Chapter 15 Role of Microbes in Sustainable Utilization of Animal Wastes

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Abstract Various human, industrial, and agricultural operations generate a wide variety of biomass wastes, ample with organic and inorganic resources along with pathogenic microorganisms. Environmental conservation is of the utmost significance. Researchers have been looking for naturally occurring technologies to improve the regulation of agricultural and animal wastes. The biggest threat to mankind is the persistent release of hazardous wastes and toxins as a byproduct of faulty industrialization. Urban and industrial wastes, toxins and animal wastes have been improperly and unscientifically managed, putting the ecology and ecosystem in jeopardy of viability. In order to establish a safe and habitable ecology for future generations, it is now necessary to repair and clean up the contaminated environment. It is well known that waste creation and economic growth are significantly associated, both in developed Western nations and in emerging nations, such as Pakistan. More efficient approaches are required for the treatment of potentially toxic wastes. Microorganisms have a potential future in this area. The distinctive characteristics of microorganisms can be efficiently employed to revive the environment. Microorganisms are used as "miracle cures" for biodegradation and the repair of polluted environments. Likewise, the application of genetically modified organisms (GMOs) in highly contaminated environments makes the microorganisms advantageous for human well-being. This chapter provides information on various types of wastes and elaborates how microorganisms can be employed productively for waste management and more eco-sustainable environments.

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Graphic Abstract

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15.1 Introduction

The global environment is under a significant strain due to rapid population growth, unplanned urbanization, industrialization, and the enormous population demand on natural resources. Multiple erroneous activities in contemporary civilization yield massive amounts of trash that causes pollution. Additionally, large-scale industries have increased the substantial quantities of radioactive and chemical wastes into the environment, causing irreversible harm to the whole biosphere (Raj et al. [2018](#page-25-0)). The primary cause of a loss of resources and energy is waste creation, which also has negative effects on the environment and costs society money to collect, treat, and manage. In India and Pakistan, the production of hazardous waste is strongly correlated with urbanization and varies greatly between cities. It is believed that as industrialization accelerates, waste production would follow suit unless scientific management practices are implemented (Jhariya et al. [2018](#page-24-0)) (Fig. [15.1](#page-2-0)).

Due to the absence of adequate trash collection and elimination facilities in poor countries, the waste management (WM) scenario in developed and developing countries is very different. WM is now given top emphasis as a result of growing concern over environmental deterioration and longevity (Brewer [2001](#page-23-0)). Regarding the management of animal wastes after 1973, the number of allegations per farm doubled, with the exception of those against cattle farms. Overall, 2590 complaints have been documented in past. The majority of them (61.6%) dealt with odor concerns, which were preceded by water pollution (40.6%) , insects (7.8%) ,

Fig. 15.1 Environmental and public health inference of animal manure

and others (5.9%) . Pork production (34.4%) , dairy (32.4%) , poultry (18.7%) , beef (12.2%), and other sources (2.3%) were the main culprits. The majority of these issues were the outcome of poor waste management. However, it is challenging for most farms to pay the necessary expenditures for manure cleanup. According to calculations, Japan produced 95 million tons of animal manure altogether in 1996, more than double the 43 million tons recorded in 1960. The total quantity of biochemical oxygen demand (BOD), one of the signs of environmental stress, was 0.84 million tons in 1960; by 1996, it had nearly tripled to 2.5 million tons. Animal excrement is significantly increasing the environmental load. Although the anticipated volume of human excrement recorded was 44 million tons, its BOD was only 0.44 million tons, significantly lesser than the volume of animal dung (Nakai [1995\)](#page-25-1).

Unplanned, unsystematic, and illogical methods of dumping waste on the outskirts of cities and villages lead to spilling landfills that are not only impossible to restore to a suitable condition but also have grave environmental consequences in the form of soil and groundwater pollution and a grant to global warming. Materials from plants, animals, humans, including their trash, are all included in the category of biomass. Food manufacturing, agriculture, and industrial effluents are additional sources of biomass wastes. These byproducts can be transformed into energy or fuel via gasification, co-firing, combustion, and potentially by anaerobic digestion, based on the properties of the wastes (Sam-Anyaoma and Anjorin [2018](#page-25-2)).

Fossil fuels such as coal, oil, and natural gas are the traditional energy sources that have so far been used to construct and maintain the highly technologically sophisticated contemporary world. However, because there are a finite number of fossil fuels, their ongoing extraction and usage have a significant negative influence on both the local ecosystem and the world's climate. Additionally, the supply of oil and gas is running out and must be replaced, reinforced, or conserved in order to prepare for the switch to more sustainable energy sources (Wilkie [2008](#page-26-0)). The amount of renewable energy needed may significantly shift as a result of anaerobic digestion of biomass wastes. It works best to transform organic byproducts from farming, raising animals, businesses, towns, and other human operations into fuel and fertilizers. Meanwhile, in South Africa, biogas digesters are mostly designed and installed in the Western and Kwa-Zulu Natal regions of the country, while anaerobic digestion has gained popularity in emerging nations like China, India, and Nepal (Mukumba et al. [2012](#page-25-3)).

Anaerobic digestion generally lessens biomass wastes, counteracts a wide range of environmental undesirables, improves sanitation, aids the management of air and water pollution, and lowers emission of greenhouse gases. Additionally, it offers a superior fertilizer full of nutrients as well as energy in the form of biogas. From developing to industrialized nations, biogas is used for a wide range of purposes. Biogas has been used as electricity, fuel, and heat at farms (Liu et al. [2009](#page-25-4)).

