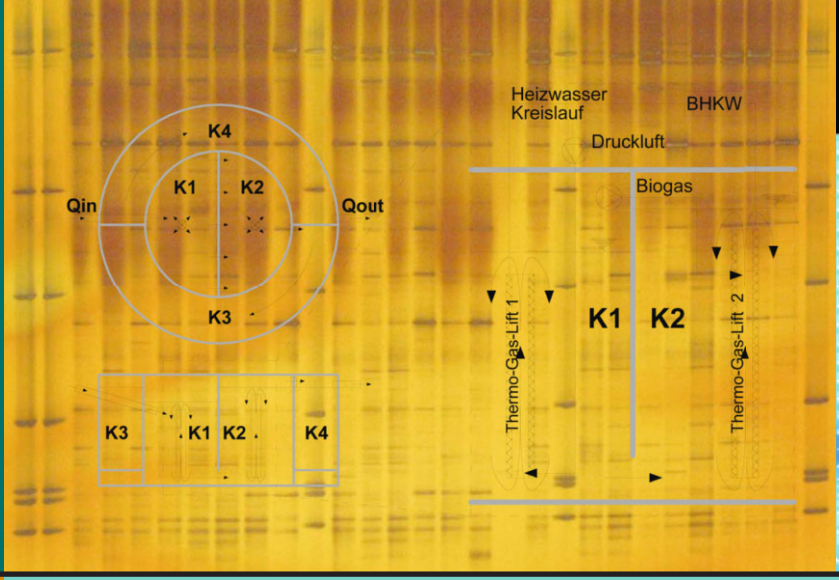

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Editors

Microbes at Work

From Wastes to Resources

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Preface

Various approaches to environmentally sound waste-treatment processes have been designed and developed in recent years. Biological treatments are the best alternative to the disposal of the putrescent fraction of separately collected biowaste, as these technologies maximize recycling and recovery of the waste components.

Composting, the biological degradation of organic wastes to humus, saves natural resources, and the application of composts may enhance the organic carbon status in soil, thereby contributing to a decrease in atmospheric CO_2. The use of compost is also known to exert positive effects on the physical properties of soil, such as increasing the soil porosity, enhancing microbial activities and the ability to suppress plant diseases, and therefore reducing the need for pesticides.

Anaerobic digestion is the biological process for the decomposition of organic waste in the absence of oxygen. This process converts the organic part of the waste into stable substances through a number of complex bacterial reactions and produces biogas, an important asset in times of decreasing fossil fuel supplies and concerns about rises in greenhouse gases, as well as an anaerobic sludge that can be used as a fertilizer and for soil conditioning. Anaerobic digestion is increasingly replacing aerobic degradation as a treatment process, since the additional benefit of gaining renewable energy is considered more and more important.

Microorganisms play a key role in both aerobic and anaerobic digestion processes of organic wastes. Until the last decade, there had been only limited research concerning the structural and functional diversity of the microbial communities involved in these digestion processes. The development of molecular tools has however allowed more detailed investigations into the microbial communities responsible for the conversion of the wastes to resources, allowing a better understanding of the dynamics of the microbial players involved.

'Microbes at work: From wastes to resources' comprises 16 chapters that discuss various aspects of digestion processes. In the first chapters, contributions focus on the communities of microorganisms involved in different types of waste treatment, including composting, vermicomposting, and anaerobic digestion. The chapters that follow discuss the presence of 'good' and 'bad' microorganisms in the final

product. The chapters occurring towards the end of the book present application-based studies or investigate the effects of application of a treated waste on the soil microbial community. Hopefully, the reader will be adequately informed about the state-of-the-art of biological waste treatment, and, with the help of the final chapter, will learn to better exploit microbial metabolic pathways.

The editors acknowledge the assistance of the many reviewers in editing the different book chapters and thank Springer Verlag for the excellent co-operation during the production of the book.

Innsbruck
September 2009

Heribert Insam
Marta Goberna
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Contents

Chapter 1
Microbes in Aerobic and Anaerobic Waste Treatment

Heribert Insam, Ingrid Franke-Whittle, and Marta Goberna

Abstract This chapter gives an overview of the materials and chemical compounds that are the subject of microbial degradation under both aerobic and anaerobic conditions. Bacteria, fungi, and archaea that are responsible for degradation or for specific phases of a degradation process are indicated. Special attention is given to two major processes of organic waste recycling involving microorganisms – composting and anaerobic digestion for biogas production. The use of classical and novel tools for investigating the involved microbiota is discussed. Also, aspects of nutrient and greenhouse gas balances are addressed. The chapter concludes by emphasizing that with microbial action, an environmentally sound recycling of organic residues is possible, and that this should be encouraged by waste management policies.

Contents

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1.1 Introduction

During biodegradation, all organic matter passes through the microbial decomposer pool. This is the reason why microorganisms have a key role to play in the recycling of matter, and why we need to facilitate their activity in order to ensure faster and proper work being done. However, very often it is the engineers who design the waste treatment facilities who are driven by empirical experience, architectural concepts, and political decisions rather than the needs of the microbes.

Microbiologists responsible for the well-being of "the workers," i.e., microbes, in a waste treatment plant would come up with a set of prerequisites that must be met for the best possible working conditions. There are essentially two different classes of workers, those that prefer fresh air with a lot of oxygen (the composters) and those that like it oxygen-free (the digesters). In some cases, however, we need both of them, and we need to offer working conditions that are acceptable for each group. This starts with the appropriate substrates.

1.2 The Substrates

Essentially, all degradable substrates are either of plant, animal, or microbial origin. Generally, plant materials comprise the largest fractions, whereas animal tissues and microbial components are only minor fractions of any mixture. The major natural compounds, as well as their potential degradability, have been summarized by Insam and de Bertoldi (2007).

1.2.1 Lignin

A major structural component of plants is lignin which may comprise up to 30% of wood. While the number of different monomers (derivatives of phenylpropane, mainly coniferyl alcohol) is small, the extraordinary variety of bondings among them make degradation a difficult task. Thus, lignin decomposition is usually of the co-metabolic type, as the energy yield from lignin degradation is negligible. Lignin is primarily degraded by fungi that are often pathogens which also thrive on living plants. Lignin-degrading fungi are known as white-rot fungi, like *Trametes versicolor* (Turkey Tail), *or Stereum hirsutum* (False Turkey Tail). They degrade the lignin and leave behind the cellulosic components. Some fungi, such as *Pleurotus ostreatus,* co-degrade cellulose and lignin.

1.2.2 Cellulose

Cellulose is the most abundant natural organic compound that is found in almost every type of organic waste. Cellulose molecules are chains of β-D-glucose with a polymerization degree of up to 40,000. The glucose molecules are joined by β-1, 4-glycosidic bonding. Three major enzymes are involved in the degradation: (1) *Endo-β-1, 4-Glucanases* cleave the *β*-1, 4-bonds within the molecule, resulting in long chains with free ends, (2) *Exo-β-1, 4-Glucanases* separate the disaccharide cellobiose from the free ends, and (3) *β-glucosidases* hydrolyze the cellobiose to leave glucose monomers.

Wilson (2008) suggests three microbial strategies for cellulolysis. Most aerobic microbes secrete cellulases that contain a carbohydrate-binding molecule (CBM) while most anaerobic microorganisms produce cellulosomes, large multi-enzyme complexes, which are bound to the outer surface of the microorganism. Gene analyses of *Cytophaga hutchinsonii*, an aerobe, and *Fibrobacter succinogenes*, an anaerobe, indicate that these organisms use a third yet unknown mechanism. They do not encode processive cellulases, and their endocellulase genes neither encode CBMs nor dockerin and cohesin domains that are the key components of cellulosomes. Fungi are, in general, more important for cellulose degradation than bacteria, which is especially the case if the cellulose is encrusted with lignin (e.g., in wood or straw). Since cellulose is rich in C but does not contain N or other essential elements, the mycelial structure gives fungi a competitive advantage in cellulose degradation as nutrients may be transported in the mycelium. Important fungal cellulose degraders include *Chaetomium*, *Fusarium*, and *Aspergillus* species. Among the bacteria, the myxomycetes and related taxonomic groups (*Cytophaga*, *Polyangium*, *Sorangium*) are important in cellulose degradation. Also, *Pseudomonas* and related genera are known to degrade cellulose, but only few Actinobacteria are involved. Under oxygen-limited conditions, cellulose is mainly degraded by mesophilic and thermophilic *Clostridia*, and also by *Fibrobacter* species.

1.2.3 Hemicelluloses (Xylan, Pectin, Starch)

Xylan is the most important of the three hemicellulosic compounds, and is found in straw, bagasse (up to 30%), and wood (2–25%). Xylan is made up of pentoses (xylose, arabinose) or hexoses (glucose, mannose, galactose), with a degree of polymerization of 30–100. The main degrading enzymes are *xylanases*, produced by many bacteria and fungi (in some cases, constitutively). Pectin comprises unbranched chains of polygalacturonic acid, making up between 2 and 35% of plant cell walls. It is degraded by *pectinases*, which are commonly produced by fungi and bacteria, many of them plant pathogens. Starch is composed of amylose (20%), unbranched chains of D-glucose and amylopectin which is branched at the

1,6 position and contains phosphate moieties and Ca and Mg ions. Three groups of enzymes are important in starch degradation. *Phosphorylases* start at the free, non-reducing end of the amylose chain, and release single glucose-1-phosphate molecules. At the 1,6 branches, the enzyme comes to a halt, and only continues after the action of *amylo-1,6-glucosidase*. The *α-1,4* bonds within the molecule are cleaved by *α-amylase*.

1.2.4 Microbial Cell Wall Components: Murein and Chitin

Murein consists of unbranched chains of *N*-acetylglucosamine and *N*-acetylmuramic acid, and muramic acid is bound through lactyl groups to various amino acids. Murein is the main component of the cell wall of most bacteria. Chitin is the most important structural compound in the cell wall of fungi, and is the substance that makes up the exoskeleton of insects and crustaceans. Fungal chitin is an important waste product from fermentation industries, and crustacean chitin is a waste product from crayfish processing. Chemically, chitin is very similar to cellulose with the glucose monomer of cellulose being replaced by *N*-acetylglucosamine in the chitin molecule. From the viewpoint of a microbe, the high N content of chitin (the C/N of chitin is approximately 5) is what distinguishes it from cellulose. Many fungi (e.g., *Aspergillus*) and bacteria (e.g., *Flavobacterium, Cytophaga, Pseudomonas*) are able to use chitin both as a N and C source. Chitin is degraded through exoenzymes to *N*-acetylglucosamine, which is resorbed, transformed to fructose-6-P, and thus incorporated into the carbohydrate metabolism.

1.3 Aerobic or Anaerobic Degradation: Four-Phase Microbially Driven Processes

Various metabolic pathways for the degradation of organic compounds exist. Aerobic processes involving oxygen as a terminal electron acceptor (composting) are thermodynamically more favorable than anaerobic processes (anaerobic digestion), so microbial degradation under oxygen is usually faster. Both composting and anaerobic digestion may be described as four-phase processes, as explained in detail below.

1.3.1 Composting

Under aerobic conditions, the degradation of organic matter is an exothermic process that produces energy in the form of heat, resulting in an increase in temperature and a high-temperature (thermic) phase. The end products of a

composting process are carbon dioxide, water, minerals, and stabilized organic matter (compost with a high content of humic acids). The transformation of fresh organic matter into compost has several advantages: it overcomes the phytotoxicity of non-stabilized organic matter, it improves the hygienic status of the materials (Chap. 9, Vinnerås et al. 2010), and it produces a stable organic material, rich in nutrients and C that is known to be beneficial to soils (Ros et al. 2006; Chap. 13, Bastida et al. 2010) and plants (Chap. 11, Fuchs 2010).

Browne (1933) was the first to prove that the self-heating of composts is due to biological activity, and Waksman (1932) was the first to publish studies on microbial community dynamics. Then, for decades, isolation and culturing procedures were the basis for studying compost microbial communities (e.g., Finstein and Morris 1975). In the 1980s and 1990s, approaches based on DNA and RNA showed that many unknown species of microorganisms were yet to be found in composts (e.g., Beffa et al. 1996). Recent molecular tools have considerably increased the knowledge on microbial communities involved in composting (Ryckeboer et al. 2003). The continuing change in habitat conditions (temperature, pH, aeration, moisture, availability of substrates) results in stages of exponential growth and stationary phases for various organisms. The microbial consortia present at any point of time are replaced by others in short intervals. On the other hand, composts are heterogeneous, and thus, not in all zones of a compost pile are similar temperatures reached. From a microbiological point of view, four major zones may be identified within a pile. The outer zone is the coolest, and well supplied with oxygen; the inner zone is poorly supplied with oxygen; the lower zone is hot, and well supplied with oxygen; while the upper zone is the hottest zone, and usually fairly well supplied with oxygen (Lott Fischer 1998).

Continuous composting processes may be regarded as a sequence of continuous cultures, each of them with their own physical (e.g., temperature), chemical (e.g., the available substrate), and biological (e.g., the microbial community composition) properties and feedback effects. These changes make it difficult to study the process, which is virtually impossible to simulate in the laboratory since temperature, aeration, moisture, etc., are directly related to the surface–volume ratio. However, it is generally accepted that composting is essentially a four-phase process, as described in Sects. 1.3.1.1–1.3.1.4.

1.3.1.1 Mesophilic Phase (25–40°C)

In the initial phase, energy-rich, abundant and easily degradable compounds like sugars and proteins are degraded by fungi and bacteria that are generally referred to as primary decomposers. During this phase, fungi compete with bacteria for the easily available substrates. Since the maximum specific growth rates of bacteria exceed those of fungi by one order of magnitude (Griffin 1985), fungi are very soon outcompeted. The importance of bacteria – with the exception of Actinobacteria – during the composting process has long been neglected, probably because of the

better visibility of organisms with a mycelial growth. An extensive review on organisms found in the first mesophile phase is given by Ryckeboer et al. (2003).

Provided that mechanical influences (like turning) are small, mesofauna, including compost worms, mites, and millipedes may thrive. From a microbiological viewpoint, these organisms may be considered as catalysts, contributing to the mechanical breakdown and offering an intestinal habitat for specialized microorganisms. Depending on the composting method, the contribution of these animals is either negligible or, as in the special case of vermicomposting, considerable (Chap. 5, Domínguez et al. 2010).

1.3.1.2 Thermophilic Phase (35–65°C)

High temperatures give a competitive advantage to thermophilic microorganisms that outcompete the mesophilic microbiota. Mesophilic organisms are inactivated by higher temperatures, and are, along with the remaining easily degradable substrates, eventually degraded by the succeeding thermophiles. The decomposition continues to be fast, and accelerates up to a temperature of about 62°C.

Thermophilic fungi grow at temperatures up to 55°C, while higher temperatures usually inhibit fungal growth. A good supply of oxygen is more important for fungi than for bacteria, and even in force-aerated systems, temporary anoxic conditions may occur. For these reasons fungi play a negligible role during the thermophilic phase. One exception is the composting of substrates that are particularly rich in cellulose and in lignin. In this case, fungi remain important degraders throughout the entire process.

At temperatures under 60°C, more than 40% of the solids are degraded within the first week, almost entirely through bacterial activity (Strom 1985). The temperature range from 50 to 65°C is of selective advantage, particularly for the genus *Bacillus*. When temperatures exceed 65°C, *B. stearothermophilus* is often dominant. Also, members of the *Thermus*/*Deinococcus* group grow on organic substrates at temperatures from 40 to 80°C, with optimum growth between 65 and 75°C. *Thermus*/*Deinococcus* group numbers in biowaste composts can be as high as 10^7 to 10^{10} g^{-1} dry weight of compost (Beffa et al. 1996). Thus, it seems that *Thermus* species, previously known only from geothermal sites, have probably adapted to the hot-compost system and play a major role in the peak-heating phase. A number of autotrophic bacteria have also been isolated from composts. These non-sporing bacteria grow at 60–80°C, with optima of 70–75°C, and closely resemble *Hydrogenobacter* strains that were previously known only from geothermal sites. They obtain their energy by the oxidization of sulfur or hydrogen, and synthesize their organic matter from CO_2 (Beffa et al. 1996).

Despite the destruction of most microorganisms at temperatures above 65°C, compost temperatures may exceed 80°C. It is probable that this final temperature rise is not due to microbial activity, but rather to the effect of abiotic exothermic reactions in which temperature-stable enzymes of Actinobacteria might be involved. Such high temperatures are important for compost hygienization (Chap. 9),

destroying human and plant pathogens, and killing weed seeds and insect larvae. The disadvantage of temperatures exceeding 70°C is that most mesophiles are killed, and thus the recovery of the decomposer community is retarded after the temperature peak. This may, however, be avoided by appropriate measures for recolonization such as inoculation with matter from the first mesophilic stage.

There is evidence that obligate anaerobic bacteria are also common in composts, but so far, very little information is available. During the preparation of *Agaricus* substrates, Eicker (1981) found evidence for sulfate reduction under thermophilic conditions. Many thermophilic or even hyperthermophilic archaea are known, but their occurrence in composts has long been negated. It was assumed that the longer generation times of archaea, when compared with bacteria, made the archaea unsuitable for the rapidly changing conditions in the composting process. Until recently, archaea had been rarely isolated from composts (e.g., Stackebrandt et al. 1997). However, since considerable methanogenesis in compost piles has recently been reported (Jäckel et al. 2005; Cabanas-Vargas and Stentiford 2006), it has been proposed that methanogenic archaea should be found if the right tools are used to search for them. Indeed, Thummes et al. (2007a, b) found that a considerable number of cultivable (*Methanosarcina thermophila*, *Methanothermobacter* sp., *Methanobacterium formicicum*, *Methanoculleus thermophilus*) and yet uncultivated archaea may be among the dominating microorganisms in composting processes.

1.3.1.3 Cooling Phase (Second Mesophilic Phase)

When the activity of the thermophilic organisms ceases due to exhaustion of substrates, the temperature starts to decrease. Mesophilic organisms recolonize the substrate, either originating from surviving spores, through the spread from protected microniches, or from external inoculation. While in the starting phase, organisms with the ability to degrade sugars, oligosaccharides and proteins dominate, the second mesophilic phase is characterized by an increased number of organisms that degrade starch or cellulose. Cellulose degraders include the bacteria *Cellulomonas*, *Clostridium*, and *Nocardia* and fungi of the genera *Aspergillus, Fusarium*, and *Paecilomyces* (as summarized by Ryckeboer et al. 2003).

1.3.1.4 Maturation and Curing Phase

During the maturation phase, the quality of the substrate declines, and in several successive steps the composition of the microbial community is entirely altered. Usually, the ratio of fungi to bacteria increases due to the competitive advantage of fungi under conditions of decreasing water potential and poorer substrate availability. Compounds that are not further degradable, such as lignin-humus complexes, are formed and become predominant.

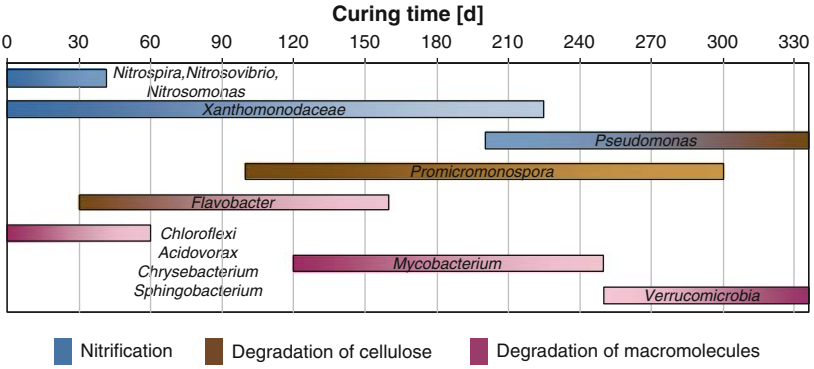

Fig. 1.1 Gantt chart of predominant compost bacteria – a model of bacterial population shifts during compost curing and their possible functions, derived from microarray, cloning and PCR-DGGE-sequencing data (Danon 2008)

Some authors have proposed a fifth composting phase, the curing phase. In this phase, also called a "storage phase," physico-chemical parameters do not change, although it has been observed that changes in the microbial communities still occur (Danon 2008). As can be seen in Fig. 1.1, during curing, i.e., after maturation, nitrifying organisms gradually disappear, and organisms involved in macromolecule degradation like Mycobacteria and Verrucomicrobia start to predominate.

1.3.2 Anaerobic Digestion

Anaerobic degradation of organic matter takes place in natural habitats where oxygen access is restricted, such as sediments, water-logged soils, and intestinal tracts. The term anaerobic digestion refers to the biotechnological process by which organic matter, be it organic waste, wastewater, or a renewable resource, e.g., purposely grown energy crops, is degraded in the absence of oxygen. Anaerobic digestion occurs in land-filled and piled wastes (Whitehead and Cotta 1999; Chen et al. 2003) and is used in engineered environments to commercially produce biogas as a source of green energy (Chap. 2, Braun et al. 2010; Chap. 3, Plugge et al. 2010; Chap. 4, Wett and Insam 2010). A co-product derived from the anaerobic digestion of wastes is the stabilized sludge, which can be applied to the soil as an organic amendment in agricultural and non-agricultural lands (e.g., rangelands, public parks). Anaerobically digested sludge seems more appropriate for land spreading than undigested products such as cattle manure. According to US-EPA (2005) this is due to its reduced organic pollutant load, malodour and pathogen content - but see Sählstrom 2003 and Bagge et al. 2005 as well as its increased nutrient bioavailability.

The process of anaerobic digestion has been extensively studied in natural and engineered ecosystems for more than a century. Until the late 1980s, the isolation of

anaerobes after enrichment culture was the approach used to investigate the microbial communities involved in a particular environmental sample. This led to the idea, as Boone et al. (1993) noted, that "because it will never be practical to study all of the innumerable different organisms in digesters, it was important to find ways to identify the numerically important microbial groups […] to understand the digestion process." In the same year, Harmsen and coworkers published the reconstructed phylogeny of *Syntrophobacter wolinii*, a fatty acid degrading bacterium common in bioreactors, based on its 16S rRNA gene (Harmsen et al. 1993). Also, Raskin and co-workers developed ^{32}P labeled oligonucleotide probes specifically targeting the 16S rRNA gene of several groups of methanogens to quantify their populations in bioreactors (Raskin et al. 1994a,b). These pioneer studies laid the basis for an avalanche of surveys using a wealth of molecular methods to phylogenetically identify the microbial drivers of the anaerobic digestion of waste (waters), as has been reviewed by O'Flaherty et al. (2006) and Talbot et al. (2008) (see Sect. 1.4). Unraveling the prokaryotic diversity in bioreactors, and understanding their physiological requirements, has allowed engineers to devise biogas plants more efficient in utilizing the organic carbon content of residues (Briones and Raskin 2003; Ward et al. 2008).

Anaerobic digestion is generally described as a four-phase process (see Sects. 1.3.2.1–1.3.2.4), in which several microbial guilds closely cooperate, forced by the low-energy yield of the anoxic degradation of organic matter (Schink 1997) and by the physical proximity that their metabolic syntrophism requires (Stams et al. 2006). Prokaryotic communities have received most of the focus and, hence, not much is known on the anaerobic eukaryotes taking part in digestion – mainly, fungi and protozoa – although some information is available about ruminal organisms (Krause et al. 2003). Bacteria are dominant in anaerobic reactors, representing over 80% of the total diversity (Krause et al. 2008). Bacterial phyla commonly detected include Firmicutes, Proteobacteria, and Bacteroidetes. Thermotogae, Chloroflexi, Fusiobacteria, Spirochaetes, Deferribacteres, Actinobacteria, and Nitrospira are also sporadically found. Archaeal representatives in reactors mostly belong to the phylum Euryarchaeota, which includes all known methanogens. However, in public databases hundreds of sequences have been stored which are phylogenetically related to yet uncultured archaea, also clustering within the phylum Crenarchaeota (Chouari et al. 2005; Collins et al. 2006; Sekiguchi 2006). Even more overwhelming is the amount of prokaryotic diversity that cannot be assigned to any known taxon. Recently, up to 15% of 1,930 identified 16S rRNA sequences could not be related to any known organism using a whole-genome-shotgun sequencing approach (Krause et al. 2008). Revealing the ecophysiological functions of this massive diversity of newly discovered microbes is the next challenge for microbial ecologists.

1.3.2.1 Depolymerization – Hydrolysis

In the first step of the anaerobic food chain (Fig. 1.2), biopolymers (polysaccharides, lipids, proteins, and nucleic acids) are depolymerized and hydrolyzed into

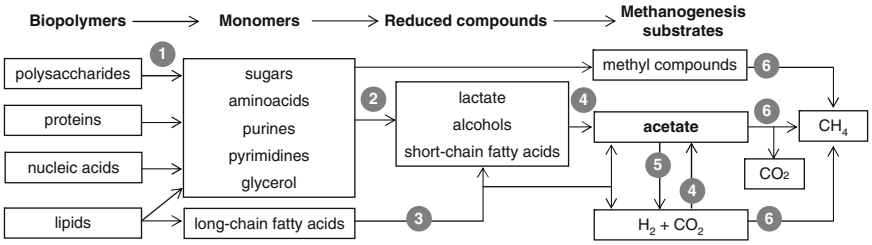

Fig. 1.2 Schematic representation of the main steps of anaerobic degradation of organic matter in the absence of inorganic electron acceptors other than H_2 and CO_2: (1) depolymerization-hydrolysis, (2) fermentation, (3) β-oxidation, (4) acetogenesis, (5) acetate oxidation, (6) methanogenesis. Modified from Sousa (2006)

monomeric compounds (sugars, amino acids, fatty acids, purines, and pyrimidines). A complex community of fibrolytic fungi and bacteria produce extracellular hydrolytic enzymes (e.g., cellulases, xylanases, proteases, lipases) that team up to dissemble the complex biopolymers into their structural units. Due to the realization that depolymerization – hydrolysis is often the rate limiting step of the anaerobic digestion process, recent strategies to optimize methane production try to foster the production and activity of extracellular enzymes or include a pre-hydrolysis step using thermal or mechanical treatments (Schwarz 2001; Mosier et al. 2005; Zhang et al. 2007; Ward et al. 2008).

Polysaccharides, particularly cellulose, are the most prominent structural and storage compounds of biomass (see Sect. 1.2). Thus, polysaccharide hydrolysis is among the most important enzymatic processes determining the efficiency of anaerobic reactors. Most aspects of the microbial utilization of cellulose have been covered by Lynd et al. (2002). In stark contrast to aerobes, most anaerobic cellulolytic bacteria have developed a multi-enzymatic complex, the cellulosome (Schwarz 2001). This includes (i) several strongly linked catalytic domains, which synergistically degrade cellulose, and (ii) substrate-anchoring domains that reduce the distance between the cell and the products of hydrolysis. In general, the hydrolysis of polysaccharides proceeds slowly under anaerobic conditions due to the heterogeneity of forms in which cellulose is present in nature and to the complexity of the hemicellulose and lignin matrices in which it is embedded (Lynd et al. 2002). The rate and efficiency of cellulose hydrolysis is related, among other factors, to the particular microbial species composition, since cellulolytic organisms differ in the structure and activity of their enzymatic complexes (Schwarz 2001) and, hence, in their cellulose degradation yields (Ren et al. 2007).

Protein hydrolysis to peptides and amino acids occurs slowly, and is the limiting step in the anaerobic digestion of proteinaceous substrates (Ortega-Charleston 2008). On the contrary, lipid hydrolysis into glycerol and long-chain fatty acids (e.g., oleic, stearic or palmitic acids) takes place rapidly in comparison to their subsequent fermentation or β-oxidation (Ortega-Charleston 2008). Lipolytic and proteolytic organisms are particularly important in industrial wastewaters rich in lipids, usually in the form of fats and oils (e.g., residues from slaughter houses and

vegetable oil refineries), or proteins (e.g., wastes from food processing industry, such as cheese, whey, beer, and fish).

Anaerobic hydrolytic fungi are slow growers compared to bacteria and, hence, fungi are less abundant in anaerobic reactors. The anaerobic fungus *Neocallimastix* sp. has been well studied in the rumen. This is a cellulase, xylanase, and esterase producer (Krause et al. 2003) that generates dihydrogen, formate, and acetate (Nakashimada et al. 2000). Co- and tri-cultures of *N. frontalis* with the methanogens *Methanobacterium formicicum* and *Methanosaeta concilii* produce methane directly from cellulose (Nakashimada et al. 2000). Other fungi commonly involved in the anaerobic digestion of cellulose are close relatives of *Neocallimastix*, namely *Orpimomyces*, *Anaeromyces*, *Piromyces*, and *Caecomyces* (Lynd et al. 2002, Gallert and Winter 2005).

Hydrolytic bacteria in bioreactors are extremely diverse, reflecting their metabolic flexibility and the range of substrates available in complex input materials. The dynamics of bacterial physiological groups is typically coupled to waste maturation. Sarada and Joseph (1994) measured an increase in the number of culturable cellulolytic, xylanolytic, pectinolytic, proteolytic, and lypolytic bacteria during the first 40 days of anaerobic digestion of tomato-processing waste, followed by a subsequent decline in all groups from days 40 to 110. Bacterial populations are very dynamic even under stable conditions (Fernández et al. 1999; Zumstein et al. 2000), probably due to the functional redundancy allowing oscillations of phylogenetic groups without changes in reactor performance (Briones and Raskin 2003; Curtis and Sloan 2004). However, bacterial phylogenetic stability has also been reported under conditions of stable (LaPara et al. 2002) and unstable reactor performance (Malin and Illmer 2008). This has been attributed to the existence of stress factors that filter the bacterial groups best adapted to harsh conditions. High salinity levels, for instance, were argued as determinants of the stability of halotolerant (*Clostridium*) and halophilic (*Halocella*) cellulolytic bacteria in a biowaste digesting reactor (Goberna et al. 2009).

Table 1.1 lists several hydrolytic bacteria that have been detected in anaerobic reactors. Members of the Firmicutes, particularly *Clostridium,* are predominant in cellulolytic anaerobic environments (Chynoweth and Pullammanappallil 1996; Schwarz 2001; Lynd et al. 2002). *Acetivibrio*, *Bacteroides, Selenomonas* or *Ruminococcus* are other common examples of hydrolytic anaerobes. Typically, the same organisms that hydrolyze biopolymers take up and ferment the resulting monomers.

1.3.2.2 Fermentation (Acidogenesis)

Monomeric compounds released after the hydrolysis of biopolymers can be taken up by cells, where they are either fermented, or, in the presence of electron donors such as nitrates or sulfates, anaerobically oxidized into alcohols, short-chain fatty acids, carbon dioxide (CO_2), and molecular hydrogen (Fig. 1.2). In engineered environments, aimed at the production of biogas – mostly composed of methane

Table 1.1 Examples of hydrolytic and fermentative bacteria detected in anaerobic reactors

Phylum	Genus	Species	Substrates	Detection	References
Actinobacteria	*Propionobacterium*	*P. acidi-propionici*	Ferment lactate	a	Sarada and Joseph (1994)
Bacteroidetes	*Bacteroides*		Cellulose, lipids, ferment citrate	a, b, c	Sarada and Joseph (1994), Ianotti et al. (1982), Shigematsu et al. (2003), Rincón et al. (2006)
Firmicutes	*Acetivibrio*	*A. cellulolyticus*	Cellulose	b	Ueno et al. (2001)
	Bacillus	*B. thermoamylovorans*	Starch, ferment sugars	b	Ueno et al. (2001)
	Caloramator	*C. proteoclasticus*	Proteins, ferment sugars and amino acids	a	Tarlera et al. (1997)
	Clostridium	*C. aldrichii*	Cellulose, xylan, ferment cellobiose	a, b, c	Boone et al. (1993), Ueno et al. (2001), Shigematsu et al. (2003)
		C. butyricum	Ferment butyrate	c	Sousa et al. (2007)
		C. cellulolyticum	Cellulose	b, c, f	Ueno et al. (2001), Krause et al. (2008), Goberna et al. (2009)
		C. cellulovorans	Cellulose	a, b	Boone et al. (1993), Ueno et al. (2001)
		C. hobsonii	(Hemi-)cellulose	a	Sharma and Hagen (1995)
		C. phytofermentans	Cellulose	f	Krause et al. (2008)
		C. propionicum	Ferment lactate	b, c	Ueno et al. (2001), Sousa et al. (2007)
		C. thermocellum	Cellulose, cellodextrins	a, b, c, f, g	Zinder et al. (1984), Ueno et al. (2001), Tang et al. (2004, 2005), Krause et al. (2008), Schlüter et al. (2008)
	Coprothermobacter	*C. proteolyticus*	Proteins	b, c	Ueno et al. (2001), Tang et al. (2004)
	Lactobacillus		Pectin, ferment sugars	a	Ianotti et al. (1982), Sarada and Joseph (1994)
	Selenomonas	*S. lipolytica*	Lipids, ferment sugars, and glycerol	a	Dighe et al. (1998)
	Ruminococcus	*R. obeum*	Cellulose	b, g	Ueno et al. (2001), Schlüter et al. (2008)
	Saccharococcus	*S. caldoxylosilyticus*	Xylose, ferment sugars	b	Ueno et al. (2001)
	Sporanaerobacter	*S. acetigenes*	Sugars, peptides, and aminoacids	c	Tang et al. (2005)
	Thermoanaerobacter	*T. ethanolicus*	Ferment sugars	f	Krause et al. (2008)
Fusiobacteria	*Fusobacterium*	*F. symbiosum*	Lipids	a	Sarada and Joseph (1994)
Proteobacteria	*Klebsiella*	*K. pneumoniae*	Ferment sugars and glycerol	a, d	Vinnerås et al. (2006)
	Salmonella		Ferment sugars	d, e	Bagge et al. (2005), Sahlström et al. (2004)

(a) culturing, (b) 16S rRNA gene PCR + DGGE or TGGE + sequencing, (c) 16S rRNA gene PCR + cloning + sequencing, (d) biochemical tests, (e) serotyping, (f) whole-genome-shotgun sequencing, (g) 454-pyrosequencing

(CH_4) and CO_2 – fermentation is the desired pathway, as it yields the major substrates for methanogenesis, i.e., acetate, formate, H_2, and CO_2.

Fermentation usually occurs through the production of an energy-rich intermediate – bearing a phosphate bond or a coenzyme A molecule – which is used to synthesize ATP. The reaction renders a fermentation product that is excreted out of the cell. Fermentation products are typically acidic substances that lower the extracellular pH. The increase in short-chain fatty acids, and the concurrent decrease in pH, is one of the most common reasons for reactor failure. Therefore, the equilibrium of fermentative-acidogenic bacteria and acid scavenging microbes is crucial for maintaining the pH balance of the system. Indeed, the volatile fatty acids/alkalinity ratio has been suggested as one of the best indicators of imbalance, its increase indicating an imminent reactor breakdown (Zhao and Viraraghavan 2004).

Bacteria are responsible for the majority of fermentative reactions, and carbohydrates generally constitute the most abundant substrates (Table 1.1). *Clostridium* sp. and enteric bacteria are common sugar fermenters in anaerobic reactors. *Streptococcus* sp. and *Lactobacillus* sp. also usually ferment sugars, yielding lactate or lactate and ethanol, plus CO_2 and molecular hydrogen. Lactate can be further fermented by organisms such as *Propionibacterium* or *Clostridium propionicum*, which yield propionate, acetate, CO_2, and water.

Amino acids are fermented via two pathways: (1) Stickland coupled deamination between a pair of amino acids, one acting as the electron acceptor and the other as the donor, and (2) deamination of a single amino acid in the presence of a H_2-scavenging partner (Ramsay and Pullammanappallil 2001). Main products of amino acid fermentation are acetate, propionate, ammonia, CO_2, and H_2. Also methylamines are formed, instead of ammonia, if betaine or sarcosine are used as hydrogen acceptors (Schmitz et al. 2006). Depending on the amino acid composition, the presence of H_2-scavenging microbes, and the availability of micronutrients in the medium, amino acid degradation can proceed through either pathway (Schink and Stams 2006). *Clostridium* sp. are the most abundant amino acid fermenters found in anaerobic digesters, and species of *Anaerobaculum*, *Caloramator*, and *Sporanaerobacter* are also commonly found (Table 1.1).

Glycerol is mainly fermented to ethanol, acetate, butyrate, propionate, succinate, formate, H_2, and CO_2 (Yazdani and Gonzalez 2007). Few microorganisms are known to catabolize glycerol fermentatively and most belong to the family of Enterobacteriaceae. This ability is well known in species of the genera *Klebsiella* and *Citrobacter*, but has also been reported in *Enterobacter*, *Escherichia*, *Clostridium*, *Bacillus*, *Lactobacillus* (Yazdani and Gonzalez 2007). Long-chain fatty acids are not fermented, but degraded via β-oxidation, their catabolism involving electron acceptors (Sousa 2006).

Fermentation of purines and pyrimidines in anaerobic environments is mostly carried out by species of *Clostridium* (Vogels and Van der Drift 1976). Fermentation of purines has been well studied in *Clostridium cylindrosporum* (Vogels and Van der Drift 1976). Typically, growing on guanine, *C. cylindrosporum* produces formate, acetate, glycine, ammonia, and CO_2 (Vogels and Van der Drift 1976).

1.3.2.3 Acetogenesis

Fermentation products are mainly oxidized to acetate, formate, H_2, and CO_2 by acetogenic bacteria (Fig. 1.2). Acetogens are phylogenetically diverse, but most of them are clustered within the low G+C branch of Firmicutes (Drake et al. 2006). Most genera encompassing acetogenic species, e.g., *Clostridium*, *Eubacterium*, or *Ruminococcus*, also include non-acetogenic members (Drake et al. 2006).

Acetogens can use diverse electron donors (e.g., carbohydrates, short-chain fatty acids, alcohols ...) and acceptors (CO_2, fumarate, pyruvate, protons …), and thus can generate multiple products apart from acetate (Drake et al. 2006). Certain acetogenic reactions are thermodynamically unfavorable under standard conditions, and require the syntrophic association between the acetogen and a H_2-scavenging methanogen (Schink 1997). This means that both organisms cooperate to degrade a substrate with a net energy gain that they would not obtain from the individual degradation of the substrate. These partners are, therefore, obligatorily inter-dependent, their metabolic linkage being mediated by H_2 and/or formate (see Chap. 3). The taxonomic groups involved in this form of microbial syntrophism have been well studied, as have been the energetics of the biochemical reactions (Schink 1997, Schink and Stams 2006) and the mechanism of inter-species H_2 (format) transfer (Stams et al. 2006). Table 1.2 lists examples of bacteria that

Table 1.2 Examples of bacteria that degrade the fermentation products through the syntrophic association with a hydrogenotrophic methanogen in anaerobic reactors

Phylum	Genus	Species	Substrates	Detection	References
Firmicutes	*Aminobacterium*	*A. colombiense*	Aminoacids	c	Tang et al. (2005)
	Aminomonas	*A. paucivorans*	Aminoacids	c	Tang et al. (2005)
	Pelotomaculum	*P. thermopropionicum*	Propionate	f, g	Krause et al. (2008), Schlüter et al. (2008)
	Syntrophobotulus	S. glycolicus	Glycolate	a	Friedrich et al. (1996)
	Syntrophomonas		Short-chain fatty acids	c, f	Krause et al. (2008), Goberna et al. (2009)
	Syntrophothermus	*S. lipocalidus*	Short-chain fatty acids	a	Sekiguchi et al. (2000)
	Moorella	*M. mulderi*	Methanol, formate, H_2/CO_2	a	Balk et al. (2003)
Proteobacteria	*Smithella*	*S. propionica*	Propionate	d	McMahon et al. (2001)
	Syntrophus	*S. buswellii*	Benzoate	a	Mountfort et al. (1984)
	Syntrophobacter	*S. wolinii*	Propionate	d	McMahon et al. (2001, 2004)
	Syntrophorhabdus	*S. aromaticivorans*	Phenol	a	Qiu et al. (2008)
Thermotogae	*Thermotoga*	*T. lettingae*	Methanol	a, b, c, e, g	Balk et al. (2002), Roest et al. (2005a), Schlüter et al. (2008)

(a) culturing, (b) 16S rRNA gene PCR + DGGE or TGGE + sequencing, (c) 16S rRNA gene PCR + cloning + sequencing, (d) 16S RNA oligonucleotide membrane hybridization, (e) DNA–DNA hybridization, (f) whole-genome shotgun sequencing, (g) 454-pyrosequencing

degrade the products of fermentation through a syntrophic relationship with H_2-consuming methanogens in anaerobic reactors.

Ideally, in an anaerobic reactor, the products of acetogenesis constitute the substrates for methanogenesis (see Sect. 1.3.2.4). However, some groups of anaerobically respiring bacteria share the substrates with methanogens, thus competing with them and reducing the efficiency of methane production (Stams et al. 2003). Sulfate-reducing bacteria (e.g., *Desulfotomaculum*, *Desulfobulbus*) are strict anaerobes that use sulfate as a terminal electron acceptor, oxidizing molecular hydrogen and organic compounds (Muyzer and Stams 2008). These can outcompete methanogens in the presence of sulfate, producing hydrogen sulfide (H_2S) that inhibits several microbial groups and causes corrosion and malodor (O'Flaherty et al. 2006). Iron-reducing (e.g., *Deferribacter*) and nitrate-reducing bacteria (e.g., *Denitrovibrio*) are also able to outcompete acetotrophic methanogens in the presence of their electron acceptors (Zinder 1993).

Three acetogenic bacteria are known to be capable of acetate oxidation in the presence of a H_2-scavenging methanogen that lowers the H_2 partial pressure: *Clostridium ultunense* (Schnürer et al. 1996), *Thermoacetogenium phaeum* (Hattori et al. 2000), and strain AOR (Zinder and Koch 1984). Also hydrogenogenic bacteria, such as *Carboxydocella* or *Thermosinus*, interact with methanogenic communities (Sokolova et al. 2002, 2004). Hydrogenogens oxidize carbon monoxide in the presence of water rendering CO_2 and H_2. These can be used by hydrogenotrophic methanogens that, in turn, produce carbon monoxide (CO) as a by-product.

1.3.2.4 Methanogenesis

The formation of methane (from acetate, H_2/CO_2, and methyl compounds) is the last step of the anaerobic degradation of organic matter (Fig. 1.2) and the most sensitive to process imbalances in engineered environments (Briones and Raskin 2003). This has been attributed to the inherently low methanogenic diversity and functional redundancy, making methanogenesis easy to inhibit (Curtis and Sloan 2004). All known methanogenic organisms are archaea belonging to the phylum Euryarchaeota. Among the five orders of known methanogens, four are commonly found in anaerobic reactors (Methanobacteriales, Methanococcales, Methanomicrobiales, and Methanosarcinales). The fifth order, Methanopyrales, includes a single hyperthermophilic species.

Members of Methanobacteriales, Methanococcales, and Methanomicrobiales use CO_2 as an electron acceptor. Hydrogen is commonly used as an electron donor, but some species can also use formate and/or secondary alcohols (e.g., isopropanol, ethanol). Members of these orders cannot use acetate or C1 compounds (e.g., methanol, methylamines), with the exception of *Methanosphaera* (Methanobacteriales) which uses H_2 to reduce methanol to methane (Bonin and Boone 2006; García et al. 2006; Whitman and Jeanthon 2006). Methanosarcinales are the most diverse metabolically. Acetate, hydrogen, formate, secondary alcohols,

and methyl compounds are used as energy sources (Kendall and Boone 2006). Among these, Methanosaetacea is the only family that includes strict acetoclastic methanogens (Smith and Ingram-Smith 2007).

Table 1.3 lists methanogens that have been found in anaerobic digesters. The predominance of hydrogenotrophic or acetotrophic methanogens seems to be mainly related to the levels of their substrates and their tolerance to diverse inhibitors, such as ammonia, hydrogen sulfide, or volatile fatty acids (Demirel and Scherer 2008). Methanogenic communities in anaerobic digesters have been found to be rather stable (Mladenovska et al. 2003; Chachkhiani et al. 2004; Leclerc et al. 2004; Goberna et al. 2009). Their dynamics has been related to main process parameters, such as volatile fatty acids (Karakashev et al. 2005; Hori et al. 2006) that are also used as indicators of process stability.

1.4 Application of Molecular Tools for Studying Microbial Community Diversity in Composts and Anaerobic Sludges

Traditional culture-based approaches to the analysis of prokaryotic diversity are only capable of the detection of less than 1% of organisms (Torsvik et al. 1990; Ward et al. 1992; Amann et al. 1995). The disparity between culturable and in situ diversity has increased the importance of culture-independent molecular approaches. Molecular techniques based on the comparative analysis of the rRNA gene sequences are now being widely used in microbial ecology and have allowed the discovery of many novel and yet unculturable microbes. Most of these methods are based on the diversity of the 16S or 18S rRNA gene sequences, although in recent years, more and more studies are being conducted using functional genes.

The first step in all molecular work is the extraction of nucleic acids, and the quality of nucleic acid (DNA and RNA) extracted from an environmental sample is crucial to be able to subsequently obtain informative results. Once good quality nucleic acid has been extracted, it can be amplified via the PCR using universal bacteria-, archaea-, or gene-specific primers, and subjected to one of the numerous community fingerprinting techniques that exist (see Chap. 12, Minz et al. 2010). The resulting pattern is reflective of the microbial diversity in the community. Included in this group of techniques are DGGE/TGGE-denaturing/temperature gradient gel electrophoresis (Muyzer et al. 1993), ARDRA – amplifed rDNA restriction analysis (Moyer et al. 1994), TRFLP – terminal-restriction fragment length polymorphism (Liu et al. 1997), ARISA – automated ribosomal inter-genic spacer analysis (Schloss et al. 2003; Nocker et al. 2007), SSCP – single strand conformation polymorphism (Talbot et al. 2004; Fracchia et al. 2006), and cloning and sequence analysis (Liesack and Stackebrandt 1992). A comparison of the methods is presented in Table 1.4. Review articles on these methods are abundant

Table 1.3 Examples of methanogenic archaea detected in anaerobic reactors

Order	Genus	Species	Substrates	Detection	References
Methanobacteriales	*Methanobrevibacter*	*M. smithii*	Formate, H_2/CO_2	g	Macario and Conway de Macario (1988)
	Methanothermobacter	*M. thermautotrophicus*	Formate, H_2/CO_2	a, b, c, f	Zinder et al. (1984), McHugh et al. (2003), Smith et al. (2004), Roest et al. (2005a), Hori et al. (2006), Goberna et al. (2009)
Methanococcales	*Methanococcus*	*M. vannielii*	Formate, H_2/CO_2	e, g	Macario and Conway de Macario (1988), McHugh et al. (2003)
Methanomicrobiales	*Methanomicrobium*	*M. mobile*	Formate, H_2/CO_2	e	McHugh et al. (2003)
	Methanocorpusculum	*M. parvum*	Formate, alcohols, H_2/CO_2	e, f	McHugh et al. (2003), Leclerc et al. (2004)
	Methanoculleus	*M. bourgensis*	Formate, H_2/CO_2	a, c	Boone et al. (1993), Tang et al. (2005), Levén et al. (2007), Goberna et al. (2009)
		M. thermophilicus	Formate, H_2/CO_2	c, e, f	McHugh et al. (2003), Chachkhiani et al. (2004), Hori et al. (2006), Levén et al. (2007)
	Methanogenium	*M. cariaci*	Formate, H_2/CO_2	e	McHugh et al. (2003)
	Methanospirillum	*M. hungatei*	Formate, alcohols, H_2/CO_2	b, c, e, f, g, h, i	Macario and Conway de Macario (1988), Huang et al. (2003), McHugh et al. (2003), Leclerc et al. (2004), Chouari et al. (2005), Levén et al. (2007), Krause et al. (2008), Schlüter et al. (2008)
Methanosarcinales	*Methanococcoides*	*M. methylutens*	Methyl compounds	e	McHugh et al. (2003)
	Methanohalobium	*M. evestigatum*	Methyl compounds	e	McHugh et al. (2003)
	Methanomethylovorans	*M. hollandica*	methyl compounds	b, c, f	Leclerc et al. (2004), Roest et al. (2005a), Laloui-Carpentier et al. (2006), Levén et al. (2007)
	Methanosarcina	*M. barkeri*	Acetate, H_2/CO_2, methyl compounds,	a, c, e, h, i	Boone et al. (1993), Godon et al. (1997), McHugh et al. (2003), Laloui-Carpentier et al. (2006), Levén et al. (2007), Krause et al. (2008), Schlüter et al. (2008)

(*continued*)

Table 1.3 (continued)

Order	Genus	Species	Substrates	Detection	References
		M. siciliae	Methyl compounds	b	Mladenovska et al. (2003)
	Methanosaeta	*M. concilii*	Acetate	b, c, d, e, f	Zheng and Raskin (2000), McMahon et al. (2001), McHugh et al. (2003), Huang et al. (2003), Shigematsu et al. (2003), Leclerc et al. (2004), McMahon et al. (2004), Chouari et al. (2005), Tang et al. (2005), Laloui-Carpentier et al. (2006), Sousa et al. (2007)

(a) culturing, (b) 16S rRNA gene PCR + DGGE or TGGE + sequencing, (c) 16S rRNA gene PCR + cloning + sequencing, (d) 16S RNA oligonucleotide membrane hybridization, (e) ARDRA + sequencing, (f) SSCP + sequencing, (g) indirect inmunofluorescence, (h) whole-genome-shotgun sequencing, (i) 454-pyrosequencing

Table 1.4 Comparison of different fingerprinting methods used in microbial ecology studies

	Method based on	Advantages	Disadvantages	Phylogenetic identification	Throughput	Time demand	Cost	Compost environment studies	AD environment studies
Clone library anaylsis	Sequence differences	Accurate phylogenetic identification of clone sequences	Time demanding cloning process, and data analysis	Yes	Low	High	High	Dees and Ghiorse (2001), Alfreider et al. (2002)	Godon et al. (1997), Fernández et al. (1999), Chouari et al. (2005), Goberna et al. (2009)
ARDRA	Sequence differences in community DNA	Detection of structural changes in relatively simple microbial communities	More labor- and time-intensive than other molecular methods	No	High	High	Low	Dees and Ghiorse (2001), Ntougias et al. (2004)	Fernández et al. (1999), McHugh et al. (2003), Klocke et al. (2008)
DGGE/ TGGE	Sequence differences	rRNA sequence information from excised DGGE bands	Limited sensitivity, gel to gel variability, partial 16S rRNA gene sequences	Yes	High	Low	Low	Ros et al. (2006), Pedro et al. (2001), Green et al. (2004), Nishida et al. (2003), Bruns et al. (2001)	Liu et al. (2002), Roest et al. (2005), Connaughton et al. (2006), Mladenovska et al. (2003)
TRFLP	Restriction site differences	High sensitivity	Overestimation of diversity due to non-specific or incomplete digestion. Multiple separate restriction digests for higher resolution	Possible	High	Medium	Low	Tiquia (2005), Michel et al. (2002), Pérez-Piqueres et al. (2006)	Collins et al. (2003), Scully et al. (2005), Lueders and Friedrich (2003)
ARISA	Differences in inter-genic spacer region length	High sensitivity, simple technique	Shorter amplicons are over-represented >1 inter-genic space region in a genome	No	High	Low	Low	Schloss et al. (2003)	

(*continued*)

Table 1.4 (continued)

	Method based on	Advantages	Disadvantages	Phylogenetic identification	Throughput	Time demand	Cost	Compost environment studies	AD environment studies
SSCP	Conformational differences	High sensitivity	High rate of reannealing of single-stranded DNA formation of more than one stable conformation resulting in the presence of extra bands	Yes	High	Low	Low	Thummes et al. (2007b), Macedo et al. (2007), Fracchia et al. (2006)	Delbes et al. (2000), Leclerc et al. (2004), Talbot et al. (2004)
Microarrays	Hybridization between complementary DNA strands	parallel detection of 16S rRNA genes	High cost, quantification not possible	Yes	High	Medium	High	Franke-Whittle et al. (2005, 2009a), Danon et al. (2008)	Goberna et al. (2008), Franke-Whittle et al. (2009a, b)
Real-Time PCR	PCR with flouroescent dyes or specific probes	Simple, reproducible, sensitive, and quantitative	Optimization can be time consuming, variation in rRNA copy number in different microorganisms	Yes	High	Low	Medium	Wéry et al. (2008), Innerebner et al. (2006), Yamada et al. (2007)	Shigematsu et al. (2003), Sawayama et al. (2006), Yu et al. (2006), Goberna et al. (2009)
FISH	Hybridization of rRNA with flourescently labeled probes	Quantiative, visualization of probed cells	Autoflouresence, necessity of metabolically active target cells	Yes	Low	Medium	Low		Raskin et al. (1994a), Montero et al. (2008), Karakashev et al. (2005)
RNA-SIP	Incorporation and metabolism by microorganisms of rare stable isotope or radioisotope	Concurrent examination of metabolic function and taxonomic identity	Not as sensitive as PFLA-SIP, biased incubation conditions	Yes	Low	High	High		Hatamoto et al. (2007, 2008), He et al. (2009)

(e.g., Juste et al. 2008; Talbot et al. 2008) and many of the methods are discussed in Chaps. 7 (Hultman et al. 2010) and 14 (Knapp et al. 2010).

Although all these molecular tools have greatly advanced microbial ecology, there are limitations with such approaches. As most molecular techniques require the extraction of DNA to be used as the template for PCR amplification and subsequent community analysis, these studies are subject to various flaws, namely, the potential formation of PCR artefacts (Wang et al 1997), the potential discrepancy between the quantitative composition of rRNA genes within the sample DNA and the amplification product (Farrelly et al. 1995; Suzuki and Giovannoni 1996; Polz and Cavanaugh 1998), and the tedium, time, and cost involved in sequencing and sequence analysis for identification purposes. As a result, studies using PCR-amplified templates may reflect a biased microbial community composition. However, the use of PCR to amplify target DNA does greatly increase the detection sensitivity of microorganisms in any environmental sample in comparison to non-PCR-based methods.

Other molecular techniques which commonly target the 16S or 18S rDNA sequence, but do not involve amplification and resolution of the gene products include microarray analysis (Guschin et al. 1997; Small et al. 2001; Loy et al. 2002), real-time PCR (Monis and Giglio 2006), fluorescence in situ hybridization (DeLong et al. 1989), and DNA and RNA stable isotope probing. These techniques will be described briefly in this section.

Nucleic acid microarrays provide a powerful tool for the parallel detection of 16S rRNA genes (or other genes of interest), thus allowing the identification of microorganisms from different environments (Franke-Whittle et al. 2005, 2009a, b; Guschin et al. 1997; Small et al. 2001; Loy et al. 2002). DNA microarrays are based on the relatively old technology of DNA hybridization between two complementary strands of nucleic acids, and offer the possibility to analyze an entire array of microorganisms concerning their presence or absence in a particular sample in a single experiment. As with all techniques, the application of microarrays for routine diagnostic work in microbial ecology and other fields is hindered by a lack of standardization and insufficient evaluation of newly developed arrays (Loy and Bodrossy 2006), and issues relating to the potentially low levels of target microorganisms in the environment have hampered the application of such diagnostic arrays (Cook and Sayler 2003). More information on the use and application of microarrays is provided in Table 1.4 (see also Chap. 7).

Real-time PCR allows a reproducible and sensitive detection and quantitation of specific microbial populations. Several technologies for performing real-time PCR exist (Chap.12, Yu et al. 2006; Monis and Giglio 2006). Despite the high level of detection sensitivity attainable by quantitative PCR, the method is not appropriate for studies requiring the enumeration of large numbers of target species in any particular sample. Quantitative PCR methods based on the analysis of rRNA genes are also subject to biases associated with PCR, as well as to the quantitative uncertainty of the numbers of rRNA genes (rrn operons) per genome. Real-time PCR has nonetheless been applied to the composting and anaerobic digestion

environments, allowing the accurate quantification of various targets of interest (Table 1.4).

The sequencing of ribosomal RNA molecules has not only facilitated the reconstruction of phylogenetic trees and allowed the development of primers for amplification of microbial DNA, but it is also the basis for the development of oligonucleotide probes which can be used to detect bacteria in situ. Whole cell hybridization with fluorescent-labeled rRNA gene-targeted oligonucleotide probes (fluorescence in situ hybridization; FISH) allows the identification of individual microbial cells (DeLong et al. 1989) in samples of natural communities. This technique is not limited by the problems inherent to PCR, although the method is influenced by the abundance and accessibility of the target intracellular rRNA molecules (Roller et al. 1994). Target cells need to be metabolically active, and hence contain large numbers of ribosomes for a strong signal to be generated (Manz et al. 1992) and microorganisms to be visualized. Although FISH has often been applied to investigate the microbial communities in the anaerobic digestion environment (Raskin et al. 1994a,b; Karakashev et al. 2005; Montero et al. 2008), it has not been commonly used in composting studies, most likely due to the problems with autofluorescence of compost particles (Table 1.4).

Stable isotope probing (SIP) is a relatively new and emerging tool, being used increasingly by environmental microbiologists (Chap. 12). It is based on the incorporation and metabolism by microorganisms of a rare stable isotope or radioisotope (Table 1.4). The method can be combined with community fingerprinting and cloning/sequencing analyses, thus allowing information on the microbial catabolic activity to be obtained (Talbot et al. 2008). Several publications on the application of SIP to the biodegradation environment have been published in the past few years (Madsen 2006), although publications in the area of anaerobic digesters and composting are limited (Hatamoto et al. 2007, 2008; He et al. 2009).

A common limitation of DNA-based community analyses is that DNA-based studies detect the occurrence of all micoorganisms irrespective of their viability or metabolic activity (Sessitsch et al. 2002). To further complicate the results, the adsorption of DNA to solid particles could potentially result in the generation of biased population profiles. RNA-based community analysis is thus considered to be a more suitable approach to describe the metabolically active community members, as the amount of rRNA produced by bacterial cells correlates well with the growth activity of bacteria (Wagner 1994). In the study of an entire bacterial community, the ribosome abundance reflects the relative contribution of each species to the protein synthesis capacity of the community (Felske et al. 2000). However, the laborious nature, and greater number of problems associated with RNA isolation and RNA studies has resulted in fewer studies based on RNA than DNA.

The newest metagenomic-based techniques being used in microbial community analyses include “shotgun” Sanger sequencing and chip-based pyrosequencing. These tools allow a mostly unbiased analysis of all genes from all members of a sampled community (e.g., Krause et al. 2008; Schlüter et al. 2008).

1.5 Carbon and Nutrient Balance

In a well-managed composting process, about 50% of the biodegradable organic matter is converted into CO_2, H_2O, mineral salts, and energy. Of the remaining organic matter, about 20% undergoes complex metabolic transformations with the final production of humic-like substances, while the other 30% is partially degraded by aerobic and anaerobic processes with the final production of less complex organic molecules. This loss of biodegradable organic matter during the composting process may vary from 30 to 60%, depending on the system of composting, length of the process, aeration system, quality (chemical and physical) of the organic matter, particle size, C/N, and temperature pattern.

The mineralization of nitrogenous organic compounds (e.g., proteins) leads to the production of free ammonia that, if not immediately oxidized by nitrifying bacteria, can be lost to the environment through volatilization. Additional nitrogen loss during composting can be caused by denitrification, an anaerobic microbial process that reduces nitrate to N_2. This can occur only in anaerobic niches which may be present even in well oxygenated material. Some denitrifying bacteria operate at thermophilic conditions (*Bacillus* sp.), while others operate at mesophilic temperatures (*Pseudomonas*, *Paracoccus*). Ideally, however, in composting, no N losses occur if the substrate composition and process conditions are set to avoid ammonia volatilization (original C/N ratio < 25, pH < 7.5, good aeration). The C/N ratio usually decreases during the process because of the loss of C as CO_2.

During the thermal stage of composting, autotrophic nitrification is inhibited (Loehr 1974; Focht and Chang 1975). Heterotrophic nitrification by *Arthrobacter* or *Actinomyces*, or by fungi-like *Aspergillus flavus* or *Penicillium*, seem to be less affected. The production of nitrate in the early phases of composting seems mostly exclusively the work of heterotrophic nitrifiers (Eylar and Schmidt 1959; Hora and Iyengar 1960; Hirsch et al. 1961; Marshall and Alexander 1962; Alexander 1977). Heterotrophic nitrifying microorganisms and those that directly assimilate ammonia for their anabolic metabolism are the most important agents in reducing the negative effects of ammonia volatilization.

Despite nitrogen losses at some stages, partial recovery takes place later in the process, due to the activity of nitrogen-fixing bacteria such as *Azospirillum, Bacillus, Clostridium, Enterobacter*, and *Klebsiella* (de Bertoldi et al. 1982, 1983). Biological nitrogen fixation is inhibited by the presence of ammonia and by the high temperature and thus, nitrogenase activity is higher during the later stages of composting.

The rate of oxygen utilization indicates biological activity. For horticultural applications, composts evolving less than 20 mg O_2 kg^{-1} compost h^{-1} are considered stable, for agricultural use, < 100 mg O_2 kg^{-1} compost h^{-1} is considered sufficiently mature. The Solvita test (Woods End Laboratories, Francou et al. 2005), is a quick test for the respiration rate and also measures the ammonia content. Less than 5 mg CO_2-C g^{-1} compost C d^{-1} indicates stable compost. Values exceeding 20 mg CO_2-C g^{-1} compost C d^{-1} indicate instability of the compost.

Table 1.5 Greenhouse gas emissions and aerobic and anaerobic waste treatment processes

Process	Compound	GHG potential	Composting	Anaerobic digestion
Respiration	CO_2	1	Low	Low
Methanogenesis	CH_4	23	Potentially high	Very low, since methane is used
Denitrification	N_2O	290	Potentially high during process	Low, only after soil application of sludge
Deammonification	NH_3	Indirect	Potentially high during process	Low, only after soil application of sludge
Degradation under partially anoxic conditions	Various volatile organic compounds (VOCs)	Unknown	When process is anaerobic: potentially high	Low
Summary			Source of GHG	Sink of GHG

Results, however, must be interpreted with care since composts that are cold, dry, or highly saline may not respire even though they are not stable.

Major concerns on greenhouse gas production from composting were raised recently (Insam and Wett 2008). On the other hand, anaerobic digestion is considered to mitigate the greenhouse gas potential of farming. Table 1.5 summarizes the potential effects of composting and anaerobic digestion on microbial greenhouse gas production.

1.6 Conclusions

Microbes are involved in various processes related to organic waste recycling. Microbial catabolic abilities are diverse, and offer exciting options for making use of various otherwise wasted materials. More specific information will be given in the remaining 15 chapters.

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References

Alexander M (1977) Introduction to soil microbiology. Wiley, New York

Alfreider A, Peters S, Tebbe CC, Rangger A, Insam H (2002) Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis. Compost Sci Util 10:303–312

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Bagge E, Sahlström L, Albihn A (2005) The effect of hygienic treatment on the microbial flora of biowaste at biogas plants. Water Res 39:4879–4886

Balk M, Weijma J, Stams AJM (2002) *Thermotoga lettingae*, sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor. Int J Syst Evol Microbiol 52:1361–1368

Balk M, Weijma J, Friedrich MW, Stams AJM (2003) Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. Arch Microbiol 179:315–320

Bastida F, Hernández T, García C (2010) Soil degradation and rehabilitation: microorganisms and functionality. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 253–270

Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M (1996) Isolation of *Thermus* strains from hot composts (60 to 80 degrees C). Appl Environ Microbiol 62: 1723–1727

Bonin AS, Boone DR (2006) The order Methanobacteriales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 3. Springer, New York, pp 231–243

Boone DR, Chynoweth DP, Mah RA, Smith PH, Wilkie AC (1993) Ecology and microbiology of biogasification. Biomass Bioenerg 5:191–202

Braun R, Drosg B, Bochmann G, Weiß S, Kirchmayr R (2010) Recent developments in bio-energy recovery through fermentation. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, New York, pp 35–58

Briones A, Raskin L (2003) Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. Curr Opin Biotechnol 14:270–276

Browne CA (1933) The spontaneous heating and ignition of hay and other agricultural products. Science 77:223–229

Bruns MA, Hanson JR, Mefford J, Scow KM (2001) Isolate PM1 populations are dominant and novel methyl tert-butyl ether-degrading bacteria in compost biofilter enrichments. Environ Microbiol 3:220–225

Cabanas-Vargas DD, Stentiford EI (2006) Oxygen and CO_2 profiles and methane formation during the maturation phase of composting. Compost Sci Util 14:86–89

Chachkhiani M, Dabert P, Abzianidze T, Partskhaladze G, Tsiklauri L, Dudauri TGJJ (2004) 16S rDNA characterisation of bacterial and archaeal communities during start-up of anaerobic thermophilic digestion of cattle manure. Bioresource Technol 93:227–232

Chen AC, Ueda K, Sekiguchi Y, Ohashi A, Harada H (2003) Molecular detection and direct enumeration of methanogenic archaea and methanotrophic bacteria in domestic solid waste landfill soils. Biotechnol Lett 25:1563–1569

Chouari R, Le Paslier D, Daegelen P, Ginestet P, Weissenbach J, Sghir A (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. Environ Microbiol 7:1104–1115

Chynoweth DP, Pullammanappallil P (1996) Anaerobic digestion of municipal solid wastes. In: Palmisano AC, Barlaz MA (eds) Microbiology of solid wastes. CRC Press, Florida, pp 71–114

Collins G, Woods A, McHugh S, Carton MW, O'Flaherty V (2003) Microbial community structure and methanogenic activity during start-up of psychrophilic anaerobic digesters treating synthetic industrial wastewaters. FEMS Microbiol Ecol 46:159–170

Collins G, Kavanagh S, McHugh S, Connaughton S, Kearney A, Rice O, Carrig C, Scully C, Bhreathnach N, Mahony T, Madden P, Enright AM, O'Flaherty V (2006) Accessing the black box of microbial diversity and ecophysiology: Recent advances through polyphasic experiments. J Environ Sci Health A 41:897–922

Connaughton S, Collins G, O'Flaherty V (2006) Development of microbial community structure and activity in a high-rate anaerobic bioreactor at 18°C. Water Res 40:1009–1017

Cook KSG, Sayler GS (2003) Environmental application of array technology: promise, problems and practicalities. Curr Opin Biotechnol 14:311–318

Curtis TP, Sloan WT (2004) Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. Curr Opin Microbiol 7:221–226

Danon M (2008) Suppression of *Sclerotium rolfsii* in compost: characterization and function of the microbial populations. The Hebrew University of Jerusalem. PhD thesis

Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y (2008) Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol Ecol 65:133–144

de Bertoldi M, Vallini G, Pera A, Zucconi F (1982) Comparison of three windrow compost systems. BioCycle 23(2):45–50

de Bertoldi M, Vallini G, Pera A (1983) The biology of composting: a review. Waste Manag Res 1:157–176

Dees PM, Ghiorse WC (2001) Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiol Ecol 35:207–216

Delbes C, Moletta R, Godon JJ (2000) Monitoring of activity dynamics of an anaerobic digester bacterial community using 16S rRNA polymerase chain reaction-single-strand conformation polymorphism analysis. Environ Microbiol 2:506–515

DeLong EF, Wickham GS, Pace NR (1989) Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science 243:1360–1363

Demirel B, Scherer P (2008) The roles of acetotrophic and hydrogenotrophic methanogens during anarobic conversion biomass to methane: a review. Rev Environ Sci Biotechnol 7:173–190

Dighe AS, Shouche YS, Ranade DR (1998) *Selenomonas lipolytica* sp. nov., an obligately anaerobic bacterium possessing lipolytic activity. Int J Syst Bacteriol 48:783–791

Domínguez J, Aira M, Gómez-Brandón M (2010) Vermicomposting: earthworms enhance the work of microbes. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 93–114

Drake HL, Küsel K, Matthies C (2006) Acetogenic prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 2. Springer, New York, USA, pp 354–420

Eicker A (1981) The occurrence and nature of sulphur crystals in phase I mushroom compost. Mushroom Sci 11:27–34

Eylar OR, Schmidt RL (1959) A survey of heterotrophic microorganisms from soil for ability to form nitrite and nitrate. J Gen Microbiol 20:473–481

Farrelly V, Rainey FA, Stackebrandt E (1995) Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl Environ Microbiol 61:2798–2801

Felske A, Wolterink A, Van Lis R, De Vos WM, Akkermans ADL (2000) Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. Appl Environ Microbiol 66:3998–4003

Fernández A, Huang S, Seston S, Xing J, Hickey R, Criddle C, Tiedje J (1999) How stable is stable? Function versus community composition. Appl Environ Microbiol 65:3697–3704

Finstein MS, Morris ML (1975) Microbiology of municipal solid waste composting. Adv Appl Microbiol 19:113–151

Focht DD, Chang AC (1975) Nitrification and denitrification processes related to waste water treatment. Adv Appl Microbiol 19:153–186

Fracchia L, Dohrmann AB, Martinotti MG, Tebbe CC (2006) Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. Appl Microbiol Biotechnol 71:942–952

Francou C, Poitrenaud M, Houot S (2005) Stabilization of organic matter during composting: influence of process and feedstocks. Compost Sci Util 13:72–83

Franke-Whittle IH, Klammer SH, Insam H (2005) Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. J Microbiol Methods 62:37–56

Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H (2009a) Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microb Ecol 57:510–521

Franke-Whittle IH, Goberna M, Pfister V, Insam H (2009b) Design and development of the ANAEROCHIP microarray for investigation of methanogenic communities. J Microbiol Methods 79:279–288

Friedrich M, Springer N, Ludwig W, Schink B (1996) Phylogenetic positions of *Desulfofustis glycolicus* gen. nov., sp. nov., and *Syntrophobotulus glycolicus* gen. nov., sp. nov., two new strict anaerobes growing with glycolic acid. Int J Syst Bacteriol 46:1065–1069

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Gallert C, Winter J (2005) Bacterial metabolism in wastewater treatment systems. In: Jördening HJ, Winter J (eds) Environmental biotechnology. Concepts and applications. Wiley-VCH Verlag Gmbh & Co KGaA, Weinheim, pp 1–48

García JL, Ollivier B, Whitman WB (2006) The order Methanomicrobiales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 3. Springer, New York, pp 208–230

Goberna M, Franke-Whittle IH, Insam H (2008) Fingerprinting the microbial communities in organic wastes using oligonucleotide microarrays and real-time PCR. In: Abstract volume of the II international meeting of soil enzimology. Recycling of organic wastes in environmental restoration and global change. Burgos, Spain, p 36

Goberna M, Insam H, Franke-Whittle IH (2009) Effect of biowaste sludge maturation on the diversity of thermophilic bacteria and archaea in an anaerobic reactor. Appl Environ Microbiol 75:2566–2572

Godon JJ, Zumstein E, Dabert P, Habouzit F, Moletta R (1997) Microbial 16S rDNA diversity in an anaerobic digester. Water Sci Technol 36:49–55

Green SJ, Michel FC Jr, Hadar Y, Minz D (2004) Similarity of bacterial communities in sawdust- and straw-amended cow manure composts. FEMS Microbiol Lett 233:115–123

Griffin DM (1985) A comparison of the roles of bacteria and fungi. In: Leadbetter ER, Poindexter JS (eds) Bacteria in nature, vol 1. Plenum Publishing, London, pp 221–255

Guschin DY, Mobarry BK, Proudnikov D, Stahl DA, Rittmann BE, Mirzabekov AD (1997) Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. Appl Environ Microbiol 63:2397–2402

Harmsen HJM, Wullings B, Akkermans ADL, Ludwig W, Stams AJM (1993) Phylogenetic analysis of *Syntrophobacter wolinii* reveals a relationship with sulphate-reducing bacteria. Arch Microbiol 160:238–240

Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2007) Diversity of anaerobic microorganisms involved in long-chain fatty acid degradation in methanogenic sludges as revealed by RNA-based stable isotope probing. Appl Environ Microbiol 73:4119–4127

Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2008) Detection of active butyrate-degrading microorganisms in methanogenic sludges by RNA-based stable isotope probing. Appl Environ Microbiol 74:3610–3614

Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. Int J Syst Evol Microbiol 50:1601–1609

He PJ, Lu F, Shao LM, Zhang H (2009) Characterization of methanogenesis using stable isotopic probing. Prog Chem 21:540–549

Hirsch P, Overrein L, Alexander M (1961) Formation of nitrite and nitrate by actinobacteria and fungi. J Bacteriol 82:442–448

Hora TS, Iyengar MRS (1960) Nitrification by soil fungi. Arch Mikrobiol 35:252–257
Hori T, Haruta S, Ueno Y, Ishii M, Igarashi Y (2006) Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. Appl Environ Microbiol 72:1623–1630
Huang LN, Chen YQ, Zhou H, Luo S, Lan CY, Qu LH (2003) Characterization of methanogenic archaea in the leachate of a closed municipal solid waste landfill. FEMS Microbiol Ecol 46: 171–177
Hultman J, Kurola J, Raininsalo A, Kontro M, Romantschuk M (2010) Utility of molecular tools for optimization of large scale composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 135–152
Ianotti EL, Fischer JR, Sievers DM (1982) Characterization of bacteria from a swine manure digester. Appl Environ Microbiol 43:136–143
Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidising bacteria in compost-treated soils. Soil Biol Biochem 38:1092–1100
Insam H, de Bertoldi M (2007) Microbiology of the composting process. In: Diaz LF, de Bertoldi M, Bidlingmaier W, Stentiford E (eds) Compost science and technology. Elsevier, Amsterdam, pp 25–48
Insam H, Wett B (2008) Control of GHG emission at the microbial community level. Waste Manage 28:699–706
Jäckel U, Thummes K, Kämpfer P (2005) Thermophilic methane production and oxidation in compost. FEMS Microbiol Ecol 52:175–184
Juste A, Thomma BPHJ, Lievens B (2008) Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiol 25:745–761
Karakashev D, Batstone DJ, Angelidaki I (2005) Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Appl Environ Microbiol 71:331–338
Kendall MM, Boone DR (2006) The order Methanosarcinales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 3. Springer-Verlag, New York, USA, pp 244–256
Klocke M, Nettmann E, Bergmann I, Mundt K, Souidi K, Mumme J, Linke B (2008) Characterization of the methanogenic archaea within two-phase biogas reactor systems operated with plant biomass. Syst Appl Microbiol 31:190–205
Knapp B, Ros M, Insam H (2010) Do composts affect the soil microbial community? In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 271–292
Krause DO, Denman SE, Mackie RI, Morrison M, Rae AL, Attwood GT, McSweeney CS (2003) Opportunities to improve fibre degradation in the rumen: microbiology, ecology, and genomics. FEMS Microbiol Rev 27:663–693
Krause L, Diaz NN, Edwards RA, Gartemann K-H, Krömeke H, Neuweger H, Pnhler A, Runte KJ, Schlnter A, Stoye J, Szczepanowski R, Tauch A, Goesmann A (2008) Taxonomic composition and gene content of a methane-producing microbial community isolated from a biogas reactor: genome research in the light of ultrafast sequencing technologies. J Biotechnol 136:91–101
Laloui-Carpentier W, Li T, Vigneron V, Mazéas L, Bouchez T (2006) Methanogenic diversity and activity in municipal solid waste landfill leachates. Anton Leeuw Int J G 89:423–434
LaPara TM, Nakatsu CH, Pantea LM, Alleman JE (2002) Stability of the bacterial communities supported by a seven-stage biological process treating pharmaceutical wastewater as revealed by PCR-DGGE. Water Res 36:638–646
Leclerc M, Delgènes JP, Godon JJ (2004) Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. Environ Microbiol 6:809–819
Levén L, Eriksson ARB, Schnürer A (2007) Effect of process temperature on bacterial and archaeal communities in two methanogenic bioreactors treating organic household waste. FEMS Microbiol Ecol 59:683–693

Liesack W, Stackebrandt E (1992) Unculturable microbes detected by molecular sequences and probes. Biodivers Conserv 1:250–262

Liu WT, Marsh TL, Cheung H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Liu WT, Chan CO, Fang HH (2002) Microbial community dynamics during start-up of acidogenic anaerobic reactors. Water Res 36:3203–3210

Loehr RC (1974) Agricultural waste management. Academic, New York, USA

Lott Fischer J (1998) Avoidance of biorisks of composting by thermohygienization: influence of the type of system and management on the occurrence of the potentially pathogenic mold *Aspergillus fumigatus* and fecal indicator bacteria. Thesis, Université de Neuchâtel, Switzerland

Loy A, Bodrossy L (2006) Highly parallel microbial diagnostics using oligonucleotide microarrays. Clin Chim Acta 363:106–119

Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer K-H, Wagner M (2002) Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microb 68:5064–5081

Lueders T, Friedrich MW (2003) Evaluation of PCR amplification bias by terminal restriction fragment length polymorphism analysis of small-subunit rRNA and mcrA genes by using defined template mixtures of methanogenic pure cultures and soil DNA extracts. Appl Environ Microbiol 69:320–326

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:507–577

Macario AJL, Conway de Macario E (1988) Quantitative inmunologic analysis of the methanogenic microflora of digestors reveals considerable diversity. Appl Environ Microbiol 54:79–86

Macedo AJ, Timmis KN, Wolf-Rainer A (2007) Widespread capacity to metabolize polychlorinated biphenyls by diverse microbial communities in soils with no significant exposure to PCB contamination. Environ Microbiol 9:1890–1897

Madsen EL (2006) The use of stable isotope probing techniques in bioreactor and field studies on bioremediation. Curr Opin Biotechnol 17:92–97

Malin C, Illmer P (2008) Ability of DNA content and DGGE analysis to reflect the performance condition of an anaerobic biowaste fermenter. Microbiol Res 163:503–511

Manz W, Amann R, Ludwig W, Wagner M, Schleifer KH (1992) Phylogenetic oligonucleotide probes for the major subclasses of Proteobacteria: problems and solutions. Syst Appl Microbiol 15:593–600

Marshall KC, Alexander M (1962) Nitrification by *Aspergillus flavus*. J Bacteriol 83:572–578

McHugh S, Carton M, Mahony T, O'Flaherty V (2003) Methanogenic population structure in a variety of anaerobic bioreactors. FEMS Microbiol Lett 219:297–304

McMahon KD, Stroot PG, Mackie RI, Raskin L (2001) Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions – II: microbial population dynamics. Water Res 35:1817–1827

McMahon KD, Zheng D, Stams AJM, Mackie RI, Raskin L (2004) Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. Biotechnol Bioeng 87:823–834

Michel FC Jr, Marsh TJ, Reddy CA (2002) Bacterial community structure during yard trimmings composting. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Heidelberg, pp 25–42

Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252

Mladenovska Z, Dabrowski S, Ahring B (2003) Anaerobic digestion of manure and mixture of manure with lipids: biogas reactor performance and microbial community analysis. Water Sci Technol 48:271–278

Monis PT, Giglio S (2006) Nucleic acid amplification-based techniques for pathogen detection and identification. Infect Genet Evol 6:2–12

Montero B, Morales JL, Sales D, Solera R (2008) Evolution of microorganisms in thermophilic-dry anaerobic digestion. Bioresource Technol 99:3233–3243

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technol 96:673–686

Mountfort DO, Brulla WJ, Krumholz LR, Bryant MP (1984) *Syntrophus buswellii* gen. nov. sp. nov.: a benzoate catabolizer from methanogenic ecosystems. Int J Syst Bacteriol 34:216–217

Moyer CL, Dobbs FC, Karl DM (1994) Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. Appl Environ Microbiol 60:871–879

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441–454

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of Polymerase Chain Reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nakashimada Y, Srinivasan K, Murakami M, Nishio N (2000) Direct conversion of cellulose to methane by anaerobic fungus *Neocallimastix frontalis* and defined methanogens. Biotechnol Lett 22:223–227

Nishida T, Fujimura T, Omasa T, Katakura Y, Suga K-I, Shioya S (2003) Changes in microflora duding composting of an aquatic plant, Brazilian elodea. J Chem Eng Jpn 36:1201–1205

Nocker A, Burr M, Camper A (2007) Genotypic microbial community profiling: a critical technical review. Microb Ecol 54:276–289

Ntougias S, Zervakis GI, Kavroulakis N, Ehaliotis C, Papadopoulou KK (2004) Bacterial diversity in spent mushroom compost assessed by amplified rDNA restriction analysis and sequencing of cultivated isolates. Syst Appl Microbiol 27:746–754

O'Flaherty V, Collins G, Mahony T (2006) The microbiology and biochemistry of anaerobic bioreactors with relevance to domestic sewage treatment. Rev Environ Sci Biotechnol 5:39–55

Ortega-Charleston L (2008) Hyperthermophilic anaerobic digestion of food waste. PhD Thesis, McGill University, Montreal

Pedro MS, Haruta S, Hazaka M, Shimada R, Yoshida C, Hiura K, Ishii M, Igarashi Y (2001) Denaturing gradient gel electrophoresis analyses of microbial community from field-scale composter. J Biosci Bioeng 91:159–165

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. Soil Biol Biochem 38:460–470

Plugge CM, van Lier JB, Stams AJM (2010) Syntrophic communities in methane formation from high strength wastewaters. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, New York, pp 59–78

Polz MF, Cavanaugh CM (1998) Bias in template-to-product ratios in multitemplate PCR. Appl Environ Microbiol 64:3724–3730

Qiu Y-L, Hanada S, Ohashi A, Harada H, Kamagata Y, Sekiguchi Y (2008) S*yntrophorhabdus aromaticivorans* gen. nov., sp. nov., the first cultured anaerobe capable of degrading phenol to acetate in obligate syntrophic associations with a hydrogenotrophic methanogen. Appl Environ Microbiol 74:2051–2058

Ramsay IR, Pullammanappallil PC (2001) Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation 12:247–257

Raskin L, Poulsen LK, Noguera DR, Rittmann B, Stahl DA (1994a) Quantification of methanogenic groups in anaerobic biological reactors by oligonucleotide probe hybridization. Appl Environ Microbiol 60:1241–1248

Raskin L, Stromley JM, Rittmann B, Stahl DA (1994b) Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. Appl Environ Microbiol 60: 1232–1240

Ren Z, Ward TE, Logan BE, Regan JM (2007) Characterization of the cellulolytic and hydrogen-producing activities of six mesophilic *Clostridium* species. J Appl Microbiol 103:2258–2266

Rincón B, Raposo F, Borja R, González JM, Portillo MC, Saiz-Jiménez C (2006) Performance and microbial communities of a continuous stirred tank anaerobic reactor treating two-phases olive mill solid wastes at low organic loading rates. J Biotechnol 121:534–543

Roest K, Altinbas M, Paulo PL, Heilig HGHJ, Akkermans ADL, Smidt H, de Vos WM, Stams AJM (2005a) Enrichment and detection of microorganisms involved in direct and indirect methanogenesis from methanol in an anaerobic thermophilic bioreactor. Microb Ecol 50: 440–446

Roest K, Heilig HGHJ, Smidt H, deVos WM, Stams AJM, Akkermans ADL (2005b) Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. Syst Appl Microbiol 28:175–185

Roller C, Wagner M, Amann R, Ludwig W, Schleifer K-H (1994) *In situ* probing of Gram-positive bacteria with high DNA G + C content using 23S rRNA-targeted oligonucleotides. Microbiology 140:2849–2858

Ros M, Klammer S, Knapp B, Aichberger K, Insam H (2006) Long term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use Manag 22:209–218

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349–410

Sahlström L (2003) A review of survival of pathogenic bacteria in organic waste used in biogas plants. Bioresource Technol 87:161–166

Sahlström L, Aspan A, Bagge E, Danielsson-Tham ML, Albihn A (2004) Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. Water Res 38:1989–1994

Sarada R, Joseph R (1994) Characterization and enumeration of microorganisms associated with anaerobic digestion of tomato-processing waste. Bioresource Technol 49:261–265

Sawayama S, Tsukahara K, Yagishita T (2006) Phylogenetic description of immobilized methanogenic community using real-time PCR in a fixed-bed anaerobic digester. Bioresource Technol 97:69–76

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280

Schink B, Stams AJM (2006) Syntrophism among prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 2. Springer-Verlag, New York, USA, pp 309–335

Schloss PD, Hay AG, Wilson DB, Walker LP (2003) Tracking temporal changes of bacterial community fingerprints during the initial stages of composting. FEMS Microbiol Ecol 46:1–9

Schlüter A, Bekel T, Diaz NN, Dondrup M, Eichenlaub R, Gartemann K-H, Krahn I, Krause L, Krömeke H, Kruse O, Mussgnug JH, Neuweger H, Niehaus K, Pnhler A, Runte KJ, Szczepanowski R, Tauch A, Tilker A, Viehöver P, Goesmann A (2008) The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology: genome research in the light of ultrafast sequencing technologies. J Biotechnol 136:77–90

Schmitz RA, Daniel R, Deppenmeier U, Gottschalk G (2006) The anaerobic way of life. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 2. Springer-Verlag, New York, USA, pp 86–101

Schnürer A, Schink B, Svensson BH (1996) *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. Int J Syst Bacteriol 46:1145–1152

Schwarz WH (2001) The cellulosome and cellulose degradation by anaerobic bacteria. Appl Microbiol Biotechnol 56:634–649

Scully C, Collins G, O'Flaherty V (2005) Assessment of anaerobic wastewater treatment failure using terminal restriction fragment length polymorphism analysis. J Appl Microbiol 99: 1463–1471

Sekiguchi Y (2006) Yet-to-be cultured microorganisms relevant to methane fermentation process. Microbes Environ 21:1–15

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (2000) *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. Int J Syst Evol Microbiol 50:771–779

Sessitsch A, Gyamfi S, Stralis-Pavese N, Weilharter A, Pfeifer U (2002) RNA isolation from soil for bacterial community and functional analysis: evaluation of different extraction and soil conservation protocols. J Microbiol Methods 51:171–179

Sharma VK, Hagen JC (1995) Isolation and characterization of *Clostridium hobsonii* comb. nov. Bioresource Technol 51:61–74

Shigematsu T, Tang Y, Kawaguchi H, Ninomiya K, Kijima J, Kobayashi T, Morimura S, Kida K (2003) Effect of dilution rate on structure of a mesophilic acetate-degrading methanogenic community during continuous cultivation. J Biosci Bioeng 96:547–558

Small J, Call DR, Brockman FJ, Straub TM, Chandler DP (2001) Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. Appl Environ Microbiol 67:4708–4716

Smith KS, Ingram-Smith C (2007) *Methanosaeta*, the forgotten methanogen? Trends Microbiol 15:150–155

Smith A, Hubbard H, Huber D (2004) Archaeal diversity of a thermophilic methanogenic pilot scale digester treating poultry farm waste. In: Proceedings of the 10th Anaerobic Digestion Conference, Montréal, Canada, 1574–1577

Sokolova TG, Kostrikina NA, Chernyh NA, Tourova TP, Kolganova TV, Bonch-Osmolovskaya EA (2002) *Carboxydocella thermautotrophica* gen. nov., sp. nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring. Int J Syst Evol Microbiol 52: 1961–1967

Sokolova TG, González JM, Kostrikina NA, Chernyh NA, Slepova TV, Bonch-Osmolovskaya EA, Robb FT (2004) *Thermosinus carboxydivorans* gen. nov., sp. nov., a new anaerobic, thermophilic, carbon-monoxideoxidizing, hydrogenogenic bacterium from a hot pool of Yellowstone National Park. Int J Syst Evol Microbiol 54:2353–2359

Sousa DZ (2006) Ecology and physiology of anaerobic microbial communities that degrade long chain fatty acids. PhD Thesis, Universidade do Minho

Sousa DZ, Pereira MA, Smidt H, Stams AJM, Alves MM (2007) Molecular assessment of complex microbial communities degrading long-chain fatty acids in methanogenic bioreactors. FEMS Microbiol Ecol 60:252–265

Stackebrandt E, Rainey FA, Ward-Rainey NL (1997) Proposal for a new heirarchic classification system, Actinobacteria classis nov. Int J Syst Bacteriol 47:479–491

Stams AJM, Elferink SJWHO, Westermann P (2003) Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. In: Sheper T (ed) Advances in biochemical engineering/biotechnology. Springer-Verlag, Berlin, pp 31–56

Stams AJM, de Bok FAM, Plugge CM, van Eekert JD, Schraa G (2006) Exocellular electron transfer in anaerobic microbial communities. Environ Microbiol 8:371–382

Strom PF (1985) Effect of temperature on bacterial diversity in thermophilic solid-waste composting. Appl Environ Microbiol 50:899–905

Suzuki M, Giovannoni S (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl Environ Microbiol 62:625–630

Talbot G, Roy C, Topp E, Palin MF, Massé D (2004) Monitoring microbial diversity and function in swine manure anaerobic digesters by length heterogeneity PCR (LH-PCR). In: Anaerobic Digestion 10th World Congress Proceedings, Aug 29th–Sept 2nd, Montreal, vol 3, pp 1594–1598

Talbot G, Topp E, Palin MF, Masse DI (2008) Evaluation of molecular methods used for establishing the interactions and functions of microorganisms in anaerobic bioreactors. Water Res 42:513–537

Tang Y, Shigematsu T, Ikbal MS, Kida K (2004) The effects of micro-aeration on the phylogenic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester. Water Res 38:2537–2550

Tang Y, Shigematsu T, Morimura S, Kida K (2005) Microbial community analysis of mesophilic anaerobic protein degradation process using bovine serum albumin (BSA)-fed continuous cultivation. J Biosci Bioeng 99:150–164

Tarlera S, Muxí L, Soubes M, Stams AJM (1997) *Calaromator proteoclasticus* sp. nov., a new moderately thermophilic anareobic proteolytic bacterium. Int J Syst Bacteriol 47:651–656

Thummes K, Kämpfer P, Jäckel U (2007a) Temporal change of composition and potential activity of the thermophilic archaeal community during the composting of organic material. Syst Appl Microbiol 30:418–429

Thummes K, Schäfer J, Kämpfer P, Jäckel U (2007b) Thermophilic methanogenic archaea in compost material: occurrence, persistence and possible mechanisms for their distribution to other environments. Syst Appl Microbiol 30:634–643

Tiquia SM (2005) Microbial community dynamics in manure composts based on 16S and 18S rDNA T-RFLP profiles. Environ Technol 26:1101–1113

Torsvik VL, Goksoyr J, Daae FL (1990) High diversity in DNA of soil bacteria. Appl Environ Microbiol 56:782–787

Ueno Y, Haruta S, Ishii M, Igarashi Y (2001) Changes in product formation and bacterial community by dilution rate on carbohydrate fermentation by methanogenic microflora in continuous flow stirred tank reactor. Appl Microbiol Biot 57:65–73

US-EPA (Environmental Protection Agency) (2006) Anaerobic digestion: benefits for waste management, agriculture, energy and environment. Available at: http://www.dcmnr.gov.ie/NR/rdonlyres/287C17F6-13D2-48B9-882C-2060512A573E/0/EPAappendix.pdf. Accessed 26 July 2006

Vinnerås B, Schönning C, Nordin A (2006) Identification of the microbiological community in biogas systems and evaluation of microbial risks from gas usage. Sci Total Environ 367: 606–615

Vinnerås B, Agostini F, Jönsson H (2010) Sanitation by composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 171–192

Vogels GD, Van der Drift C (1976) Degradation of purines and pyrimidines by microorganisms. Bacteriol Rev 40:403–468

Wagner R (1994) The regulation of ribosomal RNA synthesis and bacterial cell growth. Arch Microbiol 161:100–109

Waksman SA (1932) Principles of soil microbiology. Williams & Wilkins, Baltimore, MD

Wang LF, Rakela J, Laskus T (1997) Head-to-tail primer tandem repeats generated in hemi-nested PCR. Mol Cell Probes 11:385–387

Ward DM, Bateson MM, Weller R, Ruff-Roberts AL (1992) Ribosomal RNA analysis of microorganisms as they occur in nature. Adv Microb Ecol 12:219–286

Ward AJ, Hobbs PJ, Holliman PJ, Jones DL (2008) Optimisation of the anaerobic digestion of agricultural resources. Bioresource Technol 99:7928–7940

Wéry N, Lhoutellier C, Ducray F, Delgenès JP, Godon JJ (2008) Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. Water Res 42:53–62

Wett B, Insam H (2010) Biogas technology: Controlled gas flow for enhanced mixing, heating, and desulfurization. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 79–92

Whitehead TR, Cotta MA (1999) Phylogenetic diversity of methanogenic archaea in swine waste storage pits. FEMS Microbiol Lett 179:223–226

Whitman W, Jeanthon C (2006) Methanococcales. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 3. Springer-Verlag, New York, USA, pp 257–273

Wilson DB (2008) Three microbial strategies for plant cell wall degradation. Ann New York Acad Sci 1125:289–297

Yamada T, Miyauchi K, Ueda H, Ueda Y, Sugawara H, Nakai Y, Endo G (2007) Composting cattle dung wastes by using a hyperthermophilic pre-treatment process: characterization by physicochemical and molecular biological analysis. J Biosci Bioeng 104:408–415

Yazdani SS, Gonzalez R (2007) Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry: energy biotechnology/environmental biotechnology. Curr Opin Biotechnol 18:213–219

Yu Y, Kim J, Hwang S (2006) Use of real-time PCR for group-specific quantification of aceticlastic methanogens in anaerobic processes: population dynamics and community structures. Biotechnol Bioeng 93:424–433

Zhang B, He P-J, Lü F, Shao L-M, Wang P (2007) Extracellular enzyme activities during regulated hydrolysis of high-solid organic wastes. Water Res 41:4468–4478

Zhao HW, Viraraghavan T (2004) Analysis of the performance of an anaerobic digestion system at the Regina wastewater treatment plant. Bioresource Technol 95:301–307

Zheng D, Raskin L (2000) Quantification of *Methanosaeta* species in anaerobic bioreactors using genus- and species-specific hybridization probes. Microb Ecol 39:246–262

Zinder SH (1993) Physiological ecology of methanogens. In: Ferrry JG (ed) Methanogenesis: ecology, physiology, biochemistry and genetics. Springer-Verlag, New York, pp 128–206

Zinder SH, Koch M (1984) Non-acetoclastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. Arch Microbiol 138:263–272

Zinder SH, Cardwell SC, Anguish T, Lee M, Koch M (1984) Methanogenesis in a thermophilic anaerobic digestor: *Methanothrix* sp. as an important aceticlastic methanogen. Appl Environ Microbiol 47:796–807

Zumstein E, Moletta R, Godon JJ (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. Environ Microbiol 2:69–78

Chapter 2
Recent Developments in Bio-Energy Recovery Through Fermentation

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Abstract Bio-energy recovery through fermentation is gaining importance because of limited fossil resources. Especially, bio-ethanol and biogas production are applied worldwide and have reached industrial scale. Other options such as bio-hydrogen, microbial fuel cells, and higher alcohol and acid fermentations are still in development. Current fields of intense research are the utilization of lignocellulose substrates, intensification of the recovery of wastes and industrial byproducts for bio-energy recovery, optimization of process control (especially, in anaerobic digestion), and the optimal utilization scenario of byproducts from microbial bio-energy processes (e.g., stillage, digestate). For lignocellulose utilization, the optimal pretreatment technologies (heat, acid, enzyme, steam explosion, mechanical treatment) are being investigated. For methane production, benchmarks for fermentation parameters are presented, and current bottlenecks and deficiencies of the technology are discussed.

