given mass of microbes can degrade before they are killed by the transformation.
- Transformation yield (Ty) is the maximum mass of TCE degraded per unit mass of methane used to grow methanotrophs.
- The two are related (Ty = YTc , where Y is the yield constant for growth on methane.

END

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: *Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons* II

Aerobic Cometabolism of CAHs-II

- Another important factor is the reducing power available for cometabolism.
- Cometabolism requires NADH to provide the 2H.
- If the organism is not supplied with a donor substrate or does not have internal storage reserves, then cometabolism cannot occur.
- This results in a lower observed transformation capacity.

Aerobic Cometabolism of CAHs-II

- A broad group of primary substrates effect aerobic cometabolism of TCE.
- In addition to methane, they include aliphatics such as ethane, ethene, propane, propene, butane, isoprene;
- Aromatic compounds such as toluene, cresol, and phenol; and
- An inorganic compound, ammonium.

Aerobic Cometabolism of CAHs-II

- The susceptibility to cometabolism is different for different CAHs, different cultures, and different primary substrates.
- TCE, DCE, and VC are readily cometabolized by many different organisms, but TCA cometabolism is more restricted.

Aerobic Cometabolism of CAHs-II

- Often, if two CAHs are present, competitive inhibition between the two is involved.
- Also, since the primary substrate and CAH compete for the same oxygenase, competitive inhibition between the primary substrate and CAH is generally important.

Aerobic Cometabolism of CAHs-II

END

- Successful cometabolism can be achieved by ensuring that primary substrate is well in excess of that needed to overcome toxicity from the CAH or its transformation products.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Chlorinated Aromatic Hydrocarbons: Polychlorinated Biphenyls (PCBs) - I

Chlorinated Aromatic Hydrocarbons: PCBs – I

- Include single-ringed chlorinated benzenes, chlorinated phenols, and chlorinated benzoates; and two-ringed compounds such as the polychlorinated biphenyls.
- The single-ringed compounds generally are readily biodegraded.
- Serve as primary substrates for organism energy and growth under aerobic conditions.

Chlorinated Aromatic Hydrocarbons: PCBs – I

- Came into extensive use in the 1950s in electrical capacitors and transformers, hydraulic fluids and pump oils, and adhesives, dyes, and inks.
- Their highly desirable features were a great resistance to fire and explosive hazards and excellent electrical insulating properties.

Chlorinated Aromatic Hydrocarbons: PCBs – I

- Unfortunately, PCBs also are resistant to chemical and biological attack.
- Through inadvertent spillage and leakage, or uninformed disposal practices, PCBs were disseminated in the environment, affecting soils, rivers, lakes, estuaries, oceans, and sediments.

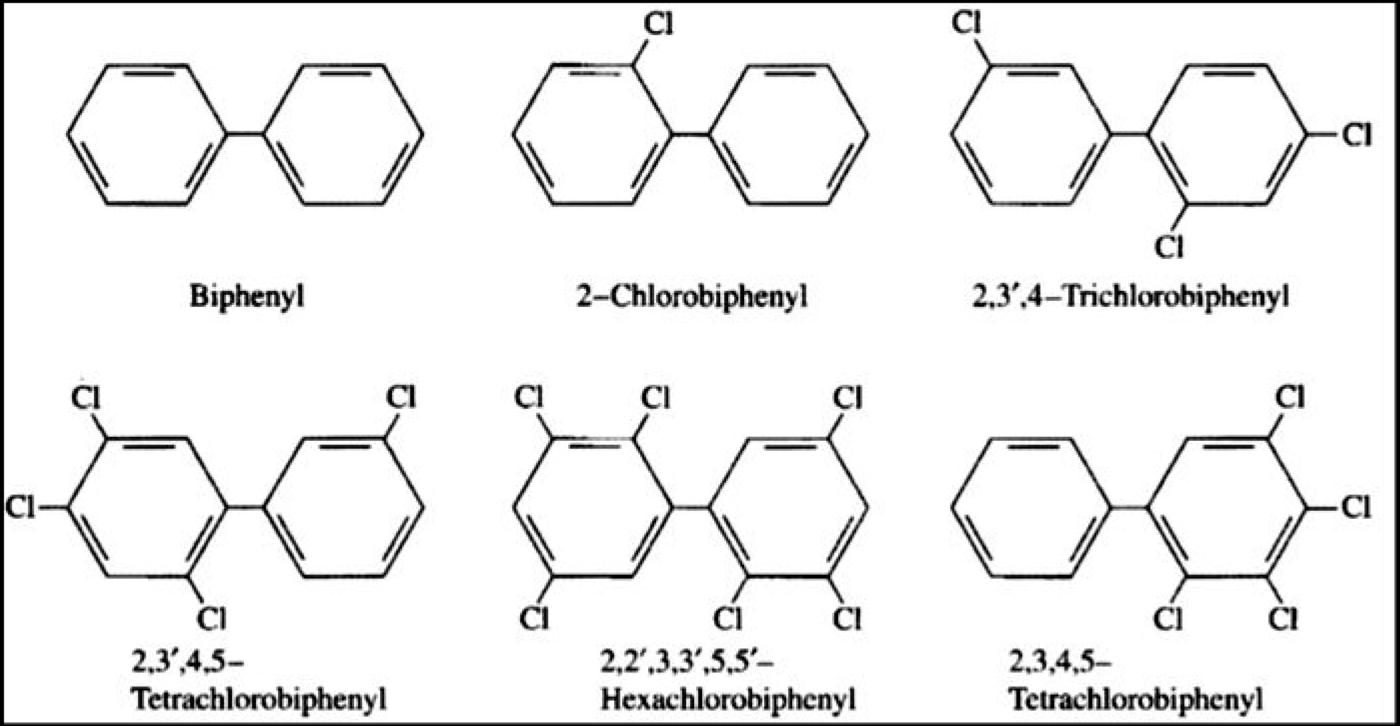
Chlorinated Aromatic Hydrocarbons: PCBs – I

