SUSTAINABLE MATERIALS | VOLUME 2

# BIOREMEDIATION FOR ENVIRONMENTAL POLLUTANTS


Editor:
**Inamuddin**



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# Sustainable Materials

## *Volume 2*

# *Bioremediation for Environmental Pollutants*

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# PREFACE

Modern civilization is experiencing an environmental catastrophe as a result of prevailing pollution issues worldwide. Scientists, researchers, environmentalists, engineers, and planners, as well as the developing nations, all need to address this problem to some extent through the introduction of microbes in order to recycle waste into useful forms that other well-beings can utilize efficiently. Varied microbial traits offer a strong substitute to get around serious issues as they can withstand practically any environmental situation because of their incredible metabolic activity. Hence, the extensive nutritional capacities of microbes can be exploited in the bioremediation of environmental contaminants. Moreover, these microorganisms can be used in bioremediation to eliminate, degrade, detoxify, and immobilize a variety of physical and chemical pollutants. Enzymes are exploited to destroy and transform pollutants like heavy metals, hydrocarbons, pesticides, oil and dyes.

Volume 2 of this book series discusses these methods, and efforts are made to eventually manipulate the remediation processes in a way that will make bioremediation economically and scientifically feasible in order to provide pertinent background information that fills in any gaps in this field of study, as microorganisms promise valuable traits to address environmental challenges and procure eco-safety. This book is focused on elaborating the application of microbial enzymes, microalgae and genetically engineered microorganisms in the bioremediation of significant pollutants, food wastes, distillery wastewater and pharmaceutical wastes. Inamuddin Department of Applied Chemistry Zakir Husain College of Engineering and Technology Faculty of Engineering and Technology Aligarh Muslim University Aligarh-202002 India.

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CHAPTER 1

# Microbial Enzymes in the Bioremediation of Pollutants

**Flávia F. Magalhães**[1], **Maria I. Bonifácio**[1], **Ana M. Ferreira**[1], **Mara G. Freire**[1] and **Ana P. M. Tavares**[1,*]

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**Abstract:** Environmental pollution is rising and becoming a major global concern for human health, public safety, and flora and fauna life. Physicochemical-based processes, traditionally applied to treat polluted environments, are usually of high cost, and low efficiency, and normally produce extra residues and/or pollutants. On the other hand, biological remediation technologies may be more environmentally friendly and may lead to a higher remediation performance. Therefore, these bio-based approaches have received significant attention in recent years. Bioremediation applies biological sources to remove or reduce pollutants from a contaminated environment. In particular, enzymatic bioremediation based on microbial enzymes is a relatively new field. It has recently received high attention over traditional methods due to its high specificity and ease of handling in the bioremediation of diverse types of pollutants. Numerous microbial enzymes with bioremediation capability have been selected and characterized in recent years. This book chapter describes the recent advances in microbial enzyme technology, namely achieved by oxidoreductases and hydrolases, in the biodegradation of environmental pollutants. Their principles, mechanisms of action, advantages, and limitations are presented and discussed. In addition, the main conditions and factors that affect the bioremediation performance, such as type of enzymes, pH, pollutants, temperature, and presence of redox mediators are also discussed. This book chapter is useful for students, researchers, and professionals in the fields of environmental sciences and biotechnology.

**Keywords:** Biocatalysis, Bioremediation, Environmental pollutants, Hydrolases, Microbial enzymes, Oxidoreductases.

## INTRODUCTION

Environmental pollution is a serious global concern; it has increased over the last decades due to the fast industrialization of different sectors, urbanization, and

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inadequate agricultural practices [1]. A vast amount of organic and inorganicpollutants, such as pesticides, dyes, plastics, heavy metals, hydrocarbons, chlorinated compounds, greenhouse gases, and nitrogen-containing compounds persist in the environment above the permissible limits [2], entering the environment by different ways causing severe pollution in air, soil and water [3]. These pollutants are highly toxic and most of them are carcinogenic. Traditionally, pollutants are treated by both chemical and physical methods of remediation, usually based on incineration, adsorption, filtration, coagulation, chemical flocculation and oxidative processes [3]. However, standard technologies are frequently not efficient to particular classes of pollutants, may be of high complexity, may involve the use of high cost chemicals and materials and, in many cases, the degrading treatment methods result in the production of intermediates or by-products that can be more toxic than the original starting compound (secondary pollution) [4].

The described shortcomings in traditional technologies have addressed efforts towards the development of biological processes as appropriate alternatives, which may be more environmentally friendly and cost-effective approaches. By bioremediation, it is possible to reduce the amount of pollutants in the environment (by degradation, detoxification, mineralization or transformation) [5, 6]. The most studied type of bioremediation of pollutants is by the use of microorganisms, such as bacteria, yeast or fungi. However, its real applicability is reduced and limited since it is a slow process dependent on the manipulation of environmental parameters, such as microbial growth and metabolic pathways [7]. Currently, due to innovations in enzymatic biotechnology processes, bioremediation based on purified or partially purified enzymes is a promising approach. The main advantage is that bioremediation does not depend on the growth of a microorganism, it depends only on the catalytic capacity of the enzyme. Besides, toxic side substances, as generated by microorganisms, may be reduced by using enzymes. Furthermore, enzymatic bioremediation has become one of the fastest approaches for environmental decontamination. There are numerous classes of enzymes, such as oxidoreductases and hydrolases, already investigated in bioremediation processes [8 - 10].

This book chapter provides a comprehensive state-of-the-art overview of the use of enzymatic bioremediation approaches. Recent developments and applications of enzymes and their mechanisms of degradation used to improve bioremediation are described. Additionally, this book chapter also provides significant information about enzymatic biotransformation pathways used in the biodegradation of pollutants from the environment.

## ENZYMATIC BIOREMEDIATION TECHNOLOGY

The bioremediation concept started in 1930 when Tausz and Donath [11] showed the use of microorganisms to treat contaminated soil with derivatives of petroleum. Since then, bioremediation has been expanding and applied for other purposes as eco-friendly and sustainable emerging technologies for the decontamination of several inorganic and organic pollutants from the environment. Most of these pollutants are highly toxic and carcinogenic, and their accumulation leads to hazardous effects on humans, and flora and fauna life [12]. Bioremediation is a biologically-friendly strategy that comprises a large range of biological processes using plants, organisms, microorganisms and/or specific enzymes to reduce or remove pollutants from the environment (namely soils, sediments, and water) into non-hazardous or harmless substances [13]. Fig. (**1**) summarizes the example of enzymatic bioremediation and its advantages.

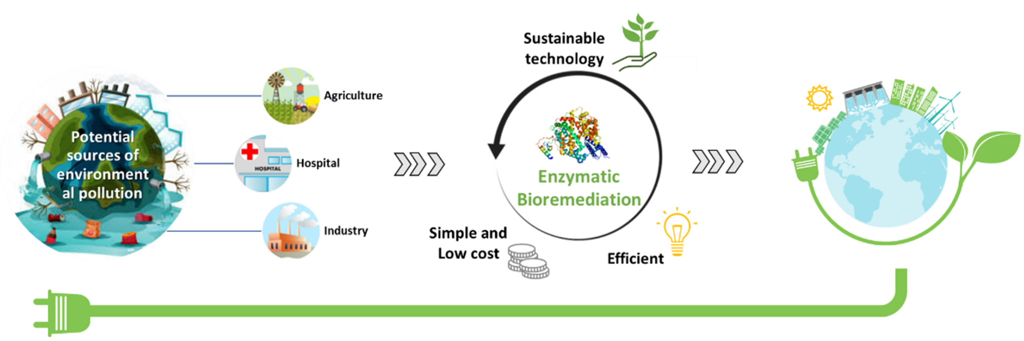


**Fig. (1).** General scheme for enzymatic bioremediation and its advantages and impact.

Bioremediation is usually considered less invasive when compared to conventional physicochemical and advanced oxidation techniques. Additionally, reduced costs of bioremediation lead it into a key position when compared to other techniques [14]. Bioremediation can be divided into two types: (i) *in-situ* bioremediation (carried out at the polluted place itself), involving the supplementation of the contaminated environment with nutrients to promote microorganisms growth and their ability to degrade pollutants; this strategy avoids damaging the place, permits the ecosystem return to its original condition and eliminates transportation costs [15]; and (ii) *ex-situ* bioremediation, involving the removal of the contaminated medium from its original environment to a different site for further pollutants treatment; *ex-situ* leads to more efficient elimination of pollutants since physicochemical parameters are better controlled [16]. Due to microbial engineering, novel strategies for enhanced bioremediation have been developed through the discovery of new metabolic pathways [17]. Despite the novel advantages of microbial remediation with modified microorganisms,

**CHAPTER 2**

# Microbial Indicators for Environmental Pollution

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**Abstract:** Environmental pollution has become a serious issue of concern across the world. Intensive agriculture, industrialization, and consumerism have resulted in the degradation of environmental quality. The presence of pollutants like fertilizers, pesticides, persistent organic pollutants (PoPs), polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals, synthetic dyes, *etc.* have not only polluted soil, air, and water but also invaded the food-chain *via* bioaccumulation and biomagnification, and have emerged as potential threats to various organisms including humans. Several organisms, including plants, animals, and microbes, indicate the presence of contaminants and environmental pollution. Among these, microbes have emerged as one of the potential indicators of environmental pollution, as they are more sensitive to trace levels of pollutants than plants/ animals/other organisms. The natural abundance of these indicator microbes has given us an opportunity to monitor environmental pollution before any major undesirable accidents occur. Based on these microbial indicators, various easy and rapid biosensors have been developed to monitor environmental pollution. Microbial indicators are the treasures of nature that have immense potential to monitor and predict environmental quality for society's safe and sustainable development.

**Keywords:** Antibiotics, Biosensors, Environmental pollution, Fertilizers, Heavy metals, Microbial indicators, PAHs, PCBs, Pesticides, PoPs, Synthetic dyes.

