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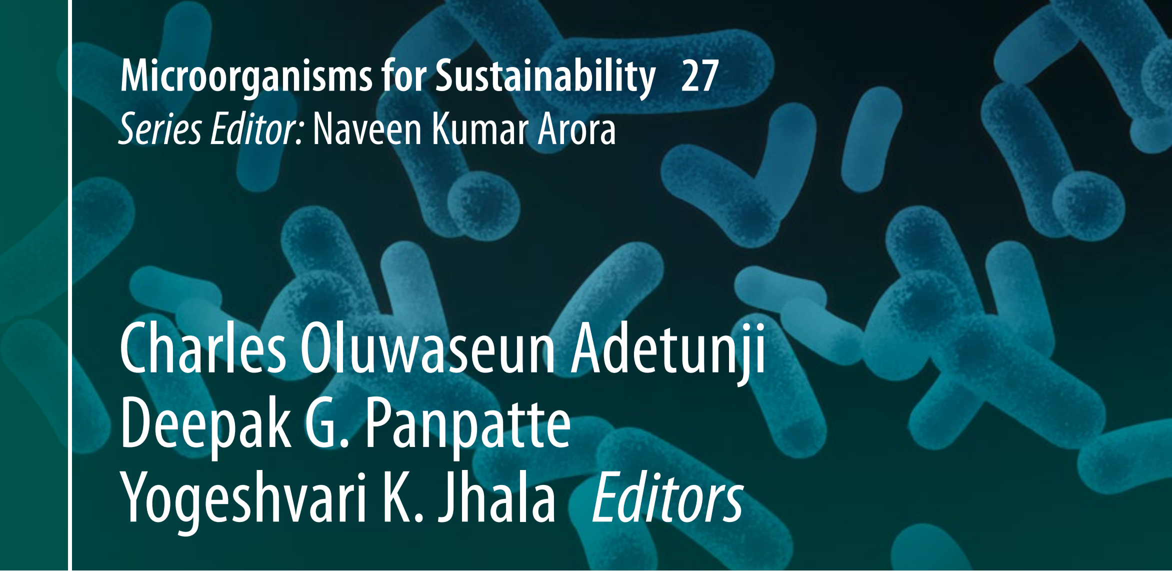

Charles Oluwaseun Adetunji
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Yogeshvari K. Jhala *Editors*

Microbial Rejuvenation of Polluted Environment

Volume 3

Springer

Microorganisms for Sustainability

Volume 27

Series Editor

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Preface

This book presents current advances in the application of bioremediation techniques that could be utilized for the rejuvenation of technogenic pressures and negative environmental consequences of anthropogenic activities. This book also provides suitable approaches through bioremediation for evaluation, monitoring, assessment, modeling risks, and consequences available in megapolises and presents successful solutions to numerous environmental challenges. This book presents state-of-the-art information on the application of bioremediation of diverse environments such as run-off purification, soil polluted with heavy metals, waste management, and rejuvenation of polluted water and soil. The quest for sustainable, safer, and eco-friendly alternatives coupled with current advances in nanotechnology, biotechnology, artificial intelligence and internet of things, and soft systems such as biodegradation plants that utilize microbial agents under controlled conditions has become a more prospective alternative compared to customary channels. This book also adopts a swift narrative and analytical approach in articulating the critical and recent advances in the exploitation of artificial intelligence and internet of things technologies in the design, deployment, and management of waste biodegradation plants. This book was written by experts from multidisciplinary discipline and will be valuable for graduate and postgraduate students, teachers, academicians from environmental science, microbiology, chemistry, and biochemistry, soil scientists, physiologists, physicists, computer scientists, and stakeholders working in urban management. Therefore, for bioremediation to be effective for successful removal of contaminants in the atmosphere, soil as well as water bodies, researchers, policymakers, and governments need to intensify more effort towards the identification of the cause, dissemination of necessary information, and provision of necessary enlightenment and education of people on the merits of bioremediation in ensuring environmental sustainability, most especially to ensure the maintenance of a cleaner environment and the safety of the ever increasing population.

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Introduction

It has been observed that there is a daily increase in the world population. Moreover, this number will increase drastically to an astronomical figure in the year 2050. The higher increase in the population also constitutes a higher level of anthropogenic activities recorded globally. These anthropogenic activities have led to a higher level of dilapidation of the ecosystem throughout the world, thereby making the environment unbearable for adequate sustainability and survival of native biological forms as well as led to a reduction in the level of the natural resources that is still available. Furthermore, the issue of environmental pollution has become a global concern over the years which affects the quality of life coupled with the uncontrolled release of contaminants into the water bodies and soil. These challenges have become aggravated also through widespread industrialization; inappropriate agricultural techniques which involve accidental discharge and excessive application of pesticides and heavy metals into the water bodies and lands have contributed to the higher level of pollution in the ecosystem on the earth. This has led to inappropriate utilization of the scarce natural resources, loss of biodiversity, enhancement in barren fields, and challenges of high level of pollution in most water bodies which have led to scarcity of potable water and enormous economic damages which are very complex to even estimate.

It has been discovered that the rate at which synthetic substances are generated day after day increases xenobiotic and recalcitrant pollutants. Most of these hazardous chemicals liberated into the environment have been estimated at 10 million tons worldwide. Typical examples of such hazardous chemicals are polychlorinated biphenyls, pesticides, polycyclic aromatic hydrocarbons, total hydrocarbons, and heavy metals which have led to a high level of contamination in water and soil. Most of these contaminants have been observed to include hazards to the environment and health impairments. Heavy metal pollution has been observed as a typical example of contaminants that have a detrimental effect on human beings and portend the potential to disrupt ecosystem. There are several processes that have been recorded to lead to a high level of heavy metal in the environment through several activities from lead-based paints, leather industries, refineries, vehicle exhausts, metal processing units, pharmaceutical chemicals, waste incineration, agrochemicals, fossil fuel burning, electronic wastes, nuclear power plants, and plastics. The high level of this heavy metal in the environment has led to a high level of contamination in the environment, which has affected the level of water and soil, making them unfit for usage as well as causing numerous adverse effects on the ecosystem.

The recent advances in agriculture have led to tremendous green revolution over the years, which have led to an increase in agricultural productivities with numerous adverse effects on the environment due to effects of pesticides. This has resulted in a high level of pesticide residues in food, water bodies, and soil as well as affects the food chain. This has resulted in nontarget effect of numerous beneficial microorganisms and detrimental effect on soil carbon, soil nutrient, and soil enzymes. Also, this has led to a high number of mortality in animals, birds, and food poisoning in human beings.

Furthermore, the incidence of oil spillage has led to a high level of pollution of several water bodies, which wreaked havoc most especially in the marine ecosystem leading to a high level of mortality of fauna and flora as well as a decrease of species, most especially the endangered ones, and a decrease in the population of beneficial microorganisms. Also, the presence of oil spillage has resulted in the development of layers leading to a decrease in oxygen level and liberation of harmful pollutants, making the mainly available agricultural soil non-fertile.

Moreover, the adventure of unstable climate changes has led to constant enhancement in the release of greenhouse gases, which has led to deprivation of ecosystems that might be linked to an increase in droughts, floods, and extreme temperatures. Moreover, this has also led to the incorporation of pollutants leading to salinization of land and higher rate of desertification which has led to a high level of agricultural productivity as well as reduction in the level of available biological resources and destruction of significant biodiversity available in the ecosystem. Therefore, since there is an increase in the human population, there is a need to search for more resources and the preservation of numerous natural resources. Hence, there is an urgent need to search for techniques that could be utilized for the maintenance of a cleaner environment, most especially the removal of toxic contaminants from the ecosystem, improvement of saline soil, reclamation of waste and marginal lands as well as rejuvenation of heavily polluted environment such as soil, ocean, and polluted water bodies.

Over the years, numerous unadventurous techniques such as thermal, physical, and chemical process have been utilized for cleaning and rejuvenation of the ecosystems. There are numerous demerits that are related to these processes, which lead to exorbitant economic implications involved in the treatment, generation of toxic intermediates, transport of polluted water/soil for treatment, and ineffective revitalization of natural flora and fauna. Therefore, the application of bioremediation and biodegradation approaches entails biological systems such as microorganisms and metabolites, and the purified compounds derived from these beneficial microorganisms could be applied for effective rejuvenation of the heavily polluted environment. This might be linked to the fact that they are cost-effective, biocompatible, sustainable, ecofriendly, and highly capable of reducing the level of contaminants in the environment.

Moreover, the application of genetic engineering has been identified to play a crucial function in the bioremediation of heavily polluted environment. This has led to enhancement in enzyme features and the cell membrane transportation, which increase the spectral degradation of contaminants. The application of pyrosequencing, a next-generation molecular approach, has been identified to play a functional role in numerous studies that involve pollutant-microbe interactions. This technique also stimulates the environmental reaction of microorganisms to pollutants, enhancement in the biodegradability potential of fungal degrading genes in soil from various diversities, and microbiome resistance against contaminants especially in heavily polluted environments. The application of exopolysaccharides, biosurfactant, and biofilm has been documented. Moreover, numerous biotechnological techniques such as microbial desalination cells, biofiltration, microbial fuel cells, bioelectrical wells, and microbial electrolysis cells have been documented. The application of recent advances in biotechnological techniques such as fluxomics, metagenomics, bioinformatics, metabolomics, and genomics has been utilized for the identification of genes that play a crucial role in the bioremediation of heavily contaminated environment. The application of bioaugmentation for the bioremediation of heavily polluted environment using beneficial microorganisms was also documented. Therefore, this book provides several biotechnological techniques that could lead to a high level of sustainability; maintenance of ecosystem and the removal of numerous anthropogenic activities could lead to the provision of a healthy planet that supports environmental sustainability without any adverse effect on the ever increasing population.

Contents

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Naveen Kumar Arora, PhD in Microbiology Fellow of International Society of Environmental Botanists (FISEB), is Professor and Head, Department of Environmental Science at Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India. He is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is plant-microbe interactions, particularly plant growth promoting rhizobacteria. He has more than 75 research articles published in premium international journals and several articles published in magazines and dailies. He is an editor of 25 books, published by Springer. He is a member of several national and international societies, Secretary General of Society for Environmental Sustainability, in editorial board of 4 journals, and reviewer of several international journals. He is also the editor in chief of the journal "Environmental Sustainability" published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has a long-standing interest in teaching at the PG level and is involved in taking courses in bacteriology, microbial physiology, environmental microbiology, agriculture microbiology, and industrial microbiology. He has been advisor to 134 postgraduate and 11 doctoral students. He has been awarded for excellence in research by several societies and national and international bodies/ organizations. Although an academician and researcher by profession he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is the President of Society for Conservation of Wildlife and has a dedicated website www.naveenarora.co.in for the cause of wildlife and environment conservation.

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Chapter 1
Strain Improvement and Mass Production of Beneficial Microorganisms for Their Environmental and Agricultural Benefit

A. M. Ugbenyen and O. P. Ikhimalo

Contents

Abstract Microorganisms are ubiquitous and are also the most abundant in nature. They have been of immense benefits in the spheres of agriculture, food, and the environment; it is thus necessary that beneficial strains be improved. The methods by which microbial strain could be improved include the conventional method and the classical or nonconventional method. Conventional strain improvement techniques include mutation, protoplast fusion and transduction, conjugation, and transformation while the nonconventional technique includes genetic engineering, genetic recombination, rDNA, DNA, and whole-genome shuffling. Some benefits of strain improvement method like mutation are that unwanted enzymatic activities are blocked and adverse regulations are eliminated. Protoplast fusion and transduction combines the beneficial traits of different strains. Improvement of microbial strains by controlling the gene products of targeted DNA molecules could be achieved using recombinant DNA technology. The goal of any strain improvement method is for the overproduction of primary metabolite otherwise needed by the

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microorganism in quantities which would be considered insufficient by the industry or for industrial purposes.

Keywords Microorganism · Strain improvement · Conventional methods · Nonconventional methods · Beneficial · Overproduction · Metabolite · Industrial purposes

1.1 Introduction

Microbes are the most phylogenetic diverse and abundant group on earth; it comprises archaea, bacterial, cyanobacteria, fungi, and viruses (Vitorino and Bessa 2017; Panizzon et al. 2015; Tsiamis et al. 2014). Consequently, considerations of a Gnotobiotic Life: a concept of the existence of animals in total isolation from microorganisms may be unrealistic (Gilbert and Neufeld 2014). There is a long history of the use of microbes dating before the use of yeast by the Egyptians in bread making and its exploitation for wine production in China around 5000 BC (Demain 2014; Vitorino and Bessa 2017). Detailed history on industrial microbiology has been outlined in the work of Demain (2014) and Kumar and Chordia (2017).

The discovery of the DNA structure and recombinant DNA technology spurred the industrial use of microbes in biotechnology and has also widened the scope to which these microbes can be exploited. In human health, microbes are vital in sustaining health and have played several roles in immunity, metabolism, and drug interaction (Kumar and Chordia 2017) with the discovery of penicillin in the Second World War stimulating the discovery of other antibiotics.

Products of microbial metabolism such as enzymes, amino acids, vitamins, and chemicals are produced in quantities sufficient for each microbe. Exploitation of such microbes for industrial scale quantity has led to industrial fermentation which uses living organisms to produce large scale commercial products.

Advances in the microbial metabolism have been of immense benefits in the spheres of health care, agriculture, food, and the environment; it is thus necessary that beneficial strains be improved. The core drive of strain improvement is to enhance the productivity of primary metabolite, to alter metabolite not utilized, to ameliorate the usage of carbon and nitrogen sources, and to separate by-products of the cell through the enhancement of the cells morphology (Ashwini et al. 2014). We discuss the application of microbes in agriculture and environment and further elucidated processes of improving these microbes for maximal production of desired products.

1.2 Strain Improvement

The goal of any strain improvement method is for the overproduction of primary metabolite otherwise needed by the microorganism in quantities which would be considered insufficient by the industry or for industrial purposes. There are two major means of improving strains, and these include the classical or nonconventional method and the conventional method. Conventional strain improvement techniques include mutation, protoplast fusion and transduction, conjugation, and transformation while the nonconventional techniques include genetic engineering, genetic recombination, rDNA, DNA, and Whole-genome shuffling.

1.2.1 Conventional Strain Improvement Techniques

1.2.1.1 Mutation

Mutation has a proven track record as an indispensable tool in the field of biotechnology; its use in the improvement of strains of microorganism for the production of antibiotics, enzymes, amino acids, and other primary metabolites is priceless. Thus, it is widely adopted in industrial fermentation and pharmaceutics. X-ray mutagenesis of *Penicillium chrysogenum* X-1612 presaged the incorporation of mutation in industrial microbiology (Adrio and Demain 2006). Most industrially important microorganism has been improved at some point with mutation. Moreover, Derkx et al. (2014) maintained that mutation and other classical techniques are the generally accepted means by consumers and producers in the food industry due to the tight regulation of the application of recombinant DNA technology for improving strains.

Mutation is a heritable change in the genetic sequence of an organism; this which is important in evolution can be used to improve strains and its performance through a number of ways. In the use of mutation as a strain improvement tool, it must be followed by the characterization of the variants and by selection to identify the desired mutant (Magocha et al. 2018). Mutation could be spontaneous or induced in which case, DNA interacts with DNA-damaging agent called mutagens. Mutagens are either physical or chemical in nature; physical mutagens include UV rays, X-rays, and gamma rays. Amongst all UV rays is reported as one of the best of physical mutagens to improve yield and has been extensively used to achieve successive mutations in microbes (Ashwini et al. 2014).

Chemical mutagens include ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), diethyl sulfate (DES), nitrosoguanidine (NTG, NG, MNNG), and Nitrous acid. Both forms of mutagens have several effects on the genetic sequence varying from single base changes, structural distortion, and DNA backbone damage (Allison 2007).

Although classical, benefits are that unwanted enzymatic activities are blocked and adverse regulations are eliminated. Time consuming and strenuous nature, development of nondesired mutant, and the limited chance to increase the gene pool are disadvantages of mutation (Fiedurek et al. 2017; Anusree and Nampoothiri 2015).

1.2.1.2 Protoplast Fusion

The term protoplast was first introduced by Hanstein in 1880; a protoplast is a cell without a cell wall. In protoplast fusion, the cell wall of the two parent of interest is digested away and the genes or genetic material is combined to generate a fusant or heterokaryon containing the genome of both parent. Selection of fusants to identify the variants of interest and its regeneration is paramount to the success of this process (Bhatia 2015).

This technology is a vital tool in hybrid and cybrid development and genetic recombination in bacteria and fungi (Patil et al. 2015, Hayat and Christias 2010), as it combines the beneficial traits of different strains (Hassan 2014; Mohamed and Haggag 2010). Isolation of organelles and mutant can be achieved effortlessly with protoplast fusion, ultrastructural studies and the study of membrane is uncomplicated; in addition, single-cell cloning can be accomplished without difficulty, it allows for the combination of genes of unrelated or distantly related species thus routing out sexual incompatibility (Tapingkae et al. 2012). It is also vital in biochemical analysis if the protoplast has also been used in hormonal study, in examining the interactions of variously labeled proteins, and in investigating the effect of stress factors (Faraco et al. 2011).

Isolation of Protoplast

The isolation and culture media used vary with the species and with the tissue from which the protoplasts were isolated. Protoplast is isolated by two major means: Mechanical and enzymatic method. In both processes, digestion is done in a hypertonic solution to plasmolyze the cell for easier removal of the cell wall. Isolation requirement is specific to species and source tissue or cell of protoplast is dependent on conditions such as culture media, temperature, and incubation time (Lakhani and Vakharia 2016). Moreover, in the work by Maren (2016) duration of exposure to enzyme is said to increase yield.

Mechanical Isolation: This is an obsolete method involving plasmolysis; however, the yield is very low and of poor quality with a poor culture performance.

Enzymatic Isolation: This is the use of lytic enzymes to digest the cell wall and can be achieved with the use of commercial enzymes such as cellulase, pectolyase, macerase, helicase, macrozyme, and rhozyme. This process can be done either sequentially or enzymatically by disaggregating cells in one step by pectinase before digesting the cell wall or simultaneously by enzymatically disaggregating with pectinase and digesting the cell wall at the same time; thus, reducing contamination and time (Bhatia 2015).

Method of Protoplast Fusion

The isolation of protoplast which is spherical in shape is subsequently followed by fusion (Fig. 1.1). In fusing protoplast, there are two major means which could be spontaneous or induced methods.

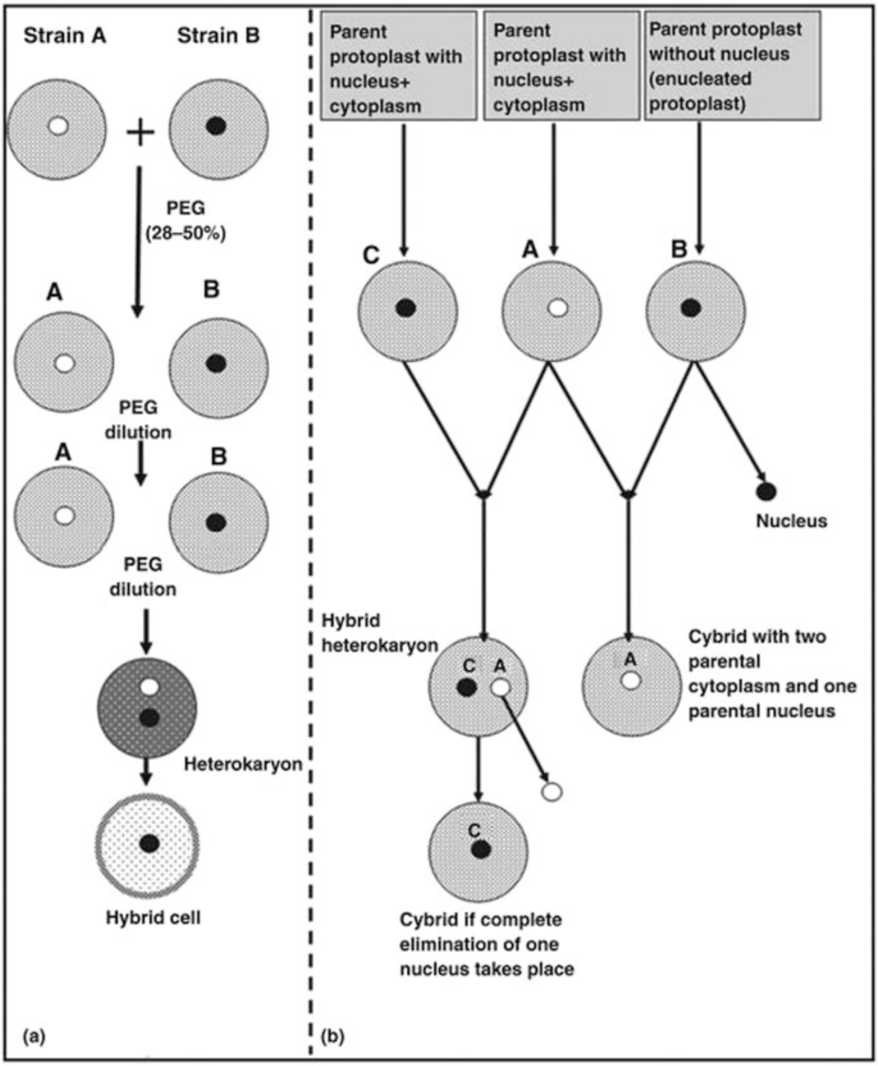

Fig. 1.1 Protoplast fusion with the use of polyethylene glycol (**a**) and hybrid production through protoplast fusion (**b**). (Adapted from Bhatia 2015)

Spontaneous

In this method, in the course of isolation, adjacent connecting protoplast fuse freely to create multinucleate protoplasts.

Induced Methods

The surface of protoplast are negatively charged and as such repel each other; therefore, a fusogen is required to overcome this charge barrier and to enhance fusion. Fusogens are fusion inducing agent and can be classified based on their nature, they include:

Chemical Fusogens: A number of chemicals have been exploited in the fusion of protoplast, examples include calcium ion (Ca^{2+}), polyethylene glycol (PEG), and sodium nitrate ($NaNO_3$). In the use of PEG by Lakhani and Vakharia (2016), attraction of pairs of protoplast was observed, followed by the breakdown of the plasma membrane and fusion of protoplasmic content to a single, large, oval or round shaped structure. Bhatia (2015) stated that a combination of PEG and calcium ion is the most effective of the chemical fusogens, the molecular weight and concentration of the PEG used is crucial to the frequency of fusion and yield, respectively (Lakhani and Vakharia 2016; Patil et al. 2015). Patil et al. (2015) generated hybrids with the combination of calcium chloride $CaCl_2$ and PEG in the protoplasts fusion of *Aspergillus oryzae* and *Trichoderma harzianum* (Table 1.1). Chemical fusogens have also been incorporated with mutagenesis in the production of hybrids as seen in Mohamed and Haggag (2010).

Electric fusogens: Induction of fusion can also be achieved by the application of a potential difference in the electrode containing protoplast suspension to generate a dielectrophoretic dipole; thus, a chain arrangement is formed. Thereafter, the application of an electric field with high strength for a period of microseconds consequently collapses the membrane leading to fusion (Verma et al. 2008). Electric fusion is favored over chemical fusion reasons being that it is easy to control, higher yield of fusants is obtainable, and cell division and regeneration are more efficient (Tapingkae et al. 2012).

1.2.2 Nonconventional Strain Improvement Techniques

1.2.2.1 Genome Shuffling

Genome shuffling (GS) originates from protoplast fusion, in this strain improvement method; recurrent protoplast fusion is done from already improved parent strains. Parent strains are improved majorly via classical method with mutagenesis being the most used; however, the use of genetic engineering is recorded (Fields et al. 2019). In this method, more than two parent strains can be used as the parent library in each generation as opposed to just two parent strains of protoplast fusion (Gong et al.

Table 1.1 Protoplast fusion method for improved product

Parent strain	Fusant	Desired products	References
T. reesei *Saccharomyces cerevesiae*	Tr x Sc	Better ethanol production than individual parent strain	El-Barkly (2006)
T. reesei *A. niger*	Tr x An	High cellulases production High endo and exoglucanase production High β-glucosidase production	El-Barkly (2006)
Trichoderma reesei strain PTr2 *Trichoderma reesei* strain SFTr2 *Trichoderma reesei* strain SFTr3	PTr2 x SFTr2 or PTr2 x SFTr3	High CMCase activity and more than twofold increment in enzyme activities when compared with high CMCase activity with 80% of fusants and more than twofold increment in enzyme activities	Prabavarthy et al. (2006)
Streptomyces griseoflavus	Intraspecific protoplastfusion	Increase production of desferrioxamine B	Nazari et al. (2005)
Saccharomyces diastaticus *Saccharomyces uvarum*	Sd x Su	Produce low carbohydrate beer of acceptable flavor in comparison with parent strain	Janderova et al. (1990)
Schwanniomyces occidentalis ATCC 48086 *Saccharomyces diaastiacus* ATCC13007 and *Saccharomyces cerevisiae*	Intraspecific protoplast fusion	Improved the ability to synthesize amylolytic enzymes	Rygielska (2004)
Nodulisporium sylviforme	Intraspecific protoplast fusion	Improved the ability to synthesize 4 enzymes lywallzyme, snailase, lysozyme, and cellulase	Zhao et al. (2004)
Helminthosporium gramineum subsp., *Echinchloae* (HGE) strain, HMI and *Curvularia lunata* (CL)	Hg x Ex Cl	Improved spore productivity and increased production of Phytotoxinophiobolin A compared with HMI	Zhang et al. (2007)
Arthrobacter simplex USA 18 *Arthrobacter simplex* US 3011	Intraspecific protoplast fusion	Cholesterol oxidase activity with 20–60% higher than that of parental strains	Liu et al. (1996)
Claviceps purpurea strains	Intraspecific protoplast fusion	Tenfold higher alkaloid production than parental strains	Brumec et al. (1993)
Thermotolerant *Saccharomyces cerevisiae* and mesophilic, xylose-utilizing *Candida shehatae*	Electrofusion	Stable and used for lignocellulosic fermentation and gave an ethanol yield of 0.459 g/g productivity of 0.67 g/L/h and fermentation efficiency of 90%	Pasha et al. (2007)
Bacillus thuringiensis spp. (H14)	Electrofusion	1.48 time more δ-endotoxins than wild type	Yari et al. (2002)

(continued)

Table 1.1 (continued)

Parent strain	Fusant	Desired products	References
Penicillium chrysogenum and *Cephalosporium acremonium*	Fusants	Produced a novel lactam antibiotic	US Patent 7241588
Trichoderma sp. Gen 9 (P1) and *Cladosporium* sp. Gen 20 (P2)	Fusants	Better hyper-yield of the enzyme L-asparaginase and L-glutaminase with powerful antimicrobial, antioxidant, and anticancerous activity was achieved from fusants than parent strain	El-Gendy et al. (2017)
Two parental isolates of *Trichoderma*	Fusant	Exhibited a potent antagonistic action against pathogens of *Macrophomina phaseolina*, *Pythium ultimum,* and *Sclerotium rolfsii*	Hassan (2014)

2009); it is a favorable technique for improving the phenotypes of microbes. Evolution of Strains of microbes with desired phenotypes can develop from this process due to the recombination or reshuffling of genes (Shi et al. 2018; Biot-Pelletier and Martin 2014). The genome diversity of population can be enhanced through this means (Fields et al. 2019). Furthermore, genetic basis of complex phenotypes of important industrial microbes has been applied in the improvement of yield of bio-substance as seen in the increase of the nucleoside yield of *Cordyceps kyushuensis* (Wang et al. 2017), used to improve the yield of Iturin of *Bacillus amyloliquefaciens* with a better potential against *Saccharomyces cerevisiae* that is tolerant to spent sulfite liquor (SSL) (Shi et al. 2018), used in a population-wide genomic survey of improved strains of *Saccharomyces cerevisiae* that is tolerant to spent sulfite liquor (SSL). Enhanced expression of the GS also provides a platform for a quick improvement of secondary metabolite (Shi et al. 2018). An example is enhanced-antifungal activity of *Lactobacillus plantarum* that was also achieved through GS (Wang et al. 2013).

GS has been applied to facilitate the uptake and conversion of substrate by microbes, thus enhancing the strain tolerance also.

In Zeng et al. (2016), an industrially satisfactory yield of Poly-γ-glutamic acid (γ-PGA) was achieved through the genome shuffling of *Bacillus subtilis* to produce strain *B. subtilis* F3-178. Metabolic analysis links up this high yield of γ-PGA to increased intracellular flux and uptake of extracellular glutamate.

Moreover, Adaptation to stress environment has also been achieved through GS. The fermentation of xylose is native to *Scheffersomyces stipitis*, its genome includes genes for the bioconversion of lignocellulose and is also able to ferment sugars (Balagurunathan et al. 2012).

Hardwood spent sulfite liquor (HSSL) rich in xylose is obtained from the by-product of acid sulfite pulping. The yeast *Scheffersomyces stipitis* which can utilize xylose to form ethanol in fermentation process is however hindered by acetic acid and lignosulfonates toxic to this microbe. *S. stipitis* C4 obtained through GS is better adapted to un-detoxified HSSL from eucalyptus than parent strain (Pereira et al. 2015). The titers of microbial products could hinder the production of such products, and hence inhibiting production. It is thus of a necessity if GS was applied in Máté de Gérando et al. (2017) to improve solvent tolerance to isopropanol/butanol/ethanol (IBE) production in *Clostridium beijerinckii* DSM 6423.

A technique known as inactivation is of necessity in GS to inactivate the fusion product, and is usually achieved by the treatment with high temperature or UV. Selection of improved variant is critical to GS process. Selection process is one of the bottlenecks of GS and the genetic basis of improvement is yet to be expounded (Skerker et al. 2009).

1.2.2.2 DNA Recombinant Technology

Improvement of microbial strains by controlling the gene products of targeted DNA molecules could be achieved using recombinant DNA technology. This technology has opened up a vast field of knowledge that has positively impact advancement to human life. Through recombinant DNA technology, crucial gene products (proteins) needed to solve human health challenges have become available and affordable. These products are also safe and could be produced in sufficient amount (Khan et al. 2016). In the work of Khan et al. (2016), these technology which includes gene therapy and gene modifications also find application in the treatment of life-threatening diseases and in environmental bioremediation. This implies that the technology could be employed in various disciplinary applications.

According to Berk and Zipursky (2000), recombinant DNA technology involves the alteration of DNA molecule outside a living cell or organism using important tools such as restriction enzymes, plasmids, and DNA ligases to obtain enhanced and desired recombinant molecule which can give desired products when expressed in living organisms. The technique involves the insertion of DNA fragments from various sources, having the desired gene sequence into an appropriate vehicle (plasmid). This gene manipulation is carried out by introducing the recombinant molecule with either one or more novel genes and regulatory elements or by reducing or completely blocking the expression of endogenous genes (Bazan-Peregrino et al. 2013). Restriction endonucleases are used to cleave DNA molecule to obtain fragments of DNAs, though the restriction enzymes cut only at specific sites with recognition sequence. It is also used to cleave circular plasmid vector. DNA ligase enzyme link or join the DNA fragments with the desired characteristic with the plasmid. The plasmid is introduced into an organism which serves as host through a process called transformation. The transformed host grows to produce many copies of the desired DNA fragment, when cultured in a plate. These multiple copies are referred to as clone which will be selected and harvested for use (Venter 2007) (Table 1.2).

Table 1.2 Applications of recombinant DNA technology

Organism	Recombinant DNA technology	Desired product	References
Microbial strains	Specific genetic engineering	Production of proteases	Olempska-Beer et al. (2006)
Strains of fungi	Genetic modification	Reduced toxic materials production	Olempska-Beer et al. (2006)
Microbial strains	Specific genetic engineering	Production of lysozymes used for inhibition of food spoiling microbes	Lian et al. (2012), Bang et al. (2011), Thallinger et al. (2013)
Microbial strains	Specific genetic engineering	Production glucose oxidase for inhibition of biofilm producing bacteria and food spoiling microbes	Torres et al. (2011)
Microbial strains	Specific genetic engineering	Derivation of recombinant proteins being used as pharmaceuticals	Ma et al. (2003)

1.3 Microbial Cell Production for Environmental Benefit

Advances in civilization also came along with perturbations to the environment some of which over time had negative consequences to human, animal, plant, and microbial lives. It is important that these perturbations be critically checkmated to halt their debilitating effect. At the crux of the human society are a number of environmental issues such as heavy metal pollution of land, air, and water; eutrophication; and waste management among others. Microbes may provide some solutions to some of these environmental dilemmas which if adequately taken care of would better the human race.

Sustainable manufacturing is hinged on clean technology with minimal negative impact on the environment. Natural endowment of some microbes which impact on them the potential in waste management has been studied and new strains are developed.

Some countries are still dependent on petrochemicals as a major source of fuel; our world is yet to recover from the debilitating effect of by-products of petrochemical combustion some of which are implicated in acid rain and global warming. There is also a concern on the finiteness of fossil fuels prompting the need for other energy alternatives. The generation of fuels from Lignocellulosic biomass can supply the energy needs of our ever-growing populations thereby reducing the concomitant dependence and adverse effect of by-products of petrochemical combustion.

Lignocellulose biomass consist of lignin, cellulose, and hemicellulose; however, ash, minerals pectin, proteins, and salts may be present (Tsegaye et al. 2019; Cortes-Tolalpa et al. 2017). Agricultural waste contributes a great deal of lignocellulose biomass in form of feedstock such as vegetable and wood residues. Pretreatment of lignocellulose biomass is done to ease the availability of cellulose and hemicellulose by the removal of lignin which sometimes constitute up to 20% (wt/wt) or more of these biomass (Tsegaye et al. 2019; Yang et al. 2009) and can be achieved by several methods including the use of heat, chemicals, and microbes. The delignification ability of bacterial species such as Actinomycetes, Nocardia, Streptomyces, Eubacteria are recognized in literature as well as that of Fungi such as *Pleurotus ostreatus*, *Trametes versicolor*, *Lentinula edodes,* and *Phanerochaete chrysosporium*. Cellulotic fungi and bacteria are the most used in biological pretreatment; however, due to the poor amount of lignases produced by bacteria, the breakdown of cellulotic material is limited to low lignin-containing biomass (De Souza 2013), thus requiring strain development to increase this enzyme.

The process of energy formation from such biomass includes the biolignification, hydrolysis, and fermentation.

Several fungi and bacterial strain such as *Brevibacterium flavum* ATCC 13826, *Pseudomonas* sp. mutant 1167, and *Corynebacterium pseudodiphtheriticum* M2128 form products from lignin and lignin-derived aromatics in lignin vasolization. These products which have application cutting across several industries includes *cis, cis*-muconate, Vanillin, Ferulate, Pyridine-2,5-dicarboxylic acid, Pyruvate, Lactate, Succinate, PHA, and Lipid (Becker and Wittmann 2019). PHA are used to produce biodegradable plastic, vanillin is used in the food industries.

Every human has a right to potable water but this is not the case in several nations; it has been reported that about four billion persons face water scarcity at least once in a year (Mekonnen and Hoekstra 2016); therefore, it is absolutely necessary to find ways of checkmating this threat to human existence. Environmental problems such as eutrophication affect the quality of water in water bodies and may also cause a shortage of the supply of potable water (Yang et al. 2009). There is potential for the use of microbial enzymes to treat wastewater. Halophytic bacteria such as *Pseudomonas aeruginosa, Bacillus flexus, Exiguobacterium homiense,* and *Staphylococcus aureus* was used to treat high saline wastewater generated from the tannery industry (Sivaprakasam et al. 2008).

Single cell protein has created an avenue to reuse nutrients and biogas in wastewater generated from several industries in the formation of other molecules of importance (Verstraete and De Vrieze 2017). SCP has been applied to activated sewage sludge (Shier and Purwono 1994).

In the use of *Rhodopseudomonas faecalis* PA2 to treat sugar wastewater, substantial amount of SCP was recovered with the obtained biomass containing all essential amino acid as well as greater amount of leucine and lysine (Saejung and Salasook 2018). This can be channeled into commercial feed production where lysine is limiting (Mukhtar et al. 2017).

Plastic is a synthetic polymer with application in wide range of industries and its nondegradable nature once celebrated is now a source of concern; however its biodegradability is continuously being researched to avert its threat which include taking long time to decompose and the release of toxins in the form of plastic additives, and plastic (de Paula et al. 2018); concerns have also been raised on microplastics from plastic disintegration. The microplastics are of size less than

5 mm and could be produced from disintegration of plastic over time due to degradation, reaction with the sun's ultraviolent rays, and oxygen (Dris et al. 2015). Microplastic are ubiquitous in the marine and fresh water and have also been isolated from fish, sea-salt, air, food, and drink. A number of concerns have been raised on microplastic and these include contamination of human food and drink. Microplastics have been isolated from drinking water; however, there is yet result to confirm the effect on humans. In pisceas, it could lead to pseudo-satiation upon injection thus causing starvation. Leaching of harmful chemicals into the environment is also a concern, also they could absorb and distribute harmful organic substances. Isolation of microplastics from other aquatic animals may be a threat to humans.

These negatives of the use of conventional plastic necessitate an alternative. There are some reports on the plastic biodegradability of some microbes. *Brevibacillus borstelensis*, *Trichoderma* sp., *Clostridium botulinum, and Penicillium roquefort* are reported to play a role in the biodegradation process of plastic waste (Pathak and Navneet 2017).

Plastic biodegradation by microbes may take a long time; thus, researchers looked on to biodegradable plastic (biopols). Biodegradable plastic provides same service as synthetic plastic with an added benefit of being able to biodegrade and not adding to the carbon footprint. Polylactic acid (PLA) and PLB are examples of biodegradable plastics (Vitorino and Bessa 2017). PLA homopolymer and copolymer was produced from direct fermentation of engineered *Escherichia coli* (Jung and Lee 2011).

PHB is a lipoidic inclusion accumulated as a carbon reserve under nutrient limitation produced from bacteria including Gram positive, Gram negative, cyanobacteria, and archaea as part of normal physiological process (Gangurde and Sayyed 2012). Its production is initiated under conditions of low nitrogen.

Bioremediation is the degradation of environmental pollutants by living organisms. Microbial remediation of contaminated land by petrochemicals (Khan 2014) and oil spills have a negative impact on soil and water health as well as the living organisms contained in them. Some organisms have potentials in the reclamation of lands and water bodies affected by oil spills and these include heavy metal contamination of soil and groundwater is prevalent around mining site, effluents of textile, brewery, and industries (Pan et al. 2018; Emenike et al. 2018). The atomic level of heavy metals renders decomposition impractical (Pan et al. 2018).

In a study by Basha and Rajaganesh (2014), effluent of textile dye abounding with cadmium, lead, and zinc was bioremediated with *Escherichia coli, Salmonella typhi, Bacillus licheniformis*, and *Pseudomonas fluorescence*. Results revealed a reduction of 98.34% of cadmium, 94.83% of lead, and 96.14% from samples.

1.4 Microbial Solutions for Advances in Sustainable Agriculture

The focus of the world today is towards sustainable agriculture. The use of improved microbial strains for agricultural activities encompasses most aspects of agriculture and plant beneficial microbes. Several amino acids are limiting in the feed industry, such as lysine, methionine isoleusine, and arginine (Mukhtar et al. 2017).

The use of microbial cell factories (MCF) to produce amino acids, enzymes, vitamins, and minerals is sustainable, environmentally friendly, and cheap (Acevedo-Rocha et al. 2019).

Overexploitation of medicinal plants has rendered some of these plants to the status of endangered and extinct in severe cases, the use MCF has also proved of immense benefit in the production of plant natural products whose utilization cuts across several industries (Liu et al. 2017). Exploitation of MCF will preserve these endangered medicinal plant species.

Investigations by Singh et al. (2017), Rashid et al. (2016), and Vassilev et al. (2015) raised concerns on the use of chemical fertilizers known to increase crop yield. These concerns include the acidification of the soil, contamination of groundwater, eutrophication of water bodies through runoff, and decrease of microbial diversity. Moreover, other than the concerns stated above, population spikes have resulted in intensive cultivation of available land and subsequent degradation of such land through erosion and other anthropological effect, thus declining the productivity and fertility of the soil.

The plant environment including the root and rhizosphere is colonized by a variety of microbes including bacteria, algae, and fungi, some of which are beneficial and are known to promote plant growth and development (Timmusk et al. 2017). These beneficial microbes known as plant growth-promoting bacteria (PGPB) and plant growth-promoting rhizobacteria (PGPR) are able to promote the the fertility of the soil and productivity of the plants directly or indirectly through their biological activity; in the direct mechanism, PGPB and PGPR either enhances nutrient bioavailability or provides plant with a beneficial exudate produced by the bacterium while in the indirect mechanism pathogenic organism of plants and their adverse metabolite are inhibited (Alori and Babalola 2018). These mechanisms have been researched on extensively leading to the development of biofertilizers, biocontrol agents, bioherbicides, and biopesticides, which are inoculated into seeds and soils; microbial inoculation of seed is a means of introducing microbial biocontrol agent into the soil (O'Callaghan 2016).

Biofertilizers are formulations of microbes in the active or latent form with the potential of enhancing the nutrient availability of the soil when applied (Fediala Abd El-Gleel et al. 2018; Suyal et al. 2017).

Biofertilizers enhances nutrient bioavailability of otherwise scarce nutrient and are grouped into nitrogen fixers, phosphorus mobilizers, and potassium and iron mobilizers (Rashid et al. 2016). Rhizosperic microbes especially the arbuscular mycorrhizal fungi (AMF) also improves the soil structure by forming soil aggregates through the production of polysaccharides and other exudates. Inoculum of plant growth-promoting bacteria are prospective fertilizers (Alori and Babalola 2018).

PBPB are able to produce and regulate the levels of phytohormones. Plant growth hormones are regulators of plant growth and development and comprise auxin, gibberellin, cytokinin, and ethylene. Their functions include root initiation, shoot elongation, and fruit ripening.

PBPB and PBPR could also synthesize plant secondary metabolites such as alkaloids, flavonoids and phenols and microbial volatile organic substances. Biocontrol agents inclusive of biopesticides, bioherbicide, biofungicide, and bionematicide are environmentally friendly and biodegradable compared to their chemical counterpart; in addition, they target specific pest. Examples of microbes used in this terrain includes *thurigenesis* which has been applied in developing plant such as the Bt cotton and the Bt maize, resistant to another is the *Trichoderma* species which is resistant to some plant pathogenic fungi. Montesinos (2003) highlighted a number of bacterial and fungi-based biocontrol agents registered in some countries; examples are: *B. subtilis* GB03 used against soil-borne and wilt caused by fungi; *Pseudomonas fluorescens* against soil-borne fungi, *Verticillium lecanii* against Whitefly; and *Trichoderma polysporum* and *T. harzianum* against soil-borne fungi. The mechanism of action of microbes used as biocontrol agents includes competitive exclusion through nutrient colonization, antibiosis, production of antimicrobial substance, induction of resistance and defense mechanism of the plant host to pathogenic microbes, and degeneration of signal messengers essential for quorum sensing (Bonaterra et al. 2011).

Seed bio-priming is a method of inoculating PBPR in seeds before sowing. The livestock industry of the agricultural sector is not left out; the amino acid lysine, methionine, and threonine are limiting factors in feed (Mukhtar et al. 2017), and microbial fermentation for the production of this amino acid is favored over chemical synthesis.

1.5 Conclusion

This chapter presents various methods of improving microbial strain for the maximal production of their metabolite which is of great benefit in agricultural and environmental sectors. Conventional and nonconventional methods of strain improvement have resulted in several benefits in various industries. Mutation and protoplast fusion were discussed in detail as a conventional method of improving microbial strain to optimize the production of the desired product of the microbial cell factory. Genome shuffling and DNA recombinant techniques were also discussed as nonconventional means of microbial strain improvement. The goal of any strain improvement method is for the overproduction of primary metabolite otherwise needed by the microorganism in quantities which would be considered insufficient by the industry or for industrial purposes.

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Chapter 2
Biochemical Role of Beneficial Microorganisms: An Overview on Recent Development in Environmental and Agro Science

Olugbemi Tope Olaniyan and Charles Oluwaseun Adetunji

Contents

Abstract The whole universe is currently under a greater pressure in order to meet the world's growing demands on food security and sustainable environment, agricultural production must double in terms of food production by the year 2050. To achieve these objectives, there must be a deliberate attempt by all the necessary stakeholders to harness every alternative method available that could be utilized towards improving the agro science sector while preserving environmental health. Recently, there has been serious attention focusing on the interaction between beneficial microorganism and plant such as acquisition and uptake of essential nutrients, plant growth and development, pathogenic control and improved soil microenvironment and structure in order to boost agro science sector. Many beneficial microorganisms are being utilized across the globe such as *Bacillus* spp., *Pseudomonas* spp., *Rhizobia* spp., *Streptomyces* spp., *Azospirillum* spp., *Mycorrhizae* spp., and *Trichoderma* spp. The utilization of molecular biology techniques will

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help to unravel the physiological and biochemical importance of beneficial microorganisms in agro science sector and also the possible advances in food biotechnology.

Keywords Beneficial microorganism · Molecular biology · Environmental health · Food biotechnology · Agro science

2.1 Introduction

The utilization of chemical fertilizers have witnessed a huge negative impact on the soil and agro products due to widespread production, circulation, utilization by farmers and government policies through subsidy and importations most especially across the world and agrochemical industries. Recently, due to the detrimental effects of these chemical fertilizers on the soil and agro produce, there is a need to search for a sustainable and a more nontoxic, eco-friendly, and cost-effective alternative biofertilizers to reduce the burden of chemical fertilizers derived from fossil fuel (Mazid and Khan 2014).

Biofertilizers are derived from beneficial microorganisms capable of generating biomolecules that can be converted to various products for the benefit of the environment and agro science sector. Even though much attention has not been given to these bioproducts, due to the dearth of information, relevant stakeholders can make much economic value from it if properly harnessed. Biofertilizers have the ability to make nutrients available for plants, enhance soil physiology and integrity, and of course protect the environment due to reduced toxicity level (Schulz et al. 2013). The production and utilization of a robust combination of agrochemical products with biofertilizers is gaining significant attention because of the potential of sustainability of environment and agricultural development through innovative research, public sensitization, and stakeholder's intervention (Jacoby et al. 2017).

Mosttafiz et al. (2012) revealed that through molecular biology techniques and biotechnology, quite a number of microbial biodiversity have not been discovered or exploited for economic value. The author also showed that microbial biodiversity represents a significant aspect of global biological diversity with a large portion of beneficial microorganisms capable of synthesizing different biomolecules for commercial gain. To develop new modern bioproducts from beneficial microorganisms careful selection of appropriate microbes and techniques must be carried out for environmental health and agricultural sustainability (Bravo et al. 2011). Different agricultural challenges such as disease control, water contamination, soil physiology and maintenance, crop yield, and quality together with environmental concerns such as waste recycling can be solved by utilizing appropriate microbial techniques (Classen et al. 2015). In the twenty-first century, global problems such as diseases, climate change, and food insecurity can be managed by employing modern development such as nanotechnology and electronics in combination with beneficial microbes (Bhattacharyya et al. 2016). These developments will ensure advancement in agricultural sustainability and environmental health.

Therefore, this chapter intends to provide a detailed information on the utilization of beneficial microorganisms for sustainable agricultural and environmental health. The various types of biochemicals that could be derived from beneficial microorganisms were also provided in detail. The application of these diverse biochemical in agroindustry and some other relevant sectors were provided in detail.

2.2 Biochemical Role of Beneficial Microorganisms

Plants and microorganisms cohabit together in their natural environment and form a rich ecosystem with diverse potentials in the soil, sea, and environments and play a significant role in mineralization, pathogenic control, and improved performance. Though there are still knowledge gaps to cover and several biomolecules to be exploited, in recent time, significant progress has been made in the functional dynamics and compositions of rhizospheric microbiomes as a potential replacement for artificial agrochemical inputs (Barea 2015). In the rhizosphere niche, microbiome structures are shaped and adapted to thrive through interactions with plants using a different alternative mechanism which are now being uncovered. Therefore, this chapter will show the interaction of root-based microbes and plant interaction enhancing nutritional status, mineralization, and recent knowledge adapted to improve our understanding.

The application of biotechnology in the area of agricultural and environmental sustainability is enormous and it is a new fast-developing filed in biological and medical sciences. Recently, there has been renewed interest on the use of bioflavonoids, biomolecules, and polyphenols from medicinal plants due to their widespread biological and pharmacological applications and benefit to health. Various functional and chemical evaluations like number of hydroxyl groups, configuration, and functional groups are ongoing to appropriately elucidate the potential metabolism, biological activity, and bioavailability of flavonoids linked with dietary supplements including vegetables and fruits (Drogue et al. 2013). Many bioflavonoids are utilized for various health issues such as in the management and treatment of cancer, inflammation, microbial infections, and cardiometabolic and endocrine disorders. Through the use of beneficial microorganisms interacting with plant system, diverse bio flavonoids have been generated from plants to combat or fight against pathogens, stress, and function as a growth modulator (Köhl et al. 2019).

Haile and Kang (2019) demonstrated that coffee is one of the most valued cash crops across the globe utilized by pharmaceutical and food biotechnology industries to produce different products. Different microbes capable of producing enzymes used in fermentation processes are employed in the production of food and beverages by food industries. Majorly in food industries during fermentation processes, meticulous attention is always given to such beneficial microbes with certain unique properties like biodegradation, suppression of pathogenic growth, mucilage tendency, tolerance to stress to enhance adequate yield, taste, aroma, and quality (Haile and Kang 2019). The utilization of beneficial microorganisms has contributed significantly to the progress and advancement of food industries most especially by increasing the neutraceutical attributes and improve the qualities of the food products such as taste, odors, and aroma.

Gupta et al. (2016) demonstrated that bioresources derived from excreta and cow dung can be a great potential source of biofuel, hence reducing the impact of fossil fuel on climate change and environmental pollution. The interaction of beneficial microorganisms with organic compounds can generate huge source of bioproducts, novel chemicals constituents, and different metabolites and if open up can serve as a new source of economy with enormous benefits such as enhancing soil fertility and nutritional status (Gupta et al. 2016). Today, many industrial sectors are now benefiting from the use of beneficial microorganisms with cow dung to generate alternative source of biofuel to increase productivity and reduce environmental pollution, hence reducing the hazardous effects of fossil fuel on climate change.

In pest management system, recent studies have revealed that to scale the challenges of using chemical in the fight against pathogens which have developed a high level of resistance, the use of biopesticides are becoming more efficient. The important role of beneficial microorganism towards the development of eco-friendly and sustainable pest management control strategies are being investigated to clarify the mode of action. Plants and animals are very susceptible to different disease pathogens that can affect agricultural yield, productivity, and environmental health (Wiesel et al. 2014). The application of beneficial microorganisms has been identified as a sustainable alternative solution to a more robust and improved biological control measures and improved products (Kachhawa 2017). Current practice of using synthetic chemicals in controlling pest and pathogens have been shown to be deleterious and hazardous to the environment health and are beginning to have decreased value. In order to improve plant growth, tolerance to biotic and abiotic stress, nutrient uptake, the utilization of biofertilizer derived from beneficial microorganism has become expedient in agro science for optimal food production and sustainable industrial sector (Bhardwaj et al. 2014).

Lily and Mary (2014) revealed that the potential role of beneficial microorganisms in the agro science sector and the biochemical importance in crop production and yield must be fully understood in terms of pathogen control, fertilization, molecular mechanism of action, side effects, and economic viability of beneficial microbes. Also Leowa et al. (2018) highlighted the important role of beneficial microorganisms in organic waste management and treatment. The authors revealed that extensive utilization of beneficial microorganisms can significantly contribute to biodegradation and elimination of pathogens. So much popularity has been witnessed in the use of beneficial microorganisms in waste treatment and management. Lignocellulosic components of cellulolytic microorganisms are particularly important in accelerating the biodegradation of waste through digestion by anaerobic mechanism (anaerobic digestion, which is described as a method in which microbes disintegrate biodegradable constituents without the use of oxygen) and composting at low-temperature method especially for heavy metal contaminates.

Microbial diversity, the use of probiotics and prebiotics and their important role in the body has become necessary for scientific study. Information on the beneficial microorganism and environment on balanced trophic chains, physiological activities, and conservation of natural habitats remains essential. It is generally known that beneficial microorganism contributes products capable of synthesizing vitamins and antimicrobial substances (Sangeetha et al. 2011). Karolina and Gajda (2018) revealed that soil represent the microenvironment in which the development and growth of plants take place together with the physiological interaction of beneficial microflora producing essential resources. Beneficial microflora are majorly responsible for biogenic element circulation, biomass decomposition making nutrient available to plants, maintenance of soil structure, and biodegradation. The survival of microorganisms in the soil depends on the moisture, pH, structure, and chemical nature. The activity of humans over the years has impacted negatively on the biological and chemical formation of ecosystem. Studies have shown that soil diversity and bioavailability can change tremendously if unsuitable agrotechnology is used (Arora and Sahni 2016). Synthetic utilization of various chemicals in agro science sector have significantly shifted and impacted negatively on the ecosystem. Girmay (2019) demonstrated that phosphorus is the major macronutrient necessary for all vital growth and developmental aspect of plants. Studies have revealed that phosphate solubilizing microbes (PSMs) are microorganisms with the ability to hydrolyze inorganic and organic degradable phosphorus molecules to soluble phosphorus type which can be integrated by plants due to scarcity of phosphorus.

Pagan et al. (2017) showed that the use of glyphosate can significantly improve microbial biomass in 14 days period of carbon and nitrogen content in about nine different soil types. These authors also showed that there was a significant increase at 30 days in soil microbial biomass treated with chlorpyrifos and cartap hydrochloride, which later reduced gradually. Soil earthworms, protozoa, and nematodes are equally influenced by the use of synthetic pesticides (Arora and Sahni 2016). Synthetic agro products are known to increase environmental CO_2 and pollutants, alter greenhouse gas emissions, stimulates loss of plant and soil biodiversity thereby affecting the ecosystems. Beneficial microorganisms are capable of stimulating organic matter decomposition and gases release playing a critical role in the soil ecosystem. Intercropping variety of plant species can increase carbon fixation by plants, and relocate carbon into the soil, via mycorrhizas, consequently modulating interplant collaborations (Pagan et al. 2017).

Rigorous agro science utilization of synthetic products to stimulate increased production of food is linked with high rate of greenhouse emission of gases resulting in climate changes and other environmental hazards. To mitigate these challenges without compromising yield is to employ the utilization of beneficial microorganisms (Bare 2015). Available studies have shown that soil microflora plays a significant function in the formation of weeds and invasive plants by linking up with microorganisms for their growth and development. Weed controlling strategies, such as the use of tillage and pesticides majorly distort the soil configuration together with alterations in the ecosystem. The establishment of new sequencing technologies for examining soil microbiomes has provided the possibility for deeper analysis. Taking advantage of the knowledge of beneficial microorganisms will offer the possibility for new improved bio control approaches against weeds based on soil and plant-linked microbes (Friederike et al. 2016).

Beneficial microorganisms' products are deployed to plants for genetic regulation of pathogens by acting through a range of pathways. It is generally known that the method of regulation may be through indirect interaction with plants via prompting resistance and preparing plants against pathogens without contact. Others may function through competitive nutrient deprivation or regulating developmental conditions in the pathogen. Drugs like antagonists function via antibiosis and hyperparasitism directly block the growth of the pathogen. These connections are modulated by series of metabolic processes and physiological signaling, often utilizing diverse mechanisms such as signaling molecules, metabolites, and enzymes produced in situ at a low amount during collaboration (Treichel et al. 2010). In recent years, studies have shown that biological method of controlling pathogens has become a very useful alternative which lowers the burden of climate change and soil ecological degradation commonly found with the use of synthetic chemicals. The mechanism by which these beneficial microflora elicit their effect could be through direct inhibition of soil-borne diseases or indirect effect on plant-controlled resistance reaction. The general mode of action is through competitive nutritional availability, parasitism or space, antibiosis, membrane degradation through hydrolyzing factors, and induction of resistance to diseases (Singh 2014).

Beneficial microorganisms like plant growth-promoting rhizobacteria (PGPR) perform an important function in the agro science sector. This is because PGPR is environmentally friendly and capable of aiding plant development via indirect or direct processes such as modulating hormonal receptor response, nutritional intake, disease resistance, and nutrient uptake, respectively. The soil nutritional level will determine the quality and yield of crops and healthy food produced (Itelima et al. 2018). Therefore, soil management strategies should be based on exploiting alternative measures to boost the integrity and physiological properties of soil using beneficial microorganisms as biofertilizers which are known to cause less threat to health and the environment. Some of the recently found microorganisms commonly used as biofertilizers include cyanobacteria, growth-promoting rhizobacteria, potassium and phosphorus solubilizers, endo and ectomycorrhizal fungi, and nitrogen fixers (Itelima et al. 2018). There are different beneficial microorganisms capable of promoting soil integrity and environmental health such as photosynthesizing bacteria, actinomycetes, lactic acid bacteria, yeasts, and fermenting fungi (Joshi et al. 2019). Also studies have shown that phosphorus is an important element in the adenosine triphosphate (ATP) transport system in cells for energy metabolism (Antouna 2012). Most of the resistance to pathogens and nutritional benefits of beneficial microorganisms are driven through high energy levels. Therefore, phosphorus solubilizers derived from microorganisms offer great benefits in this approach. Energy is released during these metabolic processes by the plants to drive the stimulation response in order to maintain the defense reactions.

Pereira et al. (2008) revealed that transgenic plant cultivated in a commercial value is the transgenic soybean which is resistant to glyphosate. These authors studied the influence of endosulfan, glyphosate, and their combinations on the microbial soil functions in soybean plant. Studies have revealed that endosulfan utilization alone or in combination with glyphosate can suppress CO_2 generation through beneficial microorganisms in the soil microenvironment. Microbial quotient plus biomass were reduced when treated using endosulfan alone and in those using endosulfan combined with glyphosate compared with glyphosate alone or control.

Šantric et al. (2018) showed the role of glyphosate or nicosulfuron herbicides on microbial function in two soils with diverse chemical and physical properties (sand and loam). Changes in urease plus dehydrogenase function, with microbial biomass carbon, were studied. The outcomes obtained by the authors revealed that the role of glyphosate or nicosulfuron depended on duration of activity, treatment rate, soil types, and test parameters. They concluded that the utilization of the herbicides increased the functions of urease plus dehydrogenase. Nicosulfuron had excitatory effects on microbial biomass carbon in loam, while both herbicides showed negative effects on the parameter in the sandy soil.

Köhl et al. (2019) revealed that microbial biological control agents (MBCAs) act in a wide range of mechanisms when used on crops to control pathogens in plants. Many MBCAs relate with plants through the induction of resistance without direct influence on the targeted pathogen. Other MBCAs act through competitive nutritional deprivation plus many other mechanisms controlling the developmental conditions for the pathogen. Hyperparasitism is the mechanism by which antagonists perform their biocontrol of pathogens and antibiosis directly inhibiting the pathogens which are sometimes controlled by a series of metabolic activities in diverse modes of action. The authors also revealed that many compounds are involved in such signaling, enzymes and other inhibiting metabolites are generated in situ at a reduced rate during collaboration. The prospect of microbes to generate compound in vitro does not essentially relate with their in situ inhibitory action. Presently, the risks of microbial metabolites implicated in antagonistic mechanism of action are sometimes assessed comparable to assessments of single-molecule fungicides. The dynamism of the mechanism of action of antagonists needs a reconsidering of data requirements for the cataloging of MBCAs.

Nielsen and Winding (2002) revealed that beneficial microbes are an important aspect of soil physiology and of extreme significance for soil health used as indicators of soil integrity. They revealed that the present plus the potential prospect use of microbes will account as an index of soil integrity and recommended particular microorganism indices for soil ecosystem parameters demonstrating policy pertinent endpoints. It was suggested that identification of a particular minimum data for exact policy pertinent endpoints, plus judiciously establishing standard values, to advance body of knowledge on biodiversity plus modeling of soil data, and to apply new indices into soil evaluation programs where applicable.

Haney et al. (2002) revealed that the use of synthetic chemical herbicides in soils can potentially destroy the integrity and physiological composition of microbial activity. The amount and incidence of Roundup Ultra (RU; *N*-(phosphonomethyl) glycine) utilization have been seen to worsen Roundup-tolerant crops. They studied the role of Roundup Ultra on soil microbial activity and biomass in diverse range soils with different fertility levels using isopropylamine salt of glyphosate. This significantly activates soil microbial activity measured by nitrogen and carbon mineralization, together with microbial biomass which increased beyond the basal level in all the verified soil. They discovered that there is a positive relationship between soil microbial mineralization and the nitrogen and carbon contents of isopropylamine salt of glyphosate suggesting that RU was the cause of the elevated microbial activity.

2.3 Recent Development in the Biochemical Role of Beneficial Microorganisms

2.3.1 Fermentation Processes

Fermentation was traditionally used as a process to preserve perishable foodstuff in the past until recent novel preservative methods have evolved; therefore, the significance of fermentation as a preservative method has reduced, and hence today the major purpose of food fermentation is to produce diverse forms of fermentation products with different aroma, taste, texture, and flavor. The use of beneficial microorganism subjected to different fermentation conditions (substrates, microorganisms, fermentation period, temperature) plus biochemical engineering (industrial fermentation) accomplishments have made it conceivable to produce thousands of different kinds of dairy products, meat products, vegetable, alcoholic beverages, bread, vinegar, other food acids and wine together with oils and cosmetics products.

Bourdichon et al. (2011) revealed that fermentation plays diverse functions in food processing and agro science industries. Some of the important roles are: maintenance of food via creation of inhibitory metabolites, like lactic acid, formic acid, acetic acid, and propionic acid which all organic acids, bacteriocins, generally combined plus decrease of water activity (use of salt or by drying), ethanol, improved food safety level via antagonizing pathogenic organism plus elimination of toxic molecules, improved nutritional status, improved sensory quality, and enlargement of the diet for more diversity. For most impulsive fermentations, beneficial microorganisms sequence takes place: more often lactic acid bacteria (LAB) will first control the process, next series of yeasts. Molds will then grow aerobically that considerably contribute by generating lipolytic and proteolytic enzymes that extremely impact the texture and flavor of the products, thus limiting their existence in certain kinds of fermented products. LAB generates antimicrobial substances and lactic acid that can block the growth of dangerous bacteria together with lowering the sugar content, hence elongating the shelf life (Treichel et al. 2010).

Vogel et al. (2011) demonstrated that the addition of bacteriocin-generating strain starter cultures alone or in mixture with certain bacteriocin-resistant strains of beneficial microorganisms have become popular in food industries. In the manufacturing of wine and beer, yeasts and molds generating single-strain starter cultures are generally used with diverse aroma and taste, and LAB for the generation of dairy products with elongated shelf life. Also for sourdough, dairy products, wine, and sausages, multiple starter cultures are often utilized. The application of this biochemical compounds offers a great improvement in food biotechnology and environmental health.

2.3.2 Food Additives

Recently, different metabolites from beneficial microorganisms have found its application and use as food additives. Microbial rennets from various microorganisms due to its proteolytic specificities have now being used for the production of cheese. Also lactase for lactose-free food or low-lactose is important for lactose-intolerant individual. Lactase-treated drinks and milk also promotes its sweetness, hence avoiding the need for adding sugars in the production of flavored drinks. Also production of yogurt, frozen desserts, and ice cream utilizes lactase to improve creaminess, scoop, digestibility plus sweetness and to decrease sandiness in the crystallization of lactose in thick preparations. In the preparation of protein hydrolysates, alkaline proteases are utilized to give high nutritional value. Protein hydrolysates are utilized in infant food preparations, strengthening of fruit juices plus drinks, and particular therapeutic dietary products. Pectinases from beneficial microbes are utilized in fruit juice clarification and extraction. Pectins add to fruit juice viscosity and turbidity. A combination of pectinases plus amylases is utilized to clarify fruit juices. β-amylase plus Glucoamylase are utilized for the manufacturing of low-calorie beer. Transglutaminase is utilized for different purposes like suppressing allergy in certain foods plus improving flavor, texture, and appearance. Xylanases are utilized alongside with pectinase plus cellulase for liquefying vegetables and fruits. β-D-glucopyranosidase plus α-L-Arabinofuranosidase are employed in food and drink processing (Sangeetha et al. 2011).

2.4 Production of Metabolites Facilitated Suppression of Pathogenic Diseases

The determination of aggressive biocontrol action against pathogens plays an important role in the generation of metabolites such as siderophores with high attraction for iron preventing pathogenic development, antibiotics like 2,4-diacetyl phloroglucinol (DAPG), Agrocin 84, and Phenazines, biosurfactants, Organic molecules, hydrolytic enzymes, and pyocyanin which act as systemic resistance against pathogens. Another mechanism by which beneficial microorganisms act in protecting against foreign pathogens is the generation of free radicals, antioxidant defense molecules, or proteins plus formation of structural barriers in the induced plant which is energy dependent (Wiesel et al. 2014). Microbe-associated molecular patterns (MAMPs) with the ability to induce pathogenic resistance in plants like bacterial proteins flagellin, glucan, chitin, and xylan are generated by many microorganisms like *Phytophthora megasperma* or *Trichoderma viride*. Some are antioxidant superoxide dismutase, 23 amino acid peptide, pep-13 transglutaminase, cellulose-binding lectins, sterol-binding elicitins; ergosterol, arachidonic acid; N-glycosylated yeast peptides, glucan, chitin, peptidoglycans which are membrane components; and lipooligosaccharides synthesized by oomycetes, gram-negative bacteria, lipophylic MAMPs, and oligosaccharide MAMPs. Microbial biological control agents (MBCAs) act as pathogenic control via the induction of priming or resistance which relies on a complex series of transduction pathway where the MBCA primarily has bound to the host, then release of particular metabolite or substrates recognized by particular receptors in plant and subsequently activating pathways within the plant causing the onset of defense response making the plant resistance to disease (Köhl et al. 2019). The initial point in this series of events is dependent on the MBCA, the next part is based on physiological and genetic composition of the plant. The MBCA activates defense response in the plant via complex signaling molecules which are low molecular weight constituents or degraded membrane products which are often generated microorganisms at low millimolar to subnanomolar concentrations for endocrine response in microorganisms with plants (Mauch-Mani et al. 2017).

2.5 Biofertilizers

Ansari et al. (2013) showed that beneficial microorganisms can generate biomolecules or substrates like auxins, ethylene, and gibberellins which function as a plant growth regulator, though ethylene is responsible for inhibition of growth in dicot plants. For instance, *Rhizobium* spp. and *Bacillus* spp. can produce auxin at different conditions in the presence of agro-waste as carrier material. Also many plant-linked microbes can synthesize biomolecules when induced with *Paenibacillus polymyxa*. Generally, in the production of biofertilizers, many physiochemical steps are being utilized to degrade complex molecules into a very simple structure through anaerobic digestion. Anaerobic biodigestion can be described as a process of biodegradation of materials or compounds into simpler structures with the use of oxygen. In the first step, complex organic matter is degraded by cellulolytic microbes to generate simple molecules like long-chain fatty acids and other constituents. In the second step, the bioproducts are fermented producing simpler intermediate metabolites like acetic acids, pyruvic acids and carbon dioxide, etc. The third step, methanogens act on the bioproducts, liberating combinations of gases called biogas and other beneficial metabolites or compounds. There are different types of organic biofertilizers based on the microbial type or class Phosphate solubilizing biofertilizer

(PSB), Potassium solubilizing biofertilizer (KSB), Nitrogen-fixing biofertilizers (NFB), Plant growth-promoting biofertilizer (PGPB), Sulfur oxidizing biofertilizer (SOB), Potassium mobilizing biofertilizer (KMB), and Phosphate mobilizing biofertilizers (PMB) (Itelima et al. 2018). Organic acid generation is the major process by which phosphate solubilizing microbes activates phosphate from sparingly soluble phosphates resulting in a drop in pH.

2.6 Conclusion and Future Recommendation

This chapter has provided a detailed information on the application of information on the utilization of beneficial microorganisms for sustainable agricultural and environmental health. The various types of biochemicals that could be derived from beneficial microorganisms were also provided in detail. The application of these diverse biochemical in agroindustry and some other relevant sectors were provided in detail. Moreover, a general overview on the interaction of root-based microbes and plant interaction enhancing nutritional status, mineralization, and recent knowledge adapted to improve our understanding were also provided. There is a need to isolate several beneficial microorganisms using metagenomonic, next generation sequencing for molecular characterization of these beneficial microorganisms. There is also a need to apply cheminformatics, informatics, and metabolomics to validate an array of biomolecules of interest that could lead to an increase in agricultural production and high level of enhancement in food production. The application of genetic engineering should be encouraged for the effective improvement of microorganism strains of interest. This will lead to an increase in the production of numerous products of interest.

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Chapter 3
Recent Advances in Application of Microbial Enzymes for Biodegradation of Waste and Hazardous Waste Material

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Abstract Biodegradation of toxic organic contaminants has been documented to involve a large number of bacterial, fungal, and plant microbial enzymes. Biodegradation is a cost-effective biotechnology powered by microbial enzymes. This chapter intends to provide detailed information on the application of microbial enzymes in the bioremediation of heavily polluted environment. Detailed information on the application of microbial enzymes and their modes of action were also provided in detail. Various types of microbial enzymes were also provided in detail. Specific information on high-rate up-flow anaerobic bed reactor and digester wastewater effluents were also highlighted while the process involved in the degradation efficiency in soil washing effluence by polycyclic aromatic hydrocarbons were stated. Techniques involved in the removal of phenanthrene, fluoranthene, and pyrene in soil washing effluent, the immobilization of specific microorganism and their enzymes with a cross-linking system when investigated using polyvinyl alcohol, sodium alginate, and nano alumina composites.

Keywords Biodegradation · Waste · Environment · Enzyme · Microorganism · Immobilization · Bioremediation · Pollutants

3.1 Introduction

Wastes are strongly associated with human activities. Research and experience have shown that urbanization, industrialization, and agricultural production activities are key players that promote the generation of wastes (Aliyu and Amadu 2017). Since man cannot be distanced from these activities it follows that waste management would continue to be relevant and could not be swept aside by individuals and governments alike (Oyebode 2018).

Consequent upon such relevance as to maintenance of healthy conducive environment, the government of every country owe its citizenry the duty to protect the environment from pollution, diseases, and other hazards which could potentially undermine the physical and/or psychological well-being of humans, animals, and the entire natural ecosystem. Various machineries are often set in motion in the process with agencies, ministries, departments created and mandated usually through legislations, laws, edicts, bye-laws, etc., to conduct activities necessary to ensure the realization of such objectives and functions contained in the respective legal provisions (Ayotamuno and Godo 2000; Kalu et al. 2009; Oyeniyi 2011).

A typical example of such a State is Nigeria, where there are presently over 20 active federal legislations all connected to environmental management especially the control and regulation of wastes including those with toxic components such as radioactive and industrial wastes from breweries, mine sites, steel production factories, etc. Federal ministries such as Environment, Health, Science and Technology, all have departments and agencies under their respective supervision with mandate to ensure healthy environment.

Agencies like the National Environmental Standards Regulation and Enforcement Agency (NESREA) in Nigeria comes to mind whenever wastes and environmental protection are in context. While such agencies and legislations are worthy interventions in their own respect and right, the need for effective techniques and technologies that could help actualize the laid down goals of such agencies is very relevant in any public or private environmental management campaign or program irrespective of the locality, geographical coverage, and jurisdiction.

In recent times, there has been a consistent drift from traditional waste management paradigms. The new paradigm shift is hinged on the developments in biotechnology, nanotechnology, and artificial intelligence among other rapidly evolving technologies. Consequent upon these developments, some factors which were hitherto not evaluated while designing waste management programs have now become issues of vital concern. In other words, cost, safety, eco-friendliness, and sustainability have become major factors considered in recent times while developing policies, guidelines, programs, and proposals on regional and urban waste management. The same is true on the design, acquisition, deployment of modern waste management infrastructure.

Notably, two decades ago, disposal of wastes including toxic wastes usually employ various methodological approaches which are shown in Fig. 3.1. Each method adopted depends on the category of waste.

These approaches include: Concentration using collection channels and subsequent disposal to remote uninhabited sites including landfills; Incineration; Recycling, etc.

While each adopted approach has its merit, there had been some attendant side-effects from such traditional methods that often results in challenging issues. These side-effects include:

(a) Pollution (Sharma et al. 2013).
(b) Health hazards arising from dumping or burying radioactive wastes in remote uninhabited places. Such hazards may manifest years after they are even forgotten. Often times they could spike disasters like explosions whose immediate cause may not be unraveled.
(c) Death of aquatic life: Unfortunately, some legislations do permit industries or companies with waste disposal license to dump industrial wastes in water bodies such as flowing rivers, sea, ocean, streams, lakes, etc. This practice is not strange in many developing countries, unlike Nigeria. While majority of these companies remain unsupervised (Agagu 2009) owing to the permit they had obtained via high license fees from the necessary authorities such as NESREA (Nigeria),

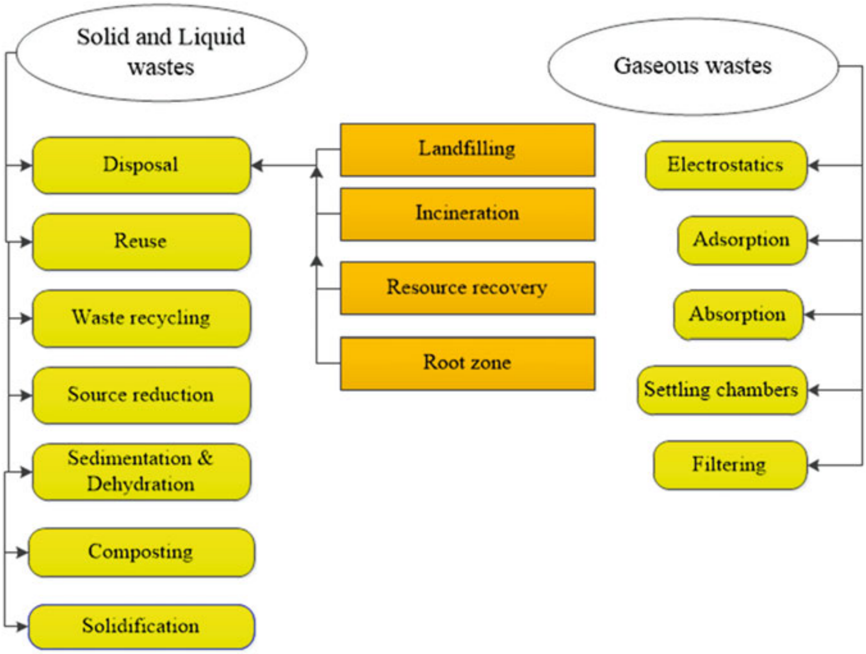

Fig. 3.1 Traditional waste management methodologies

future implications of such wastes on the aquatic ecosystem are not readily contemplated by anyone. It is widely reported that these unwholesome practices of uncontrolled release of effluents by companies have remained unabated for years (Mallak et al. 2016; Gadzama and Ayuba 2016; Emejuru 2015; Guerrero et al. 2013). The problem is worsened by endemic corruption in many of those agencies wherein the helmsmen are often settled by corrupt chief executives of those companies.

(d) Acidic rain: uncontrolled incineration had been an age-long traditional practice of disposing domestic, agricultural, and industrial wastes. Cities like Lagos and Port Harcourt among other urban areas in sub-Saharan Africa are notable for creating dump sites. Wastes are conveyed through large trucks to these sites. It may be clearly observed that combustion continues in these sites almost unabated giving rise to atmospheric pollution and resultant acidic rain.
(e) Disease and epidemics (Coppeta et al. 2019).
(f) Increase in mortality rate (Romanelli et al. 2019; Thompson and Anthony 2005).

Consequent to the downsides of customary methods of waste management, research had been consistently ongoing on safer, healthier, more sustainable, and eco-friendly alternatives.

Conceptualization along these factors have become vital owing to challenges posed by climate change in recent times (Ukhurebor et al. 2019; Ukhurebor and Azi 2019; Nwankwo and Ukhurebor 2019; Ukhurebor and Nwankwo 2020; Nwankwo

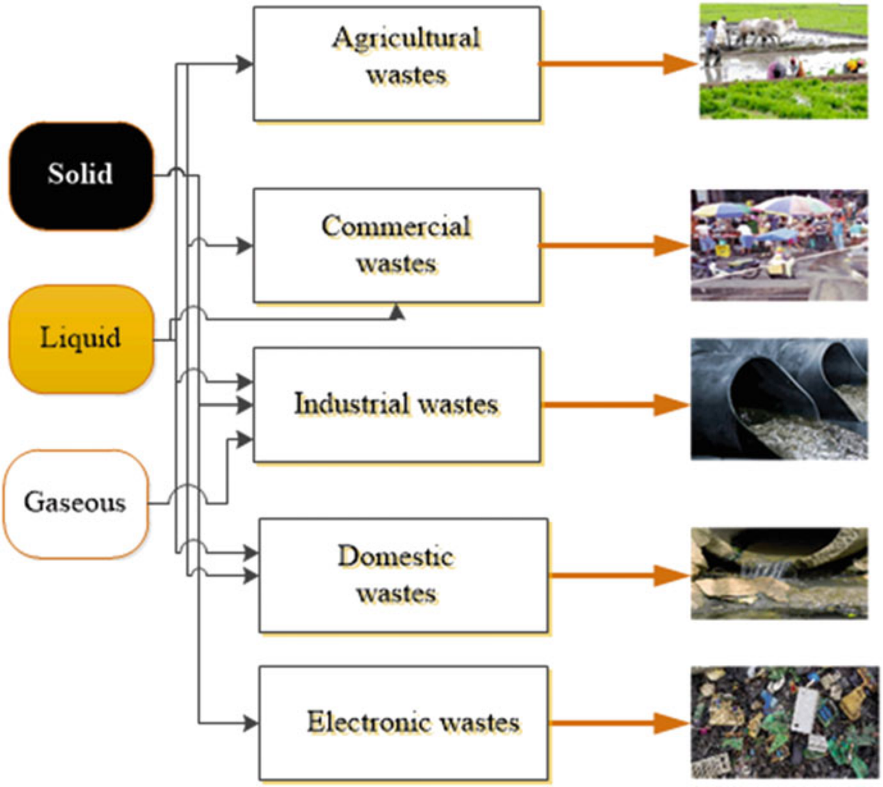

Fig. 3.2 Sources of wastes

et al. 2020a, b, c) and the need to help cleanse, revitalize, and save the environment from sudden collapse as man's industrial activities increase. With advances in nanotechnology, biotechnology, and intelligent systems, a paradigm shift towards integration of useful microbes in waste management programs and instrumentation have become a realistic venture and concern.

Microbial applications in environmental remediation and waste management have remained interesting research domain for over two decades as continuous searches for effective solutions to the menace inherently caused by waste management are ongoing and new methods, techniques, and procedures are continuously sought after (Shalaby 2011; Abatenh et al. 2017). It is on this note that this chapter is poised to present recent advances on vital applications of relevant microbial enzymes in the biodegradation of wastes and hazardous materials. Prior to rendering a detailed review of some key developments in this domain, identifying the major occurrences of wastes and possible characterization is very noteworthy. Figure 3.2 shows common sources of wastes including hazardous effluent wastes.

Characterization of wastes have been found of immense relevance in the determination of biological and/or biotechnological approaches to waste management.

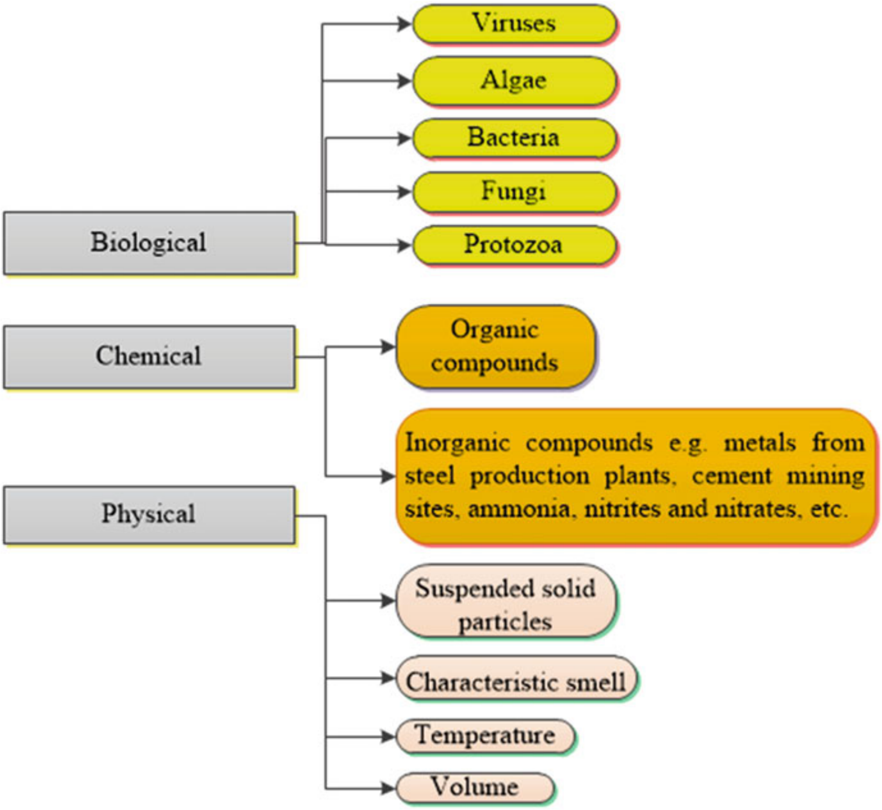

Fig. 3.3 Biophysical and chemical characteristics of wastes

Accordingly, Fig. 3.3 presents the various characteristics of wastes that could be used in the planning and implementation of appropriate microbiological systems for waste management.

As may be deduced from Fig. 3.3, one of the vital components of solid and liquid wastes are the microbes themselves. These microbes include protozoa, algae, fungi, bacteria, and viruses. Thus, the ubiquity of microbes is an important factor in strategic and tactical planning of various microbial waste processing infrastructure.