Animal manures have been recognized as appropriate sources of biogas generation in Africa due to the significant roles played by rumen bacteria in anaerobic digestion, while in Denmark and Germany they are co-digested with crop residues (Kröber et al. [2009\)](#page-25-5). Co-digestion is the term employed to describe the parallel anaerobic digestion of many organic wastes in a single digester. Due to beneficial synergisms formed

in the digestive medium, bacterial diversity in various wastes, and the co-substrate provision of deficient nutrients, this concept increases methane output (Li et al. [2011](#page-25-6)). As a result of primary variations in the digestive physiology of the various species, the components and types of diet, the stage of growth of the animal, and consequently the management system of waste accumulation and storage, the wastes generated from various animals also differ in chemical makeup and natural substances (Anunputtikul [2004\)](#page-23-1).

Significant chunks of the agricultural sector in both emerging and established nations are focused to raise poultry and cattle, thus generating tremendous quantities of animal excrement which raises public, ecological, and social issues. Currently, several digesters are designed and operated on farms for the effective handling of the wastes. It is crucial to clean up a contaminated environment in a sustainable manner; in this light, the relevance of microorganisms in wastewater cleaning and the biodegradation of pollutants have grown recently. Numerous biotechnological methods reliant on microorganisms, including bioremediation, biodegradation, biocomposting, and biotransformation, have been employed to adequately accumulate and destroy a wide variety of pollutants. *Cladophora sp.* (green algae) is a robust and efficient potential WM agent and has a high bioaccumulation capacity for hazardous metals (Maghraby and Hassan [2018\)](#page-25-7).

Finland and polar arctic regions have been extensively using archaea and bacteria in the bioreactors to treat WW. As part of a process known as nano-bioremediation, nanoparticles are now successfully used to increase the activity of microbes. Since because of the radioactive resistance and potential to withstand radiation naturally, the extremophilic bacteria (*Deinococcus radiodurans*) is commonly used in radioactive waste extraction methods (Brim et al. [2000](#page-23-2); Varma et al. [2017\)](#page-26-1).

The primary goal of this study is to suggest and encourage the use of the most practical and environmentally responsible way for treating contaminated animal waste using various microbiological agents in order to achieve environmental sustainability. In this context, biomass materials have been regarded as a means of increasing energy generation, reducing the world's rising reliance on fossil fuels, and also mitigating the environmental and health risks associated with the usage of fossil fuels in both developing and wealthy nations (Uzodinma et al. [2008](#page-26-2)).

15.2 Waste

Human activities are mostly responsible for the creation of waste material. The created trash has been worsened by the unplanned and rapid development and alteration of livelihoods around the world. The global emission of dangerous contaminants from many occupations causes the overall biosphere to progressively deteriorate. A significant amount of biomedical and agricultural waste is created as a consequence of the sudden expansion of healthcare institutions and the automation of agricultural practices, which has a detrimental impact on environment. Wastes may be divided into three major categories: solid, liquid, and gaseous waste.

15.2.1 Classification of Wastes

Waste might be in the form of solid, liquid, gas, or heat and produced from four distinct sites, such as industrial, municipal, biomedical, and electronic sources. Waste may be characterized using a variety of factors, including the kind of material, how easily it degrades, how it will affect the environment, and the source. Each category could include several kinds (Fig. [15.2](#page-5-0)).

Fig. 15.2 Classification of wastes: an inference

15.2.2 Waste Management

Storage, collection, disposal, and management of waste materials are the main components of WM. The primary goal of WM is to lessen the negative impact that wastes have on the environment and human health. Rapid population growth, industrialization, and the enormous population demand on the NR have all contributed to this problem becoming more and more of a concern for the world's environment (Fig. [15.3\)](#page-6-0).

15.3 Microbes in Waste Management

The approach to use contemporary scientific methods and procedures that employ a wide range of microorganisms under controlled conditions without disrupting the ecosystem is known as microbial biotechnology in WM. Composting, biodegradation, bioremediation, and biotransformation are the most widespread and effective WM techniques. Numerous bacteria, including *Bacillus* sp., *Corynebacterium* sp.*, Staphylococcus* sp.*, Streptococcus* sp.*, Scenedesmus platydiscus, S. quadricauda, S. capricornutum,* and *Chlorella vulgaris,* have been successfully employed for WM (Liaqat et al. [2022\)](#page-25-8) (Fig. [15.4](#page-7-0)).

Fig. 15.3 Waste management (WM) system: A schematic representation

Fig. 15.4 Various microbes involve in the waste management system

15.4 Anaerobic Digestion of Animal Wastes in Bio-Digesters

Large amounts of excrement must be managed appropriately as livestock operations expand and become more intensive. Manure emits methane, a greenhouse gas (St-Pierre and Wright [2013](#page-26-3)). Additionally, anaerobic breakdown often starts in the animal's lower digestive system and continues in the dung heaps, producing foulsmelling chemicals. These offensive substances result from inadequate decomposition of organic materials in manure by anaerobic microorganisms in uncontrolled environmental circumstances (Husfeldt et al. [2012\)](#page-24-1). An option to the correct treatment of these wastes is a farm-based anaerobic digester. Around the world, there are countless on-farm digester facilities, including Blue Spruce Farm, Green Mountain Diary, Chaput Family Farm, Cantabria Diary Plant, Buttermilk Hall Farm, Bulcote

Farm, Minnesota Mid-sized Diary Farm, etc. (Husfeldt et al. [2012](#page-24-1); Rico et al. [2011\)](#page-25-9) (Fig. [15.5\)](#page-8-0).