- The U.S. Congress banned the use of PCBs in 1976.
- Because of their great resistant to natural degradation processes, PCBs are still widely present in the environment.

Chlorinated Aromatic Hydrocarbons: PCBs – I

- The backbone of the PCB molecule is biphenyl.
- The process for chlorination of biphenyl leads to the production of a mixture of chlorobiphenyls with different numbers of chlorine atoms per molecule.

Chlorinated Aromatic Hydrocarbons: PCBs – I



Biphenyl

2–Chlorobiphenyl

2,3′,4–Trichlorobiphenyl

2,3′,4,5–
Tetrachlorobiphenyl

2,2′,3,3′,5,5′–
Hexachlorobiphenyl

2,3,4,5–
Tetrachlorobiphenyl

Biphenyl and a few of the possible chlorinated biphenyl congeners that comprise the PCBs.

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Chlorinated Aromatic Hydrocarbons: PCBs – I

END

- By substitution of chlorine atoms on any of the 10 unlinked carbon atoms on biphenyl, it is possible to produce 209 different congeners.
- However, a typical synthetic PCB mixture contains between 60 and 80 different chlorinated biphenyl congeners.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Chlorinated Aromatic Hydrocarbons: Polychlorinated Biphenyls (PCBs) - II

Chlorinated Aromatic Hydrocarbons: PCBs – II

- PCB degradation can occur under either aerobic or anaerobic conditions.
- As with chlorinated organics in general, the less chlorinated PCBs are transformed most readily aerobically.
- The more chlorinated ones are more readily transformed anaerobically.

Chlorinated Aromatic Hydrocarbons: PCBs – II

- Under aerobic conditions, the biphenyl molecule is degraded in a manner similar to other aromatic hydrocarbons:
- Oxygenase addition of molecular oxygen to one of the aromatic rings, resulting in hydroxyl groups.
- Further oxidation leads to the cleavage of one ring, and its further degradation eventually leads to benzoic acid.

Chlorinated Aromatic Hydrocarbons: PCBs – II

- Benzoic acid then goes through ring oxidation, ring cleavage, and further oxidation...
- PCBs with few chlorines can easily enter the aerobic pathway for biphenyl oxidation.
- Greater chlorination makes enzymatic attack on the ring difficult.
- PCBs most subject to aerobic degradation are those containing one to three chlorine atoms.

Chlorinated Aromatic Hydrocarbons: PCBs – II

- Reductive dechlorination of PCBs was first noted in the Hudson River in the early 1980s.
- After time in contact with actively dechlorinating sediments, the mixture composition is shifted towards less chlorinated congeners.
- As in other reductive dehalogenations, PCBs act as electron acceptors, and this requires the presence of electron donors.

Chlorinated Aromatic Hydrocarbons: PCBs – II

END

- Major barriers to more rapid dehalogenation:
- *insolubility of the higher chlorinated PCBs, and*
- *strong partitioning into sediment particles*
- This renders them less available for biodegradation.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Chlorinated Aromatic Hydrocarbons: Pentachlorophenol

Pentachlorophenol

- Pentachlorophenol (PCP) has been and is one of the most widely used wood preservatives.
- Toxic and listed as a priority pollutant by the U.S. Environmental Protection Agency.
- Because of its widespread use, PCP has contaminated many environments, such as soil and groundwater.

Pentachlorophenol

- Technical grade PCP contains 85-90 % PCP, the other components being related compounds such as tri- and tetra-chlorophenols, predioxins, and iso-predioxins.
- The latter are formed from condensation of two molecules of PCP or other chlorinated phenol isomers.

Pentachlorophenol

- Phenol is a weak acid (pKa = 9.82) and thus not ionized at pH 7.
- However, chlorine is an electron-withdrawing group that makes the hydroxyl group on the chlorinated phenols a stronger acid.
- For example, 2,4,6-trichlorophenol has a pKa of 6.13, and pentachlorophenol has a pKa of 4.75.

Pentachlorophenol

- Thus, phenols with three or more chlorine atoms on the molecule are significantly ionized at neutral pH.
- Being ionizable, the phenols are quite soluble in water and are commonly found as groundwater contaminants.

Pentachlorophenol

- However, PCP also has a high octanol/water partition coefficient (over 10^5) and partitions quite strongly into high organic content soils and sediments.
- Partitioning is pH dependent and is greater at low pH. The sodium salt of PCP is the most common commercial form.

Pentachlorophenol

- Biodegradable aerobically and anaerobically.
- Aerobic degradation occurs more readily with the less chlorinated phenols…
- Aerobic microbial degradation processes generally involve initial substitution of an -OH group for -Cl.
- The anaerobic process is generally reductive dehalogenation.