The presence of microbes in many wastes irrespective of the form may also lend credence to the high probabilities of realizing a successful microbiological system for waste management such as integrated bioreactor systems. The unique physiochemical and biological properties of these microbes in addition to the ubiquity factor stated earlier provide sufficient grounds for embarking on deployment of appropriate microbial systems for waste management. The effect is that useful microbes can easily be isolated, reproduced, stored, and utilized under controlled conditions.

Several microbial waste management methodologies have been studied and would be discussed in this chapter. They include:

(a) Biodegradation.
(b) Biostimulation.
(c) Mycoremediation.
(d) Bioaugmentation.
(e) Bioreactor systems.

Each of these microbiological systems of waste processing has its own strengths and drawbacks. It is believed that hybrid waste processing and control systems could be designed using a mixture of these microbiological procedures. With advances in AI, Machine learning, and Analytics, a much more impressive, intelligent, remote-controlled, rewarding yet cost-effective, sustainable, and eco-friendly waste treatment apparatus could be designed and deployed.

This chapter presents a detailed account on current developments on enhanced biodegradation of industrial and hazardous wastes.

3.2 Application of Microbial Enzymes for the Biodegradation of Agricultural and Industrial Wastes

Agricultural and industrial wastes can either be in solid or liquid form. Normally, we could categorize these wastes from agriculture and industries into two broad groups, viz.; biodegradable (recyclable) wastes and nonbiodegradable (nonrecyclable) wastes (Ivanov 2010; Ansari 2011; Adebayo and Obiekezie 2018).

The biodegradables or recyclable wastes which are also known as "biowastes" are those wastes produced either via agricultural or industrial activities, which can be broken down (decomposed) by microbes. Reportedly, they hardly constitute the foremost sources of effluence to the environment for an elongated period (Adebayo and Obiekezie 2018). Examples of biowastes are paper materials as well as wastes of plant and animal sources, such as fecal substance, cadaver, droppings as well as animal waste products from poultry, cattle, etc. These biodegradable wastes, despite their ability to undertake degradation without much difficulty by means of microorganisms within little period of time, still have some limitations, such as evolving belligerent aroma and it also constitutes irritation to the attractive environment when compared to the nonbiodegradable wastes. They can also institute a decent environment for the flourishing of pathogenic microbes which can contaminate fresh food produce as well as bases of fresh water in most municipal without difficulty globally (Waites et al. 2011; Semerci 2012; Adebayo and Obiekezie 2018).

The nonbiodegradable wastes, on the other hand, are usually not broken down or degradable by microbes; indicating that the medium require other procedures for the treatment and disposal of wastes (Waites et al. 2011; Semerci 2012; Adebayo and Obiekezie 2018). Examples of such procedures are landfill, incineration, and recycling. Examples nonbiodegradable wastes are wastes from metallurgical and smelting industries (such as uninhibited vehicles, machines, vehicle fragment and scuffle metals and other machine fragments) as well as wastes from construction industries (such as bitumen wastes, sand, gravel, concrete, and other waste from building and construction constituents), waste from plastic industries (such as plastic containers, cable paddings, tires, plastic sitting constituents, cellophane constituents, plastic catering ingredients, sachet, containments, etc.), and waste from glass industries (Ezeonu et al. 2012; Adebayo and Obiekezie 2018).

Appropriate management of agricultural and industrial wastes as well as other environmental contaminants will assist greatly in the reduction or elimination of the incessant adverse environmental influences on all living organisms as well as supports economic, environmental, and agricultural sustainability development and enhanced the quality of our well-being generally (Ezeonu et al. 2012; Nwankwo and Ukhurebor 2019; Aigbe et al. 2020; Nwankwo et al. 2020a, b, c).

Composting is the utmost regularly used biological or organic waste treatment process for both agricultural and industrial wastes. This process is controlled by aerobic putrefaction or decomposition of biological organic waste constituents by means of minor invertebrates and microbes (Both 2017). According to Adebayo and Obiekezie (2018), composting is a procedure whereby biological or organic waste constituents such as food and plants are putrefied or broken down and then recycled as manure for agricultural and landscaping benefits and uses (Both 2017). Adebayo and Obiekezie (2018), further reported that the furthermost composting procedures are “static pile composting, vermicomposting, windrow composting, and in-vessel composting.”

The topical scientific advancement has been attained in the environmental management of wastes from agriculture and industries via the application of microorganisms. Several agricultural and industrial activities have caused serious environmental and social complications globally; these incessant complications are a cause for serious concern that require remedial solutions by establishing physical, chemical, and biological machineries (Satyanarayana et al. 2012; Adebayo and Obiekezie 2018).

However, in this section, we will concentrate briefly on the biological procedures involving biotechnological means for the advancement of sustainable agricultural and environmental cleaning approaches that are progressively flexible for waste management procedures. Such biodegradation measures according to Adebayo and Obiekezie (2018) are “bioremediation, biostimulation, bioaugmentation, phytoremediation, etc.”

3.2.1 Biodegradation

Biodegradation involves the breaking down, dilapidation, or degradation of biological or organic matter and constituents into nutritional substances (nutrients) that could be beneficial and useful to other living organisms (Adebayo and Obiekezie 2018). According to Chahal (2015), such breakdown, decay, or degradation is carried out by means of several organisms such as fungi, bacteria, worms, insects, and some other saprophytic living organisms which consume deceased or dead substances and reprocess it into fresh forms.

Bioremediation make use of microbes as well as their resulting constituents in the presence of optimum environmental circumstances and adequate nutritional substances to breakdown, dilapidate, or degrade pollutants or contaminants as well as hazardous constituents. Bioremediation machinery make use of microbes for the reduction, elimination, monitoring, collection, management, or transformation of some categories of pollutants or contaminants contained or existing in the air, soils, or water (Chahal 2015; Adams et al. 2015).

3.2.2 Biostimulation

Biostimulation encompasses the alteration of the environment for the stimulation of prevailing bacteria that are proficient in bioremediation (Adebayo and Obiekezie 2018). The procedure is carried out by adding some forms of restrictive nutritional substances as well as electron acceptors like oxygen, phosphorus, carbon, or nitrogen that are readily accessible in measures that are small enough to oblige microbial actions and processes (Chahal 2015).

3.2.3 Bioaugmentation

Bioaugmentation involves the addition of active microbial cells proficient in breaking down, dilapidation, or degradation to supplement the original microbial inhabitants present in the environment. The microbes that are used for bioaugmentation are called "*bioremediators.*" It could take some time say days, months, or years for microorganisms to clean up a place or site, depending on some factors such as high pollutant concentrations, pollutants trapped regions, or the polluted place or region (Chahal 2015).

3.2.4 Mycoremediation

Mycoremediation is another category of bioremediation which comprises the use of fungi to disinfect a contaminated area or region. According to Adebayo and Obiekezie 2018, the secretion of "*extracellular enzyme*" and acids by "*mycelium*" is responsible for the breakdown or degradation of lignin and cellulose.

3.2.5 Bioreactor Landfills

Bioreactor landfills are topical technological and scientific research that are reportedly better off when compared to the "traditional or conventional sanitary landfills and controlled dumps." These topical technological and scientific bioreactor landfills make use of more advanced microbiological procedures in the transformation and stabilization of the readily and temperately decomposable biological or organic waste ingredients within a very short time (Ni et al. 2009; Adebayo and Obiekezie 2018). The regulatory and monitoring feature is the unceasing addition of fluid, basically liquid to be specific form for sustaining the optimum moisture or humidity for the microbial digestion process. The addition of the fluid is by the "recirculation of the landfill leachate." Peradventure that the quantity of the leachate is insufficient, the liquid or fluid waste such as sewage sludge is applied to make up for the insufficiency.

Reportedly, there are three recognized kinds of bioreactors presently, viz., "aerobic, anaerobic, and a hybrid" (Adebayo and Obiekezie (2018). These three techniques encompass the reintroduction and restoration of the composed leachate complemented or supplemented with water so as to maintain the levels of the humidity or moisture in the landfill. Microbes are responsible for the putrefaction or decomposition; hence, they are stimulated to decay at an improved or amplified rate with an attempt to diminish the injurious emissions (Adebayo and Obiekezie 2018).

In the case of the aerobic bioreactors, air is propelled or pumped into the landfill by means of either vertical or horizontal settings in the pipes. The aerobic environment putrefaction is enhanced and the quantity of unstable organic composites, noxiousness of the leachate as well as the methane are diminished accordingly (Murphy 1992; Adebayo and Obiekezie 2018).

In the case of the anaerobic bioreactors, as the leachate is being disseminated, the landfill generates methane at a rate that is faster and quicker than the conventional or traditional landfill technique. The high concentration together with the amount of the methane permits it to be applied for more resourcefully commercialized purposes, while the reduction of the time or period needed by the landfill to observe the generation of the methane (Adebayo and Obiekezie 2018).

In the case of the hybrid bioreactors which entails the combination of both the aerobic and the anaerobic techniques, as reported by "the Hinkley Center for Solid and Hazardous Waste Management (2006)," is subject to the upper regions of the landfill by means of the aerobic-anaerobic sequences to upsurge the putrefaction rate, while methane is generated via the lower regions of the landfill. The bioreactor landfills generate lesser amounts of unstable organic composites compared to the conventional or traditional landfill technique, apart from H_2S, which are reportedly generated in advanced quantities by the bioreactor landfills (Adebayo and Obiekezie 2018).

The process of anaerobic digestion is the utmost of the in-vessel treatment of waste and is normally regarded as one of the utmost inventive and beneficial technologies established by industries (Capel 2010; Adebayo and Obiekezie 2018). Not just that it avails a large-scale means of treating biological or organic waste but it also avails the ensuing gases to be converted into other forms of energy.

According to Kangle et al. (2012), anaerobic digestion is the multistep organic procedure in which anaerobic microbes translate biological or organic constituents or waste to "bio-gas and bio-fertilizer" without the use of oxygen. It comprises a sequence of metabolic connections among several kinds of microbes, and takes place in four phases of "hydrolysis/liquefaction, acidogenesis, acetogenesis, and methanogenesis."

However, there is more advanced technology presently used for huge-scale recycling of biological or organic waste, according to Adebayo and Obiekezie (2018), such technology is the use of "bio-dung composting and vermiculture technology."

Vermiculture technology is a setting that harness earthworms for the "bioconversion" of biological or organic waste into "vermicompost" which has widespread benefits in waste monitoring, management, and sustainable organic agriculture (Adebayo and Obiekezie 2018). It has been demonstrated to be an utmost efficient technique in the monitoring and management of organic wastes with minimum intricacy and economic feasibility. Ansari (2011) was the first to adopt "vermiculture technology" in his research study.

The mixture of both grass pieces, water hyacinth as well as cattle dung was applied as organic waste for the settings of "bio-dung and vermicomposting." Reportedly, from the obtained results, there were indications that the organic wastes were effectively treated by means of "partial bio-dung composting and vermicomposting" for about 86,400 s (that is 60 days); this contributed to the amount of the vital micronutrients and also encompasses the growth inspiring constituents such as "*auxins* and *cytokinins*" (Adebayo and Obiekezie 2018).

As reported by Sneha (2015), the procedures involved in the development of "vermicompost" are itemized below:

(a) Excavation of the pit of approximately 0.5 m^2 and 1.0 m depth.
(b) Facing the excavated pit with stubble or dehydrated vegetations and grass.
(c) Establishment of the dumping of organic wastes into the excavated pit as and when produced or generated.
(d) Introduction of a culture of larvae or worms (which are readily obtainable).
(e) Ensuring that the fillings are enclosed with a sprinkling of dehydrated vegetations and soil on a daily basis.
(f) Irrigating the excavated pit, at least twice a week to preserve the moisture, and revolving over the fillings of the excavated pit at least once every 2 weeks (14–15 days).

In around a month and a half (which is about 45 days) time, the waste will then be putrefied via the microbe's action or process. The resulting soil from this procedure is fertile and rich in nutritional value.

As a result of the eco-friendly and low-cost advantages of the application of microbes in the monitoring and management of waste, and also bearing in mind the decomposable (biodegradable) attributes of capacious waste produced by industrial and agricultural activities.

3.3 Types of Microbial Enzymes and Their Uses

Biodegradation of toxic organic contaminants has been documented to involve a large number of bacterial, fungal, and plant microbial enzymes. Biodegradation is a cost-effective biotechnology powered by microbial enzymes. Table 3.1 shows some

Table 3.1 Microbial enzymes and their uses

Microbial enzymes	Uses	References
Esterase	It is used to biodegrade pyrethroid insecticides used for cultivation and for household use It is used for the biodegradation of polyester and aromatic-aliphatic copolyesters	Bhatt et al. (2019), Novotný et al. (2015)
Laccase	It is used in surface flux and soil for the biodegradation and detoxification of cypermethrin	Gangola et al. (2018)
Oxygenase	It is utilized in phenol biodegradation	Mathur et al. (2008)
Peroxidase	It is used in the mycotoxin deoxynivalenol degradation process It is utilized in a sequencing batch reactor to biodegrade complete petroleum hydrocarbons	Feltrin et al. (2017), Shekoohiyan et al. (2016)
Monooxygenase	This aids in the biodegradation of C1–C8 alkanes, alkenes, and cycloalkanes It assists in the biodegradation of various aromatic and aliphatic compounds	Singh et al. (2017), Arora et al. (2010)
Dioxygenase	This helps biodegrade polycyclic aromatic hydrocarbon	Chadhain et al. (2006)
Protease	It hydrolyzes the peptide bond	Tavano et al. (2018)
Nitrilase	It helps to remove cyanide contaminated waste and toxic nitriles	Park et al. (2017)
Cellulase	This helps turn cellulosic wastes into foods	Islam and Roy (2018)
Lipase	It helps to remove cooking waste	Okino-Delgado et al. (2017), Srivastava et al. (2019)
Amylase	It is used for food waste biodegradation	Awasthi et al. (2018)
Cutinase	It is used to remove the cuticular layer cutin in the leaves or the bark suberin	Kawai et al. (2019)
Pectinase	This is used in biorefinery hydrolyzing pectin found in pectin-rich agro-industrial waste	Garg et al. (2016)
Catalase	It is used to extract H_2O_2 from the effluent of the bleaching industry and also for its future use in the food industry	Kaushal et al. (2018)

of the microbial enzymes used for the biodegradation of hazardous waste and their uses.

3.4 Application of Microbial Enzymes for Biodegradation of Effluent, Solid and Municipal Waste

Mathur et al. (2008) performed biodegradation of phenol on isolated soils and sewage samples of Rajasthan desert regions under batch cultivation using oxygenase. The oxygenase degradation assay was performed by measuring the consumption of NADH at 340 nm using whole cells of different isolates. The authors found that maximum degradation was achieved at 50 °C after 2 h. However, it was observed that four isolates out of nine phenol adapted oxygenase producing isolates showed maximum enzymatic activity.

Wu et al. (2019) examined the biodegradation of carbaryl in soil and soil quality enhancement with Rhodopseudomonas capsule in wastewater effluent. The authors found that carbaryl has triggered carbaryl hydrolase gene expression by allowing MAPKKKs, MAPKKs, and MAPKs genes to synthesize carbaryl hydrolase in the MAPK signal transduction pathway. It was also observed that the absence of soil organics and control treatment could not sustain *Rhodopseudomonas* capsule development for more than 1 day. The residual organic products in the effluent provided therefore an appropriate source of carbon and energy for the growth of *Rhodopseudomonas capsulata* after a day. The authors noted that the approach contributed to carbaryl contamination remediation and soil fertility enhancement, and to wastewater reuse and the use of *Rhodopseudomonas capsulata* as sludge.

Genethliou et al. (2020) evaluated the biodegradation of wastewater olive mill phenolic waste compounds that are fed to a high-rate anaerobic up-flow anaerobic bed reactor and digester wastewater effluents that are collected under constant operating conditions during different hydraulic retention periods. The toxicity of each sample was also measured in accordance with the microbiotest Thamnotoxkit FTM. The authors found that exceptionally high phenolic extraction by hydroxytyrosol and tyrosol was obtained, reaching approximately 94.87 ± 0.04% and 93.92 ± 0.33%, respectively.

Xu et al. (2019) isolated and purified Klebsiella sp. strain with a high degradation efficiency in soil washing effluence by polycyclic aromatic hydrocarbons. For the removal of phenanthrene, fluoranthene, and pyrene in soil washing effluent, the immobilization of the strain KL with a cross-linking system of boric acid-$CaCl^2$ was investigated based on polyvinyl alcohol, sodium alginate, and nano alumina composites. In immobilized beads, the concentration of polyvinyl alcohol, sodium alginate, and nano alumina had important effects on physicochemical and biodegrading properties. Adsorption of beads in the initial degradation stage led to the elimination of polycyclic aromatic hydrocarbons. Greater residential Tween 80 concentrations in soil washing effluent have toxic effects on KL strain growth and decrease the degradation capacity of polycyclic aromatic hydrocarbons. The requirements for the biodegradation efficiency of polycyclic aromatic hydrocarbons were between 80 and 2500 mg/L.

Mello et al. (2019) reported the biodegradation of BTEX wastewater compounds. Batch and continuous bioreactors which are supported by biofilms were studied to extract BTEX compounds. The activated loam from the local wastewater treatment plant has been modified to BTEX compounds biodegradable. The findings showed that the biofilm was well suited to the conditions under examination and was able to completely degrade BTEX compounds. BTEX compounds fed regularly in a batch bioreactor obtained kinetic parameters. The tests demonstrated that biomass could extract the BTEX compounds for up to 300 min in the bioreactor showing a possible strategy for the petrochemical industry.

Shekoohiyan et al. (2016) developed an oxidation cycle for the biodegradation of total petroleum hydrocarbons in a batch reactor, controlled by bacteria peroxidase. In the bioreactor, a small amount (0.6 mM) of H_2O_2 at 22 h of reaction time was obtained at almost complete biodegradation ($>99\%$) of the high concentrations of petroleum hydrocarbons (4 g/L). Peroxidase catalyzing hydrocarbon's catalytic activity is 1.48 U/mL biomass. The bioreactor determined a blend of bacteria, including the *pseudomonas* spp. and the *bacillus* spp., that can synthesize peroxidase and biodegrade hydrocarbon. The GC/MS effluent analysis showed that all types of hydrocarbons could be well degraded in the sequencing batch reactor induced by H_2O_2. The peroxidase-mediated cycle is thus a promising method for efficiently biodegrading saline wastewater laden with concentrated total petroleum hydrocarbons.

Ravindran et al. (2016) proposed microbial biodegradation of solid protein tannery waste for *Clostridium limosum* processing using a substrate of animal flesh. The development of optimal protease 433 U/ml was obtained and the purified enzyme was classified as monomeric acidic metalloprotease. The molecular weight of the enzyme was stated to be 71 kDa, with enhanced activity by bivalent metals like Zn^{2+} and Mg^{2+}. Clostridium limosum has been concluded as a potential candidate for simultaneous tannery waste degradation and metalloprotease production.

Sahariah et al. (2015) have identified and contrasted the ability of metal repair and biodegradation for earthworm species with Metaphire posthuma and Eisenia fetida in municipal solid waste. As a basis for comparative study, the authors used various combinations of urban solid waste and cow dung. The overall N content and availability of Phosphorus, Potassium, and Iron were substantially improved in both Metaphire and Eisenia systems, followed by major pH reductions and overall organic carbon. Both species had similar levels of urease activity and microbial breathing. In addition, the bioavailability of heavy metals as Lead, Zinc, Manganese, and Copper, regardless of earthworm types, was reduced significantly during vermicomposting. Overall findings indicate that Metaphire posthuma, an indigenous earthworm, may be used as an effective candidate for toxic waste bioprocessing.

Raaman et al. (2012) presented biodegradations of polythene isolated plastic from contaminated sites in the area of Chennai using the fungal lines *Aspergillus* spp., *Aspergillus niger* and *Aspergillus japonicus* were chosen for predominant fungal strains under laboratory conditions for polythene degradation. Further electron microscopic scanning studies confirmed the deterioration by exposing the existence of fungal degraded polythene surface porosity and fragility. *Aspergillus japonicus* showed an 8% degradation potential for 1 month compared *to Aspergillus niger.*

da Luz et al. (2013) have evaluated *Pleurotus ostreatus'* ability to degrade oxo-biodegradable plastics without being subjected to physical treatment, such as ultraviolet light or heat. The authors noticed the oxo-biodegradable plastic cracks and a small hole on the surface of the plastics after 45 days of incubation in plastic substrate-containing bags as a result of the formation of hydroxyl groups and carbon-oxygen bonds. The *Pleurotus ostreatus* degrades oxo-biodegradable plastics and uses mushrooms as a substrate using this material. The authors concluded that improvements in oxo-biodegradable plastic biodegradation may be caused by lacquer activity.

3.5 Application of Microbial Enzymes for Biodegradation of Polluted Soil with Pesticides

The continual emission of xenobiotic compounds, such as insecticides, pesticides, fertilizers, plastics, and other substances containing hydrocarbons, is the key source of contamination which must be prevented. There are many methods of contemporary restoration, such as physical, chemical, and biological, but they are not enough to clean up the environment. The biodegradation based on enzymes provides a quick, fast, eco-friendly, and socially appropriate approach to the biological degradation of recalcitrant natural xenobiotic compounds.

The biodegradation and detoxification of cypermethrin was performed by Gangola et al. (2018) in the presence of esterase and laccases present in the *Bacillus subtilis*. The authors are isolating cypermethrin from an agricultural soil polluted with pesticide using bacterial strain (MIC, 450 ppm) and distinguished by polyphasic approach. Bacteria showed a 95% degradation of cypermethrin in controlled growth conditions after 15 days. In addition, cypermethrin biodegradation end products under aerobic conditions included cyclododecylamine, phenol, 2,2-dichloroethenyl, 2,2-dimethyl cyclopropane carboxylate, 1-decanol, chloroacetic acid, acetic acid, palmitoleic acid, cyclopentane, and decanoic acid.

Dash and Osborne (2020) have shown rapid biodegradation and biofilm-mediated biological removal of organophosphorus pesticides using a strain of native Kosakonia oryzae—VITPSCQ3 in a vertical flow-packed biofilm bioreactor bed. In cultivation media, the authors centered on biodegradation of organophosphorus pesticides, Profenophos, and Quinalphos using wetland paddy isolated bacterium. The VITPSCQ3 strain showed greater resistance to pesticides, efficient biofilm formation, and the ability to synthesize enzyme-degrading organophosphate. Also used as biofilm carriers were charcoal, gravel, and mushrooms; with mushrooms, the formation of biofilms was high, with an optimal biodegradation ability up to 96% for Profenophos and 92% for Quinalphos during a 120 min reaction period.

Baczynski et al. (2010) introduced in soil polluted with methanogenic granular sludge as inoculum anaerobic biodegradation of organochlorine pesticides μ-hexachlorocyclohexane, methoxychlorine, *o*, *p*′’-DDT, and *p*, *p*′-DDT. At all temperatures, the pollutants were eliminated and their removal levels increased from 1.2 to 1.7 times as the temperature rose.

Cycoń et al. (2009) presented organophosphorus insecticide biodegradation diazinon with *Serratia* sp. and *Pseudomonas* sp. in contaminated soils. Three bacterial strains (*Serratia liquefaciens, Serratia marcescens,* and *Pseudomonas* sp.) were tested and described by the authors and they were responsible for the biodegradation of diazinon in soil by fermentation. The authors have shown that biodegradation in sterilized soil has shown an effective insecticide degradation at a constant rate of 0.032–0.085 d^{-1}. Also, it was found that degradation of diazinon was accelerated when mineral salt medium was supplemented with glucose. The process was nevertheless related to a decrease in pH values after glucose usage. Isolated bacterial strains were proposed to potentially be used for the bioremediation of diazinon-contaminated soils.

Kamei et al. (2011) indicated that the white-red fungus Trametes hirsuta degraded endosulfate and endosulfansulfate successfully. The fungus has been shown to use several pathways for endosulfan and endosulfan sulfate degradation. Strokes like *Mortierella* sp. are recorded. Strain W8 and stress Cm1-45 resulted in 50–70% degradation of endosulfan in 28 days at 25 °C, first by endosulfan diol formation and then endosulfan lactone, contributing to fertility improvements on farmland.

For the identification of endosulfan degradation, Kafilzadeh et al. (2015) isolated bacteria from sediments and water samples from high agricultural activity zones. Five bacteria such as *klebsiella, acinetobacter, alcaligenes, flavobacterium,* and *bacillus* were found to be able to effectively degrade endosulfan.

Ishag et al. (2017) investigated biodegradation from pesticidium-contaminated soils in the mineralized salt medium by the three endosulfan (α, β) and pendimethalin strains (Bacillus safensis strain FO-36bT, Bacillus subtilis subsp. inaquosorum strain KCTC 13429 T, and ATCC14579 T strains of *Bacillus cereus* strain). Endosulfan and Pendimethalin's three bacterial strains were incubated at separate intervals for GC analysis. GC–MS analysis was performed for representative samples. The loss of 0.663 mM (α endosulfan), 0.319 mM (β-endosulfan), and 1.423 mM (pendimethalin) were tracked and used in the biphasic model to measure half-lives. Endosulfan and pendimethalin were extracted by a percentage of media inoculated with bacterial strains ranging from 24 to 95% (α endosulfan), 21 to 91% (β-endosulfan), and 51 to 97% (pendimethalin), respectively. The findings usually suggest the possible mineralization of endosulfan and pendimethalin by certain microorganisms.

Diaz et al. (2016) isolated, reported, and investigated the biodegradability of high pesticides for isolated vermicomposts. This was done to enhance pesticide elimination. Three separate biobed bioremediation system mixtures consisting of greenhouse, olive, and winery waste, inoculated and incubated for a month, were polluted.

In wineries and olive mills, the highest bacterial and fungal abundance was observed. In wineries, tebuconazole, metalaxyl, and oxyfluorfen were extracted by 1.6, 3.8, and 7.7 times, respectively. Oxyfluorfen dissipation was also accelerated in the olive mill with a remaining of less than 30% after 30 days. The isolates could be used to boost bioremediation systems' performance.

The cyanobacterial degradation of organophosphorus pesticides has been studied by Vijayan and Abdulhameed (2020). The authors claimed that cyanobacteria are photoautotrophic organisms, which would resolve the need for what is supply of organic nutrients with heterotrophic substances. It was also noted that the spread of cyanobacteria in the contaminated area leads to their increase in biodegradation.

Kataoka (2018) studied chlorinated cyclodiene insecticide biodegradation processes using soil fungi. The soil infected with organochlorine pesticides was isolated from an aerobic dieldrin degrading fungus, the Mucor racemesus strain DDF and two aerobic endosulfan degrading strains, the *Mortierella* sp. strains W8 and Cm1-45. During 10 days of incubation at 25 °C, strain dieldrin degrading fungus decomposed more than 90% dieldrin and showed the development of a small amount of aldrin-transdiol. On the other hand, in liquid crops with initial 8.2μM concentrations of each element, the mortierella sp. strains W8 and Cm1-45 degraded more than 70 and 50% of α and β-endosulfan, respectively, over 28 days at 25 °C. In both cultures, only a small amount of endosulfanesulfate, a persistent metabolite, was found, whereas endosulfidesulfate did not degrade when the compound was given as the initial substratum. Both strains develop endosulfan diol in the first stage of endosulfan degradation and then begin to be converted to endosulfan lactone.

3.6 Application of Computerization and Digital World, Internet of All in Production of Microbial Enzymes and Their Application in Biodegradation of Waste and Hazardous Waste Material

Yadav (2017) presented the technological developments and applications of lignocellulosic biomass hydrolytic enzymes for valorization. The processing of lignocellulosic biomass into value-added products is a significant area of concern for the use of hydrolytic enzymes. However, process limitations because of the inadequate operation of the enzymes and stability, the limited pH range, and the optimum temperature also restrict their product usage. Innovative developments that involve enzyme activity modulation and stabilization by mutagenesis, genetic modification, and metagenomics have contributed to a major jump in all fields through the use of hydrolytic enzymes. The authors make recent progress in the separation and use of microbes for the use of lignocellulose biomass, hydrolytic bacteria, advanced technologies for managing and enhancing hydrolytic enzyme activity, and applications like enzymes in the production of value-added products based on lignocellulose biomass.

Aazam et al. (2016) provided a cloud-based, intelligent waste management solution. It deals with a solution with different containers for each category of waste (organics, plastics, bottles, and metals), which is fitted with sensors that constantly track and update its cloud status where stakeholders are linked to collect relevant information. The network works not only in waste management but also in sewage and hazardous material biodegradation.

3.7 Conclusion and Future Recommendation to Knowledge

This chapter has provided detailed information on the application of microbial enzymes in the bioremediation of heavily polluted environment. Detailed information on the various type of enzymes that could be utilized for bioremediation was also highlighted. There is a need for those concern especially agencies and government parastatals that are involved in waste monitoring and management should build on their activities and programs such as “the Integrated Solid Waste Management System (ISWM)” project that will enlighten individuals, agriculturalists, and industrialists on the appropriate and most suitable procedures in collecting, monitoring, and managing wastes. The separation of wastes at their sources should be better carried out to allow healthier, effective, resourceful collection, monitoring, and management of wastes. Microbiological techniques of monitoring and managing wastes should be established and exploited, not just for environmental cleaning alone but also for other resourceful advantages of such techniques. The collection methods of waste should be improved for sustainable and better sanitary environmental circumstances, particularly in populated regions.

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Chapter 4
Biological, Biochemical, and Biodiversity of Biomolecules from Marine-Based Beneficial Microorganisms: Industrial Perspective

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Abstract Beneficial marine microorganisms are not only of significance for the production of useful variety of substances, but also perform unique roles in element cycles with animals and plants. Beneficial marine microorganisms have been recognized as a repository of useful beneficial biomolecules with several industrial applications. Moreover, there are several challenges that mitigate against the successful determination of these molecules of special interest as well as the detection of these beneficial microorganisms from the microbial marine environments, biological and biodiversity. The application of molecular biology techniques are valuable methods for evaluating the biodiversity and biological characteristics of marine bacterial communities and its biomolecules. These techniques are generally adopted for two different types of bacterial species such as the culturable and nonculturable bacteria. Therefore, this chapter intends to explore the industrial relevance of several compounds derived from marine environment. These molecules and bioactive peptides have been identified to perform diverse biological functions such as antimicrobial activity, antioxidant activity, antihypertensive activity, anticancer activity, and anti-inflammatory activity, respectively. Moreover, more emphasis was placed on the different types of methods that could be utilized for evaluating different types of marine bioactive peptides such as organic synthesis, chemical hydrolysis, microwave-assisted extraction, and enzyme hydrolysis, respectively. Also, the modes of action of these biologically active compounds were highlighted.

Keywords Marine microorganisms · Biodiversity · Biomolecules · Physiology · Antimicrobial activity · Antioxidant activity · Antihypertensive activity · Anticancer activity

4.1 Introduction

It has been discovered that for marine-based microorganisms to survive and thrive in their natural microenvironment, there is a need for them to secrete specific bioactive secondary metabolites. Moreover, these unique molecules have been found to have a very high significant benefit for industrial, medical, pharmaceutical applications most especially for drug and vaccine production against different pathogenic diseases like infections, diabetes, cancer, infertility, and aging, respectively (Prestegard et al. 2009). Studies have shown that marine-based microorganisms and their biomolecules which could be referred to as secondary metabolites in the microenvironment are the largest of the earth (Mimouni et al. 2012). Different studies on important microorganisms called extremophilic have revealed that they possess a unique molecular ability adapted to survive in different harsh environments. In addition to that, huge opportunities have opened up through their applications in different industrial sectors owing to the fact that they can generate biocatalyst now named as extremozymes with immense biological activities such as salt allowance, thermostability, cold adaptivity, and biotransformations. In recent times, several biomolecules have been studied and new ones are gaining attention majorly because of their industrial applications such as thermophilic proteins, xylanases, proteases, acidophilic proteins, halophilic proteins, piezophilic proteins, esterases, isomerases, chitinases, cellulases, pectinases, lipases, dehydrogenases, amylases, and pullulanases (Mehbub et al. 2014).

Therefore, this chapter intends to provide a detailed information on the potential natural biologically active molecules derived from the marine environment while place more emphasis on the biological benefits, biochemical functions of beneficial marine-based microorganisms together with their biodiversity in biotechnology and agro science industries.

4.2 General Overview on the Biological Active Molecules Derived from Marine Environment

It has been observed that establishing the physiochemical properties or features of marine-based beneficial microorganisms revealed that they possess physiological adaptations which have numerous biotechnology and biological advantage. The major producer of substantial amount of biomolecules could be attributed to the capability of microorganisms, most especially fungi, to cohabiting with several other marine-based invertebrates and plants such as corals fungi, sponges, marine algae, mangroves, endophytes, and seagrass. In pulp and paper industries, studies have shown that fungi are the largest source of wood degrading enzymes used in bioremediation (Mayer et al. 2013). Also in the pharmaceutical industries, thraustochytrids can generate a large quantity of omega-3 fatty acids called docosahexaenoic acid (DHA) utilized as nutraceutical. The knowledge and exploration of deep sea fungi with high hydrostatic pressure and temperature with high metal concentration is still very little, when fully harnessed, they show promising abilities due to different genes involved in the adaptation mechanism (Lordan et al. 2011).

Furthermore, through rigorous research on interactions of different host microorganisms, it has been observed they have yielded positive outcomes in terms of ecosystem and their biodiversity. Though, there is still paucity of information concerning the molecular mechanisms through which these microorganisms execute their biological activities. Beneficial microbes offer a great deal of industrial and investigative benefits owing to their novel attractive enzymes with unique catalytic properties like thermal stability, biocatalytic efficiency, and adaptation. Many of these biomolecules derived from marine-based microorganisms are utilized in food industries for stabilizers, pigments, prebiotics, food ingredients, preservatives, gelling agents, nutraceuticals, and dietary supplements (Lee et al. 2010). For biodegradation of polymers, different approaches have been utilized to produce biomolecules from beneficial marine microorganisms. A lot of efforts have been invested towards reducing the environmental health hazard generated from increasing use of synthetic polymers. These synthetic polymers have been shown to constitute a very high level of pollution and constitute health and environmental hazards most especially when they are ingested in the form of microplastic to humans, sea, and marine dwelling animals. It has been shown that fungi and bacteria are capable of generating a huge amount of biological substances with distinctive biodegradation properties against these synthetic polymers. The common species of fungi and bacteria are *Aspergillus niger, Aspergillus flavus, Fusarium lini, Pycnoporus cinnabarinus, Mucor rouxii, Streptomyces setonii, Butyrivibrio fibrisolvens, Pseudomonas aeruginosa, Comamonas acidovorans, Streptomyces badius, Clostridium thermocellum, Pseudomonas stutzeri, Rhodococcus ruber* (Kusama et al. 2014).

Marine bioactive peptides, as a source of distinctive bioactive molecules, have the capacity to exert in diverse biological functions such as antimicrobial activity, antioxidant activity, antihypertensive activity, anticancer activity, and anti-inflammatory activity. Various methods have been utilized for the synthesis and identification of marine active peptides. Typical examples of these include organic synthesis, chemical hydrolysis, microwave-assisted extraction, and enzyme hydrolysis. As such, comparing the marine-based microbial compound against the terrestrial metabolites, significant amount of biomolecules have been discovered with new structure and physiological functions (Joint et al. 2010).

Current findings also reported the utilization of microalgae for the production of biofuels. Algae, with photosynthetic cyanobacteria, offer a great deal of ideal solutions because they can be cultivated year-round, on nonarable land, alleviating the pressure on farmland and freshwater resources that would be exerted by crops grown for biofuel purposes. Many strains of algae are suitable for producing renewable fuels (biodiesel, bioethanol, and kerosene). They have also been shown as a promising source of food and feed. The production of algae, in particular microalgae, has been documented to possess several industrial relevances most especially in the area in biotechnology development (Jaspars et al. 2015). The recent advances in biotechnology through the utilization of novel techniques such as mutation and genetic engineering have improved the application of research on the use of transgenic algae is also expanding, and commercial applications are upscaled.

Bioremediation uses living organisms for removing contaminants from the environment, for example, polluted land. To date, there have been few cases of bioremediation involving transgenic microorganisms. This is probably due to current paucity of information concerning the threats and advantages of releasing them into contaminated soils. The development of cleaners, detergents, and other similar products containing microorganisms has increased over recent years. In many cases, detailed knowledge of their composition is lacking. As far as is known, it is unlikely that any such products currently available contain transgenic forms of microorganisms, though this remains a possibility for the future (Hu et al. 2015).

Marine pharmacology could be defined as a pharmacological study that utilized marine-based biomolecules for significant advancement of chemistry study for effective deep knowledge of the pharmacology of these active metabolites. The US Food and Drug Administration has approved new product from snails known as ziconotide, v-conotoxin MVIIA used in the treatment of chronic pain. Also another product called trabectedin, ET-743 was manufactured synthetically majorly from marine-based microbes. The drugs have been utilized for the treatment of tumors and cancer such as tissue sarcomas and ovarian cancer. Subsequently, many marine-based active metabolites have been placed in the market due to increase in the number of research emanating from academic community and research institute collaborating with pharmaceutical organizations (Gupta et al. 2015).

The biodiversity and bioprospecting of marine-based metabolites in the Arctic could be linked to the huge economic potential of biomolecules in biotechnology which has translated from just academic point of view to commercial values. It should be noted that there is an increasing number of commercial companies with significant interest in undertaking research on these marine-based biomolecules. This covers significant vast areas of research and development such as enzymology for life science and industrial use, bioremediation, nutraceuticals, drugs and dietary components, health products and cosmetic industries, and antifreeze proteins, respectively.

In recent times huge success have been recorded in the research and development most especially the application of biotechnology for the transformation of biomolecules from marine-based microorganisms with enormous capability of generating enormous revenues if harness properly. Moreover, it has been discovered that traditional chemical technology using biotechnology offers great approaches of adjusting and adapting natural resources into a more environmental friendly and cost-effective products to increase its performance level (Dionisio-Se 2010). The main economic approach of the twenty-first century research and development policy is to engage in a sustainable marine-based bioproducts that utilize eco-friendly and renewable bioresources. Also, the improvement in biomass generation for industrial use and waste material for biofuel has been made possible by increasing the rate of investment dedicated to research and development to enhance the knowledge value of biomolecules in biology, biodiversity, ecology, and biotechnology in sustainable way. This advancement has resulted in a rapid increase in biodegradation of contaminants and renewable energy production through science and technology (Chiang et al. 2011). The economic impact of marine-based biomolecules is huge and can revitalize both developed and developing countries' economies if harnessed properly. Developing can effectively migrate from fossil fuel generation to a more eco-friendly biofuel-based economy from marine-based microorganisms. Also developed countries can adapt it for the bioproduction of chemicals and energy generation. This will shape the future economy and impact positively to the growth and development of quality life for all (Qian et al. 2015).

Marine-based bioresources include different kinds of microorganisms like viruses, plants, bacteria, small sea animals, and fungi capable of generating biomolecules with industrial application. This involves a rigorous search and investments to discover biomolecules and metabolites with the capability to transform many industrial sectors (Fenibo et al. 2019). There is an array of products generated from these marine-based biomolecules such as gases, oils, biofuels, food or feeds, and antioxidants. Bioprocessing is a vast field of knowledge with a multidisciplinary approach and intensive collaborations by academia, industries, and government to stimulate the course of the commercial venture towards establishing a long-term sustainable growth and development (Nigam 2013). Biodiversity is a necessary tool for bioprocessing of different species such as plants and microbial organisms but the major setback is that it is not possible to just predict the species, genes, or habitat that will result into generation of biomolecules without engaging in research and rigorous search (Corinaldesi et al. 2017). Diverse ecosystems have offered great potential and yielded products for bioprospecting such as semiarid and arid land, montane and polar regions, warm and cold seas and oceans, temperate forest, and grasslands.

Recently, due to the rapid advancement in bioprospecting which have resulted into the establishment of biobased industries like grazing, farming, forestry, fisheries, plus poultry that utilizes marine-based biomolecules, increased production in drugs, food, and drinks have been witnessed for the purpose of reduction in poverty, improvement of natural resources, and expansion in global economic growth plus development. Also there are new emerging industries such as biorestoration, biodegradation, biomimetics, and bioremediation of numerous biodiversity knowledge base. To generate huge income from bioprospecting, huge investments in conservation of biodiversity and research are needed in any given country (Pham et al. 2019). Recently, the world has witnessed significant threats to our biodiversity which will definitely impact negatively the discovery of new biomolecules and microbial varieties for medicine, agriculture, and biotechnology (Nihorimbere et al. 2011). Majorly, due to the microscopic nature of some of these biodiversities, recent global decline and lack of recognition of losses will impact heavily on bioprospecting such as change in gene expression, low productivity, knowledge loss, and gross weakening of natural biodiversity (Baskaran et al. 2017).

The use of microorganisms and its biomolecules in food biotechnology has contributed immensely to the value chain of food industries owing to the utilization of biomolecules needed to convert some perishable raw material into a palatable and more preserved manner. The utilization of biomolecules in fermentation processes has offered huge success in the beverage industries providing different taste, aroma, and flavor to different brands of products thereby increasing the nutritive value. This improvement has been made possible in fermentation due to new marine-based biomolecules utilized for these processes in recent times (Bekiari et al. 2015). Fermentation is known to improve food stability and safety through the adoption and utilization of different strains of live marine-based biomolecules to generate new ideas.

The development of probiotics came from the recognition and advanced development of fermented foods from marine-based beneficial microorganisms with their active metabolites utilized in these fermentation processes directly or indirectly. Thus, through the mechanism of its functionality, appropriate application of specific strain can exert a huge substrate level in the host. Though the realization of the industrial benefits of beneficial marine-based microorganisms have been recognized in our generation, there is still far more valuable resources to be utilized and exploited (Blunt et al. 2009). The reality of global warming and environmental effects of climate change is seen in loss of biodiversity and food insufficiency. This has alerted governments and relevant stallholders to start planning on alternative means of combating this scenario. Studies using mathematical models have projected that the impact of climate changes on desertification and traditional lifestyle such as pharmaceuticals affects about two billion people around the globe who solely rely on fermented food and microbial-based medical products (Dang and Lovell 2016). Studies have revealed that 70% of the earth is occupied by seas and oceans providing energy and biomass to significant number of life on earth (Debbab et al. 2010). The living biomass of marine ecology is dominated by microbes that have evolved billions of years ago which are specialized viruses, archaea, fungi, bacteria, and microalgae. Through the knowledge of informatics, molecular biology, and genomics their functional diversity and genetics have given immense biological functions (Gracia et al. 2015). Also through collaborative research, many essential products from marine-based microbes have been unraveled for biochemical processes. Studies have revealed that marine-based microorganism produces biomolecules essential for sustainability and existence of our planet. So many lives depend on these biomolecules to sustain life through biological processes such as feeding, storage, reproduction, and movements. Therefore, it is worthy to note that microbes are an essential element in sustaining life. The huge amount of biodiversity of microbes translate to huge amount of bioproducts of important application in agriculture, pharmaceuticals, medicines, and industries (Hay 2009).

Though very little is known about the opportunities of marine-based beneficial microorganisms and many untapped metabolites they produced despite the enormous awareness and research generated from academia. To compound this problem so many marine-based microbes cannot be cultured in a conventional laboratory making their physiological functions and applications difficult to investigate. Therefore, to change these dynamics and reduce the knowledge gap, there is a need for all the relevant stakeholders to invest massively into research and infrastructure with well-coordinated programs (Mohamed et al. 2008). Since most of these microorganisms are very small in size and difficult to recognize, there is possibility of neglecting their importance in the ecosystem and difficulty in harnessing support for them. More important is the affiliation of microorganisms to disease and pathogenic conditions in humans, plants, and animal which has over the years clouded the beneficial role of some marine-based microbes. Hence, adequate research and awareness programs must be developed to project the beneficial effects of microbiological research (Penesyan et al. 2010).

Identification and isolation of important enzymes with new biocatalytic properties from marine-based microenvironment in order to study and understand their metabolic pathways and biodiversity remains a great potential for industrial application (Rédou et al. 2015). In the past decades, different strategies have been deployed to identify the marine biotope with the purpose of unraveling untapped and unexploited rich diversity of marine physiology, biochemical and biotechnological adaptations coupled with extraction of bioactive products which has opened up new frontiers in industrial processes (Stiefel et al. 2016). Many nutraceuticals are derived from microalgae for human diet supplements and probiotics such as antibiotic and growth enhancer (Mocz 2007). Filtration systems and recirculating aquaculture systems from important microalgae biomolecules have found great industrial applications (Newman and Cragg 2004; Proksch et al. 2010). Kiuru et al. (2014) revealed that advancement in molecular biology has helped to unravel the genetic and physiological role of lipids, DNA, and proteins in the biological system. Sequencing, retrieval of nucleotide and biomolecules are now possible with molecular tools, advanced computational approaches, and technical innovations in analytical methods to study and adapt microbial functions to the industrial application (Hill and Fenical 2010; Cheung et al. 2016). The biodiversity of marine-based microbes is much larger than the terrestrial counterpart due to the fact that oceans have the ability to harbor unexplored, unique habitats (Wang et al. 2017). More gains in medicine and pharmaceutical industries can be traced to the increased exploitation of marine microbial knowledge in the development of drugs and vaccines to manage some human diseases (Anjum et al. 2016).

The chemical and biological activities of biomolecules and enzymes have been exploited as valuable agents in agriculture, pharmaceuticals and medicine, in treating and developing eco-friendly products. Now, a combined effort in a multidisciplinary approach is needed to fully access the improved yields of microbial production and generate novel compounds (Kennedy et al. 2008). The large spectrum of bioactive compound newly discovered with a plethora of industrial applications may be due to huge microbial biodiversity in the ocean; therefore, attention should shift from terrestrial-based microbes to the marine-based beneficial microorganisms with variety of biological benefits. Several products are produced from these microbes such as carotenoids, chitosan, exopolysaccharides, mycosporines, and mycosporine-like amino acids and fatty acids representing a low-cost, fast-production, and sustainable products with diverse industrial applications (Boziaris 2014; Bruno et al. 2019). In this chapter, we review the existing knowledge of these compounds produced by beneficial marine microorganisms, highlighting the marine habitats where such compounds are preferentially produced and their potential application in different industries. Some biocatalyst and bioremediation products generated from microbes have been exploited for industrial applications in bioprocessing due to the physiochemical properties and eco-friendly nature (Raghukumar 2008). Biomolecules which possess tension-active properties produced from microorganisms are referred to as biosurfactants. Also mannosylerythritol lipids, sophorolipids, emulsion, rhamnolipids, and surfactin are newly discovered biomolecules from marine-based beneficial microorganisms with huge industrial application as biosurfactants. They have been shown to have competitive advantage over the synthetic biosurfactants in performance due to their low toxicity, biodegradability, stability, and eco-friendly nature. The widespread use of biosurfactants is still lacking due to cost of production, yield, and awareness. Studies have revealed that synthetic surfactants generate recalcitrant pollutants into the environments (Dumorné et al. 2017). Actinomycetes, an antibiotic-producing microbe has gained recognition in recent times. Interesting research findings are provided in terms of metabolites generated, mechanisms, economic significance, and commercialization (Fakruddin and Mannan 2013). Other antibiotics such as amphotericin, neomycin, tetracycline, nystatin, novobiocin, gentamycin, erythromycin, vancomycin, chloramphenicol, and enzymes like lipase, amylase, and cellulases are also generated from actinomycetes and are seen to perform crucial functions in fermentation processes, bioremediation, and medical sector in treating diseases such as cancer. In agro science sector, actinomycetes have also found huge application in growth promotion and pest control. Biotechnology and biomedical research have engaged actinomycetes in the development of various novel drugs in the fight against diseases such as neurometabolic dysfunctions, cancer, and infections (Komagata 1999). The role of marine-based microbes can never be overemphasized in the advancement of pharmaceutical agents with biological functions. More eco-friendly, underutilized bioactive compounds with enormous potential solve the challenges and demands of pharmaceutical industries (Glaser and Mayer 2009).

4.3 Biological and Biochemical of Biomolecules

Several biocatalysts are known as extremozymes having great biological properties such as thermostability, salt allowance, and cold adaptivity, respectively. Extremozymes have potential application as a biocatalyst, bio-transformations, owing to their ability to resist extreme environmental conditions. The lists of important biomolecule(s) from marine-based beneficial microorganisms for industrial and environmental applications are shown in Table 4.1. Several biomolecules that are derived from these beneficial microorganisms utilized for antioxidant, anti-inflammatory, antibacterial, antimicrobial, hypolipidemic, analgesic, antitumor include the following.

4.3.1 Piezophilic Proteins

It is generally known that high hydrostatic pressure is conferred on marine-based beneficial microorganisms that has a greater ability to adapt and survive in high pressured environments. This significant thermodynamic tendency has been discovered to influence the physiochemical nature of these organisms (Allen and Bartlett 2000). Specific beneficial marine-based microorganisms like piezophiles which thrive in elevated pressure environment might be linked to their physiological characteristics such as regulatory mechanisms and molecular metabolism (Finch and Kim 2018). Also, piezophiles can survive due to revolutionized responsive genes and genetic products resulting in specific growth and developmental patterns such as homeostasis, cell division and cell cycle, and transport mechanism to cope with pressure microenvironments (Zhang and Kim 2010). Also many beneficial

Table 4.1 List of important biomolecule(s) from marine-based beneficial microorganisms for industrial and environmental applications

Biomolecule(s)	Microorganism(s)	Beneficial effects in Industries/environment	Refs
Extremozymes	Extremophilic microbes	Biocatalyst/bioremediation	Mimouni et al. (2012), Mehbub et al. (2014)
Thermophilic proteins	Piezophiles	Antioxidants/anti-inflammatory	Jin et al. (2019)
Acidophilic proteins	*Aeromonas hydrophilia* (*Chlamydomonas eustigma*)	Antioxidants/anti-inflammatory	Hilton and Buckley (1990), Hirooka et al. (2017)
Halophilic proteins	Halophilic archae	Antioxidants	Ebel et al. (2001), Madern et al. (2000)
Piezophilic proteins	Piezohiles	Analgesic/antitumor	Finch and Kim (2018), Zhang and Kim (2010)
Esterases	*Stelletta normani* *Inflatella pellicula,* *Poecillastra compressa*	Insecticides	Montella et al. (2012), Borchert et al. (2016)
Chitinases	Fungal/Crustacean	Pesticides	Rathore and Gupta (2015)
Cellulase	Cellulolytic microalgae *Fibrobacter succinogenes* *Ruminococcus flavefaciens* *Ruminococcus albus*	Biodegradation Textiles, biofuels Brewing, paper Fermentation, and food processing	Jaeger et al. (1999), Kuhad et al. (2011)
Pectinase	Fungal-*Aspergillus fumigatus* *Aeromonas hydrophilia*	Bioenergy, fermentation waste management	Tapre and Jain (2014), Emuebie et al. (2019), Kubra et al. (2018)
Amylase	*Aeromonas hydrophilia*	Insecticides	Hilton and Buckley (1990)
DHA (Docosahexaenoic)	Thraustochytrids (Microalgae)	Nutraceutical analgesic, hypolipidemic	Bradbury (2011), Mayer et al. (2013), Lee et al. (2010)
Trabectedin ET 743, Ziconotide v-conotoxin MV II A	Snails	Treatment of cancer, pain tumor	Jaspars et al. (2015), Gupta et al. (2015)
Carotenoids	Microalgae Plants (chili peppers)	Anti-inflammatory Antioxidants	Eldahshan and Singab (2013)

Chitosan	Penicillum spinulosum	Antioxidant Antitumor, Analgesic anti-thrombogenic, anti-cholesteremic, antibacterial, immunoadjuvant	Hoell et al. (2010), Younes and Rinaudo (2015)
Exopolysaccharides (Pullulan, Levan Dextra, xanthan)	Dinoflagellates Cyanobacteria	Textiles, food biotechnology cosmetics, antitumor antibacterial	Akakuru et al. (2018), Ates (2015), Patel et al. (2018)
Mycosporines and mycosporine-like amino acid	Algae/cyanobacteria	Treatment of cancer, aging	Carreto and Carignan (2011)
Amphotericin Neomycin Tetracycline Nystatin Novobiocin Gentamycin Vancomycin Chloramphenicol Lipase Amylase	Actinomycetes	Antifungal, antibiotics, insecticides Fermentation, bioremediation	Kominek (1972), Faustino and Pinheiro (2020), Chopra and Roberts (2001), Peck and Lyons (1951), Kang and Park (2015), Kunin et al. (1959), Liu et al. (2015), Fakruddin and Mannan (2013), Komagata (1999), Glaser and Mayer (2009)
Sophorolipids Emulsan Rhanolipids Surfactin	Plant and microalgae	Biosurfactants	Moussa et al. (2014)
	Jatropha	Biosurfactants	Dumorné et al. (2017), Manceau and Schoefs (2012)
	Starmerella bombicola	Biosurfactants	Shreve and Makula (2019)
		Biosurfactants	Rikalović et al. (2015)
Mannosylerithriol	Yeast-Pseudozyma	Biosurfactants	Tomotake et al. (2007)
Lipids			Kosa and Ragauskas (2011)

microorganisms found in the deep-sea habitats provide significant information useful for research into the eco-diversity and biology of life in high-temperature environments. This beneficial microorganism thrives under high temperature generally known as thermophilic proteins (Jin et al. 2019).

4.3.2 Docosahexaenoic Acid (DHA)

DHA is a high-quality marine-based compound derived from microalgae molecules which plays a crucial role in easy digestion of nutrients in human diet, excellent for brain development, and expansion with unique structure for wide variety of membrane physiology. The brain is rich in membrane tissue involved in metabolism, intelligence, and locomotor functions (Bradbury 2011). DHA precipitate the production of protectins and resolvins for analgesic and hypolipidemic activity. Studies have revealed that de novo synthesis of alpha-linolenic acid a precursor for omega-3 fatty acid synthesis in the bioproduction of DHA is lacking in mammalian cells, and further consumption of omega-6 fatty acids displaces DHA from phospholipids membrane. Researchers are looking for a way to study the role of DHA in neurodegenerative diseases and other metabolic dysfunction in humans.

4.3.3 Halophilic Proteins

High salt stability and solubility (complex salt bridges) enzymes derived from marine-based microbes are called halophilic proteins. The understanding of their adaptability and crystallization to the microenvironment needs massive research collaboration between relevant stakeholders in government, research institutes, and industrial sectors. The high acid residue in their sequence and protein folds shows significant protein-solvent interactions like hydration interactions (water and ion-binding) with high negative charge which is dependent on solvent composition and salt type (Eisenberg and Wachtel 1987). In a biological system, the induction of chaperone complex was seen in halophilic archaea due to salt induced stress (—Ebel et al. 2001; Madern et al. 2000).

4.3.4 Acidophilic Proteins

Studies were performed on acidophilic green microalga *Chlamydomonas eustigma* to determine their genetic sequence that makes them to adapt to acidic microenvironment with high content of heavy metals. The genomic analysis and transcriptomics revealed that their membrane is highly enhanced with proton pump and heat shock proteins gene when compared with its neutrophilic family member *Chlamydomonas reinhardtii* (Hirooka et al. 2017). They suggested that these acidophilic microalgae is able to survive in the acidic microenvironment because they possess multiple genes for detoxification of heavy metals, elaborated buffering system, and enhanced energy pathway.

4.3.5 Lipases

A marine-based beneficial microbe *Aeromonas hydrophila* can generate and release lipase similar in action to lecithin-cholesterola acyltransferase a mammalian enzyme. Hilton and Buckley (1990) revealed that diethyl *p*-nitrophenyl phosphate can inhibit this enzyme and also two of the histidines are implicated in the catalysis. The hydrolysis and biosynthesis of acylglycerols is derived from marine microbes capable of secreting proteases, amylases, and lipases with enantio and elevated region-selectivity. Two-component and quorum sensing systems were involved in gene expression, folding by disulfide-bond-forming proteins or foldases, and regulation and release of lipase which can progress through ABC transporters or Sec-dependent pathway. It should be noted that proper elucidation of the functional-structural connections will help to adapt new lipases for industrial applications.

4.3.6 Cellulases

It has been established that cellulosic biomaterials can be converted to products with great industrial applications. Cellulase can degrade cellulose and is considered a major industrial enzyme from cellulolytic marine-based beneficial microorganisms (Sadhu and Maiti 2013). The industrial application of cellulase enzymes ranges from textile, biofuel, brewing, paper, and pulp to agricultural feed, fermentation processes, and food. Much research in genetics and enzymology are being developed to unravel the economic value of cellulose that can be generated through modern biotechnology and molecular biology tools (Kuhad et al. 2011).

4.3.6.1 Mode of Action of Cellulase in Bacterial System

1. Adhesion through cellulosome like complexes

   The complex multienzyme capable of biodegradation of adhering cellulose is called cellulosomes made up of central noncatalytic moiety called scaffoldin and numerous attachment site referred to as cohesins where cellulose and enzymes bind to. The enzymatic submits have a central docking and catalytic domain called dockerin connecting with the scaffoldin. This is typically found in thermophilic bacteria called *Clostridium thermocellum*.

2. Adhesion via fimbriae or pili

   The surface attachment used by gram-negative bacteria in adhesion process is also called pili or fimbriae which is about 7 nm in width and 200 nm in length with various structural units. In *Streptococcus sanguis, E.coli,* and *Actinomyces viscosus*, there exist small carbohydrate receptor sites of pili (35 kDa) located in the lips or the entire length. Studies have shown that the pili protein type of cellulose-receptor protein (cbpC 17.7 kDa) with structural similarity with type IV fimbrial proteins of bacteria exist in *Ruminococcus albus*.

3. Adhesion through Carbohydrate Epitopes of Complex Bacterial Glycocalyx

   Electron microscope has enabled us to thoroughly study the complex attachment of carbohydrate epitopes. The slime layer is made up of complex glycoprotein residues that are the active adhesion site of bacteria surrounding the *Ruminococcus flavefaciens* and *Ruminococcus albus*. Treatment with dextranase plus protease a periodate oxidation will remove the complex glycoprotein residues thereby decreasing the adhesion power of bacteria *Fibrobacter succinogenes* and *R. albus* to cellulose.

4. Adhesion through cellulose-binding domains of cellulolytic enzymes

   The two domains in the cellulose are the binding domains for bacterial enzyme attachment connected to rich hydroxyl proteins having two cysteine residues and four tryptophan using either hydrophobic or hydrogen bond as a form of attachment and the catalytic domain for hydrolysis of glycosidic bonds. Studies have revealed that beneficial marine-based microbes lacking this attachment domain may be loosely bound and digestive processes for crystalloid cellulose are impaired. Several of these binding domains have been identified in different species such as *Fibrobacter succinogenes* with EGF and endoglucanase 2 (EG2) (Jaeger et al. 1999).

4.3.7 Chitinases

Chitin is a polysaccharide generally abundant in the marine microenvironment and also derived from marine-based organisms such as crustacean, fungi, and insects. Chitinase are enzymes responsible for the breakdown of chitin and its derivatives with industrial applications as pesticide in control of diseases and pathogens against plant growth and development due to the presence of glutamate on the surface (Júnior et al. 2017). Improving the catalytic capacity of chitinase through enhanced proton donor for glycosidic bond cleavage is the main focus of research for many industrial usages (Rathore and Gupta 2015).

4.3.8 *Pectinases*

Several studies have revealed that pectinase derived from beneficial marine microorganisms especially fungal strain, e.g., *Aspergillus fumigatus* have found several industrial and bioenergy applications by accumulative bioethanol level (Tapre and Jain 2014). Pectinase has the ability to breakdown pectin structure which is used in fermentation processes, drinks and food (wine) industry, and agricultural processes. They are also used in DNA extraction, liquefaction of biomass, enhancing nutrient absorption in animals, wastewater management and treatment (Kubra et al. 2018). Pectinolytic enzymes are important in drink industries for producing galacturonic, a small molecule from a complex polysaccharides structure (Emuebie et al. 2019).

4.3.9 *Esterases*

Chemical insecticides have contributed significantly to the increasing levels of resistance seen with vector control mechanisms. Esterase enzymes are present in diverse range of insecticide with the capacity to hydrolyze ester bonds (Lopes et al. 2011). At present, research on insecticide resistance on insect esterase species gene family is rapidly evolving with quite a unique detoxification genes that only genomic, microarray analysis, and biochemical study can unravel (Montella et al. 2012).

4.3.10 *Mycosporines and Mycosporine-Like Amino Acids*

The marine-based beneficial microorganism through rigorous scientific discoveries have revealed that they possess unique ability to produce mycosporine-like amino acids (MAAs), a metabolite with immense ability to protect against ultraviolet radiation that can cause cancer, aging, and many other disease conditions. Pharmaceuticals and cosmetic industries will find great usefulness in these products as there is a huge market for it if properly harnessed. Carreto and Carignan (2011) have revealed that the enzymatic processes involved in the synthesis of MAAs are through endosymbionts. Other organisms that lack this common pathway may adopt symbiotic, bacterial association or trophic transference as an alternative means.

4.3.11 *Carotenoids*

Another important metabolites secreted by marine-based microbes of multiple biological functions in plant and animal cell is called carotenoids. Recently, there is renewed effort and interest on the biological importance of carotenoids; hence, there has been a rigorous search for plants and marine-based microalgae with the potentials to secrete carotenoids. Some of the newly discovered species are chili peppers and capsicum which accumulate carotenoid pigments in large quantity causing the yellowing of fruits or seeds by β-cryptoxanthin, zeaxanthin, α- and β-carotene, and lutein. The carotenoid levels in different species are influenced by different factors such as varieties, cultivars, environmental conditions, genotypes, and disease conditions (Gómez-García and Ochoa-Alejo 2013). Another type of carotenoid that causes red coloration in plants are capsanthin-5, 6-epoxide, capsanthin, and capsorubin. In plants or microorganisms the active sites for biosynthesis and storage of carotenoids pigment are referred to as chromoplasts majorly controlled by several enzymes and loci like y, c1, and c2 which have been characterized and established but the molecular mechanisms of action regulation pathway are still missing (Eldahshan and Singab 2013).

4.3.12 Exopolysaccharides

A newly discovered biomolecules produced from marine-based microorganisms with huge industrial applications due to their unique chemical configurations and physiochemical properties are referred to as Exopolysaccharides (EPSs) such as pullulan, levan, dextran, and xanthan. They are utilized for textile production, cosmetics, packaging, agriculture, chemical industry, pharmaceuticals, and food biotechnology. Exopolysaccharides are now the center of attraction from research point of view due to their compatibility, biodegradability, and lower environmental toxicity (Ates 2015). One of the major limitations of exopolysaccharides obtaining its commercial value is due to the high cost of generation or production. Therefore, these economic challenges must be overcome before the productivity and commercial impact will be realized. Huge investment in research and development towards understanding the biosynthesis, properties, improved strains, and metabolism will boost the industrial performance and productivity. Exopolysaccharides have been shown to possess antitumor, anti-inflammatory, and antimicrobial activity. The antimicrobial activity of exopolysaccharides showed sensitivity against *B. subtilis, V. cholera, E. coli,* and *B. cereus* (Patel et al. 2018).

4.3.13 Chitosan

Using acetylation, chitin affects the biosynthesis of chitosan, other biomolecules derived from marine-based microorganisms with high compatibility, nontoxic, hydrophilic nature, nonallergic, and degradability (Hoell et al. 2010). Studies have revealed that both chitosan and chitin possess significant physiological activities such as anti-thrombogenic, antitumor, anti-cholesteremic, antioxidant, antibacterial, immunoadjuvant, bioadhesivity, wound healing and dressing properties, and antifungal properties find novel application in medical and pharmaceutical industries such as fibers (tissue engineering), sponges, powders, solutions, capsules, gels, film production, beads, and hydrating agents (Akakuru et al. 2018). The route of application of chitosan in the body include oral, ocular, nasal, and injectable form for drug and vaccine delivery at normal pH (Kafetzopoulos et al. 1993). Also material like hydroxyapatite-chitin-chitosan has shown huge promise in medical science for bone filling (self-hardening paste) and tissue regeneration such as periodontal defects. Alginate in combination with chitosan is utilized as a material for anti-thrombogenic control of drug encapsulation, gene carriers, enzyme release, and cell immobilization (Younes and Rinaudo 2015). In gene transfection and cancer treatment, chitosan derivatives *N*-lauryl-carboxymethylchitosan have less membrane toxicity and are used as hydrophobic cancer drugs carrier. Chitosan glycerophosphate, a complex mixture of citric acid and calcium phosphate can be employed as a self-hardening system in bone filling and repair with the N-alkylated form showing increased efficiency when elongated in the alkyl side chains.

4.3.14 Rhamnolipids

Rikalović et al. (2015) revealed that L-rhamnosyl-L-rhamnosyl-β hydroxydecanoyl-β-hydroxydecanoate is the only rhamnolipid found in marine-based microorganisms. Rhamnolipids are important biosurfactants capable of lowering surface tension of emulsified kerosene and water; therefore, it can be used in the development of industrial bioremediation of contaminated environment especially the aquatic microenvironment. Many important biosurfactants derived from marine organisms have found their way to industrial market such as sophorolipids, alkyl polyglycosides with huge potentials to be utilized as cleaning agents (Moussa et al. 2014). Studies have revealed that the physiochemical properties that give rhamnolipids the special strength as a biosurfactant is because it possess complex congeners of rhamnolipid when compared with the single congeners and others like reduced toxicity, renewable sources, antifungal activity, and biodegradability makes it have the largest impact in agronomy, cosmetics, nanotechnology, and food biotechnology (Shreve and Makula 2019).

4.3.15 Sophorolipids

A glycolipid biosurfactant derived from marine-based microbes with enhanced biological function and vast physiochemical activities is sophorolipids (SLs). Many marine and terrestrial plants and microalgae have shown promising properties in the biosynthesis of SLs such as edible Jatropha oil using *C. bombicola* to reduce the cost of production (Saerens et al. 2011). The industrial applications include stain removal, anti-bactericidal, and biodegradability (Joshi-Navare et al. 2013). The chemical structure derived from *Starmerella bombicola* revealed that it is made up of (*O*-β-D-glucopyranosyl-2-1-β-D-glucopyranose), a disaccharide sophorose connected with glycosidic bond to subterminal and terminal chain of fatty acid carbon. Also the mechanism of antimicrobial action includes membrane rupture, efflux of cytoplasmic substances, and death (Davila et al. 1994). The understanding of the biosynthesis is important in appreciating the industrial application which includes 5 sophorolipid genes (two glucosyltransferases, a transporter, a cytochrome P450 monooxygenase, and an acetyltransferase) are involved in the biosynthesis of sophorolipid (Silveira et al. 2018).

4.3.16 Mannosylerithritol Lipids

Marine-based yeast Pseudozyma has enormous potential to generate a glycolipid biosurfactant called Mannosylerythritol lipid (MEL). Using lipase-catalyzed esterification of a di-acylated MEL with oleic acid several other tri-acylated MEL could be produced with good interfacial properties and broad biochemical reactions. Tri-acylated MEL is the most hydrophobic form produced in culture medium having fatty acids residual (Fukuoka et al. 2007).

4.3.17 Surfactin and Emulsion

Another distinctive class of metabolites capable of growing on crude oil are known as emulsan and surfactin generated from beneficial microorganisms (Liu et al. 2015). RAG-1, an emulsan-deficient mutant grew badly on crude oil and was able to be reversed by the addition of one single revertant (Pines and Gutnick 1986).

4.3.18 Novobiocin

Another clinically useful antibiotic with a distinctive structure consisting of a unique sugar called noviose sugar (one ring), benzoic acid moiety (two rings), and a coumarin moiety (two rings) connected to an amide plus glycosidic bond is called Novobiocin. Several studies have highlighted the derivative of noviose as two methyl groups gotten from L-methionine and D-glucose. Studies have revealed that tyrosine produces coumarin through biochemical sequence and subsequent ring linkage to form novobiocin (Kominek 1972).

4.3.19 Amphotericin

Studies have shown that life-threatening invasive infections or visceral leishmaniasis are managed by an antibiotic with a wide spectrum called Amphotericin B (AmB). This molecule is combined with deoxycholate to increase its membrane permeability and solubility in water. Even though several side effects have been recorded with this antibiotic, formulation of the lipid form called liposomal AmB is still widely being utilized for several infectious diseases. The cost of production of this lipid form is high; therefore, more effort in terms of research should be focused on enhancing its biological functions and reducing the side effects in order to increase the widespread use of AmB with reduced cost and increased accessibility (Brajtburg and Bolard 1996). The antifungal properties and immunomodulatory effects are also well established due to the feature of AmB as AmB-carrier complex (Faustino and Pinheiro 2020).

4.3.20 Vancomycin

Adequate research on vancomycin, a glycopeptide antibiotics have revealed that it can be used against resistant bacteria as the last line of defense due to its ability to specifically bind cell membrane mucopeptide stopping the sequence D-Ala–D-Ala forming asymmetric antibiotic dimers which can dock two peptides of D-Ala–D-Ala in different directions. Acetate ion occupies one of the binding pockets with high flexibility and asparagine side chain on the other relatively stiff (Kang and Park 2015).

4.3.21 Gentamycin

Studies have shown that gentamycin from marine-based beneficial microbes possess the capacity to penetrate the plasma membrane, accumulate in the lysosomes, and regulate different physiological processes. Some of these physiological processes are reduction in lysosomes latency, reduction in y-glutamyl-transpeptidase and alanyl aminopeptidase activity, and reduction of lysosomal sphingomyelinase functions. Though most of these processes showed total reversal of withdrawal of gentamycin from animal exposed to gentamycin treatment after 21 days. The study is in agreement with the fact that nephrotoxicity is caused by gentamycin accumulation in the lysosomes of the kidney causing blockage of enzyme activity involved in lipid degradation, alteration of membrane permeability in the cell, and reduction in cell metabolism causing necrosis or apoptosis (Peck and Lyons 1951).

4.3.22 Chloramphenicol

Studies have shown that just like other antibiotics, chloramphenicol is also derived from marine-based beneficial microorganisms with a wide spectrum of activities blocking bacterial protein synthesis (Kunin et al. 1959). It is known to display bactericidal activity against Streptococcus pneumonia, Haemophilus influenzae, Meningitis, and Neisseria meningitides (Glazko et al. 1949). Structurally, it is a D-threo isomer, made up of *p*-nitrobenzene ring, linked with dichloroacetyl tail via 2-amino-1, 3-propanediol moiety. Though resistant to chloramphenicol has been revealed through several mechanisms of action, its side effect is a major concern that limits its usage (Dinos et al. 2016).

4.3.23 Tetracycline

Tetracyclines belong to a class of antibiotics with a wide spectrum of properties discovered about 50 years ago which are rigorously used as growth promoters and therapy for infections in animals and humans. Studies have revealed that many organisms have developed resistance to the therapeutic importance of tetracycline. For instance, many tetracycline resistance genes in gram-positive and gram-negative bacteria have been identified through transposons and plasmids. Due to this inefficacy of the present tetracycline, the use around the globe has reduced significantly and new species have acquired gram-negative genes with genetic characteristics in protein regulation and efflux (Chopra and Roberts 2001).

4.4 Conclusion

Therefore, this chapter has provided a detailed information on the utilization of biologically active compounds of industrial interest derived from the marine environment. The major part of food biotechnology involves the application of beneficial marine microorganisms based on food processing. This will go a long way towards improving the nutritional qualities of perishable raw materials to a more refined, edible, and palatable foods. Though many research have revealed the potential benefits of these microorganisms, many of these microbial species are still untapped and unexploited; therefore, leaving wide gap in knowledge and application for industrial purposes. It is therefore important for the government and other relevant stakeholders to come up with sustainable strategies to harness the biological functions and physiological adaptations of these biomolecules rich bioactive biodiversity to revitalize the economy and industrial revolution, thereby creating opportunities for growth and development across different sectors. There is a need to explore several agro ecological and marine environment for the selection of unexploited as well as novel compounds of industrial interest. There is a need to utilize numerous metabolomics techniques for the characterization of these novel and unexploited molecules derived from the marine environment. There is a need to perform structural elucidation of active constituents that are responsible for diverse biological activities using NMR, TLC, HPLC, and GCMS. The application of genetic engineering and mutation techniques could be applied for the improvement of these beneficial strains derived from the marine environment. The application of bioinformatics, proteomics, and genomics could be applied for the detection of relevant genes that regulate diverse biological activities in these marine microorganisms. Therefore, it can be anticipated that many natural metabolites and biomolecules of great industrial benefit could be generated from marine microorganisms owing to their novel structures and functional properties within the biological system against the terrestrial microbes.

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Chapter 5
Climate Change and Pesticides: Their Consequence on Microorganisms

Kingsley Eghonghon Ukhurebor, Uyiosa Osagie Aigbe, Robert Birundu Onyancha, and Charles Oluwaseun Adetunji

Contents

Abstract It has been highlighted that numerous types of pesticides could prevent the incidence of diseases and pests which normally affects the increase in

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agricultural productivity. They play a crucial role towards increment in agricultural productivity and they could also affect the climatic changes of a particular environment at a specific period of time. The influence of beneficial soil microorganisms as a component of essential soil factors also plays a vital part in boosting increase in food production. Moreover, regularized application of pesticides has led to the upsurge in the extent of health and environmental hazards and several imbalances in the ecosystem. Hence, in view of the aforementioned, this chapter will anticipate the significance of pesticides on the environment, essential soil factors, most especially beneficial microorganisms as well as the influence of climate change. Specific highlights were also given on the influence of different pesticides, their application, role of soil functions such as beneficial microorganisms on the environment. Necessary recommendations that could form a baseline study for further researches were also recommended especially those that could assist in the improvement of food production in order to meet the demand of the ever-increasing population at global level.

Keywords Climate change · Environment · Microorganism · Pesticides

5.1 Introduction

Annually, about 2.6 billion pounds of pesticides are utilized for some specific purposes such as snuffing and/or decelerating growth rate of harmful microorganisms, extinguishing and/or decelerating growth rate of unwanted plants, monitoring of germs and harmful microorganisms, and extinguishing and/or controlling of insects universally, respectively (Widenfalk et al. 2008; Muturi et al. 2017; Ukhurebor et al. 2020a). Approximately, 80% of these pesticides are used for the enhancement of agricultural proficiency while the remaining 20% are mostly used for structural and communal health in the deterrence, moderation, and obliteration of diseases and pests (Widenfalk et al. 2008; Muturi et al. 2017).

Several types of pesticides have been highlighted for specific purposes based on their activities. Examples of such pesticides include algaecides, fungicides herbicides, fungicides, antimicrobials, rodenticides, insecticides, and disinfectants (Ukhurebor et al. 2020a). According to Subhani et al. (2000), the level of impact of pesticides depends on the form of pesticides applied, the quantities applied, and the present environmental conditions when the pesticides are applied.

According to Mehjin et al. (2019), it is not easy to determine exactly the influence of the different forms of pesticides on living organisms and their activity. The swift upsurge in the world inhabitants has led to an increase in pesticide application which has led to an increase in several environmental hazards as a result of high pesticide application (Mostafalou and Abdollahi 2017; Mehjin et al. 2019). Furthermore, it has been observed that even minimal application of pesticides might still have some adverse effects most especially on biological and chemical characteristics as well as the biochemical activities of living microorganisms (Singh et al. 2008; Cycon et al. 2010).

Pesticide application and abuse have increased due to their continuous application against pests, insects, microorganisms, and diseases. Conversely, this has resulted in several undesirable side effects on the environment, food, and human welfare generally (Palikhe 2007; Mostafalou and Abdollahi 2017).

Several studies have shown that the application of pesticides could lead to several undesirable impacts on useful microorganisms in the air, land, water, and soil (Singh and Prasad 1991; Bhuyan et al. 1992; Mostafalou and Abdollahi 2017). This has been documented to have a greater effect on their activities, roles, and mode of operation (Schuster and Schroder 1990).

Several microorganisms are beneficial because of the various indispensable roles they play on the environment as a whole (Husain et al. 2009; Meena et al. 2020; Mehjin et al. 2019).

According to Prasad Reddy et al., microbes play an essential part in soil productiveness. They assist in the biodegradation of organic matter, the recycling of nutrients, the formation of humus, the stability of soil structure, the fixation of nitrogen, the preferment of plant growth, the biocontrol of diseases, and several biochemical conversion processes such as formation (ammonification) and conversion (nitrification) of ammonia and its compounds (Husain et al. 2009; Meena et al. 2020; Mehjin et al. 2019).

Considering the present incessant variations and effects of weather in our diverse daily activities which are believed to be mostly caused by human activities (Ukhurebor and Abiodun 2018). Accordingly, the influence of the application of pesticide is possibly one of the utmost potential contributing factors to the present incessant variations and effects of weather or possibly the other way around (Ukhurebor et al. 2020a; Nwankwo et al. 2020a, b). The variations in the climatic conditions could be influential on both the application of pesticides, as well as the losses of pesticides to the environment.

Specifically, several studies have revealed the ability of some species of microorganisms to distillate pesticides, as such they could be serving as a medium for pesticide conveyance to more advanced trophic stages (Cooley et al. 2007; Jo et al. 2011). Additionally, studies have shown that the unswerving effects of pesticides on one trophic stage could circuitously affect the copiousness of species at advanced or minor trophic stages (Foit et al. 2010; Relyea 2009; Staley et al. 2011).

The environmental effects of pesticides as well as the hypothetically diverse responses among some species of microorganisms could also have severe consequences for the regulation of microbial measures and the well-being of other living organisms, the health of humans in particular. However, from some previous studies, we can inferably say that the prognostication of the effects from the application of pesticides on bacterial species is the most awkward compared to other microorganisms (Staley et al. 2015). This, according to Staley et al. (2015), is due to the wide multiplicity of physiological approaches employed by bacteria. Consequently, it will be somehow difficult in drawing any general deductions concerning how the various forms of pesticides affect bacteria in particular.

It has been observed that climate changes could be greatly influenced by several human activities most especially through the application of pesticides on the environment. Therefore, this chapter will provide a comprehensive review on the recent trends on the application of pesticides and how human factors could affect the activities of some essential soil functions such as beneficial microorganisms. Necessary recommendations that could prevent several hazards and anthropogenic activities that could increase the level of pollution as a result of pesticide applications were also highlighted.

5.2 Climate Change

5.2.1 Causes of Climate Change

Climate change as rightly reported by "the Intergovernmental Panel on Climate Change (IPCC) is a statistically substantial change in either the average state of the climate or in the inconsistencies in the average state of the climate, taking place over a lengthy period. It entails a modification in the climate ensuing as a result of natural internal progressions or external influences that are ensuing by the direct or indirect activities of humans" (IPCC 2014; Field et al. 2014). These vicissitudes are integral part of the atmosphere, together with the normal discrepancy in the climate experiential over an extensive period (Ukhurebor and Umukoro 2018; Ukhurebor et al. 2019; Field et al. 2014).

The Food and Agricultural Organisation of the United Nations (FAO 2018) in their report also pointed out that there has been globe upsurge in the amount of temperature most especially from the twentieth century to the beginning of the twenty-first century. This increment in the amount of temperature according to them started at about the mid of the nineteenth century.

According to Field et al. (2014), in several regions of the world the release of the various forms of greenhouse gasses (GHGs) like Carbon (IV) Oxide (CO_2), Methane (CH_4), and Nitrous Oxide (N_2O) have amplified extremely due to the activities and influence of humans, such as agricultural and industrial activities. In their report, it was projected that in the next coming decades numerous individuals most especially those living in unindustrialized regions or areas would encounter shortage in water and food supply as well as adverse health implications ensuing as a consequence of climate change.

These gasses (GHGs) which are reportedly derived from fossil fuels (that serve a main means of global energy source) and other chemical substances including pesticides which consequently allow astrophysical radiation to go through the air in the atmosphere but prevent the reflection of heat from making their way back astronomically. This could consequently lead to an increase in the earth's temperature which may cause variations in the climate; possibly resulting in global warming (Ukhurebor and Azi 2019).

The issue of the health effects as a result of environmental effluence is now a cause of serious concern to environmental scientists globally. At the moment, there are several ongoing scientific deliberations among environmental scientists concerning the core source of global warming which consistently results in climate change (Nwankwo and Ukhurebor 2019; Herndon and Whiteside 2019; Nwankwo et al. 2020a, b). The apparent disagreement that the main source of global warming is not only CO_2 heat retention and other GHGs, but particulate pollution that engrosses radiation, heats the lower atmosphere (troposphere), and upsurge the efficiency of atmospheric-convective heat elimination from the earth's surface (Nwankwo and Ukhurebor 2019; Herndon and Whiteside 2019; Nwankwo et al. 2020a, b). Nevertheless, there have been continued research studies aimed at unraveling other tendencies which possibly cause environmental effluences that perhaps result in global warming (Nwankwo and Ukhurebor 2019; Herndon and Whiteside 2019). As reported by Nwankwo et al. (2020a, b), the continuous-increasing progression in communication and information technology via the use of computers and enormous data centers globally had also been recognized as possible sources of environmental effluence, dilapidation, and core contributing factor to the climate change issues.

5.2.2 Consequences of Climate Change

Change in climate was described by William Nordhaus in 2018 as a "Colossus that threatens our world and the ultimate challenge for economics". It was also referred to as World War III by Joseph Stiglitz in 2001. The change in climate threatens genuinely disastrous consequences across the globe and with the rights of huge numbers of humans been among the victims (Alston 2019).

Reportedly, global change in climate conditions are mostly a consequence of human activities on the natural composition of the global climate variables or elements (Ukhurebor et al. 2020b; Ukhurebor and Nwankwo 2020), especially those caused by the release of GHGs from the use of fossil fuels. The world energy consumption has been projected to grow by 28% between the years 2015 and 2040. Change in the climate could be linked to natural external forces like change in the orbital parameters/variables of the earth or the emission from the sun (solar emission) and other normal internal progressions in the earth's climate scheme. Moreover, these could also be linked to recent consequences such as record temperatures, fast melting icecaps, extraordinary wildfires, "thousand years" floods, and more frequent hurricanes, respectively (Philander 2008; Alston 2019).

In the short run, encouraging consequences of warming climate may be experienced in some parts of Russia, northern Europe, and Artic, with the extended net effects expectedly to be harmful if appropriate action is not put in place to decrease GHGs emissions. Giving the position and the inadequate governmental measures in responding to the issues like flooding, droughts, and diminishing food production, parts of Asia and Africa are more vulnerable (Busby and Busby 2007).