There are a few on-farm anaerobic digesters in South Africa. However, the manure collected, varies in accordance with the nature of animal food, on-farm activities, and the type of digester utilized (Manyi-Loh et al. [2013\)](#page-25-10). In light of the variations in animal management approaches among the farms, the crucial process of solid– liquid isolation of the manure mixture may be carried out either prior to or following anaerobic process (Tucker [2008](#page-26-4)). Additionally, for anaerobic digestion, the obtained manure may be combined with milk house waste. Manure is often collected together

Fig. 15.5 Anaerobic digestion: A schematic layout

with or without milk house trash and turned into slurry by adding water. Pumping the slurry to the separator allows the mixture to be divided into liquid and solid parts by screening. The digester then receives the filtered liquid proportion, while the solid fraction might be de-watered and distributed to places in need of nutrients, employed as bedding, or compost to provide an extra supply of carbon and nitrogen (Sakar et al. [2009\)](#page-25-11).

Intricate assemblages of bacteria in the digester catabolize larger molecules in animal manure during the course of anaerobic digestion, subsequently producing methane and carbon dioxide. Essentially, this process may be broken down into four parts: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During each of the step, certain hydrolytic, fermentative bacteria, acetogens, and methanogens contribute a vital part for the process (Lozano et al. [2009](#page-25-12)). Complicated polymers, such as carbohydrates, proteins, and lipids, are broken down into simple sugars, amino acids, and long-chain fatty acids during hydrolysis. Extracellular enzymes (cellulases, lipases, proteases, and amylases) produced by hydrolytic bacteria linked to a polymeric substrate play a major role in this decomposition process (Song et al. [2005\)](#page-26-5) (Table [15.1](#page-9-0)).

Hydrolysis byproducts are further converted into acetic acid and intermediates, such as ethanol, lactic acid, short-chain fatty acids, hydrogen, and carbon dioxide by fermentative or acidogenic bacteria. The methanogenesis process can directly use the acetate, carbon dioxide, acetone, methylamines, methyl sulfide, and methanol generated during this phase. In order to increase methane synthesis, syntrophic acetogens transform the various byproducts from acidogenesis to acetate, formate, or $CO₂$ and H2. Finally, methanogens manufacture methane during methanogenesis in two different ways: either by cleaving acetic acid molecules to form methane and carbon dioxide, or by reducing carbon dioxide with hydrogen, depending on whether they are acetotrophic or hydrogenotrophic methanogens (Franke-Whittle et al. [2009](#page-24-3)).

15.5 Anaerobic Digestion-Related Microbial Colonies

The particular bacteria and their metabolic processes during anaerobic digestion are influenced by the chemical makeup of the feedstock, ambient variables, and digester operation parameters. There are four types of concerned bacteria, and these groups are biologically closely linked. The early steps of digestion result in decreased intermediates which are used by acetogens and methanogens (Franke-Whittle et al. [2009\)](#page-24-3). But the interplay between acetogens and methanogens is quite intricate. Since these bacteria are anaerobes, oxygen poses a hazard by disrupting cellular metabolism and triggering the oxidation of cellular components that commonly arise in compact forms. Contrarily, new research has shown that certain methanogens can adapt to oxygen because their genomes contain genes that produce enzymes that protect them against oxygen toxicity, such as catalase and superoxide dismutase (Brioukhanov et al., [2006\)](#page-23-3). Methanogens, such as *Methanobacterium thermoautotrophicum, Methanobrevibacter arboriphilus*, and *Methanosarcina barkeri*, have been found to be very tolerant to oxygen and desiccation, according to a number of reports (Kiener and Leisinger [1983;](#page-24-4) Fetzer et al. [1993\)](#page-24-5).

Due to the production of thick exterior cell layers made of extracellular polysaccharide (EPS), which were combined with the buildup of cyclic 2,3 diphosphoglycerate, *M. barkeri* exhibits the natural capacity to endure prolonged periods of exposure to air and deadly temperatures. In a digester system, the microbial population may generally be divided into three types: acidogens, syntrophic acetogens, and methanogens (McInerney et al. [2009\)](#page-25-13).

15.5.1 Acidogens

According to documentation, the bacterial species that are active during the polymer hydrolysis stage are likewise active during the acidogenic phase. As a result, fermentative bacteria can also be referenced as acidogenic and hydrolytic bacteria. They can be either stringent anaerobes or facultative anaerobes, which means that they can exist in both aerobic and anaerobic environments. One of the microorganisms in charge of the initial stage in the biotransformation of carbohydrates to $CH₄$ is the family *Enterobacteriaceae*, sometimes known as enteric bacteria. This category of bacteria lives in the intestines of humans and other animals (Carbone et al. [2002](#page-23-4)).

15.5.2 Syntrophic Acetogens

Alcohols, short-chain fatty acids (C3–C6), certain amino acids, and aromatic compounds are syntrophically metabolized by syntrophic acetogens, such as *Syntrophobacter wolinii, Syntrophomonas wolfei,* and *Smithella sp.,* to produce methanogenesis precursors. The thermodynamics of converting the aforementioned substrates to produce methanogenesis precursors is unfavorable, but they are made favorable by the inclusion of a syntrophic mate such as hydrogenotrophs (McInerney et al. [2008](#page-25-14); Hori et al. [2011](#page-24-6)). However, the buildup of eruptive fatty acids forces the pH to drop, the acidification to worsen, the destruction of methanogen functionality, and finally the collapse of the digester. *Homoacetogens* also make acetate from the conversion of carbon dioxide via the acetyl Co-A reductase reaction, whereas syntrophic acetogens transform intermediate metabolites to acetate and other methanogenesis fuels (Siriwongrungson et al. [2007\)](#page-26-6).