As biomass is not only needed for energy conversion, but also for other purposes (food production, animal feed, raw material for industry), the conversion efficiency from sunlight via plants to biomass and biofuels has to be enhanced. For this reason, research also focuses on increasing yields, improving conversion technologies, usage of the entire plant biomass, and improving the entire energy conversion system. Mass and energy balances, net energy balances, and combined heat and power usage are also considered.

Current research tries to evaluate eco balances, greenhouse gas emissions, and the sustainability of the entire production process, in order to improve the entire

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conversion systems. In summary, microbial bio-energy conversion systems will be of great importance as part of a future energy mix, until alternative energy sources like direct conversion of sunlight (e.g., photovoltaics), nuclear technology (e.g., fusion), or other future energy sources can replace it.

Contents

2.1 Introduction

Due to rising prices for fossil fuels and rising energy demand, bio-energy generation concepts have gained increasing interest in recent years. Scenarios anticipating future developments (Schmid 2008) show that the usage of biomass for energy purposes will not be sufficient to provide the world's entire energy demand. Nevertheless, it will be of great importance for the next decades, until direct conversion systems for energy from sunlight are more advanced.

There are two main ways for the recovery of energy from biomass, i.e., thermal/chemical- or microbial conversion systems. This chapter covers only the bio-energy recovery through microbial fermentation. The main focus lies on biogas and bio-ethanol production processes. These technologies have been realized in many industrial applications around the world. Other concepts like bio-hydrogen production, microbial fuel cells or further alcohol and fatty acid fermentations will not be discussed in detail.

One of the main advantages of using microorganisms is that high temperature (or high thermal energy) is not needed for the conversion process. Nevertheless,

downstream processing can require high energy demand (e.g., distillation of bio-ethanol, purification of biogas to bio-methane). Additionally, the high water content of substrates (organic waste, wet biomass) makes costly effluent treatment or recovery (slops, digestate) necessary.

2.2 Bio-Ethanol Fermentation

Currently, bio-ethanol production is of great importance among microbial bio-energy transformations, providing alternative energy in the transport sector (biofuels). Industrial bio-ethanol fermentation is done by pure cultures. The fermentation is typically carried out with selected yeast strains (e.g., *Saccharomyces cerevisiae*, *S. uvarum*, *Schizosaccharomyces pombe*, *Kluyveromyces* sp.). Numerous efforts have been reported in strain improvement, aimed at improving the strain stability, pH-, ethanol-, osmo- and temperature tolerance, productivity and suppression of the respiratory chain in yeasts (Hutter and Oliver 1998; Shi et al. 1999). A stable fermentation pattern would allow prolonged continuous process cycles. Higher osmo and ethanol tolerance allows higher stillage recycling rates and a less energy consuming distillation. Higher ethanol productivity allows smaller fermentation vessels and better pH tolerance would lead to less susceptibility to infections. Finding new *S. cerevisiae* mutants with higher ethanol yield is still an issue in research (Mobini-Dehkordi et al. 2008). The alternative use of bacteria (e.g. *Zymomonas mobilis*, *Clostridium thermosaccharolyticum*, *Thermoanaerobacter ethanolicus)* for ethanol fermentation has been investigated intensively (Kosaric et al. 2001). Thermophilic bacterial fermentations would allow less energy consuming distillations. Many bacteria can also ferment pentose sugars. However, due to several reasons, i.e., a need for anaerobic and sterile fermentation conditions, accumulation of undesirable byproducts, bad centrifugability, so far no full-scale bacterial ethanol fermentation has been established. Yeast is still the only organism currently used for large-scale ethanol production. Selected yeasts have proven to be best suited in terms of stability, robustness against contaminations, and ease of separation. Most of the yeast strains applied are proprietary of plant suppliers, some selected strains are provided from culture collections. Yeast fermentations are carried out both as continuous and batch fermentations, although often the batch process is preferred due to less probability of contamination. Practical bio-ethanol fermentation plants are huge, and an optimal sized plant produces about 200,000–300,000 tons of ethanol per year (Gallagher and Brubaker 2005).

Broadening of the substrate spectrum is among the major future challenges for strain improvement for bio-ethanol fermentation (Table 2.1). In Brazil, bio-ethanol production focuses on the direct conversion from sugar (sugar cane), whereas in the United States and Europe mainly starch-containing substrates (e.g., corn, wheat, triticale) are used. In the latter, starch is broken down into sugars via amylase treatment. Future developments are now focusing on the utilization of lignocellulosic biomass as substrates (Delgenes et al. 1996; Nigam 2001; Dien et al. 2003; Mosier et al. 2005; Linde et al. 2008; Panagiotopoulos and Bakker 2008). The use

Table 2.1 Overview on typical examples of microorganisms in bio-energy recovery concepts and important issues in research

Organisms	Research issues
Bio-ethanol *(pure culture)*	
Yeasts: *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, *Schizosaccharomyces pombe*, *Kluyveromyces* sp. Bacteria: *Clostridium* sp., *Zymomonas mobilis*, *Thermoanaerobacter ethanolicus*	• Pentose sugar utilization • Strain improvement (pH, ethanol, osmo, temperature tolerance) • Suppression of the respiratory chain • Cellulose and lignocellulose utilization (pre-treatment technologies) • Cell recycle • High substrate utilization • Process integration • Stillage treatment
Biogas *(mixed culture)*	
Hydrolysis and Acidogenesis: *Bacillus* sp., *Bacteroides* sp., *Clostridium* sp., *Pseudomonas* sp. Acetogenesis: *Micrococcus* sp., *Acetobacterium woodii*, *Peptostreptococcus* sp., *Clostridium aceticum* Methanogenesis: *Methanobacterium spp*., *Methanococcus* sp., *Methanosarcina barkeri*, *Methanosaeta* sp., *Methanospirillum* sp., *Methanobacterium* sp.	• Application of pure cultures • Treatment of nitrogen-rich substrates • Fast start-up phases for treating industrial byproducts processed in campaigns • Process stability • Substrate pre-treatment technologies • Digestate treatment • Nutrient recovery from digestate
Bio-hydrogen *(pure/mixed culture)*	
Dark fermentation: strict anaerobes (e.g., *Clostridia*), thermophiles (e.g., *Pyrococcus*), facultative anaerobes (e.g., *Enterobacter*), rumen bacteria (e.g. *Ruminococcus*), aerobic bacteria (e.g., *Bacillus*) Photofermentation: purple bacteria (e.g., *Rhodobacter sphaeroides*), green algae (e.g., *Chlamydomonas reinhardtii)*	• Higher H_2-yields • Means against H_2 product inhibition • Hydrogen storage • Hydrogen distribution infrastructure
Microbial fuel cell *(mixed culture)*	
Pseudomonas aeruginosa, *Alcaligenes faecalis*, *Enterococcus gallinarum*, Proteobacteria, Firmicutes, *Planctomyces* sp.	• Higher currents • Better electrodes • Increased durability
Higher alcohol and acid fermentations *(pure culture)*	
e.g., *Clostridium acetobutylicum*	• Utilization as biofuels • Raw materials for industry

of cellulose containing raw materials would allow a major increase of renewable substrate availability, without diminishing the availability of food plants. Cellulose-containing substrates demand a more sophisticated pre-treatment to break down cellulose and hemicellulose to sugars. Enzymatic, thermal, and acid treatments are applied. Particularly, simultaneous enzymatic cellulose degradation is being intensively investigated (Pejó 2008). Unfortunately, to date the use of commercial

cellulases (e.g. from *Trichoderma reesei*) is quite expensive. Research focuses on reducing enzyme cost and increasing enzyme activity to allow economically viable, large-scale enzyme applications (Kovács et al. 2009). Also, new strains are investigated in order to detect promising alternatives, e.g. *T. atroviride* mutants (Kovács et al. 2008, 2009). In addition, thermal pre-treatment (Kovács et al. 2008, 2009; Matsumura et al. 2008) found great interest in recent research projects. Different types of heat treatment and steam explosion (Martín et al. 2002; Oliva et al. 2008) are being investigated. Mild acid treatment can be an additional conditioning step for the mentioned pre-treatment types (Panagiotopoulos and Bakker 2008). In the last few years, many processes to raise the ethanol yield and to lower the energy demand have been developed. Alkaline, acid, solvent treatment, or steam explosion offer the best opportunity to disintegrate biomass according to Cardona and Sánchez (2007). Pan and Arato (2005) describe the so-called "Lignol" process for processing softwood into ethanol and byproducts. This process uses the organosolv process for obtaining fermentable products and high quality lignin. A mixture of ethanol and water is applied as solvent at about 200°C and 30 bar. Apart from the comparatively high energy need and high costs for such pre-treatment processes, the toxicity of byproducts which occur in the pre-treatment step must be considered (Delgenes et al. 1996). Also, the simulation of the described pre-treatment processes is of importance in the latest research projects (Tsoutsos et al. 2007).

The use of genetically modified organisms (GMO) in bio-ethanol production is still restricted to laboratory scale investigations (Martín et al. 2002; Dien et al. 2003).

From a practical point of view, proper substrate selection, efficient ethanol recovery, and environmentally friendly byproduct management (stillage, CO_2) are considered essential. Main byproduct of ethanol fermentation is the residual stillage after distillation – "Distillers' dried grains with solubles." Up to now, stillage is mainly used for animal feeding, demanding a high energy consuming drying step. Due to the huge volumes and high energy demand, research currently is focused on energy recovery from stillage. It is estimated that between 75% (Pfeffer et al. 2007) and 100% (Friedl et al. 2005) of the overall process heat demand could be covered through biogas from anaerobic digestion of stillage. Anaerobic treatment of stillage and other byproducts from ethanol production has been investigated for a long time (Braun and Huss 1982). Recently, a biogas yield of 400–500 m^3 per t volatile solids (VS), at hydraulic retention times (HRT) of 20–39 days was reported for stillage from distillery. Two-stage fermentation of stillage gives an opportunity for a more stable process for the methanogenesis (Blonskaja et al. 2003).

2.3 Methane Fermentation

Different from bio-ethanol fermentation, the formation of bio-methane (Anaerobic digestion) is induced by a multi-strain mixed bacterial culture (Reith et al. 2003), usually obtained by the natural enrichment from the respective substrates applied. Depending on the substrate applied and on the fermentation conditions, constitution

and number of participating bacteria can vary considerably. According to the degradation step, different bacteria are considered (Table 2.1; see Chap. 1, Insam et al. 2010; Chap.3, Plugge et al. 2010). In the hydrolysis, the break-down phase of proteins, carbohydrates and lipids to monomers, the bacteria *Bacillus* sp., *Bacteroides* sp., *Clostridium* sp., *Pseudomonas* sp. and *Micrococcus* sp. are of greater importance. To separate the hydrolysis step as a pre-treatment step in anaerobic digestion is an important issue both in research and in constructing new biogas plants (Park et al. 2005). The same microorganism population responsible for hydrolysis also carries out the further degradation of monomers to acids in acidogenesis. In the consecutive degradation to acetate (acetogenesis), the bacteria *Acetobacterium woodii*, *Peptostreptococcus* sp., and *Clostridium aceticum* are usually involved. Whereas other species like *Methanobacterium spp*., *Methanococcus* sp., *Methanosarcina barkeri*, *Methanosaeta* sp., *Methanospirillum*, and *Methanobacterium* sp. are responsible for the last step, the methane formation (methanogenesis). The described processes take place simultaneously, provided all participating microorganisms stay in a well-balanced equilibrium.

Although there has been much research on interrelationships and growth conditions in pure cultures, little information is available on the more complex mixed culture methane fermentation or on other bio-energy fermentations carried out by mixed cultures (Kleerebezem and van Loosdrecht 2007). So far steering of the mixed population is only possible through proper control of environmental and fermentation conditions. Most important environmental parameters are temperature, pH, alkalinity, and redox conditions. Important fermentation conditions are substrate concentration, nutrient ratio (C:N:P), substrate volatile solids (VS) loading rate (kg VS m^{-3} per day), hydraulic residence time (d), as well as metabolite (volatile fatty acids, VFA), and byproduct formation (NH_4^+, H_2S). These parameters have to be properly controlled during the start-up of fermentation.

For the start-up of methane fermentation, usually no artificial inoculum materials have to be added. Normally autochthonous bacteria from the substrates applied are slowly adapted to the fermentation conditions, using gradually increasing substrate loading rates. The start-up procedure can take several weeks to months. Once established properly, mixed bacterial cultures in anaerobic digestion can stay stable for long periods of time. To accelerate the start-up process, inoculum material from operational fermentations can be used as seed sludge. Own investigations showed that commercially available inoculum materials or catalysts normally do not show a noticeable enhancement compared to naturally derived seed cultures. Future challenges for the inoculation of methane fermentations can lie in fast start-up processes. Thus, organic industrial byproducts which are processed in campaigns, like in the sugar and starch industry, can be dealt with more easily.

Since the microorganisms involved in anaerobic digestion can feed on bigger substrate spectra than ethanol fermenting yeasts, pre-treatment has not gained the same importance as in bio-ethanol production, but is still an important issue when treating energy crops. Special pre-treatment for the use of nitrogen rich or fiber-containing substrates is also being investigated. A pre-treatment of the substrates is expected to achieve shorter retention times and higher gas yields in anaerobic

digestion. Palmowski and Müller (2000) describe the influence of the size reduction of organic waste on the digestion efficiency. Smaller particle size lowers the retention time and increases the biogas yield in fibrous agricultural substrates. Various thermal, mechanical, and biochemical (enzymatic) disintegration processes have been developed (Palmowski and Müller 2000; Mao and Show 2007). Thermal disintegration processes claim an increase in biogas yields between 10 and 30% (Schiedner et al. 2000). Ultrasonic treatment also increases the digestibility through a size reduction of the organic waste particulate matter (Palmowski and Müller 2000). Ultrasonic treatment is occasionally used for sewage sludge disintegration (Mao and Show 2007).

2.4 Other Fermentation Products

Various algae and bacterial fermentations of pure and mixed cultures have been demonstrated as being capable of bio-hydrogen formation in fundamental research (Reith et al. 2003). To date none of these experiments has reached practical significance or technical scale bio-hydrogen production. While algae are cultivated in pure cultures, dark fermentation (a part of the anaerobic digestion) takes place in mixed cultures (Table 2.1). Main problems are low hydrogen concentrations, product inhibition, separation, and storage of hydrogen. The main practical problem is the missing infrastructure for hydrogen distribution.

Electric power can even be produced directly through fermentation in microbial fuel cells (MFC). In a MFC, the degradation process of the organic substrates takes place directly at an electrode which is used as an electron acceptor. By this means electricity can be produced directly, without an intermediate energy carrier (e.g., methane or hydrogen). However, only very low voltage can be achieved. So far, an industrial application seems difficult (Scholz and Schröder 2003; Schröder et al. 2003; Siegl 2006).

The fermentation of higher alcohols (e.g., butanol, butanediol) and fatty acids can also be considered part of bio-energy recovery concepts. During the Second World War, the Aceton-Butanol-Ethanol (ABE) fermentation was of great importance. Currently, this process has no industrial relevance. However, for example, butanol is interesting as an energy carrier and could, in principle, be used as fuel for cars.

2.5 Optimum Fermentation Conditions, Bottlenecks, and Process Control in Microbial Methane Production

2.5.1 Deficiencies in Methane Fermentation

Successful control of bio-ethanol fermentation with yeasts has been proved in numerous full-scale applications. Control of methane fermentations proves to be much more complicated. Due to the complex mixed culture fermentation and

substrate spectrum, further research and development is required to remove various bottlenecks in the process chain. Practical experience shows that process failures in anaerobic digestion applications may occur for several reasons: Microbiological limitations, affecting reflexively the microbial community (e.g., ammonia inhibition, trace element insufficiency etc.), and also technical weakness of the equipment, like insufficient mixing caused by inappropriate particle size, or high viscosity caused by insufficient hydrolysis. For example, after replacing half of the maize silage substrate of a biogas plant by grass and clover, the recirculation rate of the separated liquid fraction of the digestate had to be doubled. In other cases, increased viscosity caused failure of the mixing device (Resch et al. 2008). In conclusion, it may be stated that in anaerobic digestion of organic waste possible ammonia inhibition can decelerate methane production. Methane formation is, therefore, the rate limiting step in waste fermentation. In energy crop digestion, however, the rate limiting step is the hydrolysis of cellulose.

Hydrolytic, acetogenic and methanogenic prokaryotes differ considerably in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions (Pohland and Ghosh 1971; Chen et al. 2008). Imbalance between acid- and methane-forming microorganisms is frequently the primary cause of reactor instability (Speece 1996; Demirel and Yenigün 2002). Additionally, inhibitory substances are often found to be the cause of anaerobic reactor disturbance, since various contaminants are present in substantial concentrations in wastewater – and sludge – substrates. Furthermore, metabolites, such as VFA, NH_3, or H_2S frequently disturb methane fermentations. Due to the high variation of substrates applied in methane fermentations, several other environmental impacts can affect methane formation. Especially, substrates with high volatile solid (VS) contents (e.g., energy crops) can cause an upset through sudden temperature increase (Daverio et al. 2003; Lindorfer et al. 2006; Braun 2007). Even a lack in trace element supply may occur with some substrates (Hummer 2006) used in methane fermentations (e.g., exhaust vapor condensate, energy crops). Unbalanced substrate mixtures may occur occasionally in fermentations using industrial bio-wastes (e.g., slaughter house waste, pulp and paper wastes) or energy crops (e.g., maize). If the C:N ratio cannot be kept in the range of 10:1 to 30:1 (Eder and Schulz 2006) either a lack or a surplus of nitrogen can cause major disturbance of methane formation (Kirchmayr et al. 2007). Inhibition is usually indicated by a decrease of the steady state rate of methane gas production and hence, an accumulation of volatile organic acids (Kroeker et al. 1979). The concentration of low molecular free VFA (carboxylic acids C_2-C_5) as intermediate metabolites in anaerobic digestion is considered a benchmark for the equilibrium between hydrolytic microorganisms and methane-forming microorganisms (Ahring et al. 1995).

2.5.2 Ammonia Inhibition

Ammonium ions (NH_4^+) and free ammonia (NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous solution. Several mechanisms explaining ammonia inhibition have been proposed, i.e., a change in the intracellular pH,

increase of maintenance energy requirement, and inhibition of specific enzyme reactions (Whittmann et al. 1995). NH_3 is widely considered to be the main cause of inhibition, since it is easily membrane-permeable (Kroeker et al. 1979; de Baere et al. 1984). The hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency (Gallert et al. 1998; Sprott and Patel 1986). Among the four types of microorganisms involved in anaerobic digestion, the methanogens are the least tolerant and therefore, the most likely to cease growth due to ammonia inhibition. Different sensitivity to ammonia shock loadings and better tolerance to ammonia after adaptation (van Velsen et al. 1979; Hashimoto 1986; Koster and Lettinga 1984; Hansen et al. 1998) could be explained by a change in metabolic pathways as proposed by Schnürer and Nordberg (2008). The results from that study strongly suggest that the observed adaptation to higher ammonia levels corresponds to a shift in the methane-producing population.

Practical experience with full-scale bio-waste and slaughter house waste digesters show ammonium concentrations exceeding 5,000 mg L^{-1} NH_4-N which are still operated reliably (Kirchmayr et al. 2007; Resch et al. 2007b). Average HRTs in such digesters are between 50 and 100 days. Nevertheless, implementing an intermediate ammonia removal and recovery step (Resch et al. 2007b) clearly showed an increase of the biogas yield by 35% and a better reactor performance (doubling of the COD removal rate) of slaughter house waste digestion. In energy crop fermentation, ammonium concentrations were mostly negligible (Resch et al. 2007a; Gabauer et al. 2008; Lindorfer et al. 2008a). Using the formula described by Hashimoto (1986) to calculate the level of free ammonia, it is clear that the shift in this study occurred at levels above 128–330 mg L^{-1} NH_3. These values are in the same range as previously reported to be inhibitory for methanogenesis in un-adapted processes (Braun et al. 1981; de Baere et al. 1984). Resch et al. (2006) and Kirchmayr et al. (2007) reported stable operation of biogas plants up to an ammonia level of 1,000 mg L^{-1} NH_3-N which has been indicated as a critical value by Hansen et al. (1998).

2.5.3 Trace Element Supply

On some occasions, disorders in methane fermentations have been attributed to a lack in trace element supply. Better performance of anaerobic digestion of catering waste or energy crops has been described after the addition of trace elements (Preissler et al. 2007; Climenhaga and Banks 2008). There is also information available about trace metal dynamics in anaerobic granular sludge bed reactors and their influence on reactor performance (Zandvoort et al. 2006).

The key factor influencing the trace element requirement in specified reactor systems is the bioavailability of the trace elements supplied through substrate and/or supplement. Despite chelate-forming conditions (Kuo and Parkin 1996), the sulfide concentration will influence the bioavailability of trace elements, as metals will precipitate as insoluble metal sulfides (Gerardi 2003). Long-chain fatty acids can also bind with minerals such as calcium (Pereira et al. 2001).

Co-fermentation, i.e., addition of substrates rich in minerals, such as liquid manure, organic wastes or grass varieties, can help overcome lack in trace elements. Although there is little information available on actual trace element need and on positive effects in biogas fermentation, numerous commercial additives are available, usually claiming improved fermentation performance in practice.

2.5.4 Fermentation Temperature

Numerous investigations show advantages and disadvantages of thermophilic versus mesophilic methane fermentations (van Lier et al. 1993, 1996; Speece 1996; Gallert and Winter 1997; Gallert et al. 1998; Angelidaki et al. 2004; Kim et al. 2006; Zábranská et al. 2000). In most studies a specific temperature optimum was defined, either in the mesophilic range between 35 and 40°C or in the thermophilic range between 50 and 65°C (Gerardi 2003; Bischofsberger et al. 2004). For this reason most practical biogas fermentations operate either at about 35 or 55°C (Lindorfer et al. 2008b). In contrast, there are not many data available on the process temperatures between 40 and 50°C. However, deviation from the mesophilic temperature range proved to be problematic in several cases of full-scale energy crop fermentation plants (Lindorfer et al. 2008b). The exothermic carbohydrate degradation and the high energy density in the substrates, together with high loading rates, can cause a sudden temperature increase. Such self-heating effects led to an increase in process temperatures from 35–39°C to 42–49°C (Daverio et al. 2003; Lindorfer et al. 2006; Braun 2007). This effect was accompanied by a gradual cease in methane formation. This phenomenon was observed in 20 of 41 full-scale biogas plants investigated, which subsequently had to be operated at increased temperatures between 40 and 50°C (Laaber et al. 2005; Braun 2007).

The self-induced temperature increase in mesophilic digesters to sub-thermophilic levels (40–50°C) was shown to cause severe disorders of the microbial population (Lindorfer et al. 2006). In laboratory-scale experiments, complete failure in methane production for several days occurred after a sudden temperature increase (Bolzonella et al. 2003). The only way to prevent a sudden temperature increase is to change the feedstock, to reduce the organic loading rate or to install a fermenter cooling system. However, in the early planning phase of an energy crop digestion plant a thorough investigation of the optimum fermentation temperature is highly recommended.

2.5.5 Retention Time

Recent investigations have shown that considerably high HRTs of 144 days (mean values) are required in energy crop fermentations and 84 days in co-fermentation of bio-wastes (Laaber et al. 2007). Under such conditions, the degree of volatile solid

degradation can be above 90% and the final biogas yield between 0.5 and 0.8 Nm^3 kg^{-1} VS_{added} (50–60% CH_4). Because of the extended HRT, the resulting biogas productivity is just between 0.5 and 2 m^3 m^{-3} fermenter volume per day.

Apart from the substrates, the operation temperature can also be responsible for variations of the fermentation parameters. Especially, the HRT depends on mesophilic or thermophilic process control. Thermophilic temperature conditions and cofermentation lead to a shorter HRT (Schöftner et al. 2007). However, a change in substrate composition to higher proportions of fibrous and cellulosic substrates, leads to an increase in recirculation demand of diluting process liquid, which lowers the HRT and generates more solid digestion residues (Resch et al. 2008). These solid residues provide the largest source of residual methane potential (expressed as nonconverted volatile solids). Consequently, its minimization is considered the main goal (Resch et al. 2008).

So far, predominantly conventional one- and two-step continuous stirred tank reactors are applied, especially in agricultural applications, using plant byproducts, slurries, or energy crops. More sophisticated bio-reactors are occasionally applied in municipal bio-waste treatment and anaerobic industrial waste treatment (Speece 1996; Reith et al. 2003). Reactor systems such as UASB (Upflow Anaerobic Sludge Bed) reactors or anaerobic filters are based on immobilization, that is, retention of bacterial biomass within the bioreactor.

Another strategy to realize shorter retention times and high productivities in fermenters is the pretreatment of substrates as described above.

2.6 Benchmarks in Fermenter Performance

Provided that proper reaction conditions can be kept stable, the anaerobic digestion process can be performed without significant occurrence of volatile fatty acid metabolites or pH changes. According to a benchmark analysis of energy crop digesters, recommended process parameters are listed in Table 2.2. Regular methane fermentations are characterized by a volatile fatty acid concentration (as acetic acid) of 0–1.5 g L^{-1}. Nevertheless, intermediate fatty acid levels (VFA) in methane fermentations can differ widely depending on the substrate or nutrient mixture applied. While in energy crop digesters the VFA levels are found comparably low (0–1.5 g L^{-1}), in biogas plants operated with organic wastes and slaughter

Table 2.2 Recommended fermentation conditions for energy crop digesters (Laaber et al. 2007)

	Regions of values		
	Stable process	Tendency to instability	Unstable process
pH	7.5–8.1	7.1–7.5	<7.1; >8.1
Volatile fatty acids (g L^{-1})	<1.5	1.5–4.5	>4.5
NH_4-N (g L^{-1})	<5	>5	–
Total solids (% w/w)	4–8	<4; 8–9	>9
Volatile solids (% w/w)	3–6	<3; 6–7	>7

house wastes VFA levels up to 19 g L^{-1} have been reported (Kirchmayr et al. 2007). A pH range between 7.5 and 8.1 is frequently found to be stable. Ammonium levels should be below 5 g L^{-1}. Typical total solid (TS) content is between 4 and 9% and that of volatile solids (VS) between 3 and 7%.

In most cases, an adverse influence on the fermentation pattern is caused by sudden changes in substrate composition, concentration, quality, and feeding rate. Care has to be taken for a well-balanced ratio of total nutrients to be provided by the substrate. Bacterial communities producing biogas demand a ratio of carbon to nitrogen to phosphate between 75:5:1 and 125:5:1 (Eder and Schulz 2006). Practical experience (Laaber et al. 2007) as well as extended simulations and experimental studies indicate that initial concentrations of carbohydrates, proteins, and fat affect the performance of methane fermentations significantly (Angelidaki et al. 1993; Biswas et al. 2006). The methane concentration in biogas decreases with increasing concentrations of carbohydrates and protein, while fat concentration has the opposite effect (Biswas et al. 2006).

2.7 Monitoring and Control of Fermentations

It is of vital importance not only to control the environmental conditions e.g., temperature, pH, alkalinity or redox potential, but also to monitor fermentation conditions and to apply real-time analysis. Online measurement of alkali consumption and biogas production allows the detection of toxicity incidents and substrate over- or underloading. Anaerobic bio-sensors are available to monitor overload and toxicity by measuring biogas yields (Rozzi et al. 1997; Thévenot et al. 2001; Nakamura and Karube 2003).

According to Scherer (2008a), calculating the carbon balance of input and output of a biogas plant can help in evaluating the process stability. This can be achieved by following the carbon path by measuring the gas production (carbon dioxide, methane), determining carbon content of solid digestion residues and gas chromatography analysis of the metabolites, VFA and alcohols in liquid digestion residues.

An imminent plant failure can also be indicated if the ratio of VFA and buffering capacity decreases below 0.5 (Speece 1996; Gerardi 2003). Normally, the alkalinity should lie between 6,000 and 8,000 mg L^{-1} of $CaCO_3$ equivalent (Scherer 2008b).

An optional analytical parameter is the digital image analysis to estimate the ratio of methanogenic archaea in the mixed culture as well as bacterial hydrolytic activity. The methanogenic fraction represents $\geq$5% of the population (Scherer 2008b).

However, simple online methods are not always able to predict process imbalances. Offline analyses, e.g., high-performance liquid chromatography (HPLC), gas chromatography (GC), and flow injection analysis are time consuming. To obtain better control, process analytical technologies may be invoked. Thus, a wide array of process analytical methods is available (Bakeev 2005). Examples are GC determination of volatile components (Boe 2006; Diamantis et al. 2006) or

spectroscopy coupled to a flow system containing enzymes for the determination of byproducts, e.g., glycerol dehydrogenase (Fernandes et al. 2004). Also, near infrared spectroscopy (i.e., transflexive embedded near infrared sensor - TENIRS) can be applied. Such near infrared technology can be used for monitoring acetic, propionic, iso-butanoic, butanoic, iso-valeric and valeric acid, ammonium, and total VFA (Holm-Nielsen et al. 2007). The ratio between acetic acid and propionic acid in the process can provide valuable information as an early warning before a process failure occurs (Hill and Holmberg 1988; Boe 2006).

Under anaerobic conditions, H_2 is preferably used for the reduction of NO_3^- and SO_4^{2-}. Depending on the substrate used, a hydrogen sulfide (H_2S) content in the biogas from 500 to 20,000 ppm (2% v/v) can result (Woodcock and Gottlieb 2004). Because of its inhibitory, corrosive, and generally harmful properties, it has to be removed from the produced biogas. Simultaneous biological oxidation using controlled aeration or external bio-filtration is frequently applied as an alternative to conventional chemical methods (Devinny 1999; Gabriel and Deshussen 2003). Redondo et al. (2008) introduced an automated online H_2S analyzer applicable to the monitoring of methane fermentations. A single-channel flow injection analyzer detects S^- in the liquid phase and is coupled with a continuous flow analyzer including a gaseous diffusion step for detecting H_2S in the gas phase.

2.8 Sustainability of Microbial Energy Transformations

2.8.1 Process Energy Demand and Greenhouse Gas Emission

Important criteria for evaluating the sustainability are the ratio of renewable energy output to fossil energy input and the emission of greenhouse gases (GHG).

The most important issue is the amount of primary energy needed in the energy conversion process. In the first process step of plant production, primary energy input is required for ploughing, sowing, fertilizer and pesticide production, harvest and transport. Further primary energy input may be required for the pretreatment and processing of the plant material, fermentation, recovery and purification of products and byproducts. In bio-ethanol production, for example, the recovery of ethanol is a highly energy intensive step where a high percentage of the overall primary energy is spent. A standard biogas process in which biogas is used in a combined heat and power plant (CHP) requires less process energy. However, if biogas is to be upgraded to bio-methane again more primary energy will be required. Apart from the amount of primary energy required, the source of the process energy is important for the sustainability. In the bio-ethanol production process, an appreciable amount of the energy required could be provided by renewable energy, e.g., anaerobic digestion of stillage (Drosg et al. 2008b) or the use of plant byproducts (e.g., straw, bagasse).

GHG emissions occur during the whole process chain, i.e., cultivation and harvest of the biomass, during transportation, conversion processes, and through the use of products. Emissions vary considerably according to the raw materials used for fermentation. If energy crops are used for example (e.g., maize, sugar beets), N_2O-emissions from fertilization have a large negative impact on a life cycle analysis (LCA). Ecologically sound plant selection and land use are, therefore, of primary importance and consequently "Good Ecological Practise" for bio-energy projects is requested (Firbank 2008; Fritsche 2008).

Further emissions occur (e.g., NH_3), if organic waste is used as a substrate in anaerobic digestion. Incomplete substrate degradation during fermentation can often cause additional GHG emissions (e.g., CH_4) from residual substrate storage and use. Incomplete combustion of methane in CHPs can contribute to GHG emissions during product use.

2.8.2 Impacts of Byproducts on the Sustainability of Bio-Energies

Byproducts can be responsible for some of the emissions and an additional primary energy demand. In small-scale decentralized agricultural biogas plants, the digestate can be easily applied as fertilizer on nearby agricultural areas. However, the bigger the plant, the higher is the cost of storage, transportation, and application of digestate. In addition, there are often application limits for nitrogen and other nutrients. Especially, byproducts from large-scale anaerobic digestion of industrial wastes can cause considerable problems (Drosg et al. 2008b). Since most of such substrates are from supra-regional origin, the digestate has to be transported considerable distances for application.

Different technologies are applied for digestate treatment or upgrading. Usually the solid phase is separated from the liquid via screw press or decanters. The solid digestion residue subsequently can often be directly applied as fertilizer in agriculture. Increasingly, residual solids have to be composted or otherwise treated for intermediate storage and portability. For improving solid-liquid separation, flocculation or precipitation agents are commonly applied. For nutrient recovery, membrane technology, e.g., ultrafiltration and reverse osmosis, are used (Diltz et al. 2007; Fakhru'l-Razi 1994). Membrane filtration produces a nutrient concentrate and purified process water (Castelblanque and Salimbeni 1999; Klink et al. 2007). The liquid digestate can also be purified through aerobic biological wastewater treatment (Camarero et al. 1996). However, because of the high nitrogen content and low biological oxygen demand (BOD), an addition of an external carbon source may be necessary to achieve appropriate denitrification (Drosg et al. 2008a). A further possibility for concentrating digestate is evaporation and/or drying of the concentrate. For reducing the nitrogen content in the digestate, stripping (Siegrist et al. 2005), ion exchange (Sánchez et al. 1995) or struvite precipitation (Uludag-Demirer et al. 2005; Marti et al. 2008) have been proposed. Whatever the process applied, advanced digestate treatment in most cases requires high chemical and energy

inputs. Together with increased investment costs for appropriate machinery, considerable treatment costs may result. Therefore, it has to be thoroughly investigated whether the resulting overall economics allow the realization of huge centralized installations.

2.8.3 Mass and Energy Balances

For evaluating the different bio-energy recovery concepts, emphasis has to be laid on the mass and energy balance. Figure 2.1 shows the energy efficiency of a two-stage biogas plant. Manure, energy crops, and organic residues are used as substrates. In this plant about 86% of the volatile solid input is converted into biogas. The energy balance shows that in the case of such a plant a large amount of energy (51%) remains unused in the form of heat. Consequently, a combined heat and electricity utilization has to be the aim for achieving better energy efficiency in biogas plants.

The yield of plant biomass per hectare is another important contributor to the energy balance. Poor hectare yields adversely influence the energy balance. Furthermore, it makes a difference whether the entire plant is used (e.g., whole crop silage), or only parts from the plants are used (e.g., grains).

Uellendahl et al. (2008) showed that energy balances and cost-benefit analyses favor perennial crops for energy production in biogas plants. The required energy input for the cultivation of e.g., Miscanthus or Willow is significantly lower than in the case of annual crops (Heller et al. 2008; Møller et al. 2008), which are predominantly cultivated in most European countries. Due to longer growing seasons, perennials result in higher biomass yields of dry matter per hectare (Uellendahl et al. 2008). Thus, the annual solar energy conversion efficiency of perennial crops is

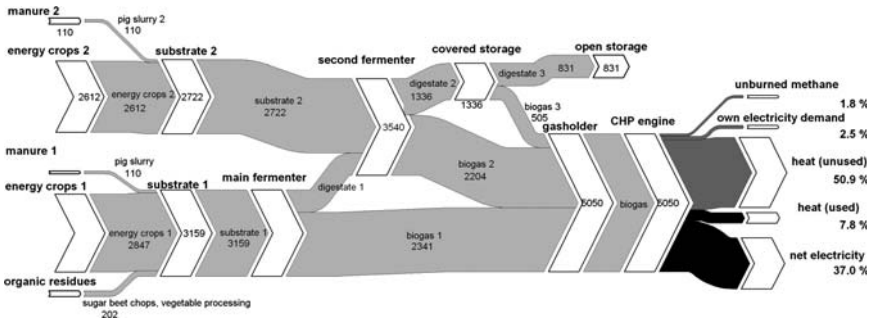

Fig. 2.1 Overall energy balance of a two-step energy crop co-digestion plant (all units are given in t volatile solids (VS) per year). A net electrical efficiency of 37% and a typical high proportion of unused heat (51%) can be seen. Just 7.8% of the heat produced can be used. Methane loss in the combined heat and power plant is 1.8%. Own process electricity demand is 2.5%

Table 2.3 Overview of energy efficiency of different microbial energy transformation systems

Substrate	Fermentation process	Energy output/input	Reference
Corn	Biogas fermentation (no pre-treatment)	7.2–9.5	[1]
Corn	Biogas fermentation (with wet oxidation pre-treatment)	6.8–8.9	[1]
Miscanthus	Biogas fermentation (no pre-treatment)	6.9–7.8	[1]
Miscanthus	Biogas fermentation (with wet oxidation pre-treatment)	11.6–13.1	[1]
Willow	Biogas fermentation (no pre-treatment)	7.3	[1]
Willow	Biogas fermentation (with wet oxidation pre-treatment)	12.3	[1]
Corn	Bio-ethanol fermentation	1.4	[2]
Corn + stover	Bio-ethanol fermentation	2.33	[2]
Alfalfa – corn (plant rotation)	Bio-ethanol fermentation	2.87–3.05	[2]
Switchgrass	Bio-ethanol fermentation	10.76–11.31	[2]
Sugar beets	Bio-ethanol fermentation	1.77	[3]
Winter cereals	Bio-ethanol fermentation	2.53–2.76	[4]

[1] Uellendahl et al. (2008), [2] Vadas et al. (2008), [3] Schäfer et al. (1997), [4] Rosenberger et al. (2001)

often higher than that of annual plants. For biogas production, the net energy yield per hectare can be increased by a properly balanced co-fermentation. Uellendahl et al. (2008) report output/input ratios ranging from 6.9 to 9.5 for corn, Miscanthus and Willow fermentation to biogas without pre-treatment (Table 2.3). However, since perennial crops show high lignocellulose concentrations, their hydrolytic pre-treatment is frequently being proposed. Akali or acid treatment, pre-digestion, ensiling, thermochemical, or ultrasonic treatment have been proposed (Yadvika et al. 2004). With wet oxidation pre-treatment, the output/input ratio can be increased up to 13.1 for biogas fermentation (Uellendahl et al. 2008).

Considering energy balances in bio-ethanol production, switchgrass results in a high energy output/input ratio ranging from 10.8 to 11.3 (Vadas et al. 2008). In comparison annual crops, e.g., corn (including straw), reach a maximum energy output/input ratio of 2.2 (without straw stover it decreases to 1.4). Alfalfa–corn rotation systems show an input/output ratio ranging from 2.9 to 3.1 (Vadas et al. 2008).

For ethanol production from lignocellulosic biomass, e.g., rye straw, oilseed rape straw, wet oxidation (Petersson et al. 2007), or steam explosion (Galbe and Zacchi 2004) were proposed for pre-treatment. For bio-ethanol, the resulting energy output/input ratios vary considerably depending on the substrates applied. Based on theoretical calculations, Hofmann (1999) reported an energy output/input ratio of 1.3. Other calculations for bio-ethanol from sugar beets (Schäfer et al. 1997) found a maximum output/input ratio of 1.8 (Table 2.3). A respective net energy gain of 77% corresponding to about 1,200 L ethanol ha^{-1} was reported. Rosenberger et al. (2001) calculated an output/input ratio ranging from 2.5 to 2.8 (depending on the process conditions) for winter cereals, i.e., triticale and winter rye.

2.8.4 Potential of Microbial Energy Transformations

Unless the direct use of solar energy or other alternative energy sources (e.g., nuclear fusion) becomes predominant, for the next decades microbial energy transformation of renewable biomass will be of increasing importance. Biomass competitively serves for a multitude of purposes, i.e., food, feed, industrial use, energetic use. Its yield and availability depends on numerous regional conditions. Different varieties of plants have to be carefully selected for different processes and uses to avoid competitive use or environmental drawbacks like water shortage, plant diseases, or greenhouse gas emissions. Biomass can have special importance in energy provision because it is storable. So imbalances between energy demand and energy provision through unsteady renewable energy sources like wind or solar energy can be adjusted.

The arable land area for the production of biomass is restricted (Hedegaard et al. 2008). Campbell et al. (2008) estimate the potential for sustainable biomass production in agriculture to be 385–472 mio ha. For this study only abandoned agricultural lands were considered, to avoid deforestation or threatening food security. Considering this area for biomass production up to 8% of the current worldwide primary energy demand could be provided by biomass. In addition, there may be a large future potential for algae production in the oceans (Orr and Sarmiento 1992).

Nevertheless, the competition between biomass for food production and for non-food purposes is increasingly discussed (Bole and Londo 2008; Cockerill and Martin 2008). Furthermore, the preservation of biodiversity is endangered by increasing monocultures (Perry et al. 2008). As a consequence, the potential of organic wastes and byproducts for energy recovery needs to be better exploited. Although an increasing alternative energy demand is pushing bio-energies, the first priority has to be food production, followed by animal feed production. Remaining land areas can be used for the production of raw materials for industry and finally as sources of renewable energy.

In transportation, there is still a big potential for bio-ethanol and biogas (or bio-hydrogen) as biofuels, since in this sector the GHG reduction is still very difficult to achieve while the fuel demand is increasing. Biogas upgraded to bio-methane shows a high potential as a biofuel because of the high hectare yields and the good energy output/input relation. Nevertheless, lacking bio-methane infrastructure and methane driven vehicles are still a big hindrance. However, the overall conversion efficiency for biofuels is still very poor as shown in Table 2.4. When using bio-methane from maize directly as biofuel in a vehicle, only 9% of the chemical energy of the whole plant is used. For biodiesel from rapeseed oil this is even worse with 6% useful energy. In Fischer Tropsch-Diesel, a second generation biofuel, the percentage of useful energy is still only 10%. According to the data from Table 2.4, electrical power generation from bio-methane in a combined cycle power plant would raise the conversion efficiency almost threefold from 9 to 24%. This can be a future scenario for biomass in the transport sector. Biomass is first converted to

Table 2.4 Comparative overall energy conversion efficiencies (after Sterner and Schmid 2008)

Energy conversion process	Useful energy at wheel as percentage of 100% primary energy/chemical energy of the whole plant
Rapeseed oil (Biodiesel)	6%
Rapeseed oil (CHP)	9%
Willow (FT-Diesel)	10%
Willow (Biomass gasification – use in combined cycle power plant)	35%
Maize (Bio-methane as biofuel)	9%
Maize (Bio-methane – use in combined cycle power plant)	24%
Wind, Hydro, solar (electric engine)	approx. 77%

electricity and then used in vehicles. However, electricity produced directly from wind or solar energy has an even higher conversion efficiency of up to 77%. All this indicates that future developments will tend to a direct use of electric power in vehicles. Nevertheless, bio-methane and bio-ethanol can be stored more easily than electricity which is a large benefit. Also, infrastructure and research for electric mobility applications is still insufficient. Bio-methane and bio-ethanol will stay, or will even become a more important factor considering GHG emission reduction and renewable energy provision for mobility in the medium term. Apart from that, microbial energy transformation from biomass will still be used for electric power generation.

References

Ahring BK, Sandberg M, Angelidaki I (1995) Volatile fatty acids as indicators of process imbalance in anaerobic digestors. Appl Microbiol Biotechnol 43(3):559–565

Angelidaki I, Ellegaard L, Ahring BK (1993) A mathematical model for dynamic simulation of an anaerobic digestion of complex substrates: focusing on ammonia inhibition. Biotechnol Bioeng 42(2):159–166

Angelidaki I, Boe K, Ellegaard L (2004) Effect of operating conditions and reactor configuration on efficiency of full scale biogas plants. Water Sci Technol 52(1–2):189–194

Bakeev KA (2005) Process analytical technologies. Blackwell, Oxford, England

Bischofsberger W, Dichtl N, Rosenwinkel KH, Seyfried CF (2004) Anaerobtechnik. Springer, Berlin

Biswas J, Chowdhury R, Bhattacharya P (2006) Kinetic studies of biogas generation using municipal waste as feed stock. Enzyme Microb Technol 38(3–4):493–503

Blonskaja V, Menert A, Vilu R (2003) Use of two-stage anaerobic treatment for distillery waste. Adv Environ Res 7(3):671–678

Boe K (2006) On-line Monitoring and control of the biogas process. Institute of Environment and Resources. PhD Thesis, Technical University of Denmark

Bole T, Londo M (2008) The changing dynamics between biofuels and commodity markets. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June, pp 226–230

Bolzonella D, Battistoni P, Mata-Alvarez J, Cecchi F (2003) Anaerobic digestion of organic solid wastes: process behaviour in transient conditions. Water Sci Technol 48(4):1–8

Braun R (2007) Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development. In: Ranalli P (ed) Improvement of crop plants for industrial end uses. Springer, Dordrecht, Netherlands, pp 335–416

Braun R, Huss S (1982) Anaerobic digestion of distillery effluents. Process Biochem 17(4):25–27

Braun R, Huber P, Meyrath J (1981) Ammonia toxicity in liquid piggery manure digestion. Biotechnol Lett 3:159–164

Camarero L, Diaz JM, Romero F (1996) Final treatments for anaerobically digested piggery slurry effluents. Biomass Bioenerg 11(6):483–489

Campbell JE, Lobell DB, Genova RCFCD (2008) The global potential of bioenergy on abandoned agriculture lands. Environ Sci Technol 42(15):5791–5794

Cardona CA, Sánchez OJ (2007) Fuel ethanol production: process design trends and integration opportunities. Bioresource Technol 98(12):2415–2457

Castelblanque J, Salimbeni F (1999) Application of membrane systems for COD removal and reuse of waste water from anaerobic digestors. Desalination 126(1–3):293–300

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresource Technol 99:4044–4064

Climenhaga MA, Banks CJ (2008) Anaerobic digestion of catering wastes: effect of micronutrients and solids retention time. Water Sci Technol 57(5):687–692

Cockerill S, Martin C (2008) Are biofuels sustainable? The EU perspective. Biotechnol Biofuels 1(9):1–6

Daverio E, Spanjers H, Bassani C, Ligthart J, Nieman H (2003) Calorimetric Investigation of anaerobic digestion. Biotechnol Bioeng 82(5):499–505

de Baere LA, Devocht M, van Assche P, Verstraete W (1984) Influence of high $CaCl_2$ and NH_4Cl salt levels on methanogenic associations. Water Res 18:543–548

Delgenes JP, Moletta R, Navarro JM (1996) Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. Enzyme Microb Technol 19:220–225

Demirel B, Yenigün O (2002) Two-phase anaerobic digestion processes: a review. J Chem Technol Biotechnol 77:743–755

Devinny JS, Deshusses MA, Webster TS (1999) Biofiltration for Air Pollution Control. Lewis Publishers, Boca Raton, 28–34

Diamantis V, Melidis P, Aivasidis A (2006) Continuous determination of volatile products in anaerobic fermenters by on-line capillary gas chromatography. Anal Chim Acta 573–574:189–194

Dien BS, Cotta MA, Jeffries TW (2003) Bacteria engineered for fuel ethanol production: current status. Appl Microbiol Biotechnol 63:258–266

Diltz RA, Marolla TV, Henley MV, Li L (2007) Reverse osmosis processing of organic model compounds and fermentation broths. Bioresource Technol 98(3):686–695

Drosg B, Fuchs W, Wirthensohn T, Konrad G, Hornbachner D, Loderer C, Wäger F, Waltenberger R, Braun R, Kirchmayr R (2008a) Comparing digestate treatment options for the anaerobic digestion of stillage at a large-scale bioethanol plant. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June, pp 2198–2200

Drosg B, Wirthensohn T, Konrad G, Hornbachner D, Resch C, Wäger F, Loderer C, Waltenberger R, Kirchmayr R, Braun R (2008b) Comparing centralised and decentralised anaerobic digestion of stillage from a large-scale bioethanol plant to animal feed production. Water Sci Technol 58(7):1483–1489

Eder B, Schulz H (2006) Biogas Praxis: Grundlagen, Planung, Anlagenbau, Beispiele, Wirtschaftlichkeit (Biogas practise: Basics, planning, plant engineering, examples, economics). Ökobuch, Staufen, Germany

Fakhru'l-Razi A (1994) Ultrafiltration membrane separation for anaerobic wastewater treatment. Water Sci Technol 30(12):321–327

Fernandes EN, Campos Moura MN, Lima JLFC, Reia BF (2004) Automatic flow procedure for the determination of glycerol in wine using enzymatic reaction and spectrophotometry. Microchem J 77:107–112

Firbank L (2008) Assessing the ecological impacts of bioenergy projects. Bioenergy Res 1(1):12–19

Friedl A, Wukovits W, Danzinger G, Pober M, Beckmann G, Hannesschläger M, Prechtl M (2005) Polygeneration Bruck/Leitha. In: Reports from energy and environment research 77/2006, Austrian federal ministry of traffic, innovation and energy, Vienna, Austria

Fritsche U (2008) Green House Gas-Emissions for Future Relevant Biomass conversion pathways. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June

Gabauer W, Voitl G, Waltenberger R, Bock P, Braun R, Kirchmayr R (2008) Microbial Monitoring of all biogas plants in the province of Lower Austria. In: Proceedings of the Fifth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunesia, May 26–28

Gabriel D, Deshussen MA (2003) Retrofitting existing chemical scrubers to biotrickling filters for H_2S emission control. Proc Natl Acad Sci USA 100(11):6308–6312

Galbe M, Zacchi G (2004) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59:618–628

Gallagher PW, Brubaker H (2005) Plant size: capital cost relationships in the mill ethanol industry. Biomass Bioenergy 28(6):565–571

Gallert C, Winter J (1997) Mesophilic and thermophilic anaerobic digestion of source-sorted organic wastes: effect of ammonia on glucose degradation and methane production. Appl Microbiol Biotechnol 48(3):405–410

Gallert C, Bauer S, Winter J (1998) Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population. Appl Microbiol Biotechnol 50 (4):495–501

Gerardi MH (2003) The microbiology of anaerobic digesters. John Wiley, Hoboken, NJ

Hansen KH, Angelidaki I, Ahring BK (1998) Anaerobic digestion of swine manure: inhibition by ammonia. Water Res 32(1):5–12

Hashimoto AG (1986) Ammonia inhibition of methanogenesis from cattle waste. Agric Waste 17:241–261

Hedegaard K, Thyø KA, Wenzel H (2008) Life cycle assessment of an advanced bioethanol technology in the perspective of constrained biomass availability. Environ Sci Technol 42 (21):7992–7999

Heller MC, Keoleian GA, Volk TA (2008) Life cycle assessment of a willow bioenergy cropping system. Biomass Bioenergy 25:147–165

Hill DT, Holmberg RD (1988) Long chain volatile fatty acid relationship in anaerobic digestion of swine waste. Biol Waste 23:195–214

Hofmann M (1999) Schnellwachsende Baumarten – zusammenfassender Abschlußbericht (Fast growing forest species - a summarising final report). Schriftenreihe Nachwachsende Rohstoffe, Landwirtschaftsverlag, Münster, Germany

Holm-Nielsen JB, Lomborg CJ, Oleskowicz-Popiel P, Esbensen KH (2007) On-line near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. Biotechnol Bioeng 99(2):302–313

Hummer A (2006) Nährstoffbedarf methanogener Bakterien und Nährstoffangebot in ausgewählten Nutzpflanzen (Nutrient demand of methanogens and nutrient supply of selected agricultural crops). Diploma Thesis, Institute for Environmental Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna

Hutter A, Oliver SG (1998) Ethanol production using nuclear petite yeast mutants. Appl Microbiol Biotechnol 49(5):511–516

Insam H, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Kim JK, Chun YN, Kim SW (2006) Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. J Biosci Bioeng 102:328–332

Kirchmayr R, Resch C, Mayer M, Prechtl S, Faulstich M, Braun R, Wimmer J (2007) Anaerobic digestion of animal by-products. In: Oreopoulou V, Russ W (eds) Utilization of by-products and treatment of waste in the food industry. Springer, Berlin

Kleerebezem R, van Loosdrecht MC (2007) Mixed culture biotechnology for bioenergy production. Curr Opin Biotechnol 18(3):207–212

Klink G, Salewski C, Bolduan P (2007) Vom Gärrest zum Nährstoffkonzentrat (From digestate to nutrient concentrate). Verfahrenstechnik 10:46–47

Kosaric N, Vardar-Sukan F (2001) Potential Source of Energy and Chemical Products. In: Roehr M (ed) The Biotechnology of Ethanol, Wiley-VCH, Weinheim, 89–220

Koster IW, Lettinga G (1984) The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. Agric Waste 9:205–216

Kovács K, Megyeri L, Szakacs G, Kubicek CP, Galbe M, Zacchi G (2008) *Trichoderma atroviride* mutants with enhanced production of cellulase and b-glucosidase on pretreated willow. Enzyme Microb Technol 43(1):48–55

Kovács K, Szakacs G, Zacchi G (2009) Comparative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of *Trichoderma reesei* and *Trichoderma atroviride*. Bioresource Technol 100:1350–1357

Kroeker EJ, Schulte DD, Sparling AB, Lapp HM (1979) Anaerobic treatment process stability. J Water Pollut Control Fed 51:718–727

Kuo WC, Parkin GF (1996) Characterisation of soluble microbial products from anaerobic treatment by molecular weight distribution and nickel-chelating properties. Water Res 30 (4):915–922

Laaber M, Kirchmayr R, Madlener R, Braun R (2005) Development of an evaluation system for biogas plants. In: Proceedings of the Fourth International Symposium on Anaerobic Digestion of Solid Waste, Copenhagen, Aug 21–Sept 2, pp 631–635

Laaber M, Madlener R, Brachtl E, Kirchmayr R, and Braun R (2007) Aufbau eines Bewertungssystems für Biogasanlagen – "Gütesiegel Biogas" (Establishing an evaluation system for biogas plants). Reports from energy and environmental research 77/2006, Austrian federal ministry of traffic, innovation and energy, Vienna, Austria

Linde M, Galbe M, Zacchi G (2008) Bioethanol production from non-starch carbohydrate residues in process streams from a dry-mill ethanol plant. Bioresource Technol 99(14):6505–6511

Lindorfer H, Braun R, Kirchmayr R (2006) Self-heating of anaerobic digesters using energy crops. Water Sci Technol 53:159–166

Lindorfer H, Corcoba A, Vasilieva V, Braun R, Krichmayr R (2008a) Doubling the organic loading rate in the co-digestion of energy crops and manure – a full scale case study. Bioresource Technol 99(5):1148–1156

Lindorfer H, Waltenberger R, Köllner K, Braun R, Krichmayr R (2008b) New data on temperature optimum and temperature rate changes in energy crop digesters. Bioresource Technol 99:7011–7019

Mao TH, Show KY (2007) Influence of ultrasonication on anaerobic bioconversion of sludge. Water Enviro Res 79(4):436–441

Marti N, Bouzas A, Seco A, Ferrer J (2008) Struvite precipitation assessment in anaerobic digestion processes. Chem Eng J 141(1–3):67–74

Martín C, Galbe M, Wahlborn F, Hahn-Hägerdal B, Jönsson LJ (2002) Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. Enzyme Microb Technol 31:274–282

Matsumura Y, Okahisa D, Petchpradab P, Yoshino K (2008) Optimisation of hydrothermal pretreatment of lignocellulosic materials for 2ND Generation Bioethanol production. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, pp 2090–2092

Mobini-Dehkordi M, Nahvi I, Zarkesh-Esfahani H, Ghaedi K, Tavassoli M, Akada R (2008) Isolation of a novel mutant strain of *Saccharomyces cerevisiae* by an ethyl methane sulfonate-induced mutagenesis approach as a high producer of bioethanol. J Biosci Bioeng 105(4):403–408

Møller HB, Nielsen AM, Murto M, Christensson K, Rintala J, Svensson M, Seppälä M, Paavola T, Angelidaki I, Kaparaju PL (2008) Manure and energy crops for biogas production. Status and barriers. Nordic council of Ministers, MJS - Milijöstrategier för jord- och skogbruk, Denmark

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technol 96:673–686

Nakamura H, Karube I (2003) Current research activity in biosensors. Anal Bioanal Chem 377 (3):446–468

Nigam JN (2001) Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. J Biotechnol 87:17–27

Oliva JM, Manzanares P, Ballesteros I, Ballesteros M, Saéz M, Negro MJ (2008) Different process configurations for bioethanol production from steam-exploded cardoon (*Cynara cardunculus*) biomass. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, pp 2135–2138

Orr JC, Sarmiento JL (1992) Potential of marine macroalgae as sink for CO_2: constraints from a 3-D general circulation model of the global ocean. Water Air Soil Pollut 64(1–2):405–421

Palmowski LM, Müller JA (2000) Influence of the size reduction of organic waste on their anaerobic digestion. Water Sci Technol 41(3):155–162

Pan X, Arato C (2005) Biorefining of softwoods using ethanol organosolv pulping: preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. Biotechnol Bioeng 90:2415–2457

Panagiotopoulos IA, Bakker RR (2008) Comparative study of different lignocellulosic feedstocks enzymatic hydrolysis for fermentable substrates production. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June, pp 1749–1752

Park C, Lee C, Kim S, Chen Y, Chase HA (2005) Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. J Biosci Bioeng 100(2):164–167

Pejó ET (2008) Simultaneous saccharification and fermentation processes for bio-ethanol production by both xylose and glucose-fermenting *Saccharomyces cerevisiae* strain. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June

Pereira A, Mota M, Alves M (2001) Degradation of oleic acid in anaerobic filters: the effect of inoculum acclimatization and biomass recirculation. Water Environ Res 73(5):612–621

Perry M, Bauen A, Rosillo-Calle F, Pearson P (2008) Food production vs. biomass export vs. land-use change in the macroeconomic level. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, pp 183–193

Petersson A, Thomsen MH, Hauggaard-Nielsen H, Thomsen AB (2007) Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. Biomass Bioenerg 31(11–12):812–819

Pfeffer M, Wukovits W, Beckmann G, Friedl A (2007) Analysis and decrease of the energy demand of bioethanol-production by process integration. Appl Therm Eng 27(16):2657–2664

Plugge CM, van Lier JB, Stams AJM (2010) Syntrophic communities in methane formation from high strength wastewaters. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, New York, pp 59–77

Pohland FG, Ghosh S (1971) Developments in anaerobic stabilization of organic wastes – the two phase concept. Environ Lett 1:255–266

Preissler D, Lemmer A, Oechsner H, Jungbluth T (2007) Anaerobic digestion of maize silage without manure addition, VDI Berichte 335–340

Redondo R, Machado VC, Baeza M, Lafuente J, Gabriel D (2008) On-line monitoring of gas-phase bioreactors for biogas treatment. Hydrogen sulfide and sulfide analysis by automated flow systems. Anal Bioanal Chem 391:789–798

Reith JH, Wijffels RH, Barten H (2003) Bio-methane & bio-hydrogen – status and perspectives of biological methane and hydrogen production. Dutch Biological Hydrogen Foundation, The Hague

Resch C, Grasmug M, Smeets W, Braun R, Kirchmayr R (2006) Optimised anaerobic treatment of house-sorted biodegradable waste and slaughterhouse waste in a high loaded half technical scale digester. Water Sci Technol 53(8):213–221

Resch C, Braun R, Krichmayr R (2007a) Mass-flow analyses and energy balance of an anaerobic digestion plant exclusively operating on solid energy crop substrate. In: Proceedings of the 15th European Biomass Conference & Exhibition, Berlin, Germany, 7–11 May, pp 1712–1717

Resch C, Wörl A, Waltenberger R, Braun R, Kirchmayr R (2007b) Changing the C:N-ratio by ammonia recovery for full scale enhance anaerobic treatment of slaughter house waste. In: Proceedings of the Eleventh International Waste Management and Landfill Symposium, Sardinia, 1–5 Oct

Resch C, Braun R, Kirchmayr R (2008) The influence of energy crop substrates on the mass-flow analysis and the residual methane potential at a rural anaerobic digestion plant. Water Sci Technol 57(1):73–81

Rosenberger A, Kaul H-P, Senn T, Aufhammer W (2001) Improving the energy balance of bioethanol production from winter cereals: the effect of crop production intensity. Appl Energy 68(1):51–67

Rozzi A, Tomei MC, Di Pinto AC, Limoni N (1997) Monitoring toxicity in anaerobic digesters by the Rantox biosensor: theoretical background. Biotechnol Bioeng 55(1):33–40

Sánchez E, Milán Z, Borja R, Weiland P, Rodriguez X (1995) Piggery waste treatment by anaerobic digestion and nutrient removal by ionic exchange. Resour Conserv Recy 15 (3–4):235–244

Schäfer V, Aufhammer W, Kübler E, Pieper HJ, Senn T (1997) Energiebilanzen zur Produktion und Verarbeitung des Kornguts von Wintertriticale und Winterweizen zu Bioethanol (Energy balances for production and processing the grains of winter triticale and winter wheat to bioethanol). Pflanzenbauwiss 1(3):133–141

Scherer PA (2008a) Efficiency analysis of biogas plants by following the carbon path. In: Proceedings of the 5th International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunesia, 25–28 May

Scherer PA (2008b) Operating analytics of biogas plants to ensure process stability. In: Proceedings of the 5th International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunesia, 25–28 May

Schiedner D, Schneider R, Bischof F (2000) Thermal hydrolysis (TDH) as a pretreatment method for the digestion of organic waste. Water Sci Technol 41(3):181–187

Schmid J (2008) Integration of biomass into future energy systems. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June

Schnürer A, Nordberg A (2008) Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci Technol 57(5):735–740

Schöftner R, Valentin K, Schmiedinger B, Trogisch S, Haberbauer M, Katzlinger K, Schnitzhofer W, Weran N (2007) Best Biogas Practise. In: Reports from energy and environmental research 45/2006, Austrian federal ministry of traffic, innovation and energy, Vienna, Austria

Scholz F, Schröder U (2003) Bacterial batteries. Nat Biotechnol 21(10):1151–1152

Schröder U, Nießen J, Scholz F (2003) A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angew Chem 115:2986–2989

Shi NQ, Davis B, Sherman F, Cruz J, Jeffries TW (1999) Disruption of the cytochrome c gene in xylose-utilizing yeast *Pichia stipitis* leads to higher ethanol production. Yeast 15(11): 1021–1030

Siegl S (2006) Betrieb und Charakterisierung von mikrobiologischen Brennstoffzellen (Operation and Characterisation of Microbial Fuel Cells). Institute for Applied Microbiology, Diploma Thesis, University of Natural Ressources and Applied Life Sciences, Vienna

Siegrist H, Hunziker W, Hofer H (2005) Anaerobic digestion of slaughterhouse waste with UF-membrane separation and recycling of permeate after free ammonia stripping. Water Sci Technol 52(1–2):531–536

Speece RE (1996) Anaerobic biotechnology for industrial wastewater. Archae Press, Nashville

Sprott GD, Patel GB (1986) Ammonia toxicity in pure cultures of methanogenic bacteria. System Appl Microbiol 7:358–363

Sterner M, Schmid J (2008) Electromobility – an efficient alternative to conventional biofuels to put biomass on the road. In: Proceedings of the 16th European Biomass Conference & Exhibition, Valencia, Spain, 2–6 June, pp 1761–1765

Thévenot DR, Toth K, Durst RA, Wilson GS (2001) Electrochemical biosensors: recommended definitions and classification. Biosens Bioelectron 16(1–2):121–131

Tsoutsos T, Bethanis D, Gekas V (2007) Simulation of fermentable sugar production from lignocellulosics to fuel bioethanol. In: Proceedings of the 15th European Biomass Conference and Exhibition, Berlin, 7–11 May, pp 1751–1760

Uellendahl H, Wang G, Møller H, Jørgensen U, Skiadas IV, Gavala HN, Ahring BK (2008) Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation. In: Proceedings of the Fifth International Symposium on Anaerobic Digestion of Solid Wastes and Energy Crops, Hammamet, Tunesia, 25–28 May

Uludag-Demirer S, Demirer GN, Chen S (2005) Ammonia removal from anaerobically digested dairy manure by struvite precipitation. Process Biochem 40(12):3667–3674

Vadas P, Barnett K, Undersander D (2008) Economics and energy of ethanol production from Alfalfa, Corn, and Switchgrass in the Upper Midwest, USA. Bioenergy Res 1(1):44–55

van Lier JB, Hulsbeek J, Stams AJM, Lettinga G (1993) Temperature susceptibility of thermophilic methanogenic sludge: Implications for reactor start-up. Bioresource Technol 43:227–235

van Lier JB, Martin JLS, Lettinga G (1996) Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. Water Res 30:199–207

van Velsen AFM, Lettinga G, den Ottelander D (1979) Anaerobic digestion of piggery waste: 3. Influence of temperature. Neth J Agric Sci 27:255–267

Whittmann C, Zeng AP, Deckwer WD (1995) Growth inhibition by ammonia and use of pH-controlled feeding strategy for the effective cultivation of *Mycobacterium chlorophenolicum*. Appl Microbiol Biotechnol 44:519–525

Woodcock KE, Gottlieb M (2004) Kirk-Othmer encyclopedia. John Wiley, Hoboken, NJ

Yadvika S, Sreekrishnan TR, Kohli S, Rana V (2004) Enhancement of biogas production from solid substrates using different techniques – a review. Bioresource Technol 95(1):1–10

Zábranská J, Stepová J, Wachtl R, Jenícek P, Dohaányos M (2000) The activity of anaerobic biomass in thermophilic and mesophilic digesters at different loading rates. Water Sci Technol 42:49–56

Zandvoort MH, van Hullebusch ED, Fermoso FG, Lens PNL (2006) Trace metals in anaerobic granular sludge reactors: bioavailability and dosing strategies. Eng Life Sci 6(3):293–301

Chapter 3
Syntrophic Communities in Methane Formation from High Strength Wastewaters

Caroline M. Plugge, Jules B. van Lier, and Alfons J. M. Stams

Abstract High-strength wastewaters from agro-industrial processes (sugar refineries, potato-processing factories, distilleries, paper mills, slaughter houses, etc.) are efficiently (pre-)treated using high rate anaerobic wastewater treatment systems. The organic pollution load can be reduced by 80–90% prior to aerobic polishing. The bio-fuel methane thus produced is generally used for the production of electricity and/or steam, thereby lowering the industrial fossil energy demand. Additional advantages of anaerobic (pre-)treatment are a distinct reduction in excess sludge production and space demand, and low operational costs, mainly owing to low sludge handling costs and low chemical costs. Anaerobic treatment in high rate anaerobic bioreactors is accomplished by mixed syntrophic communities of anaerobic bacteria and methanogenic archaea. Balanced and highly structured microbial communities are essential for high rate anaerobic wastewater treatment. This chapter deals with the syntrophic microbial associations in anaerobic sludge and their importance to high rate wastewater treatment.

Contents

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3.1 Methane

3.1.1 Sources of Methane

Methane (CH_4) is a colorless, odorless gas with a wide distribution in nature. The origin of methane is biological. Methane is produced from natural as well as anthropogenic sources (Table 3.1). It is the principal component of natural gas, a mixture that contains about 70–90% methane and other hydrocarbons (ethane, propane, butane), carbon dioxide (CO_2), oxygen (O_2), nitrogen (N_2), hydrogen sulfide (HS^-), and traces of some rare gases (He, Ne, Xe). Swamp gas, which is produced during the anaerobic microbial decomposition of plant and animal matter, e.g., under water, is also mainly composed of methane. Ruminants and other animals produce and release methane during digestion. Methane is also formed in considerable quantities during the fermentation of organic matter such as manure, wastewater sludge, municipal solid waste (including landfills), or any other biodegradable feedstock. Commonly, methane-containing gas mixtures that are formed

Table 3.1 Estimates of the global methane emissions from different sources

Sources	Methane emission (Tg CH_4 y^{-1})	Percentage (%)
Natural Sources		
Wetlands	92–237	15–40
Termites	20	3
Ocean	10–15	2–3
Hydrates	5–10	1–2
Subtotal	**127–282**	**21–47**
Antropogenic Sources		
Ruminants	80–115	13–19
Energy generation[a]	75–110	13–18
Landfills	35–73	6–12
Rice agriculture	25–100	7–17
Waste treatment	14–25	2–4
Biomass burning	40	4–9
Subtotal	**267–478**	**45–80**
Total	**400–700**	na[b]

[a]Methane deposits released by coal mining, petroleum drilling, and petrochemical production
[b]na: not applicable
Source: Adapted from Lowe (2006)

in biological processes are termed biogas or swamp gas. Methane hydrates – frozen mixtures of methane and water on the ocean floor – are present in massive amounts and are a potential source of methane to be exploited.

3.1.2 The Dual Characteristic of Methane

Methane is a greenhouse gas which, owing to its higher infrared adsorptive capacity, is over 20 times more effective in trapping heat in the atmosphere than carbon dioxide over a 100-year period (US-EPA 2008). However, its lifetime in the atmosphere is relatively short, approximately 9–15 years. On the other hand, methane is also an important energy source. Efforts to reduce methane emissions directly contribute to a reduction in greenhouse gas emissions, thereby they also contribute to energy conservation with considerable economic and environmental benefits. When methane is produced under controlled conditions and subsequently recovered as renewable fuel, it contributes to the sustainable development of our present society. In a world where the demand for fuels is rapidly increasing and fossil fuels are limited, methane can serve as a renewable energy source. With anaerobic digestion, it can be produced economically with simultaneous controlled management of biodegradable materials, such as different types of industrial wastewaters and also in (semi-)tropical areas for domestic wastewaters.

3.2 Composition of High-Strength Wastewater

Most agro-industries are based on the so-called "wet" processing of agricultural harvest products. In these industries, water is used as a transport medium for raw materials, reaction products, and waste components, and also for washing and cleaning, for cooling purposes, as well as for consumptive use. Depending on the efficiency of the industrial process, i.e., the specific water use in m^3 ton^{-1} product and the nature of the raw material, the resulting wastewater is charged with a specific amount of complex particulate organic matter. The organic compounds are, as bulk pollutant, analytically measured using oxidative techniques, which determine the amount of oxygen that is required to scavenge all possible electrons coming from the carbon atoms. The pollutant concentration is consequently expressed as chemical oxygen demand (COD). Industrial wastewaters can be arbitrarily divided into high strength, moderate strength, and low strength, with COD concentrations of >10 g O_2 L^{-1}, 2–10 g O_2 L^{-1} and <2 g O_2 L^{-1}. Typical industries discharging high-strength wastewaters are alcohol distilleries, yeast factories, cane and beet molasses producers, some pulp and paper industries, pharmaceutical industries, and chemical industries. Moderate-strength wastewaters originate from advanced beer brewers, wineries, fruit and vegetable producers, dairies, citric acid plants, soft drink producers, etc., whereas the low-strength

wastewaters originate from malteries, traditional brewers, municipalities, acid mines, etc.

With the increase in influent COD, the implementation of anaerobic (pre-) treatment is of major interest. The energy content of 1 kg COD equals about 13.5 MJ, whereas the removal of 1 kg COD, applying conventional aerobic technologies, such as activated sludge, costs about 3.5 MJ or ≈ 1 kWh-electric (kWh-e) (van Lier et al. 2008). Thus, the energy gain in applying anaerobic treatment – yielding biogas – at an industry discharging 20 tons of biodegradable COD can be easily calculated. It should be noted that biogas, after the removal of impurities, is essentially pure methane. Then the energy content of the cleaned biogas needs to be converted into effective energy. At present, modern combined heat and power plants reach up to 40% net electricity conversion. The aforementioned 20 tons of COD deliver a net electric potential of 30 MWh per day. Meanwhile, a potential of 20 MWh per day is saved by avoiding the usage of conventional activated sludge technology. At a kWh price of € 0.09, this gives a total net benefit of €4.500 per day (van Lier et al. 2008).

In the operation of an anaerobic reactor for wastewater treatment, process stability and process reliability are the key factors (see Sects. 3.3.1 and 3.3.2). To ensure stable reactor operation, various technological reactor advancements have been made and various microbiological studies have been performed to elucidate the most crucial pathways of the anaerobic conversion process.

3.3 Methane-Producing Processes

3.3.1 Methane Reactors

In anaerobic reactors, organic COD is converted stepwise to the end-products, methane and carbon dioxide (biogas), of which methane, owing to its low solubility, is automatically removed from the system. The methane formation is performed by obligate anaerobic microbial communities, which are characterized by a low growth rate (see Sect. 3.4; Schink 2006; McInerney et al. 2008). As the overall COD conversion rate of the anaerobic reactor directly depends on the number and activity of the microorganisms, reactors that are able to retain a high number of microbes will be more successful. The first anaerobic systems for waste(water) treatment appeared in the late 19th century. However, their efficiency was too low as no attempts were made to specifically retain the crucial microbial populations. Interception basins such as “Mouras fosses” and the septic tank were designed to settle solids out of wastewater streams rather than to treat the soluble COD. The potentials of the anaerobic conversion technology were better explored in the early 20th century when the completely stirred tank reactor (CSTR) was designed to treat the separated solids coming from the first activated sludge processes (McCarty 2001). However, when applying anaerobic treatment to wastewaters, the CSTR

volumes became too large as the size was directly dependent on the growth rate of the methanogenic biomass. The anaerobic contact process, a CSTR coupled to a clarifier from which the settled anaerobic flocs are returned to the reactor, enhanced the volumetric loading capacity and improved the overall conversion capacity of the system by a factor of about 5, using a similar reactor volume.

In the 1960s, it was observed that the anaerobic biomass easily adheres to inert support materials, which formed the basis for the development of upflow anaerobic filter (UAF) systems (e.g., Young and McCarty 1968). With the use of these systems, the retention of the slow growing microbial communities was fully uncoupled from the liquid retention time. Moreover, by applying the system on liquid industrial effluents, it was observed that anaerobic flocs were additionally entrapped in the applied filter material, leading to an additional increase in the organic loading capacity. Full-scale applications, however, frequently led to clogging of the filter material by excessive biomass accumulation resulting in channeling or hydraulic short cuts in the anaerobic reactor, lowering the reactor capacity. The subsequently developed downflow filter could indeed rinse these solids but the overall immobilized capacity was too low to guarantee a sufficiently high conversion capacity.

The real breakthrough was the introduction of the upflow anaerobic sludge blanket process (UASB; Fig. 3.1a), which was developed by Lettinga and his co-workers in the Netherlands in the 1970s (Lettinga et al. 1980). Important incentive for this development was the worldwide energy crises and the implementation of the first environmental law protecting the surface waters in the Netherlands. The UASB design is based on a hydrodynamic selection of anaerobic flocs when introducing wastewater in an upflow mode through the reactor. The dense particles separate automatically from the liquid leading to a complete uncoupling of the hydraulic

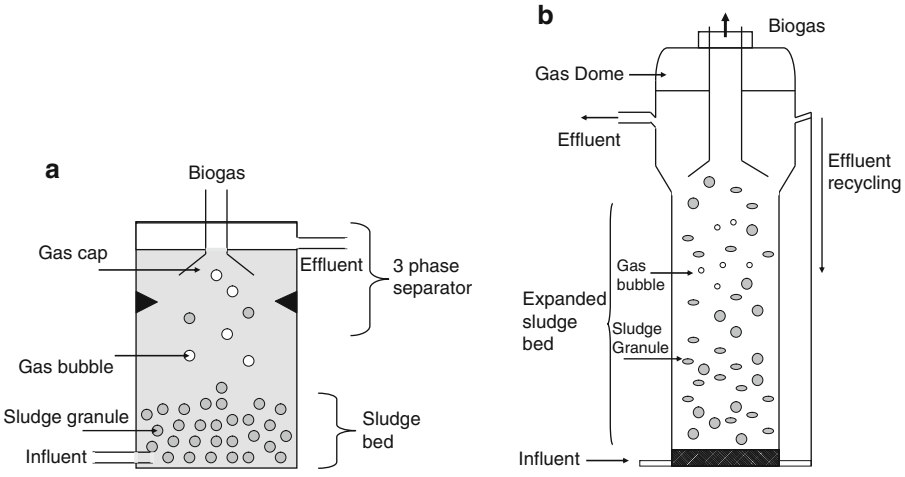

Fig. 3.1 (**a**) Upflow anaerobic sludge bed (UASB 2005) reactor concept and (**b**) expanded granular sludge bed (EGSB) reactor concept (modified from http://www.uasb.org/)

retention time (HRT) from the solid retention time (SRT). Another important feature of the UASB process is the immobilization of anaerobic microorganisms into granules without the presence of any carrier material. This microbial self-immobilization of methanogenic communities has been studied by many researchers (e.g., Lettinga et al. 1980; Liu et al. 2003; Hulshoff Pol et al. 2004). In general, the process of initial granulation and granule formation is complex and involves both physicochemical and biological interactions. It yields methanogenic granular sludge in which the microorganisms are densely packed. The sedimentation of the granules prevents the granules from being washed out of the reactors and the gas is separated from the sludge solids. In this way, a very high SRT can be achieved by applying very short HRTs. At high organic loading rates, biogas production guarantees sufficient contact between substrate and biomass, resulting in a more or less completely mixed reactor. The presence of well settling active anaerobic granular sludge gave the possibility to further improve the loading potentials of the upflow reactor by increasing the upflow velocities up to a factor of 10. By expanding the granular sludge bed, the mass transfer rates of pollutants to the microorganisms and reaction products out of the aggregates were reduced to a minimum. Currently, the so-called expanded granular sludge bed reactors (EGSB; Fig. 3.1b) are sold in the market, applying loading rates between 20 and 35 kg COD m^{-3} per day. Another high rate reactor concerns the fluidized bed (FB) system, which makes use of free floating support material such as sand or pumice on which anaerobic microorganisms are immobilized. At present, the UASB and EGSB reactors have the largest market share with regard to the application of anaerobic reactor systems for wastewater treatment. Approximately, 80% of all reactors worldwide are UASB or EGSB type systems (van Lier 2008). Many different types of high-strength wastewaters, even those that were previously believed not to be suitable for anaerobic waste treatment, are now treated by high rate anaerobic conversion processes.

Besides wastewater, many different organic waste streams are treated by anaerobic technologies with the objective to stabilize the organic matter and to reduce their environmental effects, to reduce the sludge volumes, and to recover methane as a renewable fuel. In fact, the renewed current interest in conventional anaerobic digestion is driven by the increasing energy prices and the need for renewable fuel production. Examples of feed materials are excess sewage sludge, manure, the organic fraction of municipal solid waste, industrial organic waste and, more recently, energy crops. In the digestion of (semi-)slurries and solid wastes, the hydrolysis stage of the multi-step anaerobic process (see Sect. 3.4.1) is the most important and in fact, rate limiting.

3.3.2 Factors Influencing Methane Formation

In anaerobic digestion, the microorganisms present differ in physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions. An imbalance between the different groups of microorganisms is a primary cause for reactor

instability and process failure (see Chap. 2, Braun et al. 2010). As described in a recent review by Chen et al. (2008), a wide variety of substances have been reported to be inhibitory to the anaerobic methanogenic digestion processes, such as ammonia, sulfide, salts of (earth)alkali metals, heavy metals, and organic compounds of different origins. A compound may be judged inhibitory when it causes inhibition of microbial growth and/or activity, often resulting in a shift in the microbial population. This leads to less biogas production, accumulation of unwanted products in the effluent, and in the worst case a complete destabilization of the anaerobic methanogenic digestion process. Inhibition is generally concentration-dependent. In fact, any compound will cause inhibition if present at concentrations exceeding the toxicity level of the respective microbial community. This, obviously, is also true for the organic substrates and their reaction products. If intermediate products like volatile fatty acids (VFA) and hydrogen are not removed instantaneously, their accumulation will dramatically impact the stability of the entire digestion process.

Acclimatization of microorganisms to specific wastewater constituents is possible but may take several weeks to several months (Van Lier et al. 2008). Particularly, chemical effluents require a balanced ecosystem of specialized organisms, which are responsible for crucial intermediate reactions (Razo-Flores et al. 2006). A typical example is the anaerobic treatment of purified terephthalic acid (PTA) wastewaters (Kleerebezem et al. 1999). It was shown that the decarboxylation of terephtalate to benzoic acid was only performed after a lengthy and carefully performed start-up procedure. However, compounds that are persistent organic pollutants (POPs) may not be degraded at all.

Sufficiently acclimated microbial communities have shown greater stability toward stress-inducing events such as hydraulic overloads as well as fluctuations in temperature and in volatile acid and ammonia concentrations. Not only the microbial composition for removing the (intermediate) compounds, but also the presence of matured biofilms or granular structures stabilizes the anaerobic conversion process to a great extent. As a result of immobilization, mass transfer will limit the maximum possible conversion rates. On the contrary, immobilization creates a biomass buffer at the inner side of the biofilm that can be drawn upon when the specific conversion rates are affected by toxicants and/or less appropriate environmental conditions. As a consequence, the overall capacity of the system will not be changed by the perturbation event.

3.4 Microbiology of Methane Formation from High-Strength Wastewater

3.4.1 Anaerobic Methanogenic Food Chain

The anaerobic degradation of COD (as complex, particulate organic waste) has been described as a multi-step process of series and parallel reactions performed by obligate anaerobic microbial communities (Schink 2006; McInerney et al. 2008;

see Chap. 1, Insam et al. 2010). For complete conversion, three physiological groups of microorganisms are required. One metabolic type of microorganism, in theory, might be capable of complete oxidation of COD, but these microbes have never been found in methanogenic environments. Polymeric substances, such as polysaccharides, proteins, and lipids (fat and grease) are first hydrolyzed by extracellular enzymes, secreted by microorganisms. This hydrolysis facilitates transport or diffusion of the corresponding monomers across the cell membrane. The relatively simple compounds are then fermented or anaerobically oxidized to short-chain fatty acids, alcohols, carbon dioxide, hydrogen, formate, and ammonia. The short-chain fatty acids (other than acetate) are converted to acetate, hydrogen, formate, and carbon dioxide. Methanogenesis as the final step occurs from the reduction of carbon dioxide by hydrogen and the cleavage of acetate and is performed by archaea (see Fig. 1.2 in Chap. 1 for a representation of the anaerobic food chain under methanogenic conditions). To understand microbial interactions that occur in high-strength wastewater treatment, detailed study of the physiology of the individual predominating microorganisms is necessary. A thorough understanding of the formation and structure of dense microbial aggregates is essential for application of methanogenesis. Only in bioreactors in which methanogenic communities operate in dense aggregates, anaerobic wastewater treatment can take place at a high volumetric rate (see Sect. 3.3.1).

We focus on methanogenic and acetogenic microorganisms, since they represent the core metabolism of the bio-methane producing biomass treating soluble wastewater.

3.4.2 Methanogenic Archaea in High Rate Anaerobic Digesters

Methanogenic archaea perform the final step in the overall anaerobic conversion of organic material to methane and carbon dioxide. Autotrophic methanogenic archaea utilize carbon dioxide and hydrogen, while heterotrophic methanogens convert acetate, formate, and a few other compounds to support their metabolic functions. Acetate, the absolute key intermediate in anaerobic digestion, accounts for approximately two-third of all methane produced, while the other third is produced from the reduction of carbon dioxide with electrons derived from the oxidation of hydrogen or formate (Ferry 1992, Liu and Whitman 2008). Currently, only two types of acetoclastic methanogens have been identified: *Methanosaeta* sp. and *Methanosarcina* sp. *Methanosarcina* sp. is a versatile genus of methanogens, including species capable of growing on different substrates including acetate, methanol, methylamines, and H_2/CO_2, whereas *Methanosaeta* sp. is only capable of using acetate. *Methanosaeta* sp. is widely distributed in nature and, because of its high affinity for acetate, prevails over *Methanosarcina* sp. in the low acetate environments of anaerobic waste digesters (Conklin et al. 2006). Both acetoclastic archaea are very slow growers, with doubling times of 1–12 (*Methanosaeta*) and 0.5–2 (*Methanosarcina*) days (Jetten et al. 1992). Table 3.2 summarizes the dominance of either *Methanosarcina* or *Methanosaeta* in various mesophilic reactors.

Table 3.2 Acetoclastic methanogens in anaerobic digesters

Reactor	Feed	Acetate (mM)	Dominant acetoclastic methanogen	Identification	Reference
UASB	Sugar waste	nd[a]	*Methanosaeta* ssp.	a,b	Grotenhuis et al. (1991)
UASB	Propionate	nd[a]	*Methanosaeta* ssp.	a,b	Grotenhuis et al. (1991)
CSTR	Primary	0.08	*Methanosaeta* ssp.	c	Raskin et al. (1995)
CSTR	Primary + WAS	0.8	*Methanosaeta* ssp.	c	Raskin et al. (1995)
CSTR	Primary + WAS	2	*Methanosaeta* ssp.	c	Raskin et al. (1995)
UASB	Artificial wastewater	nd[a]	*Methanosaeta concilii*	d	Sekiguchi et al. (1998)
AMBR		1 to 11	*Methanosaeta concilii*	d	Angenent et al. (2002)
Varied	Varied	nd[a]	*Methanosaeta* ssp.	d	McHugh et al. (2003)
	WAS	0.07	*Methanosaeta* ssp	d	Yu et al. (2004)
37 anaerobic reactors	Varied	nd[a]	*Methanosaeta concilii*	d,e	Leclerc et al. (2004)
5 anaerobic reactors	Varied	nd[a]	*Methanosarcina* spp. and *Methanosaeta* ssp.	d,e	Leclerc et al. (2004)
UASB	Ethanol	nd[a]	*Methanosarcina* spp.	a,b	Grotenhuis et al. (1991)
CSTR	Synthetic wastewater + primary sludge + WAS	Variable, peaked at 83	*Methanosarcina* spp.	a	Griffin et al. (1998)
FBR	Vinasse	4	*Methanosarcina barkeri*	e	Zumstein et al. (2000)
CSTR	Glucose	Variable, peaked at 66	*Methanosarcina barkeri*	e	Leclerc et al. (2001)
SBR	Whey	24.5	*Methanosarcina* spp.	f	Yu et al. (2004)
CSTR	Synthetic wastewater	5.8	*Methanosarcina* spp.	f	Yu et al. (2004)
2 anaerobic reactors	Municipal waste, vinasse	nd[a]	*Methanosarcina frisius*	d,e	Leclerc et al. (2004)

[a]nd. not determined

(a) Light microscopy, (b) Antibodies, (c) Oligonucleotide hybridization, (d) Clone libraries, (e) Fluorescence PCR, (f) Single strand conformation polymorphism, (g) Quantitative Reverse Transcriptase (RT)-PCR

Source: Modified after Conklin et al. (2006)

Under most conditions evaluated, *Methanosaeta* is the dominant acetoclastic methanogen, independent of the reactor type (Leclerc et al. 2004). However, in reactors with high acetate concentration a dominance of *Methanosarcina* was found (Conklin et al. 2006). Despite their restricted substrate range (H_2/CO_2, formate, and methylated C1 compounds), methanogens are phylogenetically diverse. They are classified in five well-established orders that are described in detail by Whitman et al. (2006). Representatives of the orders Methanobacteriales, Methanomicrobiales, and Methanosarcinales are commonly present in anaerobic bioreactors.

3.4.3 Syntrophic Acetogenic Bacteria

Saturated fatty acids, unsaturated fatty acids, alcohols, and hydrocarbons are degraded by the action of syntrophic communities in methanogenic environments. These syntrophic communities consist of an acetogen and a methanogen, that cannot grow alone on a certain organic compound, but when present together they can. Syntrophy is defined as a specialized case of tightly coupled mutual interaction (Plugge and Stams 2005; Schink 2006; McInerney et al. 2008).

The degradation of a syntrophic substrate is thermodynamically unfavorable if the product concentrations are at standard conditions (1 M concentration, or 10^5 Pa for gases). The function of methanogens is to consume hydrogen, for example, to low steady-state pressure (10^{-4}–10^{-5} atm) and to make the overall substrate conversion thermodynamically favorable (Table 3.3). An important factor in syntrophic interactions is the distance between the hydrogen-producing (acetogen) and the hydrogen-consuming (methanogen) organism. The diffusion distances for metabolite transfer should be as short as possible (Schink and Thauer 1988). The diffusion of hydrogen from producer to consumer can be described by a simple equation: $Flux H_2$ (mol s^{-1}) = –A D (c_2–c_1) per day, where A is the total surface area of the hydrogen producer ($4\pi r^2$ × number of hydrogen producers), D is the diffusion constant for hydrogen (4.9×10^{-5} cm^2 s^{-1} at 298 K), c is the concentration of hydrogen from producer (c_2) to consumer (c_1) in water, and d is the distance between producer and consumer. In general, aggregation of bacteria from different

Table 3.3 Reactions involved in propionate degradation occurring in methanogenic processes

Concept of interspecies hydrogen transfer:		$\Delta G^{0'}$	$\Delta G'$
$Propionate^- + 3H_2O$	$\rightarrow acetate^- + HCO_3^- + 3\ H_2 + H^+$	+76	–9
$4\ H_2 + HCO_3^- + H^+$	$\rightarrow CH_4 + 3\ H_2O$	–135	–12
$Acetate^- + H_2O$	$\rightarrow CH_4 + HCO_3^-$	–31	–31
Concept of interspecies formate transfer:			
$Propionate^- + 2HCO_3^-$	$\rightarrow acetate^- + 3\ formate^- + H^+$	+72	–15
$4\ formate^- + H^+ + H_2O$	$\rightarrow CH_4 + 3\ HCO_3^-$	–130	–9
$Acetate^- + H_2O$	$\rightarrow CH_4 + HCO_3^-$	–31	–31

Gibbs free energy changes under standard conditions in kJ per reaction (1 M for solutes; 10^5 Pa for gases; T = 25°C; pH 7). $\Delta G'$ is calculated for 10 μM formate and 1 Pa hydrogen
Source: From Thauer et al. (1977)

metabolic groups enables a faster degradation of complex organic material (Ishii et al. 2005).

Syntrophic interactions are usually associated with interspecies hydrogen transfer, although formate transfer is also a possible mechanism. Acetogenic bacteria are capable of producing both hydrogen and formate, and methanogens usually consume both. As hydrogen is poorly soluble in water (the maximum solubility at atmospheric pressure and room temperature is about 1 mM), it was speculated from theoretical considerations that formate may also act as an electron shuttle in methanogenic consortia (Thiele and Zeikus 1988; Boone et al. 1989). Flux calculations in suspended and aggregated biomass revealed that hydrogen transfer prevailed in aggregates (de Bok et al. 2004; Ishii et al. 2005). This may suggest that in anaerobic bioreactors, where biomass is densely aggregated, hydrogen transfer is the dominant process of interspecies electron transfer.

The syntrophic degradation of fatty acids is often the rate limiting step in methanogenic conversions. Therefore, it is essential that anaerobic wastewater treatment is operated under the conditions that favor the retention of these syntrophs. Important intermediate metabolites, besides acetate (see Sect. 3.4.2) in methanogenic processes are propionate and butyrate (McCarty 1971), which are often used as indicator for the overall performance of the process (Kida et al. 1993; Ariesyady et al. 2007a). If propionate and butyrate accumulate in the effluent, methanogenic rates decrease and the overall efficiency of COD removal decreases accordingly.

3.4.3.1 Degradation of Propionate

All currently identified syntrophic propionate-oxidizing bacteria are affiliated with the class Deltaproteobacteria within the phylum Proteobacteria (McInerney et al. 2005), or the low G+C Gram-positive bacteria in the class Clostridia within the phylum Firmicutes (Imachi et al. 2002; Plugge et al. 2002; de Bok et al. 2005). Some of the *Syntrophobacter* sp. are able to use sulfate as the electron acceptor for propionate oxidation (McInerney et al. 2005). In addition, they can grow by fermentation of pyruvate and fumarate. *Smithella propionica* is phylogenetically related to the genus *Syntrophus* (Liu et al. 1999) and lacks the ability to reduce sulfate. It also uses a different pathway to oxidize propionate from that used by *Syntrophobacter* strains, and can grow on crotonate in pure cultures (de Bok et al. 2001; Liu et al. 1999).

The concept of interspecies hydrogen versus interspecies formate transfer has been studied in detail in propionate-degrading *Syntrophobacter fumaroxidans* co-cultures (Table 3.3). Thermodynamic calculations, flux measurements in defined co-cultures, and enzyme measurements confirmed that interspecies formate transfer is an essential mechanism in syntrophic propionate degradation in suspended cultures (Dong et al. 1994; Dong and Stams 1995). The terminal reductases were studied in detail and biochemical evidence for the occurrence of the formate transfer mechanism was found (De Bok et al. 2002). Two formate dehydrogenases

were isolated from *S. fumaroxidans* and characterized. In contrast to most formate dehydrogenases, which contain molybdenum, the formate dehydrogenase (CO_2 reductase) from *S. fumaroxidans* contains tungsten and has an unusual high specific activity both in the formate oxidation and in the carbon dioxide reduction assay (Reda et al. 2008). When syntrophic co-cultures of *S. fumaroxidans* and *M. hungatei* were grown with limiting amounts of tungsten, propionate degradation decreased. This decrease coincided with decreased formate dehydrogenase activity, while the hydrogenase activities remained almost unchanged (Plugge et al. 2009). In their natural habitat, syntrophic propionate degrading bacteria form microcolonies with methanogens. In such microcolonies, interspecies distances are much shorter. We have indications that under these conditions interspecies hydrogen becomes relatively more important. In syntrophic propionate-degrading microcolonies, *Syntrophobacter* like bacteria are often surrounded by *Methanobrevibacter* sp., methanogens which can use hydrogen but not formate (Grotenhuis et al. 1991). In thermophilic sludge, interspecies hydrogen transfer also seems the preferred electron transfer mechanism (Schmidt and Ahring 1993). In addition, in highly concentrated cell suspensions with *S. fumaroxidans* and *Methanobrevibacter arboriphilus*, slow propionate degradation is observed (Dong et al. 1994).

All species described to date were isolated from anaerobic reactors, indicating the significance of these organisms in the reactors. Pure culture studies alone cannot reveal the in situ contribution of the strains in a microbial community or the diversity of propionate-oxidizing bacteria. Moreover, only a limited fraction of the total microorganisms in an ecosystem population can be cultured (Amann et al. 1995). Microbial community analysis of anaerobic sludge, based on 16S rRNA gene sequencing, is a tool to determine the microbial propionate degrading populations present in anaerobic bioreactors (see Chap. 1; Sekiguchi et al. 2000; Shigematsu et al. 2006). Harmsen et al. (1996) demonstrated the population dynamics of *Syntrophobacter* sp. by dot blot and in situ hybridization with 16S rRNA-based oligonucleotide probes in laboratory scale UASB reactors, fed with propionate or propionate plus sulfate. *Syntrophobacter* sp. in UASB reactors fed with synthetic substrates containing sucrose, acetate, propionate, and peptone or yeast extract, were detected and analyzed by 16S rRNA gene clone libraries and fluorescence in situ hybridization (FISH) (Sekiguchi et al. 1998, 1999; Ariesyady et al. 2007b). *Pelotomaculum* sp. in a UASB reactor was detected and analyzed by FISH (Imachi et al. 2000).

