

Soil Biology

Ajay Singh
Nagina Parmar
Ramesh C. Kuhad *Editors*

Bioaugmentation, Biostimulation and Biocontrol

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Soil Biology

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Preface

Soils sustain an immense diversity of prokaryotic and eukaryotic organisms. Microbial functions in ecosystems are as diverse as the microbes themselves. Microbes adapt to these microhabitats and live together in consortia, interacting with each other and with other parts of the soil biota. Microorganisms play an essential role in the functioning and sustaining of all natural ecosystems including biogeochemical cycling of nutrients and biodegradation.

Plant–microbe interactions involving plant growth-promoting rhizosphere microorganisms (PGPRs) are of beneficial agricultural importance, e.g., improve plant productivity, suppress disease-causing microbes and nematodes, and accelerate nutrient availability and assimilation. PGPRs compensate for the stress and reduction in plant growth caused by weed infestation, drought, heavy metals, salt, and other unfavorable environmental conditions and are frequently used as biofertilizers. Biochemical and molecular tools are continuously being developed in an attempt to better appreciate microbial abundance and distribution in natural environments to evaluate community structures with ecosystem functions and to develop appropriate biofertilization and remediation approaches.

Bioaugmentation, biostimulation, and biocontrol approaches using microbial inoculants, biofertilizer, bio(chemicals), and organic amendments have been used for a long time to improve soil biology, fertility, crop productivity, and soil remediation. In comparison with chemical-synthesized pesticides and fertilizers, biofertilizers have several advantages including: they are relatively more safe, potentially reduced environmental damage and human health risk, much more targeted activity, effective in small quantities, multiply themselves but are controlled by the plant and indigenous microbial populations, decompose more quickly than conventional chemical pesticides, and can be used in conventional or integrated pest management systems.

This volume, *Bioaugmentation, Biostimulation, and Biocontrol of the Soil Biology Series*, is a selection of topics related to biological processes with an emphasis on their application in improving soil health, fertility, and plant productivity. Topics include an overview of the role of bioaugmentation, biostimulation, and biocontrol in soil biology; beneficial interactions of PGPRs and their products; application of biofertilizer technology for pulse production; beneficial role of

phosphate-solubilizing microorganisms in soil, composting of lignocellulosic wastes and beneficial utilization of agro-industrial waste material for bioaugmentation and soil amendment; various bioaugmentation strategies for bio- and phytoremediation of contaminated soils, role of biosurfactants in soil biology and remediation, and various aspects of biocontrol strategies for suppression of soil-borne diseases for the protection of agricultural and horticultural plants

Experts in the area of soil science and environmental microbiology from diverse institutions worldwide have contributed to this book. This book should prove to be useful to students, teachers, and researchers in the disciplines of soil and environmental sciences, microbiology, biochemistry, and biotechnology.

We gratefully acknowledge the cooperation and support of all the contributing authors and valuable advice and encouragement provided by Prof. Ajit Varma and Dr. Jutta Lindenborn throughout the preparation of this volume.

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Ajay Singh
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Chapter 1

Bioaugmentation, Biostimulation, and Biocontrol in Soil Biology

Ajay Singh, Nagina Parmar, Ramesh C. Kuhad, and Owen P. Ward

1.1 Microbial Diversity and Function in Soil

Biological diversity (or biodiversity) can be defined as the set of animal and vegetable species, their genetic material, and the ecosystems they belong to, that is it encompasses diversity at the ecosystem, species, and gene diversity levels (Fontaine et al. 2003; Lynch et al. 2004). Soil organic matter and the associated bioactivity are major contributors to carbon and nutrient cycling in the biosphere: it is the main nutrient source for plant growth (after microbial decomposition) and impacts upon soil quality (soil structure, resistance to erosion). It also represents the major carbon reservoir of the biosphere–atmosphere system.