## INTRODUCTION

Human civilization has made its journey from a small cave in the nature's lap to large skyscrapers. There is hardly any place on the earth that is untouched by human civilisation. Intensive agriculture, industrialisation coupled with consumerism and globalisation factors have revolutionised the growth of human civilisation, but at the cost of the environment [1, 2]. Injudicious application of

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fertilizers and pesticides in agriculture has polluted various components of the environment [3, 4]. Nitrate pollution from fertilizers has created global issues like 'blue baby syndrome' in infants, gastric cancer, birth defects, heart diseases, *etc.* in ruminants, and eutrophication of aquatic systems [5]. Indiscriminate use of pesticides, especially organochlorine pesticides like DDT, HCH, aldrine, dieldrine, mirex *etc.* have contaminated soil, air and water, and they can persist for several years [6]. They are found to be a potent carcinogen and are related to various human disorders like immune suppression, hormonal imbalance, impaired intelligence, reproductive abnormalities in reproduction, *etc* [7]. The use of petroleum based products has fuelled the growth of human civilization, but at the same time, it has created the pollution issue of PAHs like fluoranthene, acenaphthene, pyrene, phenanthrene, anthracene and fluorene*etc* [8]. PHAs affect cell membrane activities by interfering with the associated enzymes. They are mutagenic, carcinogenic and suppress immunity [9, 10]. Another group of environmental pollutants is polychlorinated biphenyls [$C_{12}H_{(10-n)}Cl_n$] which have 209 individual isomers. The orientation of chlorine attached to the biphenyl ring determines the toxicity PCBs. In 2004, the Stockholm convention declared a list of 12 persistent organic pollutants (PoPs), also known as ‘dirty dozen’, which was constituted by 9 pesticides (Aldrin, dieldrin, chlordane, heptachlor, DDT, endrin, mirex, toxaphene and hexachlorobenzene), PCBs (industrial chemical), and 2 unintended by-products namely dibenzodioxins and dibenzofurans. Owing to various physic-chemical properties, these chemicals can persist for decades, travel long distances, accumulate in fatty tissues and are toxic to various organisms including humans. They have been identified as potent environmental pollutants and the list in increased since 2004. Heavy metals in environment have created a hue and cry situation across the world. Apart from geogenic sources, the other potent anthropogenic sources include metal based industrial waste and wastewaters, mines, fertilizers, domestic effluents, *etc.* They are potentially toxic and have undesirable effects on various organisms including humans. Heavy metals affect various components of cells including cell membrane, endoplasmic reticulum, mitochondrial, nuclei, and various enzymes controlling metabolic activities, detoxification, and repairing damages, *etc* [11]. Heavy metals have been reported to damage cell DNA resulting in modulation of the cell cycle, carcinogenesis or apoptosis [12]. Then comes synthetic dyes as a very important environmental pollutant. Nearly 10-20% dyes are disposed of through wastewater, and globally around 200,000 tonnes/year of synthetic dyes comes into environment [13]. Synthetic dyes are persistant, non-degrdadable and toxic to aquatic organisms, and found to disturb the food-chain [14]. Apart from these pollutants, antibiotics have emerged as a new environmental pollutant. The extensive utilization of antibiotics has resulted in the pollution of various environmental components with thousands of original antibiotic molecules or their derivatives/metabolites/transformation products [15]. All these environmental pollutants are harmful and not desirable in the environment. Table **1** represents a list of environmental pollutants covered under this chapter, along with their probable contamination sources.

**Table 1. Permissible limits of certain pollutants in drinking water.**

| Pollutant | Acceptable Limit [16] | Source of Pollution |
|---|---|---|
| **PoPs** | - | Anthropogenic sources (agricultural usage) |
| Aldrin/dieldrin | 0.03 µg/L | |
| Alpha-HCH | 0.01 µg/L | |
| Beta-HCH | 0.04 µg/L | |
| Gamma-HCH (lindane) | 1 µg/L | |
| Delta-HCH | 0.04 µg/L | |
| Total DDT (o, p and pp' isomers of DDT, DDE and DDD) | 1 µg/L | |
| **Polyaromatic hydrocarbons (PAHs)** | 0.1 µg/L | Anthropogenic sources (petroleum products/ resources) |
| **Polychlorinated biphenyls (PCBs)** | 0.5 µg/L | Anthropogenic sources (caulking, electronics, fluorescent light ballasts and other building materials petroleum products/ resources) |
| **Phenolic compounds** | 1 µg/L | Anthropogenic sources (industrial waste, agricultural waste, domestic waste, municipal waste, *etc.*) |
| **Heavy metal** | - | - |
| Lead (Pb) | 0.01 mg/L | Anthropogenic sources (industrial waste and wastewater, agricultural sources) coupled with geogenic sources |
| Cadmium (Cd) | 0.003 mg/L | |
| Total Chromium (Cr) | 0.05 mg/L | |
| Copper (Cu) | 0.05 mg/L | |
| Selenium (Se) | 0.01 mg/L | |
| Mercury (Hg) | 0.001 mg/L | |
| Zinc (Zn) | 5 mg/L | |
| Nickel (Ni) | 0.02 mg/L | |
| Silver (Ag) | 0.1 mg/L | |
| Total Arsenic (As) | 0.01 mg/L | |
| **Colour** | 5 units | Anthropogenic sources (industrial/ textile wastewater, municipal wastes) |

CHAPTER 3

# Bioaugmentation for Pollutant Removal

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**Abstract:** Environmental pollution management through conventional methods in the wake of new, recalcitrant, and increasing pollutant loads are no longer adequate or sustainable. Bioaugmentation for pollutant removal is an environmental (water and soil) decontamination approach alternative to the popular and traditional physico-chemical methods. Despite bioaugmentation's attractiveness based on being greener than the traditional methods, there are still several bottlenecks towards operating some bioaugmented processes optimally. Most bioaugmentation problems arise during upscaling successful lab-scale trials to industrial operations. In most cases, the bioaugmented micro-organisms survive for just a short span of time before their populations decrease prior to completion of the pollutant removal task. Research on various aspects meant to address this and other bioaugmentation challenges has been partly successful and such efforts are still ongoing. As part of evaluating and optimising bioaugmentation processes, sustainability concepts should always be considered at every stage of these activities. The application of bioaugmentation techniques is also gaining popularity in other industries, such as biogas production.

**Keywords:** Bioaugmentation, Biostimulation, Bioremediation, Degradation, Micro-organisms, Pollutants, Heavy metals, Hydrocarbons, Agrochemicals, Sustainability, Contamination.

## INTRODUCTION

Pollution on land and marine systems is a daunting societal challenge. Pollution is directly linked to harming all life forms with a cascade effect of biodiversity loss in all connected ecosystems and food chains. The change in the composition of polluted land and water tends to negatively impact agricultural productivity,

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part of above sentence of people and animals. Barren polluted soils are precursors of desertification and subsequent climate change effects [1]. Pollution is a consequence of various anthropogenic activities, some of which are necessary for economic and industrial development.

These activities include extraction and processing of crude oil, mining and extraction of minerals, and manufacturing of industrial and pharmaceutical chemicals, take place to cater to the needs of a growing population. When things go wrong, like accidental spillages and the generation of unwanted by-products during these processes, pollution arises. Pollutants are classified according to the pollutant priority listing standards as either organic or inorganic set out by the United States Environmental Protection Agency (EPA) [2]. Within inorganics, metals and nonmetals are sub-grouped. The broad class of organics contains the "dirty dozen", other organic pollutants and new priority emergent organic pollutants (xenobiotics). Some of these pollutants have been banned for human use, while others are produced intentionally but can be spilled accidentally, causing pollution. Additionally, the class of organic pollutants that are produced unintentionally as by-products of certain beneficial processes. Organic pollutants are predominantly products derived from fossilized fuels (crude oil, coal and natural gas). Another approach to the classification of pollutants is by their legal status [3]. Legally regulated pollutants are those that are documented in legislation, such as the EPAs toxic pollutants, the priority pollutant list and other lists in various jurisdictions. Non-regulated pollutants are those that are not listed for monitoring or control but are known to pollute ecosystems. For ease of understanding pollution challenges, Fig. (**1**) shows a general non-standardized classification of common soil and water pollutants. These pollutants are dominantly generated from anthropogenic activities such as farming (pesticides, herbicides and fertilizers), mining (heavy metals), petrochemical as well as other specific manufacturing industries.

Several strategies are employed as remedial actions for pollution management. Some of these strategies rely on mechanochemical destruction as well as removal by chemical sequestration, adsorption and photodegradation [4 - 7], while others are based on bioremediation and other phenomena. The effectiveness, cost and ease of implementation for each strategy normally determine which strategy is adopted in each specific case. Some pollutants are recalcitrant to conventional processes, while others can be transformed into by-products that are equally detrimental to the biosphere, especially during physicochemical pollution treatments. Technological developments and global pressure for environmental sustainability have recently promoted bias for the adoption of a greener approach to pollutant removal technologies, with bioremediation being one of the popular strategies [1].

