

MICROBIAL BIOTECHNOLOGY

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Lecture-27

Lec 27: Microbial waste treatment methods

Hello friends, welcome to my course on microbial biotechnology. We are in module 8, where we are discussing environmental biotechnology. Today, we will be discussing microbial waste treatment methods. So, broadly, this lecture is divided into three sections. In the first section, we begin with an introduction.

We will discuss the sources of pollutants in nature, their generation and accumulation. Then, we will learn how wastes are classified and into what categories they fall. Then, we will get an idea about projected waste generation in the future. Then, the role of microbes in waste treatment. We will also discuss natural waste degradation cycles, microbial metabolism, and biodegradation and necessary recycling systems.

Some recent research on microbes in plastic degradation will also be presented. Then, we will discuss the significance of biodegradation in waste treatment and bioremediation, and we will try to understand the differences between biodegradation and bioremediation. So, in section 2, we have microbial waste treatment methods, and in fact, there is one more section here, section 3, where we will discuss the mechanisms of microbial waste treatment. So, let us try to understand how big the problem of pollution is. So, you can see that everything on this earth today is polluted.

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We have air pollution, we have land pollution, and we have water pollution. And then you can see the gases coming out of these vehicles in some of the cities, which is a very normal sight and contributes a lot to air pollution. Then, our oceans are also today hugely polluted by the plastic waste that we use. And this waste generation and accumulation is threatening ecosystems globally.

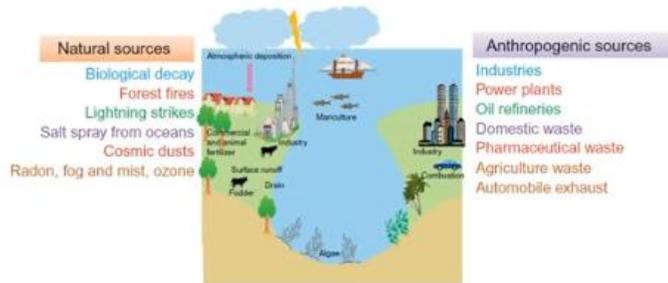
Even remote areas like Antarctica, where there is no habitation, we can find traces of pollutants reaching there. The Great Pacific Garbage Patch highlights plastic waste buildup in the ocean. And there are many such islands of plastic across the various oceans of the world. Air and water currents disperse waste worldwide, spreading pollutants across borders and affecting ecosystems far from their sources. So, places where there is no industry or no habitation are not actually safe from pollutants because they may be drifting from other places and sources.



So, what are the sources of pollution in nature? We have some natural sources of pollution, which are inevitable and not controllable, which happen due to biological decay, forest fires, and lightning strikes that sometimes cause these forest fires. Then we have the salt spray from the oceans, and we have cosmic dust. And also, many times our deserts, when exposed to strong winds and turbulence, will lift the soil, and sand will be lifted and then drifted to faraway places. Then other things like radon, fog, mist, and ozone—these all happen due to natural phenomena, and we have very little or actually no control over them.

But then there are anthropogenic sources of pollution, which come from industries, power plants, oil refineries, domestic waste, pharmaceutical waste, agricultural waste, and automobile exhaust, as we saw in the earlier slide. So, these are man-made pollutants. That's why we call it anthropogenic, due to human activities. We can control them to a large extent with proper planning. Now, waste is not a single entity.

Sources of pollutants in nature



(Das, 2014)

Some waste may be very dangerous. We call them hazardous waste, while some may not be as dangerous. We call them non-hazardous waste. For example, waste that emits radioactivity is very hazardous. We also have certain industrial waste that may contain many toxic components.

It may be discharged into water bodies or released as smoke into the air. Then we have electronic waste, which comes from electronic gadgets when they are no longer in use, damaged, or at the end of their life cycle and are disposed of into the environment. They are now a major cause of concern. Then we have medical waste, which is a mixture of many things, such as plastic, body tissues, soft waste like bandages, and metallic waste like needles from syringes or surgical equipment. These are all classified as hazardous waste because they can harm us in various ways, particularly our health.

Classification of Waste



Image generated by author

Then in the non-hazardous waste we have, say for example, general municipal waste. Then we may have some other industrial waste which are not hazardous but are coming from the industries. And then we have with these under the municipal waste we may have the

organic waste mostly dead animals or you know the vegetable wastes coming from the kitchen. And then we have the waste which are generated from the leftover of the packaging materials that's the packaging waste and others like materials, glass, plastic, metal etc. So, how much waste is currently produced and how much waste will be produced in the future?

So it's very common knowledge that with population increase the waste is going to increase because people are going to use more commodities and with the use of increase of a commodity the waste is also going to increase. So, this increase in waste is an increasing threat to nature and, of course, biodiversity. One of the challenges is that where to dump this waste. They require a lot of space. So, there is a challenge of really accommodating this waste, which also consumes a lot of land resources.

So, which could have been used for other alternative use, including parking or, you know, converting them into biodiversity parks. So, this is some projection in the past 2016 figure when you compare with 2030 which is in the center and projected waste up to 2050. So you can see that there is an increase in the waste generation in almost all the regions of the globe starting from Middle East and North Africa to South Asia which is having one of the largest population of the world and then similarly East Asia and the Pacific. So, our problems with waste is going to increase not decrease. So, therefore we have to think about handling this waste.



So, one of the ways that we can take care of this waste is by treating, handling, and processing it, and converting this waste into a product which we call a waste-to-product approach. So, in this approach, microbes play a big role. What is the role of microbes in waste treatment? We can use microbes to treat waste, and then this treated waste can be used for filling up land or as landfills. So, natural waste degradation cycles are very helpful in this approach. So, we have discussed some of these in the earlier lecture. Then,

biodegradation and bioremediation would be very essential in this entire process, which we will discuss in the next lecture.

Overall, microbial metabolism is used to break down complex compounds into simpler ones. These complex compounds may be toxic, but when broken down, they may become non-toxic, and we can use them as raw materials for other industries. So, let's start with natural waste degradation cycles or biogeochemical cycles, which are natural processes that recycle nutrients and waste products in ecosystems, essential for maintaining environmental balance. So, one of the important cycles is the carbon cycle. The decomposition of organic matter releases carbon, which plants use for photosynthesis. Then, we have the nitrogen cycle, where decomposers break down organic waste, converting nitrogen into usable forms for plants.



Then, we have the phosphorus cycle, where waste breakdown releases phosphorus, essential for DNA and ATP, which plants then absorb as a nutrient. These cycles naturally detoxify ecosystems, recycle nutrients, and sustain life by converting waste into usable forms. So, we have covered microbial metabolism in detail in Module 3. Let's have a small recap of the basics here. For example, you already know about aerobic respiration, which requires oxygen for energy production.

Natural Waste Degradation Cycles (Biogeochemical Cycles)

Biogeochemical cycles are natural processes that recycle nutrients and waste products in ecosystems, essential for maintaining environmental balance.

- **Carbon Cycle:** The decomposition of organic matter releases carbon, which plants reuse for photosynthesis.
- **Nitrogen Cycle:** Decomposers break down organic waste, converting nitrogen into usable forms for plants.
- **Phosphorus Cycle:** Waste breakdown releases phosphorus, essential for DNA and ATP, which plants then absorb.