It is believed that up to one billion bacterial species actually exist in the earth environment and yet only about 5,000 species have been described (Hunter-Cevera 1998; Curtis and Sloan 2004). Only about 1% of the soil bacterial population can be cultured by standard laboratory practices. Similarly, more than 1.5 million species of fungi are thought to exist of which only about 72,000 species have been isolated or described. Microorganisms exist in every conceivable place on earth and soil may harbor up to 10 billion microorganisms per gram. It is estimated that 1 g of soil may contain about 4,000 different bacterial “genomic units” based on DNA–DNA re-association. The tropics are considered to be richer in microbial diversity than boreal or temperate environments. Some microbiologists believe that there is a similar level of microbial diversity in the deserts. Many anthropogenic activities,

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such as city development, agriculture, dispersal of pesticides, and other chemical pollutants can potentially affect soil microbial diversity (Forney et al. 2004; Blagodatskaya and Kuzyakov 2008).

Numerous factors are known to affect diversity: trophic interactions, spatial and temporal habitat heterogeneity, disturbance, and eutrophication. Ecosystem stability, productivity, and resilience toward stress and disturbances are influenced by the microbial functional diversity (Table 1.1). Differences in microbial community structures reflect the abilities of microorganisms to respond to specific environmental factors and substrates (Kuhad et al. 2004; Little et al. 2008). For example, the fluorescent pseudomonads are attracted to plant roots and show diversity between soil and plant surfaces. Species of *Penicillium* are abundant in temperate and cold climates, whereas *Aspergillus* species predominate in warmer regions. Cyanobacteria are commonly found in neutral to alkaline soils. Depending on the nature of the metabolites present in the soil, nitrogen-fixing, sulfur- and hydrogen-oxidizing, and nitrifying bacteria are often found together with denitrifiers, sulfate-reducers, and methanogens.

Further, microbial functions in ecosystems are as diverse as the microbes themselves. Microbially digested organic materials enhance plant growth and improve soil structure and nutrient status of soil. Denitrifying bacteria utilize nitrous oxides (NO_x) as the terminal electron acceptor. These denitrifiers produce NO_x reductase and can metabolize NO_x in aerobic and anaerobic conditions. Varieties of microhabitats with different physicochemical gradients and discontinuous environmental conditions are found in soil. Microbes adapt to these microhabitats and live together in consortia, interacting with each other and with other parts of the soil biota that control microbial community structure and diversity. Competitive interactions are influenced by soil structure and water regimes. Particle size and other factors, such as pH, together with type and amount of available organic compounds, may affect microbial community structure. Soil microbes are also subjected to considerable seasonal fluctuations in environmental conditions, particularly those conditions known to affect microbial activity, such as temperature, water content, and nutrient availability.

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria of beneficial agricultural importance, for example, plant health and growth, suppress disease-causing microbes, and accelerate nutrient availability and assimilation. PGPR compensate for the stress and reduction in plant growth caused by weed infestation, drought, heavy metals, salt, and other unfavorable environmental conditions and is frequently used as biofertilizer. These bacteria belong to the genera *Acetobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Derxia*, *Enterobacter*, *Gluconacetobacter*, *Klebsiella*, *Ochrobactrum*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Zoogloea*, etc. and have been subject of extensive research for decades. PGPRs may have more than one mechanism for accomplishing plant growth by production of root exudates, repression of soil-borne pathogens (by the production of hydrogen cyanide, antibiotics, and/or competition for nutrients), siderophore production, nitrate reduction, nitrogen fixation, phosphate solubilization, production of organic

