

EFFECT OF BIOLOGICAL MATERIALS ON THE ENVIRONMENT

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ABSTRACT

Developing countries are experiencing advancements in industrial, agricultural, and other sectors, alongside a rapid increase in population, placing the environment in the spotlight. Attention to environmental cleanliness and the removal of existing pollution has become one of the most important contemporary issues. Given that the world has been advancing in biotechnology in recent years, it seems rational to utilize biological materials technology as a strong support in implementing major environmental programs in the country. The term "environment" refers to everything that surrounds us and is related to human life, including water, soil, air, and more. The environment plays a crucial role in the lives of individuals in society, and attention to its health and efforts to improve its hygiene and restore it from pollution are of great importance.

Nowadays, many people around the world live in polluted environments due to natural disasters, war, and inadequate infrastructure. On average, 5,500 children die daily from diseases caused by consuming contaminated water and breathing polluted air. All researchers strive to reduce these problems using modern methods and new technologies. One of these methods is bioremediation, the importance of which has become evident shortly after its emergence.

Keywords: Biological, Microbial, Microorganisms, Environment, pollution,

Introduction

The environment is considered a crucial factor in human life, as people have a close relationship with their surrounding environment throughout their lives. The environment is divided into three main components: water, air, and soil.

Water, being one of the essential elements of life on Earth, is vital for human survival. On average, a person drinks 2 to 2.5 liters of water daily, and water constitutes 58 to 66 percent of an individual's body weight. Breathing is also a crucial factor for human life. The human body requires oxygen to carry out all its functions, and living without clean air would be impossible.

Air pollution is the presence of one or more pollutants in the ambient air to an extent and duration that alters its quality in a way that is harmful to human health, animals, plants, or structures. The major sources of air pollution include natural and anthropogenic sources. Natural sources often exist without direct human intervention, while anthropogenic sources are human-made and the pollution they cause results from human activities.

Soil is another primary resource for providing human food. It significantly impacts human life, and soil pollution leads to many diseases in plants and animals, thereby threatening human health.



Examining how water, air, and soil become polluted, as well as addressing existing pollution, is considered crucial and is a fundamental aspect of human life.

Biological pollution

An introduction to biological pollution

Biological materials refer to substances that carry living organisms such as bacteria, viruses, fungi, algae, and others.

These tiny yet impactful organisms enter our surrounding environment through the use of biological fertilizers, herbicides, pesticides, human waste, and the skin and tissues of dead animals, among other sources, and they cause diseases and infections in people.

Since the conditions are favorable for the rapid growth and multiplication of these microscopic organisms, the initial stages of biological contamination are not easily detectable. Examining and identifying biological contamination in water, air, and soil is considered very important and is one of the methods for improving and maintaining the quality of health within a community.

Water pollution

Water is one of the most abundant and stable compounds found in nature and is known as the largest chemical solvent. It is a fundamental component of body tissues and is recognized as the most essential factor for life, without which physiological functions cannot occur. Water constitutes about 70% of the human body's weight and covers approximately 71% of the Earth's surface in the form of oceans, lakes, and rivers. The average annual rainfall worldwide is 76.2 centimeters, with about 97.3% of water in the oceans, 2.1% in polar ice, and 0.6% in lakes, rivers, and underground sources.

In principle, the total amount of water on Earth remains constant, but it is not distributed uniformly. Some regions have sufficient water resources, while others have insufficient amounts to meet the needs of human populations. Consequently, natural waters are processed through various operations to make them suitable for different uses. Although a community often obtains its water from a single source, when a single source is inadequate to meet the needs of the population, water is drawn from multiple sources, such as surface and groundwater. Surface water is sourced from large rivers or lakes, and even water from a small river can be suitable for use; in such cases, the water is collected and stored using a dam.

Surface waters vary in quality and contain microorganisms, dissolved and suspended organic and inorganic materials. Additionally, these waters may have undesirable color, taste, and odor. Surface waters are exposed to contamination from urban sewage, industrial waste, agricultural runoff, and animal and plant residues. The temperature of surface waters changes in accordance with the local climate.

Although groundwater is also subject to contamination, it is generally clearer and colorless, with lower amounts of organic materials and microorganisms compared to surface water, as the water undergoes some degree of filtration as it passes through different soil layers. In contrast, mineral content (such as calcium and magnesium ions), which is a primary cause of water hardness, may be higher in groundwater than in nearby surface waters. Generally, the quantity and type of minerals in groundwater reflect the mineral characteristics of the region's geology. Over time, the quality of groundwater tends to be more stable compared to surface water quality.