In principle, acetotrophic, hydrogenotrophic, and methylotrophic channels all contribute to the production of methane through methanogenesis. Particular methanogens belonging to the order *Methanosarcinales* are in command of the acetate breakdown to generate methane via the acetoclastic path. On the other hand, a subset of acetate-oxidizing bacteria exist in syntrophic interactions with hydrogenotrophic methanogens, where they work together to oxidize acetate to produce methane. Both mesophilic and thermophilic bacteria are included in this class of bacteria, which is also known as syntrophic acetogens. Syntrophic acetateoxidizing bacteria, which intricate in the reversed reductive acetogenesis, are being identified, using integrating flow measurement with transcriptional profiling of the formyltetrahydrofolate synthetase (FTHFS) gene, an ecological bioindicator essential for reductive acetogenesis (Hori et al. [2011](#page-24-6)).

15.5.3 Methanogens

Methanogens have been discovered in a variety of reactive environments, such as freshwater and marine ecosystems, sewage digesters, the gastrointestinal tracts of herbivores and animals, and insects that feed on humus and wood. They are members of the archaea domain and play a crucial role in the anaerobic process of digestion since it is at this stage that the lucrative methane is created (Zhu et al. 2004 ; Manyi-Loh et al. [2013](#page-25-10)). The methanogenic communities are particularly susceptible to pH, fatty acid levels, free NH₃, and NH₄⁺ in the digesting medium during an inconsistent anaerobic digestion operation in a malfunctioning anaerobic digester (Westerholm et al. [2012\)](#page-26-8). Additionally, there are six major orders of methanogens, *Methanococcales, Methanomicrobiales, Methanosarcinales, Methanocellales,* and *Methanopyrales*. Acetate, which has long been recognized as the primary source of more than 70% of the methane, generated in the majority of designed anaerobic digesters, is employed by the members of the order *Methanosarcinales* (Batstone [2006\)](#page-23-5).

The two families *Methanosarcinaceae* and *Methanosaetaceae*, which make up the *Methanosarcinales*, are also characterized as being acetoclastic. The morphology, biokinetics, and growth conditions of these two groups of acetoclastic methanogens vary, depending on the acetate content. In summation, the linkages among the numerous anaerobic microbe groups are very complex, and the harmony of these interconnections is crucial to the ability of biological process to function well (Amani et al. [2010](#page-23-6)).

15.6 Factors Affecting Anaerobic Digestion of Animal Manure

Performance parameters such as hydraulic retention time (HRT), temperature, pH, organic loading rate (OLR), free ammonia concentration, medium characteristics, biodegradability, and bio-digester layout are primary elements, impacting the effectiveness of anaerobic digester. Moreover, the anaerobic digestion of animal dung is substantially influenced by temperature, biodegradability, OLR, and HRT. Despite this, it is important to remember other factors as well (Giesy et al. [2005](#page-24-7); Cioabla et al. [2012](#page-23-7)).

15.6.1 Temperature

Anaerobic bacteria can be subdivided into psychrophiles (20 °C), mesophiles (25–37 $^{\circ}$ C), and thermophiles (55–65 $^{\circ}$ C) according to their preferred temperature ranges. Some methanogenic organisms are categorized as hyperthermophilic methanogens because they enjoy extremely hot temperatures (90–100 °C). The most significant environmental element impacting the proliferation of microorganisms might be regarded as temperature. *Methanocaldococcus jannaschii* and *Methanococcus vulcanius* are two instances. All bacteria can only reproduce and expand within a specific temperature range. The enzymatic and chemical interactions speed up as the temperature rises within a particular band, and thus, accelerates growth (Ver Eecke et al. [2012](#page-26-9); Saleh and Mahmood [2004](#page-25-15)).

Conversely, enzymes have a protein-like nature and get permanently destroyed beyond their appropriate temperature, important chemical processes which take place in many biosynthetic processes, some of which are catalyzed by enzymes, thus, cannot take place. As they enable organisms to drive desired energy-dependent events by combining them with spontaneous reactions which release energy, enzymes are essential for metabolism. Microbes will, therefore, stop growing, as well. To sudden temperature fluctuations, many microbial species react in different ways. In addition, temperature influences the metabolic rates of the microorganisms and other processing variables including OLR and ammonia content (El-Mashad et al. [2004\)](#page-24-8).

Biomass residues may typically be digested anaerobically at both mesophilic (25–37 °C) and thermophilic (55–65 °C) temperatures. At thermophilic temperature ranges, though, the proportion of free ammonia $(NH₃)$ to total ammonium ion is greater. In order to minimize ammonia-mediated suppression of methanogenesis, animal wastes that include nitrogen and ammonia molecules are digested at mesophilic temperatures (25–37 °C) (Garcia and Angenent [2009](#page-24-9)). Furthermore, thermophilic processing utilizes a significant amount of energy, which might lower the net energy gained from the entire digesting process. Despite the previously mentioned negatives, thermophilic fermentation probably destroys pathogens and weed seeds significantly while also boosts metabolism and $CH₄$ output (Campos Pozuelo et al. [1999\)](#page-23-8).