3.4.3.2 Degradation of Butyrate and Long Chain Fatty Acids (LCFA)

Bacteria capable of syntrophic butyrate metabolism include all species of the genus *Syntrophomonas* (McInerney et al. 1981; Lorowitz et al. 1989; Zhang et al. 2004, 2005; Sobieraj and Boone 2006; Wu et al. 2006a, b; Sousa et al. 2007a; Wu et al. 2007), *Thermosyntropha lipolytica* (Svetlitshnyi et al. 1996), and *Syntrophothermus lipocalidus* (Sekiguchi et al. 2000). All these acetogenic bacteria are capable of degrading anaerobically fatty acids with more than four and up to 18 carbon atoms. Syntrophic butyrate and saturated LCFA metabolism proceeds via the β-oxidation

pathway (Wofford et al. 1986). Except for *S. bryantii* and *T. lipolytica*, all strains were isolated from anaerobic reactors, indicating the significance of these organisms in the reactors. Culture-independent molecular community analyses confirm the high abundance of these butyrate fermenters in bioreactors (Roest et al. 2005; Sousa et al. 2007b, c). Moreover, recently the link between the presence and activity of syntrophic butyrate and LCFA degrading communities in methanogenic sludges was shown using the stable isotope probing (Hatamoto et al. 2007, 2008).

Wastewaters and waste streams that contain high concentrations of lipids and LCFA may yield high levels of methane in an anaerobic digestion process. However, due to their poor solubility LCFA were found to be inhibitory to methanogens (Lalman and Bagley 2000, 2001; Pereira et al. 2003, 2004). The inhibitory effects are reversible and are often associated with their physical interactions with the cell wall, preventing the conversion of other compounds (Pereira et al. 2005).

3.4.3.3 Unusual Syntrophic Conversions

Acetate, methanol, and formate are compounds that from a biochemical and thermodynamic point of view do not seem an obvious substrate for syntrophic growth. In anaerobic reactors, syntrophs have to compete with fermentative bacteria and methanogenic archaea that can directly convert these compounds, and have more net energy available than hydrogen- and bicarbonate-producing bacteria.

However, syntrophic acetate conversion was first described by Zinder and Koch (1984). The responsible bacterium was a homoacetogen and therefore nicknamed "Reversibacter." *Thermacetogenium phaeum* and *Clostridium ultunense* are characterized species that grow on acetate in coculture with hydrogenotrophic methanogens (Schnürer et al. 1996; Hattori et al. 2000). Both strains were isolated from methanogenic bioreactors. In addition, Shigematsu et al. (2004) demonstrated that the dilution rate could cause a shift in the primary pathway of acetate conversion to methane in acetate-fed chemostats. The biomass in these chemostats originated from an anaerobic methanogenic reactor. At low dilution rate, the acetate-oxidizing syntrophs, associated with hydrogen-consuming methanogens, could metabolically outcompete the acetoclastic methanogens, *Methanosarcina* and *Methanosaeta*, and played a primary role in the conversion of acetate to methane.

Pure cultures of homoacetogens ferment methanol and carbon dioxide to acetate. However, in coculture with hydrogen-consuming anaerobes, they oxidize methanol to hydrogen and carbon dioxide, and form little if any acetate (Cord-Ruwisch and Ollivier 1986; Heijthuijsen and Hansen 1986; Balk et al. 2003, 2007). Since the homoacetogens do not have an obvious energetic advantage of producing hydrogen, it could be termed as energy parasitism. Recent studies showed that hydrogen formation may result in obligate syntrophic growth as well. A homoacetogen which grew in pure culture on methanol in media that contained sufficient cobalt, has also been isolated (Jiang et al. 2009). Methanol is metabolized by means of a cobalt-containing methyltransferase. However, in the absence of cobalt the bacterium could only grow on methanol in coculture with methanogens.

Apparently, in that case methanol is metabolized by a methanol dehydrogenase, which does not contain cobalt. This process is only possible at a low hydrogen partial pressure.

Hydrolytic cleavage of formate to hydrogen and bicarbonate has been described in the past (Carroll and Hungate 1955; Guyot and Brauman 1986). Only recently it was found that bacteria were able to grow on the conversion of formate to hydrogen and bicarbonate, provided that hydrogen is consumed by a methanogen. Two different defined communities, a thermophilic *Moorella* AMP in co-culture with *Methanothermobacter* NJ1, and a mesophilic *Desulfovibrio* G11 in co-culture with *Methanobrevibacter arboriphilicus* AZ performed this type of syntrophic metabolism. The methanogens used can only use hydrogen as electron donor (Dolfing et al. 2008).

The detour via hydrogen as mentioned above calls for the introduction of new, previously unrecognized microbial guilds consisting of bacteria that sponge on substrates that can also be used by methanogens. Therefore, this type of metabolism, while apparently inconsistent in the context of the existence of acetoclastic and formate-utilizing methanogens, may actually be a more fundamental component of methanogenic organic-carbon-mineralizing systems than previously recognized.

3.5 Perspectives

To further increase our fundamental knowledge, detailed surveys of microbial communities in anaerobic sludge, in particular population changes over time, and continued challenges to cultivate relevant but fastidious anaerobes, are indispensable.

The molecular-based microbial community structure analyses may reveal biodiversity and detailed spatial organization of microbes of interest in sludge granules, and conventional cultivation techniques together with modern molecular techniques continuously enlarge our fundamental knowledge of the function and activity of significant populations in methane producing processes.

These approaches will also bridge the gaps between the engineer and the microbiologist, and will lead to novel discoveries to improve the reactor performance.

References

Amann R, Ludwig W, Schleifer K (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Angenent LT, Zheng DD, Sung SH, Raskin L (2002) Microbial community structure and activity in a compartimentalized, anaerobic bioreactor. Water Environ Res 74:450–461

Ariesyady HD, Ito T, Okabe S (2007a) Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. Water Res 41:1554–1568

Ariesyady HD, Ito T, Yoshiguchi K, Okabe S (2007b) Phylogenetic and functional diversity of propionate-oxidizing bacteria in an anaerobic digester sludge. Appl Microbiol Biotechnol 75:673–683

Balk M, Weijma J, Friedrich MW, Stams AJM (2003) Methanol utilization by a novel thermophilic homoacetogenic bacterium, *Moorella mulderi* sp. nov., isolated from a bioreactor. Arch Microbiol 179:315–320

Balk M, Weijma J, Goorissen HP, Ronteltap M, Hansen TA, Stams AJM (2007) Methanol utilizing *Desulfotomaculum* species utilizes hydrogen in a methanol-fed sulfate-reducing bioreactor. Appl Microbiol Biotechnol 73:1203–1211

Boone DR, Johnson RL, Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of Km for H_2 or formate uptake. Appl Environ Microbiol 55:1735–1741

Braun R, Drosg B, Bochmann G, Weiß S, Kirchmayr R (2010) Recent developments in bio-energy recovery through fermentation. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, New York, pp 35–58

Carroll EJ, Hungate RE (1955) Formate dissimilation and methane production in bovine rumen contents. Arch Biochem Biophys 56:525–536

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresource Technol 99:4044–4064

Conklin A, Stensel HD, Ferguson J (2006) Growth kinetics and competition between *Methanosarcina* and *Methanosaeta* in mesophilic anaerobic digestion. Water Environ Res 78:486–496

Cord-Ruwisch R, Ollivier B (1986) Interspecific hydrogen transfer during methanol degradation by *Sporomusa acidovorans* and hydrogenophilic anaerobes. Arch Microbiol 144:163–165

De Bok FAM, Stams AJM, Dijkema C, Boone DR (2001) Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. Appl Environ Microbiol 67:1800–1804

De Bok FAM, Luijten MLGC, Stams AJM (2002) Biochemical evidence for formate transfer in syntrophic propionate oxidizing cocultures of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Appl Environ Microbiol 68:4247–4252

De Bok FAM, Plugge CM, Stams AJM (2004) Interspecies electron transfer in methanogenic propionate degrading consortia. Water Res 38:1368–1375

De Bok FAM, Harmsen JM, Plugge CM, de Vries MC, Akkermans ADL, deVos WM, Stams AJM (2005) The first true obligately syntrophic propionate-oxidizing bacterium, *Pelotomaculum schinkii* sp. nov., cocultured with *Methanospirillum hungatei*, and emended description of the genus *Pelotomaculum*. Int J Syst Evol Microbiol 55:1697–1703

Dong X, Plugge CM, Stams AJM (1994) Anaerobic degradation of propionate by a mesophilic acetogenic bacterium in co- and triculture with different methanogens. Appl Environ Microbiol 60:2834–2838

Dong X, Stams AJM (1995) Evidence for H_2 and formate formation during syntrophic butyrate and propionate degradation. Anaerobe 1:35–39

Dolfing J, Jiang B, Henstra AM, Stams AJM, Plugge CM (2008) Syntrophic growth on formate: a new microbial niche in anoxic environments. Appl Environ Microbiol 74:6126–6131

Ferry JG (1992) Methanogenesis: ecology, physiology, biochemistry & genetics. Chapman & Hall, New York

Griffin ME, McMahon KD, Mackie RI, Raskin L (1998) Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnol Bioeng 57:342–355

Grotenhuis JTC, Smit M, Plugge CM, Xu YS, Van Lammeren AAM, Stams AJM, Zehnder AJB (1991) Bacteriological composition and structure of granular sludge adapted to different substrates. Appl Environ Microbiol 57:1942–1949

Guyot J-P, Brauman A (1986) Methane production from formate by syntrophic association of *Methanobacterium bryantii* and *Desulfovibrio vulgaris* JJ. Appl Environ Microbiol 52:1436–1437

Harmsen HJM, Akkermans ADL, Stams AJM, De Vos WM (1996) Population dynamics of propionate-oxidizing bacteria under methanogenic and sulfidogenic conditions in anaerobic granular sludge. Appl Environ Microbiol 62:2163–2168

Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2007) Diversity of anaerobic microorganisms involved in long-chain fatty acid degradation in methanogenic sludges as revealed by RNA-based stable isotope probing. Appl Environ Microbiol 73:4119–4127

Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2008) Detection of active butyrate-degrading microorganisms in methanogenic sludges by RNA-based stable isotope probing. Appl Environ Microbiol 74:3610–3614

Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phae*um gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. Int J Syst Evol Microbiol 50:1601–1609

Heijthuijsen JHFG, Hansen TA (1986) Interspecies hydrogen transfer in co-cultures of methanol-utilizing acidogens and sulfate-reducing or methanogenic bacteria. FEMS Microbiol Lett 144:163–165

Hulshoff Pol LW, deCastro Lopes SI, Lettinga G, Lens PNL (2004) Anaerobic sludge granulation. Water Res 38:1376–1389

Imachi H, Sekiguchi Y, Kamagata Y, Ohashi A, Harada H (2000) Cultivation and in situ detection of a thermophilic bacterium capable of oxidizing propionate in syntrophic association with hydrogenotrophic methanogens in a thermophilic methanogenic granular sludge. Appl Environ Microbiol 66:3608–3615

Imachi H, Sekiguchi Y, Kamagata Y, Hanada S, Ohashi A, Harada H (2002) *Pelotomaculum thermopropionicum* gen. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. Int J Syst Evol Microbiol 52:1729–1735

Insam H, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Ishii S, Kosaka T, Hotta Y, Watanabe K (2005) Simulating the contribution of coaggregation to interspecies hydrogen fluxes in syntrophic methanogenic consortia. Appl Environ Microbiol 72:5093–5096

Jetten MSM, Stams AJM, Zehnder AJB (1992) Methanogenesis from acetate: a comparison of the acetate methabolism of *Methanothrix soehngenii* and *Methanosarcina* ssp. FEMS Microbiol Rev 88:181–198

Jiang B, Henstra A-M, Paulo PL, Balk M, van Doesburg W, Stams AJM (2009) Atypical one-carbon metabolism of an acetogenic and hydrogenenic *Moorella thermoacetica* strain. Arch Microbiol 191:123–131

Kida K, Morimura S, Sonoda Y (1993) Accumulation of propionic acid during anaerobic treatment of distillery wastewater from barley-shochu making. J Ferment Bioeng 75:213–216

Kleerebezem R, Hulshoff Pol LW, Lettinga G (1999) The role of benzoate in anaerobic degradation of terephthalate. Appl Environ Microbiol 65:1161–1167

Lalman JA, Bagley DM (2000) Anaerobic degradation and inhibitory effects of linoleic acid. Water Res 34:4220–4228

Lalman JA, Bagley DM (2001) Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. Water Res 35:2975–2983

Leclerc M, Delbes C, Moletta R, Godon JJ (2001) Single strand conformation polymorphism monitoring of 16S rDNA Archaea during start-up of an anaerobic digester. FEMS Microbiol Ecol 34:213–220

Leclerc M, Delgènes JP, Godon J-J (2004) Diversity of the Archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. Environ Microbiol 6:809–819

Lettinga G, Van Velsen AFM, Hobma SW, DeZeeuw W, Klapwijk A (1980) Use of the upflow sludge blanket reactor concept for biological waste water treatment, especially for anaerobic treatment. Biotechnol Bioeng 22:699–734

Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterisation of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov, sp. nov. and *Syntrophobacter wolinii*. Int J Syst Bacteriol 49:545–556

Liu Y, Xu HL, Yang SF, Tay JH (2003) Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor. Water Res 37:661–673

Liu Y, Whitman WB (2008) Metabolic, phylogenetic and ecological diversity of methanogenic archaea. In: Wiegel J, Maier MJ, Adams MW (eds) Incredible anaerobe; from physiology to genomics to fuels, vol 1125. New York Academy of Sciences, New York, pp 171–189

Lorowitz WH, Zhao HX, Bryant MP (1989) *Syntrophomonas wolfei* subsp *saponavida* subsp. nov., a long chain fatty-acid degrading, anaerobic, syntrophic bacterium – *Syntrophomonas wolfei* subsp *wolfei* subsp. nov. – and emended descriptions of the genus and species. Int J Syst Bacteriol 39:122–126

Lowe DC (2006) A green source of surprise. Nature 439:148–149

McCarty PL (1971) Energetics and kinetics of anaerobic treatment. Adv Chem Ser 105:91–107

McCarty PL (2001) The development of anaerobic treatment and its future. Water Sci Technol 44:149–156

McHugh S, Carton M, Mahony T, O'Flaherty V (2003) Methanogenic population structure in a variety of anaerobic bioreactors. FEMS Microbiol Lett 219:297–304

McInerney MJ, Bryant MP, Hespell RB, Costerton JW (1981) *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty-acid oxidizing bacterium. Appl Environ Microbiol 41:1029–1039

McInerney MJ, Stams AJM, Boone DR (2005) Genus *Syntrophobacter*. In: Staley JT, Boone DR, Brenner DJ, de Vos P, Garrity GM, Goodfellow M, Krieg NR, Rainey FA, Schleifer KH (eds) Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer-Verlag, New York, pp 1021–1027

McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B, Rohlin L, Gunsalus RP (2008) Physiology, ecology, phylogeny and genomics of microorganisms capable of syntrophic metabolism. In: Wiegel J, Maier MJ, Adams MW (eds) Incredible anaerobe; from physiology to genomics to fuels, vol 125. New York Academy of Sciences, New York, pp 58–72

Pereira MA, Roest K, Stams AJM, Akkermans ADL, Amaral AL, Pons M-N, Ferreira EC, Mota M, Alves M (2003) Image analysis, methanogenic activity measurements, and molecular biological techniques to monitor granular sludge from EGSB reactors fed with oleic acid. Water Sci Technol 47:181–188

Pereira MA, Sousa DZ, Mota M, Alves MM (2004) Mineraliztion of LCFA with anaerobic sludge: Kinetics, enhancement of methanogenic activity and effect of VFA. Biotechnol Bioeng 88: 502–511

Pereira MA, Pires OC, Mota M, Alves MM (2005) Anaerobic biodegradation of oleic and palmitic acids: evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. Biotechnol Bioeng 92:15–23

Plugge CM, Balk M, Stams AJM (2002) *Desulfotomaculum thermobenzoicum* subsp. *thermosyntrophicum* subsp. nov., a thermophilic syntrophic propionate-oxidizing spore-forming bacterium. Int J Syst Evol Microbiol 52:391–399

Plugge CM, Stams AJM (2005) Syntrophism among Clostridiales. In: Duerre P (ed) Handbook on Clostridia. CRC, Boca Raton, pp 769–784

Plugge CM, Jiang B, de Bok FAM, Tsai C, Stams AJM (2009) Effect of tungsten and molybdenum on growth of a syntrophic coculture of *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei*. Arch Microbiol 191:55–61

Razo-Flores E, Macarie H, Morier F (2006) Application of biological treatment systems for chemical and petrochemical wastewaters. In: Cervantes FJ, Pavlostathis SG, van Haandel AC (eds) Advanced biological treatment processes for industrial wastewaters. IWA, London, pp 267–297

Raskin L, Zheng DD, Griffin ME, Stroot PG, Misra P (1995) Characterization of microbial communities in anaerobic bioreactors using molecular probes. Antonie Van Leeuwenhoek 68:297–308

Reda T, Plugge CM, Abram NJ, Hirst J (2008) Reversible interconversion of carbon dioxide and formate by an electroactive enzyme. Proc Natl Acad Sci 105:10654–10658

Roest K, Heilig GHJ, Smidt H, de Vos WM, Stams AJM, Akkermans ADL (2005) Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. Syst Appl Microbiol 28:175–185

Schink B (2006) Syntrophic associations in methanogenic degradation. Prog Mol Subcell Biol 41:1–19

Schink B, Thauer RK (1988) Energetics of syntrophic methane formation and the influence of aggregation. In: Lettinga G, Zehnder AJB, Grotenhuis JTC, Hulshoff Pol LW (eds) Granular anaerobic sludge; microbiology and technology. Pudoc, Wageningen, pp 5–17

Schmidt J, Ahring BK (1993) Effects of hydrogen and formate on the degradation of propionate and butyrate in thermophilic granules from an upflow anaerobic sludge blanket reactor. Appl Environ Microbiol 59:2546–2551

Schnürer A, Schink B, Svensson BH (1996) *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. Int J Syst Bacteriol 46:1145–1152

Sekiguchi Y, Kamagata Y, Syutsubo K, Ohashi A, Harada H, Nakamura K (1998) Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analysis. Microbiol 144:2655–2665

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (1999) Fluorescence in situ hybridization using 16S rRNA-targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules. Appl Environ Microbiol 65:1280–1288

Sekiguchi Y, Kamagata Y, Nakamura K, Ohashi A, Harada H (2000) *Syntrophothermus lipocalidus* gen. nov., sp. nov., a novel thermophilic, syntrophic, fatty-acid-oxidizing anaerobe which utilizes isobutyrate. Int J Syst Evol Microbiol 50:771–779

Shigematsu T, Tang Y, Kobayashi T, Kawaguchi H, Morimura S, Kida K (2004) Effect of dilution rate on metabolic pathway shift between aceticlastic and nonaceticlastic methanogenesis in chemostat cultivation. Appl Environ Microbiol 70:4048–4052

Shigematsu T, Era S, Mizuno Y, Ninomiya K, Kamegawa Y, Morimura S, Kida K (2006) Microbial community of a mesophilic propionate-degrading methanogenic consortium in chemostat cultivation analyzed based on 16S rRNA and acetate kinase genes. Appl Microbiol Biotechnol 72:401–415

Sobieraj M, Boone DR (2006) Syntrophomonadaceae. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes: an evolving electronic resource for the microbiological community, vol 4. Springer-Verlag, New York, pp 1041–1046

Sousa DZ, Smidt H, Alves MM, Stams AJM (2007a) *Syntrophomonas zehnderi* sp. nov., an anaerobe that degrades long chain fatty acids in co-culture with *Methanobacterium formicicum*. Int J Syst Evol Microbiol 57:609–615

Sousa DZ, Pereira MA, Smidt H, Stams AJM, Alves MM (2007b) Molecular assessment of complex microbial communities degrading long chain fatty acids (LCFA) in methanogenic bioreactors. FEMS Microbiol Ecol 60:252–265

Sousa DZ, Pereira MA, Stams AJM, Alves MM, Smidt H (2007c) Microbial communities involved in anaerobic degradation of unsaturated or saturated long chain fatty acids (LCFA). Appl Environ Microbiol 73:1054–1064

Svetlitshnyi V, Rainey F, Wiegel J (1996) *Thermosyntropha lipolytica* gen. nov., sp. nov., a lipolytic, anaerobic, alkalitolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. Int J Syst Bacteriol 46:1131–1137

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Thiele JH, Zeikus JG (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer during syntrophic methanogenesis in flocs. Appl Environ Microbiol 54:20–29

UASB (2005) Anaerobic granular sludge bed technology pages. Available at: http://www.uasb.org/. Accessed 25 Sept 2008

Unites States Environmental Protection Agency (2008) Climate change home pages. Available at: http://epa.gov/methane/. Accessed 25 Sept 2008

Van Lier JB (2008) High-rate anaerobic wastewater treatment: diversifying from end-of the pipe treatment to resource oriented conversion techniques. Water Sci Technol 57:1137–1148

Van Lier JB, Mahmoud N, Zeeman G (2008) Anaerobic wastewater treatment. In: Henze M, van Loosdrecht MCM, Ekama GA, Brdjanovic D (eds) Biological wastewater treatment: principles, modelling and design. IWA, London, pp 415–456

Whitman W, Bowen T, Boone D (2006) The methanogenic bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes, vol 3, 3rd edn. Springer-Verlag, New York, pp 165–207

Wofford NQ, Beaty PS, McInerney MJ (1986) Preparation of cell-free extracts and the enzymes involved in fatty acid metabolism in *Syntrophomonas wolfei*. J Bacteriol 167:179–185

Wu C, Liu X, Dong X (2006a) *Syntrophomonas cellicola* sp. nov., a novel spore-forming syntrophic bacterium isolated from a distilled-spirit-fermenting cellar and assignment of *Syntrophospora bryantii* to *Syntrophomonas bryantii* sp. nov., comb. nov. Int J Syst Evol Microbiol 56:2331–2335

Wu C, Liu X, Dong X (2006b) *Syntrophomonas erecta* subsp. *sporosyntropha* subsp. nov., a spore-forming bacterium that degrades short chain fatty acids in co-culture with methanogens. Syst Appl Microbiol 29:457–462

Wu CG, Dong XZ, Liu XL (2007) *Syntrophomonas wolfei* subsp. *methylbutyratica* subsp. nov., and assignment of *Syntrophomonas wolfei* subsp. *saponavida* to *Syntrophomonas saponavida* sp. nov comb. nov. Syst Appl Microbiol 30:376–380

Young JC, McCarty PL (1968) The anaerobic filter for waste treatment. Stanford University Technical Report No. 87

Yu Y, Lee C, Hwang S (2004) Analysis of community structures in anaerobic processes using Quantitative Real-Time PCR method. In: Proceedings of the 10th World Congress Anaerobic Digestion Conference, Montréal, Quebec, IWA London, pp 459–465

Zhang CY, Liu XL, Dong XZ (2004) *Syntrophomonas curvata* sp. nov., an anaerobe that degrades fatty acids in co-culture with methanogens. Int J Syst Evol Microbiol 54:969–973

Zhang CY, Liu XL, Dong XZ (2005) *Syntrophomonas erecta* sp. nov., a novel anaerobe that syntrophically degrades short-chain fatty acids. Int J Syst Evol Microbiol 55:799–803

Zinder SH, Koch M (1984) Non-aceticlastic methanogenesis from acetate: acetate oxidation by a thermophilic syntrophic coculture. Arch Microbiol 138:263–272

Zumstein E, Moletta R, Godon JJ (2000) Examination of two years of community dynamics in an anaerobic bioreactor using fluorescence polymerase chain reaction (PCR) single-strand conformation polymorphism analysis. Environ Microbiol 2:69–78

Chapter 4
Biogas Technology – Controlled Gas Flow for Enhanced Mixing, Heating, and Desulfurization

Bernhard Wett and Heribert Insam

Abstract History and current use of biogas utilization demonstrates that with simple approaches useful methane can be produced. From experience with small-scale plants, a new 4-chamber technology named BIO4GAS was developed that allows production of biogas in reactors that do not require mechanical stirring. On the basis of data from a demonstration plant set in operation in 2008, the 4-chamber technology and its advantages are described in detail. In particular, the low operational energy requirement and the high quality of the obtained biogas (in terms of low H_2S levels) are emphasized.

Contents

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4.1 Historical Developments

4.1.1 Europe

Plinius, always a good source for first reports on natural phenomena, noted the appearance of flickering lights emerging from below the surface of swamps and Van Helmont recorded the emanation of an inflammable gas from decaying organic matter in the 17th century (van Brakel 1980). Volta is generally recognized to have first isolated methane. He concluded as early as 1776 that the amount of gas that evolves is a function of the amount of decaying vegetation. In 1894, Gayon, a student of Pasteur, fermented manure at 35°C and obtained 100 L of methane per m^3 of manure. Toward the end of the 19th century, methanogenesis was found to be connected to microbial activity. Béchamp (1868) named the "organism" – apparently, a mixed community – responsible for methane production from ethanol. In 1876, Herter reported that acetate in sewage sludge was converted stoichiometrically to equal amounts of methane and carbon dioxide (cited from Zehnder et al. 1982). As early as 1896, gas from sewage was used for lighting streets in Exeter, England. Then, in 1904, Travis put into operation a new, two-stage process, in which the suspended material was separated from the wastewater, and was allowed to pass into a separate "hydrolyzing" chamber. Söhngen (1906) was able to enrich two distinct acetate utilizing bacteria, and he found that formate, hydrogen and carbon dioxide could be precursors for methane. Buswell and co-workers identified anaerobic bacteria and investigated the conditions that promote methanogenesis. Research focused also on the fate of nitrogen in anaerobic digestion, the stoichiometry of the reaction and the production of energy from farm and industrial wastes (Buswell and Neave 1930; Buswell and Hatfield 1936). Barker and his group performed basic biochemical studies of methanogenic archaea (then methane bacteria) and contributed significantly to the field (e.g., Pine and Barker 1956).

In Europe, biogas use has been promoted in several campaigns since the 1970s, and currently experiences a new boom. In particular, if national policies support the production of biogas by subsidies, considerable increases in plant numbers are observed. The approach, however, was often focused on agro-industrial use of energy crops in large plants and technologically sophisticated constructions. This often led to problems in terms of cost/benefit imbalances. Johansson and Azar (2007) have further shown that renewable bioenergy production based on specialized agricultural crop production will compete with the food production sector and lead to exploding food prices. This is of major concern, particularly in the less developed countries.

4.1.2 Asia

The use of biogas has a long history in Asia and dates back many centuries. There is evidence that biogas was used for heating bath water in Assyria during the tenth century BC. Marco Polo mentions the use of covered sewage tanks, a practice that

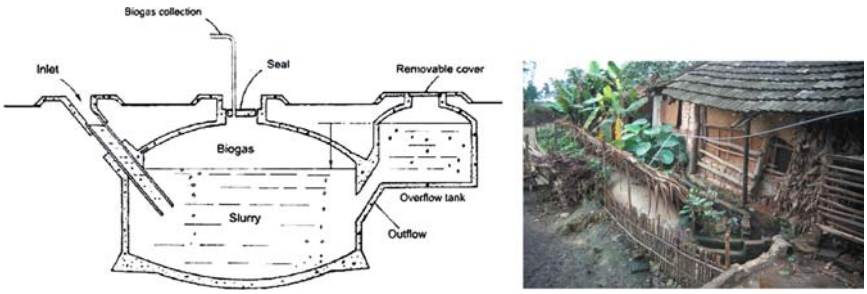

Fig. 4.1 Scheme of Chinese dome digester (*left*) and view of a small-scale manure digester in Vietnam (*right*; Foto: E. Schreckensperger)

probably goes back 2,000–3,000 years in ancient China (Chawla, 1986). India has also a quite long history of biogas development. The first unit, usually referred to in literature, is a biogas unit at a lepers asylum near Mumbai installed in 1859. The primary function seems to have been sewage treatment, but the gas was used for lighting. In China, by the end of the 19th century, simple biogas digesters had appeared in the coastal areas of southern China. Guorui invented and built an 8-m^3 biogas tank in the 1920s which formed the basis for the establishment of a successful company. *Chinese Guorui Biogas Digester Practical Lecture Notes* was published in 1935, the first monograph on biogas in China and in the world. This was the first wave of biogas use in China. The second wave of biogas use in China originated in Wuchang in 1958 in a campaign to exploit the multiple functions of biogas production, which simultaneously solved the problems of manure disposal and improvement of hygiene. The third wave of biogas use occurred between the late 1970s and early 1980s when the Chinese government enforced biogas production as an effective use of natural resources in rural areas. Biogas production was considered not only to provide energy, but also to contribute to environmental protection and improvement of hygiene. Some six million digesters were set up in China, and the "China dome" digester became the standard construction, which is followed to the present day (Fig. 4.1) for small-scale domestic use. China's *2003–2010 National Rural Biogas Construction Plan* is aimed at increasing the biogas use by a total of 50 million small-scale plants by 2010, which is 35% of all farm households.

4.1.3 Numerical Tools and Current Technologies

In parallel to the progress in biogas technology, numerical tools have been developed to analyze, understand, and simulate processes catalyzed by anaerobic microorganisms. Anaerobic digestion is a multi-step degradation process in which metabolites are transferred from one functional group of microbes to the other.

In the late 1960s and 1970s, first attempts in biokinetic modeling of anaerobic digestion processes focused on the rate-limiting process step (e.g., O'Rourke 1968; Graef and Andrews 1974; Lyberatos and Skiadas 1999) which needs to be defined for specific process conditions and substrates. Subsequently, more complex models have been developed in order to describe the digester behavior and inhibit the impacts by unionized volatile fatty acids (VFA), -acetate and -ammonia (e.g., Hill and Barth 1977; Moletta et al. 1986). The inclusion of hydrogen partial pressure as a key process parameter (Mosey 1983; Costello et al. 1991) contributed to the interactive process. Additional improvements (e.g., Angelidaki et al. 1993; Siegrist et al. 1993) led to the development of IWA's widely acknowledged Anaerobic Digestion Model ADM1 (Batstone et al. 2002a). It represents a generic description of digestion processes for various substrates like sewage sludge and manure, and considers seven functional groups of microbes (in total, more than 30 state variables) and employs more than 100 parameters (Fig. 4.2). The only nutrient that is balanced by ADM1 is nitrogen which is released as ammonia from degraded organic matter (Wett et al. 2006). Recent knowledge on methanogenesis and the involved microbiota is summarized in Chaps. 1 and 3 (Insam et al. 2010; Braun et al. 2010).

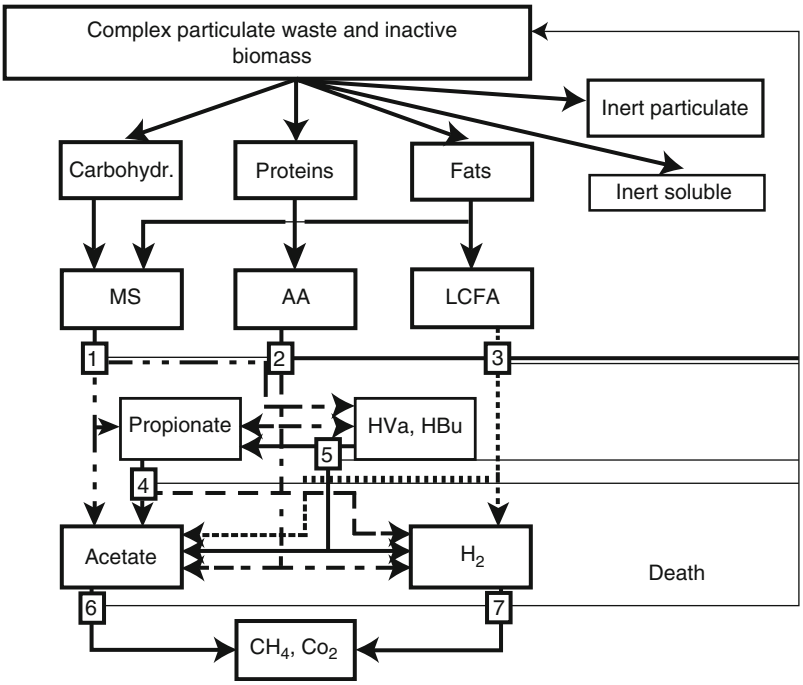

Fig. 4.2 The Anaerobic Digestion Model ADM1 considers seven groups of organisms which catalyze the following biochemical processes: (1) acidogenesis from sugars, (2) acidogenesis from amino acids, (3) acetogenesis from LCFA, (4) acetogenesis from propionate, (5) acetogenesis from butyrate and valerate, (6) aceticlastic methanogenesis, and (7) hydrogenotrophic methanogenesis (Batstone et al. 2002b)

4.2 The BIO4GAS-Approach

4.2.1 The Development of an Idea

To summarize the historical development of biogas technology till date, basically two systems of agricultural biogas plants have been established which differ significantly in size, application, and in terms of safety and general technological standards:

- Huge number of small-scale plants in developing countries for cooking gas production (energy self-sufficient households)
- Agro-industrial plants for electrical power generation (typically, >100 kW) based mainly on energy crops (renewable energy production)

The range of biogas plants between these two basic types has often been seen as economically not feasible while latest funding programs in Central Europe target smaller plants based mainly on manure as substrate. In an attempt to design a small-scale reactor for the use of agricultural wastes, in particular liquid manure, a new reactor type and mixing system called BIO4-GAS technology evolved (Wett et al. 2007). The driving factors are as given below.

1. it is known that biogas production based on agricultural and other wastes is the approach with the best performance in terms of mitigation of greenhouse gas emissions. Therefore, the size of the BIO4GAS plants should fit the size of a wide range of farms
2. often, individual planning of biogas plants makes them too costly in terms of construction and maintenance; our aim was to develop a reactor that works without any electromechanical parts inside and to reduce the construction costs by a standardized or serial implementation

The 4-chamber design was derived from a biogas plant that has been in operation since 40 years at a farm in Buch (Tyrol, Austria) (Wackerle 2005). The layout is now formed by two concentric cylinders (Fig. 4.3). The inner cylinder and the outer ring contain two chambers, each separated by baffles. During operation the substrate is pumped to chamber 1 (K1) and biogas production starts, mainly in chamber 1. Since the construction is gas-tight, gas pressure of the head space of chamber 1 displaces the liquid manure below the baffle to K2 and drives a gas-lift in K2. The gas lift also serves as a pipe-heat-exchanger for heating the reactor. The chamber K1 is also heated and mixed by a thermo-gas-lift which is driven by pressurized air injected for the desulfurization of the generated biogas. Deposits in K1 and K2 are avoided by periodical opening of a relief valve causing an oscillation and a concurrent recycle flow between K1 and K2. The determination of the optimal layout was supported by numerical models (Fig. 4.4).

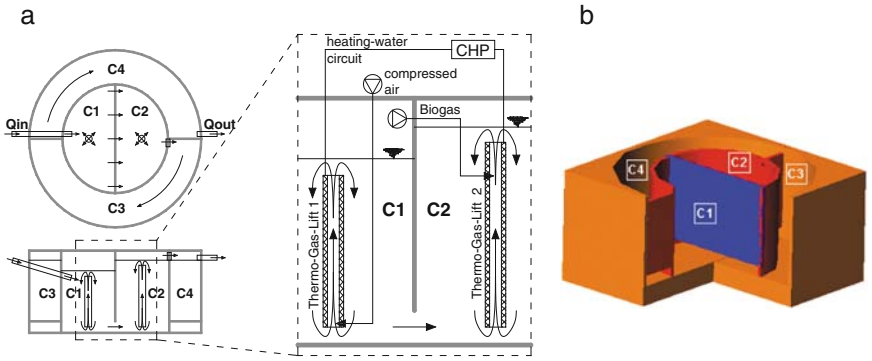

Fig. 4.3 Scheme of the BIO4GAS pilot plant with concentric shape and thermo-gas-lifts employed for heating, mixing, and desulfurization

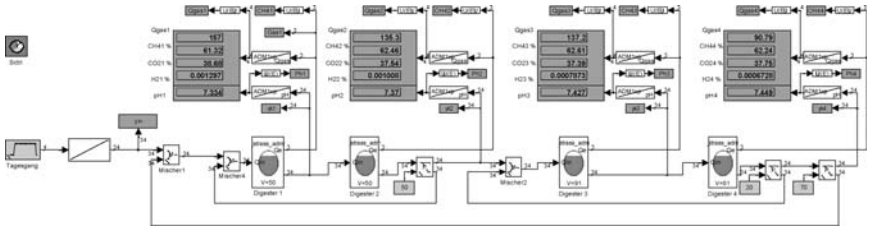

Fig. 4.4 Four-chamber system represented by serial ADM1 digesters, edited in the SIMBA-environment for the simulation of gas production in individual chambers (Wett et al. 2007)

4.2.2 Mixing and Agitation

As described above, the gas pressure is used in a twofold manner to mix the content of the reactor: Chambers K1 and K2 are individually mixed by a vertical flow induced by the thermo-gas-lifts. Rising bubbles of the injected air in K1 and biogas in K2, respectively, and heat convection reduce the density of the water column in the lift pipe, resulting in a moderate but continuous upflow current.

The difference in water level between chamber K1 and K2 represents the pressure head driving the gas-lift. A pneumatic pressure relief valve is used for a sudden equalization of the pressure difference causing an oscillation of the water columns in K1 and K2. The periodic oscillation flow shows velocity peaks at the bottom opening (Fig. 4.5), which mobilizes the occasionally heavy sludge layers. Additionally, the counterflow seeds more mature biomass back to the first chamber. Both the vertical flow from the gas-lifts and from the pressure oscillation provide sufficient turbulence to prevent stratification of the liquor in the reactor. For substrates with severe bulking sludge, an additional stirrer at the water surface for reliable scum destruction is suggested.

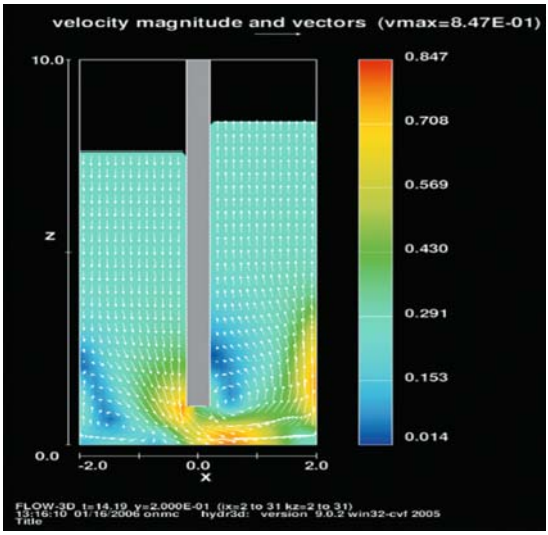

Fig. 4.5 CFD-simulation (computational fluid dynamics) of the equalization flow after the pressure relief valve between headspace of chamber K1 and K2 has been opened (colors indicating velocity magnitude and arrows directions of flow)

Several authors (e.g., Vavilin and Angelidaki 2005; Speece et al. 2006) have suggested that high mixing intensity and duration have an adverse effect on the biogas production rates in agricultural biogas plants. The sheering forces are supposed to disrupt the important spatial proximity of volatile fatty acid degrading bacteria and hydrogen-utilizing methanogens. It is generally recognized that syntrophic growth of different bacterial and archaeal species is important, and sheering forces may hamper the necessary biofilm and aggregate formation (e.g., Wu et al. 1996). The BIO4GAS approach attempts to reduce the sheering forces as much as possible, yet ensuring a sufficient substrate mixing.

4.2.3 Heating

Continuous vertical flow through the thermo-gas-lifts ensures an efficient heat transfer via the pipe walls. The actual heating medium is water circulating between the double-walled lift-pipes and the co-generation unit where the biogas is burned. The coupled heat-power system has been installed in a pre-fabricated container module hosting the complete electrical- and safety-equipment. The heat produced is distributed to three consecutive heat cycles at the following steps of hierarchy: Fermenter heating requires about one third of the heat flow and shows highest priority. The next is the external consumer, and only unused excess heat will be eliminated by an emergency cooler at the roof-top of the container. A further advancement means the installation of a boiler for redundant gas conversion, replacing the flare so that even during failure or servicing of the combined heat and power plant (CHP) unit, the thermal energy of the biogas may be used.

Table 4.1 Growth conditions of Thiobacilli

Species	pH [-]	Temperature [°C]
Acidithiobacillus		
A. albertensis, ferrooxidans	2–4	30–35
A. caldus	1–3.5	32–52
A. thiooxidans	2–4	25–30
Halothiobacillus		
H. halophilus	7	30–32
H. hydrothermalis	7.5–8	35–40
H. kellyi	6.5	37–42
H. neapolitanus	6–8	25–30
Thermithiobacillus		
T. tepidarius	6–8	40–45
Thiolcalivibrio		
T. denitrifcans, nitratus	10	25–30
T. versutus	10–10.2	25–30
Thioalcalimicrobium		
T. aerophilum	9–10	25–30
Thiomicrospira		
T. chilensis	7	32–37
T. crunogena	7–8	28–32
T. frisia	6.5	32–35
T. kuenenii	6	29–33
T. peliphila	6–8	25–30
T. thyasirae	7–8	35–40
Thiobacillus		
T. aquaesulis	7.5–8	40–50
T. denitrifcans, thioparus	6–8	25–30
Thiomonas		
T. cuprina	3–4	30–36
T. intermedia, T. perometabolis	5.5–6	30–35
T. thermosulfata	5.2–5.6	50–52.5

Source: Robertson (2004)

Microorganisms in the digester show a broad range of optimum growth conditions as Table 4.1 exemplifies for Thiobacilli (temperature optima between 25 and 52.5°C). According to Karakashev et al. (2005), the microbial diversity is higher in mesophilic than thermophilic reactors. Diversity, however, does not seem to be related to methane production rates. Pender et al. (2004) compared the microbial communities in mesophilically and thermophillically operated reactors, with and without the addition of sulfate. The reactor biomass sampled during mesophilic operation, both in the presence and absence of sulfate, was characterized by a predominance of *Methanosaeta* spp. In contrast, in the 55°C reactor an archeaon closely related to *Methanocorpusculum parvum* was dominant, when sulfate was added, *Methanobacterium thermoautotrophicum* was dominant and reactor performance decreased. The sensitivity of the acetoclastic methanogens, dominated by *Methanocorpusculum parvum*, demonstrated the fragile nature of this thermophilic ecosystem which was particularly sensitive to sulfide inhibition. An issue that has not yet adequately been addressed is the even temperature distribution within

reactors. Since methanogenesis is usually retarded in the temperature range between 40 and 50°C, a suboptimal temperature range for both mesophilic and thermophilic microorganisms, any reactor design should aim at an even temperature distribution. This is ensured in the BIO4GAS reactor by mixing and heating, accompanied by a good thermal insulation.

4.2.4 Hydrogen Sulfide Oxidation

Anaerobic digestion involves the breakdown of organic matter in an oxygen-free environment. The biogas produced contains, besides methane and carbon dioxide, hydrogen sulfide (H_2S) which is of major concern not only because of odor nuisance, but mainly because of its destructiveness to engines by impacting corrosion and oil viscosity. The concentration of H_2S in the biogas produced from chicken manure and molasses may be as high as 4,000 mg m^{-3}. Post-treatment of the biogas could be avoided by oxidation of H_2S in the reactor which can be accomplished or at least reduced by offering good conditions for the growth of sulfur oxidizing bacteria.

Sulfur oxidizing bacteria, also known as Thiobacilli, were reclassified by Kelly and Wood (2000), forming three genera, *Acidithiobacilli, Halothiobacilli*, and *Thermothiobacilli*. Besides reduced sulfur compounds as an electron donor, like H_2S, the presence of an electron acceptor like oxygen is necessary. In nature, Thiobacilli grow in the boundary layer of anaerobic sediment and aerobic water body. Many Thiobacilli are obligate or facultative autotrophs, using carbon dioxide as their only C source. A major product of H_2S oxidation is elementary sulfur. The elementary sulfur is precipitated and in biogas plants one can always find yellow sulfur excretions (Fig. 4.6). Besides reduced sulfur compounds, Thiobacilli also oxidize Fe^{++} and form Fe^{+++}, and are thus responsible for metal corrosion (Brock and Gustafson 1976):

$$H_2S + 0.5O_2 \rightarrow S + H_2O \qquad -209.4 \text{ kJ mol}^{-1}$$
$$S + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+ \qquad -587.1 \text{ kJ mol}^{-1}$$
$$H_2S + 2O_2 \rightarrow H_2SO_4 \qquad -798.2 \text{ kJ mol}^{-1}$$
$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \qquad -47.04 \text{ kJ mol}^{-1}$$

Sulfate production, as the second oxidation step, is promoted at excess oxygen conditions (Janssen et al. 2009) and causes acidification and high oxygen demand. Munz et al. (2009) point out that high pH, around 9, represents an even stronger drive for sulfate production. Fuseler et al. (1996) have shown that sulfur is not necessarily a stable intermediate but can be object to disproportionation to sulfate and hydrogen sulfide. Nevertheless moderate oxygenation (ca. 0.05 g O_2 g^{-1} COD) of anaerobic sludge environments does not lead to significant sulfate production (van der Zee et al. 2007). Obviously, sulfur production is difficult to measure due to its precipitation and attachment to surfaces, and therefore, needs to be estimated

Fig. 4.6 Surface of gas-dome and manhole cover of an agricultural biogas plant coated by precipitated elemental sulfur from biological H_2S oxidation (Foto: F. Wackerle)

from the total S balance gap. Zee et al. demonstrated that sulfide oxidation rates increased during long-term micro-aerobic operation which resulted in very low sulfide concentration in the biogas.

In agricultural biogas plants, desulfurization of the biogas is accomplished mainly in the fermenter gas space. For this purpose, small amounts of air (approximately, 5–10% of the produced biogas) are added. As in all biological processes, the availability of surfaces for bacterial settlement is most important. In the BIO4GAS technology, the admixed air fulfils a double purpose. First, the air is pressed into the reactor at the bottom of the thermo-gas-lift and thus aids the mixing of the substrate by the ascending gas bubbles. And second, majority of the formed biogas is produced in chamber 1, and is forced to move along the subsequent three chambers before it is tapped at the rear end of chamber 4. Thus, the gas has a very long passage way allowing the Thiobacilli to act. The efficiency of H_2S oxidation is demonstrated by an operational data during the start-up period when measured H_2S concentrations dropped below 200 ppm (Fig. 4.7). While the methane concentration tends to increase along the flow path through the chambers (range around 50%), the injected air (oxygen) is used up by oxidation processes.

Oechsner (1998) investigated 52 biogas plants and found that in 54% and 15% of all plants the H_2S concentration exceeded 500 ppm and 2,000 ppm, respectively. This showed that biological desulfurization did not work optimally in most cases. The explanation may be a lack of suitable surface, a passage for the gas that is too short, or a suboptimal temperature. In particular, non-insulated covers may have surface temperatures below the optimum for many Thiobacilli (see Table 4.1).

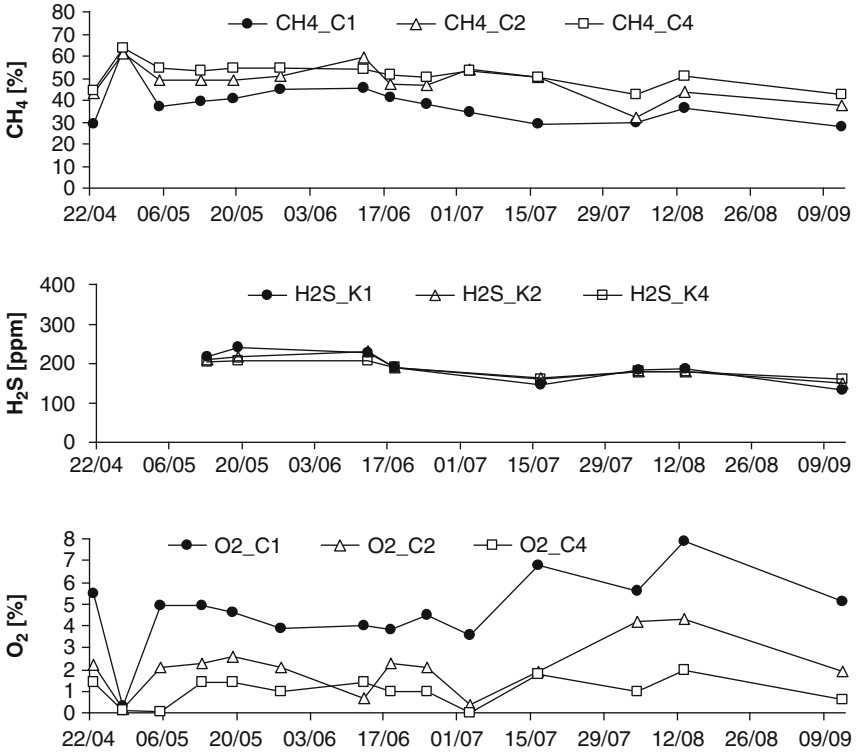

Fig. 4.7 Spatial and temporal concentration profiles of CH_4, H_2S, and O_2 along the flow-path through the head-space of BIO4GAS chambers C1, C2, and C4 of the demonstration plant in Rotholz

4.3 Conclusions

Production of biogas from organic wastes is successfully used in millions of small-scale reactors in Asia, while high-tech biogas plants in the Megawatt range are efficiently operating in many countries worldwide. Yet, medium-scale plants with a power range of 10–100 kW seem to experience a dilemma: state-of-the-art technology is too expensive in construction and maintenance, while environmental regulations and safety standards do not allow to upsize simple solutions known from developing countries. With the BIO4GAS technology, a solution has been found that optimizes the workplace of microbes in medium-scale Biogas plants.

References

Angelidaki I, Ellegard L, Ahring BK (1993) A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. Biotechnol Bioeng 42:159–166

Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA (2002a) Anaerobic Digestion Model No.1. Scientific and Technical Report, 13, IWA, London

Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA (2002b) The IWA Anaerobic Digestion Model No 1 (ADM1). Water Sci Technol 45:65–73

Béchamp A (1868) Lettre a M. Dumas. Ann. Chim. Phys. Ser. 4(13):103–111

Braun R, Drosg B, Bochmann G, Weiß S, Kirchmayr R (2010) Recent developments in bio-energy recovery through fermentation. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, New York

Brock TD, Gustafson J (1976) Ferric iron reduction by sulfur- and iron-oxidizing bacteria. Appl Environ Microbiol 32:567–571

Buswell AM, Hatfield WD (eds) (1936) Anaerobic Fermentations, State of Illinois, Dept. of Registration and Education, Div. of the State Water Survey, Urbana, IL. Bull. No. 32, 1–193

Buswell AM, Neave SL (1930) Laboratory studies of sludge digestion. Illinois Division of State Water Survey, Bulletin No. 30

Chawla OP (1986) From biodung to biogas – a historical review of European experience. In: Energy, Agriculture and Waste Management. Ann Arbour Science Publications, pp 207–260

Costello DJ, Greenfield PF, Lee PL (1991) Dynamic modelling of a single stage high-rate anaerobic reactor. Water Res 25:847–871

Fuseler K, Krekeler D, Sydow U, Cypionka H (1996) A common pathway of sulfide oxidation by sulfate reducing bacteria. FEMS Microbiol Lett 144:129–134

Graef SP, Andrews JF (1974) Stability and control of anaerobic digestion. J Water Pollut Control Fed 46:666–683

Hill DT, Barth CL (1977) A dynamic model for simulation of animal waste digestion. J Water Pollut Control Fed 49:2129–2143

Insam H, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Janssen AJH, Lens PNL, Stams AJM, Plugge CM, Sorokin DY, Muyzer G, Dijkmann H, Van Zessen E, Luimes P, Buismann CJN (2009) Application of bacteria involved in the biological sulphur cycle for paper mill effluent purification. Sci Total Environ 407:1333–1343

Johansson DJA, Azar C (2007) A scenario based analysis of land competition between food and bioenergy production in the US. Clim Change 82:267–291

Karakashev D, Batstone DJ, Angelidaki I (2005) Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Appl Environ Microbiol 71: 331–338

Kelly DP, Wood AP (2000) Reclassification of some species of Thiobacillus to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. Int J Syst Evol Microbiol 50:511–516

Lyberatos G, Skiadas IV (1999) Modelling of anaerobic digestion – a review. Global Nest Int J 2:63–76

Moletta R, Verrier D, Albagnac G (1986) Dynamic modelling of anaerobic digestion. Water Res 20:427–434

Mosey FE (1983) Mathematical modelling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. Water Sci Technol 15:209–232

Munz G, Gori R, Mori G, Lubello C (2009) Monitoring biological sulphide oxidation processes using combined respirometric and titrimetric techniques. Chemosphere 76:644–650

O'Rourke JT (1968) Kinetics of anaerobic treatment at reduced temperatures. PhD Thesis, Stanford University, California

Oechsner H (1998) Erhebung von verfahrenstechnischen Daten an landwirtschaftlichen Biogasanlagen in Baden-Württemberg Forschungsreport Baden-Württemberg

Pender S, Toomey M, Carton M, Eardly D, Patching JW, Colleran E, O'Flaherty V (2004) Long-term effects of operating temperature and sulphate addition on the methanogenic community structure of anaerobic hybrid reactors. Water Res 38:619–630

Pine MJ, Barker HA (1956) Studies on the methane fermentation. XII. The pathway of hydrogen in the acetate fermentation. J Bacteriol 71:644–648

Siegrist H, Renggli D, Gujer W (1993) Mathematical modelling of anaerobic mesophilic sewage sludge treatment. Water Sci Technol 27:25–36

Söhngen NL (1906) Waterstof en methan. Doctoral Dissertation, Technische Hoogeschool de Delft, pp 105–106

Speece RE, Boonyakitsombut S, Kim M, Azbar N, Ursillo P (2006) Overview of anaerobic treatment: thermophilic and propionate implications. Water Environ Res 78:460–473

Van Brakel J (1980) The Ignis Fatuus of biogas small-scale anaerobic digesters ("biogas plants"): a critical review of the pre-1970 literature. Delft University Press, The Netherlands

Van der Zee FP, Villaverde S, Garcie PA, Fdz-Polanco F (2007) Sulfide removal by moderate oxygenation of anaerobic sludge environments. Bioresour Technol 98:518–524

Vavilin VA, Angelidaki I (2005) Anaerobic degradation of solid material: importance of initiation centers for methanogenesis, mixing intensity, and 2D distributed model. Biotechnol Bioeng 89:113–122

Wackerle F (2005) Investigation of an agricultural 4-chamber biogas system. Diploma thesis, MCI Innsbruck, Austria

Wett B, Eladawy A, Ogurek M (2006) Description of nitrogen incorporation and release in ADM1. Water Sci Technol 54:67–76

Wett B, Schoen M, Phothilangka P, Wackerle F, Insam H (2007) Model based design of an agricultural biogas plant – application of Anaerobic Digestion Model No. 1 for an improved 4 chamber scheme. Water Sci Technol 55:21–28

Wu WM, Jain MK, Zeikus JG (1996) Formation of fatty acid-degrading, anaerobic granules by defined species. Appl Environ Microbiol 62:2037–2044

Zehnder AJ, Ingvorsen K, Marti T (1982) Microbiology of methanogenic bacteria. In: Hughes DE et al (eds) Anaerobic digestion. Elsevier, Amsterdam, pp 45–68

Chapter 5
Vermicomposting: Earthworms Enhance the Work of Microbes

Jorge Domínguez, Manuel Aira, and María Gómez-Brandón

Abstract Vermicomposting, a very efficient method of converting solid organic waste into an environmentally-friendly, useful and valuable resource, is an accelerated process that involves bio-oxidation and stabilization of the waste as a result of the interactions between some species of earthworms and microorganisms. Although microorganisms are the main agents for biochemical decomposition of organic matter, earthworms are critical in the process of vermicomposting. Complex interactions among the organic matter, microorganisms, earthworms and other soil invertebrates result in the fragmentation, bio-oxidation and stabilization of the organic matter.

Contents

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5.1 What Is Vermicomposting?

Although it was Darwin (1881) who first drew attention to the great importance of earthworms in the decomposition of dead plants and the release of nutrients from them, it was necessary to wait for more than a century until this was taken seriously as a field of scientific knowledge or even a real technology.

The cultivation of earthworms in organic wastes has been termed vermiculture, and vermicomposting, the managed processing of organic wastes by earthworms to produce vermicompost, has progressed considerably in recent years. Vermicomposting has been shown to be successful in processing sewage sludge and solids from wastewater (Domínguez et al. 2003; Clark et al. 2007; Pramanik et al. 2007; Suthar 2007), food industry waste (Nogales et al. 1999a, b, 2005), urban residues, food and animal waste (Domínguez and Edwards 1997; Atiyeh et al. 2000; Triphati and Bhardwaj 2004; Aira et al. 2006a, b; Garg et al. 2006; Suthar 2007; Lazcano et al. 2008), and in the paper industry waste (Elvira et al. 1996, 1998; Kaushik and Garg 2003; Gajalakshmi and Abbasi 2004), as well as treating horticultural residues from cultivars (Gajalakshmi et al. 2005; Pramanik et al. 2007; Gupta et al. 2007; Suthar 2007).

Vermicomposting is a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms and other soil fauna within the decomposer community, strongly affecting decomposition processes, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties (Domínguez 2004). Microorganisms produce the enzymes that cause the biochemical decomposition of organic matter, but earthworms are the crucial drivers of the process as they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter, which results in a greater surface area available for microbial colonization, drastically altering biological activity. Earthworms also modify microbial biomass and activity through stimulation, digestion and dispersion in casts (Fig. 5.1) and closely interact with other biological components of the vermicomposting system, thereby affecting the structure of microflora and microfauna communities (Domínguez et al. 2003; Lores et al. 2006). Thus, the decaying organic matter in vermicomposting systems is a spatially and temporally heterogeneous matrix of organic resources with contrasting qualities that result from the different rates of degradation that occur during decomposition (see Moore et al. 2004).

Vermicompost, the end product of vermicomposting, is a finely divided peat-like material of high porosity and water holding capacity and contains many nutrients in forms that are readily taken up by plants. High rates of mineralization occur in the organic matter-rich earthworm casts, which greatly enhances the availability of inorganic nutrients, particularly ammonium and nitrates, for plants.

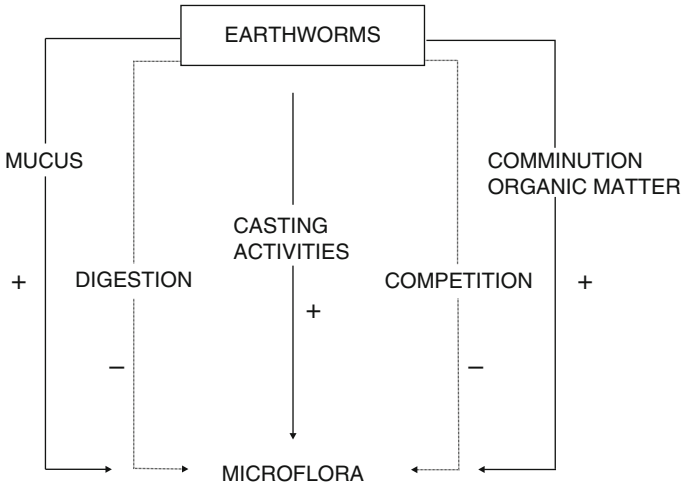

Fig. 5.1 Positive (+) and negative (–) effects of earthworms on microbial biomass and activity. Microbes are mainly dispersed through earthworm casts

5.2 Earthworms

Earthworms are macroscopic clitellate oligochaete annelids that live in soil. They are hermaphroditic animals and display indeterminate growth. Earthworms represent the major animal biomass in most terrestrial temperate ecosystems; they significantly affect soil's physical, chemical and biological properties, and play a key role in modifying soil structure and in accelerating the decomposition of organic matter and nutrient turnover (Edwards and Bohlen 1996; Lavelle and Spain 2001). More than 4,000 species of earthworms have been described, although for the great majority of these species, only the names and morphologies are known, and nothing is known about their biology, life cycles and ecology. Different species of earthworms have different life histories, occupy different ecological niches and have been classified, on the basis of their feeding and burrowing strategies, into three ecological categories: epigeic, anecic and endogeic (Bouché 1977). Endogeic (soil feeders) and anecic species (burrowers) live in the soil profile and consume a mixture of soil and organic matter, and thus excrete organo-mineral feces. Epigeic earthworms are litter dwellers and litter transformers; they live in organic horizons, in or near the surface litter and feed primarily on coarse particulate organic matter, ingest large amounts of non-decomposed litter and excrete holorganic fecal pellets. These pellets provide a higher surface to the volume ratio than the original leaf litter, which enhances the rate of decomposition (Lavelle et al. 1997; Lavelle and Spain 2001).

Epigeic earthworms, with their natural ability to colonize organic wastes, high rates of consumption, digestion and assimilation of organic matter, tolerance to a wide range of environmental factors, short life cycles, high reproductive rates, and

endurance and resistance to handling, show good potential for vermicomposting. Few earthworm species display all these characteristics, and in fact only four have been extensively used in vermicomposting facilities: *Eisenia andrei*, *E. fetida*, *Perionyx excavatus* and *Eudrilus eugeniae* (see Domínguez (2004) for details of the life cycles of these species).

5.3 Vermicomposting Food Web

Vermicomposting systems sustain a complex food web that results in the recycling of organic matter. Biotic interactions between decomposers (i.e., bacteria and fungi) and the soil fauna include competition, mutualism, predation and facilitation, and the rapid changes that occur in both functional diversity and in substrate quality are the main properties of these systems (Sampedro and Domínguez 2008). The most numerous and diverse members of this food web are microbes, although there are also abundant protozoa and many animals of varying sizes, including nematodes, microarthropods and large populations of earthworms (Monroy 2006; Sampedro and Domínguez 2008). These fauna cover a range of trophic levels – some feed primarily on microbes (microbial-feeders), organic waste (detritivores), a mixture of organic matter and microbes (microbial-detritivores), whereas others feed on animals (carnivores) or across different trophic levels (Fig. 5.2; Sampedro

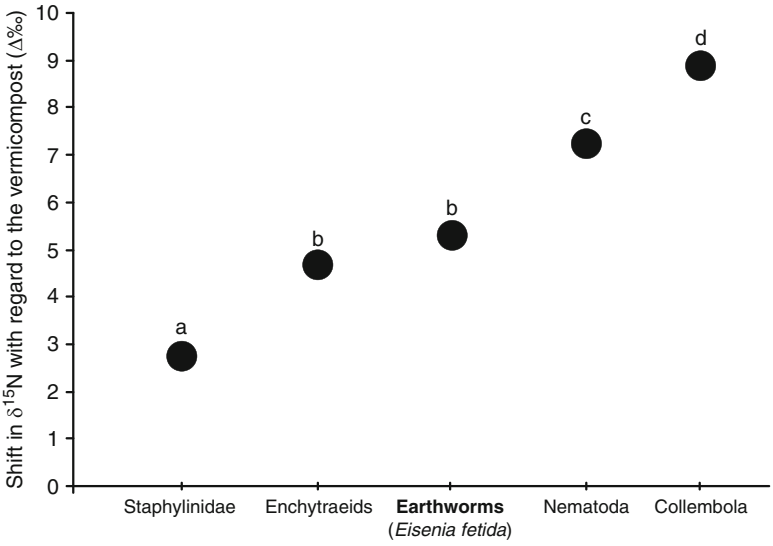

Fig. 5.2 Shift in the $\delta^{15}N$ (expressed as Δ ‰) of some components of the micro- and mesofauna of a pig slurry vermicomposting bin with respect to the fresh vermicompost in which they live. Different letters denote significant differences at $\alpha = 0.01$, Tukey HSD test. (Modified after Sampedro and Domínguez 2008)

and Domínguez 2008). A continuous range of feeding strategies from pure detritivore to pure microbivore has been proposed in detritus-based food webs (Scheu 2002), although the trophic structure and specific resource utilization are poorly understood.

The primary consumers of the vermicomposting food web are the microbes (mainly bacteria, fungi and ciliates) that break down and mineralize organic residues. Microbes are the most numerically abundant and diverse members of the vermicomposting food web, and include thousands of organisms. Secondary and higher-level consumers, i.e., the soil fauna including the earthworms, exist alongside microbes, feeding on and dispersing them throughout the organic matter. As organic matter passes through the gizzard of the earthworms, it becomes finely ground prior to digestion. Endosymbiotic microbes produce extracellular enzymes that degrade cellulose and phenolic compounds, enhancing the degradation of ingested material; and the degraded organic matter passes out of the earthworm's body in the form of casts. As earthworms feed on decaying organic wastes, their burrowing and tunneling activities aerate the substrate and enable water, nutrients, oxygen and microbes to move through it; their feeding activities increase the surface area of organic matter for microorganisms to act upon. As decomposers die, more food is added to the food web for other decomposers (Fig. 5.3; direct

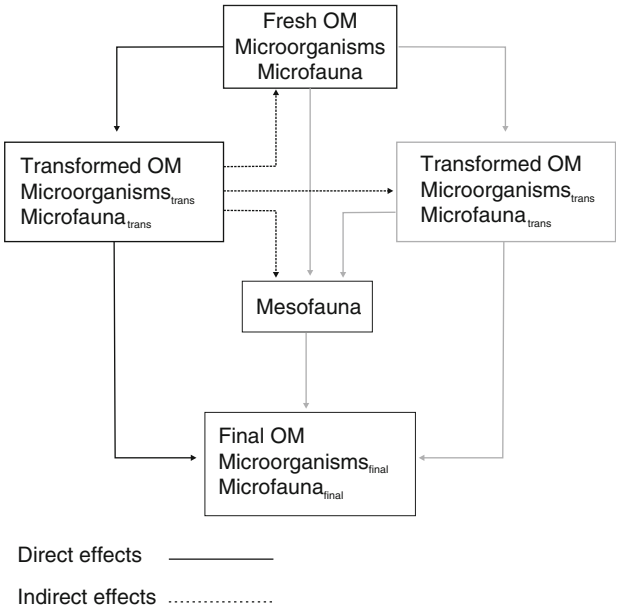

Fig. 5.3 Direct and indirect effects of earthworms on the decomposition of organic matter (OM) during vermicomposting. Here we show the two interacting pathways functioning in the process: the earthworm-mediated pathway (*black lines*) and the microbial pathway (*gray lines*). Both pathways involve intermediated stages of decomposition (here denominated transformed) which result in the final organic matter

effects are those related to direct earthworm activity such as digging, digestion and casting, which initially modify the organic matter, microorganisms and microfauna. Indirect effects are those derived from the direct ones, and include aging and mixing of casts with fresh organic matter or transport of microorganisms, i.e., the interaction of transformed substrates with fresh and transformed substrates by microorganisms).

Earthworms accelerate decomposition processes during vermicomposting (Aira et al. 2006a, 2007a), but it is not clear from where they obtain their energy inputs (decaying organic matter, microorganisms, microfauna or a combination of them). They may utilize different strategies ranging from non-selective substrate feeding to grazing strategies, and have the ability to shift between living and nonliving carbon sources (Domínguez et al. 2003; Sampedro et al. 2006).

5.4 How Vermicomposting Works

The vermicomposting process includes two different phases regarding the activity of earthworms, (i) an active phase during which earthworms process waste, thereby modifying its physical state and microbial composition (Lores et al. 2006), and (ii) a maturation-like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the earthworm's processed waste (Domínguez 2004; Fig. 5.4). As in composting, the duration of the active phase is not fixed, and depends on the species and density of earthworms (the main drivers of the process), and the rates at which they ingest and process the waste (Aira and Domínguez 2008a).

The effect of earthworms on the decomposition of organic waste during the vermicomposting process is, in the first instance, due to gut associated processes (GAPs). These processes include all the modifications that the decaying organic matter and the microorganisms undergo during the intestinal transit. These modifications include the addition of sugars and other substances, modification of the microbial diversity and activity, modification of the microfauna populations, homogenization, and the intrinsic processes of digestion, assimilation and production of mucus and excretory substances such as urea and ammonia, which constitute a readily assimilable pool of nutrients for microorganisms. Decomposition is also enhanced through the action of endosymbiotic microbes that reside in the gut of earthworms. These microbes produce extracellular enzymes that degrade cellulose and phenolic compounds, thereby further enhancing the degradation of ingested material. Other physical modifications of the substrate caused by the digging activities of earthworms, such as aeration and homogenization of the substrate also favour microbial activity and further enhance decomposition (Domínguez 2004). The proximate activity of earthworms significantly enhances the mineralization of both carbon and nitrogen in the substrate, and such effects are in proportion to the earthworm density (Aira et al. 2008). Several authors have reported

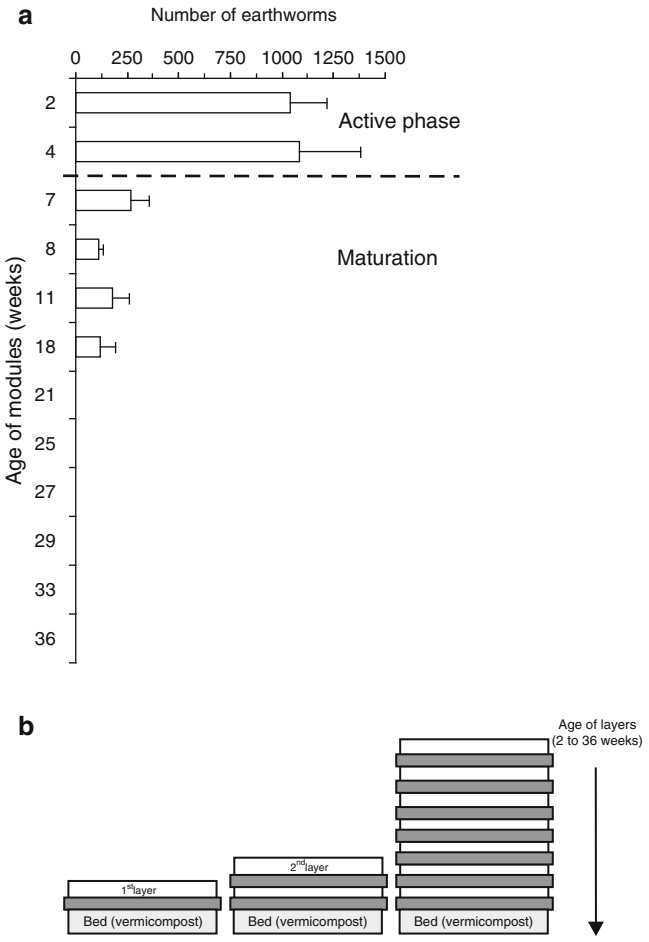

Fig. 5.4 The two phases of vermicomposting depending on earthworm presence in the substrate (active and maturation-like phase). (**a**) Earthworm population in vermireactors at different sampling times. Number of earthworms (means ± SE., n = 3) in each layer, from 2 to 36 weeks of age are shown. (**b**) Scheme of the set up and the procedure for adding new modules during the vermicomposting process. The earthworms moved upwards towards the new modules to which fresh waste had been added. (Modified from Aira et al. 2007a)

similar responses in detritivorous organisms involved in organic matter decomposition (Aira et al. 2002; Vetter et al. 2004).

Upon completion of GAPs, the resultant earthworm casts undergo cast associated processes (CAPs), which are more closely associated with aging processes, the action of the microflora and microfauna present in the substrate and with the physical modification of the egested materials (days to weeks; Parthasarathi and Ranganathan 2000; Aira et al. 2005). During these processes the effects of earthworms are mainly indirect and derived from the GAPs. It is important to note that in

vermicomposting systems, earthworm casts are always mixed with material not ingested by the earthworms, and the final vermicompost consists of a mixture of the two different fractions. During this aging, vermicompost will reach its optimum in terms of biological properties promoting plant growth and suppressing plant diseases (see Chap. 8, de Bertoldi 2010; Chap. 11, Fuchs 2010). Currently, there is insufficient information regarding when this "optimum" is achieved, how we can determine it in each case and if this "optimum" has some kind of expiration date. It is important to note that the optimal quality may only be achieved in natural ecosystems built from the correct site-specific balance of soil, plants, microorganisms, macroorganisms including earthworms and climate. However, it is not possible to easily determine when a vermicompost sample is "optimal" and thus, only after application, can this be known.

5.5 Stimulation and Acceleration of Microbial Decomposition by Earthworms during Vermicomposting

Nutrient mineralization is directly governed by the activities of bacteria and fungi and these activities are strongly affected by the soil fauna that lives alongside the microbes, and also by food web interactions that determine the transfer of nutrients through the system. Although epigeic earthworms have little direct impact on mineralization, their indirect effects on microbial biomass and activity are very important. These indirect effects include digestion and release of readily assimilable substances, such as mucus for microbiota (Brown and Doube 2004), as well as the transport and dispersal of microorganisms through casting. Earthworms ingest a mixture of organic wastes and microorganisms during vermicomposting and some of this material will be digested, but they also excrete large amounts of rather fragile fecal material in which further microbial growth is enhanced by favorable conditions of moisture and the intense mixing that has occurred in the gut. Other earthworms or members of the mesofauna may subsequently ingest those pellets and assimilate a further set of substrates made available by the most recent burst of microbial activity (Lavelle et al. 1997; Fig. 5.3). Earthworm casts play an important role in decomposition because they contain nutrients and microbiota different from those contained in the material prior to ingestion (Aira et al. 2006b; Aira and Domínguez 2008b). This enables better exploitation of resources either because of the appearance of microbial species in fresh substrate or the pool of readily assimilable compounds in the casts.

It is well known that earthworms accelerate the rate of organic matter decomposition during vermicomposting (Atiyeh et al. 2000; Domínguez et al. 2003; Domínguez 2004; Triphati and Bhardwaj 2004; Aira and Domínguez 2008a,b; Aira et al. 2006b, 2007a,b, 2008; Fig. 5.5). Although earthworms can assimilate carbon from the more labile fractions of organic wastes, their contribution to the total heterotrophic respiration is very low due to their poor capacity for assimilation.

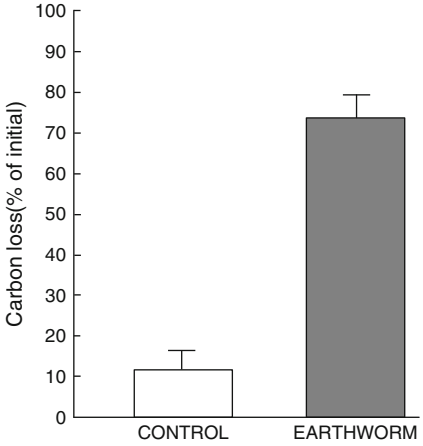

Fig. 5.5 Carbon loss (% of initial) after 1 month of vermicomposting of cow manure, as affected by the presence of the earthworm *Eisenia andrei*. Values are means ± SE. Control is the same treatment without earthworms

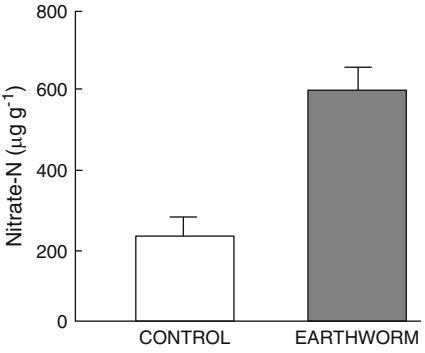

Fig. 5.6 Effect of earthworms (*Eisenia andrei*) on the amount of Nitrate-N produced after vermicomposting of cow manure (for 1 month). Values are means ± SE. Control is the same treatment without earthworms

Nitrogen mineralization is regulated by the availability of dissolved organic nitrogen and ammonium, the activity of the microorganisms and their relative requirements for carbon and nitrogen. Earthworms also have a great impact on nitrogen transformations during vermicomposting through modifications of the environmental conditions and their interactions with microbes; they enhance nitrogen mineralization, thereby producing conditions in the organic wastes that favour nitrification, resulting in the rapid conversion of ammonium-nitrogen into nitrates (Atiyeh et al. 2000; Domínguez 2004; Lazcano et al. 2008; Aira et al. 2008; Aira and Domínguez 2008b; Fig. 5.6).

The effects of microbial-feeding fauna on microbial activity and nutrient mineralization are generally positive. Enhanced C mineralization results from increased turnover rate, activity and respiration of grazed microbial populations, whereas enhanced N mineralization is mainly due to the direct excretion of excess N. In general, grazers have lower assimilation efficiencies than the microbes upon which

they graze, and therefore they excrete the excess nutrients in biologically available forms (e.g., protozoa preying on bacterial populations are assumed to release about one-third of the N consumed; Bardgett 2005). This release of nutrients in fact constitutes remobilization of the nutrients that were bound up in the microbial biomass, and has been termed the "microbial loop" (Clarholm 1994).

5.6 Effects of Earthworms on Microbial Communities during Vermicomposting

Microorganisms are the main agents of biochemical decomposition, whereas earthworms are involved in the indirect stimulation of microbial populations through comminution of organic matter, i.e., by increasing the surface area available for microbes. Earthworms also modify the microbial populations through digestion, stimulation and dispersion in casts. Therefore, it is necessary to establish the effects of earthworms on the microorganisms, because whether the earthworms stimulate or depress microbiota, or modify the structure and function of microbial communities, they would have different effects on the decomposition of organic matter**.** To address these questions we performed an experiment in our laboratory with mesocosms filled with cow manure with ten mature earthworms and without earthworms ($n = 5$ each). We used cow manure as the substrate, which is known to support a dense decomposer foodweb (Sampedro and Domínguez 2008). The mesocosms consisted of 2 L plastic jars filled with 200 g (fresh weight, fw) of substrate. We used the epigeic earthworm *E. andrei* Bouché, 1972, broadly distributed and easy to manage under lab conditions. We allowed mature individuals (375 ± 7 mg; mean individual fw ± standard error of the mean) to shed their gut contents on moistened tissue paper for 24 h at room temperature before the experiment. We covered the jars (containing the substrate and the earthworms) with perforated lids, stored them at random in a scientific incubator (20°C and 90% humidity) and after 1 month, earthworms were removed and vermicompost and control samples were collected and immediately processed for microbial analyses. Viable microbial biomass was determined as the sum of all identified phospholipid fatty acids (PLFAs). The structure of the microbial community was assessed by PLFA analysis; and some specific PLFAs were used as biomarkers to determine the presence and abundance of specific microbial groups. Microbial community function was determined measuring the bacterial and fungal growth rates by the incorporation of radioactively labelled leucine into proteins and radioactively labeled acetate into the fungal-specific lipid ergosterol, respectively. The metabolic quotient, a parameter that evaluates the efficiency of microorganisms in utilizing organic C compounds, was also determined. The data were analyzed by one-way ANOVA. Post hoc comparisons of means were performed by a Tukey HSD test at $\alpha = 0.05$ test.

5.6.1 *Effects of Earthworms on the Structure of Microbial Communities*

5.6.1.1 Microbial Biomass

Assessment of microbial communities by PLFA (Zelles 1999) revealed that earthworms impact greatly on microbial community structure and function. We found that the activity of earthworms reduced the viable microbial biomass measured as the total content of PLFAs after 1 month of vermicomposting (Fig. 5.7a); the presence of earthworms reduced total microbial biomass by approximately four to five times relative to the control without earthworms. Earthworm activity also reduced the ratio of fungal to bacterial PLFA (Fig. 5.7b) indicating, that the decrease in fungal PLFA was proportionally higher than that of the bacterial PLFA.

Certain specific PLFAs can be used as biomarkers to determine the effect of earthworms on the presence and abundance of specific microbial groups. The sum of PLFAs characteristic of Gram-positive bacteria (iso/anteiso branched-chain PLFAs), Gram-negative bacteria (monounsaturated and cyclopropyl PLFAs) and actinomycetes (10Me branched PLFAs) was chosen to represent the bacterial biomass; and the fungal biomarker 18:26,9 was used to indicate fungal biomass (Frostegård and Bååth 1996; Zelles 1997). The abundance of both bacteria and fungi was drastically reduced by the earthworms after 1 month of vermicomposting

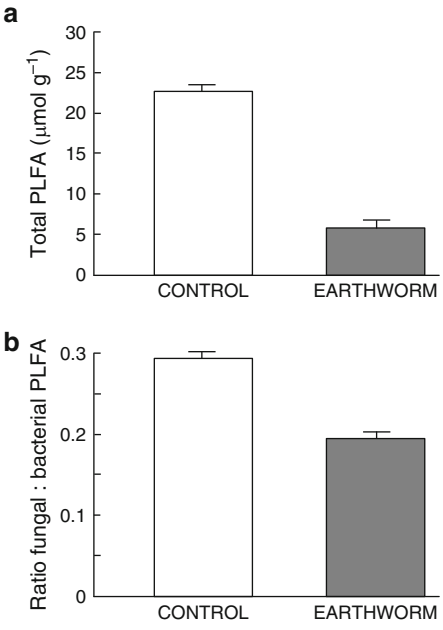

Fig. 5.7 Impact of earthworms (*Eisenia andrei*) on microbial communities after vermicomposting of cow manure (for 1 month): (**a**) Total PLFA, a measure of microbial biomass, and (**b**) the ratio of fungal:bacterial PLFA, a measure of shifts in the relative abundance of fungi and bacteria within the whole microbial community. Values are means ± SE. Control is the same treatment without earthworms

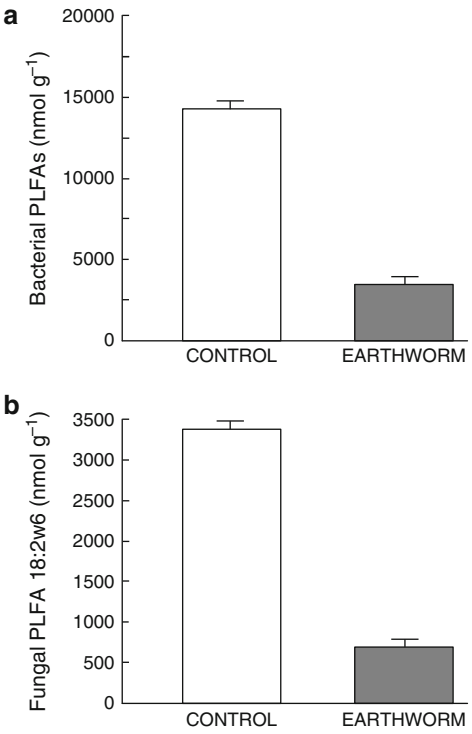

Fig. 5.8 Effect of earthworms (*Eisenia andrei*) on microbial communities after vermicomposting of cow manure (for 1 month): (**a**) Bacterial biomass, calculated as the sum of the bacterial PLFA markers: i14:0, i15:0, a15:0, i16:0, 16:1w5, 16:1w7, i17:0, a17:0, 10Me18:0, 18:1w7, cy17:0 and cy19:0 and (**b**) PLFA 18:2ω6,9, a measure of fungal biomass. Values are means ± SE. Control is the same treatment without earthworms

(Fig. 5.8). Earthworms can reduce microbial biomass directly by selective feeding on bacteria and fungi (Schönholzer et al. 1999) or indirectly by accelerating the depletion of resources for the microbes.

5.6.1.2 Bacterial and Fungal Growth

In our studies bacterial growth was estimated by the use of the leucine incorporation technique (Bååth 1994), as modified by Bååth et al. (2001), and fungal growth with the acetate-in-ergosterol incorporation technique (Newell and Fallon 1991) as modified by Bååth (2001). Earthworm activity greatly decreased the bacterial growth rate and did not affect the fungal growth rate after 1 month of vermicomposting (Fig. 5.9). Animal manures are microbiologically-rich environments in which bacteria constitute the largest fraction, with fungi mainly present as spores (Garrett 1981); moreover, the first stages of decomposition in these organic wastes are mainly dominated by bacteria because of the availability of water and easily decomposable substrates. Hence, the activity of earthworms is expected to affect the bacterial growth rate to a greater extent than the fungal growth rate. In addition, carbon availability is a limiting factor for earthworm growth and it has been

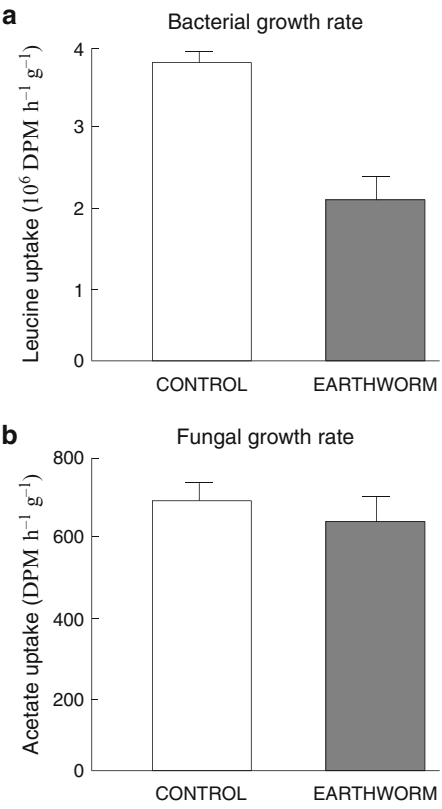

Fig. 5.9 Impact of earthworms (*Eisenia andrei*) on microbial growth after vermicomposting of cow manure (for 1 month): (**a**) Bacterial growth rate estimated as incorporation of leucine, and (**b**) Fungal growth rate estimated as incorporation of ac-in-erg. Values are means ± SE. Control is the same treatment without earthworms

reported that earthworms and microorganisms may compete for carbon resources (Tiunov and Scheu 2004); thus, earthworm activity may have reduced the quantity of resource available for microbial communities, and consequently the bacterial growth rate. The fungal growth rate is expected to decrease during the maturation stage, when depletion of more recalcitrant compounds takes place.

5.6.2 *Effects of Earthworms on the Activity of Microbial Communities*

As discussed earlier in this chapter, there is extensive evidence in the literature suggesting that earthworms and other soil animals grazing on microbes enhance microbial activity at the first instance. As a result of this, earthworm activity reduces later the availability of the resources for the microbial communities, and consequently their activity. Thus, in our experiment, the microbial activity

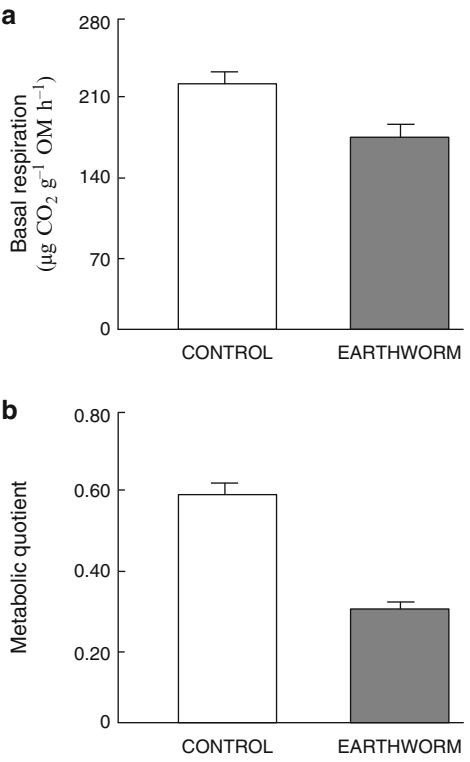

Fig. 5.10 Effect of earthworms (*Eisenia andrei*) on microbial community function after vermicomposting of cow manure (for 1 month): (**a**) Basal respiration, a measure of microbial activity, and (**b**) the metabolic quotient estimated as the amount of CO_2 released from the sample per unit of biomass. Values are means ± SE. Control is the same treatment without earthworms

measured as basal respiration decreased after 1 month of vermicomposting with the earthworm species *E. andrei* (Fig. 5.10a).

Organic carbon taken up by the heterotrophic microbial communities is partitioned between microbial cell biomass production, metabolite excretion and respiration. The proportion of substrate carbon retained as microbial biomass relative to carbon respired as CO_2 depends on the efficiency of microbial growth (i.e., the efficiency with which substrates are incorporated into biomass and by-products), as well as on the degree of protection of microbial biomass in the organic matrix and on the rate of decomposition of bacterial and fungal by-products by other microorganisms. Thus, the lower the microbial growth efficiency or the less protected the biomass, the greater the amount of carbon lost as CO_2 (Six et al. 2006). The metabolic quotient or specific activity of the microbial biomass (qCO_2; microbial respiration per unit biomass) can be used as a measure of microbial efficiency (Anderson and Domsch 1993; Wardle and Ghani 1995); higher values of qCO_2 indicate that microbial communities are under conditions of higher stress. Thus, less of the energy yielded by substrate metabolism will be used for biosynthetic purposes. An important portion of this energy will be expended on cell maintenance and lost as CO_2. Earthworm activity reduced the metabolic quotient after 1 month

of vermicomposting (Fig. 5.10b), indicating, that microbial communities used the available energy more efficiently in the presence of earthworms. As a consequence, the system functioned much better, as shown by the large increase in the rate of decomposition of the organic matter (Fig. 5.5.) and in the rate of nitrogen mineralization (Fig. 5.6). The effect of earthworms on C and N mineralization rates is density-dependent (Aira et al. 2008).

5.6.3 *Effect of Earthworms on Total Coliforms during Vermicomposting*

Earthworms also greatly reduced the presence of total coliforms during vermicomposting. The passage through the gut of the earthworm species *E. andrei*, *E. fetida* and *Eu. eugeniae* reduced the density of total coliforms by 98%, relative to fresh pig slurry (Fig. 5.11a) (Monroy et al. 2008). The same drastic reduction in the density of total coliforms was also found in another experiment after 2 weeks of vermicomposting with *E. fetida* (Monroy 2006). The reductions in total coliforms were similar to those reported by Eastman et al. (2001) for these and other human pathogens, which indicate the effectiveness of vermicomposting at reducing the levels of human pathogens during stabilization of biosolids and other organic wastes. As discussed earlier, digestion of decaying substrate by earthworms decreases the availability of nutrients for microorganisms, thereby decreasing microbial numbers in casts and altering the microbial composition (Brown 1995). There is increasing evidence that earthworms have a specific gut microflora (Karsten and Drake 1995; Horn et al. 2005), and the decrease in total coliforms also may be related to competitive interactions between coliforms and microorganisms that are specific to the earthworm gut (Brown and Mitchell 1981). Moreover, the negative effect of the passage through the earthworm gut observed in enterobacteria such as *Serratia marcescens*, *Escherichia coli* and *Salmonella enteridis* (Day 1950; Brüsewitz 1959; Brown and Mitchell 1981) suggests the occurrence of selective effects on the ingested microorganisms.

5.6.4 *Effect of Earthworms on the Composition of Microbial Communities*

The discriminant analysis of 25 PLFAs (i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, 16:19, 16:17, 16:15, 16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, 10Me17:0, 18:26,9, 18:19, 18:17, 18:0, 10Me18:0, cy19:0, 20:46, 20:53, 20:36) clearly differentiated the vermicomposts obtained with three different epigeic earthworm species (*E. andrei*, *E. fetida* and *P. excavatus*), irrespective of what manure type (cow, horse or rabbit) was used in vermicomposting (Fig. 5.12). This indicates that there were

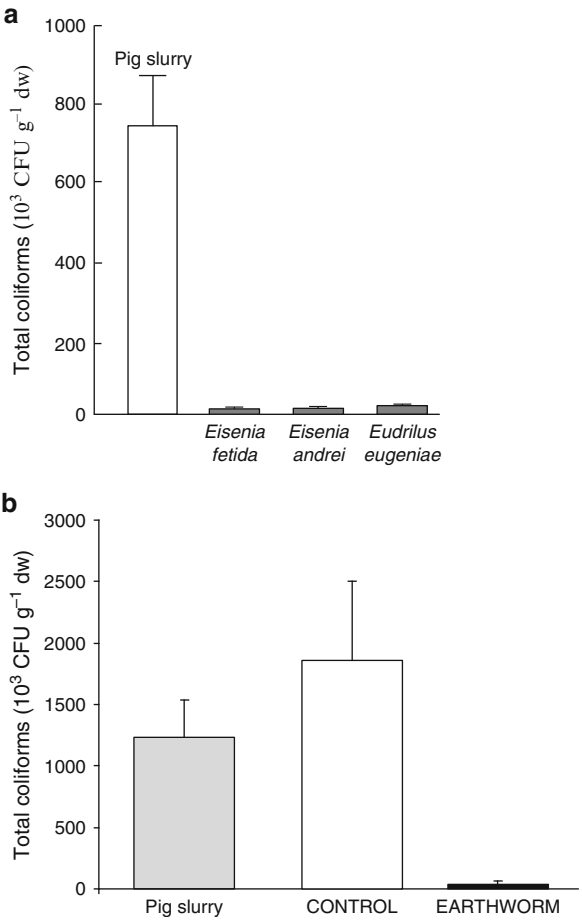

Fig. 5.11 Effect of earthworms on total coliforms in pig slurry after: (**a**) the transit through the gut of three species of epigeic earthworms, and (**b**) 2 weeks of vermicomposting with *Eisenia fetida*. Values are means ± SE. Control is the same treatment without earthworms

different PLFA profiles associated with the vermicomposts, not related to the type of animal manure used, but rather to the earthworm species and/or their endosymbiotic gut microflora. Moreover, the separation between vermicomposts and control substrates (manures processed without earthworms) was also very clear (Fig. 5.12), indicating that earthworms play a key role in shaping the structure of the microbial community in organic wastes during the vermicomposting process. Similar results were also found with fatty acid methyl ester (FAME) profiles (Lores et al. 2006). From this perspective and since different vermicomposts produced by different earthworm species and from different types of organic wastes contain an enormous and specific variety of microorganisms, it is possible to obtain specific vermicomposts for different practical applications. This may especially be important in

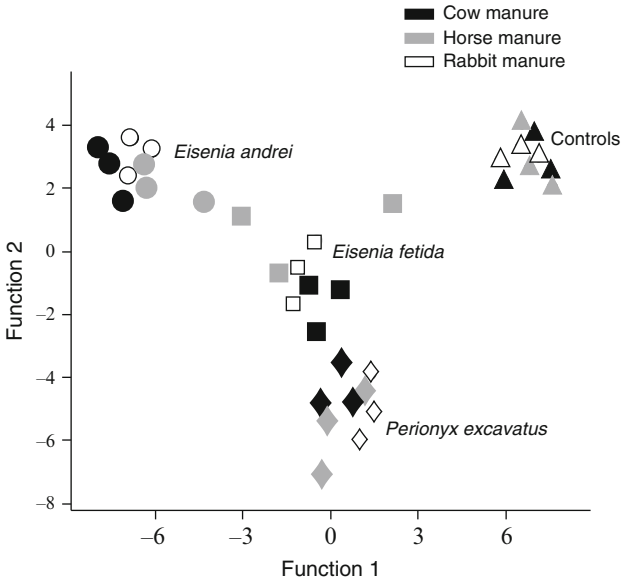

Fig. 5.12 Earthworm species-specific modification of the microbial community composition after vermicomposting of three animal manures (cow, horse and rabbit) for 1 month. Discriminant analysis of the PLFAs (Wilks´λ = 0.00099, $p < 0.0001$) from the vermicompost obtained with three epigeic earthworm species (*Eisenia andrei*, *E. fetida* and *Perionyx excavatus*). Controls are the same treatment without earthworms. Functions 1 and 2 represent 65 and 29% of the variance respectively

producing plant container media and for impoverished and/or intensively fertilized soils.