A recent report by IPCC shows that the mean land as well as the mean sea surface temperature had increased by 0.6 ± 0.2 °C. This according to them started at about the mid-nineteenth century, with the utmost alteration observed since 1976 (IPCC 2014; Field et al. 2014). Precipitation pattern has also been altered with arid and semi-arid areas becoming less moist. Other climatic zones, particularly mid to latitude, are also becoming mistier. Based on an array of other development setup and model parameterizations, the IPCC recognized that with no definite measures taken to decrease the emissions of GHGs, there was a possibility of global temperature increase between 1.4 and 5.8 °C from 1990 to 2100, with average precipitation as well as average wind speed predicted to be less reliable; however, with recommended important vicissitudes (McMichael et al. 2004; IPCC 2014; Field et al. 2014).

According to the National Research Council (2012), understanding the impact of humans on GHGs concentration is difficult due to GHGs occurring naturally in the atmosphere of the earth, as CO_2 is created and utilized in several normal activities that are a portion of the carbon cycle. But, with the mining for long-buried carbon forms like coal and petroleum/oil burning as a means of energy, extra CO_2 is emitted and released into the atmosphere more swiftly than in the normal carbon cycle. Other activities of humans like the production of cement and other chemicals as well as deforestation have also contributed to the amount of CO_2 added to the atmosphere.

More countries have experienced conflicts for the past 50 years, with nearly 70% of sub-Saharan African countries experiencing armed conflicts since 1980. Evidence shows a strong relationship between change in climate and increased chances of more conflict. According to recent estimates from the WHO, 119,000 deaths were caused by collective violence in 2012, while an estimated 505,000 deaths were caused by interpersonal violence and suicide was committed by 804,000 people (Akresh 2016).

Many features of our planet have been affected by the change in climate, with the weather being one such aspect greatly affected. From 1990 to 2000, the average global temperature has risen from 1.4 to 5.8 °C. The global mean sea level has also increased from 4 to 8 in. (that is 10–20 cm). A recent finding by IPCC showed that the concentration of CO_2 in the atmosphere in 2005 was 375 parts per million when compared to the periods (pre-industrial) when industrial activities were at levels of 280 parts per million. This according them is a major contributing factor in the increase of the earth's surface temperature to about 0.6 °C (Kaddo 2016). Also amplified surface temperature of the earth is instigating the melting of glaciers at a fast pace than winter snow can replace them, leading to an upsurge in the average sea level to about 4–8 in, during the twentieth century. The upsurge in the average sea level has the possibility of instigating a rise in the saltwater invasion into coastal aquifers, surface islands and coastal aquifers that are supportive to humans, as well as a change in the salinity circulation in estuaries, alteration in coastal circulation outline, conveyance infrastructure in truncated-lying regions, and obliteration and amplified pressure on the coastal level arrangement (Philander 2008).

Extreme weather events have multiplied since 2002 in Romania, with the country drought in 2007 being reported to be severest in 60 years. Also, hurricanes have become more destructive due to warmer temperatures resulting from GHGs emission, resulting in warmer water in the oceans. Due to these warmer oceans, tornados and hurricanes are more intense now as warmer atmosphere results in more energy in the atmosphere. Change in climate has also played a major role in ice sheets shrinking, with ice melting resulting in sea level increase; hence, endangering many islands. Greenland and West Antarctic sheets were reported to be melting approximately 130 billion tons of ice annually (Kaddo 2016; Vannuccini et al. 2018).

Poverty and inequality are aggravated by a change in climate with serious implications in underprivileged countries, locations, and places where the poverty level of people is high. According to Alston (2019), developing countries of the will reportedly bear about 75–80% of the cost to alteration in the climate. The health of humans is subtle to earthly and geographical changes in climate and weather, with weather being considered to be subject to change by actions of humans but its effects may be reduced by variation measures. While variation is an important determining factor of health consequences of change in climate, the effect of anthropogenic emission of GHG on the climate means that change in climate can be a well-thought-out risk factor that could be changed by the intervention of human with related effects on the disease problem (McMichael et al. 2004).

An estimation as reported by the World Health Organization (WHO), one-third of the world’s infection/disease problems are caused by environmental factors and that about half of these problems are bear by little children under the ages of 5 years, representing about one-tenth of the population of the world. According to estimated disability-adjusted life years metric by the WHO, children under the age of 5 years bear approximately 90% of the prevailing world infection/disease problems are due to alteration in the climate. Most of these problems are felt in unindustrialized countries and low social-economic rank populations, which directly increases the environmental justice issue. From the mid-1970s, change in climate has been estimated by the WHO to contribute to more than 150,000 deaths and in the year 2000, approximately five million lost disability-accustomed life years globally through a rise in diseases like diarrhea, malnutrition, and malaria largely in emerging countries. It was also estimated by the WHO that changes in the climate-induced excess risk of this limited number of consequences which would multiply by 2030, with areas bearing the major problem of climate-sensitive infections/diseases are those with the least capacity to adjust to risk but contribute the lowest in GHG global emissions. The major portion of the problem of infantile morbidity and mortality ascribed to alteration in climate is malnutrition and infectious disease, as children are more exposed to famine and nutritional deficiency than adults. They represent the mainstream of the world populace la-di-da by hunger as well as dysfunction from insufficient nutrition during early development can last a lifetime. In certain regions, children are more vulnerable to infectious diseases like pathogens (such as cholera and other diarrhoeal infections/diseases) from pollution resulting from plant and water from storms and floods, in addition to vector-borne infections/diseases (such as dengue fever and malaria) owing to change in the climate. In 2003, a rise in the incidence of deadly heatwaves, which is a direct effect of change in climate brought about deaths of 22,000–40,000 in Europe, with the unswerving consequence on children as a result of hyperthermia, heat stress, renal infection/disease, and respiratory disease/infection (Perera 2014; Hayes et al. 2018).

In Africa, the two furthermost problems caused by the alteration in climate are; food safety/security and human well-being (health), with both having an unswerving and critical effect on human well-being. Food safety/security deals with the accessible quantity of food for a specific purpose. Accordingly, the United Nations Organization for Food and Agriculture (FAO) reported that the four most vital factors for daily food safety/security are; food availability, food approachability, food supply steadiness, and approachability as well as the degree of which safety of food nutrients can be sustained and improved (FAO 2018). The accessibility of food is anticipated to be greatly impacted as the alteration in climate may change areas fit for agriculture, the growing season length, and yield potential. Food access and supply stability are also closely linked to infrastructure conditions, as some of these infrastructures like road and railway may struggle under growing weather pressure and air transport may be delimited owing to allowable decrease in GHGs, with IPCC expecting agricultural production, with food access in several African nations and region to be sternly compromised by inconsistency and alteration in climate (IPCC 2014).

Expectedly, about one billion hectares of land which are previously arable due to substantial activities will expand by 50–90 million hectares due to change in climate and it is a consequence rapidly spreading hunger (IPCC 2014). Another threat of change in climate is the probable rise in vector-borne diseases in some areas of Africa, where in some regions, such as the East African high ground plateaus or highlands, the possible incidence, seasonal program, and geographic extent of vector-borne infections/diseases are anticipated to increase. These infections/diseases such as various kinds of fever, malaria, different kinds of virus-related encephalitis, schistosomiasis, leishmaniasis, onchocerciasis, and lyme infection/disease. Expectedly, it is predicted that by 2100, there will be about 16–28% upsurge in the human-month exposure extent of malaria especially in the African region (Wlokas 2008).

The main drivers of agriculture response to the change in climate are biophysical influences and socioeconomic issues. Development of crops is exaggerated biophysically via weather-related (meteorological) parameters/variables which include change in temperatures, change in precipitation patterns, and surge in the extent of CO_2. These consequences exaggerated by biophysical parameters will vary through time, with some area having a positive or negative impact on agricultural production in some regions and agriculture systems. While socioeconomic factors impact the response to vicissitudes in the production extent of crops, with deviations in price and modifications in comparative benefit (Parry et al. 2004; Serdeczny et al. 2017). The IPCC (2014) report summarizes the effect of change in climate by type, possibility, and influence on diverse segments like human health and agriculture, which concludes that several weather actions and extreme will frequently and intensely become more widespread during the twenty-first century.

An extensive retreat of glaciers on five continents has been as a result of global warming. An upsurge in sea level is caused by the distribution of water to the river, sea, or ocean resulting from melting land ice and the thermal/heat expansion in water within these rivers, seas, or oceans. As the ocean water temperature rises, the rivers, seas, or oceans become less dense leading it to spreading and inhabiting more surface area on the earth. Since the end of the period of ice (about 18,000 years ago), river/sea/ocean levels have increased by about 120 m (Sharma et al. 2013).

Reportedly, some studies have shown that the influence of alteration in climate setups on the hydrology of several regions and basins, with a projected increase in temperature and a decline in rainfall results in the decrease of the net recharge and affects freshwater resources. In Africa, there has been a decrease in the water resources over time due to persistent droughts and land-use patterns, a projected 29% increase of water shortage to be experienced in Sub-Saharan Africa (SSA) by 2050, while the river flow in the Nile area expected to decline by 75% with a damaging consequence on irrigation practices ["the United Nations Environment Programme (UNEP) African regional implementation review for the 14th session of the commission on sustainable development (CDS-14), report on climate change, prepared by UNEP on behalf of the Joint Secretariat, the United Nations Economic Commission for Africa (UNECA), the United Nations Environment Programme (UNEP), the United Nations Industrial Development Organization (UNIDO), the United Nations Development Programme (UNDP), the African Development Bank (ADB), and the New Partnership for Africa's Development (NEPAD) Secretariat"].

The increasing demand for water resources also poses considerable risks to water safety/security in Sub-Saharan Africa, which is intensified via the change in climate upsetting river runoff, contribution to difficulties in the quest for water from irrigation and posing risk of surface groundwater effluence (contamination/pollution) as a result of intense rainfall. Some periods of little rainfall was observed in the 1970s and 1980s when compared to 1900–1970 leading to severe drought in the Sahel region. Also, there are reports of severe flooding amplification in the Niger Basin in the last few years, with an increased risk of flooding with temperature increase. Groundwater regeneration rates have also been predicted to decrease by about 30–70% in the western parts of southern Africa and rise by about 30% in some regions of eastern and southeastern regions of Africa for both 2 and 3 °C warmings above preindustrial extents. This estimated increase of incidence in heavy rainfall in East Africa does not lead to authentic groundwater renewal due to penetration extents which was not contemplated (Serdeczny et al. 2017).

According to Nwankwo et al. (2020a, b), some human actions have changed atmospheric structures, such as temperature, rainfall, levels of CO_2, and ground-level ozone. As precisely emphasized by IPCC, there are some doubts about vicissitudes in the climate. They emphasized that warming of the climate scheme is now explicit and it is apparent that global warming is getting higher as a result of the synthetic emissions of GHGs, particularly CO_2 by humans (Field et al. 2014).

Subsequently, there is a need for global exploitation to resist these influences that are happening incessantly. Nevertheless, as a result of global warming, the kind, amount, and extent of life-threatening events are projected to increase even with slight upsurge in temperature (Meehl et al. 2007).

5.3 Influence of Climate Change on Microorganisms

Microorganisms play a fundamental role in animal and plant nutrition and health. They are the architects of soil health and productivity given that they take part in nutrient mobilization and uptake. Notably, they aid in plant development and offer infection/disease protection in their well-known processes namely; phosphate and sulfate solubilization, siderophore formation, nitrogen fixation, denitrification, immune variation, signal transduction, and pathogen management (Prakash et al. 2015) In marine life, microbes such as bacteria, fungi, archaea, viruses, and protists are engines of the ecosystems. They not only act as food to marine life but also keep the ecosystem healthy, clean ocean waste, and protect it against diseases.

Imperatively, on earth, microorganisms exist in all environments. Human activities (effects) such as agriculture, eutrophication, and environmental changes, namely, UV radiation, salinity, water chemistry, elevation, mountain topography, and precipitation constitute factors that affect microbial well-being. These factors may lead to microbe's extinction and loss of biodiversity (loss species, communities, and habitats) (Cavicchioli et al. 2019). Accordingly, in this section, we would explore the effect of climate change on microorganisms. Noteworthy, we examine both marine and terrestrial microorganisms.

5.3.1 Effect of Climate on Marine Microorganisms

It is well established that the marine environment covers approximately 70% of the total surface of the earth. This environment includes tropics, shallow water, water coral reefs, and deep ocean trenches. The marine habitats play host to a million organisms which include many autotrophs, animals, and both autotrophic and heterotrophic microorganisms. Microorganisms are important in the well-being of coral reefs in that they recycle waste products, provide vital nutrients, carbon flow in the marine ecosystem, organic matter decomposition, and vitamins and essentially assist the immune system to fight pathogens (Bourne et al. 2016).

Human activities in the form of burning of fossil fuels and industrialization have given rise to CO_2 concentration in the atmosphere. CO_2 is the main cause of global warming and its dissolution in the ocean surface results in seawater carbonate chemistry (Bourne et al. 2016). These have resulted in an increase in acidity and lowering of pH levels thus causing a change in several chemical properties. Ocean warming, acidification, eutrophication, and overuse together cause the decline of coral reefs and may cause ecosystems to shift towards macroalgae (Hughes 1994; Mumby et al. 2007; Enochs et al. 2015). Therefore, observing an appropriate carbonate concentration is paramount for the formation of calcium carbonate which is vital in the building of skeletons and shells of marine organisms, coral reefs, and plankton.

The ratio of CO_2 concentration at the ocean surface to the atmosphere is approximately 1:1. Therefore, an increase in the atmospheric concentration results in an unexpected increase in CO_2 dissolved in ocean waters. Occasionally, if not all the time, an elevated CO_2 concentration can be detrimental to aquatic settings. For instance, it can hinder reproduction, disturb the growth process, and cause a change in skeletal and otolith development (Noorashikin and Das 2019). A study on elevated CO_2 levels on individual phytoplankton species demonstrates that an increase in the extents CO_2 offers selective benefit to noxious microalga, *vicicitus globosus*, resulting in interruption of biological/organic matter transmission across trophic stages (Riebesell et al. 2018). Furthermore, there is an increase in cell dimensions and carbon to nitrogen relations when photosynthetic green alga is subjected to elevated CO_2 levels. According to Schaum et al. (2012), CO_2 level increment results in changes in ecotypes and niche population of *O. teuri* which affects food webs and biogeochemical cycles.

For over 20 million years, the pH of the oceans was fairly constant. Apparently, in the past 200 years, it has fallen by an alarming 0.1 unit and if the atmospheric concentration of CO_2 reaches 800 ppmv, it is expected to fall by a further 0.3–0.4 unit in comparison to the present concentration level of 397 ppmv (Raven 2005). As a consequence, lower pH levels are expected which simply results in interference of intracellular homeostasis of microorganisms (Flynn et al. 2012). Moreover, lower pH causes a change in gene expression in bacteria and archaea and thus causes support in cell maintenance as opposed to growth (Bunse et al. 2016). Therefore, pH regulation is paramount and species which lack the capacity to do so are adversely affected. Regulation of pH is influenced by factors such as organism size, aggregation state, metabolic activity, and growth rate (Flynn et al. 2012).

An elevated temperature due to ocean warming triggers melting of sea ice, a rise in sea level, increased frequency of regional climate anomalies, deceased solubility of oxygen, and the diverse biological effect that influences whole ocean ecosystem and their constituent's species from microorganisms to algae to top predators (Yao et al. 2014). The latter (effect on microorganisms) which is our subject needs a clear and conscious discussion. Studies show that raising temperatures of some marine taxa could result in a poleward modification of cold-adapted communities (Follows et al. 2007; Barton et al. 2010, 2016; Thomas et al. 2012; Swan et al. 2013). Also, it has been reported that raise in temperatures upsurge protein synthesis in eukaryotic phytoplankton while tumbling cellular ribosome concentration (Toseland et al. 2013). Smaller plankton thrives well over larger ones during ocean warming resulting in change in biogeochemical fluctuations for instance particle/material spread or export (Moran et al. 2010). Furthermore, warming lessens iron constraint of nitrogen-fixing cyanobacteria, with hypothetically deep consequences for new-fangled nitrogen supplied or provided to food webs of the imminent warming oceans (Cavicchioli et al. 2019; Jiang et al. 2018).

5.3.2 *Effect of Climate on Terrestrial Microorganisms*

As aforementioned, the world today is grappling with the problems of global warming and climate change. These issues arise from the high concentration of GHGs (such as carbon dioxide, methane, nitrous oxide, and chlorofluorocarbons) in the atmosphere which leads to a warming effect. Like marine life, these affect terrestrial environments which contain approximately a total of 1029 microorganisms which is comparable to the number in marine environments (Flemming and Wuertz 2019).

Studies show that a change in climate alters microorganisms' species distribution and dictates their interactions with others. Of importance, soil microorganisms help in regulating nutrient transformation, afford plants with nutrients, permit coexistence among neighbors as well as monitor and manage plant population (Classen et al. 2015). Soil microbial connections involve interactions with each other together with living organisms' abundance, multiplicity, and configuration. Plant microbial interaction is considered undesirable when the net effects of all soil creatures diminish plant performance while interactions are desirable when aids brought about by soil communal improve plant performance such as biomass formation and existence (Classen et al. 2015).

Furthermore, climate shift is probable to upsurge the intensity, occurrence, and duration of cyanobacterial blooms in several eutrophic lakes, reservoirs, and estuaries (Paerl and Huisman 2008; Huisman et al. 2018). Imperatively, these cyanobacterial blooms yield a diversity of neurotoxins, hepatotoxins, and dermatoxins, which can be deadly to birds and mammals as well as impend the use of waters for recreation, drinking water invention, irrigational agriculture, and fisheries (Huisman et al. 2018).

Significantly, microorganisms respond differently to the change of climate because of their variance in physiology, growth rates, and sensitivity to temperature (Castro et al. 2010). It has been reported that when forest temperature was warmed by 5%, a change in soil bacterial abundance was noted whereby an increase in bacterial to the fungal proportion of the community was registered (DeAngelis et al. 2015). This kind of alteration of the microbial communal as a result of temperature modification is expected to cause a shift in ecosystem functions. Imperatively, according to Bakken et al. (2012), some microbes are responsible for "nitrogen fixation, nitrification, denitrification, and methanogenesis." Therefore, alteration in the comparative copiousness of living organisms that controls precise progressions could have an unswerving impact on the amount of that progression. Moreover, a warming study done on forest soil showed a sequential discrepancy in organic or biological matter putrefaction and CO_2 release (Melillo et al. 2017). This led to a significant change in the microorganism community and carbon use efficiency. In general, Melillo et al. (2017) in their study predicted anthropogenic climate change as a result of long-term cumulative and constant carbon release. Furthermore, carbon effect on the harmful cyanobacterial genus Microcystis was studied. Both field and laboratory experiments showed that the Microcystis spp. uptake of CO_2 and HCO_3^- was discovered to depend on the concentration of inorganic carbon (Sandrini et al. 2016).

A modification in temperature level results in a sudden change in soil moisture thus affecting the microbial community. Studies have shown that the bacterial community displays a rapid response to moisture pulses while the fungal group shows a slow response (Bell et al. 2008). Further, dry periods favor the fungal community. It has been established that a 30% reduction in water holding capacity shows fungal community dominating (Kaisermann et al. 2015). This tendency reveals that during non-dry extreme wet-dry conditions greater fungal population than bacterial community prevails (Kaisermann et al. 2015). Also, studies on the effect of elevated temperature on microbial respiration rates and mechanisms have been carried (Barton et al. 2016). It has been established that in soils with high-temperature settings registered lower respiration rates, showing that thermal or heat adaptation of microorganisms could decrease encouraging climate reactions (Bradford et al. 2019).

5.4 Pesticides: Types and Its Applications

Pesticides, according to Rani and Dhania (2014), are a massive and diverse set of constituents that are considered precisely to destroy organic/biological organisms such as weeds, insects, and rodents. Nevertheless, the prevalent applications of pesticides can result in the accrual which can affect agricultural yield, cause truncated biodegradability and this has caused pesticides to be classified as persistent toxic substances (Rani and Dhania 2014).

These are organic chemicals deliberately used for growing agricultural harvest, soil output, quality of products, reducing losses of agricultural products owing to crop pests, and for prevention of the human and animals' epidemic outbreak through insect vectors control. The use of pesticides in the field of agriculture after World War II lead to a progressive increase in world food production. Lately, more than 500 compounds were registered and used as pesticides globally (Parte et al. 2017).

Pesticides are applicable in various sectors of the economy such as health, agriculture, and industry. They are used essentially for monitoring various pests and disease carriers. They are mostly applied in agriculture for the monitoring of undesirable plants, insect plague, and diseases in both plants and animals (Meena et al. 2020; Mehjin et al. 2019). They are used in public well-being for vectors of infection/disease killing like mosquitoes and to kill pest damaging agricultural products. They vary in their chemical, physical, and undistinguishable features from one class to another. They are categorized based on their features and are studied under their specific groups. Pesticides are grouped into different categories depending on their requirements. The three common techniques of pesticide classification are based on the means of entry, pesticide purpose and the pest organism to destroy, and the pesticide chemical configuration (Yadav and Devi 2017).

The application of pesticides is of great significance in the protection, prevention, mitigation, and destruction of pests and diseases that could impair agricultural produce. They are applied essentially in the agricultural process to increase agricultural productivity to improve the standard of living (Meena et al. 2020; Mehjin et al. 2019).

Notwithstanding the benefits of the application of pesticides in agriculture and other endeavors, there have also been some consequences accompanying their applications. Consequently, the effluence resulting from the application of pesticides to living organisms as well as the environment have incessantly remained a course of apprehension (Mostafalou and Abdollahi 2017; Herndon and Whiteside 2019). However, our concern in this section is mainly on the implication's connection between climatic change and pesticides.

According to Palikhe (2007), when pesticides are used there is a possibility of the pesticides being moved from the area where they are used to other areas. Consequently, their mobility makes them occasionally move via air, land, soil, and water. Mostafalou and Abdollahi (2017), reported in their study that one of the foremost problems with the movement of pesticide from one area to another is that during such movement, they might come in contact with living organisms, and such connection can result in some impairment of both the living organisms and the environment. Pesticide exposure has led to immeasurable infections, development of cancer, and the death of thousands of people annually (Palikhe 2007; Mostafalou and Abdollahi 2017; Meena et al. 2020; Mehjin et al. 2019).

Another main problem accompanying the application of pesticides is bioaccumulation and biological magnification. Accordingly, the application of pesticides is now causing enormous menaces due to the obnoxious side effects of the chemical composition. These effects are perceptible in the environment mostly in food quality, biodiversity, human well-being, and changes in the climate (Mostafalou and Abdollahi 2017; Meena et al. 2020; Mehjin et al. 2019).

For categorization based on the means of entry, how pesticides interaction with the target are known as means of entry such as systemic, contact, stomach poisons, fumigants, and repellents. While for categorization based on pesticide purpose and pest organism they destroy, pesticides are categorized according to the target pest organism and they are given a precise name to show their action. The names of the pesticide group come from the Latin word "*cide*", which means "to kill or killer", are used as a suffix after corresponding pests name, they kill. Other pesticides are categorized according to the purpose such as development regulators that inspire or reduce pest growth, repellents which repel pests, desiccants which haste the aeration of plants for automatic harvest, or causes the dehydration or death of insects and chemosterilants, which sterilize pest. For categorization based on the chemical composition of pesticides, it is one of the furthermost techniques used for pesticide classification based on their chemical composition and dynamic ingredient nature.

This kind of categorization gives hint about the efficacy, and chemical and physical features of the various pesticides. The chemical and physical characteristics of pesticide information are greatly valuable in the determination of the application mode, application precautions, and the rates of application. According to Yadav and Devi (2017), based on chemical configuration, pesticides are categorized into four key categories which are; "organochlorines, organophosphorus, carbamates, and pyrethrin and pyrethroids."

There is a progressive increase in demand for pesticides, with more than 50% of the pesticides used are from Asia. The growing worldwide request for quality protein-rich food resources for the increasing population of the world allows for the need for the ecologically sound strategy development for sustaining soil health and evolving food security with soil biodiversity degradation (Meena et al. 2020).

Examples of commonly used pesticides include insecticides, nematicides, fungicides, herbicides, molluscicides, rodenticides, fumigants, disinfectants, wood preservatives, and antifoulants used as primary agents efficient against "insects, nematodes, fungi, weeds, slug pellets rat and mouse, etc." (Wesley et al. 2017; Rani and Dhania 2014). The application of pesticides certainly has a diverse influence on various species of microorganisms (Meena et al. 2020; Mehjin et al. 2019). It is therefore imperative to understand how these consequences of the use of pesticides affect the environment and use the resulting understanding to prognosticate the ubiquity of pesticides in such contaminated environments to confound standard regulatory procedures.

Insecticides are generally the most harmful to the environment followed by fungicides and herbicides (Yousaf et al. 2013). Despite the enormous volume of pesticides used, organophosphorus (OP) pesticides are a wide range of insecticides used on a broad spectrum of crops like vegetables, fruits, grains, and ornamentals. They are designed to repel or kill pests but can also be detrimental and deadly to other organisms including humans (Rani and Dhania 2014).

Other types of insecticides used counter to insects in all developmental forms according to Wesley et al. (2017) are "ovicides, larvicides, and adulticides used against the eggs, larvae, and adult stages of insects, respectively." However, the extensive use of insecticides has resulted in severe contamination and pollution of the environment, with the lingering effects, including carcinogenicity, mutagenicity, reproductive harmfulness, and respiratory and circulatory implications (Wesley et al. 2017).

Other research studies have shown that in some situations the use of pesticides in the environment interrupts the equilibrium or stability of the ecosystem (Mostafalou and Abdollahi 2017; Meena et al. 2020; Mehjin et al. 2019). This according to them, occurs when the said pesticide applied kills or affects the non-intended target, thereby alluringly distort the systematic stability of the ecosystem and the environment possibly will be changed for the benefit of the targeted pest. Such interruption in the stability of the ecosystem can also lead to climate inconsistency (Herndon and Whiteside 2019; Nwankwo and Ukhurebor 2019).

Several findings have shown that human-induced actions such as the application of pesticides and other chemicals (through the emission of GHGs from these chemical constituents) to the environment are enormously contributing to vicissitudes in the climate scheme (IPCC 2014; Field et al. 2014).

Report from the United Nations Framework Convention on Climate Change (UNFCCC 2007) and IPCC (2014) has stated that these GHGs (CO_2, $CH_{4,}$ and N_2O) are a consequence from the application of chemical constituents (such as pesticides) to the environment. These GHGs emissions allow radiation from the sun to transverse via the atmosphere; nevertheless, do not permit the reflected thermal/heat energy from going back into space. Hence, causing an upsurge in the temperature of the earth (Ukhurebor et al. 2020b; Ukhurebor and Nwankwo 2020) and this action will habitually cause climate variation that could as well cause to global warming (UNFCCC 2007; IPCC 2014; Field et al. 2014).

According to the statement from IPCC (2014), global warming is now a threat to living organisms globally. Presently, in Nigeria and several other nations of the world, there is a tremendous upsurge in the release of GHGs as a result of human actions (Field et al. 2014). Conversely, some reports have revealed that climate discrepancy, alteration or change could increase the risk in the diffusion losses of pesticides occurring from agriculture to the environment (Palikhe 2007). These incessant changes in the climate will not only disturb agricultural yields but could also expectedly make effects from the application of pesticides pretentious.

Notwithstanding the eminent harmful aids of pesticides on microorganisms and other living organisms as well as human well-being and the environment in general, the means of application and mode of action provides a proportionate advantage to agricultural productivity. Hence, the efficiency and application of pesticides are vulnerable to environmental effects.

The changes in the climate system, which conceivably arise from the ensuing of emission or release of GHGs, global warming, and another additional effluence such as the application of pesticides have been reported to have a significant influence on mortality rates in most parts of sub-Saharan Africa (Bakshi et al. 2019). Given these obvious environmental effects of the variations in the climate system, there could be an inference that the application of pesticides to the environment may also affect climate change impacts on the environment.

The application of pesticides certainly has a diverse influence on various species of microorganisms (Meena et al. 2020; Mehjin et al. 2019). It is therefore imperative to understand how these consequences of the use of pesticides affect the environment and use the resulting understanding to prognosticate the ubiquity of pesticides in such contaminated environments to confound standard regulatory procedures.

Due to the detrimental effects of pesticides on some microorganisms, the use of pesticides is unavoidable. According to Ukhurebor et al. (2020a), "these pesticides comprising of insecticides, herbicides, and fungicides are a group of potentially toxic substances that are capable of disrupting the microbial structure and function in aquatic habitats."

Bearing in mind the dynamicity and influence of weather in our daily activities, it is believed that the effects of pesticides used for the prevention, mitigation, and destruction of pests and diseases for the improvement of agricultural efficiency could also be a contributing factor and vice versa. Ukhurebor et al. (2020a) reported that climate change could influence both the use of pesticides, as well as the losses of pesticides to the environment. Presently, the issue of climate change is one of the furthermost intellectually stimulating issues confronting the entire human race (Nwankwo and Ukhurebor 2019).

Research findings from the study carried out by Mostafalou and Abdollahi (2017) provide a fundamental understanding by what means pesticide arbitrated vicissitudes in aquatic microbial groups could disturb aquatic groups as well as main environmental progressions such as bioremediation, putrefaction, and cycling of nutrients. These discoveries are similarly crucial in understanding the anthropogenic chemical impurities' effect on the health of humans since aquatic environments are subjugated by mosquitoes which transport a wide spread diversity of disturbing and dangerous human pathogens.

Therefore, we must continue to protect our environment from these unfavorable effects of climate change by extremely tumbling the emissions of greenhouse gases especially via the use of chemical substances such as pesticides.

5.5 Impact of Pesticides on Microorganisms

In their rightful usage, pesticides are designed to destroy and control/regulate unwanted biological organisms such as insects, weeds, fungi, and other pests to enhance crop yields and stability. However, continued usage/misuse of these substances poses serious environmental problems for both targeted and untargeted organisms. Pollution by pesticides is well facilitated by the frequency of usage, amount of rainfall, drainage pattern, properties of pesticides, and activities as a result of microbes (Mensah et al. 2014). Importantly, this pollution affects aquatic life and soil ecosystems which comprise of microorganisms, birds, invertebrates, and human beings.

5.5.1 Effect of Pesticides on Aquatic Microorganisms

The consequence of pesticides on aquatic life is immeasurable. Notably, they interfere with respiration, growth rate, reproduction, biosynthetic reactions, and photosynthesis of fundamental microorganisms such as algae, fungi, bacteria, and plankton. Owing to the importance of microorganisms, any effect on them leads to a dramatic change in marine life. For example, a compromise of zooplanktons leads to migration and death of fish which in return prompts the death of many birds that depend on fish as prey. Furthermore, contamination of breeding sites of amphibians by pesticides is another concern. Reports have shown that green frogs and latewood frogs have been affected by an application of atrazine (Storrs and Kiesiecker 2004; Delorenzo et al. 2001). Having regard to the aforementioned, here we present concised highlights on pesticides (examples of some of the insecticides, fungicides, and herbicides) and their effects on microorganisms.

5.5.1.1 Insecticide

Chlorinated Hydrocarbons: A wide response of marine organisms to chlorinated carbons have been observed and recorded. Firstly, they affect the respiratory response of algae (Vance and Drummond 1969; Clegg and Koevenig 1974; Hill and Wright 1978). At lower concentrations of less than 1 mg/L, endrin, dieldrin, and aldrin have no elaborate effect on green and blue-green algae respiration. However, at elevated concentrations of around 100 mg/L, studies have shown a lowering of adenosine triphosphate levels by aldrin and dieldrin but algal population density was not altered (Clegg and Koevenig 1974). Nineteen and 17% growth inhibition of algae by mirex and methoxychlor was reported. Nevertheless, a mixture of the two comprising of 50 μg/L each did not have any effect on their population growth (Kricher et al. 1975).

Endosulfan: Insecticide affects reproduction. It has been found that a delay of meiosis for 5 days was experienced when *Chlamydomanas reinhardtii*, which is a green alga, was exposed to a single exposure of endosulfan during its four development stages. On the other hand, exposure of 47 and 150 μg/L concentration weakened, respectively, the growth of female and tetrasporophyte of *Champia parvula* (red algae) (Thursby et al. 1985). On the bacterial population, 0.02 and 2 mg/L of endosulfan application resulted in 30.99% and 70.58% population inhibition of heterotrophic bacteria (Rajendran et al. 1990). Similarly, a reduction of bacterial abundance was reported when 1 and 10 μg/L concentrations of endosulfan were used in estuarine microbes also resulting in a decline of cyanobacterial communities (DeLorenzo et al. 1999).

Chlorpyrifos: Studies show that at a concentration of between 1 and 10 mg/L, the growth of Anabaena (blue alga) was affected by the insecticide (Lal and Lal 1988). However, the concentration of 0.24 and 0.64 mg/L administered to *Skeletonema costatum* and Minutocellus polyorpus proved toxic (Walsh et al. 1998). On bacteria species, chlorpyrifos results in a reduction of plankton in freshwater ponds with an application of 1.2 μg/L concentrations (Brown et al. 1976).

5.5.1.2 Herbicide

Brominated Herbicide Diquat: As herbicide, diquat has shown tremendous effects on aquatic life. For instance, at a concentration of ≥0.3 mg/L of diquat, a significant alteration of densities of algae and bacteria is reported. Similarly, at a concentration greater than 0.3 mg/L, the richness of protozoa is greatly reduced and there is no recovery after 21 days of exposure (Melendez et al. 1993). Furthermore, a comparative study has shown varied inhibition of algal and bacterial species. Out of this study, two algal species exhibited 53–69% inhibition of 14 C (carbon-14) uptake, another two showed 99–100% while cyanobacteria (five species) showed 100% inhibition (Peterson et al. 1994).

Phenylurea Herbicide: It affects growth, reproduction, and proved toxic. A decline in the growth rate of Chlorella sp. was experienced when the admission of monuron (a type of phenylurea) of 4 mg/L concentration was made (Cho et al. 1972). The same concentration proved toxic to marine species when diuron, a lethal phenylurea, was added (Ukeles 1962). The herbicide diuron is considered the most toxic phenylurea. Reproduction inhibition of Chlorella fusca was observed with 23.39 mg/L of chlortoluron, another type of phenylurea (Faust et al. 1994).

Atrazine: The herbicide has expansive effects on the algal community. The effects depend on exposure time, concentration, and the type of species involved. For example, 1 μg/L of atrazine results in 41–67% decrease of chlorophyll in *Chlorella vulgaris* and *Stigeoclonium tenue*. After 7 days of exposure, similar results were observed in *Oscillatoria lutea* which is a blue alga (Torres and O’Flaherty 1976). A 35% inhibition growth rate of Nannochloris oculate was reported when 50 μg/L was used (Mayasich et al. 1986). Interestingly, the same amount showed no effect on the growth rate on *Phaeodactylum tricornutum*. Conversely, a 5000 μg/L concentration suppressed five algae growth rates (Stratton 1984).

5.5.1.3 Fungicide

Fungicide has similar effects to those discussed on insecticides and herbicides. Herbicides like ethyl mercury (Ukeles 1962), zineb, and nabam are toxic to algae (Moore 1970). They inhibit growth, an example being organomercury which with a dosage of <1 μg/L reduced plankton growth and its photosynthesis (Harriss et al. 1970). Inhibition of carbon-14 uptake has also been reported in several algae (Peterson et al. 1994). This observation was witnessed on the *Scenedesmus quadricauda*, a blue alga, when 0.08 mg/L of triazole was used with the uptake of carbon-14 was from 0 to 30% experienced. Also, Rajendran et al. (2007) examined the effect of biopesticide, an insect repellent/insecticide, and a fungicide on *Tolypothrixscytonemoides*. The authors stated that the extents of the photosynthetic oxygen development reduced but the rate of respiratory oxygen utilization was enhanced in the algae cell exposed to these chemicals (pesticides). It was observed that the glutamine synthetase action was affected in all the pesticides with the exception of bavistin that improves the nitrogenis activities. Moreover, the liberation of carbohydrate and ammonia was drastically improved in the cells that were exposed to the pesticides but there was a decrease in the liberation of carbohydrate when treated with bavistin.

5.5.2 Impact of Pesticides on Terrestrial Lives

Impact on humans: Three different ways through which humans are exposed to pesticides include; direct deposition or contact of pesticides, consumption of contaminated fees (food, water, or drinks), and inhalation of polluted air (Sánchez-Bayo 2011). Pesticides have proved to be toxic and the magnitude or nature of toxicity be subject to the amount, nature of the chemical, means of exposure (whether orally, dermal, or inhaling), and organism type (Yadav and Devi 2017). The noxiousness can be acute (severe) or chronic (long-lasting). Severe effects result from a single exposure of harmful effects entering through any route. It causes severe infection/ illness and humans develop symptoms such as headaches, body aches, skin rashes, poor concentration, nausea, dizziness, impairment of vision, cramps, fright or panic attacks, and in severe cases coma. On the other hand, chronic effect emanates from the repeated dosage of small amounts extended over some time. Chronic effects include birth implications, noxiousness to a fetus, formation of benign or malignant tumors, genetic vicissitudes, blood disorders, nerve disorders, endocrine interruption, and reproduction implications (Yadav and Devi 2017). A good example summing up effects on humans is a report which indicates that a staggering number of 5000–20,000 of persons are killed and over a million get poisoned per year (Yadav et al. 2015; FAO/WHO 2000).

Impact on soil microflora: The interaction of soil with pesticides may change biochemical reactions, enzymatic activities, and microbial diversity. Microbial diversity alteration will eventually result in loss of fertility. The killing and imbalance of microbes' community may adversely compromise fundamental responses such as nitrogen fixation, nitrification, and ammonification (Yadav and Devi 2017; Hussain et al. 2009; Munoz Leoz et al. 2011). This will result in death of some organisms, reduction in nutrient cycling and decomposition, low productivity, infertility, etc.

For instance, it has been recognized that important soil microorganisms that could colonize the roots of plants such as arbuscular mycorrhizal fungi and bacteria could form a symbiotic relationship with many plants and enhances their growth. These beneficial microorganisms also possessed the capability to enhances the growth of plants even under a normal or stressed condition (Sainz et al. 2006; Saleem et al. 2007). Also, the utilization of pesticides possibly will stimulate the effects of such pesticides which could consequently affect the activities of such microorganisms.

Sainz et al. (2006) examined the influence of soil pollution with hydrogen cyanides on the vegetation and its relationship with arbuscular mycorrhizae in a noncontaminated soil and in highly contaminated soil having the same plant cover. The authors used *Plantago lanceolata* plants during the mycorrhizal evaluation which might be linked to their presence in both plots with known mycotrophy. It was observed that the hydrogen cyanide did not have any significant influence on the rate of colonization of *Plantago lanceolata* by the arbuscular mycorrhiza fungi while the density of the arbuscular mycorrhiza fungal spores and viable arbuscular mycorrhiza hyphae were minimal in HCH-polluted soil when compared to the nonpolluted soil.

Verdin et al. (2006) evaluated the effect of anthracene on the rate of colonization of chicory root by *Glomus intraradices*. The authors discovered that the utilization of anthracene decreased the growth of extra radical mycelium which led to reduction in the rate of spore germination, sporulation, and root colonization. Vieira et al. (2007) stated that the influence of sulfentrazone on rhizobial and mycorrhizal effectiveness of soybean plant. They observed that pesticides possess the capability to reduce the microbial infection of soybeans which consequently affect the rate of nitrogen fixation and the total growth of plants also. Fox et al. (2007) showed that the application of organochlorine pesticides reduced the growth of rhizobial bacteria on host plant root which consequently led to a decrease in root nodules, decrease the rate of nitrogenase activity, and decrease the yield of the harvested plant.

5.6 Significance of Dynamics that Influences Application of Pesticides and Climates Changes on Microbial Diversity

The influence of pesticides on the level of beneficial microorganisms available in the soil is regulated by numerous environmental factors such as its bioavailability, the level of toxicity of the pesticides, their persistence, and the concentration of the pesticides (Abdel-Mallek et al. 1994). Moreover, it has been observed that the process of desorption and adsorption also performs a crucial aspect in regulating the level of pollutant accessible in the soil solution (Bonczek and Nkedi-Kizza 2007; Katagi 2008) which consequently affects its bioactivity, bioavailability, and the level of its biodegradation in the soil.

Menon et al. (2004) investigated the inhibitory consequence of quinalphos and chlorpyrifos available in the sandy loam soil and loamy sand soil which might be linked to the greater bioavailability of the pesticides in loamy sand. This could be linked to the small level of organic content and clay content. The study carried out by Gundi et al. (2005) investigated the influence of three main insecticides containing cypermethrin, monocrotophos, and quinalphos on the microbial inhabitants available in the dark clay soil. It was detected that the comminatory influence of these pesticides when applied at a lower level had a detrimental effect on these microorganisms. Widenfalk et al. (2004) observed that the following pesticides containing deltamethrin, pirimicarb, captan, isoproturon were observed to have a detrimental effect on freshwater sediment microbial communities when applied a safer concentration that has been recommended to be eco-friendly.

Moreover, the presence of organic matter, soil texture, and vegetation was observed to have a greater influence on the level of toxicity on the soil microorganisms in the soil. The augmentation of different sources of carbon-containing acetate and glucose and protein source, such as tryptophan, glutamine, serine, arginine, improves the level of resistance of some soil fungi to pesticides applied (Mishra and Pandey 1989). Moreover, it has been observed that the application of tillage or no tillage does not have any effect on the effect of pesticides applied to the environment. It was observed that there was presence of more soil retention in the presence of organic matter with enhanced level of accumulation of constituent parts (Murage et al. 2007).

It was observed that these processes enhance the cycling of microbial biomass and the fate of soil biological or organic matter. Therefore, the application of pesticides under some of these conditions have a diverse influence on soil microbial biomass and their diversity. Santos et al. (2006) evaluated the influences of herbicides containing fomesafen and fluazifop-*p*-butyl as well as the combination of their mixture on microbial activities of soil when bean was planted without the application of conventional tillage and tillage system. They studied the rate of microbial respiration for a period of 12, 51, and 63 days after the application of herbicides. The following microbial variables/parameters were evaluated; microbial biomass carbon, grain yield, metabolic quotient, microbial quotient, and proportion of bean root colonization by mycorrhizal fungi at the conclusion of the cycle. The authors stated that there was an enhanced microbial respiratory rate when the process of tillage was not applied, with fluazifop-*p*-butyl showing the minimal respiration. The microbial biomass carbon and microbial quotient were negatively affected when subjected to 12 days application containing fomesafen and by combined herbicide mixtures.

Also, it was observed that the herbicides influence the rate of mycorrhizal colonization after 12 days of application under controlled tillage system. Moreover, it was observed that the herbicides applied led to reduction in the level of microbial biomass carbon and microbial quotient values after 15 days of application, while microbial quotient showed an enhanced activity under no tillage system when compared to the controlled tillage system. Furthermore, it has been observed that the presence of numerous pesticides together with other natural soil conditions and other contaminants may affect the functionality of such pesticides at a particular period of time most especially on the microbial diversity or their level of activities. In the study carried out by Wang et al. (2006) they performed a work on the influence of urea and methamidophos on the microbial multiplicity available in the soil utilizing community-level physiological profiles and integrated methods of soil microbial biomass approaches. The authors suggested that the agrochemicals possessed the capability to decrease the level of soil microbial biomass and increase the functional diversity of soil microbial groups which shows that several species of the bacteria could be improved in soil subjected to different methamidophos pressure (Wang et al. 2006).

Also, Demanou et al. (2006) evaluated the consequence of synergestic effect of mefenoxam and copper when combined on the functional multiplicity of soil microbial community when subjected to metabolic and structural profiling using RNA arbitrarily primed and capriciously primed polymerase chain reaction. It was observed that amoA, a functional molecular indicator for b-subgroup ammonia-oxidizing bacteria was affirmed in the treatment containing mefenoxamþcopper and mefenoxam containing enhanced gene copies as well as increase in the level of soil nitrification after 60 days of application. It was also resolved that Nitrosospira-like organisms constitute the significant nitrifiers under mefenoxam dealings.

Conversely, these observations could vary when pesticides are used under different conditions. For example, Sáez et al. (2006) established the influence of different pesticides containing diflubenzuron, aldrin, captan, lindane, methidathion, dimethoate, atrazine, and methyl parathion on the activity and growth of *Xanthobacter autotrophicus* CECT 7064 which is a denitrifying microorganism. It was observed that the insecticide dimethoate and herbicide atrazine totally affected the biological activity and growth of *X. autotrophicus* when applied at 10 mg/L but the remaining tested pesticides prevented the development of strain CECT 7064 without affecting the growth of bacteria after 96 h of culturing. The pesticides applied negatively affect the biological activities of *X. autotrophicus* with the exemption of fungicide captan. It was also observed that the liberation of N_2O was strongly affected by numerous pesticides containing diflubenzuron, aldrin, methyl parathion, methidathion, and lindane while simazine, dimethoate, and atrazine, affected the activities of denitrifying microorganisms available in the soil (Sáez et al. 2006).

In the study carried out by Wang et al. (2007), they examined the synergistic effect of butachlor and cadmium on microbial activity. The result obtained showed that the incorporation of a higher concentration of butachlor applied together with cadmium enhances the level of soil multiplicity of the microbial community. However, the combination of the fertilizer together with pesticides affects the action of soil microorganisms available in the soil environment.

5.6.1 Influence of Pesticides on Algae

It has been observed that pesticides have a detrimental effect on algae by affecting their metabolic activities, nitrogen fixation, growth, biochemical structure, and photosynthesis (Friesen et al. 2003; Fathi 2003; Ma et al. 2002; Mostafa and Helling 2002; Ma and Liang 2001). Moreover, small information has been recorded on the influence of pesticides on numerous algal species (Ma et al. 2004a, b).

In the study carried out by Ma (2005), they tested the effect of five pyrethroids and organotins pesticides on three different cyanobacteria containing “*Microcystis aeruginosa, Anabaena flosaquae*, and *Microcystis flosaquae*,” respectively while the five algae include *Chlorella pyrenoidosa, Selenastrum capricornutum, Chlorella vulgaris, Scenedesmus quadricauda*, and *Scenedesmus obliqnus,* respectively. The experiment was carried out for a period of 96 h acute toxicity tests. The authors observed that there was variation in the level of responses observed by different algae when exposed to the pesticides. It was observed that the level of pyrethroids pesticides toxicity was lower when compared to that of the organotin pesticides but the ecological risk of pyrethroids pesticides was more pronounced when compared to that of organotin pesticides. The difference in their variation might be linked to the variation in their rate of metabolic activities.

5.7 Conclusion and Future Recommendation to Knowledge

This chapter has provided a detailed information on the detrimental effect of pesticides on beneficial microorganisms as well as their influence on climate change, which have been highlighted with numerous activities in the ecosystem. It was observed that appropriate precaution needs to be put in place for effective application of pesticides in the environment for effective protection of all the beneficial microorganisms and other living organisms as well as human health and the protection of the ecosystems. There is a need to perform more in vitro and in vivio experimental assays on the effects of these pesticides on essential soil functions as well as the effect of climate changes. There is a need for government, policymakers, and scientists to put necessary measures in place that will regulate the application of pesticides, thereby minimizing the health and environmental hazards. The application of biopesticides could be a sustainable, eco-friendly, and cost-effective technique that could lead to the maintenance of a cleaner environment. Moreover, the application of biosensors and climate smart agriculture should be encouraged as well as adequate sensitization most especially on modalities of moderating the rates of pesticides applied in the environment.

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Chapter 6
Effect of Heavy Metals on Activities of Soil Microorganism

Abel Inobeme

Contents

Abstract Heavy metals are ubiquitous elements, and some of them play unique roles in biological systems. They are key components of soil as some of them are eco-friendly in their geochemical state. The continual increase in their concentration due to the increasing anthropogenic influences such as mining, agricultural process, and combustion of fossil fuels among others is the cause for concern. Most of these metals though present in various components of the environment are more concentrated in the soil, which acts as a natural storeroom for environmental waste from all other media. The high content of these metals in soil is toxic not only to plants and

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animals but also to microorganisms that play an indispensable role in soil and aid the sustenance of natural cycles. This chapter examines the effect of heavy metal on soil microorganisms. The effect of heavy metals on microbial biomass, population, activities, and diversity is discussed. The methods for assessing heavy metal tolerance in microorganisms are also presented, and finally a brief review on the mechanisms of adaptation of soil microorganisms to high concentration of heavy metals is also provided.

Keywords Biomass · Heavy metals · Microorganisms · Pollution and soil

6.1 Introduction

Soil is the fundamental natural resource for human existence; even in the modern social life, soil is still the most fundamental element of human production which links various activities of man together (Chu 2018a, b). Heavy metal pollution is a severe global environmental problem as it adversely affects living organisms. It also affects significantly the components and activity of soil microbial communities (Xie et al. 2016). The role of microorganisms in the environment, most especially soil, cannot be overemphasized. Bacteria, fungi, algae, and their enzymatic activities are of pertinent relevance, and factors such as those that affect microbial metabolic activity, diversity, biomass, distribution, and abundance are therefore of major concern (John 2017).

The presence of heavy metals in the environment at high concentrations has lethal effects on animals, plants, and microorganisms. Metals such as Hg, Cd, and Pb have no known physiological role in plants and animals and are highly toxic even at trace amount. Toxic metals are common contaminants of natural waters and soils and may significantly affect the vital biodegradation processes taking place naturally in the environment. The primary reason why heavy metal pollution is a pressing issue is due to their hazardous nature and unique tendency to bioaccumulate in different groups of microorganisms, plants, animals, and man, which are responsible for various metabolic and physiological disorders (Banerjee et al. 2015).

Various researchers have reported the toxicity of heavy metals on tissues of plants and animals. There are however varying documentations on the impacts of heavy metal pollutions on soil microorganisms. The harmful effect of these metals on plant growth and microbial activities would in turn influence the growth of plants (Chibuike and Obior 2014). This would no doubt constitute fear on the fast growing human population, which depends on the yield from these plants.

Safeguarding soil quality is therefore paramount for sustainable agriculture. Soil biology is a significant component of soil quality, and microorganisms are useful in the maintenance of soil fertility and primary production through organic matter decomposition and cycling in nature (Adília and Pampulha 2006). Assessing soil microbial community could be an indication of the degree of soil heavy metal pollution and the impact of heavy metal pollution on soil quality. The effects of heavy metal pollutants could be enormous especially on microbial activities (Oijagbe et al. 2019).

Due to the combined properties such as persistency, toxicity, and non-biodegradability of heavy metals, they have become an issue of much concern and have increasingly attracted the attention of researchers. This chapter examines the effect of heavy metals on soil microorganisms.

6.2 Concept of Heavy Metals

Heavy metal elements in chemistry generally refer to a metal with a density higher than 5.0 g/cm^3 which includes 45 elements such as iron, manganese, lead, copper, zinc, cadmium, and mercury (Chu 2018a, b). They are elements that are found naturally with a density that is five times that of water and a relatively higher atomic number (Oijagbe et al. 2019).

The concept of heavy metals, actually, does not avail itself to a consensus definition. Some arguments have been put forward with regard to what should constitute a "heavy metal" and what element should be accurately grouped as such. Some writers attempt their definitions from the perspective of atomic weight, while others look at it from the viewpoint of specific gravity higher than 4.0, or greater than 5.0. More currently, the term has been applied as a general term for metals and metalloids that constitute toxicity to humans or the environment (Saunders et al. 2013).

The varying distribution of these metals in soil, water, and other components of the environment is vital in the general assessment of metals in the ecosystem. The reason for the pressing interest in heavy metals among other contaminants is that they are difficult to degrade by the natural environmental processes and have high capacity to bioaccumulate in living cells (Chu 2018a, b). Agricultural produce cultivated on soil with enriched heavy metals content shows deteriorated growth, poor transpiration, yellowing of leaves, poor germination of seeds, and deformed root system (Jadwiga et al. 2013).

Some of the metals such as cerium, tin, gallium, thorium, and zircon have no known functions in biological systems. Iron, molybdenum, and manganese are useful trace minerals and have little toxicity. While nickel, copper, cobalt, zinc, and vanadium are essential nutrients but are toxic. Silver, cadmium, antimony, mercury, and lead do not have known roles and are extremely toxic (John 2017).

6.3 Heavy Metals in Soil

Heavy metals are distributed differentially in various aspects of the environment: air, water, soil, and tissues of organisms. Most of these metals have higher proportion in soil, since soil acts as a natural storehouse for all environmental waste. The content of iron and manganese naturally in soil is relatively high, and their pollution effects are not usually insignificant. Heavy metals in soil are divided into two categories from the biochemical characteristics: one is harmful to crops and humans and animals, such as Pb, Cd, and Hg; the other is beneficial when present in the desired quantity, but when present in excess would have lethal effects. Examples are Cu, Zn, Mn, and so on (Xie et al. 2016).

Metals when present in soil exist either as separate entity or combined with other components. These components may include exchangeable forms adsorbed on the surfaces of ionic solids, non-exchangeable forms and insoluble metal compounds such as calcium and magnesium carbonates and phosphates, soluble metal compound such as chlorides, metal complex of organic materials, and metals binding to silicate ores (Chibuike and Obior 2014).

Some heavy metals play an extremely important role in biochemical reactions, which are significant for the growth and development of microorganisms, plants, and animals (Kavamura and Esposito 2010). For example, heavy metals such as copper and zinc are vital components of physiological processes in all living organisms, including plants, animals, and microorganisms. Zinc (Zn), copper (Cu), etc. are vital micronutrient for growth and enzymatic activities of heterotrophic bacteria. However, excess load of the same metals show toxicity and inhibition to microbial processes (John 2017).

Although heavy metals are naturally present in the soil, geologic and anthropogenic activities increase the concentration of these elements to amounts that are harmful to both plants and animals. Some of these activities include mining and smelting of metals, burning of fossil fuels, use of fertilizers and pesticides in agriculture, production of batteries and other metal products in industries, sewage sludge, and municipal waste disposal (Chibuike and Obior 2014). Soil also gets contaminated with metals from a variety of anthropogenic inputs, most especially irrigation with wastewater. The use of contaminated water for irrigation contributes to the accumulation of chemical and biological contaminants in soils and alters the physicochemical and biological properties of soils (Kouchou et al. 2018).

Pollution of soil by heavy metals refers to the release of metals such as cadmium, lead, mercury, chromium, and other elements that are biotoxically significant in the soil, bringing about amounts that are higher than the permissible limit. Some heavy metals have been identified as priority pollutants, which include argon, arsenic, copper, mercury, nickel, lead, zinc, selenium, thallium, beryllium, and silver. These metals and metalloids are 13 in all (Sparks 2005). They are found naturally in rocks, minerals, and various anthropogenic inputs such as mining, sewage sludge, industrial waste, agricultural activities, electronics, and energy production among others (Gilmour and Riedel 2009). Heavy metals are found naturally in soils resulting from weathering of underlying bedrock or through mineral processing processes (Shakoor et al. 2015) and their contents in soils depend on the nature of the parent material, where it is found and its age. They represent less than 1% of the composition of the earth crust due to alteration of rocks and natural incidents.

More recently, the release of a large amount of these metals from industries and mining which finally goes into the soil has led to a rise in the contents of heavy metals in soil. The wide usages of pesticides and fertilizers in farms have also contributed to the increase in soil heavy metal concentrations (Park et al. 2016).

6.4 Methods for Assessing Effect of Heavy Metals on Soil Microorganisms

Different methods have been employed for the assessment of the effect of heavy metals on soil microorganisms.

6.4.1 Colony Count Method

Fungal abundance and species composition are commonly determined by the colony count technique and identification by macro- and micro-morphological observations of fungal cultures after subculture on potato dextrose agar, malt extract agar, cornmeal agar, Czapek's agar, and Sabouraud agar media. This method was adopted by Pečiulytė and Dirginčiutė-Volodkien (2009) in their study on the effect of long-term soil pollution on microorganisms' diversity.

6.4.2 Agar Diffusion Assay

Heavy metal tolerance by microorganisms can also be tested using the agar diffusion assay method. Solutions of different concentrations of heavy metals of interest are prepared from 0 to 1000 μg/ml through selected ranges of concentrations using the respective metal salts. Metal solutions are saturated in the wells dug on the culture medium in the Petri plate, and the tolerance index is then determined by studying the respective zones of inhibition (Nanda and Jayanthi 2011).

6.4.3 Respiration Rate

The respiration rate of soil microorganisms varies depending on the extent of pollution of the soil. With regard to the respiration rate of polluted soil samples in a particular area, a soil sample that is more contaminated would give a lower rate of carbon dioxide production when compared to a less polluted soil (Joonu and Divya 2017). The addition of heavy metals such as Pb and of Cr, Cd, Cu, Zn, and Mn to soil samples also caused a decrease in respiration rates. Tayebi and Ahangar (2004) reported that sandy soils gave about a 15% decrease in the rate of respiration when

375 ppm of lead was added to it, the lowest concentration used, whereas a clay soil required 1500 ppm Pb to give the same inhibitory effect. Peat soils showed no effects even when the concentration of lead was highest (7500 ppm).

6.4.4 Growth Studies

This method was employed by Nair et al. (1992) in assessing the effect of different salt concentrations on microorganisms. Growth studies of bacteria such as *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae* can be carried out in nutrient broth medium supplemented with peptone and yeast extract, etc. 10 μl of culture is inoculated into the nutrient broth medium supplemented with copper and nickel metal; then it is analyzed for growth studies. This can be done in a broad or narrow range:

Broad range: using this technique, different salt concentrations of metals are used in this technique to test the effect on the different isolates. The isolates are inoculated in a broth containing heavy metals at varying concentrations and incubated for 3 h. Then readings in UV spectrophotometer are taken at different time intervals. A plot is then drawn using the obtained readings.

Narrow range: The isolates are tested for metal tolerance using about three different concentrations of the metals. Copper chloride and ammonium sulfate salts of nickel are used in varying concentrations such as 10, 15, and 20 mM. The isolates are inoculated in a broth containing heavy metals at different concentrations and are incubated for 24, 48, and 72 h. Then readings in UV spectrophotometer are taken at different time intervals. By using the readings, graphs were plotted (Joonu and Divya 2017).

6.5 Role of Soil Microorganisms

Soil contains a variety of microorganisms including bacteria, fungi, and many others which together constitute the natural ecosystem. Microorganisms play a vital role in nutritional chains that are an important part of the biological balance in nature, where bacteria are essential for maintaining some of the natural cycles such as nitrogen, carbon, phosphorus, and sulfur (Kummerer 2004).

Microorganisms are also known to play important roles in plant nutrient recycling, soil structure conservation, poisonous chemical detoxification, and plant pest control and management (Filip 2002). In their geoactive roles in the biosphere, they are also essential, especially in the areas of biotransformation elements and biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and soil and sediment formation. All sorts of microbes, including prokaryotes and eukaryotes and their symbiotic interactions with each other and "higher species," will actively contribute to geological phenomena, and the transformations of metals and minerals are central to many such geomicrobial processes (Gaidi 2010).

It should also be emphasized that the general metabolic activities of all microbes influence the distribution and bioavailability of metals, not only because of the metabolic essential nature of many metals and the existence of unique biochemical mechanisms for their cellular accumulation but also because of the decomposition or biodeterioration of organic and inorganic substrates (Huang et al. 2004).

Microorganisms synthesize soil enzymes and serve as biological catalysts to promote various reactions and metabolic processes to break down organic contaminants and to generate important compounds for microorganisms and plants (Moreno et al. 2002).

Microorganisms also act as a bioindicator for soil contamination, particularly from pollutants such as heavy metals. If the concentration of heavy metals in the soil is above their basic level, a distinction can be made between non-contaminated and less polluted soils. Using this characteristic, however, the boundary between low pollution level and the next, medium level cannot be established. Microbiological indicators are the most appropriate for suggesting this level of pollution, because changes in the composition of the soil microbial community occur at a medium level of pollution with adverse effects (Djukic and Mandic 2000).

Studies have documented that the occurrence of negative properties in the soil is directly associated with the distribution of special component activities within the soil microorganism complex. For example, the proportion of microorganisms in the epiphytic chromogenic forms and the amount of toxinogenic micromycetes in the soil are increased. Consequently, the redistribution of active microorganisms by the degree of dominance can justifiably be regarded as a fundamental property of the pre-pathological stage of the microbial community in the soil (Jacoby et al. 2017).

Soil microorganisms are responsible for the improvement of soil fertility and structure. The major components of enzymes present in soil are contributed by soil microorganisms, which account for metabolic activity and biomass, and a short lifetime (Kuzyakov and Blagodatskaya 2015).

Soil bacteria are considered to play a very important role in maintaining soil properties although there are other microorganisms apart from them. Soil fungi are made of three functional groups: decomposers, mutualists, and the pathogenic group. Fungi, along with bacteria, are important decomposers helping to break down substances into organic matter (Kouchou et al. 2018).

Microorganisms may typically protect plants grown in metal-contaminated soils by improving root metal retention and helping plants to obtain adequate nutrients and recycle organic matter (Xie et al. 2016).

Various researchers have reported that microbial parameters are relevant indicators for assessing changing soil conditions brought about by chemical pollution (Adília and Pampulha 2006).

Soil microorganisms are the key modulators of various ecological cycles such as soil carbon, nitrogen, phosphorus, sulfur, and other elements in nature; they promote nutrient interconversion in nature. Also, soil microbes are almost vital in all biochemical reactions in the soil (Chu 2018a, b). For early indication of impact of heavy metals on soil quality, soil enzymes are used due to their sensitivity to environmental stress (Rao et al. 2014). Castro et al. (2015) in their study also reported that fungi and bacteria make up a major component of the soil microbial biomass and act as very constructive models for studying the harmful effects of metals at the cellular level (Castro et al. 2015).

In topmost layer of the ecosystems, fungi and bacteria make up about 90% of the total soil microbial population and are responsible for the regulation of soil organic matter nutrient availability (Six et al. 2006). Also, the production of mucilage by bacteria and fungi helps to make up aggregates. Moreover, soil bacteria and fungi have been reported to exhibit different responses to heavy metal pollution, which could potentially lead to differences in the distribution and diversity of microbial communities within both bulk soils and PSFs (Chen et al. 2017).

6.6 Differential Tolerance of Microorganisms to Soil Heavy Metal Contamination

Various microorganisms showed different levels of tolerance to varying concentrations of metals in the environment. For example, among bacteria, gram-negative bacteria appear to be more tolerant than gram-positive ones which is in accordance with the previous statement. Wang et al. (2010) reported the predominance of gram-negative bacteria in a polluted area that was studied and attributed this to their higher level of intrinsic metal resistance than majority of the gram-positive bacteria. Also, Kouchou et al. (2018) reported that the fungi group and actinomycetes showed a lower tolerance to heavy metals (chromium, copper, and zinc) than aerobic heterotrophic bacteria. The dehydrogenase assay is an effective primary test for assessing the potential toxicity of metals to soil microorganisms (Nweken et al. 2007). Turgay et al. (2012) reported that the strains of bacteria, obtained from Turkish serpentine soils, were able to withstand up to 34 mM Ni in the growth medium. In addition, since organisms at microscopic level are more responsive to environmental pressure than macro-organisms in soil ecosystems, their tolerance range and level reflect changes in soil environment as early as possible and are therefore considered to be reliable indicators of soil quality at a particular time (Akmal and Jianming 2009).

In their study, Ortiz-Ojeda et al. (2017) reported that 11 of the 17 strains of bacteria tested were able to grow in the presence of various heavy metals (0.5 mM Pb, Cd, and Co; 0.01 mM Hg). They also noted that the strains were better able to tolerate the heavy metals when they were grown at pH 5 instead of 4. This means the ability of the soil microorganisms to withstand different concentrations of metals also depends on soil pH. Chen et al. (2014) reported a decrease of soil basal respiration and microbial biomass carbon by 3–45% and 21–53%, respectively, as a result of high doses of heavy metal pollution.

In a laboratory study carried out by Ghorbani et al. (2002) to examine the tolerance of microbes to metals, they observed that the addition of heavy metals to soil in laboratory ecotoxicological studies leads to a decrease in the microbial biomass and an alteration in community structure. Akmal and Jianming (2009) reported that microorganisms differ in their sensitivity to metal toxicity and a high dose of exposure will lead to instant death of cells due to alteration of basic functions and to more gradual changes in population sizes due to changes in viability or ability to compete.

Microorganisms are more sensitive when compared to environmental pressure due to pollution and even trace contamination when compared to animals, plants, and molluscs among others. Through the soil microbial changes, whether the soil is contaminated, the extent of soil pollution, pollution effects, and risks can be determined scientifically (Krumins et al. 2015). Pollution due to heavy metals may produce different microbial community patterns. Even when most of the chemical and biological properties of the soil have been altered, there are many original microorganisms in the soil that are present in the microbial community (Oijagbe et al. 2019).

Long-term contamination of soil with heavy metals will select those that can specifically adapt to the microbial population of contaminated soil. The higher the organic carbon content in heavily polluted soils, the lower the performance of organic mineralization microbial populations (Xie et al. 2016). Previous studies examining the population genetics of this phenomenon found that heavy metal tolerant ecotypes evolve at an unusual level, while maintaining a high polymorphism. This phenomenon appeared to be the least variable parameter when compared to nontolerant populations despite the founder effect and selection (Mengoni et al. 2000).

There is now substantial evidence of a reduction in soil microbial biomass as a result of long-term exposure to heavy metal pollution from past sewage sludge applications as reviewed by McGrath et al. (1995).

Other studies of metal toxicity to microbes such as bacteria often report that the bacteria have a wide range of mechanisms of resistance to and intracellular absorption of marine trace metals. For example, cadmium-tolerant communities are likely to show co-tolerance to Zn (Paulsson and Nyström 2000), and organotin tolerance simultaneously occurs in association with cadmium tolerance. Such metal tolerance is a common phenomenon in the soil environment for algae and bacteria (John 2017). Oijagbe et al. (2019) in their study observe a later rise in microbial biomass carbon and attributed it to the development of tolerance and shifts in community structure to compensate for the loss of more sensitive populations.

Nonetheless, the fact that fungi tended to be the least affected by the high concentrations of heavy metals among all the microbial groups studied is not surprising, as fungi and yeasts are considered the most resistant and therefore versatile group of soil microorganisms (Iram et al. 2013).

Rajapaksha et al. (2004) in their study compared reactions of various microbial groups to soil pollution with Zn and Cu and found that the bacteria are far more prone to soil contamination than the fungi. These authors have observed varying effects of soil pH on the microbial reaction to soil contamination, whereby lower pH increased the harmful effect on bacteria.

From their studies, Teng et al. (2015) suggested that Rhizobia could tolerate high concentrations of heavy metals in various ways and could play a significant role in restoring contaminated soil. In comparison, long-term heavy metal effects can improve bacterial population tolerance as well as fungal tolerance such as arbuscular mycorrhizal (AM) pillows, which can play an important role in restoring polluted ecosystems (Jiwan and Ajay 2011).

Species of microorganisms, strains of the same species, and also activities of the same microbial species can all show noticeable differences in their resistance to toxicity of heavy metals. Because most bioassays are based on the measurement of size or activity of diverse microbial communities, microorganisms within a community structure may show differences in sensitivity to metal toxicity. Van Beelen et al. (2004) found that the sensitivity to toxicants of the microbial community responsible for the mineralization of acetate in soils with no history of exposure to elevated metal concentrations differed by many orders of magnitude between soils of similar physical and chemical properties. This suggests that differences in community structure between soils which vary in sensitivity to metal toxicity could be an important factor in explaining discrepancies between studies (Tayebi and Ahangar 2004).

Kazaure (2018) in his study on Distribution of Bacteria in Lead Contaminated Soil in Anka Local reported that the predominant bacterial isolates were identified as species of *Bacillus*, *Proteus*, *Achromobacter*, *Citrobacter*, *Corynebacterium*, *Alcaligenes*, *Pseudomonas*, *Staphylococcus*, *Klebsiella*, *Escherichia*, *Agrobacterium*, *Enterobacter*, and *Diplococcus*. They had higher tolerance to the metals investigated. *Bacillus* species had the highest frequency of occurrence (45.67%). *Achromobacter*, *Agrobacterium*, *Enterobacter,* and *Diplococcus* species had a very low frequency.

Bacteria alleviate heavy metal ion toxicity by immobilizing, mobilizing, uptake, and transformation of heavy metals (Hassan et al. 2017).

Metals without biological function are generally tolerated only in minute concentrations, whereas essential metals with biological functions are usually tolerated in higher concentrations and facilitate secondary metabolism in bacteria, actinomycetes, and fungi (Haferburg and Kothe 2007).

Lenart-Boroń and Wolny-Koładka (2015) from their study showed that fungi occurred very frequently in soils with strongly increased concentrations of heavy metals, especially cadmium, lead, and zinc.

The biomass of the total soil microbial communities is usually negatively correlated with metal stress (Wilke et al. 2005) but is less affected than community structure. Their findings obtained in a 2-year period agree with this statement. The resistance of bacteria and fungi in soils polluted with heavy metals has been studied in the field (Ramsey et al. 2005) and in laboratory studies (Rajapaksha et al. 2004), indicating that fungi are favored compared to bacteria in metal-stressed soils.

Pečiulytė and Dirginčiutė-Volodkien (2009) reported that fungi from the genera Curvularia and Fusarium, known as common in agricultural soils, were more sensitive to metals (Ni, Co, Fe, Mn, Mg) than the other fungi. Fungi that belong to the genera Monilia and geotrichum showed a relatively low tolerance to all metals in comparison to other ones.

6.7 Mechanisms of Resistance of Microorganisms to Heavy Metals

Several mechanisms are developed by microorganisms in tolerating a high concentration of heavy metals. Some of these mechanisms are dependent upon anabolic and catabolic energy of microorganisms (Banerjee et al. 2015).

Rouch et al. (1995) and Ortiz-Ojeda et al. (2017) have identified the following mechanisms for heavy metals resistance in bacteria: (1) exclusion of a metal by a permeable barrier, (2) exclusion by active transport of the metal from the inside of the cell outwards, (3) intracellular physical sequestration of the metal using a polymer to prevent metal cellular damage, (4) extracellular sequestration, (5) enzymatic detoxification of the metal to a less toxic form, and (6) cell reduction of the sensitivity to metals.

Regarding the metals essential for the proper course of cellular processes, such as copper, zinc, or iron, there are mechanisms which regulate their cellular capture. Toxic metals, however, like mercury, cadmium, or lead, do not have any specific transport methods. Disturbances of the biological balance of soil caused by excess of cadmium, copper, and zinc might be attributed to the disruption of physiological functions, denaturation of proteins, and destruction of cellular membranes of soil microorganisms (Zaborowska et al. 2006).

Heavy metal resistance in bacteria results from their primary contact with metals that appear naturally in the environment. However, intensive human activity and exploitation of natural deposits have led to the expansion of metal-resistant microorganisms (Bruins et al. 2000). Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (Soraia 2017).

Lenart-Boroń and Wolny-Koładka (2015) in their studies observed that microorganisms whose population were not affected by varying concentrations of heavy metals may possess various resistance mechanisms even to toxic heavy metal concentrations and that these properties may be applied in the removal of heavy metals from different environments (Lenart-Boroń and Wolny-Koładka 2015).

Basically, some microorganisms are able to detoxify metals by valence transformation, extracellular chemical precipitation, or volatilization. They can enzymatically reduce some metals in metabolic processes that are not related to metal assimilation (Lovley 1993). Several bacteria couple the oxidation of simple organic acids and alcohols, hydrogen, or aromatic compounds to the reduction of Fe(III) or Mn(IV). Bacteria that use U(VI) as a terminal electron acceptor may be useful for uranium bioremediation (Garbisu and Alkorta 2003).

Some microorganisms have the ability to make the metal less toxic by bringing about its conversion to a more favorable and less harmful oxidation state, through redox processes (Garbisu et al. 1995) Biomethylation to yield volatile derivatives such as dimethylselenide or trimethylarsine is a well-known phenomenon catalyzed by several bacteria, algae, and fungi (White et al. 1997). Several bacteria have been reported to reduce hexavalent chromium that is toxic and mutagenic to its trivalent form that is less toxic (Garbisu et al. 1997a, b). Bioprecipitation by sulfate reducing bacteria that convert sulfate to hydrogen sulfide, which, in turn, reacts with heavy metals to form insoluble metal sulfides such as zinc sulfide and cadmium sulfide has been reported in some bacteria (Iwamoto and Nasu 2001).

Several studies have been reported where PGPR act as potential elicitors for abiotic stress tolerance including heavy metal tolerance (Tiwari et al. 2017). They limit the bioavailability of metals by forming complexes with siderophores, particular metabolites, and bacterial transporters (Ahemad 2012). These microorganisms of agronomic importance have evolved various mechanisms to avoid heavy metal stress including:

(a) Transport of metals across cytoplasmic membrane;
(b) Biosorption and bioaccumulation to the cell walls;
(c) Metal entrapment (Tiwari et al. 2017).

Haferburg and Kothe (2007) identified some mechanisms and adaptive strategies developed by bacteria in combating lethal effects associated with heavy metals presence in the environment:

1. Metal resistance is achieved via intra- and extracellular mechanisms;
2. Metals can be excreted via efflux transport systems;
3. Cytosol sequestration compounds can bind and detoxify metals in the cell;
4. The release of chelators in the extracellular medium capable of binding and fixing metals;
5. The structure of the cell envelope which is capable of binding large quantities of metals by sorption thus preventing impulses.

Li (2004) and Soraia (2017) categorized the bioprocess of metal accumulation into two major group:

1. Bisorption (passive) using non-living cells
2. Bioaccumulation using living cells

El Baz (2015) reported that metal resistance can be widespread among live actinobacteria in contaminated environments. Several bacteria have resistance mechanisms such as superoxide dismutases, efflux transporters, and metallothioneins. *Streptomyces* are the most common among actinomycete isolates in polluted water habitats and artificial swamps for the treatment of industrial effluents. *Streptomyces* and *Amycolatopsis* can survive in environments contaminated by heavy metals; this is probably due to their remarkable resistance to extreme environmental conditions and various pollutants.

Álvarez et al. (2013) reported that the presence of heavy-metal-resistant strains in different *Streptomyces* clades can have two different explanations:

(a) Resistance is already present in the most recent common ancestor (MRCA) and was then inherited by the different lineages;
(b) The different lines inherited from the MRCA have developed new mechanisms or have modified those already existing, in order to generate resistance to heavy metals.

Some microorganisms developed specific mechanisms also depending on the metals and the nature of the environment. For Pb(II) contamination some microorganisms have evolved strategies that help them to survive exposure. The main mechanisms of lead resistance involve adsorption by extracellular polysaccharides, cell exclusion, sequestration as insoluble phosphates, and ion efflux to the cell exterior (Jaroslawiecka and Piotrowska 2014). A number of microorganisms inhabiting soil and water can transform inorganic and organic lead compounds into volatile forms, which diminishes their toxic effect (Thayer 2002).

Hao et al. (2014) showed that rhizobia heavy metal tolerance mechanisms may include: (1) adsorption and accumulation of heavy metals and (2) microbial secretion of enzymes and bioactive metabolites to increase their bioavailability and sequester their toxicity.

Sonil and Jayanthi (2011) in their study observed that maximum resistance to heavy metals is exhibited by *Pseudomonas*, followed by *Azotobacter* and *Rhizobium*. Tolerance to Cr was found up to 200 μg/ml by *Pseudomonas* and *Azotobacter* and 300 μg/ml by *Rhizobium*, while tolerance to As was almost equal in the three bacteria but less. *Pseudomonas* and *Azotobacter* were able to tolerate higher concentrations of Cu and Mg. They inferred from their study that comparatively higher concentration of Cu and Mg did not affect microbial growth to a larger extent but those of As and Cr had an adverse effect.

6.8 Mechanisms of Accumulation of Heavy Metals in Microorganisms

This approach relies on components on the cell surface and the spatial structure of the cell wall on one side and on the other, the physicochemical conditions of the environment where the cell develops are vital in the accumulation of heavy metals in microorganisms, which have formed the basis for replacement when natural affinity of biological compounds is necessary for metallic elements (Wang and Chen 2009). It is recognized that pH, ionic strength, temperature, and the presence of other metals and organic compounds play an important role in this process (Violante et al. 2010).

There is a wide variety of microbial mechanisms in the accumulation of xenobiotics and metals on their cells, one of which includes efflux transporters that excrete the excess metal outside the cell (Nies 2003).

Fungi can also transform Pb(II) into pyromorphite, the most stable lead mineral, indicating their significant role in the lead cycle in the environment (Clipson and Gleeson 2012; Rhee et al. 2012). In turn, *Aspergillus niger* is capable of solubilizing this mineral and subsequently immobilizing Pb(II) as lead oxalates (Sayer et al. 1999).

Some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microorganisms can also excrete extracellular polymeric substances (EPS), such as polysaccharides, glucoprotein, siderophores, lipopolysaccharide, soluble peptide, etc. These substances possess a substantial quantity of functional groups which can coordinate with metal(loid) ions (Seshadri and Naidu 2015).

Many microorganisms synthesize extracellular polymers (EPs) that bind cations of toxic metals, thus protecting metal-sensitive and essential cellular components (Bruins et al. 2000). Little is known about lead resistance mechanisms in fungi, but even low concentrations of this metal can affect their growth and reduce biomass. The growth of *S. cerevisiae* is disturbed at 0.05 mM Pb(II) (Donmez and Aksu 1999).

Escherichia coli are found to adsorb more heavy metals than other species of bacteria, and biosorption, which depends on the special structure of the cell wall, is found to be the primary mechanism for this (Jin et al. 2018).

Microbes can accumulate heavy metals by either adsorption or absorption, which are two main ways to increase metal ions in soil. The process of adsorption differs from absorption, in that a fluid (the absorbate) is dissolved by or permeates a liquid or solid (the absorbent). Thus, adsorption is a surface phenomenon, while absorption involves the entire volume of material. The overall sorption mechanisms, include precipitation, chemical adsorption and ion exchange, surface precipitation, the formation of stable complexes with organic ligands, and redox reaction (Singh and Prasad 2015).

Wang et al. (2001) showed that the primary mechanism by which microbes accumulate heavy metal ions is adsorption, which normally does not depend on energy metabolism, rather than absorption, which depends on energy metabolism and almost exclusively occurs in living cells. Generally, microbes adsorb large amounts of heavy metal ions rapidly. It has been found that at pH 7.2, *Bacillus* can adsorb 60% of its Cu^{2+} capacity within the first minute and reach adsorption equilibrium within 10 min (He and Tebo 1998).

Adsorption involves complexation of heavy metals on the cell surface, from which they can be absorbed into the cell. Because of the cell surface structure principally the cell wall and mucus layer—heavy metals can be adsorbed and absorbed relatively easily. Many ions in the cell surface functional groups, such as nitrogen, oxygen, sulfur, and phosphorus, can be complexed with metal ions as coordination atoms. In addition, phosphoric acid anions and carboxyl anionic groups on the surface of the microbial cell wall are negatively charged, and most heavy metal surfaces carry a cationic group that interacts with the cell wall and allows the metal ions to bind or pass through the cell membrane (Jin et al. 2018).

Heavy metal ions bind to the surface of the cell not only by electrostatic interaction and complexation but also by ion exchange to the cell surface; for example, the non-living brown algae (*Ascophyllum nodosum*) exchanges the original cell wall adsorption of K^+, Ca^{2+}, and Mg^{2+} to adsorb Co^{2+} (Freitas et al. 2006). Brady and Duncan showed that yeast releases approximately 70% of K^+ rapidly and 60% of Mg^{2+} slowly in the process of adsorbing Cu^{2+} (Aly 2018).

Numerous microbial species, including bacteria and fungi from *Bacillus*, *Pseudomonas*, *Streptomyces*, *Aspergillus, Rhizopus,* and *Penicillium*, have significant removal ability. At present, it has been found that a variety of bacteria can absorb soil heavy metals. Among them, *Escherichia coli* K-12 can absorb the widest variety of metal ions; the outer membrane of this stain can absorb more than 30 different kinds of metal ions. *Rhizopus* can absorb Zn, Cu, Cd, Pb, and other heavy metal ions, and *Thiobacillus* can absorb heavy metal ions as well as inorganic ions, such as S, which combines with the metal ions to form a precipitate that can be separated from the soil.

Mechanisms involved in the detoxification and transformation of metals, include the mechanism that restricts entry into the cell and intracellular detoxification or organellar compartmentation, the latter occurring in some eukaryotes, e.g., algae and fungi. Operation of a number of mechanisms is possible depending on the organism and the cellular environment; mechanisms may be dependent on and/or independent of metabolism. A variety of mechanisms may be involved in transport phenomena contributing to decreased uptake and/or efflux. A variety of specific or nonspecific mechanisms may also affect redox transformations, intracellular chelation, and intracellular precipitation. Biomineral formation (biomineralization) may be biologically induced, i.e., caused by physicochemical environmental changes mediated by the microbes, or biologically controlled (solid rectangles) (Gadd 2009).

6.9 How Microorganism Are Used to Effect the Process of Metal Remediation

The utilization of organisms, primarily microbes, to clean up contaminated soils, aquifers, sludges, residues, and air, known as "bioremediation," is a rapidly changing and expanding area of environmental biotechnology that offers a potentially more effective and economical clean-up technique than conventional physicochemical methods (Garbisu and Alkorta 2003). The use of microbes to change the concentration of heavy metals in soil and improve the ability of plants to deal with elevated metals concentrations has significant economic and ecological benefits (Jin et al. 2018).

Microbes are known for enhancement of plant growth and survival under heavy metal stress condition as they have the capability of consuming waste and converting the complex waste into simple nontoxic by-products/compounds. This is feasible because microorganisms have developed many resistance mechanisms for survival in the presence of toxic heavy metals in their environment (Mustapha and Halimoon 2015).

These microbes interact with metals and minerals in natural and synthetic environments, altering their physical and chemical state, with metals and minerals also able to affect microbial growth, activity, and survival (Gadd 2010).