15.6.2 pH and Alkalinity

It is more pertinent to speak about alkalinity and pH in relation to anaerobic digestion because the former may be employed to modulate pH, buffering the system acidity resulting from the acidogenesis phase. As an outcome, the buffering capability of an anaerobic digester is represented by the quantity of alkalinity in the system (Gerardi [2003\)](#page-24-10). The OLR (which relies on reactor system) and the substrate buffering ability determine the pH range of anaerobic digestion, which typically takes place in the vicinity of neutral pH value. Cow, swine, and poultry dung are examples of livestock wastes with good buffering potential because, when microbes break them down, they create alkalinity (Molinuevo-Salces et al. [2010\)](#page-25-16). The anaerobic digestion of these wastes is frequently kept at elevated pH levels of 7.6, though. Increased volatile fatty acid buildup owing to increased acidity of the digesting media can be caused by an increase in OLR with a matching reduction in HRT (Veeken et al. [2000](#page-26-10)).

15.6.3 Ammonia

Biological breakdown of organic nitrogen induces high percentage of total ammonium ions and free ammonia. The amount of ammonia generated during digestion is influenced by the nitrogen content of the medium, reactor loads, the C/N proportion, buffering ability, and temperature (Benabdallah et al. [2009](#page-23-9)). In water solutions, inorganic ammonia and nitrogen may be found in two main states which rely on pH, ammonium ions (NH_4^+) , and unionized ammonia or free ammonia (NH_3) . The operational pH and heat have a significant impact on ammonia toxicity. Since a greater portion of total ammonia nitrogen will be in the form of free ammonia, which is known to be hazardous, a rise in pH will make the ammonia poisoning of the system more worse (Chen et al. [2008](#page-23-10)).

A decrease in pH value, on the contrary end, will assist to balance out the levels of free ammonia and bringing it closer to the ideal pH range desired for the growth

of microbes. But at the other perspective, process, destabilization brought on by oxidative stress frequently causes a rise in volatile fatty acid levels and a commensurate reduction in methane output. Elevated ammonia content causes deficient biogas integrity, lower COD competence, impaired biogas output, and stinking, in addition to stifled operation. As free ammonia inhibits methanogen growth, it has often been linked to poor performance characteristics and a higher likelihood of process failure. As a result of greater ammonia levels in a fermenter, the biomethanation process switches from acetoclastic methanogenesis (carried out by methanogens that use acetate) to syntrophic acetate oxidation, which is carried out by syntrophic acetogens working with hydrogenotrophs (Westerholm et al. [2012\)](#page-26-8).

Additionally, the operating temperature (mesophilic and thermophilic temperatures) of the digester unit might have an impact on chemical equilibriums, notably of free ammonia concentration at a fixed total ammonium concentration. Despite the fact that temperature plays a crucial role in the kinetics and thermodynamics of microbial activities in methanogenesis, treating biomass components rich in ammonium, urea, and proteins at thermophilic temperatures (56–65 \degree C) might be difficult because of a larger supply of free ammonia (Angelidaki and Ahring [1993](#page-23-11)). Since free ammonia inhibits methane production, the ratio of total ammonium to free ammonia gets substantially higher at warmer temperatures. Nevertheless, the ammonia lethality of digester system got reduced by a rise in temperature within the mesophilic ranges. Therefore, mesophilic range of temperatures gives greater process stability to anaerobic digestion of animal manure and a better performance than thermophilic temperatures (Campos Pozuelo et al. [1999](#page-23-8)).

It has been shown that combining animal manure with carbon-rich co-substrates would aid the avoidance of inhibition, imposed by both volatile fatty acids and ammonia. However, due to inconsistent findings from various investigations carried out in various environments with distinct substrates and buffers, along with the intricate nature of the process of anaerobic digestion and acclimation times, the inhibitory ammonia threshold concentration is not standardized (Chen et al. [2008](#page-23-10)).

15.6.4 Hydraulic Retention Time (HRT) and Organic Loading Rate (OLR)

The HRT refers to the average time the substrate spends in the anaerobic digester, while the OLR measures the quantity of organic matter added to the digester per reactor volume and unit time. HRT and OLR have an inversely proportional relationship and convey valuable information on the design and operation of the reactor (Bolzonella et al. [2005](#page-23-12)).

The holding duration of animal manure in the reactor has a significant impact on its biological breakdown. Heat, the kind of reactor utilized for the processing, and the solid concentration of the excrement, all affect retention duration. More specifically, fixed film reactors often have a short residence period of a few hours to a few days,

whereas CSTR and plug flow reactors for animal manure treatment generally take retention duration of 20–30 days (Karim et al. [2005](#page-24-11)). The holding period for covered lagoons must be 60 days. The integrity of waste is likewise impacted by HRT in terms of nutrient concentration, methane output, and microbial load. It has been looked at how HRT affects anaerobic batch process reactors and found that when HRT increases, methane output and effluent clarity both improve (Umaña et al. [2008](#page-26-11)).

In contrast, OLR is influenced by HRT and temperature. System failure is triggered by a rapid rise in OLR and is attributable to declines in pH, methane generation rate, and COD removal effectiveness. More specifically, a larger OLR above the optimum potential increases the rate at which acidogenic and hydrolytic bacteria produce intermediates such as fatty acids. Owing to the sluggish pace at which these fatty acids are consumed by methanogens, they would eventually build, causing the pH to decrease and reducing methanogenic operations. The elevated OLR affects the microbial communities in the digester circuit. With the genus *Clostridium* predominating at reduced OLR, the classes and phyla *Gammaproteobacteria, Deferribacteres, Actinobacteria,* and *Bacteroidetes* prevail at high OLR (Rincón et al. [2008\)](#page-25-17).