5.6.5 *Molecular Tools Applied to Vermicomposting Studies*

Molecular tools are commonly used for investigating microbial communities in ecological studies (see Hultman et al. 2010, Insam et al. 2010, Knapp et al. 2010, Minz et al. 2010). Such tools include clone libraries, fluorescent in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) analysis. Each of these methods measures different aspects of the community such as diversity, in situ detection, and community dynamics, and all of them are based on 16S rRNA gene sequences (Deutschbauer et al. 2006). Although these techniques nowadays are frequently used in composting research (Danon et al. 2008; Franke-Whittle et al. 2009), their application in vermicomposting is very scarce (Fracchia et al. 2006;

Vivas et al. 2009; Sen and Chandra 2009). Application of these techniques has shown that compost and vermicompost differ greatly in their microbial communities, and that a higher microbial diversity exists in vermicompost relative to the initial substrate than in compost (Fracchia et al. 2006; Vivas et al. 2009; Sen and Chandra 2009).

5.7 Conclusions

Vermicomposting is a bio-oxidative process in which detritivore earthworms interact intensively with microorganisms in decomposition processes, accelerating the stabilization of organic matter and greatly modifying its physical and biochemical properties. Earthworms are crucial drivers of the process as they are involved in the indirect stimulation of microbial populations through fragmentation and ingestion of fresh organic matter. Earthworms reduce microbial biomass and activity during the vermicomposting process. The activity of epigeic earthworms drastically reduces the viable microbial biomass during the vermicomposting process and this reduction is proportionally higher for fungi than for bacteria. After 1 month of vermicomposting the bacterial growth rate decreases in the substrate whereas the fungal growth rate is not affected. Microbial activity measured as basal respiration decreases after vermicomposting. Earthworm activity helps microbial communities to use the available energy more efficiently and plays a key role in shaping the structure of the microbial community in organic wastes during the vermicomposting process. These evidences indicate that detritivorous earthworms directly modulate the decomposer community composition in the short term accelerating the decomposition of organic matter.

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References

Aira M, Domínguez J (2008a) Optimizing vermicomposting of animal wastes: effects of dose of manure application on carbon loss and microbial stabilization. J Environ Manage 88: 1525–1529

Aira M, Domínguez J (2008b) Microbial and nutrient stabilization of two animal manures after the transit through the gut of the earthworm *Eisenia fetida* (Savigny, 1826). J Hazard Mater. doi:10.1016/j.jhazmat.2008.04.073

Aira M, Monroy F, Domínguez J, Mato S (2002) How earthworm density affects microbial biomass and activity in pig manure. Eur J Soil Biol 38:7–10

Aira M, Monroy F, Domínguez J (2005) Ageing effects on nitrogen dynamics and enzyme activities in casts of *Aporrectodea caliginosa* (Lumbricidae). Pedobiologia 49:467–473

Aira M, Monroy F, Domínguez J (2006a) Changes in microbial biomass and microbial activity of pig slurry after the transit through the gut of the earthworm *Eudrilus eugeniae* (Kinberg, 1867). Biol Fertil Soils 42:371–376

Aira M, Monroy F, Domínguez J (2006b) *Eisenia fetida* (Oligochaeta, Lumbricidae) activates fungal growth, triggering cellulose decomposition during vermicomposting. Microb Ecol 52:738–746

Aira M, Monroy F, Domínguez J (2007a) *Eisenia fetida* (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure. Microb Ecol 54:662–671

Aira M, Monroy F, Domínguez J (2007b) Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermicomposting independently of the application rates of pig slurry. Sci Total Environ 385:252–261

Aira M, Sampedro L, Monroy F, Domínguez J (2008) Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. Soil Biol Biochem 40:2511–2516

Anderson JPE, Domsch KH (1993) The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem 25:393–395

Atiyeh R, Dominguez J, Subler S, Edwards CA (2000) Changes in biochemical properties of cow manure during processing by earthworms (*Eisenia andrei*) and the effects on seedling growth. Pedobiologia 44:709–724

Bååth E (1994) Measurement of protein synthesis by soil bacterial assemblages with the leucine incorporation technique. Biol Fertil Soils 17:147–153

Bååth E (2001) Estimation of fungal growth rates in soil using ^{14}C-acetate incorporation into ergosterol. Soil Biol Biochem 33:2011–2018

Bååth E, Pettersson M, Söderberg KH (2001) Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biol Biochem 33:1571–1574

Bardgett RD (2005) The biology of soil: a community and ecosystem approach. Oxford University Press, Oxford

Bouché MB (1977) Strategies lombriciennes. In: Lohm U, Persson T (eds) Soil organisms as components of ecosystems, vol 25. Biology Bulletin, Stockholm, pp 122–132

Brown GG (1995) How do earthworms affect microfloral and faunal community diversity? Plant Soil 170:209–231

Brown GG, Doube BM (2004) Functional interactions between earthworms, microorganisms, organic matter, and plants. In: Edwards CA (ed) Earthworm ecology, 2nd edn. CRC, Boca Raton, pp 213–224

Brown BA, Mitchell MJ (1981) Role of the earthworm, *Eisenia foetida*, in affecting survival of *Salmonella enteriditis* ser. *typhimurium*. Pedobiologia 22:434–438

Brüsewitz G (1959) Untersuchungen über den einfluß des regenwurms auf zahl und leistungen von mikroorganismen im bodem. Arch Microbiol 33:52–82

Clarholm M (1994) The microbial loop. In: Ritz K, Dighton J, Giller KE (eds) Beyond the biomass. Blackwell, Oxford, pp 355–365

Clark WP, Taylor M, Cossins R (2007) Evaluation by respirometry of the loading capacity of a high rate vermicompost bed for treating sewage sludge. Bioresour Technol 98:2611–2618

Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y (2008) Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol Ecol 65:133–144

Darwin C (1881) The formation of vegetable mould through the action of worms with observations on their habits. Murray, London

Day GM (1950) Influence of earthworms on soil microorganisms. Soil Sci 69:175–184

de Bertoldi M (2010) Production and utilization of suppressive compost: environmental, food and health benefits. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 153–170

Deutschbauer AM, Chivian D, Arkin AP (2006) Genomics for environmental microbiology. Curr Opin Biotechnol 17:229–235

Domínguez J (2004) State of the art and new perspectives on vermicomposting research. In: Edwards CA (ed) Earthworm ecology, 2nd edn. CRC, Boca Raton, pp 401–424

Domínguez J, Edwards CA (1997) Effects of stocking rate and moisture content on the growth and maturation of *Eisenia andrei* (Oligochaeta) in pig manure. Soil Biol Biochem 29:743–746

Domínguez J, Parmelee RW, Edwards CA (2003) Interactions between *Eisenia andrei* (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia 47:53–60

Eastman BR, Kane PN, Edwards CA, Trytek L, Gunadi B, Stermer AL, Mobley JR (2001) The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. Compost Sci Util 9:38–49

Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms. Chapman and Hall, London

Elvira C, Goicoechea M, Sampedro L, Mato S, Nogales R (1996) Bioconversion of solid paper pulp mill sludge by earthworms. Bioresour Technol 57:173–177

Elvira C, Sampedro L, Benítez E, Nogales R (1998) Vermicomposting of sludges from paper mill and dairy industries with *Eisenia andrei*: a pilot-scale study. Bioresour Technol 63:205–211

Fracchia L, Dohrmann AB, Martinotti MG, Tebbe CC (2006) Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. Appl Microbiol Biotechnol 71:942–952

Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H (2009) Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microb Ecol 57:510–521

Frostegård Å, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Gajalakshmi S, Abbasi SA (2004) Vermiconversion of paper waste by earthworm born and grown in the waste-fed reactors compared to the pioneers raised to adulthood on cow dung feed. Bioresour Technol 94:53–56

Gajalakshmi S, Ramasamy EV, Abbasi SA (2005) Composting–vermicomposting of leaf litter ensuing from the trees of mango (*Mangifera indica*). Bioresour Technol 96:1057–1061

Garg P, Gupta A, Satya S (2006) Vermicomposting of different types of waste using *Eisenia foetida*: a comparative study. Bioresour Technol 97:391–395

Garrett SD (1981) Soil fungi and soil fertility. Pergamon, Oxford

Gupta R, Mutiyar PK, Rawat NK, Saini MS, Garg VK (2007) Development of a water hyacinth based vermireactor using an epigeic earthworm *Eisenia foetida*. Bioresour Technol 98:2605–2610

Horn MA, Ihssen J, Matthies C, Schramm A, Acker G, Drake HL (2005) *Dechloromonas denitrificans* sp. nov., *Flavobacterium denitrificans* sp. nov., *Paenibacillus anaericanus* sp. nov. and *Paenibacillus terrae* strain MH72, N_2O-producing bacteria isolated from the gut of the earthworm *Aporrectodea caliginosa*. Int J Syst Evol Microbiol 55:1255–1265

Hultman J, Kurola J, Raininsalo A, Kontro M, Romantschuk M (2010) Utility of molecular tools for optimization of large scale composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg pp 135–152

Insam I, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Karsten GR, Drake HL (1995) Comparative assessment of the aerobic and anaerobic microflora of earthworm guts and forest soils. Appl Environ Microbiol 61:1039–1044

Kaushik P, Garg VK (2003) Vermicomposting of mixed solid textile mill sludge and cow dung with the epigeic earthworm *Eisenia foetida*. Bioresour Technol 90:311–316

Knapp B, Ros M, Insam H (2010) Do composts affect the soil microbial community? In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 271–292

Lavelle P, Spain AV (2001) Soil Ecology. Kluwer, London
Lavelle P, Bignell D, Lepage M, Wolters V, Roger P, Ineson P, Heal OW, Ghillion S (1997) Soil function in a changing world: the role of invertebrate ecosystem engineers. Eur J Soil Biol 33:159–193
Lazcano C, Gómez-Brandón M, Domínguez J (2008) Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chemosphere 72:1013–1019
Lores M, Gómez-Brandón M, Pérez-Díaz D, Domínguez J (2006) Using FAME profiles for the characterization of animal wastes and vermicomposts. Soil Biol Biochem 38:2993–2996
Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–230
Monroy F (2006) Efecto das miñocas (clase Oligochaeta) sobre a comunidade descompoñedora durante o proceso de vermicompostaxe. PhD Thesis, Universidade de Vigo, Spain
Monroy F, Aira M, Domínguez J (2008) Changes in density of nematodes, protozoa and total coliforms after transit through the gut of four epigeic earthworms (Oligochaeta). Appl Soil Ecol 39:127–132
Moore JC, Berlow EL, Coleman DC, de Ruiter PC, Dong Q, Johnson NC, McCann KS, Melville K, Morin PJ, Nadelhoffer K, Rosemond AD, Post DM, Sabo JL, Scow KM, Vanni MJ, Wall DH (2004) Detritus, trophic dynamics and biodiversity. Ecol Lett 7:584–600
Newell SY, Fallon RD (1991) Toward a method for measuring instantaneous fungal growth rates in field samples. Ecology 72:1547–1559
Nogales R, Elvira C, Benitez E, Thompson R, Gómez M (1999a) Feasibility of vermicomposting dairy biosolids using a modified system to avoid earthworm mortality. J Environ Sci Health B 34:151–169
Nogales R, Melgar R, Guerrero A, Lozada G, Benítez E, Thompson R, Gómez M (1999b) Growth and reproduction of *Eisenia andrei* in dry olive cake mixed with other organic wastes. Pedobiologia 43:744–752
Nogales R, Cifuentes C, Benítez E (2005) Vermicomposting of winery wastes: a laboratory study. J Environ Sci Health B 40:659–673
Parthasarathi K, Ranganathan LS (2000) Ageing effect on enzyme activities in pressmud vermicasts of *Lampito mauritii* (Kingberg) and *Eudrilus eugeniae* (Kingberg). Biol Fertil Soils 30:347–350
Pramanik P, Ghosh GK, Ghosal PK, Banik P (2007) Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresour Technol 98:2485–2494
Sampedro L, Domínguez J (2008) Stable isotope natural abundances ($\delta^{13}C$ and $\delta^{15}N$) of the earthworm *Eisenia fetida* and other soil fauna living in two different vermicomposting environments. Appl Soil Ecol 38:91–99
Sampedro L, Jeannotte R, Whalen JK (2006) Trophic transfer of fatty acids from gut microbiota to the earthworm *Lumbricus terrestris* L. Soil Biol Biochem 38:2188–2198
Scheu S (2002) The soil food web: structure and perspectives. Eur J Soil Biol 38:11–20
Schönholzer F, Dittmar H, Zeyer J (1999) Origins and fate of fungi and bacteria in the gut of *Lumbricus terrestris* L. studied by image analysis. Fems Microbiol Ecol 28:235–248
Sen B, Chandra TS (2009) Do earthworms affect dynamics of functional response and genetic structure of microbial community in a lab-scale composting system? Bioresour Technol 100:804–811
Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555–569
Suthar S (2007) Vermicomposting potential of *Perionyx sansibaricus* (Perrier) in different waste material. Bioresour Technol 98:1231–1237
Tiunov AV, Scheu S (2004) Carbon availability controls the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization. Oecologia 138:83–90

Triphati G, Bhardwaj P (2004) Decomposition of kitchen waste amended with cow manure using an epigeic species (*Eisenia fetida*) and an anecic species (*Lampito mauritii*). Bioresour Technol 92:215–218

Vetter S, Fox O, Ekschmitt K, Wolters V (2004) Limitations of faunal effects on soil carbon flow: density dependence, biotic regulation and mutual inhibition. Soil Biol Biochem 36:387–397

Vivas A, Moreno B, García-Rodríguez S, Benítez E (2009) Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. Bioresour Technol 100:1319–1326

Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem 12:1601–1610

Zelles L (1997) Phospholipid fatty acid profiles in selected members for soil microbial communities. Chemosphere 35:275–294

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biol Fertil Soils 29:111–129

Chapter 6
Compost Microbial Activity Related to Compost Stability

An Ceustermans, Jozef Coosemans, and Jaak Ryckeboer

Abstract In this chapter, compost stability has been reviewed in general and in relation to microbial activity. The evolution of microbial activity during composting is discussed. Another issue that has been reviewed is the relationship between temperature and microbial activity during the composting of biowaste. Different methods to measure compost stability, as well as chemical, physical and biological methods have been considered. Moreover, an overview is given about the implications of the use of non-stabilized compost and about the benefits of the use of compost in agriculture.

Contents

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6.1 Introduction

Due to the intensive agricultural systems, the organic matter content of soils lowers continuously (Baize et al. 1999). Moreover, landscape preservation hampers the production of traditional organic matter, i.e., peat (Rivière et al. 2008). Therefore, an enhanced interest in the use of compost in agriculture is certainly desirable. Addition of compost to soil not only improves soil structure, soil pore system characteristics and lowers bulk density (Pagliai et al. 2004; Lynch et al. 2005; Tsadilas et al. 2005; Wahba 2007), but can also increase the C/N ratio, increases soil organic carbon concentration (Lynch et al. 2005) and leads to an enhanced enrichment of organic nitrogen in the soil (Amlinger et al. 2003).

The principal requirement of compost for a safe use in soil is its degree of stability, implying a stable organic matter content and the absence of phytotoxic compounds and plant or animal pathogens (Bernal et al. 1998; Ryckeboer 2001), as the application of non-stabilized compost to soil may cause several phytotoxicity effects and can adversely affect the environment (Butler et al. 2001). Consequently, compost stability is an important issue to guarantee a safe use of this end-product and thus benefit both agriculture and the environment (Baffi et al. 2007). The level of stability and maturity of composts is an essential aspect of compost quality (McAdams and White 1996; Lasaridi and Stentiford 1998a,b; Benito et al. 2003; Huang et al. 2006; Said-Pullicino et al. 2007; Gómez-Brandón et al. 2008). In this chapter, compost stability is reviewed in general and in relation to microbial activity. Different methods that have been proposed to determine compost stability based on physical, chemical and biological parameters are summarized. At the end of the chapter, stability is also linked to the agricultural use of composts.

6.2 Important Factors During Composting

Composting is a common method to treat organic waste and is based on the partial oxidation of readily oxidizable organic matter, creating a more stable, humic, organic matrix in the process (Bio-Logic Environmental Systems 2001). The composting process is generally characterized by a short mesophilic period initially and then a rapid transition to the thermophilic period. After the subsequent decrease in temperature, the curing period or compost stabilization stage starts (Tang et al. 2007).

The principal factors that contribute to making an optimum environment for the microbial processes in composting are oxygen, temperature, moisture, C/N ratio and pH (de Bertoldi et al. 1983; Ryckeboer et al. 2003b; Neklyudov et al. 2008). Interstitial O_2 concentrations should not fall below 10% to maintain aerobic microbial metabolism (Vallini et al. 2002). Lower O_2 concentrations can delay organic matter decomposition, what might lead to anaerobic conditions (Steger et al. 2005) and thus, an increased methane emission (Beck-Friis et al. 2003).

Moreover, inadequate O_2 levels lead to the establishment of an anaerobic microflora, which can produce odoriferous compounds and phytotoxic metabolites (Vallini et al. 2002). The effects of C/N ratio on composting have been investigated by many researchers, who generally recommend the range between 20 and 30 as the optimum C/N ratio (Sadaka and El-Taweel 2003). Vallini et al. (2002) reported that a C/N ratio of 25/1 to 30/1 is considered ideal for faster compost stabilization. Higher values slow down the rate of decomposition while nitrogen becomes the limiting factor, and lower ones result in nitrogen loss in the form of ammonia whereas the available carbon may be fully utilized (de Bertoldi et al. 1983; Vallini et al. 2002; Sadaka and El-Taweel 2003). Microbes driving compost stabilization operate best in the range of pH between 6.5 and 8.0. Nevertheless, the natural self-correcting or buffering capacity of the process makes it possible to proceed over the much wider range of 5.5 to 9.0 (Vallini et al. 2002). Besides these factors also temperature and moisture content play an important role during composting and will be discussed in relation to microbial activity (see Sect. 6.3, Chap. 1, Insam et al. 2010; Chap. 11, Fuchs 2010).

6.3 Microbial Activity During Composting

Own work shows that the total biological activity follows a typical evolution during the composting process (Fig. 6.1). At the start of the process, there is a pronounced decrease in biological activity together with increasing temperatures during the heat peak. Also Saludes et al. (2007) observed a low microbial activity (indicated by low ATP content) on day 1 probably caused by a very high composting temperature

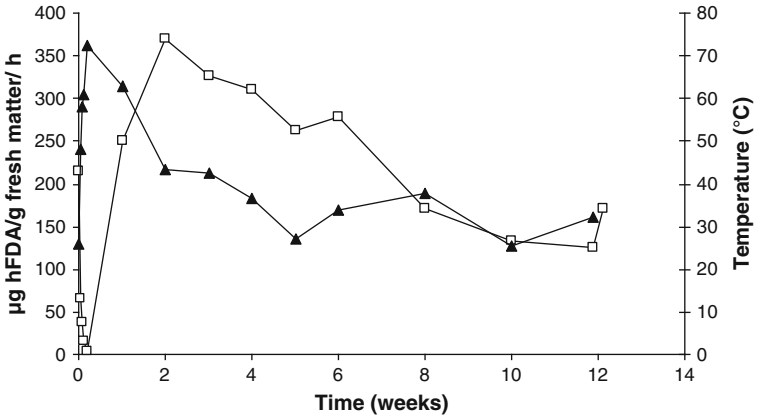

Fig. 6.1 Typical evolution of biological activity (expressed as µg hFDA g^{-1} fresh matter per h) in function of time (weeks) and temperature (°C). Parameters: ▲ = temperature, □ = total biological activity

(about 67°C), resulting in the inactivation of fungi, actinomycetes and bacteria. Miyatake and Iwabuchi (2005) also observed a decrease in bacterial activity at temperatures over 60°C, not attributable to microbial extinction but to the decrease in metabolic activity. As temperature drops, there is an increase in biological activity. This is followed by a decrease in both temperature and biological activity. At the end of the composting process the total biological activity gets stable and reaches a basic level with low and stable end-values. The drop in biological activity at the end of the process is probably due to a lack of easy decomposable organic compounds and points to compost stability (Benítez et al. 1999; Ryckeboer et al. 2003a). Also Tiquia (2005) found no further decomposition towards the end of the composting process: O_2 consumption rate, ATP content and dehydrogenase activities (all indicators of microbial activity) dropped and stabilized to low levels.

During composting metabolic heat is produced as a byproduct through the activity of the indigenous microbial community, which decomposes the usable matter for energy and growth substrates (Vestal and McKinley 1986). Temperature is probably the most important factor affecting microbial metabolism during this process (Vallini et al. 2002) and therefore the most critical parameter influencing the rate of composting and the quality of the product (Vestal and McKinley 1986).

Temperature is simultaneously a consequence and a determinant of the microbial activity (Vallini et al. 2002; Gea et al. 2007). On the one hand, temperature is a consequence as heat production during composting is almost completely derived from biological activity and because it changes in direct response to heat production (Yu et al. 2008). On the other hand, microbial activity is dependent on the temperature. Several researchers have tried to define the optimal temperature for composting (Nakasaki et al. 1985). However, many of the findings on optimum temperatures for maximum decomposition rate during composting are contradictory. These discrepancies may be partly due to the indirect and incomplete nature of many of the studies concerning microbial activity and biomass in composting material (Vestal and McKinley 1986). Moreover, substrate composition influences the optimal composting temperature, that is, there is a difference in optimum compost temperatures between food wastes that contain easily degradable compounds and more resistant substrates (Eklind et al. 2007). For rapid composting high temperatures for long periods must be avoided, as ammonia emissions increase at higher composting temperatures, resulting in higher N losses (Eklind et al. 2007). However, the waste material must be exposed to a sufficiently high temperature for a sufficiently long period of time to ensure an effective inactivation of pathogens (de Bertoldi et al. 1983; Ryckeboer 2001; Vinnerås et al. 2003; Haug 1993). For further details on pathogen inactivation see Chap. 9, Vinnerås et al. (2010).

After this stage optimal temperatures vary from 45 to 55°C (de Bertoldi et al. 1983). Some researchers indicate 60°C as the optimal temperature during composting according to maximal respiration rates (O_2 uptake rate and CO_2 evolution rate) (Suler and Finstein 1977; Nakasaki et al. 1985; Cronjé et al. 2004) while others demonstrated that lower temperatures might allow higher microbial activities (McKinley and Vestal 1984, 1985; Vestal and McKinley 1986; Miyatake and Iwabuchi 2006). The effect of high compost temperatures on enzymatic activity

was studied by Miyatake and Iwabuchi (2005). When the temperature increased to 63°C, an overall reduction in bacterial diversity was observed. At 66°C, bacterial diversity increased again, and a diverse group of bacteria including *Thermus* spp. and thermophilic *Bacillus* spp. appeared to adapt to the higher temperature. At 70°C, the activity of these diverse bacteria increased, but the decomposition of manure organic matter was reduced due to the higher temperature.

The decrease in temperature at the end of the composting process is not always observed. Metabolic heat generation is combined with the thermal inertia effect found in compost materials due to their self-insulating properties (Haug 1993). As water holds more heat and transfers heat more effectively than air, the thermal properties of compost increase with an increasing number of water-filled pores. Especially the water content and density of the compost, has a significant effect on the temperature of the composting process (Ahn et al. 2006). Thermophilic temperatures can be maintained during the maturation stage in the core of the pile, because of the self-insulating properties of the compost (Barrena et al. 2006). The heat generated by the biological activity is retained in the composting mass. This phenomenon is not observed in laboratory or pilot scale studies, in which temperature decreases rapidly when the easily biodegradable organic matter is consumed and where the insulating effect is less (Ryckeboer et al. 2003a). For this reason, temperature is not a good parameter to predict biological activity and compost stability at full scale composting plants.

Moisture content has been referred to as a critical factor to optimize compost-engineering systems because decomposition of organic matters depends on the presence of water to support microbial activity (Luo et al. 2008), as water provides a medium of transport of dissolved nutrients required for the metabolic and physiological activities of microorganisms (McCartney and Tingley 1998). Thus, the evolution of the moisture content during composting and the moisture content of the final compost give an idea about how well a composting process is managed. In the work of Liang et al. (2003) the moisture content had an even greater influence on microbial activity than the temperature. Also the study of Margesin et al. (2006) indicated that low moisture content is a more limiting factor for composting than low temperature. Optimum metabolic rates can be achieved by reaching the maximum water content that does not restrict O_2 transfer and utilization (Vallini et al. 2002). Additionally, optimal moisture content differs between various starting materials (Madejón et al. 2002), which depends on the physical state and size of the particles (de Bertoldi et al. 1983; Forshell 1993) and is related to maintenance of a certain free air space (Madejón et al. 2002). Many investigators have conducted experiments and identified that 50–60% moisture content is suitable for efficient composting (Suler and Finstein 1977; Tiquia et al. 1998; Lin 2008; Mohee et al. 2008). Moreover, Liang et al. (2003) reported that 50% moisture content is the minimal requirement for obtaining adequate microbial activities during biosolid composting. On the other hand, their experimental design could not detect an upper limit. However, when the initial moisture content is too high, this situation can lead to the production of biologically unstable compost, as high water content could influence gaseous

exchange by limiting diffusion and thus restricting oxygen utilization by microorganisms, resulting in decreased microbial activity (Tiquia et al. 1996; Sundberg and Jönsson 2008). In addition, biosolids showed significant compressive behaviour and lower air permeabilities with increasing moisture content (Das and Keener 1997).

Also, when the moisture content is too low, microbial activity is reduced, as shown by the lower CO_2 evolution rate (Nakasaki et al. 1994; Ryckeboer et al. 2003b) and this can cause early dehydration, which arrests the biological process, resulting in physically stable but biologically unstable compost (de Bertoldi et al. 1983). Decomposition slows dramatically in mixtures below 40% moisture (Vallini et al. 2002, Liang et al. 2003). Adding a water absorbent (an organic high polymer) seems to be a good method to adjust the initial moisture content of the compost, because it has been shown to induce the longest duration of thermophilic stage and inhibitive temperature stage at the bottom of the pile (Luo et al. 2008).

6.4 Compost Stability

6.4.1 Definition of Compost Stability

First of all, it is important to differentiate between compost stability and compost maturity; often these two terms are used synonymously. Compost stability reflects the degree of decomposition of the organic matter (Chen 2003). McAdams and White (1996) defined compost stability as the point where readily degradable substrate is diminished so that its decomposition rate does not control the overall rate of decomposition. According to Albrecht et al. (2008) compost stability refers to the degree to which composts have been decomposed to more stable organic materials. Also Lasaridi and Stentiford (1998a) defined stability as the extent to which readily biodegradable organic matter has decomposed. A material is considered unstable if it contains a high proportion of biodegradable matter that may sustain high microbial activity (Tiquia 2005; Barrena Gómez et al. 2006). In most publications, compost stability is related to biological activity (Butler et al. 2001; Tiquia 2005; Zmora-Nahum et al. 2005), because stability increases as biological activity decreases (ADAS Consulting Limited 2005). Since composting is characterized by a high degree of microbiological activity, which ceases when all available nutrients have been consumed, compost with a low biological activity is described as being stable (Pearson et al. 2004). In this way, compost stability refers to the level of activity of the microbial biomass and can be determined by the O_2 uptake rate, the CO_2 production rate or by the heat released as a result of microbial activity (Iannotti et al. 1993, 1994). On the other hand, compost maturity is often characterized by germination indexes, which are a measure of phytotoxicity (Cunha Queda et al. 2002), by the degree of decomposition of phytotoxic organic substances produced during the active composting stage

(Wu et al. 2000) or by nitrification (Bernal et al. 1998) and has been associated with the degree of compost humification (Jouraiphy et al. 2005). A compost which is mature is likely also stable. Yet stable compost may not always be at a level of maturity adequate for use as a medium for growing a certain species of plant (McAdams and White 1996).

6.4.2 Compost Stability Evaluation

Several authors agree that no single technique or method can successfully be used to evaluate compost stability because the differences between raw materials used to produce compost are so great that these may affect biological stability itself (Mondini et al. 2003). Many parameters, with varying degrees of reliability and technical complications, are used for the assessment of compost stabilization (Lasaridi and Stentiford 1998b).

6.4.2.1 Physicochemical Parameters

Physical parameters include temperature, odor and color (Iglesias Jiménez and Pérez García 1989; de Guardia et al. 2004). Temperature has been suggested to be one of the most convenient parameters for stability evaluation (Huang et al. 2003). However Gea et al. (2004) observed that temperature is not always useful for monitoring biological activity and thus biological stability (see Sect. 6.3). A problem with using temperature as a stability indicator is the influence of other factors like pile size and weather conditions (McAdams and White 1996). The generally unpleasant odor of refuse decreases during the first stages of the bio-oxidation phase and practically disappears by the end of the composting process (Iglesias Jiménez and Pérez García 1989). The end-product, after a sufficiently long period of maturation, has a dark brown or almost black color (Iglesias Jiménez and Pérez García 1989). As odor and color are two subjective parameters, these are not considered as accurate stability indicators (de Guardia et al. 2004). Physical characteristics give a general idea of the decomposition stage reached, but give little information as regards the degree of maturation (Bernal et al. 1998).

Chemical parameters such as pH, electrical conductivity (EC), cation exchange capacity (CEC), C/N ratio, thermal analyses and humification parameters have been applied as indicators of stability (Iglesias Jiménez and Pérez García 1989; Mondini et al. 2003; de Guardia et al. 2004; Melis and Castaldi 2004; Gómez et al. 2007; Gómez-Brandón et al. 2008). Although pH is a parameter that is often determined, it does not seem to offer any information on the course of the process or the quality of the compost according to Lasaridi and Stentiford (1998b). Also Francou et al. (2005) concluded that pH was not a good indicator of compost stability as little variations of pH were observed. According Wu et al. (2000), pH as well as EC, may

be used to monitor compost stabilization processes, but with relatively consistent source waste composition and calibration of other stability tests. A low value of final pH may signify that organic acids remain in the material and that composting time was insufficient. However, pH around neutrality is not sufficient to consider that the organic matter is stable (de Guardia et al. 2004). The absence of a rigorous tendency of the evolution of electrical conductivity during composting, the influence of the drying effect, which may be responsible for conductivity increases without any real increase of the quantity of ions, the expected dependency on the initial composition of the material and the complexity of reactions between ions and organic matter prevent to use conductivity as a stability indicator (Lasaridi and Stentiford 1998b; de Guardia et al. 2004). During efficient composting, the C/N ratio is expected to decrease as a consequence of degradation of organic matter and mineralization (Harada et al. 1981; Margesin et al. 2006). Bernal et al. (1998) reported that the C/N ratio was the most suitable parameter they studied for describing the stability of different composts as it correlated with many chemical characteristics of the material during composting. However, according to Namkoong et al. (1999) the C/N ratio could not be considered as a reliable index of compost maturity, as it changed irregularly with time. Moreover, as the variation of the C/N ratio during composting depends on the type of compost, it is of limited usefulness (Lasaridi and Stentiford 1998b). In the study of Pascual et al. (1997) for instance, the C/N ratio varied between wastes from 6.9 to 24.8, which suggests that a C/N ratio of less than 15, proposed by other authors as an indicator of organic matter stability, cannot always be used, particularly in the case of sewage sludges, since their high nitrogen content leads to C/N ratios of below 15 although the organic matter is fresh.

Cation-exchange capacity (CEC) increases as compost approaches stability (McAdams and White 1996). This increase is a function of humification due to the formation of carboxyl and phenolic functional groups (Lax et al. 1986). But the wide variations of CEC of initial substrates prevent to define threshold values and to use it as a stability indicator (de Guardia et al. 2004).

Determination of the humification rate (HR) and the humification index (HI) demonstrated to be effective indicators of the formation of humic-like substances during compost maturation, being able to establish with accuracy the moment of complete stabilization (Ciavatta et al. 1990). Humification parameters, based on the fractionation of the extractable humic-like and non-humic organic C, have been successfully used for evaluating the stability level of compost (Tittarelli et al. 2002). The time course of the humification parameters – humification index (HI) and degree of humification (DH) – allowed to detect different rates of organic matter transformation during the process in the study of Mondini et al. (2003). After 19 days the variations were less pronounced, indicating a slower transformation rate, as a function of the decrease of substrate available for biological processes and the increased stability of the organic matter in the compost.

Thermal analyses seem to be useful for the characterization of the compost organic matter, because of their rapid determination and simplicity. They are based on a programmed heating of the samples under a controlled atmosphere which finally provides qualitative and quantitative information regarding the compost

humic matter content. In the study of Melis and Castaldi (2004), thermoanalytical data resulted in being useful in integrating quantitative information coming from chemical analysis of humified fraction of compost organic matter. Differential scanning calorimetry (DSC) curves allowed distinguishing between well and poorly stabilized organic matter. Information deriving from weight losses, registered by the thermogravimetry (TG) and differential TG (DTG) curves enable to individuate the evolution state of the organic matter and therefore its stability.

In the study of Castaldi et al. (2005) the water soluble carbon (WSC) concentration showed a significant correlation with time, which makes it a suitable parameter to indicate the degree of compost stabilization. Also the water soluble carbon to nitrogen ratio (WSC/N) can be used as a parameter to indicate the stability of the tested materials. The lowest mean values for WSC/N were presented by the mature compost in the work of Pascual et al. (1997).

6.4.2.2 Biological Parameters

Also biological parameters, such as oxygen and CO_2 respirometry and enzyme activities have been proposed to measure stability (Lasaridi and Stentiford 1998a). Respiration (CO_2 evolution rate and/or O_2 uptake rate) can be considered as a general measure of microbial activity and has been widely used to evaluate the stability of the compost (Lasaridi and Stentiford 1998a,b; Heerenklage and Stegmann 2005; Barrena Gómez et al. 2006; Scaglia et al. 2007; Kalamdhad et al. 2008). The basis of these methods is that immature compost has a strong demand for O_2 and high CO_2 production rates due to the intense development of microorganisms as a consequence of the easily biodegradable compounds in the raw material. Conversely, at late composting stages, both processes decline as the amount of degradable organic matter decreases (Barrena Gómez et al. 2005). As a consequence, the respiration rate in stable composts is significantly lower than in raw waste (Lasaridi and Stentiford 1998b).

Carbon dioxide evolution is the most direct technique of compost stability because it measures carbon derived directly from the compost being tested (Kalamdhad et al. 2008). CO_2 production is directly correlated with the aerobic respiration (Barrena Gómez et al. 2006). However, monitoring of CO_2 evolution presents two major drawbacks: (1) the solubility of CO_2 in aqueous solutions and (2) this solubility is pH-dependent (Barrena Gómez et al. 2006).

Methods based on O_2 uptake rate (OUR) have been classified into two different classes: dynamic and static methods. Dynamic methods are those in which a continuous supply of air is used throughout the assay minimizing O_2 diffusion limitations (Barrena Gómez et al. 2006). When static methods are used, underestimation of oxygen uptake is possible, as they do not allow the oxygen to be dispersed throughout the biomass (Adani et al. 2001). The problem can be solved by continuous biomass stirring and periodical aeration to replenish the oxygen consumed by the microorganisms (specific oxygen uptake rate or SOUR test;

Lasaridi and Stentiford 1998a). The SOUR is a simple technique for the assessment of compost stability (Lasaridi and Stentiford 1998a; Gazi et al. 2007). Oxygen consumption of an aqueous compost suspension is measured instead of a solid matrix, as in most traditional respiration tests. By doing this, the test is not affected by variations in the matric water potential of the samples, since there is immediate contact between substrate, microbes and oxygen leading to maximum reaction rates; and the gas-liquid barrier for oxygen diffusion at the surface to the compost particles is omitted (Lasaridi and Stentiford 1998a).

Another way to solve the problem of underestimation of oxygen uptake by static methods is the continuous aeration of the biomass (dynamic respiration index DRI; Adani et al. 2001). The DRI measurement represents a reproduction of the full-scale process using a laboratory approach (Adani et al. 2006) and determines the biological stability of composts because it is able to measure the easily degradable organic fraction (Baffi et al. 2007). Oxygen uptake is ascertained by measuring the difference in oxygen concentration between the inlet and outlet airflows passing through the biomass. Two DRIs are defined depending on the operating conditions: (1) the real dynamic respiration index (RDRI) and (2) the potential dynamic respiration index (PDRI), without or with adjustment to optimal moisture content respectively (Adani et al. 2001). The static respiration index (SRI) is determined using the same scientific apparatus as that used for determining the DRI. The oxygen uptake rate is ascertained by following the changes in oxygen concentration with time, in the air-space on top of the solid sample in the sealed container, after which aeration is stopped (Adani et al. 2001).

Barrena Gómez et al. (2005) built a static respirometer to determine the respiration index (RI) of composting samples at different temperatures. Respiration indices were determined at 37°C (RI_{37}) and at the in situ temperature of the composter at sampling (RI_T).

In Europe, the respiration activity after 4 days (AT_4) and the DRI are recommended in the Second Draft of the Working Document on the Biological Treatment of biowaste as parameters for the estimation of compost stability (European Commission 2001). AT_4 respiration activity may be determined in a respirometer (Sapromat), in which the CO_2 produced by microorganisms is absorbed by a base so that a negative pressure builds up. A sensitive pressure sensor gives a signal to an electrolytical oxygen production cell which produces oxygen until normal pressure conditions are restored (Heerenklage and Stegmann 2005). Alternatives such as the Oxitop method exist (Veeken et al. 2003; Wagland et al. 2009).

In addition to the aerobic respirometric methods, there is also the Dewar self-heating test, which measures the heat produced by the sample under aerobic conditions, rather than the gases consumed or produced (Wagland et al. 2009). The principle of the method is to precisely record the highest temperature achieved after placement of compost into the vessel for several days (Brinton et al. 1995). The rate of microbial respiration affects the heat output which is reflected in the rise in temperature. The higher the temperature reaches, the less stable the material is (Cabañas-Vargas et al. 2005). The self-heating test is widely adopted at solid waste composting plants in Europe to determine the biological stability of the compost

produced. It is a simple test and a good indicator of compost organic matter stability and does not require sophisticated equipment (Francou et al. 2005).

The Solvita maturity index provides a simple, relatively inexpensive relative test of compost stability and NH_3 emission for diverse samples of compost. Solvita measures CO_2 evolution and ammonia emissions simultaneously. A compost sample of optimum water content is allowed to equilibrate at 25°C in partially closed plastic bags and loaded into jars up to the fill line. CO_2 and NH_3 test gel-paddles are inserted into the compost. After a 4 h incubation at 25°C the gel color change is observed and used to determine CO_2 values on a scale of 1 to 8 and NH_3 values on a scale of 1 to 5 (Changa et al. 2003).

Enzymatic activities have been proposed as a simple, quick and a cheap measure for compost stabilization (Mondini et al. 2004; Tiquia 2005). Enzymes play a role during composting as they are implicated in the biological and biochemical processes (Vuorinen 2000; Tiquia 2002). Important enzymes during composting include cellulases, β-glucosidases, amidohydrolase, proteases, urease, phosphatases and arylsulphatase (Mondini et al. 2004). Therefore, enzymatic activities are suitable as indicators of the state and evolution of the organic matter during composting (Benítez et al. 1999; Castaldi et al. 2008) and as stated earlier the stability of the end-product (Mondini et al. 2004; Gómez-Brandón et al. 2008). According to Tiquia (2005) dehydrogenase activity is the simplest, quickest and cheapest method to monitor compost stability compared with respiration rate, ATP content and microbial biomass procedures. In her study the decrease in dehydrogenase activity to low levels towards the end of composting indicated that there was no more active decomposition going on. Mondini et al. (2004) reported that the formation of a stable enzymatic activity, either in moist or air-dried compost samples, could represent a useful index of stabilization. Moreover, the use of enzymatic activity as an indicator of compost stability was supported by the final values of conventional chemical parameters. Also FDA (Fluorescein diacetate) hydrolysis activity, as a parameter of overall hydrolytic activity, has been suggested by García-Gómez et al. (2003), Ryckeboer et al. (2003a) and Cayuela et al. (2008) as a valid parameter for measuring the degree of biological stability of the composting material. In the study of Cayuela et al. (2008) FDA increased during the composting of two-phase olive mill waste and was able to classify compost samples with different degree of stability. In addition, FDA correlated with important stability indices.

6.4.2.3 Spectroscopic Methods

Nuclear magnetic resonance spectroscopy of carbon (^{13}C NMR) is a valid tool for examining the chemical structure of natural organic matter (OM) and the chemical changes associated with OM decomposition. Changes can be measured on the bulk OM either fresh or composted, on humic substances extracted from the compost or on dissolved OM (Chen 2003). According to Gómez et al. (2007) the carbon

distribution determined from ^{13}C NMR spectra can be used as a mean for quantification of the conversion experienced by the organic matter under biological stabilization.

Fourier transform-infrared spectroscopy (FT-IR) is a technique that is efficient in providing comprehensive information on chemical composition of heterogeneous materials (Wagland et al. 2009). FT-IR characterizes the principal classes of chemical groups that compose the OM (Chen 2003). Several indicator bands that are referred to functional groups represent components or metabolic products. Their presence and intensity or their absence shed light on the phase of degradation or stabilization. Therefore, the rapid assessment of the stage of OM decomposition in waste materials is a very important field of application. Moreover, the infrared spectrum that is used for the qualitative assessment is less affected by the heterogeneity of the material than single parameters due to diverse indicator bands that provide information. Classification of waste materials regarding quality properties (stability) is possible by means of the spectral pattern (Smidt and Meissl 2007).

6.5 Stability and Agricultural Use of Composts

6.5.1 The Role of Compost in Preserving Soil Quality

Compost has the unique ability to improve soil properties and the growing media physically (structure), chemically (nutrition) and biologically (Wahba 2007; Chap.13, Bastida et al. 2010). The degree of stability required may depend on the end-use of the compost. Complete stability is not readily attainable and not likely desirable as there would be no soil amendment value due to low or nonexistent organic content. On the other hand, compost with a high potential for continuing decomposition can adversely affect crop growth (see Sect. 6.5.2). There is, therefore, a level of stability which must be met based on the end-use of the product (Ministry of Environment, Ontario 2004).

Addition of compost to soil improves soil structure and soil pore system characteristics while it lowers bulk density (Lynch et al. 2005; Pagliai et al. 2004; Tsadilas et al. 2005; Wahba 2007). In the study of Bazzoffi et al. (1998) results showed the positive lasting effect of compost in ameliorating physical properties of the soil and reducing water runoff and soil erosion. Compost can increase the C/N ratio, increases soil organic carbon (Lynch et al. 2005) and leads to an enhanced enrichment of organic nitrogen in the soil (Amlinger et al. 2003). Tsadilas et al. (2005) found that after three years of biosolids application, organic matter content, water retention capacity, available water and infiltration rate significantly increased, whereas aggregate instability index decreased. In a study of Speir et al. (2004), the soil's total C, N and P and CEC and exchangeable cations increased after the previous application of compost indicating a potential long-term enhancement of

nutrient storage and supply. Total-extractable and EDTA-extractable metals (Cd, Cr, Cu, Ni, Pb and Zn) were also elevated, which is due to the impurity of the waste feedstocks of this type of composting; however none of the heavy metals approached soil limit concentrations except for Cu. According to Albrecht et al. (2008) several studies showed that compost application to the soil stimulated beneficial biological activity by increasing microbial biomass and enzyme activities. Also Albiach et al. (2000) observed that the annual application of organic residues led to a significant increase of soil enzyme activities. Guerrero et al. (2007) reported that as well as composted, noncomposted manure increased microbial biomass, but that the use of composted manure is preferred in order to avoid an excessive inorganic N production. The finished compost product can be beneficial to plants, i.e. through improvement of plant development in e.g. *Arachis hypogaea* (Ravindran et al. 2007) and leek and cauliflower (De Rooster 2006) or can induce suppression of diseases e.g. *Fusarium* crown and *Fusarium* wilt of tomato (Cotxarrera et al. 2002; Cheuk et al. 2005) and pathogens including *Pythium ultimum* (Erhart et al. 1999) and *Rhizoctonia solani* apart from many others (Ryckeboer 2001, De Clercq et al. 2004). Mulching with compost (of vegetable, fruit and garden waste) suppressed *Colletotrichum acutatum* in strawberry plant nursery (Meurrens and Demeyer 2003). Disease-suppression of composts has been reviewed by Bailey and Lazarovits (2003) and De Clercq et al. (2004), and is further discussed in Chapters. 8 and 11.

6.5.2 Implications of the Use of Non-Stabilized Compost

One problem associated with immature or unstable compost is its continued decomposition in soil. This can induce anaerobic conditions as the microbial biomass utilizes oxygen in the soil pores to break down the material (Butler et al. 2001). This in turn can deprive plant roots of oxygen and lead to production of hydrogen sulphide (H_2S) and nitrite (NO_2^-) (Mathur et al. 1993).

Immature compost with a high C/N ratio can induce nitrogen starvation in plants as microbes scavenge soil N to make up for the deficit (Iglesias Jiménez and Pérez García 1989; Bernal et al. 1998; Butler et al. 2001; Gómez-Brandón et al. 2008), starved roots of oxygen due to a high microbial activity, support growth of pathogens as *Salmonella* and *Pythium* and create high levels of organic acids (Inbar et al. 1990). Moreover, N immobilization in soil is thought to have caused low yield of ryegrass plants (Bernal et al. 1998). On the other hand, immature composts with a low C/N ratio create high ammonia concentrations, resulting in ammonium toxicity in plants (Bio-Logic Environmental Systems 2001).

The immaturity of compost can also result in phytotoxicity and the use of this kind of material as a soil amendment causes phytotoxic reactions to the crops (Reinikainen and Herranen 2001). On the one hand, phytotoxicity may be of internal origin due to the temporarily production of harmful byproducts during some of the intermediate stages in the composting process, i.e. an excess of NH_4^+,

the presence of phenolic substances or organic acids such as acetic acid, propionic acid and n-butyric acid (Smith and Hughes 2004). The inhibitory effects of acetic acid are stated on weed suppression (Ozores-Hampton et al. 1999), seed germination and root length of cucumber (Shiralipour et al. 1997). Phytotoxic substances of internal origin can be eliminated for a large part thanks to the composting process (Pascual et al. 1997). On the other hand, phytotoxicity can be of external origin: the bioavailability of heavy metals is a function of the degree of maturity of the compost, since the humic material is capable of binding them. Experiments have shown that metals become less available with increasing maturity (Déportes et al. 1995). A degradation of environmental contaminants has also been reported by the use of spent mushroom compost (Lau et al. 2003). Phytotoxic effects from compost of different maturity degree were also observed on germinating ryegrass. In this study phytotoxicity was mainly related to pH and electrical conductivity of the compost extract (Zubillaga and Lavado 2006).

6.6 Conclusion

Optimal compost use in agriculture needs an appropriate determination of compost stability in relation to microbial activity. Stability prevents nutrients from becoming tied up in rapid microbial growth, allowing them to be available for plant needs (Kalamdhad et al. 2008). However, evaluating compost stability is difficult due to the widely different properties of the organic substrates (Mondini et al. 2003) and the degree of stability required may depend on the end use of the compost (Ministry of Environment, Ontario 2004).

To date there is no single parameter that can give a sure indication of the stability of composts from different starting materials (Mondini et al. 2003). Therefore, one has to use a combination of methods depending on the intended compost use (Reinikainen and Herranen 2001). Numerous authors have suggested the use of different indices (Mondini et al. 2003, Baffi et al. 2007), e.g. FDA hydrolysis rate in addition to measurement of CO_2-evolution (Levanon and Pluda 2002), or e.g. CO_2-evolution in combination with ammonia emissions (Solvita; Changa et al. 2003).

References

Adani F, Lozzi P, Genevini P (2001) Determination of biological stability by oxygen uptake on municipal solid waste and derived products. Compost Sci Util 9:163–178

Adani F, Ubbiali C, Generini P (2006) The determination of biological stability of composts using the dynamic respiration index: the results of experience after two years. Waste Manage 26: 41–48

ADAS Consulting Limited (2005) Assessment of options and requirements for stability and maturity testing of composts. The Waste and Resources Action Programme, Banbury

Ahn H, Sauer TJ, Richard TL, Glanville, TD (2006) Determination of thermal properties of composting bulking materials. In: Agricultural and Biosystems Engineering Conference Proceedings. Available at: http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=204730. Accessed 27 Apr 2009

Albiach R, Canet R, Pomares F, Ingelmo F (2000) Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Bioresource Technol 75:43–48

Albrecht R, Joffre R, Gros R, Le Petit J, Terrom G, Périssol C (2008) Efficiency of near-infrared reflectance spectroscopy to assess and predict the stage of transformation of organic matter in the composting process. Bioresource Technol 99:448–455

Amlinger F, Götz B, Dreher P, Geszti J, Weissteiner C (2003) Nitrogen in biowaste and yard waste compost: dynamics of mobilisation and availability – a review. Eur J Soil Biol 39:107–116

Baffi C, Dell'Abate MT, Nassisi A, Silva S, Benedetti A, Genevini PL, Adani F (2007) Determination of biological stability in compost: a comparison of methodologies. Soil Biol Biochem 39:1284–1293

Bailey KL, Lazarovits G (2003) Suppressing soil-borne diseases with residue management and organic amendments. Soil Tillage Res 72:169–180

Baize D, Bidoglio G, Cornu S, Brus D, Breuning-Madsen H, Eckelmann W, Ernstsen V, Gorny A, Jones RJA, King D, Langenkamp H, Loveland PJ, Lobnik F, Magaldi D, Montanarella L, Utermann J, van Ranst R (1999) Heavy metals (trace elements) and organic matter content of European soils. A feasibility study. Report by the European Soil Bureau – Scientific Committée, Ispra

Barrena R, Canovas C, Sánchez A (2006) Prediction of temperature and thermal inertia effect in the maturation stage and stockpiling of a large composting mass. Waste Manage 26:953–959

Barrena Gómez R, Vázquez Lima F, Gordillo Bolasell MA, Gea T, Sánchez Ferrer A (2005) Respirometric assays at fixed and process temperatures to monitor composting process. Bioresource Technol 96:1153–1159

Barrena Gómez R, Vázquez Lima F, Sánchez Ferrer A (2006) The use of respiration indices in the composting process: a review. Waste Manage Res 24:37–47

Bastida F, Hernández T, García C (2010) Soil degradation and rehabilitation: microorganisms and functionality. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 253–270

Bazzoffi P, Pellegrini S, Rocchini A, Morandi M, Grasselli O (1998) The effect of urban refuse compost and different tractors tyres on soil physical properties, soil erosion and maize yield. Soil Tillage Res 48:275–286

Beck-Friis B, Smars S, Jonsson H, Eklind Y, Kirchmann H (2003) Composting of source-separated household organics at different oxygen levels: Gaining an understanding of the emission dynamics. Compost Sci Util 11:41–50

Benítez E, Nogales R, Elvira C, Masciandaro G, Ceccanti B (1999) Enzyme activities as indicators of the stabilization of sewage sludges composting with *Eisenia foetida*. Bioresource Technol 67:297–303

Benito M, Masaguer A, Moliner A, Arrigo N, Palma RM (2003) Chemical and microbiological parameters for the characterization of the stability and maturity of pruning waste compost. Biol Fertil Soils 37:184–189

Bernal MP, Paredes C, Sánchez-Monedero A, Cegarra J (1998) Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresource Technol 63:91–99

Bio-Logic Environmental Systems (2001) Report on assessing compost maturity: Appendix A – Preliminary study. Responsible Waste & Resource Management, Dartmouth

Brinton WF, Evans E, Droffner ML, Brinton RB (1995) Standardized test for evaluation of compost self-heating. Biocycle 36:64–69

Butler TA, Sikora LJ, Steinhilber PM, Douglass LW (2001) Compost age and sample storage effects on maturity indicators of biosolids compost. J Environ Qual 30:2141–2148

Cabañas-Vargas DD, Sánchez-Monedero MA, Urpilainen ST, Kamilaki A, Stentiford EI (2005) Assessing the stability and maturity of compost at large-scale plants. Ingeniería 9–2:25–30

Castaldi P, Alberti G, Merella R, Melis P (2005) Study of the organic matter evolution during municipal solid waste composting aimed at identifying suitable parameters for the evaluation of compost maturity. Waste Manage 25:209–213

Castaldi P, Garau G, Melis P (2008) Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. Waste Manage 28:534–540

Cayuela ML, Mondini C, Sánchez-Monedero MA, Roig A (2008) Chemical properties and hydrolytic enzyme activities for the characterization of two-phase olive mill wastes composting. Bioresource Technol 99:4255–4262

Ciavatta C, Govi M, Vittori Antisari L, Sequi P (1990) Characterization of humified compounds by extraction and fractionation on solid polyvynilpyrrolidone. J Chromatogr 509:41–146

Changa CM, Wang P, Watson ME, Hoitink HAJ, Michel FC Jr (2003) Assessment of the reliability of a commercial maturity test kit for composted manures. Compost Sci Util 11:125–143

Chen Y (2003) Nuclear magnetic resonance, infra-red and pyrolysis studies of solid waste composts. Compost Sci Util 11:152–168

Cheuk W, Lo KV, Copeman R, Joliffe P, Fraser BS (2005) Disease suppression on greenhouse tomatoes using plant waste compost. J Environ Sci Health 40:449–461

Cotxarrera L, Trillas-Gay MI, Steinberg C, Alabouvette C (2002) Use of sewage sludge compost and *Trichoderma asperellum* isolates to suppress *Fusarium* wilt of tomato. Soil Biol Biochem 34:467–476

Cronjé AL, Turner C, Williams AG, Barker AJ, Guy S (2004) The respiration rate of composting pig manure. Compost Sci Util 12:119–129

Cunha Queda AC, Vallini G, Agnolucci M, Coelho CA, Campos L, de Sousa RB (2002) Microbiological and chemical characterization of composts at different levels of maturity, with evaluation of phytotoxicity and enzymatic activities. In: Insam H, Riddech N, Klammer S (eds) Microbiology of Composting. Springer Verlag, Heidelberg, pp 345–355

Das K, Keener M (1997) Moisture effect on compaction and permeability in composts. J Environ Eng 123:275–281

de Bertoldi M, Vallini G, Pera A (1983) The biology of composting: a review. Waste Manage Res 1:157–176

De Clercq D, Vandesteene L, Coosemans J, Ryckeboer J (2004) Use of compost as suppressor of plant diseases. In: Lens P, Hamelers B, Hoitink H, Bidlingmaier W (eds) Resource recovery and reuse in organic solid waste management. IWA, London, pp 317–337

de Guardia A, Tremier A, Martinez J (2004) Indicators for determination of stability of composts and recycled organic wastes. In: Lens P, Hamelers B, Hoitink H, Bidlingmaier W (eds) Resource recovery and reuse in organic solid waste management. IWA, London, pp 338–376

Déportes I, Benoit-Guyod J-L, Zmirou D (1995) Hazard to man and the environment posed by the use of urban waste compost: a review. Sci Total Environ 172:197–222

De Rooster L (2006) Compostgebruik in de groenteteelt (Compost use in horticulture). Proeftuinnieuws 16:17–19

Eklind Y, Sundberg C, Smårs S, Steger K, Sundh I, Kirchmann H, Jönsson H (2007) Carbon turnover and ammonia emissions during composting of biowaste at different temperatures. J Environ Qual 36:1512–1520

Erhart E, Burian K, Hartl W, Stich K (1999) Suppression of *Pythium ultimum* by biowaste composts in relation to compost microbial biomass, activity and content of phenolic compounds. J Phytopathol 147:299–305

European Commission (2001) Working Document, Biological Treatment on Biowaste, Second Draft, Brussels

Forshell LP (1993) Composting of cattle and pig manure. J Vet Med 40:634–640

Francou C, Poitrenaud M, Houot S (2005) Stabilization of organic matter during composting: influence of process and feedstocks. Compost Sci Util 13:72–83

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

García-Gómez A, Roig A, Bernal MP (2003) Composting of the solid fraction of olive mill wastewater with olive leaves: organic matter degradation and biological activity. Bioresource Technol 86:59–64

Gazi AV, Kyriacou A, Kotsou M, Lasaridi KE (2007) Microbial community dynamics and stability assessment during green waste composting. Glob NEST J 9:35–41

Gea T, Barrena R, Artola A, Sánchez A (2004) Monitoring the biological activity of the composting process: oxygen uptake rate (OUR), respirometric index (RI), and respiratory quotient (RQ). Biotechnol Bioeng 88:520–527

Gea T, Barrena R, Artola A, Sánchez A (2007) Optimal bulking agent particle size and usage for heat retention and disinfection in domestic wastewater sludge composting. Waste Manage 27: 1108–1116

Gómez X, Diaz MC, Cooper M, Blanco D, Morán A, Snape CE (2007) Study of biological stabilization processes of cattle and poultry manure by thermogravimetric analysis and ^{13}C NMR. Chemosphere 68:1889–1897

Gómez-Brandón M, Lazcano C, Domínguez J (2008) The evaluation of stability and maturity during the composting of cattle manure. Chemosphere 70:436–444

Guerrero C, Moral R, Gómez I, Zornoza R, Arcenegui V (2007) Microbial biomass and activity of an agricultural soil amended with the solid phase of pig slurries. Bioresource Technol 98: 3259–3264

Harada Y, Inoko A, Tadaki M, Izawa T (1981) Maturing process of city refuse compost during piling. Soil Sci Plant Nutr 27:357–364

Haug RT (1993) The practical handbook of compost engineering. Lewis, Boca Raton

Heerenklage J, Stegmann R (2005) Analytical methods for the determination of the biological stability of waste samples. In: Proceedings of Tenth International Waste Management and Landfill Symposium, S Margherita di Pula, Cagliari, Italy

Huang G, Zhong L, Zhang Z, Wu Q (2003) Physiochemical changes and maturity evaluation of solid organic waste compost. Ying Yong Sheng Tai Xue Bao 14:813–818

Huang GF, Wu QT, Wong JWC, Nagar BB (2006) Transformation of organic matter during co-composting of pig manure with sawdust. Bioresource Technol 97:1834–1842

Iannotti DA, Pang T, Toth BL, Elwell DL, Keener HM, Hoitink HAJ (1993) A quantitative respirometric method for monitoring compost stability. Compost Sci Util 1:52–65

Iannotti DA, Grebus ME, Toth BL, Madden LV, Hoitink HAJ (1994) Oxygen respirometry to assess stability and maturity of composted municipal solid waste. J Environ Qual 23:1177–1183

Iglesias Jiménez E, Pérez García V (1989) Evaluation of city refuse compost maturity: a review. Biol Waste 27:115–142

Inbar Y, Chen Y, Hadar Y, Hoitink HAJ (1990) New approaches to compost maturity. Biocycle 31:64–69

Insam H, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Jouraiphy A, Amir S, El Gharous M, Revel J-C, Hafidi M (2005) Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste. Int Biodeterior Biodegradation 56:101–108

Kalamdhad AS, Pasha M, Kazmi AA (2008) Stability evaluation of compost by respiration techniques in a rotary drum composter. Resour Conserv Recycl 52:829–834

Lasaridi KE, Stentiford EI (1998a) A simple respirometric technique for assessing compost stability. Water Res 32:3717–3723

Lasaridi KE, Stentiford EI (1998b) Biological parameters for compost stability assessment and process evaluation. In: Proceedings of the International Symposium on Composting and Use of Composted Materials for Horticulture, Ayrshire, Scotland, pp 119–128

Lau KL, Tsang YY, Chiu SW (2003) Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52:1539–1546
Lax A, Roig A, Costa F (1986) A method for determining the cation-exchange capacity of organic materials. Plant Soil 94:349–355
Levanon D, Pluda D (2002) Chemical, physical and biological criteria for maturity in composts for organic farming. Compost Sci Util 10:339–346
Liang C, Das KC, McClendon RW (2003) The influence to temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. Bioresource Technol 86:131–137
Lin C (2008) A negative-pressure aeration system for composting food wastes. Bioresource Technol 99:7651–7656
Luo W, Chen TB, Zhen GD, Gao D, Zhang YA, Gao W (2008) Effect of moisture adjustments on vertical temperature distribution during forced-aeration static-pile composting of sewage sludge. Resour Conserv Recycl 52:635–642
Lynch DH, Voroney RP, Warman PR (2005) Soil physical properties and organic matter fractions under forages receiving composts, manure or fertilizer. Compost Sci Util 13:252–261
Madejón E, Díaz MJ, López R, Cabrera F (2002) New approaches to establish optimum moisture content for compostable materials. Bioresource Technol 85:73–78
Margesin R, Cimadom J, Schinner F (2006) Biological activity during composting of sewage sludge at low temperatures. Int Biodeterior Biodegradation 57:88–92
Mathur SP, Owen G, Dinel H, Schnitzer M (1993) Determination of compost biomaturity. I. Literature review. Biol Agric Hortic 10:87–108
McAdams MW, White RK (1996) Compost stability determination. In: Composting in the Carolinas – Proceedings of the 1996 Conference, Myrtle Beach, South Carolina, 23–25 October. Available at: http://www.p2pays.org/ref/12/11501
McCartney D, Tingley J (1998) Development of a rapid moisture content method for compost materials. Compost Sci Util 6:14–25
McKinley VL, Vestal JR (1984) Biokinetic analyses of adaptation and succession: microbial activity in composting municipal sewage sludge. Appl Environ Microbiol 47:933–941
McKinley VL, Vestal JR (1985) Physical and chemical correlates of microbial activity and biomass in composting municipal sewage sludge. Appl Environ Microbiol 50:1395–1403
Melis P, Castaldi P (2004) Thermal analysis for the evaluation of the organic matter evolution during municipal solid waste aerobic composting process. Thermochim Acta 413:209–214
Meurrens F, Demeyer A (2003) GFT-compost veelbelovend tegen *Colletotrichum* in de opkweek van plantgoed van aardbeien (Biowaste-compost promising against *Colletotrichum* in the plant nursery of strawberry). Proeftuinnieuws 13:33–34
Ministry of Environment (2004) Interim guidelines for the production and use of aerobic compost in Ontario. Ontario Ministry of the Environment
Miyatake F, Iwabuchi K (2005) Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost. Bioresource Technol 96:1821–1825
Miyatake F, Iwabuchi K (2006) Effect of compost temperature on oxygen uptake rate, specific growth rate and enzymatic activity of microorganisms in dairy cattle manure. Bioresource Technol 97:961–965
Mohee R, Driver M-FB, Sobratee N (2008) Transformation of spent broiler litter from exogenous matter to compost in a sub-tropical context. Bioresource Technol 99:128–136
Mondini C, Dell'Abate MT, Leita L, Benedetti A (2003) An integrated chemical, thermal, and microbiological approach to compost stability evaluation. J Environ Qual 32:2379–2386
Mondini C, Fornasier F, Sinicco T (2004) Enzymatic activity as a parameter for the characterization of the composting process. Soil Biol Biochem 36:1587–1594
Nakasaki K, Shoda M, Kubota H (1985) Effect of temperature on composting of sewage sludge. Appl Environ Microbiol 50:1526–1530
Nakasaki K, Aoki N, Kubota H (1994) Accelerated composting of grass clippings by controlling moisture level. Waste Manage Res 12:13–20

Namkoong W, Hwang E-Y, Cheong J-G, Choi J-Y (1999) A comparative evaluation of maturity parameters for food waste composting. Compost Sci Util 7:55–62

Neklyudov AD, Fedotov GN, Ivankin AN (2008) Intensification of composting processes by aerobic microorganisms: a review. Appl Biochem Microbiol 44:6–18

Ozores-Hampton M, Stoffella PJ, Bewick TA, Cantliffe DJ, Obreza TA (1999) Effect of age of cocomposted MSW and biosolids on weed seed germination. Compost Sci Util 7:51–57

Pagliai M, Vignozzi N, Pellegrini S (2004) Soil structure and the effect of management practices. Soil Tillage Res 79:131–143

Pascual JA, Ayuso M, García C, Hernández T (1997) Characterization of urban wastes according to fertility and phytotoxiciy parameters. Waste Manage Res 15:103–112

Pearson J, Lu F, Gandhi K (2004) Disposal of wool scouring sludge by composting. AUTEX Res J 4:147–156 http://www.autexrj.org/No3-2004/0093.pdf

Ravindran KC, Venkatesan K, Balasubramanian T, Balakrishnan V (2007) Effect of halophytic compost along with farmyard manure and phosphobacteria on growth characteristics of *Arachis hypogaea* Linn. Sci Total Environ 384:333–341

Reinikainen O, Herranen M (2001) Different methods for measuring compost stability and maturity. In: Proceedings of the International Symposium on Composting of Organic Matter 549:99–104

Rivière L-M, Morel P, Michel J-C, Charpentier S (2008) Growing media in French Horticulture. In: Proceedings of the International Symposium on growing media 779:33–38

Ryckeboer J (2001) Biowaste and yard waste composts: Microbiological and hygienic aspects – Suppressiveness to plant diseases. PhD Dissertation, Katholieke Universiteit Leuven, Belgium, 278 pp

Ryckeboer J, Mergaert J, Coosemans J, Deprins K, Swings J (2003a) Microbiological aspects of biowaste during composting in a monitored compost bin. J Appl Microbiol 94:127–137

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003b) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349–410

Sadaka S, El-Taweel A (2003) Effects of aeration and C:N ratio on household waste composting in Egypt. Compost Sci Util 11:36–40

Said-Pullicino D, Erriquens FG, Giliotti G (2007) Changes in the chemical characteristics of water-extractable organic matter during composting and their influence on compost stability and maturity. Bioresource Technol 98:1822–1831

Saludes RB, Iwabuchi K, Kayanuma A, Shiga T (2007) Composting of dairy cattle manure using a thermophilic-mesophilic sequence. Biosyst Eng 98:198–205

Scaglia B, Erriquens FG, Gigliotti G, Taccari M, Ciani M, Genevini PL, Adani F (2007) Precision determination for the specific oxygen uptake rate (SOUR) method used for biological stability evaluation of compost and biostabilized products. Bioresource Technol 98:706–713

Shiralipour A, McConnell DB, Smith WH (1997) Phytotoxic effects of a short-chain fatty acid on seed germination and root length of *Cucumis sativus* cv. "poinset". Compost Sci Util 5: 47–52

Smidt E, Meissl K (2007) The applicability of Fourier transform infrared (FT-IR) spectroscopy in waste management. Waste Manage 27:268–276

Smith DC, Hughes JC (2004) Changes in maturity indicators during the degradation of organic wastes subjected to simple composting procedures. Biol Fertil Soils 39:280–286

Speir TW, Horswell J, van Schaik AP, McLaren RG, Fietje G (2004) Composted biosolids enhance fertility of a sandy loam soil under dairy pasture. Biol Fertil Soils 40:349–358

Steger K, Eklind Y, Olsson J, Sundh I (2005) Microbial community growth and utilization of carbon constituents during thermophilic composting at different oxygen levels. Microb Ecol 50:163–171

Suler DJ, Finstein MS (1977) Effect of temperature, aeration, and moisture on CO_2 formation in bench-scale, continuously thermophilic composting of solid waste. Appl Environ Microbiol 33:345–350

Sundberg C, Jönsson H (2008) Higher pH and faster decomposition in biowaste composting by increased aeration. Waste Manage 28:518–526

Tang J-C, Shibata A, Zhou Q, Katayama A (2007) Effect of temperature on reaction rate and microbial community in composting of cattle manure with rice straw. J Biosci Bioeng 104:321–328

Tiquia SM (2002) Evolution of extracellular enzyme activities during manure composting. J Appl Microbiol 92:764–775

Tiquia SM (2005) Microbiological parameters as indicators of compost maturity. J Appl Microbiol 99:816–828

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. Bioresource Technol 55:201–206

Tiquia SM, Tam NFY, Hodgkiss IJ (1998) Changes in chemical properties during composting of spent pig litter at different moisture contents. Agric Ecosyst Environ 67:79–89

Tittarelli F, Trinchera A, Intrigliolo F, Benedetti A (2002) Evaluation of organic matter stability during the composting process of agroindustrial wastes. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Heidelberg, pp 397–406

Tsadilas CD, Mitsios IK, Golia E (2005) Influence of biosolids application on some soil physical properties. Commun Soil Sci Plant Anal 36:709–716

Vallini G, Di Gregorio S, Pera A, Cunha Queda ACF (2002) Exploitation of composting management for either reclamation of organic wastes or solid-phase treatment of contaminated environmental matrices. Environ Rev 10:195–207

Veeken AHM, de Wilde V, Hamelers HVM, Moolenaar SW, Postma R (2003) Oxitop® measuring system for standardized determination of the respiration rate and N-mineralization rate of organic matter in waste material, compost and soil. Availablet at: http://www.nmi-agro.nl/_Public/artikel/oxitop/Oxitop.pdf. Accessed 27 Apr 2009

Vestal JR, McKinley VL (1986) Microbial activity in composting municipal sewage sludge. Project Summary US EPA, EPA/600/S2-86/025

Vinnerås B, Björklund A, Jönsson H (2003) Thermal composting of faecal matter as treatment and possible disinfection method – laboratory-scale and pilot-scale studies. Bioresour Technol 88:47–54

Vinnerås B, Agostini F, Jönsson H (2010) Sanitation by composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 171–192

Vuorinen AH (2000) Effect of the bulking agent on acid and alkaline phosphomonoesterase and β-D-glucosidase activities during manure composting. Bioresource Technol 75:133–138

Wagland ST, Tyrell SF, Godley AR, Smith R (2009) Test methods to aid in the evaluation of the diversion of biodegradable municipal waste (BMW) from landfill. Waste Manage 29:1218–1226

Wahba MM (2007) Influence of compost on morphological and chemical properties of sandy soils, Egypt. J Appl Sci Res 3:1490–1493

Wu L, Ma LQ, Martinez GA (2000) Comparison of methods for evaluating stability and maturity of biosolids compost. J Environ Qual 29:424–429

Yu S, Clark OG, Leonard JJ (2008) A statistical method for the analysis of nonlinear temperature time series from compost. Bioresource Technol 99:1886–1895

Zmora-Nahum S, Markovitch O, Tarchitzky J, Chen Y (2005) Dissolved organic carbon (DOC) as a parameter of compost maturity. Soil Biol Biochem 37:2109–2116

Zubillaga MS, Lavado RS (2006) Phytotoxicity of biosolids compost at different degrees of maturity compared to biosolids and animal manures. Compost Sci Util 14:267–270

Chapter 7
Utility of Molecular Tools in Monitoring Large Scale Composting

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Abstract Composting is an aerobic biological process in which solid organic matter is degraded by microorganisms. The microbiology of composting has been of interest for decades, and microbes in composting have been characterized in many types of composing processes using traditional culture-based methods. In recent years, an immense diversity of bacteria, archaea, and fungi has been found to occupy many different habitats using culture-independent molecular biological methods. Molecular methods which can detect both the culturable and non-culturable fractions of the microbial community are under constant development. In this chapter, several new molecular tools for characterising the microbes present in different composting processes are described, and the advantages and limitations of the application of these methods in studying composting microbiology are discussed.

Contents

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7.1 Introduction

Composting is the aerobic degradation of organic waste into humus-like material. It is not only a waste treatment technique but also a recycling method as the end product can be used in agriculture as a fertiliser, in gardening, or in landscaping. The composting process can be divided into four different stages based on temperature (Gray et al. 1971). In the initial mesophilic stage, the temperature is the same as in the environment, and the pH is low. As the material starts to degrade, the numbers of mesophilic microbes, often lactic acid bacteria and yeasts, increase. The proliferation of these acid-producing microbes causes a further drop in the pH-level. As the temperature rises, a thermophilic microbial flora take over the degradation, and normally this event coincides with the pH turning alkaline. The temperature starts to fall as the resources for the thermophilic microbes become scarce, signifying the beginning of the cooling phase, which is followed by the maturation phase (see also Chap. 6, Ceustermans et al. 2010; Chap. 1, Insam et al. 2010).

Although the principle of composting is rather simple, there are intrinsic problems in industrial large scale composting. When increased amounts of solid organic waste should be composted efficiently, controlling the process or the quality of the end-product may become difficult. For example, if the composting process is not proceeding optimally and the temperature does not rise spontaneously, there is a risk of the survival of pathogens that are often abundant in organic waste material (see Chap. 9, Vinnerås et al. 2010). These microorganisms pose a health risk to the workers in the compost facilities, users of the end product, and also to the general population. According to the European Commission (EC) health rules concerning animal by-products not intended for human consumption, the principal method of ensuring disinfection during composting is by recording time-temperature relationships that destroy pathogens (EC 2002, 2006). Linked to problems with temperature in the large scale composting processes, is a high moisture content, which can lead to low oxygen levels and anaerobic conditions. This, in turn, can cause the decomposition rate to decline and also results in severe odor problems (Tiquia et al. 1996).

Compost microbiota have been studied in different composting scales, from 0.5 L laboratory scale batch units (Schloss et al. 2003), and 30 L synthetic composts made from dog food (Dees and Ghiorse 2001), to large scale (25 m^3 drum to a 136.5 m^2 floor area) composting drums (Ishii and Takii 2003) and piles (Vuorinen and Saharinen 1997; Steger et al. 2007a). In a typical laboratory scale experiment, the process is a closed batch, the material used is usually known, and factors such as rate of compacting, surface to volume ratio, and heat dissipation characteristics differ from those in large scale composting. The laboratory setups (Petiot and de Guardia 2004), although valuable tools (Peters et al. 2000; Schloss et al 2003; Hansgate et al. 2005), cannot, therefore, be used alone to predict the succession and the behavior of microbial communities in the large scale processes. In such processes, waste materials with varying composition are used and the microbes already present in the composting facility function as a seed for the process or alternatively, the composted mass is recycled to act as a seed for the process.

7.1.1 Microbes in the Composting Process

The study of compost microbes has mainly focused on bacteria, although it has been reported that composting is most efficient when both bacteria and fungi are present (Gray et al. 1971). In the mesophilic stages of composting, bacteria from the genera *Lactobacillus* and *Bacillus* are often detected (Ryckeboer et al. 2003). When the temperature rises, the community changes so that thermophilic and thermotolerant bacteria such as *Actinobacteria* (Fergus 1964), *Bacillus* (Blanc et al. 1997) and *Thermus* (Beffa et al. 1996) become abundant (see also Chap. 11, Fuchs 2010).

Fungi have been found to play an important role in the composting process as degraders of lignin and cellulose (Tuomela et al. 2000) and the Eukarya have been found to be numerically abundant in the early stages of composting as well (Schloss et al. 2005). Yeasts have been detected in the mesophilic stages, (Choi and Park 1998) while thermophilic fungi belonging to the Pezizomycota (Fergus 1964) and Zygomycota (von Klopotek 1962; Kane and Mullins 1973) have been found in the thermophilic stages of the process. Basidiomycota become abundant in the cooling and maturation phases of composting (Von Klopotek 1962).

It is now widely accepted that with cultivation-based methods less than one percent of microbes are detected because the growth conditions on any plate or in any cultivation situation are favorable for only a very small portion of the microbes present (Amann et al. 1995; Amann and Ludwig 2000). The development of culture-independent molecular methods for ecological studies of microorganisms (Insam 2001) has however expanded the potential for characterization of microbial community composition in composts. Advantages and limitations of different cultivation dependent and independent methods which have been utilised in studying compost microbiology are described in Table 7.1. Since the development of the polymerase chain reaction (PCR, Saiki et al. 1985) to amplify DNA, the molecular methods for studying microbes and the knowledge on microbial diversity have substantially increased. Many of these methods have been applied to the study of compost microbiology, and thus conversely, monitoring of the composting process could be based on observing changes in microbial composition in different phases of the process. In this chapter, we describe a set of culture-independent molecular tools which can be utilised in fine-scale monitoring of the composting process.

7.2 Molecular Methods to Study Microbial Diversity

Microbial diversity in many different environments has been studied by amplifying the gene of interest (often ribosomal genes or genes representing metabolic functions of interest) with PCR, followed by cloning of the amplified fragments and sequencing. Because of the superior sensitivity of PCR, there is no need to cultivate the microbes as the gene of interest can be amplified directly from the sample (see Chap. 12, Minz et al. 2010).

Table 7.1 Comparison of different methods used in determining microbial community composition in composts.

Method	Advantages	Limitations
Plating	Widely available, relatively easy to use, produces quantitative data	Heavily biassed towards cultivable strains, high detection limit
Plating + pure culturing	Specific identification, provides isolate to work with	Time consuming
MPN[a]	Widely available, relatively easy to use, produces quantitative data	Biased towards cultivable strains, high detection limit
PCR + DGGE	Relatively widely available and easy to use, produces fingerprint profile of the studied community	Requires dedicated equipment, species identification not achieved
PCR+DGGE + sequencing	See PCR + DGGE. Excised sequenced DNA bands give positive identification	High diversity observed may obscure resolution. Requires possibility to sequencing. See also text
Microarrays	Very sensitive (new versions), fairly robust, and fast. Possibility for simultaneous detection of many species.	Expensive machinery, design of the array is time consuming.
Quantitative PCR	Excellent for quantification, fast, and very sensitive detection of target species	Requires skills, targeted for one species at a time, expensive machinery
PLFA[a]	Major microbial groups can be quantified, easy to use, protocol takes a few days	Expensive machinery, low resolution, microbes can not be identified to species level
Random amplification and cloning	PCR fairly sensitive, microbial identification up to species level	Requires skills, takes several days + data-analysis

[a]Most probable number (MPN) and phospho-lipid fatty acid (PLFA) analysis are not described in this chapter

The genes coding for ribosomal RNA (rRNA) have been broadly used in studying microbial diversity with molecular methods. There are several explicit reasons for focusing on rRNA: (i) rRNA is the key element of the protein synthesising machinery and it is present in all organisms, (ii) the rRNA genes are extremely conserved in secondary structure and in nucleotide sequences, which allows alignment of disparate sequences, so that they can be used in phylogenetic analyses, (iii) there is a large amount of rRNA in most cells, and it is easily recovered from all types of organisms, and (iv) the availability of huge rRNA databases which is required for comparative sequence analysis (Amann et al. 1995; Amann and Ludwig 2000; Amaral-Zettler et al. 2008).

The genes coding for the bacterial and the archaeal small and large ribosomal subunits, 16S and 23S rRNA genes, respectively, (Fig. 7.1.), and eukaryal 18S and 28S rRNA genes, contain both conserved and variable regions, and the differences in these genes can be used to infer the relationships between RNA

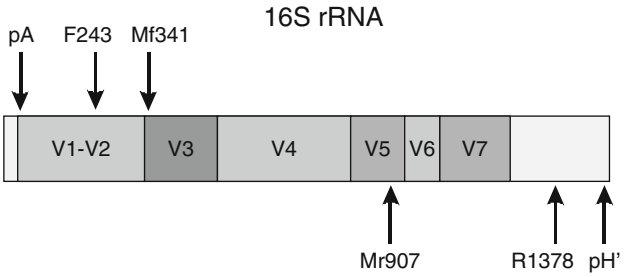

Fig. 7.1 The bacterial 16S rRNA gene and the location of the various primers used for amplification. pA and pH' (Edwards et al. 1989), F243 and R1378 (Heuer et al. 1997), Mf341 and Mr917 (Muyzer et al. 1998). The different variable regions (V1-V7) are marked

Fig. 7.2 Graphical illustration on the location of the internal transcribed spacer areas (ITS) flanking the 18S rRNA gene and 28S rRNA gene. The arrows represent the location of the widely used ITS-primers for PCR amplification (White et al. 1990)

gene sequences from different microbial species (Van de Peer et al. 1996). Several domain and phylum specific primers have been developed for the PCR amplification of rRNA gene regions from bacteria, fungi and archaea (e.g. Edwards et al. 1989; White et al. 1990; Jürgens et al. 1997; Anderson et al. 2003; Baker et al. 2003).

For fungi, the gene coding for 18S rRNA does not provide enough taxonomic resolution and therefore the internal transcribed spacer (ITS) area allocated between the 18S and 28S rRNA gene is used. Primers that are situated within the 18S gene, spanning the ITS1, the 5.8S gene, and the ITS2, all the way into the upstream part of the 28S gene are often utilised (White et al. 1990; Fig. 7.2). Since the ITS area varies in length between different species, the PCR-amplicon produced is between 500 and 1,200 base pairs (bp) in size.

Methods which are based on PCR amplification face certain inherent biases. PCR does not provide amplification products exactly in the same ratios as the genomic templates that are present in the reaction (Poltz and Cavanaugh 1998) and problems with primer selection and the formation of PCR artefacts have been reported (see Chap. 1). The possible bias that has emerged with the use of PCR-based methods could be avoided by using whole genome amplification instead of PCR prior to the ligation detection reaction and microarray hybridisation. This amplification has been successfully applied in a soil microbiome study in which community composition was further studied with cloning and sequencing (Abulencia et al. 2006), as well as in a microarray study by Wu et al (2006).

7.2.1 Sequencing

As described above, the rRNA gene has been widely used in the determination of the microbial contents and diversity of environmental samples, and as a result, several 16S rRNA gene sequence databases have been developed and maintained (Amaral-Zettler et al. 2008). By aligning newly generated sequences to those stored in the databases it is possible to perform a taxonomical annotation, and to determine whether the same sequences have been detected elsewhere. In order to make sense out of the sequence data, several programmes are available for sequence analyses. According to common practise, sequences with $\geq$99% similarity in fungal ITS sequences and $\geq$96% similarity in bacteria can be joined into contigs or phylotypes, and aligned sequences can be further used in phylogenetic analyses.

Partanen and collaborators (2009) studied bacterial diversity in a large scale and a pilot scale composting facility by amplifying the bacterial 16S rRNA gene with the primers pA and pH' (Edwards et al. 1989), then cloning and sequencing over 1,500 complete 16S rRNA gene sequences. After comparing the results between the suboptimally functioning large scale facility and an optimised pilot scale facility, it was found that the frequency of certain bacterial families or genera differed between the phases and conditions of the composting process, suggesting that the bacterial community profile or certain key bacterial species may be used as indicators of process efficiency. For example, a high incidence of lactic acid and acetic acid bacteria was found to correlate with insufficient aeration and low pH, while the rise of *Bacilli* and *Actinobacteria* would indicate a transfer from the mesophilic to the thermophilic phase. The fact that bacteria from the anaerobic genus *Clostridium* were detected in almost all types of processes showed, however, that targeting only one or several bacterial species may give an incomplete picture of the situation in the composting process. The recovered number of different bacterial (566, Partanen et al. 2009) and fungal (166, Hultman et al. 2009a) phylotypes in the studied compost samples was lower than that found in farm soils (3,000 phylotypes, Tringe et al. 2005) and urban air (1,500–1,800 phylotypes, Brodie et al. 2007). The reason may be that the conditions during composting change very rapidly and can reach extremes: the pH can drop below four and the temperature may rise in a relatively short time from below zero (temperature of the incoming frozen waste) up to +80°C. This may restrict the diversity of bacteria and fungi in the processes as the rapidly changing conditions favor the growth of a limited number of microorganisms in each phase. When examining different composting processes, the bacterial species found may vary greatly, while dominating types/groups may correlate to the conditions, and may therefore be used for diagnosing the process. Presently, however, not enough data are available to confirm this assumption.

Studies using molecular techniques (Hultman et al. 2009a) revealed that the fungal community residing in industrial composting plants, and in pilot and laboratory scale reactors, differed from what has been reported earlier. In previous studies, fungal communities were dominated by Pezizomycotina members, such as *Aspergillus* and *Penicillium* species (e.g. Anastasi et al. 2004), fungi from the

genera *Mucor* and *Rhizomucor* (Von Klopotek 1962; Fergus 1964; Anastasi et al. 2004), and basiodiomycetes such as *Coprinus cinereus* (Von Klopotek 1962). Yeasts have rarely been detected (von Klopotek 1962; Peters et al. 2000) in previous studies although they are known to be important in the first phases of the composting process to overcome the acidophilic early stages (Choi and Park 1998). However, in industrial composting processes in Nordic countries Ascomycetous, yeasts were in some cases found to dominate the fungal community, even at later stages of the process (Hultman et al. 2008a, 2009a).

In recent years, the study of microbial communities has expanded and both phylogenetic and metagenomic approaches have been applied (Hugenholtz and Tyson 2008). The microbial diversity found in these phylogenetic and metagenomic studies has been high and the results have advanced microbial ecology. Nonetheless, some problems exist in approaches that are based on the cloning of DNA from environmental samples. For example, it is challenging to get a representative sample to study the diversity and abundance of microbes in certain environments (Ranjard et al. 2003). Another challenge is that microbial groups that are abundant in samples can obscure organisms present in lower numbers that consequently may not be sampled and sequenced (Curtis and Sloan 2005). Moreover, studying the diversity of natural microbial communities using the Sanger sequencing method (Sanger et al. 1977) is slow and laborious as it has been estimated that over 40,000 sequencing reactions are required to reach 50% coverage of the diversity in a soil sample (Dunbar et al. 2002). Therefore the sampling needs to be carefully planned in order to get a good picture of the microbes in the heterogeneous environment.

In recent years, several next generation sequencing techniques, such as 454 tag sequencing (Margulies et al. 2005), have been developed. These methods have been introduced to microbial ecology as sequencing of specific short (~100 to 210 bp) rRNA or functional gene tag-sequences that have been isolated from environments such as the deep marine biosphere (Sogin et al. 2006; Huber et al. 2007) and soil (Leininger et al. 2006; Roesch et al. 2007). The amount of data gained with this approach is massive – the number of sequences in the above examples was from 26,000 to 900,000. The technology in this area is developing rapidly. Both the length of the reads, as well as the number of fragments processed in parallel is increasing, leading to an exponential growth of raw sequence data to be analysed. In particular, an increase in the length of the fragments is welcome in view of the fact that phylogeny based on very short fragments is often unreliable. The utility of these novel sequencing techniques is in the vast amount of sequence data, which can be used in other applications, such as microarrays (see Sect. 7.2.4) for compost monitoring.

7.2.2 Fingerprinting Methods

Various molecular fingerprinting methods, such as denaturing gradient gel electrophoresis (DGGE, Ishii et al. 2000; Steger et al. 2007b), single stranded conformation pattern (SSCP; Peters et al. 2000), amplified ribosomal DNA restriction analysis

(ARDRA; Schloss et al. 2003) and restriction fragment length polymorphism (RFLP) analysis and sequencing (Dees and Ghiorse 2001) have been used to study the compost microbial communities to find out which microbes are present in certain stages of the composting process (see Chaps. 1, 12 and 14, Knapp et al. 2010). These methods can also be used prior to sequencing to determine how much sequencing is required for a sample of interest, and also which sequencing technique to use as the diversity in samples varies. Figure 7.3 shows an example of compost process monitoring using DGGE for investigating the genus *Bacillus*.

Although DNA fingerprinting methods give a profile of the community composition of different environmental samples (Liu et al. 1997; Osborn et al. 2000; Brodie et al. 2003; Steger et al. 2007a,b) they may underestimate the true microbial diversity. Microbes from different species or genera can share an identical restriction pattern (Dunbar et al. 2002), a single DGGE band can contain several different ribotypes (Costa et al. 2006) and numerically rare phylotypes may not be detected (Bent and Forney 2008). These methods are therefore not suitable if the aim is to detect rare phylotypes or certain species. While bacterial species abundance curves are generally log-normal distributed with very long tails (Martiny et al. 2006) and dominating phylotypes make up a minority of the diversity (Curtis and Sloan 2005),

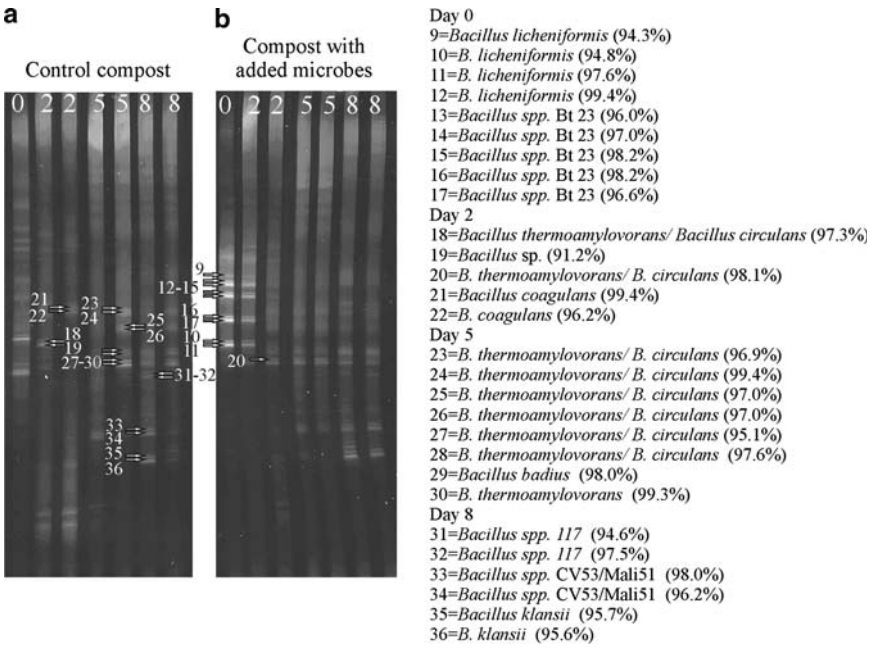

Fig. 7.3 Example of use of DGGE-analysis in compost monitoring. Genus specific primers were used to study community structure of *Bacillus* ssp. during the early stages of biowaste composting. (**a**) Control compost, days 0, 2, 5 and 8. (**b**) Compost with added microbes, days 0, 2, 5 and 8. The changes of *Bacillus* ssp. community within the process development can be seen in the different banding patterns in the different days

capturing the tail-end of those communities is difficult with fingerprinting methods. With a cloning approach they may be captured, but also then the abundant types may prevent this. Moreover, it is possible that in some cases the phylogenetic denomination changes when the complete gene coding for 16S rRNA gene is used instead of a shorter subfragment of the gene (Hultman 2009b).

Dot-blotting methods have been applied to study microbes in different environments. In this method, species specific probes are blotted on a nylon membrane and a labeled sample is hybridised on the membrane (e.g. Valinsky et al. 2002). The species present are detected with either colorimetric or radioactive staining. These methods lack specificity and sensitivity and are therefore not in wider use in microbial diagnostics (Bodrossy and Sessitsch 2004). Novel approaches, based on the dot-blot concept have, however, been developed (see Sect. 7.2.3).

7.2.3 Macroarrays for Negative Selection

As mentioned above, capturing the tail-end of communities is difficult with fingerprinting methods (Bent and Forney 2008). A novel method based on dot blotting hybridizations was recently published (Hultman et al. 2008a). A macroarray test was developed to find sequences representing a minor fraction of the total population. The method was used for 1,536 fungal ITS clones that were gridded on a nylon membrane. With six probes representing the most common fungal phylotypes, the identity of 900 clones was determined, and thus not chosen for sequencing. The remaining 41% were sequenced. In addition, 384 clones were sequenced to verify the hybridization results and the specificity and sensitivity of the method and the probes used. The rate for the false negatives (i.e. sequenced clones among the 384 that did not hybridize with the six probes used, despite representing common phylotypes) was 5.2%. False positives (clones that were recognized in the hybridization as common despite representing new phylotypes) were found in fifteen cases (n = 384, 3.9%). The false positives were mainly from species that were present in high numbers in these samples and therefore, the amount of probe was not sufficient as it did not attach to all of these samples. Part of the false positives proved to be impure PCR-products that contained PCR-products from two clones. With the membrane hybridization method (Hultman et al. 2008a) the common phylotypes can be detected allowing only the novel clones to be sequenced. New probes can be added to the probe pool when the studied sample is further characterized and more of the abundant phylotypes are detected.

7.2.4 Diagnostic Microarrays

Microbial diagnostic microarrays, also known as phylochips or phylogenetic oligonucleotide arrays, became a popular high-throughput tool for microbial detection

from various environments over a decade ago (Guschin et al. 1997). With these microarrays (see Chap. 1), the parallel detection of hundreds to thousands of microorganisms can be achieved and arrays have been applied in the study of microbes in different environments such as soil (Small et al. 2001), landfills (Bodrossy et al. 2003), water (Rich et al. 2008), compost (Franke-Whittle et al. 2005; Franke-Whittle et al. 2009) and urban air (Brodie et al. 2007). Both short (18–28 mer) and long (50–70 mer) oligonucleotide probes specific for certain species, genus or strains, can be printed on a glass slide. Short probes have been shown to be more specific than the long ones, but they suffer from a high detection limit (from 1 to 5%). With the longer probes, a lower detection limit is achieved but they are not as specific as the short probes (reviewed in Bodrossy and Sessitsch 2004). Fluorescently labeled total DNA from the sample or a PCR-amplified gene of interest is hybridized on the array and the results are read with a specific scanner.

Although in microarray probe design attention is paid to reaching the highest possible nucleotide specificity for the probes, the thermodynamic properties of the probes need to be checked as well. The microarray probes are printed on the same glass slide and therefore, to avoid cross hybridization, their thermodynamic properties have to be identical. The design of only one probe for the identification of a certain species is not sufficient due to the fact that ribosomal genes do not always have enough discrimination power. Therefore, microarrays with several probes for each species are widely used (e.g. Loy et al. 2004; Brodie et al. 2007). The nested-probe approach relies on parallel or hierarchical probe specificity. These microarray platforms are usable for the study of the microbiota in the sample, but as the microbes cannot be distinguished at the species level and as there are problems with detection limits (Bodrossy et al. 2003; Loy et al. 2004; Franke-Whittle et al. 2005; DeSantis et al. 2007), novel platforms are being developed for the study of microbial communities in a specific and sensitive manner.