The temperature of groundwater is generally more stable than that of surface water and is usually close to the annual average air temperature of the region, whereas surface water temperatures vary with the local climate. Groundwater may only need microbial treatment to be suitable for general use. Depending on the type of contamination, certain substances that cause complaints among consumers should be removed from the water or reduced to levels that are tolerable for consumers and do not have adverse effects on their health.

Surface waters generally require more treatment compared to groundwater to be made suitable for public use. Engineering and environmental sciences emerged in the latter half of the 19th century due to findings that indicated water could be a cause of certain dangerous diseases affecting humans. Engineers involved in water supply sought ways to ensure that the provided water was safe and palatable.

Fundamental measures included coagulation and filtration in the late 19th century, followed by disinfection with chlorine in the early 20th century. Despite these fundamental measures, waterborne diseases are still prevalent in both developed and developing countries and remain a major cause of illness.

Additionally, some waterborne diseases in developed countries are now caused by organisms that previously did not pose a concern. New pathogens have emerged for which there is no historical basis to design preventive measures, and in some cases, there are no suitable methods for tracking these pathogens.

Given the fundamental importance of pathogenic microorganisms associated with water, engineers and scientists involved in water and sewage treatment need to be aware of pathogens transmitted through fecal or food sources.

The water test to find indicator organisms is a practical method for determining microbial contamination in water. Indicator organisms are used to indicate contamination and possess the following characteristics:

1. They are always present in contaminated waters.
2. They are not seen in uncontaminated waters.
3. They survive in water with more pathogenic factors.
4. They are easily identifiable.

All forms belong to a group of bacteria that exhibit all the above characteristics and are used as indicators of microbial contamination in water. Typically, coliform bacteria are not pathogenic themselves but are often found alongside infectious microbes in water. Moreover, the number of coliform bacteria is much higher than pathogenic microbes, and they often survive longer in water. Coliform bacteria are easily identified in laboratory tests.

According to a rule, if coliform bacteria are present in water, it is assumed that pathogenic microbes may also be present, indicating the water is microbiologically unsafe. Conversely, if coliform bacteria are absent, the opposite of the above rule holds true, meaning pathogenic microbes are not present in the water.

Many waterborne diseases are well-known for causing gastrointestinal illnesses because they result from ingesting water or consuming food contaminated with human feces. This contamination can be prevented through proper hygiene practices. These diseases are prevalent in developing countries where people lack the economic means for prevention or have insufficient knowledge about disease transmission methods. Many biological agents are responsible for gastroenteritis, a term used for diseases

caused by water or food where the causative agent is unidentified. Gastroenteritis leads to stomach and intestinal inflammation, followed by diarrhea and severe discomfort. Pathogens associated with water and food cover a wide range of microorganisms from viral particles to multicellular organisms. Since all microorganisms are particulate, they can be removed from water through processes like sedimentation or filtration. Some pathogens, particularly bacteria, can be eliminated using disinfectants such as chlorine or ozone. Only a small number of viruses capable of causing infections in humans are found in water. Poliovirus was previously considered a significant waterborne disease agent due to its prolonged lifespan in water, but its primary mode of transmission is undoubtedly direct human contact.

Currently, a concerning waterborne virus causing infectious hepatitis is the Hepatitis A virus. The most significant issue arises from consuming hard-shelled seafood like shellfish, which can concentrate viral particles from fecally contaminated water through filtration.

An important epidemic of infectious hepatitis that infected nearly 30,000 people occurred in Delhi, India, in 1955, with the cause being the entry of sewage discharged into the river into the inlet of the water treatment plant. Apparently, the disinfection process used was not sufficient to eliminate the viruses, resulting in a widespread epidemic. Viruses like Cox sackie, Norwalk, Rotavirus, and Echo are agents of gastroenteritis and diarrhea. Contaminated food, shellfish, or water can transmit these viruses. Waterborne bacterial pathogens fall into the group of purple bacteria, which belong to the gamma subdivision of enteric organisms. This group consists of relatively homogeneous Gram-negative bacteria that lack the ability to produce spores, are aerobic, have rod shapes, are oxidase-negative fermenters of glucose, and produce a variety of end products. Another new disease caused by protozoa is Cryptosporidium, which has been the cause of the largest waterborne epidemic to date.

Cryptosporidium is a common intestinal pathogen in dairy cattle, and its sudden presence in water sources is partly due to spring rains and runoff from agricultural lands, increasing the organism's load in water treatment systems. Meanwhile, coagulation-filtration processes were not optimizing effectively, and the removal of turbidity was not feasible. The cysts of this organism are resistant to chlorination, requiring removal through coagulation and filtration methods. As a result, some water treatment processes are undergoing changes to enhance the removal of turbidity through filtration.