Table 1.1 Functional diversity of microbes in natural environment

| Microbial process in soil | Examples of microbes |
|--|---|
| Organic matter decomposition | <i>Trichoderma, Fusarium, Bacillus, Streptomyces, Clostridium</i> |
| Nitrogen fixation | <i>Rhizobium, Bradyrhizobium, Frankia, Anabaena, Azotobacter, Beijerinckia, Aerobacter, Chlorobium, Nostoc</i> |
| Nitrogen cycles | <i>Bacillus, Pseudomonas, Serratia, Nitrobacter, Nitrosomonas, Achromobacter, Pseudomonas</i> |
| Phosphate solubilization | <i>Azotobacter, Enterobacter, Bacillus, Aspergillus, Penicillium, Rhizoctonia, Trichoderma, Irwinia</i> |
| Sulfur transformation | <i>Desulfovibrio, Thiobacillus</i> |
| Iron transformation | <i>Ferribacterium, Leptothrix</i> |
| Siderophore production | <i>Neurospora, Trichoderma, Agaricus, Fusarium, Penicillium, ericoid mycorrhizal fungi, Nocardia, Pseudomonas, Bacillus, Aeromaonas, Erwinia</i> |
| Phytohormone production (auxin, gibberellin, cytokinin) | <i>Azotobacter, Azospirillum, Pseudomonas, Rhizobium, Bacillus, Flavobacterium, Actinomyces, Nocardia, Fusarium, Gibberella, Aletrnaria, Penicillium</i> |
| Vitamins production (biotin, thiamin) | <i>P. fluorescens, P. putida</i> |
| Antibiotics (kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin and zwittermycin A) production | <i>Bacillus</i> spp. |
| Enzymes production (chitinase, cellulase, glucanase, protease, lipase, dehydrogenase, phosphatase, nitrogenase) | Plant growth-promoting rhizobacteria and fungi |
| Lipopeptide biosurfactants (viscosinamide, tensin) | <i>Pseudomonas fluorescens</i> |
| Metabolites production (HCN, diacetylphloroglucinol) | <i>P. fluorescens</i> |
| Volatile compounds (2,3-butanediol, acetoin, pyoluteorin, auxofuran) production | <i>Pseudomonas</i> spp. |
| Biocontrol | <i>Agrobacterium, Pseudomonas, Bacillus, Streptomyces, Trichoderma, mycorrhizal fungi</i> |
| Bioremediation | <i>Acinetobacter, Alcaligenes, Ochrabactrum, Pseudomonas, Flavimonas, Rhodococcus, Stenotrophomonas, Comamonas, Arthrobacter, Burkholderia, Ralstonia, Moraxella, Nocardia, Klebsiella, Phanerochaete, Penicillium, Aspergillus, Fusarium, Cladosporium</i> |
| Phytoremediation | <i>Pseudomonas, Agrobacterium, Enterobacter, Rhizobium, Kluyvera, Glomus, Rhizobacteria, mycorrhizal fungi</i> |

acids, and phytohormones (indole acetic acid or IAA), NH_3 , release of enzymes (dehydrogenase, phosphatase, nitrogenase, 1-aminocyclopropane-1-carboxylate (ACC) deaminase), and the induction of systemic disease resistance (Figueiredo et al. 2010).

Plant-associated bacteria include endophytic, phyllospheric, and rhizospheric bacteria. Endophytes are bacteria or fungi that colonize healthy plant tissue inter- and/or intracellular without causing any apparent symptoms of disease (Wang and Dai 2010). They are found in almost every host plant studied so far and the relationship between endophytes and host plants involves both mutualism and antagonism, which beneficially impact upon the symbiotic system. The phyllosphere refers to the above ground external regions of plant parts including leaves, stems, flowers, and fruits. Bacteria residing in the phyllosphere are exposed to large and rapid fluctuations in temperature, solar radiation, and water availability.

Root exudates are believed to have a major influence on the diversity of plant growth-promoting rhizosphere microorganisms. Root exudates are chemical compounds such as photosynthates, organic acids, sugars, polyamine putrescine excreted from root tissues. Indirect interactions between plants and microbes occur in the rhizosphere due to root exudates (Yang 2009).

Plant growth-promoting mycorrhizal fungi play a major role in the induced resistance against diseases and uptake of P, Zn, Fe, and N in organically grown crops (Raviv 2010). Simultaneously, mycorrhizae provide additional benefits, not the least of which being their positive effect on gradual improvement in soil aggregate stability, resulting from the direct effect of mycorrhizae mycelia.

Plant-microbe interactions such as biofertilization, rhizoremediation, biocontrol, and phytostimulation may be quorum sensing (QS) dependent. In QS behavior, small diffusible extracellular signaling molecules mediate cell-cell communication. The signaling molecules for gram-negative bacteria are named autoinducers, usually acylated homoserine lactones (AHLs). The gram-positive bacteria use peptide-signaling molecules for QS. The catabolic response profile (CRP), a measure of short-term substrate-induced respiration, has been used to calculate the catabolic diversity in soil (Gil-Sotresa et al. 2005). After a major disturbance (landslides, volcanic eruptions, chemical or petroleum oil spills, etc.), significant changes in catabolic functional diversity have been reported in soil ecosystems. The major sources of carbon input for soil organisms are the plant roots and organic residues contributed during and following plant growth. The proportions of nitrogen, carbon, and other organic matter alter microbial activity and diversity (Bending et al. 2002). Microorganisms play an essential role in the functioning and sustaining of all natural ecosystems including biogeochemical cycling of nutrients and biodegradation. The types of nutritional substrates available are different in soils with varying soil organic matter quality, and they directly affect the microbial community active in the soil. Native soil organic matter content may also significantly affect enzyme diversity, which is greater in high organic-containing soils.