Methods that rely on enzyme assisted detection methods have been developed in combination with microarray hybridization in order to improve the specificity of the oligonucleotide microarray (Busti et al. 2002; Banér et al. 2003; Castiglioni et al. 2004). A method based on the ligation detection reaction (LDR; Busti et al. 2002; Castiglioni et al. 2004) has recently been adapted for compost samples (Hultman et al. 2008b). Previously the method had been used for detecting single base mutations associated with genetic diseases (Landegren et al. 1988; Barany 1991) but has been adapted to characterize microbial communities (Busti et al. 2002; Rantala et al. 2008). Two target specific probes are used in LDR. These probes hybridize to the template DNA adjacently and are ligated with a thermostable ligase if there is a perfect complementarity between the probes and the target DNA. The probes are designed so that the nucleotide in the junction point of the probes distinguishes the target from other species. After ligation, the ligation products are linearly amplified (Barany 1991). The first of the two probes is fluorescently labeled (Gerry et al. 1999; Busti et al. 2002) while the second probe contains a 3' tag sequence (zip code,) which directs it to the right address on the microarray containing a complementary zip code-sequence. These zip sequence pairs have

uniform hybridization conditions and the same array platform can be used with multiple ligation probe sets. For use in characterization of composting fungi, the microarray was first optimised with pure cultures and clones, after which real environmental samples were used. When comparing to fungal diversity results that were obtained by cloning and sequencing the same samples, it was concluded that the results of the LDR microarray test appeared to give reliable results. Since the chip is still a prototype, further testing is needed for demonstration of reproducibility (Hultman et al. 2008b). The detection limit was 0.04% (target DNA/total DNA), and the sensitivity was similar to that of quantitative-PCR (qPCR). With qPCR, the presence of one species or phylotype can be analysed at a time, while with a microarray thousands of species can be detected in one run. In many oligonucleotide studies, a detection limit from 1 to 5% have been calculated (e.g. Loy et al. 2004; Franke-Whittle et al. 2005) so the increase in sensitivity observed with the LDR-based microarray is considerable. Furthermore, the microarray was species or phylotype specific despite the close relatedness of the target microbes. Thus, species that are highly similar based on the rRNA small subunit sequences, but that may occupy different niche spaces (Jaspers and Overmann 2004) can be distinguished with LDR-chips.

7.2.5 Quantitative Real-Time PCR

When the presence and concentration of specific microorganisms needs to be monitored, qPCR (also known as real-time PCR) is a good choice. qPCR approaches (see Chap. 1) are now widely applied in microbial ecology to quantify the abundance and expression of taxonomic and functional gene markers within the environment (Smith and Osborn 2009). In qPCR, the amplification of the target gene is measured in the terms of the increment in the quantity of fluorescence which is determined at the end of each amplification cycle. The threshold cycle C(t), at which the sample's fluorescence trace crosses the threshold line, is then used for the calculation of the amount of template DNA in a reaction. Quantification of the initial target sequences of an unknown concentration is determined from the C(t) values and can be described either in relative or in absolute terms.

In relative quantification, changes in the unknown target are expressed relative to a coamplified steady state (typically housekeeping) gene. Using quantification standards (absolute quantification) with a range of known amounts of template DNA, a linear standard curve of the log of the template DNA versus C(t) is generated. Unknown template quantity can then be calculated by interpolating the samples threshold cycle against the standard curve. Melting curve analysis is then used in product identification, determination of product homogeneity, and certification of the PCR amplification.

Two detection chemistries are commonly used, namely, the intercalating SYBR green assay and the TaqMan probe system (Smith and Osborn 2009, Chap. 1).

The detection of microbial DNA does not indicate the presence of live or intact microorganisms due to the persistence of DNA within or released from cells that once lived in the investigated site. This must be kept in mind when DNA-based techniques are used in analysing microbial numbers in the environment.

The qPCR method has been used successfully in samples from different environments, e.g. the enumeration of pathogenic bacteria and indicator bacteria during wastewater treatment and sludge composting (Novinscak et al. 2007; Wery et al. 2008). The qPCR method is not officially approved or in regular use for monitoring hygienization of compost. This is despite the fact that the sensitivity of qPCR is equivalent, and sometimes surpasses that of the cultivation-based methods which are currently used for determining compost quality.

Although free from the effect of cultivation bias, qPCR like all PCR-based methods involves sources of error related to DNA extraction and PCR amplification (see Chap. 1). In addition, variation in the number of RNA operons (1 to 14 per genome) precludes direct conversion of 16S and 18S rRNA gene numbers into bacterial and fungal cell numbers, and even the exact number of copies of the 16S rRNA gene in given bacterial and fungal species may vary (Klappenbach et al. 2000).

7.3 Conclusions

The fast development of modern DNA-based techniques has opened up new possibilities for determining microbial diversity and succession in composts. The methods described here, large scale clone library sequencing, 454-tag sequencing, DNA fingerprinting methods, negative selection of rare phylotypes from the clone libraries, diagnostic microarrays and qPCR have been used in our laboratory independently and combined together. The information on the diversity of compost microbes can be collected by cloning and sequencing of PCR-amplified indicator regions, and with the use of the macroarray method for the negative selection of already sequenced fragments. Major changes in the microbial community structure can be monitored with fingerprinting methods and the confirmation of presence of particular (pathogenic) microbes can be achieved with the use of specific microarrays or with qPCR. As more detailed information on the microbial communities present in the different stages of typical well functioning composting processes, as well as information on microbes that indicate specific process disturbances is gathered, this knowledge can be used in monitoring the composting process. With the tools presented above, the microbial community composition can be determined in greater detail, and in a shorter time than what previously has been possible. Nevertheless, the study of the microbial profile should always be complemented with and compared to the physical and chemical properties of the samples, and as always when studying complex environmental processes, utmost attention has to be paid to sampling – the results are at best only as representative as the sampling has been.

References

Abulencia CB, Wyborski DL, Garcia JA, Podar M, Chen W, Chang SH, Chang HW, Watson D, Brodie EL, Hazen TC, Keller M (2006) Environmental whole-genome amplification to access microbial populations in contaminated sediments. Appl Environ Microbiol 72:3291–3301

Amann R, Ludwig W (2000) Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiol Rev 24:555–565

Amann R, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Amaral-Zettler L, Peplies J, Ramette A, Fuchs B, Ludwig W, Glockner FO (2008) Proceedings of the international workshop on Ribosomal RNA technology, April 7–9, 2008, Bremen, Germany. Syst Appl Microbiol 31:258–268

Anastasi A, Varese G, Voyron S, Scannerini S, Morchisio VF (2004) Characterization of fungal biodiversity in compost and vermicompost. Compost Sci Util 12:185–191

Anderson IC, Campbell CD, Prosser JI (2003) Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environ Microbiol 5:36–47

Baker GC, Smith JJ, Cowan DA (2003) Review and re-analysis of domain-specific 16S primers. J Microbiol Methods 55:541–555

Banér J, Isaksson A, Waldenstrom E, Jarvius J, Landegren U, Nilsson M (2003) Parallel gene analysis with allele-specific padlock probes and tag microarrays. Nucleic Acids Res 31:e103

Barany F (1991) Genetic disease detection and DNA amplification using cloned thermostable ligase. Proc Natl Acad Sci USA 88:189–193

Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M (1996) Isolation of Thermus strains from hot composts (60 to 80 degrees C). Appl Environ Microbiol 62: 1723–1727

Bent SJ, Forney LJ (2008) The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity. ISME J 2:689–695

Blanc M, Marilley L, Beffa T, Aragno M (1997) Rapid identification of heterotrophic, thermophilic, spore-forming bacteria isolated from hot compost. Int J Syst Bacteriol 47:1246–1248

Bodrossy L, Sessitsch A (2004) Oligonucleotide microarrays in microbial diagnostics. Curr Opin Microbiol 7:245–254

Bodrossy L, Stralis-Pavese N, Murrell JC, Radajewski S, Weilharter A, Sessitsch A (2003) Development and validation of a diagnostic microbial microarray for methanotrophs. Environ Microbiol 5:566–582

Brodie EL, Edwards S, Clipson N (2003) Soil fungal community structure in a temperate upland grassland soil. Fems Microbiol Ecol 45:105–114

Brodie EL, DeSantis TZ, Parker JP, Zubietta IX, Piceno YM, Andersen GL (2007) Urban aerosols harbor diverse and dynamic bacterial populations. Proc Natl Acad Sci USA 104:299–304

Busti E, Bordoni R, Castiglioni B, Monciardini P, Sosio M, Donadio S, Consolandi C, Rossi Bernardi L, Battaglia C, De Bellis G (2002) Bacterial discrimination by means of a universal array approach mediated by LDR (ligase detection reaction). BMC Microbiol 2:27

Castiglioni B, Rizzi E, Frosini A, Sivonen K, Rajaniemi P, Rantala A, Mugnai MA, Ventura S, Wilmotte A, Boutte C, Grubisic S, Balthasart P, Consolandi C, Bordoni R, Mezzelani A, Battaglia C, De Bellis G (2004) Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. Appl Environ Microbiol 70:7161–7172

Ceustermans A, Coosemans J, Ryckeboer J (2010) Compost microbial activity related to compost stability. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 115–134

Choi MH, Park YH (1998) The influence of yeast on thermophilic composting of food waste. Lett Appl Microbiol 26:175–178

Costa R, Gotz M, Mrotzek N, Lottmann J, Berg G, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol Ecol 56:236–249

Curtis TP, Sloan WT (2005) Microbiology. Exploring microbial diversity – a vast below. Science 309:1331–1333

Dees PM, Ghiorse WC (2001) Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiol Ecol 35:207–216

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 53:371–383

Dunbar J, Barns SM, Ticknor LO, Kuske CR (2002) Empirical and theoretical bacterial diversity in four Arizona soils. Appl Environ Microbiol 68:3035–3045

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853

European Parliament and of the Council Commission regulations (EC) (2006) Amending annexes VI and VIII to regulations (EC) No 1774/2002 as regards processing standards for biogas and composting plants and requirements for manure. Official Journal of the European Communities 8 February 2006, No 208/2006, L 36/25-31

European Parliament and of the Council Regulations (EC) (2002) Health rules concerning animal by-products not intended for human consumption. Official Journal of the European Communities 10 October 2002, No 1774/2002, L 273/1-95

Fergus CL (1964) Thermphilic and thermotolerant molds and actinomycetes of mushroom compost during peak heating. Mycologia 56:267–284

Franke-Whittle IH, Klammer SH, Insam H (2005) Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. J Microbiol Methods 62:37–56

Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H (2009) Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microb Ecol 57:510–521

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Gerry NP, Witowski NE, Day J, Hammer RP, Barany G, Barany F (1999) Universal DNA microarray method for multiplex detection of low abundance point mutations. J Mol Biol 292:251–262

Gray KR, Sherman K, Biddlestone AJ (1971) A review of composting – part 1. Process Biochem 6:32–36

Guschin DY, Mobarry BK, Proudnikov D, Stahl DA, Rittmann BE, Mirzabekov AD (1997) Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. Appl Environ Microbiol 63:2397–2402

Hansgate AM, Schloss PD, Hay AG, Walker LP (2005) Molecular characterization of fungal community dynamics in the initial stages of composting. FEMS Microbiol Ecol 51:209–214

Heuer H, Krsek M, Baker P, Smalla K, Wellington EM (1997) Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63:3233–3241

Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML (2007) Microbial population structures in the deep marine biosphere. Science 318:97–100

Hugenholtz P, Tyson GW (2008) Microbiology: metagenomics. Nature 455:481–483

Hultman J (2009b) Unpublished information

Hultman J, Pitkäranta M, Romantschuk M, Auvinen P, Paulin L (2008a) Probe-based negative selection for underrepresented phylotypes in large environmental clone libraries. J Microbiol Methods 75:457–463

Hultman J, Ritari J, Paulin L, Romantschuk M, Auvinen P (2008b) The application of ligase-detection-reaction microarray for compost samples. BMC Microbiol 8:237

Hultman J, Vasara T, Partanen P, Kurola J, Kontro M, Paulin L, Auvinen P, Romantschuk M (2009a) Determination of fungal succession during municipal solid waste composting using a cloning-based analysis. J Appl Microbiol DOI: 10.1111/j.1365-2672.2009.04439.x

Insam H (2001) Developments in soil microbiology since the mid 1960's. Geoderma 100: 389–402

Insam I, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Ishii K, Takii S (2003) Comparison of microbial communities in four different composting processes as evaluated by denaturing gradient gel electrophoresis analysis. J Appl Microbiol 95:109–119

Ishii K, Fukui M, Takii S (2000) Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J Appl Microbiol 89:768–777

Jaspers E, Overmann J (2004) Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Appl Environ Microbiol 70:4831–4839

Jürgens G, Lindström K, Saano A (1997) Novel group within the kingdom Crenarchaeota from boreal forest soil. Appl Environ Microbiol 63:803–805

Kane BE, Mullins JT (1973) Thermophilic fungi in a municipal waste compost system. Mycologia 65:1087–1100

Klappenbach JA, Dunbar JM, Schmidt TM (2000) rRNA operon copy number reflects ecological strategies of bacteria. Appl Environ Microbiol 66:1328–1333

Knapp B, Ros M, Insam H (2010) Do composts affect the soil microbial community? In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 271–292

Landegren U, Kaiser R, Sanders J, Hood L (1988) A ligase-mediated gene detection technique. Science 241:1077–1080

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Loy A, Kusel K, Lehner A, Drake HL, Wagner M (2004) Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal co-occurrence of recognized genera and novel lineages. Appl Environ Microbiol 70:6998–7009

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Ovreas L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112

Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252

Muyzer G, Brinkhoff T, Nűbel U, Santegoeds C, Schäfer H, Wawer C (1998) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (eds) Molecular microbial ecology manual, vol 3.4.4. Kluwer, Dordrecht, pp 1–27

Novinscak A, Surette C, Filion M (2007) Quantification of Salmonella spp. in composted biosolids using a TaqMan qPCR assay. J Microbiol Methods 70:119–126

Osborn AM, Moore ER, Timmis KN (2000) An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ Microbiol 2:39–50

Partanen P, Hultman J, Paulin L, Auvinen P, Romantschuk M (2009) Bacterial diversity in the different stages of composting process based on the 16S rRNA analysis. Submitted

Peters S, Koschinsky S, Schwieger F, Tebbe CC (2000) Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation polymorphism-based genetic profiles of small-subunit rRNA genes. Appl Environ Microbiol 66:930–936

Petiot C, de Guardia A (2004) Composting in a laboratory reactor: a review. Compost Sci Util 12:69–79

Poltz MF, Cavanaugh CM (1998) Bias in template-to-product ratios in multitemplate PCR. Appl Environ Microbiol 64:3724–3730

Ranjard L, Lejon DP, Mougel C, Schehrer L, Merdinoglu D, Chaussod R (2003) Sampling strategy in molecular microbial ecology: influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environ Microbiol 5:1111–1120

Rantala A, Rizzi E, Castiglioni B, de Bellis G, Sivonen K (2008) Identification of hepatotoxin-producing cyanobacteria by DNA-chip. Environ Microbiol 10:653–664

Rich VI, Konstantinidis K, DeLong EF (2008) Design and testing of "genome-proxy" microarrays to profile marine microbial communities. Environ Microbiol 10:506–521

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FA, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349–410

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Schloss PD, Hay AG, Wilson DB, Walker LP (2003) Tracking temporal changes of bacterial community fingerprints during initial stages of composting. FEMS Microbiol Ecol 46:1–9

Schloss PD, Hay AG, Wilson DB, Gossett JM, Walker LP (2005) Quantifying bacterial population dynamics in compost using 16S rRNA gene probes. Appl Microbiol Biotechnol 66:457–463

Small J, Call DR, Brockman FJ, Straub TM, Chandler DP (2001) Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. Appl Environ Microbiol 67:4708–4716

Smith CJ, Osborn AM (2009) Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbiol Ecol 67:6–20

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci USA 103:12115–12120

Steger K, Sjogren AM, Jarvis A, Jansson JK, Sundh I (2007a) Development of compost maturity and *Actinobacteria* populations during full-scale composting of organic household waste. J Appl Microbiol 103:487–498

Steger K, Jarvis A, Vasara T, Romantschuk M, Sundh I (2007b) Effects of differing temperature management on development of *Actinobacteria* populations during composting. Res Microbiol 158:617–624

Tiquia SM, Tam NF, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen K, Chang HW, Podar M, Short JM, Mathur EJ, Detter JC, Bork P, Hugenholtz P, Rubin EM (2005) Comparative metagenomics of microbial communities. Science 308:554–557

Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183

Valinsky L, Della Vedova G, Scupham AJ, Alvey S, Figueroa A, Yin B, Hartin RJ, Chrobak M, Crowley DE, Jiang T, Borneman J (2002) Analysis of bacterial community composition by oligonucleotide fingerprinting of rRNA genes. Appl Environ Microbiol 68:3243–3250

Van de Peer Y, Chapelle S, De Wachter R (1996) A quantitative map of nucleotide substitution rates in bacterial rRNA. Nucleic Acids Res 24:3381–3391

Vinnerås B, Agostini F, Jönsson H (2010) Sanitisation by composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 171–192

Von Klopotek A (1962) Über das vorkommen und verhalten von Schimmelpilzen bei der Kompstierung Städtischer Abfallstoffe. Antonie van Leeuvenhoek 28:141–160

Vuorinen AH, Saharinen MH (1997) Evolution of microbial and chemical parameters during manure and straw co-composting in a drum composting system. Agric Ecosyst Environ 66:19–29

Wery N, Lhoutellier C, Ducray F, Delgenes JP, Godon JJ (2008) Behaviour of pathogenic and indicator bacteria during urban wastewater treatment and sludge composting, as revealed by quantitative PCR. Water Res 42:53–62

White TJ, Bruns T, Lee S, Taylor J (eds) (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic, San Diego

Wu L, Liu X, Schadt CW, Zhou J (2006) Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Appl Environ Microbiol 72:4931–4941

Chapter 8
Production and Utilization of Suppressive Compost: Environmental, Food and Health Benefits

Marco de Bertoldi

Abstract Since many centuries, compost has been utilized in agriculture to replace organic matter and nutrients for different crops. Only in recent decades, particular composts, produced with selected starting material and with controlled processes, have been applied to suppress phytopathogenic agents. Such composts can be used to control soil-borne or air-borne pathogens. This has been tested, to control diseases, both in the field and laboratory on many different crops and conditions: greenhouse, horticulture, floriculture, apple, grapes, container systems, pot culture, turf grass, plant nursery, etc. The mechanisms of disease suppression are still not fully understood and include a complex interplay of abiotic (pH, temperature, C/N, organic matter quality, etc.) and biotic (predators, antagonists, competition for nutrients, antibiosis, production of lytic enzymes, microbial metabolites like siderophores, etc.) factors. In this chapter, compost characteristics related to disease suppression, the use of suppressive compost and economical benefits are discussed, in particular the possible reduction of pesticide use.

Contents

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8.1 Introduction

The first scientific reports on composting, use of compost and benefits for the growth and the health of plants had been published more than eight centuries ago. The Knight Templars were a military order in the time of Crusades. When the Moslems occupied the holy places in Palestine, they moved away and settled in Spain and in France where they devoted themselves to agriculture. They had the donations of many farms and others had been rented by them. These farms had been devastated by Moslems during their retreat from Spain and some remained uncultivated for many years; the Knight Templars, drawing up the contracts, had to describe clearly the methods used to recover these soils. These contracts and respective clauses, how to recover the depleted soils, were written and still exist in the Historic National Archives of Madrid. Manuscripts also exist in the Cistercian Abbeys of Fitero, Poblet, Santes Crues, Huerta, Archives of Caceres Deputation, the biggest "Commanderie" farm of Templars in Alcanedre, Extremadura (Dailliez 1981; de Bertoldi 1999). In these manuscripts of the thirteenth century, the techniques used by Templars to recover fertility in arid and depleted soils are reported in detail. Composting technologies are, for the first time in the history, scientifically described. The descriptions start with the preparation of different starting materials in order to obtain different composts to be used in different crops. Particle size, quality and moisture are carefully evaluated. After the preparation of the starting mixture, exact indications on windrow dimension follow. Treatment times for different materials are clearly reported. Finally, they suggest the use (quantity and time of application) of any compost produced for different crops. They assure that, with the use of compost, the plants will grow better and without diseases!

To have well-documented results with respect to the production and use of disease suppressive compost we have had to wait until 1975 (Malek and Cartner 1975) and 1977 (Hoitink et al. 1977). As a substitute for peat, composted hardwood has been produced in container systems. *Phytophtora cinnamoni* (Hoitink et al. 1977) and nematodes as *Meloidogyne*, *Pratilenchus* and *Trichodorus* species where inhibited by this kind of compost (Malek and Cartner 1975). As a result of these interesting researches, bark compost has been widely used in the United States, Israel, and Switzerland to suppress phytopathogenic agents. Two schools have deeply worked in this field: the Ohio Agricultural Research and Development Centre, Wooster, USA and The Hebrew University of Jerusalem. In these two countries, since many years, an industrial production of suppressive compost exists and it is utilized to produce horticultural crops and flowers without pesticides. After

1980, there has been a huge increase in scientific research on suppressive compost and its use, both against soil-borne and air-borne pathogenic agents.

8.2 The Composting Process

Composting is a bioxidative microbial process leading to highly stabilized organic matter, which may contribute directly to soil conditioning and fertility (de Bertoldi et al. 1983b). The starting material for composting (see Table 8.1) must be physically and chemically conditioned in order to guarantee a good performance of the process. The specific weight should be around 0.5 and water content around 60%. The structural strength of the material should permit to have interstices large enough to allow air to pass through the mass. The dimension of the particles should be around 3–5 cm, in order to be degraded by microorganisms for a time no longer than 50 days. For high quality compost, maximum process efficiency is required, which in turn is a reflection of our capability to manage the microbial biomass growth and activity. The main factors which need to be optimized are: oxygen supply to the mass, moisture and temperature control, C/N ratio, pH, organic matter (quantity and quality), physical structure and balance of nutrients. Production of phytotoxic metabolites characterizes the intermediate phase of the process. Management of the process must take into account the end-product value and compatibility with plant growth. Many detailed analyses of the main parameter which govern composting have already been reported (de Bertoldi et al.1982, 1983a, 1985, 1988; Finstein and Morris 1975; Finstein et al. 1985, 1986; de Bertoldi and Zucconi 1986; Diaz et al. 2007). Stabilization implies oxidation, mineralization and humification of organic matter. The stable products that remain after composting are still degradable, but at a much reduced rate compared to the raw material. Very important is to evaluate how much compost should be stabilized before utilization both as an organic fertilizer and as a suppressive medium (de Bertoldi and Schnappinger 2001). The rate of oxygen uptake by the compost is one of the most important parameters both for stability and suppressivity control. Several procedures for

Table 8.1 Starting materials utilized in suppressive compost production

Bark
Chipped wood
Forestry waste
Agricultural waste (animal dejections, straw, corn cobs, pruning, etc.)
Marc grape
Olive mill waste
Food processing waste
Garden waste
Organic fraction of municipal solid waste, from source separation
Sludge from anaerobic digestion
By products from bioenergy production (bioethanol, biodiesel)

testing stability and maturity of the compost have been proposed. Negative plant responses to compost can be due to high cellulose content, resulting in N immobilization, allelopathic chemicals, high salt content (Hoitink and Kuter 1986). The most widely used methods to assay maturity level are plant bioassays (Chanyasak et al. 1983; Zucconi et al. 1981a,b), oxygen uptake and CO_2 evolution. The self-heating method is also utilized to determine stability; it is very simple and still valuable (van Elsas and Postma 2007).

8.3 Controlling of Composting Process

The control of the composting process in order to obtain suppressive compost is essential. Parameters like oxygenation of the mass, temperature, ventilation and moisture are strictly connected. Their control during the process is very important to maintain favorable conditions for achieving suppressivity.

Microbial oxidation and humification of organic matter during composting are the result of microbial catabolism. About 50% of the total energy recovered is used for the growth of new microorganisms (anabolism), the remaining 50% is lost as heat (de Bertoldi and Civilini 2006). The microorganisms involved in the process are mainly aerobic, with most of them being mesophilic and some thermophilic.

Some of the earliest composting strategies were developed by researchers working in the US Department of Agriculture in Beltsville, MD. The system used were static piles with vacuum aeration from the bottom. In such experiments lasting 28 days (Epstein et al. 1976) the pile temperature from the tenth day until the end was between 60 and 80°C. The maximum oxygen consumption occurs in the first 10 days when temperature was lower: a confirmation that in the thermophilic conditions microbial activity was depressed and that the aeration provided was insufficient to cool the pile to mesophilic temperatures. Finstein and co-workers at the Rutgers University developed a new strategy (oxygen feed-back control), blowing air not at a fixed rate but with a configuration which maintains a temperature ceiling that provides a high decomposition rate through on-demand removal of the heat by ventilation. Compared with the approach in widespread use at that time, the Rutgers strategy was said to yield high rate composting that decomposes four times more organic matter in half the time (Finstein et al. 1985).

A new strategy for controlling the composting process has been developed, based on O_2 feedback control (de Bertoldi et al. 1988). More air is required to remove heat than to supply oxygen. Calculations show that it takes nearly nine times more air to remove the heat than to replenish the oxygen (Finstein et al. 1986). For this reason temperature has been considered the primary control parameter with regard to oxygen. The optimal values for these parameters are respectively 40°C (temperature) and 15% (oxygen).

A good control of the composting process, on order to respect the requirements for suppressivity, can be based on these two systems: a computerized system

(based on temperature and oxygen) manages air ventilation of the composting mass, maintaining temperature, oxygenation and moisture at the optimal values for inducing suppressivity. Ventilation of piles can be regulated at 0.1 m^3 of air per ton of organic matter per minute. This rate will be automatically varied by the computer on receiving values of temperature and of oxygen level different from those planned in the computer program. Oxygen levels and temperature are directly controlled by ventilation of the mass; moisture will be regulated spraying water over the composting piles during turning. The computer will stop water supply when the values in the mass will reach the most convenient value (about 40–45%).

8.4 Suppressivity of Compost

There are two kinds of suppressivity that can be obtained: (1) by compost (soil-borne phytopathogens); (2) by water extracts of compost, called compost tea (air-borne phytopathogens). Compost is not an inert material and it contains living microorganisms. During the process it evolves through a thermophilic phase that kills most of the pathogenic agents (to man and plant). At the same time, antagonistic microorganisms develop during compost maturation. Not all composts have the same capacity to protect plants against diseases. The loss of suppressivity in compost, after a thermal treatment (90°C), indicates that disease suppression is also due to the microbiological activity of compost. Also physiochemical and biological properties of compost can also influence suppressivity.

To obtain suppressive compost it is important to select the starting material and to condition it chemically and physically. In general, bark, hardwood, lignocellulosic compounds, yard waste, agricultural waste, food processing waste (olive and grape marc) are very good starting materials. The particle size must not be too small to hinder the mass aeration and not too large because of microbial degradation problems (size of 2–8 cm). The C/N ratio should be not too high and not too low (25–40). Nitrogen at the end of the process should be mostly organic; high levels of mineral nitrogen reduce the suppressive effect of compost. The maturity (biological stability) influences the potential for plant disease control. The oxygen supply during the process of composting is very important. Periodical turning of windrows is therefore not enough to guarantee a constant supply of oxygen. It must be operated with ventilation of the mass (forced or passive aeration from the bottom) in conjunction with periodical turning (Finstein et al. 1986). To obtain a high quality compost it seems very important to operate with a feed-back control system, utilizing as a parameter oxygen and/or temperature (de Bertoldi et al. 1988). Compost quality is not stable, therefore the mature compost has to be stored appropriately and never in anaerobic conditions (no plastic packaging) in order to maintain suppressivity. Thermal treatments (pelletting) should also be avoided because they reduce suppressivity.

8.5 Mechanisms of Suppression

In spite of the many efforts to find indicators of disease suppressivity, there is still a general lack of understanding the disease-suppressive status of the compost (van Elsas and Postma 2007). However the suppressivity of compost can be caused by a complex range of abiotic and biotic factors. Prediction of compost suppressivity is complex, mainly due to the different soil-borne pathogens: fungi, bacteria, nematodes. Although general indicators for suppression are not available, several promising examples of disease suppression have been described for specific diseases (Klose and Mohnl 2010; van Elsas and Postma 2007). In Table 8.2 the main factors which can affect inactivation and destruction of pathogenic agents are reported.

8.5.1 Biotic

The different sensitivity of the various pathogens to heat is reported in the previous reviews/chapters (Bollen 1993; Bollen and Volker 1996). The quantity of heat generated during the first phase of composting of crop residues and organic household waste exceeds the level that is needed for the thermal kill of most pathogens.

Unfortunately, beneficial as well as detrimental microorganisms are killed during the thermophilic phase of composting. Therefore, suppression of pathogens and/or disease is largely induced during maturation and curing of compost (mesophilic phase) as biocontrol agents recolonize compost after peak heating. In the curing phase of composting, the concentration of readily biodegradable components declines. At this time mesophilic microorganisms recolonize the compost from the outer low temperature layer into the pile (e.g., Danon et al. 2008). *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum*, *Pseudomonas* spp., *Streptomyces* spp., as well as fungi like *Penicillium* spp., *Trichoderma* spp., *Gliocladium virens* and other fungi have been identified as biocontrol agents in the compost

Table 8.2 Mechanisms of suppression

Abiotic	Biotic
• Process heat generated	• Competition for nutrients
• pH	• Production of lytic enzymes
• C/N	• Microflora of compost
• Organic matter quality	• Biocontrol (competition, antibiosis, hyperparasitism)
• Chemical molecules (like siderophores)	• Toxicity caused by decomposition of some products during composting
• Low molecular weigh molecules able to degrade fungal wall	• Antagonistic microorganisms (Actinobacteria, Fungi and Bacteria)
	• Decomposition level of organic matter
	• Beneficial microorganisms in compost

(Chung and Hoitink 1990; Hadar and Gorodecki 1991; Hardy and Sivasithamparam 1991; Hoitink and Fahy 1986; Nelson and Hoitink 1983; Nelson et al. 1983; Phae et al. 1990; Hoitink et al. 1993, 1996, 1997; Hoitink and Boehm 1999; Hoitink and Krause 2001).

The moisture of compost critically affects the potential for bacterial mesophiles to colonize the substrate after peak heating. Dry composts with lower moisture than 35% become colonized by fungi and are conducive to Pythium diseases. The moisture content must be high enough, at least 45%, so that bacteria can colonize the substrate after peak heating and induce biological control. Often, water must be added during composting as well as curing to avoid this problem (Hoitink et al. 1996).

Compost produced in the open, near a forest, an environment that is high in microbial species diversity, is colonized by a greater diversity of biocontrol agents than the same produced in a closed reactor (Kuter et al. 1983). Field compost is therefore more consistently suppressive to Rhizoctonia diseases.

Two mechanisms of biological control, based on competition, antibiosis, hyperparasitism, and induced systemic resistance in the host plant, have been described for compost amended substrates. Plant pathogens as *Pythium* spp. and *Phytophtora* spp. are suppressed through a mechanism known as "general suppression" (Chen and Hadar 1999; Chen et al. 1988a,b; Cook and Baker 1983; Hardy and Sivasithamparam 1991; Boehm et al. 1993, 1997).

Diseases caused by *Phytophtora* spp. and *Phythium* spp. have been suppressed by many types of microorganisms present in compost correctly produced. High microbial activity prevents both germination of conidia of many pathogens and infection of the host through microbiostasis (Chen et al. 1988a; Mandelbaum and Hadar 1990).

The biological control for *Rhizoctonia solani* in compost is different from that of *Pythium* and *Phytophtora* spp. Few microorganisms are able to eradicate sclerotia of *R. solani* and this type of suppression is referred as "specific suppression" (Hoitink et al. 1991, 1996). *Trichoderma* spp is the predominant hyperparasite recovered from compost prepared with lingo-cellulosic substrates. These fungi interact with various bacterial strains in biological control of Rhizoctonia damping-off (Kwok et al. 1987). *Penicillium* species are the dominant hyperparasites recovered from sclerotia of *Sclerotium rolfsii* in composted grape by-products (Hadar and Gorodecki 1991).

In fresh composts that still contain undecomposed organic matter, in particular cellulose, biological control does not occur because pathogens grow as saprophytes and remain capable of causing disease. Also the biosynthesis of lytic enzymes, involved in hyperparasitism, is repressed due to the high glucose concentration. The same may apply to antibiotic production which plays an important role in biocontrol (Hoitink et al. 1996). In mature compost, where concentration of free nutrients is low and biological control prevails, e.g., sclerotia of *R. solani* are killed by the hyperparasites (Chen et al. 1988a; Nelson and Hoitink 1983). Also excessively stabilized composts, where most of the organic matter is humified and highly mineralised, do not support adequate activity of biocontrol agents (Workneh et al. 1993).

Table 8.3 Microorganisms correlated with suppressiveness of compost

Fungi	Bacteria
Acremonium spp.	*Bacillus cereus*
Chaetomium spp.	*Bacillus mycoides*
Gliocladium virens	*Bacillus subtilis*
Penicillium spp.	*Burkholderia* spp.
Trichoderma spp.	*Chryseobacterium gleum*
Trichoderma hamatum	*Enterobacter cloacae*
Trichoderma viride	*Enterobacter agglomerans*
Verticillium spp.	*Flavobacterium balustinum*
Zygorrhynchus spp.	*Janthinobacterium lividum*
	Paenibacillus spp.
	Pantoea spp.
	Pseudomonas aeruginosa
	Pseudomonas fluorescens
	Pseudomonas putida
	Pseudomonas stutzeri
	Serratia spp.
	Streptomyces spp.
	Streptomyces aureofaciens
	Xanthomonas maltophilia

Source: Hoitink and Fahy (1986), Boulter et al. (2000, 2002), van Elsas and Postma (2007), Hoitink et al. (1993)

Four mechanisms have been described for the activity of biocontrol against soil-borne plant pathogens. They are: (1) competition for nutrients (carbon and/or iron), (2) antibiosis, (3) hyperparasitism and (4) induced protection (Hoitink et al. 1993). In Table 8.3 a list of microorganisms correlated with suppressiveness of compost is reported. Some phytopathogenic microorganisms that can be controlled by suppressive compost are reported in Table 8.4.

8.5.2 Abiotic Action

Various products, originated from decomposing crop residues, are toxic to phytopathogens. Ammonia is often formed early during the composting process. In relatively high concentration, it has a detrimental effect on some pathogenic fungi as *Phytophtora cinnamoni* (Bollen 1993). High pH and low C/N ratio in composting material, due to ammonia presence, will increase suppression of phytopathogenic agents (Gilpatrick 1969; Bollen 1993). Fungitoxic compounds have been detected in the extract of bark compost. Water extracts of fresh composted hardwood bark contain ethyl esters of hydroxyl-oleic acids that inhibit development of *Phytophtora* spp. (Hoitink and Fahy 1986). Compost from helmlock bark releases fungitoxic substances with selective activity (Kai et al. 1990).

Compost produced with grape marc which contains siderophores (microbial iron chelators) has a suppressive effect on nine phytopathogens: *Rhizoctonia solani,*

Table 8.4 Phytopatogenic agents that can be controlled by the use of suppressive compost

Fungi		
Alternaria spp.	*Plasmopara viticola*	*Sclerotinia* spp.
Botrytis spp.	*Pythium* spp.	*Sphaerotheca* spp.
Botrytis cinerea	*Pythium aphanidermatum*	*Sphaerotheca pannosa*
Cylindrocladium spp.	*Phytophtora* spp.	*Stromatina* spp.
Erysiphe spp.	*Phytophtora parasitica*	*Taphrina deformans*
F. oxysporum	*Pseudopeziza* spp.	*Typhula* spp.
Fusarium spp.	*Ralstonia solanacearum*	*Uncinula necator (Oidium tuberi)*
Microdochium spp.	*Rhizoctonia* spp.	*Venturia inequalis*
Monilia spp.	*Rhizoctonia solani*	*Verticillium* spp.
Olpidium spp.	*Sclerotium rolfsii*	*Verticillium dahliae*
Nematodes		
Globodera rostochiensis, Meloidogyne spp., *Pratylenchus* spp., *Trichodurus* spp.		

Boen et al. (2006), Scheuerell and Mahaffee (2002), Bollen (1993), Hoitink et al. (1993), van Elsas and Postma (2007), Hoitink et al. (1977)

Fusarium oxysporum f. sp. *radicis-lycopersici, Fusarium oxysporum* f.sp. *lycopersici* race 0 and race 1, *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, *Verticillium dahliae*, *Pythium aphanidermatum*, *Phytophthora parasitica* and the mycopathogen *Verticillium fungicola* (Diànez et al. 2006). Grape marc is the remaining product once grapes have been pressed to produce vine. In the European Union (EU) more than 20 mio tons per year of grape marc are produced; these can be used as lignocellulosic bulking agents in composting processes to obtain a very active suppressive compost. The microorganisms present in the grape marc compost produce siderophores which capture iron, stopping its availability, thus preventing the phytopathogen development. The suppressivity shown by grape marc compost extracts is a combination of various factors, such as competition for nutrients, antibiosis, production of lytic enzymes outside the cell and of low molecular weight molecules, which, are capable of degrading the fungus wall (Diànez et al. 2006).

Allelopathy toxin, the available carbon-to-nitrogen ratio, and the concentration of soluble salts, and possibly also the chloride ion concentration in composts, are the principal chemical properties identified so far that affect biological control induced by compost (Hoitink et al. 1993). Tree bark compost releases inhibitors (natural fungicides) that lyse zoospores and sporangia of *Phytophthora* spp. Also the presence of mineral nitrogen in compost must be taken into account to avoid severe epidemic causes by plant pathogens. In well stabilized compost most of the nitrogen should be in the organic form.

8.6 Compost Tea

To obtain compost tea, compost is diluted in water; the mixture is homogenized and maintained for different periods of time (from 1 day to 2 weeks). Subsequently it can be filtered, centrifuged and sterilized by filtration. The final liquid is then

sprayed on the leaves of the plant to control air-borne phytopathogens (Scheuerell and Mahaffee 2002).

Soil and plant sprays, based on compost and on different plants, have been in practice since the 1920s (Koepf 1992). An increasing body of experimental evidences indicates that plant disease can be suppressed by treating plant surfaces with a variety of water-based compost extracts (Weltzien 1990, 1991; Diver 1998). Two methods to produce compost tea have being described: with aeration and without aeration. Irrespective of aeration, both methods intentionally ferment compost in water for e defined period of time. Fermentation is intended as a cultivation of microorganisms (Hilton 1999). Both methods require a fermentation vessel, compost and water, incubation and filtration. Nutrients may be added before or after fermentation and various spray adjuvants can be added prior to the application of undiluted or diluted tea (Scheuerell and Mahaffee 2002). There is a debate over the necessity to aerate during compost tea production (Brinton et al. 1996; Ingham 2003). Aeration methods are associated with reduced process time. Non aerated methods are associated with low cost, with low energy input and with many documented reports of plant disease control (Weltzien 1991).

There are several reasons why compost tea use is expanding: Tea has been tested on potatoes, wheat, calla liles, cherries, pears, delphiniums, papayas, sugar beets and turfs in many states, enhancing plant growth, disease suppression, soil health and tilth (Touart 2000). Professional landscapers, golf courses are assessing compost tea for fertility and disease control. Municipal parks and recreation departments are using compost tea for grounds maintenance. A number of individuals and companies are selling compost tea at farmers, retail outlets, internet sites and through application services (Scheuerell and Mahaffee 2002).

8.7 Tests to Evaluate Suppressivity of Compost

In vitro tests can be performed with Petri dishes containing potato dextrose agar on which a layer of water extract from suppressive compost is spread. As a control, Petri dishes are used without water extract of suppressive compost. Both series of plates are inoculated with propagules of phytopatogenic fungi and incubated at 28°C. After some days the growth of the phytopatogen is measured and compared with that occurred in the plate with the water extract from suppressive compost (Diànez et al. 2006). Experiments carried out by these authors in vitro have suggested that microorganisms present in grape marc compost produce siderophores and inhibit several pathogens (see above; Diànez et al. 2006).

Similar tests can be performed using small pots containing suppressive compost or peat with soil. All the pots are irrigated with a suspension containing propagules of a phytopathogenic fungus. Seedlings from different vegetables are then planted in these pots. The pots with suppressive compost should have healthier plants during the cropping cycle. Chen et al. (1988a,b) and Inbar et al. (1991) have established a direct correlation between microbial activity, based on the rate of

hydrolysis of fluorescein diacetate, and the severity of damping-off of cucumber induced by *Pythium ultimum* and *P. irregulare*. This procedure is now used in the United States as a quality control test for natural suppression.

Randomly amplified polymorphic DNA analysis and PCR (polymerase chain reaction) fingerprinting have proven to be a valuable tool for studying DNA polymorphism in fungal organisms (Abbasi et al. 1999). It is a simple and reliable technique that can be used to detect genetic differences at the isolate level in compost (Civilini et al. 2000). Using this approach it is possible to identify molecular markers that can be used to detect a specific isolate of a microorganism that gives specific biological control against phytopathogens.

The utilization of these molecular biological methods as a diagnostic tool for suppressive microorganisms can be very useful for tagging a particular isolate and verify its presence in compost, without transforming the organism. This, highly specific combination techniques allow detection and enumeration of beneficial microorganisms in compost-amended substrates.

8.8 Use of Suppressive Compost

The European strategy on waste management has established a hierarchy on the different treatment options for biowaste which in order are: (1) reduction of waste production; (2) recycling and composting; (3) termovalorisation; (4) landfill. This hierarchy is based on the effects that each option has on the environment and the intent to reach the general aim of sustainability. To improve the integrated management of organic waste and to contribute to an increasing sustainability, it is mandatory to respect the hierarchy above mentioned. A European Directive (EC 31 1999) says that Member States shall set up a national strategy for implementation of the reduction to 35% of the biodegradable waste going to landfill. This will result in a big increase of composting plants in Europe, but if compost would play an important role in this scenario, it would be of prime importance to produce high quality products. Factors that affect the compost quality are: (1) starting material and its conditioning; (2) the composting system and process; (3) the control of the process. Only respecting all the requirements that a composting process needs, it is possible to obtain products compatible with agricultural crops and beneficial to biological fertility of soils.

In the Mediterranean area soils have been depleted since 2,000 years; climatic conditions and intensive agriculture reduce every year the organic matter content in soils. The introduction of compost in agriculture can solve most of these problems (Hoitink et al. 1996). The potential production of compost in the EU is high due to the huge quantity of available organic wastes. The realization depends only by the politics of the single Nations and a good coordination of the EU. Composts can partially substitute chemical fertilizers and pesticides (suppressive compost) (Chen and Hadar 1999; Hoitink and Boehm 1999; van Elsas and Postma 2007), contributing to maintain a sustainable agriculture with renewable resources. In Table 8.5 the

Table 8.5 Compost utilization and benefits

• Biological agriculture (no pesticides)	• Organic fertilizer, soil conditioner
• Contribute to chemical fertilization	• Preparation of growth media
• Plant nurseries (pot and container cultures)	• Horticultural and floricultural substrates
• Green house (peat substitute)	• Green fields (golf, soccer, turfgrass)
• Control of plant diseases (suppressivity)	• Beneficial effect on mycorrhiza and nitrogen-fixation
• Mushroom production	• Reclamation of sandy soils
• Viticulture, pomology	• Recovery of landfills
• Biofilters for air depuration and odor control	• Improvement of soil organic matter status, soil porosity and texture
• Increase in water retention	• Increase of biological fertility of soil
• Benefit to microbial activity in soil	• Improvement of plant nutrient availability
• Prevention of desertification and soil erosion	• Prevention of pollution caused by improper waste disposal
• Reduced leaching of nutrients	• Enhancement of sustainability in agriculture
• Prevention of replant disease	

main benefits and uses of compost are summarized (de Bertoldi and Schnappinger 2001).

8.9 Pesticides in Agriculture

More than 3 mio t of pesticides are produced worldwide each year to be used in agriculture; 30% of this is used in the EU. A wide range of different chemical compounds are currently in use as insecticides, fungicides and herbicides. About 1,000 active ingredients are utilized in the EU (Stanners and Bourdeau 1995). According to World Health Organization, more than 20,000 fatalities per year in the EU may result, only from acute poisoning by pesticides (WHO 1990). Among the different classes of chemicals present in the environment and to which the human population is massively exposed, pesticides play an important role due to their wide use and their toxicity. Most of pesticides used in agriculture are fungicides to suppress soil and air-borne plant diseases. Fungi are eukaryote organisms, genetically more similar to the human being than to bacteria. Therefore, fungicides have a particularly toxic effect to man.

Only recently, besides the acute toxicity, the potential genotoxic effect of pesticides has been considered; newly developed pesticides are currently screened for their potential genotoxic effects and it is mandatory for manufacturers to present the results of mutagenicity studies to Health Authorities. Extensive research carried out by different authors confirm the high mutagenic, carcinogenic and teratogenic activity of a large number of tested pesticides (Siebert et al. 1970; Bridges 1975; Gibel 1975; Shirasu et al. 1976; de Bertoldi et al. 1981, 1983a; de Bertoldi 1996; Ames and Gold 1988).

For these reasons it is of the utmost importance to test pesticides for their potential genetic and carcinogenic activity before they come into contact with man; still more urgent seems the task of testing the effect of those compounds that have already been in use. Moreover, there is an increasing tendency to produce systemic pesticides, some of which may persist in the plant tissues and fruits for some months. The risk in using these compounds is therefore not only confined to the farmers, but also to the people who consume the treated products. The most persistent products may enter the food chain in different way: fruits, vegetables, meat, fish, animal derived products (milk, cheese, eggs, etc.) and water.

The evaluation of the potential risks posed by cancer-causing pesticides in our foods is uncertain. Most of the epidemiological analyses have been done on mammalian species different from man. Nevertheless, the US National Academy of Sciences estimates that 20% of cancer deaths are due to the consumption of pesticide residues in food in the United States in our life times (Winter 1992). The US Environmental Protection Agency (EPA 1989) and the US National Resource Council (NRC 1987) have classified pesticides according to cancer risk for humans. Most of the classified pesticides belong to high risk classes (Ekstrom and Akerblom 1990). Other authors (Gold et al. 2001) minimize the genotoxic effect of synthetic pesticides in food considering the larger amount of natural pesticides (produced by plants) present in the human diet.

A high level of public concern from pesticide residues in food currently exists, and much of this concern is due to the high genotoxic effects of some molecules and to the difficulty to evaluate the persistence in foods of their residues or of their metabolites. Finally, it is possible to assert that epidemiological analyses and dietary pesticide risk assessment are very important parameters to evaluate and to correlate with carcinogenic risk in humans.

The utilization of suppressive compost in agriculture could help to reduce the use of pesticides and to obtain a healthier environment and food.

8.10 Concluding Remarks

A huge amount of scientific papers on disease suppressivity of composts has been produced in the past 40 years. All the aspects have been investigated: starting materials for suppressive compost, how to control a composting process, compost storage, phytopathogenic agent suppressed, microorganisms that induce suppressivity, mechanisms of suppression and so on.

In spite of this abundant literature on disease suppressiveness, there is still insufficient insight into the general principles of disease suppression by compost. Prediction of efficacy of compost application to the field is still premature. Still research is needed to be able to produce specific compost for the suppression of certain pathogens in a specific cropping system.

However, many positive and concrete results have been achieved and production and application in agriculture of suppressive compost is a concrete fact not fiction

(Fuchs 2002, 2010; Fuchs et al. 2006). However, today only two countries, USA and Israel, produce industrially suppressive compost and utilize it to produce horticultural products, fruits and flower without pesticides and free of diseases. In many other countries the utilization of suppressive compost is still in an experimental phase. The potential starting material to produce suppressive compost is very impressive. In the EU, 4,000 mio t of organic refuses are produced (de Bertoldi 2008). Their transformation into compost, suppressive to plant pathogens, would be a splendid result, not only for agriculture but also for the environment and, above all, human health. We must thus reconsider our view of these organic materials not as a waste item to be disposed, but as a valuable resource, put to the highest and best use possible. Simply by keeping compostable organics out of landfill and incineration, we can prevent methane and CO_2 emissions and build healthier soils, recycling organic matter and nutrients. These in turn replenish carbon stocks and provide sustainably healthier foods for the population. Through composting these organic wastes can be transformed into a humified organic fertilizer, compatible with plant growth. The benefits to agriculture soils are: the improvement of soil organic matter status, substitution of chemical fertilizers (N, P, S, K and trace elements), increase in water retention, reduction of nutrient leaching, prevention of soil erosion and desertification, control of plant disease (suppressivity) and sustainability in agriculture.

It is worthwhile to mention also the economical benefits. Recycling organic wastes into compost prevents the cost of land filling and incineration. Besides, the utilization of compost in agriculture saves money to buy chemical fertilizers and pesticides.

References

Abbasi PA, Miller SA, Meulia T, Hoitink HAJ, Kim JM (1999) Precise detection and tracing of *Trichoderma hamatum* 382 in compost-amended potting mixes by using molecular markers. Appl Environ Microbiol 65:5421–5426

Ames BN, Gold LS (1988) Carcinogenic risk estimation. Science 240:1045–1047

Boehm M, Madden LV, Hoitink HAJ (1993) Effect of organic matter decomposition level on bacterial species diversity and composition in relationship to Phythium damping-off. Appl Environ Microbiol 59:4171–4179

Boehm MJ, Wu W, Stone A, Kraakman B, Iannotti DA, Wilson GE, Madden LV, Hoitink HAJ (1997) Cross-polarized magic-angle spinning 13C nuclear magnetic resonance spectroscopic characterization of soil organic matter relative to culturable bacterial species composition and sustained biological control of Pythium root rot. Appl Environ Microbiol 63:162–168

Boen A, Hammeraas B, Magnusson C, Aasen R (2006) Fate of potato cyst nematode *Globodera rostochiensis* during composting. Compost Sci Util 14(2):142–146

Bollen GJ (1993) Factors involved in inactivation of plant pathogens during composting of crop residues. In: Hoitink HAJ, Keener HM (eds) Science and engineering of composting. Renaissance Publications, Ohio, pp 301–319

Bollen GJ, Volker D (1996) Phytohygienic aspects of composting. In: de Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The science of composting. Blackie A&P, Glascow, pp 233–246

Boulter JI, Boland GJ, Trevors JT (2000) Compost: a study of the development process and endproduct potential for suppression of turfgrass disease. World J Microbiol Biotechnol 16:115–134

Boulter JI, Trevors JT, Boland GJ (2002) Microbial studies of compost: bacterial identifications and their potential for turfgrass pathogen suppression. World J Microbiol Biotechnol 18:661–671

Bridges BA (1975) The mutagenicity of Captan and related fungicides. Mutat Res 32:3–34

Brinton WF, Trankner A, Droffner M (1996) Investigations into liquid compost extracts. Biocycle 37(11):68–70

Chanyasak V, Katayama A, Hirai MF, Mori S, Kubota H (1983) Effects on compost maturity on growth of Komatsuna (*Brassica rapa* var. Pervidis) in Neubauer's pot. Soil Sci Plant Nutr 29:251–259

Chen Y, Hadar Y (1999) Compost as a substitute of pesticides. In: Proceedings of International Symposium on Modern Agriculture and Environment, Jerusalem

Chen Y, Hoitink HAJ, Madden LV (1988a) Microbial activity and biomass in container media predicting supressiveness to damping-off caused by *Pythium ultimum*. Phytopathology 78: 1447–1450

Chen W, Hoitink HAJ, Schmitthenner AF, Touvinen OH (1988b) The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. Phytopathogy 78:314–322

Chung YR, Hoitink HAJ (1990) Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of Rhizoctonia damping-off in a bark compost amended container medium. Phytopathology 80:73–77

Civilini M, Venuti F, de Bertoldi M, Damante G (2000) Recovery of *Salmonella typhimurium* from compost with IMS-PCR method. Waste Manag Res 18:1–6

Cook RJ, Baker KF (1983) The nature and practice of biological control of plant pathogens. American Phytopatological Society, St. Paul, MN 539pp

Dailliez L (1981) Les Templiers et l'Agriculture ou les Composts Templiers. Alpes Méditerranée Edition Impress'Sud, Nice, France

Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y (2008) Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol Ecol 65:133–144

de Bertoldi M (1996) Genotoxic effects of pesticides. Eur J Cancer Prev 5:397–399

de Bertoldi M (1999) The control of the process and compost quality. In: ORBIT99. Rombos, Berlin, pp 47–54

de Bertoldi M (2008) The recycling of nutrients by means of organic residues. In: Proceedings of the 6th International Conference ORBIT, Wageningen, pp 1–17

de Bertoldi M, Civilini M (2006) High rate composting with innovative process control. Compost Sci Util 14(4):290–295

de Bertoldi M, Schnappinger U (2001) Correlation among plant design, process control and quality of compost. In: Proceedings of International Conference ORBIT 2001: biological processing of waste. Spanish Waste Club (ed), Sevilla, Spain, pp 3–13

de Bertoldi M, Zucconi F (1986) Composting of organic residues. In: Wise DL (ed) Biotechnology applied to environmental problems. CRC, USA, pp 95–141

de Bertoldi M, Vallini G, Pera A (1981) Mutagenicity of pesticides evaluated by means of gene-conversion, crossing-over and non-disjunction in *Aspergillus nidulans*. In: Proceedings of Third International Conference on Environmental Mutagens, Tokyo

de Bertoldi M, Vallini G, Pera A, Zucconi F (1982) Comparison of three windrow compost systems. Biocycle 23:45–50

de Bertoldi M, Vallini G, Pera A (1983a) Genotoxic effects of some agricultural pesticides in vitro tested with *Aspergillus nidulans*. Environ Pollut 30:39–58

de Bertoldi M, Pera A, Vallini G (1983b) The biology of composting: a review. Waste Manag Res 1:157–176

de Bertoldi M, Vallini G, Pera A (1985) Technological aspects of composting including modelling and microbiology. In: Gasser JKR (ed) Composting of agricultural and other waste. Elsevier, London, pp 27–41

de Bertoldi M, Rutili A, Citerio B, Civilin M (1988) Composting management: a new process control through O_2 Feedback. Waste Manag Res 6:239–259

Diànez F, Santos M, Boix A, de Cara M, Trillas I, Avilès M, Tello JC (2006) Grape mark compost tea suppressiveness to plant pathogernic fungi: role of sideriphores. Compost Sci Util 14(1): 48–53

Diaz LF, de Bertoldi M, Bidlingmaier W, Stentiford E (2007) Compost science and technology. Elsevier, Amsterdam

Diver S (1998) Compost teas for plant disease control. ATTRA, Fayetteville, AR

Ekstrom G, Akerblom M (1990) Pesticide management in food and water safety: international contributions and national approaches. Rev Environ Contam Toxicol 114:23–55

EPA (1989) Reference dose list. Health Effects Division, Office of Pesticides Programs, U.S. Environmental Protection Agency, Washington, DC, pp 1–62

Epstein E, Willson WD, Burge WD, Mullen DC, Enkibi NK (1976) A forced aeration system for composting wastewater sludge. J Water Pollut Control Fed 84(4):687–694

Finstein MS, Morris ML (1975) Microbiology of municipal solid waste composting. Adv Appl Microbiol 19:113–151

Finstein MS, Miller FC, MacGregor ST, Psarianos KM (1985) The Rutgers strategy for composting: process design and control. EPA Research & Development 600/S2-85/059

Finstein MS, Miller FC, Strom PF (1986) Waste treatment composting as a controlled system. In: Schoenborn W (ed) Biotechnology, vol 8. VCH, Weinheim, Germany, pp 363–398

Fuchs JG (2002) Practical use of quality compost for plant health and vitality improvement. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer, Berlin, pp 435–444

Fuchs JG (2010) Interactions between beneficial and harmful microorganisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Fuchs JG, Baier U, Berner A, Mayer J, Tamm L, Schleiss K (2006) Potential of different composts to improve soil fertility and plant health. In: E Kraft, W Bidlingmaier, M de Bertoldi, LF Diaz, J Barth (eds) Biological Waste Management: from Local to Global. ORBIT 2006, pp 507–518

Gibel W (1975) Carcinogenic, hematotoxic and hepatotoxic effects of organic phosphate pesticides. In: Coulston F, Korte F (eds) Environmental quality and safety, pesticides, supplement, vol 4. G. Thieme, Stuttgart, pp 232–234

Gilpatrick JD (1969) Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59:973–978

Gold LS, Slone TH, Ames BN, Manley NB (2001) Pesticide residues in food and cancer risk: a critical analysis. In: Krieger R (ed) Handbook of pesticide toxicology, 2nd edn. Academic, San Diego, CA, pp 799–843

Hadar Y, Gorodecki B (1991) Suppresion of germination of sclerotia of *Sclerotium rolfsii* in compost. Soil Biol Biochem 23:303–306

Hardy GE, Sivasithamparam K (1991) Antagonism of fungi and actinomycetes isolated from composted eucalyptus bark to *Phytophtora drechsleri* in a steamed and non-steamed composted eucalyptus bark-amended contained medium. Soil Biol Biochem 27:243–246

Hilton MD (1999) Small scale liquid fermentation. In: Demain AL, Davies JE (eds) Manual of industrial microbiology and biotechnology. ASM, Washington, DC, pp 49–60

Hoitink HAJ, Boehm MJ (1999) Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu Rev Phytopathol 37:427–446

Hoitink HAJ, Fahy PC (1986) Basis for the control of soilborn plant pathogens with compost. Annu Rev Phytopathol 24:93–114

Hoitink HAJ, Krause MS (2001) Systemic resistence induced in plants against diseases by composts. In: Orbit and Spanish Waste Club (ed) Proceedings of ORBIT 2001, May 9–12, Sevilla, Spain, pp 143–146

Hoitink HAJ, Kuter GA (1986) Effects of compost in growth media on soil born plant pathogens. In: Chen Y, Avnimelech Y (eds) The role of organic matter in modern agriculture. Academic, Dordrecht, The Nederland, pp 289–306

Hoitink H, van Doren DM, Schmitthenner AF (1977) Suppression of *Phytophtora cinnamoni* in a composted hardwood bark mixture. Phytopathology 67:561–565
Hoitink HAJ, Inbar Y, Boehm MJ (1991) Status of composted-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Dis 75:869–873
Hoitink HAJ, Boehm MJ, Hadar Y (1993) Mechanisms of suppression of soilborne plant pathogens in compost-amended substrates. In: Hoitink K (ed) Science and engineering of composting. The Ohio State University, Ohio, pp 601–621
Hoitink HAJ, Stone AG, Grebus ME (1996) Suppression of plant disease by compost. In: de Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The science of composting. Blackie Academic & Profesional, London, pp 373–381
Hoitink HAJ, Stone G, Han DY (1997) Suppression of plant diseases by compost. Hortscience 32:184–187
Inbar Y, Boehm MJ, Hoithink HAJ (1991) Hydrolysis of fluoreshin diacetate pear container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Soil Biol Biochem 23:479–483
Ingham ER (2003) The compost tea brewing manual, 4th edn. Soil Foodweb Inc, Corvallis, OR
Kai H, Ueda T, Sakaguchi M (1990) Antimicrobial activity of bark-compost extracts. Soil Biol Biochem 22:983–986
Klose V, Mohnl M (2010) Microbial antagonists in animal health promotion and plant production. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 193–212
Koepf HH (1992) Biodynamic farming: principles and practice. Anthro Press, New York
Kuter GA, Nelson EBB, Hoitink HAJ, Madden LV (1983) Fungal population in container media amended with composted hardwood bark suppressive and conductive to Rhizoctonia damping off. Phytopathology 73:1450–1456
Kwok OC, Fahy PC, Hoitink HAJ, Kuter GA (1987) Interactions between bacteria and *Trichoderma hamatum* in suppression of Rhizoctonia damping-off in bark compost media. Phytopathology 77:1206–1212
Malek RB, Cartner JB (1975) Hardwood bark as a soil amendment for suppression of plant parasitic nematodes in container grown plants. HortScience 10:33–35
Mandelbaum R, Hadar Y (1990) Effect of available carbon source of microbial activity and suppression of *Pythium aphanidermatum* in compost and peat container media. Phytopathology 80:794–804
Nelson EB, Hoitink HAJ (1983) The role of microorganisms in the suppression of *Rhizoctonia solani* in container media amended with composted hardwood bark. Phytopathology 73: 274–278
Nelson EB, Kuter GA, Hoitink HJ (1983) Effect of fungal antagonists and compost age on suppression of *Rhizoctonia* damping-hoff in container media amended with composted hardwood bark. Phytopathology 73:1457–1462
NRC (1987) Regulating Pesticides in Food: the Delaney paradox. National Research Council, National Academy Press, Washington, DC
Phae CG, Shoda M, Kubota H (1990) Characteristics of *Bacillus subtilis* isolated from composts suppressing phytopathogenic microorganisms. Soil Sci Plant Nutr 36:575–586
Scheuerell S, Mahaffee W (2002) Compost tea: principles and prospects for plant disease control. Compost Sci Util 10(4):313–338
Shirasu Y, Moriya M, Kato K, Furuhashi A, Kada T (1976) Mutagenicity screening of pesticides in the microbial system. Mutat Res 40:19–30
Siebert D, Zimmermann FK, Lemperle E (1970) Genetic effects of fungicides. Mutat Res 10: 533–543
Stanners D, Bourdeau P (1995) Chemicals and genetically modified organisms, In: "Europe's Environment: the dobris assessment," European Environment Agency, Copenhagen, pp 375–382
Touart AP (2000) Time for compost tea in the Northwest. Biocycle 41(10):74–77

van Elsas JD, Postma J (2007) Suppression of soil-born phytopathogens by compost. In: Diaz L, de Bertoldi M, Bidlingmaier W, Stentiford E (eds) Compost science and technology. Elsevier, Amsterdam, pp 210–214

Weltzien HC (1990) The use of composted material for leaf disease suppression in field crops. Monograph Br Crop Prot Counc 45:115–120

Weltzien HC (1991) Biocontrol of foliar fungal diseases with compost extracts. In: Andrews JH, Hirano SS (eds) Microbial ecology of leaves. Springer-Verlag, New York, pp 430–450

WHO (1990) Cancer: causes, occurence and control. In: Tomatis L (ed) IARC Scientific Publication 100. International Agency for Research on Cancer, Lyon

Winter CK (1992) Dietary pesticide risk assessment. Rev Environ Contam Toxicol 127:23–67

Workneh F, van Bruggen AHC, Drinkwater LE, Sherman C (1993) Variables associated with a reduction in corky root and phytophthora root rot of tomato in organic compared to conventional farms. Phytopathology 83:581–589

Zucconi F, Forte M, Monaco A, de Bertoldi M (1981a) Biological evaluation of compost maturity. Biocycle 22:27–30

Zucconi F, Pera A, Forte M, de Bertoldi M (1981b) Evaluating toxicity of immature compost. Biocycle 22:54–57

Chapter 9
Sanitation by Composting

Björn Vinnerås, F. Agostini, and Hakan Jönsson

Abstract In composting, there is always a risk of pathogens and unwanted plant seeds being present. The risk of pathogen content varies with substrate and is the highest in sewage and excreta products. There are several functions regulating pathogen inactivation in composting, with the main effect deriving from the heat produced. Most pathogen inactivation starts at temperatures above 50°C, and it increases with increasing temperature. The compost conditions needed for reaching high temperatures are the available energy and oxygen. The temperature distribution within the compost varies depending on factors such as moisture content and external cooling from incoming air. In areas with lower temperatures, inactivation decreases and in some cases where the material is fresh, growth of pathogenic bacteria can occur. In a composting process, it is impossible to monitor all polluting pathogens and plant seeds, and therefore, the most appropriate management option is the validation of the process regarding its efficiency for pathogen inactivation. Thereafter, the sanitisation effect can be monitored via process parameters such as temperature, pH and product stability.

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9.1 Introduction

If one of the aims of composting is the recycling of organic matter and nutrients to the soil, then the hygiene quality of the product is *critical* for its safe use. Product stability is not a guarantee of compost hygiene quality, though compost looks and smells like soil, it is not necessarily as safe as soil. This chapter looks at the factors regulating compost hygiene quality and the measures that ensure such quality, allowing the nutrient loop to be closed while leaving the pathogens behind.

9.1.1 Hygiene, an Important Factor in Composting

There are several factors that have to be taken into consideration regarding the hygiene safety in composting. The most discussed factor is the final product quality, which is included in most quality assurance programmes and which is also strongly related to consumer-acceptance of the product. The main organisms that pose a threat to compost hygiene are presented in Table 9.1. As it is impossible indeed to monitor the quality of each and every kilogram of a compost produced, a safety assurance system is very much needed whereby the efficiency of the process is evaluated mainly by monitoring process parameters in combination with an initial validated process to verify the final quality of the products. However, the risk remains of disease transmission before and during treatment to workers, who in some cases handle unsanitised material. The more manual labour included in the treatment, the higher the risk of disease transmission. Of course, there is also

Table 9.1 Pathogenic agents that can be present in the starting material for composting

Pathogen	Disease
Viruses	
Enterovirus	Gastroenteritis, meningitis
Rotavirus	Gastroenteritis
Norwalk virus	Gastroenteritis
Parvovirus	Gastroenteritis
Adenovirus	Respiratory infections
Hepatitis A virus	Viral hepatitis
Hepatitis E virus	Viral hepatitis
Polio virus	Poliomyelitis
Ecovirus	Meningitis
Coxsackie virus	Meningitis
Bacteria	
Salmonella (1,700 types)	Typhoid fever
Mycobacterium tuberculosis	Tuberculosis
Vibrio cholerae	Cholera
Verotoxin producing *Escherichia coli* (VTEC)	Haemorrhagic colitis
Yersinia enterocolitica	Gastroenteritis
Listeria monocytogenes	Meningo-encephalitis
Shigellae spp.	Sigellosis
Yersinia enterocolitica	Yersinosis
Campylobacter jejuni	Gastroenteritis
Protozoa	
Cryptosporidium parvum	Cryptosporidiosis
Cyclospora cayetanensis	Gastroenteritis
Entamoeba histolytica	Amoebiasis
Guardia lambdia	Guardiasis
Balantidium coli	Balantidiasis
Naegleria fowleri	Meningo-encephalitis
Acanthamoebae	Meningo-encephalitis
Helminths	
Ascaris lumbricoides	Ascariosis
Enterobius vermicularis	Enterobiasis
Strongyloides stercolaris	Strongylaidiasis
Toxocara spp.	Toxocariasis
Taenia spp.	Tapeworm
Hymenolepis spp.	Hymenolepiasis (tapeworm)
Echinococcus spp.	Echinococcosis (tapeworm)
Thrichuris thrichuria	Thricuriasis

the risk of transmitting diseases to the surrounding environment, e.g., water recipients (by runoff water), wild animals and aerosols.

Most diseases only infect one species, although some can infect several. These are called zoonoses, and examples are salmonella and verotoxin-producing *Escherichia coli* (VTEC). There are two main types of transmissions to vectors staying at the site for shorter or longer time periods, e.g., flies, birds and rodents, and

to larger wild animals such as deer and boar, which can become a reservoir, thus potentially affecting humans and animals in the surrounding area (Albihn and Vinnerås 2007).

Other factors that need to be included in the assessment of compost quality are with the presence of plant pathogens, which behave similar to animal and human pathogens, and the presence of seeds from unwanted plants that can pollute the end-product, e.g., tomato, papaya, melon. However, the content and germination capacity of plant seeds can also be used as an indicator of product quality regarding plant and animal hygiene as discussed below.

9.1.2 Barriers – Use of Product

When waste fractions are treated for reuse, several barriers should be included to prevent disease transmission. The treatment is one of the most important barriers as it is here that the actual numbers of pathogens can be reduced. Another barrier that affects the risk of disease transmission is the source of the incoming material, e.g., material from hospitals, toilet waste, sewage sludge, etc. Finally, selection of the end-use of the compost produced is itself a further barrier, as material of uncertain quality can be used for application to non-food crops or to crops for animal feed if the quality is slightly higher.

9.2 Risk Factors

9.2.1 Incoming Material

Depending on the source of the incoming material, the risks of contamination of unwanted organisms such as pathogens vary. The main risk material for transmission of disease via the faecal-oral route is animal manure and fractions that contain human excreta, such as latrine or sewage sludge, which from a large population usually contain pathogenic pollutants (Sahlström et al. 2004). By controlling and monitoring the treatment process, there is no problem in managing the increased risk of these fractions. Only including food waste in the composting process does not ensure a safe material, since there can be faecal contaminants present and since the food waste itself can contain and also promote growth of pathogens such as *Salmonella* spp.

9.2.2 Contamination of Composting Material

During the treatment process, pathogenic bacteria can contaminate and grow within the composting material. The source of contamination can be vector organisms such

as birds and rodents. Another important source is the use of contaminated equipment. If pathogenic bacteria contaminate the composting material before it is well stabilised, and when the temperature is below sanitising levels, then there is a high risk of contamination and growth of these bacteria.

9.3 Organisms That Pose a Risk

The main risk of disease-transmission from compost is posed by pathogens that follow the faecal-oral route. These pathogens can be divided into four categories: non spore-forming bacteria, spore-forming bacteria, viruses and parasites. In addition, these categories can be divided into two, organisms specific to one species and zoonoses that can infect both animals and humans, e.g., *Salmonella* spp. The main human pathogenic agents are listed in Table 9.1.

9.3.1 Enteric Pathogens

The enteric Gram-negative bacteria behave relatively similarly in compost material and, therefore, the reduction in these organisms can be monitored by analysis of Gram-negative indicator bacteria, e.g., faecal coliforms, especially thermotolerant coliforms.

Salmonella spp., are Gram-negative, rod-shaped bacteria, and are zoonoses that infect both humans and animals. The infective dose can be as small as 20 cells, but in most cases 10^4–10^6 cells are required for infection (FDA 1992). However, as for all organisms, the infective dose varies from host to host depending on factors such as age, time of infection, nutritional status, etc., and the most severe infections occur in young and old people and in immuno-suppressed individuals. Growth of *Salmonella* spp. has been reported in sewage sludge (Sahlström et al. 2004), in compost material (Elving et al. 2009) and in manured soil (Gibbs et al. 1997).

VTEC produce verotoxins, a shiga-like toxin. The behaviour of such strains is similar to that of the non VTEC-producing *E. coli,* which means that they have high survival in the environment. The infective dose of VTEC is as low as ten organisms, depending on the receptivity of the host (FDA 1992).

Vibrio cholera is a Gram-negative, curved-rod bacterium motile with polar flagella. It is mainly found in water and most outbreaks are related to water (FDA 1992). It has a high infective dose ($>10^6$) and the organism has a low thermal resistance (Feachem et al. 1983; Vinnerås et al. 2003). It should, therefore, not pose any major risk within compost management.

Shigella spp. is a Gram-negative, rod-shaped bacterium closely related to *E. coli* and *Salmonella*. Some of the *Shigella* strains produce enterotoxin and shiga toxin, which is similar to the toxin found in VTEC.

Campylobacter jejuni is a Gram-negative, slender, curved and motile rod. It is a micro aerophilic bacterium that is sensitive to high O_2 levels and to most other

factors related to composting, such as heating, drying and acids. Therefore, this organism should *not* be of any great concern in compost management.

9.3.2 Other Pathogenic Bacteria

Listeria monocytogenes is a Gram-positive bacterium, motile by means of flagella. It is normally found in the environment and is relatively hardy, as it can withstand heat and drying. Most people infected do not show any symptoms at all and the risk is only related to the foetus during pregnancy and immuno-suppressed individuals (FDA 1992). This organism should not be of any great concern regarding compost management.

Mycobacterium tuberculosis is an obligate aerobe, acid-fast, Gram-positive bacterium that causes tuberculosis. It is slow-growing in bacterial terms but very resistant to chemicals and drying (FDA 1992). *M. tuberculosis* is relatively stable in the environment but is thermally inactivated, with a decimal reduction of less than one day in compost at 55°C (Grewal et al. 2006).

9.3.3 Pathogenic Viruses

Several viruses are included in the group that cause gastroenteritis, but only a few of these viruses are considered to be zoonotic, e.g., Hepatitis E infecting both humans and pigs. Other viruses commonly included in this group are Hepatitis A, Rotaviruses, Noroviruses, Astroviruses, Caliciviruses, Enteric adenoviruses, Enteroviruses and Parvovirus. In total, more than 130 pathogenic viruses may be present in faeces-contaminated material. The infective dose of viruses is generally low, even as low as one single viral unit (FDA 1992). Viruses do not proliferate outside the body, as they need their host for growth. Some of these viruses are very thermoresistant and studies have shown slow reduction at high temperatures (Sahlström et al. 2008), but treatment in thermophilic composting including mixing should be sufficient for appropriate reduction (Feachem et al. 1983; Vinnerås et al. 2003; Wichuk and McCartney 2007). Viruses in stool samples are mainly detected via electron microscopy or immunoassays, but such analyses are complicated and costly to perform for compost material. One alternative for monitoring the viral count is to use naturally occurring bacterial viruses, i.e., coliphages, and correlate their thermal inactivation in the material and use them for monitoring the viral reduction during the process.

9.3.4 Pathogenic Parasites

Parasites can be divided into two groups, protozoa and helminths. Both groups have a low-infective dose but they cannot increase in numbers outside the host.

The protozoa, mainly the zoonotic *Giardia* and *Cryptosporidium*, are relatively sensitive to heat treatment and are rapidly reduced within thermophilic composting (Feachem et al. 1983; Wichuk and McCartney 2007).

The helminths, on the other hand, include very heat-tolerant microorganisms, with *Ascaris* spp. regarded as the most thermoresistant pathogen (Feachem et al. 1983; Haug 1993). The inactivation of complex organisms such as *Ascaris* cannot be regarded as linear (Nordin et al. 2009), as most studies show an initial lag phase before inactivation starts. Even if the organism is resistant, it shows a reduction that increases in speed with temperature for thermophilic conditions over 50–55°C (Feachem et al. 1983; Vinnerås et al. 2003; Wichuk and McCartney 2007).

9.4 Inactivation Process

There are several factors that regulate the inactivation of unwanted microorganisms and seeds in compost. A number of chemical substances affect microorganisms, especially in combination with heat. Ammonia is a well-known antibiotic agent (Warren 1962), which in its uncharged form inactivates most organisms, especially bacteria and parasites. The inactivation of viruses is somewhat slower, but at the temperatures found in compost the effect is high for all organisms (Vinnerås et al. 2003, 2008; Pecson et al. 2007; and Nordin et al. 2009). Ammonia is one of the end-products during degradation of proteins and peptides. Most of it is lost as gaseous emissions (Haug 1993) but to some extent, the ammonia gas can affect the sanitation process. Other products found in the compost are volatile fatty acids (VFA), which have an effect on microorganisms especially when the temperature increases (Sundberg et al. 2004). During the initial stage of composting and when areas of the compost become anaerobic, organic acids are produced as explained further below. Another factor that can have an influence is the competition with other organisms for energy consumption, but also via antagonistic relationships. The combination of these factors leads to a natural decay over time, as the environment is not optimised for growth of pathogenic bacteria. In most cases, these factors cannot regulate the inactivation of pathogenic organisms alone. They are also difficult to monitor for an estimation of reliable inactivation. Therefore, heat is the single easily monitored factor within the composting process for reliable inactivation to ensure that a properly sanitised end-product is produced.

The following sections, therefore, mainly focus on the hygiene quality of compost in relation to heat production and distribution in the material, in combination with the actual inactivation process.

Regardless of the type of organism, the inactivation during the composting process is mainly driven by heat inactivation. To ensure reduction of the pathogens by heat, an understanding of the microbial population's resistance to heat is crucial. The heat stresses the organisms, with the end result that proteins are denatured. During mild heat stress, the effect on microorganisms is a reversible inactivation that is negated when the temperature decreases. The effect of the temperature is that

the organisms start producing heat-shock proteins that assist other proteins to maintain their integrity and avoid being denatured by the heat. At a certain point, the heat becomes too strong for the organisms to withstand and the proteins are irreversibly denatured. The rule of thumb used in most situations is that a temperature of at least 50–55°C is needed for successful inactivation of pathogens. Generally, no pathogenic bacteria grow at these temperatures but some pathogenic organisms *can* withstand high temperature stress for a long time, when the temperature is up to 50°C and sometimes above, before being inactivated. The higher the temperature, the more efficient the inactivation, and the better the heat transfer, the faster the inactivation, i.e., moist heat is more efficient than dry heat. Thermotolerant organisms such as those active in the compost during the thermophilic phase can withstand high temperatures but are normally not considered to be pathogenic.

The main exception regarding inactivation of pathogens at high temperatures is some spore-forming bacteria, which in their spore form withstand high temperatures without inactivation (FDA 1992). The relevant organisms mainly affect animals, e.g., *Clostridium schauvoei*, which causes black leg. In addition, some organisms mainly affect farming due to toxin production, e.g., *C. botulinum*. The main effect of these toxins occurs when they are present in the feed, e.g., in silage, or in the bedding, e.g., on chicken farms. As *Clostridium* spp. are obligate anaerobes, they only increase during anaerobic conditions, which can develop in anaerobic parts of a compost mass.

The other group of spore-forming organisms, *Bacillus* spp., includes the pathogen *Bacillus anthraci*. However, anthrax is not commonly associated with waste and manure and is thus beyond the scope of this paper. Spore-forming organisms, in general, are very heat-resistant in their spore form and are probably not significantly reduced during composting treatment. On farms experiencing problems due to spore-formers, extra measures need to be taken regarding manure management to reduce the spread of these organisms within the farm and to the surrounding environment.

9.5 Heat Generation in the Compost

Composting can be defined as the *aerobic degradation of organic substrates mediated by microbes*. The organic substrates are ideally oxidised completely, which leads to the generation of large amounts of heat. The heat generated in the complete oxidation of a molecule of fatty acid and a molecule of glucose is given in (9.1) and (9.2), respectively.

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O \quad \Delta H = -39.2 \text{ MJ kg}^{-1} \tag{9.1}$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \Delta H = -15.7 \text{ MJ kg}^{-1} \tag{9.2}$$

As can be seen from these equations, complete oxidation of the organic substrates releases large amounts of heat. The actual heat released during the build-up phase of composting is not as large as that shown by (9.1) and (9.2), because much of the energy from the substrate is spent on the growth of the bacterial population.

During this build-up phase, the degradation activity can occur very quickly. During short periods activity increases exponentially, before it is rate-limited by substrate deficiency. Turning the compost material can relieve this rate limitation and the activity can again increase very quickly provided that the environmental conditions are optimal, i.e., that the most limiting factor for degradation is the size of the bacterial population and no other environmental condition (Eklind et al. 2007; Fig. 9.1). The exponential increase in degradation is mainly due to two factors, the exponentially increasing bacterial population and the rapidly increasing temperature.

Under such optimal degradation conditions, the peak of the degradation activity is relatively sharp, at least in a substrate consisting of fine particles (Fig. 9.1). In such a substrate there is a sharp peak, after which the degradation activity only continues for 1–2 days before starting to fall exponentially. This decrease is probably mainly due to decreasing access to available and easily degradable substrates, as by this time sugars are getting depleted, the starch is rapidly decreasing and the main substrate is rapidly turning into hemicellulose and cellulose (Eklind et al. 2007; Fig. 9.2).

Often, however, the degradation activity is limited to far less than the maximum by non-optimal environmental conditions such as temperature (Haug 1993; Eklind et al. 2007), temperature in combination with pH below 6–6.5 (Nakasaki et al. 1993; Choi and Park 1998; Smårs et al. 2002; Sundberg et al. 2004), limited oxygen

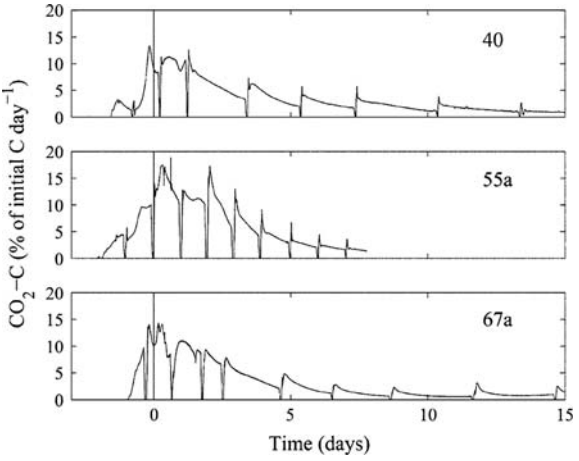

Fig. 9.1 Degradation measured as CO_2-C evolution when composting source-separated kitchen waste. Day 0 was when the pH was high enough to allow the temperature to increase above 37°C. The sharp peaks and troughs in the diagrams are due to daily turning of the compost. The numbers 40, 55a and 67a denote runs at 40, 55 and 67°C, respectively. (Figure from Eklind et al. 2007)

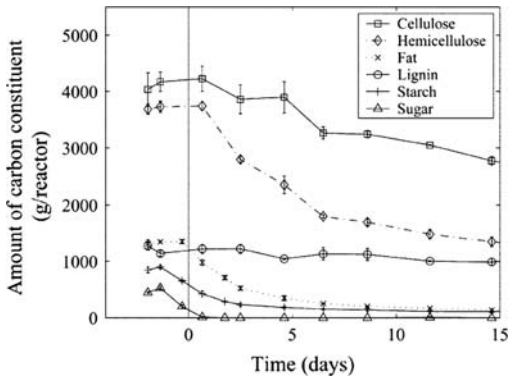

Fig. 9.2 Substrate constituents remaining in the reactor in run 67a from Fig. 9.1. (Figure from Eklind et al. 2007)

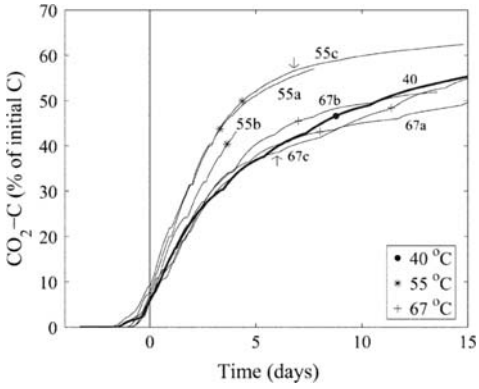

Fig. 9.3 Cumulative carbon turnover when composting at 40, 55 and 67°C. (Figure from Eklind et al. 2007)

availability (e.g., Richard et al. 2002; Beck-Friis et al. 2003) or limited water availability (e.g., Haug 1993; Richard et al. 2002).

The optimal temperature for degradation of most substrates is somewhere around 55°C (Haug 1993; Eklind et al. 2007; Fig. 9.3). In large compost masses, the temperature can easily rise too high, up to 70°C and even 80°C. While this is very good for the reduction of pathogens, it seriously decreases the rate of degradation of the organic substrates, which can have a negative or positive influence depending on the specific circumstances. It is negative if the goal is to optimise the degradation, i.e., if the goal is to produce mature compost in the shortest available time, while it is positive if the amount of easily available substrates is borderline for safe sanitisation, as it slows down the degradation of the substrates and preserves them. At this high temperature, pathogen reduction is fast, and thus a high temperature can be a good way to economise on a limited amount of degradable substrates in such a way that complete sanitisation is achieved.

In many small composting units, the degradation activity is limited by low temperature, much below 55°C. This is a very serious limitation as regards sanitisation, as for many pathogens there is little or no reduction at temperatures below 50°C. The seriousness of this condition is due to the fact that the degradation

activity at around 40°C is as large as it is around 65–70°C (Fig. 9.3), but while the pathogen reduction is very high at 65–70°C, for most pathogens the reduction at around 40°C is very low or even negligible.

At a pH of 6–6.5, the degradation activity is also much more sensitive to temperature. It is seriously inhibited if the temperature increases to about 38°C or above (Smårs et al. 2002; Sundberg et al. 2004). These conditions are common when substrates with a large proportion of kitchen waste are composted, e.g., in municipal plants composting source separated household waste in the Nordic countries (Sundberg and Jönsson 2008). There is hardly any garden waste in the source-separated kitchen waste in these countries and the reason for the low pH is that there is a high concentration of lactic acid bacteria in fresh kitchen waste (Sundberg et al. 2008). The decrease in pH is mainly from the activity of *Lactobacter* spp., which uses the available sugars for production of lactic acid. During the initial collection and storage phase in the household, the activity of these organisms is high, resulting in a low pH, sometimes as low as 4.5 (Eklind et al. 1997), in the incoming waste. In the initial stage of composting, the pH often sinks even further due to the continued activity of the acid-producing bacteria, as when there are ample supplies of sugars some bacteria prioritise incomplete degradation, producing organic acids, instead of full degradation to carbon dioxide and water.

In composts with only small heat losses, i.e., large composts, the inhibition due to low pH (<6–6.5) and high temperature (>40°C) can become semi-permanent. Any degradation that still goes on under these conditions lowers the pH rather than increases it (Sundberg et al. 2004, 2008). This means that the compost can linger at a temperature around 40–50°C for several months with minimal degradation (Sundberg et al. 2008). At the same time, both the degradation activity and the pathogen reduction are low.

If the availability of oxygen is low or totally lacking, the initial tendency for the pH to decrease is enforced due to anaerobic degradation, initially mainly acidification (Beck-Friis et al. 2003). Low oxygen availability is a sign of the aeration being inadequate. While the most common cause of this is inadequate capacity of the aeration system, another very common cause is excessive water content in the substrate, resulting in too low a volume of air-filled pores. A rule of thumb is that an air-filled pore volume of about 30% is needed for well-functioning aeration of large composting units (Haug 1993). If the moisture content is too high, then too many of the pores become waterlogged, which inhibits the oxygen supply to the microbes, limiting aerobic oxidation and thus heat generation. The relationship between moisture level and air-filled pores depends on the structural strength and water-absorption capacity of the substrate and thus, the optimal moisture content is substrate-dependent.

On the other hand, if the moisture level becomes too low, for most substrates below 40–45%, then the degradation activity is hampered by limited access to water. If water addition is possible, as it is on household and small scale, it is often advisable to start with the substrate being a little bit too dry to ensure well-aerated conditions and to minimise anaerobic zones and thus the production of VFA and odour. Furthermore, the heat capacity of the material is lower with lower moisture

content (Haug 1993), facilitating a more rapid increase in temperature. As the temperature and activity increase, the substrate dries and becomes too dry, particularly if it is energy-rich, like kitchen waste (Sundberg and Jönsson 2008). At this stage, water should be added. The degradation activity can actually be regulated by the moisture level in the substrate, and ensuring that the moisture level is always below optimum can minimise the risk of anaerobic zones and odour production.

In large composting plants, addition of sufficient amounts of water to the substrate is often difficult and thus, it is tempting to allow the initial moisture level of the substrate to be a little too high. However, there is a sharp divide between a moisture level that is slightly high but still gives a high-rate composting process, and a moisture level that is too high and seriously inhibits the aerobic process (Richard et al. 2002; Niwagaba et al. 2009). Such anaerobic conditions often result in production of VFA, leading to a decrease in pH and in a long period of inhibited degradation due to the combination of high temperature and low pH. Studies of wet composting substrates show that the temperature never reaches much above 40°C at a pH below 6.5, even when the moisture level is adjusted (Niwagaba et al. 2009). Thus, it is critical to avoid starting with too high a moisture level, as this easily results in a vicious circle for the process (Sundberg et al. 2008). When the material is not degraded but rather preserved, changes in the composition, e.g., buffering the compost, can lead to high regrowth of pathogenic bacteria (Elving et al. 2009), even if these are not initially detected (Gibbs et al. 1997).

9.6 Distribution of Heat in the Compost

Heat generation and transport within the composting mass are critical events for the whole composting process, especially if one of its purposes is heat inactivation of pathogens. It is not possible to design a successful composting process that ensures the complete treatment of the whole substrate with predetermined temperature gradients *without* being able to model the heat distribution within such a substrate, accounting for all the thermodynamic factors involved (Mason and Wilke 2005).

Compost substrates are extremely heterogeneous in composition and in physical and chemical properties. However, they always consist of an organic solid component, the majority of which is easily degradable, a microbial community, and gas and liquid phases, which provide the water and the oxygen demanded by the microbial degradation (Haug 1993). In such a context, the distribution of heat within the compost cannot be based on a single or few physical models, since it is itself the result of complex interactions between concurrent and dependent physical processes: heat conduction through the solid matrix, heat conduction by the non-solid phases and within them, heat exchange between the phases, heat convection, vapour air diffusion, water movement due to capillarity, air-vapour equilibrium and evaporation. Such a scenario increases further in complexity because the substrate changes radically during the composting process in its chemical composition and physical structure. The main change occurs in the ratio

between the three physical phases, with volume and mass decreasing due to water and gas losses. Therefore, the evolution of porosity, bulk density and water content within the compost must also be accounted for when investigating the heat distribution during composting (Hogan et al. 1989; Sarwar and Majumdar 1995; Izadpanah et al. 1998).

In such a multiphase environment, the heat distributed along the solid matrix, even if this is heterogeneous in composition, follows the fundamental Fourier Law on heat transfer:

$$\overrightarrow{\phi_q} = -k\overrightarrow{\nabla}T$$

where $\overrightarrow{\phi_q}$ is the local heat flux (W m^{-2}), k is the material's conductivity (W m^{-1} K^{-1}), and ∇T is the temperature gradient (K m^{-1}).

In this case, the flux is a function of the thermal conductivity (k) of the components of the solid matrix, which is also a function of the contact surface between the particles of the matrix. More precisely, the matrix stores a part of such heat according to its specific heat capacity (c_p), so the thermal diffusivity (α) coefficient, ($k/\rho c_p$), is the parameter to consider. The other distribution pathways are the conduction–convection mix transfers *between* the three phases and *within* them. The weight of these distribution flows can be assumed to range from small, and nearly negligible, to prevalent with the increase in empty spaces, fluid contents and circulation within the solid matrix.

Different approaches can be adopted to quantify the heat distribution in such a scenario, which is essentially a case of heat distribution in a porous medium where the solid, liquid and gas phases do not maintain constant conditions, but continuously change with the progression of the composting process. A general simplification is to look only at the conductive transfer and to neglect all the convection and mix transfers.

In the simplest scenario of all, the solid component is prevalent and relatively stable during the process, the ideal case of a non-aerated low porosity substrate and with low moisture content. The temperature gradient can then be calculated (9.3) as the product of thermal diffusivity coefficient of the matrix for the second derivative of the difference in temperature between points in relation to their internal distances (Carslaw and Jaeger 1958):

$$\frac{\partial T}{\partial t} = \alpha \frac{\partial^2 T}{\partial d^2} \tag{9.3}$$

However, since the solid matrix is of variable chemical composition and the effect of the porosity (at least 30% in most compost substrates, according to Haug 1993) is relevant, an α_{eff} (effective thermal diffusivity coefficient), which accounts for these should be calculated as:

$$\alpha_{\text{eff}} = \varepsilon\alpha_{\text{air}} + (1-\varepsilon)\alpha_{\text{average solid components}} \tag{9.4}$$

where ε is porosity.

A further step is to account for the conduction occurring along all three phases and as a function of the contact surfaces between them (Sarwar and Majumdar 1995). A weighted harmonic average of α of the different phases is then used in the basic differential equation, assuming α_{sol} to be the diffusion coefficient of the solid particles and α_{Por} to be that of the pores filled by air–water mix (9.5):

$$\alpha_{eff} = \frac{2\alpha_{sol} + \alpha_{por} - 2\varepsilon(\alpha_{sol} - \alpha_{por})}{2\alpha_{sol} + \alpha_{por} - \varepsilon(\alpha_{sol} - \alpha_{por})}\alpha_{sol} \tag{9.5}$$

Considering instead the more realistic conductive and convective scenario, the parameters to be accounted for and therefore, measured or extrapolated increase in number because the heat distribution and movement within the fluid mix (air, water, and vapour) must also be described. The heat transfer coefficient between the solid phase (or more accurately, between its different components) and the non-solid phases must then be considered, i.e., the convective heat transfer coefficient and the thermal conductivity of the fluid mix. In particular, the ratio between these two terms or the Biot number (which indicates whether heat moves faster within the fluid and is then dispersed, in which case the heat gradient within the fluid is negligible) should be determined (Erdogu 2005, 2008).

When the heat distribution is greatly affected by the fluid dynamics within the composting mass, e.g., the case of forced air ventilation to control temperature and moisture, then the fluid mix properties determine whether the heat is mainly transferred by fluid convection or by fluid conduction (Izadpanah et al. 1998; Seki 2000). All such parameters and their relationships are summarised and expressed by specific dimensionless numbers expressing the effects of viscosity (Prandtl number), flow paths among the solid components (Rayleigh number) and types of flow (laminar, turbulent or mix) determined through viscosity, buoyancy or inertia (Grashof, Reynolds or Nusselt numbers, respectively). The values of these parameters must be known in order to understand heat distribution in complex systems, such as natural and forced ventilated systems. The fluid dynamics within the compost can be even more difficult to describe if the heterogeneity of the substrate leads to the formation of areas with different affinity to water or different porosity. In these cases, the mathematical models of heat distribution increase their complexity depending on the accuracy with which the substrate and its phases are described, necessitating the use of partial differential equations through the need to couple two or more physical models from different physical fields (e.g., Darcy flow dynamics and heat convection, evaporation and capillarity, etc.). The study of heat distribution in such conditions is based on different computational approaches in functions of pore dimensions and evaporation rates, e.g., finite element method, finite differential method or finite volume method (Datta 2007a, b).

Heat distribution within a composting substrate is a very complex phenomenon, which can be studied with different levels of accuracy and precision. If the aim of the investigation is the assessment of time and temperature gradients to establish a selective microbial environment against microbial pathogens, a minimum-risk

approach involves not relying on pure theoretical modelling, which cannot account for the presence of spot cold zones generated by drastic changes in thermal conductive properties within the substrate due to its heterogeneity and to associated events such as evaporation or unpredictable changes in porosity. These untreated areas could generate undesired health hazards. Instead, composting design should always consider technical strategies such as mass turning and mixing and/or extended treatment periods, which ensure the complete, uniform and thorough exposure of the substrate to heat fluxes.

9.7 Function of Mixing

The general method for management of the temperature distribution in the compost material, and thereby the hygiene in the process, is *mixing* during the high temperature phase. As the inactivation is temperature-related, only the compost areas where high temperatures are reached are sanitised. Therefore, mixing is required for transportation of unheated material into regions where the material is hot. By performing temperature measurements, it is possible to estimate the temperature distribution in the compost and from that calculate the proportion of compost that can be considered to have a sanitising temperature and the proportion that does not achieve such a temperature.

Based on the distribution of hot and cold areas in the compost, it is possible to calculate the extent to which the material needs to be turned for sufficient reduction of unwanted organisms and pathogens. The simplest assumption is that there is total inactivation in the high temperature area, which is achieved if the minimum temperature of 50°C is combined with a treatment time allowing several days between turnings, thereby ensuring high levels of inactivation (Feachem et al. 1983; Vinnerås et al. 2003). The higher the temperature, the shorter is the possible time between turnings while still the same level of safety is maintained. The other assumption is that there are no changes at all in the microbial population in the cold zone. Any changes there can have a major effect on the overall inactivation. If there is a decrease in the number of organisms, the total effect is simply an increase in the safety level, as the total number of pathogens is the sum of those surviving in the hot and cold zones (9.6).

$$n_t = n_0 (f_l)^{N+1} \tag{9.6}$$

where n_t = number of organisms at time t, n_0 = number of organisms at time 0, f_l = proportion of compost in cold zones and N = number of turnings of the pile.