Table 1. Some pathogens that are transmitted by water

Disease	Cause of Disease	Source Organism	Symptoms of the Disease
Gastroenteritis	Various pathogens	Animal or human feces	Acute diarrhea and vomiting
typhoid	Salmonella typhoza	Human feces	Intestinal swelling, enlarged spleen, high body temperature, fatal
Microbial diarrhea	Shigella	Human feces	Diarrhea, rarely fatal

cholera	Vibriocoma	Human feces	Vomiting, severe diarrhea, rapid loss of body water and minerals, high mortality
Infectious hepatitis	Virus	Human feces	Yellow skin, enlarged liver, abdominal pain, low mortality, the disease lasts about 4 months
Amoebic diarrhea	Antambahistolitica	Human feces	Mild and chronic diarrhea
Giardiasis	Giardia lamblia	Feces of domestic and wild animals	Diarrhea, dysentery, nausea and general weakness - the disease lasts from 1 to 30 weeks, it is not fatal.
Balantidiasis	Ballantidium coli	Human feces	Bloody diarrhea, similar to diarrhea caused by Antambast

Biochemical oxygen demand (BOD)

The proliferation and abundant reproduction of microbial communities lead to the consumption of dissolved oxygen in water. If bacterial activity increases excessively, the reduction of available oxygen in the water can reach a level where it may result in the death of fish.

An aquatic environment that lacks sufficient oxygen is considered contaminated for organisms that require more oxygen than what is available. The amount of oxygen required for this biochemical breakdown is referred to as Biochemical Oxygen Demand (BOD). BOD, which is a common measurable parameter in water quality management, is measured in milligrams per liter of oxygen consumed over a day at a temperature of 20 degrees Celsius. Approximately 33% of the BOD in aquatic environments, especially in rivers, is related to agricultural activities. When BOD levels are high, dissolved oxygen decreases to a point where it does not support aquatic life (Bouwer & Zehnder, 1993).

According to the definition of the Environmental Quality Council, the pollution alert threshold for water is where dissolved oxygen is less than 5 milligrams per liter. If the amount of organic matter increases and its breakdown occurs rapidly, almost all dissolved oxygen in the water is consumed. In this case, anaerobic fermentations and reductive reactions occur, leading to the production of sulfides, H₂S, CH₄, and NH₃. These reactions mostly occur in flowing waters.

The continuous addition of mineral nutrients derived from organic matter can ultimately lead to the phenomenon of eutrophication in lakes or aquatic environments. In this scenario, the biomass of phytoplankton in lakes or ponds increases, leading to a decrease in water transparency. As a result, plankton accumulates more in the surface layers of the water.

The accumulation of plankton at the water surface increases the amount of organic matter, which in turn leads to an increase in heterotrophic organisms. Consequently, the dissolved oxygen in the water decreases. Thus, following the initial pollution caused by the increase in organic matter input, secondary pollution stemming from internal sources occurs.

Air pollution

Air is essential for life and is needed more than water and food. An average adult breathes approximately 15 kilograms of air in a day, while their

consumption of food and water is about 1.5 and 2.5 kilograms, respectively. The importance of air lies in the fact that a person can survive without food for about 5 weeks and without water for approximately 5 days, but without air, they will not be able to continue living after just a few minutes.

Air is a mixture of various gases, with the majority composed of nitrogen and oxygen. Healthy and natural air typically consists of about 78% nitrogen, 21% oxygen, 0.93% argon, 0.03% carbon dioxide, and very small amounts of gases like neon, helium, krypton, xenon, radon, ozone, hydrogen, and others. Oxygen, in particular, is the most crucial element for the continuation of human life.

The air that is so crucial for human life and whose impact on human health is undeniable, unfortunately, is constantly exposed to pollution due to natural factors, especially human activities. Considering the significance of clean air and the dangers of its contamination, in this section, we will briefly examine the sources and factors of air pollution, the effects of air pollution on humans, plants, animals, and various materials, and methods to combat air pollution.

Air pollution is the presence of one or more pollutants in the ambient air to an extent and duration that alters its quality in a way that is harmful to human health, animals, plants, or structures. The major sources of air pollution include natural and anthropogenic sources. Natural sources often exist without direct human intervention, while anthropogenic sources are human-made and the pollution they cause results from human activities.

Plant and animal sources such as pollen, biological particles, and gases resulting from the decay and fermentation of substances, if released into the surrounding environment, can be effective in polluting the air.

Microorganisms present in the air are divided into two categories:

1. Bacteria: The most important bacteria present in the air are Gram-negative cocci, filamentous bacteria such as spore-forming micrococci, corynebacteria, streptomycetes, and actinomycetes.