Microbial functional diversity analysis is important when considering the ability of ecosystems to respond to changing environmental conditions, the need to conserve the microbial gene pool, and utilization of the selective gene pools for

useful biotechnological applications relevant to bioremediation and phytoremediation (Ward et al. 2003; Ohtsubo et al. 2004; Zhuang et al. 2007). Fortunately, with development of advanced molecular in situ methods and improved cultivation procedures, better estimates of the microbial functional diversity on earth can be predicted and its role in soil ecosystem can be thoroughly evaluated.

1.2 Characterization of Natural Microbial Communities in Soil

Characterization of natural microbial communities is a daunting task due to the interactions, including those involving substrates and metabolites, possible in soil. Methods for studying microbial diversity and community function can be broadly divided into culture-dependent and culture-independent methods (Dahllöf 2002). Culture-dependent methods are generally based on differential morphological, metabolic, and physiological properties, including use of techniques for isolation and cultivation on solid media, determination of most probable number (MPN), and characterization of substrate utilization patterns. Culture-independent methods include various biochemical and molecular approaches of community analysis involving direct examination of metabolically active microbes using differential stains, phospholipids fatty acid analysis (PFLA), polymerase chain reaction (PCR), and application of DNA microarray to study specific microorganisms or groups of microorganisms, specific genes, and to evaluate overall community profiles.

Soil biochemical properties related to the biocycling of elements (C, N, P, and S) are generally useful indicators of soil quality (Gil-Sotresa et al. 2005). These properties include both general biochemical parameters such as microbial biomass C, dehydrogenase activity and N mineralization potential, and specific biochemical parameters like activities of hydrolytic enzymes, such as phosphatase, urease, and β -glucosidase. Biochemical properties can be used both individually, as simple indices, or in combinations using complex equations derived from mathematical combinations or the application of statistical programs.

Due to the diversity of compounds contained within the soil organic matter, a great diversity of enzymes exists in soil. Because of the diversity of the soil community and of the physical soil matrix, multiple soil enzymes are required to efficiently degrade different compounds. With the recent advances in molecular ecology, the genetic potential of microbial communities to produce enzymes can be identified by the genomic studies targeting functional genes coding for extracellular enzymes (Wallenstein and Weintraub 2008).

Metagenomics is a fast growing and diverse field directed at obtaining knowledge on genomes of environmental microbes and entire microbial communities, omitting the cultivation step (Chistoserdova 2010). Other terms such as environmental genomics, ecogenomics, community genomics, and megagenomics are also used to describe this area of biology. Function-based metagenomics relies on cloning environmental DNA into expression vectors and propagating them in

appropriate hosts (Craig et al. 2010). Following appropriate activity screens, an active clone is identified and the sequence of the clone is determined. The gene of interest and its respective product are further analyzed, and their biotechnological potential is explored. Transcriptomic studies of mRNA and emerging proteomic tools can now be used to assess the microbial regulation of extracellular enzymes, pool sizes, diversity, and microbial source of soil enzymes. Furthermore, new mass-spectrometry approaches can be used to quantify the enzymatic degradation products and develop improved models of decomposition.

Biochemical and molecular tools are continuously being developed in an attempt to better appreciate microbial abundance and distribution in natural environments, to evaluate community structures with ecosystem functions, determine the community structure and function in soil, long-term effects of pollution (Prosser 2002; Singh and Ward 2005) and to develop appropriate remediation approaches (Siciliano et al. 2003; Van Hamme et al. 2003; Singh et al. 2009). Molecular methods for the analysis of microbial diversity and community analysis will be greatly advanced if genome projects are initiated to sequence environmentally important microorganisms.

1.3 Microbial Inoculants and Biofertilizers

Traditional use and importance of chemical fertilizers in agricultural production cannot be over-emphasized, but with fertilizer costs going up, generally in parallel to increase in energy costs, these need to be supplemented or substituted with cheaper available alternatives such as beneficial microbial inoculants, biofertilizers, and organic amendments to improve soil quality, fertility, biology, and agricultural productivity (Saleem et al. 2007; Ray et al. 2008; Babalola 2010).