The use of microbial remediation has become more common, and it is generally considered promising owing to its many advantages, including retention of soil structure, and the fact that the pollutants and microbes can be almost completely removed from the soils, and secondary pollution can be avoided (Singh and Prasad 2015). Microbial remediation therefore presents new techniques for addressing the problem of heavy metal pollution in soil, and it has become a focus of new research and development in bioremediation technology (Jin et al. 2018).

Sodango et al. (2018) reported algae to be a more efficient biosorbent (with a reported rate of 15.3–84.6%) compared to other microbial biosorbents (bacteria and fungi). Microbes have a variety of properties that can effect changes in metal speciation, toxicity, and mobility, as well as mineral formation or mineral dissolution or deterioration.

Microbes possess transport systems for essential metals; inessential metal species can also be taken up. Microbes are also capable of mediating metal and mineral bioprecipitation, e.g., by metabolite production, by changing the physico-chemical microenvironmental conditions around the biomass, and also by the indirect release of metal-precipitating substances from other activities, e.g., phosphate from organic decomposition or phosphate mineral solubilization.

Many different metal-containing minerals formed as a direct or indirect result of microbial activity, e.g., various carbonates, phosphates, etc., are omitted from the table. Microbial cell walls, outer layers, and exopolymers can sorb, bind, or entrap many soluble and insoluble metal species as well as, e.g., clay minerals, colloids, oxides, etc. which also have significant metal-sorption properties. Redox transformations are also widespread in microbial metabolism; some are also mediated by the chemical activity of structural components (Gadd 2010).

Fungal cell wall contains different materials which proved to be efficient metal biosorbents. Due to their properties, some fungal species, such as *Aspergillus niger* or *Mucor rouxii*, have been successfully investigated for their usefulness in adsorption of heavy metals and therefore metal removal from various environments (Javaid et al. 2011; Joshi and Sahu 2014).

6.10 Effect of Heavy Metals on Soil Microorganisms

Monitoring the structure of microbial communities and animals is a sensitive tool for the assessment of soil quality and health. Generally, the microbial parameters in the soil ecosystems are considered to be the best indicators reflecting changing soil quality and properties, and thus, enabling early detection of soil degradation (Fazekašova and Fazekaš 2019).

Heavy metals also have large effects on processes important for soil fertility by affecting the structure and function of microbial communities (Sandaa et al. 1999).

The high concentration of heavy metals can directly affect the microbiotope by modifying the population size, diversity, and activity. These modifications are expressed by the blocking of the main functional groups, the displacement of the essential metal ions, or by the modification of the active aspect of the biological molecules.

Soraia (2017) reported that there was a clear mutual influence in the mining areas from which microbes in the soil are not only directly and indirectly affected by their environment, but they control in particular the soil parameters. Haferburg and Kothe (2007) also suggest that growth and metabolism can lead to changes in pH and ionic strength of the soil.

A large number of laboratory and field studies have been carried out on the effects of heavy metals on soil microorganisms which play an important role as decomposers in the soil ecosystems. Some problems have, however, been pointed out in both laboratory and field studies. The laboratory conditions, where a high concentration of heavy metals is added to soil on a single occasion, are not representative of the field conditions where heavy metal pollution has usually been occurring at a low concentration for a long period of time (Hiroki 1992).

Microorganisms are the first group that undergoes any direct and indirect effect of heavy metals in the soil environment. Adverse effects of metals on soil microorganisms may cause decreased decomposition of organic matter, reduced soil respiration, decreased microbial diversity, as well as they reduce the activity of some soil enzymes (Lenart-Boroń and Wolny-Koładka 2015).

They can have inhibitory effect on the development of bacteria, fungi, and actinomycetes. Heavy metals reduce the biomass of microorganisms and lower their soil activity. Several studies have shown a negative relationship between heavy metal concentration and microbial activities, such as respiration (Sonil and Jayanthi 2011).

Earlier reports suggest that heavy metals inhibit the growth of specific microbial groups, especially nitrifiers and nitrogen fixers (Bääth 1989).

6.10.1 Effect of Heavy Metals on Microbial Genetic Structure

Heavy metals alter the conformational structures of nucleic acids and proteins and consequently form complexes with protein molecules which render them inactive. Those effects result in disruption of microbial cell membrane integrity or destruction of entire cell (Bong et al. 2010).

Sensitive microorganisms are negatively affected by heavy metals, but it should also be noted that heavy metals lead to the development of tolerant species that can survive and adapt due to their genetic characteristics (MacNaughton et al. 1999).

Heavy metal contamination is a major environmental threat worldwide due to its adverse effects (toxicity) on natural biota and humans which are manifested as DNA damage, lipid peroxidation and other deleterious effects on living cells (Kazaure 2018).

Heavy metals affect soil microorganism production, morphology, and metabolism by functional disruption, protein denaturation, or loss of cell membrane integrity. The metals do this by altering the conformational structures of nucleic acids and proteins, thereby forming more stable complexes with protein molecules, which render them inactive (John 2017). It is reasonable to assume that any changes in the microbial properties of the contaminated soil sample can be attributed to the effects of the metal contamination and the presence of organic matter in soil (Kouchou et al. 2018).

Evidence from the field indicates that under longtime metal tension there is a change in the genetic structure of the soil microbial community, which brings about an increase in metal tolerance. A decrease in the total soil microbial biomass under persistent metal stress has been perceived in many field studies, but is perhaps to be preceded by changes in community structure. A decreased size of the microbial biomass can likely at least partially be described by physiological reasons such as a decrease in the microbial substrate utilization efficiency and an increased preservation energy requirement (Tayebi and Ahangar 2004).

6.10.2 Effect of Heavy Metals on Microbial Biomass

There is now a considerable amount of evidence documenting a decrease in the soil microbial biomass as a result of long-term exposure to heavy metal contamination from past applications of sewage sludge as reviewed by McGrath et al. (1994). It is generally accepted that accumulation of metal(loids) reduces soil microbial biomass and various enzyme activities, leading to a decrease in the functional diversity in the soil ecosystem (Crowley 2008) and changes in the microbial community structure (Yang et al. 2007).

A drop in the population of beneficial soil microorganisms due to high contamination by metals can result in a decrease in organic matter decomposition leading to a decline in soil nutrients. Enzyme activities necessary for plant metabolism may also be affected due to heavy metal influence on the activities of soil microorganisms (Chibuike and Obior 2014). The heavy metal ions inhibit the activities of these enzyme by forming a bond with the substrate or combining with the protein-active sites (Wu et al. 2011).

Several studies, depending on the isolation-based techniques used, have revealed that heavy metal contamination gave rise to shifts in microbial populations (Roane and Kellogg 1996). However, heavy metals when present in amounts equal to the geochemical background do not interfere with the soil metabolism, which is associated with the growth and development of soil microorganisms as well as the processes of synthesis and re-synthesis, governed by intra- and extracellular enzymes (Jadwiga et al. 2013). However, isolation-based techniques are limited because they only represent a small component of the microbial community. This limitation could be attributable to the fact that only a small percentage of soil microbes are culturable for such study.

For instance, fungi constitute a high proportion of the microbial biomass in soil. Their large surface-to-volume ratio and high metabolic activity are used to change the heavy metal dynamics in soil (Ayangbenro and Babalola 2017).

Markowicz et al. (2016) in their study reported that the biomass of gram-negative bacteria and fungi was affected by heavy metals; a drop in microbial activity was observed and was attributed to high metal concentrations. Also a positive relationship was observed between microbial biodiversity and metal content, with the genetic diversity decreasing with a rise in metal content.

Heavy metal contamination may lead to a reduction of total microbial biomass, to a decrease in numbers of specie populations such as infecting rhizobia or mycorrhizae, or to shifts in microbial community structure (Sandaa et al. 1999).

It is shown that Cd has much more significant effect in decreasing bacterial population than Pb that has little effect. It is also clear that the higher concentration of Pb and Cd causes decreases in total bacterial population (Abdousalam 2010).

Adverse effects of heavy metal contamination of the environment can be reflected not only in the accumulation of metals in plants but also in the alteration of soil microbial community (Lenart-Boroń and Wolny-Koładka 2015).

Cr (VI) has been reported to cause shifts in the composition of soil microbial populations and known to cause detrimental effects on microbial cell metabolism at high concentrations (Shun-hong et al. 2009).

Analysis of soils contaminated with heavy metals from other sources such as Cu and Zn in animal manures, run-off from timber treatment plants, past applications of Cu-containing fungicides, and analysis of soils in the vicinity of metal-contaminated army disposal sites confirm that a decrease in the microbial biomass occurs at a relatively modest, and sometimes even at a surprisingly low, metal loading. The widespread occurrence of this effect of metal toxicity suggests that there may be a common physiological explanation (Tayebi and Ahangar 2004).

Lenart-Boroń and Wolny-Koładka (2015) in their study observed that the correlation between the concentration of heavy metals and microbial numbers was very weak and statistically insignificant. High numbers of fungi were observed in the contaminated sites, which was particularly interesting in one of the sites, strongly contaminated with Cd, Pb, and Zn. Cd-contamination can decrease the taxonomic species of microbes in soil and change the soil microbial composition. The functional pathways involve changes in the soil microbial structure, many of which are related to the heavy metal tolerance of soil microbes.

6.10.3 Effect of Heavy Metals on Microbial Diversity

Microorganism communities in soil seem to be extremely diverse, with estimates of as many as 13,000 species of bacteria present in a single gram of soil and an unknown diversity of soil fungi and algae. Gross measurements of microbial diversity have been used to estimate environmental stress (Tayebi and Ahangar 2004). Until now only few studies have tried to examine more subtle effects of heavy metal pollution on the structure of microbial communities or on the genetic diversity of particular groups of organisms.

The influence of heavy metals on the size of microbial communities changes, depending upon which group of microorganisms is being discussed, on the metal involved, and on the particular environment. For instance, on the phylloplane, bacteria appear to be more sensitive to metal pollution than fungi (Tayebi and Ahangar 2004).

Chen et al. (2010) suggested that heavy metals caused a decrease in bacterial species richness and a relative increase in soil actinomycetes or even decreases in both the biomass and diversity of the bacterial communities in contaminated soils.

Sandaa et al. (1999) in their study reported that compared to non-contaminated soil, a pronounced reduction of bacterial diversity as well as changes in bacterial community structure were obtained even in the presence of low metal concentrations below the upper legal limits set by the European Union.

However, Lenart-Boroń and Wolny-Koładka (2015) from their study observed that despite increased concentrations of some heavy metals, particularly zinc and lead, the recorded contamination did not have a significant impact on the numbers of the selected microbial groups in soils. Lenart-Boroń and Wolny-Koładka (2015) attributed the possible reason for the previously mentioned decreased microbial diversity in heavy metal-containing soils to the selection of tolerant strains.

Pollution affects microbial diversity both in terms of species richness due to the extinction of species which have no sufficient resistance to the stress imposed, and can potentially lead to the enrichment of new species which survive well in the stress condition. These studies have caused to highlight that subtle effects of heavy metals on the diversity of microorganisms in soils are happening which may disturb the potential response of the soil microbial community to new stresses (i.e., it may decrease the resilience of the soil ecosystem). Evidence from the field indicates that under longtime metal tension there is a change in the genetic structure of the soil microbial community thereby bringing about an increase in metal tolerance (Tayebi and Ahangar 2004).

6.10.4 Effect of Heavy Metals on Microbial Activity

Heavy metals indirectly affect soil enzymatic activities by shifting the microbial community which synthesizes enzymes (Jiwan and Ajay 2011). Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms (Jiwan and Ajay 2011).

Enzymatic activities are frequently used for determining the influence of various pollutants including heavy metals on soil microbiological quality (Shen et al. 2005). Heavy metals can inhibit enzymatic activities by interacting with the enzyme-substrate complexes, denaturing the enzyme protein, and interacting with its active sites (Megharaj et al. 2003).

Microbial enzymes might be affected by heavy metals due to the potential inhibition of both enzymatic reactions and complex metabolic processes. Heavy metals decrease the phosphatase synthesis during the composting process. Microorganisms have to cope with toxic Pb during their growth in the Pb-contaminated substrates, and the exposure of microorganisms to metals always inhibits microbial growth and activity (Huang et al. 2010).

In the soil environment, almost all reactions are catalyzed by enzymes that are largely of microbial origin and associated with viable cells (Sardar et al. 2007). Heavy metals exhibit toxic effects towards soil biota by affecting key microbial processes and decrease the number and activity of soil microorganisms (Jiwan and Ajay 2011).

Contamination of soil by heavy metals does not only directly affect the microorganisms but also the enzymatic processes in the soil. The microorganism population can be affected seriously by Pb, Cd, Zn, and Cu, which in turn leads to a decrease in the activities of invertase, urease, cellulase, and acid phosphatase. As a result of this, the decomposition rate of carbon, nitrogen, and phosphorus in soils would be affected and the natural cycles thereby disrupted (Li et al. 2018).

Xie et al. (2016) in their study reported that the total bioactivity, richness, and diversity of microorganisms decreased with increasing heavy metal concentrations. The excessive amounts of metals such as cadmium, copper, and zinc alter the homeostasis of soil by affecting the control mechanisms on the level of genes, thus inhibiting the activity of microbial enzymatic proteins. They cause damage to metabolic pathways, often resulting in the apoptosis of cells. Hence, the species diversity of soil microorganisms decreases. Natural processes such as nitrification and ammonification are inhibited, alongside the activity of soil enzymes (Jadwiga et al. 2013).

Bong et al. (2010) investigated the effect of heavy metals on soil organisms and enzymatic processes and showed that the addition of Zn did not cause any significant changes on the bacteria abundance. However, the addition of Zn clearly decreased the aminopeptidase activity compared to control. They further observed that Zn contamination seems to have an effect on the microbial activities and transformation of proteins in the aquatic system. Although high concentrations of heavy metals inhibit bacterial growth, there are some species which are resistant and could grow up to 5000 ppm of Pb (Kumar et al. 2015).

Hagman et al. (2015) showed declines in enzymatic activity associated with soil metal load. In a study to determine the effect of heavy metals on enzymatic activity, Kandeler et al. (2000) found that metals resulted in decreased enzyme activities.

Although in water and sediments almost all heavy metal studies have been directed towards various aspects of carbon cycling, in the case of soil the effects of heavy metals on nitrogen transformations have also received a considerable amount of attention. Rother et al. could find little or no effect of various metals on nitrogenase activity (acetylene reduction) in a number of polluted and uncontaminated soils. In fact they concluded that in their investigation soil moisture was probably more limiting than heavy metal toxicity.

Moreira et al. (2008) in their study on associative diazotrophic bacteria in grass root and soil from heavy metal-contaminated sites reported that nitrogenase activity decreased as metal concentrations increased, but it varied depending on both metal concentration and isolates/strains.

Ashraf and Ali (2007) also reported that the heavy metals exert toxic effects on soil microorganism and hence result in the change of the diversity, population size, and overall activity of the soil microbial communities and observed that the heavy metal (Cr, Zn, and Cd) pollution influenced the metabolism of soil microbes in all cases. In general, an increase of metal concentration adversely affects soil microbial properties, e.g., respiration rate and enzyme activity, which appear to be very useful indicators of soil pollution. The presence of heavy metals in the environment changed microbial communities and activities (Jansen et al. 1994; Matyar et al. 2008).

An increase in metal concentration also influences the soil microbial properties, especially respiration and enzymatic activity that serve as good indicators of metal pollution (Sonil and Jayanthi 2011). Dahlin et al. (1997) found reduced nitrogenase activity by heterotrophic soil bacterial populations in HM-contaminated soil treated with sewage sludge.

Heavy metals may inactivate enzyme reactions by complexing the substrate, by reacting with protein-active groups of enzymes, or by reacting with the enzyme-substrate complex or indirectly by altering the microbial community which synthesizes enzymes (Jiwan and Ajay 2011). However, in some studies no correlation has been found between microbial parameters and heavy metal contamination (Trasar-Cepeda et al. 2000).

Also, the microbial metabolic entropy of metal-polluted soil is higher, and the content of organic carbon converted to bio-carbon is reduced. Finally, heavy metals can result in physiological dysfunction and plant malnutrition as metal exposure can be passed to plant seed (Jin et al. 2018).

6.11 Conclusion

The effect of heavy metals on soil microorganisms has been unveiled in this chapter. Although some heavy metals have unique roles in soil as trace nutrient, their effect on soil microorganism and other organisms is an issue of concern since their accumulation in living cells has detrimental effect. Generally toxic metals cause enzyme inactivation and also cause damage to cells by acting as antimetabolites or form precipitates or chelates with essential metabolites. The response of soil microorganisms to heavy metals has also been employed as an indicator of the extent of soil contamination by these metals.

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Chapter 7
Microbial Community Dynamics in Anaerobic Digesters for Biogas Production

Darshan M. Rudakiya and Madhuri Narra

Contents

Abstract Microbial communities including bacteria and archaea in anaerobic digestion (AD) process play a crucial role in biogas production. In microbial communities, a deep understanding is required related to microbial diversity, composition, abundance, interactions, and its behavior to produce biogas. In addition, their active genes, proteins, and active metabolic products also enhance the

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productivity of AD process. Increased valuable products and biogas production can be carried out by optimizing the process parameters. The optimization can be visually seen by microbial diversity by performing metagenomic, meta-transcriptomic, meta-proteomic, and metabolomic data. However, the abundance and diversity of microbial communities are easily evaluated by high-throughput sequencing and a suitable bioinformatics analysis which are critically important for overall process. This chapter includes the microbial diversity and abundance presented in various bioreactors, process optimization factors and its impact on the biogas production. Thus, metagenomic data-based fermentation or AD can be proposed by integrating the bioinformatic data of microbial communities with their performance on the anaerobic digesters to facilitate the process improvement and higher generation of energy and value-added products.

Keywords Anaerobic digestion · Archaea · Biogas · Bacterial diversity · Methanogens

7.1 Introduction

Bio-methanation is a microbiological process of anaerobic digestion (AD) or degradation of organic materials which causes the production of biogas. It comprises a mixture of methane and carbon dioxide. It mainly occurs in natural environments, such as landfills, rice fields, sediments, and intestinal tracts of animals, where light and inorganic electron acceptors (oxygen, nitrate, sulfate, iron, etc.) are not present or limiting (Hattori 2008). The AD process is a multistep complex process performed by the combined action of three major physiological groups of microorganisms (Hattori 2008): hydrolytic–acidogenic bacteria, syntrophic–acetogenic bacteria, and methanogenic archaea. These fermenting microorganisms decompose the biopolymers (lipids, proteins, nucleic acids, carbohydrates, etc.) to soluble monomers (long-chain fatty acids, glycerol, amino acids, purines, pyrimidines, monosugars, etc.) that are further converted to short chain fatty acids (butyrate, propionate, acetate, etc.), alcohols (ethanol and methanol), hydrogen, and carbon dioxide by the same microbes. Short chain fatty acids and also alcohols are oxidized by proton-reducing syntrophic acetogens to hydrogen, acetate, formate, and carbon dioxide. These end products are ultimately transformed to methane and carbon dioxide by the methanogenic archaea (Thauer et al. 2008).

As shown in Fig. 7.1, AD is a multistep process which includes the number of microbial interrelationships and dependencies. Individual processes are kinetically nonlinear with respect to substrate concentration and inhibitors, and under most circumstances, either hydrolysis or acetolactic methanogenesis is the rate-limiting process. This is not a fixed rule, and under certain conditions (e.g., highly loaded

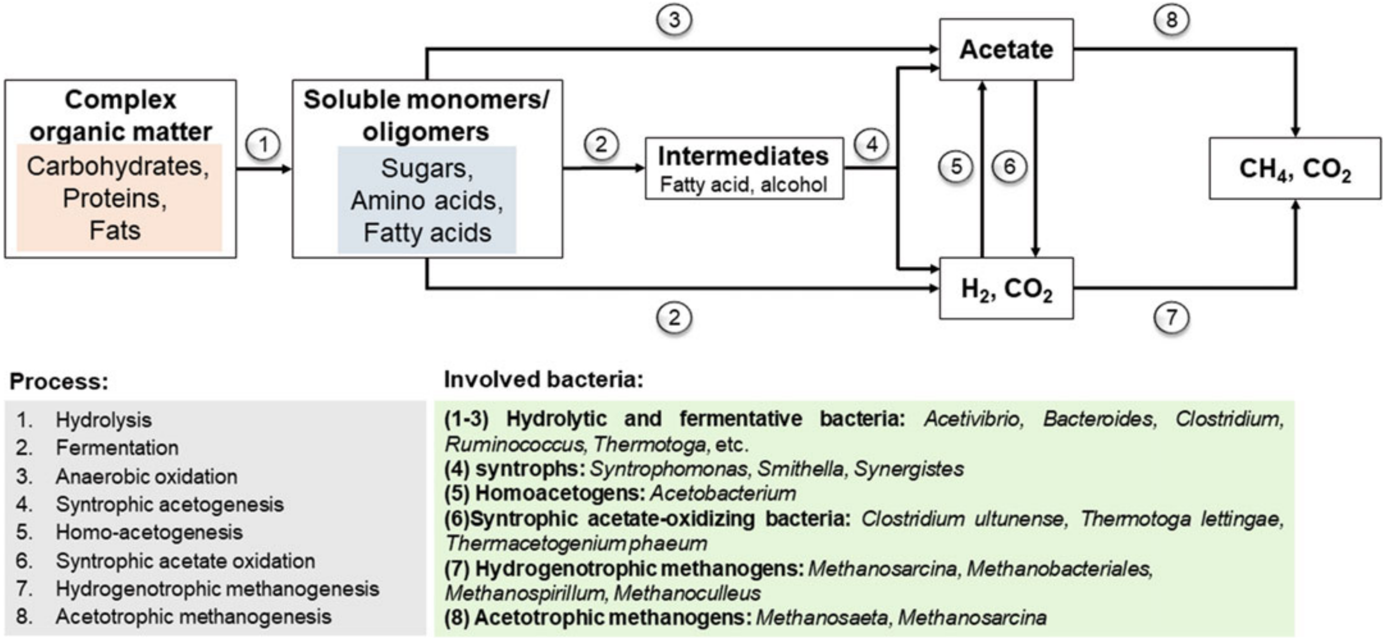

Fig. 7.1 Detailed process flow of anaerobic digestion with involved bacterial communities (Khanal 2011)

glucose-fed systems), a buildup of hydrogen can prevent acetogenesis from occurring (Angelidaki et al. 2011).

7.1.1 Hydrolysis

In AD of complex materials, the term hydrolysis is used to describe a wide range of depolymerization and solubilization processes by which complex polymeric organic compounds are broken down into soluble monomers. Most of these reactions, such as carbohydrate, polypeptide, triglyceride, and nucleic acid hydrolysis, are true hydrolysis processes, while others (e.g., scission of disulfide bonds) are reductive or oxidative bio-transformations (Sevier and Kaiser 2002). The three main primary substrates (biopolymers) for hydrolysis are carbohydrates, lipids, and proteins, which hydrolyze to monosaccharides, long-chain fatty acids plus glycerol, and amino acids, respectively. In waste or feedstreams to anaerobic digesters, materials can either be a mixture of the three substrates. Hydrolysis is widely regarded as the rate-limiting step of degradation of particulate organic matter (e.g., manure, sewage sludge, crop residues, etc.) (Pavlostathis and Giraldo-Gomez 1991). Therefore, the overall rate of the process is determined by the hydrolysis rate of this complex substrate. There are also particular considerations for different primary materials, classified as biofibers, carbohydrates, proteins, and fats. The most common composite feed is waste-activated sludge from sewage treatment plants. This is a mixture of microbial material, decay products, and inert materials originating from the feed. The degradation of decay products and inerts is generally limited in anaerobic processes, and hence the degradability of waste-activated sludge depends heavily on upstream properties (Ekama et al. 2007). Proteins and lipids are generally found together in streams such as originating from meat processing. Protein degradation depends on protein structure, with semi-soluble globular proteins being highly degradable, and fibrous proteins being relatively difficult to hydrolyze (McInerney et al. 2008). Lipids are normally triglycerides, which are hydrolyzed by lipases. Hydrolysis rates of lipids depends less on the chemical properties of the substrate and more on particle size and environmental conditions such as pH and surface tension (Yang et al. 2009).

7.1.2 Fermentation

Fermentation is anaerobic conversion of organic materials in the absence of inorganic electron acceptors such as sulfate, nitrate, or oxygen. Reduction of protons to form hydrogen may take place, but this is generally facultative. This is in contrast to degradation of propionate or butyrate to acetate and hydrogen, a process more properly referred to as anaerobic oxidation. A wide range of substrates can be fermented, including monosaccharides, amino acids, unsaturated fatty acids, glycerol, and halogenated organics (Madigan et al. 2008). However, the most abundant substrates for fermentation, and a primary route for carbon flux, are monosaccharides and amino acids. Fermentation of amino acids and monosaccharides has common elements, in that both fermentative processes are relatively energy rich and rapid. They have a wide range of operating conditions in terms of pH and oxidation/reduction potential (Batstone et al. 2002; Madigan et al. 2008; Ramsay and Pullammanappallil 2001).

Monosaccharides: Monosaccharides ferment via the Embden–Meyerhof–Parnas (EMP) or Entner Doudoroff (ED) pathway, and subsequently to C3 products (lactate and propionate) or C2/C4/C6 products (acetate/butyrate/caproate) via acetyl-CoA. C3 products are uncommon, except under overload conditions, and the most common products are acetate, butyrate, and ethanol, with waste carbon converting to carbon dioxide, and excess electrons to hydrogen gas (Rodríguez et al. 2006). There is some dispute as to whether ethanol production is enhanced at low (Ren et al. 1997) or high pH (Temudo et al. 2008).

Amino acids: Amino acids can act as electron acceptor, donor, or in some cases as both (Ramsay and Pullammanappallil 2001). Glutamate fermentation is an example of uncoupled amino acid degradation (Buckel 2001).

7.1.3 Acetogenesis

Acetogenesis refers to the synthesis of acetate, which includes the formation of acetate by the reduction of carbon dioxide and the formation of acetate from organic acids. Hydrogen-utilizing acetogens, previously also termed homoacetogens, are strict anaerobic bacteria that can use the acetyl-CoA pathway as (1) their predominant mechanism for the reductive synthesis of acetyl-CoA from carbon dioxide, (2) terminal electron-accepting, energy-conserving process, and (3) mechanism for the synthesis of cell carbon from carbon dioxide (Drake 1994). These bacteria compete with methanogens for substrates like hydrogen, formate, and methanol.

Organic acids (such as propionate and butyrate) and alcohols (such as ethanol) produced during the fermentation step are oxidized to acetate by hydrogen-producing acetogens. Electrons produced from this oxidation reaction are transferred to protons to produce hydrogen or bicarbonate to generate formate. The term obligate means that the primary substrate cannot be used as alternative electron acceptor, and electrons must be wasted to hydrogen ions or carbon dioxide, with generally unfavorable energetics (Stams and Plugge 2009).

Acetogens that oxidize organic acids obligately use hydrogen ions and carbon dioxide as electron acceptor. Acetogenic bacteria are limited by the unfavorable energetics of the conversion processes (Schink and Stams 2006). Figure 7.1 illustrates the conversion of propionate and butyrate, important intermediates in anaerobic fermentations of complex organic matter, to the methanogenic substrates, acetate and hydrogen. Figure 7.2 shows the involvement of multi-species for the conversion of acid to methane. It is evident that bacteria can only derive energy for

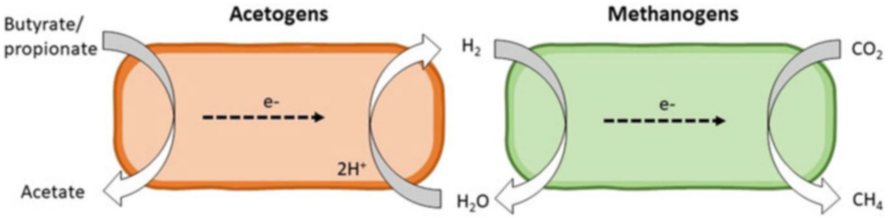

Fig. 7.2 Schematic for electron transfer within inter-bacterial species, i.e., acetogens to methanogens

growth from these conversions if the concentration of the products is kept low. This results in an obligate dependence of acetogenic bacteria on methanogenic archaea or other hydrogen scavengers (e.g., sulfate reducers) for product removal (McInerney et al. 2008; Sousa et al. 2009; Stams and Plugge 2009).

7.1.4 Methanogenesis

Methanogenic bacteria and archaea are responsible for the final step in AD process which is methane formation from acetate and/or from carbon dioxide and hydrogen, formate, alcohols, and methylated C1 compounds (Thauer et al. 2008). These types of organisms are strictly anaerobic microorganisms and their abundance in environment is varied from place to place. It is because of the need of external electron acceptors such as O_2, NO_3, Fe^{3+}, and $SO_4{}^{2-}$, which are limited. Common habitats for those archaea are anoxic marine and freshwater sediments, gastrointestinal tracts of ruminants and insects, anaerobic digesters, hot springs, and flooded soils (Nguyen et al. 2019).

Methanogens have a unique metabolism involving a number of unique enzymes and coenzymes (Deppenmeier 2002). The most interesting feature is that none of the methanogenic archaea can utilize energy from substrate level phosphorylation, and ATP is probably generated by a proton motive force and, for hydrogenotrophic methanogens, by a sodium motive force (Boone et al. 1993).

7.2 Microbial Communities and the Process

In AD process, mainly bacterial and archaeal communities are observed which are mainly responsible for the fermentation or degradation of polymeric substances and its final conversion into methane gas.

7.2.1 *Bacteria*

Various genuses of Firmicutes such as *Acetovibrio, clostridium, leuconostoc* and *lactobacillus* are generally observed in the AD process. Proteobacteria, *Spirochaetes, Cloacimonetes,* and *Bacteroides* are also most prominent phyla which are presented in the AD process. The major function of these phyla is to convert the polymers into various monomers and monomers to organic acids such as acetic acid, lactic acid, etc. Some bacteria in the AD process are responsible for various other metabolisms, i.e., sulfur reduction, nitrate reduction, metal stress, contaminant reduction, and antibiotic-resistant metabolisms which are not linked or related to the energy or biomethane metabolisms (Angelidaki et al. 2011).

7.2.2 *Archaea*

Mostly methanogenic microorganisms belong to the Archaea domain, phylum Euryarchaeota. Six phyla of methanogens have been identified, which are: *Methanosarcinales, Methanobacteriales, Methanomicrobiales, Methanococcales*, and *Methanopyrales*. *Methanocellales* (Angelidaki et al. 2011; Sakai et al. 2008). Methanogens are remarkably diverse with respect to cell morphology—from regular and irregular coccoidal cell shape (*Methanococcales, Methanomicrobiales* sp.) to short rods (*Methanobacteriales*, *Methanopyrales*) and long filaments (*Methanosaetacea* sp. within *Methanosarcinales*) (Gao et al. 2019).

7.2.3 *AD Process*

AD process is comparatively complex which includes more than three types of bacteria or archaea which convert the complex organic matter to methane. The whole process is shown in Fig. 7.3. Initially, hydrolytic bacteria hydrolyze the complex organic matter and then convert into soluble monomers or oligomers. These compounds directly engulfed by the fermentative bacteria which convert into volatile fatty acids and other intermediates. Thereafter, acetogens convert it into acids like acetic acid, lactic acid, propionic acid, etc. Other syntrophic acetate oxidizing bacteria degrade the acids into carbon dioxide and hydrogen gas which is further utilized by hydrogenotrophic methanogens. Sometimes, homoacetogens use carbon dioxide and hydrogen to synthesize the acetic acid which is further degraded by acetomorphic methanogens (Angelidaki et al. 2011; Nguyen et al. 2019; Sun et al. 2016).

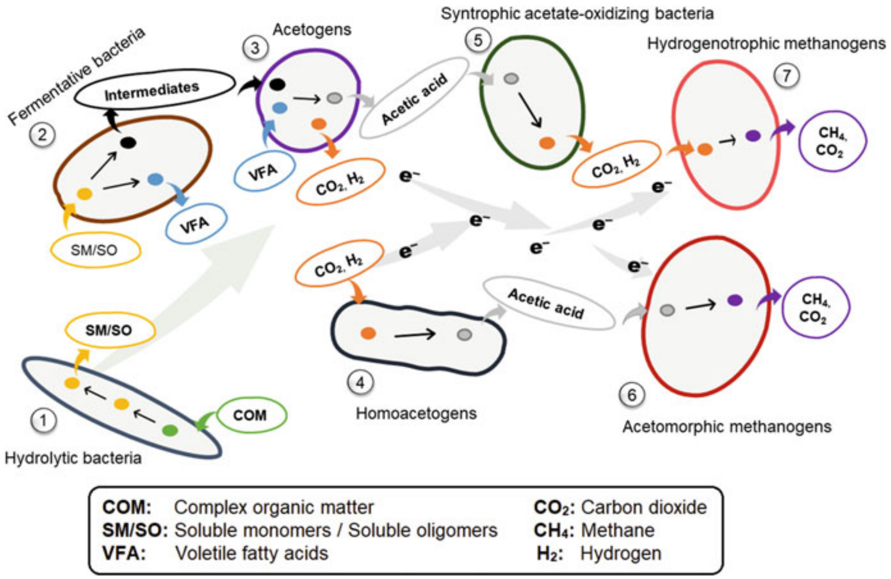

Fig. 7.3 AD process is conducted from complex organic matter to methane synthesis via a series of bacteria and intermediate process, wherein process is started from 1 to 6 intermediate process. (1) conversion of organic complex matter to soluble monomers/oligomers by hydrolytic bacteria, (2) conversion of soluble monomers/oligomers to volatile fatty acids by fermentative bacteria, (3) conversion of intermediate metabolites and volatile fatty acids to acetic acid, CO_2 and H_2 by synotrophs or acetogens, (4) conversion of CO_2 and H_2 to acetic acid by homoacetogens, (5) conversion of acetic acid to CO_2 and H_2 gases by syntrophic acetate oxidizing bacterial, (6) conversion of acetic acid to CH_4 and CO_2 by acetomorphic methanogens, and (7) conversion of CO_2 and H_2 to CH_4 and CO_2 gases by hydrogenotrophic methanogens

7.3 Bioinformatics Analysis

Microbial communities of anaerobic digester can be analyzed via metagenomic and bioinformatics approaches which include a series of steps (Fig. 7.4) (Zhang et al. 2019).

7.3.1 Sample Collection

Sample collection can be carried out from various AD processes which is mainly dependent on the different feedstock. The most dominant feedstock are food waste (>30%), sludge waste (22.8%), manure (20.3%), agricultural and horticultural waste (15.2%) (Suhartini et al. 2014; Zhang et al. 2019).

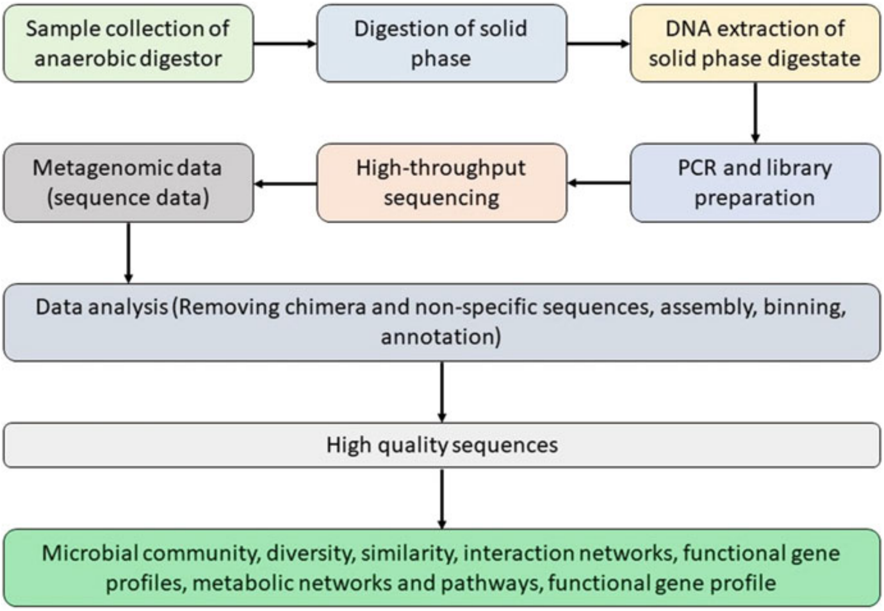

Fig. 7.4 Flowchart of microbial community analysis of anaerobic digesters which is carried out using metagenomic and bioinformatics approaches

7.3.2 DNA Extraction, PCR, and Library Preparation

DNA extraction is a prerequisite for performing bioinformatics analysis of metagenomic data of microbial communities. Prior to DNA extraction, the digestate should be centrifuged for 10 min and the supernatant should be decanted. Then, a certain amount (0.3–0.5 g) of wet weight sludge material is used to extract the DNA of microbial communities. Thereafter, PCR can be done using 16S rRNA or specific primers which are provided by the distributor. After obtaining the PCR products, they are cloned into specific vectors and the vector library has been prepared using vector cloning methods (Ju and Zhang 2015; Zhang et al. 2019).

7.3.3 Sequencing

Next-generation sequencing (NGS) technologies are competent to perform qualitative and quantitative analysis of microbial communities from different environments. The analysis is carried out fast and is cheap. The most frequently used DNA sequencing method is based on the Roche GS FLX454 pyrosequencing platform (Sun et al. 2016). However, some NGS techniques are based on Illumina sequencing platform (Zhang et al. 2017), ABI SOLiD™ short-read DNA sequencing platform (Sträuber et al. 2016), and ABI analysis reagents coupled with Applied Biosystems 3130xl (Zhang et al. 2019).

7.3.4 Sequence Analysis

Raw NGS reads are obtained after acquisition of the metagenomic data. The pretreatment of raw sequences is an overly critical step to attain high quality reads for downstream analysis. Various bioinformatics tools have been developed to pretreat the sequences which are Trimmomatic software (Campanaro et al. 2016), ACE Pyrotag Pipeline (Ho et al. 2014), HMMER (Azizi et al. 2016), MG-RAST (Wirth et al. 2012), ChimeraSlayer (Martínez et al. 2014), etc. The sequence pretreatment generally includes (1) removing adapters and linkers, (2) excluding chimeras and replication, and (3) demultiplexing of barcoded samples and quality control. UCHIME is the most cited tool to check and remove chimeras from the raw sequences while MOTHUR and QIIME (http://qiime.org/) are currently the two most frequently used platforms to denoise the metagenome data(Zhang et al. 2019).

7.3.5 OUT Clustering Analysis

After cleaning the sequences, all sequences are aligned using sequence aligners such as MOTHUR (Martínez et al. 2014), INFERNAL aligner (Cardinali-Rezende et al. 2016), and ClustalW (Zhang et al. 2019). Subsequently, the aligned sequences are clustered into operational taxonomic units (OTUs) with average neighboring clustering algorithm via Usearch software or various sequence classifiers like RDP Bayesian Classifier (Cardinali-Rezende et al. 2016), UCLUST-RDP classifier (Pope et al. 2013), and MEGA/MEGA5 (Cardinali-Rezende et al. 2016; Rudakiya et al. 2019).

7.3.6 Diversity Analysis

For investigating the biological richness/diversity of microbial communities in various biogas-producing systems, the OTUs-based alpha diversity analysis in terms of Chao1 richness estimator (Chao1), abundance coverage-based estimator of species richness (ACE), Shannon-Weaver diversity index (Shannon), and Simpson diversity index (Simpson) can be performed using the MOTHUR package (Zhang et al. 2019), Rsoftware package with VEGAN library (Oksanen et al. 2007), and the RDP Pipeline (Cardinali-Rezende et al. 2016). Straightforward calculation using the given equations to rapidly estimate biological diversity of microbial communities is a big advantage for various diversity indices. However, the diversity indices like Simpson index and Shannon index combine richness and evenness components into a simple index through a single measure, leading to a loss of relative roles of other different variables such as potential economic, ecological, and social importance of individual species (Barrantes and Sandoval 2009).

7.3.7 Taxonomic Composition Analysis

Taxonomic composition analysis is one of the most frequently used bioinformatics analyses for anaerobic microbial communities. Generally, this analysis can be performed through two steps: (1) database comparison and filtration and (2) taxonomic classification of sequences. Particularly, sequences are firstly filtered through a BLAST search against the sequence database which are SILVA database, EzTaxon database, GenBank NT/NR database, and RDP database (Zhang et al. 2019).

7.3.8 Statistical Analysis

Based on a brief survey of the literature concerning anaerobic microbial community analysis, the most common multivariate analysis techniques include principal component analysis (PCA), principal coordinate analysis (PCoA), non-metric multi-dimensional scaling (NMDS), redundancy analysis (RDA), and canonical correspondence analysis (CCA) (Barrantes and Sandoval 2009; Khanal 2011; Sun et al. 2016). The similarity of these multivariate techniques is that each one is essentially regarded as an ordination analysis, with a basic aim of depicting similar objects near to each other and dissimilar objects further apart from each other (Ramette 2007). Generally, these techniques are classified into two groups: unconstrained ordination analysis (PCA, PCoA and NMDS) and constrained ordination analysis (RDA and CCA) based on the types of used data sets and computing methods.

7.4 Factors Affecting AD Process

To operate the AD process effectively, various environmental as well as operational factors are important for the diversity and abundance of microbial communities. Important factors such as feedstock characteristics (composition, C:N ratio, particle size, total solids, etc.), process monitoring (pH, temperature, inhibitors, etc.), type of bioreactor (suspended growth anaerobic digester, attached growth anaerobic digester, solid-state anaerobic digester, household anaerobic digester), and process management (batch or continuous process) are important to achieve higher methane production

(Nguyen et al. 2019; Rocamora et al. 2019). These factors are discussed as follows in brief.

In process monitoring factors, pH and temperature of AD process, total solids, volatile fatty acids, alkalinity, organic acid concentration, chemical oxygen demand, C:N ratio, hydrogen and ammonia gas content are the major factors. For AD process, optimal conditions are mainly affected the microbial communities, wherein pH (6.8–7.4), temperature for mesophilic (35–40 °C) and thermophilic (50–60 °C), total solids for solid state (15–30%) and liquid state (<15%), total volatile fatty acids (50–250 mg/L), acetic acid (<1000 mg/L), propionic acid (<250 mg/L), total alkalinity (1500–3000 mg/L), ratio of volatile fatty acid and alkalinity (0.1–0.2), carbon to nitrogen ratio (20–40), total ammonia nitrogen (50–1000 mg-N/L), hydrogen gas content (<100 ppm), and ratio of chemical oxygen demand to nitrogen to phosphorous (350:7:1) should be within the range (Nguyen et al. 2019).

7.5 Microbial Communities of Anaerobic Digesters

Microbial communities of different AD process are described as follows:

Anaerobic digester treating the conventional and vacuum toilet flushed blackwater exhibited the methanation rates were 0.23–0.29 and 0.41–0.48 g CH_4-COD/g feed COD and the COD removal rates were 72% and 89%, respectively (Gao et al. 2019). Archaeal genera in the inoculum and reactors were different in composition which are *Methanospirillaceae, Methanoculleus, Methanospirillum,* and *Methanogenium*. The enriched bacteria were linked with high ammonia conditions, including *Porphyromonadaceae, Fibrobacteraceae, Ruminococcaceae, Bacteroidaceae, Clostridiales,* etc. (Gao et al. 2019).

Anaerobic digester treating the steam exploded food showed lower methane yield, however more organic acids like lactic acid, propionic acid, and acetic acid were produced (Svensson et al. 2018). Microbial community such as Tenericutes (42%) dominated in frequently fed digester but Firmicutes (31%) was most abundant in the Daily Fed Digester (Svensson et al. 2018).

Microbial community of anaerobic mono-and co-digesters treating food waste and animal waste is described by Koo et al. (2019). *Methanobacterium beijingense, Methanobacterium petrolearium, Methanoculleus bourgensis, and Methanoculleus receptaculi* are the major species found in the anaerobic digester. In digester, 32 bacterial genera had relative abundance >0.5% which accounted for 77.1% of total reads. *Fastidiospila* (16.4 ± 5.2%), *Petrimonas* (14.7 ± 5.1%), *Rikenellaceae* RC9 gut group (11.1 ± 6.2%), *Candidatus Cloacamonas* (4.7 ± 9.5%), *Christensenellaceae* R-7 group (3.5 ± 0.9%), *Proteiniphilum* (3.3 ± 2.3%), and *Sedimentibacter* (3.0 ± 1.0%) were dominant bacteria in anaerobic digester.

Microbial communities of mesophilic anaerobic digesters treating food wastewater or sewage sludge were shown by Lee et al. (2018). Major methane producing communities observed are *Methanoculleus* (78.6 ± 17.0%), *Methanobacterium* (7.3 ± 13.6%), *Methanomassiliicoccus* (4.1 ± 3.9%), *Methanomethylophilaceae*

(1.8 ± 3.1%), *Candidatus Methanoplasma* (2.6 ± 3.6%), uncultured *Methanosarcina* (1.2 ± 4.7%), and *Methanimicrococcus* (0.9 ± 2.0%) in anaerobic digester (Lee et al. 2018).

Microbial communities of mesophilic and thermophilic anaerobic digesters treating food waste-recycling wastewater were shown by Kim et al. (2018). In the mesophilic digester, *Fastidiosipila*, *Petrimonas*, *vadinBC27*, *Syntrophomonas*, and *Proteiniphilum* were dominant bacterial genera; they may contribute to hydrolysis and fermentation. In the thermophilic digester, *Defluviitoga*, *Gelria,* and *Tepidimicrobium* were dominant bacteria; they may be responsible for hydrolysis and acid production. In the mesophilic digester, dominant methanogens changed from *Methanobacterium* (17.1 ± 16.9%) to *Methanoculleus* (67.7 ± 17.8%) due to the increase in ammonium concentration. In thermophilic digester, dominant methanogens changed from *Methanoculleus* (42.8 ± 20.6%) to *Methanothermobacter* (49.6 ± 25.9%) due to the increase of pH (Kim et al. 2018).

Methane production was observed during the bioconversion of rice straw which is shown by Wachemo et al. (2019). Major bacterial communities observed *Enterobacteriaceae, Clostridiaceae1, Prevotellaceae,* and *Peptostreptococcaceae.* Archaeal *Methanosaeta* is dominant among all digester samples. However, the *Methanobacterium* is predominant (34.88–59.40%) in samples obtained at late stages of AD period (Wachemo et al. 2019).

Maximum methane generation rates in dry anaerobic co-digestion of rice straw and cow manure were found to increase by 30.5 ± 2.2%, 20.5 ± 1.9%, and 18.7 ± 1.8% in the reactors with limonite concentrations of 1%, 5%, and 10%, respectively. Hydrogen-consuming methanogenesis by *Methanofollis* was dominant in the reactors digesting the rice straw and cow manure with added limonite (Xu et al. 2019).

Microbial communities of thermophilic digester treating the dairy manure were shown by Lv et al. (2013). Three temperatures (50, 55, and 60 °C) were tested on the thermophilic digester and 50 °C was found to be the optimal temperature for overall performance with 31% VS removal and 0.22 L methane/g VS fed. *Methanobacterium* and *Methanoculleus* were the most predominant methanogen genera which produced the methane gas (Lv et al. 2013).

7.6 Biogas Applications

Biogas produced from AD process comprises methane and carbon dioxide. In addition to this, some traces of moisture, hydrogen sulfide, ammonia, nitrogen, and hydrogen gas are detected. In biogas cleaning, majority of unwanted gases like hydrogen sulfide, hydrogen, nitrogen, and moisture have been eliminated using various methodologies wherein scrubber and other porous materials have been used. Biogas upgrading is the enhancement of the methane content by reducing the carbon dioxide content. After biogas upgradation, methane content is above 90%. The biogas has many different applications depending on its quality. Cleaned biogas (CH_4 50–75%, $H_2S < 1000$ ppm) is suitable for cooking, burning in boiler, or generating electricity and heat via CHP units (Nguyen et al. 2019). Upgraded biogas, often termed biomethane, is more than 95%. It can be injected into natural gas grid ($H_2S < 4$ ppm) or converted into CNG as transportation fuel ($H_2S < 16$ ppm). Biogas can also be reformed to produce syngas (mixture of H_2 and CO_2), which can be converted into methanol or various liquid fuels using different catalysts via Fischer–Tropsch process (Munasinghe and Khanal 2011).

7.7 Concluding Remarks

Microbial communities involved in AD process are most phylogenetically and functionally diverse among different environments. In the last 15 years, substantial research on the microbial communities of various anaerobic digester is conducted and concluded that certain methanogen communities produce methane, acetogens and fermentative bacteria produce acid, and other bacteria assist in the bio-methanation process. Day by day, many new applications of bioinformatics technology and tools are emerged, which offer the potential analysis that can be benefited for the biogas industries (Zhang et al. 2019). In addition to metagenomics, other omics branches strengthen the microbial diversity data which is also essential for further and in-depth application of AD process. This use of complementary techniques will allow the simultaneous identification of phylogeny, interspecies interactions and function, and improve the operation of anaerobic digesters to fully utilize their potential as an effective waste management strategy and resource recovery process, and for the production of high-value products (Vanwonterghem et al. 2014). However, research is still limited to the bacterial communities and abundance. Majority of research are focused on the identification of the communities and metabolites which does not show the actual potential of metagenomics. The research should focus more on the following areas:

(a) More and precise databases should be created for bacterial identification, so that identification of bacterial and archaeal communities is error free. It also helps to identify the bacteria/archaea as majority (above 90%) of them belong to the unidentified group.
(b) More precise sequencing techniques should be evolved to identify the bacteria error free with less nucleotide sequence.
(c) More statistical tools and techniques should be created to validate the data obtained by sequencing.
(d) Utilization of omics approach or integration of multi-omics approach should be mandatory to obtain perfect and balanced data of AD system (Vanwonterghem et al. 2014).
(e) Isolation and screening of genes and proteins which are responsible for potential industrial application should be explored.

(f) Extraction and purification of industrially important molecules generated during biogas production should be explored.
(g) After identification of bacteria, more research is needed to integrate these molecular methods to develop the molecular microbiology for industrial applications.

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Chapter 8

Effect of Microbially Produced Silver Nanoparticles on Bioremediation of Waste Dye: Nanobioremediation

Abir M. Partila

Contents

Abstract The dyes, like methylene blue and azo dyes, are soluble organic compounds, toxic, mutagenic and carcinogenic agents. These dyes enter water bodies causing water pollution and health problems. Dyes are hard to evacuate by ordinary

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water treatment procedures. The bioremediation of dyes by bioproduced AgNPs was defined as nanobioremediation (NBR). Among nanomaterials, silver nanoparticles AgNPs have been focused due to their distinctive properties. Parameters must be adjusted during AgNPs preparation to increase the percentage of dye removal; these parameters are particle size, the concentration of silver nanocomposites, effect of incubation time, pH, and temperature.

Keywords Methylene blue · Azo dyes · Nanobioremediation · AgNPs

8.1 Introduction

Nanotechnology presents a number of potential environmental benefits. This could be divided into three categories: treatment and remediation, sensing and detection, and pollution prevention. Besides the applications for soil, groundwater, and wastewater, a number of nanotechnologies for air remediation are also in development. Watlington (2005) also as mentioned by Suleiman et al. (2015) wastewater remediation using nanoparticles is one of the areas of concentration among the various applications of the nanotechnology.

8.1.1 Effects of Dyes on Health and Environment, Problems Related Dyes' Environmental Contamination

The textile dyes significantly compromise the aesthetic quality of water bodies, increase biochemical and chemical oxygen demand (BOD and COD), impair photosynthesis, inhibit plant growth, enter the food chain, provide recalcitrance and bioaccumulation, and may promote toxicity, mutagenicity, and carcinogenicity (Lellis et al. 2019).

Robinson et al. (2001) one of the essential contaminant to be perceived in waste water is color which is highly visible and undesirable even in very low concentrations of dyes (Banat et al. 1996). The used water contains various constituents such as dyes and chemicals which are directly released into the sources of water which gets contaminated and thus resulting in water pollution (Punnoose and Mathew 2018). Dyes are a major class of synthetic organic compounds used in many industries especially textiles (Annamalai et al. 2011) which consume about 60% of total dye production for coloration of different fabrics. Moreover, after the completion of their use nearly 15% of dyes are washed out. These dye compounds dissolve in water bodies with a concentration in between 10 and 200 mg/L, which results in major water pollution worldwide (Gonawala and Mehta 2014). The release of dye effluents in aquatic systems is chief environmental concern because coloration not only decreases sunlight penetration and dissolved oxygen in water bodies, but also releases toxic compounds during chemical or biological reaction pathway that affects aquatic flora and fauna (Dutta et al. 2014). Despite its undeniable importance, this industrial sector is considered one of the biggest global polluters and it consumes high amounts of fuels and chemicals (Bhatia 2017).

The special emphasis is placed on the enormous use of drinking water in various operations of its production chain, such as washing, bleaching, dyeing, among others (Hossain et al. 2018).

The textile industry is spread globally, generating around one trillion dollars, contributes with 7% of the total world exports and employs around 35 million workers around the world (Desore and Narula 2018).

The textile industry is responsible for an extensive list of environmental impacts (Muthu 2017). The textile sludge, on the other hand, reveals problems related to surplus volumes and unwanted composition, often presenting high loads of organic matter, micronutrients, heavy metal cations, and pathogenic microorganisms (Bhatia 2017).

The main damages caused by the textile industry to the environment, however, are those resulting from the discharge of untreated effluents into the water bodies (Bhatia 2017). The greater emphasis should be attributed to the large amount of non-biodegradable organic compounds, especially textile dyes (Orts et al. 2018).

The dyes are soluble organic compounds (Mahapatra 2016), especially those classified as reactive, direct, basic, and acids. They exhibit high solubility in water making it difficult to remove them by conventional methods (Hassan and Carr 2018). One of its properties is the ability to impart color to a given substrate (Shamey and Zhao 2014) because of the presence of chromophoric groups in its molecular structures.

The color associated with textile dyes not only causes aesthetic damage to the water bodies (Setiadi et al. 2006), but also prevents the penetration of light through water (Hassan and Carr 2018), which leads to a reduction in the rate of photosynthesis (Imran et al. 2015) and the dissolved oxygen levels which affect the entire aquatic biota (Hassan and Carr 2018). The textile dyes also act as toxic, mutagenic, and carcinogenic agents (Khatri et al. 2018), persist as environmental pollutants and cross entire food chains providing bio-magnification (Sandhya 2010), such that organisms at higher trophic levels show higher levels of contamination (Newman 2015). In this sense, special mention should be made to azo-type textile dyes which, around 15–50%, do not bind to the fabric, during the dyeing process, and are released into waste water which is commonly used, in developing countries, for the purpose of irrigation in agriculture (Rehman et al. 2018). The use of these azo compounds is very negative to soil microbial communities (Imran et al. 2015), and to germination and growth of plants (Rehman et al. 2018).

The toxins generated by waste dyes from several industrial sectors in water bodies are hard to evacuate by ordinary water treatment procedures (Partila and El-Hadedy 2020). There are many kinds of dyes used in different industries on the large scale, among them the Methyl Orange (MO), Methyl Red (MR), and Congo Red (CR) are of common class because of their ease of application and basic colors (Nithya and

Ragunathan 2009). Long-term exposures, in particular, bring profound unfoldings to aquatic biota and to human health, as is the case with complexed metal dyes. This category of dyes is widely used by the textile industry, given their resistance, and exhibits half-lives of 2–13 years (Copaciu et al. 2013). Once released in the aquatic environment, the heavy metal cations can be assimilated by the fish gills, because they present negative charges, allowing their accumulation in certain tissues (Vargas et al. 2009). Thus, through the food chain, they can reach the human organs causing a series of pathologies (Khan and Malik 2018). Another problem associated with recalcitrant character, which is the damaging of the plant growth and development of plants, especially to photosynthesis and CO_2 assimilation (Copaciu et al. 2013). And it causes high incidence of bladder cancer, especially benzidine and 2-naphthylamine. The diseases provided by textile dyes comprise from dermatitis to disorders of the central nervous system (Khan and Malik 2018). The acute toxicity to textile dyes is caused by oral ingestion and inhalation, especially by exposure to dust, triggering irritations to the skin and eyes. The latter are the result of the formation of a conjugate between human serum albumin and the reactive dye, which acts as an antigen. Tiwari et al. (2016) point to the existence of studies made in *Allium cepa* root cells exhibiting chromosomal aberrations. Azure-B, widely used in the textile industry, is able to intercalate with the helical structure of DNA and duplex RNA, as it can be partitioned to the lipid membrane of the cells (Li et al. 2014).

It is noteworthy that this dye can reveal cytotoxic effects by acting as a notable reversible inhibitor of monoamine oxidase A (MAO-A), according to *in vitro* tests (Petzer et al. 2012), which is an intracellular enzyme of the central nervous system (Factor and Weiner 2007) that plays an important role in human behavior (Di Giovanni et al. 2008). Its potential for enzyme inhibition also concerns glutathione reductase (Paul and Kumar 2013) which plays an essential role in cellular redox homeostasis (Couto et al. 2016).

The Disperse Red 1 dye is also used by the textile industry and exhibits mutagenic potential (Chequer et al. 2009). When used *in vitro* in human lymphocyte and human hepatoma (HepG2) and when used *in vivo*, in human hepatocyte imitative cells (Will et al. 2016), it is capable of increasing the frequency of micronuclei (Fernandes et al. 2015), which are indicative of mutagenic activity at the chromosome level (Duarte and Rai 2016) which, in the case of humans, constitute a mutagenic event that is key to the characterization of cancer (Hsu and Stedeford 2010). In its turn, the Disperse Orange 1 dye exhibits similar mutagenic behavior (Chequer et al. 2009) inducing DNA damage, found in the *Salmonella* spp. assays, involving base pair substitution and frame shift mutations that alter the reading frame. In addition, it has a cytotoxic effect, with apoptosis, in contact with HepG2 cells (Ferraz et al. 2011).

The textile dyes may offer carcinogenicity, especially those of the azo and nitro type, and its effects manifest themselves over time (Mondal et al. 2018).

Once present in the bodies of animals or humans, it is enzymatically transformed, through the action of the intestinal flora, into carcinogenic aromatic amines (Piatkowska et al. 2018). Especially in the case of azo dyes, carcinogenicity can be produced by both the dye itself and its own metabolized compounds.

The Basic Red 9 dye, used in the textile, leather, paper, and ink industries (Duman et al. 2015), offers carcinogenic potential in humans (Lacasse and Baumann 2012) and high environmental toxicity (Foguel et al. 2015). It breaks down, under anaerobic conditions, into carcinogenic aromatic amines and their disposal in water bodies has the potential for allergic dermatitis, skin irritation, mutations, and cancer itself (Sivarajasekar and Baskar 2014). The latter, according to the tests performed on rats, may comprise local sarcomas and tumors in the liver, bladder (Pohanish 2017), mammary glands, and hematopoietic system (National Toxicology Program 1986).

The Crystal Violet dye, a member of the cationic triphenyl methane group, shows a very intense color (Ali et al. 2016) and is responsible for mitotic poisoning, which is associated with abnormal accumulation of metaphases (Mani and Bharagava 2016) as well as the *in vitro* clastogenic effects observed in Chinese hamster ovules (Azmi et al. 1998), which induce chromosomal damage (Newman 2015). This powerful carcinogenic agent (Bharagava et al. 2018) promotes fish tumors (Mani and Bharagava 2016), as well as hepatocarcinoma and reticular cell sarcoma in various organs, such as the vagina, uterus, ovary, and bladder (Littlefield et al. 1985), hardened gland adenoma and ovarian atrophy in rats. In humans, it is capable of generating chemical cystitis, irritation of the skin and digestive tract, respiratory and renal failure, among others (Mani and Bharagava 2016).

The water from industries with potential hazardous organic matters when discharged into the water bodies causes undesirable effects to the environment, aquatic life, and human health. So it is necessary to treat waste water prior to their disposal. Degradation of organic pollutants is a major concern in the present scenario due to its stability, fastness to sunlight, resistance to degrading agents, or microbial attack. It is mainly achieved by silver metal nanoparticles synthesized by green route (Punnoose and Mathew 2018).

CR is highly toxic and carcinogenic anionic dye belonging to a group of azo dyes, derived from benzylamine. The presence of CR dye in the waste water would be very harmful to the aquatic environment. An efficient dye degradation has become a challenging task for environmental engineers as well as scientists. Different methods have been adopted by scientists for degradation of dyes in effluent which includes biological degradation, photocatalysis and adsorption tactics, etc. (Xiao et al. 2019).

Environmental remediation of toxic organic pollutants on catalytic reduction has gained much attention. The discharge of untreated effluents into the aquatic environment leads to an unwanted change in ecological balance. Recently, effluents of organic dyes and nitroarenes have been identified as a new threat because the unwanted dyes and nitro compounds are toxic to fauna, flora, and humans. Effective and selective removal of organic dye from water systems is a pressing global issue for both drinking water and wastewater purification.

Partila and El-Hadedy (2020) reported that the AgNPs polymer was consolidated and cross-linked by gamma radiation to prevent the dissolution of sodium alginate in water. This strategy was utilized for adsorption and reduction of two different waste dyes methylene blue and Fantacell dyes, and found the maximum reduction percentage for Fantacell dye was 87%, detected at 45 min, and the maximum reduction percentage of bromothymol blue was 87%, detected at 150 min.

8.2 Nanobioremediation (NBR)

The removal of environmental contaminants (such as heavy metals, organic and inorganic pollutants) from contaminated sites using nanoparticles/nanomaterial formed by plant, fungi, and bacteria with the help of nanotechnology is called nano-bioremediation. NBR is still a new area but growing rapidly in the field of nanotechnology. This chapter speculates on biosynthesis of nanoparticles from plants, bacteria, yeast and fungi and bioremediation of waste dye. The biosynthetic route of nanoparticle synthesis could emerge as a better and safer alternative to conventional methods. The area is often referred to as the "Next Industrial Revolution" (Roco 2005). A combined approach involving nanotechnology and biotechnology could overcome this limitation: in which complex organic compounds would be degraded into simpler compounds (Singh 2010).

Biosynthesis of nanoparticles using microorganisms has emerged as rapidly developing research area in green nanotechnology across the globe, this is an alternative for conventional chemical and physical methods. Optimization of the processes can result in synthesis of nanoparticles with desired morphologies and controlled sizes (Iravani 2014).

8.2.1 Nanoparticles and Its Unique Properties

As a particle decreases in size, a greater proportion of atoms are found at the surface compared to those inside. For example, a particle of size 30 nm has 5% of its atoms on its surface, at 10 nm 20% of its atoms, and at 3 nm 50% of its atoms. As growth and catalytic chemical reactions occur at surfaces, this means that a given mass of material in nanoparticulate form will be much more reactive than the same mass of material made up of larger particles (Lubick and Betts 2008).

To put that scale in another context, the comparative size of a nanometer to a meter is the same as that of a marble to the size of the earth. In the "bottom-up" approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are constructed from larger entities without atomic-level control. "quantum size effect" is when the electronic properties of solids are altered with great reductions in particle size. Diffusion and reactions at nanoscale, nanostructures materials and nanodevices with fast ion transport are generally referred to nanoionics (Lubick and Betts 2008).

Materials reduced to the nanoscale can show different properties compared to what they exhibit on a macroscale, enabling unique applications. For instance, opaque substances become transparent (copper); stable materials turn combustible (aluminum); solids turn into liquids at room temperature (gold); insulators become conductors (silicon). A material such as gold, which is chemically inert at normal scales, can serve as a potent chemical catalyst at nanoscales. Much of the fascination with nanotechnology stems from these quantum and surface phenomena that matter exhibits at the nanoscale (Lubick and Betts 2008).

It is possible to prepare small molecules to almost any structure. These methods are used today to produce a wide variety of useful chemicals such as pharmaceuticals or commercial polymers. These approaches utilize the concepts of molecular self-assembly and/or supramolecular chemistry to automatically arrange themselves into some useful conformation through a bottom-up approach. The Watson–Crick base pairing rules are a direct result of this, as is the specificity of an enzyme being targeted to a single substrate, or the specific folding of the protein itself. Thus, two or more components can be designed to be complementary and mutually attractive so that they make a more complex and useful whole (Edelstein Alan and Cammarata Robert 1998).

The challenge for nanotechnology is whether these principles can be used to engineer novel constructs in addition to natural ones (Levins and Schafmeister 2005).

Molecular nanotechnology, sometimes called molecular manufacturing, is a machine that can produce a desired structure or device atom-by-atom using the principles of mechano-synthesis. The conventional technologies are used to manufacture nanomaterials such as carbon nanotubes, nanoparticles (Lubick and Betts 2008), molecular wire or nanowires (Mihailovic 2009), and switches. They are the fundamental building blocks for molecular electronic devices. Their typical diameters are less than three nanometers, while their bulk lengths may be macroscopic, extending to centimeters or more. Nanotechnology is characterized by the use of very small manufactured particles (<100 nm), called nanoparticles (NPs) or ultrafine particles. They are more reactive and more mobile in nature. NPs are broadly in two groups of organic and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles (fullerenes) while some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (e.g., gold and silver), and semiconductor nanoparticles, e.g., titanium dioxide and zinc oxide (Ruffini-Castiglione and Cremonini 2009). Smaller particle size enables the development of smaller sensors, which can be deployed more easily into remote locations. Recently, nanomaterials (NMs) have been suggested as efficient, cost-effective and environmentally friendly alternatives to existing treatment materials, in both resource conservation and environmental remediation (Dastjerdi and Montazer 2010) and stable with great importance in wider application in the areas of electronics, medicine, and agriculture (Kavitha et al. 2013). Although nanoparticles can be synthesized through an array of conventional methods, the biological route of synthesizing it has advantages because of ease of rapid synthesis, controlled toxicity, control of size characteristics, low cost, and eco-friendly approach (Ingale and Chaudhari 2013). Nanoparticles are extensively used for removal of biological contaminants and chemical contaminants including organic pollutants (Okhovat et al. 2015) also. Bioremediation of radioactive wastes from nuclear power plants and nuclear weapon production, such as uranium, has been achieved using nanoparticles. Cells and S-layer proteins of *Bacillus sphaericus* JG-A12 have been found to have special capabilities for the cleanup of uranium contaminated wastewaters (Duran et al. 2007). A unique study on plants suggests that some nanomaterials may inhibit seed germination and root growth (Lin and Xing 2007).

8.2.2 Silver Nanoparticles

In recent years, silver nanoparticles (AgNPs) have greatly focused the researcher's attention because of their important application as antimicrobial, catalytic, textile fabrics and plastics to eliminate microorganisms (Fouad et al. 2019). They are small enough to confine their electrons and produce quantum effects. Silver nanoparticles have distinctive physico-chemical properties, such as high electrical and thermal conductivity, chemical stability, catalytic activity, and nonlinear optical behavior (Xu et al. 2006; Tran and Le 2013).

Besides these silver nanoparticles have also been used as antimicrobial agents, silver nanoparticles used as anti- microbial material and in waste water purification, etc. (Park 2014; Banerjee et al. 2014).

The biomolecules might be acting as reducing and stabilizing agents. According to the previous studies, the band appeared in the ranges of the band spectrum indicates in the spectrum indicates the formation of AgNPs and capped with different bio-moieties (Sharma et al. 2017).

Among nanomaterials, silver nanomaterials have been focused due to their distinctive physical, chemical, and biological properties as compared to macroscopic materials (Dwivedi and Gopal 2010).

Earlier studies reported that nanoparticle synthesized from silver was effective in enhancing the dye removal (Harsha et al. 2011). Seventy percent decolorization was achieved by the nanoparticles of both bacteria against the dye Acid Orange 5 and Black 7984 at 100 and 200 ppm concentration. Similar results were reported by Modi et al. (2015) where the silver nanoparticles from Bacillus pumills decolorize Congo red. A slower rate of decolorization was attributed to higher molecular weight and structural complexity of the dyes (Hu and Wu 2001).

8.3 Factors Affecting Dye Removal by Nanoparticles

8.3.1 Particle Size

Solanum nigrum assisted silver nanocomposites are better in dye removal as compared to *Cannabis sativa* as the efficiency of dye removal for *Solanum nigrum* is two times more as compared to *Cannabis sativa*; this may be due to small size of

Solanum nigrum assisted silver nanocomposites, in which the DLS pattern reveals that silver nanocomposites synthesized by this method have average diameter of 34.13 ± 3.10 and 70.93 ± 3.57 nm for *Solanum nigrum* and *Cannabis sativa* leaf extract, respectively. The TEM data reveals that biosynthesized silver nanocomposites are spherical in shape having 5–17 nm for *Solanum nigrum* and 12–22 nm for *Cannabis sativa* (Khatoon and Sardar 2017).

8.3.2 Concentration of Silver Nanocomposites

As the concentration of silver nanocomposites increases from 1 to 4 μg/mL, the efficiency of dye removal increases up to 3 μg/mL and after this the dye removal efficiency becomes constant. The *Solanum nigrum* mediated silver nanocomposites at 3 μg/mL for yellow, blue, and mixture of both the dyes are 68.2%, 77.5%, and 54.23%, respectively, while *Cannabis sativa* mediated silver nanocomposites at same concentration are 27.02%, 45.7%, and 25.5% (Banerjee et al. 2014).

8.3.3 Incubation Time

Incubation time has a significant role in synthesis. The results show that absorption increases up to 2 h for *Solanum nigrum* and 5 h for *Cannabis sativa*, after that it is stable (Khatoon and Sardar 2017).

8.3.4 pH

The maximum dye removal (yellow, blue, and mixture) was obtained at basic pH that is at pH 9 for both the nanocomposites. This may be due to electrostatic interaction of dyes with nanocomposites which varies with the change in pH (Banerjee et al. 2014). The biosynthesized silver nanoparticles are most effective in dye removal at alkaline pH 9 and at 60 °C. The textile industry effluents have high pH and temperature which makes these nanoparticles more appropriate in treatment of these industry effluents, in which the decolorizing efficiency of biosynthesized silver nanocomposites was investigated up to three cycles (Khatoon and Sardar 2017).

8.3.5 Temperature

Rise in temperature may also have resulted in a higher mobility of the dye molecules accompanied by a reduction in the retarding forces and the adsorbent efficiency. By keeping all other conditions constant, an increase in temperature led to an increase in the percentage of dye adsorption up to 60 °C. The decrease of dye removal beyond 60 °C may be due to denaturation of biomolecules present on silver nanocomposites at higher temperature. Finally, the visual observations of dye removal done, when incubated with Solanum nigrum and Cannabis sativa that assisted silver nanocomposites at a concentration of 3 μg/mL, *pH* 9 at 60 °C for 2 h (Khatoon and Sardar 2017).

8.4 Methods for Production of AgNPs

8.4.1 Physical

Adsorption is the most efficient method for the removal of dyes. Adsorption does not require an additional pre-treatment step before its application and process does not result in the formation of any harmful substance (Dabrowski 2001). In ion exchange treatment of dye-containing effluents, wastewater is passed over the ion exchange resin until the available exchange sites are saturated (Slokar and Le Marechal 1998) the polymer was over saturated by AgNPs (Partila and El-Hadedy 2020). Membrane separation including ultrafiltration, nanofiltration, and reverse osmosis have been increasingly used recently for the treatment of effluents due to its ease of operation (Kurniawan et al. 2006).

8.4.2 Chemical

Chemical methods include coagulation or flocculation combined with flotation and filtration, precipitation–flocculation with $Fe(II)/Ca(OH)_2$, electroflotation, electrokinetic coagulation, and conventional oxidation methods by oxidizing agents (ozone) (Pereira and Soares 2003). These chemical techniques are often expensive, and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem (Ghoreishi and Haghighi 2003).

8.4.3 Biological

Traditionally, nanoparticles were produced only by physical and chemical methods. The need for biosynthesis of nanoparticles arose due to the high cost of physical and chemical processes. In the search of cheaper pathways for nanoparticle synthesis, microorganisms and then plant extracts were used for synthesis, where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles (BCC Research 2007). Most of scientists utilized toxic chemicals and also include the use of enormous energy. This scenario is not economic and causes serious pollution to environment. Synthesis of nanomaterials using biological entities is gaining attention because biological methods are less expensive, nontoxic and involve environmentally acceptable procedures (Logeswari et al. 2013). The diversity of microorganisms is being used as eco-friendly nanofactories for biosynthesis of nanoparticles. Southham and Beveridge (1996) such as silver, cadmium sulfide, gold, tin and (Sastry et al. 2003). The biosynthesis of microbial metal nanoparticles shows several applications including the fields of bioremediation, bio-mineralization, bio-leaching, and microbial corrosion (Singh et al. 2011).

8.4.3.1 Nanoparticles Produced by Plants and Bioremediation

Green synthesis of nanoparticles by plants is gaining importance nowadays because of single step biosynthesis process, absence of toxicants, and occurrence of natural capping agents (Gurunathan et al. 2009). The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction (Manzer et al. 2015). They proved the biological synthesis of silver nanoparticles using ethanolic extracts of two medicinal plants, *Ocimum sanctum* and *Artemisia annua* leaf extracts. After exposing the silver nitrate solution to the leaf extracts, the rapid reduction of silver ions led to the formation of silver nanoparticles in solution. The *Ocimum sanctum* and *Artemisia annua* assisted silver nanoparticles were studied for their potential to remove phenols, textile dyes, and microbial contaminants from water. The silver nanoparticles from *Ocimum sanctum* can catalyze the reduction of 4NP within 20 min in presence of $NaBH_4$. The synthesized nanoparticles can efficiently adsorb toxic textile dyes (Reactive Blue 4, Reactive Orange 4, and Reactive Red 120) from aqueous solutions. The nanoparticles show excellent antimicrobial properties and can be reused repeatedly (Sardar and Khatoon 2016). Silver nanoparticles (AgNPs) are useful in various fields such as catalysis, optics, biomedical, pharmaceutical, and sensor technology (Khatoon et al. 2017).

AgNPs were synthesized using *Duranta erecta* leaves' extract as a reducing agent. Organic pollutants were reduced such as 4-nitrophenol (4-NP), 2-nitrophenol (2-NP), 2-nitroaniline (2-NA), trinitrophenols (TNP) as well as rhodamine B (RhB) and methyl orange (MO) dyes.

Percentage of dye degradation was estimated by the following formula:

$$\%\text{Decolorization} = (C0 - C)/C0 \times 100$$

where

$C0$ is the initial concentration of dye solution and
C is the concentration of dye solution after catalytic degradation.

The average size of the AgNPs synthesized is approximately to be ~13 nm spherical and uniform nature produced during 20 min boiling.

The degradation of methylene blue was noted by color change from the initial first day deep blue color to light blue at the end of fifth day. Take absorbance value at 660 nm. Of various concentrations of AgNPs, 10 mg was found effectively degrading the dye at the end of fifth day (75%). Also, the phytosynthesis of AgNPs was accomplished by using the leaf extract of *cynodon dactylon* (L.) Pers and dye degradation, in which the synthesis of AgNPs was confirmed by color change from pale yellowish reaction mixture to dark brown after 20 min of boiling. And it showed an absorption peak at 350 nm in UV–visible spectrum corresponding to the plasmon resonance band of the synthesized AgNPs, the silver nanoparticles was around 13 nm, AgNPs concentration (10 mg/1000 mL), absorbance at 660 nm for the dye. The percentage of dye degradation was increased with increasing the day. Of various concentrations of AgNPs used, dye solution containing 10 mg AgNPs showed 75% dye degradation after 5 days of incubation at room temperature (Anjana and Geetha 2019).

The use of plant and plant extract in nanoparticles synthesis is advantageous compared to microbial based system because it eliminates the intricate process of maintaining cell cultures (Monda et al. 2011). Nowadays biosynthesized nanocatalysts are widely used for the efficient removal of dye contaminants. Plant contains a complex network of metabolites and enzyme that can be used to synthesize nanoparticles. The presence of different chemical compounds in plant such as polyphenols, flavonoids, sterols, triterpenes, reducing sugar like glucose and fructose, and protein could help produce metallic nanoparticles (Bonnia et al. 2016). Green synthesized AgNPs were found to be stable for 6 months; this stability may be due to the presence of phytochemicals present in the leaf extract of *C. dactylon* which acts as stabilizing agents (Anjana and Geetha 2019).

The AgNPs are synthesized via the green synthesis method by using extracts of tomato (T), onion (O), acacia catechu (C), and combined extracts of COT as reducing and stabilizing agents. The formation of AgNPs synthesized by onion and tomato extracts showed the well-defined absorbance band at 432 and 450 nm (Chand et al. 2013).

8.4.3.2 Nanoparticles Produced by Bacteria and Bioremediation

Alcaligenes faecalis AZ26, *Bacillus cereus* AZ27, and *Bacillus* sp. AZ28 were isolated from effluents of textile industries. These bacterial isolates grew well in the presence of up to 500 mg/L of Novacron Super Black G (NSB-G) and showed decolorization of approximately 90% at 200 mg/L of NSB-G after 96 h of cultivation at 37 °C and pH 8.0 under static condition, which have potential in the biological treatment of dyeing effluents (Hossen et al. 2019). Synthesize AgNPs using endophytic bacterium *Bacillus siamensis* strain C1, which was isolated from the medicinal plant *Coriandrum sativum*.

Bacteria are capable of mobilization and immobilization of metals and in some cases, the bacteria which can reduce metal ions show the ability to precipitate metals at nanometer scale. Bacteria are considered as a potential "biofactory" for the synthesis of nanoparticles like gold, silver, platinum, palladium, titanium, titanium dioxide, magnetite, and cadmium sulfide. The use of bacteria as a source of enzymes that can catalyze specific reactions leading to inorganic nanoparticles (Iravani 2014). Extracellular secretion of enzymes offers the advantage of producing large quantities of nanoparticles, and the further purification of nanoparticles is successfully achieved by filtering. The special metal binding abilities of the bacterial cells and S-layers make them useful for technical applications in bioremediation and nanotechnology. The properties of nanoparticles are controlled by optimization of important parameters which control the growth condition of organisms, cellular activities, and enzymatic processes. The large-scale synthesis of nanoparticles using bacteria is appealing because it does not need any hazardous, toxic, and expensive chemical materials for synthesis and stabilization processes (Iravani 2014). Immobilization of AgNPs with polyacrylamide and sodium alginate gave the best results for dye decolorization, the polymer polymerized by 3Mega gamma rays; as shown by Partila and El-Hadedy (2020) percentage of decolorization for Fantacell dye was 87%, detected at 45 min, and the maximum reduction percentage of bromothymol blue was 87%, detected at 150 min. as a combination of two methods biological and physical.

8.4.3.3 Nanoparticles Produced by Fungi and Dye Bioremediation

Fungi are the best candidates in the synthesis of metal nanoparticles due to their ability to secrete large amount of enzymes, high protein contents, its biomass, economic livability, tolerance, and metal bioaccumulation capability; moreover, a number of species grow fast and therefore culturing and maintaining them in the laboratories are very simple (Castro-Longoria et al. 2012; Dhillon et al. 2012).

Also, in addition to monodispersity, nanoparticles with well-defined dimensions can be obtained using fungi. Compared with bacteria, fungi could be used as a source for the production of larger amounts of nanoparticles. This is because fungi secrete a greater volume of proteins which directly translate to higher productivity of nanoparticle formation (Mohanpuria et al. 2008). The fungus *Aspergillus flavus* was capable of synthesizing extracellular silver nanoparticles (AgNPs) (Vighneshwaan et al. 2007).

Fusarium oxysporum is known to secrete the nicotinamide adenine dinucleotide (NADH) enzyme, especially nitrate reductase, which might be responsible for the bioreduction of Ag+ to Ag° through electron shuttle enzymatic metal reduction process (Duran et al. 2005). Hamad (2018) reported the extracellular synthesis of AgNPs by using two filamentous fungi *Penicillium citreonigrum Dierckx* and *Scopulariopsis brumptii Salvanet-Duval*, isolated from east of Lake Burullus. Nithya and Ragunathan (2011) reported that silver nanoparticle synthesized by *Pleurotus sajor caju* can effectively decolorize congo red dye (78%) in 24 h incubation and the dye was fully decolorized after 35 h incubation.

Bioremediation occurs by biosorption washing water from cotton fabric processing, by silver nanoparticles (Duran et al. 2010). The process also allowed recovery of silver leached into the effluent for reutilization, avoiding any effect to the environment and reducing cost.

8.4.4 Photocatalytic Degradation

Sunlight is an abundantly available natural source of energy which can be conveniently exploited for the photodegradation of pollutants (Wang et al. 2014). Dyes can be degraded in the presence of photocatalyst such as metal nanoparticles; UV irradiation acts as another source for organic dye degradation by photocatalysts including TiO_2 (El-Kemary et al. 2011) and ZnO. ZnO is unstable due to incongruous dissolution to yield $Zn(OH)_2$ on the ZnO particle surfaces, thus leading to catalyst inactivation (Bahnemann et al. 1987). Photocatalysts like SnO_2, ZrO_2, CdS, MoO_3, WO_3, RuO_2, Co_3O_4, Cu_2O, SiO_2, α–Fe_2O_3, Mn_2O_3, and Fe_3O_4 have shown negligible activity (Han et al. 2004). The photocatalytic activity of these could be improved under UV and visible illumination by incorporation of metal nanoparticles of silver, gold, or iron to broaden the absorption of solar radiations (Anpo 2000).

8.4.5 Reducing Agent

An alternative method for organic dye reduction utilizes the electron donating capacity of transition metal nanoparticles such as silver, gold, iron, nickel, palladium, and platinum in the presence of reducing agents like $LiAlH_4$, $NaBH_4$, or H_2O_2. Silver metal nanoparticles have high efficient catalytic activities because of their unique properties such as extreme small dimensions, large surface to bulk ratio, large dispersivity and ability to transfer electrons between the donor and acceptor electron relay system (Ghosh et al. 2002). Among these, plant products is the most adopted method as phytochemicals are the natural resource for the synthesis of metal nanoparticles, much safer to handle, widely available and ability to act as both stabilization and reduction agent (Shankar et al. 2004). *Azadirachta indica* leaves, *Camellia sinensis, Capsicum annuum L., Cinnamomum camphora* leaves, *Datura metel, Emblica officinalis* fruit, and *Ocimum sanctum* leaves are few among them (Krishnaraj et al. 2010).