However, if the organisms increase in number in the cold zone (see below) the total number of microorganisms can actually increase. Studies performed on regrowth risk have linked the degree of inactivation to the maturity of the compost material, with e.g., Sidhu et al. (2001) showing a decreased risk of regrowth with increasing compost maturity. This is due to decreased availability of nutrients and

energy in the form of short-chain, easily available organic molecules. Later studies performed by (Elving et al. 2009) have been able to link the maturity of the compost (measured using Solvita and Rottegrad maturity tests) to the pattern of regrowth of faecal indicator bacteria and *Salmonella* spp. In that study, a good correlation was found between low compost maturity and pathogen growth, with microorganisms added to immature compost growing to a high extent (increase corresponding to 1–3 $\log_{10}$ growth) during the first 3 days. Studies of the survival of pathogens in compost/manure-amended soil have shown that even when no pathogens were detected initially, after a change in climate, such as increased moisture due to rain, pathogens had re-grown (Gibbs et al. 1997).

The potential of microbial growth in compost cold zones generates a risk of the total number of organisms increasing. If 10% of the compost is not hot enough for inactivation and there is a growth corresponding to 2 $\log_{10}$ in this area, the total number of pathogenic bacteria in the compost, even though all pathogens are inactivated in 90% of the compost, will increase by 1 $\log_{10}$. Therefore, the inactivation outcome for pathogenic bacteria is a combination of the inactivation in the hot area and the possible growth in the cold area. As discussed above, the risk of pathogen growth in the compost is related to the maturity of the material, with more mature material carrying a lower risk of regrowth or recontamination. It is important to consider the risk of false stability, e.g., due to moisture deficiency, where the material shows stability and no detectable pathogens but where changes in the environment such as increased moisture can result in an increase in bacterial pathogens.

9.8 Calculation of Inactivation

The time required at a certain temperature to inactivate 90% of the microbial population is called the decimal reduction time, or D-value. The D-value is given in minutes together with the temperature studied, e.g., D60 = 40 min, meaning that the population number has been reduced by one decimal place at a temperature of 60°C when heated for 40 min, and thus 10% of the original population still remains. For the treated material to be considered safe from a hygiene point of view, the reduction in pathogens has to be related to the hygiene status of the incoming material. A rule of thumb is provided by the EU animal by-product (ABP) directive and the WHO recommendations on reuse of faeces. The ABP directive demands a reduction in thermotolerant *Salmonella senftenberg* W775 S- or the indicator *Enterococcus faecalis* corresponding to 5 $\log_{10}$ and, in cases of risk for viruses, a 3 $\log_{10}$ reduction in thermotolerant viruses. The WHO guideline on faecal management recommends a 6 $\log_{10}$ reduction in potential risk organisms.

The resistance characteristics of microorganisms can be determined by exposing the microorganisms to heat at a defined temperature and plotting the survival curves or by using fraction negative methods. Ideally, the survival curve is linear (since microbial death is an exponential function), but most microorganisms do not

show such behaviour over the full plotted range. Plotting semi-logarithmic survival curves ($\log_{10}$ of the viable part of the population against time) involves enumeration (the method is also referred to as the direct enumeration method), while the fraction-negative method is a statistically based calculation of surviving micro-organisms in the test population. The fraction-negative method usually requires more samples than a survivor curve test since it is applied in the lower range of microbial counts and uses observation of growth in a fluid growth medium as a +/– result. In the survivor curve, the slope of the plotted line is calculated (least squares; see international standard ISO 11138-1 for extended guidance on test methodology).

The inactivation of pathogens differs in different materials. This is mainly due to the different characteristics of the material. The main inactivation factor is the heat transfer from the surrounding material to the organisms themselves. This transfer is mainly related to the moisture content of the material as the heat transfer is much higher when it is accompanied by moisture. This is easily shown by the difference in time and temperature requirements during dry sterilisation compared with moist sterilisation. The difference in heat transfer can be shown by the difference in heat inactivation on egg albumin, where the coagulation temperature at 0% moisture is 165°C, while at 50% moisture, it is 56°C (Haug 1993).

In addition to this, the heat transfer can be affected by other factors such as non-homogeneous moisture distribution, with dry or isolated parts of the compost. Furthermore, organisms closely attached to particles in the compost can have microclimates as the material to which they are attached can act as an insulator or can share its heat with the attached organisms.

One factor that can improve the inactivation is repeated temperature peaks, tyndallisation or intermittent sterilisation. The repeated heat peaks add more stress to the organisms and in some cases this can even affect the spore formers, as the spores can be encouraged to germinate by the periods of decreased temperature. Therefore, turning of the compost, with decreases in temperature after turning and in some parts for longer periods, can actually have a positive effect on sanitisation compared with having a constant high temperature.

The advantage of using D-values for calculating inactivation of different organisms in the material is that these make it possible to estimate the potential safety margins for inactivation of the organisms. The D-values for some important organisms are presented in Table 9.2. These values are based on equations for total inactivation presented by Vinnerås et al. (2003) as 12 $\log_{10}$ reduction (including a safety margin of 6 $\log_{10}$ reduction), using inactivation data presented in a review of inactivation studies by Feachem et al. (1983). By combining the D-value for the individual organisms (Table 9.1) with temperature measurements and estimates of the required turning interval for adequate reduction (9.6), a management plan for production of hygienically safe end-products can be developed.

With the D-values shown above (Table 9.2), a treatment of 13 h at 55°C would be sufficient to achieve the goal of a 6 $\log_{10}$ reduction in accordance with WHO recommendations for excreta treatment. However, WHO also states that the treatment should last for 1 week with temperatures above 50°C. The difference between the actual time of inactivation and the recommended time of treatment is due to

Table 9.2 D-values (hours) for thermal inactivation of indicator pathogens during biological treatment at 55, 60, and 65°C based on calculations by Vinnerås et al. (2003) using data presented by Feachem et al. (1983)

Temperature	55°C	60°C	65°C
Enteroviruses	1.70	1.02	0.62
Salmonella spp.	1.45	0.70	0.33
Vibrio cholerae	0.0089	0.0031	0.0011
Shigella spp.	0.293	0.148	0.074
Entamoeba histolytica	0.11	0.03	0.006
Ascaris spp.	2.11	0.80	0.30

differences in heat distribution within the compost material, as discussed above. Therefore, it is important to combine the two factors of heat treatment long enough for inactivation, in combination with mixing. Thus, to reach the goal of inactivating 6 $\log_{10}$ of potential pathogens, at least $1–10^{-6}$ (99.9999%) of the material must be treated at the same time as the reduction in the hot section of the compost is above 6 $\log_{10}$.

9.9 Validation of the Hygiene Level in the Composting Process

The composting process requires validation to assure the hygiene quality of the final compost, e.g., regarding its efficiency for inactivation of pollutants, i.e., unwanted plant seeds, plant pathogens and human and animal pathogens. Analysis of the actual unwanted organisms in the final product is not a feasible method for determining the product quality, mainly because the numbers of different organisms present would require different methods of analysis and in some cases are not even possible to analyse, and therefore the cost of analysis would be unreasonably high. Another reason is that even if such analyses are performed, their results express assurance only for the actual samples, which are some grams from a pile of several tonnes, i.e., perhaps some ppm of the treated material. Therefore, the process needs to be validated regarding hygiene quality in connection with factors that are actually *easy* to monitor. The validation should be performed during an initial stage where several factors such as pH, moisture content and temperature are monitored at several points within the compost. In addition, analysis regarding the hygiene quality of the incoming material should be performed. This should then be re-validated at regular intervals to assure the functioning of the system and the actual points of measurements, especially if the process changes (e.g., amounts and sources of added material).

Considering naturally occurring pathogens and plant seeds for valuing, the inactivation can sometimes be complicated, as the natural occurrence varies and their concentrations are often below the required reduction for evaluating the compost as a high quality end-product. WHO guidelines recommend a reduction corresponding to 6 $\log_{10}$ reduction, while the EU ABP directive recommends 3 $\log_{10}$ reduction of thermoresistant viruses. In most cases, the natural levels in

the compost material are below this and, in combination with the detection limits, it would be impossible to validate the process to these levels of reduction. At the same time, adding pathogenic organisms to the fresh material in the large-scale compost system is not an option due to the risk of contamination and the large volumes treated. However, several other alternatives are available. Firstly, there are data on the thermal inactivation levels for most pathogens, either as actual inactivation energy (Haug 1993) or as field data measurements with detection of organisms and temperatures in full-scale systems as presented in Table 9.1 (Feachem et al. 1983; Vinnerås et al. 2003).

One method for adding actual pathogens to the compost is the use of carriers, small vessels/bags containing organisms, which are added to the test material at the start of the process and then analysed at the end. Some studies have used contaminated pieces of meat in monitoring (Ceustermans et al. 2006). By analysing the outgoing material, it is possible to evaluate the process quality. This can be combined with analysis of indicator organisms that have similar behaviour to the pathogens. Examples of such indicators are total coliforms for pathogenic enterobacteria, bacteriophages for pathogenic viruses, etc. One additional indicator that can be used is plant seeds, both naturally occurring and added. The German standard for compost quality (BioAbfallverordnung 1998) includes analysis of tomato seed inactivation, with the requirement that 99% of 200 seeds should be inactivated during the process for good compost quality.

After a validation test, it is possible to correlate the measurements of the inactivation of indicators and/or pathogens with parameters crucial for the process, e.g., temperature/temperature distribution, number of turnings, pH, moisture content and particle size. The set of parameters should then be examined within an expected interval, which can be decided on in laboratory studies where the impact from one parameter can be isolated and determined for the full scale. From this, a standard procedure should be developed where the most crucial parameter/s listed above are monitored during the process to ensure the quality. This means that if e.g., a certain pH together with the temperature improves the safety of the end-product, then these parameters should be monitored during the composting process. Based on laboratory evaluations combined with the actual validation, a set or range of acceptable deviations from the standard process procedure also needs to be developed. Furthermore, an action plan needs to be developed for actions to be taken with material treated in a process not reaching the required parameters for acceptable quality.

References

Albihn A, Vinnerås B (2007) Biosecurity and arable use of manure and biowaste. Livest Sci 112:232–239

Beck-Friis B, Smårs S, Jönsson H, Eklind Y, Kirchmann H (2003) Composting of source-separated organic household waste at different oxygen levels: gaining an understanding of the emission dynamics. Compost Sci Util 11(1):41–50

BioAbfallverordnung (1998) *Verordnung über die Verwertung von Bioabfällen auf landwirtschaftlich, forstwirtschaftlich und gärtnerisch genutzten Böden (Bioabfallverordnung – BioAbfV). BGBl. I S. 2955*. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Bundesministerium für Ernährung, Landwirtschaft und Forsten und Bundesministerium für Gesundheit. Germany

Carslaw HS, Jaeger JC (1958) Conduction of heat in solids. Oxford University Press, London

Ceustermans A, De Clercq D, Aertsen A, Michiels C, Coosemans J, Ryckeboer J (2006) Inactivation of *Salmonella* Senftenberg strain W 775 during composting of biowastes and garden wastes. J Appl Microbiol 103:53–64

Choi MH, Park YH (1998) The influence of yeast on thermophilic composting of food waste. Lett Appl Microbiol 26:175–178

Datta AK (2007a) Porous media approaches to studying simultaneous heat and mass transfer in food processes. I: problem formulations. J Food Eng 80:80–95

Datta AK (2007b) Porous media approaches to studying simultaneous heat and mass transfer in food processes. II: property data and representative results. J Food Eng 80:96–110

Eklind Y, Beck-Friis B, Bengtsson S, Ejlertsson J, Kirchmann H, Mathisen B, Nordkvist E, Sonesson U, Svensson BH, Torstensson L (1997) Chemical characterization of source-separated organic household waste. Swed J Agr Res 27:167–178

Eklind Y, Sundberg C, Smårs S, Steger K, Sundh I, Kirchmann H, Jönsson H (2007) Carbon turnover and ammonia emissions during composting of biowaste at different temperatures. J Environ Qual 36(5):1512–1520

Elving J, Ottoson J, Vinnerås B, Albihn A (2009) Growth of *Salmonella* in cold zones in compost material. Journal of Applied Microbiology (in press)

Erdogu F (2008) A review on simultaneous determination of thermal diffusivity and heat transfer coefficient. J Food Eng 86:453–459

Erdogu F (2005) Mathematical approaches for use of analytical solutions in experimental determination of heat and mass transfer parameters. J Food Eng 68:233–238

Feachem RD, Bradley DJ, Garelick H, Mara DD (1983) Sanitation and disease health aspects of excreta and wastewater management. World Bank studies in water supply and sanitation 3. The World Bank, Washington

FDA (1992) Bad bug book. U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition. Available at: http://www.foodsafety.gov/~mow/intro.html. Accessed November 2008

Gibbs RA, Hu HJ, Ho GE, Unkovich I (1997) Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. Water Sci Technol 35:269–275

Grewal SK, Rajeev S, Sreevatsan S, Michel FC (2006) Persistence of *Mycobacterium avium* subsp. *Paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing, and liquid storage of dairy manure. Appl Environ Microbiol 72:565–574

Haug RT (1993) The practical handbook of compost engineering. Lewis, New York

Hogan JA, Miller FC, Finstein MS (1989) Physical modeling of the composting ecosystem. Appl Environ Microbiol 55:1082–1092

Izadpanah MR, Muller-Steinhagen H, Jamialahmadi M (1998) Experimental and theoretical studies of convective heat transfer in a cylindrical porous medium. Int J Heat Fluid Flow 19:629–635

Mason IG, Wilke MW (2005) Physical modelling of the composting environment: a review. Part 1: reactor systems. Waste Manag 25:481–500

Nakasaki K, Yaguchi H, Sasaki Y, Kubota H (1993) Effects of pH control on composting of garbage. Waste Manag Res 11(2):117–125

Niwagaba C, Nalubega M, Vinnerås B, Sundberg C, Jönsson H (2009) Substrate composition and moisture in composting source separated human faeces and food waste. Environ Technol 30(5): 487–497

Nordin A, Nyberg K, Vinnerås B (2009) Inactivation of *Ascaris* eggs in source-separated urine and faeces by ammonia at ambient temperatures. Appl Environ Microbiol 75(3):662–667

Pecson BM, Barrios JA, Jiménez BL, Nelson KL (2007) The effects of temperature, pH, and ammonia concentration on the inactivation of *Ascaris* eggs in sewage sludge. Water Res 41:2893–2902

Richard TL, Hamelers HVM, Veeken A, Silva T (2002) Moisture relationships in composting processes. Compost Sci Util 10(4):286–302

Sahlström L, Aspan A, Bagge E, Danielsson-Tham M-L, Albihn A (2004) Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. Water Res 38:1989–1994

Sahlström L, Bagge E, Emmoth E, Holmqvist A, Danielsson-Tham M-L, Albihn A (2008) A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants. Bioresour Technol 99:7859–7865

Sarwar MK, Majumdar RP (1995) Thermal conductivity of wet composite porous media. Heat Recov Syst ClIP 15(4):369–381

Seki H (2000) A new deterministic model for force-aeration composting processes with batch operation. Trans ASAE 45(4):1239–1250

Sidhu J, Gibbs RA, Ho GE, Unkovich I (2001) The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. Water Res 35:913–920

Smårs S, Gustafsson L, Beck-Friis B, Jönsson H (2002) Improvement of the composting time for household waste during an initial low pH phase by mesophilic temperature control. Bioresour Technol 84:237–241

Sundberg C, Smårs S, Jönsson H (2004) Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. Bioresour Technol 95:145–150

Sundberg C, Jönsson H (2008) Higher pH and faster decomposition in biowaste composting by increased aeration. Waste Manag 28(3):518–526

Sundberg C, Romantschuk M, Jönsson H, Kauppi S, Norgaard E, Smårs S (2008) Reducing odour by process control when composting food waste with low pH. In: ORBIT 2008 Conference Proceedings – Compost Emissions, Paper no 199, Wageningen, Netherlands, Oct 13–15, 12 pp

Vinnerås B, Björklund A, Jönsson H (2003) Disinfection of faecal matter by thermal composting – laboratory scale and pilot scale studies. Bioresour Technol 88(1):47–54

Vinnerås B, Nordin A, Niwagaba C, Nyberg K (2008) Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. Water Res 42:4067–4074

Warren KS (1962) Ammonia toxicity and pH. Nature 195:47–49

Wichuk KM, McCartney D (2007) A review of the effectiveness of current time-temperature relations on pathogen inactivation during composting. J Environ Eng 6:573–586

Chapter 10
Microbial Antagonists in Animal Health Promotion and Plant Protection

Viviana Klose, Markus Neureiter, Michaela Mohnl, Herbert Danner, and Christina Donat

Abstract The use of agrochemicals in animal husbandry and crop cultivation is well established, but the public acceptance is generally low and in some cases, substances have already been legally banned because their application poses risks for public health. Microbes that are able to suppress the growth of pathogens have been shown to be an effective alternative to maintain animal or plant health. Isolation and screening of potent strains as well as the characterization of their mode of action and the assessment of potential risks play an important role in order to obtain a safe and acceptable biological product. The development of a commercial production process, product formulation, and the requirements for the registration process are further critical items, which will determine over the commercial success of the final product.

Contents

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10.1 Health Promoting Microbial Antagonists

The application of antibiotic growth promoters in animal nutrition is no longer acceptable and legally banned in the European Union. Gastrointestinal microbes have the ability to suppress harmful microbes and to strengthen the resistance against certain diseases in farm animals and, therefore, represent a viable alternative to the use of antibiotics. Current husbandry techniques, however, restrict the contact between younger and older animals, which is the usual way to establish a protective microflora. Therefore, the supplementation of animal feed with beneficial strains appears to be a promising strategy to maintain animal health. The combination of multiple strains in one product may enhance the effect. The screening of microbes used as animal feed additive should preferentially focus on isolates from the target of action. A thorough characterization of the mode of action, metabolites and potential risks is necessary in order to obtain an effective product that meets the legal requirements and is widely accepted.

10.1.1 The Competitive Power of Gut Bacteria

Humans and animals coexist with complex communities of commensal microbes in their gastrointestinal tract (GIT), predominantly facultative and anaerobic organisms (Blaut et al. 2002). Gastrointestinal microbes achieve one of the highest densities and diversities recorded for an ecosystem, made up of several hundred species and with estimated numbers of 10^{10}–10^{12} bacteria in a gram of gut sample (Zoetendal et al. 2004). The composition and physiology of the gut microbiota of food producing animals is of important scientific and economic interest, because of its impact on the health and well-being of animals. Most of the microbes are "working for the host" by playing an important role in digestive processes and in maintaining animal health (Hooper and Gordon 2001). They provide the necessary nutrients or protection from pathogens and disease by limiting the ability of harmful bacteria to colonize the intestinal environment. Stress or changes in feed management practices (e.g. diet changes, antibiotic supplementation, inadequate feed hygiene) can significantly influence the microbial population in the GIT of food-producing animals and their effects on performance and health (Savage 1981). In order to enhance animal growth and feed efficiency and to reduce the frequency of bacterial diseases under certain conditions, antimicrobial compounds have been fed

to various food-producing animals for more than five decades. Nowadays, however, the emergence of antibiotic resistance in the human commensal bacteria has raised concerns about the impact of the subtherapeutic use of these antibiotic growth promoters for animal farming (van den Bogaard and Stobberingh 2000). Because of the general problem of increased resistance of bacteria and the decreasing acceptance of consumers for this type of additive, the feeding of antibiotics has been banned step by step throughout the European Union, and this has accelerated the search for biological strategies. A successful alternative to antimicrobial promoters must not only enhance growth but also protect animals from a myriad of enteric pathogens and be devoid of adverse side effects. Infectious diseases in young animals are a major cause of morbidity and mortality, and this increased susceptibility is especially pronounced in animals kept at high density such as chickens and pigs. Most important infections are caused by a great variety of bacterial pathogens, such as *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., and enterotoxigenic, enterohemorrhagic or toxin-producing *Escherichia coli* serotypes, causing not only severe human diseases (zoonoses), but also large economic losses in livestock (Bertschinger and Fairbrother 1999). Nowadays, the most promising protective strategy is to feed high quality diets including health-promoting additives such as live bacteria acting as GIT competitors to diverse harmful bacteria in their natural environment. The concept of feeding native gut bacteria to improve pathogen colonization resistance of young food-producing animals goes back to Nurmi and Rantala (1973) who noticed that feeding of intestinal content (feces) from adult chickens could protect newly hatched chicks against *Salmonella* infection. Commercially produced poultry lack the natural contact between chicks and mother hens, resulting in a delayed development of the intestinal microflora. As a consequence, day-old chicks that do not establish a protective microflora immediately after hatching are susceptible to pathogen colonization, particularly to the genera *Salmonella* and *Campylobacter*. The phenomenon by which adult gut flora protects young birds against invasion by enteropathogens is called competitive exclusion (CE) or the Nurmi concept (Nurmi et al. 1992). The microbes of the GIT environment are thought to enhance resistance to infection by competing with pathogens for nutrients or attachment sites, or more directly by antagonistic action against non-desirable microorganisms (barrier effect). Several inhibitory compounds can be produced by lactic acid bacteria, including well-characterized bacteriocins (e.g. nisin, reuterin), bacteriocin-like substances and other antagonists such as hydrogen peroxide and certain organic acids (Juven et al. 1991). Although little is known about their mode of action in the intestine, their biological significance is thought to be that of amensalism, a means of one bacterium gaining advantage over another competing microbe. A very simple approach, but in fact, in many countries it is impossible to register such an undefined feed supplement for placement on the market. In Europe, for example, only well-defined microbial feed additives are accepted by legislation. Preparations of unknown bacterial composition pose the risk of containing pathogenic bacteria or viruses (Collins and Gibson 1999). Regarding the foreseen problems associated with the total ban of antibiotic growth promoters (e.g. increased use of therapeutic antimicrobials), several studies

have been performed in order to find out alternative ways of preventing and treating animal infections that are also a risk to humans. The intestinal microflora of the adult animal is, in itself, capable of resisting the establishment of intestinal pathogens (Hillman et al. 1994). It has often been shown that certain members of the commensal gut microbiota, including lactic acid bacteria (LAB) and bifidobacteria, possess an inhibitory activity towards enteric pathogens and that the in vitro addition of large numbers of certain bacteria to the animal´s microflora results in suppression or removal of the pathogen (Deprez et al. 1986; Xuan et al. 2001). In this context, the idea of strengthening the protective microbiota by key gut bacteria thereof is a promising approach because it offers a biological and safe alternative, which should find acceptance by both the producers and consumers. The microbial feed additives are applied especially during critical key times in animal production, e.g. for chicken, in the first days of life either by spraying in the hatchery or in the farm via the first feed or drinking water. The classical example of competitive exclusion – the introduction of a gut microflora originating from adult birds for *Salmonella* reduction in young, growing chickens – has with time being expanded for protecting other animals against a variety of pathogenic species. For all animals, the development and colonization of the GIT at young age is a critical time period. In pigs, for example, the microbial colonization of the intestine begins after birth and follows a rapid succession during the neonatal and weaning period (Moughan et al. 1992). Weaning is a very stressful process in which piglets are separated from the mother sow at an early age, mixed with other piglets and gradually introduced to solid feed. Hence, during the weaning period piglets are highly susceptible to develop gastrointestinal disturbances. The balance between the development of a beneficial microbiota and the establishment of intestinal pathogens can easily shift towards disease and increased mortality. Weaning is typically accompanied by changes in morphology and histology of the small intestine of pigs resulting in maldigestion and proliferation of enterotoxemic bacteria causing high incidence of diarrhea (Nabuurs 1998). Supplementing beneficial microbes to weaning diets is, therefore, a practical strategy to reduce disease outbreaks by providing highly competitive bacteria such as lactobacilli and bifidobacteria at times of need, thereby reducing overgrowth of intestinal pathogens. Of the present possibilities, multispecies or strain combinations of gut bacteria are considered to be highly effective in suppressing various harmful bacteria, but compared to antibiotics with the added benefit of acting naturally and fully biologically in their habitat without leaving any residues.

10.1.2 Finding an Alternative to Antibiotics

In recent years, multicomponent feed additives consisting of a range of well-defined and carefully selected bacterial strains have been developed for chicks and piglets that show promise as dietary intervention tools. Several authors suggested that multiple strains may be more useful than a single strain because they may act at

different sites, in various modes and probably in a synergistic manner (Dunne et al. 1999; Rolfe 2000; Sanders and Huis in't Veld 1999). Mixed cultures may contain bacteria that complement each others' effect. Indeed, it was reasoned that distinct members of the gut flora may exert a protective role against different pathogens. Furthermore, it has also been hypothesized that microbial preparations are more effective when they contain both, obligate anaerobic and facultative anaerobic members of the gut flora, respectively, because the oxygen scavengers might create more favorable conditions for growth and activity of strict anaerobic microorganisms like bifidobacteria (Timmerman et al. 2004). In the complex microflora of the intestine, these bacteria are, by nature, in constant competition with other microbial species for nutrients so that the production of compounds (e.g. bacteriocins) inhibitory to other species confers a survival advantage (Abee et al. 1995). However, to obtain physiological and host-adapted microbial strains with a broad range of competitive features, the microbes are preferentially isolated from their target of action, the GIT of the host animal. Having in mind the use of various beneficial microorganisms of different GIT compartments (e.g. ileum, colon, caecum), it seems logical that their modes of action will probably be based on different principles. Depending on the strain, the gut microbes may counteract pathogens through competitive exclusion based on aggregation with pathogenic bacteria, competitive adhesion to epithelial receptors, production of specific substances (organic acids, bacteriocins, hydroxid peroxide), or competition for nutrients (Kirjavainen et al. 1998). As additional effects, modifications of the structure and function of the intestinal epithelium as well as of the immune response have been described (Erickson and Hubbard 2000).

Microbes used in animal feed should bear several important features:

- Digestion of nutrients and detoxification of toxic molecules
- Antagonistic action against non-desirable microorganisms (barrier effect)
- Protection of the intestinal mucous membrane against invading microorganisms
- Contribution to maturation and stimulation of the hosts' immune system

However, for their use as feed supplement, the bacterial strains should be selected carefully in terms of identity, efficacy and safety. Scientific work has been carried out, which aimed at the research and development of a well-defined multispecies feed additive for chickens. The feed additive development was focussed to control the problems associated with increased occurrence of infectious disease due to the ban of antibiotics (e.g. performance losses in animal husbandry, food-borne disease in humans, increased use of therapeutic antibiotics) and on the other hand, to meet the requirements in the European Community to guarantee safety in animal production (European Commission 1994) and safe animal-derived products, free of contaminants (e.g. antibiotics, hormones, toxins).

The main purposes of the EU-promoted competitive exclusion research are:

- To provide European animal producers with a biological alternative to antimicrobials
- To decrease the occurrence of antibiotic-resistant microorganisms

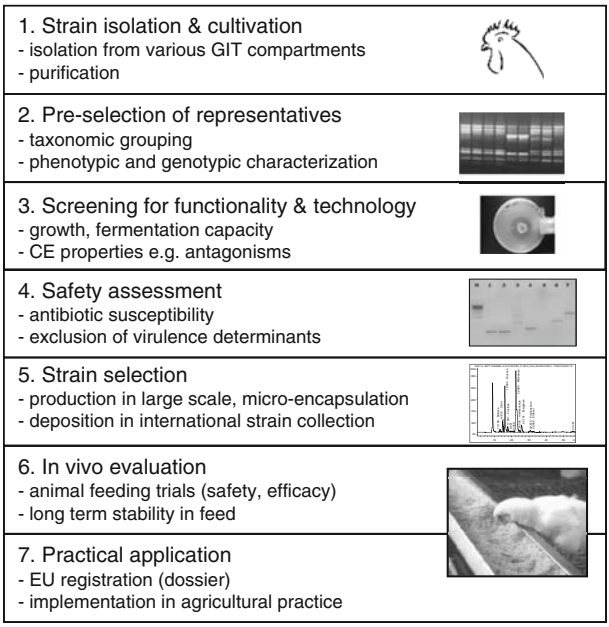

Fig. 10.1 General steps for the development of a microbial feed additive

- To provide sound production processes based on natural resources
- To reduce zoonotic burden (e.g. *Salmonella* spp., *E. coli* spp.) in the meat

In our laboratory, the development of the multispecies preparation followed the technological steps from the naturally derived source to the refined product (Fig. 10.1). The feed additive was designed as a five-strain product using various bacterial species derived from different niches from the gut of chickens (Klose et al. 2006). These strains were selected out of 477 strains, originally isolated from the crop, jejunum, ileum and caecum of healthy chickens, and according to their ability to inhibit common poultry pathogens such as *Salmonella enterica*, *E. coli*, *Clostridium perfringens* and *Campylobacter*. After isolation, the strains were subjected to microbiological studies in order to select those with the potential of stabilizing the indigenous microflora by competitive exclusion (CE) against pathogenic microorganisms. For classifying the strains, a polyphasic approach was carried out combining pheno- and genotypic methods as described by Klose et al. (2006). Finally, 121 of the isolated strains were selected as representatives of chicken intestinal strains based on differences in whole cell protein patterns and screened for antagonistic properties against common poultry pathogens. By using a co-cultivation assay, a reduced number of 90 strains exhibited the ability to inhibit a *Salmonella enteritidis* pathogenic indicator strain. A range of 20 strains showed antagonism to a series of pathogenic strains affiliated to *E. coli* serotypes O157:H7, O147:H19, *S. choleraesuis* ssp. *choleraesuis*, *C. jejuni* and *C. perfringens*, respectively. On the basis of these data, five well-defined strains belonging to the genera

Enterococcus, *Pediococcus*, *Lactobacillus* and *Bifidobacterium* were selected. All strains derived from the natural intestinal flora of healthy chicken, thus providing a rationale for their combined use as feed additive for chickens. Further, selection criteria were growth and fermentation performance, pH reduction, formation of different organic acids, survival in simulated GIT passage, adhesion capability to mucosal cell lines, and immunological activity (Joelli 2005). Further laboratory studies focused on the biosafety of the feed additive strains. Concerns about the increased prevalence of antibiotic resistance were the important reason that led to the European ban of antibiotic feed additives. As a matter of fact, one of the focuses in safety evaluation of microbes used in animal feed was put on antibiotic resistances and the potential carryover among bacteria. Therefore, the feed-additive strains were subjected to a critical evaluation of the risks associated with the genetic transfer of antibiotic resistances from animals to humans via the food chain. Using a combination of pheno- and genotypic methods following the European guidances for risk assessment (European Commission 2005), it was verified that the strains do not harbour easily transferable, plasmid-linked resistances to clinically relevant antibiotics.

10.1.3 Microbial Feed Additives: To Be Accepted

Bringing a feed additive to the European market involves a step-wise evaluation process in order to provide a well-studied functional and safe animal feed supplement. First, the success of the practical approach depends on technological aspects. Maintaining the viability, stability and functionality of the selected strains during processing, formulation and storage is essential for delivering the health benefits of these microbes to animals. To protect the bacterial strains of the multispecies feed additive against the harsh conditions of the stomach (low pH, gastric acid, detergent effect of bile salts), in our laboratory, a microencapsulation process was developed and optimized for every strain. The coated strains were then subjected to long-term stability investigations (up to 24 months) and to feeding trials.

Lactic acid bacteria and bifidobacteria have been consumed in fermented foods or feeds for several centuries without any obvious adverse effects (Salminen et al. 1998). They are, therefore, classified “Generally Recognized As Safe” (GRAS). Nevertheless, risks associated with a potential repertoire of virulence determinants are known to be strain-dependent and among enterococci few strains have been involved in septicemia (Franz et al. 1999). Therefore, in our experiments, the GRAS status of newly isolated organisms with no previous history was confirmed by safety studies using target animals prior to being incorporated into feed products, as recommended by the European Commission (2003). In addition, several feeding trials were conducted at various European farms (e.g. Austria, Hungary, Greece, Romania) to confirm the efficacy and safety of the feed additive for the target animal, the chicken (Mountzouris et al. 2007). A challenge trial performed at the Sao Paulo State University in Brazil with *S. enteritidis* showed the protective

potential of the multispecies additive in vivo (Mohnl et al. 2006). In this field experiment, the five-strain combination was fed to chicks for 3 days. Control birds received the same unsupplemented feed. Thereafter, the birds were orally inoculated with *S. enteritidis* (10^5 colony forming units). Significant differences were obtained when comparing the group treated with supplemented feed and the control, indicating that the common poultry pathogen was successfully reduced within the birds' intestine by the multispecies feed additive. Although long-term challenge studies will be required to determine its efficacy and the optimal application conditions (e.g. time, form of intake) for achieving a long-lasting protective effect, the results from this study showed that well-designed microbial preparations of native, host-adapted gut bacteria have a strong potential for their use as a biological feed additive for the reduction of *Salmonella* carriage in the chicken GIT, thus demonstrating the success of the bacterial isolation and selection process. Usage of the natural competition of certain gut bacteria is a powerful principle to protect newly hatched chicks from common enteric infections. Furthermore, studies in our laboratory have recently demonstrated antagonistic properties of selected porcine gut-derived strains against *Brachyspira hyodysenteriae* spp., the main agent causing the swine dysentery (Taylor 2005). Further investigations of the mode of action of these bacteria are currently underway. The pathogen inhibition by some strains of *Enterococcus* sp., *Lactobacillus* sp. and *Pediococcus* sp. may indicate the possible production of bacteriocins. Future work will first involve monitoring the survival of the introduced bacteria through their GIT journey and examination of changes in the gut microbiota and immune response of the host after feeding of microbial preparations by using DNA-based methods, with later work focussing on the ability of the microbes to inhibit important pathogenic organisms (e.g. *Salmonella*, *Brachyspira*) under these circumstances.

10.2 Microbial Plant Protection Agents

The massive use of synthetic pesticides for the control of plant diseases entails well-known disadvantages: residues of synthetic plant protection products are found in the environment and on food and feed (European Commission 2008), development of resistance against several active ingredients in plant pathogens, e.g. *Botryticides* (Kretschmer et al. 2008) is enhanced, and also human pathogens can develop resistance against the active ingredients, resulting in e.g. multi-resistant strains against antibiotics (Witte 1998). Therefore, the public acceptance of these products is low. Microbiological plant protection agents have been developed as an alternative. The effect of these agents is based on their different modes of actions, which have to be carefully evaluated with respect to their impact on the target organism and the environment. Regarding the screening of microbial antagonists, it is crucial to isolate microbes that are able to survive in the conditions of the host environment. It, therefore, appears to be a useful approach to take isolates directly from the host plant and to use culture-independent methods to characterize the indigenous

microflora on the plant. For commercial applications, the effect has to be proven in field trials and a production process that yields an effective, storable, and applicable product needs to be established. Currently, the success of these products is limited because of requirements for the registration process and the acceptance by the farmers due to the economical framework.

10.2.1 Use of Antagonists in Plant Protection

When humans began to settle and switched from hunting and collection of food to the use of farmland, our natural competitors changed from large animal predators to weeds, insects and microorganisms. Therefore, it was necessary to develop techniques to fight these new enemies, and in the early beginning of agriculture, biocontrol of plant disease and storage losses was the only existing possibility. Records on the use of natural antagonists in plant protection date back to the eleventh century, when Chinese farmers moved nests of predatory insects and birds into their storages (Liu 1939). In the middle of the eighteenth century, the search for beneficial organisms began on a scientific basis, and with the discovery of the causal pathogen of potato late blight (*Phytophthora infestans*) in 1845, the research in microbe-plant interactions started. In 1877, J.T. Burril discovered the causal pathogen of fire blight and thus described the first bacterial plant pathogen (Börner 1990).

In the beginning of the twentieth century and especially after World War II, synthetic pesticides overstocked the plant protection market, with a remarkable increase in turnover each vegetation period. Regarding all plant diseases, the main losses in yield are due to microbial plant pathogens, whereof 80% are caused by mycosis (Krieg and Franz 1989). The trend went from broad-spectrum active ingredients to the implementation of very specific active ingredients. Both strategies, however, have their problems:

- Unspecific active substances tend to affect all living organisms, not distinguishing between beneficial and harmful (from the human point of view)
- Very specific active substances have shortened the time between first use of the products and the occurrence of the first resistances in target (plant pathogenic) and non-target (human pathogenic) microorganisms

As a consequence, modern plant protection should focus on the development of biological substitutes for pesticides (see Chap. 8, de Bertoldi 2010, and Chap. 11, Fuchs 2010), relying on a stable ecosystem and a stable predator-prey relationship. The time is now to initiate a comeback to biological plant protection. Disregarding the environmental and economical need for alternative plant protection products, however, the overall contribution of biological control agents (BCAs) to plant health management is still less then 1% of chemical sales in the USA (Fravel 2005). Due to high costs for product development, problems with patent application, and high costs and long time for product registration, unfortunately, only a

small amount of scientifically well-developed products succeed in reaching the market.

10.2.2 Targets and Soldiers, Microbial Ecology in and on Plants

The plant surface is a sophisticated and complex ecosystem and the microbial communities in this microhabitat depend on a large set of interactions between plant and microorganism and among microorganisms themselves. Microorganisms are able to colonize all parts of a plant: The parts above the ground, including stem, leaves and blossoms, are referred to as the phyllosphere (Lindow and Brandl 2003), while the habitat below the ground surrounding the roots constitutes the rhizosphere. In addition, there are endophytic microbes which live inside the plant tissue. Endophytes can be found in the phyllosphere as well as in the roots, where they comprise the endorhiza. Since the rhizosphere provides more protection and a higher amount of nutrients, it is more densely colonized than the phyllosphere (Brencic and Winans 2005). However, since both the rhizosphere and the phyllosphere are subject to the attack of pathogens, both habitats are of importance for biocontrol agents.

Very close relationships between plants and microorganisms have been the topic of intensive research for a long period of time and such interactions will, in most cases, strengthen the plant and promote the growth by improving the nutrient supply. Well-established examples are mycorrhiza, which is a general term used for a huge number of different symbiotic interaction between plants and fungal species, and the symbiosis of plants belonging to the familiy *Leguminosae* with nitrogen-fixing bacteria of the genera *Rhizobium* and *Bradyrhizobium*. It is estimated that about 80% of all plants are involved in some kind of mycorrhiza (Paul and Clark 1996). A mycorrhizza results in an enlargement of root surface for the plant, which leads to a more effective nutrient uptake and thus effectuates an improved performance of the plants in terms of yield and resistance against pathogens. The symbiosis between plant and fungus is, in many cases, obligatory for both partners, which implies that the fungi are obligatory biotrophic and therefore, not able to grow saprophytic in the soil without nutrient supply from their plant partners (Gisi 1990). Similarly, the uptake of Rhizobia in nodula on the roots of legumes provides nitrogen to the plant independently from soil nitrogen supply. However, these plant-strengthening effects due to microorganisms in the rhizosphere of plants can, usually, not be attributed to a distinct antagonistic reaction of the involved beneficial microorganisms against plant pathogens. In fact, the improved performance is mainly the result of a close relationship in the metabolism (nutrient uptake) of the participating organisms. Products to force this kind of symbiosis are available in the market, for nitrogen fixation as well as for the establishment of mycorrhiza.

Regarding the mode of action of microbial antagonists against microbial plant pathogens, a differentiation can be made between antibiosis, hyperparasitism, competition, and induced resistance of the plant against the pathogen. Microbial

plant protection products focusing on an antibiotic mode of action produce compounds that are inhibitive or toxic for the pathogenic microorganism. This mechanism is common in many fungi and yeasts as well as in bacterial preparations based on e.g. *Bacillus* sp. or *Pseudomonas* sp. (Punja and Utkhede 2003, Montesinos 2007). Examples of antibiotic agents are: *Pseudomonas fluorescens* ("Blight Ban"), which is used against fire blight on apple and pear and produces an antibiotic toxin against *Erwinia amylovora* (Temple et al. 2006); and *Bacillus amyloliquefaciens* FZB42 ("RhizoVital"), claimed to have a plant-strengthening effect due to antagonistic efficacy against soil-born pathogens. In the latter case, more than 8.5% of the genome is devoted to synthesizing antibiotics and siderophores, including the polyketides bacillaene and difficidin, respectively (Chen et al. 2007).

Hyperparasitism means that the pathogen, which is to be regarded as a plant parasite, is affected by another microorganism, which is living at its expense. Based on hyperparasitism is the effect of *Trichoderma harzianum* ("Trichodex"), which produces hydrolytic enzymes (chitinases) and physically attacks the fungal mycelium (Schubert et al. 2008). The same mode of action is used by *Coniothyrium minitans* ("Contans"): sclerotia, the resting states of the pathogen *Sclerotinia sclerotium* causing sclerotia rot are penetrated by the hyphae of the antagonist, the wall collapses and the cytoplasma disintegrates. Conidia of the antagonist germinate when sclerotia are in the surrounding (Grendene and Marciano 1999). The antagonist sporulates on the surface and in the sclerotium. Hyperparasitism is often combined with the production of cell wall lysing hydrolytic enzymes. These enzymes can also interact with pathogenicity factors involved with the invasion of the host plant by the pathogen. This could be considered as an additional mode of action (Punja and Utkhede 2003).

Competition for nutrients involves epiphytic or saprophytic microorganisms, which are able to use nutrients more efficiently than a potential pathogen in a certain ecological niche. An example is the effect of *Aureobasidium pullulans* ("Blossom Protect", "Boni Protect") against fire blight (*E. amylovora*) and storage diseases on pome fruits (e.g. *Botrytis cinerea*, *Monilia* spp., *Penicillium* spp.) as shown in Fig. 10.2. The antagonist is a saprophytic yeast-like fungus. During growth, *A. pullulans* produces organic acids and thereby gains an additional advantage in the development of colony-forming units in the blossoms or in wounds. The pathogens have not enough space and nutrients available to start the infection process (Kunz 2006). For the efficacy of *A. pullulans* against storage diseases, it has been demonstrated that providing additional nutrients in the pathosystem (apple-*B. cinerea*-*A. pullulans*) reduces the antagonistic effect of the yeast-like fungus, which indicates that competition for nutrients is an important factor in this case (Wandmacher 1996).

Induced-resistance of the plant against pathogens due to the presence of beneficial microorganisms cannot be clearly defined as an antagonistic action mode. In most cases, it may be that the mode of action of the beneficial microorganism is not known in detail and only an overall plant-strengthening effect is noticed. However, two systems are to be distinguished: systemic acquired resistance (SAR), typically induced by less virulent pathogens, and induced systemic resistance (ISR), typically induced by non-pathogenic bacteria (van Loon et al. 1998). In order to produce

Fig. 10.2 Untreated (*left*) and with Boni-Protect® treated (*right*) apples (variety Cox Orange) after storage

induced-resistance, it is not necessarily required to add living cells; in some cases, it appears to be sufficient to add only cell fractions or active components (e.g. proteins) produced by the microbes to induce resistance (Gerhardtson 2002). As an example, *Penicillium simplicissimum* GP17-2 is reported to induce resistance and activate multiple defence signals in *Arabidopsis thaliana* (Hossain et al. 2007). Some commercial products are already in the market under the name of plant strengtheners; these may be sold as fertilizers in some countries.

In most cases, two or more modes of action are combined, as has been shown for *Trichoderma* strains (Benítez et al. 2004) and for *A. pullulans* (Wandmacher 1996). It has been described that the presence of *A. pullulans* forces wounded apples to produce more β-1,3-glucanase, chitinase and peroxidase; these enzymes are known to inhibit the growth of several fungal pathogens. The fact that *A. pullulans* itself is not affected by these substances raises the hypothesis whether this is a symbiotic interaction to protect the apple against fruit rot (Ippolito et al. 2000). This system of plant pathogen interaction may be one of the advantages of microbial plant protection agents, preventing the target pathogens from becoming resistant.

Plant-protection products with microorganisms, as active ingredients, are applied in several ways: depending on the location where the effect is most likely to occur, they can be sprayed aerially on parts of the plants like leaves or blossoms as well as on or in the soil. Some agents are used as seed coatings and only recently a "stubble treatment" has been proposed for antagonists against the pathogen *Fusarium* ssp in wheat and maize in order to reduce the risk of infection with the pathogen for the next generation of crops on that site (Luongo et al. 2005).

10.2.3 Out of Nature – Back to Nature: Screening for New Antagonistic Microorganisms

In order to develop a microbial plant-protection product with a high efficacy, one possible strategy will be to search for new antagonists at infection time on

infection site. Since the antagonist should be efficient in the field of use, it has to cope with all environmental factors on site. In the case of the phyllosphere, the microorganism has to endure UV radiation and tremendous fluctuations in temperature and moisture. Traditional culture-dependent methods have been shown to underestimate the size of the microbial population on plants (Yang et al. 2001). Therefore, the microbial community should be screened by cultivation-independent molecular biological methods as far as possible, in order to guarantee that potential candidates are not excluded. Changes in microbial community composition under pathogen pressure may be monitored; for example, with the help of denaturing gradient gel electrophoresis (DGGE) or similar approaches focusing on the genomes of active microorganisms (Timms-Wilson et al. 2007). If dominating species or strains occur in correlation with a low number of infected plants, these microorganisms can be identified and with the help of cultivation methods tailored to their particular needs they can be cultivated. In another approach, existing knowledge of a pathosystem or an antagonistic behavior of already known species may be used for pathogen control on several plants. Since e.g. *A. pullulans* is highly effective against storage diseases on apples including *B. cinerea* (Wandmacher 1996), it is an obvious step to develop applications of the same preparation against *B. cinerea* on grapes.

Further knowledge on the mode of action of the microbial antagonist is gained by dual-culture tests on nutrient agar plates, in order to investigate if the antagonist produces antibiotic substances that inhibit the growth of the target pathogen. While these substances prove to be very effective in many cases (Gullino et al. 2000), their application is to some extent disputable. On the one hand, all microorganisms, and especially antagonists, will communicate with their environment including the plant and other microorganisms through signal molecules. On the other hand, biological control agents with antibiosis main mode of action may have some characteristics in common with traditional chemical pesticides because the effect is mainly due to a defined chemical compound. Therefore, the same problems concerning toxicity and resistance may pose a hazard, although these antibiotic metabolites can be found in the natural environment and are usually harmless to mammals. An example of this is the group of chemical pesticides named strobilurins, originally based on fungal metabolites extracted from the fungus *Strobilurus tenacellus* (Gullino et al. 2000) or the usage of streptomycin, extracted from the fungus *Streptomyces griseus*, against the bacterial plant pathogen *E. amylovora*. Current approaches include the identification of the genes that are responsible for the production of the effective compounds (Chen et al. 2007), allowing the optimization of the expression system and the transference of the respective genes to other organisms (Filutowicz et al. 2008). A further step would be the production of these compounds by biotechnological or chemical means including purification and modification in order to improve their effectiveness. Such an approach may be helpful for the development of new and more effective pesticides; however, in this case, the term "biocontrol agent" should be avoided, since such products are similar to conventional pesticides and pose the same problems.

In order to demonstrate an effect which is not mainly based on antibiosis, a test system that involves the plant is required. Usually, this involves greenhouse and

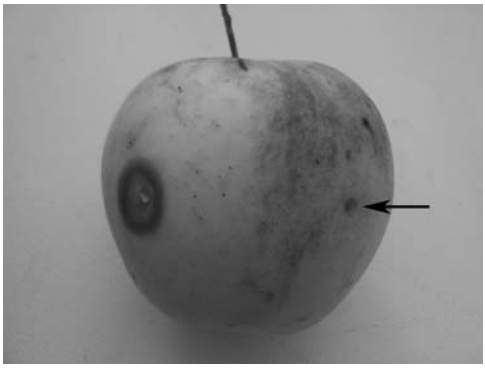

Fig. 10.3 In vitro test of antagonists against storage disease with apples (variety Golden Delicious) after 6 week of incubation. Wounded apples were artificially infected with *Pezicula malicorticis*, the arrow highlights the wound which was protected by adding 0.15% Boni-Protect® (active substance: *Aureobasidium pullulans*) to the inocula

field trials. In some cases, tests on parts of the plant are sufficient; e.g. antagonists against fire blight can be tested on infected blossoms (Johnson and Stockwell 2000), the effect on storage diseases can be evaluated on wounded and artificially infected apples (Fig. 10.3) and the cultivation of detached wheat spikelets in liquid culture medium can be used for studying plant-microbe interactions in wheat (Trione and Stockwell 1989). Such laboratory test systems have the advantage that they are easy to handle and a high number of potentially antagonistic strains can be tested at steady environmental conditions.

While greenhouse and laboratory trials may be adequate for screening purposes, the final product has to prove its efficacy in field trials. This has to be done on different sites with different plant varieties over several vegetation periods. For microbial antagonists, several methods of applications are in use: spraying like "classic" plant protection products and seed coatings. Furthermore, insects can be used as vectors, e.g. microbial antagonists against fire blight are brought to the blossoms of apple and pear trees by honey bees (Moosbeckhofer et al. 2008).

Another hurdle on the way to the market is to overcome critical technological steps, such as the establishment of a stable large scale production; the output has to be a product that can be stored over a sufficient time period (at least more than one vegetation period) with a minimum number of colony-forming units of the microbial antagonist to ensure a high efficacy, and free of contaminations. Commercial calculations and trials of different formulations concerning the production price versus the possible end-user price have to be done necessarily at this point of the developing process.

10.2.4 The Hard Way to Commercial Application

Registration of microbial plant-protection products is a long story. For authorities and applicants, it is a hard challenge to ensure human and environmental safety and the need to bring such products as fast as possible to the market in view of their

utility and substitutability of harmful chemical pesticides. The registration practice for chemical substances can not easily be applied for microbial plant-protection agents. Studies need to be adapted to the specific microorganism.

Currently, studies concerning pathogenicity and infectivity for mammals are necessary for the registration more or less worldwide. Ecotoxicity is investigated by studies with birds, fish, arthropods like bees and predatory mites, water flees and earthworms. Investigations have to be provided on the fate and behavior of the microbial plant-protection agent in the environment. It can be concluded that the registration costs to launch a product in the market in one European country with the final registration for 10 years may be about €1,000,000. The registration process lasts for about 10 years. Some countries offer national provisional registrations to allow the owner of a new biological product to reach the market earlier, but this registration forms are always coupled with some clear disadvantages for marketing. This may include registration that is restricted to only a short period of time (120 days). In some countries, it is also possible to sell products as "plant strengtheners," which involves that no advertising addressing the efficacy of the product against specific plant diseases is allowed.

10.2.5 Perspectives: Acceptance in the Market, Competing Products

One fact which is often overestimated during research for new antagonistic products is the environmental concern of the end-user. Agriculture is, nowadays, a question of economical success and aside from farmers who work under organic farming labells, the decision for the use of plant-protection products is basically an economical one. Until society is not willing to take part in indirect additional costs for sustainable plant-protection systems, e.g. by supporting development and registration of microbial plant-protection products, success for such products in competition with chemical pesticides will, furthermore, take a long period of time.

10.3 Conclusions

Preparations that are mainly based on microbial antagonism can be used in animal nutrition to promote animal health as well as in plant protection to prevent the growth of microbial plant pathogens. Screening appears to be a crucial step during product development. In addition to the effectiveness, aspects concerning safety also gain importance and therefore, it is essential that the exact mode of action is known and that potential product strains are evaluated with regard to hazardous properties. From the technological point of view, the challenge lies in the development of a production process that provides a sufficient number of living

microorganisms. Furthermore, a proper product formulation is necessary to ensure shelf life, applicability and effectiveness of the product.

While registration is essential to guarantee a proven effect and safety for the consumer, especially for products based on microorganisms, the legal requirements often pose a large obstacle on the way to commercialisation. This may be due to the fact that current regulations have been mainly developed for chemical agents and the responsible authorities still lack experience with microbial products.

Microbial antagonists for agricultural application have been proven to be effective and an increasing number of products is available in the market. Over the long term, these products can help to reduce the use of the undesirable and potentially hazardous agrochemicals.

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References

Abee T, Krockel L, Hill C (1995) Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int J Food Microbiol 28:169–185

Benítez T, Rincón AM, Limón MC, Codón AC (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7(4):249–260

Bertschinger HU, Fairbrother JM (1999) *Escherichia coli* infections. In: Straw BE, D'Allaire S, Mengeling WL, Taylor DJ (eds) Diseases of swine, 8th edn. Iowa State University Press, Ames, IA, pp 431–467

Blaut M, Collins MD, Welling GW, Dore J, van Loo J, de Vos W (2002) Molecular biological methods for studying the gut microbiota: the EU human gut flora project. Br J Nutr 87: S203–S211

Börner H (1990) Pflanzenkrankheiten und Pflanzenschutz, 6th edn. Eugen Ulmer GmbH&Co, Stuttgart, p 518

Brencic A, Winans SC (2005) Detection of and response to signals involved in host-microbe interactions by plant associated bacteria. Microbiol Mol Biol Rev 69(1):155–194

Chen XH, Koumoutsi A, Scholz R, Eisenreich A, Schneider K, Heinemeyer I, Morgenstern B, Voss B, Hess WR, Reva O, Junge H, Voigt B, Jungblut PR, Vater J, Süssmuth R, Liesegang H, Strittmatter A, Gottschalk G, Borriss R (2007) Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. Nat Biotechnol 25:1007–1014

Collins MD, Gibson GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr 69:1052S–11057S

Commission E (2005) Opinion on the scientific panel on additives and products or substances used in animal feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance. EFSA J 223:1–12

de Bertoldi M (2010) Production and utilization of suppressive composts: environmental, food and health benefits. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 153–170

Deprez P, Van den Hende C, Muylle E, Oyaert W (1986) The influence of the administration of sow's milk on the post-weaning excretion of hemolytic *E. coli* in the pig. Vet Res Commun 10:469–478

Dunne C, Murphy L, Flynn S, O'Mahony L, O'Halloran S, Feeney M, Morrissey D, Thornton G, Fitzgerald G, Daly C, Kiely B, Quigley EM, O'Sullivan G, Shanahan F, Collins J (1999) Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. Antonie Van Leeuwenhoek 76:279–292

Erickson KL, Hubbard NE (2000) Probiotic immunomodulation in health and disease. J Nutr 130:403–409

European Commission (1994) Commission Directive 94/40/EC of 22 July 1994 amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition, pp 15–19

European Commission (2003) Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition, pp 29–43

European Commission (2008) Monitoring of pesticides residues in products of plant origin in the European Union, Norway, Iceland and Liechtenstein 2006. Commission Staff Working Document SEC(2008) 2902

Filutowicz M, Burgess R, Gamelli RL, Heinemann JA, Kurenbach B, Rakowsky SA, Shankar R (2008) Bacterial conjugation-based antimicrobial agents. Plasmid 60:38–64

Franz CMAP, Holzapfel WH, Stiles ME (1999) Enterococci at the crossroads of food safety? Int J Food Microbiol 47:1–24

Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Gerhardtson B (2002) Biological substitutes for pesticides. Trends Biotechnol 20(8):338–343

Gisi U (1990) Bodenökologie. Ulrich Thieme Verlag, Stuttgart ISBN 3-13-747201-6

Grendene A, Marciano P (1999) Interaction between *Sclerotinia sclerotiorum* and *Coniothyrium minitans* strains with different aggressiveness. Phytoparasitica 27(3):201–206

Gullino ML, Leroux P, Smith CM (2000) Uses and challenges of novel compounds for plant disease control. Crop Prot 19:1–11

Hillman K, Murdoch T, Spencer R, Stewart CS (1994) Inhibition of enterotoxigenic *Escherichia coli* by the microflora of the porcine ileum, in an *in vitro* semicontinuous culture system. J Appl Bacteriol 76:294–300

Hooper LV, Gordon JI (2001) Commensal host-bacterial relationships in the gut. Science 292:1115–1118

Hossain M, Sultana F, Kubota M, Koyama H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17–2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol 48:1724–1736

Ippolito A, El Ghaouth A, Wilson CL, Wisniewsky M (2000) Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol Technol 19:265–272

Joelli D (2005) Bestimmung von Effizienzkriterien für ausgewählte probiotische Stämme zur Verwendung in der Geflügelproduktion. Diploma Thesis, BOKU-University of Natural Resources and Applied Life Sciences, Vienna, Department Institute for Agrobiotechnology Tulln, Austria, pp 1–110

Johnson KB, Stockwell VO (2000) Biological control of fire blight. In: Vanneste JL (ed) Fire blight. The disease and its causative agent, *Erwinia amylovora*. CABI, Wallingford, pp 319–337

Juven BJ, Meinersmann RJ, Meinersmann RJ (1991) Antagonistic effects of lactobacilli and pediococci to control intestinal colonization by human enteropathogens in live poultry. J Appl Bacteriol 70(2):95–103

Kirjavainen PV, Ouwehand AC, Isolauri E, Salminen SJ (1998) The ability of probiotic bacteria to bind to human intestinal mucus. FEMS Microbiol Lett 167:185–189

Klose V, Mohnl M, Plail R, Schatzmayr G, Loibner A (2006) Development of a competitive exclusion product for poultry meeting the regulatory requirements for registration in the European Union. Mol Nutr Food Res 50:563–571

Kretschmer M, Walker A-S, Leroch M, Schoonbeck H-J, Fillinger S, Hahn M (2008) Vermehrtes Auftreten von Multidrug Resistenz (MDR) bei *Botrytis cinerea* Isolaten in Weinbergen der Champagne und entlang der deutschen Weinstraße. In: Bundesforschungsinstitut für Kulturpflanzen (JKI) (ed.): 56. Deutsche Pflanzenschutztagung, 22–25 September 2008, Kiel, Germany

Krieg A, Franz JM (1989) Lehrbuch der biologischen Schädlingsbekämpfung. Paul Parey Verlag, Berlin, Hamburg

Kunz S (2006) Fire blight control in organic fruit growing – systematic investigation of the mode of action of potential control agents. Mitt Biol Bundesanst Land- Forstwirtsch 408:249–253

Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69 (4):1875–1883

Liu G (1939) Some extracts from the history of entomology in China. Psyche 46(1):23–28. doi:10.1155/1939/74632

Luongo L, Galli M, Corazza L, Meekes E, De Haas L, Van Der Plas CL, Köhl J (2005) Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci Technol 15(3):229–242

Mohnl M, Berchiere A, Klimitsch A, Klose V, Plail R, Schatzmayr G (2006) Development of a competitive exclusion product with defined cultures and its efficacy on reduction of *S. Enteritidis* in broiler. In: CD ROM Proceedings XII European Poultry Conference, Verona, Italy, 10–14 September 2006

Montesinos E (2007) Antimicrobial peptides and plant disease control. FEMS Microbiol Lett 270:1–11

Moosbeckhofer R, Loncaric I, Oberlerchner J, Persen U, Ertl C, Donat C (2008) Use of honeybees (*Apis mellifera*) as vectors for fire blight antagonists in field experiments. Acta Hortic (ISHS) 793:461–464

Moughan P, Birtles M, Cranwell P, Smith W, Pedraza M (1992) The piglets as a model animal for studying aspects of digestion in milk-fed human infants. In: Sinopoulos AP (ed) Nutritional triggers for health and in disease. Karger, Basel, pp 40–113

Mountzouris KC, Tsirtsikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K (2007) Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci 86:309–317

Nabuurs MJ (1998) Weaning piglets as a model for studying pathophysiology of diarrhea. Vet Q 20:42–45

Nurmi E, Rantala M (1973) New aspects of *Salmonella* infection in broiler production. Nature 241:210–211

Nurmi E, Nuotio L, Schneitz C (1992) The competitive exclusion concept: development and future. Int J Food Microbiol 15:237–240

Paul EA, Clark FE (1996) Soil microbiology and biochemistry. Academic, San Diego, CA

Punja ZK, Utkhede RS (2003) Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol 21:400–407

Rolfe RD (2000) The role of probiotic cultures in the control of gastrointestinal health. J Nutr 130:396–402

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fonden R, Saxelin M, Collins K, Mogensen G (1998) Demonstration of safety of probiotics – a review. Int J Food Microbiol 44:93–106

Sanders ME, Huis in't Veld J (1999) Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. Antonie Van Leeuwenhoek 76:293–315

Savage DC (1981) The effect of stress, diet and environment on the stability of the gastrointestinal microflora. Fortschr Veterinarmed [Adv Vet Med] 33:23–31

Schubert M, Fink S, Schwarze FWMR (2008) Evaluation of *Trichoderma* spp. as a biocontrol agent against wood decay fungi in urban trees. Biol Control 45:111–123

Taylor DE (2005) Swine dysentery, spirochaetal diarrhoea. In: Taylor DJ (ed) Pig diseases. St Edmundsbury Press, Bury St Edmund's, Suffolk, UK, pp 119–128

Temple TN, Stockwell VO, Johnson KB, Loper JE (2006) Bioavailability of iron to *Pseudomonas fluorescens* strain A506 on flowers of pear. Acta Hortic (ISHS) 704:301–306

Timmerman HM, Koning CJM, Mulder L, Rombouts FM, Beynen AC (2004) Monostrain, multistrain and multispecies probiotics – a comparison of functionality and efficacy. Int J Food Microbiol 96:219–233

Timms-Wilson TM, Griffiths RI, Whiteley AS, Prosser JI, Bailey MJ (2007) Detection of active bacterial populations in soil. In: van Elsas JD, Jansson JK, Trevors JT (eds) Modern soil Microbiology, 2nd edn. CRC, Boca Raton, pp 387–407

Trione EJ, Stockwell VO (1989) Development of detached wheat spikelets in culture. Plant Cell Tissue Organ Cult 17:161–170

van den Bogaard AE, Stobberingh EE (2000) Epidemiology of resistance to antibiotics: links between animals and humans. Int J Antimicrob Agents 14:327–335

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Wandmacher S (1996) Untersuchung der Wirkungsmechanismen mikrobieller Antagonisten bei der biologischen Bekämpfung der Lagerfäule bei Äpfel. Diploma Thesis, University of Konstanz, pp 1–98

Witte W (1998) Medical consequences of antibiotic use in agriculture. Science 279:996–997

Xuan ZN, Kim JD, Heo KN, Jung HJ, Lee JH, Han YK, Kim YY, Han IK (2001) Study on the development of a probiotics complex for weaned pigs. Asian-australas J Anim Sci 14: 1425–1428

Yang C-H, Crowley DE, Borneman J, Keen NT (2001) Microbial phylosphere populations are more complex than previously realized. Proc Natl Acad Sci USA 98:3889–3894

Zoetendal EG, Collier CT, Koike S, Mackie RI, Gaskins HR (2004) Molecular ecological analysis of the gastrointestinal microbiota: a review. J Nutr 134:465–472

Chapter 11
Interactions Between Beneficial and Harmful Microorganisms: From the Composting Process to Compost Application

Jacques G. Fuchs

Abstract Numerous microorganisms are involved in the composting process, but their precise roles are often unknown. Compost microorganisms are influenced by the composition of the substrate and by the temperature in the compost pile. In addition, different microorganisms also influence each other, e.g. through competition. In the first phase of composting, microbial activity increases drastically, leading to a rise in temperature. The initial bacterial dominance is replaced by a fungal one during compost maturation.

Compost management aims to achieve favourable conditions for beneficial and unfavourable conditions for harmful microorganisms. The type of input substrate, the size of compost piles, the frequency of turning, particle size, aeration and moistening all affect the microbial processes. They influence microorganisms mainly by affecting nutrient, oxygen and water supply. Sometimes, composts are inoculated with selected microorganisms. Harmful microorganisms are introduced into the compost mainly with the input substrate. They are mainly inactivated by high temperatures, but other mechanisms of inactivation have also been demonstrated, e.g. certain plant-derived compounds and antagonistic interactions. Beneficial microorganisms are capable of outcompeting harmful ones during the process and/or have a beneficial effect on crops after field application. Application of compost increases the microbial activity of soils, and crops are less sensitive to diseases after compost application (disease suppressiveness); the mechanisms are largely unknown. Better knowledge in this field would certainly allow optimizing the composting process to enhance disease suppressiveness.

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Contents

11.1 Introduction

Numerous microorganisms have been shown to be associated with composts (Ryckeboer et al. 2003b). It is evident that the microbial community, as a whole, plays an important role in the decomposition of organic materials and in the build-up of stabilized compounds. For most microbial species, however, the precise role in the composting process is unknown.

Among the microorganisms found in composts, there are not only beneficial and useful ones (i.e. microorganisms responsible for the regular composting process), but also those that are potentially harmful for humans, animals, plants or the environment. For example, plant pathogens are a normal component of crop residues, and if household waste is composted, human pathogens such as *Salmonella* are not uncommon. One of the most important goals of composting is the *inactivation* of these harmful microorganisms and the *development* of a beneficial microbial community. To achieve this, operators can adapt the compost management process in a way that is favourable for beneficial microorganisms and unfavourable for harmful microorganisms. This is described in a separate section below.

One of the challenging difficulties of studying microbial populations in compost is the interpretation of the results in relation to the methods used. For example, Dees and Ghiorse (2001) used three different methods to determine microbial populations (fluorescent direct counting, plate counts, and molecular methods), and their results differed 100-fold. A similar difference was observed by Atkinson et al. (1996). The difference may be explained by the fact that only active microorganisms can be counted, while also inactive microorganisms are detected with molecular methods. However, we do *not* know at present which of the methods gives the most adequate result in terms of compost microbiology.

11.2 Microorganisms at the Beginning of the Process

The microorganisms present at the beginning of the process are introduced with the original mixture of organic materials, with which the composting process is started. These microorganisms are all found in the natural environment. The most frequent

in numbers are bacteria, and in particular actinobacteria, but fungi are also important members of the community. The composition of the input substrate influences the microbial communities (Klammer et al. 2008). This is particularly true for the populations found at the beginning of the composting process. Input substrates are often very heterogeneous and so are the initial microbial communities found on them. However, initial microbial communities of input substrates have rarely been investigated.

In source-separated household waste, only few mesophilic fungi but numerous thermophilic bacteria and fungi were found (Ryckeboer et al. 2003a). Food wastes containing vegetable residues have a low-initial pH, which favours the proliferation of fungi and yeasts and slows down bacterial growth (Choi and Park 1998; Ryckeboer et al. 2003b). On leaves, grass and brush compost samples at the first day of composting, Michel et al. (2002) found primarily Gram-negative, α-, β- and γ-proteobacteria.

Especially, the harmful organisms are substrate dependent. For example, animal manure and food wastes contain significant quantities of potential human and animal pathogens like *Escherichia coli*, *Listeria* sp. and *Salmonella* sp. (Grewal et al. 2007; Heinonen-Tanski et al. 2006; Hess et al. 2004; Jiang et al. 2004; Lemunier et al. 2005; Wichuk and McCartney 2007). Vegetable and crop residues may contain various plant pathogens (Bollen 1993; Hoitink et al. 1976).

Immediately after the start of the composting process, the microbial community changes drastically, and soon does not resemble the initial community any more as will be described in the next section.

11.3 Succession of Microorganisms During the Process

Immediately at the beginning of the composting process, the microbial biomass increases drastically (Hellmann et al. 1997; Narihiro et al. 2004). For example, Klamer and Baath (1998) observed a six-fold increase during the first day of composting shredded straw of *Miscanthus* with added pig slurry. Not all microorganisms multiply equally fast, and there are complex interactions between individual species. This results in significant changes of the microbial community (Klamer and Baath 1998).

The physical and chemical properties of the substrate change during the composting process. The microorganisms which are active first, degrade the original substrate, produce metabolites and create a new physico-chemical environment. This can then be used by other microorganisms (Ryckeboer et al. 2003a). Ishii and Takii (2003) postulate that the main factor affecting microbial communities in the composting process is the concentration and composition of dissolved organic materials. Quantitatively, the main components of organic matter are carbohydrates, proteins, lipids and lignin (Ryckeboer et al. 2003b). Different microorganisms produce different enzymes needed for degradation of the different substrates (Hu and van Bruggen 1997; Ryckeboer et al. 2003b; Tuomela et al. 2000).

Bacteria dominate the microbial community during the degradation phase (Beffa et al. 1996; Hu and van Bruggen 1997; Ryckeboer, et al. 2003a; van Heerden et al. 2002). During this phase, large quantities of dissolved organic carbon are usually available in the substrate. Depending on the input substrate (e.g. green waste, household waste), nitrogen may also be available in significant quantities. At a C/N ratio from 25 to 40, microbial activity is intense. This activity causes a rise in temperature, particularly in the centre of the compost pile. This phase is, therefore, also called "thermophilic phase." In general, the highest microbial numbers and enzymatic activities occur during this phase (Cunha-Queda et al. 2007). In composting of household biowaste, Narihiro et al. (2004) found that bacteria increased in two phases up to 10^{11} cells per gram dry weight, and then stayed stable during the process. Although the bacterial community was quantitatively stable, the authors observed a drastic *shift* from ubiquitous proteobacteria in the first phase to actinobacteria in the second phase. They attribute this community shift to antagonistic interactions between the different bacteria.

The increase of temperature that causes significant changes in the microbial communities (Hassen et al. 2001; Sundh and Rönn, 2002) is essential for the autosterilization of the compost (see Sect 11.4). Guo et al. (2007) found different microbial communities at different locations within the compost pile, which were related to the temperature at this precise location. Thambirajah et al. (1995) observed that during the peak heating phase, fungal activity was almost completely suppressed. Klamer and Baath (1998) observed that Gram-positive bacteria increased when compost heated up, and decreased against when the compost cooled down. Gram-negative bacteria and fungi increased with rising temperature up to approximately 50°C, but decreased at higher temperatures. After cooling to <50°C, these two groups increased again.

During the maturation phase, the number of bacteria decreases, but their diversity increases, as demonstrated by phospholipid-fatty acid profiling (Ryckeboer et al. 2003a). At the same time, the populations of fungi increase in quantity and in diversity (Ryckeboer et al. 2003a). Fungal activity is mainly important in the maturation phase of the composting process (Hu and van Bruggen 1997; Klamer and Baath 1998).

In summary, the microbial populations present in compost during different phases are the result of dynamic, complex interactions between the microorganisms and their environment. In the short term, high temperature is probably the major selective factor influencing the composition of microbial communities. High temperature is, itself, the result of high microbial activity, which depends on the substrate availability. However, the composition of the substrate is also greatly influenced by the metabolic activity of microorganisms.

11.4 Influence of Compost Management on Compost Microflora

The aim of compost management is to influence the microbial processes in a way that the input substrate is well decomposed, stable humus compounds are formed, harmful microorganisms are destroyed and beneficial microorganisms are

promoted. All four aims must be achieved simultaneously, but this chapter is concerned only with the microorganisms.

Composting plants differ in the substrate which they use, as well as in the size of piles they make (composting system). They have several management tools at hand to influence oxygen level and moisture within the compost pile. Aggregate structure can be influenced by adapting the shredding of the input substrate. Further, oxygen levels can be influenced with forced aeration and/or by the frequency of turning the pile. If moisture is too low, the compost pile can be moistened artificially. The practical aspects of compost management are described elsewhere in Chap. 10 of this book (Klose et al. 2010). To some extent, the microbial interactions can be *influenced* by managing the environmental conditions. Oxygen and moisture are the two factors, which can be managed by the operator and have the greatest influence on microbial communities.

The oxygen level is known to be an important factor influencing compost quality. Enticknap et al. (2006) observed a growth stimulation of aerobic bacteria by oxygenation of a compost pile. Facultatively anaerobic microorganisms grow also in aerated composts (Atkinson et al. 1996). These authors postulate that anaerobic microorganisms in microenvironments within substrate particles may be responsible for a significant proportion of the metabolic activity in aerobic composts also in the later phases of the composting process. Thus, the size and structure of the substrate particles can greatly influence the activity of anaerobic bacteria. Watanabe et al. (2008) observed that populations of the family Bacillaceae clearly dominate under optimal composting conditions (98%), but that they were significantly decreased when the substrate was aggregated. Anaerobes or facultative anaerobes were dominant in the aggregates, but were *not* found in the non-aggregated substrate. The oxygen level in the composting material also has an indirect influence on the microflora, for example through the ammonia concentration in the substrate. If the aeration of the compost pile is poor, levels of ammonia increase, and the communities of ammonia-sensitive microorganisms decrease (de Guardia et al. 2008).

The moisture content in the composting material also greatly influences the microbial activity and the composition of the microbiota. Liang et al. (2003) observed that a minimal moisture content of 50% (w/w) is necessary for optimal aerobic microbial activity. However, too high a moisture content has a negative effect on the biological activity through the increased compaction of the material and the diminution of oxygen diffusion through the matrix (Das and Keener 1997). Not all microorganisms have similar needs in terms of water availability, and a change in moisture content can cause a shift in the composition of the microbial community (Takebayashi et al. 2007). In general, high water content favors bacteria over fungi (Finstein and Morris 1975).

The inoculation with selected microorganisms can also influence the biological processes and modify the microbial community. For example, Sasaki et al. (2006) added a commercial microbial additive composed of the genera *Alcaligenes*, *Bacillus*, *Clostridium*, *Enterococcus* and *Lactobacillus* to cattle manure compost. As a result, the temperature increased more quickly and the ammonia emission

from the compost pile decreased more quickly. Also, the microbial composition of the manure changed and contained 10 to 100 times more mesophilic and thermophilic, aerobic bacteria, but a smaller number of thermophilic anaerobes. Inoculation is especially useful in unilateral raw mixtures, for example containing high amounts of woody material. However, the choice of the microorganisms is very important (Vargas-Garcia et al. 2005). They have to be competitive enough to colonize the material, and the quantity of inoculum must be sufficient. Otherwise, the competition of the native microorganisms does *not* allow the inoculum to develop in the compost.

The influence of inoculation on composting depends on the conditions of the process and on the characteristics of the raw material (Vargas-Garcia et al. 2007). The difficulty of inoculation is that each situation is very specific, and the inoculation strategy has to be adapted to each situation. Thus, it is not possible to give precise advice here. More research is clearly needed in this area. Our preliminary observations suggest that inoculation is not needed and has no effect if a balanced input substrate is used. In this context, a balanced substrate has a C/N ratio between 30:1 and 40:1, and a good structure allowing for adequate moisture and aeration.

11.5 Destruction of Harmful Microorganisms

With the initial substrates, microorganisms which are harmful for humans, animals or plants can be introduced into the compost (Wichuk and McCartney 2007; Noble and Roberts 2004). Noble and Roberts (2004) describe more than 60 plant pathogens, which potentially survive the composting process and which can be found in green wastes. Hence, the inactivation of harmful organisms is essential to obtain a safe compost.

High temperature is the most important factor for the "hygienization" (i.e. elimination of pathogens) of compost (Downer et al. 2008; Elorrieta et al. 2003; Suarez-Estrella et al. 2003). However, not all pathogens have the same sensitivity to high temperature (Bollen and Volker 1996; Wichuk and McCartney 2007). Effectivity of hygienization depends not only on the maximum temperature achieved within the compost pile, but also on the duration of the heat period (Bollen 1993; Elorrieta et al. 2003; Fayolle et al. 2006; Katan 2000; Suarez-Estrella et al. 2003). In addition, moisture also interacts with temperature in the hygienization process (Fayolle et al. 2006).

Contrary to common belief, high temperature is *not* the only mechanism for the hygienization. Even when the composting process does not reach the temperature level required for thermal kill, pathogens can be inactivated in compost. A number of compounds have been shown to be capable of pathogen inactivation.

Their occurrence varies with the substrate used. For example, Elorrieta et al. (2003) showed that the release of phenolic compounds during the composting process could be responsible for hygienization. Ammonia is generally present at relatively high concentrations at the beginning of the composting process. According to Gilpatrick (1969) and Lazarovits (2001), ammonia is well known to have some effect on different pathogens. Hoitink and Fahy (1986) showed that young composted hardwood bark contains ethyl esters of hydroxyl-oleic acids, which inhibit the development of the pathogen *Phytophthora* spp. Crucifers contain substances, which are toxic to some pathogens (Cohen et al. 2005; Koike and Subbarao 2000). Crucifers can be used for biofumigation, which can be considered as a specific mode of composting within the soil.

Antagonistic interactions may also lead to hygienization (Elorrieta et al. 2003). Different mechanisms can be involved. Competition for substrate has already been described above. Saprophytic microorganisms are often more competitive on dead substrates than pathogenic microorganisms. Some microorganisms produce volatile substances (Seewald et al. 2010), often secondary metabolites, which can be toxic for other microorganisms including pathogens (Wheatley 2002). For example, some isolates of *Trichoderma* spp. produce hydrolytic enzymes, which may destroy the cell wall components of many microorganisms (Krupke et al. 2003; Savoie et al. 2001; Williams et al. 2003). *Bacillus subtilis* can be an important inhabitant of composts (Ashraf et al. 2007; Kim et al. 2008; Phae et al. 1990; Yangui et al. 2008). This species is known for its production of antifungal substances, which are particularly active against plant pathogens.

It is generally assumed that the majority of the pathogens are destroyed during the composting process. An important question concerning the quality of the final product is to know whether pathogens from the environment are able to re-colonize the compost after the hygienization phase. Lemunier et al. (2005) tested the re-infestation risks of mature compost with *E. coli*, *Salmonella* serovar *enteridis* and *Listeria monocytogenes* by artificial inoculation. While *L. monocytogenes* was never detected in the different composts, *E. coli* and *S.* serovar *enteridis* survived between 3 and 90 days, but did not grow in the substrate. In sharp contrast, all three pathogens were able to proliferate after inoculation to a sterilized compost. This illustrates the importance of natural microbial populations in the compost for preventing re-colonization by pathogens. Jiang et al. (2002) observed a similar pattern: after artificial inoculation, *E. coli* declined more rapidly in manure-amended soil than in autoclaved soil. Cayuela et al. (2009) found that composts that were prepared with hoof or meat meal as a nitrogen source showed elevated abundance of *Acinetobacter calcoaceticus*, a bacterium that is suspected to trigger bovine spongioform encephalopathy (BSE).

In conclusion, good hygienization of composts *can* be achieved in most cases. Only very few pathogens, e.g. the tobacco mosaic virus (TMV) and *Xanthomonas malvacearum*, are critical in terms of their potential to survive a well-managed composting process (Bollen 1993).

11.6 Development of Beneficial Microflora During the Composting Process

As described above, a microbial succession occurs during the composting process, which is influenced by several factors (Ryckeboer et al. 2003a). In the case of good management practice, the population shift leads to a product containing mainly beneficial microorganisms, while the harmful microorganisms are eliminated during the process (see Sect.12.6, Minz et al. 2010).

In practice, crops growing on soil that had received compost are often less susceptible to diseases than plants growing on soil without compost (Arora et al. 2005; Boulter et al. 2000, 2002; Hoitink and Boehm 1999; Hoitink et al. 1997a; Suarez-Estrella et al. 2007). This phenomenon is known as "disease suppressiveness" (see also Sect. 12.7). Microorganisms are assumed to play an important role in disease suppression (Fuchs 2002; Hoitink et al. 1993; Noble and Coventry 2005; Tilston et al. 2002). There is evidence that beneficial microorganisms are more competitive in colonizing organic residues during the composting process than pathogens are. For example, Thornton (2004) observed that antagonistic *Trichoderma* species were able to outcompete the pathogenic fungus *Rhizoctonia solani* for nutrients, and thereby prevent its saprophytic growth. Cohen et al. (1998) could not attribute disease suppressiveness to the community of beneficial microorganisms alone.

Hygienization is not evenly effective throughout a compost pile. The outer zones of the compost pile do not reach temperatures high enough for hygienization. In these zones, mesophilic, heat-sensitive microorganisms including pathogens are present. During turning of a pile, the substrate from the outer zones is mixed with the hygienized material. Subsequently, the beneficial microorganisms grow faster than the pathogens. After a few cycles of turning and composting, the beneficial microorganisms outcompete the pathogens completely.

During the final process of compost maturation, the amount of the readily available nutrients is limited and the microbial community is stabilized. For example, the beneficial effect of green manure was more constant when it was composted than when it was not composted, independent of whether or not it contained pathogens (Bonanomi et al. 2007). Not all composts have the same ability to protect the plants against disease (Fuchs et al. 2008). Various authors observed that composts can show two different suppressive reactions: a broad, modest, suppressivity, or a specific suppressivity (Fuchs 2002; Fuchs et al. 2008; Hoitink et al. 1997b).

Addition of antagonistic microorganisms to compost is a promising technique to improve its suppressivity. Already in 1983, Nelson et al. (1983) increased the suppressive potential of compost by adding selected *Trichoderma* strains. They found that not only the addition of the antagonist is important, but also the strategy of inoculation of the antagonist, so that it can establish itself and develop its antagonistic activity. Chung and Hoitink (1990) also state that the inoculation of an antagonist must be optimized. Otherwise it cannot efficiently colonize the

substrate, because the autochthonous microbial community inhibits it. Kwok et al. (1987) demonstrated that bacterial antagonists could establish themselves and protect specifically cucumber against *R. solani* better in sterilized bark compost media than in the medium with a broad suppressive effect.

11.7 Influence of Compost Amendments on the Soil Microflora

The interactions between microorganisms are not limited to the composting process alone. They continue also in the soil after the application of the compost. The microbiological activity of soils is increased by compost amendments. Angers et al. (1993) studied a soil, which was amended annually with 30 m^3 of compost of horse manure and wood shavings. In the second year of the experiment, they observed 50% more microbial biomass C and an ammonification rate increase by 30%. Crecchio et al. (2001) studied an amendment with 12 tons of municipal solid waste compost per hectare. This increased dehydrogenase activity by 20%. Fuchs et al. (2008) tested the effect of eight different composts and digestates on soil microbial activity. Six months after the amendment, the fluorescein diacetate (FDA) hydrolytic activity in the soil amended with compost or digestate was 20 to 40% higher than in the control, independent of the tested products. Other authors made similar observations (Nayak et al. 2007; Rumberger et al. 2004; Serra-Wittling et al. 1995; Tiquia et al. 2002). The overall quantity of microorganisms in the soil increases between 5 and 60% after addition of compost (Angers et al. 1993; Fliessbach et al. 2005; Ros et al. 2006a; Rumberger et al. 2004; Tabuchi et al. 2008; Tiquia et al. 2002; Zaman et al. 2004).

The diversity of the soil microbial community is also increased by compost amendments (Buckley et al. 2006; Dambreville et al. 2006; Drenovsky et al. 2004; Inbar et al. 2005; Kong et al. 2004). For unknown reasons, Cherif et al. (2008) did not observe a significant shift in bacterial community after the application of municipal solid waste compost. The influence of compost amendments on soil microorganisms depends on their quality. For example, the composting level (i.e. compost maturity) of cow manure had a significant effect on the microbial diversity (Kong et al. 2004). Compost application selectively influences the populations of soil microorganisms. For example, Roe and Ozores-Hampton (2003) observed that compost applications decreased the populations of aerobic and anaerobic bacteria in the soil, but increased the numbers of fungi, actinobacteria, pseudomonads and N-fixing bacteria.

The ways of compost influence on the soil microbial community are not fully understood. In many cases, it is not a simple multiplication of the microorganisms in the compost. In contrast, there is evidence that the addition of compost promotes the growth of indigenous soil microorganisms (Innerebner et al. 2006; Chu et al. 2007; Saison et al. 2006). However, the mechanisms of promotion are unknown in most cases. For example, Chu et al. (2007) conclude that compost promotes indigenous *Bacillus* sp. in the soil. Possibly, the supply of organic matter activates

certain soil microorganisms (Buckley et al. 2006; Fliessbach et al. 2005). However, the quantity of organic matter applied with the compost is very small in comparison with the total organic matter present in the soil. This could explain why different types of composts had a similar effect on the bacterial community and activity in soil (Ros et al. 2006b; Fuchs et al. 2008). It would also explain the observation of Ros et al. (2006a) that the soil itself influences the microbiological activity and the community diversity more strongly than the compost treatments. Saison et al. (2006) report that compost affects the soil microbial community mainly through the physicochemical characteristics of the compost matrix. In conclusion, the establishment of microorganisms in the soil after compost application is still poorly understood, and more research is clearly needed in this area. A better knowledge of the mechanisms of establishment would allow to optimize compost application in practice.

Disease suppressiveness is obviously connected with the soil microbial community (Van Elsas et al. 2002). However, the respective roles of the native microflora in the soil and of the microflora added with the compost are not well understood, and the two are possibly working together. For example, Serra-Wittling et al. (1996) tested the influence of municipal waste compost on the suppressiveness of a loamy field soil against *Fusarium* wilt of flax. Compost addition did increase the suppressiveness of the soil, regardless of whether the compost was heat-treated or not, suggesting thereby that the microorganisms of the compost did not play a significant role in the soil. However, if the soil was heat-treated, non-treated compost could restore its suppressiveness. In conclusion, it seems that the microflora of the soil and the compost were both involved in the suppressiveness, and they mainly acted through nutrient and space competition with the pathogen. Such a complex interaction was also found by Inbar et al. (2005) for streptomycetes on cucumber roots.

Observations from practice support the role of microorganisms in disease suppressiveness (Bruns and Schüler 2000; Fuchs 2002; Hoitink and Boehm 1999; Reuveni et al. 2002; Tilston et al. 2002). In many cases, the suppressive effect disappears when the compost is sterilized (Chen and Nelson 2008; Craft and Nelson 1996; Malandraki et al. 2008). The majority of these experiments were performed with potted plants, where up to 50% of compost was added to the substrate. In the field, much smaller quantities of compost can be added. Therefore, findings obtained with potted plants cannot be extrapolated to field crops, and there are severe knowledge gaps concerning the effects of composts on field crops, including disease suppressiveness. Disease suppressiveness in the field is not always correlated with suppressiveness in the laboratory (Craft and Nelson 1996). In general, field conditions are much more variable than the conditions in the laboratory (e.g. meteorological conditions, indigenous microbial populations). For example, Santos et al. (2008) could demonstrate the role of compost microorganisms in the suppression of *Pythium aphanidermatum*. However, the in vitro inhibition of pathogens by isolated compost microorganisms did not correlate with the suppressive effect in the plant-soil-pathogen system. This indicates that mechanisms other than

antagonistic relationships between compost and soil microorganisms could also be involved in compost-induced disease suppression.

11.8 Conclusions

There are complex interactions among different microorganisms, as well as among microorganisms, compost substrate and environment. In addition, these interactions vary in different phases of the composting process and after compost application to the soil. Although we know the outcome of the composting process, we are far from understanding all the interactions, mechanisms and processes leading to the end result. One of the more important factors in this relationship seems to be the competition for different organic substrates. As a result of microbial breakdown, the substrates change during the process, leading to a succession of microorganisms. Another important factor influencing the relationship between harmful and beneficial microorganisms is the temperature evolution during the composting process. Harmful microorganisms are more sensitive to heat than beneficial microorganisms, and the beneficial organisms seem to be more effective in re-colonizing the compost after the hot period. With appropriate management practices, the operator can affect some of the physical and chemical conditions within the compost pile, and thereby influence the balance between different microorganisms.

Compost application can have a positive influence on plant health (disease suppressiveness). To some extent, this can be attributed to beneficial microorganisms present in compost. More importantly, however, composts alter the microbial community of soils, or the two mechanisms interact.

With respect to practical application of composts, it is clear that compost quality, compost microorganisms, soil microorganisms and soil parameters are all involved in disease suppressiveness. However, the interactions and mechanisms are largely unknown. The processes need to be studied in depth not only in the short, but also in the long term. Research indicates that compost has a huge potential for disease suppression. In practice, however, this potential is not fully exploited. With a better understanding of these relationships, the practical use of compost for disease suppression could be optimized. In the long term, it can be imagined to produce specific "designer composts" for specific applications in specific soils, on specific crops and against specific diseases too. With such composts, high levels of disease suppressiveness are likely to be achieved.