Pentachlorophenol

- Aerobically, microbes obtain cell carbon and energy from PCP oxidation and mineralization.
- First step is oxygenolytic dehalogenation, or the substitution of an -OH group for a -Cl.
- The first step could occur through hydrolysis or through a fortuitous action of an oxygenase enzyme, molecular oxygen, and two NAD(P)Hs.

Pentachlorophenol

- Different organisms appear to have different pathways for aerobic metabolism, including steps that involve hydrolysis and, reductive dechlorination.
- Anaerobic degradation of PCP also occurs.
- This is of critical importance, because groundwaters contaminated by PCP are generally anaerobic.

Pentachlorophenol

- PCP serves as an electron acceptor in reductive dehalogenation in the same manner as for other aliphatic and aromatic halogenated organic compounds.
- An electron donor, such as acetate or molecular hydrogen, needs to be present.

Pentachlorophenol

END

- *Ortho* dechlorination occurs most readily, followed by *para* and then *meta* dechlorination.
- Complete anaerobic dehalogenation to phenol is possible, and phenol biodegrades anaerobically.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Dioxins - I

Dioxins - I

- Dioxins are chemicals of significant environmental concern.
- Although not produced commercially as marketable products.
- Formed as unwanted by-products of pesticide manufacturing, combustion and incineration, chlorine bleaching, disinfection, and controlling dust and sediments.

Dioxins - I

- Major incidences:
- Contamination of 1.44 million tons of soil at Times Beach, Missouri.
- widespread contamination due to the explosion of a pesticide plant in Seveso, Italy, in 1976.
- Numerous human exposures due to the use of 2,4,5-T herbicide, which formerly contained dioxin as a by-product of manufacture.

Dioxins - I

- Many possible congeners.
- The most toxic is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is thought to be the most poisonous synthetic chemical.
- Human responses include chloracne, weight loss, insomnia, liver dysfunction, spontaneous abortion, and possibly cancer.

Dioxins - I

END

- The toxicity of the different dioxin congeners varies widely from low to extreme.
- Thus, in order to evaluate possible toxic effects, congener-specific analysis is required.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Dioxins - II

Dioxins - II

- Excavation and incineration of soils, but expensive.
- Biodegradation is a desirable alternative.
- Highly resistant to biodegradation.
- Many microorganisms can bring about their conversion under aerobic and anaerobic conditions.

Dioxins - II

- In many studies of aerobic biodegradation, only limited biotransformation of dioxins is observed.
- Involves addition of oxygen with oxygenase enzymes, resulting in the unproductive formation of mono- and di-hydroxylated analogs.
- These accumulate with no further degradation.

Dioxins - II

- Many bacteria contain selective dioxygenases that simultaneously substitute an -OH group at an ether bond-carrying carbon and an adjacent unsubstituted carbon.
- This leads to the cleavage of ether bonds and compound mineralization.

Dioxins - II

END

- Reductive dechlorination of dioxin under anaerobic conditions.
- TCDD congener can be formed through reductive dehalogenation of other congeners.
- Dehalogenation of dioxins is a slow process.
- Very low solution concentrations and the high tendency to partition to sediments reduces biological availability.

Environmental Biotechnology

Biodegradation of Problem Environmental Contaminants: Explosives

Explosives

- Contamination of soils and groundwater from the manufacture, use, and disposal of explosives.
- A long-standing challenge.
- Interest in decommissioning military bases for other uses has led to an intensified need to remediate explosives-contaminated environments.

Explosives

- Incineration…
- Impossible for wastewaters and expensive in general.
- A characteristic of explosives is the presence of nitro (-NO_2) groups.
- Transformation of TNT results in the reduction of a nitro group to form an amino group.
- The reductions can be enzymatically catalyzed or,
- Abiotic under proper reducing conditions.

Explosives

- Commonly found in TNT-contaminated soils are intermediate products such as
- *4-amino-2,6-dinitrotoluene (4ADNT)m*
- *2-amino-4,6-dinitrotoluene (2ADNT).*
- Less frequently found are the further reduction products
- *2,4-diamino-6-nitrotoluene (2,4DANT),*
- *2,6-diamino-4-nitrotoluene (2,6DANT).*

Explosives

- The transformation products are subject to further interactions with each other and with components of soils.
- These form stable complexes.
- Resulting products are large and insoluble.
- Thus, disappearance of TNT is often observed but mineralization does not commonly occur.

Explosives

- Toluene, benzene, and phenols with one or two nitro groups are more subject to biodegradation.
- Initial monooxygenation or dioxygenation reactions can lead to the release of – NO_2 groups and substitution of -OH.
- This can lead to mineralization.

Explosives

- RDX is more readily mineralized than TNT.
- Slower rates...
- HMX biotransformation occurs primarily under anaerobic conditions.
- Its biotransformation is slower than that of RDX.
- The explosives are also transformed, at least partially, by plants.
- Potential for phytoremediation.

END

Environmental Biotechnology

General Fate Modeling for Organic Chemicals - I

General Fate Modeling for Organic Chemicals - I

- Many hazardous organic chemicals are hydrophobic.
- They partition to solid or gas phases.
- Not directly available for biodegradation…
- Can come into contact with human through contact.
- One must account for partitioning to understand their fate in treatment reactors or in the environment.