8.5 Mechanisms of Dye Degradation by Silver Nanoparticles

It is confirmed that the reduction of silver ion occurred due to different functional groups: terpenoids, flavones, phenolics, and polysaccharides compounds present into the plant extracts (Ahmed et al. 2016). Terpenoids and flavonoids compounds are responsible for the stabilization of AgNPs and also potential constituents of onion and tomato extracts are responsible for the reduction of silver ion (Akter et al. 2018).

8.5.1 Catalytic Degradation by Silver Nanoparticles Using Reducing Agents

The process of dye degradation by silver nanocatalyst through reducing agent like $NaBH_4$ can be explained by electron transfer mechanism. During degradation, the catalysis process occurs on the surface region of metals, therefore the surface area availability will increase significantly and in turn improve the efficiency of the catalyst (Grogger et al. 2004). The reductant molecules and dye molecules are probably adsorbed on the large surface of silver nanoparticles without affecting their activity. When the reducing agent $NaBH_4$ is adsorbed on the nanoparticles, its reductive potential decreases, as $NaBH_4$ is a strong nucleophile. On the other hand, when dye molecules get adsorbed on nanoparticles, their reduction potential increase, as the molecules are electrophilic in nature and hence, when both the species are adsorbed on nanoparticles they become more negative for $NaBH_4$ molecules and more positive for dye molecule. The biogenic silver nanoparticles support the "electron shuttling" from the donor to the acceptor molecules and thus act as an effective substrate for the electron relay process (Pradhan et al. 2002). During electron transfer reaction, the BH_4^- ions are simultaneously adsorbed on the surface of metal nanoparticles and thus electron transfer occurs from BH_4^- ions to dye through the nanoparticles, resulting in the destruction of the dye chromophore structure to form small species such as acetamide, CO_2, and H_2O, which are less hazardous than the organic pollutant (Mallick et al. 2006).

8.5.2 Photocatalytic Degradation of Dyes by Silver Nanoparticles

In the utilization of leaf and calyx extracts of *Plumbago auriculata* for the biosynthesis of silver nanoparticles (AgNPs), the water-soluble components of the extracts were responsible for the reduction of Ag^+ ions. Stabilization properties of these particles due to the nature of the capping agent FTIR spectra revealed that the –OH and –C=O groups present in the biomolecules were responsible for the stabilization and reduction of the AgNPs (Singh et al. 2018). The photodegradation of methylene blue dye by silver nanoparticles from *Helicteres isora* (Bhakya et al. 2015) and *Anas platyrhynchos* (Sinha and Ahmaruzzaman 2015) showed decrease in the absorption peak at 664 nm in the presence of light. In the proposed mechanism, the excited surface electron interacts with dissolved oxygen which produced hydroxyl radicals which helped in the interaction of Ag^+ ions with dyes resulting in effective degradation (Kumari et al. 2016). Smaller particle size, large surface area, and capping phytochemicals effectively reduce the recombination of electrons leading to increase in the photodegradation efficiency of the biosynthesized *Dimoncarpus longan*, silver nanoparticles. The silver nanoparticles acted as a catalyst by lowering the activation energy (Khan et al. 2016a, b). Absorption peak of the dye decreased with increase in time of exposure to sunlight (Mendhulkar et al. 2016). The visible light irradiation of silver nanoparticles promoted electron from valence band to conduction band (Khan et al. 2016a, b). The excited electrons in the conduction band were scavenged by lattice oxygen molecules on the surface of the catalyst to form a superoxide radical anion (O_2^-) (Karthik et al. 2017). The newly formed oxygen anions further react with water to generate many oxidative species which causes dye degradation (Ashok et al. 2017). *Parkia roxburghii* (Paul et al. 2015) silver nanoparticles showed effective degradation of methylene blue and rhodamine B through $NaBH_4$, indicating that silver nanoparticles caused structural changes and removed chromophore group from the dye (Paul et al. 2015). The high potential difference among the donor and acceptor groups made the reaction kinetically unsuitable. The kinetic barrier could be removed by using silver catalyst that mediates the transfer of electron from donor (BH_4^-) to acceptor molecules (Khan et al. 2016a, b). The probable mechanism of photocatalytic degradation by silver nanoparticles could be attributed to the surface plasmon resonance effect where the excited surface electrons might interact with the dissolved oxygen molecules and ultimately produce hydroxyl radicals while allowing Ag+ ions to interact with the anionic dyes (Kumari et al. 2016).

The photocatalytic mechanism can be related by two parts, namely, photo and catalysis. The first portion is related to photon absorption. The second portion is related to surface radical formation and surface reactivity between O_2, H_2O, and organic molecules (Sinha and Ahmaruzzaman 2015). Upon photon absorption by silver nanoparticles, an electron gains energy and gets excited from valence band (VB) to conduction band (CB), leaving a positively charged hole in the valence band (h+VB). The valence band holes react with the chemisorbed H_2O molecules to form reactive species such as $OH^{\bullet}$ radicals. An electron in the conduction band (e–CB) of the nanoparticles reacts with dissolved oxygen molecules in the reacting medium and converted into oxygen anion radicals (da Silva and Faria 2003). $HO_2^{\bullet}$ radicals were generated on protonation of superoxide radical anions $O_2^{\bullet-}$.The hydroxyl radicals ($OH^{\bullet}$) and super oxide radical anions ($O_2^{\bullet-}$) are powerful oxidizing agents capable of attacking dye molecules and degrade them into small molecules such as CO_2, H_2O, and NH_3, which are not particularly toxic.

8.6 Types of Dyes and Its Degradation

8.6.1 Degradation of Methylene Blue

Methylene blue is a model cationic dye employed by industries such as textile industry for a variety of purposes. It is a heterocyclic aromatic chemical compound with a molecular formula $C_{16}H_{18}CIN_3S$. It causes eye burn which may be responsible for permanent injury to the eyes of human as well as aquatic animals. It can also cause irritation to the gastrointestinal tract with symptoms of nausea, vomiting, and diarrhea. Methylene blue also causes irritation to the skin when in contact with it (Oliveira et al. 2008). Methylene blue degradation at 664 nm is studied using UV–vis. spectrophotometer, in the presence and absence of different biogenic silver nanocatalysts. The color change from blue to colorless leuco methylene blue infers the complete degradation of dye. Silver nanoparticles act as an electron mediator between donor ($NaBH_4$) and electron acceptor (methylene blue dye) (Naraginti and Li 2017). Reduction of dye by $NaBH_4$ without nanocatalyst is not effective and required months for the degradation. The reduction of dye by sodium borohydride in the presence of *Soymida febrifuga* silver nanocatalyst was completed in less than 20 min.

8.6.2 Degradation of Azo Dyes

Methyl orange, methyl red, and Congo red are the commonly used azo dyes in textile industries and are also used as indicators in various industries. These are highly toxic and are harmful to plants and human beings. So its effective degradation is a need. The aqueous solution of methyl orange is orange red in color. The UV–vis. spectrum of aqueous solution of the methyl orange and methyl red showed strong absorptions at 465 and 490 nm, respectively. Congo red in water medium shows surface plasmon resonance bands at 498 and 338 nm (Meena Kumari and Philip 2015). An increase in the rate of methyl orange degradation by *Punica granatum* nanocatalyst with the help of $NaBH_4$ was observed because of the excellent catalytic activity of nanoregime particles due to their relative high surface to volume ratios. Catalytic degradation by the reductant $NaBH_4$ alone results no change in the characteristic absorption peak at 465 nm even up to 6 h. Addition of green synthesized silver nanoparticles using *Mussaenda erythrophylla* into the solution containing dye and $NaBH_4$ caused a rapid dip in the absorbance peak at 465 nm (Varadavenkatesan et al. 2016). Surface plasmon resonance band showed a blue shift in absorption peak from 465 to 440 nm during the dye degradation due to the surface alterations arising due to electron relay process (Varadavenkatesan et al. 2016). The catalyzed reaction found to be faster due to electron relay of silver catalyst from BH_4^- ions to the methyl orange dye. *Curcuma longa* mediated silver nano showed complete degradation within 3 min (Vadivu et al. 2017). The degradation of methyl orange and Congo red not even started by the reducing agent $NaBH_4$ without nanocatalyst. Only after the addition of silver nanoparticles, catalytic reductive degradation of the dyes commenced and completed within 30 min for both dyes. Silver nanocatalyst was the electron transfer mediator and provided more catalytic sites due to high surface to volume ratio; *Aglaia elaeagnoidea* (Manjari et al. 2017) mediated silver nanocatalysts in the presence of $NaBH_4$ degraded almost 80 and 99% of Congo red. Degradation in the absence of nanocatalyst is very slow due to the inefficient transfer of electron from the reductant to the dye which was usually done by the catalyst (Kolya et al. 2015).

Catalytic reduction of Allura red and Congo red, Eosin Y, Green Pls, Bromo phenyl blue under visible light was done using $NaBH_4$ in presence of silver nanoparticles synthesized from *Camellia japonica* (Karthik et al. 2017), *Dichrostachys cinerea* (Paau et al. 2010), and *Cirsium japonicum* (Khan et al. 2016a, b) extracts, respectively. $NaBH_4$ or sunlight alone is unable to bring about effective dye degradation. The presence of silver nanoparticles caused enrichment of the rate of reaction due to its electron relay effect for borohydride ions (Bhuyan et al. 2017).

8.6.3 Degradation of Nitro Compounds

Plant-based metal nanoparticles are effective for the degradation of nitro compounds. Characteristic absorption peaks of *o*-nitroanilines are at 283 and 412 nm. In the presence of sodium borohydride, degradation was very slow. Upon the addition of *Indigofera tinctoria* mediated nanocatalyst in presence of the reducing agent, *o*-nitroaniline was reduced to 1, 2 benzenediamine. Intensity of the characteristic peak of *p*-nitroaniline at 380 nm decreased and a new peak was formed at 240 nm which indicates the complete conversion of *p*-nitroaniline to phenelenediamine (Sunkar et al. 2013). The nanocatalyst took part in the electron relay (Naraginti and Li 2017). *Elephantopus scaber* mediated silver nanoparticles showed effective reduction of 4-nitrophenol, *o*-nitroaniline, *p*-nitroaniline by sodium borohydride. The aqueous solution of 4-nitrophenol is pale yellow in color and its UV–vis (Francis et al. 2018). Absorption spectrum showed peaks at 317 and 227 nm respectively due to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. Majority of organic pollutants like nitrophenol and their respective derivatives are chiefly formed during the production of pesticides, insecticides, herbicides, and synthetic dyes in their industries (Gangula et al. 2011).

In the presence of $NaBH_4$ as reducing agent, the color of 4-nitrophenol solution instantly changed to greenish yellow and the absorption maximum at 317 nm was red shifted to 400 nm due to the formation of 4-nitrophenolate ions in the alkaline condition caused by the addition of $NaBH_4$ (Kundu et al. 2004). In the presence of catalyst, peak at 400 nm vanished along with the decoloration of bright yellow color of 4-nitrophenolate ions and a new peak at 298 nm emerged due to the formation of 4-aminophenol (Joseph and Mathew 2015). The presence of the kinetic barrier due to large potential difference between donor and acceptor molecules decreased the feasibility of this reaction (Naraginti and Li 2017). The metal nanoparticles catalyzed this reaction by facilitating electron relay from the donor BH_4^- to acceptor 4-nitrophenol to overcome the kinetic barrier. Both BH_4^- and 4-nitrophenolate ions are adsorbed on the catalytic surface (Ajitha et al. 2016). Joseph and Mathew (2015) capped silver nanoparticles was effective catalyst for the reduction of 4-nitrophenol. The concentration of $NaBH_4$ stayed practically constant throughout the course of the reaction. The *Hyphaene thebaica*, 61 *Dillenia indica* (Mohanty and Jena 2017), and *Actinidia deliciosa* (Naraginti and Li 2017) mediated silver nanoparticles could facilitate electron transfer from BH_4^- ion to the 4-nitrophenol, leading to 4-aminophenol formation. Fast electron transfer occurred in presence of catalyst, which gave rise to fast reaction process (Mohanty and Jena 2017).

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Chapter 9
Bioremoval of Fluoride: Process and Mechanism

Shubha Dwivedi, Naveen Dwivedi, and C. B. Majumder

Contents

Abstract Fluoride is one of the major pollutants found in all forms of water in some concentration. The concentration of fluoride below 1 mg/L has valuable effect on the tooth and bone health. But excessive fluoride results in the development of life menacing effects on over all kind of lives in the form of several types of fluorosis. Thus, there is a pressing need for the removal of the fluoride from industrial wastewater, surface water, and groundwater. The population of rural India depends

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on groundwater resources which are highly contaminated with fluoride through leaching. Among all the techniques used, the bioadsorption/bioremoval is found to be the most developing technique. Over the past few years, the use of biological adsorbents primed by fruit peels, different parts of trees such as leaf, bark, and the microbial cell mass has remained of great interest. Removal of fluoride by microorganism involves two basic mechanisms, i.e., bioadsorption and bioaccumulation. This chapter gives an insight on the statistics of fluorosis, diverse methods adopted by the various researchers in the field of fluoride removal from water and mechanism of removal of fluoride.

Keywords Fluoride · Adsorption · Fluorosis · Bioremoval · Bioadsorption · Bioaccumulation · SABA

9.1 Introduction

Contamination of groundwater by fluoride has become a global crisis in the twenty-first century. Large number of people in countries like India, Sri Lanka, China, Rift Valley countries in East Africa, Turkey, parts of South Africa, Iran, Jordan, Pakistan, Thailand, and Japan are affected by various types of health disorders due to the consumption of fluoride contaminated groundwater for drinking purpose. The maximum permissible limit of fluoride in drinking water has been set as 1.5 mg/L by many regulatory authorities like WHO, US EPA, CPCB, etc.

Fluoride enters into groundwater due to dissolution from minerals/rocks like topaz, fluorite, fluorapatite, cryolite, phosphorite, theorapatite, etc. present at the aquifer bottom (Murray 1973). It enters the soil through weathering of rocks, precipitation or waste runoff. Further, a number of industrial processes such as coal combustion, steel production, and other manufacturing processes (aluminum, copper, and nickel production, phosphate ore processing, phosphate fertilizer production, glass, brick and ceramic manufacturing), etc. also contribute to upgrade fluoride levels in water.

Various physicochemical processes such as adsorption, ion exchange, electrodialysis, coagulation/precipitation, reverse osmosis, nanofiltration, ultrafiltration, etc. (Tor et al. 2009; Popat et al. 1994; Meenakshi and Viswanathan 2007; Haron and Yunus 2001; Sundaram et al. 2008; Chubar et al. 2005; Kabay et al. 2008; Sourirajan and Matsurra 1972; Simons 1993) have been used for the removal of fluoride from water. All these processes have their inherent advantages and limitations such as less efficiency, sensitive operating conditions, production of secondary sludge in application. Among the above processes, adsorption is a simple and attractive method for the removal of metal from the effluents due to its high efficiency, easy handling, and economic feasibility. Further, agro-based adsorbents are getting more attention nowadays due to their abundant availability and low cost. Some literature are available on the removal of fluoride from water using various agro-based adsorbents like rice husk ash, neem leaf, peepal leaf, khair leaf, tamarind fruit shell, etc. (Mondal et al. 2012; Jamode et al. 2004; Sivasankar et al. 2010). In most of these literature the concentration of fluoride is in between 1.5 and 5 mg/L, normally available in groundwater. However, industrial wastewater normally contains higher fluoride concentration (World Bank Group 1998). Fluoride acts as double-edged weapon, uptake of it up to 1 mg/L has beneficial effects on to the plants, animals, and human beings. In humans, fluoride exhibits the bone formation, remineralization, and protection from demineralization of teeth. Fluoride is extremely effective in protecting cavities and making teeth stronger. However, it is much less effective if cavities have already formed. According to the National Health Service, the limited amount of fluoride acts as a nutrient and prevents tooth decay by changing enamel structure resulting in remineralization and inhibits bacterial action. Fluoride uptake in limited levels has variable effects on the different species of animals. In cows, the food supplemented with fluoride results in increase in milk production.

9.2 Severity of the Issues Related with Fluoride

68.84% population of India resides in rural areas and is facing several problems, viz. food, shelter, drinking water, education, transportation, etc. Groundwater contamination is one of the most important problems facing by rural India's population. The groundwater comes from number of sources, such as rainfall and snow. Groundwater is used for the domestic and industrial purposes. There is a large amount of water present on the earth even then it is very precious because the whole amount of water reserves are divided into two parts, marine water which covers the 97.4% of total water reserves and fresh water which covers only 2.6%. Our all basic needs like drinking, irrigation, transportation, washing, etc. depend on our freshwater reserves. But over the past few years, there seems to be a reduction in water quality because of urbanization, ever growing population, and unskilled utilization of water resources. India is known as the country of villages. A great part of the human population is residing in villages. Villagers fulfill their need like drinking, washing, etc. by using groundwater resources. But since the last few decades, these groundwater resources have been polluted by various natural and anthropogenic contaminants like heavy metals, fluoride, arsenic, lead, mercury, etc. Among these, fluoride contamination of groundwater has now become a major issue in most of the parts of the world because of its toxic effects. The striking fact about fluoride is that it affects the majority of children who are living in the contaminated areas. India is among 24 nations which are suffering from fluoride problem.

9.3 Statistics of Fluorosis in India

In India, around 20 million people were severely affected by fluorosis and around 40 million are exposed to its risk. The number of people getting affected, the number of villages, blocks, districts, and states endemic for fluorosis have been steadily increasing ever since the disease was discovered in India during the 1930s. About 62 million people in India suffer from dental, skeletal, and non-skeletal fluorosis. Out of these, six million are children below the age of 14 years (Susheela 2001). Fluorosis is an endemic disease prevalent in 20 states out of the 36 states and Union Territories of the Indian Republic. In India, 40–70% districts are affected in Bihar, National Capital Territory of Delhi, Haryana, Jharkhand, Karnataka, Maharashtra, Madhya Pradesh, Odisha, Tamil Nadu, and Uttar Pradesh (NPPCF 2018). According to the data available as on 1 January, 2019, on the official site of government of India, there are nearly more than 15 districts are affected with the fluoride as number of habitations depicted in pie chart. The fluoride levels of the drinking water in Uttar Pradesh range from 0.2 to 25.0 mg/L, the maximum level of which is well above the normal range of 1.5 mg/L as stated by World Health Organization. The apparent variation in the prevalence of dental fluorosis can be attributed to the source of drinking water. In most parts of India, groundwater is found to be the major source of drinking water. Fluoride level depends primarily on the groundwater level; the deeper the underground level from where the water is derived, the higher the fluoride level is. The ministry of health & family welfare has identified at least 132 districts in 19 states severely affected by high fluoride content in drinking water, a leading cause of fluorosis. Uttar Pradesh has 75 districts, Madhya Pradesh 51, Bihar 38, and Rajasthan 33 districts whose people are at a very high risk of fluorosis (Press release updated on 17 Jul 2017, on Live Mint). The union health ministry, under its National Programme for Prevention and Control of Fluorosis (NPPCF), in association with the ministry of drinking water and sanitation, has started a baseline survey to assess the actual burden of the disease in identified states. Meenakshi and Maheshwari (2006) reported that there were 17 states in India affected by fluoride as range of fluoride found in groundwater is given in Table 9.1.

"Fluoride prevalence was earlier reported in 230 districts of 19 states. As per present data from ministry of drinking water and sanitation, there are 14,035 habitations (as on 1 April, 2016) from 17 states which are yet to be provided with safe drinking water. The population at risk based on population in habitations with high fluoride in drinking water is 11.53 million." Table 9.2 focused on the assumed cases of dental and skeletal fluorosis and the survey done by the NPPCF. Some major statistics given in the subsequent pie charts (Figs. 9.1, 9.2, and 9.3) show the number of habitations affected by fluoride in India in consecutive years of 2016, 2017, and 2018.

Percentage analysis based on the total no of people examined in community as well as in school on the basis of data mentioned in Table 9.2 is given in Fig. 9.4.

Table 9.1 Ranges of fluoride in different states of India (Meenakshi and Maheshwari 2006)

S. no.	States	Range of fluoride concentration (mg/L)
1	Andhra Pradesh	0.11–20.0
2	Assam	0.2–18.1
3	Bihar	0.6–8.0
4	Delhi	0.4–10.0
5	Gujarat	1.58–31.0
6	Haryana	0.17–24.7
7	Jammu and Kashmir	0.05–4.21
8	Karnataka	0.2–18.0
9	Kerala	0.2–2.5
10	Maharashtra	0.11–10.2
11	Madhya Pradesh	0.08–4.2
12	Orissa	0.6–5.7
13	Punjab	0.44–6.0
14	Rajasthan	0.2–37.0
15	Tamil Nadu	1.5–5.0
16	Uttar Pradesh	0.12–8.9
17	West Bengal	1.5–13.0

9.4 Consequences of High Level of Fluoride on to Human Beings

Fluoride poisoning and the biological response leading to ill effects depend on the following factors:

- Excess concentration of fluoride in drinking water.
- Low calcium and high alkalinity in drinking water.
- Total daily intake of fluoride.
- Duration of exposure to fluoride.
- Age of the individual.

Expectant mothers and lactating mothers are the most vulnerable groups as fluoride crosses the placenta because there is no barrier and it also enters maternal milk. De-arrangement in hormonal profile either as a result of fluoride poisoning or as a cause aggravates the disease.

9.4.1 Mechanism of Fluoride Toxicity

There are several mechanisms of fluoride toxicity. Ingested fluoride initially acts locally on the intestinal mucosa. It can form hydrofluoric acid in stomach which leads to GI irritation. Fluoride also exerts effects on glucose metabolism. Results have shown that fluoride exposure may contribute to impaired glucose intolerance.

Table 9.2 Assumed cases of dental and skeletal fluorosis as reported by States under National Programme for Prevention & Control of Fluorosis (NPPCF) (National health profile, 2018) with % analysis

		Community			School		Community		
S. no.	Name of the state	Total no examined	No. of case dental fluorosis	No. of case skeletal fluorosis	Total no examined	No. of case dental cases	% Dental cases	% Skeletal cases	% of Dental cases in school
1	Karnataka	1,317,088	549,988	197,563	1,098,723	399,062	41.75	14.99	36.32
2	Rajasthan	506,552	237,560	37,783	302,431	–	46.89	7.45	–
3	Odisha	98,382	3815	1966	–	–	3.87	1.99	–
4	Maharashtra	84,218	5512	1334	393,783	4450	6.54	1.58	1.130
5	Andhra Pradesh	79,433	15,901	309	20,847	2215	20.01	0.388	10.62
6	Tamil Nadu	41,437	19,948	13,834	24,671	16,890	48.14	33.38	68.46
7	Punjab	38,631	4494	58	31,901	7007	11.63	0.15	21.96
8	Assam	36,004	5236	480	2068	811	14.54	1.33	39.21
9	West Bengal	28,638	8143	8257	22,133	13,053	28.43	28.83	58.97
10	Madhya Pradesh	20,297	7861	35	55,224	26,078	38.72	0.17	47.22
11	Kerala	15,268	1145	5	39,986	9802	7.49	0.03	24.51
12	Uttar Pradesh	12,588	2626	267	2055	1365	20.86	2.12	66.42
13	Telangana	10,772	4635	3191	4953	2692	43.02	29.62	54.35
14	Bihar	8740	5270	1606	6250	1650	60.29	18.37	26.4
15	Haryana	4413	1269	45	5476	2669	28.75	1.01	48.73
16	Gujarat	3535	1193	656	25,851	3580	33.74	18.55	13.846
17	Jharkhand	3754	401	281	8416	852	10.68	7.48	10.12
18	Chhattisgarh	2879	2540	252	482	482	88.22	8.75	100
	Grand total	*2,312,629*	*877,537*	*267,922*	*2,045,250*	*492,658*	*37.94*	*11.58*	*24.08*

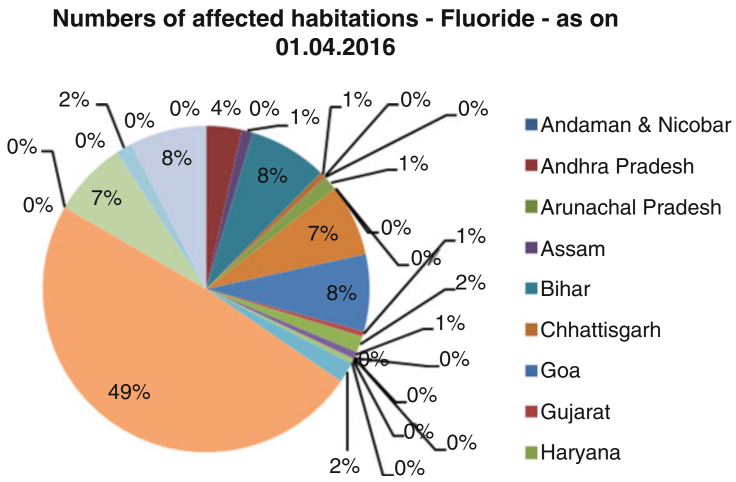

Fig. 9.1 Pie chart shows the no of affected habitations in India by fluoride as on first April, 2016 (Ministry of Drinking Water & Sanitation 2016)

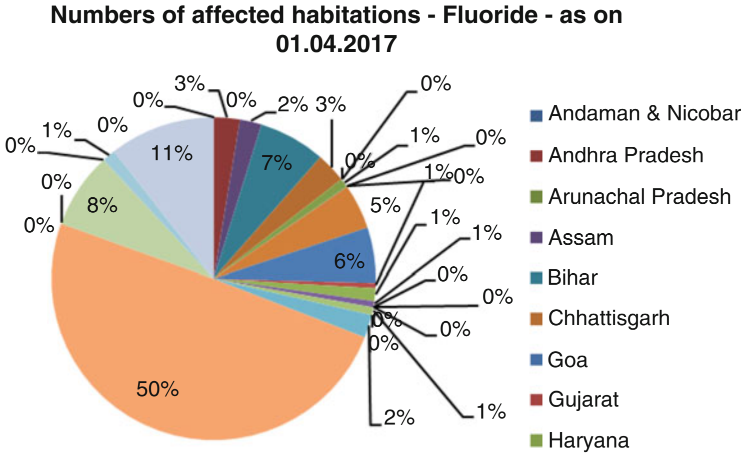

Fig. 9.2 Pie chart shows the no of affected habitations in India by fluoride as on first April, 2017 (Ministry of Drinking Water & Sanitation 2016)

Fluoride inhibits Na^+/K^+-ATPase, which may lead to hyperkalemia by extracellular release of potassium. Fluorides influence the secretary pathway which leads to the dental fluorosis.

Several other cellular processes are also affected by the molecular mechanism of inorganic fluoride. Fluoride can induce oxidative stress, homeostasis, and lipid peroxidation as well as also alters gene expression and causes cell death.

Redox status: Fluoride alters mitochondria rich cells such as those of the human kidney.

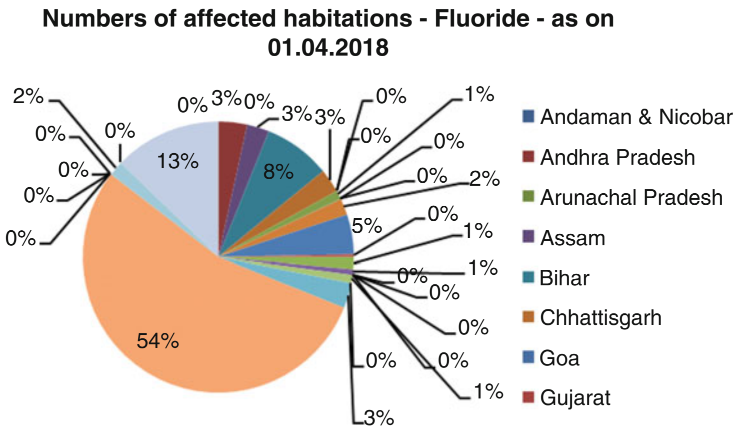

Fig. 9.3 Pie chart shows the no of affected habitations in India by fluoride as on first April, 2018 (Ministry of Drinking Water & Sanitation 2016)

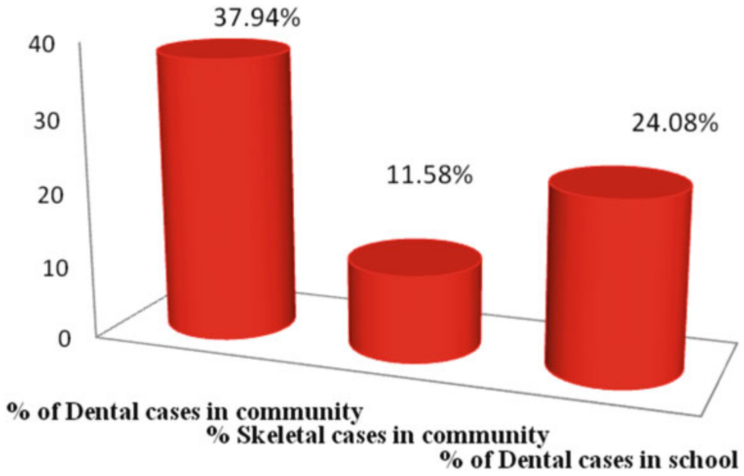

Fig. 9.4 Assumed cases of dental and skeletal fluorosis (% analysis) as reported by NPPCF (National health profile, 2018)

Cellular Respiration: Fluoride inhibits cellular respiration. Fluoride ions bind to the functional amino acid groups surrounding the active center of an enzyme as in case of enzyme of glycolytic pathways and Krebs cycle which is sensitive to fluoride.

Generation of ROS: Fluoride exposure increases the generation of anion superoxide (O_2^-), increased O_2^- concentration, and downstream consequences such as hydrogen peroxide. Fluoride also increases NO generation. Fluoride inhibits the antioxidant activity.

Apoptosis: Fluoride induces the apoptosis by elevating the oxidative stress induced lipid peroxidation, thus causing mitochondrial dysfunction and activation of downward pathways observed as primary mechanism of cell death in presence of relatively high fluoride concentrations. Fluoride acts as enzyme inhibitor but fluoride ions occasionally stimulate enzyme activity (Barbier et al. 2010).

9.4.2 Deficiency by Excess of Fluoride in Human Being

As the fluoride is highly electronegative in nature, it possesses strong affinity for the positively charged ions. The fluoride present in the drinking water firstly brings alternations in the tooth as the fluoride replaces tooth enamel hydroxyl group with fluoride resulting in formation of more stable compound said to be fluorapatite and get deposited as calcium-fluorapatite crystals. Various types of fluorosis occur due to excess of fluoride in the body as summarized in Table 9.3 (Dwivedi et al. 2017).

Dental Fluorosis: Disease is generally seen in the children on age group of 5–8 years due to excessive fluoride intake, enamel losses its luster, and fluorosis characterized by white, opaque areas on the tooth surface and in several forms as shown in Fig. 9.5. This sometimes results in the development of yellow brown to black stains and severe pitting of the teeth. Dental fluorosis is caused by continuous exposure to high concentrations of fluoride during tooth development, leading to enamel with low mineral content and increased porosity. The critical period for risk to dental fluorosis is between 1 and 4 years of age. After the age of 8 when permanent teeth have established, there is lesser risk to dental fluorosis.

Skeletal Fluorosis: Excessive exposure to fluoride can cause a debilitating bone disease known as skeletal fluorosis as shown in Fig. 9.6. The skeletal fluorosis occurs in all age group people, i.e., in children as well as adults. Fluoride accumulates in the joints of neck, knee, pelvic, and shoulder bones resulting in difficulty in movement or walking. The symptoms observed during this disease in very similar to

Table 9.3 Stages of fluorosis in human being

Types of fluorosis	Responsible range of fluoride in mg/L (Meenakshi and Maheshwari 2006)	Age	Symptoms
Dental fluorosis	1.0–3.0	Children (5–7 years)	White opaque area on the tooth surface
Skeletal fluorosis	3.0–4.0	Children (5–7 years) Adults (<18 years)	Accumulation of fluoride in the joints of neck, knee, pelvic, and shoulder bones
Crippling fluorosis	4.0–6.0	Children (5–7 years) Adults (<18 years)	Arthritis, sclerosis, Muscle fiber degeneration, low hemoglobin level

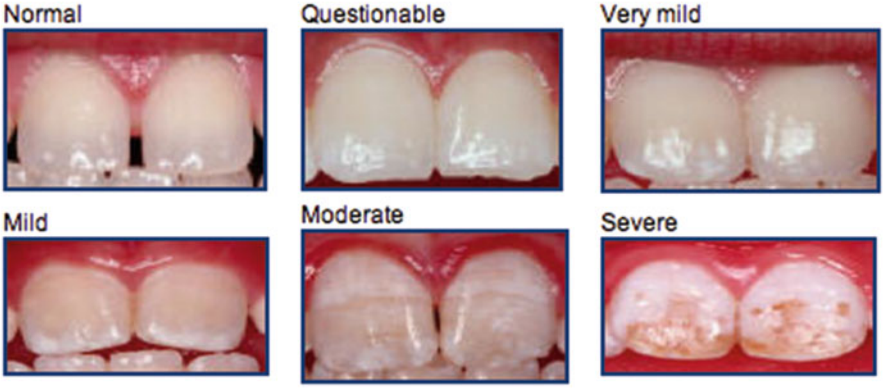

Fig. 9.5 Stages of dental fluorosis

the symptoms seen in arthritis or spondylitis which are calcium deposits in bones, muscle weakness, etc. In the initial stages, known as "pre-skeletal" fluorosis, a patient may suffer a variety of symptoms in the absence of any detectable bone charges, including joint pain, joint stiffness, and gastric distress. The absence of bone charge makes this pre-skeletal phase difficult to diagnose, because the symptoms are indistinguishable from common forms of arthritis such as osteoarthritis and rheumatoid arthritis. Skeletal fluorosis is developed by the disturbance of calcium metabolism in the formation of bones of the body. It results in softening and weakening of bones resulting in deformities leading to crippling. It can also aggravate calcium related disorders such as rickets in children and osteoporosis mainly in adults. For people who are exposed to high fluoride levels for decades, severe cases of crippling can occur.

Most common symptoms to identify skeletal fluorosis are as

- Severe pain and stiffness in neck, backbone (lumbar region), shoulder, knee, and hip region. Pain may commence either in 1 or 2 or more joints. Patient has restricted mobility of cervical and/or lumbar spine and has to turn the whole body towards that side to see.
- Knock knee/Bow leg (in children, adolescents).
- Inability to squat (advanced stage of skeletal fluorosis).
- Ugly gait and posture (advanced stage of skeletal fluorosis.

Non-Skeletal Fluorosis: Any case with a history of residing in an endemic area along with one or more of the following health complaints. It is important to identify non-skeletal fluorosis as it is the earliest sign of fluoride toxicity.

It includes symptoms like gastro-intestinal problems, viz. consistent abdominal pain, intermittent diarrhea/constipation, bloated feeling, nausea, loss of appetite.

Neurological manifestations like nervousness and depression, tingling sensation in fingers and toes, excessive thirst, and tendency to urinate frequently (polydipsia and polyuria) have also been seen in affected persons.

Fig. 9.6 Stages of skeletal fluorosis

Muscular manifestations like muscle weakness and stiffness, pain in the muscle and loss of muscle power, unable to walk or work have also been seen in affected persons.

9.4.2.1 Crippling Fluorosis

Crippling fluorosis is the advanced stage of fluorosis resulting in osteoporosis and bony outgrowths can also occur as shown in Fig. 9.7. Vertebrae may fuse together and eventually the victim may be crippled. It may even lead to a rare bone cancer—

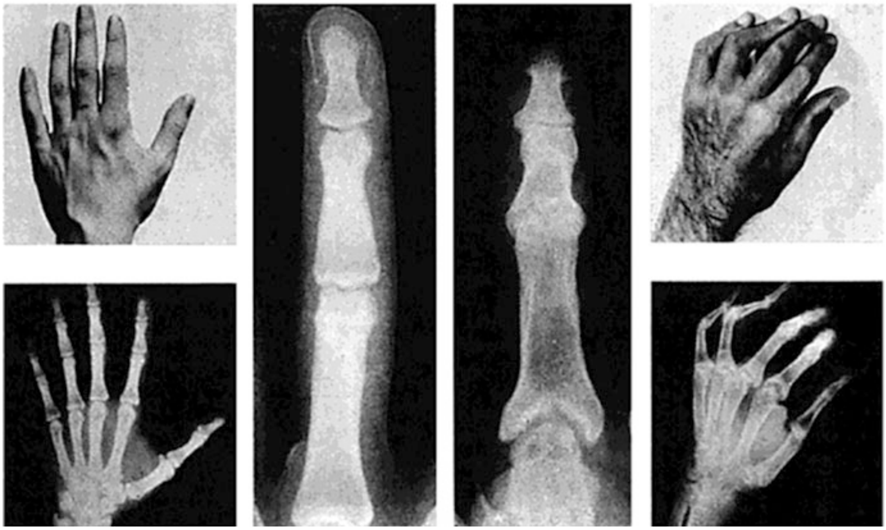

Fig. 9.7 Stages of crippling fluorosis

osteosarcoma and finally spine, major joints, muscles, and nervous system get damaged.

9.4.2.2 Other Problems

Besides dental, skeletal, and crippling fluorosis, excessive fluoride can result in muscle fiber degeneration, deformities in RBCs, low hemoglobin levels, excessive thirst, headache, skin rashes, nervousness, neurological man, etc. A brief of other effects of fluoride on microbes, aquatic microorganisms, flora and fauna is summarized in Table 9.4.

9.5 Properties of Fluoride and Its Natural Distribution

Fluoride belongs to halogen family, the most electronegative element which is the ionic form of fluorine and does not occur in the elemental state in nature because of its high reactivity. In the elemental form, fluorine is a flammable, irritating, and is the most powerful oxidizing agent known. Fluoride occurs in two forms in nature, viz. inorganic fluoride and organic fluoride. There are a number of sources from where fluoride is generally generated and added to the environment. The sources of fluoride are characterized into two categories: natural sources which include the fluorine already present in nature and distributed in Earth's crust, mainly as the minerals, fluorspar, fluorapatite, and cryolite, and anthropogenic sources which include

Table 9.4 Effect of fluoride on microbes, aquatic microorganisms, flora and fauna

References S. no	Effect of Fluoride on microbes (Herrera et al. 2009)	Effect of Fluoride on flora (Fornasiero 2001)	Effect of fluoride on fauna (Suttie 1964)	Effect of Fluoride on aquatic organisms (Camargo 2003)
1	Fluoride has low inhibitory effect on the nitrifying bacteria in activated sludge process	The effect of fluoride on plants depends upon a number of factors such as the concentration, time of exposure, type and age of plant, temperature, type of light and intensity, composition of the air, and its rate of circulation, concentrations of the gas	Young animals are generally more susceptible to harmful or toxic substances than older ones	Some of the algae have high negative effect at high level of fluoride. Rate of respiration of *Chlorella pyrenoidosa* was reduced by 50% with 570 mg/L concentration of fluoride. 25–27% growth inhibition was seen in *Amphidinium carteri* and in *Dunaliella tertiolecta*
2	Fluoride presents inhibitory effects towards glucose fermenting microorganisms	Vital plant processes such as respiration and photosynthesis may be influenced	Acute intoxication develops local, affecting the gastro-intestinal tract (thirst, vomiting, abdominal pain, diarrhea)	Fluoride tends to accumulate in the exoskeleton and bone tissues of fishes. Toxicity of fluoride to aquatic invertebrates increases with the increasing level of fluoride
3	Anaerobic microorganisms that utilize propionate and butyrate were found to be very sensitive to fluoride. Fluoride is the inhibitor of pyrophosphatase in various microorganisms	Varying degrees of injury to leaf tissues may occur until the entire leaf is affected and it falls from the plant	Death is attributed primarily to respiratory paralysis	It exerts poisonous effect on the health of aquatic animals by inhibiting the enzyme activity and finally interrupting metabolic process

industrial activities. Other sources include glassworks, exhaust fumes, and the production of metals (e.g., steel, copper, and nickel), bricks, ceramics, and adhesives. Generally fluoride found in groundwater is naturally occurring from the breakdown of rocks, soils or weathering and deposition of atmospheric volcanic particles. Fluoride found in both the forms inorganic and organic. Organic fluoride is

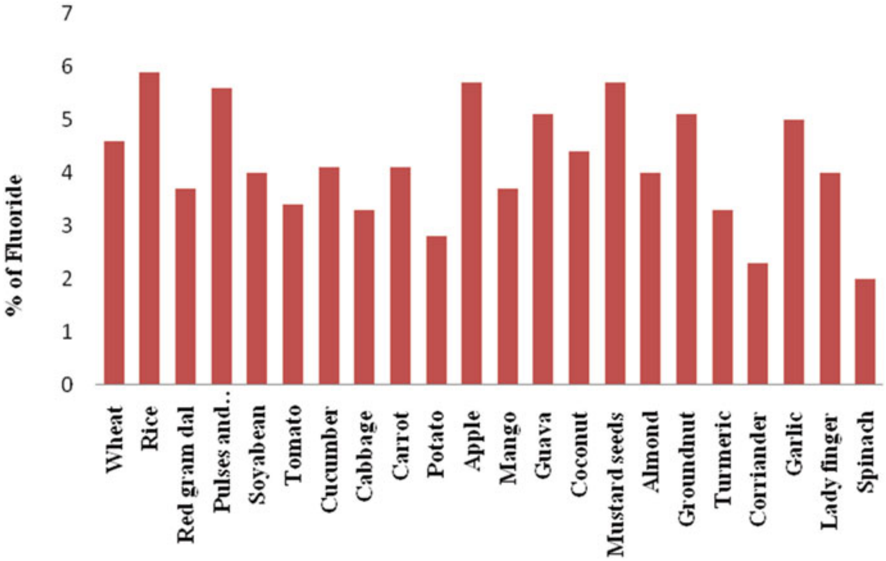

Fig. 9.8 Fluoride concentration in mg/kg in different food stuffs

Table 9.5 Fluoride concentration in different food stuffs

Food item	Fluoride concentration (mg/kg)
Beef	4.0–5.0
Pork	3.0–4.5
Mutton	3.0–3.5
Fishes	1.0–6.5
Tobacco	3.2–38
Beetle leaf (pan)	7.8–12.0
Rock salts	200.0–250.0
Areca but (supari)	3.8–12.0
Tea	60–112

present in vegetables, fruits, and nuts as % fluoride concentration shown in Fig. 9.8 and in Table 9.5.

Fluoride does not exhibit any color, taste, or smell when dissolved in water. Hence, it is not easy to determine it through physical examination. Only chemical analysis of the water samples can determine the concentration of this ion. The widely used method for the estimation of fluoride in groundwater sample is colorimetric SPANDNS (sodium 2-(parasulfophenylazo)-L, 8-dihydroxy-3,6-naphthalene disulfonate) method. Ion selective electrodes are available to measure fluoride concentration in water, which can be used both in the field and in laboratory. Fluoride is one of the important micronutrients in humans which is required for strong teeth and bones. In humans, about 95% of the total body fluoride is found in bones and teeth.

9.6 Need of Hour

Due to the detrimental and injurious effects of excess fluoride, there is an urgent prerequisite of methods for its removal from drinking as well as forms of groundwater. The methods used for fluoride removal are distillation, membrane separation processes, precipitation, ion exchange, and adsorption. In distillation water is purified by boiling it. Ion exchange uses the resins. Precipitation involves chemical results in precipitate formation and is separated. The membrane filtration supports the use of membrane for separation such as reverse osmosis and electrodialysis. The adsorption method complies with the use of physical or the biological adsorbents. The process removes toxic chemicals such as fluorides, pesticides, phenols, cyanides, and organic dyes that cannot be treated by conventional treatment methods. Most commonly used adsorbent for treatment is activated carbon. Activated carbons are more effective in the removal of heavy metals and fluorides due to some specific characteristics that enhance the use of activated carbon for the removal of contaminants including heavy metals from water supplies and wastewater. Defluoridation of water by using various other adsorbents and bioadsorbents also reported in this paper. Use of bioadsorbents and microorganisms is the cheapest and most reliable among all methods. Bioadsorbent defined as naturally occurring biomass or spent biomass or the processing of waste for efficient usage. The use of agro-based adsorbents (rice husk ash, neem leaf, peepal leaf, khair leaf, tamarind fruit shell, etc.) and biological microorganisms (bacteria, fungi, etc.) comes under the category of bioadsorbent. The use of biosorbents came to existence due to their low cost and high removal efficiencies with no pollutant generation.

9.7 Process and Mechanism

Mainly three types of treatment process are found which are described below. These methods include physical treatment, chemical treatment, and biological treatment.

9.7.1 Physical Treatment

The physical methods used for the removal of fluoride from drinking water are divided in the following categories:

9.7.1.1 Membrane Filtration Process

Filtration is the most relied water treatment process to remove particulate material from water. Coagulation, flocculation, and settling are used to assist the filtration process to function more effectively. The coagulation and settling processes have become so effective that sometimes filtration may not be necessary. However, where filtration has been avoided, severe losses in water main carrying capacity have occurred as the result of slime formation in the mains. Filtration is still essential.

These processes involve the use of permeable membrane for the removal process of number of contaminants. Reverse osmosis (Sourirajan and Matsurra 1972), ultrafiltration (Guo et al. 2001) nanofiltration, and electrodialysis are membrane filtration processes which can be used for removal of fluoride. This process occurs against concentration gradient. This method is highly effective for fluoride removal. No chemicals are required and at wide pH range. But the process is very expensive in nature.

Advantages

- No need of chemicals (coagulants, flocculants, disinfectants, pH adjustment).
- Constant quality of treated water in terms of chemicals and microbial removal.
- Simple automation.
- Process and plant compactness.
- Remove nearly all contaminant ions and most dissolved non-ions.
- An activated carbon filter to trap organic chemicals and chlorine, which will attack and degrade thin film composite membrane reverse osmosis membrane.

Limitations

- Household reverse osmosis units use a lot of water because they have low back pressure.
- The remainder is discharged as wastewater.
- Large-scale industrial/municipal systems recover typically 75–80% of the feed water, or as high as 90%.
- Approximately 2.4 L of water containing the fluoride and other chemicals and minerals are wasted for every 1 L of purified water produced.

9.7.1.2 Adsorption/Ion Exchange Method

In the adsorption method, influent in the form of water is passed through a bed containing defluoridating material. The material retains fluoride either by physical, chemical, or ion exchange mechanisms. The adsorbent gets saturated after a period of operation and requires regeneration. Adsorbents used for removal of various contaminants include activated alumina, granular activated carbon, bone charcoal, and synthetic ion exchange resins (Wasewar et al. 2010). This process involves the passage of water through a contact bed where fluoride is removed by ion exchange or surface chemical reaction with the solid bed matrix. After operation, a saturated column must be refilled or regenerated (Feenstra et al. 2007). Recently, amorphous alumina supported on carbon nanotubes, aligned carbon nanotubes, selective ion exchanger and an ion exchanger polymeric fiber, based on a double hydrous oxide of

Al and Fe ($Fe_2O_3Al_2O_{3x}H_2O$), acid type ion exchanger have been assayed for removal of fluoride from drinking water as well as industrial wastewater (Alagumuthu et al. 2010 and Popat et al. 1994).

Advantages

- Selective cations exchange may provide economically mean of removing mixed heavy metals from contaminated water.
- Removal of up to 98% contaminants.
- No wasted water.
- Electricity is not required.
- Flow rate is fast.
- No need for a storage tank.

Limitations

- Capacity of these methods is lessened when used on water supplies with a high mineral content so it does not remove bacteria, viruses or parasites.
- If resin is not regenerated at proper intervals, contaminants can return to the water.

9.7.1.3 Distillation

Distillation units can also be used for treating the drinking water. Distillation is a technique to separate binary or multivalent liquid mixture by the virtue of difference in their relative volatility. During this process, water is heated to its boiling point and being separated in the vapor form. At last the vapor is condensed and is ready for use. There are two types of distillation apparatus used in industries:

- Tray tower.
- Packed tower.

Advantages

- Distilled water is free of dissolved solids.
- It is short time process of purification of water.
- Distilled water is active absorber and when it comes in contact with air, it absorbs carbon dioxide, making it acidic.

Limitations

- It is expensive.
- It is energy intensive.
- Draws out chemicals and metal contaminants from whatever container it's stored in.
- Leaches minerals from your body which can lead to health problems.

9.7.2 Chemical Treatment

There are many processes available to remove fluoride from contaminated water, i.e., these methods involve the addition of soluble chemicals to the water. Fluoride is removed either by precipitation, co-precipitation or by Donnan dialysis (Hichour et al. 2000). Chemicals include lime used alone or with magnesium or aluminum salts along with coagulant aids. Treatment with lime and magnesium makes the water unsuitable for drinking because of the high pH after treatment. The use of alum and a small amount of lime has been extensively studied for defluoridation of drinking water. The method is popularly known as the *Nalgonda technique*, named after the town in India where it was first used at waterworks level. It involves adding lime (5% of alum), bleaching powder (optional), and alum ($Al_2(SO_4)_3{\cdot}18H_2O$) in sequence to the water, followed by coagulation, sedimentation, and filtration. A much larger dose of alum is required for fluoride removal (150 mg/mg F^-), compared with the doses used in routine water treatment.

9.7.2.1 Precipitation

Precipitation processes involve addition of chemicals and formation of fluoride precipitates. Among these are precipitations with calcium and aluminum salts. Precipitation chemicals must be added daily in batches and precipitation techniques produce a certain amount of sludge every day (Feenstra et al. 2007).

Advantages

- It is simple to do.
- Cost-effective.
- Does not need great expertise but some skill and practice are required.
- Instruments are easily available.
- It is oldest one and used commonly.

Limitations

- Need skill and practice for effective results.
- Instruments have to be properly calibrated because it will affect the final results
- Reactivity of the elements to be titrated should be well researched since this may affect the end point.
- Time consuming if done manually.

9.7.2.2 Contact Precipitation

Contact precipitation is a recently reported technique in which fluoride is removed from water through the addition of calcium and phosphate compounds. The presence of a saturated bone charcoal medium acts as a catalyst for the precipitation of fluoride either as CaF_2, and/or fluorapatite. Tests at community level in Tanzania have shown promising results of high efficiency. Reliability, good water quality, and low cost are reported advantages of this method.

9.7.3 Biological Treatment

Biological removal and biosorption is an emerging technique for water treatment utilizing abundantly available biomaterials. Various biosorbents have been developed for fluoride removal. Among various biosorbents, chitin and chitosan-derivatives, neem bark, neem leaves, peepal leaves, etc. (Sundaram et al. 2008; Maheshwari and Gupta 2015; Jamode et al. 2004) have gained wide attention as effective biosorbents due to their low cost and high contents of amino and hydroxyl functional groups which show significant adsorption potential for the removal of various aquatic pollutants. The applicability of chitin and chitosan as adsorbents for the removal of excess fluoride from drinking water was evaluated (Rangel-Mendez et al. 2010).

The biosorption can be also classified according to the location where the metal removed from solution is found:

1. Extracellular accumulation/precipitation.
2. Cell surface sorption/precipitation.
3. Intracellular accumulation.

9.7.3.1 Treatment Using Microorganisms

Conventional and less effective physicochemical methods are being replaced by the more effective biological methods for example, bioreduction for the fluoride contamination in soil and groundwater by bacteria *Shewanella putrefaciens* etc., supports the use of eco-friendly ways for the bioremediation (Chubar et al. 2008). These bacteria were found very effective in bioremediation of heavy metals and iron. In present scenario, bioremediation/bio-treatment/bioremoval of contaminants from wastewater has also proved to be a fresh technology for wastewater treatment (Mandal et al. 2010).

9.7.3.2 Treatment Using Bioadsorbent

New approaches are discovered to minimize or even eliminate the defects and disadvantages of the water purification techniques. Bioadsorption is one of the significant techniques in which fluoride adsorbed onto a membrane, or a fixed bed packed with bed or other mineral particles. Some low cost and natural materials such as zirconium impregnated coconut shell carbon, red mud, ground nut shell carbon, cashew nut shell carbon, mosambi peels, orange peels, bark of babool, tamarind shell, peepal leaf powder, pine apple peel, and clays have been used as adsorbents for removal of fluoride from drinking water. In recent years, considerable attention has been focused on the study of fluoride removal using natural, synthetic, and biomass materials such as activated alumina, fly ash, chitosan beads, zeolite, calcite, red mud, alum sludge, hydrated cement, and acid-treated spent bleaching earth (Gandhi et al. 2012).

Bioadsorbent is defined as the adsorbent prepared using the biological substances such as different parts of the trees. For example, leaf, bark, fruit peelings, husk like rice husk, etc. (Jamode et al. 2004; Naiya et al. 2009). Bioadsorbent could be used as a high potential and biodegradable bioadsorbent to remove anionic compounds such as reactive dyes from textile industry wastewater. High adsorption capacity, biodegradability, biocompatibility, and non-toxicity are among the unique properties of adsorbents. A brief review has been provided in the following sections.

9.8 Reviews on the Basis of Different Adsorbent/ Bioadsorbent Available for the Removal of Fluoride

Application of adsorption process with commercial available adsorbents as well as bioadsorbents is gaining momentum in recent years for the removal of fluoride from water. Hence, literature survey has been carried out on the removal of fluoride from wastewater with conventional and natural bioadsorbents as discussed below:

Ramanaiah et al. (2007) worked on the removal of fluoride from aqueous phase using the waste fungus (*Pleurotus ostreatus* 1804) biosorbent obtained from laccase fermentation process. Batch sorption studies were performed and followed pseudo-first-order rate equation. The adsorptive data fitted isothermal data fitted well with the Langmuir isotherm adsorption model. Fluoride removal aqueous phase pH and the fluoride uptake were observed to be greater at lower pH. The fluoride sorption phenomena on fungal biosorbent might be attributed to the chemical type of interaction. These biosorbents used under batch studies were performed to evaluate their efficiency for real field application using eight water samples collected from the fluorosis-affected area.

Mohan et al. (2007) reported that non-viable algal *Spirogyra* IO1 was studied for its fluoride sorption potential in batch studies. The results demonstrated the ability of the biosorbent for fluoride removal. The sorption interaction of fluoride on to non-viable algal species obeyed the pseudo-first-order rate equation. The intra-particle diffusion of fluoride molecules within the *Spirogyra* was identified to be the rate-limiting step. It was also found that the adsorption isotherm followed the rearranged Langmuir isotherm adsorption model. Fluoride sorption was dependent on the aqueous phase pH and the fluoride uptake was greater at lower pH.

Kumar et al. (2008) investigated the use of thermally activated carbon prepared from neem (*Azadirachta indica)* and kikar *(Acacia arabica)* leaves for the treatment of fluorinated aqueous solution. In this study, the neem leaves carbon and kikar leaves carbon were subjected to heating at 400 °C in electric furnace. The adsorbents of 0.3 and 1.0 mm sizes of neem and kikar leaves carbon were prepared by standard sieve. The process was carried under batch mode. The effect of pH, adsorbent dose, and contact time on adsorption efficiency was also studied. The optimum pH was found to be 6 for both and the optimum dose was found to be 0.5 g/100 mL for ANC (activated neem leaves carbon) and 0.7 g/100 mL for AKC (activated kikar leaves carbon). The optimum time was found to be 1 h for both the adsorbents. The adsorption process followed Freundlich adsorption isotherm. All optimized conditions were used for four natural water samples.

Deshmukh et al. (2009) reported that the batch adsorption studies were undertaken to assess the suitability of inexpensive adsorbent prepared from agricultural waste, rice husk. The adsorbent was prepared by chemical impregnation method followed by physical activation. Static studies have aimed for investigation of fluoride removal efficiency under the varying conditions of the major parameters of adsorption, viz. pH, dose of adsorbent, rate of stirring, contact time, and initial adsorbent concentration, and optimized by batch procedure in the mixture of known concentration of fluoride solution. The optimum sorbent dose was found to be 10 g/L by varying the dose of adsorbent from 0 to 16 g/L; equilibrium was achieved in 120 min for the optimum pH. It has been observed that the optimum adsorption takes place at lower pH by varying pH from 2, 4, 6, 8, and 10. Maximum fluoride removal was observed to be 75% at optimum conditions. Freundlich as well as Langmuir isotherms were plotted and constants of isotherms were determined.

Alagumuthu et al. (2010) studied the application of *Cynodon dactylon* based thermally activated carbon for fluoride toxicity. The batch adsorption techniques were followed at neutral pH as the functions of contact time, adsorbent dose, adsorbate concentration, temperature and the effect of co-anions. The data indicate that the prepared adsorbent surface sites are heterogeneous in nature and that fits into a heterogeneous site-binding model. The present system followed the Redlich–Peterson isotherm as well as Langmuir adsorption isotherm model. Lagergren pseudo-first-order, pseudo-second-order, intra-particle diffusion and Elovich kinetics were modeled to describe the adsorption rate of fluoride and determined as this scheme followed pseudo-second-order kinetics. The calculated enthalpy change, $\Delta H°$, and entropy change, $\Delta S°$, for the adsorption process are +8.725 kJ/mol and +0.033 J/mol K, respectively, and shows endothermic experience. Instrumental analysis of XRD, FTIR, and SEM gives the idea about the fluoride binding ability of adsorbent.

Sivasankar et al. (2010) performed the defluoridation of water using activated (ATFS) and MnO_2-coated (MTFS) tamarind fruit *(Tamarindus indica)* shell in batch as well as column manner. In the batch technique, the rate of adsorption studied with respect to pH, $[F]_o$, and sorbent dose was studied. The adsorption followed pseudo-first order for ATFS and Ritchie-second order for MTFS. The kinetics data were found to fit well with Temkin isotherm for ATFS and Langmuir for MTFS. Column experiments were carried out under a constant fluoride concentration of 2 mg/L, flow rate and different bed depths. The capacities of the breakthrough and exhaustion points increased in the bed depth for ATFS unlike MTFS and the Thomson model were applied to the column experimental results. The FTIR, SEM, and XRD techniques are used for sorption characterization. Values of q_T for ATFS and MTFS were found to be 1.032 and 0.954 at bed height 12 cm, respectively.

Abburi et al. (2012) reported that the experiment has been carried out at 23–26 °C under 7–7.5 pH in the laboratory followed by pH meter. Using Orion Fluoride meter, all the observed diminutions of concentrations of fluorides at PPM/L have been reported. This research has been carried out for the welfare of the society which is being affected by fluorides in not only drinking water but also wastewaters around the Aksum of Ethiopia. After adding different quantities of adsorbents in all stopper bottles, these bottles had been vigorously shaken off on certain timings by mechanical machinery. On time intervals, we had different diminutions of fluorides in different bottles. After adding 10 g of adsorbent in Bottle No.1, it showed the concentrations of fluorides after 24 h, from 10 to 7.6 PPM. The total quantity of fluoride inclined to 2.4 PPM on 24 hrs, time interval. The second bottle showed after 24 h time interval as the total quantity of descended fluoride concentration is 3.1 PPM. Similarly bottle No.3, bottle No.4, and bottle No. 5 showed their diminutions of fluorides at 4.1 PPM, 3.2 PPM, and 6.0 PPM, respectively, after 24 h by adsorbent of *Cordia africana*. The 50 g of adsorbent added in bottle No. 5 after vigorous shaking, and it gives more descending concentration of fluorides.

Merugu et al. (2013) investigated that fungal biosorbent prepared from *Aspergillus nidulans* was used for removal of fluoride from water. Calcium and alkali treated biomass was effective in removal of fluoride. Defluoridation was dependent on the initial pH of fluoride-containing water and decreased with increasing pH. At pH 4.0 fluoride removal capacity was found to be 29% while it was 14% at pH 8.0. Presence of chloride and sulfate did not affect fluoride removal while fluoride decreased with increased bicarbonate concentration. The kinetics of fluoride removal exhibited a rapid phase of binding for a period of 1.5 h and a slower phase of binding during the subsequent period.

Shubha Dwivedi et al. (2014) investigated the removal of fluoride by *Citrus Limetta* in batch experiment. Batch experiments were conducted to study the impacts of agitation time and initial fluoride ion concentrations. The adsorption kinetics is presented well by pseudo-second-order rate equation and the estimated equilibrium concentration falls within ~6% error limit. Freundlich isotherm gives well prediction of the equilibrium adsorption ($R^2 = 0.996$). The specific uptake increases from 0.089 to 1.35 mg/g with the increase in initial fluoride concentration from 1 to 20 mg/L. Maximum specific uptake obtained from Langmuir isotherm is found to be 1.82 mg/g. When the initial fluoride concentration is 5 mg/L, the removal efficiency of mosambi peel is 82.5%, so that the fluoride concentration at the treated water is below the permissible limit.

Shubha Dwivedi et al. (2014) investigated the removal of fluoride by *Ficus religiosa* in batch experiment. Batch experiments were conducted to study the impacts of agitation time and initial fluoride ion concentrations. The optimum pH for the removal of fluoride by the investigated adsorbents is ~7. The optimum adsorbent dose for the investigated adsorbent is 10 g/L for the removal of fluoride from water. The optimum temperature for fluoride removal is ~30 °C. Freundlich isotherm gives well prediction of the equilibrium adsorption ($R2 = 0.995$). The specific uptake increases from 0.09 to 1.48 mg/g with the increase in initial fluoride concentration from 1 to 20 mg/L. Maximum specific uptake obtained from Langmuir isotherm is found to be 2.24 mg/g. When the initial fluoride concentration is 5 mg/L, the removal efficiency of peepal leaf powder is 85.7% so that the fluoride concentration at the treated water is below the permissible limit.

Goswami et al. (2015) investigated on the use of leaf powder from neem (*Azadirachta indica*) trees for the defluoridation of water. The efficiency of the sorption of fluoride ion is affected by contact time, pH, and particle size of adsorbents. Treated leaf powder was studied at various pH and contact time with aqueous solutions containing 10 mg F^-/L. Results show that these low-cost bioadsorbent could be fruitfully used for the removal of fluoride over a wide range of concentrations. Treated bioadsorbents were observed to be efficient for the uptake of fluoride ions between 2.0 and 8.0 pH. Fluoride removal for a given bioadsorbent size increased with time attaining equilibrium within 1.5 h. The percentage of fluoride removal was found to be a function of adsorbent particle size and time at a given initial solute concentration. It increased with time, and higher initial solute concentration decreased with time. The adsorption capacity of treated biosorbents was studied by varying the particle size. With the largest particle size of 1.4 mm, the amount of fluoride ions adsorbed was found to be 50%. With smallest particle size of 600 μm for an initial fluoride ion concentration of 10 mg/L, 90% adsorption was observed. Small particle size provides more active surface area and hence such results.

Amin et al. (2015) investigated the potential of white rot fungus *Pleurotus eryngii* ATCC 90888 for the removal of fluoride in aqueous solution as a function of pH, initial fluoride concentration, biosorbent dose, temperature, and contact time. Langmuir model showed better data interpretation then Freundlich model. The monolayer biosorption capacity of *P. eryngii* biomass for fluoride ions was found to be 66.6 mg/g. Biosorption study was carried out at different varying parameters, viz. pH (2–7), initial fluoride concentration (5–25 mg/L), and contact time (60–300 min). At pH 2.0, initial fluoride concentration 5 mg/L, bioadsorbent dose 0.2 g, maximum fluoride removal, i.e., 97.03%, were achieved.

Koshle et al. (2016) investigated the potential of *Trichoderma hezardium* for the removal of fluoride. Study was carried out at different pH ranges, viz. 6.5, 7.5, 8.5, and 9.5 and initial fluoride concentration varies to 2, 4, 6, and 8 mg/L. Biosorption studies were carried out at different temperatures, viz. 30, 40, and 50 °C. Bioadsorbent dose varied as 0.4, 0.6, 0.8, and 1.6 g. Results showed maximum removal of 38% at adsorbent dose 1.6 g. In this study, adsorption of fluoride on to fungus bioadsorbent followed the Freundlich isotherm which revealed the heterogeneous nature of surface binding sites. The value of Freundlich constant K_f was found to be 1.14 which shows the greater affinity for fluoride-fungal system.

Desorption studies indicate 82% desorption achieved. A brief of above discussed reviews are summarized in Table 9.6.

9.9 Biological Pathways for Fluoride Removal

Mainly two pathways such as bioadsorption on bacterial cell surface and bioaccumulation in bacterial cells have been proposed for the removal of fluoride from water using bacterial whole cells (Chubar et al. 2008). Mechanisms of cell surface sorption are independent of cell metabolism; they are based upon physicochemical interactions between fluoride and functional groups of the cell wall. The microorganism's cell wall mainly consists of polysaccharides, lipids, and proteins, which have many binding sites for halides. This process is independent of the metabolism and metal binding is fast.

Bioaccumulation, in contrast, is an intracellular fluoride accumulation process which involves dehalogenation and it is mediated only by periplasmic and membrane fractions, not by cytoplasmic fractions of the cells (Picardal et al. 1993). Since it depends on the cell metabolism, it can be inhibited by metabolic inhibitors such as low temperature and lack of energy sources. Various processes of bioadsorption as well as bioaccumulation are shown in Fig. 9.9.

Based on the above prescribed mechanism, three recent researches have been reported here in sequential order. Removal of fluoride from wastewater based on simultaneous adsorption and bioaccumulation basically involves two types of mechanism, i.e., adsorption and accumulation. The availability of adsorbents increased the surfaces of liquid-solid phase. Microbial cells, pollutants, enzymes, and oxygen are adsorbed. Physicochemical reaction is also possible due to surface catalysis on the surface of adsorbent. Microorganisms like *Shewanella putrefaciens* and *Actinobacter* immobilized to surface of adsorbent bring extracellular biodegradation/bioaccumulation on adsorbed pollutants. SABA approach enhanced the removal efficiency of fluoride.

Mohammad and Kumar (2019) worked on the *Actinobacter* species immobilized on sweet lemon peel. *Actinobacter* is a water living microorganism which survives in wastewater. Microorganism (*Actinobacter*) immobilized on the surface of sweet lemon peel. Removal efficiency of fluoride increased from 59.59 to 94.47% in optimum conditions of contact time (87 h), pH 4.0, and dose 14 g/L for 20 mg/L initial concentration of fluoride. Simple adsorption process removal efficiency was 59.59% at optimum conditions of contact time (60 min), pH 4.0, and dose 16 g/L.

Shubha Dwivedi et al. (2017) worked on bacteria *Shewanella putrefaciens* in bulk phase as well as immobilized phase in batch reactor. Growth of the bacterial cells, its acclimatization in fluoride media and in under substrate stress has been investigated. Optimization of process parameters for the removal of fluoride in bulk phase has been investigated and the performance under optimum condition has been compared with the simultaneous adsorption bioaccumulation (SABA) process using *Citrus limetta* (mosambi peels) and *Ficus religiosa* (peepal) leaves as adsorbent

Table 9.6 Comparative tabulation of adsorbents used in fluoride removal from aqueous solution

Bioadsorbent	IFC (mg/L)	Temp.	pH	Adsorbent dose (g/L)	RPM	Time (h)	% Removal	Adsorption capacity (mg/g)	References
Spirogyra IO1	5	Room temp	2.0	10	100	2	64	1.27	
Pleurotus ostreatus 1804	5	30	7	0.1	100	2	52	1.27	Ramanaiah et al. (2007)
Neem and Kiker leaves	5	27	6	5 (Neem) 7 (Kiker)	–	1	58	–	Kumar et al. (2008)
Rice husk	5	29 ± 0.5	2	10	30	2	75	–	Deshmukh et al. (2009)
Cynodon dactylon Activated carbon	3	30	5–5.5	12.5	250	1.75	84	4.70	Alagumuthu et al. (2010)
MnO_2-coated Tamarind Fruit Shell (MTFS)	2–5	Room temp	6.5	1	125	0.5	98	1.99	Sivasankar et al. (2010)
Cordia Africana seed powder	10	26	7	10–50	–	1–24	60	–	Abburi et al. (2012)
Aspergillus nidulans	–	40	4	1.5	–	20	29	–	Merugu et al. (2013)
Citrus limetta peel	20	30	7	10	125	0.5	67.5	1.82	Dwivedi et al. (2014a, b)
Ficus religiosa leaves	20	30	7	10	125	0.75	74	2.24	Dwivedi et al. (2014a, b)
Neem (*Azadirachta indica*)	10	27	2–8	10	–	1.5	90	–	Goswami et al. (2015)
Pleurotus eryngii ATCC 90888	5	30	2	0.2	100	4	97.03	66.6	Amin et al. (2015)
Trichoderma hezardium	4	30	7	0.4	100	1.0	20	1.14	Koshle et al. (2016)

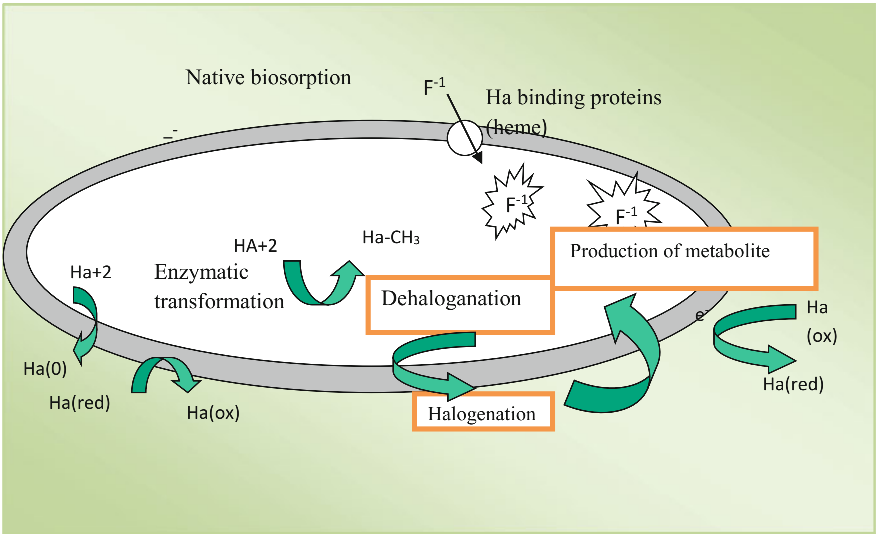

Fig. 9.9 Various processes of bioadsorption as well as bioaccumulation for the removal of halides by bacterial cell (Dwivedi et al. 2018)

along with the bacteria. The simultaneous adsorption and bioaccumulation (SABA) process shows maximum fluoride removal capacity followed by bioaccumulation in bulk phase and adsorption shown in Fig. 9.10. 91.7% removal of fluoride occurred by bioaccumulation process. Both bioaccumulation and SABA are able to reduce the initial concentration of fluoride from 20 to below 1.5 mg/L.

Chubar et al. (2008) investigated the sorption of fluoride and phosphate to viable cells of *Shewanella putrefaciens.* Here, uptake is measured over a different concentration range. In addition, sorption of the metals was compared to that of two anions (fluoride and phosphate), and both viable cells and autoclaved cells are used as a sorbent phase what was rarely done in past. Batch potentiometric titration and Boehm method were applied to investigations of bacterial surfaces. FTIR absorption spectra and pH-dependent zeta potentials were similar for the viable and bacterial cells. Sorption of fluoride and phosphate is not pH dependent, although an initial addition of acid and base was needed to activate the anion binding sites. Uptake of fluoride is comparable for viable and killed cells. Its physiological tolerance makes it a promising microorganism for bioremediation applications in a wide variety of environments.

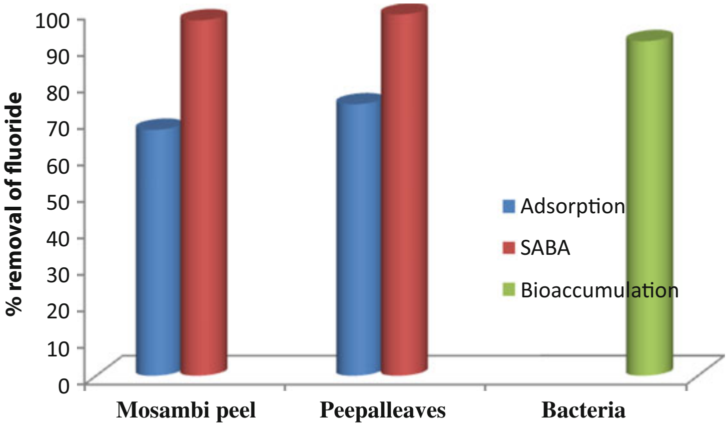

Fig. 9.10 Comparison between the % removal of fluoride by using adsorption, bioaccumulation in bulk phase and SABA process for bioadsorbents

9.10 Conclusion

One of the highlights of this study is that there is an urgent need to remove fluoride from groundwater, as it is the vital source of fresh water. According to the statistics mentioned in above review, more than 15 states are suffering from fluoride in which Rajasthan and Andhra Pradesh are at more alarming condition. Though several physical and chemical technologies are available for the removal of fluoride, the future lies in green technological approach which is undoubtedly the bioadsorption/ bioaccumulation. It is also a well-known fact that physical and chemical technologies have their own merits and demerits and produce some side effects too, but bioremoval has no harmful effects on the environment. As discussed in the previous sections, various bioadsorbents are available that have the capacity to adsorb fluoride. Also some studies focus on microbial removal of fluoride by using microbial species; certainly it is a more potential route to diminish the level of fluoride in water.

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Chapter 10
A Critical Review of Microbial Transport in Effluent Waste and Sewage Sludge Treatment

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Abstract The biological wastewater treatment processes include bioremediation of wastewater which involves aerobic treatment and anaerobic treatment, phytoremediation of wastewater and mycoremediation of wastewater using technologies such as activated sludge and biofilm systems. On the other hand, the chemical wastewater treatment processes include chemical precipitation, ion exchange, neutralization, adsorption and disinfection. Therefore, this chapter intends to provide a detailed information on the treatment of effluent waste and sewage sludge with respect to its associated microorganisms and transport activities. In a bid to achieve this, this chapter will address the various biological waste treatment processes in relation to the activities or role of microorganisms. Due to the convolute interface interaction between microbes and material surfaces, the critical review will also entail taking a cue from microbial transport in alluvial streams in order to understand the microbial transport in effluent waste (liquid) and sewage sludge (solid).

Keywords Environment · Effluent waste · Sewage sludge · Microorganisms · Aerobic treatment and anaerobic treatment · Microbial transport

10.1 Introduction

Wastewater has its origin from several sources including human sewage, animal wastewater, industrial wastewater, municipal wastewater, domestic wastewaters, and agricultural wastewaters. The composition of wastewater is about 99.9% water by mass while the rest contains suspended and dissolved matter (Lin 2007; Gray 2005). The primary purpose of wastewater treatment is to eradicate pollutants that are delirious to destructive to the marine surroundings. Most of these pollutants that are organic in nature tend to reduce the dissolved oxygen (DO) demand in the marine waters as the aerobic microorganisms act on them during their metabolism. Also, due to the disproportionate vegetal and algal growth in the water bodies as a result of the release of nutrients such as nitrogen and phosphorus, there is need to design waste treatment structures to reduce the effect of these pollutants or nutrients using an effectual and cost-effective technique. Pollutants may be characterized as soluble or insoluble, organic or inorganic, biodegradable or non-biodegradable, toxic or non-toxic, biogenic or anthropogenic, etc. However, some pollutant may possess more than one characteristics.

Essentially, wastewater largely holds macro- and microscopic organisms and its treatment undergoes different processes involving physical, chemical and biochemical operations or combinations of these practices dependent on the mandatory discharge criteria. However, the biochemical operations involve the use of microorganisms to alter noxious waste through enzymatically catalysed biological reactions. More so, the biological wastewater treatment processes include bioremediation of wastewater that includes aerobic treatment and anaerobic treatment, phytoremediation of wastewater and mycoremediation of wastewater using technologies such as activated sludge and biofilm systems. On the other hand, the chemical wastewater treatment processes include chemical precipitation, ion exchange, neutralization, adsorption and disinfection (Mohamed 2015).

According to Raeesossadati et al. 2014, the temperature of wastewater plays a major role in the biological activities of microorganism. Other parameters include pH, conductivity, saturation level of gases and various form of alkalinity. Physical characteristics such as colour are influenced by the source of the produced products. However, the odour is determined by several factors such as the effervescence of gases and decomposition of organic matter (Metcalf and Eddy 1987). Wastewater generally comprises components from protein, lipids, carbohydrates, urea and other traces of organic materials (Jiang et al. 2011). On the other hand, organic materials are made up carbon, hydrogen and oxygen (Sahu et al. 2013) with the presence of inorganic mixtures such as chloride, hydrogen and iron.

Wastewater treatment usually involves the initial handling plant in which constituents like fats, grease and solid particle are eradicated. However, the biological treatment of wastewaters is carried out in static media or suspended growth reactors with the aid of activated sludge, biofiltration, revolving biological contactors or modifications of these methods which consequently yields sludge solids as its effluent. This chapter also addresses the sludge handling which consist of the coagulating and dewatering of sludge before disposal.

The biological handling of wastewater is centered on the ingestion of biological material by microorganisms such as bacteria, viruses, algae and protozoa. Thus, there is need to understand the metabolism for effective control of the process. Of all the microorganisms, bacteria are the most common microorganisms used in the handling wastewater. While aerobic bacteria require oxygen in breaking down the substrate, anaerobic bacteria function in the absence of oxygen and usually occur in under septic environments like sewers or in sludge tanks where there is limited oxygen (Environmental Protection Agency 1997). In the biological treatment processes, there is a segregation of solid matter and nutrients such as nitrogen and phosphorus by the microorganisms which grow actually in the reactor. The microbes consume the carbonaceous matter in the sewers and thus diminish the organic materials in wastewater (Rosen et al. 1998). One of the merits of adopting the biological processes include the fact that it is a natural process in which no chemicals are needed. It is worthy to mention that this practice can also remove both phosphorus and nitrogen with concurrent sequestering carbon while producing oxygen for enhanced Biochemical Oxygen Demand (BOD). In addition, the residue biomass can be utilized as an alternative for energy or production of compost fertilizer (Senthil Kumar and Saravanan 2018).

Most wastewater treatment plants depend on anaerobic microbial treatment processes. Besides the eradication of contaminants, contemporary concern on anaerobic wastewater handling has diverted towards the repossession of resources, for sustainable creation of bioenergy and biochemical (Angenent et al. 2004). Anaerobic methods entail system of multifaceted biological and chemical reactions with the degradation of biological materials in the presence of different microorganisms resulting in low energy output (Sengor 2019).

Nonetheless, there is an assembly of activated quantity of micro-organisms capable of steadying the biological matter of waste in the activated sludge process. Several factors such as micro-organisms, oxygen and food have a great impact on the operation of the activated sludge method. The quantity of micro-organism present affects the outcome of the mixed liquor and consequently the inbound wastewater. As such, the handler needs to offer optimum conditions for the growth of microorganisms. With the aid of a microscope, it was reported that flagellates prevail at low sludge age when the flocs are dispersed. However, due to low effluent value and overoxidized sludge, the rotifers and worms overcome at prolonged sludge ages (Environmental Protection Agency 1997).

This chapter aims to provide a vivid review on the treatment of effluent waste and sewage sludge regarding its related microorganisms and transport activities. In a bid to achieve this, this chapter will address the various biological waste treatment processes in relation to the activities or role of microorganisms. Due to the convolute interface interaction between microbes and material surfaces, the critical review will also entail taking a cue from microbial transport in alluvial streams in order to understand the microbial transport in effluent waste (liquid) and sewage sludge (solid).

10.2 Modes of Action in Bioremediation of Wastewater

10.2.1 Growth of Microorganism in Effluent

This section elucidates the growth of microorganism (bacteria) with respect to the contact time with the substrate. Initially, the microorganism starts to absorb the substrate before undergoing an exponential growth as it uses the readily available energy for replication and then reaches a limiting growth level. At this limiting level the growth is static. Due to deficiency of substrate, bacteria start to die off while some other bacteria forage on the dead cells, resulting to an overall decline in the mass of the microorganism. This process is referred to as endogenous respiration or cryptic growth. Thus, biological processes require effective control so as to maintain the population of the microorganism under unfavourable conditions (Environmental Protection Agency 1997). In an attempt to understand bacteria growth, the reactants comprise the organic matter, bacteria, nutrients and oxygen, which combine together to yield a product of new bacteria, carbon dioxide, water, residual organic matter and inorganic matter. Thus, the rate of bio-reaction is strongly affected by certain factors such as temperature, dissolved oxygen, pH, toxic matter and nutrient content which when upheld under favourable conditions tends to promote bacterial growth within the bioreactor. In general, the biological breakdown increases proportionally with higher temperatures (with typical temperature ranges from 20 to 40 °C). Although, aeration tanks and percolating filters function at temperature between 12 and 25°C (Mohamed 2015).

10.2.2 Inhibition of Microorganism

The treatment of the wastewater is reliant on the actions of microorganisms and their breakdown which can be inhibited by the existence of toxic matter like organic/ inorganic solvents, heavy metals and biocides. The toxicity of industrial effluent inhibits to microorganisms and this is measured by a drop in the oxygen consumption rate. Parameters such as pH and temperature also impacts the biological activity in the waste treatment plant (Environmental Protection Agency 1997).

10.2.3 Transport of Microorganism in Sewage Discharge

Kay et al. (2008) claimed that there is little empirical data in the literature on fecal indicator organism (FIO) concentrations in sewage discharge. However, Kay et al. (2008) investigated and reported different FIO data: total coliforms (TC), faecal coliforms (FC) and enterococci (EN) from several types of sewage-related effluent in the United Kingdom in which the tertiary waste treatment particularly for the eradication to microbes or fecal indicator organism (FIO). The result shows the impact the concentration of FIO in sewage effluent for different flow conditions. According to Tian et al. (2002), Steets and Holden (2003), Kay et al. (2005), Haydon and Deletic (2006), there is limited research in the development of predictive models to determine the transport of microbes. However, Walker et al. (1999), Medema and Schijven (2001), Dorner et al. (2006) and Ferguson et al. (2007) have also reported the growing interest in this field of area in recent times.