Biofertilizers contain different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes in soil. Biofertilizers have emerged as a potentially important component of the integrated soil nutrient supply system and hold great promise to improve crop yields. Microbial inoculants and biofertilizers are an important component of organic farming accounting for about 65% of the nitrogen supply to crops worldwide. In comparison with chemical/synthesized pesticides and fertilizers, microbial inoculants or biofertilizers have several advantages (Berg 2009) including:

- (a) Greater relative safety
- (b) Potentially reduced environmental damage and human health risk
- (c) Much more targeted activity
- (d) Effectiveness in small quantities
- (e) Capacity for self-multiplication while being controlled by the plant as well as by the indigenous microbial populations
- (f) Faster decomposition than conventional chemical pesticides
- (g) Ability to be used in conventional or integrated pest management systems

Biofertilizers containing N-fixer (*Rhizobium* spp., *Bradyrhizobium* spp., *Azotobacter chroococcum*), P-solubilizer (*Bacillus megaterium*) and K-solubilizer (*Bacillus mucilaginosus*), and arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) have been developed for commercial applications. However, in the current economic situation there is a need to get maximum output with minimum cost, which is possible only if chemical fertilizers are supplemented with organic- and bio-fertilizers.

Inoculation of legume seed by dusting with peat culture in the presence of adhesives is an efficient and convenient way to introduce effective rhizobia to soil and subsequently to the rhizosphere of legumes (Deaker et al. 2004). Lime-pelleting of inoculated legume seed with superfine limestone (CaCO_3) is used to counteract the acidic effects of soil or superphosphate on the survival of the rhizobia. Co-inoculation studies with PGPR and *Rhizobia* have shown increased plant nodulation and N fixation (Figueiredo et al. 2010). Co-inoculation of some *Bacillus* strains with effective *Bradyrhizobium* resulted in enhanced nodulation and plant growth of green gram (*Vigna radiata* L.).

1.4 Fate of Genetically Modified Organisms

Determining the impact and fate of genetically modified organisms (GMOs) or non-modified organisms on the environment are of great concern today. Genetic exchange between microbes, plants and animals may be promoted by transformation. In general, release of DNA from different organisms occurs by cell lysis after death. However, some microorganisms possess active mechanisms for releasing large amounts of chromosomal or plasmid DNA which can reach concentrations that could support horizontal gene transfer by transformation (Singh et al. 2006). Plant DNA enters the soil continuously, predominantly from the sloughing off of root cap cells, as a result of pathogen colonization of below-ground biomass, through pollen dispersal, and during crop residue decomposition.

Bacteria are the only organisms capable of natural transformations and considered for the genuine bacterial gene transfer process. In bacteria, gene transfer can occur by three mechanisms in the natural environment:

- (a) Transformation – extracellular DNA is taken up by recipient bacteria
- (b) Conjugation – genetic material is transferred from one bacterium to another by cell to cell contact
- (c) Transduction – the transfer of genetic information between bacteria is mediated by bacteriophages

Both bacteria and free DNA may be dispersed by percolation and flow of water, air and dust, and other soil organisms. Upon entering the soil environment, extracellular DNA is subjected to dynamic biological, physical, and chemical factors that determine its fate (Levy-Booth et al. 2007; Pietramellara et al. 2009). Extracellular DNA up to 20 kb in size may persist through cation bridging onto soil minerals

and humic substances, and may be enzymatically degraded and restricted by DNases of microbial origin, and/or enter the microbial DNA cycle through natural transformation of competent bacteria. Lateral gene transfer may disseminate DNA through the microbial community. DNA also tends to adsorb to the clay and sand particles.

The potential risks associated with the release of GMOs into the environment has led to the development and construction of active biological containment systems in which bacteria are killed in a controlled suicide process (Ronchel and Ramos 2001). This strategy has been developed to prevent the undesirable spread of genetically modified microorganisms in the environment after they have completed their intended tasks.