General Fate Modeling for Organic Chemicals - I

- Concern about the release of volatile organic chemicals (VOCs) from wastewater treatment plants led to general fate models in the late 1980s and 1990s.
- Excellent examples of how phase-transfer processes are integrated with the microbial reactions.

General Fate Modeling for Organic Chemicals - I

- Consider an activated sludge treatment plant whose influent contains normal BOD and two types of hydrophobic organic chemicals: VOCs and strongly sorbable chemicals.
- Assume that the hydrophobic compounds are present in the influent at low concentrations and are utilized by the microorganisms through secondary utilization.

General Fate Modeling for Organic Chemicals - I

The steady-state mass balance on a hydrophobic chemical (indicated by concentration C) in the water is

$$0 = \text{Advection In} - \text{Advection Out} - \text{Volatilization} - \text{Sorption} - \text{Biodegradation}$$

Eq. 1

Each of the terms is derived.

Advection in and out are simply stated by the product of the flow rate times concentration in that flow:

$$\text{Advection In} = Q \cdot C^0$$

Eq. 2

$$\text{Advection Out} = Q \cdot C$$

Eq. 3

where Q is the influent flow rate (L^3T^{-1}], C^0 is the influent concentration (M_cL^{-3}], and C is the effluent soluble concentration [M_cL^{-3}].

General Fate Modeling for Organic Chemicals - I

Volatilization is comprised of two parts: volatilization at the surface and volatilization to bubbles in diffused aeration. Together, the volatilization rate is

$$\text{Volatilization} = k_L a_c C V + Q_A C H_c$$

Eq. 4

in which $k_L a_c$ is the surface gas transfer rate coefficient [T^{-1}], V is the volume being aerated [L^3], QA is the aeration gas volumetric flow rate [L^3T^{-1}], and H_c is the Henry's constant in units L^3 water (L^3 gas)$^{-1}$.

General Fate Modeling for Organic Chemicals - I

The loss rate by sorption is equal to the density of the compound in the wasted sludge times the sludge-wasting rate, as long as adsorption is at equilibrium. In that case,

$$\text{Sorption} = X_v V K_p C / \theta_x \qquad \textbf{\textit{Eq. 5}}$$

in which X_v is the MLVSS concentration $[M_x L^{-3}]$, V is the volume in which X_v is contained $[L^3]$, θ_x is the solids retention time [T], and K_p is a linear partitioning coefficient $[L^3 M_x^{-1}]$.

MLVSS: Mixed liquor volatile suspended solids

General Fate Modeling for Organic Chemicals - I

Since the concentration of the target compound is low, we assume that the rate of biodegradation is first-order in C:

$$\text{Biodegradation} = k_1 X_a C V \qquad \textit{Eq. 6}$$

in which k_1 is the mixed second-order rate coefficient $[L^3M^{-1}T^{-1}]$, and X_a is the concentration of active biomass degrading the contaminant $[M_xL^{-3}]$.

Environmental Biotechnology

General Fate Modeling for Organic Chemicals - II

General Fate Modeling for Organic Chemicals - II

The steady-state mass balance on a hydrophobic chemical (indicated by concentration C) in the water is

$$0 = \text{Advection In} - \text{Advection Out} - \text{Volatilization} - \text{Sorption} - \text{Biodegradation}$$

Eq. 1

General Fate Modeling for Organic Chemicals - II

Assume that all of the active biomass utilizes the target compounds. Substituting Equations 2 - 6 into Equation 1 gives

$$0 = QC^0 - QC - k_L a_c CV - Q_A C H_c - X_v V K_p C/\theta_x - k_1 X_a CV$$

Eq. 7

Equation 7 can be rearranged to solve for C:

$$C = C^0/[1 + k_L a_c \theta + Q_A H_c/Q + X_v K_p \theta/\theta_x + k_1 X_a \theta]$$

in which θ= V/Q is the hydraulic retention time [T]. ***Eq. 8***

Equation 8 is very simple to use, as long as we can measure or predict the parameters.

Each of the five terms in the denominator is the relative rate compared to advection in the effluent.

Thus, if we know k_1X_aV, we know immediately how important it is compared to advection (value of 1) or bubble volatilization (Q_AH_c / Q).

The actual rates can be computed immediately by substituting the value of C into Equations 2 to 6.

General Fate Modeling for Organic Chemicals - II

- The assumptions behind Equation 8 must not be overlooked, because they are not always valid.
- E.g., adsorption is not necessarily at equilibrium.
- If that is the case, then Equation 5 usually overestimates the removal.

General Fate Modeling for Organic Chemicals - II

- Likewise, a target compound may not be biodegraded by secondary utilization alone or at all.
- Then, the fraction of the biomass active in degrading the target compound must be predicted separately from X_a.

General Fate Modeling for Organic Chemicals - II

END

- Biodegradation kinetics are not always first-order in C.
- Monod kinetics or some form of inhibition kinetics or cometabolic kinetics may be more appropriate.
- Even when the simple approach leading to Equation 8 is not completely correct, the general mass balance equation (Equation 1) remains the right starting point.