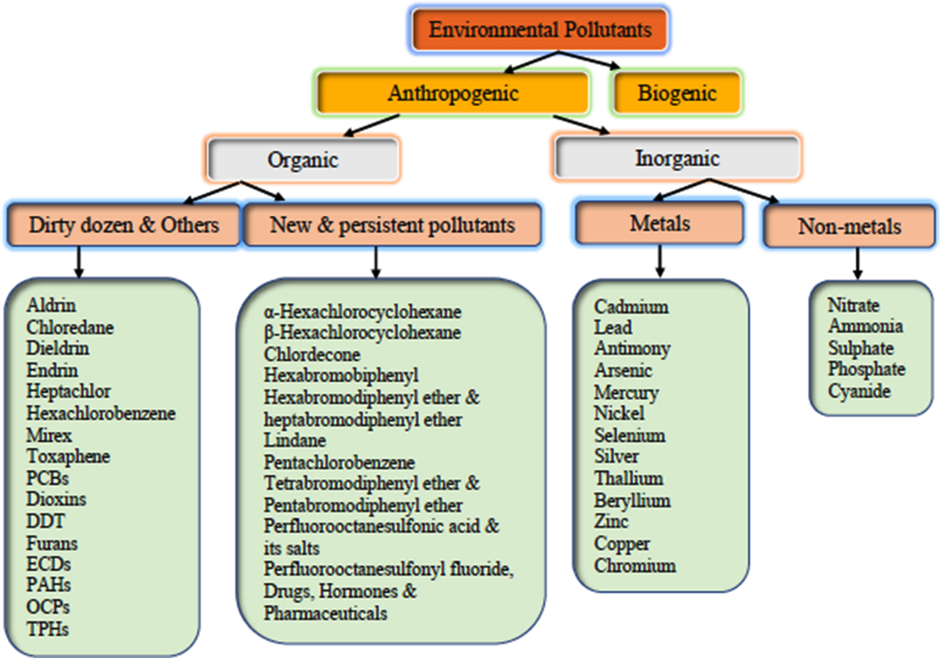


**Fig. (1).** Pollutants classified by their properties. *Adapted from* [2, 3].

Bioremediation employs micro-organisms for pollutant removal or toxicity reduction through biotransformation, bioaccumulation, bioleaching, biosorption, biodegradation and other mechanisms [8]. Generally, bioremediation processes consume less energy, are more cost-effective and less environmentally damaging than physicochemical pollutant removal methods [9]. Bioremediation is achieved through three sub-processes of bioattenuation, bioaugmentation and biostimulation [10]. When pollutant degradation proceeds naturally by micro-organisms indigenous to the polluted area without any human intervention, this is referred to as bioattenuation. On the other hand, biostimulation, involves human intervention to improve the polluted environment's conditions so as to promote rapid growth of indigenous pollutant degrading micro-organisms [11, 12]. Bioattenuation and biostimulation as bioremediation processes are generally slow or may be hampered by the non-availability or low populations of the specific pollutant degrading species in the polluted environment. Again, poor solubility of the pollutant in the polluted environment and poor bioavailability of the pollutant may impede these bioremediation strategies. Under these circumstances, bioaugmentation which involves inoculation of externally (adding micro-organisms to the polluted area) sourced micro-organisms into a polluted ecosystem for pollutant removal, will become expedient.

# CHAPTER 4

# Bioremediation by Microalgae: Current Progress and Future Perspectives

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**Abstract:** Algae can provide sustainable food and non-food energy biomass. Economically favorable strategies are in continuous development to produce renewable energy from this biomass. The integration of the bioremediation process using algae and the products that can be obtained from the biomass along with the favorable carbon life cycle have the capability of a self-sustained remediation process. In natural or industrial environments, microalgae utilize the pollutants present in the medium and this interaction affects the microalgal physiology and metabolism. The metabolic products such as dissolved organic or inorganic matter, extracellular polymeric substances and signaling molecules exude interactions that define the efficiency of detoxification of hazardous pollutants. In-depth analysis at a multi-omics platform can provide some valuable breakthroughs in the bioremediation process using algae. This chapter points out the present and evolving applications of microalgae-based bioremediation. The self-sustaining synergetic interactions not only detoxify the pollutants but also lead to the production biomass, high-value chemicals or biogas.

**Keywords:** Microalgae, Bioremediation, Sustainable, Contaminants, Omics.

## INTRODUCTION

The use of modern technology in chemical processes has increased environmental toxicity beyond its self-cleaning limits. These contaminants, primarily of anthropogenic origin, include various metals, nonmetals, organic, and inorganic compounds [1]. These pollutants are transferred *via* rivers into the marine ecosystem, particularly the coastal aquatic environment. Various natural processes, such as volatilization and condensation, leading to the accumulation of such compounds in the rain, fog, and snow, all the while impacting the general

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biogeochemical stability of the environment [2, 3]. Strong or indirect exposure to these contaminants causes harmful health conditions and negatively impacts all life forms [3]. Despite global efforts to reduce pollution, there are still major challenges in improving the air, water and soil quality and maintaining the environmental balance [4].

These contaminants must be eliminated from the environment, and current heavily contaminated areas must be of prime focus. The generation of methods for the elimination of environmental pollutants is of considerable importance [5]. However, due to the high costs of physicochemical strategies, biological methods can be a more applicable option, using the capability of biological agents such as bacteria, yeasts, fungi, microalgae and higher plants for the degradation of persistent pollutants [6]. This type of practice is termed as bioremediation. The rapid increase in global pollution rate as a result of industrialization and anthropological outcomes have resulted in research towards advanced bioremediation. The process of bioremediation decomposes or eradicates pollutants by exploiting biological catalysts residing at various levels of the network. Bioremediation process enriches the environmental quality at all levels, water, soil as well as air. Bioremediation aids the environment and benefits the world health and the economy as well. Another advantage of bioremediation is lower energy input as well as moderate operating processes, along with certain useful output products. A wide range of wastewater and solid pollutants are treated through bioremediation using diverse microbes and the process has been modified for the production of energy and other products such as biohydrogen, bioethanol, *etc*. This feature of bioremediation pushed the search for renewable energy as well [7 - 9]. The energy produced from waste is the result of the oxidation of organic pollutants; hence the efficiency of energy production is relative to the pollutant removal [10].

For the selection of a sustainable bioremediation technique, various criteria are considered, such as the contaminant's nature, extent and degree of contamination, environmental quality, location, cost and environmental policy [11, 12]. Apart from selection criteria, performance criteria (concentrations of oxygen and nutrients, temperature, pH and other abiotic factors), which decide the success of bioremediation processes, are also given major considerations prior to the bioremediation project [1]. Bioremediation is a flexible procedure that can be performed in-situ, *i.e.*, at the pollution site, or ex-situ, with the elimination of contaminated material on a processing site [13]. A number of bioremediation processes that involve microbes usually clean up the metal and organic pollutants [14 - 16]. Photosynthetic organisms such as microalgae can also help in cleaning off carbon dioxide ($CO_2$), a greenhouse gas (GHG) that is discharged in huge quantities due to fuel consumption in industries and transportation systems. This process of using algae as a means of bioremediation is known as phytoremediation. Utilizing sewage water and flue gases is a promising concept for sustainable development, where both remediations, as well as energy and product generation, can be unified.

## PHYCOREMEDIATION

Recently, bioremediation using microalgae for decontamination of crude oil, hydrocarbonates, organophosphate pesticides, and heavy metals, among other contaminants, has been explored as a new environmental biotechnological approach [17]. Indigenous microbes present at contaminated sites may help in solving many of the challenges connected with biodegradation and bioremediation of pollutants, given that conditions are suitable for their growth and metabolism [4]. Multiple studies have demonstrated that cultures of numerous algae from different pollution sites can be used to treat the commonly detected pollutant. Microalgae are photosynthetic species that can consume and use solar energy to grow biomass composed of lipids, proteins, carbohydrates, hydrocarbons and other high value compounds such as pigments and omegas. Microalgae can consume nitrogen, phosphate, and certain other toxic chemicals, as well as carbon dioxide, hence they are an attractive choice for pollution reductions and $CO_2$ sequestration [18, 19]. The biomass produced may contain more than 50% of dry weight as triacylglycerols (TAGs) that can be converted to liquid fuel such as biodiesel, jet fuel *etc*., through esterification [20, 21]. Using other processes, the microalgal biomass can also be used to produce biohydrogen, methane, syngas and even electricity. Few microalgae may accumulate a significant amount of carbohydrates that can be fermented to bioethanol as well. Microalgal biomass can also be a source of a few other compounds useful in cosmetics, food additives and fertilizers [22 - 25]. Microalgae have also been used for recombinant protein expression as well [26]. Hence, microalgae provide an attractive platform for sustainable production of various commodities when integrated with bioremediation to mitigate $CO_2$, nitrogen, phosphorous and other toxic elements from industrial and municipal wastes (Fig. **1**).

## WASTEWATER REMEDIATION

### Nitrogen and Phosphorous

Nitrogen and phosphorous form the essential elements and are key to plant growth and sustenance. However, excess nitrogen and/or phosphorous may be toxic to the environment in many ways, such as algal blooms and loss of oxygen that hamper the aquatic life leading to loss of biodiversity [27]. These elements form the major pollutants from waste water coming through municipal and agricultural wastes [28]. As the food demand is increasing with the increasing population, it is

**CHAPTER 5**

# Bioremediation using Genetically Engineered Microorganisms

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**Abstract:** The applications of biotechnology, especially genetic modification, are varied and concern multiple fields, as they can provide appropriate and radical solutions to many problems of agriculture, the environment, and human and animal health. The promoters of these technologies present them as the best and only solution to the problems of famine in the world in the twenty-first century, especially in underdeveloped and developing countries. The astonishing development of crops in developed countries is mainly due to the introduction of massive genetic improvement programs with intensive agricultural systems (irrigation, fertilizers, health care) until the matter in these countries reach genetically modified products.

In this chapter, we will present the importance of the techniques of genetic mutation of micro-organisms and the negative effects of genetically modified species and products for experts, decision-makers, and decision-makers to protect human, animal, and plant health. We will also shed light on the future of these genetically modified organism - based treatments.

**Keywords:** Biotechnology, Genetic mutation, Micro-organisms, Biotreatment, Modified species.

## INTRODUCTION

The beginning of the era of genetic engineering was 1970, when restriction enzymes were discovered for the first time, which enabled scientists in 1972 to synthesize the first hybrid DNA consisting of two different sources [1]. Genetic engineering came in the mid-seventies, with many scientists aspiring to give new solutions to several problems that prevented the growth and expansion of agricultural production [2]. It made a paradigm shift in the production of new strains, providing a set of technologies that allow the transfer of some individual

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and selected genes between living species that are not related to each other. These technologies helped produce high-yielding, productive crops that are resistant to disease, pests, and harsh environmental and climatic conditions. Genetic engineering refers to several techniques that are used to change the genetic makeup of cells and transfer genes across species barriers to produce new organisms [3]. These techniques include highly complex treatments for genetic elements and chemicals of vital importance. Through genetic engineering techniques, new organisms are given unique creatures, leading to the creation of new traits as well, which cannot usually be found by natural means [4]. This industrial technology is fundamentally different from conventional plant and animal breeding. Biotechnologists are working to introduce / process genes in plants, animals, and micro-organisms to find the required properties in the recipient organisms. Proponents of biotechnology believe that they have the potential to increase elements of food security, reduce pressures to use the soil, water, and agricultural chemicals, and increase the permanence of output in new areas, but opponents of them fear their potential negative impact on biodiversity and human health [5]. The common way to insert the gene is by using a gene pistol to transfer it to the cells prepared to receive it, which is not a sufficiently accurate method. The gene may be inserted in other genes, and multiple copies may be scattered in the Deoxyribonucleic acid (DNA), which may lead to the phenomenon recognized to be unstable [6]. Other techniques use vector capabilities such as bacteria, plasmids, and viruses to facilitate random transfer in the genetic code of an organism. Genetically modified organisms (GMOs) can be defined as living organisms whose genetic material has been altered in a method that does not naturally occur [7]. This technique is called “modern biotechnology” or “genetic technology,” and it is sometimes called “DNA remodeling” or “genetic engineering.” It allows individual genetics to be transferred from one organism to another, as well as between organisms of different origins. Such methods are used in the production of genetically modified plants, which are used in the cultivation of genetically modified food crops [8]. The source of what is known as a transgene may be an unrelated plant or a completely different species.