Genetically modified plants (GMPs) have great potential for future agricultural, but also require a well-defined risk assessment. Most of the studies that have been conducted in order to determine the effects of GMPs on soil microorganisms and processes have been able to detect some sort of effect (Bruinsma et al. 2003). GMPs have been found to affect bacteria, non-target fungi, target fungi, enzyme activities, substrate utilization, and decomposition. Natural transformation is the most likely mechanism for horizontal transfer of genes from transgenic crops to bacteria. The single-stranded DNA taken up by the bacteria can either integrate into the bacterial genome by homologous recombination or form an autonomous replicating element. From laboratory experiments, >40 bacterial species from different environments are known to be naturally transformable. Transgenic plant DNA can be degraded during plant senescence and during microbial degradation of the plant residue in soil. However, measured amounts of transgenic plant DNA can escape these degradation processes and the long-term persistence, even of a small percentage of released plant DNA, is assumed to enhance the likelihood of bacterial transformation.

However, the effects of transgenic crops on soil microbial populations are expected to be low or at least less important compared to other biosafety issues of transgenic crops such as out-crossing to weedy species, effects on non-target organisms or the appearance of new viruses (Mercier et al. 2006; Icoz and Stotzky 2008).

1.5 Organic Amendments

Typical organic wastes and amendments that are applied to soil are pulp and paper industrial sludge, municipal wastewater sludge, animal manure, abattoir waste, and compost. Direct application of raw organic wastes is inappropriate for land use due to their unknown compositions with respect to pathogens, toxic compounds, weed seeds, heavy metals, and foul odors. These materials, if not appropriately treated or processed to reduce environmental risks and disposal constraints, may pose a serious threat to the environment and human health and cause toxicity to beneficial microflora in soil. The practice of using landfills for organic waste disposal has to

diminish due to large quantities of waste generation, and reduced availability of dumping sites and the associated environmental hazards. Similarly, incineration is expensive and causes air pollution. In contrast, land application of treated organic wastes has emerged as an attractive and cost-effective strategy. These materials have been proved to supply plant nutrients and organic matter to the soil for improved crop production. The beneficial impacts of organic amendments to soil and nutrient composition of a range of organic material are shown in Tables 1.2 and 1.3, respectively.

Use of organic soil amendments is a traditional cultural practice to improve soil fertility and structure. It is also known as a control method for soil-borne diseases, including plant–parasitic nematodes. Organic amendments have also been proposed to control diseases caused by soil-borne pathogens such as *Aphanomyces euteiches*, *Gaeumannomyces graminis*, *Macrophomina phaseolina*, *Rhizoctonia solani*,

Table 1.2 Beneficial impact of organic amendments to soil

| Soil property | Beneficial effect to soil |
|---------------|--|
| Biological | Microorganisms, earthworm, decomposition, humus production, nutrient availability, production of beneficial chemicals (hormones, amino acids, vitamins, organic acids, antibiotics), suppression of plant pathogens, crop productivity |
| Chemical | Buffering capacity, chelating capacity, cation exchange capacity, pH |
| Nutritional | Micronutrients (B, Cu, Mn, Mo, Zn) and macronutrients (Ca, Fe, Mg, K, P, C, N, O, H) |
| Physical | Soil aggregation, texture, porosity, bulk density, crusting, erosion, water holding capacity, water infiltration and percolation |

Table 1.3 Nutrient values of organic waste material

| Organic matter | % Nitrogen | % Phosphorus | % Potassium | Availability of nutrients |
|-----------------|------------|--------------|-------------|---------------------------|
| Alfalfa hay | 2–3 | 0.5–1 | 1–2 | Medium |
| Cottonseed meal | 6 | 3 | 1 | Slow |
| Compost | 1.5 | 0.5 | 1 | Slow |
| Bone meal | 1 | 11 | 0 | Slow |
| Dried blood | 12 | 1.5 | 0.5 | Rapid |
| Feather meal | 12 | 0 | 0 | Medium |
| Fish meal | 10 | 4 | 0 | Slow |
| Grass clippings | 1–2 | 0–0.5 | 1–2 | Medium |
| Horn meal | 12–14 | 1.5–2 | 0 | Medium |
| Kelp | 1 | 0.5 | 9 | Rapid |
| Leaves | 1 | 0–0.5 | 0–0.5 | Slow |
| Legumes | 2–4 | 0–0.5 | 2–3 | Medium |
| Cow manure | 0.25 | 0.15 | 0.25 | Medium |
| Horse manure | 0.3 | 0.15 | 0.5 | Medium |
| Sheep manure | 0.6 | 0.33 | 0.75 | Medium |
| Swine manure | 0.3 | 0.3 | 0.3 | Medium |
| Pine needles | 0.5 | 0 | 1 | Slow |
| Poultry manure | 2 | 2 | 1 | Rapid |
| Sewage sludge | 2–6 | 1–4 | 0–1 | Moderate |
| Wood ashes | 0 | 1–2 | 3–7 | Rapid |