15.6.5 Heavy Metals and Features of Substrate

The contents of excrement strongly influence the rate of biological pathways which occurs in the digester unit and the generation of biogas. The nutrition, waste handling, and storage strategies used in farming will all have an impact on the makeup of the manure. There is a demand for easily accessible energy sources including carbon for the creation of fresh biomaterials, inorganic materials like sulfur, nitrogen, phosphorus, magnesium, potassium, and calcium as well as organic nutrients, for the effective functioning and continuous reproductive success of microbes involved in the anaerobic digestion. Therefore, before the digestion process begins, the chemical and physical features of the feedstock, including all the moisture contents, total solid subject matter, volatile solids substances, phosphorus, nitrogen, and carbon values, must be assessed (Ganorkar et al. [2014\)](#page-24-12).

Volatile manure matter is a highly important factor since it is made up of two sections: the biodegradable half, which comprises carbohydrates, lipids, and proteins, while the resistive or lignocellulosic portion, which cannot be decomposed anaerobically. The phrase "biodegradability of manure" is determined by the production of biogas or methane and the proportion of solids (total or volatile solids) which have been eliminated. For maximum growth and functioning, microorganisms need a trace quantity of certain metals, such as iron, nickel, copper, zinc, cobalt, and molybdenum (Zhang et al. [2007](#page-26-12)). Coenzymes and cofactors contain these essential trace nutrients, which are additionally considered as the stimulatory micronutrients. Methane output, substrate utilization, and unit consistency all raise as a result of these metals as they have stimulatory impacts on the functionality of the biogas operation. The researchers have determined that variances in OLR, pH, HRT, substrate properties, and the intricate biochemical and biological mechanisms involved to regulate

trace metal accessibility are to blame for the large variations in the amount of these metals which have stimulatory affects for biogas production (Zhang et al. [2007\)](#page-26-12).

Likewise, various kinds of trace metals have unique enhancing effects. It is confirmed that adding a well-measured trace metal solution (made up of Ni, Co, and Mo) promotes a rise in methane yields. Yet, the removal of Ni from the solution resulted in a larger reduction in methane production and process stability. Additional findings indicate that methane productivity grew by 10% at $0.4-2 \mu$ m values of Co but did not substantially change with the addition of molybdenum (Mo). It is indeed worthwhile to know that animal dung has been shown to have high levels of micro and macronutrients. However, anaerobic digestion of single feedstock like maize silage has shown that digestion disruption (induced by the lack of trace elements) can occur.

Certain single feedstock such as maize silage, potato, or even food wastes cannot offer both the micronutrients and macronutrients necessary for the development of anaerobic bacteria that are essential for the anaerobic digestion. As a rule, essential nutrients must be supplied before the digestive process can start. Even better, they can be co-decomposed with animal manure such that the animal excrement offers strong buffering capacity and necessary nutrients while the energy crop boosts the fuel production (Facchin et al. [2013\)](#page-24-13). Alterations in the composition of the microbial population can result from a lack of certain metals. Furthermore, a proportion of these heavy metals that is too high would be hazardous to the system and impede the biological mechanisms via disrupting the structure and function of relevant enzymes. They reportedly have the potential to replace naturally existing metals in the prosthetic group of enzymes or by interacting with the Sulfhydryl groups on enzymes (Chen et al. [2008](#page-23-10)).

15.6.6 Blending and Mixing of Animal Wastes with Bacteria

The degree of interaction between the flowing animal waste and a viable bacterial population and consequence of blending in the reactor are crucial for a better anaerobic digestion of animal manure. The advantages of mixing the entire content of the fermenter during the anaerobic treatment have been noted by several authors, and they include: It inhibits the emergence of filth inside the digester, guarantees homogenous distribution of microorganisms and substrate throughout the mixture and intensifies contact between them, hinders stratification within the digester so that balanced dispersion of heat is conceivable, and lastly, it aids in the discharge of gas from the concoction (Rojas et al. [2010\)](#page-25-18). Nonetheless, as anaerobic digestion advances, stirring may cause a decrease in the substrate particle size. When contemplating the possibility of using various modalities (mechanical mixers and recirculation pumps), the intensity and duration of mixing are what remain uncertain. Total and volatile solids play a critical role in the classification of manure because, beyond a certain point, the manure ceases to be slurry, which complicates mixing and pumping activities. It has been noted as a conclusion that combining anaerobic digestion of dairy manure

with low volatile solids added to extended HRT reduces the necessity for doing so (Rico et al. [2011\)](#page-25-9).

15.7 Composting

A diversified population of microbes aids the composting, which is an aerobic breakdown activity. The metabolic reactions of microbiota aids to decompose various types of wastes. Organic waste has been transformed and consolidated by composting into a form that may be beneficial for a variety of agricultural techniques. It is a waste management strategy that is both inexpensive and sustainable. Humus and plant nutrients are the primary end products of composting, whereas carbon dioxide, water, and heat are the contaminants. This process involves a variety of microorganisms, including bacteria, *actinomycetes*, yeasts, and fungus. The three stages of composting are the mesophilic phase, the thermophilic phase, and the cooling and maturation phase. The kinds of composting organic matter (OM) and the efficacy of the technique, which is determined by the level of aeration and agitation, are the two variables that control the longevity of the composting stages (García-Gómez et al. [2005;](#page-24-14) Abbasi et al. [2000](#page-23-13)).