Today, we eat and use how much we are surrounded by genetically modified foods, plants, chemicals, and medicines. Corn, soybeans, canola, and cotton are the main plant species genetically modified for commercial purposes and in the human environment. About 17 million hectares of land in 18 countries are currently producing wheat, soy, and genetically modified cotton. Other crops are still subject to experiments, such as bananas, cowpea, millet, sweet potatoes, sorghum, and strawberries [9].

Biodiversity is the totality of the genetic makeup of all living things in all ecosystems on Earth and includes the differences between species and within each species. It not only resulted from the changes that the planet has witnessed throughout history but was also affected by human activities, through human care, certain types that serve its interests, development, and manipulation (using natural biological phenomena) and neglecting other types [10]. Perhaps most importantly, humans developed methods for using the Earth that destroyed entire ecosystems (and their biological diversity) and were replaced by innovative systems, such as monocultures used in agricultural and animal production methods [11].

Over thousands of years, but particularly in recent centuries, biodiversity has declined. Fears about this trend have been expressed, and environmental groups have issued calls for remedial action. Conserving biodiversity and natural ecosystems is crucial for them [12]. But there is also another aspect which is responsible for the multiplication of the "economic types" on which the continuation of human development depends on obtaining food, fiber, and medicines. Propagation and crossbreeding programs required varieties and family breeds that could only be obtained from traditional farms and wild ecosystems, which had come to be called "genetic resources". Modern biotechnologists also required all kinds of natural genetic materials since they could not "invent" synthetic genetic materials [13].

## Bioremediation in Promoting the Principle of Sustainability

Biological treatment is a process in which the use of biological processes regulates environmental emissions. People use this manner to accelerate the cleaning process without damaging the environment or species [14]. Biological treatment primarily focuses on converting poisonous or dangerous chemicals into biologically harmless or non-toxic products [15]. When applying these approaches, micro-organisms are substantial concern since they are easy to use and exhibit different reactions. Biological therapy is used for soil, land, water, *etc* [16]. Biological treatment has different methods; the utilization of genetically modified micro-organisms, the use of local micro-organisms, phytotherapy, biostimulation, and bioremediation [17]. Micro-organisms and plants are used in biological treatment to break down pollutants into less toxic compounds, which is an environmentally friendly process that is performed to cleanse the environment and reduce risks [18]. Phytotherapy is a kind of technique for biological treatment using green plants. Plants capable of converting or breaking down contaminants are used for environmental clean-up.

It is an on-site biological treatment method, a solar-based and energy-saving technology. This is the difference between biological treatment and phytotherapy [19].

# CHAPTER 6

# Microbial Degradation of Agricultural and Food Wastes into Value-Added Products

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**Abstract:** According to the Food and Agriculture Organization (FAO), one-third of the food produced globally for human consumption is lost within the food supply chain. In many countries, food and agricultural wastes are dumped in landfills. Plastic wastes from agriculture have become a major concern, especially with increasing pollution associated with the microplastics and nanoplastics in the ocean and marine ecosystem. Microbial biodegradation of the agricultural wastes and the conversion into value-added products could meet the economic and environmental demands to reduce land pollution, whilst benefiting from the generated products. Furthermore, energy together with other combustible municipal wastes can be recovered. Food wastes have attracted much interest for conversion into bioenergy such as biogas, hydrogen, ethanol, and biodiesel, and the residues are further used as animal feed or fertilizer. This review highlights the use of plastics in agriculture, their disposal, and degradation. Factors affecting biodegradation are also discussed. The production of bioenergy from agro-waste and food waste is elaborated.

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**Keywords:** Agricultural waste, Biodiesel, Biogas, Chitosan, Degradation, Ethanol, Food waste, Hydrogen, Valuable products.

## INTRODUCTION

Residues generated from the processing and growing of raw agricultural products (meat, vegetables, fruits, dairy products) are termed as agricultural wastes. The composition depends upon the system and type of agricultural activities and can be in liquid, slurry, or solid forms. The agro-wastes include crop waste (sugarcane bagasse, corn stalks, prunings, residual stalks, leaves, roots, straws and husks from vegetables and fruits), food processing waste (80% of maize is waste and only 20% is canned), animal waste (animal carcasses, manure) and hazardous chemical waste (herbicides, insecticides and pesticides). Agricultural practices have been expanded, resulting in increased output of agro-industrial byproducts, crop residues, and livestock wastes. A significant increase in agricultural waste in developing countries can be attributed to intensified farming systems. Approximately, 998 million tonnes of agricultural waste is generated annually. Out of the total solid waste produced in any farm, 80% consists of organic waste [1]. Agro-waste is therefore the most widely available and renewable source with a significant economic potential to be converted into steam, charcoal, ethanol, methanol and raw materials (composting, animal feed and energy) [2].

Food waste (FW) is the biodegradable organic waste released from the degradation of the pre-cooked or residual form of foods. According to the Food and Agriculture Organization (FAO), food waste is 'the food loss (either in quantity or quality), occurring at the different stages of the food supply chain such as post-harvest, production and processing level'. Most FW is generated at the production level [3]. FW can be generated from food processing plants, domestic kitchens, and restaurants. The end-products of various food processing industries have not been completely utilized for any other useful purposes. FAO estimates that about 1.3bn tonnes of food (such as vegetables, fruits, meat, dairy, and bakery products) are wasted annually globally, representing around one-third of the food produced for human consumption [4, 5]. In the next 25 years, with economic and population growth, there will be greater food waste in Asia. Globally, the annual production of food waste is 1.3 billion tonnes, and out of these, Asia is the highest contributor at 278 million tonnes [6].

Food waste may come from the wastes of industries, households, and kitchens and contains approximately 30% organic and 70% moisture [6]. FW consists of nutrients, primarily of 60% carbohydrate polymers (cellulose, starch and hemicellulose), proteins (20%), lipids (10%), organic acids, inorganic parts and other different compounds. This makes it a rich resource of raw organic materials for conversion into valuable end products (enzymes and organic fertilizers) [7, 8]. Considering rapid economic development, global climatic change, and depletion of fossil fuel, FW can be the major sustainable source of renewable carbon for the generation of industrial products such as chemicals, polymers, fuels, energy and other materials. Food waste has been a microbial feedstock for the production of useful by-products like methane, ethanol, hydrogen, bio-plastics, bio-polymers, organic acids and enzymes [7].

A huge population of naturally occurring microorganisms and effective microorganisms (EM) is capable of converting FW into plant nutrients that help to reduce carbon-nitrogen ratio to enhance soil productivity, maintain the flow of nutrients in the eco-system and minimize ecological imbalance [9]. Microbial degradation of FW, in a process called composting, is economical, resulting in lesser damage to the environment. Composting is an aerobic solid phase degradation process of organic materials under specified, and controlled conditions [10]. Another process, anaerobic digestion (AD), is a biological process for the conversion of organic waste into useful products by making use of metabolic versatility of microorganisms [5]. Composting of organic wastes (paddy straw and sugarcane trash) and other agricultural waste into other useful products make use of the natural succession of microflora like bacteria and fungi [10]. Many cellulolytic bacteria, under appropriate conditions, degrade cellulosic content in agricultural and food wastes. *Trichoderma harzianum*, *Polyporous ostriformis*, *Phanerochaete chrysosporium* and *Pleurotus ostreatus* are examples of various fungi playing a vital role in the degradation of lignocellulosic compounds [5, 10].

## PLASTIC-BASED AGRO-WASTES AND FACTORS AFFECTING DEGRADATION

Polymers and plastics have been in great demand in the agriculture industry [11]. Plasticulture method has assisted farmers with enhanced crop production, better yields, on-time harvesting, water conservation and reliable protection of food products for longer shelf-life [12]. The on-ground uses of plastic films protect the crops during growth. Plastic films have also been utilized in greenhouses, silage covering, and for wrapping on bales. Plasticulture enhances the quality of goods especially by maintaining the growth factors, reducing the effects of extreme weather conditions, and protecting the plants from diseases [12]. Greenhouses are mainly located in China, Japan and Korea, comprising 80% of the world's greenhouses, with 15% being in the Mediterranean basin. In China, the numbers increase from 4200 ha (1981) to 1,250,000 ha in 2002, which is 29-30% per annum, suggesting rapid utilization of plastic films [13, 14]. Approximately 3 tons

## CHAPTER 7

# Bioremediation of Environmental Contaminants and Their Impact on Food Safety in the Food Production Chain

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**Abstract:** Bioremediation is critical in eliminating and controlling environmental contaminants in the ecosystem. This is crucial in the food industry where pollutants are vast. The industry is moving towards novel sustainable food safety strategies and techniques. Also, the nature-based solution should be at the forefront of innovations in the food industry. This paper focuses on the application of bioremediation techniques on chemical environmental contaminants and how the pollutants enter the food production chain. Moreover, the impact of pollutants and the food safety risks on humans and agricultural land are discussed.