2. Fungi: Aspergillus, Penicillium, Fusarium, Cladosporium, yeast fungi

To identify the bacteria, present in the air, the following test is conducted:

First, create a Nutrient agar medium in a sterile Petri dish and expose it to the air without a cover for 48 hours. If Gram-negative cocci or filamentous bacteria like spore-forming bacteria are present in the environment, white colonies will form.

If Micrococcus or Corynebacterium is present in the environment, colored colonies are produced. However, if Streptomyces or Actinomycetes are present, convex and thick colonies are formed. It should be noted that bacteria with pigments in the surrounding air live better and more abundantly because their pigments protect them from the harmful effects of light and UV radiation. Additionally, Gram-positive bacteria, due to their thick and simple cell walls, tend to survive better in the air.

Bacteria in the air do not have any active mechanisms for dispersal in the air and become active in the environment through the following factors. Dust and Particles: Dust and particles facilitate the transport and dispersal of microorganisms in the environment. Fine Water Particles in the Environment cause their dispersion. Dead skin cells shed by humans and animals entering the environment contribute to their dispersal. The most important fungi present in the air are Aspergillus and Penicillium. Similar to bacteria, fungi do not have an active mechanism for dispersal in the environment. Fungi have tiny

and dry spores that are released into the air. These spores are lightweight and are unaffected by temperature and humidity conditions (Zuhara & Isaifant, 2018)

Cladosporium fungi demonstrate their response through two mechanisms: swelling and dormancy, dispersing their spores in the air. Yeast fungi are a group of true yeasts that release their spores in high relative humidity during the night air. Therefore, the majority of fungi present in the night air belong to this type. The highest concentration of fungi in the air during the day is Cladosporium. They are called yeast fungi because when they grow in malt extract agar medium, they give colonies the same appearance regardless of the type of medium used for cultivation.

An important point to note is that the rate of microbial growth is always higher during the night compared to during the day. The reason for this phenomenon is not clear, but it is possible that daytime light has a detrimental effect on the growth of biological organisms.

Soil pollution

From a global perspective, after air and water, soil is considered the third major component of the environment. As long as humans were hunting for a living, there was no need to think about nature or the value of the soil beneath their feet. However, when they shifted from hunting to cultivation and the nature of the soil had a direct impact on their livelihood, they were forced to consider the soil as well.

Over time, as the general perception of the value of soil and the hidden world within it increased, soil acquired a deeper meaning, becoming essential for the survival of millions of hungry people. Although humanity now fully believes that soil is life-giving and the savior of millions of hungry people, excessive exploitation of it, in the name of civilization, has not only polluted the air and water but also led to the contamination of soils (Espinoza & Dendooven, 2003).

Soil pollution is usually the result of unhygienic practices, various agricultural activities, and improper methods of disposing of solid and liquid waste. Additionally, the deposition of air pollutants due to atmospheric precipitation can also contribute significantly to soil pollution. Soil can become contaminated by chemical substances, including heavy metals and petroleum industry by-products, due to severe carelessness. Through this contamination, soil pollutants can enter the food chain, surface waters, or groundwater and eventually enter the human body. In many countries worldwide, especially in industrialized nations, soil pollution by pathogenic microorganisms still holds special significance.

Despite the advancements that have taken place in various fields by humans in recent centuries, large masses of people can still be found near big and modern cities whose quality of life, level of education and awareness, and ultimately their economic and social status have undergone very slight changes. Particularly, their excrement, urine, and other secretions are still in a condition where the cycle of infectious diseases through soil transmission remains preserved. In many parts of the world, due to shortages in the distribution of chemical fertilizers, human excrement is still used as a valuable fertilizer.

In regions where water scarcity is noticeable, the reclamation and reuse of sewage are often used as an additional water source for irrigation purposes. If necessary, precautions are not taken in this regard, this practice can be hazardous to health and hygiene. In some areas where there is not strict monitoring of sewage disposal and raw sewage is still transported, it leads to numerous health problems. For example, one of the causes of the spread of diseases in the Middle East is the consumption of agricultural products and vegetables irrigated with raw sewage. Although in suspicious cases, vegetables are

consumed cooked, one can imagine that surfaces of utensils and kitchens may become contaminated during the preparation of these vegetables, leading to the contamination of other raw food items that are consumed. Therefore, among soil pollutants, what is most concerning is biological contamination, which we will briefly discuss in this chapter.

Soil contamination by pathogenic biological agents

Biological factors that lead to soil contamination and result in human diseases can be divided into the following 3 groups:

1. Pathogenic organisms excreted by humans, which are transmitted to humans through direct contact with contaminated soil or the consumption of fruits and vegetables grown in contaminated soil (human-soil-human transmission). This category includes pathogenic intestinal bacteria, some protozoa, and certain worms such as *Ascaris* and hookworms. The dissemination of these factors occurs due to the improper disposal of human feces or their use as fertilizer, as well as irrigation of fields with sewage.