15.7.1 Factors Influencing the Rate of Composting

15.7.1.1 Microbes

Various biochemical molecules can be effectively oxidized or digested by a number of microorganisms into more obvious and stable byproducts. A pile of biodegradable solid waste may be colonized by specific microorganisms, including mesophilic bacteria, *actinomycetes*, fungus, and protozoa (Gajalakshmi and Abbasi [2008\)](#page-24-15). These microorganisms can flourish between 10 and 45 °C and efficiently break down biodegradable materials. The active stage of composting is known as the thermophilic phase, and it can be present for several weeks. The majority of the waste is decomposed in the thermophilic stage (Meena et al. [2018\)](#page-25-19).

15.7.1.2 Temperature

According to reports, optimum composting occurs between the ranges of 52–60 °C and proceeds at temperatures as high as $60-70$ °C, when the majority of microorganisms are less active. Below 20 \degree C, the composting process might halt or become much more stable. Additionally, it has been shown that temperatures exceeding 60 °C can lower microbial activity since they go beyond the optimal thermophilic boundary for microbes (Gajalakshmi and Abbasi [2008](#page-24-15)).

15.7.1.3 pH

The pH value has a significant impact on the composting. Varying pH ranges are preferred by various composting bacteria. For the growth of bacteria, a pH range from 6.0 to 7.5 is optimum, whereas fungi prefer a pH range between 5.5 and 8.0. If the pH value somehow exceeds 7.5, nitrogen is lost (Gajalakshmi and Abbasi [2008](#page-24-15)). A large variety of bacteria presumably thrive best at a pH somewhere around 6.5 and 7.5. Bacterial activity is reported substantially hindered or even abolished below pH 5.0.

15.7.1.4 Moisture Content

The recommended and optimal moisture level for composting is often between 60 and 70%. However, the ideal moisture content is between 50 and 60% at the finishing stage. Higher than 75% and lower than 30% moisture ratio greatly lower the microbial activity. The moisture content is efficiently managed by striking a balance between microbial activity and the amount of oxygen accessible. Anaerobic conditions caused by too much moisture produce unwanted compounds and a foul smell (de-Bertoldi et al. [1983](#page-24-16)).

15.7.1.5 Carbon and Nitrogen (%)

Both C and N are vital for microbes. The primary form of energy is carbon, and N is crucial for microbial development. A substrate ability to humify quickly and completely is largely dependent on the C to N ratio, which is generally from 25 to 35% (Gajalakshmi and Abbasi [2008](#page-24-15)).

15.7.1.6 Size and Nature of the Particles

During composting, nature and particle size are crucial factors. Both oxygen saturation in the heap and microbiological infiltration to the substrates are impacted by particle size. Smaller particles need more surface space for microbial assault, whereas bigger particles reduce the surface area available for microbial invasion, slowing down or even stopping the composting equipment (Zia et al. [2003](#page-26-13)).

15.8 Role of Microbes in Biodegradation of Plastic

Plastics are regarded as a significant waste material. Plastic trash recycling is a significant issue nowadays. Plastic is a polymer, and depending on the nature of the causative agents, polymers can degrade in a variety of ways, including thermally, photo-oxidatively, mechanochemically, catalytically, and biologically. The biodegradation method, among these, is the most promising because of its efficiency and ecological beneficial approach. The inherent capacity of microorganisms to start the process of breaking down via enzymatic activity is known as biodegradation (Albertsson et al. [1987](#page-23-14)) (Table [15.2](#page-20-0)).

Microbes play a big part in how natural and manmade polymers disintegrate and deteriorate. Plastics degrade gradually, and a variety of environmental conditions, including temperature and pH, are necessary for this cycle. The principal organisms that break down plastic are bacteria and fungus. One of most significant of the successive enzymatic processes that occur during the biodegradation of plastic is hydrolysis. Typically, several variables, such as the availability of microbial enzymes and adequate abiotic conditions, affect how biodegradable polymeric compounds behave (Gu and Gu [2005;](#page-24-17) Schink et al. [1992](#page-26-14)). The impurities are used by microbes for growth, feeding, and development. This is the primary driver underlying the microbial transformation of many organic pollutants. Carbon is obtained by microbes from organic compounds. C is crucial for bacteria because it serves as the foundation for new cells (Chapelle [1993](#page-23-15)).

15.9 Bioremediation

A natural procedure known as bioremediation employs microorganisms to clean trash or pollution from soil and water (Fig. [15.6\)](#page-21-0). This technique enables ecofriendly bacteria to treat solid waste, making it biodegradable and beneficial to the environment (Kensa [2011](#page-24-18)). There are two types (Fig. [15.6\)](#page-21-0).

15.9.1 **In-Situ** *Bioremediation*

In this, wastes are removed from the soil or water without extraction or transportation. Bacteria conduct biological treatment on the waste interface. It is an alternate way of treating groundwater and soil. Non-toxic microorganisms are used in this method. There are three categories of this sort of bioremediation.

15.9.1.1 Biosparging

It is a procedure for treating waste at locations where petroleum products like diesel, gasoline, and lubricating oils are present. This technique involves pumping compressed air below ground water to raise the oxygen content. To prevent the release of volatile particles into the environment, which causes air pollution, the air pressure needs to be properly managed.