**Keywords:** Antibiotics, Bioremediation, Chemical contaminants, Food safety, Heavy metals, Inorganic compounds.

## INTRODUCTION

The global demand for food due to the ever-increasing population numbers is a major challenge. Industrialization is a continuing threat to food safety and security because of the increasing chemical pollutants accumulating in the environment. Legislative requirements have been set by authorities in many countries, however significant concentrations of hazardous chemical compounds are still detected in the food chain [1 - 4]. As a consequence, the environment and human health are compromised due to the acute and chronic effects of toxic substances. The implication of such pollution is observed chiefly in agriculture dependant countries. This happens due to the technologies introduced in the agriculture

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sector through the usage of pesticides, herbicides, and fertilizers to increase agricultural production [2, 5].

Abiotic environmental stresses which encompass pollution in soils by heavy metals and other environmental contaminants can decrease agricultural yield due to the toxic effects caused by the soil-plant transfer of pollutants [6, 7]. In addition, they significantly influence the safety and nutritional value of food production in various stages of the food production chain to the consumers. Decontamination through degrading polluted media using microorganisms has been explored as an alternative to the existing chemical solutions. Bioremediation technologies are widely used in water treatment due to their economic viability and low-cost maintenance [8 - 12]. Therefore, bioremediation is regarded as a nature-based solution because it encompasses native microorganisms that are beneficial in remediating polluted environments.

Industrial waste from food processing activities can cause health hazards even though the waste from processing factories is deemed non-hazardous generally [2, 13]. The management of waste in the food industry requires sustainable solutions that are environmentally friendly and cost-effective. In food processing plants, biofilms are a major challenge. Biofilms are formed when a cluster of microbial species attach themselves to a surface thus promoting cell-to-cell interaction to optimize growth [14, 15]. Consequently, they may lead to contamination of food thus causing foodborne disease outbreaks. Various chemical substances have been used effectively to control the formation of biofilms. More recently, innovations have led to the use of bio-solutions as an alternative. The bio-solutions contain microorganisms that can outperform each other regarding energy usage. Some bacteria, such as *Bacillus Subtilis* can produce biosurfactants that are used to disperse *Proteus mirabilis*, *E. coli*, and *Salmonella enterica* [16 - 18].

Research shows that vermitechnology is an efficient technique in reducing the toxicity of pollutants [2]. In the past, innovations, such as vermicomposting have been used as an effective method of managing organic waste. During vermicomposting, mesophilic microorganisms and earthworms are used to degrade organic residues into a finished product of value. Furthermore, this process produces nutrient-rich manure for crops in the agricultural sector. The richness of vermicompost in microbial populations makes it an effective catalyst for food plant growth due to the abundant enzymes and hormones that are required for optimal growth. Furthermore, this helps to reduce the need for inorganic fertilisers, which are more harmful to the environment and can contaminate food sources.

## AGRICULTURAL FARMLANDS AND FOOD SAFETY

Environmental degradation and food safety are two critical issues faced by the world. In China, these two challenges are complex in that the effects of one phenomenon directly affect the other [19]. Environmental pollution affects the soil, water, and air and these elements are vital in food production. The soil serves as a medium for environmental contaminants. Persistent Organic Compounds (POPs) such as polychlorinated dibenzo-p-dioxins accumulate in the soils due to pollutants building up over the years or during chemical spills. These result in hot spots of pollution which may be on agricultural land or nearby water sources [19, 20]. This type of pollution goes against a declaration signed by member states of the WHO/FAO at the Stockholm convention where a debate on the use of POPs was discussed together with the global agenda on sustainable development [21]. Pollution affects the biotic system and the impact manifests in the food chain. The pollution is further exacerbated by the widespread distribution of heavy industries that continue to be a threat to available agricultural land. Therefore, novel technologies need to be introduced to safeguard the health of consumers and the environment at large.

According to Weber *et al.* [21], 80% of human exposure to persistent organic pollutants is through food. Also, the food derived from food animals such as meat, dairy, chicken, and fish is largely the source of exposure. Soil contaminants are taken up by outdoor food animals when they feed. Pollutants such as Polychlorinated biphenyls (PCBs) have been reported to be in high concentrations in free-range chickens. This is potentially due to the free-range chickens being able to consume 30g/day of soil [21]. Livestock is exposed through their grass feed. Moreover, the exposure chain continues when they are breastfeeding. In other scenarios of contamination, pesticide residues and fertilizers affect agricultural soils by accumulating in the food chain through crops.

Rice and wheat are considered staple foods globally. Rice in particular forms a significant portion of half of the global population's dietary intake [22, 23]. In China, soil contamination has been enacted due to increased numbers of food safety issues reported in the past. This burden of contamination is attributed to the industrialized regions of the country. Despite legislative interventions in China, the total usage of pesticides and fertilizers increased significantly within the study period of 10 years (1991-2011) [19]. Therefore, stringent measures should be taken to control and minimize the impact of contaminants on agricultural soils.

# CHAPTER 8

# Bioremediation in Food Waste Management

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**Abstract:** A growing population implies an increasing demand for food. Consequently, the processing industry related to it generates large amounts of waste. This problem arose due to the delayed development in establishing effective and advanced waste management technologies, which must be developed and used to lower the cost of producing processed foods and minimizing pollution risks. Recent studies on the valorization of residues in the food chain have focused on obtaining value-added products such as biofuels, enzymes, bioactive compounds, biodegradable plastics, and nanoparticles. Regulatory agencies and the food processing industries can work together to develop new waste management and use processes that are commercially viable. This chapter presents an introduction to bioremediation and management of various residues from the food industries (beverages, dairy, fruit, vegetables, oil and meat), as well as some characteristics, advantages, and limitations of some methods. New possibilities for using food waste are also described.

**Keywords:** Aerobic processes, Anaerobic digestion, Anaerobic processes, Biological treatment, Bioremediation, Brewery wastewater, By-products, Cleaner environment, Dairy wastewater, Distillery wastewater, Environmental sustainability, Food waste, Meat wastewater, Oil wastewater, Organic waste, Phenolic compounds, Solid wastes, Vegetable wastewater, Waste management, Winery wastewater.

## BEVERAGE INDUSTRIES

Beverage industries are considerable wastewater producers. Between the production, washing, and sometimes cooling, a distillery produces about 15 L of

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wastewater per litre of alcohol produced; 1 kg of coffee beans results in 5-15 L of water, while coffee powder generates 45 L of wastewater per kilogram of the product; in the same sense, each liter of beer produces 4-10 L of wastewater [1 - 4]. When these untreated effluents are discharged, eutrophication is caused by the augmentation of nutrients, like carbon, nitrogen, and phosphorus, which alters the pH, reduces the dissolved oxygen and increases the algae concentration, and consequently the death of many species [5].

Environmental sustainability is a concern for industries, especially for market competitiveness. The ideal residue treatment would be optimum nutrient removal performance with the lowest operation costs [6]. The vast majority of the bioremediation processes in industries wastewaters make use of the microorganism already situated in there to degrade the organic matter, only supplementing nutrients for their metabolism when needed [7].

One of the greatest issues when analyzing wastewaters is the large variability between the contents of the products (*e.g.*, beer, wine, soft drinks) and the unevenness of the material in different regions or seasons (*e.g.*, wine terroir). It is also necessary to observe at which steps of production are they generated to understand the composition of wastewaters (Table **1**).

**Table 1. Characterization of different beverages wastewaters.**

| Type of Wastewater | pH | COD (mg/L) | TS (mg/L) | TDS (mg/L) | TSS (mg/L) | TN (mg/L) | TP (mg/L) | Nitrate (mg/L) | Ammonia (mg/L) | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Brewery | 6.1 | 32,000 ± 3000 | NI | NI | NI | 1238 | 95.2 | <10 | 11.3 | [8] |
| | 7.7 | 1420.0 | NI | NI | 420.0 | 84.0 | 6.6 | 26.5 | 5.2 | [4] |
| | NI | 565–7,837 | NI | NI | NI | NI | 56.98–325.75 | 1.86–11.16 | 3.07–106.44 | [9] |
| Wine distillery | 3.9 | 25,500 | NI | NI | NI | NI | NI | NI | NI | [10] |
| | 3.5-4 | 25,000-30,000 | 15,505 | 10,155 | 722.5 | NI | NI | 310.5-473,75 | 0.2-0.25 | [11] |
| | 4 | 15,000-35,000 | 18,000 | NI | NI | 350 | 150 | NI | 10 | [12] |
| Winery | 3.98-5.17 | 320-5,760 | NI | NI | NI | NI | NI | NI | NI | [13] |
| Molasses distillery vinasse | 4-4.1 | NI | 2,82 | NI | NI | 2.02 | 240 | NI | 125,4 | [14] |
| Beer sugar molasses distillery vinasse | 5.1-5.7 | 115,200-176,344 | 109,275-145,200 | NI | 5,390-8,120 | 1050-8,759 | 355-1030 | NI | 114-380 | [15] |
| Coffee | 4.98 | 20,000 | NI | 1520 | NI | NI | NI | NI | NI | [3] |
| | 3.57 | 8,079.0 | NI | 3,933.0 | 2,019 | 350 | 14.7 | NI | NI | [2] |
| | 4.49 ± 0.16 | 16, 684.33 ± 1683 | 7,754.08 ± 321.16 | 5, 442.59 ± 1100.97 | 2, 390 ± 765.14 | NI | NI | NI | NI | [16] |
| Soft drink | NI | 1200–8000 | NI | NI | 0–60 | NI | 20–40 | 150–300 | NI | [17] |

*(Table 1) cont.....*

| Type of Wastewater | pH | COD (mg/L) | TS (mg/L) | TDS (mg/L) | TSS (mg/L) | TN (mg/L) | TP (mg/L) | Nitrate (mg/L) | Ammonia (mg/L) | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Malt Whiskey distillery | 2.9-3.85 | 15,000-58,500 | NI | NI | NI | 4.9-24.1 | 740-1960 | NI | NI | [18] |

COD: Chemical Oxygen Demand; TDS: TS: Total Solids; Total Dissolved Solids; TSS: Total suspended solids; TN: Total nitrogen; TP: Total phosphorus; NI: not informed.