As a result, the soil and food products obtained from the fields are contaminated with pathogens such as cholera, typhoid, such as typhoid, and bacillary diarrhea and make humans sick. The transmission to humans occurs through contaminated water, direct person-to-person contact, contaminated food, contact of flies with human feces, and food.

2. Pathogenic organisms from animals that are transmitted to humans through direct contact of individuals with animal excreta-contaminated soil (animal-soil-human transmission). Some common diseases shared between humans and animals, such as leptospirosis, anthrax, Q fever, and migratory worm larvae, for which soil plays a fundamental role in their transmission, fall into this category.

The distribution of leptospirosis is related to specific environmental hygiene conditions, especially involving the animal vector, water, soil, and human contact. Vector animals sometimes excrete the pathogenic agent abundantly through urine, which can survive for weeks in environments such as water or moist soil with a neutral or slightly alkaline pH (Cerqueira et al. 2014).

Infection is more common among individuals who come into contact with animal feces. Children playing on seashores, grassy areas, and play areas where worm larvae are present are at risk. In some cases, soil disinfection is recommended.

3. Pathogenic organisms naturally found in soil can infect humans through direct contact with such contaminated soils (soil-human transmission). The most important diseases in this category include fungi, tetanus, and botulism.

Most fungi that naturally grow as saprophytes in soil or on decaying matter can become pathogenic under specific conditions and attack specific tissues.

Tetanus is an acute disease that can occur due to the toxin produced by the anaerobic growth of the bacterium *Clostridium tetani* at the site of injuries. The causative agent of tetanus (*Clostridium tetani*) is excreted through the feces of infected animals, especially horses, and the primary sources of contamination may be soil, dust, human or animal feces, which can lead to disease transmission following bodily injury and contact with these sources. Botulism is often a deadly form of poisoning caused by the toxin produced by the bacterium *Clostridium botulinum*. The reservoir of the disease agent is soil and the intestines of animals. The toxin is produced as a result of anaerobic spore growth in a food environment, which is considered the main source of poisoning. The disease

usually occurs due to the consumption of canned foods that have not been completely sterilized during preparation or canned foods that have previously come into contact with soil contaminated with *Clostridium botulinum*. It is important to note that ensuring the absence of contamination in organic matter in the soil before its application will significantly contribute to increasing soil fertility and productivity.

Bioremediation

All organisms grow by utilizing stored energy in organic and inorganic molecules or available radiant energy such as sunlight. To obtain energy, organisms convert chemical substances through oxidation and reduction reactions. Some of this energy is used for organism reproduction. In environmental biotechnology, oxidation/reduction reactions and the production of new biomass by microorganisms remove pollutants (Van Gestel et al, 2003).

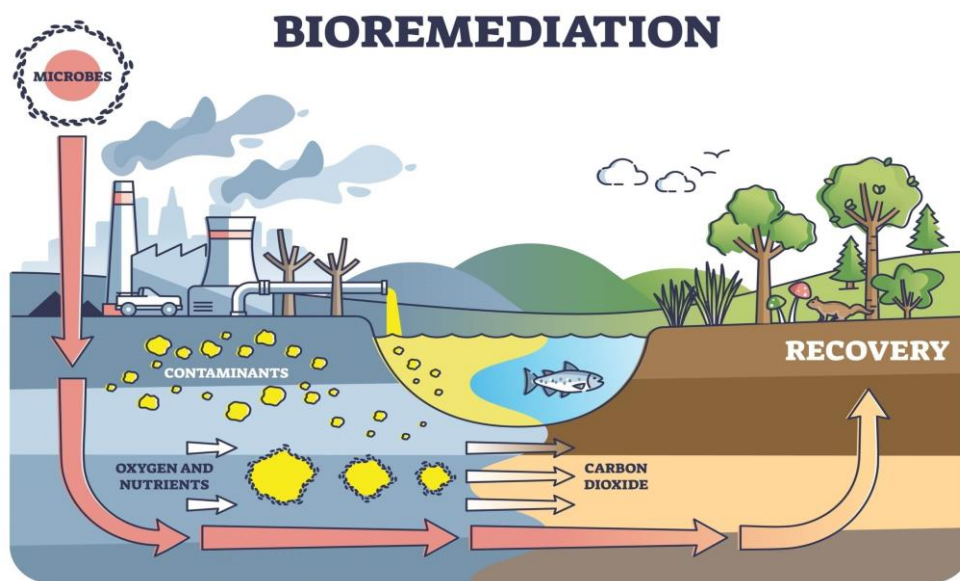


Figure 1. Bioremediation cycle in the ecosystem (Epcmholdings, 2024)

Examples of environmental benefits that can be achieved through microbial systems include:

- Sewage treatment using activated sludge
- Conversion of organic waste into methane as a useful fuel
- Conversion of biodegradable organic matter into harmless inorganic compounds
- Decomposition of toxic pollutants in soil and water

Understanding the energy-producing and consuming reactions of microorganisms can create conditions that result in environmental protection and improvement.