Environmental Biotechnology

Inorganic Elements - I

Inorganic Elements - I

- Microbial processes can affect the fate of inorganic contaminants.
- Although inorganic elements cannot be destroyed, microbes can change their speciation.
- This alters their mobility and toxicity.
- Precipitation, volatilization, sorption, and solubilization…

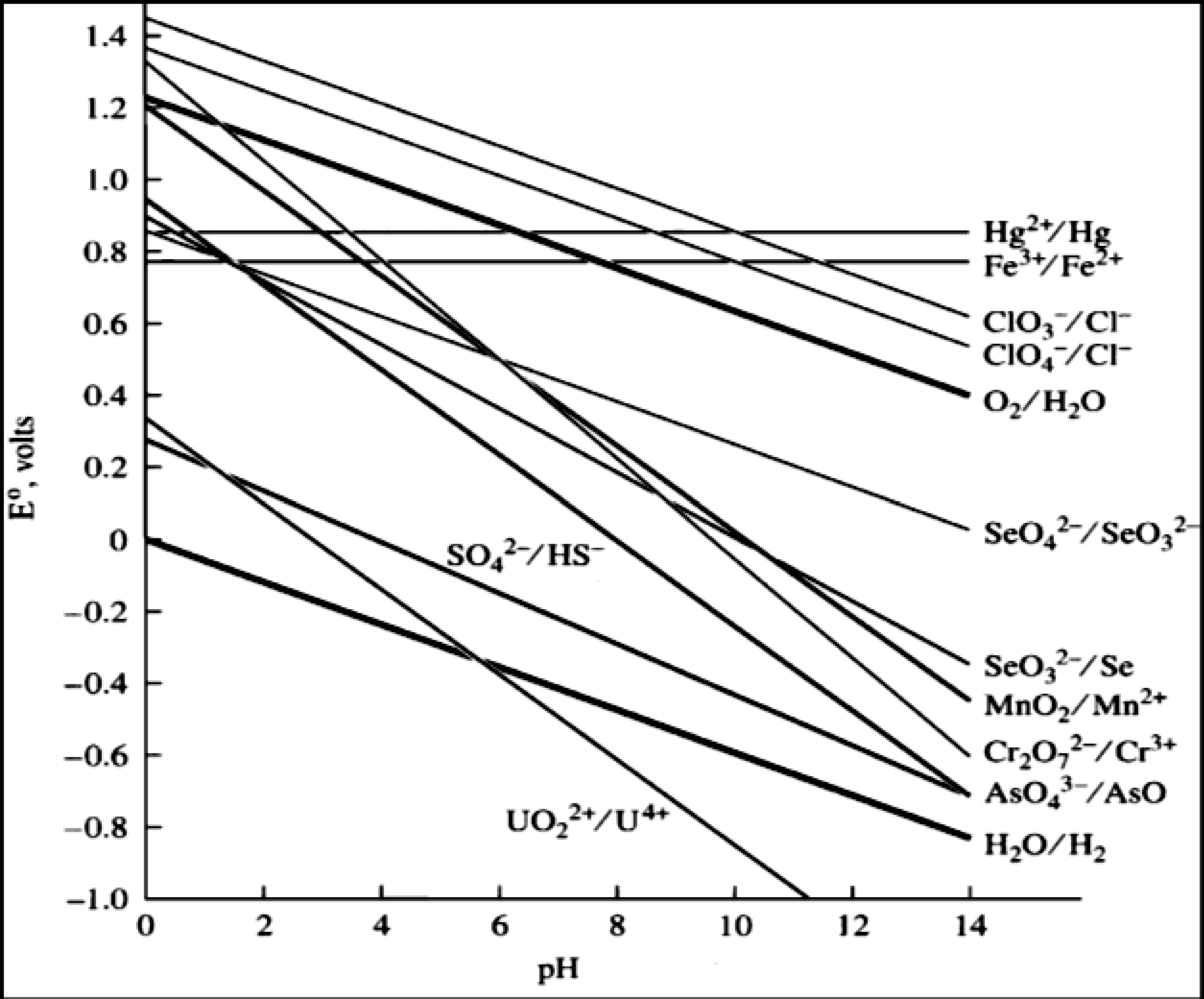
Inorganic Elements - I

- Microbially catalyzed redox reactions can also lead directly to the reactions that alter mobility.
- In other cases, the effects are indirect when the microbes alter the geochemistry of their environment.

Inorganic Elements - I

- Immobilization in microbial biomass and/or microbial exopolymers…
  - *Hydrophobic sorption,*
  - *complexation, or*
  - *incorporation.*
- The biomass must be removed and disposed of.
- Oxidation-reduction reactions mediated by microorganisms.

Inorganic Elements - I



Effect of pH on reduction potentials for various inorganic species.
pH-dependent half-reaction potentials for various redox couples.

Environmental Biotechnology: Principles and Applications, by Bruce

Inorganic Elements - I

- Any of the oxidized species (the left member in the previous figure) could be an electron acceptor in the oxidation of some organic or inorganic electron donor.
- They would be converted to the reduced species (the right member in the previous figure).

Inorganic Elements - I

END

- Oxygen is a more favored electron acceptor than any species with lines below the O_2/H_2O line.
- E.g., SO^{2-}_4/HS^- line lies well below the O_2/H_2O line.
- Less energetically favorable than oxygen
- SO^{2-}_4 can serve as an electron acceptor only under highly reducing conditions.