In soft drinks production, the main sources of waste are losses on syrup and the soft drink itself, water from the bottle and can decontamination and grease from machinery [17]. The most widely used wastewater treatment technique for soft drinks is the biological treatment, especially the anaerobic since the chemical (COD) and biological oxygen demand (BOD) is reasonably lower than in other beverages.

Soft drinks wastewater is rarely treated by the aerobic process. It is most commonly used in treatments with lower organic content; still, a suspended or attached aerobic treatment can be employed, only requiring enough time of contact between the microorganisms and wastewater [17]. The anaerobic process generates methane which can be recirculated in the process to feed boilers instead of fossil sources.

Coffee wastewater has high acidity, turbidity, total dissolved solids (TDS) and COD [2, 3, 16]. While still in bean processing, the major wastewater (approx. 50%) income is from the de-pulping process [19]. This wastewater, when treated, can be transformed into biogas, animal feed or disposed of on land as compost, which is the cheapest alternative despite it has disadvantages such as no added value and releasing carbon dioxide methane (greenhouse gases) in the atmosphere [2]. Due to its high COD, TS, TDS and TSS, the anaerobic digestion can also be used for coffee wastewater treatment, with the estimative of energy recovery with methane production from 4 to 10 million KJ/day.

Winery effluent is one of the most variables between fermented beverages wastewaters, given the production method, type of wine and seasonality of wine production. The winery and wine distillery wastewaters, comparatively with other wastewaters, have a substantial amount of phenolic compounds, the first varying from 5.1 to 27.2 and the second 470 to 1200 mg/L [10, 11, 13]. The winery wastewater is comprised of grapes and wine residue, remains of yeast, bacteria, clarifying substances and cleaning and sterilizing materials [12]. The biomass is filtrated after the fermentation of wine along if other remaining substances known as wine lee. This effluent has a high TS content (about 10%), and a larger COD, and its characteristics are nearest to wine distillery effluent. Unlike the winery effluent, the wine distillery wastewater also has a higher COD content; it contains

## CHAPTER 9

# Treatment of Distillery Wastewater by Bioremediation Technique: A Green and Sustainable Alternative

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**Abstract:** Distillery industries generate a considerable amount of wastewater, having a high percentage of inorganic matter and organic compounds. Wastewater generated from distillery industries is dark brown in colour. Untreated wastewater from distillery industries have an adverse impact on the sustainability of the environment due to its high pollutant concentration. Hence, distillery industry effluent requires urgent attention for the minimization of toxic waste generation. Several technologies used in the elimination of pollutants from wastewater include physico-chemical and bioremediation techniques. Bioremediation technique is a simple, economical, and the most potential technique. Among bioremediation methods, anaerobic, aerobic, and various kinds of phytoremediation processes have been discussed here. Further, the removal of contaminants by bacteria, fungi, and algae has also been mentioned. A large amount of sludge generation by the anaerobic process also needs attention and proper management. It also outlines the mechanism of the decolourization of melanoidin by microorganisms. The role of different bioreactors in bioremediation technique has also been discussed in detail. Keeping in view the applicability of different bioremediation techniques discussed here for removal of melanoidin high biological oxygen demand (BOD), high chemical oxygen demand (COD), a heavy concentration of suspended solids, polysaccharide, lignin, protein and waxes, it is expected that this technique can be useful for further treatment in a variety of wastewater from distillery industries.

**Keywords:** Aerobic, Algae, Anaerobic, Bacteria, Biological oxygen demand, Bioreactors, Bioremediation technique, Chemical oxygen demand, Distillery industry, Fungi, Inorganic matters, Lignine, Melanoidin, Microorganisms, Organic compounds, Physico-chemical technique, Polysaccharide, Protein, Suspended solids and waxes.

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## INTRODUCTION

Distillery industry is one of the most contaminated industries as most of its raw materials are transformed into waste and it is discharged into an aqueous medium, which leads to water pollution [1]. For the production of one litre of alcohol, approximately fifteen litres of spent wash is released. Alcohol is the basic need for many chemical industries. That's why alcohol is in huge demand so is the distillery industries. Most of the distillery industries co-exist with sugar industries and utilize sugar cane molasses which is starting material for the production of alcohol [2]. Sugar cane molasses contributes 61% as a raw material in distillery industries. Corn is the second most significant raw material used in distillery industries. Grapes, grains, sugarcane juice and barley malt are also used in distillery industries as raw material. Raw materials used in distillery industries vary from one country to another. It depends upon availability, cost of raw materials and carbohydrate content. India is having more than 330 distillery industries that produce approximately 3 billion litres of alcohol annually [3].

The distillery industries play an important role in India's economy. Distillery effluent is known to exhibit variable pH (acidic or alkaline depending upon the method used), high biological oxygen demand (BOD), high chemical oxygen demand (COD), high temperature, high concentration of suspended solids and large quantity of heavy metals [3, 4]. So there must be some treatment [5 - 7] before final discharge to achieve the legal and aesthetic standards. Leaching of inorganic and organic minerals also adversely affects the quality of ground water by changing electrical conductivity, pH and colour [8]. The production of alcohol in distillery industries is having the following steps:

- Feed preparation
- Fermentation
- Distillation
- Packaging

The process of formation of alcohol in distillery industries has been shown in Fig. (**1**).

## CHARACTERISTICS OF DISTILLERY INDUSTRY EFFLUENTS & ITS HAZARDOUS EFFECTS

The characteristics of wastewater depend upon raw materials used in alcohol production. The physico-chemical properties of distillery industry effluents have been presented in Table **1** [9]. The BOD and COD are high in distillery industry wastewater due to the presence of organic matter *viz*; polyphenol, protein and polysaccharide. Most of the contaminants of distillery industry wastewater are non- biodegradable in nature.

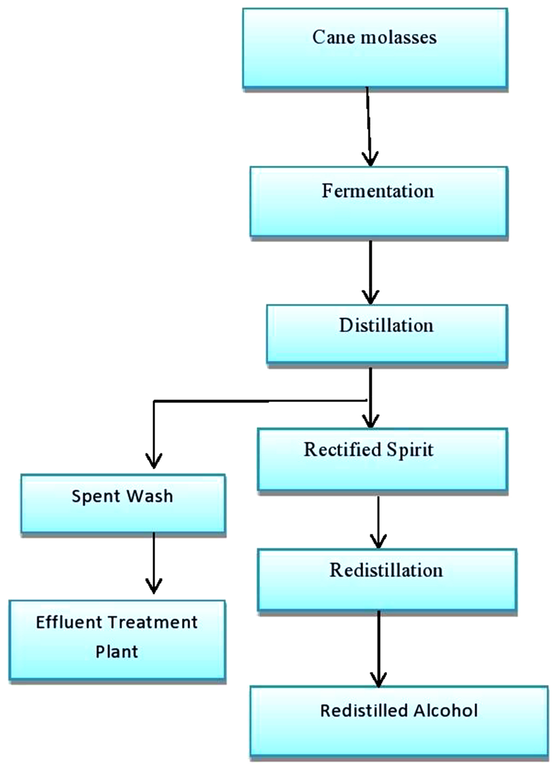


**Fig. (1).** Steps involved in formation of alcohol in distillery industries.

**Table 1. Physico-chemical characteristics of distillery industry effluent [9].**

| Characteristics | Values | Units |
|---|---|---|
| Temperature | 71 to 81 | ºC |
| pH | 4.0 to 4.5 | - |
| Colour | Dark colour | - |
| Total solids | 59,000 to 82,000 | mg/l |
| Total suspended solids | 2400 to 5000 | mg/l |
| Volatile solids | 38,000 to 66,000 | mg/l |
| BOD | 35,000 to 50,000 | mg/l |
| COD | 100,000 to 150,000 | mg/l |
| Nitrogen | 1660 to 4200 | mg/l |
| Phosphorus | 225 to 308 | mg/l |

## CHAPTER 10

# Bacterial Resistance to Antibiotics in Groundwater. Impact to the Public Health

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**Abstract:** Antibiotics are defined as medicines used for the prevention and treatment of bacterial infections. They act as growth inhibitors or destroy the bacteria by destructing the bacterial cell wall or inhibiting energy generation from glucose within the cell. When bacteria adapt to resistance or develop the ability to resist the factors & survive after being exposed to antibiotics, they become resistant to antibiotics. Antibiotic resistance occurs due to several changes (physical, chemical, environmental) or undergoing mutation in bacterial DNA. There are many ways through which antibiotics may reach groundwater. The increased use of antibiotics leads them to transfer from one place to another. They can be transferred from hospitals to humans and from human waste to groundwater. Agriculture practices, poultry farming, and industrial techniques also allow antibiotics to reach groundwater, which further affects aquatic lives and also causes antibiotic resistance in bacteria.

**Keywords:** Antibiotics, Antibiotic resistance, Mutation, groundwater, Human waste, Poultry farming, Agriculture practices.

## INTRODUCTION

Antibiotics are examined as the sensational discovery of the 20th century and the real wonder is the rise of antibiotic resistance in hospitals, communities, environment due to their excessive use [1]. Penicillin was the first antibiotic discovered by Alexander Fleming in 1928 [2]. It was able to stop infectious pathogens like *Staphycoccus aureus* by inhibiting the biosynthesis of the cell wall

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of pathogenic bacteria. *S. aureus* became the main cause of death in European hospitals. Even after great success in controlling and reducing mortality & morbidity due to such infections, it was further observed that bacteria was able to resist and tolerate the antibiotic actions and become resistant [3]. A wide range of physiological & biochemical mechanisms became responsible for resistance [1]. Antibiotic resistance occurs when bacteria produce some changes which banish the effectiveness of drugs, chemicals or some other agents and as a result bacteria survive, grow rapidly, replicate themselves and form antibiotic-resistant colonies, which later cause harm. Man's overuse of antibiotics to prevent further infection became one of the sources of transfer of resistance genes. Antibiotics are introduced into agriculture practices (Bt gene), in poultry farming (pigs, chickens), and clinical methods (drugs) allowing the mechanism of transfer of antibiotic resistance from one place to another and affecting lives [4]. These antibiotics, when to get transferred to groundwater, and due to biomagnification, the concentration of toxins get modified, which affects aquatic lives (phytoplankton, fishes), and the bacteria which have undergone mutations do not get affected by antibiotics present in water and become resistant then further multiplies [5]. When this toxic water reaches our households, and by using toxic, unfiltered water in cooking, cleaning or drinking or due to lack of sanitation or unhygienic activities, human health gets affected [6]. It is estimated by the European Centre for Disease Prevention and Control (CDC) that each year around 25,000 people lose their lives due to drug-resistant bacterial infections [3]. Antibiotic resistance can affect people's health at any stage of life, as well as in the veterinary, and agriculture industries making it the biggest public health problem [7].