Biogas

The energy crisis and its shortage due to limited oil reserves have prompted scientists to recently compensate for this shortage by utilizing energy sources derived from sunlight, wind, water, atoms, and also biological energies. One of the most successful studies to obtain new and natural energy is the application of animal waste and organic material residues, which has now resulted in the presentation of biogas as very simple and cost-effective methods.

In many developing countries and even in many parts of Iran, the issue of fuel scarcity has always been present. For example, rural populations have dealt with very simple and basic issues to sustain their livelihoods, and the dispersion of population centers and the lack of reliable communication pathways have

exacerbated their problems, including energy shortages. Additionally, while electricity shortages still persist for industrial centers in the country, in many Iranian villages, heating, cooking, and lighting are often provided through the burning of oil and gas, and many also fulfill their energy needs by burning animal waste or firewood (Atlas & Bartha, 1973).

Although all organic biological materials have the capability to ferment and produce biogas, animal and human waste are more suitable for this process. Therefore, in areas where animal husbandry is possible, such as villages, farms, and poultry farms, some of the required energy can be obtained by fermenting animal and human waste.

The fermentation process takes place in tanks called digesters, and on average, about 150 liters of biogas is produced per cubic meter of useful volume in a digester. The retention time of biological materials in the digester, or in other words, the time required for the reaction to complete, varies from 30 to 90 days depending on the ambient temperature.

In addition to creating energy, biogas has other important advantages, the most important of which are:

1. Preserving forests is important because the substitution of biogas for firewood eliminates the need to cut down trees for firewood supply. The biogas produced provides more energy compared to directly burning these materials.
2. Burning gas is significantly simpler than burning firewood; additionally, it does not produce smoke, which means air pollution is not a concern. Moreover, there is no need to collect or cut firewood, thus requiring less human labor for energy production.
3. The fermented materials from the fermentation tank are rich in nitrogen and phosphorus, and not only do they retain the fertilizing properties of animal waste, but they also become denser and richer. The raw fermented materials create an unfavorable environment for the growth of mosquitoes, flies, and various parasites.
4. Due to the non-burning of plant waste such as tree branches, straw, and rice husks, livestock farming will also benefit, as these materials can be utilized as fodder and animal feed.
5. Weed seeds present in animal manure are destroyed during fermentation, so using fermented materials as fertilizer will not lead to the growth of unwanted plants.
6. The production and consumption of biogas will result in significant savings in the use of other forms of energy, such as oil and electricity, which in turn will enable energy supply to more areas.

Biological treatment of sewage using Activated Sludge method

Biological treatment of sewage using activated sludge was first developed in 1913 in Manchester by Anderson and Lockett, and soon this method was adopted in other countries. Today, activated sludge treatment is considered the most important and fundamental unit in sewage treatment plants. In this method, sewage is introduced into a basin where the retention time is approximately 6 to 8 hours. In this basin, a large number of active microorganisms in the form of activated sludge is added, and the required oxygen is continuously supplied through aeration. In this basin, both soluble and insoluble organic materials are decomposed, converting them into carbon dioxide, water, and occasionally ammonia. If the basin is well-designed, about 90 to 95 percent of the organic materials will be removed from the sewage.

The following factors can be mentioned to ensure biological treatment using activated sludge:



1. The activated sludge rich in microorganisms must be sufficiently present.
2. A sufficient surface area of sewage should be in contact with air.
3. Adequate mixing and agitation occur during aeration and increase of activated sludge.
4. The aeration time of the mixture of sewage and activated sludge must be sufficient.

The mixture of sewage and activated sludge is then directed to a secondary settling basin. In this basin, the microorganisms and digested organic materials settle out, with a portion of the resulting sludge being returned to the activated sludge basin to maintain the active sludge, while the excess is disposed of. The remaining effluent in the upper part of the settling basin is also directed to the disinfection basin for final disposal. Below are some of the advantages and disadvantages of this method (Makadia et al, 2011).