These cycles naturally detoxify ecosystems, recycle nutrients, and sustain life by converting waste into usable forms.

Glucose is broken down into pyruvate, then into carbon dioxide, water, and ATP via the Krebs cycle and electron transport chain. These are used in activated sludge processes and bio-trickling filters, which we will discuss in a later part. Then you have anaerobic respiration, which does not require oxygen or occurs at very low concentrations of oxygen. This uses alternative electron acceptors like sulfate, nitrate, and fumarate instead of oxygen.

Less efficient ATP production occurs here compared to aerobic respiration, and it drives processes like methanogenesis in anaerobic digesters. Then we have facultative anaerobes, which can switch between aerobic and anaerobic respiration based on oxygen availability. Some examples are *E. coli* and *Pseudomonas* species. These are versatile and adaptable for various waste treatment scenarios. Let us take a peek into nature's own recycling system, which is basically what we call biodegradation—a natural process driven by the activity of bacteria and fungi.

Microbial metabolism

We've covered this topic in detail in Module 3 Lecture 1. We'll recap the basics here.

Aerobic Respiration	Anaerobic Respiration	Facultative anaerobes
<ul style="list-style-type: none">• Requires oxygen (O_2) for energy production.• Glucose is broken down to pyruvate, then to CO_2, H_2O and ATP via Krebs cycle and electron transport chain.• Used in activated sludge processes and biotrickling filters (will be discussed in later slides).	<ul style="list-style-type: none">• Does not require oxygen.• Uses alternative electron acceptors like sulfate (SO_4^{2-}), nitrate (NO_3^-), or fumarate ($C_4H_4O_4^{2-}$) instead of O_2.• Less efficient ATP production compared to aerobic respiration.• Drives processes like methanogenesis in anaerobic digesters.	<ul style="list-style-type: none">• Can switch between aerobic and anaerobic respiration based on O_2 availability.• Examples: <i>E. coli</i>, some <i>Pseudomonas</i> species.• Versatile and adaptable for various waste treatment scenarios.

You can see here in the image one fungus. This serves as a vital means of decay in the environment. It is like nature's built-in recycling system. These microorganisms act as decomposers, breaking down large, complex organic molecules into smaller, simpler ones through the action of enzymes. This decomposition process is essential for replenishing ecosystems and ensuring the continuous cycling of nutrients.

Biodegradation: Nature's Recycling System

Biodegradation is a natural process driven by the activity of bacteria and fungi (shown in the image) that serves as a vital means of decay in the environment. It is like nature's built-in recycling system.

These microorganisms act as decomposers, breaking down large, complex organic molecules into smaller, simpler ones through the action of enzymes. This decomposition process is essential for replenishing ecosystems and ensuring the continuous cycling of nutrients.

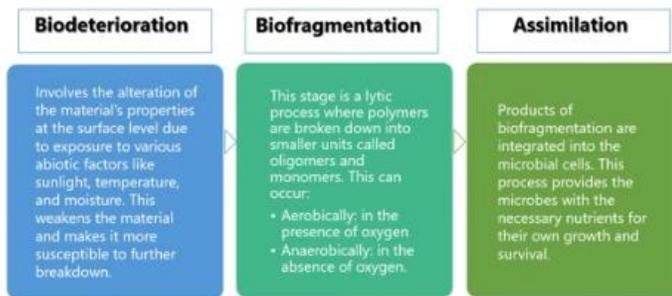


Image adopted from Bernard Spragg, NZ CC0 1.0

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This process of biodegradation can be divided into three stages. The first one is the biodeterioration involves the alteration of the materials properties at the surface level due to exposure to various abiotic factors like sunlight, temperature and moisture. This weakens the material and makes it more susceptible to further breakdown. Next comes the biofragmentation. This stage is a lytic process where polymers are broken down into smaller units called oligomers and monomers. This can occur aerobically in the presence of oxygen or anaerobically in the absence of oxygen or in low oxygen concentration. The third stage is assimilation. The products of biofragmentation are integrated into the microbial cells. The process provides the microbes with the necessary nutrients for their own growth and survival.

The Biodegradation Process: Three Stages



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Let us now take the example of biodegradation of plastics. Plastics we know are a big challenge today from the pollution point of view and today the microplastics are contaminating the soil and that microplastics are also being absorbed by plants and some of them have been reported to land up in the flowers and the honey bees have while sucking the honey from the flowers actually also suck in the microbes and there are reports where microplastics are also been found to be now present in honey. So, that is the level of challenge we have today with plastics. So it is very very essential that we think about degrading plastics which is actually a challenge.

However, biodegradation of plastics has come as a very interesting idea. Most conventional plastics are highly resistant to degradation. Some microorganisms have evolved the ability to break down these tough polymers, which is a big area of research today. So, now let's look into the figure, which tells us about the process of biodegradation of plastics. These enzymes attach to the surface and cleave the polymer chains.

This could be organic waste, plastic, or inorganic waste. Then, there is excretion of extracellular enzymes by the microbes, and these enzymes attach to the surface of these plastics. In this process, it releases carbon dioxide, water, and other metabolic byproducts. Surface erosion actually takes place by this enzymatic action and may release water-soluble intermediates. These intermediates may assimilate into the cell, or there are certain salt degradation intermediates that dissolve into the media.

The plastic-degrading microbes are therefore able to utilize the plastic as a food source, converting it into smaller molecules that can be further decomposed and incorporated into the environment. Some of the recent research on microbes in plastic degradation includes a study published in 2024 on microbial decomposition of biodegradable plastics on the deep sea floor. We know that oceans are now full of plastics, and some of them have sunk

down to the ocean floor. This team has studied the decomposition of such deep-sea floor plastics. Then, we have this study on the biodegradation of polyethylene by the marine fungus *P. album*.

Biodegradation of Plastics

The biodegradation of plastics is a crucial example of this natural process in action. Although most conventional plastics are highly resistant to degradation, some microorganisms have evolved the ability to break them down.

These plastic-degrading microbes are able to utilize the plastic as a food source, converting it into smaller molecules that can be further decomposed and incorporated into the environment.

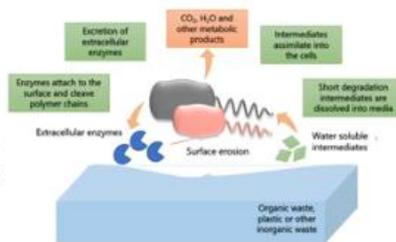


Fig: Process of biodegradation of Plastic
Image generated by author

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So, here you can see that *P. album* is actually placed into an incubator, and in nine days, it degrades some of the plastics that are in this incubator. So basically, this *P. album* was isolated from plastic debris in the North Pacific subtropical Gyre. This is one of those plastic islands that I mentioned in the beginning. Then, this organism was found to be able to mineralize UV-treated polyethylene into carbon dioxide. Over a time interval of nine days, mineralization of the UV-treated polyethylene occurs at a rate of 0.44%

per day, which is low but still a very good rate considering the challenge of plastic degradation. Despite the high mineralization rate, incorporation of PE-derived carbon into fungal biomass is only minor, but now there are other ideas of genetically engineering these organisms to enhance biodegradation. Then, there are reports of plastic-degrading bacteria in the gut microbiome of marine benthic polychaetes, which support enhanced digestion of plastic fragments. So that's a very interesting and active area of research currently. So, we are trying to solve the problem of plastic pollution by first identifying and isolating naturally occurring microbes that can do the job for us.