Environmental Biotechnology

Inorganic Elements - II

Inorganic Elements - II

- Perchlorate used in rocket fuels.
- Contaminates soil and water around…
- Chlorate (ClO_3^-) and perchlorate (ClO_4^-)lie well above oxygen.
- Theoretically, they provide more energy if used as electron acceptors.
- Are used by some microbes in energy metabolism of organic electron donors.

Inorganic Elements - II

- Whether they would be selected as electron acceptors in the presence of oxygen is debatable.
- Biological reduction to chloride using readily available organic donors, such as acetate or H_2…

Inorganic Elements - II

- Mercury reduction…
- The Hg^{2+} /Hg line intersects the oxygen line.
- Reduction of Hg^{2+} to elemental mercury occurs quite readily.
- Enhanced by bacterial enzymes.

Inorganic Elements - II

- Iron oxidation and reduction…
- At high pH, Fe(III) could be a more favorable electron acceptor than O_2.
- However, Fe(III) quite insoluble at high pH.
- Soluble at neutral or lower pH.
- Serves as electron acceptor for under anaerobic conditions.
- Fe(II) serves as an electron donor under aerobic conditions.

Inorganic Elements - II

- The potential for using Mn(IV) (e.g., MnO_2) as an electron acceptor…
- Like for iron, the reduced manganese (e.g., Mn^{2+}) can be an electron donor.
- Manganese cycling between the (II) and (IV) states is important in sediments, lakes, and some groundwaters…

Inorganic Elements - II

- Oxidized forms of selenium, chromium, arsenic, and uranium.
- All could serve as electron acceptors under anaerobic conditions.
- In general, the reduced forms are much less soluble and mobile.
- Potential to reduce these elements and remove from the water as solids.

Inorganic Elements - II

- Indirect changes in the oxidation states of inorganic species when microbes create oxidizing or reducing conditions.
- E.g., microbial reduction of sulfate produces sulfide.
- This acts as a chemical reductant for many species.
- Likewise, reduced iron and manganese, produced microbially, can be electron donors for abiotic reduction of other inorganic elements.

END

Environmental Biotechnology

Inorganic Elements
- III

Inorganic Elements - III

- Potential for microbes to methylate inorganic species, often yielding highly toxic and mobile compounds.
- E.g., methlyation of mercury, arsenic, and selenium.
- Methylmercury and dimethylmercury are highly toxic compounds
- Formed from Hg(II) under anaerobic conditions, generally by sulfate-reducing bacteria.

Inorganic Elements - III

- Methylmercury is a detoxification method for the organisms.
- To move the mercury away, they form the more mobile methylated form.
- This form can diffuse from the sediments into overlying waters.
- There it can be concentrated by planktons and then concentrated further in the fatty tissue of fish.

Inorganic Elements - III

- Further concentrated in the fatty tissues and brains of those consuming the fish, including humans,
- This leads to Minamata disease.
- A disease of the central nervous systems that claimed many victims around the Minamata River delta in Japan during the 1950s as a result of mercury discharges from an organic chemical plant.

Inorganic Elements - III

- ➢ Biological reduction of Cr(VI) to Cr(Ill)
- *Conversion of a highly toxic, soluble, and mobile species into a species having much lower toxicity and solubility*.
- ➢ This desirable outcome of reduction reactions occurs for many metals.

Inorganic Elements - III

- A few species are more mobile in the reduced form.
- E.g., biological reduction of Fe(III), which leads to the formation of the more soluble and mobile Fe(II).
- Plutonium is also more mobile in the reduced state.

Inorganic Elements - III

- Other Microbe-mediated chemical changes to inorganic elements:
- Microbial reactions produce acids and bases,
- *alter the pH and affect speciation*.
- E.g., most cationic metals species form hydroxide or carbonate solids.
- A lower pH decreases concentrations of OH^- and CO_3^{2-} , thereby making the cationic metals more soluble and mobile.

Inorganic Elements - III

- Microbial reactions also produce and consume ligands that complex metals.

END

Environmental Biotechnology

Biosensors:
Introduction

Biosensors: Introduction

- Defined as analytical devices incorporating a biological material, a biologically derived material, or biomimic, intimately associated with or integrated within a physicochemical transducer or transducing microsystem.
- Should be distinguished from a bioassay or a bioanalytical system, which require additional processing steps.

Biosensors: Introduction

- For conventional “off-site” analysis, samples need to be sent to a laboratory for testing.
- Conventional methods reaching highest accuracy with low detection limits, but are
- *expensive,*
- *time-consuming, and*
- *require the use of highly trained personnel.*

Biosensors: Introduction

- Biosensors as new analytical tools to provide fast, reliable, and sensitive measurements with lower cost.
- Many of them aimed at on-site analysis.
- Do not compete with official analytical methods.
- Can be used both by regulatory authorities and by industry to provide enough information.

Biosensors: Introduction

- Biosensors are being developed for different applications, including
- *environmental monitoring,*
- *bioprocess control,*
- *quality control of food,*
- *agriculture,*
- *military, and*
- *medical applications.*

Biosensors: Introduction

- In the food industry,
  - *detection of contaminants,*
  - *verification of product content,*
  - *monitoring of raw materials conversion, and*
  - *product freshness*

Biosensors: Introduction

- Can be also a defense tool through the early detection of hazardous materials such as germs or chemical warfares.
- The detection of illicit drugs and explosives, with airport security purposes.