Thielaviopsis basicola, *Verticillium dahlia*, etc. (Bonanomi et al. 2010). Application of organic soil amendments is a traditional control method for plant-parasitic nematodes as well (Oka 2010). A variety of organic amendments, such as animal and green manures, compost, nematicidal plants, and proteinous wastes, are used for this purpose. Combinations of different mechanisms appear to produce nematode suppression in amended soils. Possible mechanisms involved in nematode suppression are:

- (a) Release of pre-existing nematicidal compounds in soil amendments
- (b) Generation of nematicidal compounds, such as ammonia and fatty acids, during degradation
- (c) Enhancement and/or introduction of antagonistic microorganisms
- (d) Increase in plant tolerance and resistance
- (e) Changes in soil physiology those are unsuitable for nematode behavior

1.5.1 Conventional Compost and Vermicompost

Composting is considered one of the most appropriate options for addressing the constraints associated with organic solid waste materials for agricultural use. However, according to an estimate (Ahmad et al. 2007), 827 million tons of compostable materials are produced each year, largely by agriculture, municipalities, and industry. However, only 140 million tons, or 17%, of those are collected for composting. Composting is a biological process which converts heterogeneous organic wastes (manure, sludge, yard wastes, leaves, fruits, vegetables, and food wastes) into humus-like substances by mixed microbial population under controlled optimum conditions of moisture, temperature, and aeration. Composts provide plant nutrients and improve soil biophysical properties, soil organic matter, and crop yields. Decomposers include bacteria, actinomycetes, and fungi that are widespread in nature and are indigenous to soil, dust, fruit and vegetable matter, and wastes of all sorts, so special organisms are not required.

Vermicompost, like conventional compost, provides many benefits to agricultural soil, including increased ability to retain moisture, better nutrient-holding capacity, better soil structure, and higher levels of microbial activity. Vermicompost may sometimes be superior to conventional aerobic compost in the levels of plant-available nutrients, beneficial microorganisms, ability to stimulate plant growth, ability to suppress diseases, and ability to repel pests. This is a relatively new area and not much information is available. There seems to be strong evidence that worm castings may repel hard-bodied pests probably due to the production of the chitinase enzyme by the worms, which breaks down the chitin in the insects' exoskeleton.

Climate change is one of the most serious and pressing environmental problems of our time. Farms are a significant contributor to climate change, largely through the release of carbon from soils and the generation of methane gas from livestock

and their manures. Both composting and vermicomposting address these issues through carbon sequestration, a process of locking up carbon in organic matter and organisms within the soil. Because composts are stable, more carbon is retained in the soil than would occur if raw manure or inorganic fertilizer were applied. The consistent application of compost or vermicompost gradually raises the level of carbon in the soil.

Since, the composting process results in the same level of greenhouse gas (GHG) emissions as if the materials were allowed to decay naturally, it is considered to be neutral with respect to GHG generation. The potential advantages of composting described above also apply to vermicomposting. In theory, however, vermicomposting should provide some potentially significant advantages over composting with respect to GHG emissions. The vermicomposting process does not require manual or mechanical turning, as the worms aerate the material as they move through it. This should result in fewer anaerobic areas within the piles, reducing methane emissions from the process. It also reduces the amount of fuel used by farm equipment or compost turners. It has been suggested that the increased effectiveness of vermicompost relative to compost in promoting plant growth and increasing yield can result in the displacement of 5–7 times as much fertilizer per unit of vermicompost, thereby decreasing the GHG emissions proportionately. Finally, analysis of vermicompost samples has shown generally higher levels of nitrogen than analysis of compost samples made from similar feedstock. This implies that the process is more efficient at retaining nitrogen, probably because of the greater numbers of microorganisms present in the process. This, in turn, implies that less nitrous oxide is generated and/or released during the process, such that less free ammonia is generated. Since N_2O is 310 times as potent a GHG as CO_2 , this could be a significant benefit.