Type of Plastic	Bacteria	Fungi	Algae	References
Polyethylene bags	Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis	Phanerochaete chrysosporium, Aspergillus niger	Algae is not involved in the biodegradation of polyethylene bags	Nwachukwu et al. (2010). Aswale and Ade (2009)
Low density Polyethylene	Rhodococcus ruber C208, Brevibacillus borstelensis 707, Rhodococcus ruber C208, Staphylococcus epidermidis, Bacillus cereus C1	Aspergillus niger, Penicillium sp., Chaetomium globosum, Pullularia <i>pullulans,</i> Fusarium sp. AF4, Aspergillus oryzae	Not Involved	(Gilan et al. 2004) Shah et al. (2009) Chatterjee et al. (2010) Sivan et al. (2006)
High density Polyethylene	Bacillus sp., Micrococcus sp., Vibrio sp., Arthrobacter sp., Pseudomonas sp.	Aspergillus terreus MF12, Trametes sp.	Not Involved	Balasubramanian et al., (2014) Fontanella et al. (2013) Iiyoshi et al. (1998)
Polyurethane	Corynebacterium sp., Pseudomonas sp., Arthrobacter globiformis, Bacillus sp.	Chaetomium globosum, Aspergillus terreus. Curvularia senegalensis, Fusarium solani	Acinetobacter calcoaceticus, A. gerneri	Howard et al. (2012) Crabbe et al. (1994)
Degradable Plastic	Pseudomonas sp., Micrococcus luteus, Bacillus subtilis, <i>Streptococcus</i> lactis, Proteus vulgaris	Phanerochaete chrysosporium, Penicillium sp., Aspergillus sp.	Streptomyces sp.	El-Shafei et al. (1998) Seneviratne et al. (2006) Priyanka and Archana (2011)
Degradable Polyethylene bags and Polyethylene carry bags	Serratia marcescens, Bacillus cereus, Pseudomonas aeruginosa, Streptococcus aureus, Micrococcus lylae, Pseudomonas sp., Micrococcus luteus, Bacillus subtilis. Streptococcus lactis, Proteus vulgaris	Phanerochaete chrysosporium, Aspergillus niger, A. glaucus, Pleurotus ostreatus	Not Involved	Priyanka and Archana (2011) Aswale and Ade (2009)

Table 15.2 Numerous bacterial, fungi, and algae strains that degrade plastics

Fig. 15.6 Flow diagram of bioremediation

15.9.1.2 Bioventing

It is an aerobic method for the degradation of waste materials. When oil resources are mined for petroleum and gasoline, various solid wastes are produced that can be treated via bioventing. To speed up the cleanup procedure, oxygen, nutrients like phosphorus and nitrogen are delivered to the polluted spot during the procedure.

15.9.1.3 Bio-augmentation

In this instance, cultivated microorganisms are introduced to the contaminated area with the intention of causing the pollutants in a particular environment to degrade. As a result of this process, pollutants in the groundwater and soil are converted to non-toxic compounds by microbes.

15.9.2 **Ex-Situ** *Bioremediation*

It defines the procedure of removing polluted soil or water. The many forms of ex situ bioremediation are listed here.

15.9.2.1 Composting

Composting is an aerobic process that involves mixing polluted soil with safe organic fillers. Microorganism community is greatly increased with the use of organic additives.

15.9.2.2 Land Farming

In this bioremediation technique, polluted soil is combined with green manure before being tilled into the ground. Enhancing native bio-degradative bacteria is the major goal in order to allow for the aerobic breakdown of pollutants.

15.9.2.3 Bio-Piling

Bio-piling is a composite method that combines composting with on-site gardening. This method creates an environment that is ideal for both aerobic and anaerobic microbial growth. With the aid of biodegradation, bio-piles are used to reduce the quantities of petroleum elements (Fig. [15.6](#page-21-0)).

15.10 Conclusions

The protection and sustainable development of the environment are acknowledged as having the greatest degrees of significance and calling for immediate assistance on a worldwide scale. The main areas which require a focus of concentration are waste management, conservation of NR and biodiversity, and treatment of contaminants and pollutants if sustainability is to be ensured. To protect the environment from degradation nowadays, it is not only necessary to remove toxins and pollutants, but also necessary to recycle and control hazardous chemicals by converting various wastes into a wealth of usable items in an aesthetically pleasing and environmentally beneficial way. As human struggles to find a durable way to clean up contaminated surroundings and garbage, awareness in the employment of microbes has grown and gained importance in the recent decades. The potential of microbes for specific applications has drawn more interest and curiosity with the development of biotechnology. The nature of microorganisms is unusual and even unforeseen. Numerous environmental issues may be effectively solved by using microorganisms. The ethical and scientific reliance of microorganisms results in a stunning progression of knowledge and cutting-edge equipment that offers a practical solution to protect our world as well as contemporary methods of biological WM and environmental sensing. Finally, it can be said that the application of microorganisms and microbiological techniques has created new opportunities for sustainable prosperity, notably in the fields of the environment and other significant health issues. Animal manure digestion by anaerobic bacteria is viewed as a potent alternative for properly recycling animal wastes or turning them into useful products and fuels. The potential advantages of the biodegradation method that occurs in a confinement include lessening the biological oxygen demand (BOD) and chemical oxygen demand (COD) of wastes, wrecking pathogenic microorganisms to mitigate the microbial load to a level that humans could handle safely with marginal health risks, and destroying volatile fatty

acids and many odorous compounds present in the feedstock and lowering emissions. Fundamentally, it promotes the idea of turning waste into riches by producing biogas and high-quality, nutrient-rich fertilizer from animal dung. Likewise, the other methods for the controlled management of animal wastes discussed in the chapter have a promising future to overcome the public and environmental health concerns.

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