Significance of Biodegradation in Waste Treatment

- Eliminate harmful pollutants
- Recycle nutrients
- Reduce waste volume
- Generate renewable energy (Module 11)
- Cost-effective waste treatment

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Bioremediation

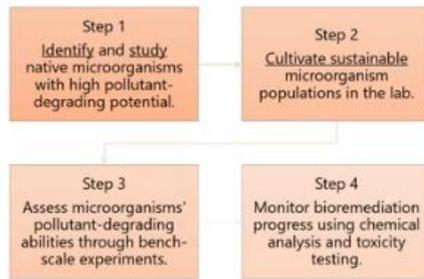
Bioremediation uses microorganisms to break down environmental pollutants in soil, water, and air. Both natural and introduced microbes are employed, but optimal conditions, including nutrients, pH buffers, and minerals, are needed. The process relies on oxidation-reduction reactions, with oxygen, nitrate, sulfate, etc. as the electron acceptor and organic substrates as donors.

We'll discuss bioremediation in the next lecture.

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Some of the steps involved in bioremediation techniques are as follows. In step one, we identify and study native microorganisms with high pollutant-degrading potential. In the second step, we cultivate microorganisms sustainably in laboratory conditions. In step 3, we assess microorganisms' pollutant-degrading abilities and their efficiencies through bench-scale experiments. In step 4, we monitor bioremediation progress using chemical analysis and toxicity testing of the products produced by this process.

Steps involved in bioremediation techniques



(Christopher Chibueze Azubuikwe et al., 2016)

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Let us now try to understand the differences between biodegradation and bioremediation. So biodegradation is a naturally occurring phenomenon. Bioremediation is developed by humans, so it is man-made or an engineered process. The target of bioremediation is a wide range of organic matter. In bioremediation, since it is man-made, the target is specific pollutants or contaminants.

Biodegradation is a passive approach and does not require human intervention. If we allow some waste to stay in a space, natural forces will take care of it. The process may be slow, but over time, biodegradation will start decomposing that material, which may range from days to weeks to months to years to decades. Then we have these bioremediation processes. Here, the approach is active.

It requires human intervention. Most of the time, we will be developing reactors where we treat the waste, or we also construct dump sites and use various gadgets and infrastructure for degrading waste by this approach. So, that's why it is an active approach. Some examples of biodegradation, which is a natural process, are composting and decaying leaves.

The leaves fall from the tree; we don't touch them. They stay there for a long time and decay on their own over time. Some examples of bioremediation, which is man-made and an active process involving human intervention, are cleaning up oil spills and treating wastewater, which may be generated by industries or from city waste. Let us now move on to Section 2, where we will discuss microbial waste treatment methods like suspended growth systems. Under that, we will study the activated sludge method, sequencing batch reactors (SBRs), Then we will discuss attached growth systems, where we deal with bio-trickling filters and rotating biological contactors.

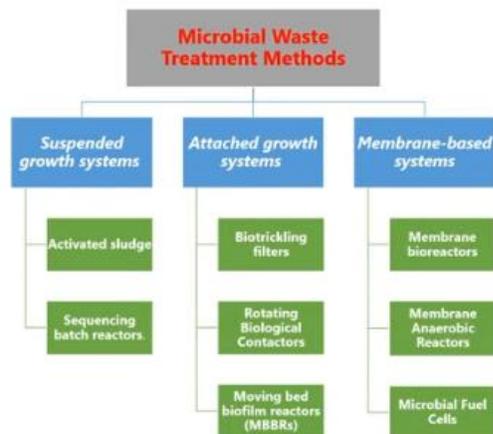
Biodegradation v/s bioremediation

Feature	Biodegradation	Bioremediation
Natural or man-made?	Natural	Man-made (Engineered)
Target	Wide range of organic matter	Specific contaminants
Approach	Passive (don't require human intervention)	Active (requires human intervention)
Examples	Composting, decaying leaves	Cleaning up oil spills, treating wastewater

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Then we have moving bed biofilm reactors. Next, we move to membrane-based systems, where we discuss membrane bioreactors, membrane anaerobic reactors, and microbial fuel cells. Then we have another section, Section 3. Let us move to microbial waste treatment methods. We can broadly divide them into three types: suspended growth systems, attached growth systems, and membrane-based systems.

Under suspended growth systems, we have the activated sludge method and sequencing batch reactors. In attached growth systems, we have bio-trickling filters, rotating biological contactors, and moving bed biofilm reactors. In membrane-based systems, we have membrane bioreactors, membrane anaerobic reactors, and microbial fuel cells. Let's look into suspended growth systems, which rely on microorganisms free-floating in wastewater to break down organic matter and other pollutants. The advantages of suspended growth systems are that they are highly efficient and help in the rapid degradation of organic matter.



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Adaptability is another advantage, suitable for various waste streams with different compositions and strengths. Comparatively, they are very simple to operate and require less complex infrastructure compared to other systems. Flexibility is one of the advantages, making it easy to modify and scale up or down systems, and they are very cost-effective. So, we have lower operational costs compared to some other technologies. However, they also suffer from certain disadvantages, such as high energy consumption, requiring continuous aeration for aerobic systems.

There is also excess sludge production, which requires disposal or further treatment of the excess biomass. They may be sensitive to shock loads; sudden changes in waste characteristics can disrupt microbial activity and decrease efficiency. These systems are susceptible to bulking; the formation of filamentous bacteria can hinder settling and reduce treatment efficiency. What are the different types of suspended growth systems? Number one is the activated sludge method.



This is the most widely used system. It employs aeration and mixing to maintain a high concentration of active microbes. Then we have the sequencing batch reactors, or SBRs. These SBRs are advanced wastewater treatment systems that operate in batch mode, allowing for a sequential process of filling, reacting, settling, and decanting within a single reactor.

Types of Suspended Growth Systems

Activated Sludge Method

The most widely used system, employing aeration and mixing to maintain a high concentration of active microbes.

Sequencing Batch Reactors (SBRs)

Sequencing Batch Reactors (SBRs) are advanced wastewater treatment systems that operate in a batch mode, allowing for a sequential process of filling, reacting, settling, and decanting within a single reactor.

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How activated sludge works? So, here you can see the schematic of an activated sludge system and here is the inlet through which we will send in the waste and this is the aeration tank where we send in compressed air. You can see here from this point. And then the waste will be degraded in this particular tank and then the degraded products will go to the clarifier and here the sludge will be deposited. And this is the outlet through which the overflowing liquid will go out.

And this sludge is extracted through this opening. Now this sludge may contain material which is not fully degraded or treated. So, we may actually recirculate it and put it back into the aeration tank and further we expose it to air for aerobic degradation. And this process can continue for a couple of times until and unless we get a very good degradation. So, now let us see into the operations in the aeration tank we introduce air to support the microbial growth microorganisms consume and break down the organic pollutants in the aeration tank and then there is the sedimentation where settling of the activated sludge takes place.