References

Angers DA, Lafrance P, Simard RR, Pelletier F, Legere A, Cook HF, Lee HC (1993) Temporal variation in soil microbial biomass and activity as influenced by compost and atrazine applications. Soil management in sustainable agriculture. In: Proceedings of Third International

Conference on Sustainable Agriculture, Wye College, University of London, UK 31, pp 538–543
Arora T, Eklind Y, Ramert B, Alstrom S (2005) Microbial analysis and test of plant pathogen antagonism of municipal and farm composts. Biol Agric Hortic 22:349–367
Ashraf R, Shahid F, Ali TA (2007) Association of fungi, bacteria and actinomycetes with different composts. Pakistan J Bot 39:2141–2151
Atkinson CF, Jones DD, Gauthier JJ (1996) Putative anaerobic activity in aerated composts. J Ind Microbiol 16:182–188
Beffa T, Blanc M, Marilley L, Fisher JL, Lyon PF, Aragno M, de Bertoldi M, Sequi P, Lemmes B, Papi T (1996) Taxonomic and metabolic microbial diversity during composting. In: de Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The science of composting: part 1. Blackie, London, pp 149–161
Bollen GJ (1993) Factors involved in inactivation of plant pathogens during composting of crop residues. In: Hoitink HAJ, Keener HM (eds) Science and engineering of composting: design, environmental, microbiological and utilization aspects. Renaissance, Worthington, OH, pp 301–318
Bollen GJ, Volker D (1996) Phytogenic aspects of composting. In: de Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The science of composting: part 1. Blackie, London, pp 233–246
Bonanomi G, Antignani V, Pane C, Scala F (2007) Suppression of soilborne fungal diseases with organic amendments. J Plant Pathol 89:311–324
Boulter JI, Boland GJ, Trevors JT (2000) Compost: A study of the development process and end-product potential for suppression of turfgrass disease. World J Microbiol Biotechnol 16: 115–134
Boulter JI, Trevors JT, Boland GJ (2002) Microbial studies of compost: bacterial identification, and their potential for turfgrass pathogen suppression. World J Microbiol Biotechnol 18: 661–671
Bruns C, Schüler C (2000) Suppressive effects of yard waste compost amended growing media on soilborne plant pathogens in organic horticulture. In: Paper read at 13th International IFOAM Scientific Conference, Basel-Switzerland
Buckley DH, Huangyutitham V, Nelson TA, Rumberger A, Thies JE (2006) Diversity of Planctomycetes in soil in relation to soil history and environmental heterogeneity. Appl Environ Microbiol 72:4522–4531
Cayuela M, Mondini C, Insam H, Sinicco T, Franke-Whittle I (2009) Plant and animal wastes composting: effects of the N source on process performance. Bioresour Technol 100: 3097–3106
Chen M-H, Nelson EB (2008) Seed-colonizing microbes from municipal biosolids compost suppress *Pythium ultimum* damping-off on different plant species. Phytopathology 98: 1012–1018
Cherif H, Ouzari H, Marzorati M, Brusetti L, Jedidi N, Hassen A, Daffonchio D (2008) Bacterial community diversity assessment in municipal solid waste compost amended soil using DGGE and ARISA fingerprinting methods. World J Microbiol Biotechnol 24:1159–1167
Choi MH, Park Y-H (1998) The influence of yeast on thermophilic composting of food waste. Lett Appl Microbiol 26:175–178
Chu H, Lin X, Fujii T, Morimoto S, Yagi K, Hu J, Zhang J (2007) Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biol Biochem 39:2971–2976
Chung YR, Hoitink HAJ (1990) Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in a bark compost-amended container medium. Phytopathology 80:73–77
Cohen R, Chefetz B, Hadar Y (1998) Suppression of soil-borne pathogens by composted municipal solid waste. In: Brown S, Angle JS, Jacobs L (eds) Beneficial co-utilization of agricultural, municipal and industrial by-products. Kluwer, Dorcrecht, The Netherlands, pp 113–130

Cohen MF, Yamasaki H, Mazzola M (2005) *Brassica napus* seed meal soil amendment modifies microbial community structure, nitric oxide production and incidence of *Rhizoctonia* root rot. Soil Biol Biochem 37:1215–1227

Craft CM, Nelson EB (1996) Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Appl Environ Microbiol 62: 1550–1557

Crecchio C, Curci M, Mininni R, Ricciuti P, Ruggerio P (2001) Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol Fertil Soils 34:311–318

Cunha-Queda AC, Ribeiro HM, Ramos A, Cabral F (2007) Study of biochemical and microbiological parameters during composting of pine and eucalyptus bark. Bioresour Technol 98:3213–3220

Dambreville C, Hallet S, Nguyen C, Morvan T, Germon J-C, Philippot L (2006) Structure and activity of the denitrifying community in a maize-cropped field fertilized with composted pig manure or ammonium nitrate. FEMS Microbiol Ecol 56:119–131

Das K, Keener HM (1997) Moisture effect on compaction and permeability in composts. J Environ Eng 123:275–281

de Guardia A, Petiot C, Rogeau D, Druilhe C (2008) Influence of aeration rate on nitrogen dynamics during composting. Waste Manag 28:575–587

Dees PM, Ghiorse WC (2001) Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiol Ecol 35:207–216

Downer AJ, Crohn D, Faber B, Daugovish O, Becker JO, Menge JA, Mochizuki MJ (2008) Survival of plant pathogens in static piles of ground green waste. Phytopathology 98:547–554

Drenovsky RE, Vo D, Graham KJ, Scow KM (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb Ecol 48:424–430

Elorrieta MA, Suarez-Estrella F, Lopez MJ, Vargas-Garcia MC, Moreno J (2003) Survival of phytopathogenic bacteria during waste composting. Agric Ecosyst Environ 96:141–146

Enticknap JJ, Nonogaki H, Place AR, Hill RT (2006) Microbial diversity associated with odor modification for production of fertilizers from chicken litter. Appl Environ Microbiol 72: 4105–4114

Fayolle L, Noble R, Coventry E, Aime S, Alabouvette C (2006) Eradication of *Plasmodiophora brassicae* during composting of wastes. Plant Pathol 55:553–558

Finstein MS, Morris ML (1975) Microbiology of municipal solid waste composting. Adv Appl Microbiol 19:113–151

Fliessbach A, Dubois D, Esperschütz J, Gunst L, Mäder P, Oberholzer H, Schloter M, Gattinger A (2005) Soil microbial community structure and organic matter transformation processes in organic and integrated farming systems. In: Paper read at Researching Sustainable Systems – International Scientific Conference on Organic Agriculture, Adelaide, Australia, 21–23 September 2005

Fuchs JG (2002) Practical use of quality compost for plant health and vitality improvement. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin Heiselberg, pp 435–444

Fuchs JG, Berner A, Mayer J, Smidt E, Schleiss K (2008) Influence of compost and digestates on plant growth and health: potentials and limits. In: Paper read at CODIS 2008: Compost and digestate: sustainability, benefits, impacts for the environment and for plant production, Solothurn, Switzerland

Gilpatrick JD (1969) Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59:973–978

Grewal S, Sreevatsan S, Michel FC Jr (2007) Persistence of *Listeria* and *Salmonella* during swine manure treatment. Compost Sci Util 15:53–62

Guo Y, Zhu N, Zhu S, Deng C (2007) Molecular phylogenetic diversity of bacteria and its spatial distribution in composts. J Appl Microbiol 103:1344–1354
Hassen A, Belguith K, Jedidi N, Cherif A, Cherif M, Boudabbous A (2001) Microbial characterization during composting of municipal solid waste. Bioresour Technol 80:217–225
Heinonen-Tanski H, Mohaibes M, Karinen P, Koivunen J (2006) Methods to reduce pathogen microorganisms in manure. Livest Sci 102:248–255
Hellmann B, Zelles L, Palojarvi A, Bai Q, Bai QY (1997) Emission of climate-relevant trace gases and succession of microbial communities during open-windrow composting. Appl Environ Microbiol 63:1011–1018
Hess TF, Grdzelishvili I, Sheng H, Hovde CJ (2004) Heat inactivation of *E. coli* during manure composting. Compost Sci Util 12:314–322
Hoitink HAJ, Boehm MJ (1999) Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu Rev Phytopathol 37:427–446
Hoitink HAJ, Fahy PC (1986) Basis for control of soilborne pathogens with compost. Annu Rev Phytopathol 24:93–114
Hoitink HAJ, Herr LJ, Schmitthenner AF (1976) Survival of some plant pathogens during composting of hardwood tree bark. Phytopathology 66:1369–1372
Hoitink HAJ, Boehm MJ, Hadar Y (1993) Mechanisms of suppression of soilborne plant pathogens in compost-amended substrates. In: Hoitink HAJ, Keener HM (eds) Science and engineering of composting : design, environmental, microbiological and utilization aspects. Wooster, Wooster, OH, pp 601–621
Hoitink HAJ, Grebus ME, Hayes MHB, Wilson WS (1997a) Composts and the control of plant diseases. In: Hayes MHB, Wilson WS (eds) Humic substances in soils, peats and waters: health and environmental aspects. Royal Society of Chemistry, Cambridge, pp 359–366
Hoitink HAJ, Stone AG, Han DY (1997b) Suppression of plant diseases by composts. Hortscience 32:184–187
Hu S, van Bruggen AHC (1997) Microbial dynamics associated with multiphasic decomposition of 14C-labeled cellulose in soil. Microb Ecol 33:134–143
Inbar E, Green SJ, Hadar Y, Minz D (2005) Competing factors of compost concentration and proximity to root affect the distribution of streptomycetes. Microb Ecol 50:73–81
Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidizing bacteria in compost-treated soils. Soil Biol Biochem 38:1092–1100
Ishii K, Takii S (2003) Comparison of microbial communities in four different composting processes as evaluated by danaturing gradient gel electrophoresis analysis. J Appl Microbiol 95:109–119
Jiang X, Morgan J, Doyle MP (2002) Fate of *Escherichia coli* O157:H7 in manure-amended soil. Appl Environ Microbiol 68:2605–2609
Jiang X, Islam M, Morgan J, Doyle MP (2004) Fate of *Listeria monocytogenes* in bovine manure-amended soil. J Food Prot 67:1676–1681
Katan J (2000) Physical and cultural methods for the management of soil-borne pathogens. Crop Prot 19(8–10):725–731
Kim WG, Weon HY, Lee SY (2008) In vitro antagonistic effects of bacilli isolates against four soilborne plant pathogenic fungi. Plant Pathol J 24:52–57
Klamer M, Baath E (1998) Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiol Ecol 27:9–20
Klammer S, Knapp B, Insam H, Dell'Abate MT, Ros M (2008) Bacterial community patterns and thermal analyses of composts of various origins. Waste Manag Res 26:173–187
Klose V, Neureiter M, Mohnl M, Danner H, Donat C (2010) Microbial antagonists in animal health promotion and plant protection. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Hdidelberg, pp 231–252
Koike S, Subbarao KV (2000) Broccoli residues can control *Verticillium* wilt of cauliflower. Calif Agric 54:30–33

Kong W, Liu K, Liao Z (2004) Effects of different organic materials and their composting levels on soil microbial community. Ying Yong Sheng Tai Xue Bao 15:487–492

Krupke OA, Castle AJ, Rinker DL (2003) The North American mushroom competitor, *Trichoderma aggressivum* f. *aggressivum*, produces antifungal compounds in mushroom compost that inhibit mycelial growth of the commercial mushroom *Agaricus bisporus*. Mycol Res 107:1467–1475

Kwok OCH, Fahy PC, Hoitink HAJ (1987) Interactions between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. Phytopathology 77:1206–1212

Lazarovits G (2001) Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. Can J Plant Pathol 23:1–7

Lemunier M, Francou C, Rousseaux S, Houot S, Dantigny P, Piveteau P, Guzzo J (2005) Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. Appl Environ Microbiol 71:5779–5786

Liang C, Das KC, McClendon RW (2003) The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. Bioresour Technol 86:131–137

Malandraki I, Tjamos SE, Pantelides IS, Paplomatas EJ (2008) Thermal inactivation of compost suppressiveness implicates possible biological factors in disease management. Biol Control 44:180–187

Michel FC, Marsh TJ, Reddy CA (2002) Bacterial community structure during yard trimmings composting. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin Heiselberg, pp 25–42

Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252

Narihiro T, Abe T, Yamanaka Y, Hiraishi A (2004) Microbial population dynamics during fed-batch operation of commercially available garbage composters. Appl Microbiol Biotechnol 65:488–495

Nayak DR, Babu YJ, Adhya TK (2007) Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biol Biochem 39:1897–1906

Nelson EB, Kuter FA, Hoitink HAJ (1983) Effects of fungal antagonists and compost age on suppression of *Rhizoctonia* damping-off in container media amended with composted hardwood bark. Phytopathology 73:1457–1462

Noble R, Coventry E (2005) Suppression of soil-borne plant diseases with composts: a review. Biocontrol Sci Technol 15:3–20

Noble R, Roberts SJ (2004) Eradication of plant pathogens and nematodes during composting: a review. Plant Pathol 53:548–568

Phae CG, Sasaki M, Shoda M, Kubota H (1990) Characteristics of *Bacillus subtillis* isolated from composts suppressing phytopathogenic microorganism. Soil Sci Plant Nutr 36:575–586

Reuveni R, Raviv M, Krasnovsky A, Freiman L, Medina S, Bar A, Orion D (2002) Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Prot 21:583–587

Roe N, Ozores-Hampton M (2003) Compost application increase some soil microbiological populations. Hortscience 38:728

Ros M, Klammer S, Knapp B, Aichberger K, Insam H (2006a) Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use Manag 22:209–218

Ros M, Pascual JA, Garcia C, Hernandez MT, Insam H (2006b) Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biol Biochem 38:3443–3452

Rumberger A, Yao S, Merwin IA, Nelson EB, Thies JE (2004) Rootstock genotype and orchard replant position rather than soil fumigation or compost amendment determine tree growth

and rhizosphere bacterial community composition in an apple replant soil. Plant Soil 264:247–260

Ryckeboer J, Mergaert J, Coosemans J, Deprins K, Swings J (2003a) Microbiological aspects of biowaste during composting in a monitored compost bin. J Appl Microbiol 94:127–137

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003b) A survey of bacteria and fungi occurring during composting and self-heating processes. Ann Microbiol 53:349–410

Saison C, Degrange V, Oliver R, Millard P, Commeaux C, Montange D, Le Roux X (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environ Microbiol 8: 247–257

Santos M, Dianez F, Gonzalez del Valle M, Tello JC (2008) Grape marc compost: microbial studies and suppression of soil-borne mycosis in vegetable seedlings. World J Microbiol Biotechnol 24:1493–1505

Sasaki H, Kitazume O, Nonaka J, Hikosaka K, Otawa K, Itoh K, Nakai Y (2006) Effect of a commercial microbiological additive on beef manure compost in the composting process. Anim Sci J 77:545–548

Savoie J-M, Iapicco R, Largeteau-Mamoun ML (2001) Factors influencing the competitive saprophytic ability of *Trichoderma harzianum* Th2 in mushroom (*Agaricus bisporus*) compost. Mycol Res 105:1348–1356

Seewald M, Bonfanti M, Singer W, Knapp BA, Hansel A, Franke-Whittle IH, Insam H (2010) VOC emissions from compost amended soils. Soil Biol Biochem (in press)

Serra-Wittling C, Houot S, Barriuso E (1995) Soil enzymatic response to addition of municipal solid-waste compost. Biol Fertil Soils 20:226–236

Serra-Wittling C, Houot S, Alabouvette C (1996) Increased soil suppressiveness to *Fusarium* wilt of flax after addition of municipal solid waste compost. Soil Biol Biochem 28:1207–1214

Suarez-Estrella F, Vargas-Garcia MC, Elorrieta MA, Lopez MJ, Moreno J (2003) Temperature effect on *Fusarium oxysporum* f.sp. melonis survival during horticultural waste composting. J Appl Microbiol 94:475–482

Suarez-Estrella F, Vargas-Garcia C, Lopez MJ, Capel C, Moreno J (2007) Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. Crop Prot 26:46–53

Sundh I, Rönn S (2002) Microbial succession during composting of source-separated urban organic household waste under different initial temperature conditions. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin Heiselberg, pp 53–64

Tabuchi H, Kato K, Nioh I (2008) Season and soil management affect soil microbial communities estimated using phospholipid fatty acid analysis in a continuous cabbage (*Brassica oleracea* var. *capitata*) cropping system. Soil Sci Plant Nutr 54:369–378

Takebayashi S, Narihiro T, Fujii Y, Hiraishi A (2007) Water availability is a critical determinant of a population shift from *Proteobacteria* to *Actinobacteria* during start-up operation of mesophilic fed-batch composting. Microbes Environ 22:279–289

Thambirajah JJ, Zulkali MD, Hashim MA (1995) Microbiological and biochemical changes during the composting of oil palm empty-fruit-bunches. Effect of nitrogen supplementation on the substrate. Bioresour Technol 52:133–144

Thornton CR (2004) An immunological approach to quantifying the saprotrophic growth dynamics of *Trichoderma* species during antagonistic interactions with *Rhizoctonia solani* in a soilless mix. Environ Microbiol 6:323–334

Tilston EL, Pitt D, Groenhof AC (2002) Composted recycled organic matter suppresses soil-borne diseases of field crops. New Phytol 154:731–740

Tiquia SM, Lloyd J, Herms DA, Hoitink HAJ, Michel FC (2002) Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure

determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes. Appl Soil Ecol 21: 31–48

Tuomela M, Vikman M, Hatakka A, Itavaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183

Van Elsas JD, Garbeva P, Salles J (2002) Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegradation 13:29–40

van Heerden I, Cronjé C, Swart SH, Kotzé JM (2002) Microbial, chemical and physical aspects of citrus waste composting. Bioresour Technol 81:71–76

Vargas-Garcia MC, Lopez MJ, Suarez F, Moreno J (2005) Laboratory study of inocula production for composting processes. Bioresour Technol 96:797–803

Vargas-Garcia MC, Suarez-Estrella F, Lopez MJ, Moreno J (2007) Effect of inoculation in composting processes: modifications in lignocellulosic fraction. Waste Manag 27:1099–1107

Watanabe K, Nagao N, Toda T, Kurosawa N (2008) Changes in bacterial communities accompanied by aggregation in a fed-batch composting reactor. Curr Microbiol 56:458–467

Wheatley RE (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek 81:357–364

Wichuk KM, McCartney D (2007) A review of the effectiveness of current time-temperature regulations on pathogen inactivation during composting. J Environ Eng Sci 6:573–586

Williams J, Clarkson JM, Mills PR, Cooper RM (2003) Saprophitic and mycoparasitic of aggressivness of *Trichoderma harzianum* groups toward the commercial mushroom *Agaricus bisporus*. Appl Environ Microbiol 69:4192–4199

Yangui T, Rhouma A, Triki MA, Gargouri K, Bouzid J (2008) Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Prot 27:189–197

Zaman M, Matsushima M, Chang SX, Inubushi K, Nguyen L, Goto S, Kaneko F, Yoneyama T (2004) Nitrogen mineralization, N2O production and soil microbiological properties as affected by long-term applications of sewage sludge composts. Biol Fertil Soils 40:101–109

Chapter 12
Compost Microbial Populations and Interactions with Plants

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Abstract The compost environment consists of complex organic materials that form a habitat for a rich and diverse microbial community. The fate and role of these microorganisms, when introduced into agricultural soils or potting mixes, depend on a suite of environmental factors that include biological and chemical properties of the soil and plant type and growth stage. In this review, we broadly consider the state-of-knowledge regarding compost microorganisms and their fate in plant-soil-compost systems. We explicitly consider microbial populations during the final stages of composting and in the mature product. The changes in the soil microbial community as affected by compost amendment and interactions with plant surfaces are the main focus of this chapter. We also consider important technical advances in the field of microbial ecology that have greatly improved our understanding of compost and rhizosphere microbiology. These advanced molecular biology techniques have allowed a comprehensive description of *in situ* microbial communities and have started to link microbial community structure with community function, even in the absence of relevant microbial isolates.

Contents

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12.1 Methods in Microbial Ecology

Many of the current methods in microbial ecology are cultivation-independent. Most of them are based on amplification of DNA fragments using the polymerase chain reaction (PCR; Saiki et al. 1985). The PCR (see Chap. 1, Insam et al. 2010; Chap. 7, Hultman et al. 2010; Chap. 14, Knapp et al. 2010) rapidly produces a huge number of copies of a desired DNA fragment, often a region encoding for a specific gene. For the purposes of microbial ecology, this technique is frequently used to amplify the gene encoding for the small subunit of the ribosomal RNA (SSU rRNA). This conserved gene is ubiquitous in all organisms (Olsen et al. 1986; Woese 1987; Pace 1997; Watanabe et al. 2001). The entire 16S rRNA gene is not equally well-conserved, and different regions of the gene can be targeted with PCR primers that target different taxonomic groups (from domain to species). Certain highly conserved regions of the gene allow the targeting of domains, but between these conserved regions the gene is variable enough to facilitate phylogenetic comparisons (Woese 1987). It has become the most commonly used gene for community composition studies and, when coupled with one or more profiling techniques, can allow a rapid and informative characterization of the predominant microbial members within a sample.

PCR amplification is dependent on the extraction and purification of adequate amounts of high-quality DNA from environmental samples. In samples of or containing compost, the recovery of DNA is frequently confounded by the co-extraction of substances, such as humic acids, that are inhibitory to downstream enzymatic reactions such as PCR (e.g. Howeler et al. 2003; Tebbe and Vahjen 1993; Tsai and Olson 1992). Humic acid contamination has been shown to result in PCR inhibition, and the presence of humic acids in DNA extracts may result in a strongly biased measure of microbial diversity (Martin-Laurent, et al. 2001; Miller et al. 1999; Stach et al. 2001). A number of manuscripts describe techniques to circumvent the co-extraction of humic acids (e.g. Lamontagne et al. 2002; Inbar et al. 2005; Arbeli and Fuentes 2007), and commercial DNA extraction kits have been successful in extracting clean DNA from some composts. Proper sampling and sample handling (i.e. temperature, humidity, oxygen concentration, storage conditions and duration) prior to nucleic acid extraction are also important to avoid introduction of bias into microbial analysis of native communities (Jackson et al. 1997; Schneegurt et al. 2003; Wintzingerode et al. 1997).

Standard PCR analyses are not strictly quantitative; at best, they produce results that can be construed as semi-quantitative. The non-quantitative nature of PCR is an

essential part of the widespread application of PCR – namely, generating a great number of copies of a DNA target without strong sensitivity to the number of original copies introduced into the reaction. The polymerase chain reaction can be modified to allow quantitation, in a process called quantitative or real-time PCR (see Chaps. 1, 7 and 14). This approach, in which the increase in DNA target copy number is monitored during every cycle of the reaction, allows the user to accurately measure gene copy numbers present in a given sample (e.g. Higuchi et al. 1993; Heid et al. 1996). Real-time PCR has been used in several environmental studies to monitor and measure the relative abundance of specific groups of organisms in complex environments such as compost (e.g. Innerebner et al. 2006; Halet et al. 2006; Pedro et al. 2003). This methodology is also exquisitely sensitive to PCR inhibitors, and in samples with suspected humic contamination, a range of dilutions of the sample should be tested to determine that inhibitors are not affecting the quantification.

While standard rRNA gene clone library analysis is effective for characterization of the most abundant members of a sample and for generating diversity indices, such an approach, however, does not provide much information regarding more minor constituents of microbial communities nor does it provide any direct information regarding the physiology of the detected microorganisms. The first concern – the inability to detect minor constituents of a microbial community – is a significant one in the study of compost. Dramatic shifts in microbial community structure are routinely observed in composting – from the community associated with the raw ingredients, to the rapid die-off during peak-heating, to re-colonization, shifts associated with maturation, and finally shifts associated with incorporation of compost into soil or potting mixes and associated plant-soil interactions. Thus, the inability to detect minor constituents during one phase of this development can impede the determination of provenance for dominant members of microbial communities during later stages.

The second concern – the inability to directly identify cell physiology based on rRNA gene sequences, and the inability to identify the key physiological activities being conducted at the time of sampling – may be overcome by way of large-scale sequencing efforts. The recently developed methods for metagenomic study of community DNA, via construction of genomic DNA libraries and subsequent high-throughput sequencing or library screening (e.g. Riesenfeld et al. 2004; Handelsman 2004) were recently applied to study soil (Daniel 2004; van Elsas et al. 2008) and rhizosphere (Mirete et al. 2007). We can expect that it will, in the near future, also be applied to compost systems and reveal the communities and functions involved in shaping and affecting rhizosphere ecology. One limitation to this approach is the high microbial diversity in compost samples. In a metagenome analysis of another high diversity ecosystem, hypersaline microbial mats, a modest metagenome of roughly 100 Mb was clearly inadequate for a deep characterization of the system (Kunin et al. 2008). However, key insights into the community structure were nonetheless possible, and such analyses may reveal unexpected physiologies or commonalities among the microbial communities in compost.

Finally, stable isotope probing (SIP) also offers an additional molecular approach to enable linking physiology to phylogeny. In this method (see also Chap. 1), an exogenous compound containing a stable isotope (usually ^{13}C) is amended to a sample of interest. Microorganisms capable of utilizing the labeled compound incorporate the stable isotope into cellular biomarkers that can be used to identify organisms assimilating the substrate (e.g. Boschker and Middelburg 2002). The analysis of labeled biomarkers such as phospholipid fatty acids (PLFA; Bull et al. 2000; Boschker and Middelburg 2002), rRNA (Manefield et al. 2002) and DNA (Radajewski et al. 2000) have been used in microbial ecological studies. Stable isotope probing studies on plant roots have already been employed (e.g. Rangel-Castro et al. 2005; Lu and Conrad 2005). These studies have examined microbial growth on plant exudates by providing plants with ^{13}C-carbon dioxide. During plant growth and exudation, heavy carbon exudates are fed to the root-associated microbial community. In other contexts, cross-feeding can confound results, as the primary consumer transfers heavy carbon to the rest of the microbial community. Additional advances in the SIP approach have been developed to limit the incubation period to reduce cross-feeding (Binga et al. 2008). While DNA remains the primary information molecule for SIP studies, lipid analysis remains a viable approach as well. Lipid, such as PLFA, analyses have been routinely utilized for community analysis (see below), and have been coupled with SIP for analysis of active rhizosphere microbial communities (Treonis et al. 2004). Analysis of PLFAs allows the quantitative estimation of total microbial biomass, coupled with lower-resolution phylogeny and community structure; such analyses have previously been applied to compost studies (Herrmann and Shann 1997; Klamer and Bååth 1998; Carpenter-Boggs et al. 1998; Steger et al. 2003; Steger et al. 2007).

12.1.1 Microbial Community Profiling

The application of molecular strategies has allowed scientists to accurately examine microbial diversity in a wide range of environmental samples. These approaches have been successful in characterizing microbial communities, and have also encouraged the discovery and identification of novel organisms and genes in the environment (Murrell and Radajewski 2000; Milcic-Terzic et al. 2001; Stokes et al. 2001; Greene and Voordouw 2003). Many of the molecular approaches described above, however, represent only the first stage in the characterization of microbial communities. Further analysis of PCR yields and informational molecules separated by density gradients (i.e. SIP) must be conducted to characterize microbial communities. Microbial community composition can be analyzed based on profiles generated, for example, from the physical separation of rRNA gene sequences on an acrylamide gel (Muyzer et al. 1993). In this regard, several techniques based on the amplification and comparisons of PCR-amplified DNA sequences have been developed and used to characterize microbial communities from many environments, including soil, compost and rhizosphere. These separation techniques have several

key advantages and disadvantages, and are increasingly used in conjunction with other molecular techniques (see Chaps. 1, 7, 14). The ability to process multiple samples rapidly and economically is the primary advantage of these separation techniques. These methodologies have also become useful companions to other molecular techniques such as SIP, and direct comparison of DNA- and RNA-based fingerprints can be used to compare active and predominant community members (e.g. Cytryn et al. 2005). Conversely, the methods suffer from several potential technical pitfalls. These pitfalls include the limited detection of minor constituents of the microbial community and the lack of resolution of the approaches for distinguishing between different sequences, and conversely, the generation of multiple peaks or bands from nearly-the same sequence. Some of the commonly used techniques include (i) Amplified ribosomal DNA restriction analysis (ARDRA; e.g. Heyndrickx et al. 1996); (ii) denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993); (iii) Terminal-restriction length polymorphism (T-RFLP; Liu et al. 1997); (iv) generation of clone libraries; and (v) application of DNA microarrays. Most of these methods have been applied to compost studies, and have been dealt with thoroughly elsewhere. We take a moment to consider some of the issues relating to the generation of clone libraries and microarrays here.

12.1.2 Clone Libraries

Construction of clone libraries, a common approach in microbial ecology, is used to identify key constituents of microbial communities (and increasingly to also identify extremely minor constituents), to estimate population diversity, and to perform direct comparisons of microbial communities (Lozupone and Knight 2005; Neufeld et al. 2004; Ramette and Tiedje 2007). The approach involves PCR amplification, cloning, sequencing, and subsequent phylogenetic analysis. With the constant improvement of high-throughput sequencing, future studies will, undoubtedly, cover the diversity of even highly complex environments such as soils, composts and roots (Hughes et al. 2001). Soil (Liesack and Stackebrandt 1992; Borneman and Triplett 1997) and compost microbial communities (Dees and Ghiorse 2001; Hansgate et al. 2005; Thummes et al. 2007; Danon et al. 2008) have been already studied using SSU rRNA clone libraries. We note a few developments that have encouraged this approach. Not only did the capacity to sequence genes increase (in part, due to the development of rolling circle amplification, obviating the need for plasmid extractions from each clone, and robotic approaches for high-throughput sequencing; e.g. Dean et al. 2001), but a whole suite of online software packages were developed, including software to (a) detect chimeric sequences (e.g. Huber et al. 2004); (b) auto-align 16S rRNA gene sequences (e.g. Greengenes; DeSantis et al. 2006); (c) give rapid identification of an unaligned set of sequence data (RDP, Wang et al. 2007); (d) calculate a suite of diversity indices at all levels of taxonomic groupings (e.g. DOTUR, FastGroupII; Schloss and Handelsman 2005; Yu et al. 2006); and (e) compare microbial community structure based on phylogenetic trees

generated from sequence data (e.g. Unifrac, Libshuff; Lozupone and Knight 2005; Singleton et al. 2001). Recently, it has been demonstrated that short regions of the 16S rRNA gene, roughly 100–200 bases, are suitable for identification purposes down to a genus or species level (Wang et al. 2007). This bioinformatic advance has been coupled with sequencing platforms that generate massive quantities of short (50–250 base reads) sequences (e.g. Ronaghi et al. 1998). This approach has previously been utilized in deep-sequencing efforts of environmental samples from a variety of environments (e.g. Edwards et al. 2006; Acosta-Martínez et al. 2008), and would be welcome for characterizing shifts in microbial community structure during and after composting.

12.1.3 DNA Microarrays

DNA microarrays are a recent addition to the molecular ecology toolbox (see Chap. 1). DNA microarrays consist of small, solid supports onto which known DNA sequences from thousands of genes are immobilized in an array at fixed locations. DNA is printed, spotted, or synthesized directly onto the support. Nucleic acid samples to be analyzed are fluorescently labeled and hybridized to the array, allowing the parallel analysis of thousands of genes. Microarrays have been used for microbial ecology research in several habitats (Loy et al. 2002; Stralis-Pavese et al. 2004; Small et al. 2001). This technology provides a rapid, high-throughput means to analyze the complex and highly diverse microbial community found in compost, thus providing a tremendous tool for process monitoring, detection of pathogens, and detection of beneficial microbial populations in compost. A DNA microarray was recently designed and used for the investigation of microbial communities in compost (Franke-Whittle et al. 2005, 2009; Danon et al. 2008). This approach is limited, however, by requiring *a priori* knowledge of potential community members or target genes of interest.

12.2 Compost Communities

It is difficult to construct a specific “compost” microbial community. Composts undergo a series of dramatic changes in microbial community composition during the composting process and during maturation and application (see Chap. 1), and composts are produced from a wide variety of feedstocks and in a wide variety of composting processes from simple bins to industrial-scale composting plants. In addition, microorganisms in mature compost are present in high numbers and are quite diverse. Considerable effort and a variety of techniques, as described above, have been applied to the study of compost microbial populations (e.g. Cahyani et al. 2003; Schloss et al. 2003; Ryckeboer et al. 2003). The initial phase of composting is

the most dynamic part of the process and is characterized by rapid increases in temperature, large changes in pH, and the rapid degradation of labile organic compounds. These physio-chemical changes in the compost strongly influence the constituent microbial community, and there are multiple significant shifts in the microbial community structure during the composting process (e.g. Schloss et al. 2003; Peters et al. 2000). In general, the peak-heating stage is most likely to select for similar microbial communities by way of selecting for thermophilic microorganisms; as a result, Gram-positive bacteria, such as bacteria from the genus *Bacillus* (low G-C Gram-positive phylum Fimicutes), can dominate (e.g. Strom 1985a,b; Peters et al. 2000; Michel et al. 2002; Ryckeboer et al. 2003). Actinobacteria are rarely detected in the post-peak heating compost without the use of phylum-specific PCR primers, but can become abundant in maturing composts (Green et al. 2004; Michel et al. 2002; Tiquia et al. 2002b; Dees and Ghiorse 2001; Danon et al. 2008). After peak heating, the compost is re-colonized by microorganisms present in the production environment, and bacteria from the phylum Bacteroidetes often dominate (Cahyani et al. 2003; Green et al. 2004, 2006, 2007; Takaku et al. 2006). Sometimes, the effect of the source material for the compost is evident in the final product (Green et al. 2004). Mature composts can be rich in phytopathogen antagonistic species, or more likely, these organisms can be amended to the compost for colonization purposes (e.g. Hoitink et al. 1993; Hoitink and Boehm 1999).

Ryckeboer et al. (2003) attempted to determine the microbial succession of the dominating cultivable taxa and functional groups of microorganisms, as well as the total microbial activity during composting of vegetable, fruit and garden waste. They reported that bacteria dominated the thermophilic phase, while fungi and bacteria from the family *Streptomycetes*, were below detection limits. The bacterial community differed between the thermophilic and mesophilic composting phases. During the peak-heating phase of fresh waste, the only bacterium isolated was Gram-positive Firmicutes of the genus *Bacillus*; however, during the cooling and maturation phases, a wide spectrum of Gram-positive and Gram-negative bacteria was isolated (Ryckeboer et al. 2003). Similarly, the diversity and succession of microbial communities were studied using bacterial isolation, DGGE and clone library analysis during composting of municipal waste mixed with rice hull (Takaku et al. 2006). The dominant microbial taxa changed from organisms belonging to the phylum Firmicutes during the thermophilic phase to the Bacteroidetes in the maturation phase. Likewise, Cahyani et al. (2003) reported a bacterial community transition during the composting of rice straw: Alphaproteobacteria in the raw materials, Bacilli and Actinobacteria during the thermophilic stage, and Cytophaga and Clostridia in the middle and curing stages. Members of the phylum Bacteroidetes have been shown to be critical components of plant-associated microbial communities for plants grown in compost-amended soils or media, and they have been shown to be large contributors to nutrient recycling in plant environment by way of production of degradative enzymes (Hallmann et al. 1997; Mahaffee and Kloepper 1997a, b; McSpadden Gardener and Weller 2001; Green et al. 2006, 2007). While the different stages of composting do appear to

select for specific taxonomic groups, it should be noted that the studies reveal similarity at very high taxonomic levels, and that the full diversity of these communities varies more significantly when examined at a genus or species level.

The maturation, or curing stage of compost is a critical period, and the length and conditions of curing can strongly influence not only the microbial community within the compost, but also physical and chemical properties. The successful application of compost is considerably dependent on the selection of an appropriate curing period, and a number of prior studies have emphasized the importance of achieving compost maturity to ensure balanced plant nutrition and for the biological control of soil-borne plant disease (Fuchs 2002; Noble and Coventry 2005). Non-cured composts may be phytotoxic, whereas extensively cured composts can lose their plant-disease-suppressive properties (e.g. Hoitink and Fahy 1986; Danon et al. 2007; Zmora-Nahum et al. 2008). For instance, loss of compost suppressiveness toward *Sclerotium rolfsii* has been demonstrated during prolonged curing time (Danon et al. 2007). In general, a shift in community structure is observed during maturation or curing of compost although Cahyani et al. (2003) reported that the microbial community remained stable during the curing phase. Using a suite of cultivation-independent analyses, including DGGE, 16S rRNA gene sequencing, and microarray analysis, Danon et al. (2008) observed statistically significant shifts in bacterial community structure during an extended maturation period. These data indicated that bacteria from the phylum *Bacteroidetes* and from the *Gammaproteobacteria* were ubiquitous, but their relative dominances were inversely related; at the beginning of the compost-curing process, *Bacteroidetes* were dominant. Later, during the mid-curing stage, *Actinobacteria* became more abundant. After a lengthy curing period, *Gammaproteobacteria* were more abundant.

12.3 Effect of Compost Application on Soil Microbial Community

The utility of compost materials lies in their amendment to soils and potting substrates as a source of organic matter, of abiotic nutrients, and of diverse and beneficial microorganisms. The latter include plant-growth promoting and pathogen-antagonistic bacterial species. The enormous range of composts and of the soils to which they are added, however, limits the ability to predict *a priori* the impact of such amendments. The biological and physiochemical characteristics of the native soil are, nonetheless, critical in determining the resulting microbial community. For example, in soils containing very limited organic matter and consequently low microbial biomass, even modest amendments of compost can radically affect the total soil and rhizosphere microbial community (e.g. Inbar et al. 2005).

Pérez-Piqueres et al. (2006) systematically studied the amendment of three composts of differing origins to two different soils under controlled conditions. They observed that shifts in the soil biological properties depended on both the

compost amendment and the native soil. All the composts produced shifts in the microbial community composition, as determined by T-RFLP fingerprinting. Similar observations were also made by Ros et al. (2006) who studied different compost types in a long-term field experiment. The results indicated that the shifts induced in the microbial community compositions were mainly due to the amendment of the soil with new community members originating from the compost, along with some stimulation of the native soil microflora. Interestingly, Pérez-Piqueres et al. (2006) observed that the compost amendments did not radically change the physiological capabilities of the soil, as inferred by Biolog community level physiological profiling functional analysis, and attributed this to functional redundancy of soil and of compost microorganisms.

Elsewhere, Calbrix et al. (2007) showed only a minor and transient effect for compost on soil microbial community composition and functions. Similarly, Crecchio et al. (2004) found that amendment of cropped plots with compost increased the soil organic C and total N content and enhanced a variety of soil microbial activities. However, they found that changes in the microbial community, as a consequence of compost amendment (determined using DGGE, rRNA gene internal spacer analysis and ARDRA of bacteria, Archaea, Actinobacteria, and ammonia-oxidizing bacteria), were minimal. This may have been due to the relatively small increase in organic matter introduced by way of the compost amendment (12% increase) relative to that in the Pérez-Piqueres et al. (2006) study (39–260% increase).

12.4 Effect of Compost Application on Root and Rhizosphere Microbial Community

The amendment of compost to soils represents a fundamentally distinct biological intervention in comparison with inoculation with specific microorganisms such as *Rhizobium* spp. or other growth-promoting bacteria. Compost introduces to soils and potting mixes an abundant and highly diverse microbiota and rich organic matter (Tiquia et al. 2002a, b). Thus, via a combination of physical, chemical and biological factors, composts can simultaneously influence a wide range of processes affecting soil, rhizosphere and root-associated bacterial communities (see also Chap. 6, Ceustermans et al. 2010; Chap. 11, Fuchs et al. 2010; Chap. 13, Bastida et al. 2010; Chap. 14). We note that even a simple inoculation of soil with a single bacterial or fungal species may have an effect that extends beyond the target organisms (Lottmann et al. 2000; Johansen et al. 2005) and may involve multiple mechanisms of action (Zhang et al. 1998; Compant et al. 2005).

Spermosphere and rhizosphere microbial populations are influenced by the type and quantity of depositions from plant seeds and roots, which include sugars, organic acids, polymers and sloughed cells (Campbell and Greaves 1990; Whipps 2001; Baudoin et al. 2002). These depositions vary in quantity and type with, among

other factors, plant development status (Andrews and Harris 2000; Lugtenberg et al. 1999). Thus, development from seed to root shifts exudate quantity and quality that, in turn, impacts soil microorganisms (Buyer et al. 1999, 2002). Naturally, the rhizosphere bacterial community is derived from that of the bulk soil. Even so, the composition of the dominant bacterial population in the rhizosphere may differ considerably from that of the bulk soil (e.g. Inbar et al. 2005; Weisskopf et al. 2005). Moreover, different plant species and even cultivars select for unique bacterial communities under identical soil conditions (Smalla et al. 2001; Kowalchuk et al. 2002). The resilience of the rhizosphere bacterial community to different anthropogenic and other disturbances has previously been discussed (Lynch 2002; Baumgarte and Tebbe 2005). Generally, artificial modifications such as introduction of new microorganisms are not sustained (Nautiyal et al. 2002; Götz et al. 2006). Currently, only a few basic research studies examining the effects of compost amendments on root and the rhizosphere microbial community composition have been conducted (de Brito et al. 1995; Tiquia et al. 2002a; Inbar et al. 2005; Green et al. 2006, 2007). There is only limited research in this area, but we note that there is a wide range of findings that is most likely a result of the different soils, different composts and compost amendment rates, and different plants.

For example, in the study of Tiquia et al. (2002a), total microbial biomass (as indicated by soil respiration and microbial N) increased in rhizosphere samples from plots mulched with compost compared to un-amended or wood-mulched plots. Consistent with the biomass and cultivation data, they observed that the rhizosphere communities in compost-mulched plots differed significantly from the rhizosphere communities of the un-amended and wood-mulched plots. Despite shifts in community composition, significant differences in T-RFLP-based diversity indices were not observed. A higher number of terminal restriction fragments (T-RFs) corresponding to possible biocontrol organisms such as *Pseudomonas* and *Pantoea* spp. were found in compost-mulched plots.

Conversely, de Brito et al. (1995) examined four commercial composts and their effect on plant growth, total rhizosphere microflora, and incidence of plant growth-promoting rhizobacteria (PGPR) in the rhizosphere of tomato plants. In this study, compost amendments caused only small variations in the total numbers of bacteria, actinobacteria, and fungi in the rhizosphere of the tomato plants. On the other hand, the addition of composts to soil increased the incidence of bacteria exhibiting antagonism towards several plant pathogenic fungi in the rhizosphere. Elsewhere, the role of native soil organic matter and compost amendment rate on plant-associated microbial communities was examined in a study of *Streptomyces* on the roots of cucumber plants (Inbar et al. 2005). Although actinobacteria are generally present in low abundance in composts after peak heating, *Streptomyces* spp. are often present in the rhizospheres of plants (Weller et al. 2002; Smalla et al. 2001; Korn-Wendisch and Kutzner 1999). The apparent rhizosphere competence of this bacterial group has been attributed to their production of and resistance to antibiotics, and their ability to consume a variety of organic carbon sources, including complex and recalcitrant plant-derived compounds (Goodfellow and Williams 1983). In the study of Inbar et al. (2005), a single compost was amended

at three different rates to an organic-poor sandy soil used for cultivation of cucumber, and samples of bulk soil, rhizosphere and roots were analyzed via cultivation-independent techniques. The *Streptomyces* community composition was influenced both by proximity to the root and by the rate of compost amendment, and the interaction of these two factors was most readily visualized in community profiles of the rhizosphere where the two factors overlapped to the greatest extent (Inbar et al. 2005). Compost amendment produced a dramatic shift in the composition of the bulk soil *Streptomyces* community, even at low rates. However, establishment of those populations in the rhizosphere and at the rhizoplane, required substantially higher amendment rates.

Inbar et al. (2005) observed that all root community profiles were predominantly composed of a single *Streptomyces* strain closely related by 16S rRNA gene sequence to non-pathogenic, plant tissue-associated and antibiotic-producing *Streptomyces*. The predominance of a single population is consistent with a rhizosphere effect, a strong plant activity-induced selection for bacterial communities in close proximity to roots (Smalla et al. 2001; Whipps 1990). At the highest compost amendment rate, the dominant compost-*Streptomyces* population was detected in association with the roots. Overall, the results presented by Inbar et al. (2005) suggest a significant "rhizosphere buffering," in which organisms applied as soil amendments can establish high population levels but ultimately are not sustained in the rhizosphere or rhizoplane due to plant selection (Weller et al. 2002). The apparent insulation of the root *Streptomyces* community from the effects of compost amendment, as observed in the bulk and rhizosphere soils, must be considered in the employment of compost amendments for modifying plant-associated microbial communities. Nonetheless, as compost organisms were detected in root samples at the highest amendment rate applied, it is possible that with high-enough amendments rates, beneficial populations can be forced into these communities. This is consistent with the current understanding of the influence of plant depositions on microbial population dynamics in the root and rhizosphere. Cheng et al. (1993) showed that water-soluble carbon concentrations varied inversely with distance from the rhizoplane. Likewise, Wieland et al. (2001) found that the influence of soil on microbial population distribution was reduced with increasing proximity to the rhizoplane. This diminishing influence is presumably due to the increasing predominance of exudates closer to the roots, which in turn increases competition among copiotrophic bacteria. However, Semenov et al. (1999) observed a decrease in such a rhizosphere effect in wheat plants grown in high organic soils, compared to soil poor in organic matter. This may account for the increasing similarity of rhizosphere and root community profiles in compost treatments. Specifically, plants grown in compost-amended soils can be expected to have a less-drastic rhizosphere effect due to the higher microbial carrying capacity of the soil itself. Clearly, soil characteristics and proximity to the root surface are critical in determining plant-associated microbial populations.

Plant-associated microbial community structure can also be expected to change during the growth of the plant. One of the general observations in the study of Green et al. (2006) was of dramatic shifts in plant-associated bacterial populations during

plant development from seed to root. While Green et al. (2006) observed relatively little overlap between seed- and root-colonizing bacterial communities, seed surfaces were consistently colonized by a subset of active microbial populations present at high levels in the potting mix at the time of sowing. Normander and Prosser (2000) also observed such a disparity between seed and root microbial communities and proposed that this difference was an indication that emerging plant roots are colonized by soil-borne microbial populations rather than by seed-borne populations. Presumably, in the longer term, the development of the plants with accompanying shifts in exudation pattern allows more competitive microbial populations to develop from relatively low levels of soil and compost. In this facet, compost amendments – which deliver a diverse array and great abundance of microorganisms – have the potential to be a successful means of introduction of beneficial bacteria to the plant environment and multiple stages of development.

The transition from seed to root adds additional complexity to the soil-compost-plant system, and the development of roots further extends the zone impacted by plant exudates. Green et al. (2007) hypothesized that the interaction between exogenous soil amendments such as compost and the influence of the plant or rhizosphere effect does not impact all microorganisms equally. To explore this issue, the community composition of two distinct taxa, one consisting of bacteria within the family *Oxalobacteraceae* (Betaproteobacteria), and the second from the genus *Chryseobacterium* (Bacteroidetes), both consistently detected in seed and root communities, were characterized by the application of population-specific analyses (Green et al. 2007). These analyses revealed that the two groups of seed- and root-colonizing taxa responded to compost and root effects in different ways. The response of *Oxalobacteraceae* populations to plant-growth stage and proximity to roots was consistent with the response of saprophytic bacteria largely influenced by plant exudates, resulting in a strong rhizosphere effect (Green et al. 2007). Furthermore, the *Oxalobacteraceae* community composition changed dramatically during plant development, as seed communities resembled the initial potting mix while the root communities comprised a single dominant population, which differed from those detected on the seeds (Green et al. 2007). The shift in the *Oxalobacteraceae* population composition from seed to root was consistent with prior observations that the seed-colonizing bacterial communities differed substantially from the root microbial communities (Green et al. 2006).

Conversely, the community composition of compost-derived bacteria of the genus *Chryseobacterium* was not sensitive to the seed or root environment, and was consistent regardless of proximity to the plant surface (Green et al. 2007). This ecological behavior is more consistent with organisms that are influenced by bulk soil (e.g. soil organic matter). Soil organic matter can be a significant source of carbon for rhizosphere organisms (Toal et al. 2000), and high levels of organic matter and nutrients, such as those that are supplied via compost amendment, can influence the strength of the observed rhizosphere effect (Semenov et al. 1999). Although *Chryseobacterium* spp. are found frequently in organic-rich environments such as composts (Ryckeboer et al. 2003), these organisms are not particularly well known as rhizosphere organisms, though they have been previously

detected in or isolated from rhizosphere environments (McSpadden Gardener and Weller 2001;Young et al. 2005; Park et al. 2006). The consistent presence of *Chryseobacterium* spp. on seed and root surfaces may be a result of their persistence in the compost-amended potting mixes. The ability of bacteria to survive in large numbers in soil can be a major determinant of their ability to subsequently colonize rhizosphere environments (Jjemba and Alexander 1999; De Ridder-Duine et al. 2005), and compost amendments have been shown to sustain *Chryseobacterium* in peat-based potting mixes for longer than in unamended potting mixes (Krause et al. 2001).

Thus, although root-associated bacterial communities can be heavily influenced by the plant, some microorganisms can be sustained in root environments with relative insensitivity to the rhizosphere effect (Green et al. 2007). The persistence of the same *Chryseobacterium* populations in potting mixes and on seed and root surfaces demonstrates that the cucumber rhizosphere selection is not the exclusive factor involved in determining the composition of the spermosphere and rhizosphere communities. Although it seems unlikely that this is a plant-specific phenomenon, these types of studies must be expanded to different plant systems. Such research may allow us to more specifically characterize the multiple factors that can mitigate the rhizosphere effect, and allow the persistence of 'non-traditional' rhizosphere microorganisms in close proximity to plant seed and root surfaces.

12.5 Conclusions

The study of plant-associated microbial communities, even without consideration of compost, is a daunting task. A wide range of factors influence the structure of microbial communities, a fact that is reflected in the extensive rhizosphere literature. The current literature on factors affecting plant-associated microbial communities is at best confusing, and at worst, downright contradictory. This reflects, in a sense, the difficulty in attempting to extrapolate from single system analyses to trends present in all rhizospheres. In general, each researcher employs an entirely different plant-soil system and different sampling methodology, and it can be difficult to relate the results of one study to another. Nonetheless, it is clear that microbial interactions (microbe–microbe and microbe–plant) on plant surfaces are influenced by plant, soil and environmental conditions and the interactions among them. In a sense, the distribution of microorganisms on plant surfaces fall into the dialectic of "nature" versus "nurture." Soil microorganisms can be classified as "nature" – all the potential plant surface colonizing microorganisms, while plant effects can be considered "nurture," as the plant environment selects for certain members of the soil microbial community. As with the debate over "nature" versus "nurture," the real question is not which factor determines the ultimate outcome, but to what extent each factor controls the outcome. The addition of compost to soil or potting mixes is a decided effort to try and tilt the balance against plant selection. Elsewhere, researchers are modifying plant genes, not to reduce plant selection of microorganisms, but to modify those organisms for which they select

(Oger et al. 2004). Ultimately, the goal is the same – to be able to control, reproducibly, the structure and function of seed- and root-associated microbial communities, be it for disease control or plant growth promotion.

Compost amendment to soils is a common agricultural technique, and has not yet received its deserved attention, particularly with regard to molecular analyses. Composts, of course, exert more than a biological effect; such amendments can substantially modify soil chemistry, soil structure, and soil organic matter. These effects are not always positive, and some composts may indeed be inhibitory to plant growth, perhaps due to the presence of high levels of phytotoxins and high C/N ratio, particularly in immature composts (Craft and Nelson 1996; de Brito et al. 1995). Despite studies on the beneficial effects of compost physical and chemical characteristics, in some cases for disease control (Pascual et al. 2002), the literature suggests that it is the great biological diversity of compost materials that is most responsible for plant growth promotion and disease control. However, compost minerals and organic matter can serve an important role in sustaining compost microorganisms (Boehm et al. 1997). The driving concept is to be able to use compost amendments to introduce and sustain specific beneficial microbial populations. The high native diversity of compost microbial communities, coupled with the high metabolic diversity, may also greatly assist in introducing into the plant genes environment multiple organisms that are highly competitive and antagonistic to pathogens.

We believe that major progress in understanding the effect of compost amendments on soil and plant-associated communities will come from better knowledge of the function and diversity of the microbial populations involved (see also Chap. 11, Fuchs 2010). This is now feasible with the application of molecular techniques already available. High-throughput rRNA gene and functional gene sequence analyses will help define more clearly shifts in microbial community structure and function during the composting process and after amendment to soil and plant environments. We propose the need for deep-sequencing of rRNA and mRNA transcripts from many composts during multiple stages of development, and for metagenomic data from these composts. Such data will enable us to track compost microorganisms more effectively, and to ultimately understand the effect of compost amendments on both the microbial community structure and activity in plant-associated environments.

References

Acosta-Martínez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem 40:2762–2770

Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant surfaces. Annu Rev Phytopathol 38:145–180

Arbeli Z, Fuentes CL (2007) Improved purification and PCR amplification of DNA from environmental samples. FEMS Microbiol Lett 272:269–275

Baudoin E, Benizri E, Guckert A (2002) Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl Soil Ecol 19:135–145

Baumgarte S, Tebbe CC (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mol Ecol 14:2539–2551

Binga E, Lasken R, Neufeld JD (2008) Something from (almost) nothing: the impact of multiple displacement amplification on microbial ecology. ISME J 2:233–241

Boehm MJ, Wu TY, Stone AG, Kraakman B, Iannotti DA, Wilson GE, Madden LV, Hoitink HAJ (1997) Cross-polarized magic-angle spinning C-13 nuclear magnetic resonance spectroscopic characterization of soil organic matter relative to culturable bacterial species composition and sustained biological control of *Pythium* root rot. Appl Environ Microbiol 63:162–168

Borneman J, Triplett EW (1997) Molecular microbial diversity in soil from Eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl Environ Microbiol 63:2647–2653

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol Ecol 40:85–95

Bull ID, Parekh NR, Hall GH, Ineson P, Evershed RP (2000) Detection and classification of atmospheric methane oxidizing bacteria in soil. Nature 405:175–178

Buyer JS, Roberts DP, Russek-Cohen E (1999) Microbial community structure and function in the spermosphere as affected by soil and seed type. Can J Microbiol 45:138–144

Buyer JS, Roberts DP, Russek-Cohen E (2002) Soil and plant effects on microbial community structure. Can J Microbiol 48:955–964

Cahyani VR, Matsuya K, Asakawa S, Kimura M (2003) Succession and phylogenetic composition of bacterial communities responsible for the composting process of rice straw estimated by PCR-DGGE analysis. Soil Sci Plant Nutr 49:619–630

Calbrix R, Barray S, Chabrerie O, Fourrie L, Laval K (2007) Impact of organic amendments on the dynamics of soil microbial biomass and bacterial communities in cultivated land. Appl Soil Ecol 35:511–522

Campbell R, Greaves MP (1990) Anatomy and community structure of the rhizosphere. In: Lynch JM (ed) The rhizosphere. John Wiley, New York, pp 11–34

Carpenter-Boggs L, Kennedy AC, Reganold JP (1998) Use of phospholipid fatty acids and carbon source utilization patterns to track microbial community succession in developing compost. Appl Environ Microbiol 64:4062–4064

Cheng W, Coleman DC, Carroll CR, Hoffman CA (1993) In situ measurement of root respiration and soluble C concentrations in the rhizosphere. Soil Biol Biochem 25:1189–1196

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Craft CM, Nelson EB (1996) Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Appl Environ Microbiol 62: 1550–1557

Crecchio C, Curci M, Pizzigallo MDR, Ricciuti P, Ruggiero P (2004) Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biol Biochem 36:1595–1605

Cytryn E, van Rijn J, Schramm A, Gieseke A, de Beer D, Minz D (2005) Identification of bacteria potentially responsible for oxic and anoxic sulfide oxidation in biofilters of a recirculating mariculture system. Appl Environ Microbiol 71:6134–6141

Daniel R (2004) The soil metagenome – a rich resource for the discovery of novel natural products. Curr Opin Biotechnol 15:199–204

Danon M, Zmora-Nahum S, Chen Y, Hadar Y (2007) Prolonged compost curing reduces suppression of *Sclerotium rolfsii*. Soil Biol Biochem 39:1936–1946

Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y (2008) Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol Ecol 65:133–144

de Brito AMA, Gagne S, Antoun H (1995) Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Appl Environ Microbiol 61:194–199

De Ridder-Duine AS, Kowalchuk GA, Klein Gunnwiek PJA, Smant W, van Veen JA, de Boer W (2005) Rhizosphere bacterial community composition in natural stands of *Carex arenaria* (sand sedge) is determined by bulk soil community composition. Soil Biol Biochem 37: 349–357

Dean FB, Nelson JR, Giesler TL, Lasken RS (2001) Rapid amplification of plasmid and phage DNA using Phi29 DNA polymerase and multiply-primed rolling circle amplification. Genome Res 11:1095–1099

Dees PM, Ghiorse WC (2001) Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiol Ecol 35:207–216

DeSantis TZ Jr, Hugenholtz P, Keller K, Brodie EL, Larsen N, Piceno YM, Phan R, Andersen GL (2006) NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes. Nucleic Acids Res 34:W394–W399

Edwards RA, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson DM, Saar MO, Alexander S, Alexander EC Jr, Rohwer F (2006) Using pyrosequencing to shed light on deep mine microbial ecology. BMC Genomics 7:57

Franke-Whittle IH, Klammer SH, Insam H (2005) Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. J Microbiol Methods 62:37–56

Franke-Whittle IH, Knapp B, Fuchs J, Insam H (2009) Changes in the microbial community of different Swiss composts over time, investigated using the CompoChip DNA microarray and DGGE. Microb Ecol 57:510–521

Fuchs JG (2002) Practical use of quality compost for plant health and vitality improvement. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin, pp 435–444

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Goodfellow M, Williams ST (1983) Ecology of actinomycetes. Annu Rev Microbiol 37:189–216

Götz M, Gomes NC, Dratwinski A, Costa R, Berg G, Peixoto R (2006) Survival of gfp-tagged antagonistic bacteria in the rhizosphere of tomato plants and their effects on the indigenous bacterial community. FEMS Microbiol Ecol 56:207–218

Green SJ, Michel FC Jr, Hadar Y, Minz D (2004) Similarity of bacterial communities in sawdust- and straw-amended cow manure composts. FEMS Microbiol Lett 233:115–123

Green SJ, Inbar E, Michel FC Jr, Hadar Y, Minz D (2006) Succession of bacterial communities during early plant development: transition from seed to root and effect of compost amendment. Appl Environ Microbiol 72:3975–3983

Green SJ, Michel FC Jr, Hadar Y, Minz D (2007) Contrasting patterns of seed and root colonization by bacteria from the genus *Chryseobacterium* and from the family Oxalobacteraceae. ISME J 1:291–299

Greene EA, Voordouw G (2003) Analysis of environmental microbial communities by reverse sample genome probing. J Microbiol Methods 53:211–219

Halet D, Boon N, Verstraete W (2006) Community dynamics of methanotrophic bacteria during composting of organic matter. J Biosci Bioeng 101:297–302

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685

Hansgate AM, Schloss PD, Hay AG, Walker LP (2005) Molecular characterization of fungal community dynamics in the initial stages of composting. FEMS Microbiol Ecol 51: 209–214

Heid CA, Stevens J, Livak KJ, Williams PM (1996) Real time quantitative PCR. Genome Res 6:986–994

Herrmann RF, Shann JF (1997) Microbial community changes during the composting of municipal solid waste. Microb Ecol 33:78–85

Heyndrickx M, Vauterin L, Vandamme P, Kersters K, De Vos P (1996) Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. J Microbiol Methods 26:247–259

Higuchi R, Fockler C, Dollinger G, Watson R (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 11:1026–1030

Hoitink HAJ, Boehm M (1999) Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Annu Rev Phytopathol 37:427–446

Hoitink HAJ, Fahy PC (1986) Basis for the control of soilborne plant pathogens with composts. Annu Rev Phytopathol 24:93–114

Hoitink HAJ, Boehm MJ, Hadar Y (1993) Mechanisms of suppression of soilborne plant pathogens in compost-amended substrates. In: Hoitink HAJ, Kenner HM (eds) Science and engineering of composting: design, environmental, microbiological and utilization aspects. Renaissance, Worthington, pp 601–621

Howeler M, Ghiorse WC, Walker LP (2003) A quantitative analysis of DNA extraction and purification from compost. J Microbiol Methods 54:37–45

Huber T, Faulkner G, Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics 20:2317–2319

Hughes JB, Hellmann JJ, Ricketts TH, Bohannan BJ (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. Appl Environ Microbiol 67:4399–4406

Inbar E, Green SJ, Hadar Y, Minz D (2005) Competing factors of compost concentration and proximity to root affect the distribution of streptomycetes. Microb Ecol 50:73–81

Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidizing bacteria in compost-treated soils. Soil Biol Biochem 38:1092–1100

Jackson CR, Harper JP, Willoughby D, Rodin EE, Churchill PF (1997) A simple, efficient method for the separation of humic substances and DNA from environmental samples. Appl Environ Microbiol 63:4993–4995

Jjemba PK, Alexander M (1999) Possible determinants of rhizosphere competence of bacteria. Soil Biol Biochem 31:623–632

Johansen A, Knudsen IMB, Binnerup SJ, Winding A, Johansen JE, Jensen LE (2005) Non-target effects of the microbial control agents *Pseudomonas fluorescence* DR54 and *Clonostachys rosea* IK726 in soils cropped with barley followed by sugar beet: a greenhouse assessment. Soil Biol Biochem 37:2225–2239

Klamer M, Bååth E (1998) Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. FEMS Microbiol Ecol 27:9–20

Korn-Wendisch F, Kutzner HJ (1999) The family Streptomycetaceae. In: Dworkin M (ed) The prokaryotes: an evolving electronic resource for the microbiological community. Springer-Verlag, New York

Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PGL, van Veen JA (2002) Effects of above-ground plant species composition and diversity on the diversity of soilborne microorganisms. Antonie Van Leeuwenhoek 81:509–520

Krause MS, Madden LV, Hoitink HAJ (2001) Effect of potting mix microbial carrying capacity on biological control of *Rhizoctonia* damping-off of radish and *Rhizoctonia* crown and root rot of Poinsettia. Phytopathology 91:1116–1123

Kunin V, Raes J, Harris K, Spear JR, Walker JJ, Ivanova N, von Mering C (2008) Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat. Mol Syst Biol 4:198

LaMontagne MG, Michel FC Jr, Holden PA, Reddy CA (2002) Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. J Microbiol Methods 49:255–264

Liesack W, Stackebrandt E (1992) Occurrence of novel groups of the domain bacteria as revealed by analysis of genetic material isolated from an Australian terrestrial environment. J Bacteriol 174:5072–5078

Liu WT, Marsh TL, Cheng H, Formey LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Lottmann J, Heuer H, Mahn VJA, During K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33:41–49

Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer KH, Wagner M (2002) Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microbiol 68: 5064–5081

Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235

Lu Y, Conrad R (2005) In situ stable isotope probing of methanogenic Archaea in the rice. Science 309:1088–1090

Lugtenberg BJ, Kravchenko LV, Simons M (1999) Tomato seed and root exudates sugars: composition, utilization by *Pseudomonas* biocontrol strains and role in rhizosphere colonization. Environ Microbiol 1:439–446

Lynch JM (2002) Resilience of the rhizosphere to anthropogenic disturbance. Biodegradation 13:21–27

Mahaffee WF, Kloepper JW (1997a) Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). Microb Ecol 34:210–223

Mahaffee WF, Kloepper JW (1997b) Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth promoting rhizobacterium or its genetically modified derivative. Can J Microbiol 43:344–353

Manefield M, Whitely AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking community function to phylogeny. Appl Environ Microbiol 68:5367–5373

Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G, Catroux G (2001) DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol 67:2354–2359

McSpadden Gardener BB, Weller DM (2001) Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. Appl Environ Microbiol 67:4414–4425

Michel FC Jr, Marsh TJ, Reddy CA (2002) Bacterial community structure during yard trimmings composting. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin, pp 25–42

Milcic-Terzic J, Lopez-Vidal Y, Vrvic MM, Saval S (2001) Detection of catabolic genes in indigenous microbial consortia isolated from a diesel-contaminated soil. Bioresour Technol 78:47–54

Miller DN, Bryant JE, Madsen EL, Ghiorse WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microbiol 65:4715–4724

Mirete S, de Figueras CG, González-Pastor JE (2007) Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl Environ Microbiol 73:6001–6011

Murrell JC, Radajewski S (2000) Cultivation-independent techniques for studying methanotroph ecology. Res Microbiol 151:1–8

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Nautiyal CS, Johri JK, Singh HB (2002) Survival of the rhizosphere-competent biocontrol strain *Pseudomonas fluorescence* NBRI2650 in the soil and phytosphere. Can J Microbiol 48: 588–601

Neufeld JD, Yu Z, Lam W, Mohm WW (2004) Serial analysis of ribosomal sequence tags (SARST): a high-throughput method for profiling complex microbial communities. Environ Microbiol 6:131–144

Noble R, Coventry E (2005) Suppression of soil-borne plant diseases with composts: a review. Biocontrol Sci Technol 15:3–20

Normander B, Prosser JI (2000) Bacterial origin and community composition in the barley phytosphere as a function of habitat and presowing conditions. Appl Environ Microbiol 66:4372–4377

Oger PM, Mansouri H, Nesme X, Dessaux Y (2004) Engineering root exudation of lotus toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Microb Ecol 47:96–103

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365

Pace NR (1997) A molecular view of microbial diversity and the biosphere. Science 276:734–740

Park MS, Jung SR, Lee KH, Lee MS, Do JO, Kim SB, Bae KS (2006) *Chryseobacterium soldanellicola* sp. nov. and *Chryseobacterium taeanense* sp. nov., isolated from roots of sand-dune plants. Int J Syst Evol Microbiol 56:433–438

Pascual JA, Garcia C, Hernandez T, Lerma S, Lynch JM (2002) Effectiveness of municipal waste compost and its humic fraction in suppressing *Pythium ultimum*. Microb Ecol 44: 59–68

Pedro MS, Haruta S, Nakamura K, Hazaka M, Ishii M, Igarashi Y (2003) Isolation and characterization of predominant microorganisms during decomposition of waste materials in a field-scale composter. J Biosci Bioeng 95:368–373

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. Soil Biol Biochem 38:460–470

Peters S, Koschinsky S, Schwieger F, Tebbe CC (2000) Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation polymorphism-based genetic profiles of small-subunit rRNA genes. Appl Environ Microbiol 66:930–936

Radajewski S, Ineson P, Parekh NH, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403:646–649

Ramette A, Tiedje J (2007) Biogeography: an emerging cornerstone for understanding prokaryotic diversity, ecology, and evolution. Microb Ecol 53:197–207

Rangel-Castro JI, Killham K, Ostle N, Nicol GW, Anderson IC, Scrimgeour CM, Ineson P, Meharg A, Prosser JI (2005) Stable isotope probing analysis of the influence of liming on root exudate utilization by soil microorganisms. Environ Microbiol 7:828–838

Riesenfeld CS, Schloss PD, Handelsman J (2004) Metagenomics: genomic analysis of microbial communities. Annu Rev Genet 38:525–552

Ronaghi M, Uhlén M, Nyrén P (1998) A sequencing method based on real-time pyrophosphate. Science 281:363–365

Ros M, Klammer S, Knapp B, Aichberger K, Insam H (2006) Long term effects of compost amendment of soil on functional and structural diversity and microbial activity. Soil Use Manag 22:209–218

Ryckeboer J, Mergaert J, Coosemans J, Deprins K, Swings J (2003) Microbiological aspects of biowaste during composting in a monitored compost bin. J Appl Microbiol 94:127–137

Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Amheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71: 1501–1506

Schloss PD, Hay AG, Wilson DB, Walker LP (2003) Tracking temporal changes of bacterial community fingerprints during the initial stages of composting. FEMS Microbiol Ecol 46:1–9

Schneegurt MA, Dore SY, Kulpa CF Jr (2003) Direct extraction of DNA from soils for studies in microbial ecology. Curr Issues Mol Biol 5:1–8

Semenov AM, van Bruggen AHC, Zelenev VV (1999) Moving waves of bacterial populations and total organic carbon along roots of wheat. Microb Ecol 37:116–128

Singleton DR, Furlong MA, Rathbun SL, Whitman WB (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl Environ Microbiol 67: 4374–4376

Small J, Call DR, Brockman FJ, Straub TM, Chandler DP (2001) Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. Appl Environ Microbiol 67:4708–4716

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751

Stach JEM, Bathe S, Clapp JP, Burns RG (2001) PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. FEMS Microbiol Ecol 36:139–151

Steger K, Jarvis Å, Smårs S, Sundh I (2003) Comparison of signature lipid methods to determine microbial community structure in compost. J Microbiol Methods 55:371–382

Steger K, Jarvis A, Vasara T, Romantschuk M, Sundh I (2007) Effects of differing temperature management on development of Actinobacteria populations during composting. Res Microbiol 158:617–624

Stokes HW, Holmes AJ, Nield BS, Holley MP, Nevalainen KMH, Mabbutt BC, Gillings MR (2001) Gene cassette PCR: sequence independent recovery of entire genes from environmental DNA. Appl Environ Microbiol 67:5240–5246

Stralis-Pavese N, Sessitsch A, Weilharter A, Reichenauer T, Riesing J, Csontos J, Murrell JC, Bodrossy L (2004) Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers. Environ Microbiol 6:347–363

Strom PF (1985a) Effect of temperature on bacterial species diversity in thermophilic solid-waste composting. Appl Environ Microbiol 50:899–905

Strom PF (1985b) Identification of thermophilic bacteria in solid-waste composting. Appl Environ Microbiol 50:906–913

Takaku H, Kodaira S, Kimoto A, Nashimoto M, Takagi M (2006) Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and -independent approaches. J Biosci Bioeng 101:42–50

Tebbe CC, Vahjen W (1993) Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant-DNA from bacteria and yeast. Appl Environ Microbiol 59:2657–2665

Thummes K, Kämpfer P, Jäckel U (2007) Temporal change of composition and potential activity of the thermophilic archaeal community during the composting of organic material. Syst Appl Microbiol 30:418–429

Tiquia SM, Lloyd J, Herms DA, Hoitink HAJ, Michel FC Jr (2002a) Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes. Appl Soil Ecol 21: 31–48

Tiquia SM, Wan JHC, Tam NFY (2002b) Microbial population dynamics and enzyme activities during composting. Compost Sci Util 10:150–161

Toal ME, Yeomans C, Killham K, Meharg AA (2000) A review of rhizosphere carbon flow modelling. Plant Soil 222:263–281

Treonis AM, Ostle NJ, Stott AW, Primrose R, Grayston SJ, Ineson P (2004) Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biol Biochem 36:533–537

Tsai YL, Olson BH (1992) Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Appl Environ Microbiol 58:2292–2295

van Elsas JD, Costa R, Jansson J, Sjöling S, Bailey M, Nalin R, Vogel TM, van Overbeek L (2008) The metagenomics of disease-suppressive soils – experiences from the METACONTROL project. Trends Biotechnol 26:591–601

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267

Watanabe K, Kodama Y, Harayama S (2001) Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. J Microbiol Methods 44:253–262

Weisskopf L, Fromin N, Tomasi N, Aragno M, Martinoia E (2005) Secretion activity of white lupin's cluster roots influences bacterial abundance, function and community structure. Plant Soil 268:181–194

Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Whipps JM (1990) Carbon economy. In: Lynch JM (ed) The rhizosphere. John Wiley, New York, pp 59–97

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wieland G, Neumann R, Backhaus H (2001) Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development. Appl Environ Microbiol 67:5849–5854

Wintzingerode FV, Gobel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21: 213–229

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Young CC, Kampfer P, Shen FT, Lai WA, Arun AB (2005) *Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). Int J Syst Evol Microbiol 55:423–426

Yu Y, Breitbar M, McNairnie P, Rohwer F (2006) FastGroupII: a web-based bioinformatics platform for analyses of large 16S rDNA libraries. BMC Bioinformatics 7:57

Zhang W, Han DY, Dick WA, Davis KR, Hoitink HAJ (1998) Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Photopathology 88:445–450

Zmora-Nahum S, Danon M, Hadar Y, Chen Y (2008) Chemical properties of compost extracts inhibitory to germination of *Sclerotium rolfsii*. Soil Biol Biochem 40:2523–2529

Chapter 13
Soil Degradation and Rehabilitation: Microorganisms and Functionality

F. Bastida, T. Hernandez, and C. Garcia

Abstract The maintenance of soil quality is critical for ensuring the sustainability of the environment and biosphere. However, soil degradation and desertification affect many areas of the planet, particularly the Mediterranean area where the climatological and lithological conditions, together with anthropological activity, are responsible for increasing desertification. It is, therefore, considered to be of paramount importance to be able to measure soil degradation quantitatively. In this chapter, the key role that microorganisms play in the maintenance of soil structure and functionality, as well as their great sensitivity to soil changes, which make microbiological parameters the most suitable ones for elaborating soil quality indices is highlighted. Short- and long-term experiments of soil rehabilitation carried out in semiarid areas by the addition of organic amendments are also discussed in this chapter.

Contents

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13.1 Soil Quality

When referring to air or water, the term "quality" usually involves the analysis of specific contaminants which are known to have well-defined threshold values. There is no need to specify dynamic, chemical, physical and biological properties that would define an ideal state for which there are an almost limitless number of environmental scenarios, as would be expected when referring to soil quality.

A variety of definitions were proposed for the term soil quality during the 1990s (Parr et al. 1992; Doran and Parkin 1994), ranging from a purely agricultural point of view to a more environmental perspective. In 1995, the Soil Science Society of America (SSSA) suggested soil quality as *the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal production, maintain or enhance water quality and support human health and habitation*. From that moment, soil quality emerged as a branch of environmental studies since soil is a critically important component of the Earth's biosphere. However, nowadays, the term continues to lack definition, and individual investigators feel free to give it whatever meaning suits their research at any moment – not that the meaning will necessarily be incorrect – on an arbitrary basis, including those parameters of interest for each scientist. Indeed, Bouma et al. (1998) suggested that emphasis of soil research is still on a disciplinary rather than on an interdisciplinary approach, and a recommendation was made to continue the effort to expand soil research into a more interdisciplinary domain enabling to develop the concept of soil quality in a holistic way.

However, despite the difficulty involved in providing a definition, the maintenance of soil quality is critical for ensuring the sustainability of the environment and the biosphere, though this is a complex theme because of the importance of climate, soil, plants, anthropic factors and their interactions. Indeed, soils are subject to natural or environmental degradation, often accompanied by erosion, even without human intervention (Popp et al. 2000).

Soil quality and its degradation depend on a large number of physical, chemical and biological properties, of which the last two are the most sensitive since they respond rapidly to changes that are directly linked to soil functionality (Trasar-Cepeda et al. 1998; Ros et al. 2002). The microbial activity of a soil directly influences the ecosystem stability and fertility, and it is widely accepted that a good level of microbiological activity is essential for maintaining soil quality. However, it should be remembered that soil microbiological activity is a mosaic of metabolic processes that cannot be assessed from a single parameter, but only

from a variety of parameters (Nannipieri et al. 1990). Some fractions of the organic matter, for example, water-soluble carbon (WSC), are easily degraded by microorganisms since they act as energy source, and so it is useful to study them when analysing the soil's microbial activity (Garcia et al. 2000; Pascual et al. 2000). Microbial biomass carbon is useful for evaluating microbial population size and this parameter has been considered in the evaluation of natural as well as degraded systems (Ross et al. 1982), whereas microbial activity can be evaluated by parameters like ATP and respiration (Nannipieri et al. 1990). Enzymatic activities are biological catalysts of essential processes in the life cycle of microorganisms, and the simultaneous measurement of several of these activities may be useful when studying the level of bioactivity in a soil (Nannipieri et al. 1990). Among these activities are those related with the N (urease and BAA-protease), P (phosphatase) and C (β-glucosidase) cycles. Other enzymatic activities provide a more general knowledge of microbiological activity. For example, Garcia et al. (1994) showed that dehydrogenase activity is a clear indicator of the microbiological status of soils in semiarid zones subject to degradation and desertification.

However, as soil microorganisms are influenced by the surrounding microenvironment, only one of these parameters is not enough to assess the soil quality status. In the past years, there has been a tendency to "quantify" soil quality and degradation by means of soil quality indices (Andrews et al. 2002; Sharma et al. 2005; Bastida et al. 2006b, 2008a; Zornoza et al. 2007). *A soil quality index could be defined as the minimum set of parameters that, when interrelated, provides numerical data on the capacity of a soil to carry out one or more functions. A soil quality indicator is a measurable property that influences the capacity of a soil to carry out a given function* (Acton and Padbury 1993). All studies on soil quality indices point to the complexity of the subject since a diversity of physical, chemical, microbiological and biochemical properties need to be integrated to establish such quality.

13.2 Soil Degradation in Semiarid Areas

Desertification is the degradation of soils in arid, semiarid and dry subhumid areas, resulting from several factors, including climatic change and human activity (Article 1, UN Convention of the fight against desertification). This process is of global importance since it affects about 40% of continental areas. Soil degradation is a serious problem because the soil fulfils several vital functions: (i) it is the growth medium of plants, acting as a physical support and reservoir of water and essential nutrients; (ii) it regulates the flow of water in the environment and (iii) it has a certain capacity to attenuate the harmful effects of contaminants by its physical, chemical and biological processes.

The real scenario that we find nowadays is that there is a clear trend to desertification and degradation along the world because of climatic factors and human actions such as contamination, inadequate agricultural practices, etc.

This world soil state is reflected in the global desertification vulnerability map established by the United States Department of Agriculture which shows wide areas with a high and very high risk for desertification, corresponding to areas with very low organic carbon content. Thus, the main point to note is that the organic matter losses are responsible for a great part of the soil degradation processes over the world.

Under natural conditions, soils are in a steady state when their pedogenetic factors are balanced. However, this equilibrium is easily upset by human activity (e.g. agricultural abandonment) and this effect is aggravated in semiarid regions by climatic conditions. Under such conditions, the scarcity of water limits vegetation and, consequently, the input of organic matter to soil (Garcia et al. 1997), triggering in many cases degradation (Bastida et al. 2006a). Therefore, the increase of the organic matter content of soils is one approach to improve the quality of degraded soils (Garcia et al. 1998). In semiarid areas, where soil organic matter content is scarce, the increase of the organic matter content may play a key role on the establishment of a vegetal cover.

13.3 Microbial Activity and Soil Degradation

It is well known that soil organisms, particularly the microbiota, play an essential role in the cycling of elements and stabilisation of soil structure. The mineralization of organic matter is carried out by a large community of microorganisms and involves a wide range of metabolic processes. For this reason, it is important to relate ecosystem structure and function to genetic and functional diversity. Microbial functional diversity is related both to the use or not of specific substrates and to the rates of substrate utilisation.

To study biological processes in soils, various parameters have been used. Because of the complex dynamic of soil ecosystems, no single parameter is satisfactory. The search for indicators, which can be used as quantitative tools to assess the health of the soil, has thus become a major challenge for both scientists and land managers. Indicators need to be robust and meaningful, and easy to measure and interpret. To date, emphasis has been given to physical and chemical soil properties which are generally regarded as more difficult to measure, predict or quantify. However, biological processes are intimately linked with the maintenance of soil structure and fertility and are potentially more sensitive to changes in the soil than indicators based on physical and chemical properties (Nannipieri et al. 1990; Brookes 1995). Biological indicators, therefore, may provide an early warning of system collapse and allow us react before irreversible damage occurs. However, problems such as the inherent temporal and spatial heterogeneity of soil biological communities and the unpredictable interaction of soil organisms with the climatic factors, can limit the use of these indicators.

13.4 Indices for Soil Quality Based on Microbial Parameters

Microbial functional diversity depends on many metabolic reactions and interactions of microbiota and for this reason can be determined by measuring simultaneously a range of microbial and biochemical parameters (Nannipieri et al. 1990). In theory, an index integrating the enzyme activities that catalyse the reactions limiting the rate of the main metabolic processes could be used to measure microbial functional diversity (Nannipieri et al. 1990). The selection of the enzyme activities can be restricted to those involved in a particular process, such as the degradation of organic matter, N mineralization or nitrification.

Many researchers have proposed the use of simple indices of soil quality based on a few parameters. Probably, the most straightforward index used in the literature is the metabolic quotient (qCO_2; respiration to microbial biomass ratio), which has been widely used to asses changes in soil microbial activity because of heavy metal contamination (Liao and Xiao 2007), deforestation (Bastida et al. 2006a), temperature (Joergensen et al. 1990) or changes in soil management practices (Dilly et al. 2003). However, the index has also been criticised for its insensitivity to certain disturbances and to the ecosystem's development whenever stress increases along successional gradients (Wardle and Ghani 1995).

The percentage of organic C present as microbial biomass C has been proposed as a more sensitive index of soil changes than total organic C, since the microbial biomass of a soil responds more rapidly to changes than organic matter (Anderson and Domsch 1990). Nevertheless, changes in this ratio because of different manuring or cropping practices can be masked by climatic changes (Insam and Haselwandter 1989).

The metabolic ratio of Masciandaro et al. (1998), calculated dividing the dehydrogenase activity by the amount of water-soluble C, relates the potential sources of C available in soil for microbial metabolism to microbial activity. This ratio was initially used to get quantitative information about soil degradation due to the intensive soil use with a higher organic matter mineralization in cultivated than in undisturbed soils.

Other biochemical and microbiological indices, such as the biological index of fertility (BIF; Stefanic et al. 1984) which offers information on soil fertility, use two enzyme activities (dehydrogenase and catalase). Others, such as the enzymatic activity number (EAN; Beck 1984), take into consideration more enzyme activities (dehydrogenase, phosphatase, protease and amylase activities); however, this index considers amylase instead of cellulase activity and it has been seriously criticised because cellulose is more prominent than starch in vegetal residues (Kang et al. 2005).

Although they are easy to apply, the use of two parameters in a soil quality index has almost the same limitations as the use of one parameter: the lack of information. Therefore, to obtain indices that provide and integrate more information on the quality of a soil multiparametric indices have been developed for agro-ecosystems and for non-agricultural soils.

Trasar-Cepeda et al. (1998) chose three Umbrisols covered by climax vegetation in Galicia (NE Spain), analyzed during a year (one sample per month) and established an equation to define the total N from several microbial parameters (microbial biomass C, mineralized N, and phosphatase, β-glucosidase and urease activities). From the resulting equation, it can be concluded that there was a relationship between total N and several biochemical parameters. This model points to the closeness to an ideal quality level of the chosen soils, for which they used the quotient between the N estimated by the model and the total N calculated by the Kjedahl method.

By using the same approach followed by Trasar-Cepeda et al. (1998), Armas et al. (2007) calculated biological quality index for volcanic Andisols and Aridisols from Canary Island (Spain). Ten experimental plots were established in arid coastal, humid midland and xeric highland areas, both at mature and degraded stages. Sampling was carried out seasonally during one year. The expression obtained by Armas et al. (2007) related total C to different enzyme activities and hot-water-soluble carbon: Total C = –2.924 + 0.037 × extC – 0.096 × cellulase + 0.081 × dehydrogenase + 0.009 × respiration, where: extC (hot water soluble C) is expressed in g C m^{-1}, cellulase activity is expressed in mmol glucose m^{-2} h^{-1}, dehydrogenase activity in mmol INTF m^{-2} h^{-1}, and respiration in mg CO_2-C m^{-2} h^{-1}. The ratio between the values predicted for this model and the measured total C values were considered the soil biological quality index. Values close to 1 were observed for climax soils, while lower values were detected when vegetation differed from climax conditions.

In four different forest sites under semiarid conditions in Spain, Zornoza et al. (2007) found two equations by multilineal regression systems for evaluating the environmental quality of mollisols, i.e. mature soils under natural vegetation with minimum human disturbance and entisols, i.e. young soils similar to the lithological material. Thirty soil samples were taken for each forest site within an area of 5 km^2. The equation established for mollisols related total N content to different enzyme activity (phosphatase, urease and β-glucosidase activity), microbial biomass C, available P and water-holding capacity; while the equation for entisols related soil organic C content to available P and different enzyme activities (urease and β-glucosidase activity). Results confirmed that these models accurately expressed deviations from natural equilibrium in undisturbed soils.

Bastida et al. (2006b) used the approach by Andrews et al. (2002) to calculate the microbiological degradation index (MDI) of natural soils under semiarid climate (Murcia, SE Spain), where the climatic conditions may accelerate soil degradation. Samples were taken during one year in eleven different areas and under different type of plant cover, from *Pinus halepensis* Miller to shrublands based on xerophytic species. This index suggests the use of biochemical and carbon-related parameters to set up a soil quality index according to the following steps: (i) selection of appropriate parameters, (ii) transformation and weighing of values and (iii) combining the scores into an index. The polynomial relationship for calculating the MDI is:

$$\begin{aligned}\mathrm{MDI} = {} & [0.89(1/(1+(a/4.87)^{-2.5})] + [0.86(1/(1+(b/11.09)^{-2.5})] \\ & + [0.84(1/(1+(c/1.79)^{-2.5})] + [0.75(1/(1+(d/95.03)^{-25})] \\ & + [0.72(1/(1+(e/18.01)^{-25})],\end{aligned}$$

where a is the dehydrogenase activity in μg INTF g^{-1} h^{-1}, b is the value of water-soluble carbohydrates in mg kg^{-1}, c is the urease activity in μmol NH_3 g^{-1} h^{-1}, d is the value of water soluble C in mg kg^{-1} and e is the respiration in mg CO_2-C kg^{-1} soil.

Different levels of degradation (null, low, high or very high) were established for this index, so as to have a threshold value below which actions should be taken to counter possible negative effects that may lead to desertification in a semiarid climate.

13.5 Soil Rehabilitation by Applying Organic Amendments. A Case of Study in Semiarid Areas

As commented in the previous sections, soil degradation can occur in semiarid areas because of the soil organic matter loss and the application of organic amendments from municipal wastes to semiarid soils can be a suitable biotechnological management to restore soil quality in these areas. Changes in microbial activity and in microbial community structure have been observed after short- and long-term application of these materials (Bastida et al. 2007, 2008a,b) and these changes should be considered for soil rehabilitation and restoration of these soils.

13.5.1 Short-Term Effects of Organic Amendments in Semiarid Areas: Effects on Soil Microbial Community Size, Activity and Structure

Applying organic amendments to soil can increase its total organic carbon content and fractions, and can positively affect both microbial growth and microbial activity of soils (Ros et al. 2003; Tejada et al. 2006). In general, such amendments improve microbial growth and activity (Chap. 6, Ceustermans et al. 2010; Chap. 11, Fuchs 2010). However, the composition of these materials is important for the effect on the composition of soil microbiota. Indeed, changes in the composition of microbial communities as a result of incorporating inorganic or organic amendments have been observed in soil (Marschner et al. 2003). The phospholipid fatty acids profile (PLFAs) can give quantitative information about community structure (Ebersberger et al. 2004) and is sensitive to the soil type and management (Bossio and Scow 1998), heavy metal contamination (Frostegard et al. 1993),

vegetal composition (Tscherko et al. 2005) and organic amendments (Petersen et al. 2003).

Despite the extensive bibliography on the effects of organic amendments on the recovery of degraded soils (Pascual et al. 1999; Ros et al. 2003), the effects of organic matter on the size, composition and activity of the soil microbial communities has been poorly studied (but see Ros et al. 2006; Chap. 12, Minz et al. 2010; Chap. 14, Knapp et al. 2010). We postulated that a single amendment can stimulate soil microbial growth and activity and can promote vegetation cover because of the huge amount of nutrients and carbon added to soil.

In experimental plots, located in Murcia (Southeast Spain) in an area greatly affected by soil degradation processes, to which 12 kg m^2 of fresh or composted sewage sludge were added, a higher plant cover was recorded compared to unamended soils, as well as a higher microbial biomass and higher overall and specific enzyme activities, suggesting that the large amount of nutrients provided by these organic materials favours the increases on carbon fractions, microbial activity and microbial growth (Borken et al. 2002; Ros et al. 2003). The presence of a stable plant cover is important for improving the input of C and for the development of soil microbial communities, both of them contributing to the improvement of soil fertility and avoiding soil erosion processes (Garcia et al. 2002; Tejada et al. 2006).

Hydrolase activities are indicators of microbial activity related to the cycles of elements and their general increase in the amended soils with respect to the control may be due to the increase in the microbial biomass, since these enzymes are synthetised by soil microorganisms (Liang et al. 2005; Tejada et al. 2006).

As already mentioned, organic matter decline in soil is of particular concern in Mediterranean areas and for this reason research has been mainly focused on organic C dynamics. Conversely, N has received less attention despite being a limiting nutrient crucial to plant survival in semiarid regions. Nitrification is a two-step process which involves the transformation of ammonia to nitrite and the oxidation of nitrite to nitrate (Innerebner et al. 2006). The first step is considered the bottleneck of N cycle in soil, since only a limited number of bacteria and archaea are able to perform this reaction (Leininger et al. 2006). As the turnover rates of ammonia oxidising in archaea are much lower than those of ammonia oxidising in bacteria (Könneke et al. 2005), we decided to focus on the bacterial amoA gene. This gene encodes a subunit of the ammonia oxygenase (the subunit carrying the active site) which catalyses the oxygenation of ammonia to hydroxylamine. We found a higher number of amoA copies per gram of soil and hence of potential ammonia oxidizers in amended compared to control soils (Fig. 13.1). Probably, the higher amount of ammonia in amended than untreated soils stimulated ammonia oxidisers.

Denitrification is a respiratory process by which oxidised N compounds are used as alternative electron acceptors for energy production when oxygen is limited. It is the most important process by which fixed N returns to the atmosphere in the form of N_2 from soil and water, thus completing the N cycle. The process includes several reactions, each of them controlled by various environmental factors, of which the most important are oxygen limitation, availability of NO_3^- and organic C (Mahmood et al. 2005; Dambreville et al. 2006). Genes involved in denitrification,

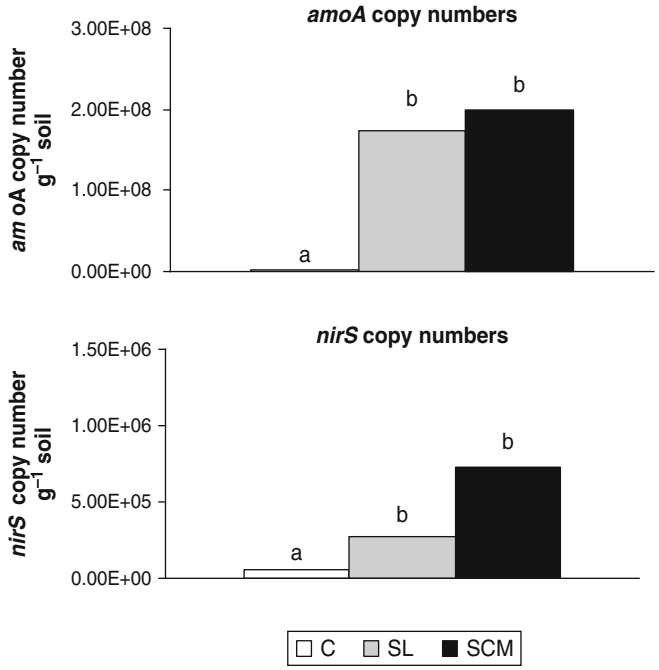

Fig. 13.1 *amoA* and *nirS* gene copy numbers in control and amended soils during the experiment. Bars with the same letter are not significantly different according to the LSD test ($P \leq 0.05$)

such as nitrate reductase (*narG* and *napA*), nitrite reductases (*nirS* and *nirK*) and nitrous oxide reductase (*nosZ*), contain highly conserved DNA regions which have been successfully exploited for developing gene probes. The conversion of nitrite to nitric oxide (or nitrous oxide) is the limiting reaction of denitrification and the reaction product is a gaseous compound (Sharma et al. 2005). Denitrifying bacteria possess either a cytochrome cd_1 (cd_1 NIR) encoded by nirS or a Cu-containing enzyme (Cu NIR) encoded by nirK to perform this reaction. In our semiarid soils, nirK only occurred in low numbers ($<10^5$ copies per gram soil dry weight), which was not affected by the amendments described above. In contrast, nirS showed a significant response to the incorporation of amendments (Fig. 13.1) and was detected in larger amounts than nirK (Bastida et al. 2009). These results contrast with those of other authors, who could amplify successfully nirK from soil samples but not nirS (Wolsing and Priemé 2004). It is difficult to explain these differences since current knowledge of the environmental response of nirS- and nirK-containing denitrifying bacteria is very limited because of the difficulties to culture most of these organisms. Apart from the fact that organic amendments enriched the soil with a potential positive effect on soil denitrifiers, the direct incorporation of denitrifiers with the amendments has been proposed by You (2005), but not by Saison et al. (2006), because the survival of exogenous microbial populations in these soils could be constrained by edaphoclimatic properties.

The effect of different organic amendments on soil microbial communities was evaluated by measuring their PLFA profiles. The organic amendments stimulated both bacterial and fungal proliferation, as observed by Marschner et al. (2003), because of the nutrient inputs by the organic amendments. After a primary succession, an increase in fungal biomass is generally observed because of increases in organic matter content or C/N ratio (Wardle et al. 2004). After the rapid fungal proliferation, the fungal biomass is stabilised (secondary succession) and this can be due to changes in pH, N content and both quantity and quality of organic matter (Van der Wal et al. 2006). In this sense, the different chemical nature of sludge and compost can be responsible for the higher proliferation of fungi in the sludge treatment with respect to the compost-amended soil two years after amendment (Bastida et al. 2008b).

The ratio between fatty acid 16:1ω5, an indicator of mycorrhizal biomass (Gormsen et al. 2004), and fungal biomass can provide information on the variation of the mycorrhizal communities with respect to the total fungal community of the soil. Compost amendment increased the presence of mycorrhizal communities with respect to the addition of the fresh sewage sludge, probably as a result of the nature of the organic matter incorporated with the compost (Fig. 13.2). Although most soil microorganisms are thought to be C limited, a significant group, the mycorrhizal fungi, does not experience C limitation unless their plant hosts decrease the below ground C allocation in response to high N availability and/or low light intensity, defoliation and other stresses (Högberg et al. 2007). Because of the dependence on recent photosynthate, it is very likely that mycorrhizal fungi are sensitive to responses of their plant hosts to variations in N supply; indeed, it has been postulated that N loading decreases the C supply to mycorrhizal fungi (Högberg et al. 2007). In our study, a lower mycorrhizal to total fungi ratio was observed in sludge-amended versus compost-amended soil which could be related to the lowest C/N ratio found after the sludge treatment. It is probable that, once symbiotic fungi have diminished their community size, non-mycorrhizal fungi may have competitive advantages for their development.

The Gram-positive/Gram-negative ratio pointed to the preferential development of the Gram + bacteria in the compost and sludge-amended soils compared to the control. The bigger development of this group may be interpreted as a shift from chemolithotrophic microbial communities, many of them Gram-negative (Schlegel 1992) towards a heterotrophic community with the increase in C (Tscherko et al. 2004). Many of these Gram-positive bacteria have a capacity to sporulate, for example Actinomycetes, *Bacillus*, *Clostridium*, etc. (Schlegel 1992). This is unsurprising in Mediterranean soils since the environmental conditions would favour the presence of the most resistant bacteria, the endosporulating ones.

It can be concluded that the addition of organic amendments can increase fungal and bacterial biomass and their activity, as well as the different carbon fractions. The growth of bacteria and fungi was parallel for both amendments, but the growth of Gram-positive bacteria was higher than that of Gram-negative bacteria in both the amended soils. It is not only to consider the changes in the content of organic C, microbial biomass and microbial activity but also the changes in the structure of

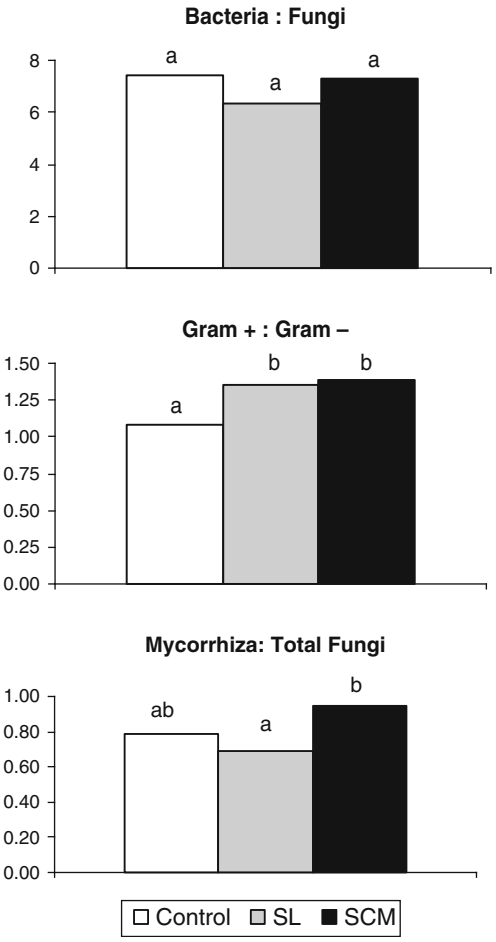

Fig. 13.2 Bacteria:Fungi, Gram-positive: Gram-negative, and mycorrhiza PLFA: total Fungi ratios in control and amended soils. For each ratio, bars followed by the same letter are not significantly different according to the LSD test ($P \leq 0.05$)

microbial and plant community (e.g. decrease in plant diversity). The chemical composition of sewage sludge is an important issue when developing logical recommendations for the application rates of sludge to agricultural soils and minimising the risks of groundwater contamination, odour, etc. (Singh and Agrawal 2008). The possible emergence of pathogens from sludge and the presence of higher levels of heavy metals in this fresh material (Singh and Agrawal 2008) could limit the use of sludges in agriculture. However, for restoration purposes, a single dose of 12 kg m^{-2} was not negative for soil microbial populations and vegetal development (Bastida et al. 2008b).

In addition, despite the general absence of differences in activity indicators between compost- and sludge-amended soils, the longer-lasting effect of compost carbon compounds could be related to long-term positive effects on microbial properties and even on soil physical structure. Both the emergence of pathogens

in sludges and the carbon nature of compost (subject to a stabilization process) lead us to conclude that compost could be preferred for soil restoration when compared to sludge. Both materials are useful for stimulating microbial growth and microbial activity, thus promoting soil restoration in semiarid areas, but long-term experiments are necessary to assess the long-term effects of these materials in semiarid regions.

13.5.2 Long-Term Effects of Organic Amendments in Semiarid Areas: Effects on Soil Microbial Community Activity and Structure

Despite increasing understanding of the links between gene expression and process at the microscale, there is still much progress to be made when relating these processes at the macroscale (Standing et al. 2007). This fact should be considered both in spatial and temporal terms. Thus, once we have clearly showed that organic amendment application is a way to valorize these organic materials in a short-time scale, we will consider the effects of the application of these materials in a longer period of time. Studies are usually focused on short or medium time scales but the long-term effects on the ecosystem health are poorly known. Here, we will discuss some long-term results in semiarid plots of southeast Spain, concluding the suitability of these applications in a broad scale in the restoration of degraded soils.

In an area of SE Spain, which is greatly affected by degradation processes, experimental plots were amended only once in 1988 with the organic fraction of fresh municipal solid waste (MSW), without composting or grinding, at different rates (65, 130, 195 and 260 tonnes ha^{-1}) (Albaladejo et al. 1994); soils were sampled and monitored 17 years later. Amended soils showed higher C fraction content than unamended soil, which is not surprising since the incorporation of waste 17 years ago has led to the development of a stable plant cover that is still evident today. This vegetation would have led to an increase in carbon inputs as a result of plant remains and root exudates, thus favouring the formation of organic matter (Garcia et al. 1992).

Plant cover can increase the content of organic C in soil, microbial biomass, microbial activity and the formation of humus–enzyme complexes. In fact, the activity of different immobilised enzymes (β-glucosidase, urease and alkaline phosphatase) extracted with 0.1M pH 7 sodium pyrophosphate showed an increased in the amended experimental plots, showing an increase in the amended soils with respect to the unamended soil.

The fact that the 195 tonnes ha^{-1} application rate lead to higher density of plant cover and higher immobilized enzyme activity than the treatment with higher dose rate (260 tonnes ha^{-1}) was one of the most noteworthy findings and suggests that the long-term biochemical and biological response of soil to the addition of organic wastes is linear up to a given threshold value. The lower plant cover observed in the

latter treatment was probably responsible for the lower humic substance C concentration, probably resulting in a reduced enzyme immobilisation in this C fraction. There is a clear match between humic substance C and the enzyme concentration of the extract. Likewise, the addition of MSW to soil did not induce a proportional response of microbial biomass and microbial activity to the dose, and the same occurred for C fractions studied after 17 years. For many of the parameters studied, a threshold level was detected above which the addition of more waste does not increase the measured parameter. This threshold level varied in our study according to the selected parameter; in some cases, it was 130 tonnes ha^{-1} and in others 195 tonnes ha^{-1}. Under the prevailing semiarid conditions of the studied area, the soil may have reached a long-term equilibrium, with respect to both the different carbon fractions and microbial activity. Enhancement of soil quality with addition of MSWs allows the recovery of a stable vegetation cover, which in turn provides organic inputs to soil, positively influencing the microbial growth and activity in soil. The addition of these organic materials to the degraded soil is a valid strategy for soil rehabilitation and counteracts the soil degradation and desertification on a long-term scale (Bastida et al. 2007).

13.6 Metaproteomics of Organic Wastes

In this section, we analyse the prospect of the future soil quality improvement thanks to the use of organic wastes. A further characterization of these materials is needed, for what DNA- and RNA-based methods are now available. It should be noted that these materials are highly rich, not only as regards their high content of nutrients but also their high content and diversity of microorganisms, with a broad enzymatic system that remains undiscovered. For this, the identification of microoganisms and genes or transcripts is not enough to drive the bioremediation approach. Furthermore, it is necessary to characterise biochemical pathways and the presence of important enzymes involved in element cycling and pollutant degradation.