## SPREAD OF ANTIBIOTIC RESISTANCE

Extreme use of antibiotics in various fields and daily life will encourage the exposure of resistant bacteria. Using antibiotics in advance to prevent further infection or treat non-bacterial infection and neglecting the doctor's prescription enhances the spread of such bacteria. Using antibiotic genes in crops to get modified crops like Bt cotton, Bt brinjal, *etc*, promotes the transfer of antibiotic genes to animals and humans [8]. Due to the high demand for non veg food or high protein animals, poultry farmers give antibiotics to animals (pigs, cattle, chickens) to control or prevent disease, making them look healthy without knowing the cause, and promote the spread [9].

### Human Activities

When people self-medicate themselves by consuming drugs or antibiotics to treat self-identified disorders or symptoms or by using the same drug for every treatment without consulting a doctor or without any prescriptions, or over-prescribing of drugs results in the exposure to pathogenic resistance [10], antibiotics are made to kill bacteria, but when people take antibiotics to treat any type of infection, this may cause the risk of inducing antibiotic resistance. Due to incomplete metabolism in human beings or because of unused antibiotics, a huge amount of antibiotics gets released into wastewater *via* human waste (Fig. **1**) [11].


**Fig. (1).** Spread of antibiotics through humans.

## Agriculture Practices

Antibiotics are used in agriculture to control bacterial disease in high value fruits, vegetables or crops. The most commonly used antibiotic is streptomycin and oxytetracycline [12]. These antibiotics are used for the better production of crops that must be disease resistant, resistant to bacteria and other external factors like environmental factors and get genetically modified crops [13]. Some of the antibiotics (insecticides, pesticides) are sprayed over the fruit trees and vegetable trees. When the sewage water gets mixed with animal waste, it turns into manure which is used as fertilizer in crops. Widespread use of antibiotics led to multidrug-resistant pathogens, and through the consumption of these crops, fruits and vegetables, antibiotic resistance gets transmitted (Fig. **2**).

# CHAPTER 11

# Bioremediation of Pharmaceutical Waste

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**Abstract:** Industrial production of pharmaceutical products is rising simultaneously with the increase in the world population and urbanization. They are vital in the treatment, prevention and control of diseases. Although pharmaceutical products play a pivotal role worldwide, their disposal and subsequent toxic metabolites are causing havoc in the environment. Accumulation of these hazardous pollutants in the environment increases the chances of reaching and affecting communities. The potential toxicity of these compounds includes the ability to be mutagens, carcinogens and genotoxins. Remediation methods currently available to rejuvenate nature from such wastes are generally expensive and may convert one toxin to another. Therefore, the use of microorganisms for the bioremediation of pharmaceutical and toxic waste has become an economical and effective alternative. Bioremediation techniques further detoxify the waste into useful or harmless products that can be beneficial to the ecosystem.

**Keywords:** Aerobic, Anaerobic, Analgesic, Antibiotics, Anti-inflammatory, Biodegradation, Bioremediation, Degradation, Ecosystem, Enzymes, Hormones, Metabolites, Microbes, Microbial remediation, Microorganisms, Pharmaceutical waste, Sewage, Sludge, Phenols, Wastewater.

## INTRODUCTION

Pharmaceutical waste includes used and unused/expired prescribed medicines, over the counter medication, and even the home-use personal care products. Pharmaceutical products cater to various needs, ranging from diagnosis, prevention, and treatment of diseases to management of livestock and many other uses. Pharmaceuticals and personal care products (PPCPs) contain substances used for health or cosmetic benefits. Medicines or any therapeutic compounds

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subjected to regulations as a medicine or drug by food and drug regulatory authorities under the department of health are considered pharmaceutical products. Pharmaceutical products are comprised of a variety of synthetic, semi-synthetic, and natural chemicals intended for the use and benefit of humans, plants, and animals.

Pharmaceuticals are chemicals specifically designed to act on living cells. They include oral, parenteral, topical, and other kinds of therapeutic drugs. They regulate major metabolic pathways such as the endocrine system, nutrient metabolism, and central nervous system. They also assist the body in fighting diseases, thus preserving life. Administration of drugs is *via* several routes, including injections, nasal and oral routes being the most used. To be effective, drugs need to resist the stomach's acidic environment so the compounds can reach their designated destinations inside the body. Hence, they are designed to degrade very slowly or not degrade at all.

They are directly released with the normal industrial, hospital, and household sewage. They also leach from the waste dumps into various systems, especially water. Active pharmaceuticals pass through our bodies and enter the environment through the sewage system. For instance, a commonly used pain killer (paracetamol) is excreted at 58-68% from the body during therapeutic use [1]. Also, pharmaceutical products find their way into the environment through direct release from the pharmaceutical industries and disposal of unused medicines from the health care facilities and pharmacies [2, 3]. Moreover, they are found in minute concentrations, ranging from ng/ml to µg/ml, in soil and water [4, 5]. For that reason, they have gone undetected and have become environment persistent pharmaceutical pollutants (EPPPs).

Pathogenic bacteria exposed to EPPPs may become more resistant to medication. Microbial resistance caused by such chemicals in the environment is on the rise. Currently, microbial resistance caused by exposure of bacteria to EPPPs, especially antimicrobial, is one of the threats faced by public health care. Antimicrobials may also reach the environment *via* an indirect release from treated humans and animals. Once these active compounds are in the environment, they can stimulate the growth of the already existing resistant microbes and increase the genes responsible for resistance. These substances also promote gene mutation and adversely promote the increase in new resistant strains.

## PHARMACEUTICAL WASTE

Pharmacy practice has been around since the beginning of time with the use of medicinal plants as one of the first drugs. The art of pharmacy started with the first person who discovered curing a wound by applying succulent leaf juices to the wound. In recent years, there has been an astonishing increase in the discovery and production of new pharmaceutically active compounds.

The International Society of Doctors for the Environment suggested the term EPPP as an emerging issue in pharmaceuticals and the environment. This suggestion was raised in 2010 at the Strategic Approach to International Chemicals Management (SAICM). SAICM is a committee that aims to achieve the management of chemicals throughout their lifecycle, ensuring that chemicals are produced and used in ways that minimize significant adverse impacts on the environment. The concern around pharmaceutical pollutants in the environment has been around since the 1970s, with just less than a decade in the spotlight. EPPPs are a major emerging concern to communities due to their complex and hazardous nature. Pharmaceutical waste is characterized by a high concentration of chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids, dissolved solids, total phosphorous, oil content, pharmaceutical active compounds, *etc* [2, 6 - 8].

**Environmental Dangers: Pharmaceutical Waste**

Pharmaceutical industries generate vast quantities of waste [4]. Alongside this, global urbanization and increased use of pharmaceutical drugs are adding to the rising concern of pharmaceutical waste pollutants. It has been difficult to effectively remove pharmaceutical waste components as they contain heavy chemical compositions. Drugs that are regularly found in waste are antibiotics, hormones, painkillers, anti-inflammatories, *etc.* [9, 10]. These substances are pharmaceutically active, toxic, and volatile. The major concern across the globe is the removal and degradation of hormonal drugs and their derivatives.

Conventional methods used in waste treatment plants fail to remove some of the active pharmaceutical compounds, especially hormonal drugs. Incomplete removal of pharmaceutical compounds results in contamination of surface and groundwater. Continuous exposure to low concentrations of these biologically active compounds from drinking water leads to major health concerns and subsequent damage to aquatic life [11]. These compounds accumulate in the body and cause adverse effects such as acute or chronic damage to tissues, behavioural change, cell proliferation inhibition and reproductive damage [12, 13]. Industrial wastewater presents a concentration of about 1μg/L of EPPPs [14]. The degradation of these compounds should be a priority due to their increased damage to the ecosystem. There are several methods used in the treatment of pharmaceutical waste.

# CHAPTER 12

# Antibiotics Bioremediation

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**Abstract:** Antibiotics are in high demand across the world in a variety of industries . The use of antibiotics has become a global threat, being called an emerging pollutant. Thus, they are incorrectly disposed of in wastewater and their bioremediation becomes indispensable. In this context, the objective of this chapter is to explore the bioremediation of antibiotics. First, information was provided on the sources of antibiotics in wastewater and their possible impacts on the environment and humans. Then, the antibiotic removal process performed by microorganisms and microalgae-based processes was discussed, showing the factors that interfere with their performance in the removal of antibiotics. Finally, future prospects for fostering development in this area were analyzed.

**Keywords:** Antibiotic, Bioremediation, Climatic conditions, Conventional process, Environmental impacts, Microalgae-based processes, Pharmaceutical pollutants, Primary treatment, Secondary treatment, Tertiary treatment, Wastewater.

## INTRODUCTION

With population growth accelerating compared to the expansion of the livestock sector, the global demand for antibiotics is steadily increasing. In the middle of 1928, Alexander Fleming discovered penicillin, and consequently, later, several antibiotics were synthesized for human, plant, and animal health [1]. In addition, antibiotics are also used to expand food capacity as facilitators of development in livestock and aquaculture [2, 3].

Unfortunately, the increasing use of antibiotics produces an abundance of effluents, becoming an emerging contaminant [4].

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Antibiotics are classified as natural, synthetic, or semi-synthetic compounds, where they have antibacterial, antifungal, and antiparasitic action. Thus, they are strongly applied in veterinary and human treatment to treat and prevent bacterial diseases. Therefore, based on their modes of action, they can kill bacteria or inhibit their growth. It is worth mentioning that approximately 15% of all drugs used worldwide are concentrated in antibiotics [5, 6].