The sludge is recirculated, a portion of settled sludge is recirculated for sustained microbial activity. Then we have a secondary clarifier where separation of treated water from the settled sludge takes place. Let us look into the working of a sequencing batch reactor. So, here we have the influent mixture and air. So, we fill it up which is steady, mixed or aerated.

How Activated Sludge Works?

- **Aeration Tank:** Introduction of air to support microbial growth.
- **Microbial Digestion:** Microorganisms consume and break down organic pollutants.
- **Sedimentation:** Settling of activated sludge.
- **Sludge Recirculation:** A portion of settled sludge is recirculated for sustained microbial activity.
- **Secondary Clarifier:** Separation of treated water from settled sludge.

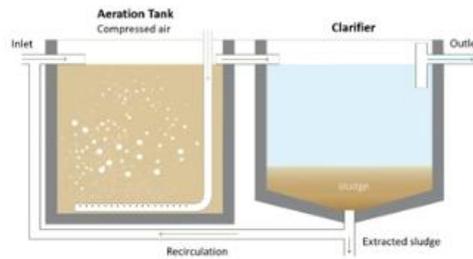


Figure: Schematic of an Activated Sludge System
Adapted from Lawag, Swiss Federal Institute of Aquatic Science and Technology
Technical drawings: designport, Paolo Monaco, Zurich, CC 3.0

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And then we have a in the step to the reaction where the mixing or aeration takes place and we allow the reaction to happen for some time interval after which the sludge will settle down. and then we thicken the top water and separate out the sludge and then this waste sludge is allowed to be idle and then we again may fill up with additional influent material and this cycle can actually go on. So, in the first step, which is a filling step, inflow waste water into the reactor. Then in the next process, there is a biological treatment and neutral removal and settling or sedimentation of the solids take place. And by decanting, we remove the treated water and the idling period is between draw and fill, the time interval.

How Sequencing Batch Reactors (SBRs) Work?

- **Fill:** Inflow of wastewater into the reactor.
- **React:** Biological treatment and nutrient removal.
- **Settle:** Sedimentation of solids.
- **Decant:** Removal of treated water.

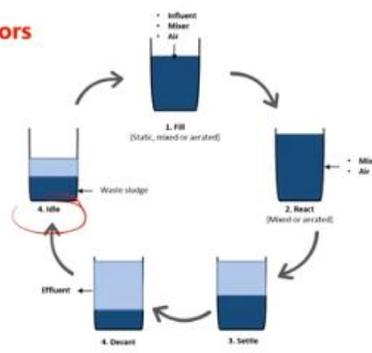


Fig: Processes involved in SBRs

Image generated by SBR.

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And if we compare the both the two methods of activated sludge versus SBR, we can see the two processes are different. So, from the operational point of view, activated sludge is a continuous operation. But here it is not continuous. This is basically batch wise cycle. From vessel point of view, we have separate aeration and settling tanks.

In the case of SBRs, single reactor for all the phases. We have moderate flexibility in the case of activated sludge. In the case of SBRs, we have highly flexible cycles or phases.

then we have less control on activated sludge process. But in SBR, more precise control over each phase.

From the point of view of energy consumption, activated sludge requires very high energy because of the continuous aeration. But in SBR, the energy consumption is lower because aeration takes place only during the reaction phase. Then we have the effluent quality, which is high in both cases, but in the case of SBR, there is potential for higher quality. Then, excess sludge production is very high in the case of activated sludge, but it is comparatively lower in the case of SBRs. From the point of view of cost, activated sludge requires lower capital cost but higher operational cost because of the continuous need for energy.

In SBR, we have a higher capital cost but a lower operational cost. From the application point of view, activated sludge is most common in municipal and industrial settings for water treatment. SBRs are suitable for smaller communities, fluctuating influent flows, and high effluent quality requirements. Let us now discuss the attached growth systems. These systems are a powerful and versatile category of biological wastewater treatment methods.

Activated Sludge Method v/s SBRs

Feature	Activated Sludge	SBRs
Operation	Continuous flow	Batch wise cycles
Vessels	Separate aeration and settling tanks	Single reactor for all phases
Flexibility	Moderately flexible	Highly flexible (adjust cycle phases)
Control	Less precise	More precise control over each phase
Energy consumption	High (continuous aeration)	Lower (aeration only during reaction phase)
Effluent quality	High	High (potential for higher)
Excess sludge production	High	Lower
Cost	Lower capital cost, higher operational cost	Higher capital cost, lower operational cost
Applications	Most common municipal and industrial wastewater treatment	Suitable for smaller communities, fluctuating influent flows, and high effluent quality requirements

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However, unlike suspended growth systems, where the microorganisms are freely suspended in the wastewater, attached growth systems utilize a fixed surface for microbial colonization. This leads to the formation of biofilms, communities of microorganisms encased in a self-produced extracellular matrix. So, what are the advantages of using attached growth systems? They are also highly efficient. Biofilms can achieve high removal rates for organic matter, nutrients, and other pollutants.

Attached growth systems

Attached growth systems are a powerful and versatile category of biological wastewater treatment methods.

Unlike suspended growth systems, where the microorganisms are freely suspended in the wastewater, attached growth systems utilize a fixed surface for microbial colonization.

This leads to the formation of biofilms, communities of microorganisms encased in a self-produced extracellular matrix.

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They consume very low energy and require less aeration compared to suspended growth systems. The small footprint requires less space than some suspended growth systems due to high biomass density. They are resilient to shock loads. They can tolerate fluctuations in water flow and pollutant concentrations because of their biofilm nature. They are simple to operate and maintain, requiring minimal maintenance in fact.

Again, like any other system, they also have certain disadvantages. For example, they have a slower startup. Biofilm development takes time until the microorganisms are arranged and encased in an extracellular matrix to form a biofilm. They won't be able to show their metabolic activity as a group. So, this leads to longer startup times than some suspended growth systems where degradation may start quite early.



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There is a clogging potential. Biofilm growth can potentially clog the media, requiring periodic cleanup or replacement. Then, they are sensitive to toxic compounds. High concentrations of toxic chemicals can harm the biofilm. Limited control over microbial population.

The composition of the biofilm is determined by environmental conditions and may not be easily controlled. How does this attached growth system work? Number one, there is a wastewater distribution. Then, secondly, biofilm formation and growth take place. Then, the organic matter degradation by the diversity of microorganisms in the biofilm happens.

Then, we have the oxygen supply and aeration. These are typically open systems that allow natural air circulation in some cases. Forced aeration systems supplement natural air circulation and optimize treatment performance. The treated water collection happens after this, and we have to maintain the biofilm. As the biofilm grows thicker, a portion of it naturally sloughs off the media and exits the filter with the treated waste.

How Attached Growth Systems work?

1. Wastewater Distribution

2. Biofilm Formation and Growth

3. Organic Matter Degradation by the diversity of microorganisms in the biofilm

4. Oxygen Supply and Aeration: Typically open systems, allow natural air circulation. In some cases, forced aeration systems supplement natural air circulation and optimize treatment performance.