Some disadvantages of biological treatment using activated sludge compared to similar methods include the following:

1. Activated sludge treatment requires constant maintenance and, consequently, the employment of skilled and knowledgeable personnel. Therefore, in cities where it is difficult to find qualified individuals, the use of this method is not advisable.
2. The flexibility of the treatment plant is lower, meaning that changes in the quantity and quality of incoming wastewater also affect the degree of treatment.
3. The storage tanks required for this method are extensive.
4. Dewatering the sludge produced by this method is generally more challenging.

The role of microorganisms in removing oil pollution from water

The battle against oil pollution has been a significant part of scientific research since the emergence of this dark yet valuable substance. While it received much less attention in the past, it is increasingly capturing the interest of specialists and experts today. Among the proposed solutions that yield better and faster results, the use of microorganisms is a method widely adopted in most developed countries, known as bioremediation or biodegradation.

Crude oil, with over 340 products, is one of the main sources of energy and a driving force in the global economy, with Iran holding 9 percent of the world's total oil reserves. Crude oil is a complex mixture of hundreds of different compounds, including hydrocarbons, nitrogen, sulfur, and vanadium, with the hydrocarbon portion comprising aromatic, aliphatic, and asphaltene compounds. Oil pollution is almost an unavoidable consequence of rapid population growth and energy consumption based on oil technology. In recent years, experts' attention has primarily focused on oil pollution in the oceans caused by tanker accidents, the largest of which occurred in 1967 in the waters off England (Moslemi et al,2005).

In 1975, approximately one thousand gallons of crude oil leaked from a storage tank in North Carolina into the sea. Additionally, in 1991, over 5 tons of crude oil were spilled into the waters of the Persian Gulf, leading to the destruction of coastal plants and ecosystems. Due to the passage of oil tankers, the drilling of multiple wells, and oil extraction, the Persian Gulf contains about 160,000 tons of oil and petroleum materials annually, making it one of the most polluted seas in the world. Generally, the events mentioned have led to increased attention toward developing various methods for removing oil pollution from the environment, the most important of which include:

1. Manual collection of oil pollution from the water surface.
2. Containment of oil pollution using physical barriers.

3. Utilization of materials such as feathers and straw that absorb oil particles.
4. Burning.
5. Use of biphasic solvents.
6. Bioremediation or biodegradation.

Cleanup or biodegradation

Twenty-five percent of the oil released into water is eliminated through evaporation, while the rest is broken down by photooxidation and microorganisms, a process known as bioremediation. The presence of hydrocarbon-degrading microorganisms in seawater and soils has made biodegradation one of the most effective methods for removing oil pollution. Bioremediation accelerates the use of biological systems to destroy or reduce the concentration of toxic substances, such as petroleum hydrocarbons. This process is enhanced in the presence of oxygen and nutrients, particularly nitrogen and phosphorus. The products of biodegradation are typically CO₂ and small organic compounds with very low toxicity.

Sulfur combined with oil produces SO₂ when burned. Generally, the heavier the fossil fuels, the more SO₂ is produced from their combustion. For example, burning one ton of coal with a certain percentage of sulfur generates 38 kilograms of SO₂. This gas, after being released into the atmosphere, forms compounds that return to the ground as acid rain. Such rains create numerous problems for agricultural lands and forest and aquatic ecosystems. Given the severe pollution that sulfur-containing compounds cause for the environment, environmental organizations impose special restrictions on oil refining industries (Atlas & Bartha, 1993).

Desulfurization operations and biological methods

Historically, desulfurization operations were carried out using chemical methods. Chemical desulfurization imposes significant costs on the oil industry and, on the other hand, these methods cannot remove all the sulfur present in oil. For this reason, scientists in the fields of oil and environmental science have long been considering ways to enhance this process. The idea of separating sulfur using biological methods emerged nearly fifty years ago, particularly with the discovery of microorganisms capable of extracting sulfur from petroleum compounds. Research in this area has been extensive (Das & Murkherjee, 2007).

It should be noted that currently, the exclusive use of biological methods for desulfurization is not cost-effective. Since a significant percentage of sulfur can be removed using more economical chemical methods, it is wiser to utilize biological methods as a complement to chemical ones. When these two methods are used in conjunction, operational costs can be significantly reduced.

Removal of heavy metals using microorganisms

Heavy metals are classified into three categories based on bioaccumulation: toxic metals (such as Hg, Pb, Zn, Cr, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, etc.), and radionuclides (such as U, Ra, Am, etc.), which typically have a specific gravity greater than 5 grams per cubic centimeter. Lead is a soft, heavy metal with a bluish-gray color. It is introduced into the environment by various industries, including ceramics, glassmaking, textiles, battery production, and the manufacture of lead bullets for printing types. Lead enters the human body through air, water, and food. The effects and symptoms of lead poisoning appear to be related to its ability to bind to the sulfhydryl groups of various enzymes necessary for heme production, thereby inhibiting this process. Additionally, lead inactivates pyrimidine 5-nucleotidase (which is involved in RNA breakdown), resulting in the accumulation of ribosomal RNA. Lead also prevents the production of 1 and 25-dihydroxyvitamin D.