Associated with this, humans and animals contain antibiotics that are not fully metabolized and are then discarded simultaneously in municipal wastewater [7]. Thus, antibiotic pollution poses serious threats to human health and the environment. They can generate antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) and unregulated endocrine effects in the environment [1, 8, 9]. Consequently, these compounds have been recognized as emerging pollutants in recent decades [10]. Therefore, its removal becomes a challenging activity [11].

In fact, the effective and adequate reduction of this pollutant is of profound need and interest, although conventional remediation biotechnologies, such as chemical or physical, have been presented and used. Unfortunately, these systems pose a high demand for chemicals, which not only makes them an expensive means of treatment but also creates problems with secondary disposal. Consequently, for the sustainable reduction of various polluting compounds, it is not satisfactory [12].

Thus, biological removal is being used to reduce contamination by antibiotics, ich recently received significant attention [13]. Notoriously, biological treatments are considered promising. For several factors, including its performance efficiency, related to cost-benefit, and the generation of important products and bioenergies [14]. Thus, microorganisms become important degraders of organic matter and xenobiotics [15]. Recently, the implementation of microalgae has been used because it is a potential strategy for a sustainable recovery, where it uses light energy, is environmentally friendly and, in addition, is more flexible than antibiotics [4].

In this sense, the chapter aims to explore the bioremediation of antibiotics. Initially, we provided information on the main sources of antibiotics in wastewater and their possible impacts on the environment and humans. Then, the antibiotic removal process performed by microorganisms and microalgae-based processes was discussed. In addition, factors that affect the performance of microalgae in removing antibiotics have been presented. Finally, prospects for fostering development in this area are analyzed.

## Wastewater Antibiotic Sources

Mass production, misuse, and overuse of antibiotics, even in low concentrations, overburden the waste disposal and, consequently, harm the environment [16]. Wastewater treatment plants (WWTPs) receive discharges from many sources and are generally indicated as a prevalent flow of antibiotics because most of them have not yet been designed to remove this category of waste [17, 18].

Today, hospitals and health centers are classified as important sources of the spread of antibiotics in wastewater treatment plants. Due to widespread consumption and elimination by man [19]. Thus, it was found that the body metabolizes only a part of these pollutants, while about 10 to 90% is excreted unchanged or transformed by feces and urine [20, 21]. In fact, some studies report that a high accumulation of antibiotics has been detected in hospital wastewater and that fluoroquinolones, tetracycline, and ciprofloxacin have been found in higher concentrations [22, 23]. At the same time, improper storage and disposal of unused drugs in domestic environments makes it likely that antibiotics will accumulate in wastewater in varying amounts [24].

Among the main properties of drugs, solubility is one of the causes that most influence the accumulation of drugs in treatment plants [25]. In view of this, manufacturers of pharmaceutical products are also responsible for increasing the total concentration of antibiotics in wastewater since most antibiotics are low molecular weight and easy to dissolve in water [26]. Several authors have already proven in their research that in the locations of the pharmaceutical industry and their accumulations in the affluent, there is a direct relationship between them [27 - 29]. In addition, the unusual flow on rainy and humid days can exceed the volume of the treatment plant or cause medications, such as antibiotics and their isomers, to flow directly into the environment [30].

Regions influenced by agriculture and veterinary facilities, such as animal feedlots and fishponds, are also known as significant sources of antibiotics [31]. In these places, the large use of specific drugs to promote animal growth and increase food efficiency indirectly pollutes the soil and water resources [32].

In comparison with the human body, antibiotics are also not completely absorbed by animals, where more than 70% are excreted in animal manure [33]. Animal manure is popularly known for the fact that it is often adapted with fertilizers on arable land, and therefore antibiotics can penetrate agricultural land or drain into watercourses or groundwater in the environment through the spread of manure [34].

# CHAPTER 13

# Microbial Degradation of Sodium Polyacrylate Present in Diapers

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**Abstract:** Sodium polyacrylate is widely used in many fields, including agriculture, sanitary products, drug delivery, wastewater treatment plants, *etc.* Since polyacrylamides (PAM) are produced and used in bulk quantities, they have higher rates of mobility in the environment when polymers are degraded. They can create potential challenges for water supplies as well as wastewater treatment plants, and hence their disposal and degradation in nature is a significant issue. Various modes of degradation include mechanical, chemical, photolytic, and biological methods. In the biological model, microorganisms are used, either aerobic or anaerobic. Both bacteria and fungi seem to contribute to this cause; however, the enzymology and detailed pathway of their degradation steps are still being researched, and various hypotheses are available. Although enzyme amydases are found to be involved, there are speculations about the role of other enzymes as well. Various methods, including fenton oxidation, low glucose exposure, zinc oxide, and UV irradiation, are tried to enhance the biodegradation of polyacrylamide and polyacrylates. In this book chapter, we have included our own preliminary study results in this area. Further studies are required to fully elucidate the mechanisms and create a suitable consortium of microorganisms.

**Keywords:** Polyacrylamide, Polyacrylate, Sodium polyacrylate, Disposable diaper, Degradation, Microbes, Aerobic degradation, Anaerobic degradation, Waterlock, Amydases, Carbon source, Nitrogen source, *Proteus*, *Pseudomonas*, PAM.

## INTRODUCTION

Polyacrylamides or PAMs are high molecular weight, swellable organic polymers that are water-soluble and are formed of acrylamide subunits. Initially, they are formed as a simple, repeating, linear chain structure which later gets modified to form branched, highly structured, and cross-linked variants. PAMs are compar-

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atively economical and were first used in the paper industry in the mid-1950s [1]. The acrylamide-acrylic acid copolymer, also known as Poly(acrylamide-co-acrylic acid) and its sodium salts (APAM) have many applications such as a binder, thickening agent, super absorbent, filtering aid, soil conditioner, crosslinker, flocculating agent, lubricant, suspending agent, and oil recovery agent. Sodium polyacrylate is also known as waterlock and has widespread applications in many consumer products as it can absorb 100- 1000 times its mass in water and is soft tough and rubbery in nature [2]. Sodium polyacrylate is also used in plant nurseries nowadays due to their higher water holding capacity.

It is used as an absorbent polymer and has also been used as a viscosity agent, a fixative for hair, an emulsion stabilizer, skin conditioner and also in baby diapers as gel crystals [3]. As water is highly attracted to sodium ions, when water is poured into sodium polyacrylate, it moves into the individual powder particles and results in the expansion of polymer particles into a solid-like gel [4]. Various methods used to fabricate sodium polyacrylate include solution polymerization in water, inverse polymerization as suspension, emulsion or plasma, polymerization under pressure and these various production methods determine the swelling capacity, absorbency and various mechanical properties [5]. Superabsorbant nanofibers [6], and composites, such as clay, chitosan and metal ions have uses in tissue engineering, agriculture, foods, hygiene products, wastewater treatment and drug delivery [7 - 9]. Superabsorbant sodium polyacrylate composites are also developed for heavy metal remediation [10].

A study conducted on the degradation of polyacrylates in soil by burying them in soil for 12 months revealed that it could in the end may become part of soil by the action of the inorganic and organic components of the soil [11]. Degradation studies under controlled temperatures using thermogravimetry revealed dimers or trimers as fragments, nevertheless only smidgeons of monomers were found [12]. Studies by Chiaudani [13] showed that only lower molecular weight fractions of sodium poly acrylate are degraded by microbes though not complete and further investigations by Kawai and Larson indicated that the polyacrylate below 7 monomer units can initiate metabolic utilization by microbes and complete biodegradation may take place only with further lower monomer numbers [14, 15]. Several studies have been conducted to detail the biodegradability of sodium polyacrylate and the microorganisms associated with it. The acrylamide monomer is identified as a neurotoxic substance as depicted in many studies [16 - 18], but sodium polyacrylate is considered to be nontoxic to plants and animals [19], but it can cause adverse effects on the environment mainly and thereby directly or indirectly human and animal health also. Hence, environment friendly bioremediation is considered one of the solutions to this problem [20]. Since they are more hydrophilic, they have higher rates of mobility in the environment when the polymers are degraded and it will create potential challenges for water supplies as well as wastewater treatment plants [21].

## Properties of Sodium Polyacrylate

The molecular formulae of sodium polyacrylate are $(C_3H_3O_2)$.Na or $C_3H_3NaO_2$, with a molecular weight of 94.04g/mol. It has a melting point of >300ºC and has good solubility in water. It can be in a liquid physical state or as an odourless powder that is white in color. It has a density of 1.32g/mL at 25ºC; refractive index of *n*20/D 1.43; specific gravity-1.23 and pH range of 6 to 9 [22, 23]. Though it is considered slightly irritating for skin and eyes on acute contact and ingestion and inhalation, there are no available reports of chronic exposure health hazards as a carcinogen, mutagen, teratogen, or causing developmental toxicity [24].

## Polyacrylamide and Polyacrylate Degradation

### *Degradation Mechanisms Include*

1. Mechanical degradation: This type of degradation occurs in the process such as tillage abrasion, freezing and thawing. It is frequently found in the oil and gas processing industry. This is attributable to the augmented shear along with elongation rates that impinge under the turbulent flow along with the small pores and splintering inside the porous media of these conceptions [25]. Usually, in PAM degradation, an abiotic process like mechanical stress breaks the polymer into smaller fragments. The polymer segments of 6 - 7 monomers in length are then expended by soil microbes.

2. Chemical degradation: This kind of degradation will lead to chain breaks which involve activation of polymers by means of free radicals and this kind of scission occurs much faster under high temperature conditions. But thermal degradation is not usually occurring in the natural environment however, the temperature is one of the rates limiting factors of chemical and photolytic degradation [26].

3. Photolytic degradation: It is similar to chemical degradation which occurs in the presence of oxygen. During light exposure, free radicals are generated, causing chain scission [27].

4. Biological degradation: PAM and its derivatives, and acrylamides will act as the singular source of Carbon and/or nitrogen for microorganisms in the presence or absence of air. This degradation process starts with diminished viscosity and polymer molecular weight along with ammonification.

# SUBJECT INDEX



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