(Oakley, 2017)

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As a result, trickling filters require secondary sedimentation to remove that sloughed biofilm, measured as suspended solids. Additionally, periodic cleaning or replacement of the media may be necessary to prevent excessive clogging and ensure optimal performance. So, what are the different types of attached growth systems? We have trickling filters, rotating biological contactors, and moving bed biofilm reactors. In trickling filters, wastewater trickles over a bed of media, allowing biofilm formation and degradation.

5. Treated Water Collection

6. Biofilm Maintenance: As the biofilm grows thicker, a portion of it naturally sloughs off the media and exits the filter with the treated water. As a result, trickling filters require secondary sedimentation to remove the sloughed biofilm measured as suspended solids.

Additionally, periodic cleaning or replacement of the media may be necessary to prevent excessive clogging and ensure optimal performance.

(Oakley, 2017)

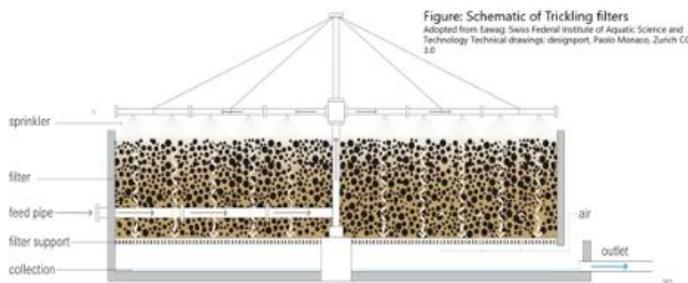
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So, here is the schematic of a trickling filter. So, you have these sprinklers on the top, which are sprinkling the media wastewater. Then there is the filter layer here, as you can see. Then there is a feed pipe over here. Then this is the filter support, which separates the collection chamber from the media chamber into which we have this filter.

Then natural air is allowed in, as discussed earlier, and this is the outlet through which the treated wastewater will exit. So, this is a simple arrangement in a way. Now we have the rotating biological contactors. So, this system is a static system. It does not move.

Trickling filters

Wastewater trickles over a bed of media, allowing biofilm formation and degradation.



In the RBCs, we have rotating discs with biofilm growth. These are partially submerged in the wastewater for treatment. So, some of the system components are: the rotating biological system consists of a circular tank containing a series of rotating discs mounted on a central shaft. So, this is the side view of this system, and this is the front view of this system. So, you have these media disks or panels stacked next to one another, and from the front view, you can see this is the central shaft, and this is the central shaft, and there is a motor which drives this shaft.

So, here you can see the media support around the disc, and this disc part is in the air, with around 35 to 40% submerged. So, the disc is rotating alternately between air and liquid, which is being treated. So, this is partial submergence of the disc, allowing the formation of biofilms on its surfaces. The rotation speed of the discs can be controlled to meet specific treatment needs. So, that has to be optimized.

Rotating biological contactors (RBCs)

Rotating discs with biofilm growth are partially submerged in the wastewater for treatment.

System Components:

- An RBC system consists of a circular tank containing a series of rotating discs mounted on a central shaft.
- The discs are partially submerged in the wastewater, allowing the formation of biofilms on their surfaces.
- The rotation speed of the discs can be controlled to meet specific treatment needs.

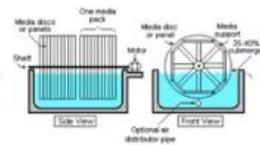


Image: Schematic of a Rotating Biological Contactors
Adopted from Mbeychok CCD

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So, since the disc is moving here, this is a rotating biological contactor. Now we have the moving bed biofilm reactor. This is a system where freely moving media with biofilm growth are fluidized in the wastewater. Biofilms grow on media within a fixed bed or packed column reactor. So, this is the schematic of a moving bed biofilm reactor.

Basically, you have reactor 1 and reactor 2. So, in reactor 1, we introduce the raw effluent for pre-treatment, and then we have the air blowers for aeration from below. In reactor number 2, we have sedimentation, and there we also have this air distribution grid. So, here we have the specially developed media loaded, and the treated effluent is taken out from this outlet. So, this is one of the special kinds of designs, which we call the moving bed biofilm reactor.

Moving bed biofilm reactors (MBBRs)

Freely moving media with biofilm growth are fluidized in the wastewater. Biofilms grow on media within a fixed bed or packed column reactor.

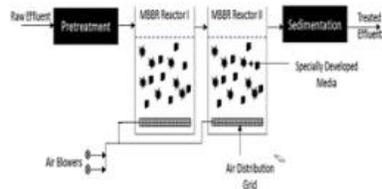


Image: Schematic of a MBBR
Adopted from <https://www.amintech.com/products/moving-bed-bio-reactor-mbbbr.html>

Now, what is the difference between a trickling filter versus an RBC and an MBBR? So, from the design point of view, we have already shown you that a trickling filter is a stationary bed or media. A rotating biological contactor has rotating discs with biofilm. In a moving bed biofilm reactor, we have freely moving media with biofilm. Some of the advantages of a TF are its simple design.

Low energy, resilient, and cost-effective. RBCs have a high surface area, are compact, have good nitrification, and require low energy. In an MBBR, we have the capacity for high biomass density. It is compact, efficient, and adaptable. However, each of them has certain disadvantages: a trickling filter has a slow startup, is order-limited, and nutrient removal is a challenge; there is also a problem of clogging.

An RBC also has mechanical complexity; disc breakage may happen, and there may be bulking of the filaments. Then, in the MBBR, we have a very high cost of operations. There is media loss, and we need to mix things regularly. And then, from a suitability point of view, trickling filters are suitable for small to medium communities, industrial settings, and bioremediation applications, while rotating biological contactors are useful for municipal and industrial high-strength wastewater treatment.

Similarly, MBBR is also useful for municipal, industrial, and upgrading plants, as well as for high-strength wastewater treatment. Let us now have a discussion on membrane-based systems. These combine biological treatment with membrane filtration for high-quality effluent and increased biomass retention. Some of the advantages are high-quality effluent that meets stringent discharge standards and can be reused or discharged into sensitive environments. It has a reduced environmental impact, minimizes sludge production, and requires less land compared to conventional methods.

Trickling Filter v/s RBC v/s MBBR

Feature	Trickling Filter	Rotating Biological Contactor (RBC)	Moving Bed Biofilm Reactor (MBBR)
Design	Stationary bed of media	Rotating discs with biofilm	Freely moving media with biofilm
Advantages	Simple, low energy, resilient, cost-effective	High surface area, compact, good nitrification, low energy	High biomass density, compact, efficient, adaptable
Disadvantages	Slow startup, odor, limited nutrient removal, clogging	Mechanical complexity, disc breakage, filamentous bulking	Higher cost, media loss, mixing requirement
Suitable applications	Small/medium communities, industrial, bioremediation	Municipal, industrial, high-strength wastewater	Municipal, industrial, upgrading plants, high-strength wastewater

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Improved public health reduces the risk of waterborne diseases by effectively removing pathogens. Resource recovery enables the extraction of valuable resources such as biogas and water from waste streams. However, there are certain disadvantages, such as high initial capital costs, as membranes and associated equipment can be very expensive. Membrane fouling may occur, where membranes get clogged by organic matter. Additionally, there is high energy consumption, requiring energy for both aeration and membrane filtration. There is sensitivity to toxic compounds. High concentrations of toxic chemicals can damage the membranes.