Jamieson et al. (2004) reports that developing a typical model for microbial water quality watershed scale will go to provide further insight to microbes associated contaminants. To further shed more light, Jamieson et al. (2004) proffered certain steps for it to come to fruition. The method comprises the characterization of different wastes and the allied microorganisms, simulation of microbes from soil surface to receiving streams and within stream networks. It was reported that the limitation on the previous microbial water quality watershed scale models is the assumption that the transport of microorganisms on the earth crust is simply related with sediment attrition. However, the transport of microbes in sediments stream or slurry is convolute by interface interaction between the sediment stream and the microbes. Thus, there is need to further research on the microbial transport in effluent waste, sewage sludge and alluvial streams.

10.3 Effluent Waste and Microbial Transport in Perspective

Wastes occur in different forms and may be broadly traced to humans, animals, plants, and even non-natural sources. The above assertion is borne out of the very fundamental characteristics of substances or compounds regarded as wastes by man. A critical examination of the various forms in which wastes exist as identified above would reveal that some have remote roots. Ideally, majority of wastes that pose serious health and environmental concern do not emanate from remote sources but have strong nexus with human actions and activities.

Wastes date back to creation hence are not really separable from human existence. However, unmanaged wastes may exert some effects that are worse than the worst taboos in various sociopolitical jurisdictions regardless of geolocation on earth's surface.

Due to the hazards and deleterious effects, wastes may exhibit on humans and its environment, every government across the globe is in continuous search for enhanced and safe approaches towards eliminating the dangers posed by wastes.

Among the various categories of wastes, effluent wastes are commonplace. The reason is not far-fetched. Human activities at every point be it home, industry, factory, field, etc. do generate some of effluent waste. However, some effluent wastes may be generated via actions which may not have been contemplated ab initio as potential channels or players through which effluent wastes could result. Effluent wastes vary in their composition and degree of harmfulness. Generally, their toxicity may depend on its sources. Figure 10.1 shows the general categories of effluent wastes based on sources. Five major sources are identified:

(a) Domestic (with origin from around the home and other residential areas)
(b) Commercial (emanating from commercial and/or industrial settings such as shops, restaurants and markets)
(c) Industrial (with origin in industrial production enclaves such as factories and manufacturing plants)
(d) Agricultural effluents, generated during and/or following agricultural operations
(e) Stormwater effluent, a hybrid effluent produced when rainwater comes into contact with various wastes. Stormwater may contain wastes from differing sources and may include wastes from all residential houses, commercial environments, industrial and agricultural sources.

In addition to other sources, the increasing industrialization and urbanization activities across the globe especially in developing countries had been noted as the

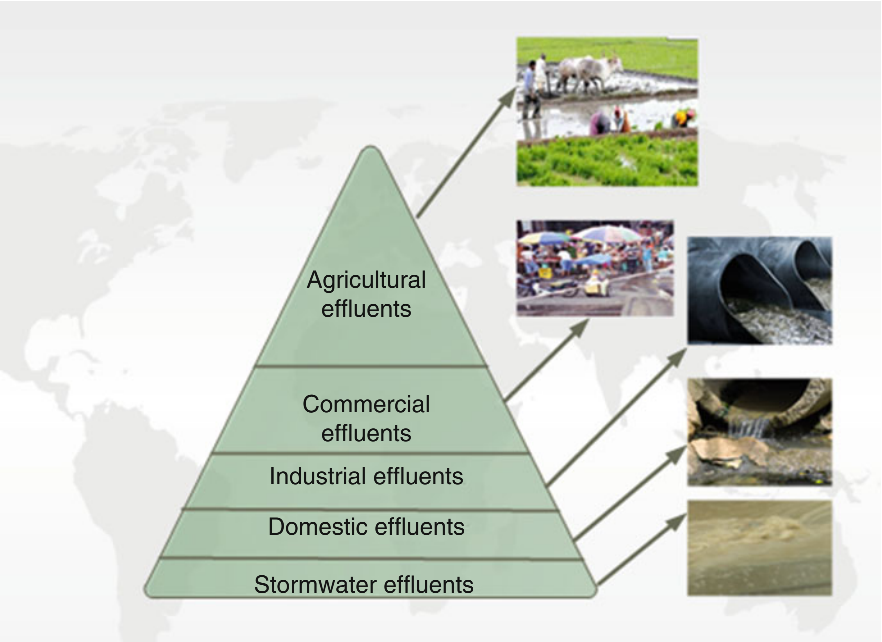

Fig. 10.1 Sources of effluent wastes

key contributors to the generation of effluent wastes (Ghosh 2005; Coles et al. 2012; Aliyu and Amadu 2017). According to findings, urbanization has the potential to produce unlimited wastes with resultant critical effects of water bodies and the environment (Dhania and Rani 2014; McGrane 2016; Shaharoona et al. 2019; Yuan et al. 2019).

Figure 10.2 shows the various forms the effluents in Fig. 10.1 may take. It is very evident from the diagram the wide scope effluent waste could take hence the reason why no country shies away from designing and putting into effect necessary infrastructure to control these wastes.

Effluent wastes are very significant when compared to other wastes in that they cover a wider spectrum with varying physicochemical characteristics hence their heavy impact on the environment (da Rosa et al. 2015; Redouane and Mourad 2016; Blanco et al. 2019; Venturoti et al. 2019). Characterization of effluent wastes is important in that it would enhance the deployment of the right infrastructure for managing such wastes. Figure 10.3 reflects the various characteristics of effluent wastes. Evaluation of the characteristics of each source of effluent waste enables decision takers in marshalling the right waste management alternative. Waste management may involve various machineries including legislations, policies, procedures, guidelines, etc.

In most jurisdictions, mechanisms in the form of policies and guideline exist that provide framework for handling effluent wastes. Often, these mechanisms categorize

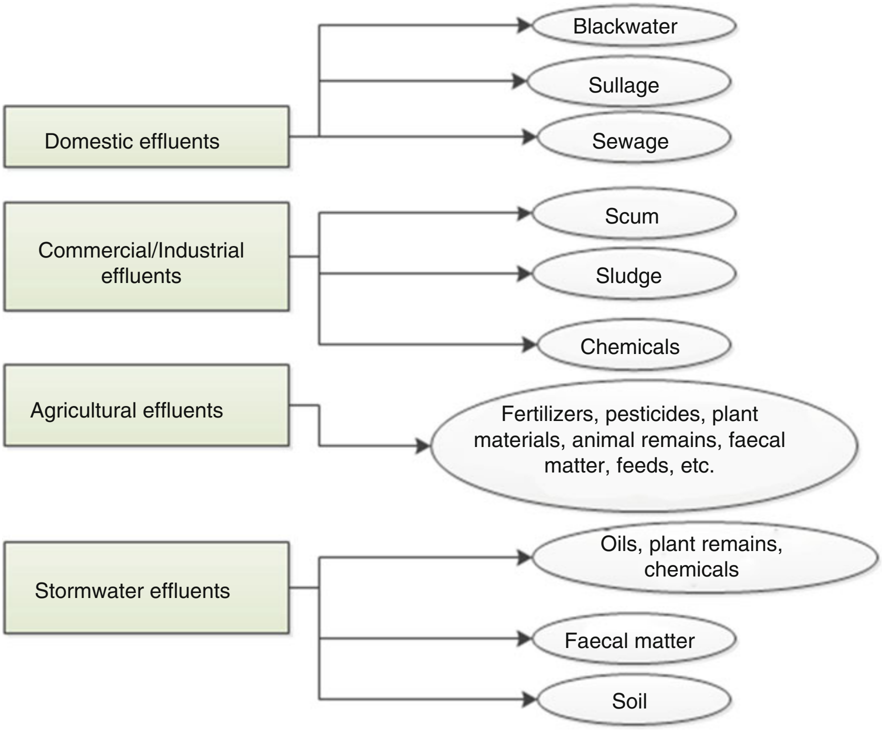

Fig. 10.2 Various forms of effluents

wastes and also grant legitimate powers and authority to specific agencies (public/private) to regulate, control, and manage effluent wastes. The commonest approach undertaken in managing wastes is treatment. It is interesting to note that waste treatment in itself has become a challenging task in most large cities. The essence of treating effluent wastes is to deactivate them hence making them less hazardous to the environment and human health. Various treatment options are available ranging from flocculation, biofiltration, adsorption, ion exchange to membrane technology. While each technology has proved attractive, several factors (such as cost and sustainability) affect their choice of deployment in effluent waste treatment plants.

In recent times, ecofriendly alternatives of treating effluent wastes have come under limelight. One of such option is microbial transport. Microbial transport explores the use of specific microbes as agents of waste treatment.

In the context of effluent waste management, microbial transport involves the controlled use of microorganisms such as bacteria to enforce biodegradation and biotransformation of effluent wastes thereby deactivating or detoxifying the wastes. In other words, controlled quantities of such microorganisms under specific determinable conditions are relayed or relocated from a microbial bank to various sites of actions where they act on effluent wastes. Microbial transport could aid in reducing the burden of handling wastes in urbanized and industrial cities as microbes are easy

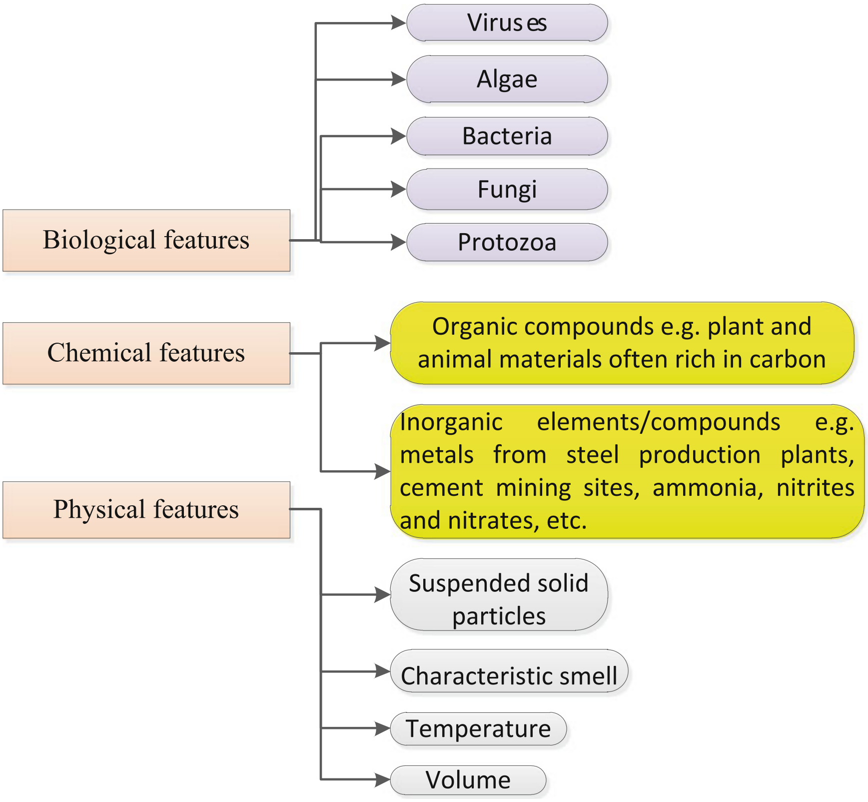

Fig. 10.3 Characterization of effluent wastes

to produce and their ubiquity in effluents is another advantage that could be exploited in the management of such wastes.

10.4 Application of Wastes in Production of Crops and Foods

The importance of effluent and sludge waste in crop and food production has long been widely recognized where water supplies are insufficient (National Research Council 1996). Treated or untreated effluent or sludge waste is widely used in crop and food production because it is a rich source of nutrients, providing all the moisture required for crop growth. It has been stated that crops yield higher when effluent or sludge waste is used for irrigation (Hussain et al. 2002). In addition, the acceptance of usage of wastes influential and sludge was generally embraced and implemented in other counties, such as the United States (National Research Council

1996) and European countries (Kirchmann et al. 2017), whereas the acceptance and application of water effluent and sludge was slow due to religious factors in countries such as Pakistan.

For example, waste effluent is applied to irrigate crops in the United States so as to avoid nutrient intrusion through sensitive water reception. The beneficial reuse of this wastewater is therefore more economic and technologically viable than using advanced technologies for treating wastewater that meet the surface water disposal requirement (National Research Council 1996).

Effluent was also used in Brazil to supplement wastewater and nutrient treatment methods associated with savings in mineral fertilizers and high crop yields (Fonseca et al. 2007). The use of secondary effluents in Botswana has been documented by Emongor (2006). The effluent waste is used for the processing of food depending on the components in the waste. Effluent pollution requirements for the constituents are the same as usual irrigation water.

In India, farmers use even untreated sewage effluents for irrigation purposes as a cheap alternative for other irrigation solutions when they are needed. In Central India, the experiment shows that the wastewater effluent saves chemical fertilizer NPK by 60 kg N, 13 kg P and 25 kg K per hectare per year. In addition, the study shows that approximately 18 kg N, 7 kg P and 49 kg K of nutrients per hectare per year could be recovered by waste effluent (Dotaniya et al. 2019).

On the other hand, sludge is a result of wastewater treatment that includes many wastewater contaminants. The experience with the use of human excrement, sewage and animal manure in food and crop production was already applied. But it is associated with its challenges, such as the composition of trace elements and large amounts of water depending on sludge treatment causing handling difficulties. Farmers in the United States urged the cost savings of replacing sludges with chemical fertilizer to be negligible (National Research Council 1996).

Council Directive No. 86/278/EEC regulates the European countries requiring prior testing and standard sludge and soil components to be applied on agricultural soil (Mininni et al. 2015). Nevertheless, the country's national standards were set in other countries such as Denmark, Germany, Norway, Sweden, Austria and France. The study shows that 39% of European Union wastewater is recycled into agriculture (Lamastra et al. 2018). The demand, however, varies across the countries of Europe, whereas some countries like Wallonia, Belgium, Denmark, France, Ireland, Spain and the UK use less than five per cent of the sludge produced for agriculture in the other parts of Europe (Lamastra et al. 2018). It may be due to the danger associated with the components in the sludge waster like heavy metals. For example, 70% of Norway's total sewage sludge is recycled as fertilizer used in crop and food production. Officials have set up special monitoring systems for identified pollutants to ensure that the necessary sludge waste portion levels are maintained to avoid environmental contamination (Eriksen et al. 2009).

Throughout China, sludge waste is further treated for biochar which is later used in the production of garlic. The results from biochar cultivation have been shown to increase significantly in comparison with when used in normal soils (Song et al. 2014).

10.5 Application of Computerization and Digital World, Internet of All Things in Bioremediation of Heavily Polluted Environment

The studies by Dlodlo (2012) revealed that the benefits of Internet of Things (IoT) applications could lead to sustainable environmental management. On the other hand, Saha et al. (2017) showcased the use of IoT in the identification of environmental contaminants. However, Toma et al. (2019) explain the use of IoT in smart emissions control environments. It is necessary to use information technology to reduce threatening health risks and raise awareness of air pollution exposure impacts, consequently helping to boost environmental pollution regulation.

In order to test Chromium(VI) removal efficiencies in nanoscale zero-valent iron cycle, Yu et al. (2014) conducted a study on BP-ANN with input variables including pH control, dissolved oxygen, initial Chromium(VI) concentrations, oxidation-reduction potential, contact time and nanoscale zero-valent iron. Three samples were installed in the nanoscale zero-valent iron batch reactor monitoring variations in dissolved oxygen, oxidation-reduction potential, and pH and online data was retrieved. Therefore, the datasets constructed from Chromium(VI) batch removal experiments were randomly selected as testing and training subsets. It was shown that the well-trained MLP-NN models provided accurate results, which demonstrated the capacity to optimize the nanoscale zero-valent iron cycle for removing Chromium(VI).

Dolatabadi et al. (2018) used a multilayer perceptron neural network 5-7-2 model and an adaptive neuro fuzzy inference system model to estimate sawdust adsorption capabilities by simultaneous removal from polluted solution of Copper(II) and Basic Red 46 (BR46). For the creation of prediction models, experimental data of 50 samples were used. The studies showed that both the multilayer perceptron neural network and adaptive neuro fuzzy inference system models had outstanding predictive performance for both copper and dye ($R2$ values of 0.98–0.99).

In order to optimize removal efficiency using multilayer perceptron neural network, Khandanlou et al. (2016) developed a predictive model for the identification of optimal values for the adsorption of Lead(II) and Copper(II), with 20 test data. The assessment of adsorption efficiencies of the artificial neural network training samples of Lead(II) and Copper(II) at 74.04% was found to be similar to the real value of 75.54% under optimal conditions, indicating that the model could generate accurate prediction without abundant experimental data.

Nag et al. (2018) have provided the MSME sector with effective and affordable emission control means. The effect of operating parameters, i.e. aqueous phase pH, initial cadmium(II) concentration, adsorbent dose, time, temperature of cadmium (II) ion removal, on these green biomaterial are performed via batch experiments. The bioremediation process was strongly pH-based, spontaneous and followed kinetics of the second order. Complex sorption modelling was performed using the combined genetic algorithm with artificial neural network technique to accurately predict the efficiency of metal ion removal, and the results obtained are well-compatible with the experimental data with the correlation coefficient (*R*) of 0.97–0.99.

10.6 Application of Microorganism for the Treatment of Effluent, Liquid and Municipal Wastes

The treatment of effluent, liquid and municipal waste is a procedure wherein contaminants are detached from the effluent, liquid and municipal waste (Zhao et al. 2013; Ma et al. 2015; Aigbe et al. 2020). The primary reason for the treatment of effluent, liquid and municipal wastes, like other environmental contaminants is to produce wastes that will have no adverse environmental influences; this will in no doubt assist in mitigating the incessant environmental issues globally (Nwankwo and Ukhurebor 2019; Nwankwo et al. 2020a, b, c). If these effluent and liquid wastes are not treated appropriately, the results can be devastating as effluent, liquid and municipal wastes can cause several environmental consequences, that could lead to loss of lives of both plants and animals (Adebayo and Obiekezie 2018; Aigbe et al. 2020).

Essentially, chemical and biological effluent, liquid and municipal wastes need to be degraded before they released in the environment. This degradation process can efficiently be monitored and controlled by the management of the microbial inhabitants in waters thus causing microorganisms to digest the biological or organic matter. Such water should be disinfected before it is considered suitable for domestic purposes. It is also advisable to treat underground water before making use of them domestically or otherwise (Ma et al. 2015; Adebayo and Obiekezie 2018; Aigbe et al. 2020).

Canler and Perret (2011) identified three major procedures for the treatment of effluent, liquid and municipal wastes, viz. primary treatment, secondary treatment and tertiary treatment.

The primary treatment procedure entails the physical separation of effluent, liquid and municipal wastes to form solid and liquid by means of a settling sink or basin, after which the effluent, liquid and municipal wastes are at that point moved to the secondary treatment stage; in this stage the removal of the dissolved organic or biological composite occur through micro-organisms or microbes application; the microbes frequently apply aerobic metabolism for the degradation of the biological or organic matter in the liquid mud. At that point, the tertiary treatment procedure is required to sterilize the effluent and liquid wastes so that they could be released and delivered into the environment. As of the solid effluent, liquid and municipal wastes which are detached during the primary treatment stage are moved into a reservoir of tank for mud assimilation which comprises anaerobic degradation by means of micro-organisms (Canler and Perret 2011; Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018).

10.7 Microbial Processes for the Treatment of Wastes

Apparently, there are several microbial procedures for the treatment of effluent, liquid and municipal wastes that have been reported and these microbial procedures can be broadly grouped into two groups, known as aerobic procedure and anaerobic procedure (Satyanarayana et al. 2012; Kangle et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Ma et al. 2015; Adebayo and Obiekezie 2018).

10.7.1 Aerobic Procedure

As stated earlier, during the primary treatment procedure, the liquid and solid stages are detached physically. The liquid stage is treated by exposing the liquid to air in order for aerobic breakdown (degradation) of the nutrients in a process that is known as aeration (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). According to Wagner (1996), the two vital microbial procedures at this phase are nitrification and phosphorous elimination.

Nitrification ensues in two distinct phases; firstly, the ammonium is oxidized to nitrite by means of "*Nitrosomonas* spp." Furthermore the nitrite is oxidized to form nitrate by means of "*Nitrobacter* spp." (Wagner 1996; Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). The phosphorous elimination phase occurs by a biological process known as improved biological phosphorous elimination; this procedure is established by the cell embracing phosphorous within their compartment as well as the filtration of the biomass (Zou 2013).

10.7.2 Anaerobic Procedure

In the liquid constituent of the effluent, liquid and municipal wastes, denitrification of bacteria diminishes the nitrate to form dinitrogen gas that can release nitrate from the effluent and liquid wastes (Chen and Lin 1993; Adebayo and Obiekezie 2018). Hernon et al. (2006) reported that the solid constituent of the effluent and liquid wastes which are detached during the primary treatment procedure is fermented anaerobically by microorganism such as bacteria (Hernon et al. 2006; Kangle et al. 2012; Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018).

10.8 Microorganisms for Treatment and Management of Wastes

Despite the fact that microorganisms could cause some detrimental consequences to the environment, they however play vital roles in the conservation and preservation of several environmental progressions both naturally and artificially by performing some inspiring roles that make human existence more comfortable (Satyanarayana et al. 2012; Zhao et al. 2013; Ma et al. 2015; Adebayo and Obiekezie 2018). According to Adebayo and Obiekezie (2018), the foremost of such roles are in the management of wastes both effluent and liquid wastes as well as municipal wastes.

The appropriate removal and discarding of the capacious wastes that are produced domestically and industrially by human actions on a daily basis is a great environmental threat globally, as such the government and environmental agencies of any nation are incessantly in search of the appropriate and improved means for its management (Adebayo and Obiekezie 2018; Aigbe et al. 2020).

However, several studies have demonstrated that one of the best techniques of effectively managing this threat is via the use of microorganisms (Satyanarayana et al. 2012; Zhao et al. 2013; Ma et al. 2015; Adebayo and Obiekezie 2018).

According to Adebayo and Obiekezie (2018), microbes that potentially inhabit the aerobic organic or biological treatment procedures of wastes are: bacteria, protozoa, fungi, rotifers, algae and some other complex living organisms.

It has been reported that the development of microorganisms in a given industrial waste removal technique depends on the following (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018):

- The chemical properties of the industrial wastes
- The environmental restrictions of the specific waste technique
- The properties (biological and chemical; biochemical) of the microorganisms

However, microorganisms which are cultivated in a presumed industrial waste removal technique contribute to its properties generally.

It is therefore imperative to identify the contributions made by each kind of microorganism to the entire maintenance of the biological or organic wastes if the waste treatment procedure is to be appropriately planned and operated for almost effectiveness (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018).

10.8.1 Application of Bacteria for the Treatment of Wastes

Bacteria has proven to be one of the foremost biological or organic components in aerobic procedure for the treatment of effluent, liquid and municipal wastes (McKinney 1957; Satyanarayana et al. 2012; Adebayo and Obiekezie 2018); this is attributed to the fact that bacteria exhibit various biochemical attributes, which makes it possible for them to metabolize the biological or organic composites in such wastes whether it is liquid waste, municipal wastes, domestic wastes or industrial wastes (Satyanarayana et al. 2012; Adebayo and Obiekezie 2018)

According to Adebayo and Obiekezie (2018), the development of any specific kind or species of bacteria depends on the competitivity capability of such bacteria to attain a portion of the accessible biological or organic constituent in the procedure.

It has been reported that bacterial prevalence would customarily split itself into two main categories (McKinney 1957; Adebayo and Obiekezie 2018): the bacteria using the biological ororganic composites in the waste and the bacteria using or inducing lysis (the breaking down of the molecules into smaller molecules) of the initial set of bacteria.

The bacteria using the biological or organic composites present in the waste are the foremost set and they control the properties of the treatment procedure. Studies have shown that the bacteria whose growth rate is faster and the capability to use most of the organic or biological matter would prevail (Satyanarayana et al. 2012; Adebayo and Obiekezie 2018).

The limit of secondary preponderation as rightly reported by Adebayo and Obiekezie (2018) is dependent on the extent of malnourishment. Reduction of the biological or organic composite results in death as well as the breakdown of the molecules of the primary set of bacteria of the preponderate bacteria (Satyanarayana et al. 2012; Adebayo and Obiekezie 2018).

The release of cellular composites of the bacteria assists the growth rate of the other bacteria. Subsequently, all biological or organic treatment procedures are ordinarily over-premeditated as a protection influence, secondary preponderation would ensue (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). Apart the bacteria's metabolic properties, the furthermost property is their capability to collect all loose aggregates together (flocculate). Aerobic biological or organic waste treatment procedures are subject to flocculation of the microbes as well as their extraction from the liquid stage for comprehensive stabilization (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018).

Previously, it was alleged that flocculation was instigated by only one kind or species of bacteria known as *Zoogloe ramigeria*. However, new research studies have postulated and revealed that there are several other kinds or species of bacteria which exhibit flocculation under some certain environmental circumstances (McKinney 1957; Adebayo and Obiekezie 2018).

The main issues affecting flocculation according to Adebayo and Obiekezie (2018) are: "the surface charges of the bacteria and their energy level". Reportedly, the electrical surface charge of bacteria grown in low concentration organic or biological waste procedures has been discovered to be less than the value of the critical charge for auto-accretion which is 0.020 V (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). This implies that Brownian movement affords adequate energy to withstand the resisting electrical forces as soon as two bacteria come close to each other and to allow the Van der Waal forces to preponderate as well as embrace the two bacteria together

(Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). Normally, auto-accretion hardly occurs if the energy level of the procedure is adequately high to allow the bacteria to tremendously increase as well as having the swift potential to move spontaneously (motile).

Auto-accretion or flocculation only takes place after when the bacteria do not have enough energy of motility in withstanding the Van der Waal forces (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). Once the formation of soft particles suspended in the liquid (floccule or floc) is in progress, reportedly at this stage according to Adebayo and Obiekezie (2018), some of the bacteria would perish and break down into smaller molecule (lysis). An unsolvable fragment in the cell of the bacteria is left, that is predominantly polysaccharide. The floccule or floc at this stage grows into more advanced polysaccharide.

10.8.2 Application of Fungi for the Treatment of Wastes

Fungi play a vital part in bringing equilibrium to organic or biological wastes. Like bacteria, fungi could metabolize virtually all kind of organic composite contained in industrial wastes (Adebayo and Obiekezie 2018). The fungi have all it takes to preponderate over other microorganisms like the bacteria. However, they only do so only under certain unusual environmental circumstances. As a result of the filamentous attribute of some of the fungi contained in industrial wastes makes them obnoxious because of their inability to form compacted floc and settle without difficulty. Hence, substantial efforts are used to assure more approving environmental circumstances for bacteria preponderation than for filamentous fungi preponderation. The filamentous fungi preponderate needed for bacteria at truncated oxygen strains, at truncated pH as well as at truncated nitrogen (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018).

The truncated oxygen strain could be as a result of the truncated oxygen that is supplied or consequently due to the high biological or organic weight instigating the quest to surpass the supply. Under condensed oxygen levels, metabolism hardly turn to carbon dioxide and water; however, it could stop by means of the formation of biological or organic alcohols, aldehydes as well as acids. If there is insufficient buffer in the process, the biological or organic acids reduce the pH to a better approving range for fungi. Consequently, it could be seen that truncated oxygen strain as well as pH could be interconnected.

Several of fungi develop properly at pH ranging from 4 to 5, whereas limited bacteria are able to develop properly. Fungi need smaller amount of nitrogen compared to the amount needed by bacteria per unit mass of protoplasm (McKinney 1957). In wastes with deficient nitrogen, the fungi will be able to synthesize additional energetic masses of protoplasm from such wastes compared to bacteria and preponderate. Average number of bacteria is approximately ranged from 10 to 12% of nitrogen, whereas the average number of fungi range from 5 to 6% of nitrogen (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). Under usual environmental circumstances, fungi would be existing and would assist in stabilizing organic matter. Nevertheless, fungi are of secondary significance and would not preponderate (Akpor et al. 2014; Adebayo and Obiekezie 2018).

10.8.3 Application of Viruses for the Treatment of Wastes

They are units collected from the "biopolymers", which are proficient in increasing and accumulating as fresh virus units inside "living prokaryotic or eukaryotic cells" (Ivanov 2010). In our surrounding, viruses are significant for the following purposes (Adebayo and Obiekezie 2018):

- Pathogenic viruses should be detached, reserved or demolished during the process of treatment of water and wastewater.
- Viruses of bacteria known as "bacteriophages" could contaminate and degrade the bacterial cultures in our surrounding.
- "Bacteriophages" could assist in the detection of precise microbial effluence of environmental waste.

10.8.4 Application of Protozoa for Treatment of Wastes

Protozoa is reportedly one of the simplest living organisms which are contained in waste disposal processes (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). According to study carried out in ascertaining the stabilization of biological or organic wastes, show that they are accountable for the reduction the quantity of "*free-swimming bacteria*", accordingly, they assist in the generation of simplified effluent (Satyanarayana et al. 2012; Zhao et al. 2013; Akpor et al. 2014; Adebayo and Obiekezie 2018). The sequence of protozoa had extensive due to it observance in biological or organic waste disposal processes (McKinney 1957). However, there are presently limited explanation of the details for this sequence (Adebayo and Obiekezie 2018). The sequence of protozoa is influenced by similar factors which influence the preponderation of any organic or living species. The category of food as well as the struggle for food have been reported to be the foremost factors for the determination of the preponderation of protozoa (Akpor et al. 2014; Adebayo and Obiekezie 2018).

The "*Sarcodina*" are reportedly found in the treatment processes of waste aerobically, this is as a result of the insufficient sufficient food to contend with other living organisms like bacteria (Adebayo and Obiekezie 2018).

The "*Phyto-Mastigophora*" subsist slightly longer more than the "*Sarcodina*" since they inject soluble biological or organic food. However, they are incapable to contend against bacteria as such they are rapidly displaced (Adebayo and Obiekezie 2018).

The “*Zoo-Mastigophora*” preponderates over the “*Phyto-Mastigophora*” such that they are capable of utilizing bacteria as food instead of competing with bacteria for nourishment. However, the “*Zoo-Mastigophora*” give way to the “*free-swimming Ciliata*” which have an improved machinery for obtaining bacteria as well as other nourishment mechanisms (Adebayo and Obiekezie 2018). As the process becomes further steady, there are fewer and less “*free-swimming Ciliata*” (Adebayo and Obiekezie 2018). However, the process rapidly becomes so steady that the “stalked Ciliata” cannot attain sufficient energy, so they pass away from the process (Akpor et al. 2014; Adebayo and Obiekezie 2018).

The sequence of protozoa brings a decent index of firmness for the treatment process of biological or organic waste. According to Adebayo and Obiekezie (2018), few numbers of “*free-swimming Ciliata*” ensue at both a little degree of decontamination ranging from 20 to 40%, while at high decontamination ranging from 75 to 95%. The comparative categories of protozoa and comparative numbers could be used for any specific for estimating the irregular efficiency of ±10%, for any biological or organic treatment process (McKinney 1957). The protozoa has been reported to having more intricate metabolic procedures compared to other microorganisms such as bacteria and fungi; this make protozoa more delicate to noxious organic composites (Adebayo and Obiekezie 2018).

In the processes comprising of noxious organic or biological composites, steady observations of protozoa could be applied as indicator of the noxious concentration as well as to caution possible noxiousness to the bacteria that would have been responsible for stabilizing the organic or biological wastes. Protozoa could as well be used for the indication of deficits of some crucial elements like nitrogen and phosphorus. According to Adebayo and Obiekezie (2018), nutrient deficits would decrease both quantity of species and the quantity of any specific species.

10.9 Conclusion and Future Recommendation

This chapter has provided a detailed information on the use of handling of effluent waste and sewage sludge with respect to its accompanying microorganisms and transport activities. The various types of techniques utilized in their management were also provided most especially the application of beneficial microorganisms. The application of genetic engineering and mutation could lead the generation of novel strains that could be used for the management of effluent waste and sewage sludge. The application of these techniques need to be performed on a larger scale especially when tested on a field trial. There is a need to adopt the application of cheap agricultural waste resources for the mass production of these beneficial microorganisms.

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Chapter 11
Recent Trends in Utilization of Biotechnological Tools for Environmental Sustainability

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Abstract The liberation of numerous organic and inorganic chemicals derived from numerous industries, such as tanneries, textiles, agro-based industries, pharmaceuticals, and petrochemicals, has been identified to produce several pollutants that could constitute several hazards to the environment and humans. Different types of technology and processes such as advanced oxidation techniques physical and chemical processes are utilized for the management of these contaminants. However, there are several drawbacks associated with these techniques, and the majority of the end products are also poisonous in nature. Hence, there is a need to identify sustainable and eco-friendly techniques that could lead to the maintenance of a cleaner environment which is economical feasible, non-poisonous, and without any adverse effect. The utilization of these beneficial microorganisms has been identified as a sustainable biotechnological technique that could lead to the maintenance of a healthy environment. These microorganisms portend capability to reduce these contaminates into non-toxic components. Therefore, this chapter intends to provide detailed information on the application of beneficial microorganism for the achievement of environmental sustainability. Special emphasis was laid on some specific examples of sustainable biotechnology techniques that could lead to the maintenance of a cleaner environment. The different types of beneficial microorganisms are also highlighted.

Keywords Microorganism · Environment · Sustainability · Bioremediation · Eco-friendly · Health · Hazards

11.1 Introduction

Environmental pollution is linked to the action of anthropogenic activities and natural sources, which might be linked to the availability as well as persistence of numerous pollutants that are toxic in nature (Adetunji et al. 2020; Adetunji et al. 2019; Adetunji et al. 2018a, b, c, d, e; Adetunji et al. 2017a, b, c, d). Numerous decontamination technologies and processes have been identified over the years in order to achieve a high level of sustainability for effective bioremediation of the environment (Doble and Kumar 2005; Gavrilescu and Macoveanu 1999, 2000; Khan et al. 2004).

Most of the actions employed for the maintenance of environmental sustainability possess the capability to continue with a lower long-term influence on the environment, which are determined for a non-impact objective by placing a higher priority on economic and social factors (Gavrilescu 2011).

It has been discovered that sustainable development and sustainability are two different multifaceted concepts that possess numerous definitions, which might indicate the way the concept could be effectively used (Gavrilescu 2011; WCED 1987). Hence, sustainability was defined in a report compiled by the World Commission on Environment and Development as “the capability needed for the demand of the present capability, without conceding the future cohorts that would come across their own requirements”. Moreover, the World Bank also defines sustainability as “the condition or a process that could preserve the environment indeterminately without advanced dwindling of the esteemed qualities of the external or internal system in which the process could take place based on the condition that need to be overcome” (Holdren et al. 1995).

The process involved in the reduction of the environmental footprint entails air emissions, ecological impacts, materials, greenhouse gases (GHGs), land, waste, water as well as the application of the eco-friendly and biodegradable materials that are protected by the “green” characteristics (Hurst 2010). Also, a sustainable remediation could be compared to a green remediation, because it entails the reduction of environmental impacts at minimum cost, decreases GHG emissions, as well as promotes some sustainable practices that could lead to the reduction in cost, and these could enhance social acceptability (Hurst 2010).

Furthermore, it has been identified that green and sustainable remediation entails a wider range of economic impacts, as well as social and environmental remediation procedures (Reddy and Adams 2010). This indicates that green and sustainable remediation discourses the defence of human health together with the maintenance of a cleaner environment, as well as the reduction of the numerous side effects that could affect the environment (Ellis and Handley 2009; Reddy and Adams 2010; USEPA 2008).

Biotechnology tools have been identified as a process that involves the application of living organisms for a successful achievement of more sustainable products that could lead to the maintenance of a cleaner environment. The application of biotechnology has been identified as a sustainable process when compared to the conventional chemical synthesis of products. This is due to the fact that they are environmental friendly, as the synthetic techniques portend the capacity to pollute the environment. Chen et al. (2005) clearly define environmental biotechnology as the application of microorganisms for effective enhancement and maintenance of quality in the environment. Therefore, this chapter intends to provide detailed information on the application of beneficial microorganism for the achievement of environmental sustainability. Special emphasis has been given to some specific examples of sustainable biotechnology techniques that could lead to the maintenance of a cleaner environment. The different types of beneficial microorganism are also highlighted.

11.2 Specific Examples of Microorganisms for Attainment of Environmental Sustainability

11.2.1 Bioremediation

Bioremediation of wastes, also known as biotreatment, is a subdivision of biotechnology that employs the use of living organisms especially microorganisms/microbes such as bacteria for the removal of impurities, contaminants, pollutants, and/or chemical toxins from the environment (soil and water in particular). Bioremediation is used for the cleansing of environmental complications arising from petroleum/oil spills and/or groundwater pollution from industries and other sources (Abatenh et al. 2017; Adams et al. 2015; Couto et al. 2014; Jain and Bajpai 2012; Das and Chandran 2011).

According to Adams et al. (2015), microorganisms are well known for their capability to disintegrate a massive array of organic or biological composites and absorb or engross inorganic constituents. Presently, microorganisms have been used for the treatment of pollution/contamination and the process is termed bioremediation. As reported by the United States Environment Protection Agency (USEPA), bioremediation activities during the year 2018 has recovered about 1507 sites (Adams et al. 2015). Bioremediation made use of microorganisms for the reduction of pollution/contamination by means of biological degradation or breakdown of contaminants/pollutants into non-noxious constituents/materials. This process could either encompass aerobic or anaerobic microbes that habitually use degradation process as a source of energy (Abatenh et al. 2017).

Bioremediation techniques or procedures are mainly grouped into three categories, viz. (Jain and Bajpai 2012):

(a) In situ land treatment for soil and groundwater.
(b) Biofiltration of the air.
(c) Bioreactors (which are mostly involved in water treatment).

Bioremediation can be viewed as:

- A biotechnological field where microorganisms and other living organisms eradicate contaminants or pollutants from the environment (soil, water, and air).
- A conventionally used procedure for cleaning oil/petroleum spills or/and contaminated or polluted groundwater.
- A means of "in situ" at the polluted or contaminated sites, or "ex situ" means away from the polluted or contaminated sites. This may be essential if the weather or climate is so cold for sustaining the microbial action, or if the soil is over dense for even distribution of nutrients. Ex situ bioremediation may entail exhuming or excavating and cleaning the soil directly above the ground, and this could add substantial costs to the procedure.

Bioremediation depends on the stimulation in the development of certain microorganisms that exploit pollutants or contaminants such as petroleum/oil, solvents, and pesticides for various sources such as food and energy. These microorganisms translate the pollutants and contaminants into small quantities of water and other non-toxic gases like carbon dioxide and nitrogen (Abatenh et al. 2017; Jain and Bajpai 2012). Bioremediation involves the combination of appropriate temperature, nutrients, and foods. The deficiency of these essential components could prolong the cleansing procedure of the pollutants or contaminants (Jain and Bajpai 2012; Macaulay 2014; Adams et al. 2015). Some conditions that are not suitable for bioremediation procedure could be enhanced by the addition of some environmental alterations, such as molasses, vegetable oil, or simple air. These alterations enhance the conditions for microorganisms to flourish, thus accelerating the achievement procedure (Adams et al. 2015). The bioremediation procedure could take several months or years to complete, depending on some parameters such as the extent of the contaminated or polluted region, the concentration of pollutants or contaminants, temperature, density of the soil, and whether the bioremediation procedure will take place in situ or ex situ (Jain and Bajpai 2012; Macaulay 2014; Adams et al. 2015; Abatenh et al. 2017).

11.2.2 Benefits of Bioremediation

Bioremediation provides several benefits compared to other clean-up procedures. By relying exclusively on natural procedures, it is a comparatively green technique that reduces impairment to the ecosystems (Adams et al. 2015; Abatenh et al. 2017). Habitually, bioremediation takes place underground, where alterations and microorganisms could be impelled, so as to eliminate pollutants or contaminants in groundwater and soil. Subsequently, bioremediation does not disturb neighbouring locations as compared to other clean-up procedures (Abatenh et al. 2017).

Bioremediation procedure generates comparatively few injurious by-products, primarily as a result of the fact that the pollutants or contaminants are transformed into water and other harmless gases such as nitrogen and carbon dioxide (Adams et al. 2015; Abatenh et al. 2017). Also, bioremediation is less expensive than most clean-up procedures, as it does not need extensive instruments or efforts.

11.3 Bioremediation in Soil

Industrially, soils could be polluted or contaminated through various sources, such as spill from petroleum/oil, chemicals, or/and heavy metals accrual from industrial emanations (Adams et al. 2015; Abatenh et al. 2017; Aigbe et al. 2020). Agriculturally, soils could be polluted or contaminated owing to the use of pesticides and other chemicals used for agricultural activities or by means of the heavy metals that are contained within agricultural products (Couto et al. 2014; Abatenh et al. 2017; Aigbe et al. 2020). Also, bioremediation has been used for effective bioremediation of heavily polluted environment where 2012 Olympics games was performed (Couto et al. 2014; Abatenh et al. 2017). Bioremediation was used for the cleansing of about 1.7 million cubic metres of severely contaminated soil to turn this brownfield location into one comprising sports infrastructure bounded by about 45 ha of wildlife habitats. Groundwater contaminated with ammonia was cleaned by means of a novel bioremediation procedure that saw archaeal microorganisms breaking down the ammonia into innocuous nitrogen gas (Couto et al. 2014; Macaulay 2014; Abatenh et al. 2017). The rehabilitated and transformed park marked the London 2012 Olympic Games as well as the Paralympic Games as the "greenest" and most sustainable games ever held; this was made possible by bioremediation procedures (Couto et al. 2014; Macaulay 2014; Abatenh et al. 2017).

While some soil cleansing procedures entail the introduction of new microorganisms, biostimulation procedures upsurge natural degradation procedures by inspiring the development of microorganisms that are previously existing (Adams et al. 2015). Natural or ordinary biodegradation procedures could be restricted by several factors, such as nutrient accessibility, temperature, or nature of soil moisture content. However, biostimulation procedures overcome these restrictions, providing microorganisms with the resources required, which upsurges their proliferation and results in improved rate of degradation (Das and Chandran 2011; Adams et al. 2015; Burghal et al. 2016).

Cleansing of oil/petroleum-contaminated soil is an illustration of where stimulating microbial development could be used for good outcome (Das and Chandran 2011; Adams et al. 2015; Burghal et al. 2016). Some research studies have revealed that poultry droppings could be used as a biostimulating agent, offering nitrogen and phosphorous to the process which enhances the natural development rate of oil/petroleum-degrading bacteria. Processes like these could demonstrate cost-effective and more eco-friendly alternatives to existing chemical treatment opportunities (Das and Chandran 2011; Adams et al. 2015; El-Borai et al. 2016; Burghal et al. 2016).

11.4 Bioremediation in Air

Air is contaminated by a diversity of volatile and explosive organic or biological composites formed by a series of industrial procedures (Jain et al. 2010, 2011; Abatenh et al. 2017). While organic or chemical cleansing has been applied for the cleansing of gases emitted from funnels, chimneys, or pipes, the newer procedure of biofiltration has been of great assistance for the cleansing of industrial gases. This technique encompasses passing contaminated air over a disposable matrix encompassing microbes that degrade pollutants into products that are non-toxic such as water, carbon dioxide, or salts. Presently, biofiltration is one of the biological organic procedures available for the remediation of airborne contaminants (Jain et al. 2010, 2011; Abatenh et al. 2017).

11.5 Bioremediation of Water

In some advanced western countries accessibility to clean, drinkable water and contemporary public health (sanitation) is something they do not handle with levity. Nevertheless, there are millions of persons especially in developing countries involved in the task to produce clean, drinkable water and contemporary public health (sanitation) is like a luxury (Demnerova et al. 2005; Aigbe et al. 2020).

From reports from the World Health Organization (WHO), it is estimated that about 842,000 persons die annually as a result of diseases (such as diarrhoea) caused by contaminated water. According to WHO, several of such deaths can be prevented by providing drinkable and potable water as well as appropriate contemporary public health sanitation. Approximately, 2.6 billion persons are deficient in any contemporary public health sanitation, with more than 200 million tons of industrial and human waste which are not treated annually (Adams et al. 2015; Abatenh et al. 2017).

Sewage treatment plants are the major and further most imperative bioremediation inventiveness in most part of the world especially developed countries (Adams et al. 2015). For example, in the United Kingdom, about 11 L of wastewater are collected and treated daily (Adams et al. 2015). Major ingredients of raw sewage are organic matter, suspended solids, phosphorus, and nitrogen. Wastewater circulated in a treatment plant is aerated so as to make oxygen available to bacteria that degrade organic material and contaminants. Microorganisms consume the organic pollutants and bind the less soluble portions, which could then be filtered off; the noxious ammonia is reduced to non-toxic nitrogen gas and is released into the atmosphere (Adams et al. 2015).

11.6 Influence of Microbes on Bioremediation of Wastes

Microbes are generally circulated on the biosphere; this is as a result of their inspiring metabolic capability as well as their capability to grow without difficulties under diverse environmental circumstances (Abatenh et al. 2017).

The nourishing adaptability of the microbe's community is another aspect for the exploitation for biodegradation of contaminants by means of bioremediation. According to Tang et al. (2007), microbial bioremediation is based on the capability of some variety of microorganisms to translate, change toxic contaminants to acquire energy, and also generate biomass in the progression. The process of bioremediation is a microbiologically efficient procedure, which degrades, breaks down, or transmutes pollutants to less noxious, essential, and composite forms by the collection and storage of the pollutants. The microbes which are basically the biological agents of bioremediation are known as "bioremediators"; distinctive leading examples of bioremediators are archaea, bacteria, and fungi (Strong and Burgess 2008; Abatenh et al. 2017).

Bioremediation entails the degradation, removal, alteration, immobilization, or detoxification of several physical and chemical wastes in the form of liquid, solid, or gases from the environment by means of the action of microbes such as bacteria, fungi as well as some other organism like plants. Microbes take part in this procedure by means of their enzymatic trails performance as "biocatalysts" and ease the development of the biochemical reactions for the degradation of the anticipated contaminant. The microbes perform these actions by accessing several composites that assist them in the generation of energy and nutritional substances for the development of more cells.

The effectiveness of any bioremediation procedure is influenced by some factors as well as the chemical attribute and concentration of the contaminants (El Fantroussi and Agathos 2005; Abatenh et al. 2017). The environmental physicochemical features and their accessibility to the microbes also influence bioremediation procedure (El Fantroussi and Agathos 2005; Abatenh et al. 2017).

The rate at which microbes carry out degradation is influenced by interaction nature of the microbes and contaminants as well as their environmental distribution, nature of the microorganisms, and contaminants. The monitoring, management, and optimization of bioremediation procedures are intricate processes, resulting from several factors. As reported by Abatenh et al. (2017), these factors include but not limited to the following:

- The presence of microbial inhabitants which are proficient for the degradation of the contaminants.
- The accessibility of pollutants to the microbial inhabitants.
- Environmental factors such as the kind of soil, the temperature range, pH value, existence of oxygen or additional electron acceptors, and available nutritional components.

11.7 Biological Influences on Bioremediation Procedures

Biological factors influence the degradation of organic composites as a result of the competition among microbes for inadequate carbon sources, unfriendly relations among microbes, or the predation of microbes by protozoa and bacteriophages. The rate at which the pollutants get degraded is habitually dependent on the concentration of the pollutant and the quantity of available "catalysts". Hence, the quantity of "catalyst" signifies the number of organisms which are capable of metabolizing the pollutant and the quantity of enzymes(s) generated by each of the cell (Abatenh et al. 2017).

The countenance of the specific enzymes by the cells could surge or reduce the pollutant degradation rate. Additionally, the level of contaminant, metabolism specification by enzymes, their "affinity" for the pollutant as well as the accessibility of the required pollutant will also contribute. The foremost biological influences as reported by Boopathy (2000), Madhavi and Mohini (2012), and Abatenh et al.

(2017) are "mutation, horizontal gene transfer, enzyme activity, interaction (competition, succession, and predation), its own growth until critical biomass is reached, population size and composition".

11.8 Environmental Influences on Bioremediation Procedures

The metabolic physiognomies of the microbes as well as the physicochemical physiognomies of the targeted pollutants are the foremost possible interactive determinant during the procedure. However, this effective foremost possible interaction is regulated by environmental circumstances of the location. The microbial development and action are influenced by the pH value, temperature conditions, moisture content, soil structure, soluble nature of the water, available nutritional substances, site physiognomies, chemical reactions and available oxygen as well as inadequate technical expertise in this field, and physicochemical bioavailability of contaminants. This physicochemical bioavailability of the contaminants are pollutant concentration, category, solubility, chemical structure, and noxiousness (Madhavi and Mohini 2012; Adams et al. 2015; Abatenh et al. 2017). According to Adams et al. (2015), these factors regulate the kinetics of the degradation procedure.

Biodegradation could take place under a high pH range of 6.5–8.5, which is normally ideal for biodegradation in water (aquatic systems) and land (terrestrial systems). Moisture or humidity affects the rate of contaminants metabolism. According to Cases and de Lorenzo (2005), it is as a result of its influence on the type and quantity of soluble constituents which are present and the osmotic pressure as well as the pH range of the environment. Several authors have reported various categories of microbes and the composites used for bioremediation procedures, some of selective ones are summarized in Tables 11.1, 11.2, 11.3, and 11.4 as revised from Abatenh et al. (2017).

Heavy metals hardly degrade or breakdown biologically, but vicissitudes could take place in the nuclear structure of some metals. They can however be converted from one form to another by oxidation or by some biochemical reactions. Nevertheless, bacteria have been proven effective for some heavy metals' bioremediation. According to Abatenh et al. (2017), most microbes have the capabilities to shield themselves from heavy metal harmfulness by means of some mechanisms, such as "adsorption, ingestion, methylation, oxidation and reduction reactions". Microbe's ingestion of heavy metals is by means of bioaccumulation procedure (active) and/or by means of adsorption procedure (passive). Microbial methylation is a vital procedure in bioremediation of heavy metals; the methylated composites are normally unstable. For instance, Jaysankar et al. (2008) reported that Mercury, Hg (II), could be biomethylated by means of diverse bacterial types such as "*Alcaligenes faecalis,*

Table 11.1 Microbes used for oil bioremediation

Microbes	Composites	References
Fusarium sp.	Oil	Hidayat and Tachibana (2012)
Alcaligenes odorans, Bacillus subtilis, Corynebacterium propinquum, Pseudomonas aeruginosa	Oil	Singh et al. (2013)
Bacillus cereus A	Diesel oil	Maliji et al. (2013)
Aspergillus niger, Candida glabrata, Candida krusei, and Saccharomyces cerevisiae	Crude oil	Burghal et al. (2016)
B. brevis, P. aeruginosa KH6, B. licheniformis, and B. sphaericus	Crude oil	El-Borai et al. (2016)
Pseudomonas aeruginosa, P. putida, Arthrobacter sp.*, and Bacillus* sp.	Diesel oil	Sukumar and Nirmala (2016)
Pseudomonas cepacia, Bacillus cereus, Bacillus coagulans, Citrobacter koseri, and Serratia ficaria	Diesel oil, crude oil	Kehinde and Isaac (2016)

Table 11.2 Microbes involved in dyes bioremediation

Microbes	Composites	Authors
B. subtilis strain NAP1, NAP2, NAP4	Oil-based based paints	Phulpoto et al. (2016)
Myrothecium roridum IM 6482	Industrial dyes	Jasinska et al. (2012, 2013, 2015)
Pycnoporus sanguineous, Phanerochaete chrysosporium, and Trametes trogii	Industrial dyes	Jasinska et al. (2015)
Penicillium ochrochloron	Industrial dyes	Shedbalkar and Jadhav (2011)
Micrococcus luteus, Listeria denitrificans, and Nocardia atlantica	Textile azo dyes	Hassan et al. (2013)
Bacillus spp. *ETL-2012, Pseudomonas aeruginosa, Bacillus pumilus HKG212*	Textile dye (remazol black B), sulfonated di-azo dye, reactive red HE8B, RNB dye	Maulin et al. (2013), Yogesh and Akshaya (2016), Das et al. (2015)
Exiguobacterium indicum, Exiguobacteriumaurantiacums, Bacillus cereus, and Acinetobacter baumannii	Azo dye effluents	Kumar et al. (2016)
Bacillus firmus, Bacillus macerans, Staphylococcus aureus, and Klebsiella oxytoca	Vat dyes, textile effluents	Adebajo et al. (2017)

Table 11.3 Microbes used for some heavy metals

Microbes	Composites	References
Saccharomyces cerevisiae	Heavy metals, lead, mercury, and nickel	Chen and Wang (2007), Talos et al. (2009), Infante et al. (2014)
Cunninghamella elegans	Heavy metals	Tigini et al. (2010)
Pseudomonas fluorescens and, Pseudomonas aeruginosa	Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu^{2}	Paranthaman and Karthikeyan (2015)
Lysinibacillus sphaericus CBAM5	Cobalt, copper, chromium, and lead	Peña-Montenegro et al. (2015)
Microbacterium profundi strain Shh49T	Fe	Wu et al. (2015)
Aspergillus versicolor, A. fumigatus, Paecilomyces sp., *Paecilomyces* sp., *Trichoderma* sp., *Microsporum* sp., *Cladosporium* sp.	Cadmium	Soleimani et al. (2015)
Geobacter spp.	Fe (III), U (VI)	Mirlahiji and Eisazadeh (2014)
Bacillus safensis (JX126862) strain (PB-5 and RSA-4)	Cadmium	Priyalaxmi et al. (2014)
Pseudomonas aeruginosa, Aeromonas sp.	U, Cu, Ni, Cr	Sinha et al. (2011)
Aerococcus sp., *Rhodopseudomonas palustris*	Pb, Cr, Cd	Sinha and Paul (2014), Sinha and Biswas (2014)

Table 11.4 Potential biological agents for bioremediation of pesticides-contaminated environment

Microbed	Composites	References
Bacillus, Staphylococcus	*Endosulfan*	Mohamed et al. (2011)
Enterobacter	Chlorpyrifos	Niti et al. (2013)
Pseudomonas putida, Acinetobacter sp., *Arthrobacter* sp.	Ridomil MZ 68 MG, Fitoraz WP 76, Decis 2.5 EC, malation	Mónica et al. (2016), Hussaini et al. (2013)
Acinetobacter sp., *Pseudomonas* sp., *Enterobacter* sp., *and Photobacterium* sp.	Chlorpyrifos and methyl parathion	Ravi et al. (2015)

Bacillus pumilus, Bacillus sp., *P. aeruginosa, and Brevibacterium iodinium* to gaseous methyl mercury”.

11.9 Forms of Bioremediation Procedures

Presently, we have various forms of techniques of wastes treatment by means of bioremediation procedures. The foremost bioremediation techniques are “biostimulation, bioattenuation, bioaugmentation, bioventing, and biopiles”.

11.9.1 Biostimulation

This form of bioremediation procedure is by means of the injection of some specific nutritional substances into the location which could be soil or groundwater for the stimulation of the activity of indigenous microbes. This technique focuses more on the stimulation of indigenous or microbes (fungus and bacteria) inhabitants that exist naturally, primarily by providing fertilizers and some nutritional supplements as well as trace minerals. After which, this provides other environmental necessities such as "pH, temperature, and oxygen" that would assist to accelerate the metabolism rate and trail (Kumar et al. 2011; Adams et al. 2015; Abatenh et al. 2017). According to Abatenh et al. (2017), the existence of little quantity of contaminants could as well serve as stimulants by revolving the operons meant for bioremediation enzymes. This type of premeditated trail in most cases unstained additional nutritional substances and oxygen to assist the natural microbes. These nutritional substances are the fundamental part of life and assist the microbes in the generation of other basic necessities such as cell biomass and energy as well as enzymes that breakdown the contaminants. However, phosphorous, nitrogen, and carbon are all required for this process (Madhavi and Mohini 2012; Abatenh et al. 2017).

11.9.2 Bioattenuation

This is the natural attenuation, which involves the annihilation of contaminant concentrations from the environment. Abatenh et al. (2017) reported that it normally places within biological procedures which could sometimes include aerobic and anaerobic biodegradation, plant and animal ingestion, as well as some physical tendencies such as "advection, dispersion, dilution, diffusion, volatilization, and sorption/desorption". It also includes some chemical reactions such as "ion exchange, complexation, and abiotic transformation". Moreover, it has been observed that natural attenuation also involved some process such as intrinsic remediation or biotransformation of heavily polluted environment (Mulligana and Yong 2004). According to Li et al. (2010), whenever the environment is contaminated with some constituents, they could naturally clean up by the following:

1. Microbes that exist in soil and groundwater apply some chemical composites for nourishment. These chemical composites could modify them into water and innocuous gases, as soon as they entirely digest the chemical composites.
2. Chemical composites can stick or sorb to soil, which holds them in place. This does not clean up the chemicals, but it can keep them from polluting groundwater and leaving the site.
3. When the contamination is transmitted via soil and groundwater, it could combine with uncontaminated water and this will cause a reduction dilution of the contamination.

4. Several chemical composites, such oil and diluents or solvents, could dissolve, which implies that they could change from one state to another like liquids to gases or vice versa within the soil. If these substances in the soil dissolve in the air, sunlight could extinguish them. If the natural attenuation is not fast or complete in time, bioremediation would need to be improved by means of biostimulation or bioaugmentation.

11.9.3 Bioaugmentation

This is another technique of biodegradation which involves the addition of contaminant degrading microbes to enhance the biodegradative dimensions of natural microbial inhabitants on the contaminated regions. This is done to swiftly increase the growth rate of the natural microbe inhabitants as well as to improve the degradation procedure that favourably accommodates the contaminated areas. In this technique, collection of the microorganisms is from the remediation areas, and these microorganisms are cultured separately, genetically improved, and brought back to the location (Niu et al. 2009). This procedure involves the addition of engineered microorganisms so as to swiftly and completely eradicate composite contaminants. Furthermore, genetically modified microbes have proven to have the ability which could upsurge the degradative efficacy of several environmental contaminants; this is as a result of having various metabolic outline that could change into less intricate and innocuous end products (Malik and Ahmed 2012; Alwan et al. 2013; Gomez 2014). Natural microbial species are reportedly not that fast in breaking down some composites, to facilitate the speed; such natural microbes should be genetically modified by mean of “DNA manipulation”; genetically engineered microbes could be applied in this regard (Sayler and Ripp 2000; Thapa et al. 2012).

11.9.4 Genetically Engineered Microbes

These are microbes whose genetic constituents have been previously altered by means of genetic engineering procedures, stimulated by natural or artificial genetic exchange among microbes and it is mostly called “recombinant DNA technology”. Genetic engineering has enhanced the utilization and eradication of hazardous wastes under laboratory circumstances by generating genetically modified organisms (Jain et al. 2011). Genetically engineered microorganisms are obtained by “genetic recombination of DNA techniques or by natural genetic material exchange between organisms”. Currently we are able to supplement the appropriate gene for the production of some enzymes which could degrade some contaminants (Jain et al. 2010, 2011). This technique has demonstrated some great potential for bioremediation applications in “the soil, groundwater, and stimulated sludge environments”, demonstrating improved degradative abilities encompassing several chemical contaminants.

There are presently several opportunities for the improvement of degradative performance using genetic engineering approaches as we can see in “the rate-limiting steps known as metabolic pathways” which could be genetically deployed to produce enhanced degradation rates, or entirely “new metabolic pathways” that could be merged into “the bacterial strains” for the previously degraded recalcitrant composites.

For creation of genetically engineered microbes, four basic approaches are carried out:

(a) The alteration of the enzyme specificity as well as the enzyme affinity.
(b) The creation and regulation of the trail.
(c) The development, monitoring, and management of the bioprocess.
(d) The application of the bioaffinity and bioreporter sensor for chemical sensing, reduction of the toxicity as well as evaluation of the end point.

The required genes of the microbe are present on a single chromosome but the genes stipulating the enzymes which are required for the catabolism reactions of some of these unstable composites are present on plasmids which are drawn in the catabolism reaction. Consequently, genetically engineered microbes could be used efficiently for biodegradation and also represent future research frontier with wide consequences (Kulshreshtha 2013; Abatenh et al. 2017).

Also, the benefits of using the genetically engineered microbes in contaminated environment entail high catalytic effectiveness with the use of little quantity of cell mass and are eco-friendly while it has some limitations such as the low survival rates of cells in some cases. At some instant, there could be a delay in the growth and composites degradation process, periodic discrepancy and some other abiotic factors could also cause the instability, and this could cause direct and indirect effect on the association of the microbial action. It could also introduce some external modified strain to the settings, making it inactive and could cause several antagonistic effects on the physical, structural, chemical, and functional properties of the microbial inhabitants’ configuration and existence.

11.9.5 Bioventing

This technique has to do with the uttering of oxygen via the soil in order to enhance the growth of natural or the introduced microorganisms such as bacteria and fungus in the soil via the provision of oxygen to existing microorganisms present in soil. Efficient aerobically degradable composites get degraded in the presence of little airflow rates due to the provision of oxygen for the sustenance of microbial actions. Oxygen is mostly provided by means of direct air injection to the remaining adulteration in soil via wells. By this means, adsorbed fuel residuals are biodegraded as well as unstable composites are biodegraded as vapours and slowly move by means of biologically active soil. Contemporary bioremediation of crude oil contaminated in the soil has been demonstrated by several researchers by the application of bioventing (Lee et al. 2006; Agarry and Latinwo 2015).

11.9.6 Biopiles

This technique involves the treatment of contaminated soil by the excavation of the contaminated soil with "aerobically remediable hydrocarbons". Biopiles could also be called "biocells, bioheaps, biomounds, and compost piles". They are applied for the reducing concentrations of petroleum contaminants in the excavated soils during the biodegradation process. This procedure entails that air is provided to the biopile structure via piping and pumps air into the pile under "positive pressure" or by drawing air via the pile under "negative pressure" (Delille et al. 2008). The microbial activity is enhanced through microbial respiration that results in the degradation of adsorbed petroleum pollutant at a higher rate (Emami et al. 2012).

11.10 The Future of Bioremediation

Microbes play a significant role in bioremediation procedure; at the moment, it is an evolving research area that requires appropriate attention because microbes are eco-friendly and auspicious valuable inherent substance for the mitigation and management of environmental contaminations and hazards that have incessantly threatened human existence. Microorganisms serve as the crucial alternative solution for overcoming these challenges by means of bioremediation. Although bioremediation is not a new procedure, nevertheless, as our acquaintance of the causal microbial reactions grow, the capability to apply them to our benefit upsurges (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016). Normally, bioremediation involves a smaller amount of resources and energy compared to the conventional or traditional technology. Also, bioremediation does not accrue harmful by-products as waste. Bioremediation has methodological and cost benefits, even though the procedure normally takes more time compared to the conventional or traditional procedures.

Bioremediation could be channelled to the needs of the contaminated location in question, and the precise microorganisms required for the degradation or breaking down of the contaminant are encouraged by choosing the restraining factor required for the promotion of their development. This channelling could be further enhanced by means of biological or organic synthetic tools of microorganisms' pre-adaptation in environmental pollution to which they remain included.

Environmental contamination or pollution is a threat not only to human health but also could cause serious environmental damages to other living organisms, wildlife as well as globe sustainability. Impairment to the soils affects food production and safety. Bioremediation has been recognized as a sustainable tool for effective reduction and removal of pollution or contamination water as well as to ensure a non-toxic air, and maintenance of soil to be in good physical and healthy shape for upcoming generations.

11.11 Biomonitoring

Biological monitoring “is the measurement and assessment of agents or their metabolites either in tissues, secretes, excreta, espied air or any combination of these to evaluate exposure and health risk compared to an appropriate reference”. According to Berlin et al. 1984, this definition was from “the seminar of 1980, which was jointly sponsored by the European Economic Community (EEC), National Institute for Occupational Safety and Health (NIOSH) and Occupational Safety and Health Association (OSHA)”. Monitoring is a monotonous, consistent, and precautionary activity which is intended to lead, if required, to counteractive activities; it ought to not be confused with investigative diagnostic measures (Hamza et al. 2016).

Biological monitoring belongs to one of the three vital tools for preventing diseases and infections as a result of noxious agents that are present in the occupational/industrial or overall environment. The other two are environmental monitoring and health surveillance.

The categorization in the potential growth of such infection or disease is source, exposed chemical agent, internal dose, biochemical or cellular effect (reversible), health effects, and infection or disease (Berlin et al. (1984).

When noxious or harmful constituents such as chemicals from the industry are present in the environment, it pollutes and infects food, air, water, or even living organisms when they come in contact with the body (Dadrasnia et al. 2017). The quantity of noxious agents in these media is normally assessed and evaluated by means of environmental monitoring. Due to the absorption, circulation (distribution), metabolism (breakdown process), and emission (excretion), precise internal quantity of the noxious agent that is the net or remaining quantity of a contaminant absorbed in or passed through the microorganism over a precise time interval is efficiently brought to the body and becomes noticeable in fluids context of the body (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016). Consequently upon its interaction with a receptor in the critical organs (which are under precise situations of exposure, displays the initial or the furthermost significant adverse consequence), biochemical and cellular proceedings ensue. Both the internal quantity absorbed and the produced biochemical and cellular effects could be quantified by means of biological monitoring (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016).

According to the 1980 seminar of EEC/NIOSH/OSHA, health surveillance is “the periodic medico-physiological examination of exposed workers with the objective of protecting health and preventing disease” (Berlin et al. 1984).

Both biological monitoring and health surveillance are portions of a continuum that involves the measurement and quantification of agents or their metabolites in the body by way of assessment and evaluation of biochemical as well as the cellular consequences, for detecting the indications of initial reversible consequence of the critical organ. However, detecting reputable and established infection or disease is not within the scope of these assessments and evaluations.

11.11.1 Goals of Biological Monitoring

There are basically two categories of biological monitoring, viz. (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016):

(a) Monitoring of exposure
(b) Monitoring of consequence

The main purpose of biological monitoring of exposure is to measure and evaluate the amount of health hazard by assessing and evaluating the internal amount intake; this is done so as to achieve approximate value of the biologically or organically active body problem of the contaminant in question. The rationale behind this is to make sure that the exposure of employee or members of staff of such industry does not exceed the levels capable of causing adverse effects (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016). It is adverse in the sense that there is a damage of functional capacity, a diminished capability to compensate for further stress, a diminished capability to sustain homeostasis (a steady state of equilibrium), or an improved vulnerability to other influences of the environment (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016).

Biological monitoring of consequences is expectedly for the purpose of identifying initial and reversible modifications which advance in the critical organ, and which can simultaneously detect persons with indications of adverse health consequences (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016). Consequently, biological monitoring of consequences signifies the major device for the health surveillance of employees or workers.

11.11.2 Major Monitoring Devices in Biomonitoring

Biological monitoring of exposure is built on the determination of indicators of internal amount intake of the contaminants by means of measuring the following (Cydzik-Kwiatkowska and Zielińska 2016; Dadrasnia et al. 2017; Hamza et al. 2016):

- The quantity of the contaminants, to which an individual is exposed to.
- The quantity of contaminants that are available in the same fluids content of the body.
- The concentration of explosive biological or organic composites in alveolar air.
- The biologically or organically effective amount of intake of composites which have generated adducts to "DNA" or other bulky particles and which consequently have a possible genotoxic consequence.

Biological monitoring of consequences is achieved via the determination of indicators of consequence, that is, those that could detect initial and reversible modifications. This method can offer an indirect approximation of the quantity of chemical bound to the locations of action and provides the opportunity for the assessment and evaluation of functional modifications in the critical organ in an initial stage (Refs).

Some examples of the application of this method are:

1. The inhibition of pseudo-cholinesterase by organophosphate insecticides,
2. The inhibition of δ-aminolaevulinic acid dehydratase (ALA-D) by inorganic lead, and
3. The increased urinary excretion of *d*-glucaric acid and porphyrins in subjects exposed to chemicals inducing microsomal enzymes and/or to porphyrogenic agents (e.g. chlorinated hydrocarbons).

11.11.3 Benefits of Biological Monitoring

For constituents that apply their harmfulness after entering living organisms, biological monitoring offers a more intensive and targeted assessment and evaluation of health hazard than those of environmental monitoring and management.

Biological or organic parameters reflecting the internal amount of intake bring a proper systemic understanding of the adverse effects when compared to any environmental measurement procedure. Biological monitoring provides several benefits when compared to environmental monitoring and management procedure. Particularly, biological monitoring permits the assessment and evaluation of the following:

- Exposure over a prolonged period.
- Exposure on account of worker mobility in the employed environment.
- Absorption of a constituent by means of several routes, as well as the skin.
- Complete exposure as a consequence of diverse sources of effluence; both work-related and non-work-related.
- The amount of a constituent absorbed by an individual depending on influences other than the level of exposure, like the physical effort needed by the occupation, ventilation, or weather/climate.
- The amount of a constituent absorbed by an individual depending on individual influences that could influence the toxic kinetic of the noxious agent in the living organism, such as sex, age, genetic features, or functional state of the body part

(organ) where the noxious constituent experiences biotransformation and abolition.

11.11.4 Limitations of Biological Monitoring

Notwithstanding these benefits, biological monitoring still accounts for some substantial limitations. The following are the furthermost substantial limitations of biological monitoring:

- The quantity of possible constituents which could be monitored biologically or organically is at present still somewhat small.
- In the circumstance where there is a severe exposure, biological monitoring needs valuable information only for exposure to constituents that are swiftly metabolized, for instance, aromatic diluents or solvents.
- The consequence of biological or organic indicators has not been evidently distinct.
- Usually, biological or organic indicators of internal amount of intake allow assessment and evaluation of the extent of exposure, but do not provide information that will assist adequately for the measurement of the definite quantity present in the critical body part (organ).
- Frequently, there is no information of possible interference in the metabolism of the constituents being monitored by other exogenous constituents to which the living organism is concurrently exposed in the place of work and the environment in general.
- Inadequate knowledge of the associations existing between the degrees of environmental exposure and the degrees of the organic or biological indicators on the one hand, and between the degrees of the organic or biological indicators and possible health consequences on the other hand.
- The quantity of biological or organic indicators for which biological or organic exposure indices (BEI) occur at present is somewhat limited. Supplementary information is needed to ascertain whether a constituent, currently known is not capable of instigating an adverse consequence, could in future be revealed to be detrimental.
- Biological or organic exposure indices habitually signify a degree of an agent that is most probable to be detected in a specimen collected from a healthy person who has been exposed to the contaminant to the same level as a person with an inhalation exposure to the threshold limit value (TLV) time-weighted average (TWA).

11.11.5 Essential Information for the Development of Procedures and Measures for Choosing Biological Tests

The following are some of the basic conditions for programming organic or biological monitoring:

- Toxicokinetics: Information about the metabolism of the exogenous constituent living organisms.
- Toxicodynamics: Information about the modifications that ensue in the critical organ.
- Presence of indicators.
- Presence of appropriate and accurate investigative procedures.
- Opportunity of using available and accessible biological or organic samples on which the indicators can be quantified.
- Presence of intake-effect and intake-response relationships as well as information of these relationships.
- Prognostic validity (the extent to which the variable under contemplation envisages the circumstances as it actually is) of the indicators. The validity is determined by combining the sensitivity and specificity. If the assessment possesses a lofty sensitivity, this implies that it will give limited incorrect negatives; if it possesses lofty specificity, it will give limited incorrect positives.

11.12 Conclusion and Future Recommendation to Knowledge

This chapter has provided comprehensive information on the application of beneficial microorganism for the maintenance of a cleaner environment as well as detailed information on the application of beneficial microorganism for the achievement of environmental sustainability. Special emphasis was laid on some specific examples of sustainable biotechnology techniques that could lead to the maintenance of a cleaner environment. The different types of beneficial microorganism were also highlighted. Hence, there is a need to perform the application of these bioremediation techniques on a larger scale, most especially on field trial by placing more emphasis on the environmental outcomes of the end products. There is a need to identify some unique microorganisms that have not been exploited in the environment through the application of metagenomics as well as to identify necessary gene that regulates the bioremediation activities that could lead to the maintenance of a cleaner environment. There is a need to intensify more effort on some novel biomimetic practices and bioremediation that could lead to more effective sustainable environmental management, which might be applied for the sustainable treatment of polluted soil, groundwater, recalcitrant chemicals, contaminated air, and surface water.

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Chapter 12
Artificial Intelligence and Internet of Things in Instrumentation and Control in Waste Biodegradation Plants: Recent Developments

Kingsley Eghonghon Ukhurebor, Wilson Nwankwo, Charles Oluwaseun Adetunji, and Ayodeji Samuel Makinde

Contents

Abstract In recent times, millions of researches have been directed to exploring the potentials of Artificial Intelligence (AI) and Internet of Things (IoT). With enhanced capabilities offered through the integration of artificial intelligence, big data, and the cloud, the future might be descending into a new era defined and determined by artificial intelligence and Internet of Things. Across various jurisdictions and socio-political systems, the perspective on artificial intelligence technologies is rapidly converging. Vladimir Putin, the Russian President, in a statement published on

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4 September 2017 by the Verge, had stated unequivocally that “Artificial intelligence is the future and that the nation that leads in artificial intelligence research would certainly dominate in global affairs.” Thus, it appears that every facet of governance including environmental management is very likely to be redefined by artificial intelligence and allied technologies. In today’s traditional and highly urbanized cities, waste management has remained a matter of great concern among decision makers and public sector operatives. In the past, a number of traditional approaches such as incineration, concentration and subsequent deposition in landfills, recycling, gasification, etc. had been employed but are becoming increasingly unsustainable owing to side-effects they pose ranging from secondary pollution, diseases and epidemics, high cost of maintenance, climate change, etc. The quest for sustainable, safer, and eco-friendly alternatives coupled with current advances in nanotechnology, biotechnology, artificial intelligence and internet of things, soft systems such as biodegradation plants that utilize microbial agents under controlled conditions, have become a more prospective alternative compared to customary channels. This chapter adopts a swift narrative and analytical approach in articulating the critical and recent advances in the exploitation of artificial intelligence and internet of things technologies in the design, deployment, and management of waste biodegradation plants.

Keywords Artificial Intelligence · Internet of things · Biodegradation · Wastes · Techniques

12.1 Introduction

Wastes generation will be an inevitable aspect of human endeavor, as long as we continue to make use of the various natural resources for the development and advancement of our societies. Nevertheless, the techniques and means by which we dispose, monitor, control, and manage these wastes is another issue entirely. Several studies have reported that there are adverse influences of these wastes that are customarily generated via agricultural and industrial activities as well as some domestic activities (Aigbe et al. 2020; Hamza et al. 2016; Mittal 2011; Sneha 2015; Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017). As a result of these adverse influences of these domestic, agricultural, and industrial wastes, which are contributing to several environmental and climatic problems as well as telecommunication challenging issues (Ukhurebor and Umukoro 2018; Ukhurebor and Azi 2019; Ukhurebor et al. 2019, 2020a, b; Ukhurebor and Nwankwo 2020), there has been continuous effort to find the most appropriate and suitable as well as eco-friendly techniques that will not contribute further to the challenges ensuing from these wastes. Several conventional or traditional techniques were initially employed for disposing, monitoring, controlling, and management of these wastes but some of these conventional or traditional techniques were found to be inappropriate, unsuitable, and non-eco-friendly (Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017; Adebayo and Obiekezie 2018). However, of recent the use of microbial treatment processes for the wastes has attained increasing consideration due to its capability and high proficiency compared to conventional techniques (Adebayo and Obiekezie 2018; Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017). Customarily, these wastes could be categorized into two broad groups, viz. biodegradable (recyclable) wastes and non-biodegradable (non-recyclable) wastes (Adebayo and Obiekezie 2018; Ansari 2011; Ivanov 2010).

The biodegradables wastes are also known as "biowastes." They are those wastes that are generated either via domestic, agricultural, or industrial activities, that can be broken down in a process known as decomposition by means of microorganisms (Adams et al. 2015; Adebayo and Obiekezie 2018; Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017). Supposedly, they are reported to barely constitute a main source of contamination to the environment for a lengthened period (Adebayo and Obiekezie 2018).

These biodegradable wastes, even with their capability to undertake degradation without much effort via the use of microorganisms within a short period of time, are also faced with some constraints. Such limitations are the budding aggressive aroma and irritation defects to the environment when compared to the non-biodegradable wastes as well as their ability in instituting the environment with several healthy pathogenic microbes which can infect fresh food produce (Adebayo and Obiekezie 2018; Waites et al. 2011; Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017).

The non-biodegradable wastes are those wastes that are habitually not broken down or degradable by means of microorganisms. This implies that such wastes require other measures for their disposal, monitoring, controlling, and management (Waites et al. 2011; Adebayo and Obiekezie 2018; Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017).

Appropriate disposal, monitoring, controlling, and management of domestic, agricultural, and industrial wastes as well as other environmental contaminants will assist greatly in the reduction or elimination of the incessant adverse environmental influences on all living organisms as well as supports economic, environmental and agricultural sustainability development and enhanced the quality of our well-being generally (Ezeonu et al. 2012; Nwankwo and Ukhurebor 2019; Aigbe et al. 2020; Nwankwo et al. 2020a, b, c).

Undoubtedly, several techniques that have been developed for the proper disposal, monitoring, controlling, and management of both the biodegradables and non-biodegradables wastes have yielded some potential solutions. These various techniques are geared toward the achievement of quality standards that will ensure environmental protection and sustainability. In order to have a proper understanding and control procedures of wastes treatment techniques, it is necessary and paramount to comprehend the role that is carried out by microbes' community and structure that are involved in these treatment procedures. However, the biodegradation procedure which has been considered one of the most suitable in terms of cost-effectiveness and eco-friendly can be enhanced by the application of Artificial Intelligence (AI) and Internet of Things (IoT) in the instrumentation, control monitoring and management of wastes (Zhang et al. 2014; Cydzik-Kwiatkowska and Zielińska 2016; Meerbergen et al. 2016; Dadrasnia et al. 2017; Waites et al. 2011; Adebayo and Obiekezie 2018).

Hence, this chapter will attempt to give a comprehensive account on the applications of Artificial Intelligence and Internet of Things for the control, monitoring, and management of biological or organic wastes by means of biodegradation technique in plants and the recent developments and future advancement in order to have an inclusive understating of microbial features needed in the control, monitoring, and management of biological or organic wastes so as to exploit its numerous benefits and advantages in attaining the much anticipated environmental sustainability and eco-friendly environment.

12.2 Application of Artificial Intelligence and Internet of Things in Waste Biodegradation

Since its birth of artificial intelligence in 1958 at Darmouth College, artificial intelligence has undergone a series of excellent developments and has emerged not only a discipline but also a suite of technologies and machineries that have inspired other technologies (Ramos et al. 2013; Serdeczny et al. 2017; Ye et al. 2020). Artificial intelligence is rapidly changing the trend and schemes in all socioeconomic, biological, biochemical, medical as well as political climes (Ramos et al. 2013; Serdeczny et al. 2017; Ye et al. 2020). Several governments across the globe are sponsoring artificial intelligence researches, as it is evident that the future control of socioeconomic activities and governance are more probably affected in all respects by this influential technology called artificial intelligence (Ramos et al. 2013; Serdeczny et al. 2017; Ye et al. 2020). From manufacturing, production, agriculture, biological, medical to business and finance, artificial intelligence has gained a weighty foothold and more subsectors are being remodelled and refined by artificial intelligence (Ramos et al. 2013; Serdeczny et al. 2017; Ye et al. 2020).

Artificial intelligence finds very useful application in controlled system platforms. In recent times, platforms whether hardware or software are simply intelligent or unintelligent depending on whether or not some level of artificial intelligence algorithms or functionalities are incorporated into the platform (Ramos et al. 2013; Serdeczny et al. 2017; Ye et al. 2020). As decision makers and public operatives contend with the challenges of governance, platforms that could assist in providing solutions to socioeconomic challenges usually emerge the center of focus.

In urban areas in some West Africa nations such as Lagos (Nigeria), Accra (Ghana), and Lomé (Togo), waste management is a serious business for any responsive government. It is commonplace to have agencies with legitimate mandates directed toward ensuring that the society is not bedevilled by the menace of waste accumulation in and around the environment (Adewuyi et al. 2014; Ola-Adisa et al. 2015; Kofoworola 2016; Omole et al. 2016; Ogunmakinde et al. 2019). In Lagos (Nigeria), for instance, the Lagos Waste Management Authority (LAWMA), a public agency conducts all tasks around waste management within the geographical environment of Lagos State. In such agency, various units and divisions exist that undertake periodic process re-engineering to ensure that as the society evolves into a megacity, sophisticated platforms are deployed to cater for such challenges especially in the realm of waste management. Though, in various quarters, LAWMA has been tagged an efficient and proactive agency, it is however not uncommon to notice the several challenges it faces in ensuring the timely management of wastes. Solid waste disposal by the agency is by way of routine collection and transportation to dumpsites (Omole et al. 2016; Ogunmakinde et al. 2019). The agency operates eight dumpsites scattered across various locations. These sites present some awful sights to anyone who visits such locations. The effectiveness of the agency's programs is marred by the growing population, which are not in any dimension proportional to the infrastructure managed by the agency. The pattern in Lagos is similar to what obtains in many urban cities in developing countries especially in sub-Saharan Africa.

12.3 Biodegradation as Alternative Waste Management Approach

Biodegradation involves the application of biological agents especially microbes (viruses, protozoa, bacteria, fungi, algae) to the reduction of waste burden in the environment. Biodegradation is one silent eco-friendly and sustainable approach to management of liquid wastes and a range of solid wastes. Often, the agents of degradation, i.e., microbes, are derived from the waste itself making the entire process a consistent and continuous cycle that could be sustained without incurring heavy running expenses (Abatenh et al. 2017; Adams et al. 2015; Couto-Mendoza et al. 2014; Jain and Bajpai 2012; Das and Chandran 2011).

However, in biodegradation processes, specificity and precision are very important factors in that attention is paid to specific microbes in precise quantities, right combinations or microbial dosing, and specific optimal conditions. Such conditions are not limited to temperature, luminosity, pH, reduction-oxidation potentials, etc. of the bioreactor tank. Without strict adherence to such considerations, efficiency and/or efficiency in the entire process becomes doubtful. Accordingly, to meet such specificity requirements, a controlled environment would offer the best results.

Bioremediation made use of microbes for the reduction of contamination by means of biological or organic degradation or breakdown of contaminants into non-noxious constituents/materials. This process could either encompass aerobic or anaerobic microbes that habitually use this degradation or breakdown as a source of energy (Emami et al. 2012; Abatenh et al. 2017).