The first organs affected by lead exposure include the brain, peripheral nervous system, bone marrow, kidneys, and liver. Due to increasing consumption and high intrinsic toxicity, heavy metal pollution has become one of the most significant environmental challenges today. Conventional methods for removing metal ions from liquid solutions have been extensively studied, including chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, and adsorption on activated carbon, among others (Schaefer & Juliane, 2007).

The adsorption of heavy metals by microbial cells results from biosorption and bioaccumulation mechanisms. The term biosorption refers to the ability of living, inactive, and dead biomass to bind to heavy metals or pollutants present in dilute solutions. This property is primarily attributed to the cell wall. The term bioaccumulation pertains to the metabolic uptake of substances by actively living cells. It has been reported that the bioaccumulation of heavy metals in yeast cells occurs in two stages. The first stage is rapid and independent of metabolism, requiring binding to the cell wall surface. In most cases, the metal-binding capacity of *Saccharomyces cerevisiae* and fungi is attributed to the electric charge resulting from the weak dissociation of acidic carboxyl groups and other functional groups, such as amino groups, that constitute the cell wall. The second stage is slower and metabolism-dependent, involving the accumulation of larger amounts of metal cations compared to biosorption by inactive biomass. However, heavy metals are toxic to microorganisms. Their high affinity for forming complexes with membrane components can damage membrane integrity and reduce functionality. Yeasts are preferred over other microorganisms for the removal of heavy metals due to their ability to survive and grow in metal-contaminated environments, their high metal-binding capacity to the cell wall, and their significant intracellular absorption rates.

Biological treatment of wastewater containing toxic metal zinc (Zn^{2+}) by seaweed

Metals such as nickel, cadmium, lead, copper, and zinc contaminate the environment through industrial activities. Therefore, wastewater pollution by toxic metal ions is not merely a problem; the accumulation of these toxic heavy metals in fertile agricultural soils renders them unusable, primarily due to the bioaccumulation of these metals by plants and their subsequent entry into the food chain. The most common physical and chemical methods for separating these metals include sedimentation, chemical oxidation and reduction, ion exchange, reverse osmosis, membrane separators, electrochemical reactions, and evaporation. Human concerns increase when existing treatment methods for such wastewater fail to comply with environmental standards, as many of these methods are ineffective or uneconomical when heavy metal concentrations in contaminated environments range from 10 to 100 ppm, while permissible concentrations are below 1 ppm. In recent decades, extensive research has been conducted on the binding of metals by bacteria, yeasts, fungi, and algae. As a result, biosorption, particularly the uptake of toxic metals by algae, can be an effective alternative for large volumes with low contamination levels of toxic metals. Alginate plays a key role in the absorption of heavy and toxic metals, constituting about 10% to 40% of the dry weight of the algal cell wall. The biosorption mechanism by living and dead biomass, bacteria, polysaccharides, and various biological products has been studied. The aim of this research is to investigate the biological treatment efficiency of wastewater containing zinc using the brown algae *Fucus* and the impact of physical factors on zinc absorption (Broda. P, 1992).

Conclusion

In the future, countries will face crises related to water, soil, and air pollution due to the increase in urban populations and the advancement of

industry and agriculture. The use of modern methods for environmental purification has become a focus due to limited resources. Fecal pollution and the introduction of oil spills into water, the presence of pesticides and herbicides in soil, and the creation of biological contamination in its surface layers, as well as air pollution caused by fungi, bacteria, and others, have increased significantly today.

In the past, physical methods such as ion exchange, electrodialysis, and reverse osmosis, as well as chemical methods like chemical reduction, electrolysis, and ion exchange, were commonly used. However, due to their relatively low efficiency, high costs, and other associated issues, alternative methods have now replaced these approaches. Biological methods have emerged as a modern solution for environmental pollution remediation. This method involves the use of fungi, algae, bacteria, yeast, and plant cells to remove pollution. Considering that Iran has been making significant strides in biotechnology in recent years, it seems rational to utilize biotechnology as a supporting tool in large-scale environmental programs in the country.

Now, it is crucial for universities to seriously engage in biotechnology research alongside new developments in engineering and sciences. This will allow us to pass on the frontiers of contemporary knowledge to future generations and contribute to the emerging structures of existence. We must awaken promptly, appreciate the profound miracles of human thought, and move away from short-sightedness and outdated ideas.

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