Then there is operational complexity. It requires more sophisticated operation and maintenance. What are the different types of membrane-based systems? We have membrane bioreactors, which combine activated sludge treatment with membrane filtration for high-quality effluent and a reduced footprint. Then we have membrane anaerobic reactors.

Membrane Based Systems

Combine biological treatment with membrane filtration for high-quality effluent and increased biomass retention.

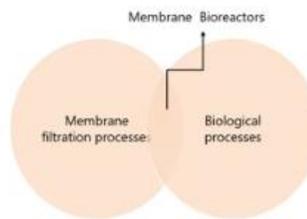


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They utilize anaerobic digestion with membrane separation to treat organic waste and produce biogas. So, this is the schematic of a membrane bioreactor. So, this is a submerged membrane bioreactor here, and this is the side-stream membrane bioreactor. Then, we have a rotating disc membrane filter over here. And you can see here the biological membrane is inside in this case, while here it is outside. Then, you can see the inlet position from here, but in this side-stream membrane bioreactor, the sludge is recycled, and the sludge passes through this biological membrane.

Types of Membrane-based systems

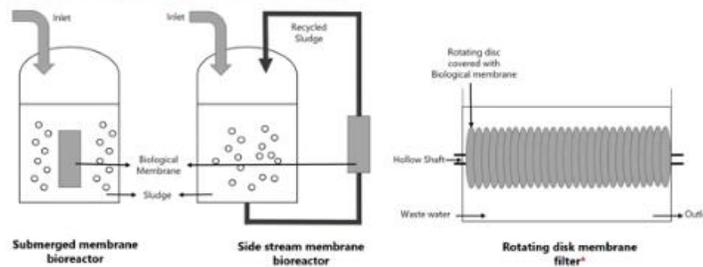
- **Membrane Bioreactors (MBRs):** Combine activated sludge treatment with membrane filtration for high-quality effluent and reduced footprint.
- **Membrane Anaerobic Reactors (MARs):** Utilize anaerobic digestion with membrane separation to treat organic waste and produce biogas.



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In the case of a rotating disc membrane filter, we have a rotating disc covered with a biological membrane, and there is a shaft. Of course, there will be a motor which will help us in rotating it. Then, the wastewater enters from one side, and this disc keeps on rotating, and treated waste is taken out through the outlet. So, this rotating membrane filter was developed, which consisted of a pressurized membrane with a hollow rotating shaft and hollow membrane-covered discs stacked along with it. This design led to the reduction of membrane fouling and cost. How does a membrane bioreactor work? Wastewater enters the biological reactor, as you can see in both cases, from the top.

Membrane Bioreactors (MBRs)



*A rotating disk membrane filter was developed by Reed et al. (1997) which consisted of a pressurized membrane with hollow rotating shaft and hollow membrane-covered disks stacked along with it. This design led to the reduction of membrane fouling and cost.

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(Reed et al. 2017)

Here, microorganisms in the activated sludge degrade organic matter and pollutants. Mixed liquor is pumped to the membrane filtration unit. This contains the treated wastewater and the activated sludge. Pressure is applied on the mixed liquor. This forces the water and dissolved molecules through the membrane, leaving behind a concentrated stream of solids and microorganisms.

MBR: How it works?

- 1. Wastewater enters the biological reactor:** Here, microorganisms in the activated sludge degrade organic matter and pollutants.
- 2. Mixed liquor is pumped to the membrane filtration unit:** This contains the treated wastewater and the activated sludge.
- 3. Pressure is applied to the mixed liquor:** This forces the water and dissolved molecules through the membranes, leaving behind a concentrated stream of solids and microorganisms.

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Then, the treated water is collected; the permeate, or treated water, is collected and can be discharged or reused. Concentrated solids are recycled or disposed of. The retentate, or concentrated stream containing the biomass, is recycled back to the biological reactor to maintain the desired concentration of microorganisms. Excess biomass is then removed. A portion of the concentrated solids is periodically removed to prevent excessive accumulation and maintain treatment efficiency.

MBR: How it works?

- 4. Treated water is collected:** The permeate, or treated water, is collected and can be discharged or reused.
- 5. Concentrated solids are recycled or disposed:** The retentate, or concentrated stream, containing the biomass, is recycled back to the biological reactor to maintain the desired concentration of microorganisms.
- 6. Excess biomass removed:** A portion of the concentrated solids is periodically removed to prevent excessive accumulation and maintain treatment efficiency.

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Membrane anaerobic reactor and its working mechanism. So, here you can see the schematic of a membrane anaerobic reactor. So, you have a membrane here, and then this is the anaerobic bioreactor, and these pumps will force this material to be treated through

this membrane. The permeate will go out, while the retentate will be recycled back into this system.

So, organic waste like food scraps and agricultural residue enters the anaerobic digester. So, this is the feed which may be any of these wastes. Then, the microbes will decompose the waste, producing biogas, methane, and carbon dioxide, and also the digester. Then, the digester is sent to the membrane filtration unit, separating it into permeate, which is the treated effluent.

Free of solids and microbes for discharge or reuse. The retentate, the concentrated solids and microbes, is recycled back to the digester to maintain microbial concentration. Biogas is collected for use in electricity generation, heating, or as a fuel. Let us now briefly discuss microbial fuel cells. We will discuss these in detail later.

3. Digestate is sent to a membrane filtration unit, separating it into:

- **Permeate:** Treated effluent, free of solids and microbes, for discharge or reuse.
- **Retentate:** Concentrated solids and microbes.

4. Retentate is recycled back to the digester to maintain microbial concentration.

5. Biogas is collected for use in electricity generation, heating, or as fuel.

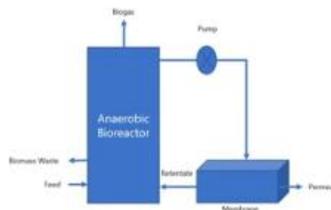


Fig: Schematic of a MAR
Image generated by author

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In later lectures. Microbial fuel cells are recognized for their capacity to generate electricity through microbial metabolism, utilizing various degradable wastes, which is quite an interesting technology. Here, you can see the schematic of a microbial fuel cell with the cathode at one end and the anode at the other end. Electricity is produced due to the reactions over here. This is basically loaded with microbes.

The concept of microbial fuel cells dates back to 1912 when Potter demonstrated electricity production from *Saccharomyces* and *Escherichia coli*. Later, in 1931, Cohen claimed nearly 35 volts of electricity production. Let us now discuss an interesting system produced as a result of membrane filtration processes combined with biological and electrochemical processes. We call this system a bioelectrochemical membrane reactor system. There is growing interest in combining microbial fuel cells and membrane bioreactors for water treatment and electricity generation.

Microbial Fuel Cell (MFC)

MFCs are recognized for their capacity to generate electricity through microbial metabolism, utilizing various degradable wastes.

The concept dates back to 1912 when Potter demonstrated electricity production from *Saccharomyces* and *Escherichia coli*, and later in 1931, Cohen claimed nearly 35 V electricity production.