Biosensors: Introduction

END

- For environmental control and monitoring.
- Provide fast and specific data of contaminated sites.
- Offer other advantages over current analytical methods such as the
- *possibility of portability,*
- *working on-site.*
- Possibility of determining not only specific chemicals, but also their biological effects.

Environmental Biotechnology

Biosensors: Configurations

Biosensors: Configurations

- Classified according to the signal transduction and the biorecognition principles.
- On the basis of the transducing element, categorized as electrochemical, optical, piezoelectric, and thermal sensors.

Biosensors: Configurations

- Electrochemical biosensors, and among them the amperometric and the potentiometric ones, are the best described in the literature.
- Those based on optical principles are the next most commonly used transducers.

Biosensors: Configurations

- Various types of optical transducers exploit properties such as
- *Simple light absorption,*
- *Fluorescence / phosphorescence,*
- *Bio/ chemiluminescence,*
- *Reflectance,*
- *Raman scattering, and*
- *Refractive index.*

Biosensors: Configurations

- Surface plasmon resonance (SPR) with main advantage that the analyte presence can be determined directly, without the use of labeled molecules.
- Cantilever biosensors are based on the bending of silicon cantilevers caused by the adsorption of target molecules onto the cantilever surface, where receptor molecules are immobilized.

Biosensors: Configurations

- According to the biorecognition principle, biosensors classified into
- *immunochemical,*
- *enzymatic,*
- *nonenzymatic receptor,*
- *whole-cell, and*
- *DNA biosensors.*
- Immunosensors present the advantages of sensitivity and selectivity.
- Limitations are the regeneration of the immunosurface, and cross-reactivity.

Biosensors: Configurations

- Enzymes act as recognition elements because of their specificity.
- In general, enzymatic biosensors are based on the selective inhibition of specific enzymes by different classes of compounds.

Biosensors: Configurations

- Biosensors based on natural receptors can be built by
  - *integrating the specific receptor within a membrane, and*
  - *by coupling it to a transducing device.*
- The binding signal is detected as a structural change or an associated enzyme activity.

Biosensors: Configurations

- ➢ Whole cells of living organisms
- • *bacteria, yeast, fungi, plant and animal cells, or even tissue slices*
- ➢ Used as the recognition component by interrogating their general metabolic status.
- ➢ Useful to determine whether a substance is toxic to certain cells.

Biosensors: Configurations

- In the case of DNA biosensors, two strategies:
  - hybridization detection of nucleic acid sequences from infectious microorganisms, and
  - monitoring of small pollutants interacting with the immobilized DNA layer (drugs, mutagenic pollutants, etc.)

Biosensors: Configurations

- A step in the development of biosensors is the immobilization of the biological component at the transducer surface.
- Procures both the stabilization and the proximity between the biomaterial and the transducer.

Biosensors: Configurations

END

- Immobilization methods mostly employed :
- *physical adsorption at a solid surface,*
- *cross-linking between molecules,*
- *covalent binding to a surface,*
- *entrapment within a membrane,*
- *surfactant matrix,*
- *polymer or microcapsule…*

Environmental Biotechnology

Environmental Applications of Biosensors: Toxicity

Applications of Biosensors: Toxicity

- Chemical analysis does not provide sufficient information to assess the ecological risks of polluted waters and wastewaters.
- Much efforts to develop and use different bioassays and biosensors for toxicity evaluation of water samples.

Applications of Biosensors: Toxicity

- Whole organisms to measure the potential biological toxicity of a water or soil sample.
- Toxicity assays Microtox® (Azure, Bucks, UK), or ToxAlert® (Merck, Darmstadt, Germany).
- Based on the use of luminescent bacteria, *Vibrio fischeri*, to measure toxicity from samples.

Applications of Biosensors: Toxicity

- Cellsense®, an amperometric sensor that incorporates *Escherichia coli* cells, for rapid ecotoxicity analysis.
- Uses ferricyanine, a soluble electron mediator, to divert electrons from the respiratory system of the immobilized bacteria of a suitable carbon electrode.
- The resulting current is, a measure of bacterial respiratory activity.

Applications of Biosensors: Toxicity

- Perturbation by pollutants can be detected as a change in the magnitude of the current.
- To investigate the toxicity of
- *3,5-dichlorophenol and other phenols,*
- *for the determination of nonionic surfactants and*
- *benzene sulfonate compounds,*
- *for the analysis of wastewater treatment, etc.*

Applications of Biosensors: Toxicity

- Most environmental biosensors focus on bacterial systems.
- Eukariotic biosensors are rare.
- Mammalian cell can give more sensitive response when compared to bacteria…

END

Environmental Biotechnology

Environmental Applications of Biosensors: Endocrine Effect Biosensors

Endocrine Effect Biosensors

- Many environmental contaminants produce adverse effects by interfering with endogenous hormone systems, the so-called EDCs.
- EDCs constitute a class of substances not defined by chemical nature, but by biological effect.
- “Endocrine effect biosensors” take advantage of this feature.

Endocrine Effect Biosensors

- Steroid hormones induce different effects in mammalian cells after binding to specific intercellular receptors (ligand-dependent transcription factors).
- Many endocrine disruptors also believed to bind to the estrogen receptor (ER) as agonists or antagonists.

Endocrine Effect Biosensors

- Binding ability of the chemicals toward the ER crucial for screening or testing their potential environmental toxicity.
- Several biosensors are based on ER.
- Simple to perform and allow identification of all endocrine disruptors that act through the corresponding receptor.