In major applications, the targeted goal is to ensure that the interaction of those specific microbes and the host environment does not create an additional burden on the entire degradation process. Good ethical principles would demand that prior to implementing such projects, the decision makers ought to be fully acquainted as to the efficacy of each selected bacteria because the rate of biodegradation across different species vary and this is also affected by prevailing environmental conditions.

For instance, prior to utilizing nitrobacter in bioreactors, the project designers ought to conduct an in-silico modelling of the ecosystem to reasonably understand the interaction between the microbes and specific elements and/or compound contained in the wastes, e.g., Cl^-, Br^-, NH_3, N_2, Mn^{2+}, H_2O, Fe^{2+}, etc. Understanding anaerobic and anaerobic processes would entail good knowledge of bio-physicochemical conditions, growth kinetics (of microbes), substrate utilization, and synthesis of products. The complexity of these processes entails sophisticated models (Ramachandran et al. 2019).

Bioremediation techniques or procedures are mainly grouped into three categories, viz. (Asira 2013; Jain and Bajpai 2012):

- In situ land treatment for soil and groundwater.
- Biofiltration of the air.
- Bioreactors (which is mostly involved in water treatment).

Bioremediation can be viewed as:

- It is biotechnological field where microorganisms and other living organisms eradicate contaminants or pollutants from the environment (soil, water, and air).
- It is conventionally used to clean oil/petroleum spills or/and contaminated or polluted groundwater.
- It could be by means of “in situ” at the site of the pollution or contamination, or “ex situ” away from the site of the pollution or contamination. This may be essential if the weather or climate is so cold for sustaining the microbe action, or if the soil is overdense for even distribution nutrients. Ex situ bioremediation may entail exhuming or excavating and cleaning the soil directly above the ground, and this could add substantial costs to the procedure.

Bioremediation depends on the stimulation in the development of certain microorganisms that exploit pollutants or contaminants such as petroleum/oil, solvents, and pesticides for various sources such as food and energy. These microorganisms translate the pollutant contaminants into small quantities of water and other non-toxic gases like carbon dioxide, nitrogen, etc. (Abatenh et al. 2017; Emami et al. 2012; Jain and Bajpai 2012).

Bioremediation involves the combination of the appropriate temperature, nutrients, and foods. The deficiency of these essential components could prolong the cleansing procedure of the pollutants or contaminants (Jain and Bajpai 2012; Macaulay 2014; Adams et al. 2015). Some conditions that are not suitable for bioremediation procedure could be enhanced by the addition of some environmental alterations, such as molasses, vegetable oil, or simple air. These alterations enhance the conditions for microorganisms to flourish, thus accelerating the achievement procedure (Adams et al. 2015). The bioremediation procedure could take several months or years to complete, depending on some parameters such as the extent of the contaminated or polluted region, the concentration of pollutants or contaminants, temperature, density of the soil, and whether the bioremediation procedure will take place in situ or ex situ (Jain and Bajpai 2012; Macaulay 2014; Adams et al. 2015; Abatenh et al. 2017).

Microbes are generally circulated on the biosphere; this is as a result of their inspiring metabolic capability as well as their capability to grow without difficulties under diverse environmental circumstances (Abatenh et al. 2017).

The nourishing adaptability of the microbe's community is another aspect for the exploitation for biodegradation of contaminants by means of bioremediation; this according to Tang et al. (2008) is based on the capability of some variety of microorganisms to translate, change, and apply toxic contaminants so as to acquire energy and also generate biomass in the progression. The process of bioremediation is a microbiological efficient procedural action which are use to degrade, breakdown, or transmute pollutes to less noxious essential and composite forms by the collection and storage of the pollutants. The microbes which are basically the biological agents of bioremediation are known as "bioremediators"; distinctive leading examples of bioremediators are archaea, bacteria, and fungi (Strong and Burgess 2008; Abatenh et al. 2017).

Bioremediation entails the degradation, removal, alteration, immobilization, or detoxification of several physical and chemical wastes both in the form of liquid, solid, or gases from the environment by means of the action of microbes such as bacteria, fungi as well as some other organism like plants. Microbes take part in this procedure by means of their enzymatic trails performance as "biocatalysts" and ease the development of the biochemical reactions for the degradation of the anticipated contaminant. The microbes perform these actions by accessing several composites that assist them in the generation of energy and nutritional substances for the development of more cells.

The effectiveness of any bioremediation procedure is influenced by some factors as well as the chemical attribute and concentration of the contaminants (El Fantroussi and Agathos 2005; Abatenh et al. 2017). The environmental physicochemical features and their accessibility to the microbes also influence bioremediation procedure (El Fantroussi and Agathos 2005; Abatenh et al. 2017).

The rate at which microbes undergo degradation is influenced by interaction nature of the microbes and contaminant as well as their environmental distribution nature of the microorganisms and contaminants. The monitoring, management, and optimization of bioremediation procedures is an intricate process, resulting from several factors. As reported by Abatenh et al. (2017), these factors include but not limited to the following:

- The presence of microbial inhabitants which are proficient for the degradation of the contaminants.
- The accessibility of pollutants to the microbial inhabitants.
- Environment influences such as the kind of soil, the temperature range, the pH value, the existence of oxygen or additional electron acceptors, and the available nutritional components.

12.4 Control of Biodegradation Processes Using Artificial Intelligence Technologies and Internet of Things

Artificial intelligence provides enormous tools for modelling, simulation, and validation of the potentials of specific microbes in biodegradation processes (Ruan et al. 2017). Not only are they valuable tools for modelling, they are also part of an integrated system in the waste processing cycles owing to intelligent interventions they offer in control and monitoring especially in system that are subject to uncertainties. Vox Creative (2018) notes that machine learning, robotics, internet of things, drones, etc. offer potential and unaided technologies that could enable the society achieve better results on understanding, monitoring, and control of waste degradation processes and other aspects of waste management.

The following applications have been employed in biodegradation in waste treatment:

(a) Use of artificial neural networks to predict the efficiency of bacteria in dislodging pollutants from wastewater (Ye et al. 2020).
(b) Optimization of environmental conditions.
(c) Intelligent control in aerobic phase of waste treatment (Ye et al. 2020).
(d) Detection of the progress of the aerobic stage vis-à-vis optimization of the reactor as against the conventional fixed timing that is susceptible to inaccuracies owing to uncertainties in biological processes (Ramos et al. 2013).
(e) AI-driven biosensors have been deployed for online and off-line sensing and control of uncertain parameters in environmental installations and wastewater plants (Sanders 2008; Sinha and Kaur 2019; Mao et al. 2019). Various sensors have been deployed for AI operations and they include:

    - Pressure sensors
    - Biosensors
    - Velocity sensors
    - Position sensors
    - Flow sensors
    - Optical sensors
    - Temperature sensors
    - Current and voltage sensors
    - Gage and force sensors

Table 12.1 Biosensors used in artificial intelligence-controlled biodegradation plants

Sensor	Reference parameters	Deployment
Amperometric sensors	Biochemical Oxygen Demand (BOD)	Water/wastewater treatment plants
Microbial BOD	BOD	Biodegradation plants/fermentation processes
Fuel cell	BOD, pH, COD, temperature, organic content, etc.	Biodegradation plants/waste treatment
Geobacter-enriched biofilm BOD	BOD	Biodegradation plants/waste treatment
Urease	Chromium ion	Industry effluent, heavy metal detection
Alkaline phosphatase	Concentration of toxic chemical	Heavy metal detection

- Gas sensors
- Chemical sensors
- Torque and strain

Biosensors are highly relevant in modern waste treatment. Sensors generate huge data, which could be used to control decision-making in these plants. IoT provides a formidable platform for relaying such data in real time to enable the control of mission-critical installations.

Several biosensors are appropriate in the design and deployment of treatment plants for biodegradation of liquid, gaseous, and solid wastes. Their deployment is not restricted to pre-treatment phases (in bioreactors and sedimentation tanks) but also in the post-treatment phases. Feedback from these sensors to the control software platforms is reasonably processed using AI tools such as fuzzy logic and neural networks. Table 12.1 presents some of these sensors.

(f) Estimation of concentrations of pollutants and monitoring performance of microbes in treatment plants.

12.5 Specific Examples of Artificial Intelligence and Internet of Things for Waste Biodegradation

Saminathan et al. (2019) present an automated municipal scrapper based on IoT that can automatically isolate dumped waste to create more recyclable material. They are developing an intelligent bin that can be configured to transmit information on waste biodegradation.

AI-based intelligent software is designed by Sottara et al. (2007) to recognize events analyzing the profiles of certain available signals, such as pH, and dissolved oxygen, allowing the process to maximize performance and detecting potential shortcomings. The authors demonstrate also how a decentralized network of experts who are managers and consultants to run an urban wastewater treatment plant fed with real sewage on an SBR pilot scale could be used with the various AI technology. In addition, the system is trained to remember an acceptable collection of reference signals, which are given context using Bayesian confidence techniques, via a SOM neural network.

Huang et al. (2017) suggest a smart hybrid approach to effectively modelling and simulating the biodegradation cycle of dimethyl phthalate in an anaerobic/anoxic/oxic wastewater process based on a fluid wavelet neural network. The proposed hybrid intelligent model will derive dynamic behavior and complex interrelationships from various water quality variables through self-learning and the memory capacities of neural networks, the management of ambiguity of fuzzy logic, the study of local information of wavelet transformations, and the global search for genetic algorithms.

Nasr and EL Shahawy (2016) studies the electrocoagulation method for the treatment of wastewater from an olive mill using bipolar aluminum electrodes. Surface response methodology and adaptive neurofuzzy inference system were used to study the effect on the removal of the chemical oxygen demands by operational parameters. The predicted chemical oxygen demand efficiency of 40.4% was close to the experimental outcome with a deciding coefficient $r^2 = 0.92$ at the predictable status of initial pH 4, current density 83 mA cm^{-2}, and an electrolyte period of 20 min. Tests of the adaptive neurofuzzy inference method showed that the order of operating parameters influencing the removal of chemical oxygen needs was pH > current density > electrolysis time. This research shows that the neurofuzzy adaptive infusion system can be used as a tool to explain the factors that affect the process of electrocoagulation.

12.6 Advantages and Disadvantages of Artificial Intelligence and IoT in Instrumentation and Control in Waste Biodegradation

In order to enhanced the effectiveness and the process involved in the maintenance of high level of cleaning process in the environment. Most of these techniques are built on IoT and artificial intelligence which are adequately executed and carried out.

The major objective of incorporating smart cities constitutes a major goal of developing and developed countries. The incorporation of data access networks, IoT, geographic information systems, combinatorial optimization and their execution by the action of electronic engineering have been identified to play a crucial role in the waste management of a particular city through the application of necessary organization scheme (Reyes-Gutiérrez et al. 2015).

The application of IoT integrated trash cans has persuaded the capability of the can to sense and disseminate all the collected data most especially from the trash volume back to the server most especially through the application of internet. The application of graph theory optimization methodology and related algorithms have enabled the data to be processed for effective dynamic as well as effective management of wastes.

The application of these techniques through the application of this techniques could lead to the discovery of innovative solutions for the achievement of smart cities. This system entails the application of geographic information systems, the application of graph theory optimization, and the incorporation of machine learning.

The utilization of sensor has been identified for the dissemination of trash volume data back to the server which is then utilized in the treatment and for effective stimulation of the process involved in cleaning. This could also play an effective role toward the maintenance of a cleaner environment (Adetunji et al. 2017, 2018, 2019a, b, 2020; Adetunji and Adejumo 2017, 2018, 2019). The merits of this approaches are that it gives a guarantee that the trash is cleared properly before it become filled to the bream and also guarantee that unwanted trash pickup wherever they cannot be filled. The application of open date is recognized to be the initiator of big data analytics which could improve with cyber systems (Smith et al. 2013).

The incorporation of sensor most especially the weight sensors and temperature sensors together with the metal detectors are utilized for the categorization of the reusable, biodegradable, and recyclable (Reyes-Gutiérrez et al. 2015). The addition of microcontroller plays a crucial role in the regulation of some components such as memory, economic cost, minimal power consumption, and processing.

The application of artificial intelligence enables the collation of data, the utilization of statistical evaluation in the appraisal of necessary rates involved in the filling of the trash as well as the necessary routes that needs to be followed most especially through the help of cleaning team. The timing involved in the gathering of data from the trash can accentuate on the locations to be given priority most especially during the various period of the day.

Some possess the potential to visualize regular filling of the trash cans in the evening while the other late in the nights. Moreover, it allows the application of location such as house, school, and offices which are in greater needs of cleaning when compared to open field that involves minimal human interaction. The moment the artificial intelligence is boosted to the system, the numerous routes are well stated and optimized depending on the timing as well as the corresponding needs (Sinha et al. 2014).

The utilization of optimization methodology and artificial intelligence enables the system to utilize the data recorded to minimize the transportation expenses involved in the time and the cost through directing the best routes involved in the emptying of the filled trash can. This process is normally optimized bearing in mind the time and driving distance. The finalization of the optimization results in directing the driver the best route to be followed most especially with improved GPS location services (Kim et al. 2005).

12.7 Conclusion and Future Recommendations

This chapter has provided a detailed information on swift narrative and analytical approach in articulating the critical and recent advances in the exploitation of artificial intelligence and internet of things technologies in the design, deployment, and management of waste biodegradation plants. The application of artificial intelligence and internet of things in instrumentation and control in waste biodegradation plants will ensure innovation of simple techniques that are sustainable, safer, and eco-friendly alternatives coupled with current advances in nanotechnology, biotechnology, artificial intelligence and internet of things, soft systems such as biodegradation plants that utilize microbial agents under controlled conditions, have become a more prospective alternative compared to customary channels. There is a need for the government and policy maker at numerous countries to adopt the application of artificial intelligence and internet of things in instrumentation and control in waste biodegradation plants. There is a need to train students and scientists on the significance of this technology. This will go a long way toward the maintenance of a cleaner environment.

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Chapter 13
Bioremediation of Polythene and Plastics Using Beneficial Microorganisms

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Abstract Pollution caused by plastics is one of the greatest threats to the environment. This is because of their non-degradability, persistence, and recalcitrant nature. This contamination can result to severe health and ecological pressures to humans. Specifically, microplastics which are of great concern have been associated to cause instant death when consumed by aquatic organisms. The use of physical and chemical methods to degrade plastic wastes has been denounced, consequence of the increase in environment concerned problems they emanate. Therefore, there is a need to search for a sustainable remediation technique that will preserve the constituents of the ecosystem without any ecological and health risks issues. Recently, the biological breakdown (biodegradation) of plastic employing bacteria and fungi had increased prominence. Due to their efficiency, cost-effectiveness, environmental friendly and sustainability they portend. They do this by the secretion of metabolites such as polyhydroxyalkanoate depolymerases, which aids in the plastics breakdown. In view of this, this chapter intends to screen the potentials of some polythene and

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plastic degrading microorganisms from marine sediment and water, evaluate the degradation, environmental fate and risk implications of polythene and plastic in marine environment, and highlight the role of microbes in the degradation of synthetic polythene and plastics in marine sediment and water. We recommend that effective disposal measure method should be put in place such as provision of waste bins that distinguish biodegradable wastes from non-biodegradable ones. This will pave a sustainable way of wastes conversion and leaching in marine environment. There is also a need to employ the services of well-engineered native microorganisms that will play active roles in the remediation of microplastics when the other options fail.

Keywords Bioremediation · Microplastics · Marine plastics · Sediments · Microbes · Sustainable remediation

13.1 Introduction

Plastics are artificial and semi-artificial materials that can be remade into various shapes and forms such as polyvinyl chloride, polyurethane, polytetrafluoroethylene, polystyrene, polypropylene, polyethylene, polyethylene terephthalate, polycarbonate, and nylon that are utilized in our daily living.

Basically, these plastic constituents are excavated from natural gas, crude oil, and coal. However, their monomer and polymer chain structures consist of chloride, nitrogen, hydrogen, oxygen, and carbon. Recent data have shown that about one hundred and forty (140) million tons of plastics are generated by industries globally every year, where higher quantities of them are leached as wastes into the ecosystem (Shimao 2001). Thirty (30) percentage of these tons is utilized as chemicals, detergents, cosmetics, pharmaceuticals and for packaging foodstuffs (Sangale et al. 2012). About 64% of artificial plastics is made of polyethylene derived from polyethylene with high molecular weight and hydrophobic level (Vatseldutt and Anbuselvi 2014). Globally, about 500 billion to one trillion polythene materials are produced, with great amount accumulated in the environment (land and water). This provokes serious environmental concern. About 10% of wastes generated from municipal globally have been accredited to polythene, packaging plastics. Nonetheless, only 5% of the wastes are recycled while the rest are buried underground and take about 100 years to break down naturally, without the influence of microbes (Barnes et al. 2009).

Pollution caused by plastics is one of the greatest threats to the environment. This is because of their non-degradability, persistence, and recalcitrant nature (Mukherjee and Chatterjee 2014). This contamination can result to severe health and ecological pressures to humans. Specifically, microplastics which are of great concern have been associated to cause instant death when consumed by aquatic organisms

(Krueger et al. 2015; Acampora et al. 2017). When serious death does not ensue, the microplastics residues in the gastrointestinal system of the aquatic biota are being transferred along the food chain to cause adverse chronic impact along with the trophic level of feeding. However, Pathak and Navneet (2017) and Moharir and Kumar (2019) in their studies recounted that most plastics that are recalcitrant can be partially digested or metabolized by some specific species or strains of degrading fungi and bacteria. For example, *Ideonella sakaiensis* a microbe isolated from landfill, has been proven to efficiently degrade PET (polyethylene terephthalate). Evidences of the degradation of HDPE (high-density polyethylene) and PE (polyethylene) are still under study (Joo et al. 2018; Zampolli et al. 2018).

The use of physical and chemical methods to degrade plastic wastes has been denounced, consequence of the increase in environment concerned problems they emanate. Recently, the biological breakdown (biodegradation) of plastic employing bacteria and fungi had increased prominence. Due to their efficiency, cost-effectiveness, environmental friendly and sustainability they portend. They do this by the secretion of metabolites (polyhydroxyalkanoate depolymerases) which aids in the plastics breakdown (Mukherjee and Chatterjee 2014). Abiotic factor combined with the bacterial consortia also aid in the fragmentation, depolymerization, assimilation, and mineralization of plastic wastes in the environment into tiny droplet materials, monomers, dimers, oligomers, molecules, water, methane, nitrogen, and carbon dioxide (Mueller 2005).

Some specific strains used in the degradation of plastic wastes since the 1970s are of the genus *Aspergillus*, *Penicillium*, *Moraxella*, *Nocardia*, *Brevibacterium*, *Streptomyces*, *Streptococcus*, *Staphylococcus*, *Pseudomonas,* and *Bacillus* (Jones et al. 1974; Pegram and Andrady 1989; Krueger et al. 2015).

However, more studies are still required to ascertain specific microbes that are involved in the degradation of plastics. This chapter intends to X-ray the bioremediation of polythene and plastics using specific beneficial microorganisms, the screening of polythene and plastic degrading microorganisms from marine sediment and water, the role of microbes in the degradation of synthetic polythene and plastics in marine sediment and water and the environmental, health implications and fate in the degradation of polythene and plastic by microorganisms in marine environment.

13.2 Screening of Polythene and Plastic Degrading Microorganisms from Marine Sediment and Water

Delacuvellerie et al. (2019) screened the potential impacts of *Alcanivorax borkumensis* on plastic degradation in marine plastisphere (plastic environment). The authors stated most plastic wastes are released mainly on land that about 32% of the plastics fate are in the ocean. This can elicit serious health and ecological influence in the hydrosphere. The results of the 16S rRNA micro-biome sediment and surface water profiles showed that there were significant bacterial biofilms and plastic relationship with indications of Bacteroidetes and Gamma proteobacteria individually. The findings from the study established that the plastic environment and the bacterial consortia had distinct ecological niche. The structure of the microorganism conglomerates was fundamentally plastic hooked on. Microbes in the genus such as Arenibacter and Marinobacter, are able to breakdown PET and LDPE. In conclusion, the authors suggest the utilization of *Alcanivorax borkumensis* as the best candidate in the breakdown of plastic wastes from petroleum source. More so, they are able to form dense biofilms on low-density polyethylene (LDPE) which is the reason for their active potentials.

Divyalakshmi and Subhashini (2016) isolated and screened some microbes sourced from different soil environments and their degradation capacity on polyethylene in MSM (mineral salt medium). After incubation at 37 °C for 30 days, the results showed an extreme weight depreciation (13.6%) of the polyethylene. The results of the Fourier Transform Infrared (FTIR) analysis indicated chemical variations in the polyethylene properties because of the degradation activities of the microbes. The results of the 16S rRNA micro-biome performed on the Fourier Transform indicated that the most active microbes were strain KX344032 (*Staphylococcus arlettae*) out of the 32 isolated and 12 screened strains investigated. In conclusion, the authors suggested that for shortening period of degradation of plastics, the isolation of vigor genes from *Staphylococcus arlettae* for plastic degradation might give way for an enhanced biotechnology of recombinant DNA.

It is a known fact that polythene and plastic wastes disposed in the environment indiscriminately pose a serious ecological problem. However, the degradation of these wastes by microbes has also been in the forefront in sustainable environmental management. Usha et al. (2011) tested and screened some microbes and fungi sourced from refuse soil in the degradation of polyethylene and plastics. Results obtained after two, four, and 6 months incubation periods showed that the population of actinomycetes, fungi, and heterotrophic microbial counts in the polythene and plastics, range as follows: 72.54 × 104 and 64.75 × 104, 44.32 × 102 and 35.62 × 102, and 62.71 × 104 to 56.52 × 104 correspondingly. It was observed that the degradation of the plastics was associated to the various actinomycetes, fungi, and bacterial species; *Streptomyces*, *Aspergillus flavus*, *Aspergillus nidulans*, *Staphylococcus sp*. *Bacillus sp*., and *Pseudomonas*. The results obtained from the efficiency of the bacteria breakdown revealed that 28.42% of plastics and 37.09% of polythene were broken down by the strain of Pseudomonas after the 6-month incubation period. While 35.78% of plastics and 46.16% of polythene wastes were degraded by the fungi strain Streptomyces, the finding from the study showed that Streptomyces had more degrading potentials on polythene and plastics when related to other strains of fungi and bacteria.

It is known facts over the last decade that the impacts of plastic on marine community, especially the benthic region have established environmental and general problems. Currently, studies have been focusing on the propensity of microorganisms' growth on plastic surfaces (plastisphere) as well as their toxicological impacts on marine lives. Jacquin et al. (2019) in a review looked at the biodegradation and colonization of marine plastic-polluted community (plastisphere) by microorganisms and their ecotoxicological impacts. The issues of the influence of microbial consortia on the degradation of plastics in marine environment were discussed. The influence of plastic on marine microbe's communities was also established. The authors also recounted the influence of biofilms on the superficial region of plastics as well as their potential function as carriers of dangerous organism and plastic debris. A detailed view on how microbes partake in the breakdown of marine plastic as well as their importance in standard protocol tests for breakdown of marine plastics was discussed. Some specific examples of marine polymer degradation by microbes and metabolic pathways were highlighted. The authors stated that no current evidence of the pathogenicity of microbes in humans and animals as it pertain to plastic consumption in the oceans has risen so far based on the proclivity of plastic wastes being alleged as portfolio for invasive species. They reported that an improved understanding on the issues that pervade the microbial role in plastic degradation and plastisphere should be put into cognizance. In conclusion, the authors stated that an integration of the scientist (biologists, chemists, and physicists) need to collaborate in order to answer the problems environment faced as well as the reverberation in the society. Mores so, assessment of research gaps is pertinent in other to forestall future ecorestoration.

Urbanek et al. (2018) in a review looked at the degradation of plastics by plastic microbes in marine habitat that is very cold. The authors recounted that man-made plastics constitute the major human debris in the earth's crust. That changes as a result from plastic influence in marine ecosystem can destabilize the water resources, energy, and food. Hence, plastic pollution currently has resorted to sharp dramatic changes in a global scale. However this problem permeate, there have been several methods that these plastic impact on the environment can be reduced. The utilization of microorganisms to degrade plastic waste has been in the vanguard of biotechnological approaches specifically in cold regions. They reported that microbes can be isolated from cold marine environment to degrade plastic in situ. In the justification of this context, the authors nevertheless stated that the relationship between microbes and plastic wastes in cold environment are yet to be ascertain immensely. However, current knowledge of the breakdown of plastic wastes in cold environment was discussed. Highlights of some advantages of the isolation of specific microbes employed in the degradation and elimination of marine plastic wastes were itemized. In conclusion, the authors stated that in as far as the biofouling consortia of microbes are not well understood, there should be an urgent clarion call to study microbes and plastic wastes in the arctic marine ecosystem as well as human influence on the environment because the plant and future depend on our sole responsibility. Without this, the natural ecosystem might be depleted and microbes will not be able to adapt to the irreversible corruption of the environment by plastic wastes.

Different consortia of microbes in aquatic environment usually live in colonies. This consortia usually colonize plastic remains (plastisphere) which are gotten from fossil fuels. The degrading impact of bacteria (coerce hydrocarbon-degrading forms) alongside with biofilm has been proven to be efficient in plastic wastes decontamination. Erni-Cassola et al. (2019) isolated, tested and screened coastal marine seawater microbes in the colonization and the breakdown of polyethylene. The results from the 16S rRNA protein sequence showed a positive relative abundance enrichment of non-weathered (5.8%) and weathered (3.7%) polyethylene after about 2 day incubation period of the coastal water as compared to the controls (0.6%). However, there was a decrease in the relative abundance of the matured biofilms (<0.3%) after a 9 day incubation period. Associates of the genus *Aestuariibacter* and *Roseobacter* strongly improved the microbes in the primary stage of colonization with the percentage mean of 1.8 and 26.9 respectively in the consortia. This might be as a result of the presence of small chains of oxidization emanated from the weathering process. The findings from their study showed that the breakdown of plastic materials depends on the level the colonized consortia that enriched by the early coerce hydrocarbon-degrading microbes. They concluded that collective non-hydrolyzable plastic larger chains (polymers) might not aid as basis of carbon advance plastispheres which is gotten from the depletion and weathering of labile substrates.

Marine sediment has been seen of recent as deposit or trash can of many residual contaminants. Artificial microplastics polyethylene of size greater or equal to 5 mm has been reported to impact on both pelagic and benthic microcosms. Harrison et al. (2014) tested and screened the potential impact of selected rapid microorganisms in the degradation and colonization of microplastics polyethylene in marine sediment consortia. After a 14 day assessment period of the bacterial and marine microcosm of the LDPE (low-density polyethylene) in the sediment, the result obtained using a CARD-FISH (catalyzed reporter deposition fluorescence in situ hybridization) revealed that the microbial connection on the LDPE around the sediment improved in the richness of 16S rRNA protein sequence genes on day 7. The results of the T-RFLP (terminal-restriction fragment length polymorphism) showed a rapid medley of linked microbial consortia whose composition and structure varied considerably from those sourced from the immediate sediment environment. More so, the terminal-restriction fragment length polymorphism analysis showed a sequence of the conjunction of the low-density polyethylene linked communities from the varied sediment at the end of the studied regime or experiment (14 days). The findings from their study showed that the 16S rRNA protein sequence genes confirmed the perfect dominance of microbes (*Colwellia* and *Arcobacter*) in the sediment-polyethylene communities, amounting to a sum percentage of 84–93 of the sequences. In addition, the connexion of strains *Colwellia* in the sediment-LDPE was also collaborating by the catalyzed reporter deposition fluorescence in situ hybridization. In conclusion, the authors stated that since *Colwellia* and *Arcobacter* have been used to degrade hydrocarbons in recent studies, they suggested the enlistment of hydrocarbonoclastic microorganisms such as *Colwellia* and *Arcobacter* in the degradation of marine microplastics as perfect candidates in marine pelagic and benthic ecorestoration of pollutants.

Over the last four and half decades, microplastics marine ecosystem has been known to be transporters of microbial consortia. However, their connection with marine microorganisms has recently showed the poor paucity in literatures related to their biodegradability utilization. Oberbeckmann and Labrenz (2019) in a review looked at the role, adaptation, and diversity of coastal bacteria consortia on microplastics degradation. Their research objectives focused on the breakdown of microplastics in marine environment, the pathogenic and vector role of bacteria from the genus Vibrio, and the plastisphere/biofilms relationship with already established plastics. Nonetheless, the deduction from recent literatures established the degradation potentials and relationship between particles from microplastics and microbes as well as their influence in the marine ecosystem. The authors recounted that this relationship of the microbial eating plastics and the microplastics appear to belong to an adaptable migrant that do not discriminate between the artificial and natural surfaces of any media. Therefore, the microplastics do not cause any hazard when compared to natural particles found in higher aquatic biota, but however, possibly harboring disease-causing microbes. In other vein, they represent a level of marine recalcitrant materials that play no supporting function to unicellular metabolism as well not relevant to animals and humans in the top food chain. Because of the poor bioavailability of microplastics in the marine environment, the efficiency of microbes in the total degradation of them cannot be significantly resourceful, because of the amount of plastics generated day in day out, human time-scale factors, and degree of degradability. In conclusion, the authors recommend alternative methods in reducing plastic inputs in coastal water bodies and enhanced biotechnological in vitro methods in microplastics degradation.

Vague et al. (2019) isolated and screened Pseudomonas and associated biofilms in the degradation of PET (polyethylene terephthalate) and related plastic chains. The authors recounted that in landfills and coastal waters, there have been a resounding increase of plastic wastes. They noted some positive lipase *Bacillus* (two) and *Pseudomonas* (three) strains as part of the assemblages in the degradation of PET in marine environment. The authors reported that when the positive bacterial consortia were placed alongside with the control (*E. coli*) during the incubation period under ultraviolet radiation, there was a high propensity of degradation of LDPE, HDPE, and PET (low-density polyethylene, high-density polyethylene, and polyethylene terephthalate) and carbon was the major source of wastes. However, in the results of the spectroscopy, there was an observed superficial degradation of the PET as well as molecular vibrations by the bacteria and associated biofilms. This was also consonant with the observation from the SEM (scanning electron microscope), spotting an induced preliminary plastic degradation stage. The authors in their findings found out that some strains of Pseudomonas were able to degrade LDPE rapidly. In conclusion, they suggested further advance screening of lipase positive bacteria in other to harness hidden potentials for future PET and related plastics chain degradation.

The fate of the devastating nature of plastic in the aquatic environment cannot be overemphasized. Polypropylene and polyethylene are the most common plastics found in oceanfront and superficial region of aquatic ecosystem sourced from benthic sediment, cellulosic fibers waste treatment, and polyester. Icon et al. (2018) did a review of the degradation of aquatic plastics by microbes as well as their occurrence and physicochemical features. The authors stated three major reasons why plastics are missing on the superficial layer of the ocean is because of the level of seepage and buildup of floating and non-floating polymers along river banks, sediments, and sewage treatment tanks. Secondly, the relaxation or settling of floating and non-floating polymers in the deepest part of the sea. And thirdly, disintegration of the floating and non-floating polymers into minute particles. They stated that some separation techniques may represent a bigger and more stable particles which require an enhanced method in capturing full size of different ranges of litter of plastics. The authors stated further that fragments of microplastics are neutrally stable and therefore are probably scattered in water bodies both vertically and horizontally. Eventually, it will take a longer time to successively and possibly biodegrade plastics; this reason is based on the amount of plastic litters in different environmental sections.

It is a known fact that plastic wastes in aquatic bodies can impede biological process. Brunner et al. (2018) isolated and tested the capability of some fungi strains isolated from floating debris sourced from shoreline in an aquatic body in the degradation of plastic wastes. The authors recounted that these fungi strains have the potential to degrade complex cellulose lignin such as C-polymers. This clarion call has opted the need for them to be used in the degradation of plastic wastes which have similar polymeric structures. They isolated and tested over 100 plastic degrading fungi, of which, one of them belongs to the species Oomycota and was spotted out to be a very good degrader. The results from the biodegradation test of the fungi on the polyurethane and polyethylene showed that four strains out of which one was plant pathogen (*Leptosphaeria sp.)* and the other three were saprophytes (*Penicillium griseofulvum*, *Xepiculopsis graminea*, and *Cladosporium cladosporioides*), were able to degrade polyurethane, while no strains of fungi were unable to break down polyethylene. Further assessment revealed that a part of the four degrading fungi, two saprotrophic strains (*Marasmius oreades* and *Agaricus bisporus*) showed more potential to break down polyurethane. It was also observed from their study that ectomycorrhizal and wood-saprotrophic fungi were the key polyurethane degraders. The findings from their study showed that the saprophytes have well-enhanced enzymes for degradation process. In conclusion, the authors recommend saprotrophic fungi as candidate for polyurethane degradation in aquatic environment.

Pathak and Navneet (2017) in a review looked at the recent state of microbial degradation of plastic polymers. The authors recounted the indiscriminate use of artificial polymers and the increased ineptness of the environmental control of water and land pollution. Plastics being one of the most widely used polymers in household, agriculture, and industrial packaging have been shown not to degrade easily, they stated. This recalcitrant polymers can take hundreds of years to be broken down by natural process. However, recent biotechnological approach using microbes has been employed in the degradation of plastic wastes within the shortest period of time. The distribution and occurrence of microorganisms in the degradation of artificial and natural polymers were discussed. The exploitation of enzymes generated by some biological control messengers as instrument of plastic degradation was highlighted. The authors recounted that fungal and bacterial species are well-known biological messengers found in the environment that have the capability to degrade both artificial and natural polymers. Specific examples of them are *Butyrivibrio* *fibrisolvens*, *Clostridium thermocellum*, *Comamonas acidovorans*, *Rhodococcus ruber*, *Streptomyces setonii*, *Streptomyces badius*, *Pseudomonas stutzeri,* and *Pseudomonas aeruginosa* for the bacterial species. And *Mucor rouxii*, *Pycnoporus cinnabarinus*, *Fusarium lini*, *Aspergillus flavus,* and *Aspergillus niger* for the fungal species. However, *Pseudomonas aeruginosa* has been reported as one of the effective microbes that degrades polymer through the formation of biofilm with the aid of alginate-like quorum detecting motion and chemicals system. They stated that strain CA9 (Pseudomonas aeruginosa) has been recounted to degrade LDPE and strain AKS2 (Pseudomonas aeruginosa) has been reported to form biofilms in the degradation of LDPE, thereby increasing the superficial hydrophobicity by 26% as well as the hydrolytic action by 31%. Pseudomonas stutzeri has been recounted to degrade high molecular weight polyethylene glycol (PEG) of about 4000–20,000 g. Strains 75Vi2 and 252 (Streptomyces setonii and Pseudomonas stutzeri), respectively, were reported to be more in effect against treating heat degraded plastics. More so, they stated that Rhodococcus rubser degrade and colonize polyethylene by producing hydrolytic chemical substances and biofilms as well as enhancing the peroxidant essences which makes it susceptible to both photochemical mineralization and in vitro thermal processes. Strain TB-35 (*Comamonas acidovorans*) has been reported to be valuable for the degradation of polyurethane-polyester via polyurethane (PUR) enzymatic hydrolysis activity and production of esterase using structural genetically material (pudA). They reported the action of the following fungi *Mucor rouxii*, *Pycnoporus cinnabarinus*, *Fusarium lini*, *Aspergillus flavus,* and *Aspergillus niger*. That *Aspergillus niger* manufactures acetyl xylan esterase which functions with the blend of endo-xylanase for an effective breakdown of xylan. *Aspergillus flavus* and *Aspergillus niger*, respectively, have been reported to be useful for the fast mineralization of average units of monomers. *A. flavus* has been recounted to active polythene degrader as well as strain NRRL 1835 (*A. flavus*). *F. lini* and *P. cinnabarinus* have been recounted to produce dehydratase for the degradation and biotransformation of PVA (polyvinyl alcohol) to H_2O and CO_2. In conclusion, the authors recommend that further works should be carried out on the evaluation and screening of different unharnessed microbes and fungi for the utilization of polymer breakdown.

13.3 Degradation, Environmental Fate, and Risk Implications of Polythene and Plastic in Marine Environment

About 350–400 shedload of plastics are generated annually in marine and terrestrial ecosystem, of which they are poorly recycled. In today's environmental watch, it has become clearer that the adverse health and ecological influence of plastics in the biosphere of animals and humans has compromised environmental safety standards. Danso et al. (2019) in a review looked at the biotechnological and environmental standpoints of microbial breakdown of plastics. The authors stated that current biotechnological approach using beneficial microorganisms; fungi and bacteria in degrading such recalcitrant pollutants are employed. The issues of enzymatic activity secreted by microbes on PET and PUR (polyethylene terephthalate and polyurethane) were discussed in detail. The authors stated that previous reports only stated the consortia of microbes and their additives degrading chemicals, there has been paucity in the enzymatic action on high structural weight polymers like polyethylene, ether-based polyurethane, polypropylene, polyvinylchloride, polyamides, and polystyrene. In sum, these polymers accounted for 80% of the yearly plastic manufacturing. They also highlighted the core importance of some enhanced variation of enzymes as well as the microbial activity on the above stated polymers. In conclusion, they reported that black material, proteins, and worldwide metagenomes of microbes not yet cultured can bridge the gap of these enhanced variations. That only this new biocatalysts and enhanced microbes can cause fast recycling and degradation of huge majority of natural and human made plastic polymers.

Hayden et al. (2013), in a review, looked at the environmental consequences of plastic degradation by microbes with special focus on polyethylene terephthalate (PET). The authors recounted the natural resistance of plastics to degradation, its ever accumulation to the environment and the health attendant and risk concerns. The present state of awareness as it relates to biodegradation of plastic polymers was discussed. The authors highlighted some problems linked to plastic contamination in the coastal environment with special features on the degradability, commercial production, and properties of PET. The adsorption and accumulation of PET in a coastal environment as well as the hazardous impacts to coastal wildlife were discussed. The authors stated that presently, only three main plastic discarding techniques (recycling, incineration, and landfill) is invoked. They highlighted their drawbacks and demerits. They recommend an eco-friendly approach using beneficial bacteria and fungi to degrade PET. This method has been proven to be effective and sustainable. It does not generate environmental wastes that are toxic. The fate of the pollutants is well known. In conclusion, the authors recommend extensive research in the area of plastic biodegradation and enhancement of the metabolic capabilities of microbes.

Due to the durability of plastics in the environment and poor recycling, there is an urgent call to address the probable life and ecosystem threat posed with it. WWTPs (Wastewater treatment plants) have been linked as one of the source points of the release of plastic into the ecosystem. Okoffo et al. (2019) in a review looked at the fate, quantification, and occurrence of treated plastic wastes plants in the environment. A compressive analysis of the present knowledge on the contamination of plastic via influent into the wastewater treatment plants, source apportionment of sludge from sewage, effluents and influents occurrences as well as the various techniques employed in their usage were discussed. In addition, the route of transfer and fate of plastics from wastewater treatment plants into the terrestrial and aquatic ecosystem was also argued. The authors recounted some research gaps such as quantification, identification, and healthy systematic methods that will bridge recent research works. They advocated research harmonization via improved techniques such as the utilization of nano-size plastics.

Billions of tonnes (6.3 billions) of wastes from plastics are released globally, which about 9–12% are either incinerated or recycled. Due to the rise of human population, day in and year in, the generation and utilization of plastics are accompanied by serious environmental wastes as well as ecosystem degradation. Alabi et al. (2019), in a review, looked at the environmental and public health impacts of wastes from plastics as well as their disposal methods. The effects of plastic wastes on the soil, water, and air were looked on to. Different disposal techniques, lethal chemical composition of plastics and various types of plastics fabrication were highlighted. The authors also recounted the public and health impacts associated with plastic contamination. The lists of some economical consumable materials of plastics (phenanthrene, dichlorodiphenyl dichloroethylene, polychlorinated biphenylethers, nonylphenol, brominated flame retardants, bisphenol, heavy metals, and phthalates) used in water flasks, food wrapping materials, and medical devices which are noxious were stated. The authors opined that eight million tonnes of plastics wastes are discharged into marine ecosystem which ultimately affect the aquatic life forms therein. That when there is intense exposure of plastics to high temperature, it can result to leaching of noxious chemical wastes into water, drinks, and food. The authors in conclusion suggested that for proper health and environmental safety, governmental agencies should prohibit the use of noxious chemicals such as BPA and phthalates in the manufacture of plastic consumables that are in straight contact with children, beverages, water, and food through environmental laws.

Globally, plastic production has reached over 300 million tons annually. It has been also reported that for over five decades, the pollution of the marine ecosystem by plastic has risen greatly. Currently, rapid plastic production commercially has skyrocketed due to the rise in human needs fingered to population and technological boom. Serious concern has been geared towards the risks, both health and ecological pose by plastic wastes to coastal wildlife. Law (2017) in a review looked the pollution of the coastal environment by plastics and its attendant risks both ecological and health to life forms. The issues of influences of plastic to the coastal ecosystem, fate of plastics, their distribution and source apportionment were highlighted and discussed. The ever increasing indication of plastics in the marine ecosystem and their significant impact on the marine environment were also discussed in detail. In conclusion, the authors suggested the implementation of the point source 3Rs (reduce, reuse, and recycle) techniques in decreasing the potential ecological and health risks already foreseen in the coastal environment for a sustainable prevention of life forms and ecosystem structures.

Ilyas et al. (2018) did a review of the probable significant threat caused by plastic wastes and the attendant threat posed on the marine coastal ecosystem. The authors recounted the ecological imbalance brought as a result of plastic wastes in the aquatic environment. That plastics do not degrade naturally in the environment, as a recalcitrant pollutant it poses a serious environmental threat. The authors highlight some systematic protocols to curb plastic wastes threat. Various disposal methods, management, the influence of wastes from plastics, and their environmental fate in the natural ecosystem, were highlighted and discussed. In conclusion, the authors suggested some five key methods for managing wastes from plastics such as translation into useable materials, degradation by fungi and bacteria, burning in incinerator, and disposal by landfill and recycling.

13.4 Role of Microbes in Degradation of Synthetic Polythene and Plastics in Marine Sediment and Water

One of the major environmental pollution in marine environment has been linked to the increase in the rate of plastic wastes. It has been stated that some plastic that could be degraded by microorganisms could be referred to as eco-friendly in nature with numerous utilization in packaging industries. Jumaah (2017) evaluated the rate of breaking down of plastic material for a period of 1 month using submerged fermentation. The microorganism discovered that was related with degradation effectiveness includes two gram negative and three gram positive bacteria. The microorganisms detected with high potential for plastic degradation are Pseudomonas putida, Bacillus subtilis, Bacillus amylolyticus, Pseudomonas fluorescens, and *Bacillus firmus.* It was discovered that the effectiveness of microorganisms with high potential for plastic degradation when performed in the submerged fermentation techniques include *Pseudomonas putida* which showed a high capability that it could break down plastic containing materials with a period of 1 month (30% weight loss/month) in comparison with *Bacillus subtilis* (22% weight loss/month). Their work indicated that *Pseudomonas putida* possess a greater potential to degrade plastic when compared to other microorganisms isolated during this study.

Begum et al. (2015) isolated beneficial soil bacteria that could be found in polluted soil samples. The authors discovered that *Pseudomonas alcaligenes* and *Desulfotomaculum nigrificans* were the two isolates found in the plastic contaminated area after they were subjected to biochemical and morphological characterization. The author assessed the biodegradation effectiveness of *Pseudomonas alcaligenes and Desulfotomaculum nigrificans* when performed in polythene bag. The result obtained indicates that *Pseudomonas* alcaligenes was detected to be more active in terms of biodegradation of polythene bag when compared to *Desulfotomaculum nigrificans* during the period of 30 days. Moreover, it was discovered that the high level of increment in the incubation period led to increase in weight loss of polythene bag.

Trivedi et al. (2016) wrote a comprehensive review on the significance of microorganisms and their role in the production of bioplastic. Several microorganisms have been identified as several sources of bioplastic production and they could be utilized for the degradation of plastics. However, the production of bioplastic has been shown to be more expensive than the synthetic plastic because it possesses numerous benefits when compared with them. Several number of microorganisms are utilized for breaking down of plastic and they also play a critical role in the production of bioplastics. It has been stated that member of the public have a greater preference to biopolymer produced by beneficial microorganism which is presently gaining public acceptance.The authors also highlighted the role of beneficial microorganisms in the degradation of plastic as well as in the production of plastic.

Microplastics show a world global distribution and have been discovered in also most all level of the marine environment. Microplastics have been highlighted as a dangerous portion of a degraded plastic and could lead to suffocation of hundreds of marine species. Moreover, it has been discovered that microplastic could lead to the movement of non-indigenous marine species to different area which could constitute threat to food web and marine biodiversity. They could be dangerous because they possess the capability to build up poisonous substances on their surface which could serve as a hazard which could affect the ecosystem with numerous health challenges. Hence, the evaluation of the status, influence, and implementation of necessary action that could lead to their prevention are necessary. In view of the aforementioned, Ogunola and Palanisami (2016) wrote a comprehensive review of the prevalence of microplastics in the marine environments that were performed by evaluating the literature pooled from several scientists that have performed extensive work in this regard.. The authors emphasized on novel methodology for removal and enumerating microplastics from marine matrices though, it is a complex and enormous job in resolving the challenges of microplastics in the ocean, selected methodologies were also explained by the authors.

Microplastics have been identified to spread rapidly in sediment and marine environment and possess the potential to settle in the sediments. The authors stated that over 100 manuscripts were written for a period of 50 years with the following aims: (1) to establish holistic evaluation of possible adverse effect of microplastic to marine environment, (2) to deliberate the incidence and global distribution of microplastics in sediments, (3) to assess the current microplastic extraction methodology. The authors also proposed the need to perform some further research and determine that there is an urgent need for a standardized methodology, incorporated reporting units, and more accurate influence evaluation.

Plastic polymers are extensively used in industry and agriculture and play a crucial role in the daily utilization of human being because they are cost-effective and they are easy to use.

It has been highlighted that polyethylene has several adverse effects on human and animal health, most especially when they settle down in environment because they could not degrade easily most especially under natural condition. Ren et al. (2019) isolated *Enterobacter* sp. D1 from the guts of wax moth (*Galleria mellonella*) which was later utilized for the biodegradation experiment. It has been stated that several pollution could be linked to plastic polymer most especially polyethylene which has adverse effect on human and animal health because of their high availability in the environment. This might be linked to the fact that they could not be easily degraded in a natural environment. The result obtained indicated that there was presence of microbial colonies development around a polyethylene film after 14 days of culturing most strain D1. Moreover, cracks, roughness, and depressions were observed on the exterior of polyethylene film when subjected to atomic force microscopy and scanning electron microscopy. The presence of function groups available after the biodegradation was evaluated using Fourier transform infrared spectroscopy which indicated the presence of ether groups and carbonyl functional groups. Moreover, the presence of other chemical constituents was detected by liquid chromatography-tandem mass spectrometry which shows the presence of acids, alcohols, and esters. The bioproduct or chemical constituents might be linked to the oxidation reaction which happened on the surface of polyethylene film most especially with strain D1. Their study indicated that strain D1 possess the capability to be utilized for the biodegradation of polyethylene.

It has been stated that there is increase in the buildup of plastic most especially from polystyrene and polyethylene terephthalate which constitute increase in ecological disturbance as a result of massive utilization in everyday of mankind. Therefore, in order to mitigate against this ecological threats there is a need to isolate beneficial microorganisms that could biodegrade plastic mainly from garbage soil, garden soil, soil near petrol pump, forest soil, and mangrove soil. Their study showed that there was loss in weight of polystyrene and polyethylene terephthalate most especially garbage soil and garden soil in comparison to other soil in terms of Gram positive *coccobacillus*, Gram negative *cocci*, Gram negative rod shaped bacillus, Gram positive *cocci* (in clusters) in garden soil and Gram negative *cocci* (in singles) in garbage soil. Moreover, the polystyrene and polyethylene terephthalate were broken down by the following bacteria *Aspergillus niger, Pseudomonas aeruginosa, Streptococcus pyogenes, Bacillus subtilis, Staphylococcus aureus*. Furthermore, it was also stated that *Bacillus subtilis* exhibited the highest percentage loss in weight of polystyrene most especially by *Bacillus subtilis* when cultured inside Bushnell Hass and Nutrient and Bushnell Hass broth, whereas in the case of PET, percentage loss in the weight was maximum by *Pseudomonas aeruginosa* in Bushnell Hass broth, *Bacillus subtilis* in Nutrient broth, and *Aspergillus niger* in Rose Bengal broth.

The high rate in the level of polyethylene waste building up in the environment has led to enhanced ecological challenges. It has been realized that polymer of ethylene constitute a major part of our daily requirement used in various activities. They are utilized in different sectors for numerous applications such as wrapping of foods, consumer products, and packaging materials. It has been stated that the request for these synthetic polymers has risen to 500 billion to 1 trillion tonnes which constitutes what are utilized globally. The structure of these synthetic plastics entails high molecular hydrocarbons with complex structures which could not be degraded easily.

Several findings have shown that the application of biodegradation could be used for the mitigation of these all aforementioned environmental challenges. In view of these, Senthilkumar et al. (2016) wrote a comprehensive review on the isolation of beneficial microorganisms that possess the capability to degrade synthetic polymer from the soil. These soil bacteria possess the capability to degrade these polymers through the help of enzymes and the cloning of genes for biodegradation. Typical examples of such enzymes include manganese peroxides and lignin peroxidase. The authors suggested that there is a need to still isolate some other soil microorganisms that possess the capability to degrade numerous plastics and polymers most especially from various sources.

Kumar et al. (2016) established that six various types of packaging plastics with various composition in their starch (low-density polyethylene [LDPE], 10, 20 30, 40, and 50% of LDPE starch) were utilized for degradation experiment in collaboration with microorganism in compost reactor. The experiment was performed in a small scale bioreactor which entails two degraded samples in compost and soil. The result obtained indicated that in the compost bioreactor, the weight loss in 50% starch-blended low-density polyethylene was 10.06% and was 0.0343% for pure low-density polyethylene on day 65. It was later revealed that the biodegradability of all the commercial plastic in the soil was detected that they could biodegrade low-density polyethylene. The rate of the outcome observed showed that the degradation was estimated to be around 19.8% on day 100 while the loss in tensile strength was 81.24% on day 97. Moreover, the percentage elongation also decreased by 13.70% and 68.27% on day 71 and 100, respectively, while the total organic carbon was reduced by 13.70 and 68.27% on day 71 and 100, respectively.

Kathiresan (2003) performed an experiment on the degrading potentials of microbes sourced from mangrove soil on polythene and plastics. The rate of biodegradation of plastic cups and polythene bags was performed after 2, 4, 6, and 9 months of incubation in the mangrove soil. It was detected that level of biodegradation of the polythene bags was enhanced up to 4.21% in 9 months when compared to than that of plastic cups (up to 0.25% in 9 months). Moreover, it was detected that the rate of microbial count in the plastic material degraded by these microbes was recorded up to 55.33 × 102 per gram for fungi and 79.67 × 104 per gram for total heterotrophic bacteria. The microbial species that were detected that possess the potential to degrade these synthetic material were identified as eight fungal species of *Aspergillus,* two Gram negative bacteria, and five Gram positive bacteria. Examples of such species that were isolated include two species of fungi (*Aspergillus glaucus* and *A. niger*), *Streptococcus*, *Micrococcus* (Gram +ve), *Moraxella, and Staphylococcus*.

The effectiveness of these microorganisms in the biological degradation of these polythene and plastic was assessed in shaker cultures. It was stated that *Pseudomonas* species degraded 20.54% of polythene and 8.16% of plastics in 1-month period. Among the fungal species, *Aspergillus glaucus* besmirched 28.80% of polythene and 7.26% of plastics in 1-month period. Their study indicated that the microorganism isolated during this study could be used for the bioremediation of heavily polluted environment.

It has been highlighted that synthetic polymers are one of the pollutants that constitute high rate of anthropogenic pollutants of the terrestrial ecosystems, lentic and marine environment. These synthetic polymers most especially microplastic possess that capability to affect various natural processes such as natural food webs, wildlife, ecosystem, human health, and the environment. The application microorganism has been identified as a sustainable means of remediating a heavily polluted environment with synthetic plastic. It was stated that numerous microorganism possess the capability to biodegrade many polymers through the process of oxidation or enzyme hydrolysis. It has been further proven that microorganisms portend that capability to degrade plastic and have been validated to be highly recalcitrant even under environments known to enhance microbial degradation. In view of the aforementioned, Krueger et al. (2015) wrote a comprehensive review on the recent knowledge on the application of microbiological techniques that could be utilized for the biodegradation of synthetic plastic. Additionally, the authors established the analytical defies regarding the estimation of plastic biodegradation as well as restrictions likely that could prevent effective biodegradation pathways.

Plastic has been identified as one of the highly persistent pollutants that couldn't biodegrade easily. It also proves to be resistant to the attack of most microorganisms and remains non-degradable. Their buildup in the environment could be hazardous which could lead to numerous environmental challenges. Biodegradation has been identified as the chemical and physical alteration induced on any material by any environmental factors such as heat, wind, and light together with the action of any biological agents such as fungi and bacteria. Therefore, in view of the aforementioned, Muthukumar and Veerappapillai (2015) wrote a review on the application of numerous bacteria and their application for the bioremediation of plastic when exposed to different environment. The authors stated that the bioremediation of plastic using non-conventional techniques could guarantee a future free of several hazards due to microplastic and plastics, most especially those used in packaging and commercial polymers, which are the most abundant form of plastic wastes.

It has been highlighted that petroleum-based plastic pollution has been recognized as worldwide environmental challenges which persist for several decades. This has led to buildup of plastics and microplastics in the aquatic environment. Therefore, the application of cheaper and sustainable techniques should be put in place for effective biodegradation of these synthetic plastics. It has been highlighted that polystyrene (PS) wastes which is a recalcitrant plastic polymer are among the list of heavy pollutants that constitute a lots of hazards to mankind. Therefore, the application of bioremediation has been highlighted as a sustainable and effective and cheaper means for the bioremediation of polystyrene. Therefore, since 1970 the role of microbial biodegradation on polystyrene most especially when mixed with manure, sludge, soil, and trash has been highlighted but the result obtained is very slow. Yang et al. (2018) describe the effectiveness of microorganisms in the biodegradation of polystyrene. Their study established that the application of larvae of yellow mealworms (*Tenebrio molitor linnaeus*) has been shown to be an effective bioremediation technique.

The utilization of mealworms has been established to possess the potential to degrade polystyrene foam as food and poses the potential to break them down and mineralized into carbon iv oxide through microbe-dependent activities within the gut in less than the 12–15 h gut retention time. Their study infers that mealworms could be utilized as a potential and sustainable tool for the biodegradation and bioremediation of plastic.

Cole et al. (2011) in a review extensively looked at microplastics as one of the major marine pollutants. The sources, nomenclature, and properties of microplastics were discussed. The routes and methods of detection were evaluated. The temporal and spatial variations of microplastics were highlighted as well as their influence to the biological organisms and physical and chemical constituents in the marine ecosystem. The authors stated that the rate of microplastic abundance in the marine and coastal fringes cannot be quantified. That health risk via the ingestion of this pollutant (hydrophobic or additives forms) in aquatic biota can facilitate acute or chronic situations and even cause trophic influence across the food chains. In conclusion, they stressed possible research areas for both legislators and scientist.

Chemically synthesized plastics are utilized in our everyday activity as human being and they have been recognized as a source of anthropogenic debris entering the earth's ocean. It has been highlighted that the ocean could be a source of significant and necessary resources such as energy, food, and water. They are also recognized as the major means of international trade and the main stabilizer of the climate. It has been stated that the alteration in the marine ecosystem constitute the major reasons for the various anthropogenic effects which include contamination by plastic. Although it has been highlighted that the biodegradation of plastic has become very difficult, there is a need to minimize all the highlighted environmental challenges associated with it. The utilization of microorganisms has been recognized as a powerful tool that could be used for effective biodegradation of these plastic. In view of the aforementioned, Urbanek et al. (2018) wrote a comprehensive review on potential microorganisms that possess the capability to biodegrade plastic and plastic–microorganism interactions in cold marine habitats. Furthermore, the authors highpointed the merits of microorganisms obtained from this environment for eradicating plastic waste from ecosystems.

It has been stated that majority of the microplastic detected in the ocean could be linked to the assemblage due to microorganisms which has been observed for almost 45 years.

Hence, only the function of the microbial relationship with microplastic in the marine ecosystem has been studied widely. In view of this, Oberbeckmann and Labrenz (2020) wrote a comprehensive review by carrying out investigation regarding microplastic extensively in three fields which entail: (1) The formation of plastic-specific biofilms which could also be called plastisphere (2) Enhancement of pathogenic bacteria, predominantly members of the genus *Vibrio*, together with a vector function of microplastics (3) The breaking down of microplastic by microorganism majorly in the marine environment. There is also a need to provide more insight on the relationship between microplastic and microorganisms. The popularity of microorganisms developing on microplastics appears to belong to opportunistic colonists that do not differentiate between artificial and natural surfaces. Consequently, microplastic doesn't pose any higher risk when compared to the natural particles to higher life forms by hypothetically protecting pathogenic bacteria. Moreover, it has been observed that microplastic available in the ocean constitutes recalcitrant substances for microorganisms that are inadequate for the maintenance of prokaryotic metabolism which could not be broken down by the action of microorganisms in any period of time relevant to human society. The author also suggests that necessary precaution should be put in place to prevent microplastics from entering the environment.

The utilization of polyethylene has been highlighted to increase day by day and recycling it has become a great challenge. It has been highlighted that almost 500 billion to 1 trillion polythene are made of bags that are used in different parts of the world. It has been stated that polythene has a longer durability and might take a longer time for its breaking down up to 1000 years. In view of this aforementioned, Sangale et al. (2012) did a review of the biodegradation of pollution, which was later grouped into the following: (1) the extent of pollution does to polythene; (2) to reveal the cost implication of the methodology; (3) to highlight probable microorganism that could biodegrade polyethylene; (4) to establish the modes of action of these biodegrading microorganisms; (5) to point out the techniques utilized for the biodegradation of polythene; (6) to elucidate the evaluation of polythene degradation by proficient microbes; (7) to highlight numerous compounds or constituents that are derived after polythene has been subjected to the process of degradation process; (8) to analyze level of the toxicity level of the products of the degraded polythene, and (9) to deliberate the upcoming features of polythene degradation.

The increase in the utilization of plastic has increased tremendously since the mid-twentieth century, as a source of pollutant for the contamination of the environment. The high rate of spreading of the plastic to the marine and terrestrial environment showed that they are major geological indicator of the anthropocene, as a distinctive striatal component. Moreover, it has been stated that high level of microplastic is now increasing on a high level of widespread marine sedimentary deposits in both shallow- and deep-water settings. Also, it has been stated that biological and physical processes play a crucial role in the breaking down of plastic towards the formation of microplastic. These microplastics have a means of entering into the food chain via the ‘fecal express’ route from the surface to the seafloor. In view of this, Zalasiewicz et al. (2016) wrote a review on the significance of microplastic as a pollutant in the marine environment. Plastic is majorly found in the sedimentary deposits and they increased to larger amount to several folds over the next few decades. They will remain to be moved into the sedimentary cycle over approaching millennia as provisional stores—landfill sites—are eroded. Plastics previously allow fine time determination within anthropocene deposits through the expansion of their dissimilar types and through the artifacts (“techno fossils”) they become shaped into, and numerous of these may have long-term safeguarding effectiveness when buried in strata.

Muhonja et al. (2018) carried out the biochemical and molecular characterization of low-density polyethylene bacteria and fungi isolated from Dandora dumpsite, Nairobi. The authors isolated 10 fungi and 20 bacteria using 18S rDNA and 16S rDNA, respectively. The fungal strain that showed the maximum biodegradation potentials on low-density polyethylene was *Aspergillus oryzae* strain A5, while the bacterial strain that showed maximum biodegradation was *Brevibacillus borstelensis* strain B2,2, and *Bacillus cereus* strain A5,a respectively. These microorganisms were later subjected to screening of their potential for their capability to generate extracellular esterase and laccase. *Aspergillus oryzae* strain A5,1 exhibited the highest presence of esterase (14.33 mm), *while Aspergillus fumigatus* strain B2,2 exhibited the highest presence of laccase (15.67 mm). The alkane hydroxylase-encoding genes were evaluated for using primer AlkB 1 which was used in the amplification of the fragment of size 870 bp while only four out of the bacterial strain showed a positive result for the presence of the gene. The optimum temperature that enhances the biodegradation capability of the tested isolated was found at 30 °C. Their result also shows that the presence of alkane esterase, hydroxylase, and lactase showed that they represent the major molecular basis for the biodegradation of low-density polyethylene. The authors also suggested that there is a need to perform further investigation of some other microorganisms that has capability to be utilized for biodegradation purposes most especially when combined with the process of bioaugumentation.

13.5 Conclusion and Further Recommendation to Study

This chapter has provided thorough facts on the bioremediation of polythene and plastics using beneficial microorganisms. Comprehensive facts of the screening of polythene and plastic degrading microorganisms from marine sediment and water, the degradation, environmental fate and risk implications of polythene and plastic in marine environment and the role of microbes in degradation of synthetic polythene and plastics in marine sediment and water were highlighted. There is a necessity for environmentalist, engineers, scientist and associated environmental fields to come together to address the clarion call of wastes reduction, recycling, and reuse. This will in a great extent reduce the plastics as well as the minute forms (microplastics) from source generation points. More so, effective disposal measure method should be put in place such as provision of waste bins that distinguish biodegradable wastes from non-biodegradable ones. This will pave a sustainable way of wastes conversion and leaching in marine environment. There is also a need to employ the services of well-engineered native microorganisms that will play active roles in the remediation of microplastics when the other options fail.

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Chapter 14
Recent Advances in the Application of Genetically Engineered Microorganisms for Microbial Rejuvenation of Contaminated Environment

Charles Oluwaseun Adetunji and Osikemekha Anthony Anani

Contents

Abstract Globally, it has been recognized that pollution preclusion constitutes more economical and environmental challenges. Several types of research over the years have fingered uncontrolled human activities as the major cause of environmental pollution. The role of indigenous microbial consortia in the bioremediation of such heavily polluted soils has been reported but with several challenges. However, the application of genetically modified organisms developed from the wild-type or the indigenous strains will go a long way in the determination of the general success during in situ bioremediation program as well as their active role in the reuse or recycling in pollution prevention. Therefore, this chapter intends to discuss recent advances in the application of genetically modified microorganism and their role in the biodegradation of a heavily polluted environment with activated sludge, synthetics chemicals, petroleum hydrocarbon, heavy metal, etc. The mode of action

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through which this genetically modified microorganism performs its role was also highlighted. The advantages and demerits of genetically modified microorganisms were also highlighted. The future recommendation that could promote the acceptability and effectiveness of genetically modified microorganisms was also discussed.

Keywords Genetically modified organisms · Environment · Microorganisms · Modes of action

14.1 Introduction

Organisms whose genetic constituents have been improved with the use of cell or gene technology are called genetically modified organisms (GMOs). The genes of microorganisms were the first to be transformed or modified in the laboratory (Melo et al. 2007). These GMOs are currently used in different fields, especially in food science and medicine (Leader et al. 2008). Today, with more improvement in the recombinant DNA of microorganisms, the metabolic capacities have been harnessed and explored in more innovative ways, such as in textiles and paper industries and the degradation of environmental concerned pollutants. So, it is pertinent to have a proper understanding on both the microbes and the process (bioremediation) so as to effectively harness their hidden potentials. Many microbes such as yeast, bacteria, and fungi play a pivotal role in bioremediation. This process is of different forms, but the end material of degradation (carbon dioxide) is similar. Several microorganisms including fungi, bacteria, and yeasts are involved in the biodegradation process. Organic materials have been established to possess the capacity to become degraded anaerobically (without O_2) and aerobically (with O_2) by microbes (Mrozik et al. 2003; Das and Chandran 2011; Pramila et al. 2012).

However, bioremediation, which is a biological process of the degradation and removal of pollutants from the environment by microbes, through the action of the secretion of metabolic enzymes has been identified as one the cheapest, green, and safe alternative tool in environmental rejuvenation or eco-restoration of contaminated environment. Still, microbes lack some level of efficiency in the removal of wastes or any noxious compound in any system. This entire process is rather dawdling, which might cause contaminants to amass in the ecological or biological community. The amassed residues of the pollutants might pose a latent health risk (s) to the ecological or biological community. Heavy metals and pesticides which are one of the most recalcitrant pollutants are typical in this context. Their ecological role has generally lowered the diversity and metabolic activities of living cells, and highly noxious to fauna and flora (Perpetuo et al. 2011). Moreover, microbes have several pathways they applied in the reduction of highly toxic nature of most pollutants; how toxic compounds are taken up by microbial cells (bio-sorption), and the process through which toxic compounds are accumulated in the body cell of microbes (bio-adsorption or bioaccumulation) correspondingly.

It has been discovered that several elements portend the capability to influence the way microbes can degrade pollutants effectively. Typical examples of such factors include phosphorus and nitrogen sources, pH, temperature, humidity, and ionic balance. These factors have spurred various researches on the persistence, competition, and survival of GMOs in the natural ecosystem in which they are introduced and the likely ecological and health risks that may pervade their usage.

This chapter discusses extensively important GMOs that could be applied for bioremediation, a general overview of the molecular proteomics and genomics of microorganism used for bioremediation and specific review on GMOs used for bioremediation and their modes of actions during bioremediation of contaminants. The merits and demerits of GMOs in the ecorestoration of heavily polluted environments were also highlighted. Recommendations for future research will also be highlighted.

14.2 Application of Omics Techniques for the Biodegradation of the Polluted Environment

The utilization of omic techniques and systems biology has given rise to novel algorithms and computational tools that can pave way for a better understanding of the biological activities and reactions that occur with and outside the cell of microorganisms during the degradation of pollutants (Bouhajja et al. 2016). The utilization of the 16S rRNA technique has been applied in the characterization of microbes that have the efficiency to degrade xenobiotic substances (Shapir et al. 2007; Chakraborty et al. 2012).

The application of proteomic, metabolomic, and transcriptomic has been identified to play a crucial role in the biodegradative potential of bacterial hosts (Sohn et al. 2010). A typical illustration could be found in the fluxomic evaluation used on *P. Putida* KT2440 where it was discovered that the effective carbon source pathways could release NADPH, which supports and enhances resistance to oxidative stress (Chavarría et al. 2013; Nikel and de Lorenzo 2013). Examination of transcriptomic data uncovered the significant portion of the *P. Putida* genome ($\geq$20%) that could be differentiated when the cells are grown on diverse substrates and emphasized the role of a collection of global regulators (Kim et al. 2013). Winsor et al. (2016) stated that 215 *Pseudomonas* genomes were completed and 3133 drafted that are present in the *Pseudomonas* Genome Database (http://www.pseudomonas.com/), which is one of the most holistic genomic databases that is devoted to a single bacterial genus.

Moreover, the complete genomic sequences presently available for other beneficial microorganisms that could biodegrade pollutants such as *Cupriavidus necator* JMP134 and *Acidovorax* sp. strain KKS102 contain 300 genes associated with catabolism of aromatics (Lykidis et al. 2010; Ohtsubo et al. 2012). *Oleispira* *antarctica*, *Alicycliphilus denitrificans* strains BC and K601, which are soil degrading microorganisms, have been fingered to possess significant genes that have the capacity to adapt, degrade hydrocarbon, and prevent oil spills in the environment that are cold (Kube et al. 2016).

Bacteria possessing the capability to convert oil-derived aromatics and alkanes into biomass were detected and characterized using metagenome sequencing of the DNA derived from the stable-isotope-probing experiments (Damborsky and Brezovsky 2014; Gutierrez et al. 2013). The consortium roles of the following bacteria such as *Rhodospirillales*, *Cycloclasticus*, *Alteromonas,* and *Alcanivorax* assisted in the biodegradation of oil spill degradation of a complex mixture which consists of almost 1000 compounds. The application of metagenomic approaches also gives a proper understanding of the dynamics and composition of consortia involved in the biodegradation of polycyclic aromatic hydrocarbons in soils (Guazzaroni et al. 2013). The natural broken-down form of chlorinated ethenes available in the polluted groundwater (Adetutu et al. 2015).

Some databases such as MicroScope (www.genoscope.cns.fr/agc/microscope/home/) and NCBI genome database (https://www.ncbi.nlm.nih.gov/genome/) contain several genomic sequences of numerous potential microorganisms that possess the capability to degrade pollutant (Vallenet et al. 2010). It provides a holistic avenue for the comparative evaluation and annotations of almost 6000 microbial genomes out of which 100 were manually curated for accuracy.

14.3 Application of Engineering Genetic in Microorganism for Bioremediation of Heavily Contaminated Environment

The recent advances in protein engineering have provided new opportunities to move towards the wider application of genetically engineered microorganisms to play a crucial role as "designer biocatalysts" in which some biodegradation pathways or the enzyme derived from microorganisms utilized for some of these activities. Several studies have been performed to established that microorganisms and their metabolites could be applied for bioremediation of heavily polluted environment (Adetunji et al. 2017, 2018, 2019a, b, 2020; Adetunji and Adejumo 2017, 2018, 2019). Timmis and Piper (1999) also highlighted several available opportunities that permit the enhancement of utilizing microorganisms for numerous biodegradation opportunities using genetically engineered microorganisms.

Masai et al. (1995) stated that the enzyme 2,3-dihydroxy biphenyl 1,2-dioxygenase derived from strain RDC1 (*etbC*-encoded) of *Rhodococcus* when cultivated on ethylbenzene having the largest substrate specificity of any *meta*-cleavage dioxygenase which have been identified as *Rhodococcus* strains.

Hauschild et al. (1996) discovered that genes *bphC* and *etbC* present in *Rhodococcus* strain RHA1 cultivated on biphenyl or ethylbenzene have been observed to enhance the spectrum of PCB substrates when encoded by the expression of two 2,3-dihydroxy biphenyl 1,2-dioxygenases. Chen and Mulchandani (1998) stated that a gene that regulates the process of deamination of de-ethylatrazine, de-isopropylatrazine, and dechlorination was moved from *R. Corallines* to *Rhodococcus* TE1. This led to the total degradation of atrazine to cyanuric acid through the recombinant *Rhodococcus*.

Fujita et al. (1995) reported that the presence of the phenol hydroxylase gene isolated from *Pseudomonas putida* BH with the recombinant *Escherichia coli* possesses the capability to proficiently degrade trichloroethylene. Yee et al. (1998) tested and evaluated the rhizoremediation potential effects of *P. fluorescens* on trichloroethylene. Moreover, Hauschild et al. (1996) observed that genes derived from catechol 2,3-dioxygenase and phenol hydroxylase were cloned and that their manifestation was established under many conditions in *Alcaligenes eutrophus* JMP134. The manifestation of the gene(s) encoding phenol hydroxylase was detected with the possible potential for oxidative removal of TCE. It was observed that the cloning of the *tod* gene encoding toluene dioxygenase inside *Deinococcus radiodurans* which stimulates the bacterium to play a crucial role in the oxidization of 3,4-dichloro-1-butene, indole, toluene, and chlorobenzene. It was discovered that the engineering of *D. radiodurans* enhances the process of bioremediation which entails mixed wastes containing both organic solvents and radionuclides (Lange et al. 1998).

Erb and Wagner-Dobber (1993) stated that genetically engineered pseudomonad portends the capacity to improved degradation of a mixture of phenols when performed at laboratory scale using a sewage plant fed with chemicals containing 4-methyl phenol and chlorophenols, respectively. They also stated that microbiota can prevent the effects of the pollutants on the sewage tank by guaranteeing the up-keep. Some scientists from the University of Tennessee in cooperation with Oak Ridge National Laboratory recently perform a bioremediation field evaluation utilizing a genetically modified strain of *P. Fluorescens* strain HK44 (Sayler et al. 1999; Ripp et al. 2000). It was discovered that strain HK44 possesses the capability to sense the presence of a contaminant in an environment by reacting to it through bioluminescence signaling to be applied as an online tool for the evaluation and assessment of the eco-restoration process (Sayler and Ripp 2000). Table 14.1 shows the details of genetically engineered microorganisms used in bioremediation.

14.4 Genetically Engineered Microorganisms Used for Bioremediation

Abatenh et al. (2017) did a review of the utilization of microbes in the biodegradation of pollutants. The authors reported that microbes are important elementary tool to stop or reduce environmental degradation as a result of pollution. These microbes are used as bioremediation agents because of the astonishing qualities they have: strong metabolic activities, ability to withstand environmental stress, great genetic

Table 14.1 List of genetically engineered microorganisms reported for bioremediation

SN	Lists of GMOs	Role	Contaminated compounds used for	References
1	Fungi	Bioremediation	Polycyclic aromatic hydrocarbons (PAHs)	Atagana (2009) and Kang (2014)
2	*Rhizobium* sp., *Rhodococcus erythropoli*	Bioremediation	Polychlorinated biphenyl (PCBs)	Damaj and Ahmad (1996) and Kang (2014)
3	*Sphingonomas*, *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Agrobacterium*	Bioremediation	Polycyclic aromatic hydrocarbons (PAHs) and phenanthrene	Aitken et al. (1998) and Kang (2014)
4	*Fomitopsis palustris*, *Pleurotus ostreatus*, *Coriolus versicolor*, *Pycnoporus sanguineus,* and *Pseudomonas* spp.	Bioremediation	Polycyclic aromatic hydrocarbons (PAHs)	Arun et al. (2008) and Kang (2014)
5	*Clostridium acetobutylicum* and *Acetobacterium paludosum*	Bioremediation	Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	Sherburne et al. (2005) and Kang (2014)
6	*Rhizobium meliloti* and strain B-14 (*Enterobacter*)	Bioremediation	Chlorpyrifos	Kang (2014)
7	*Pseudomonas* spp.	Bioremediation	Atrazine	Kang (2014)
8	*Rhodopseudomonas palustris* and *Aerococcus* spp.	Bioremediation	Cd, Cr, and Pb^{2+}	Mohamed et al. (2011) and Sinha and Biswas (2014)
9	*Aromona* spp. and *Pseudomonas aeruginosa*	Bioremediation	Cr, Ni, cu, and U	Sinha et al. (2011)
10	Strains RSA-4, PB-5, and JX126862	Bioremediation	Cd	Priyalaxmi et al. (2014)
11	*Geobacter* sp.	Bioremediation	U (VI) and Fe (III)	Mirlahiji and Eisazadeh (2014)
12	*Cladosporium* sp., *Microsporum*, *Terichoderma*, *Paecilomyces* sp., *Paecilomyces* sp., *A. fumigatus,* and *Aspergillus versicolor*	Bioremediation	Cd	Soleimani et al. (2015)
13	Strain $shh_{49}T$	Bioremediation	Iron (Fe)	Wu et al. (2015)
14	Strain $CBAM_5$	Bioremediation	Pb, Cr, cu, and co	Peña-Montenegro et al. (2015)
15	*Pseudomonas aeruginosa* and *Pseudomonas fluorescence*	Bioremediation	Cu^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, and Fe^{2+}	Paranthaman and Karthikeyan (2015)
16	*Cunninghamella elegans*	Bioremediation	Ni, hg, and Pb^{2+}	Tigini et al. (2010)

(continued)

Table 14.1 (continued)

SN	Lists of GMOs	Role	Contaminated compounds used for	References
17	*Saccharomyces cerevisiae*	Bioremediation	Ni, hg, and Pb^{2+}	Chen and Wang (2007), Talos et al. (2009) and Infante et al. (2014)
18	*Serratia ficaria*, *Citrobacter koseri*, *Bacillus coagulans*, *Bacillus cereus*, *Pseudomonas cepacia*	Biodegradation	Crude and diesel oils	Kehinde and Isaac (2016)
19	*Bacillus* sp., *Arthrobacter* sp., *P. putida,* and *pseudomonas aeruginosa*	Biodegradation	Diesel oil	Sukumar and Nirmala (2016)
20	*B. sphaericus*, *B. licheniformis*, strain KH_6 (*P. aeruginosa*), and *B. brevis*	Biodegradation	Crude oil	El-Borai et al. (2016)
21	*Saccharomyces cerevisiae*, *Candida krusei*, *Candida glabrata*, and *Aspergillus niger*	Biodegradation	Crude oil	Burghal et al. (2016)
22	*Bacillus cereus*	Biodegradation	Diesel oil	Maliji et al. (2013)
23	*Pseudomonas aeruginosa*, *Corynebacterium propinquum*, *Bacillus subtilis,* and *Alcaligene sodorans*	Biodegradation	Oil	Singh et al. (2013)
24	*Fusarium* sp.	Biodegradation	Oil	Hidayat and Tachibana (2012)
25	*Photobacterium* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Acinetobacter* sp.	Biodegradation	Methyl parathion and chlorpyrifos	Mulligana and Yong (2004)
26	*Arthrobacter* sp., *Acinetobacter*, and *Pseudomonas putida*	Biodegradation	Decis 2.5 EC, malation, Fitoraz, and Ridomil MZ 68 MG	Mónica et al. (2016) and Hussaini et al. (2013)
27	*Enterobacter*	Biodegradation	Chlorpyrifos	Niti et al. (2013)
28	*Bacillus, Staphylococcus*	Biodegradation	Endosulfan	Mohamed et al. (2011)
29	*Klebsiella oxytoca*, *Staphylococcus*, *Bacillus macerans*, and *Bacillus firmus*	Biodegradation	Textile effluents and vat dyes	Jaysankar et al. (2008) and Adebajo et al. (2017)
30	*Acinetobacter baumannii*, *Bacillus cereus*, *Exiguobacterium aurantiacum*, *Exiguobacterium indicum*	Biodegradation	Azo dye effluents	

(continued)

Table 14.1 (continued)

SN	Lists of GMOs	Role	Contaminated compounds used for	References
31	Strain HKG_12 (*Bacillus pumilus*), *Pseudomonas aeruginosa*, and strain ETL_{-2012} (*Bacillus* spp.)	Biodegradation	RNB dye and sulfonated di-Azo dye reactive red HEBB	Shah et al. (2013), Das et al. (2015), and Yogesh and Akshaya (2016)
32	*Nocardia atlantica*, *Listeria denitrificans*, *Micrococcus luteus*	Biodegradation	Textile Azo dyes	Hassan et al. (2013)
33	*Penicillium ochrochloron*	Biodegradation	Industrial dyes	Shedbalkar and Jadhav (2011)
34	*Trametes trogii*, *Phanerochaete chrysosporium*, and *Pycnoporus sanguineus*	Biodegradation	Industrial dyes	Yan et al. (2014)
35	Strain NAP_1, NAP_2, and NAP_4 (*B. subtilis*)	Biodegradation	Oil-based paints	Jasińska et al. (2012)

ability that is retained in the population and high nutritional qualities. They stated the process involved in the bioremediation of hazardous and waste materials as degradation, eradication, and immobilization/detoxification. Besides, they itemized various bioremediation techniques (bioattenuation, biopiles, bioaugmentation, and biostimulation) that are currently applied in the degradation of pollutants in the environment. However, each technique has its own merits and demerits based on its utilization. For microbes to be efficient in their biological role in the ecosystem, they must be able to tolerate the level of abiotic and biotic factors influencing them. In conclusion, the authors stated that bioremediation is a very attractive and fruitful alternative for recuperating, managing, cleaning, and decontaminating environmental pollutants. The action of microorganisms on wastes and hazardous compounds will be more effective if the environmental factors allow their activity and growth. The merits of using microorganisms surpassed their demerits.

Kang (2014) did a review on the elimination of natural pollutants using phytoremediation and bioremediation processes. The authors recounted the ecological and health impacts of dangerous organic contaminants to the environment and humans at large. They also stressed that the application of biotechnology using phytoremediation and bioremediation processes is the best natural method in decontaminating wastes in the environment. Hence, they are green, cheap to purchase, and sustainable in usage. The benefits of their usage surpassed what is obtainable from conventional methods. In conclusion, the authors recommend both the biological degrading processes as promising biotechnology for environmental sustainability. Moreover, to expand on their utilization, the technology must be couturier to the level of pollutants, the site of application, and the indigenous locality. In conclusion, they recommend that further study should be done on the biological activities of plant and microbial remediators. The role of a specific DNA of the microbes and plants that are involved in the transport of specific contaminants, uptake, and metabolism should also be looked into to be utilized for successful eco-friendly biotechnology. However, there is great latency in the field of biotechnology. With the increase of substance and effective management from governmental bodies, there may be novel breakthroughs via research and pilot studies on phytoremediation and bioremediation.

Urgun-Demirtas et al. (2006) in a review critically evaluate the bioremediation potentials of GMOs on contaminants. The authors stated some important aspects of utilizing GMOs such as enzyme affinity and specificity and the improvement of new strains that have desirable qualities. A typical example is the genetic engineering of a microbe using the red blood cell (VHb). This engineered microbe can be utilized for the decontamination of polyaromatic hydrocarbons (PAHs) under low oxygen state. This will enhance the decontamination of polluted sites where bacteria cannot thrive under oxygen limiting environment. This is because some bacteria need oxygenated enzymes (oxygenases) for the mineralization of some natural pollutants on site. Notwithstanding the enormous merits of this, there are a series of worries that their application for the bioremediation process might cause serious environmental and health risks via gene transmission. In conclusion, they proposed the genetic enhancement of native microbial species to increase the efficiency of bioremediation.

Das and Chandran (2011) did a review of the bioremediation of pollutants in petroleum hydrocarbon compounds. They recounted that most neurotoxic and carcinogenic impacts are a source of hydrocarbon contaminants. Conventional methods (chemical and mechanical) used in the degradation of hydrocarbon pollutants are too expensive and not environmentally friendly. An alternative approach (bioremediation) serves as a promising biological tool because of the enormous benefits derived from its utilization: cheap, green, non-noxious, and the ability to totally mineralize (biodegrade) pollutants into cell protein, inorganic compounds, water, and carbon dioxide. Specific microbes in soil and water have been known to have the potential in decontaminating hydrocarbon pollutants. In conclusion, the authors recommend the utilization of GMO microbes as the first tier in the decontamination or clean-up of hydrocarbon pollutants in soil and water because of the significant potential it possesses.