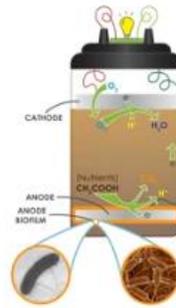


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Bioelectrochemical membrane reactors efficiently generate electricity and remove organic pollutants, with recent design advancements enhancing performance and reducing costs. Let us now proceed to section 3, where we will discuss the mechanisms of microbial waste treatment, including biomineralization, biosorption, biotransformation, bioaccumulation, and bioleaching. The various mechanisms of microbial waste treatment fall into biomineralization, biosorption, biotransformation, bioaccumulation, and bioleaching. However, we have various types of biomineralization, which include microbial-induced carbonate precipitation, microbial-induced phosphate precipitation, and microbial-induced sulfate precipitation. Then, we have heavy metal biosorption, heavy metal biotransformation, and heavy metal bioaccumulation.

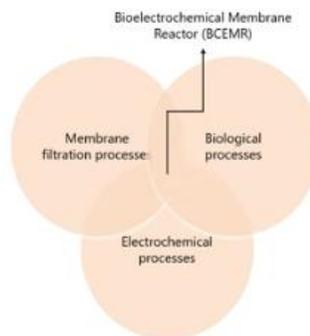
Bioelectrochemical Membrane Reactors (BECMR)

MFC + MBR → BECMR

There is growing interest in combining Microbial Fuel Cells (MFC) and Membrane Bioreactors (MBR) for water treatment and electricity generation.

Bioelectrochemical Membrane Reactors

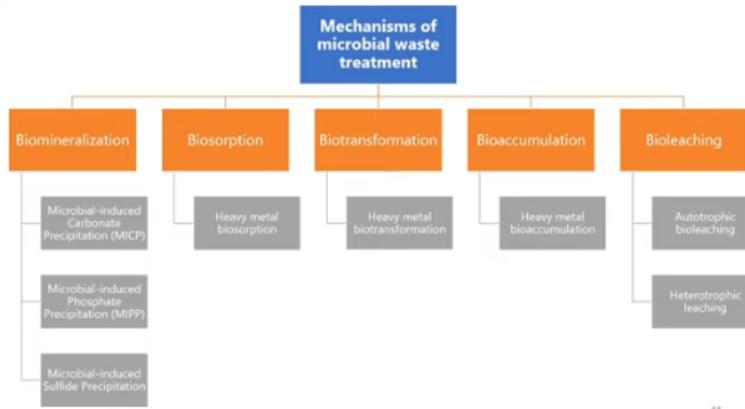
(BECMR) efficiently generate electricity and remove organic pollutants, with recent design advancements enhancing performance and reducing costs.



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Additionally, we have auxotrophic bioleaching and heterotrophic leaching. In microbial-induced carbonate precipitation, urea hydrolysis by urea-producing bacteria generates carbonate, which reacts with heavy metal ions in the soil, forming precipitates that immobilize heavy metals. Here, cellular metabolism produces urease, which helps hydrolyze urea, forming precipitates and immobilizing heavy metals. These are the heavy

metals, as you can see here. In microbial-induced phosphate precipitation, phosphate mineralization is caused by bacteria that produce phytase or phosphatase, hydrolyzing organic phosphorus into phosphate.



Biomineralization

1. Microbial-induced Carbonate Precipitation (MICP):

Urea hydrolysis by urease bacteria produces carbonate, which reacts with heavy metal ions in the soil, forming precipitates that immobilize heavy metals.

Biomineralization

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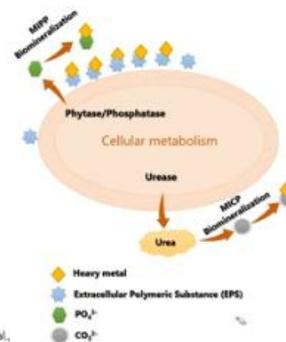


Fig: Process of Biomineralization
Image redrawn by author with adaption from Zheng et al., 2021

Phosphate biominerals then form with heavy metal ions in the soil, fixing the heavy metals. You can observe the production of phytase and phosphatase, which aids in MIPP

biomineralization. In microbial-induced sulfide precipitation, sulfate-reducing bacteria utilize sulfate as an electron acceptor. Here, sulfate serves as the electron acceptor, while organic substrates act as electron donors, oxidizing organic compounds to produce sulfide and increase alkalinity. The generated sulfides react with metal ions, forming metal sulfides that precipitate and immobilize the heavy metals.

2. Microbial-induced Phosphate Precipitation (MIPP):

Phosphate mineralization bacteria generate phytase or phosphatase, hydrolyzing organic phosphorus into phosphate. Phosphate biominerals then form with heavy metal ions in the soil, fixing heavy metals.

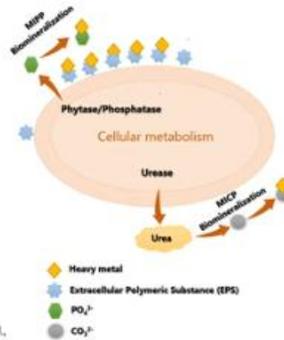


Fig. Process of Biomineralization
Image redrawn by author with adaption from Zheng et al., 2021

3. Microbial-induced Sulfide Precipitation:

Process: Sulfate-reducing bacteria (SRB) utilize sulfate as an electron acceptor to oxidize organic compounds, producing sulfide and increasing alkalinity. The generated sulfides react with metal ions, forming metal sulfide precipitates that immobilize heavy metals.



(Zheng et al., 2021)

Let us now discuss biosorption. Bacteria, algae, and fungi act as biosorbents, with bacteria excelling due to their surface-to-volume ratio and binding sites like carboxyl, phosphate, and hydroxyl groups. Several studies have found that dead bacteria often outperform living ones in biosorption, as shown by *Bacillus asparagus*, which had 13 to 20% higher chromium ion absorption when dead. This may be due to resilience to toxicity, no nutrient requirement, storage without degradation, and cost-effectiveness. The heavy metal biosorption by bacterial biomass figure shows the mechanism of biosorption.

Biosorption

Bacteria, algae, and fungi act as bio-sorbents, with bacteria excelling due to their **surface-to-volume ratio and binding sites (e.g., carboxyl, phosphate, hydroxyl groups)**.

Several studies have found that dead bacteria often outperform living ones in biosorption, as shown by *Bacillus sphaericus*, which had 13–20% higher chromium ion absorption when dead (Aravindhana et al., 2011). This is due to:

- Resilience to Toxicity
- No Nutrient Requirement
- Storage without degradation
- Cost-Effectiveness

(Pande et al., 2022; Velázquez & Dussan, 2009)

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There is precipitation, which involves chemical interaction between the cell surface and the metal surface in step A. Then, in step B, there is complexation of heavy metals that occurs by interaction of the metal ions with the surface active groups of bacterial biomass. Then, in step C, we have the exchange of bivalent metal ions, which occurs with the counterions present on the bacterial cell in the ion exchange mechanism. And then we have diffusion in step D. This is a simple process of biosorption without the involvement of any rate-limiting step. So, this enters the cell interior.