Endocrine Effect Biosensors

- Constitute a similar approach as that for toxicity biosensors.
- Effects as a general parameter, and not a specific substance, are monitored.
- By employing the commonly utilized human estrogen receptor, the SPR biosensor BIAcore has been applied in the determination of estrogens and xenoestrogens.

END

Environmental Biotechnology

Environmental Applications of Biosensors: Biocides

Applications of Biosensors: Biocides

- Widespread presence of pesticides in natural waters.
- Concerns about their toxicity and persistence led the European Community to set limits on the concentration.
- A limit of 0.1 μg/l for individual pesticides and of 0.5 μg/l for total pesticides.

Applications of Biosensors: Biocides

- Though techniques such HPLC/MS and GC/MS give satisfactory analytical results for pesticide determination.
- New assays and sensors for cheaper and faster on-site analysis.
- Enzymatic sensors, based on the inhibition of a selected enzyme, are the most extended biosensors used for the determination of these compounds.

Applications of Biosensors: Biocides

- Various biosensors, based on the inhibition of acetyl cholinesterase (AChE) and colin oxidase, for the detection of organophosphorous and carbamate pesticides.
- Although sensitive, biosensors based on AchE inhibition are not selective.
- One approach to solve the lack of specificity of AchE involves the genetic engineering of cholinesterase enzyme.

Applications of Biosensors: Biocides

- The organophosphorous hydrolase (OPH) is able to hydrolyze a number of OP pesticides.
- Generates p-nitrophenol, which is an electroactive and chromophoric product.
- OPH could be combined with an optical transducer to measure the absorbance of p-nitrophenol
- Or with an amperometric transducer to monitor the oxidation or reduction current.

Applications of Biosensors: Biocides

- Photosynthesis inhibition rapidly reflects the toxic effect of certain pollutants.
- Some biosensors based on Photosystem II (PSII) have been reported to be able to detect herbicides in the environment…

Applications of Biosensors: Biocides

- Biosensors based on immunological assays.
- 2,4-dichlorophenoxyacetic (2,4-D) acid determined in water by an amperometric immunosensor.
- Enzyme alkaline phosphatase (AP) catalyzes the conversion of p-aminophenyl phosphate (PAPP) to p-aminophenol (PAP) allowing a limit of detection of 0.1 µg/l.

END

Environmental Biotechnology

Environmental Applications of Biosensors: Hormones

Applications of Biosensors: Hormones

- Endogenous hormones, of human or animal origin, have been reaching the environment for thousands of years…
- Exogenous steroids, used as growth promoters in several countries, have also become a matter of concern.

Applications of Biosensors: Hormones

- Endocrine-disrupting activity in aquatic fauna or even terrestrial.
- Their widespread use and their capability to induce responses in fish at concentrations as low as ng/l or even pg/l level, have alerted scientists to the potential dangerous consequences.

Applications of Biosensors: Hormones

- An optical immunosensor to determine estrone, along with other organic pollutants (atrazine and isoproturon).
- New biosensing strategies for the control of hormone residues in an effort to improve food quality controls and to protect public health.

END

Environmental Biotechnology

Environmental Applications of Biosensors: PCBs & Dioxins

Applications of Biosensors: PCBs & Dioxins

- Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants.
- Widely used as industrial chemicals, particularly as dielectric fluids in electrical transformers and capacitors.
- Though the production of PCBs has been banned, these are still present in the environment.

Applications of Biosensors: PCBs & Dioxins

- The high toxicity represents a risk for public health.
- Different biosensor configurations to determine PCBs in the environment.
- DNA biosensor with chronopotentiometric detection, and various immunosensors with fluorescence, SPR, and electrochemical detection principles.

Applications of Biosensors: PCBs & Dioxins

- Dioxins released as by-products in a number of chemical processes.
- Production of some pesticides, the manufacture of PVC plastics, the chlorine bleaching of pulp and paper, and waste incineration.
- Carcinogenic.

Applications of Biosensors: PCBs & Dioxins

END

- A significant number of immunoassays for dioxins.
- Biosensors developed for PCBs have been used for Dioxins too.
- Biosensor for detection of dioxin-like chemicals (polyhalogenated dioxins, furans, and biphenyls) based on a recombinant mouse hepatoma cell line.

Environmental Biotechnology

Environmental Applications of Biosensors: Phenols

Applications of Biosensors: Phenols

- Originate from the paper and pulp industry, from the production of drugs, dyes, and antioxidants.
- Important pollutants because of their high toxicity and possible accumulation in the environment.
- Also considered as precursors of the dioxins.

Applications of Biosensors: Phenols

- An amperometric biosensor, with tyrosinase (a polyphenol oxidase with a relatively wide selectivity for phenolic compounds) immobilized in a higrogel on a graphite electrode.
- Correlated satisfactorily with the official method for the determination of the phenol index in environmental samples.

Applications of Biosensors: Phenols

END

- Chlorophenols detected with a flow-injection chemiluminescence fiber optic biosensor.
- *Exploiting the ability of certain substituted phenols to enhance the chemiluminescence reaction of luminol.*
- *Catalyzed by horseradish peroxidases.*
- A Lux-based biosensor to assess the toxicity of a paper mill sludge.