Price and Cotter (2014) did a pilot survey of the contagion episodes linked to GMOs from 1997 to 2013. Since the introduction of GMOs in 1996 into cultivated land, there has been a great concern that native plants (non-GMOs) can become contaminated by the recombinant engineered DNA. A total of 396 incidences were documented from 1997 to 2013, in the survey piloted in 63 countries. The results of their pilot survey showed that *Oryza sativa* (rice) has the highest contaminated level from GMO occurrences regardless of that genetically modified rice has not yet been commercialized anywhere in the world. The major incidences occurred from two clear illicit rice-contaminated scenarios: BT63 rice from China and LLRICE from the USA (75% contamination level). *Zea mays* (maize) accounted for about 25% of the polluted episodes while *Brassica napus* (oilseed rape) and *Glycine max* (soya bean) had roughly 10% of the contaminated incidents. However, several biological, ecological, and economic factors such as the level of international trade, a description of the nature of food, the biology of the plant, and the span of acre cultivated, might have influenced the degree of the contamination. The authors in conclusion stated that GMO contamination can ensure self-reliance from commercialization. A typical case is pawpaw, maize, and grass in Thailand, Mexico, and the USA, respectively, which is still ongoing. Other special cases are pharmaceutical producing genetically modified and Bt10 maize crops. GMO contamination relies solely on both besieged observing regimes and routine which might not be consistent from one nation to the other, even in the European Union. They recommend that a rapid assessment should be done at field trials before commercialization. This will hinder the transfer of the genetic line of the modified organisms.

Liu et al. (2019) did a review on the alleviation of the contaminated environment of GMOs as well as the present challenges and prospect standpoints. The authors stated that environmental toxic wastes are increasing due to the global increase in population and technological boom. These have generated a lot of chemical residues in the biosphere which might be carcinogenic and hazardous to life in water, land, and air. The physical and conventional chemical means of decontamination of wastes in the ecosystem have been proven to be inefficient for waste-chemical degradation. However, a sustainable approach using a biological approach (bioremediation), a less noxious, social acceptance, and eco-friendly method has received a significant level of acceptability for the abatement and mitigation of pollutants in the environment. The authors looked at the merits derived from GMOs when utilized in the degradation of pollutants like agrochemicals, phenazines, PCBs, PAHs, heavy metals, and artificial dyestuff. However, the ecological and health risk associated with the genetic exchange of materials with the microbes at the site of actions has been one of the limitations and challenges faced in the applications. The authors in conclusion propose that an integrated ecological, biological, and microbiological association should go alongside the methodology of the engineering designs to attain a desirable and effective in situ remediation process without a trace of recombinant bacteria contamination. Besides, the introduction of protoplast fusion and plasmid technical know in the breeding of GMOs will serve as a pivotal stand view for future bioremediation processes.

Benjamin and Ashok (2016) did a review on the mode of action for the bioremediative capability of GMOs. The authors recounted that the application of conventional method in the remediation of wastes and hazardous compounds in the ecosystem has failed in so many aspects like the generation of the more noxious compound, the complication of the biological process, and very expensive to employ. An environmental welcoming biological method, bioremediation, has been chosen as the best candidate for the natural decontamination of wastes and hazardous wastes because it is cheaper and more effective in environmental clean-up. The authors recommend an integrated method between bioremediation and

GMOs, to fathom their correlation for effective management of pollutants at the sites.

Wastewater effluents obtained from metal refineries and mines have been highlighted to be contaminated with heavy metal ions which could eventually constitute environmental and health hazards. The application of conventional technologies has been recognized for the removal of heavy metal ions, but there are several disadvantages such as ion exchange resins are manufactured from unsustainable nonrenewable resources, chemical precipitation produces activated carbon and sludge waste. The utilization of biomass derived from some beneficial microorganisms has been identified as an alternative for the bioremediation of heavily contaminated metal ion present in an environment. Precisely, bioaccumulation has been identified as a natural biological process where microorganisms could be utilized to sequester metals and proteins available in the intracellular space, for specific utilization in various cellular processes such as stabilizing charges on the biomolecules, cell signaling, and enzyme catalysis. However, the application of genetic engineering and recombinant expression enables improved sequestration and uptake of heavy metal ions.

Because of the aforementioned, Diep et al. (2018) wrote a comprehensive review on the application of bioaccumulation that could be utilized for bio-extractive applications which entail the absorption and recovery of heavy metal ions for downstream purification as well as the process of refining instead of disposal. The authors also validated various import-storage systems into a biochemical framework and pointed out significant effort that could prevent all the highlighted limitation that could affect a greater result especially at the industrial level while gaps and other future recommendation that could promote the process of bioaccumulation.

14.4.1 Application of Recombinant Bacteria for Metal Removal

The application of recombinant bacteria in the removal of certain metal from heavily polluted water has been established. This includes the application of genetically engineered *E. coli* which has been acclimatized to possess the capability to express metallothionein which is a metal-binding protein and Hg^{2+} transport system as well as possess the capability to build up 8 μmole Hg^{2+}/g cell dry weight. It has been observed that the presence of chelating agents such as Ca^{2+}, Na^{+}, and Mg^{2+}.

14.4.1.1 Significant of Genetically Modified Biosorbents in the Eco-Restoration of Polluted Environment

The application of genetic engineering has been applied for the enhancement of beneficial microorganisms in a way to enhance some intrinsic potentials and adaptabilities to numerous environmental conditions (Bae et al. 2000; Majáre and Bülow

2001). It has been observed that virgin biosorbents normally do not have specificity in the metal binding which may induce problems in the recovery and especially the process of reuse of certain metal of high significance. Therefore, the utilization of genetic engineering could enhance the level of specificity and build up potentials of microbial cells for the effective restoration of the heavily polluted environment (Pazirandeh et al. 1995). The application of genetic engineering could also assist in the production of microbial biomass especially from the fermentation process. In the bioprocess, several nucleic acids and amino acids are generated on an industrial scale. It has been observed that some strains react and are sensitive to the presence of metals with enhanced generation of cysteine-rich peptides which entails metallothioneins (MTs), phytochelatins (PCs) (Mehra and Winge 1991), and glutathione (GSH) (Singhal et al. 1997). They all possess the capability to combine with metal ions biologically, in inactive forms (Bae et al. 2000).

It has been highlighted that the overexpression of MTs in bacterial cells will lead to the improvement of metal accumulation as well as strategic enhancement in the rate of metal build-up and microbial-based biosorbents for the eco-restoration of the heavily polluted environment (Pazirandeh et al. 1995). The presence of intracellular expression of MTs may obstruct the reuse of biosorbents because it possesses the capability to prevent the release of the build-up metals (Gadd and White 1993). However, Chen and Georgiou (2002) infer that these challenges could be overcome through the manifestation of MTs on the cell surface.

Sousa et al. (1996) evaluated the probable introduction of MTs into accommodating site 153 of the *LamB* sequence. It was observed that the manifestation of hybrid protein on the cell surface vividly improved the whole-cell build-up of cadmium. The presence of this manifestation or the presence of proteins on the surface offers an alternative method for the preparation of cost-effective affinity adsorbents. The application of PCs in the same way in comparison to MTs has been recommended (Bae et al. 2000). PCs are cysteine-rich peptides, with short size, and they possess a general structure (γGlu-Cys)nGly ($n = 2$–11). One of the merits of PCs, when compared to MTs, includes exceptional structural features, predominantly the incessantly recapping γGlu-Cys units. Moreover, it has been noted that PCs possess a more enhanced metal-binding capability which is based on a cysteine basis when compared to MTs (Mehra and Mulchandani 1995). Conversely, the growth of overexpressing PC organisms necessitates a comprehensive understanding of the mechanisms applied in the production and chain elongation of these peptides.

Numerous biosorbents, demonstrating metal-binding peptides on the cell superficial, have been efficaciously engineered. An example of this entails a repetitive metal-binding motif, having (Glu-Cys)nGly (Bae et al. 2000). These peptides resemble the structure of PCs but with little difference in the peptide bond that joins the cysteines and the glutamic acids. It was discovered that phytochelatin analogs were available on the outer surface of the bacterial cell which enhances the build-up of 20-fold (Bae et al. 2001) and Cd^{2+} and Hg^{2+} by 12-fold (Bae et al. 2000).

The application of *E. coli has* been recognized as a recombinant bacterium that possesses an enhanced metal absorption capacity because the bacteria has an enhanced surface area per unit of cell mass which assists in the uptake of more metal from solutions (Chen and Wilson 1997). Moreover, it has been observed that the system has its own advantages when compared to Gram-negative bacteria (Malik et al. 1998; Samuelson et al. 2000). The process of translocation occurs only through one membrane. During the process of translocation, the Gram-positive bacteria are more rigid with minimal sensitivity to stress because of the presence of a thick cell wall that can encircle the microbial cells, which enable the process of bio-adsorption to be more feasible (Kelemen and Sharpe 1979). Samuelson et al. (2000) also utilize *Staphylococcus carnosus* strains and recombinant *Staphylococcus xylosus* with a surface-exposed chimeric protein. It was revealed that the two strains of Staphylococcaceae possess the capability to enhance nickel-binding potentials which might be linked to the introduction of the H1 or H2 peptide into their surface proteins. This showed that genetically engineered biosorbents may be utilized in the separation of pollutants from toxins and other contaminants from heavily contaminated solutions.

14.4.1.2 Modes of Action Utilized by Genetically Engineered Microorganisms for the Bioremediation of Heavily Contaminated Environment

The application of biostimulation and bioaugmentation has been identified as the technique utilized for the rapid bioremediation of heavily polluted sites. Several tremendous signs of progress had been made in the late 1970s and early 1980s where the bacterial gene responsible for the encoding catabolic enzymes for bioremediation of compounds were identified, cloned, and characterized and used for the eco-restoration of the heavily polluted environment (Cases and de Lorenzo 2005). A genetically modified microorganism (GMM) and genetically engineered microorganism (GEM) could be defined as microorganism whose genetic material has been changed utilizing genetic engineering approaches stimulated by a regular genetic exchange between microorganisms. This approach is referred to as recombinant DNA technology. The application of genetically engineered microorganisms has special applications for eco-restoration of activated sludge, groundwater, soil, and the heavily polluted environment with synthetics chemicals (Sayler and Ripp 2000).

The specific effort has also been considered on the application of biosafety and risk assessment especially when genetically modified microorganisms are utilized for the bioremediation of polluted soil (Cases and de Lorenzo 2005). The following five potential modes of action that govern the process of genetically modified microorganisms for the bioremediation of heavily polluted environment.

1. Alteration of enzyme affinity and specificity.
2. Construction of necessary pathway and their regulation.

3. Bioprocess improvement, observing, and control.
4. Bioaffinity.
5. Use of bioreporter sensor for toxicity decrease, chemical sensing, and endpoint evaluation (Menn et al. 2008).

14.4.1.3 Genes Responsible for Bioremediation Process in Genetically Engineered Microorganisms

Molecular biology has been able to provide more insight on the application of tools that could be used to optimize the biodegradation potentials of microorganisms, through the pool of catabolic segments sourced from them (Ramos et al. 1994). Several genes have been identified to be responsible for the biodegradation of a heavily contaminated environment that consists of halogenated pesticides, toxic wastes, chlorobenzene acids, and toluene. It has been highlighted that every compound required a particular plasmid for the bioremediation. These plasmids are grouped into (1) NAH plasmid which reduces naphthalene; (2) OCT plasmid which reduces hexane, octane, and decane; and (3) CAM plasmids that decompose camphor and XYL plasmid which reduces xylene and toluene (Ramos et al. 1994). The capability of the genetically modified microorganisms to degrade toxic compounds and hydrocarbon has been highlighted by Markandey and Rajvaidya (2004). They utilize multiplasmid-containing *Pseudomonas* strain that could oxidize terpenic, aliphatic, aromatic, and polyaromatic hydrocarbons. Moreover, the presence of XYL and NAH plasmid together with a hybrid plasmid from the recombination parts of CAM and OCT derived through the process of conjugation showed that they could biodegrade naphthalene, camphor, salicylate, and octane (Sayler and Ripp 2000). It was also observed that they possess the capability to grow on crude oil because it could metabolize hydrocarbon more proficiently when compared to another plasmid (Markandey and Rajvaidya 2004).

Moreover, *P. putida* has been highlighted as a microorganism that possesses the capability to degrade several chemical compounds such aspKF439 (for salicylate toluene), TOL (for toluene and xylene), pAC 25 (for 3-chlorobenzoate), and RA500 (for 3,5-xylene).

It was observed that plasmid WWO of *P. putida* which belongs to the sets of plasmids which is referred to as TOL plasmid. It has been observed that molecular approaches through sheer genetic engineering and plasmid breeding could lead to the generation of microbes with enhanced catalytic potential that could play an active role in the biodegradation of a heavily polluted environment (Sayler and Ripp 2000).

Typical examples of the genetic engineering technologies utilized by genetically engineered microorganisms include alteration of substrate specificity by *Comamonas testosterone* VP44 and changes in pathway (Hrywna et al. 1999). In this regard, *Alcaligenes eutrophus* AE104 (pEBZ141) was applied for the removal of chromium available in industrial waste (Srivastava et al. 2010) while the application of recombinant photosynthetic bacterium, *Rhodopseudomonas palustris*, was applied to remove mercury (Hg^{2+}), and metallothionein for Hg^{2+} removal was available in heavy metal wastewater (Xu and Pei 2011). Moreover, the following microorganisms possess the capacity to break down polychlorinated biphenyls such as *A. denitrificans* JB1, *Achromobacter* sp. LBS1C1, and *R. eutropha* A5 which were discovered to possess chromosomally located PCB catabolic genes that were transferred into a heavy metal–resistant strain *R. eutropha* CH34 using natural conjugation (Menn et al. 2008).

The utilization of rhizospheric and endophytic bacteria for the biodegradation of toxic compounds available in the soil represents a new technology for the eco-restoration of heavily contaminated sites (Divya and Deepak Kumar 2011). Three procedures are involved in the selection of a suitable strain for gene recombination and inoculation into the rhizosphere. First, the probable strain must be stable after the process of cloning, and the targeted gene must possess a high expression; second, the strain must be insensitive and tolerant to pollutant, and third, the stains must be able to survive in some certain specific plant rhizosphere (Huang et al. 2004).

Glick (2010) stated that numerous bacteria available in the rhizosphere possess the limited capability to break down organic pollutants but the application of molecular biology through the application of genetically engineered rhizobacteria which possess the capability to break down pollutants because of the presence of contaminant-degrading gene are constructed for rhizoremediation. A typical example of these molecular modes of action involved the breakdown of some contaminants which include polychlorinated biphenyls and trichloroethylene.

Sriprang et al. (2003) inserted *the Arabidopsis thaliana* gene which is responsible for phytochelatin synthase (PCS; PCSAt) into *Mesorhizobium huakuii* sub sp. *rengei* strain B3 which later establish a robust symbiosis between *Astragalus sinicus* and *M. huakuii* subsp. *rengei* strain B3. The gene was inserted to perform some specific function such as the build-up of Cd^{2+}, produce phytochelatins, and regulation of bacteroid specific promoter gene (Sussman et al. 1988). Moreover, there is a need to factor in some biosafety issues and many field trials before the eventual release of genetically engineered microorganisms into the environment. This will go a long way in resolving several ecological damages (Wackett 2004).

14.4.1.4 Disadvantages of Genetically Engineered Microorganisms in the Eco-Restoration of Polluted Environment

The application of genetic engineering has led to the production of many useful strains that possess a huge capability to break down contaminants in a bioreactor or a petri dish. However, the translation of these techniques on a larger scale is a very big problem especially in situ bioremediation practices (Sayler and Ripp 2000). It has been observed that some of these strains possess little action during the field trial. The application of equivalent methods has been applied to established that recombinant genes play an active role in the biodegradation of a heavily contaminated environment. Moreover, some strains are fast grower with high biodegradative capability. However, they could build up a high level of biomass which could affect the rate of their bioremediation potentials. The alternative route to this involves the application of potential strain that could exhibit a very high catalytic potential with the lowest of cell mass. The manifestation of biodegradation genes can be preciously separated with the application of starvation promoters or stationary phase promoters (Matin 1994).

Furthermore, the recent development in the utilization of recombinant DNA technologies has surfaced the manner for hypothesizing “suicidal genetically engineered microorganisms” (S-GEMS) that could mitigate the predicted hazards and eventual achievement of a cleaner environment. Also, some protozoa have been identified to engulf and prevent the growth of the introduced recombinant or bacteria beyond a certain level (Iwasaki et al. 1993; Foster et al. 2002). On the whole, there is a need to evaluate the effectiveness of a strain with necessary in situ catalytic activity for the biodegradation of pollutants which must be carried out on a larger scale in the field. Moreover, there is a need to establish the risk associated with engineered bacteria. This will go a long way in their establishment as a potential bioremediation tool for the treatment of a heavily polluted environment (Sayler and Ripp 2000; Ripp et al. 2000). There is also a need to develop some other stains apart from *B. subtilis*, *E. coli*, and *P. putida*, as an engineered microbe for their application as an effective bioremediation tool. Conversely, the persistence of the genetically engineered bacteria in complex environmental conditions is still to be addressed in light of state-of-the-art outcomes (Singh et al. 2011).

14.5 Conclusion and Future Recommendations

This chapter has provided a detailed information about the application of genetically engineered microorganisms for the eco-restoration of the heavily polluted environment such as activated sludge environments, groundwater, and soil, and detailed information was provided on their utilization for the bioremediation of polluted environment with heavy metal and several synthetics chemicals. The application of omic and system biology in the enhancement was also highlighted in detail while the mode of action through which these genetically engineered microorganisms perform their biodegradative activity was also established. There is a need to carry out several field trials such as long-term field release studies to validate all the laboratory-based experimental data. The biosafety precaution and safety of genetically engineered microorganisms need to be establishing before their eventual release.

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Chapter 15
Efficacy of Microorganisms in the Removal of Toxic Materials from Industrial Effluents

Naveen Dwivedi, Shubha Dwivedi, and Charles Oluwaseun Adetunji

Contents

Abstract Industrial wastewater creates major environmental trouble if released without proper treatment. Large volumes of water contaminated with various industrial and anthropogenic activities can produce hazardous effects on the environment and the living organisms. Various industries discharge toxic materials, heavy metals, and anions into the environment that considerably enhanced the deterioration of environment, flora, and fauna, and significantly pose threats to the ecosystem. These

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noxious materials cause serious health issues, if they surpass the acceptable limit in water. There is a big challenge to remove toxic pollutants from water and wastewater. Some traditional methods such as coagulation, chemical precipitation, carbon adsorption, oxidation, ion exchange, evaporations, and membrane processes are found to be helpful in the treatment of wastewater. However, these methods are unlikable from both environmental and cost-effective viewpoints because these require utilizing chemical compounds, huge energy and also do not degrade the complete range of pollutants. Therefore, novel and more effective treatment methods of removing toxic compounds from water and wastewater needed to be develop. In this regard, the efforts have been made toward bioremoval applications and its efficiency for the removal of hazardous materials from water and wastewater by using microorganisms. Among all treatment methods introduced above, the biological treatment method is a proficient, inexpensive, simple, and environment friendly process for treating pollutants. Use of microbial technology in the treatment of pollutants gained a momentum of efficient degradation ability, simple technical operation, lower process time, low energy requirements, no secondary pollution, and long-term viability.

In this chapter, we are aimed to make comprehensive description about microorganisms and their effectiveness on the removal of various hazardous materials which are released from different industrial effluents.

Keywords Microbial technology · Toxic pollutants · Cyanide · Heavy metals · Sewage · Enzymatic treatment

15.1 Introduction

Environmental pollution has been increasing in the past few decades due to increased human activities on unsafe agricultural practices, energy reservoirs, and rapid industrialization. The increasing pollution of water resources by hazardous industrial wastewater poses a critical ecological trouble (Giraldo and Moreno-Piraján 2010). Most hazardous contaminants like phenol, cyanide, fluoride, xenobiotics, and heavy metals are commonly toxic and carcinogenic doer, including discharges from different industries such as chemical, pharmaceutical, paint, agricultural, herbicides, pesticides, plastics, pulp and paper mills, textile, coal refineries, dying, petrochemical, etc. When these toxic materials are released from industrial effluents into the water tributary, it makes a serious threat to the human population, aquatic life, flora, and fauna. Today, humans are affected by the devastating environment and polluted water became a greater issue of concern. In general, from about 1970 to 1990s, treatment of wastewater concentrated on the reduction and elimination of floatable and suspended material, removal of BOD, and the abolition of disease-causing pathogens from wastewater. From 1990, due to improved technical knowledge and scientific awareness, treatment of industrial effluents has started to believe on the environmental issues and the health concerns associated to hazardous and potentially lethal substances released into the water and environment (Rajasulochana and Preethy 2016). However, the early treatment objectives remain applicable at present, but currently the essential level of treatment has improved extensively and further treatment principles and goals have been added. In this regard, key pursuits were taken throughout the world, to achieve more effective and prevalent methods for the improvement in the quality of wastewater treatment. For this, the efforts should be done on an increased understanding and facts about the adverse effects on the environment due to the hazardous industrial effluents discharge in water bodies.

There are various treatment techniques available in the removal of hazardous contaminants from industrial wastewater such as physical and chemical processes, i.e., adsorption, ion exchange, coagulation, precipitation, evaporation, chemical oxidation, reverse osmosis, membrane processing, and electrochemical treatment (Bakkaloglu et al. 1998; Matsumotto et al. 2007a, b; Hamdan and El-Naas 2014; Lin et al. 2014). However, some treatment techniques have given very positive results but they are disapproved due to the high processing cost and unfavorable in the environmental point of view. Scarcity of clean water has become more common, therefore environmental regulations has become stricter day by day. Hence, there is a need to search for a sustainable, reliable, eco-friendly, cost-effective solution that could prevent environmental pollution and challenges. Considering the high treatment cost and eco-unfriendly methods, the extensive work has been done on the recent advancement in the bioremoval techniques using microorganisms with the ultimate goal being to effectively restore polluted environments in an inexpensive and environment friendly manner. The microorganisms used to eliminate and recover harmful or valuable metals from industrial wastewaters has grabbed attention in the recent past due to improved performance, low cost of raw materials, and availability (Ahluwalia and Goyal 2007, b; Benaissa and Elouchdi 2007; Bunluesin et al. 2007, b). Microorganisms act an important role in the environmental outcome of hazardous compounds and toxic metals with a diversity of mechanisms carrying out transformations between insoluble and soluble forms. These mechanisms are essential parts of real biogeochemical cycles and are of possible for both in situ and ex situ biological treatment processes for liquid and solid wastes (Gadd 2000). Researchers have developed and designed different bioremediation techniques; however, according to nature and/or type of contaminants, various microbial species are found to be capable to remove toxic materials from industrial wastewater and restore polluted environments. The present chapter describes the neoteric mechanisms of contaminants removal from wastewater and effectiveness of microbial transformations of contaminants through bioremediation and biotechnological approach.

15.2 Major Toxic Pollutants Their Sources and Effects on Environment and Human Beings

From many years it has been observed, the inappropriate disposal of industrial wastewater becomes a foremost trouble and a prime concern to both industrialists and government. The wastewater produced from industrial activities and domestic comprise the key sources of the natural water pollution. Following is the major toxic pollutants which are released from various industrial effluents.

15.2.1 Heavy Metals

Heavy metals are one of the most stable metallic elements discharge from industrial waste, having a comparatively high density, i.e., more than 5 g/cm^3 and are toxic even at low concentrations.

As we know that human activities and wrong industrial discharge practices are the major cause of heavy metal production, pollution, and causative to disease as well as poverty on a worldwide level. Depending on the exposure of heavy metals, it can be noxious to human health (Costa et al. 2006). The metalloids and metals that pollute water and most frequently occur in environment comprise mercury, arsenic, chromium, lead, zinc, uranium, selenium, silver, nickel, gold, and cadmium. Some of them are recognized micronutrients, such as iron, cobalt, zinc, chromium, copper, iodine, manganese, molybdenum, and selenium, while they are crucial for the synthesis of certain biomolecules. Industrial wastewater containing heavy metals include automobile exhaust, acid mine drainage, effluent from storage batteries and the manufacturing and too little use of pesticides, fertilizers, and loads of others. The metallurgical industries to make batteries such as lithium, lead, and Ni-Cadmium batteries leads to the environmental pollution. Smelters and factories that practice enormous quantities of metals may also be cause of metal pollution via smoke stacks.

Arsenic is also one of such highly toxic contaminant, which have utmost threats to the microorganisms and human beings. It is expected that approximately ten million people suffer from high arsenic menace worldwide (Li et al. 2014; Essadki et al. 2009). Arsenic is occurring natively in a large quantity in the Earth's crust and in little amounts in rock, soil, air, and water. The normal concentration of arsenic in the continental crust is 1–2 mg/kg. The mean concentration of arsenic in igneous rocks ranges from 1.5 to 3.0 mg/kg, while in sedimentary rocks it ranges from 1.7 to 400 mg/kg. Around one-third of the arsenic in the atmosphere comes from natural sources (NIH and CGWB 2010). Volcanic activity is the most significant natural source of arsenic. Another most important natural source is the arsenic-containing vapor that is produced from solid or liquid forms of arsenic salts at low temperatures. The rest two-third arsenic comes from anthropogenic sources which include the major industrial processes such as metal smelting, coal-fired power plants, mining,

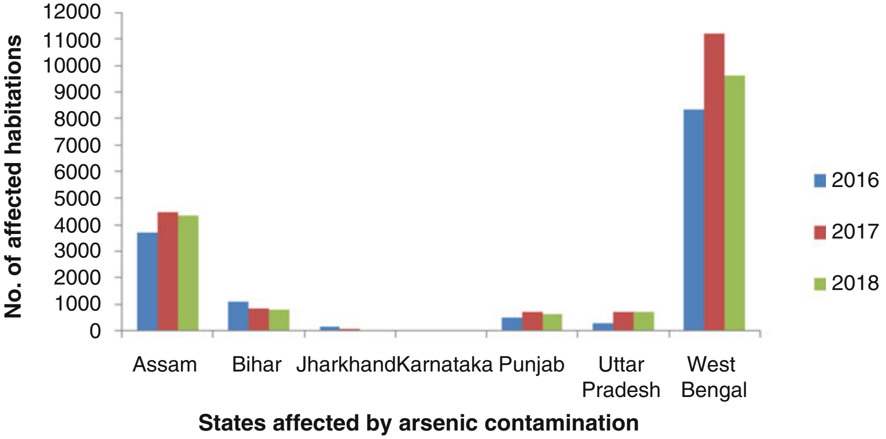

Fig. 15.1 Number of habitation affected by arsenic

burning of fossil fuels and that provide arsenic pollution to water, air, and soil (NIH and CGWB 2010). Arsenic pollution in groundwater was reported in the Ganga-Brahmaputra fluvial plains in India and Padma-Meghna fluvial plains in Bangladesh. In India, the groundwater arsenic pollution was earliest surfaced from West Bengal in 1983, many other States, namely, Punjab, Uttar Pradesh, Bihar, Jharkhand, Assam, Manipur, and Rajnandgaon village in Chhattisgarh state have chronically been exposed to arsenic contamination in groundwater beyond the permissible limit of 50 μg/L. The arsenic menace in India has been recently reported, according to the data available as from April 2016 to April 2018, the state-wise several habitations influenced by arsenic pollution in ground water is shown in Fig. 15.1 and the number of affected habitations by heavy metals is depicted in Fig. 15.2 (Ministry of Drinking Water and Sanitation 2019).

15.2.1.1 Effect of Heavy Metals

Pollution by heavy metals generates several harmful outcomes, which affect microorganisms, human health as well as flora and fauna (Chakraborty et al. 2013; Ilyina et al. 2003). Among heavy metals, lead is one of the most hazardous elements which affects the pregnant women and children.

The World Health Organization (WHO) declares that there is no identified level of lead exposure that is considered safe, and lead poisoning in childhood is linked to cognitive impairment, aggressive offense in adulthood, and loss of financial productivity (Landrigan et al. 2017). Arsenic, cadmium, lead, and mercury are listed among the ten chemicals of foremost public concern by the WHO for their promising to be carcinogenic and impose severe organ injury (Tchounwou et al. 2012). The heavy metals guidelines for drinking water by the World Health Organization (WHO) are given in Table 15.1. Arsenic affected people suffering from different deadly diseases

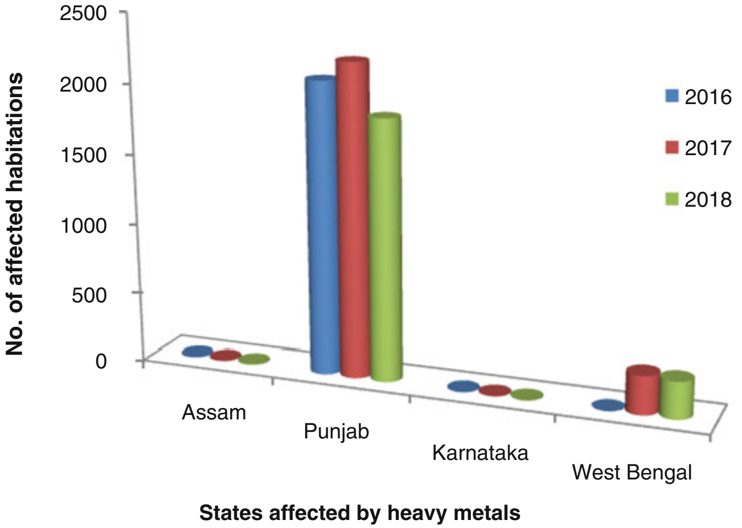

Fig. 15.2 Number of habitation affected by heavy metals

Table 15.1 The World Health Organization (WHO) standards for the most hazardous heavy metals in drinking water

S. no.	Heavy metals	Max. acceptable conc. (WHO)
1	Arsenic	0.01 mg/L
2	Zinc	5 mg/L
3	Cadmium	0.003 mg/L
4	Magnesium	50 mg/L
5	Calcium	50 mg/L
6	Mercury	0.001 mg/ L
7	Silver	0.0 mg/L
8	Lead	0.01 g/L

are being reported. Arsenic groundwater pollution has distant getting consequences including its ingestion via food chain which are in the form of social chaos, health risks, and socio-economic disintegration. Several kinds of skin traits and other arsenic toxicity were identified from hyperkeratosis, melanosis, dorsal, keratosis, keratosis, and non-pitting edema to gangrene. Arsenicals have been associated with cancers of the bladder, lung, and skin (Ng et al. 2003). Arsenic expands its toxicity by deactivating up to 200 enzymes, particularly those concerned in cellular energy pathways and DNA synthesis and repair. Severe arsenic poisoning is linked primarily with nausea, vomiting, abdominal pain, and severe diarrhea. Peripheral neuropathy and encephalopathy are reported due to arsenic toxicity. Persistent arsenic toxicity results in multisystem disease (Ratnaike 2003). The lethal impacts of some heavy metals, as they do not have any biological role, stay present in a few or the other form risky for the human being and its exact performance. Occasionally they work as a pseudo element of the body whereas at certain times they may still intervene with metabolic routes. Some metals, like aluminum, can be removed through elimination procedures, whereas some metals get accumulated in the body and food chain, showing a chronic character. Metal toxicity depends upon the absorbed quantity, the mode of exposure and level of exposure, i.e., acute or chronic. This can lead to diverse disorders and can also consequence in extreme damage because of oxidative stress induced by free radical formation. Heavy metal ions have a strong electrostatic attraction and high binding affinities with the similar sites that essential metal ions usually bind to in different cellular structures, causing destabilization of the structures and biomolecules (DNA and RNA, enzymes of cell wall), therefore inducing replication fault and subsequent hereditary genetic disorders, mutagenesis, and cancer (Perpetuo et al. 2011). Unlike organic pollutants, the hazardous heavy metals cannot be biodegraded and accumulate in living organisms, causing critical diseases and disorders (Liu et al. 2014), but they can change their chemical properties through a unexpected array of mechanisms.

In order to combat over heavy metals menace, various remedial actions and research studies have been encouraged and applying mostly in highly contaminated areas, even as in other areas, where they are inadequate.

15.2.2 *Fluoride*

Fluoride is the 13th most copious element present in the earth's crust. It is the elemental form, fluorine is a flammable, irritating, and is the most powerful oxidizing agent. There are number of sources from where fluoride is generally generated and added to the environment. The sources of fluoride are characterized into two categories; natural sources which includes the fluorine already present in nature and disseminated in Earth's crust, mostly as the minerals fluorspar, fluorapatite, and cryolite (Mamilwar et al. 2012). Sivasankar (2016) and Tebutt (1983) have described the profusion of fluorine in earth's crust as 0.05–0.1% (500–1000 mg/kg) and 300 mg/kg respectively. Studies reported the concentration of fluorine may differ from 100 to 1000 mg/kg in the three rock types (igneous, sedimentary, and metamorphic rocks) (Sivasankar 2016). Fluoride exists in igneous and sedimentary rocks, mainly in carbonated-type sedimentary and phosphate beds of volcanic layers. Geochemistry, mineralogical composition of rocks and level of alteration of sediments direct the concentration of fluoride in groundwater (Sivasankar 2016). A huge range of fluoride-containing minerals have been recognized these include sellaite [MgF_2], fluorite or fluorspar [CaF_2], cryolite [Na_3AlF_6], fluorapatite [$3Ca_3(PO_4)_2Ca(F,Cl_2)$], apatite [$CaF_2 \cdot 3\text{-}Ca_3(PO_4)$], topaz [$Al_2SiO_4(F,OH)_2$], fluormica (phlogopite) [$KMg_3(Si_3Al)O_{10}(F,OH)_2$], biotite [$K(Mg,Fe)3AlSi_3O_{10}(–F,OH)2$], epidote [$Ca_2(Al_2Fe^{3+})(SiO_4)(Si_2O_7)O(OH)$], amphibole such as tremolite [$Ca_2Mg_5Si_8O_{22}(OH)_2$], and hornblende [$(Ca,Na)_2(Mg,Fe,Al)_5(Al,Si)_8O_{22}(OH)_2$], mica, clays, villuanite, and phosphorite can be given as outstanding examples

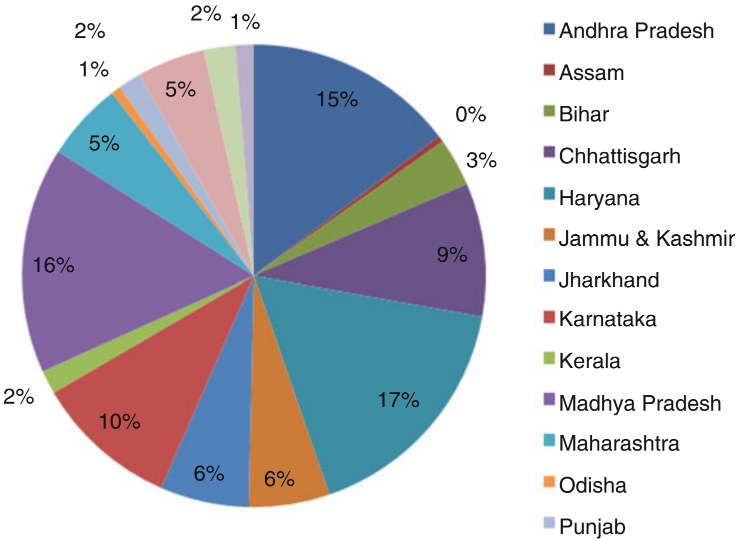

Fig. 15.3 Percentage of affected habitations by fluoride (Ministry of Drinking Water and Sanitation 2019)

(Sivasankar 2016; Datta et al. 1996; Mohapatra et al. 2009; Kundu and Mandal 2009). The other source of fluoride is anthropogenic which includes industrial activities. Moreover, several industrial procedures as coal combustion, steel production, and other manufacturing processes like aluminum, nickel and copper production, phosphate fertilizer production, phosphate ore processing, glass, brick and ceramic manufacturing, etc. also contribute to enhance fluoride levels in water. Organic fluorine is present in vegetables, fruits, and nuts. Concentrations of fluoride are found almost in all types of natural waters. Sea water contains about 1 mg/L whereas lakes possess concentration of less than 0.5 mg/L. In ground water, concentration of fluoride depends on the nature and regularly contacts with ores, rocks, and minerals; as a result, high fluoride concentration may yield in groundwater (Jacks et al. 2005; Vithanage and Bhattacharya 2015; Kumar et al. 2016; Raghav and Kumar 2018; Ali et al. 2019; Kimambo et al. 2019). The contamination of fluoride and its related diseases has been reported in many countries including Kenya, Ethiopia, Libya, Algeria, Benin, Cameroon, Egypt, Ghana, Ivory Coast, Malawi, Morocco, South Africa, Nigeria, Rwanda, Sierra Leone, Sudan, Tanzania, Togo, Tunisia, Uganda, Zimbabwe, China, India, Indonesia, Iran, Pakistan, Saudi Arabia, South Korea, Turkey, Yemen, Argentina, Brazil, Estonia, Sweden, UK (England and Wales), Norway, USA, Canada, and Mexico (Yadav et al. 2013; Kimambo et al. 2019). The problems associated to fluoride contamination are more severe in various states of India like Rajasthan, Andhra Pradesh, Odisha, Haryana, Punjab, Uttar Pradesh, Gujarat, Maharashtra, Karnataka, and Tamil Nadu (Meenakshi et al. 2008; Arlappa et al. 2013; Ali et al. 2018). State/Union territories wise number of fluoride contaminated habitations with population is reported by the states in IMIS of the government of India (Ministry of Drinking Water and Sanitation 2019) are shown in Fig. 15.3. Though, from various studies, an approximate estimate of overall daily fluoride exposure in a moderate climate would be around 0.6 mg per adult per day in a region in which no fluoride is present in to the drinking water and 2 mg per adult per day in a fluoridated area (WHO 1984).

15.2.2.1 Effect of Fluoride

Critical, high-level exposure to fluoride can lead to abdominal pain, nausea and vomiting, seizures and muscle spasms and excessive saliva. The WHO guideline limit for fluoride in drinking water is 1.5 mg/L (Fawell et al. 2006). CPCB (Central Pollution Control Board) India sets the limit of fluoride for different forms of water which are 2.0 mg/L for inland surface water, 15 mg/L for public sewers and marine coastal areas. Fluoride toxicity and the biological response leading to ailing effects depend on various factors such as high concentration in drinking water, low calcium and excess alkalinity in drinking water, total daily intake of fluoride, duration of exposure to fluoride, and age of the individual. Expectant mothers and lactating mothers are the most vulnerable groups as fluoride crosses the placenta because there is no blockade and it also enters maternal milk. Derangement in hormonal profile either as a result of fluoride poisoning or as a cause increases the disease. However, fluoride defends teeth from decay by demineralization and remineralization, but excess of fluoride can lead to dental fluorosis or skeletal fluorosis, which can damage bones and joints. Since the fluoride is highly electronegative in nature, it possesses strong affinity for the positively charged ions. The fluoride present in the drinking water firstly brings alternations in the tooth as the fluoride replaces tooth enamel hydroxyl group with fluoride resulting in formation of more stable compound said to be fluorapatite and get deposited as calcium-fluoropatite crystals. Some of the algae have high negative effect at high level of fluoride. Rate of respiration of *Chlorella pyrenoidosa* was reduced by 50% with 570 mg/L concentration of fluoride. Many of the aquatic animals like fishes and invertebrates directly take up fluoride from water. Fluoride tends to accumulate in the exoskeleton and bone tissues of fishes. Toxicity of fluoride to aquatic invertebrates increases with the increasing level of fluoride. It exerts poisonous effect on the health of aquatic animals by inhibiting the enzyme activity and finally interrupting metabolic process (Camargo 2003).

15.2.3 Cyanide

Cyanide is also one of the most hazardous nitrogenous compounds, and it occurs in environment by both natural and anthropogenic sources. In First World War, cyanide has been used as chemical weapon. Hydrogen cyanide vapor has been applied to fumigate ships and buildings. Although some organisms synthesize cyanide, there are about 3000 plants species, animals, microbes, and fungi that contain cyanide. Various food crops (cassava, lima beans, and corn), forages (alfalfa, sorghum, and Sudan grasses) and horticulture plants (ornamental cherry and laurel) produce cyanogenic glucosides that are stored in plant vacuoles and during hassle situation or when the plant tissues are damaged they are released as nitrogen source. In general HCN is produced by the plant as a defense mechanism against herbs. Various algae like *Cyanobacteria*, *Chlorella valgaris*, *Scenedesmus* and *Nostoc muscorum* (Gurbuz et al. 2004, 2009) bacteria like *Chromobacterium violaceum* and certain *Pseudomonas species* (Fairbrother et al. 2009) and fungi like *Actinomycetes* and *Tricholoma* produce and metabolize cyanide. In bacteria normally HCN is produced during transition stage of growth and in fungus when fruiting bodies are formed or during damaged or stress condition as a mechanism to provide nitrogen and carbon source (Ezzi and Lynch 2005). In animals arthropods also produce HCN (Barclay et al. 1998; Gupta et al. 2010). Cyanide enters into water stream from different industrial activities, such as mining (extraction of metals such as gold and silver), electroplating and metal finishing, steel tempering, automobile parts manufacturing, photography, pharmaceuticals, synthesis of acrylic plastics, organic nitriles, nylon, paints, dyes, drugs, and chelating agents and coal processing units (Monser and Adhoum 2002; Aksu et al. 1999; Patil and Paknikar 2000; Dwivedi et al. 2016b, c). Besides that, cyanide has also been produced in the jewelry and metal industries which in turn lead to the generation of wastes having high cyanide concentrations. The release of cyanide from industries worldwide has been estimated to be more than 14 million kilogram per year (Dwivedi et al. 2011). The extraction of gold from a mine is called cyanidation process, this process showed the result higher than 20% of worldwide cyanide production (Mudder and Botz 2004). The process of leaching the cyanide compound will lead to the production of extensive range of metal–cyanide complexes in the overall cyanidation reaction. During cassava processing in industries, abundance of natural cyanoglucosides released from the roots of cassava is hydrolyzed enzymatically to form cyanide which is frequently discharged in waste (Balagopalan and Rajalakshmy 1998). Cyanide is discharge into the environment from agriculture practices also by the application of nitrile pesticides such as bromoxynil and chlorothalonil. Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is used as a post-emergence herbicide for the control of diseases of broad leaf crops (Grover et al. 1997).

15.2.3.1 Effect of Cyanide

Global industrial exploitation of cyanide is calculated to be 1.5 million tons per year, and professional exposures account for a considerable number of cyanide poisonings (Cummings 2004). Assimilation of cyanide can also result in either acute poisoning (including death) or chronic poisoning to human beings and animals (Dwivedi et al. 2016b, c). Acute cyanide exposure results primarily in central nervous system, cardiovascular, and respiratory effects; thyroid function abnormalities also have been noted in people who are chronically exposed to cyanide. Cyanide is a leading metabolic inhibitor by inactivation of respiration due to its firm binding to cytochrome C oxidase. In this process, electron transport chain is seized by the cyanide by binding with the iron ion in terminal electron acceptor cytochrome C oxidase as a result of rapidly declining respiration rates and ATP synthesis in mitochondria is inhibited (Gupta et al. 2010).

An acute cyanide exposure influences mainly the CNS, firstly producing stimulation, which may be followed rapidly by depression. Stimulation of peripheral chemoreceptors makes improved respiration, while stimulation of the carotid body receptors slows the heart rate. These early changes are frequently transient and may be followed by hypoventilation progressing to apnea and myocardial depression. The outcome is hypotension and shock, which are rapidly fatal if untreated. Because of the brain's vulnerability to cyanide, electrical activity may cease even as the heart is still beating. Predominance of anaerobic metabolism within a cyanide-poisoned cell induces a decrease in the ATP/ADP ratio and therefore changes energy-dependent procedures such as calcium homeostasis. Disruption in calcium regulation with resultant changes in neurotransmitter releases can change the electrical movement in the brain and may be a significant factor in the manifestation of cyanide-induced neurotoxic effects such as tremors and convulsions. Delayed onsets Parkinson-like syndromes have been expressed after severe cyanide poisoning as well as after carbon monoxide poisoning, involving that the basal ganglia are susceptible to the neurotoxic effects of both agents. With acute inhalation of HCN gas, death may take place within seconds. Usually, fish and other aquatic life are killed by cyanide concentrations in the microgram per liter range (part per billion), whereas bird and mammal deaths result from cyanide concentrations in the milligram per liter range (part per million). Inhalation of 270 ppm hydrogen cyanide results lethal effects on humans immediately (Larsen et al. 2005). Cyanide has also been associated with syndromes affecting the central nervous system in animals (Kaewkannetra et al. 2009). Skin contact with cyanide salts can result in burns, which allow for enhanced absorption of cyanide through the skin. The amalgamation of cyanide salts and acid, as utilized in electroplating, results in the release of cyanide gas, which can lead to fatal inhalational exposures. Splashes of cyanide solutions can result in dermal as well as mucosal absorption (Sullivan and Krieger 2001; Megarbane et al. 2003). Considering the lethal impacts of cyanide, the acceptable limits of cyanide in drinking and surface water have been fixed by regulatory bodies. According to Indian standard (CPCB), a minimal national standard (MINAS) limit for cyanide in effluent is 0.2 mg/L for drinking water. USEPA (US Environmental Protection Agency) standard for cyanide in drinking and aquatic-biota waters are 200 and 50 ppb, respectively (Young and Jordan 2004; USEPA 1985). To protect the environment and our water resources, cyanide containing wastewaters must be treated before being discharged into the environment.

15.2.4 Phenol

Phenol is a naturally occurring organic compound with hydroxyl (OH) group substitution on a benzene ring (Gayathri and Vasudevan 2010). This structure provides phenol its aromatic nature and also permits it to form the phenolate ion in aqueous solution. Though phenol occurs naturally (during the decomposition of organic materials and as a constituent of coal tar) its existence in the environment is mainly the result of human activities. Phenol is most frequently manufactured from the oxidation of cumene (also known as isopropylbenzene), however it can be produced from chlorobenzene and toluene, and from the distillation of petroleum. Phenol is generally used in the manufacture of bisphenol A, phenolic resins, caprolactam, alkyl, and chlorinated phenols. Phenol is also utilized in the production of synthetic fibers such as nylon, and in the production of algaecides and fungicides. Phenol also has antibacterial properties and therefore it is extensively used in pharmaceutical products such as ointments, ear and nose drops, disinfectant, antiseptic agent, and in medicinal products such as mouth washes, throat lozenges, and cough drops (Darisimall 2006). Phenol is one amid the most prevalent chemical and pharma pollutants, owing to its toxicity even at lower concentrations and formation of substituted compounds during oxidation and disinfection processes. Due to its extensive uses, phenol has been identified in at least 595 of 1678 National Priorities List (NPL) hazardous waste sites in the United States (HazDat 2006). There are several malpractices done by individuals, which are responsible for the release of phenol into the environment. The most imperative source of phenol discharges is the use of phenolic resins as binding materials in insulation products, chipboard, paints, and casting sand molds (used by metal foundries), pharmaceuticals, textile wood, dying, pulp mill, petrochemical (Fleeger et al. 2003; Mukherjee et al. 1990, 1991). Phenol discharges can also take place from vehicle exhaust (where it is present as a component and a breakdown product of benzene via hydroxyl radical initiated reactions), wood pulp manufacture, cigarette smoke, and in landfill leachate. As a result, aquatic lives also suffer including fish or other animals are subjected to these pollutants (Shalaby et al. 2007).

15.2.4.1 Effect of Phenol

Phenol pollution represents a menace against natural environment and also to human health (Hori et al. 2006). When phenol is available in the aquatic environment, the consumption of fish food, mean weight, and fertility are considerably reduced (Saha et al. 1999). Disclosure of fish to unusual sorts of pollutants (heavy metals, industrial effluents, and pesticides) effect several biochemical alterations in blood parameters. Phenol, especially in high concentrations, is an irksome and corrosive substance, making mucosal membranes targets of toxicity in humans and animals. Victims due to ingestion of phenol have been reported (Boatto and Carta 2004; Tanaka and Kita 1998). expected toxic oral doses of phenol in adults fluctuate usually, from 1 g

(14 mg/kg, assuming an adult body weight of 70 kg) to as much as 65 g (930 mg/kg, assuming an adult body weight of 70 kg) (Bruce and Neal 1987; Deichmann and Keplinger 1981). Direct effects of phenol on the environment include depletion of ozone layer, impact on the earth's heat balance, lower visibility and adding acidic air pollutants to the atmosphere (Jame et al. 2010).

15.2.5 Dyes

Dyes are chemicals which combine with material and impart color to that material. The color of a dye is because of the presence of chromophore group. They are broadly used to color the substrate like leather, textile fiber, paper, hair, fur, wax, plastic material, a cosmetic base and food stuff. Based on chemical structure of chromophore there are 20–30 different groups of dyes. Azo (monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine, and triarylmethane dyes are the most significant groups (Mohammad 2005). The majority of industrial important azo dyes belong to the following classes: acid dyes, basic dyes, direct dyes, disperse dyes, mordant dyes, reactive dyes, and solvent dyes. Dyes contain at the minimum one nitrogen-nitrogen (N=N) double bond, nevertheless many different structures exist. For example, in the azo dyes, monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds, respectively. The azo groups are commonly linked to benzene and naphthalene rings. These side groups are essential for forwarding the color of the dye, with several different shades and intensities being feasible (Zollinger 1991).

Textile industry is one of the major water consumers in the world that creates the wastewater including several unmanageable agents like dye, dying aid, and sizing agents. A considerable amount of these dyes enter to the environment through contaminated water and create a high level of environmental contamination leading to severe health hazards. Around 10,000 different dyes and pigments are utilized by industries and over 0.7 million tons of synthetic dyes are produced yearly, worldwide (Rafi et al. 1990). In the removal of textile wastewater, color is really important because of the attractive deterioration as well as the impediment of diffusion of dissolved oxygen and sunlight into natural water bodies. The textile industry discards about 50% of the textile azo dyes in free state to be discharged in the factory effluent and ultimately to the nearby environment. Azo compounds comprise the leading and the most diverse group of synthetic dyes and are broadly used in a number of industries such as food, textile, cosmetics, and paper printing (Maulin 2013).

15.2.5.1 Effect of Dyes

The uncontrolled expulsion of the effluents from the textile industries includes toxic chemicals such as azo dyes and reactive dyes which negatively affect the natural resources, soil fertility, and aquatic organisms and disturb the integrity of the ecosystem by changing the pH, raising the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and critically affecting water quality (Mester and Tien 2000; Puvaneswari et al. 2006). Toxic compounds of azo dyes freely mix with water bodies and enter into aquatic organisms through food chain and finally reach to human beings and cause physiological disorders such as hypertension, sporadic fever, renal damage, and cramps (Fang et al. 2004; Asad et al. 2007). Most of the azo dyes are water soluble and easily gets absorbed through skin contact and inhalation leading to the threat of cancer and allergic reactions, an irritant for the eyes and permanent blindness also, if inhaled or consumed. The dyes containing effluents cause serious environmental pollution. Therefore, industrial effluents containing different dyes particularly azodyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents.

15.3 Treatment Techniques

It is expected that around 1.1 billion people globally drink unsafe water. The World Bank estimates that 21% of the communicable diseases, in India, are water-borne. Keeping in wits the havoc of environmental problem, many physicochemical techniques have been proposed for the treatment of toxic pollutants which are released from the industrial effluents. These include adsorption on different matrix or materials, electrolysis, oxidation, ion exchange, reverse osmosis, coagulation, alkaline breakdown, chlorination, photolysis, precipitation, electro-oxidation, photo degradation, and membrane filtration (Wang and Chen 2009; Dwivedi et al. 2014; Matsumotto et al. 2007a, b). All these physical or chemical methods are really expensive and result in the production of huge quantities of sludge and other unwanted materials, which generates the secondary stage of land and water pollution. Thus, cost-effective and safe removal of the toxic pollutants is still a vital problem. Hence, providing sustainable and affordable solutions to tackle this menace is a need of the hour.

Bioremediation with microorganisms has been recognized as a cost-effective and environment friendly alternative for disposal of industrial effluent. By using the biological materials including living and nonliving microorganisms, toxic materials or precious metals from industrial effluents can be removed and recovered. In fact, biological methods have gained recognition over the years due to increased performance, availability, and low cost of raw materials (Ahluwalia and Goyal 2007, b; Bunluesin et al. 2007, b). Microorganisms such as algae, fungi, and yeasts are able to effectively accumulate heavy metals from their external environment (Pan et al. 2007).

In recent years, several studies have focused on various microorganisms which are able to degrade and accumulate toxic pollutants from industrial effluents. A wide range of microorganisms are reported in Table 15.2 to be capable of removing hazardous materials from industrial effluents.

Table 15.2 Microorganisms are capable to remove toxic materials from industrial effluents

S. no.	Microorganism	Pollutants/heavy metals	References
1	*Pseudomonas chlororaphis, Azotobacter vinelandii*	Cu	He et al. (2010)
2	*Bacillus cereus, Psychrobacter* sp.	Ni	Ma et al. (2009)
3	*Escherichia coli*	Cr (VI), Cd(II), Fe(III) and Ni(II)	Quintelas et al. (2009)
4	*Alcaligenes faecalis, Gallionella ferruginea, Leptothrix ocracia, Pseudomonas putida, Pseudomonas arsenitoxidans*	Arsenic	Mondal et al. (2008)
5	*Aerococcus* sp., *Rhodopseudomonas palustris*	Pb, Cr, Cd	Sinha and Paul (2014)
6	*Shewanella putrefaciens*	Fluoride	Dwivedi et al. (2016d)
7	*Rhizopus arrhizus*	Zinc	Sag and Kutsal (2007)
8	*Pseudomonas putida*	2,4-dichlorophenol	Ullhyan and Ghosh (2014)
9	*Alcaligenes* sp.	Phenol	Indu and Shashidhar (2004)
10	*Pseudomonas putida*	Phenol	Der and Humphrey (1975)
11	*Pseudomonas* spp.	Fluorobenzoate	Goldman (1972)
12	*Bacillus subtilis*	Acid blue 113	Gurulakshmi et al. (2008)
13	*Pterocladia capillacea*	Hexavalent chromium	El Nemr et al. (2011)
14	*Enterobacter agglomerans*	Methyl Red	Keharia and Madamwar (2003)
15	*Aspergillus ochraceus*	Reactive blue 25	Parshetti et al. (2006)
16	*Streptomyces ipomoea*	Orange II	Molina-Guijarro et al. (2009)
17	*Bacillus cereus*	Potassium cyanide	Dwivedi et al. (2016a)
18	*P. arcularius*	KCN	Ozel et al. (2010)
19	*P. pseudoalcaligenes*	NaCN	Huertas et al. (2010)
20	*Citrobacter* sp.	K, Zn, Cu-cyanide	Patil and Paknikar (2000)

15.4 Bioremoval Mechanism of Toxic Materials

Generally two types of mechanisms involve in remediation of toxic materials, first is the bioremediation and other is bioaccumulation. Bioremediation is a pollution control technology that uses natural biological species to catalyze the degradation or transformation of various toxic chemicals to less harmful forms while in bioaccumulation soluble materials or metals are actively transported through the cell membrane and accumulated within the cells as solid particles or in vacuoles.

15.4.1 Heavy Metals

Since heavy metals are nonbiodegradable, removal of contaminants from industrial effluents is quite challenging. Bioremediation is employed in order to transform toxic heavy metals into a less harmful state using microbes or its enzymes to clean-up polluted environment (Abbas et al. 2014; Okoduwa et al. 2017). Microorganisms take up different mechanisms to interact and survive in the presence of metals. In response to metals in the environment, microorganisms have developed ingenious mechanisms of metal resistance and detoxification. The mechanism involves several procedures, together with ion exchange, electrostatic interaction, surface complexation, redox process and precipitation (Yang et al. 2015). Microorganisms can decontaminate metals by valence conversion, volatilization, or extracellular chemical precipitation. Microorganisms have negative charge on their cell surface because of the presence of anionic structures that empower the microbes to bind to metal cations. The negatively charged sites of microbes involved in adsorption of metal are the hydroxyl, alcohol, phosphoryl, ester, sulfhydryl, sulfonate, thiol groups and amine, carboxyl group (Gavrilescu 2004).

15.4.1.1 Biosorption and Bioaccumulation Mechanism

Sorption process has been extensively used to remove toxic metals from wastewater using microorganisms and low cost adsorbents such as agriculture wastes and activated carbon developed from agriculture wastes. Biosorption can be carried out by living or dead cells biomass as passive uptake through surface complexation onto the cell wall and surface layers (Fomina and Gadd 2014). The uptake of heavy metals by microbial cells through biosorption mechanisms can be classified into metabolism-independent biosorption, which generally occurs on the cells exterior and metabolism-dependent bioaccumulation, which includes sequestration, redox reaction, and species-transformation methods (Vijayaraghavan and Yun 2008; Godlewska-Żyłkiewicz 2006). Bioaccumulation depends on a variety of physical, chemical, and biological mechanisms (Fomina and Gadd 2014). The biosorption can also be classified according to the pollutant characteristics and the location where the metal removed from solution is found extracellular accumulation/ precipitation, cell surface sorption/precipitation and intracellular accumulation. Extracellular sequestration is the accumulation of metal ions by cellular components in the periplasm or complexation of metal ions as insoluble compounds. Copper-resistant *Pseudomonas syringae* strains produced copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) which bind copper ions and microbial colonies (Cha and Cooksey 1991). Bacteria can expel metal ions from the cytoplasm to grab the metal within the periplasm. Zinc ions can cross from the cytoplasm by efflux system where they are accumulated in the periplasm of Synechocystis PCC 6803 strain (Thelwell et al. 1998). Iron-reducing bacterium such as *Geobacter* spp. and sulfur reducing bacterium like *Desulfuromonas* spp. are capable of reducing toxic metals to less or nontoxic metals. *G. metallireducens* is an anaerobe having the capability of reducing manganese (Mn), from lethal Mn (IV) to Mn (II), and uranium (U), from toxic U (VI) to U (IV) (Gavrilescu 2004). Intracellular sequestration is the complexation of metal ions by various compounds in the cell cytoplasm. The concentration of metals within microbial cells can result from interaction with surface ligands followed by slow transport into the cell. The ability of bacterial cells to accumulate metals intracellular has been exploited in practices, predominantly in the treatment of effluent treatment. Cadmium-tolerant *Pseudomonas putida* strain possessed the ability of intracellular sequestration of cadmium, copper, and zinc ions with the help of cysteine-rich low molecular weight proteins (Higham et al. 1986). Also, intracellular sequestration of cadmium ions by glutathione was revealed in *Rhizobium leguminosarum* cells.

Mondal et al. (2008) reported some of the bacteria having arsenic removal capability: *Alcaligenes faecalis, Agrobacterium tumefaciens, Bacillus subtilis, Bacillus indicus, Corynebacterium glutamicum, Desulfovibrio desulfuricans, Gallionella ferruginea, Leptothrix ocracia, Pseudomonas putida, Pseudomonas arsenitoxidans, Ralstonia pickettii, Thiomonas ynys1, Acidithiobacillus ferrooxidans, Ralstonia eutropha,* etc. Among the above bacteria *Ralstonia eutropha* and *Pseudomonas putida* have *ars*R and *ars*C gene in their plasmid. Presence of *ars*R and *ars*C gene in bacterial strain makes them capable for producing ArsR protein and arsenate reducing enzyme respectively. ArsR protein selectively captures As(III) whereas arsenate reducing enzyme reduces As(V) to As(III) which is further captured by ArsR protein. Mondal et al. (2008) reported that arsenic removal efficiency of *Ralstonia eutropha MTCC 2487*, *Pseudomonas putida MTCC 1194,* and *Bacillus indicus MTCC 4374* from simulated acid mine drainage are ~67%, 60%, and 61%, respectively. Industrial heavy metals containing wastewater treatment by using algal biomass is also a new approach. Soltmann et al. (2010) were immobilized the algal biomass Macro (Fucus) and Micro algae (*spirulina,* etc.) on different types of silica sols. The efficiency of the immobilized biocers was tested for absorbance of nickel, chromium, copper, and lead in drinking water.

15.4.2 Fluoride Bioaccumulation Mechanism

Mainly two pathways such as bioadsorption on bacterial cell surface and bioaccumulation in bacterial cells have been proposed for the removal of fluoride from water using bacterial whole cells (Chubar et al. 2008). Mechanisms of cell surface sorption are independent of cell metabolism; they are based upon physico-chemical interactions between fluoride and functional groups of the cell wall. The microorganism's cell wall mainly consists of polysaccharides, lipids, and proteins, which have many binding sites for halides. This process is independent of the metabolism and metal binding is fast. Bioaccumulation, in contrast, is an intracellular fluoride accumulation process which involves dehalogenation and it is mediated only by periplasmic and membrane fractions, not by cytoplasmic fractions of the cells. (Picardal et al. 1993) Since it depends on the cell metabolism, it can be inhibited by metabolic inhibitors such as low temperature and lack of energy sources.

15.4.3 Cyanide Biodegradation Mechanism

Biodegradation of cyanide compounds may take place through various pathways. Generally degradation of cyanide is induced by the presence of cyanide in the media which is followed by conversion of cyanide into carbon and nitrogen. Various researchers have described various organisms, which use different pathways for cyanide degradation (Dubey and Holmes 1995). Some times more than one pathway can be applied for cyanide biodegradation in some organisms (Mufaddal and James 2002). Five general pathways as reported in literature for the biodegradation of cyanide, these are: hydrolytic pathway, oxidative pathway, reductive pathway, substitution/transfer pathway, and syntheses pathway (Sexton and Howlett 2000). First three pathways are degradation pathways in which enzymes catalyze the conversion of cyanides into simple organic or inorganic molecules and converts it to ammonia, methane, CO_2, formic acid, and carboxylic acid. Last two pathways are for the assimilation of cyanide in the microbe as nitrogen and carbon source (Baxter and Cummings 2006). All these pathways depend on the cyanide tolerance mechanism in microbes and that process is used to dissociate the cyanide metal complexes or for chelating metals (Gupta et al. 2010).

15.4.3.1 Hydrolytic Pathway

Hydrolytic pathway of cyanide degradation is catalyzed by various enzymes present in microbial system, such as cyanide hydratase, nitrile hydratase, cyanidase, and nitrilase (Ebbs 2004; Kwon et al. 2002; Baxter and Cummings 2006). First two enzymes have specific substrate and directly hydrolyze and cleave the carbon–

nitrogen triple bond to form formamide, and the last two convert it to ammonia and carboxylic acid, which are utilized in their metabolic activity (Gupta et al. 2010). Cyanide compounds are degraded by the following enzymes through different reactions as discussed below (Ebbs 2004):

Cyanide Hydratase

Cyanide hydratase is primarily a fungal enzyme; the most frequently encountered cyanide conversion takes place through this inducible enzyme, resulting in the formation of formamide, which subsequently decomposes to carbon dioxide and ammonia by another enzyme formamide hydratase (FHL).

$$HCN + H_2O \rightarrow HCONH_2 \tag{15.1}$$

This enzyme belongs to the family of lyases, specifically the hydrolyases, which cleaves carbon-nitrogen bonds. The systematic name of this enzyme class is formamide hydrolyase (cyanide-forming). Other names in common use include formamide dehydratase, and formamide hydrolyase. This enzyme participates in cyanoamino acid metabolism (Gupta et al. 2010). Cyanide hydratase was first partially purified by *Stemphylium loti* and is highly conserved between species (Barclay et al. 2002).

Cyanidase

Cyanidase is also known as cyanide dihydratases. It comprises a group of bacterial enzymes that are available in *Pseudomonas stutzeri* AK61, *Alcaligenes xylosoxidans* subsp. *Denitrificans* DF3, and *Bacillus pumilus* C1 (Meyers et al. 1993; Ingvorsen et al. 1992). Cyanide dihydratases readily convert cyanide to relatively nontoxic formate directly as shown below:

$$RCN + H_2O \leftrightarrow RCOOH + NH_3 \tag{15.2}$$

Nitrile Hydratase

Primarily aliphatic nitriles can be effectively degraded by nitrile hydratases. Nitrile hydratases convert cyanides to their corresponding amides as shown in following reaction:

$$R - CN + H_2O \rightarrow R - CONH_2 \tag{15.3}$$

The nitrile hydratase, isolated by *Pseudonocardia thermophila* shows high activity compared to other microorganism known for the production of nitrile hydratase, i.e., *Rhodococcus rhodochrous, Pseudomonas*, *Corynebacterium*, *Klebsiella,* and *Rhizobium*. Some new bacterial strains, *Pseudomonas putida* MA113 and *Pseudomonas marginales* MA32, containing nitrile hydratases was isolated from soil samples by an enrichment procedure. This isolated microbe could tolerate up to 50 mM cyanide and also has broad substrate range small substrates like acrylonitrile, nitriles with longer side chains and even nitriles with quarternary alpha-carbon atoms. *Pseudomonas putida* MA113 and *Pseudomonas marginales* MA32 were used as a whole cell biocatalyst for the hydration of acetone cyanohydrin to a hydroxylisobutyramide, which is a precursor of methacrylamide (Nawaz et al., 1989). Nitrile hydratase are composed of two types of subunits, α and β, which are not related in amino acid sequence. Nitrile hydratase exist as $\alpha\beta$ dimers or α_2 β_2 tetramers and bind one metal atom per $\alpha\beta$ unit (Gerasimova et al. 2004).

Nitrilase

Nitrilase enzymes catalyze the hydrolysis of nitriles to carboxylic acids and ammonia, without the formation of "free" amide intermediates.

$$R - CN + 2H_2O \rightarrow R - COOH + NH_3 \tag{15.4}$$

Nitrilases are involved in biosynthesis of proteins and there posttranslational modifications in plants, animals, fungi, and certain prokaryotes. The structure of nitrilases is usually inducible enzymes composed of one or two types of subunits of different size and number, and these subunits of nitrilase self-associate to convert the enzyme to the active form. *Nocardia* sp. nitrilase was reported to be induced by enzonitrile (Goldhust and Bohak 1989). Acetonitrile has been used as an inducer for the formation of nitrilase in *Fusarium oxysporum* (Collins and Knowles 1983).

15.4.3.2 Oxidative Pathway

In oxidative pathway, cyanide conversion involves oxygenolytic conversion to carbon dioxide and ammonia. This pathway requires NADPH to catalyze this degradation pathway. Microorganisms which prefer cyanide conversion by this pathway also require additional carbon source with cyanide. There are two types of oxidative mechanism involving three different enzymes:

1. Cyanide monooxygenase and cyanase.

   Cyanide monoxygenase (Ebbs 2004; Raybuck 1992) converts cyanide to cyanate which is further catalyzed by cyanase, resulting in the overall conversion of cyanate to ammonia and carbon dioxide as given in the following reaction.

$$\begin{aligned} &HCN^- + O_2 + 2H^+ + NADPH \rightarrow CNO^- + NADP + 2H_2O \\ &\qquad\qquad\qquad\qquad\qquad\qquad \downarrow \\ &CNO^- + 3H^+ + HCO_3{}^- \rightarrow NH_4{}^+ + 2CO_2 \end{aligned} \tag{15.5}$$

2. Cyanide dioxygenase

   This oxidative pathway utilizes cyanide dioxygenase to form ammonia and carbon dioxide directly (Ebbs 2004).

$$HCN + O_2 + 2H^+ + NADPH \rightarrow NADH + CO_2 + NH_3 \tag{15.6}$$

Immobilized cells of *P. putida* can effectively use oxidative pathway to produce ammonia and carbon dioxide (Chapatwala et al. 1998). The cyanide degradation in three white rot fungi, *Trametes versicolor* ATCC 200801, *Phanerochaete chrysosporium* ME 496 and *Pleurotus sajor-caju*, were achieved by an oxidative reaction that results in the end products of ammonia and CO_2.

15.4.3.3 Reductive Pathway

The reductive pathways of degradation of cyanide are generally considered to occur under anaerobic conditions. This pathway is mediated by an enzyme nitrogenase. The enzyme utilizes HCN and produce methane and ammonia as end product.

$$HCN + 2H^+ + 2e^- \rightarrow CH_2 = NH + H_2O \rightarrow CH_2 = O \tag{15.7}$$

$$CH_2 = NH + 2H^+ + 2e^- \rightarrow CH_3 - NH + 2H^+ + 2e^- \rightarrow CH_4 + NH_3 \tag{15.8}$$

K. oxytoca is able to degrade cyanide compounds to methane and ammonia through this path.

15.4.3.4 Substitution/Transfer Pathway

The activity of this pathway involves cyanide assimilation and usably this tends to increase the growth of the microorganism by providing extra nitrogen source and preventing it from cyanide toxicity. There are two types of enzymes that catalyze cyanide assimilation through this pathway such as rhodanese and mercaptopyruvate sulfurtransferase. Both enzymes are widely distributed in living organisms and catalyze the formation of pyruvate and thiocyanate from mercaptopyruvate (Burton and Akagi 1971; Roy and Trudinger 1977). Rhodaneses are extremely conserved and prevalent enzymes presently regarded as one of the mechanism evolved for cyanide detoxification. In vitro rhodaneses catalyze the irreversible transfer of a sulfur atom from a suitable donor (i.e., thiosulfate) to cyanide, leading to formation of less toxic sulfite and thiocyanate. The enzyme activity is modulated by phosphate ions and divalent anions that found to interact with the active site (Bordo et al. 2001).

$$\text{Thiosulfate} + \text{cyanide} \rightarrow \text{sulfite} + \text{thiocyanate} \qquad (15.9)$$

Rhodaneses has been also recognized in a variety of bacterial species including *Escherichia coli* (Cipollone et al. 2004), *Azotobacter vinelandii* (Ezzi et al. 2003), and several species of *Thiobacillus.*

The second enzyme mercaptopyruvate sulfurtransferase belongs to the family of transferases specifically the sulfurtransferases, which transfer sulfur-containing groups. The systematic name of this enzyme class is 3-mercaptopyruvate: cyanide sulfurtransferase. This enzyme participates in cysteine metabolism. It catalyzes the following chemical reactions:

$$\text{HSCH}_2\text{COCOO}^- + \text{E} \leftrightarrow \text{CH}_2\text{COCOO}^- + \text{ES}\ (\text{step1}) \qquad (15.10)$$

$$\text{ES} + \text{CN}^- \leftrightarrow \text{E} + \text{SCN}^-\ (\text{step2}) \qquad (15.11)$$

3-mercaptopyruvate is converted to thiosulfate by the two-step reaction as mentioned above. In step 1 (ES) the enzyme-sulfur intermediate is formed and in step 2 intermediate reacts with cyanide to E (mercaptopyruvate sulfurtransferase) and thiocyanide (Gupta et al. 2010).

15.4.3.5 Syntheses Pathway

This pathway is also a cyanide assimilation pathway by using enzyme β-cyanoalanine synthase and γ-cyano-α-aminobutyric acid synthase. It involves the synthesis of amino acid, β-cyanoalanine, and γ-cyano-α-aminobutyric acid by using amino acid residues as precursor that react with cyanide compounds. β-Cyanoalanine synthase is believed to play the important role in the removal of endogenous cyanide and is produced at highly active growth period in the microbe (Nagahara et al. 1999). This enzyme belongs to the family of lyases, specifically the class of carbon–sulfur lyases. The systematic name of this enzyme class is L-cysteine hydrogen-sulfide-lyase (Miles 1986). The growth of *Bacillus megaterium* is by converting cyanide to β-cyanoalanine and then to asparagines (Dunnil and Fowden 1965). β-Cyanoalanine synthase is induced by various amino acids as serine, cysteine, asparagines, etc. Cyanide produced by *C. violaceum* can first convert cyanide to β-cyanoalanine then into asparagine as shown in reaction below (Fowden and Bell 1965).

$$\begin{aligned}\underset{\text{(Cysteine)}}{\text{HCN}} + \text{HS} - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \rightarrow \text{H}_2\text{S} + {} & \text{NC} - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \\ & \downarrow (\beta\text{-cyanoalanine}) \\ & \underset{\text{(Asparagine)}}{\text{H}_2\text{NCO}} - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}\end{aligned} \tag{15.12}$$

γ-Cyano-α-aminobutyric acid synthase is alternative pathway for cyanide assimilation. This pathway requires pyridoxal phosphate for function and induced by glutamate or glysine. Ones this γ-Cyano-α-aminobutyric acid is synthesized it is slowly converted to amino acid glutamate. A thermophilic and cyanide ion tolerant bacterium, *Bacillus stearothermophilus* CN$_{3,}$ isolated from a hot spring in Japan, was found to produce thermostable γ-Cyano-α-aminobutyric acid synthase.

15.4.4 Phenol Biodegradation Mechanism

For the biodegradation of phenol, the presence or absence of molecular oxygen plays a crucial role. In general, phenol can be transformed both under aerobic and anaerobic conditions. Two types of metabolic reactions can take place in the aerobic degradation step meta and ortho degradation pathways. In microbial degradation of phenol under aerobic conditions, the degradation is initiated by oxygenation in which the aromatic ring is initially monohydroxylated by a mono oxygenase phenol hydroxylase at a position ortho to the pre-existing hydroxyl group to form catechol. This is the main intermediate resulting from metabolism of phenol by different microbial strains. Depending on the type of strain, the catechol then undergoes a ring cleavage that can occur either at the ortho position therefore initiating the ortho pathway that leads to the formation of succinyl Co-A and acetyl Co-A or at the meta position thus initiating the meta pathway that leads to the formation of pyruvate and acetaldehyde. Leonard and Lindley (1998) have described the biodegradation or metabolism of phenol by *Pseudomonas cepacia, Pseudomonas putida, Pseudomonas pickettii,* and *Alcaligenes eutrophus* respectively via the meta cleavage pathway, while Paller et al. (1995) described the biodegradation of phenol by *Rhodotorula rubra, Trichosporon cutaneum,* and *Acinetobacter calcoacetium,* respectively through the ortho cleavage pathway.

An anaerobic degradation of phenol is based on the similarity with the anaerobic benzoate pathway proposed for *Paracoccus denitrificans* (Williams and Evans 1975). In this pathway phenol is carboxylated in the para position to 4-hydroxybenzoate which is the first step in the anaerobic pathway. Here the enzyme involved is the 4-hydroxybenzoate carboxylase. The anaerobic degradation of several other aromatic compounds has been shown to include a carboxylation reaction. Carboxylation of the aromatic ring in para position to the hydroxyl group of *o*-cresol resulting in 3-methyl 4-hydroxybenzoate has been reported for a denitrifying

Paracoccus like organisms, in addition to methogenic consortium was later shown to travel a verity of phenolic compounds including *o*-cresol, catechol and ortho halogenated phenols through para carboxylation followed by dehydroxylation.

15.4.5 Dyes Biodegradation Mechanism

The use of microbes and their enzymatic treatment method for the complete decolorization and degradation of textile dyes from their effluent is advantageous in many ways like being cost-effective, less sludge forming, environmentally friendly, yielding nontoxic end products and less water consumption. The effectiveness of microbial decolorization depends on the adaptability and activity of the selected microorganisms. Several pure cultures of bacteria have been reported to decolorize azo dyes under aerobic condition. A bacterial strain *Klebsiella* sp. VN-31 was reported to decolorize monoazo dye RY107 and RR198 in 72 and 96 h, respectively; the diazo dye RB5 and triazo dye DB71 were decolorized by the same culture in 120 and 168 h, respectively (Franciscon et al. 2009). Similarly, Wang et al. (2009) reported decolorization of Reactive Red 180 (200 mg/L) by *Citrobacter* sp. (CK3) within 36 h of incubation. The mechanism of bacterial degradation of dyes involves various oxidoreductive enzymes which utilize these complex xenobiotic compounds as substrates and convert them to less complex metabolites. Most of the bacteria having the dye degradation mechanism are generally obtained from actual sites of dye disposal or from real textile effluents. The bacterial decolorization of dyes is being facilitated by various reductive enzymes such as azoreductase, NADH-DCIP reductase, and MG reductase and oxidative enzymes like lignin peroxidase and laccase (Kalme et al. 2007; Kalyani et al. 2009). The process of bacterial azo dye degradation consists of two stages. The first stage involves the reductive cleavage of the dyes' azo bond (–N=N–), resulting in the formation of aromatic amines, that is usually colorless but potentially hazardous. The second stage involves degradation of the aromatic amines under aerobic conditions. Azo dye reduction with the help of azoreductase under anaerobic conditions, involves transfer of four-electrons (reducing equivalents), which proceed through two stages at the azo linkage and in each stage two electrons are transferred to the azo dye, which acts as a final electron acceptor resulting in dye decolorization. The resulting intermediate metabolites are further degraded aerobically or anaerobically. The presence of oxygen usually inhibits the azo bond reduction activity since aerobic respiration may dominate utilization of NADH; therefore hindering the electron transfer from NADH to azo bonds (Chang et al. 2004). Anaerobic bio-reduction of an azo dye includes three different mechanisms as illustrated by earlier investigators namely: direct enzymatic reduction, indirect/mediated reduction, and chemical reduction (organic and inorganic compounds). A direct enzymatic reaction or a mediated/indirect reaction is catalyzed by biologically regenerated enzyme cofactors or other electron carriers. The azo dye chemical reduction can also result from purely chemical reactions with biogenic reductants like sulfide. These azo dye reduction mechanisms have been exposed to be intensely accelerated by the addition of various redox-mediating compounds, such as anthraquinone-sulfonate (AQS) and anthraquinone-disulfonate (Guo et al. 2007).

15.5 Advances on Removal of Toxic Industrial Effluents

Removal of hazardous metals and other toxic compounds from industrial effluent through biological process has gained substantial attention in the last few decades. However, only a small part of these studies employed growing microbial cells that keep multiple mechanisms of metal sequestration and consequently may hold better metal uptake capacities. With the advancement in genetic engineering, microbes are engineered with desired characteristics such as ability to tolerate highly toxic materials and metal stress, over expression of metal-chelating proteins and peptides and metal accumulation capability. Frederick et al. (2013) has reported to genetically engineered microorganisms are able to produce trehalose and found it to reduce 1 mM Cr (VI) to Cr (III). Genetically engineered microorganism such as *Chlamydomonas reinhardtii* produced significant increase in tolerance to Cd toxicity and its accumulation (Ibuot et al. 2017). Genetically engineered microbes for heavy metal remediation involve the use of *Escherichia coli* (*E. coli* ArsR (ELP153AR)) to target As(III) and *Saccharomyces cerevisiae* (CP2 HP3) to target Cd^{2+} and Zn^{2+} (Kostal et al. 2004; Vinopal et al. 2007). *Corynebacterium glutamicum* was genetically modified using over expression of ars operons (*ars*1 and *ars*2) to decontaminate As-contaminated sites (Mateos et al. 2017).

Immobilization of cells improves the degradation rate by preventing washing of cells and it also increases the cell density. The immobilization technique was used by Kowalska et al. (1998). They used ultrafiltration membranes made of polyacronitrile and carried out simultaneous degradation of cyanides and phenol using *Agrobacterium radiobacter, Staphylococcus sciuri,* and *Pseudomonas diminuta*. The efficiency of phenol and cyanide biodegradation was dependent on transmembrane pressure. The immobilization technique was earlier studied by Babu et al. (1992) where immobilized cells of *P. putida* were able to degrade sodium cyanide as a sole source of carbon and nitrogen. Various immobilization matrixes are available like granular activated carbon (GAC), alginate beads, zeolite, which has shown very high efficiency and improved degradation rate (Babu et al. 1992; Chapatwala et al. 1998; Dursun et al. 1999). Among the commercial available matrixes, various agro-based materials have been widely reported for their ability to remove different toxic contaminants from water and wastewater (Moussavi and Khosravi 2010; Sahranavard et al. 2011; Gonen and Serin 2012; Dwivedi et al. 2016a, b). Simultaneous adsorption and biodegradation (SAB) is the most recent development in cyanide removal. Dwivedi et al. (2016d) reported high removal efficiency using *Bacillus cereus* immobilized on almond shell in SAB process. One novel study has proposed a transcription-based assay for monitoring the biodegradation of both simple and metal cyanides (Barclay et al. 2002). The gene encoding cyanide hydratase (chy) in *F. solani* has been sequenced and primers utilized in reverse transcription-polymerase chain reaction (RT–PCR) to demonstrate transcription of this gene. The *chy* gene from *F. solani* displays significant homology to the corresponding cyanide hydratase gene from *Gloeocercospora sorghi, F. lateritium,* and *Leptosphaeria maculans*. This observation implies that the assay could be utilized in different contexts, provided that expression of the *chy* gene can be conclusively linked with activity of cyanide hydratase. This assay could provide an important tool to site managers, regulators, and industries that generate cyanide wastes (Ebbs 2004).

Extensive research continues to be carried out on toxic contaminants removal from industrial wastewater. In this regard, enzymatic treatment taking motivation from biological treatment uses a biocatalyst, an enzyme, to carry out a transformation on toxic compounds that leads to their removal from wastewater. The microbial enzymatic treatment method is a new treatment technique for the degradation of toxic pollutants from industrial effluents, and this process can have significant advantages (including cost-effectiveness, easy to harvest, readily mobilizable and simply downstream process) over conventional physical and chemical treatments. Recent research trends on nanoparticle-microbial enzyme conjugates are also highly efficient to remove the azo dye from textile waste within a few minutes. But unfortunately, due to some gap between academia and industry, these methods remain only limited up to laboratory and its industrialization is still a challenge.

15.6 Conclusion

Microorganisms are an extremely potential bioremediation tool for industrial effluents and related pollutants. The use of microorganisms provides a cost-effective, efficacious, and eco-friendly alternative over the traditional techniques for the degradation of toxic industrial effluents. The uptake of heavy metals by microorganisms take place through bioaccumulation is an active process and through adsorption, it is a passive process. Metal-resistant microorganisms in immobilized, consortium and alone have given better efficiency in metal removal, however the immobilized form could have more chemosorption sites to absorb heavy metals. Enzymatic treatment has also proven to be an effective method to remove contaminants from industrial wastewater. Improvements have been seen in enzymatic treatment to overcome low efficiencies and high operational costs of conventional techniques. The future prospect looks promising on microbial genetic technologies and the advancement of using biofilms, nanomaterials, and nanocomposites which could be attained by immobilization and optimization process. Therefore, additional efforts should be made toward genetically modified microbes and microbial fuel cell (MFC) in the bioremediation of heavy metals and other toxic materials from the industrial effluents.

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