1.5.2 Wastewater Biosolids

Biosolids are the residual solids remaining after wastewater or sludge has been treated. The need for solids reduction is becoming more evident as the volume of generated wastewater biosolids is growing and municipal plants are choosing to dispose of the nutrient-rich solids through recycling to agriculture fields and thus helping to save space in landfills. Biosolids contain significant amounts of nutrients required by plants, including nitrogen, phosphorus, potassium, and micronutrients, making them an excellent fertilizer for use in agriculture and forestry. Addition of biosolids to soil improves bulk density, increases porosity, soil aggregation, moisture and nutrient retention, and organic carbon. Biosolids have been used in conjunction of phytoremediation technology for landfill remediation as landfill or phytocapping to stabilize soil and simultaneously remediate landfill leachate (Kim and Owens 2010). However, long-term application of biosolids to cultivated land may raise concerns for food safety from contaminants, such as the pathogens, heavy

metals, and endocrine disruptive compounds (EDCs) present in the biosolids that may find their way in water streams or accumulate in plant tissues.

A large number of human pathogens, which primarily originate from human feces, can find their way into biosolids. The diversity and number of pathogens in biosolids depend upon the general health of the contributing population, presence of hospitals, farm animals, and abattoir industries in the area (Girones 2006; Sidhu and Toze 2009). Enteric virus, protozoa, and parasites are obligatory parasites and hence unable to multiply in biosolids, whereas bacteria may multiply under favorable conditions. Generally, pathogenic viruses and bacteria die within 1–3 months, whereas protozoan oocysts and helminth ova can survive for up to a year in wastewater and possibly much longer in untreated biosolids. The inactivation of pathogens in the biosolids depends upon a number of factors such as temperature, moisture content and competition from indigenous microflora. Other factors such as predation, pH adjustment, sunlight, oxygen and oxidants, mechanical shearing or abrasion, soil type, and texture also influence pathogen inactivation. A number of different types of pathogens can be present in the biosolids but only a small portion of them are a cause of concern including bacteria: *Escherichia coli* O157:H7, *Listeria*, and *Helicobacter pylori*; the viruses: coxsackievirus, echovirus, hepatitis A, rotavirus, and norovirus; and the parasites: *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, *Microsporidia* and *Giardia*. Currently, the lack of well-developed methods for the detection and enumeration of viral, protozoan, and helminth pathogens is the main cause of non-availability of the data on pathogen behavior in biosolids.

Various stabilization processes for the treatment of wastewater biosolids to remove pathogens have been used commercially; including composting, heat drying, pelletizing, incineration, mechanical and thermal destruction, enhanced thermophilic digestion and chemical (alkali, ammonia, sulfamic acid, fly ash, etc.) treatment (Table 1.4). Treatment technologies involving high temperature or pressure systems are generally more energy extensive and expensive to operate and maintain with high capital/operating costs as compared to technologies involving only chemical and pasteurization processes. Chemical stabilization and heat-drying processes have been used that produce pathogen-free nutrient-rich high solids liquid or dry soil-like organic product for soil enrichment, topsoil blend, and as organic fertilizer amendments.

Municipal wastewater may contain a complex mixture of EDCs, originating from personal care products, pharmaceuticals, excreted hormones, household and industrial chemicals, etc. Different environmental agencies have classified the following compounds as EDC or potential EDC: steroids (17 β -estradiol, ethynil estradiol, estrone, diethylstilbestrol), some alkylphenols (nonylphenol, nonylphenol ethoxylate, octylphenol, octylphenol ethoxylate), polychlorinated biphenyls (PCBs), brominated flame retardants, polyaromatic hydrocarbons (PAHs), di-(2-ethyl hexyl) phthalate, bisphenol A (BPA), hexachlorobenzene, pentachlorophenol, polychlorinated dibenzodioxins/furans (PCDD/F), tributyltin, and many pesticides including, atrazine, lindane, and dieldrin. Some pre-treatment methods (enzymatic, thermal, oxidation) may facilitate EDC biodegradation in subsequent biological