Then there is also surface adsorption. This is a rapid and reversible process in which metal cations bind to the bacterial surface and ions via nonspecific attraction forces. And then we have intracellular accumulation of heavy metals, which takes place by the transport of metal ions across the cell membrane. Let us now discuss the biotransformation process, which involves the active metabolic conversion of contaminants into less toxic forms by microorganisms through metabolic pathways. Bacteria and fungi break down organic pollutants like hydrocarbons, pesticides, and industrial chemicals.

Heavy metal biosorption by bacterial biomass

- (a) Precipitation
- (b) Complexation
- (c) Ion exchange
- (d) Diffusion
- (e) Surface adsorption
- (f) Intracellular accumulation

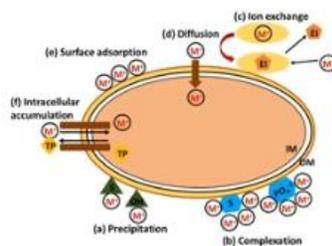


Fig: Mechanism of biosorption
IM- Inner Membrane, OM- Outer Membrane, EI- Exchangeable Ions, TP- Transport Proteins.

Image adopted from (Priyadarshani & Das, 2021)

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This process can result in complete degradation, mineralization, or conversion to less harmful substances, facilitating easier removal. So here, this figure shows the biotransformation of heavy metals, during which they are converted to less toxic forms. So, these heavy metal ions may be accumulated inside, which is called intracellular bioaccumulation, or they may be immobilized onto the cell by covalent bonding, ionic bonding, or there may be complex formation and chelation, and that can happen after internalization or that may lead to the internalization. There is also cationic change happening over here, and then many reactions are shown over here where inorganic arsenic is converted to organic arsenic, chromium-4 is converted to chromium-3, mercury ion is converted to elemental mercury, and these are considered as the less toxic forms.

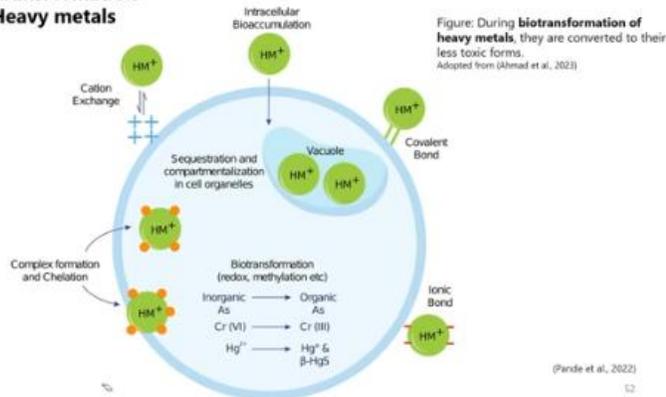
Biotransformation

Biotransformation involves the active metabolic conversion of contaminants into less toxic forms by microorganisms.

Through metabolic pathways, bacteria and fungi break down organic pollutants like hydrocarbons, pesticides, and industrial chemicals. This process can result in complete degradation (mineralization) or conversion to less harmful substances, facilitating easier removal.

(Pande et al., 2022)
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Biotransformation of Heavy metals



Now, another method is microbial bioaccumulation, which is a metabolically active process where heavy metal ions are transported into the cell via transporter proteins, involving mechanisms like endocytosis, ion channels, and carrier-mediated transport, and we have discussed these in module 3 earlier. So, this is the schematic for the process of bioaccumulation where these heavy metals are internalized, and this is the extracellular

polymeric substance as shown in the blue color. This process allows microbes to store heavy metals, thus remediating polluted environments. However, extremely high concentrations of contaminants can overwhelm microbial defense systems, leading to cell damage or death. Let us now have a brief discussion about bioleaching.

Bioaccumulation

Microbial bioaccumulation is a metabolically active process where HM ions are transported into the cell via transporter proteins, involving mechanisms like **endocytosis, ion channels, and carrier-mediated transport** (Discussed in Mod 3 Lecture 1).

This process allows microbes to store heavy metals, thus remediating polluted environments.

However, extremely high concentrations of contaminants can overwhelm microbial defense systems, leading to **cell damage or death**.

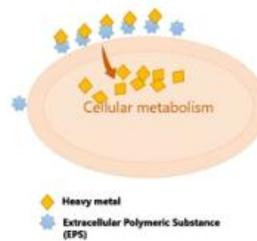


Fig: Process of Bioaccumulation
Image generated by author

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Bioleaching is mainly performed by acidophilic microbes and involves the oxidation of ferrous to ferric and sulfur to sulfuric acid in low pH environments. This produces ferric ions and protons, dissolving metal sulfides and oxides from ores. Microbes are used to extract and recover heavy metals or other soluble contaminants from soil by converting them into soluble substances for removal. The process can be direct bioleaching or indirect bioleaching, and both have different kinds of mechanisms. So, in direct bioleaching, microorganisms directly attach to the metal surface, as you can see here in this picture.

Bioleaching

- Bioleaching, mainly performed by acidophilic microbes, involves the oxidation of Fe(II) to Fe(III) and sulfur to sulfuric acid in low pH environments.
- This produces ferric ions and protons, dissolving metal sulfides and oxides from ores.
- Microbes are used to extract and recover heavy metals or other insoluble contaminants from soil by converting into soluble substances for removal.

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The bacterial cell interacts with the mineral, enzymatically breaking down the sulfide bonds. Electrons are transferred from the metal sulfide to the bacterial cell during the oxidation process. This leads to the release of soluble metal ions, for example, ferrous and

copper ions, into the solution. This process is driven by enzymatic activity on the mineral surface, in brief. In the indirect mechanism, microbes generate ferric ions and other oxidizing agents in the surrounding solution.

These oxidants, for example, ferric ions, react chemically with the metal sulfides, as you can see here. Causing them to oxidize and release metal ions. The role of bacteria here is primarily to regenerate ferric ions from ferrous via metabolic processes. This ensures a continuous supply of oxidizing agents for metal solubilization. So with this, we come to the end of today's lecture.

Direct Bioleaching Mechanism

In this pathway, microorganisms directly attach to the metal sulfide (MS) surface.

The bacterial cell interacts with the mineral, enzymatically breaking down the sulfide bonds.

Electrons are transferred from the metal sulfide to the bacterial cell during the oxidation process.

This leads to the release of soluble metal ions (e.g., Fe^{2+} , Cu^{2+}) into the solution.

The process is driven by enzymatic activity on the mineral surface.



Comparison of direct and indirect bioleaching mechanisms (M=metal).

Figure taken from DOI: 10.3390/fermentation8030115 License: CC BY 4.0

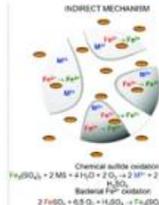
Indirect Bioleaching Mechanism

In this pathway, microbes generate Fe^{3+} ions and other oxidizing agents in the surrounding solution.

These oxidants (e.g., ferric iron, Fe^{3+}) react chemically with the metal sulfides (MS), causing them to oxidize and release metal ions.

The role of bacteria here is primarily to regenerate Fe^{3+} ions from Fe^{2+} via metabolic processes.

This ensures a continuous supply of oxidizing agents for metal solubilization.



Thank you for your patient hearing. God bless you.