The extraction and identification of proteins of these organic materials is a challenge because their higher organic matter content interferes with many methods (Roberts and Jones 2008). Nevertheless, Wilmes and Bond (2006) showed that the metaproteome analysis of these materials is possible. Metaproteomics can help in finding the microorganisms and enzymes responsible for a given process without the need for previous metagenomic studies, though we should not forget that such studies have permitted the compilation of the databases on which protein identification is based which nowadays remains uncompleted especially for environmental samples (Keller and Hettich 2009).

Metaproteomics development for heterogeneous matrices opens the door for new applications of organic materials. Identification of functional proteins in wastes can be useful for choosing the specific amendment. Indeed, such studies may be more valuable in certain organic wastes, which practically act as microbial

culture media and in which certain microbial species predominate, while some of these enzymes may have a biotechnological, industrial, environmental and/or pharmaceutical value (Schmeisser et al. 2007). In the coming years, metaproteome studies may help to understand the richness of these organic materials, providing a definitively valorization and usefulness as biocatalyzers in soils.

13.7 Conclusions and Final Remarks

The use of organic materials is needed to restore degraded soils since the high organic carbon and nutrient content of these materials have more benefits than the negative effects that they can exert in semiarid ecosystems where soil organic content is very low. In this sense, organic materials can improve the organic carbon content and microbial activity. However, at this stage we should consider that the effects of such applications are dose-dependent and not always an improvement of soil quality is reached by increasing the dose. This fact means that, if restoration programs are made rationally, soil restoration is very cheap and problems related to soil contamination should not be expected when using high-quality organic amendments. Furthermore, the effect of these applications last in time even with only one addition to a semiarid soil. Nevertheless, monitoring amended soils should be carefully made because these practices may suppose an alteration on the ecosystem related to the structure of microbial and plant communities. These changes need to be evaluated in long-term scales since they can affect the biogeochemical cycling in soil. Nevertheless, there are a wide range of soil quality indexes that implement diverse microbiological indicators helping to understand and summarise the response of soil management. These indexes include parameters related to soil microbial biomass and its activity, organic C and nutrients availability. In future years, scientists should be able to use the biological capacity of organic wastes not only as a source of organic matter and nutrients to soil, but also as a source of enzymes. However, efforts at molecular level should be carried out in order to provide enough genome database information, which is the base for proteomic characterization.

References

Acton DF, Padbury GA (1993) A conceptual framework for soil quality assessment and monitoring. In: A program to assess and monitor soil quality in Canada. Soil quality evaluation summary, Res Branch Agric, Ottawa, Canada

Albaladejo J, Stocking M, Díaz E, Castillo V (1994) Land rehabilitation by urban refuse amendments in a semi-arid environment: effect on soil chemical properties. Soil Technol 7:249–260

Anderson TH, Domsch KH (1990) Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from of soils from different cropping histories. Soil Biol Biochem 22:251–255

Andrews SS, Karlen DL, Mitchell JP (2002) A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agric Ecosyst Environ 90:25–45

Armas CM, Santana B, Mora JL, Notario JS, Arbelo CD, Rodríguez-Rodríguez A (2007) A biological quality index for volcanic Andisols and Aridisols (Canary Islands, Spain): variations related to the ecosystem development. Sci Total Environ 378:238–244

Bastida F, Moreno JL, Hernandez T, Garcia C (2006a) Microbiological activity in a soil 15 years alter its devegetation. Soil Biol Biochem 38:2503–2507

Bastida F, Moreno JL, Hernandez T, Garcia C (2006b) Microbiological degradation index of soils in a semiarid climate. Soil Biol Biochem 38:3463–3473

Bastida F, Moreno JL, Garcia C, Hernandez T (2007) Addition of urban waste to semiarid degraded soil: long-term effect. Pedosphere 17:557–567

Bastida F, Zsolnay A, Hernandez T, Garcia C (2008a) Past, present and future of soil quality indices: a biological perspective. Geoderma 147:159–171

Bastida F, Kandeler E, Moreno JL, Ros M, Garcia C, Hernandez T (2008b) Application of fresh and composted wastes modifies structure, size and activity of soil microbial community under semiarid climate. Appl Soil Ecol 40:318–329

Bastida F, Pérez de Mora A, Babic K, Hai B, Hernandez T, Schloter M, Garcia C (2009) Role of amendments on N cycling in Mediterranean abandoned semiarid soils. Appl Soil Ecol 41: 195–205

Beck T (1984) Methods and application of soil microbiological analysis at the Landesanstalt für Bodenkultur und Pflanzenbau (LBB) in Munich for the determination of some aspects of soil fertility. In: Nemes MP, Kiss S, Papacostea P, Stefanic C, Rusan M (eds) Fifth symposium on soil biology, pp 13–20

Borken W, Muhs A, Beese F (2002) Changes in microbial and soil properties following compost treatment of degraded temperate forest soils. Soil Biol Biochem 34:403–412

Bossio DA, Scow KM (1998) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35:265–278

Bouma J, Finke PA, Hoosbeek MR, Breeuwsma A (1998) Soil and water quality at different scales: concepts, challenges, conclusions and recommendations. Nutr Cycl Agroecosyst 50:5–11

Brookes PC (1995) The use of microbial parameters in monitoring soil pollution by heavy-metals. Bio Fertil Soils 19:269–279

Ceustermans A, Coosemans J, Ryckeboer J (2010) Compost microbial activity related to compost stability. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 115–134

Dambreville C, Hénault C, Bizouard F, Morvan T, Chaussod R, Germon JC (2006) Compared effects of long-term pig slurry applications and mineral fetilization on soil denitrification and its end products (N_2O, N_2). Biol Fertil Soils 42:490–500

Dilly O, Blume HP, Sehy U, Jiménez M, Munich JC (2003) Variation of stabilised, microbial and biologically active carbon and nitrogen soil under contrasting land use and agricultural management practices. Chemosphere 52:557–569

Doran JW, Parkin TB (1994) Defining and assessing soil quality. In: Doran JW, Coleman DC, Bezdicek DF, Stewart BA (eds) Defining soil quality for a sustainable environment. SSSA Special Pub 34. Soil Science Society of America, Madison, WI, pp 3–21

Ebersberger D, Werrnbter N, Niklaus P, Kandeler E (2004) Effects of long term CO_2 enrichment on microbial community structure in calcareous grassland. Plant Soil 264:313–323

Frostegard A, Tunlid A, Baath E (1993) Phospholipid fatty-acid composition, biomass, and activity of microbial communities from 2 soils types experimentally exposed to different heavy-metals. Appl Environ Microbiol 59:3606–3617

Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230

Garcia C, Hernandez T, Costa F (1992) Variation in some chemical parameters and organic matter in soils regenerated by the addition of municipal solid-waste. Environ Manage 16:763–768

Garcia C, Hernandez T, Costa F (1994) Microbial activity in soils under Mediterranean environmental conditions. Soil Biol Biochem 26:1185–1191

Garcia C, Roldán A, Hernandez T (1997) Changes in microbial activity after abandonment of cultivation in a semiarid Mediterranean environment. J Environ Qual 26:285–291

Garcia C, Hernandez T, Albaladejo J, Castillo V, Roldán A (1998) Revegetation in semiarid zones: influencing of terracing and organic refuse on microbial activity. Soil Sci Soc Am J 62: 670–676

Garcia C, Hernandez T, Roldán A, Albaladejo J, Castillo V (2000) Organic amendment and mycorrhizal inoculation as a practice in aforestation of soils with *Pinus halepensis* Miller: effect on their microbial activity. Soil Biol Biochem 32:1173–1181

Garcia C, Hernandez T, Roldán A, Martín A (2002) Effect of plant cover decline on chemical microbiological parameters under Mediterranean climate. Soil Biol Biochem 34:635–642

Gormsen D, Olsson PA, Hedlund K (2004) The influence of collembolans and earthworms on AM fungal mycelium. Appl Soil Ecol 27:211–220

Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601

Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidising bacterial in compost-treated soils. Soil Biol Biochem 38:1092–1100

Insam H, Haselwandter K (1989) Metabolic quotient of the soil microflora in relation to plant succession. Oecologia 79:174–178

Joergensen RG, Brookes PC, Jenkinson DS (1990) Survival of the soil microbial biomass at elevated-temperatures. Soil Biol Biochem 22:1129–1136

Kang GS, Beri V, Sidhu BS, Rupela OP (2005) A new index to assess soil quality and sustainability of wheat-based cropping systems. Biol Fertil Soils 41:389–398

Keller M, Hettich R (2009) Environmental proteomics: a paradigm shift in characterizing microbial activities at the molecular level. Microbiol Mol Biol Rev 73:62–70

Knapp B, Ros M, Insam H (2010) Do composts affect the soil microbial community? In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 271–292

Könneke M, Bernhard AE, De la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxiding marine archaeon. Nature 437:543–546

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442: 806–809

Liang YC, Si J, Nikolic M, Peng Y, Chen W, Jiang Y (2005) Organic manure stimulates biological activity and barley growth in soil subject to secondary salinization. Soil Biol Biochem 37:1185–1195

Liao M, Xiao XM (2007) Effect of heavy metals on substrate utilization pattern, biomass, and activity of microbial communities in a reclaimed mining wasteland of red soil area. Ecotox Environ Safe 66:21–223

Mahmood T, Ali R, Malik KA, Aslam Z, Ali S (2005) Seasonal pattern of denitrification under an irrigated wheat-maize cropping system fertilized with urea and farmyard manure in different combinations. Biol Fertil Soils 42:1–9

Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol Biochem 35:453–461

Masciandaro G, Ceccanti B, Gallardo-Lancho JF (1998) Organic matter properties in cultivated versus set-aside arable soils. Agric Ecosyst Environ 67:267–274

Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252

Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of the biological activity in soils. In: Bollag JM, Stotzky G (eds) Soil biochemistry. Marcel Dekker, New York, pp 293–355

Nannipieri P, Kandeler E, Ruggiero P (2002) Enzyme activities and microbiological and biochemical processes in soil. In: Burns RG, Dick RP (eds) Enzymes in the environment. Marcel Dekker, New York, pp 1–33

Parr JF, Papendick RI, Hornik SB, Meyer RE (1992) Soil quality: attributes and relationship to alternative and sustainable agriculture. Am J Alternative Agr 7:5–10

Pascual JA, Garcia C, Hernandez T (1999) Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. Biol Fertil Soils 30:1–6

Pascual JA, Garcia C, Hernandez T, Moreno JL, Ros M (2000) Soil microbial activity as a biomarker of degradation and remediation processes. Soil Biol Biochem 32:1877–1883

Petersen SO, Henriksen K, Mortensen GK, Krogh PH, Brandt KK, Sorensen J, Madsen T, Petersen J, Gron C (2003) Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. Soil Tillage Res 72:139–152

Popp JH, Hyatt DE, Hoag D (2000) Modeling environmental condition with indices: a case study of sustainability and soil resources. Ecol Model 130:131–143

Roberts P, Jones DL (2008) Critical evaluation of methods for determining total protein in soil solution. Soil Biol Biochem 40:1485–1495

Ros M, Hernandez MT, Garcia C (2002) Bioremediation of soil degraded by sewage sludge: effects on soil properties and erosion losses. Environ Manage 31:741–747

Ros M, Hernandez MT, Garcia C (2003) Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biol Biochem 35:463–469

Ros M, Pascual JA, Garcia C, Hernandez MT, Insam H (2006) Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biol Biochem 38:3443–3452

Ross DJK, Tate R, Cairns A, Meyrick KF, Parsic EA (1982) Restoration of pasture after topsoil removal: effect of soil carbon and nitrogen mineralization, microbial biomass and enzyme activities. Soil Biol Biochem 14:575–581

Saison C, Degrange V, Oliver R, Millard P, Commeaux C, Montange D, Le Roux X (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environ Microbiol 8: 247–257

Schlegel HG (1992) General microbiology. Cambridge University Press, Cambridge, UK

Schmeisser C, Steele H, Streit WR (2007) Metagenomics, biotechnology with non-culturable microbes. Appl Environ Microbiol 75:955–962

Sharma KL, Mandal UK, Srnivas K, Vittal KPR, Mandal B, Grace JK, Ramesh V (2005) Long-term soil management effects on crop yields and soil quality in a dryland Alfisol. Soil Tillage Res 83:246–259

Singh RP, Agrawal M (2008) Potential benefits and risks of land application of sewage sludge. Waste Manag 28:347–358

Standing D, Baggs EM, Wattenbach M, Smith P, Killham K (2007) Metting the challenge of scaling up processes in the plant-soil-microbe system. Biol Fertil Soils 44:245–257

Stefanic F, Ellade G, Chirnageanu J (1984) Researches concerning a biological index of soil fertility. In: Nemes MP, Kiss S, Papacostea P, Stefanic C, Rusan M (eds) Fifth Symposium on Soil Biology, pp 35–45

Tejada M, Hernandez MT, Garcia C (2006) Application of two organic amendments on soil restoration: effects on the soil biological properties. J Environ Qual 35:1010–1017

Trasar-Cepeda C, Leirós C, Gil-Sotres F, Seoane S (1998) Towards a biochemical quality index for soils: an expression relating several biological and biochemical properties. Biol Fertil Soils 26:100–106

Tscherko D, Hammesfahr U, Marx MC, Kandeler E (2004) Shifts in rizosphere microbial communities and enzyme activity of *Poa alpine* across an alpine chronosequence. Soil Biol Biochem 36:1685–1698

Tscherko D, Hammesfahr U, Zeltner G, Kandeler E, Bocker R (2005) Plant succession and rizosphere microbial communities in a recently deglaciated alpine terrain. Basic Appl Ecol 6:367–383

Van der Wal A, van Veen JA, Smant W, Boschker HTS, Bloem J, Kardol P, van der Putten WH, de Boer W (2006) Fungal biomass development in a chronosequence of land abandonment. Soil Biol Biochem 38:51–60

Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient qCO_2 as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem 27:1601–1610

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystems properties and forest decline in contrasting long-term chronosequences. Science 305:509–5512

Wilmes P, Bond PL (2006) Metaproteomics: studying functional gene expression in microbial ecosystems. Trends Microbiol 14:92–97

Wolsing M, Priemé A (2004) Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of *nir* gene fragments. FEMS Microbiol Ecol 48:261–271

You SJ (2005) Identification of denitrifying bacteria diversity in an activated sludge system by using nitrite reductase genes. Biotechnol Lett 27:1477–1482

Zornoza R, Mataix-Solera J, Guerrero C, Arcenegui V, Garcia-Orenes F, Mataix-Beneyto J, Morugán A (2007) Evaluation of soil quality using multiple linear regression based on physical, chemical and biochemical properties. Sci Total Environ 378:233–237

Chapter 14
Do Composts Affect the Soil Microbial Community?

Brigitte A. Knapp, Margarita Ros, and Heribert Insam

Abstract Compost amendments have been shown to provide manifold benefits, as long as compost of good quality is used and care is taken not to accumulate heavy metals or organic pollutants as a consequence of repeated applications. Among the advantages of compost as soil amendment is its potential to maintain soil organic matter, foster nutrient availability, suppress plant diseases and increase soil microbial abundance and activity, thus enhancing soil quality and fertility. However, only little is known about how compost amendments act as microbial inoculum to the soil and if the compost-borne microflora leaves a long-term imprint on soil microbial communities. In this chapter, it will be analysed if and to what extent soil microbial biomass, activity and community structure are affected by compost amendments. A long-term field study, in which four different composts have been applied annually since 1991, will be presented in detail.

Contents

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14.1 Introduction

The biological process of composting involves the complete or partial degradation of organic materials by a consortium of microorganisms, the composition of which changes as the composting process progresses (Ryckeboer et al. 2003). In recent years, the transformation of organic waste (e.g. municipal and industrial organic waste, green waste, animal manure and sewage sludge) to compost has become increasingly popular across Europe. Composting decreases the amount of waste being deposited, and, by the application of the mature compost to agricultural soils, reduces the use of artificial fertilisers (Hüttl and Fussy 2001). Amendment of high-quality composts to soil is considered to be an agronomically advantageous practice, using a natural recycling product that is able to enhance soil fertility and productivity. The effects of compost application to agricultural soils range from an increase in nutrient availability (mainly N and P) and soil organic matter content (Garcia-Gil et al. 2000) to the suppression of pathogens (see Chap. 8, de Bertoldi 2010; Chap. 11, Fuchs 2010) and changes in the composition and activity of soil microorganisms (Ros et al. 2003). Although microbial activity and succession during composting have been analysed in various studies (e.g. Kowalchuk et al. 1999; Alfreider et al. 2002; Ryckeboer et al. 2003), little is known about how compost amendments act as microbial inoculum to the soil and if the compost-borne microflora leaves an imprint on soil microbial communities in the long-term (see Chap. 13, Bastida et al. 2010; Chap. 12, Minz et al. 2010).

In this chapter, whether and to what extent microbial abundance, activity and community structure are affected by the amendment of composts to soils will be analysed. Furthermore, a selected long-term field study, in which four different composts as well as combined mineral fertiliser and compost treatments were applied annually for more than 15 years, will be presented in detail.

14.2 Compost as Biological Fertiliser

Composting is an aerobic process, during which a mixture of organic substrates is degraded by a diverse microbial community, transferring the organic matter into a stabilised end-product (Insam and de Bertoldi 2007) and reducing human, animal

and plant pathogens (see Chap. 9, Vinnerås et al. 2010) as well as phytotoxins (Beffa et al. 1995). Various microbial groups are, thereby, involved, changing in abundance and composition during the composting process (see Chap. 6, Ceustermans et al. 2010; Chap. 1, Insam et al. 2010). At the beginning, different species of yeasts and mesophilic bacteria dominate, utilising soluble substances such as sugars, amino acids and fatty acids. As a consequence of microbial activity, the temperature rises and the mesophilic microorganisms are inactivated and replaced by a wide range of thermotolerant and thermophilic organisms (Ryckeboer et al. 2003). The hot rotting phase may last a few days to several weeks or even months, depending on the source material used, and temperatures up to 80°C are reached. In this stage, organic material is actively degraded by bacteria, whereas fungi are inhibited by temperatures exceeding 60°C (Beffa et al. 1995). Good management is required during this stage, since heat may inactivate enzymes or limit oxygen supply and thus cause inhibitory effects on the composting process (Ryckeboer et al. 2003). When the easily available organic matter is metabolised, temperature decreases and mesophilic bacteria and fungi recolonise the substrates, starting to degrade the more recalcitrant organic compounds (Beffa et al. 1995). Moreover, this last step of the composting process, the maturation or curing phase, is characterised by the humification of ligno-cellulosic compounds, leading to stabilised organic matter as an end-product (Insam and de Bertoldi 2007).

In previous studies, microbes characteristic for the individual stages of composting were described (Ryckeboer et al. 2003); however, owing to the inhomogeneity of compost (different nutrients, oxygen, water and pH), the diversity of microorganisms can vary considerably between different niches. As compost is produced from starting materials of different quality and is affected by temperature, pH, nutrient content, O_2 supply, moisture content, turning frequency and other factors (Diaz and Savage 2007), process parameters and characteristics of the end-product differ widely (Dimambro et al. 2007). If compost is inadequately processed, it can contain animal and plant pathogens as well as toxic compounds, which may cause damage to crop plants upon application (Chaps. 6 and 9, Fuchs et al. 2006). Different parameters have been proposed for assessing the maturity and stability of composts, and include physico-chemical properties, phytotoxicity tests (Ranalli et al. 2001) as well as microbiological parameters such as microbial respiration, ATP content, microbial counts, enzyme activity (Tiquia 2005) and community level physiological profiles (Belete et al. 2001).

14.3 The Use of Compost as Soil Amendment

In the twentieth century, the concept of sustainable agriculture has contributed to the popularity of composting, thereby making use of this technique's potential for closing nutrient cycles (Tittarelli et al. 2007), restoring organic matter to the soil (Marmo 2008), and at the same time reducing the application of artificial fertilisers and pesticides (Hargreaves et al. 2008). Political initiatives aiming at a reduction of

the landfill of bio-waste have furthermore enforced the conversion of the degradable fraction of solid wastes to compost. The European Union addressed this issue in 1999, releasing the Council directive 1999/31/EC on the landfill of waste, in which goals targeting the stepwise reduction of biodegradable municipal waste being deposited in landfills were clearly defined. As a consequence of this measure, methane emissions from landfills are expected to decrease considerably (Marmo 2008). With the increasing interest in compost, research activities towards understanding this complex process have been intensified, as only a profound knowledge of composting and the use of compost as soil amendment guarantees an efficient and safe use in agriculture.

The virtue of composts for agricultural and horticultural use has been shown previously in numerous studies. Benefits include

- compost helps to increase soil organic matter levels and renders soils a sink for atmospheric CO_2, thus contributing to the goals of the Kyoto Protocol (Marmo 2008)
- compost increases the soil sorption capacity for pollutants such as heavy metals on the one side as well as water and nutrients on the other (Traulsen et al. 1997)
- compost treatment is known to exert a positive effect on soil physical properties (increased porosity and aggregate stability; Hüttl and Fussy 2001)
- compost may affect plant growth (Zhang et al. 2000) and induce suppressiveness against soil-borne diseases (Hoitink and Fahy 1986; Noble and Coventry 2005; Yogev et al. 2006)
- compost application increases microbial activity (Guisquiani et al. 1995; Ros et al. 2006a,b)
- compost amendments are known to change soil microbial communities either directly (through the indigenous compost microbiota) or indirectly through the above mentioned mechanisms (Ros et al. 2003)

Studies on the effect of compost applications on soils have mostly concentrated on physico-chemical parameters (Shindo et al. 2006; Tejada et al. 2009), as well as plant productivity (Zhang et al. 2000; Mantovi et al. 2005; Fuchs et al. 2006). Little is known about how composts impact on soil microbial communities, although they play an important role in soil ecosystem functioning and maintenance. Moreover, microbiological parameters are more responsive to soil management changes than physico-chemical properties, thus providing more accurate information on the soil status (Tejada et al. 2008).

14.4 How Compost Amendments Affect the Soil Microbiota

A variety of studies have tried to elucidate the complex interactions between compost application and soil microbial properties. These investigations, however, vary considerably in the applied experimental designs, displaying differences in soil physico-chemical properties, land-use and compost type (e.g. different starting

material, process parameters), frequency and dosage of application, plant cover on the research site, the duration of experiments as well as the parameters chosen for analysis. Thus, different studies are frequently not comparable to each other and general conclusions about the effectiveness of compost as for soil amelioration or fertilisation are difficult to draw (Postma and Kok 2001). Despite these limitations, some interesting findings have been made, shedding light on the impact of compost amendments on the microbial biomass, activity and diversity in soil ecosystems (see Chap. 12).

14.4.1 The Effect of Compost Amendments on Microbial Biomass

A classical tool for assessing microbial abundance is plate counting, which is either used for determining the total cultivable bacteria and fungi in the soil or for studying a specific microbial group by applying selective media and growth conditions. The number of colony-forming units (CFU) has also been used for investigating the effect of compost applications on soils. In a laboratory study in which two types of agricultural soils were amended with three different composts (green waste compost and two different types of mushroom compost), it was revealed that the impact of the organic amendments on the soil microbiota was dependent on the type of compost used (Perez-Piqueres et al. 2006). When examining compost produced from turkey manure and ligneous waste, Calbrix et al. (2007) were not able to detect a higher bacterial abundance after application of compost, whereas manure and especially sewage sludge led to a significant increase in CFU. However, this effect was transient and 6 months after application, CFUs were similar to the numbers found before addition of organic amendments. In this study, CFUs were not correlated with microbial biomass carbon (C_{mic}), which is another classical biological parameter for characterising soils and is commonly determined by chloroform-fumigation extraction (CFE; Jenkinson et al. 2004) or substrate-induced respiration (SIR; Anderson and Domsch 1978). As microorganisms adapt rapidly to changing environmental conditions such as the incorporation of organic materials, microbial biomass carbon is a suitable indicator of changes in soil ecosystems (Pascual et al. 1997). In a long-term experiment applying municipal solid-waste compost (MSWC) on an agricultural soil low in organic matter content, Garcia-Gil et al. (2000) were able to show that soils amended with MSWC contained higher levels of C_{mic} as compared to the non-fertilised control plots. This effect was, on the one hand, attributed to the input of microbial biomass as part of the amendments and, on the other hand, it was assumed that additional C contained in these organic substrates activated the indigenous soil microbiota. This finding was also confirmed for compost amendments derived from green waste (Okur et al. 2007; Tejada et al. 2008) and sewage sludge (Zaman et al. 2004). However, other factors were reported to show a more pronounced effect on soil microbial biomass than compost treatments, especially the type of soil the compost was applied to (Nendel and Reuter 2007).

In contrast to these traditional parameters, quantitative polymerase chain reaction (qPCR) is an approach that detects microorganisms by targeting their DNA or RNA, thus being independent of the growth requirements of the microorganisms involved. By using primers that specifically amplify a selected group of bacteria or fungi, it is possible to gain insights into their abundance within a soil sample (see Chap. 7, Hultman et al. 2010). Furthermore, a specific biological process in the soil can be studied, as was demonstrated for ammonia oxidation in soil by He et al. (2007). These authors analysed the effect of different fertilisers on ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) after 16 years of fertiliser treatment. Different responses of the AOB and AOA communities on the various fertiliser combinations were found; however, no compost application was included in this study. In another long-term field experiment, Innerebner et al. (2006) were not able to reveal significant differences between soil plots that had been amended with four different compost types (green manure compost, organic waste compost, manure compost and sewage sludge compost) over 15 years. However, all compost-amended soils obtained a higher abundance of AOB than plots receiving mineral fertiliser or control plots. This finding was explained by the fact that the organic N in the composts was liberated by microorganisms through ammonification, thus leading to an increase in AOB cell density within the compost-amended soil plots. By using reverse transcriptase (RT)-qPCR, based on the detection of RNA, information not only on the microbial cell numbers, but also on the microbial activity can be obtained (Nielsen and Winding 2002), as metabolically active cells contain higher levels of intracellular RNA than dormant ones. Thus, RNA may be used as an indicator for microbial activity (Felske et al. 1998).

14.4.2 The Effect of Compost Amendments on Microbial Activity

Microbial respiration is a frequently measured parameter in soils, reflecting the basic turnover rates (Insam et al. 1991) and responding rapidly to perturbations or soil management changes (Gilani and Bahmanyar 2008). Basal soil respiration as an indicator for microbial activity was determined in a long-term study (20 years), in which organic farming was compared to conventional farming (Fließbach et al. 2006). As an outcome of this investigation, higher microbial activities were found in the soils fertilised with farmyard manure compost than in the control plots. An increase of soil microbial respiration upon application of municipal solid waste compost and vermicompost was also demonstrated in an investigation on the effect of compost applications on a silty clay soil in Iran. However, the level of soil microbial respiration was more dependent on application rates than on fertiliser type, and was linked to total organic C (Gilani and Bahmanyar 2008).

As microorganisms play an essential role in maintaining biogeochemical cycles in the soil and enzymatic activity is mainly of microbial origin (Nielsen and Winding 2002), soil enzymes can be used as indicators of microbial activity (Chaps. 6 and 13, Marcote et al. 2001). Moreover, enzyme activity reacts quickly

to changes in the soil ecosystem, and thus it has frequently been used to assess the impact of treatments on microbial communities. Typically, enzymatic activities referring to either the C-cycle (e.g. cellulase, β-glucosidase, β-galactosidase), the N-cycle (e.g. protease, urease, amidase) and/or the P-cycle (e.g. phosphatase) are measured, as these enzymes contribute essentially to plant nutrient availability (Galli 2004). In two studies dealing with the effect of MSWC application at an annual rate of 20 and 80 tonnes ha^{-1}, it was found that soil enzyme activities varied depending on the treatment, dose of compost application and sampling date (Garcia-Gil et al. 2000; Marcote et al. 2001). As a drawback of MSWC application, heavy metals were incorporated into the soil and even led to inhibitory effects when compost was applied in large doses. The same effect was revealed in a field study using composted yard waste in combination with sewage sludge as soil amendment, whereby available metal concentration (Cu, Zn, Mn) increased with compost application (He et al. 1995). Heavy metal accumulation in agricultural soils as a consequence of compost amendments, especially of MSWC and sewage sludge compost, has frequently been reported to be one of the main risks of long-term application (Pinamonti et al. 1997; Wei and Liu 2005; Madrid et al. 2007; Cherif et al. 2009).

14.4.3 The Effect of Compost Amendments on Microbial Community Structure and Diversity

Besides having an influence on microbial biomass and activity, compost amendments may also impact microbial community structure and diversity within the soil. One cultivation-independent method for characterising the structure of soil microbial communities is phospholipid fatty acid analysis (PLFA; Elfstrand et al. 2007). This technique is based on the measurement of fatty acids within the membrane of viable microbial cells, making it possible to distinguish several microbial groups according to their specific PLFA composition (Zelles 1999). Saison et al. (2006) revealed that PLFA profiles were significantly affected by compost amendments. Successions in microbial community structure were strongly dependent on the compost application rate, showing that higher amounts of compost led to a more pronounced and faster effect. Fatty acid analysis was also used by Carrera et al. (2007), demonstrating that fatty acid profiles, especially in regard to the relative amount of polyunsaturated fatty acids (including the biomarkers for fungi), were both influenced by treatment with poultry manure compost as well as sampling date. In another study, Bastida et al. (2008) applied this method to analyse the microbial community structure in a degraded semiarid soil 2 years after compost application and revealed a change in the plots that had received sewage sludge compost as compared to the control. This shift in community structure was connected to an increase in microbial activity and biomass as well as elevated carbon content, thus indicating a beneficial effect of compost application.

Another frequently applied tool for revealing the structure of microbial populations in the soil is the use of molecular fingerprinting techniques such as automated rRNA intergenic spacer analysis (ARISA; Ranjard et al. 2001), temperature or denaturing gradient gel electrophoresis (TGGE/DGGE; Muyzer et al. 1993), terminal restriction fragment length polymorphism (T-RFLP; Liu et al. 1997) or single-strand conformation polymorphism (SSCP; Schwieger and Tebbe 1998). These tools (discussed in Chaps. 1, 7 and 12) allow for the rapid and simultaneous analysis of multiple samples and are especially useful for investigating microbial succession in a habitat. DGGE and SSCP moreover offer the possibility to identify distinctive bands from the fingerprinting profiles by excising and sequencing bands of interest from the gel (Nocker et al. 2007). A prerequisite for the application of these methods is the extraction and amplification of DNA from the soil, on the one hand, providing also insight into the non-cultivable fraction of the soil microflora, but, on the other hand, exposing the analysis to an additional bias (von Wintzingerode et al. 1997).

Several studies have been conducted using molecular techniques to investigate the influence of compost amendments on the structural composition of the soil microbiota. Whereas some analyses discovered differences between compost- and non-compost-amended soils (Innerebner et al. 2006, using DGGE; Perez-Piqueres et al. 2006, using T-RFLP; Ros et al. 2006a, b, using DGGE; Saison et al. 2006, using ARISA), others were not able to detect compost effects (Crecchio et al. 2001, using ARISA and DGGE; Calbrix et al. 2007, using T-RFLP; Cherif et al. 2009, using DGGE). Therefore, general statements on the usefulness of fingerprinting techniques for analysing the impact of compost amendments on soils are difficult to draw and combination with other methods is recommended.

To investigate the potential functional diversity of the soil ecosystem, community level physiological profiles (CLPPs; Garland and Mills 1991; Insam and Goberna 2004) have been widely used. This method allows an assessment of the microbial metabolic activity in respect to specific carbon sources; however, it is again a tool that is restricted to the cultivable fraction of the microbial community, as only aerobic heterotrophic microbes that are able to rapidly grow on the various substrates can be detected (Carrera et al. 2007; Ros et al. 2008). CLPP was also applied for analysing the effect of compost amendments on the soil microflora, as microbial substrate utilisation patterns are considered to be suitable indicators for detecting soil management changes (Bending et al. 2000). Different kinds of vermicompost applications (vermicompost from source-separated household solid waste and vermicompost from horse and rabbit manure) were demonstrated to modify substrate utilisation patterns of the soil microbial community in comparison to unamended control soils (Gomez et al. 2006). In contrast, Perez-Piqueres et al. (2006) pointed out that the impact of compost application on bacterial metabolism was dependent both on the type of compost used as well as on the soil type it was applied to. Other studies found that sampling date was more influential for CLPP results than compost treatments (Calbrix et al. 2007; Carrera et al. 2007), highlighting the need for repeated analysis applying a multi-parameter approach.

Calbrix et al. (2007) discovered that treatment effects found directly after compost application could not be retrieved 6 months later, which was not only true for CLPP analysis, but also for CFU numbers and total DNA contents. In a similar study investigating not only the effect of compost amendments on the abundance, activity and structural composition of soil microorganisms, but also the resilience of compost applications to soils, Saison et al. (2006) found that composts made from grape skins and other winery residues had an influence on all three domains of microbial life. However, although high application rates were used in this experiment, the effects on microbial biomass, activity and community structure were only observed shortly after application, and were not detectable 6 months later. Moreover, the initial impact of compost amendments on soil microbiota was mainly attributed to an additional input of organic matter, whereas compost-derived microorganisms seemed to be outcompeted by the indigenous soil microbiota (Saison et al. 2006). These findings indicate that besides analysing if and to what extent compost applications have an influence on the soil microbiota, the issue of persistency of the initially observed effects is of great importance (Bhattacharyya et al. 2003; Gomez et al. 2006; Calbrix et al. 2007) and emphasises the need for long-term investigations. Still, the number of long-term field experiments (>5 years) investigating the impact of compost amendments on agricultural soils are rare (Table 14.1). This is why, in the following section, a selected long-term field trial will be presented and the use of a multi-parameter approach will be demonstrated.

14.5 Long-Term Effects of Different Compost Amendments on the Soil Microflora on an Agricultural Site – a Case Study

In order to conduct a full survey on the fertiliser value of different composts (source-separated urban organic waste, green waste, manure and sewage sludge compost), a field experiment was set up in 1991 by the Austrian Federal Agency for Food Safety (AGES). The initial aim of the study was to observe long-term effects of compost amendments on crop yield and compare compost-treated plots to mineral-fertilised and control plots. In recent years, the analyses have however been extended to investigate soil physico-chemical properties as well as microbial activity, biomass and structure of microbial communities. The primary aim of the present investigation was to address two major questions:

1. Does the long-term application of composts affect soil microbial biomass and activity?
2. Does the application of different composts result in divergent structural fingerprints in the soil?

Table 14.1 Research studies dealing with the long-term (>5 years) application of compost on agricultural soils

Authors	Duration of experiment	Compost type	Application rate per year	Investigated parameters	Effect of compost amendment
Canali et al. (2004)	6 years	Compost from distillery by-products, MC	Corresponding to 160 kg N ha^{-1}	CLPP, soil microbial biomass and basal respiration	Microbiological soil properties were only weakly influenced
Crecchio et al. (2004)	6 years	MSWC	Corresponding to 120 or 240 kg N ha^{-1}	PCR-DGGE, ARISA, ARDRA, soil enzyme activity	Increase in soil enzyme activity, but only minimal changes in microbial community structure as revealed by molecular tools
Fließbach et al. (2006)	20 years	MC	Corresponding to 1.78 mg organic carbon ha^{-1}	Soil microbial biomass and basal respiration, dehydrogenase activity	Microbial biomass and activity was enhanced
Garcia-Gil et al. (2000)	9 years	MSWC	20 or 70 tonnes ha^{-1}	Soil microbial biomass, soil enzyme activity	Compost application increased microbial biomass
Innerebner et al. (2006)	12 years	GC, MC, OWC, SSC	Corresponding to 175 kg N ha^{-1}	PCR-DGGE, qPCR	Abundance of ammonia-oxidising bacteria was higher with compost application
Mäder et al. (2002)	20 years	MC with slurry	Corresponding to 1.78 mg organic carbon ha^{-1}	Soil microbial activity and biomass, qCO_2, enzyme activity, CLPP	Increase in functional microbial diversity caused by organic amendment associated with a decrease in qCO_2
Ros et al. (2006a)	12 years	GC, MC, OWC, SSC	Corresponding to 175 kg N ha^{-1}	Soil microbial activity and biomass, CLPP, PCR-DGGE	Compost amendments influenced soil microbiota; however, the effects caused by different composts could not be distinguished except for SSC
Zaman et al. (2004)	23 years	SSC mixed with rice husk or sawdust	240 kg ha^{-1}	Soil microbial activity and biomass, soil enzyme activity	Soil microbial activity and biomass as well as enzyme activity was significantly higher in compost-treated soils

GC: Green waste compost; MC: Manure compost; MSWC: Municipal solid waste compost; OWC: Urban organic waste compost; SSC: sewage sludge compost

14.5.1 Experimental Design

A crop rotation (maize, summer-wheat, legumes and winter-barley) field experiment was started in 1991 at the research site Ritzlhof near Linz, Austria. The soil was loamy silt (17.4% clay, 69% silt, 13.6% sand) with a pH (H_2O) of 6.8. The soil contained 1.9% organic matter, 260 mg kg^{-1}available P and 300 mg kg^{-1} available K.

The experiment was performed using a randomised block of 12 plots (5 m × 6 m) with 4 replicates. Treatments were applied annually after harvest as follows:

1. Soil without fertilisation (control)
2. Compost amendment corresponding to 175 kg N ha^{-1}
   - urban organic waste compost (OWC) from source-separate collection of domestic organic waste
   - green waste compost (GC) from roadside and park leaves, tree prunings, yard clippings, and other bulky cellulose and lignin-rich materials
   - cattle manure compost (MC) containing straw bedding impregnated with liquid and solid manure
   - sewage sludge compost (SSC) from anaerobically stabilised sewage sludge of a municipal waste-water treatment plant with wood chips and bark as bulking agents
3. Composts plus mineral fertiliser treatments: 175 kg N ha^{-1} from compost plus 80 kg mineral N (NH_4NO_3) ha^{-1} (OWC+80N; GC+80N; MC+80N, SSC+80N)
4. Mineral fertilisation treatments corresponding to 80 kg N (NH_4NO_3) ha^{-1} (80N)

Soils from the field experiment were sampled in October 2003 after harvest of winter barley and in August 2004 after harvest of legumes. Three random soil cores (20 cm depth, 6 cm diameter) were taken from each plot (four replicate plots for each treatment), bulked and sieved (<2 mm). Samples for DNA analysis were taken separately with a spatula and transferred into sterile 50-ml Greiner tubes. The samples were analysed immediately (soil microbial respiration and microbial biomass) or frozen at –18°C prior to molecular analysis.

14.5.2 Results and Discussion

As has been illustrated in the last section, composts may impact the soil microbiota on three levels, leading to a change in microbial biomass, activity and/or community structure. Therefore, all the three dimensions were investigated in this survey, analysing microbial biomass using SIR (Anderson and Domsch 1978), microbial activity by measuring microbial basal respiration using a continuous flow infrared gas analysis system (Heinemeyer et al. 1989) and revealing microbial community structure by performing PCR-DGGE analysis (Muyzer et al. 1993) on bacterial and fungal communities.

14.5.2.1 Microbial Biomass and Activity

Whereas microbial biomass increased by only a small amount as a consequence of the annual application of organic amendments or combined compost and mineral fertiliser treatments, the effect of compost amendment on soil microbial respiration was more pronounced (Table 14.2). The highest basal respiration was measured for the SSC and the SSC + 80 kg N treatment, which was significantly different to the unamended control soil, and moreover displayed an elevated metabolic quotient (qCO_2). Since this finding was connected to an accumulation of Zn and Cu in the SSC-amended soil plots, heavy metal concentration may have lead to a stress-induced qCO_2 rise (Insam et al. 1996). Moreno et al. (1999) previously demonstrated that the metabolic quotient rose because of the incorporation of heavy metal-contaminated sewage sludge compost, making it a sensitive indicator for stress-induced responses of the soil microbiota. Sewage sludge compost is generally considered to be a more problematic soil amendment than other types of compost, not only displaying elevated heavy metal contents, but also elevated contents of xenobiotics (Pascual et al. 2008).

14.5.2.2 Soil Community Analysis Based on Microbial DNA

Molecular fingerprinting tools have proven useful for simultaneously investigating the structural community composition of multiple samples (Sect. 14.4.3). PCR-DGGE analysis was performed in the framework of this study to reveal if composts leave imprints in the soil after they had been applied for many years. Control plots and soils treated with mineral fertiliser were compared to soils amended with four different types of compost.

Table 14.2 Microbial biomass (Cmic), basal respiration and metabolic quotient (qCO_2) for compost-amended (GC, MC, OWC, SSC), mineral fertiliser amended (80N), compost + mineral fertiliser amended (GC+80N, MC+80N, OWC+80N, SSC+80N) and unamended soil plots (control)

Treatments	C_{mic} (μg C g^{-1} soil)	Basal Respiration (μg CO_2-C g^{-1} soil h^{-1})	qCO_2 (mg CO_2-C g^{-1} C_{mic} h^{-1})
Control	245.73 (6.20)a	0.44 (0.13)a	0.48 (0.15)a
80N	229.86 (4.42)a	0.37 (0.10)a	0.44 (0.07)a
GC	277.78 (7.94)a	0.58 (0.08)ab	0.57 (0.08)ab
MC	250.55 (4.27)a	0.40 (0.08)a	0.44 (0.05)a
OWC	284.90 (7.13)a	0.55 (0.07)ab	0.52 (0.10)ab
SSC	260.16 (5.91)a	0.66 (0.14)ab	0.69 (0.16)ab
GC + 80N	280.47 (7.65)a	0.52 (0.12)ab	0.50 (0.04)a
MC + 80N	270.28 (3.29)a	0.59 (0.06)ab	0.60 (0.15)ab
OWC + 80N	283.90 (6.04)a	0.65 (0.13)ab	0.62 (0.14)ab
SSC + 80N	271.26 (6.48)a	0.82 (0.10)b	0.81 (0.13)b

Numbers in parentheses are standard deviations, $n = 4$. Mean values followed by the same letter are not significantly different ($P \leq 0.05$) between different treatments

PCR-DGGE analyses were performed using universal bacterial (Ros et al. 2006a) and fungal primer sets (Vainio and Hantula 2000) as well as Streptomycetes group-specific primers (Monciardini et al. 2002). Cluster analyses of fingerprinting patterns showed that compost amendments, especially the combined compost and mineral fertiliser treatments, had an impact on the bacterial community structure, both on the universal and the Streptomycetes group-specific level (Fig. 14.1). In contrast, fungal community structure was more influenced by seasonal variations than by treatment (Fig. 14.2). Soil samples from October 2003 formed a separate cluster in the dendrogram, while samples taken the following year formed a second cluster. The influence of seasonal changes on microbial community structure in a fertiliser experiment on agricultural soil was confirmed by Calbrix et al. (2007), showing that the date of sampling contributed more to modifications in microbial community structure than treatment effects. In contrast, DGGE fingerprints of Streptomycetes, which are of interest because of their function as essential biodegraders and enzyme-producers in soil (Williamson et al. 2000), clustered according to the treatment. Seasonality seemed to have nearly no impact. Cluster analysis showed that the different treatments separated into two main groups, with all combined compost and mineral fertiliser treatments in one cluster and the mineral fertiliser only treatment (80) and control treatments in the second cluster. Composts without additional mineral fertiliser were either found in the cluster with the

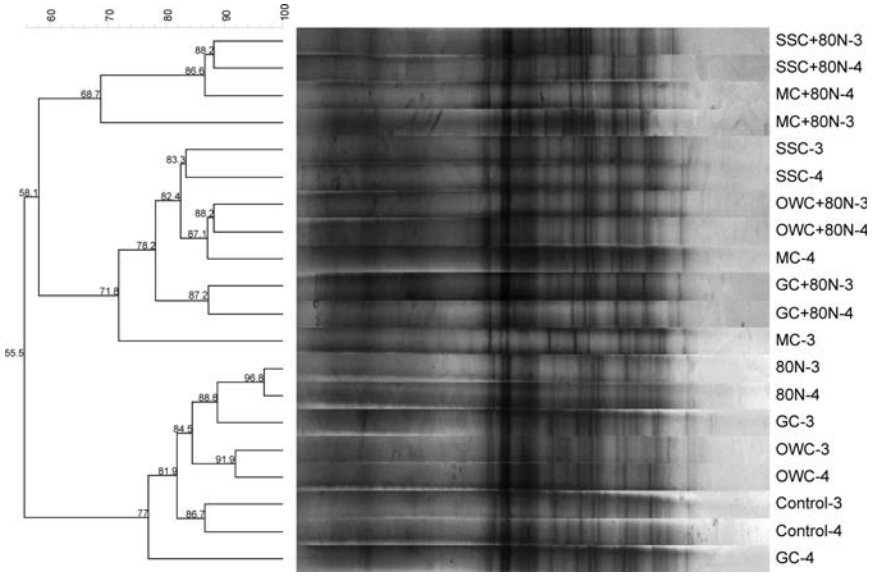

Fig. 14.1 Cluster analysis of bacterial DGGE fingerprints based on 16S rRNA gene extracted from compost-amended (GC, MC, OWC, SSC), mineral fertiliser amended (80N), compost + mineral fertiliser treated (GC + 80N, MC + 80N, OWC + 80N, SSC + 80N) and unamended control soil plots (control). The number at the end of the labels (–3 resp. –4) indicates the year of sampling, 2003 resp. 2004. Values at the branches of the dendrograms show the percentage of similarity, based on the Dice correlation coefficient

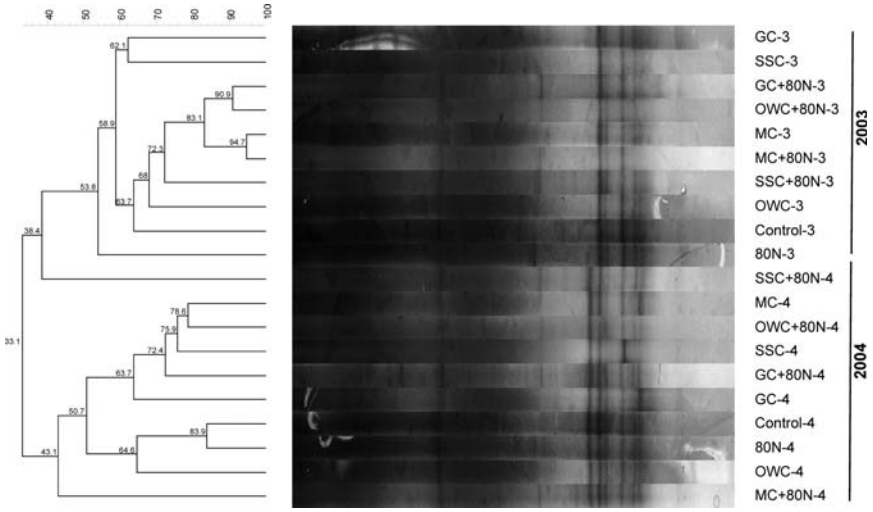

Fig. 14.2 Cluster analysis of fungal DGGE fingerprints based on 18S rRNA gene extracted from compost-amended (GC, MC, OWC, SSC), mineral fertiliser amended (80N), compost + mineral fertiliser treated (GC + 80N, MC + 80N, OWC + 80N, SSC + 80N) and unamended control soil plots (control). The number at the end of the labels (–3 resp. –4) indicates the year of sampling, 2003 resp. 2004. Values at the branches of the dendrograms show the percentage of similarity, based on the Dice correlation coefficient

combined organic and mineral fertiliser amendments (MC, SSC), or grouped with the control and 80 kg N mineral fertiliser treatment (MC, OWC). In general, fingerprinting profiles of the Streptomycetes-DGGEs were highly similar to each other, and did not show distinctive bands characteristic for a certain amendment. Therefore, it was difficult to clearly distinguish between the four compost types according to their banding patterns. Since DGGE has the limitation that its profiles represent only the most dominant phylotypes in the investigated samples (Muyzer et al. 1993), the similar fingerprinting profiles of the differently amended soils may be attributed to the presence of some predominant microbes in all the samples, whereas differences in less abundant representatives of the microbial community cannot be detected by this fingerprinting technique.

14.5.3 Outlook

A multi-parameter approach applying diverse methods for investigating the microbial biomass and activity as well as microbial community structure and diversity has proven valuable for assessing the interactions between compost and soil microbiota (Ros et al. 2006a). This is why the experiment on the research site is still ongoing, using modern tools for analysing the effects of different fertiliser treatments on soil microbial communities. In a current project, the impact of

different compost amendments as compared to non-amended and mineral fertiliser amended agricultural soil is being investigated by measuring volatile organic compounds (VOCs). VOCs are a group of substances with a high diversity including alcohols, ketones, acetates, alkenes monoterpenes, terpenes, isoprenes and aromatic compounds as well as sulphur- and nitrogen-containing compounds. VOCs are ubiquitous in the environment (e.g. spoiling foods, composts, soils, plant surfaces) and produced by plants, animals and fungi as well as by bacteria and archaea. VOCs develop as intermediate products of the decomposition of organic matter such as litter or organic household biowastes (Leff and Fierer 2008; Mayrhofer et al. 2006) and can be detected before microbial growth can be measured, making them an interesting parameter for investigating changes in a soil ecosystem. Although microorganisms in the soil release a considerable proportion of VOCs and thus influence soil processes, only little is known about the amounts and types of VOCS emitted from soils (Leff and Fierer 2008). This is why these compounds are currently being investigated by proton transfer reaction mass spectrometry, a method that enables the on-line detection of trace components present at very low levels (Lindinger et al. 1998). Since it is assumed that soil amendments induce changes in the microbial community structure, distinguishable VOC emission patterns are likely to occur as a consequence of the different treatments. If specific VOCs were detected for the organic amendments, it would moreover be possible to distinguish between the four types of composts (Seewald et al., submitted for publication).

Several studies have demonstrated that composts may induce suppressiveness against soil-borne diseases (see Chap. 8, Hoitink and Fahy 1986; Noble and Coventry 2005; Yogev et al. 2006). This is why a further emphasis of this survey will be placed on the suppressiveness of different composts against soil-borne phytopathogens (Fuchs 2002). If suppressive effects of a certain compost treatment towards specific plant diseases are found, a microarray approach (Franke-Whittle et al. 2005, 2009) may make it possible to identify specific microorganisms linked to the disease suppressiveness of the soil.

14.6 Conclusions

Compost amendments have been shown to provide manifold benefits, as long as composts of good quality are used and care is taken not to accumulate heavy metals or organic pollutants as a consequence of repeated application. Among the advantages of compost as soil amendment is its potential to maintain soil organic matter, foster nutrient availability and increase soil microbial abundance and activity, thus enhancing soil quality and fertility (Tejada et al. 2009).

Despite the intensive efforts to reveal the effects of compost applications on the soil microbiota using microbial and molecular techniques, these investigations provide only restricted information since the effects found directly after compost amendment may differ considerably from those observed in the long term. Thus,

long-term field studies on this topic are essential to gain a deeper knowledge on the impact of compost amendments on the soil microbiota. In our experiment conducted near Linz, Austria, we demonstrated that composts may impact the soil microbiota and leave a distinct imprint on the soil.

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References

Alfreider A, Peters S, Tebbe CC, Rangger A, Insam H (2002) Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis. Compost Sci Util 4:303–312

Anderson JPE, Domsch KH (1978) Mineralisation of bacteria and fungi in chloroform fumigated soils. Soil Biol Biochem 10:207–213

Bastida F, Kandeler E, Moreno JL, Ros M, Garcia C, Hernandez T (2008) Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Appl Soil Ecol 40:318–329

Bastida F, Hernández T, García C (2010) Soil Degradation and rehabilitation: microorganisms and functionality. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 253–270

Beffa T, Blanc M, Lott Fischer J, Lyon PF, Marilley L, Aragno M (1995) Composting: a microbial process. In: Barrage A, Edelman X (eds) Recovery, Recycling and Re-integration. EMPA, Dübendorf, pp 139–144

Belete L, Egger W, Neunhäuser C, Caballero B, Insam H (2001) Can community level physiological profiles be used for compost maturity testing? Compost Sci Util 9:6–18

Bending GD, Putland C, Rayns F (2000) Changes in microbial community metabolism and labile organic matter fractions as early indicators of the impact of management on soil biological quality. Biol Fertil Soils 31:78–84

Bhattacharyya P, Chakrabarti K, Chakraborty A (2003) Effect of MSW compost on microbiological and biochemical soil quality indicators. Compost Sci Util 11:220–227

Calbrix R, Barray S, Chabrerie O, Fourrie L, Laval K (2007) Impact of organic amendments on the dynamics of soil microbial biomass and bacterial communities in cultivated land. Appl Soil Ecol 35:511–522

Canali S, Trinchera A, Intrigliolo F, Pompili L, Nisini L, Mocali S, Torrisi B (2004) Effect of long term addition of composts and poultry manure on soil quality of citrus orchards in Southern Italy. Biol Fertil Soils 40:206–210

Carrera LM, Buyer JS, Vinyard B, Abdul-Baki AA, Sikora LJ, Teasdale JR (2007) Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. Appl Soil Ecol 37:247–255

Ceustermans A, Coosemans J, Ryckeboer J (2010) Compost microbial activity related to compost stability. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 115–134

Cherif H, Ayari F, Ouzari H, Marzorati M, Brusetti L, Jedidi N, Hassen A, Daffonchio D (2009) Effects of municipal solid waste compost, farmyard manure and chemical fertilizers on wheat

growth, soil composition and soil bacterial characteristics under Tunisian arid climate. Eur J Soil Biol 45:138–145
Crecchio C, Curci M, Mininni R, Ricciuti P, Ruggiero P (2001) Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol Fertil Soils 34:311–318
Crecchio C, Curci M, Pizzigallo MDR, Ricciuti P, Ruggiero P (2004) Effects of municipal solid compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biol Biochem 26:1595–1605
de Bertoldi M (2010) Production and utilization of suppressive compost: environmental, food and health benefits. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 153–170
Diaz LF, Savage GM (2007) Factors that affect the process. In: Diaz LF, de Bertoldi M, Bidlingmaier W, Stentiford E (eds) Compost science and technology. Elsevier, Oxford, pp 49–65
Dimambro ME, Lillywhite RD, Rahn CR (2007) The physical, chemical and microbial characteristics of biodegradable municipal waste derived composts. Compost Sci Util 15:243–252
Elfstrand S, Båth B, Mårtensson S (2007) Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. Appl Soil Ecol 36:70–82
European Council (1986) Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Official Journal L 181: 6–12
European Union. Landfill directive 1999/31/EC. Official Journal of the European Communities, L182/1, 1999.
Felske A, Wolterink A, van Lis R, Akkermans ADL (1998) Phylogeny of the main bacterial 16S rRNA sequences in drentse a grassland soils (The Netherlands). Appl Environ Microbiol 64:871–879
Fließbach A, Oberholzer H-R, Gunst L, Mäder P (2006) Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agric Ecosyst Environ 118:273–284
Franke-Whittle IH, Klammer SH, Insam H (2005) Design and application of an oligonucleotide microarray for the investigation of compost microbial communities. J Microbiol Methods 62:37–56
Franke-Whittle IH, Knapp BA, Fuchs J, Kaufmann R, Insam H (2009) Application of COMPOCHIP microarray to investigate the bacterial communities of different composts. Microb Ecol 57:510–521
Fuchs JG (2002) Practical use of quality composts for plant health and vitality improvement. In: Insam H, Riddech N, Klammer S (eds) Microbiology of composting. Springer-Verlag, Berlin, pp 435–444
Fuchs JG (2010) Interactions between beneficial and harmful micro-organisms: from the composting process to compost application. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 213–230
Fuchs JG, Baier U, Berner A, Mayer J, Tamm L, Schleiss K (2006) Potential of different composts to improve soil fertility and plant health. In: Kraft E, Bidlingmaier W, de Bertoldi M, Diaz LF, Barth J (eds) ORBIT 2006: Biological Waste Management. From Local to Global. In: Proceedings of the International Conference ORBIT 2006. Verlag ORBIT e.V., Weimar, pp 507–517
Galli U (2004) Einfluss von Kompost auf die Bodenorganismen. In: Fuchs JG, Bieri M, Chardonnens M (eds) Auswirkungen von Komposten und Gärgut auf die Umwelt, die Bodenfruchtbarkeit sowie die Pflanzengesundheit. Zusammenfassende Übersicht der aktuellen Literatur. Forschungsinstitut für biologischen Landbau (FiBL), Frick, pp 81–105
Garcia-Gil JC, Plaza C, Soler-Rovira P, Polo A (2000) Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol Biochem 32:1907–1913

Garland J, Mills AL (1991) Classification and characterisation of heterotrophic microbial communities on the basis patterns of community level sole carbon source utilization. Appl Environ Microbiol 57:2351–2359

Gilani SS, Bahmanyar MA (2008) Impact of organic amendments with and without mineral fertilizers on soil microbial respiration. J Appl Sci 8:642–647

Gomez E, Ferreras L, Toresani S (2006) Soil bacterial functional diversity as influenced by organic amendment application. Bioresour Technol 97:1484–1489

Guisquiani PL, Pagliai M, Gigliotti G, Businelli D, Benetti A (1995) Urban waste compost: effects on physical, chemical, and biochemical soil properties. J Environ Qual 24:175–182

Hargreaves JC, Adl MS, Warman PR (2008) A review of the use of composted municipal solid waste in agriculture. Agric Ecosyst Environ 123:1–14

He ZL, Alva AK, Calvert DV, Li YC, Stoffella PJ, Banks DJ (1995) Nutrient availability and changes in microbial biomass of organic amendments during field incubation. Compost Sci Util 4:293–302

He J, Shen J, Zhang L, Zhu Y, Zheng Y, Xu M, Di H (2007) Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ Microbiol 9:2364–2374

Heinemeyer O, Insam H, Kaiser EA, Walenzik G (1989) Soil microbial biomass and respiration measurements: an automated technique based on infra-red gas analysis. Plant Soil 116:191–195

Hoitink HAJ, Fahy PC (1986) Basis for the control of soil-borne plant pathogens with composts. Annu Rev Phytopathol 24:93–114

http://ec.europa.eu/environment/waste/pdf_comments/040119_proceedings.pdf. Cited 07 Oct 2008

http://ec.europa.eu/environment/waste/pdf_comments/040119_proceedings.pdf. Cited 07 Oct 2008

http://www.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/FR388.pdf. http://www.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/FR388.pdf. Cited 13 Oct 2008

Hultman J, Kurola J, Raininsalo A, Kontro M, Romantschuk M (2010) Utility of molecular tools for optimization of large scale composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 135–152

Hüttl RF, Fussy M (2001) Organic matter management – A contribution to sustainability. In: Applying compost – benefits and needs. Seminar Proceedings Brussels, 22–23 November 2001, pp 9–18

Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidizing bacteria in compost-treated soils. Soil Biol Biochem 38:1092–1100

Insam H, de Bertoldi M (2007) Microbiology of the composting process. In: Diaz LF, de Bertoldi M, Bidlingmaier W, Stentiford E (eds) Compost science and technology. Elsevier, Oxford, pp 25–48

Insam H, Goberna M (2004) Use of Biolog for the community level physiological profiles (CLPP) of environmental samples. In: Kowalchuk GA, de Bruijn FJ, Head IM, Akkermans ADL, van Elsas JD (eds) Molecular microbial ecology manual. Kluwer, Dordrecht, pp 853–860

Insam H, Mitchell CC, Dormaar JF (1991) Relationship of soil microbial biomass and activity with fertilisation practice and crop yield of tree ultisols. Soil Biol Biochem 32:1131–1139

Insam H, Hutchinson TC, Reber HH (1996) Effects of heavy metal stress on the metabolic quotient of the soil microflora. Soil Biol Biochem 28:691–694

Insam I, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Jenkinson DJ, Brookes PC, Powlson DS (2004) Measuring soil microbial biomass. Soil Biol Biochem 36:5–7

Kowalchuk GA, Naoumenko ZS, Derikx PJL, Felske A, Stephen JR, Arkhipchenko IA (1999) Molecular analysis of ammonia-oxidizing bacteria of the ß subdivision of the class Proteobacteria in compost and composted materials. Appl Environ Microbiol 65:396–403

Leff JW, Fierer N (2008) Volatile organic compound (VOC) emissions from soil and litter samples. Soil Biol Biochem 40:1629–1636

Lindinger W, Hansel A, Jordan A (1998) On-line monitoring of volatile organic compounds at pptv levels by means of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS): medical applications, food control and environmental research. Int J Mass Spectrom 173:191–241

Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterisation of microbial diversity by determining terminal restriction length polymorphisms of genes encoding 16S rDNA. Appl Environ Microbiol 63:4516–4522

Mäder P, Fließbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. Science 296:1694–1697

Madrid F, Lopez R, Cabrera F (2007) Metal accumulation in soil after application of municipal solid waste compost under intensive farming conditions. Agric Ecosyst Environ 119:249–256

Mantovi P, Baldoni G, Toderi G (2005) Reuse of liquid, dewatered, and composted sewage sludge on agricultural land: effects of long-term application on soil and crop. Water Res 39:289–296

Marcote I, Hernández T, Garcia C, Polo A (2001) Influence of one or two successive annual applications of organic fertilisers on the enzyme activity of a soil under barley cultivation. Bioresour Technol 79:147–154

Marmo L (2008) EU strategies and policies on soil and waste management to offset greenhouse gas emissions. Waste Manag 28:685–689

Mayrhofer S, Mikoviny T, Waldhuber S, Wagner A, Innerebner G, Leuenberger J, Franke-Whittle IH, Märk T, Hansel A, Insam H (2006) Correlation of microbial community and VOC emission in household biowaste. Environ Microbiol 8:1960–1974

Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252

Monciardini P, Sossio M, Cavaletti L, Chiocchini C, Donadio S (2002) New PCR primers for the selective amplification of 16S rDNA from different groups of actinomycetes. FEMS Microbiol Ecol 42:419–429

Moreno JL, Hernández T, Garcia C (1999) Effects of a cadmium-contaminated sewage sludge compost on dynamics of organic matter and microbial activity in an arid soil. Biol Fertil Soils 28:230–237

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analyses of polymerase chain reaction-amplified genes for 16S rRNA. Appl Environ Microbiol 59:695–700

Nendel C, Reuter S (2007) Soil biology and nitrogen dynamics of vineyard soils as affected by a mature biowaste compost application. Compost Sci Util 15:70–77

Nielsen MN, Winding A (2002) Microorganisms as indicators of soil health. Technical report 388. National Environmental Research Institute, Denmark

Noble R, Coventry E (2005) Suppression of soil-borne plant diseases with composts: a review. Biocontrol Sci Technol 15:3–20

Nocker A, Burr M, Camper AK (2007) Genotypic microbial community profiling: a critical technical review. Microb Ecol 54:276–289

Okur N, Kayikcioglu HH, Okur B, Delibacak S (2007) Organic amendment based on tobacco waste compost and farmyard manure: influence on soil biological properties and butter-head lettuce yield. Turk J Agr Forest 32:91–99

Pascual JA, Hernandez T, Ayuso M, Garcia C (1997) Changes in the microbial activity of arid soils amended with urban organic wastes. Biol Fertil Soils 24:429–434

Pascual JA, Avilés M, Aguirreolea J, Sánchez-Díaz M (2008) Effect of sanitized and non-sanitized sewage sludge on soil microbial community and the physiology of pepper plants. Plant Soil 310:41–53

Perez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. Soil Biol Biochem 38:460–470

Pinamonti F, Stringari G, Gasperi F, Zorzi G (1997) The use of compost: its effects on heavy metal levels in soil and plants. Resour Conservat Recycl 21:129–143

Postma J, Kok H (2001) Effect of compost application on the soil microflora. In: Applying compost – benefits and needs. Seminar Proceedings Brussels, 22–23 November 2001, pp 157–162

Ranalli G, Bottura G, Taddei P, Garavani M, Marchetti R, Sorlini C (2001) Composting of solid and sludge residues from agricultural and food industries. Bioindicators of monitoring and compost maturity. J Environ Sci Health A36:415–436

Ranjard L, Poly F, Lata J-C, Mougel C, Thioulouse J, Nazaret S (2001) Characterization of bacterial and fungal soil communities by automated ribosomal intergenic spacer analysis fingerprints: biological and methodological variability. Appl Environ Microbiol 67:4479–4487

Ros M, Garcia C, Hernandez T (2003) Soil microbial activity after restoration of a semiarid soil by organic amendments. Soil Biol Biochem 35:463–469

Ros M, Klammer S, Knapp BA, Aichberger K, Insam H (2006a) Long term effects of soil compost amendment on functional and structural diversity and microbial activity. Soil Use Manag 22:209–218

Ros M, Pascual JA, Garcia C, Hernandez MT, Insam H (2006b) Hydrolase activities, microbial biomass and bacterial community in a soil after long-term amendment with different composts. Soil Biol Biochem 38:3443–3452

Ros M, Goberna M, Pascual JA J, Klammer S, Insam H (2008) 16S rDNA analysis reveals low microbial diversity in community level physiological profile assays. J Microbiol Methods 72:221–226

Ryckeboer J, Mergaert J, Vaes K, Klammer S, De Clercq D, Coosemans J, Insam H, Swings J (2003) A survey of bacteria and fungi occuring during composting and self-heating processes. Ann Microbiol 53:349–410

Saison C, Degrange V, Oliver R, Millar P, Commeaux C, Montange D, Le Roux X (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environ Microbiol 8:247–257

Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870–4876

Seewald MSA, Singer W, Hecker-Denschlag N, Knapp BA, Hansel A, Franke-Whittle IH, Insam H. VOC emissions from compost amended soils. Submitted for publication.

Shindo H, Hirahara O, Yoshida M, Yamamoto A (2006) Effect of continuous compost application on humus composition and nitrogen fertility of soils in a field subjected to double cropping. Biol Fertil Soils 42:437–442

Tejada M, Gonzalez JL, Garcia-Martinez AM, Parrado J (2008) Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Bioresour Technol 99:4949–4957

Tejada M, Hernandez MT, Garcia C (2009) Soil restoration using composted plant residues: effects on soil properties. Soil Tillage Res 102:109–117

Tiquia SM (2005) Microbiological parameters as indicators of compost maturity. J Appl Microbiol 99:816–828

Tittarelli F, Petruzzelli G, Pezzarossa B, Civilini M, Benedetti A, Sequi P (2007) Quality and agronomic use of compost. In: Diaz LF, de Bertoldi M, Bidlingmaier W, Stentiford E (eds) Compost science and technology. Elsevier, Oxford, pp 119–157

Traulsen BD, Schönhard G, Pestemer W (1997) Risikobewertung der Anwendung von Bioabfallkomposten auf landwirtschaftlichen Nutzflächen. Agribiol Res 50:102–106

Vainio EJ, Hantula J (2000) Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol Res 104:927–936

Vinnerås B, Agostini F, Jönsson H (2010) Sanitisation by composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 171–192

von Wintzingerode F, Göbel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229

Wei Y, Liu Y (2005) Effects of sewage sludge compost application on crops and cropland in a 3-year field study. Chemosphere 59:1257–1265

Williamson N, Brian P, Wellington EMH (2000) Molecular detection of bacterial and streptomycete chitinases in the environment. Antonie Van Leeuwenhoek 78:315–321

Yogev A, Raviv M, Hadar Y, Cohen R, Katan J (2006) Plant waste-based composts suppressive to diseases caused by pathogenic *Fusarium oxysporum*. Eur J Plant Pathol 116:267–278

Zaman M, Matsushima M, Chang SX, Inubushi K, Nguyen L, Goto S, Kaneko F, Yoneyama T (2004) Nitrogen mineralization, N_2O production and soil microbiological properties as affected by long-term applications of sewage sludge composts. Biol Fertil Soils 40:101–109

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fertil Soils 29:111–129

Zhang M, Heaney D, Solberg E, Heriquez B (2000) The effect of MSW compost on metal uptake and yield of wheat, barley, and canola in less productive framing soils of Alberta. Compost Sci Util 8:224–235

Chapter 15
Production and Application of Bioorganic Fertilizers for Organic Farming Systems in Thailand: A Case Study

N. Teaumroong, C. Wanapu, Y. Chankum, W. Arjharn, S. Sang-Arthit, K. Teaimthaisong, and N. Boonkerd

Abstract As organic farming in Thailand has gained more momentum in recent years, many organic production projects have been initiated. Owing to the regulation of organic farming, synthetic chemical fertilizers are not allowed to be used. Therefore, the combination of organic fertilizers and plant growth-promoting rhizobacteria has been developed, commonly known as bioorganic fertilizers. Amendment of mature compost with mixed microbe cultures (*Azotobacter* sp. + *Azospirillum* sp. + *Trichoderma harzianum*) was conducted. In addition, the use of *Azolla microphylla* as a green manure together with bioorganic fertilizer for rice cultivation showed a satisfactory yield. In the case of vegetable (Chinese kale), *Sesbania rostrata* and cowpea cultivation were also used as green manures prior to bioorganic fertilizer application. The combination of green manures and bioorganic fertilizers demonstrated that green manures enhance the effect of bioorganic fertilizers so that it can become a potential alternative to conventional farming systems.

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15.1 Introduction

15.1.1 Aim of Organic Farming

Organic agriculture is a production system concerned with healthy and environmentally friendly food production. The inputs should be reduced by reusing, recycling and the efficient management of materials and energy, in order to maintain and improve environmental quality and conserve resources. The Food and Agricultural Organization (FAO) considers organic farming an essential strategy to improve the quality of food and to prevent health effects as a result of exposure to residual agrochemicals. Therefore, organic farming can be referred to as a farming system that does not use synthetic chemicals such as chemical fertilizers, pesticides and plant hormones or genetically modified organisms (GMOs). Organic farming systems need complex and integrated biological systems in order to achieve their goal of sustainable crop and livestock production. Improving soil fertility supports a diverse and active biotic community, and allows for an undisturbed decomposition (Foth and Ellis 1997; Mäder et al. 2002). It is dependent upon an understanding of the effects of management practices on soil fertility. This requires the provision of good on-farm advice by advisors who fully understand the complexity of managing soil fertility in organic farming systems. The development and widespread accessibility of appropriate tools to support decision-making is also important.

The term "organic" as applied to farming was first used in the United States by J.I. Rodale in 1940, who developed his idea based on the works of Sir Albert Howard (England), Rudolph Steiner (Germany) and Dr. William Albrecht (USA). In England, the term was developed at about the same time by Lord Northbourne

who described an integrated farm as a "dynamic living organic whole" (Scofield 1986). Organic farming is developing rapidly and is now practiced in more than 120 countries around the world. According to the latest survey on worldwide organic farming (Willer and Yussefi 2007), almost 31 million hectares are currently managed organically by at least 633,891 farms. This constitutes 0.7% of the agricultural land of the countries covered in the survey in 2007. In total, Oceania holds 39% of the world's organic land, followed by Europe (23%) and Latin America (19%). The countries with the greatest organic areas are Australia (11.8 million hectares), Argentina (3.1 million hectares), China (2.3 million hectares) and the United States (1.6 million hectares). The number of farms and the proportion of organically managed land when compared to conventionally managed systems, however, are highest in Europe.

The most important factors for production of crops under organic farming systems are soil fertility management and pest management. In this paper, soil fertility management through the use of biofertilizers (BFs) and bioorganic fertilizers (BOFs) will be discussed. To produce healthy crops, essential plant nutrient elements must be available in the soil throughout the period of cultivation.

15.1.2 Organic Agriculture in Thailand

In Thailand, organic agriculture is a part of the larger sustainable agricultural movement, initiated by farmers and local non-governmental organizations (NGOs) in the 1980s. The Alternative Agricultural Network (AAN) was established in 1984 as a national network, and provides a main discussion forum of experience sharing and policy advocacy for sustainable agriculture. As organic agriculture has gained more and more momentum in recent years, several organizations specializing in organic agriculture have also emerged. Many organic production projects have been initiated by government sectors, private company exporters and even NGOs. Thailand exports moderate amounts of organic rice, spices, fruits and vegetable, tea, coffee, cotton, cereals and honey (Thilmany et al. 2006), and approximately 25,000 hectares of farmland is now under organic management (www.actorganic.cert.or.th). The government policy promoting organic farming is generally favourable. The Thai government has placed its attention mainly on developing national standards, certification and accreditation. The NGO's standard as Organic Agricultural Thailand Certificate (ATC) also received a certificate of ISO Guide 65 from IOAS (International Organic Accreditation Services Inc.) in 2005 and a certificate of Accredited Organic Body of IFOAM (International Federation of Organic Agriculture Movements). In addition, research in the fields of soil fertility management, pest management and assessment of long-term impacts of whole farms is also encouraged.

The principal objections to the proposition that organic agriculture can contribute significantly to the global food supply are low yields and insufficient quantities of organically acceptable fertilizer. The main limiting macronutrient in agricultural

production is biologically available nitrogen (N) in most areas. Generally, nitrogen amendments in organic farming derive from crop residues, animal manures, organic fertilizers or composts, and biologically fixed N from leguminous plants. Organic fertilizers from manures and crop residues can be used as an alternative fertilizer source in organic farming where the use of manufactured chemical is prohibited. Therefore, the development of organic fertilizer formulations has increasingly become the center of attention.

15.1.3 BOF Perspectives

BF is a product comprised of beneficial microorganisms which enhance soil fertility and crop production either by fixing atmospheric nitrogen, by solubilizing soil phosphorus, or by stimulating plant growth through the synthesis of growth promoting substances (Shetty et al. 1994; Vessey 2003; Wu et al. 2005). In addition, the induction of systemic resistance against phytopathogens such as defense-related enzymes, L-phenylalanine ammonia lyase (PAL), peroxidase (Pox) and polyphenol oxidase (PPO) was also recognized in plants applied with BF (Dutta et al. 2008).

BOF is a product arising from the combination of a good quality organic fertilizer with an appropriate BF. *Azotobacter* sp. and *Azospirillum* sp. were used as the BF microorganisms in this study. These organisms belong to the group of plant growth-promoting rhizobacteria (PGPR), and are associative nitrogen-fixing bacteria and phytohormone (indole acetic acid: IAA) producing bacteria. In addition, *Trichoderma harzianum* was also amended as a biological control agent. In this study, we aim to demonstrate the production of 100 tons per month BOF and its application in agricultural farming and organic farming systems in Thailand. The quality control systems such as nutrient analysis of BOF, phytotoxicity tests and PGPR root colonization will also be discussed.

15.2 Materials and Methods

15.2.1 Materials Selection for BOF Production

Cassava peel and chicken manure were selected to produce organic fertilizer. The chemical properties analyses were summarized in Table 15.1. Seventy five tonnes of each raw material was loaded into an organic fertilizer production pilot plant (Fig. 15.1). Composting was conducted by mixing raw materials with a screwing operation unit prior to adjusting the moisture content to 60% water-holding capacity. The turning program was operated using the screwing operation unit for 7 h per day. Aeration was conducted underneath the pile when necessary. Temperature and C/N ratio changes were measured every 7 days. After maturation (27–30 days),

Table 15.1 Chemical properties of raw material used in organic fertilizer production

Raw materials	OM (%)	C (%)	N (%)	P (%)	K (%)	EC (dS m^{-1})	pH
Cassava peel	46.42	26.92	0.85	0.19	0.63	0.91	7.90
Chicken manure	45.69	26.5	2.90	2.80	3.68	13.3	6.90

Values are averages of three replicates

Fig. 15.1 The organic fertilizer pilot plant production with two sets of ribbon screws

1% (W/W) of each inoculum (*Azotobacter* sp. and *Azospirillum* sp.) was applied to the matured organic fertilizer via pop-up sprinklers.

15.2.2 Characterization and Production of PGPR

The *Azotobacter* and *Azospirillum* strains selected as PGPR for the study were chosen for their nitrogen-fixing ability. The acetylene reduction assay (ARA) was conducted to determine nitrogenase activity (Somasegaran and Hoben 1994). IAA production (Nuntagij et al. 1997), ACC-deaminase (Shah et al. 1998) and bioefficacy were also determined (Table 15.2). The bioefficacy test of PGPR with seeds of Pakchoi (*Brassica campestris* L. ssp. Chinese Lowr) and tomato (*Solanum lycopersicum*) were carried out as follows. Both Pakchoi and tomato seeds were surface sterilized for 2 min first with 1% sodium hypochlorite, followed by 1 min with 70% ethyl alcohol at room temperature. The seeds were washed extensively with sterile distilled water and allowed to germinate for 2 days in sterile dish plates with wet paper in the dark at 30°C. For inoculation, *Azotobacter* sp. and *Azospirillum* sp. were grown and diluted to 10^7 cfu ml^{-1}. Seedlings of approximately 1 cm in length were transferred to Petri dishes containing the cell suspensions and inoculated for 2 h. A sterile 0.8% NaCl solution was used for control seedlings. The seedlings were then transferred to a Leonard jar containing sterile sand and Hoagland's solution (Hoagland and Arnon 1938). Plants were grown at 25°C under light with a flux density of 450 $\mu Es^{-1}m^{-2}$ and 12–12 h light-dark regime. Plants were harvested 4 weeks after inoculation. The root length and total biomass were determined.

Table 15.2 Characteristics and bioefficacy tests of *Azotobacter* sp. and *Azospirillum* sp.

Bacteria	Bioefficacy test				Root colonization		IAA (μM mg^{-1} protein)	ARA (nM C_2H_2 d^{-1} mg^{-1} protein)	ACC-deaminase (μM mg^{-1} protein)
	Tomato (*Solanum lycopersicum*)		Pakchoi (*Brassica campestris*)		Tomato (*Solanum lycopersicum*)	Pakchoi (*Brassica campestris*)			
	Root length (cm)	Total Biomass (g dw)	Root length (cm)	Total Biomass (g dw)	(cfu g^{-1} root dw)	(cfu g^{-1} root dw)			
Azotobacter sp.	11.5 ± 2.82[b]	0.25 ± 0.02[c]	20.6 ± 1.02[c]	0.27 ± 0.08[b]	3.59×10^9 ± 1.12×10^6	1.03×10^9 ± 2.7×10^6	139.64 ± 2.11	1.166 ± 0.82	0.55±0.02
Azospirillum sp.	14.75 ± 0.35[c]	0.16 ± 0.03[b]	15.7 ± 1.27[b]	0.35 ± 0.04[c]	5.73×10^8 ± 8.2×10^5	2.41×10^9 ± 1.5×10^6	41.25 ± 6.9	0.64 ± .001	ND
Control	4.46 ± 1.41[a]	0.07 ± 0.01[a]	10.3 ± 0.30[a]	0.208 ± 0.07[a]	ND	ND	–	–	–

ND: Not detected

Values are averages of three replicates ± standard error.

Different letters within the same column and experiment indicate a significant difference at $P \leq 0.05$

The root colonization assay was performed as described by Vande Broek et al. (1998). To reduce the risk from contamination, *Azotobacter* sp. and *Azospirillum* sp. were grown in the nitrogen free media LG and NFB, respectively. For large scale production, each was cultivated in a 500-L bioreactor for 2 days. *Trichoderma harzianum* was cultivated by a solid-state fermentation. Briefly, 10^6 spores ml^{-1} were inoculated into 100 g of sterilized sorghum seed for 5 days prior to scaling up in 1 ton of sterilized saw dust containing the necessary nutrients for growth (incubation was at room temperature for 7 days). The inocula of *Azotobacter* sp., *Azospirillum* sp. and *T. harzianum* were applied to matured organic fertilizer and stored for 3 days inside the pilot plant before use.

15.2.3 Application of BOFs to Agricultural Cropping Systems

15.2.3.1 Application of BOFs to Vegetable Cropping Systems

The vegetable cropping system experiments were conducted over a total area of 16.8 m^2 per treatment in the Wangnamkeaw district (Nakhon Ratchasima province). The soil characteristics were 1.17% organic matter, 18.4 ppm P, 321 ppm K, pH 6.3 and EC. 2.8 (dS m^{-1}). In order to demonstrate the effect of the combination of BOF and nitrogen fixed via rhizobia-leguminous plants symbiosis, *Sesbania rostrata* and *Vigna unguiculata* (cowpea) were employed. The main experimental plot lay out was split plot. The main plots included plots planted with *S. rostrata*, plots planted with *V. unguiculata* and plots without green manure. The experimental variants were: (1) control (non-fertilized), (2) fertilized at recommended rate with chemical fertilizer for vegetable (N, P and K in the ratio 15:15:15) and (3) fertilized with BOF (5 kg per subplot). All plots were set up in triplicate.

The seeds of *S. rostrata* and cowpea were mixed with *Azorhizobium* sp. and *Bradyrhizobium* sp., respectively, prior to being planted in the experimental plots. After growing *S. rostrata* and cowpea for 60 and 45 days, respectively, fields were plowed, and left for 2–3 weeks before cultivation with Chinese kale.

15.2.3.2 Application of BOF to Rice Cultivation System

Rice field experiments were carried out over a total area of 80 m^2 per treatment in the Burirum province. The soil characteristics were 0.42% organic matter, 2.73 ppm P, 106.93 ppm K, pH 5.7 and EC 1.9 (dS cm^{-1}). The experimental layout was a randomized complete block design with seven treatments and three replicates per treatment. The treatments were : (1) control (non-fertilized), (2) fertilized with chemical fertilizer at the recommended rate for rice (7.5:3.75:2.5 ($\times 10^3$) kg N: P_2O_5:K_2O m^{-2}) (3) fertilized with farmer organic fertilizer (1.20% N, 0.95% P, 1.20% K, pH 7.75 and EC 5.25 dS m^{-1}) 36 kg per treatment, (4) fertilized with BOF 36 kg per treatment, (5) fertilized with *Azolla microphylla* 5 kg fresh weight per

treatment, (6) fertilized with half the amount of chemical fertilizer and BOF and (7) fertilized with BOF and Azolla. The data of rice yield were collected and analyzed by ANOVA and Duncan's multiple range test (DMRT)

15.2.4 Quality Control of BOFs

The plant nutrient contents of BOFs were analyzed by the Department of Soil Sciences, Faculty of Agriculture, Khonkaen University, as shown in Table 15.3. Total Kjeldahl nitrogen (TKN) and total organic carbon (TOC) were estimated by using the Micro-Kjeldahl method (Singh and Pradhan 1981) and Walkey and Black's Rapid Titration method (Walkley and Black 1934), respectively. The C/N ratio was derived from TOC/TKN values. Total phosphorus was determined spectrophotometrically (Olsen and Sommers 1982) while total potassium was detected by the flame emission technique and calculation according to Okalebo et al. (2002). The viable number of *Azotobacter* sp., *Azospirillum* sp. and *T. harzianum* cells was enumerated on LG, NFB and PDA media, respectively.

To detect the persistence of the applied PGPR in cultivation systems, the fluorescent antibody technique (FA) and denaturing gradient gel electrophoresis (DGGE) approaches were employed (see Chap. 7, Hultman et al. 2010; Chap. 1, Insam et al. 2010; Chap. 14, Knapp et al. 2010). For bacterial root colonization determination, the FA technique was applied using FITC conjugated goat anti-rabbit IgG (whole molecule), and observations were made under the fluorescent microscope (450–480 nM). The roots of Chinese kale were taken from the Suranaree University of Technology organic farm (BOF application for 3 years) and from the first inoculation in pot experiments. For DGGE analysis, DNA was extracted directly from the rhizosphere soil using the Ultraclean soil DNA kit (MoBio 101 Laboratories kit, Qbiogene Inc., Irvine, CA, USA.) The primers and PCR conditions were as described by Olivares et al. (1997).

15.3 Results of BOF Production and Application in Agricultural Cropping Systems

15.3.1 BOF Production and Its Characteristics

Cassava peel was chosen as the energy-providing material for the microbes in the composting process (Table 15.1). As there are more than ten big tapioca starch factories in the area of Nakhon Ratchasima province (30% production of whole country), the cost of cassava peel as a raw material was lower than when compared with other materials in this area, and it was considered an appropriate material for organic fertilizer production in this study. The C/N ratio of both raw materials

Table 15.3 Chemical properties and population numbers of PGPR and *Trichoderma harzianum* in BOF product

	OM (%)	N (%)	P (%)	K (%)	EC (ds cm^{-1})	pH	C/N ratio	*Azotobacter* sp. (cfu g^{-1})	*Azospirillum* sp. (cfu g^{-1})	*T. harzianum* (cfu g^{-1})
BOF	34.58	2.96	1.67	1.92	6.12	7.2	19.1:1	$1.87 \times 10^6 \pm 2.1 \times 10^4$	$4.3 \times 10^6 \pm 8.2 \times 10^3$	$1.3 \times 10^5 \pm 2.6 \times 10^2$
Thai organic fertilizer standard 2005*	≥30.0	1.0	0.5	0.5	≤6.0	5.5–8.5	≤20:1	–	–	–

Values are averages of three replicates

*The regulation announced by Ministry of Agriculture and Cooperatives

(mixed before composting process) was 30/35. The final C/N ratio of the mature compost was 19.1:1 and the temperature was 35 °C. The characteristics of mature BOF are summarized in Table 15.3. The matured compost could be obtained within 27–30 days of operation in the pilot plant.

The pilot plant was capable of production of 100 tons compost per month in the rectangular agitated bin with forced aeration. In addition, since *Azotobacter* sp. and *Azospirillum* sp. are capable of producing the IAA phytohormone and as *T. harzianum* has potential soilborne disease suppressiveness capacity, this process could produce 100 tons of BOF within 30 days. From the results of plant nutrient elements and seed germination in the final product of BOF (Tables 15.3 and 15.4), it could be preliminarily concluded that BOF is a good organic fertilizer when compared with the standard quality certified by Department of Agriculture, Thailand (Table 15.3). Nevertheless, the percentage seed germination from BOF application seemed to be better than that of the control and organic fertilizer treatments. This was especially the case for cucumber. Prior to applying BOF in the field experiment, a bioefficacy test under sterilized conditions was conducted (Table 15.2).

Figure 15.2 shows the effect of PGPR on root promotion. *Azotobacter* sp. and *Azospirillum* sp. provide IAA to plants. Inoculation with these organisms could enhance root growth and development. This might be explained by IAA being able

Table 15.4 Seed germination index of various vegetables

Vegetable seed	Vegetable seed germination index (%)		
	Distilled water	Organic fertilizer	BOF
Chinese kale	73.66 ± 3.09^{a}	71.0 ± 1.41^{a}	76.0 ± 2.4^{a}
Spinach	53.33 ± 3.67^{a}	61.33 ± 2.57^{b}	63.66 ± 1.17^{b}
Cucumber	76.66 ± 0.99^{b}	60.0 ± 2.07^{a}	86.66 ± 1.36^{c}

Values are average of three replicates ± standard error
Different letters with in the same row indicate significant difference was established at $P \leq 0.05$

Fig. 15.2 Effect of PGPR inoculation on root growth promotion. The plants were grown in axenic culture condition. On the left is Pakchoi (*Brassica campestris*) and on the right is Tomato (*Solanum lycopersicum*)

to ramify more root systems, which implies that there will be root proliferation absorbing a larger volume of nutrients, resulting in higher yields.

15.3.2 Application of BOF to Agricultural Cropping Systems

In order to compare the plant yields obtained by using BOF and chemical fertilizers in the field experiment, vegetables and rice plants were used. The results in Table 15.5 indicated that application of BOF could provide almost the same yield of rice as chemical fertilizer amendment. The use of BOF together with chemical fertilizer (half of recommended amount of each) resulted in the highest yield. The symbiotic nitrogen-fixing cyanobacterium, *Anabaena* sp., which lives in the leaf cavities of the aquatic fern *Azolla,* is capable of high rates of nitrogen fixation, and the application of *Azolla* as a green manure to rice fields is well documented. Combination of *Azolla microphylla* with BOF gave a higher yield than that of chemical fertilizer or BOF alone. This shows that different farming alternatives exist in order to reduce the application of chemical fertilizers, and still produce high yields of rice.

In the case of vegetable cultivation, results demonstrated that the inoculation of the leguminous plants *S. rostrata* and cowpea (*V. unguiculata*) with the appropriate rhizobial strains as green manures followed by plowing before BOF application could enhance the yield of Chinese Kale (Table 15.6). This demonstrates that application of green manure could enhance the effect of BOF to become more advantageous, thus confirming its replacement of chemical fertilizer application.

In addition, to ensure the effect of *T. harzianum* as a biological control agent, the experiments were carried out in a field where plants were affected by the cucumber wilting disease caused by *Fusarium*. The plot of cucumbers amended with BOF was located near the actual plot of farmer cultivation (no BOF application). Figure 15.3 illustrates plants affected by the wilt disease, and shows cucumber plants amended with BOF to be healthy. This indicates that the application of BOF can reduce the need for chemical fertilizers and some fungicides. Thus, BOF would be a beneficial production factor for organic farming practice.

Table 15.5 Rice yields obtained with different fertilizer treatments in field experiment (Burirum province)

Treatments	% Seed fill	Weight 100 seeds (g)	Rice yields (kg ha^{-1})
Control	75.22^{a}	2.087^{d}	2,505.87^{d}
Farmer organic fertilizer	79.85^{b}	2.342^{c}	3,318.87^{c}
Chemical	80.83^{b}	2.412^{bc}	3,813.43^{bc}
BOF	80.85^{cd}	2.455^{bc}	3,965.93^{bc}
Azolla microphylla	82.70^{c}	2.397^{bc}	3,667.18^{bc}
½ chemical + ½ BOF	85.34^{d}	2.605^{a}	4,980.25^{a}
BOF + *Azolla mycropyhlla*	84.72^{d}	2.470^{b}	4,146.75^{b}

Different letters within the same column and experiment indicate a significant difference at $P \leq 0.05$

Table 15.6 Effect of leguminous plants as green manure and BOF on the yield of Chinese Kale in a field experiment (Wangnamkeaw, Nakhon Ratchasima province)

Leguminous plants	Fertilizer	Fresh weight (g plant^{-1})	Chinese Kale Yield (kg ha^{-1})
Sesbania rostrata	Bioorganic fertilizer	52.00[a]	5,559.00[a]
	Chemical fertilizer	60.33[a]	6,492.75[a]
	Control (no fertilizer)	33.33[b]	3,555362[a]
Vigna unguiculata (cowpea)	Bioorganic fertilizer	71.66[a]	7,647.75[a]
	Chemical fertilizer	54.00[a]	5,760.00[a]
	Control (no fertilizer)	24.00[a]	2,559.81[b]
Non	Bioorganic fertilizer	55.00[a]	5,862.06[a]
	Chemical fertilizer	20.33[b]	2,180.75[b]
	Control (no fertilizer)	11.33[b]	1,208.93[b]

Different letters with in the same column and experiment indicate that significant difference was established at $P \leq 0.05$

Fig. 15.3 Wilting symptom of cucumbers in plots amended with bioorganic fertilizer (BOF) containing *Trichoderma harzianum* (*left*) and without BOF amendment (*right*)

15.3.3 Quality Control of BOF

In order to assess the persistence of PGPR in an agricultural system after BOF application, two approaches were used. To detect whether both *Azotobacter* sp. and *Azospirillum* sp. colonized the root plants, an FA-based technique was conducted. Figure 15.4 clearly demonstrates that roots of inoculated plants were heavily colonized by the inoculated PGPR when long-term BOF applications in organic farming were conducted, whilst the roots of Chinese Kale in the pot experiment were less colonized following a single BOF application. This again confirms that PGPR must firstly need an appropriate niche, such as the root for colonization. The persistence of both PGPR strains in the root rhizosphere was elucidated using DGGE (Fig. 15.5). Thus, both methodologies should be applied for quality control of BOF production and commercialization.

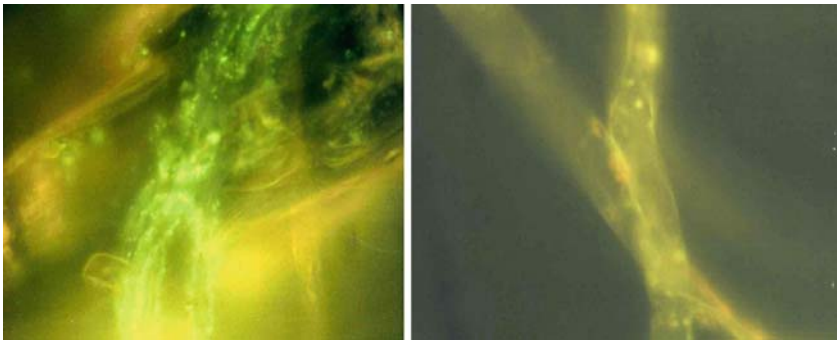

Fig. 15.4 Detection of PGPR root colonization by FA-based technique. Left is the root sample from organic farming where BOF has been applied for 3 years. Right is the root sample in a pot experiment (3 days after inoculation) with the roots of Chinese Kale

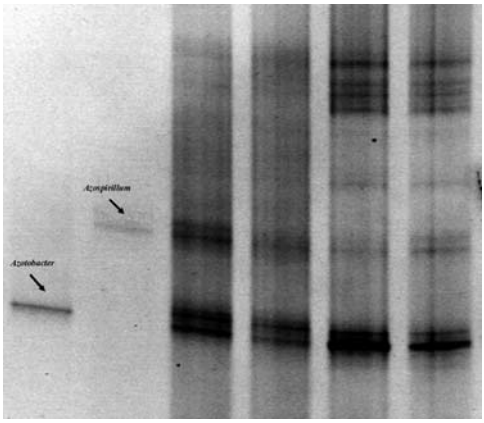

Fig. 15.5 DGGE of PCR-amplified 16S rDNA gene fragments from soil amended with bioorganic fertilizer (BOF) in organic farming system

15.4 Discussion

15.4.1 Factors Involved with the Production of a Good Quality Organic Fertilizer

To obtain a good quality organic fertilizer, several factors have to be considered. Low quality compost essentially arises from an excess of heavy metals, salts and a low degree of stabilization (Badgley et al. 2007). Some composts can have increased electrical conductivity (EC) because of high salt concentration and this can restrict seedling performance (Moral et al. 2008). Moisture is also one of the limiting factors not only necessary for composting but also for the control of the amount of greenhouse gas emissions. Tamura and Osada (2006) demonstrated that manure containing lower moisture control gave better composting conditions. They also found that NO_2 and CH_4 emissions during the composting process were estimated at 3.4–3.8 N_2O-N $kg^{-1}N$ and 3.1–53.7 CH_4 kg^{-1} organic material of the

initial materials. High greenhouse gas emission was observed from samples with higher moisture content.

Organic fertilizer amendment to soil is often viewed as a way to improve soil fertility and physical structure particularly because it can contribute to the stabilization of the aggregate erosion process. It also increases the amount of soil organic carbon and other major nutrients such as N and P. Bastida et al. (2008) demonstrated the long-term (17 years) effect of municipal solid waste application on microbial abundance and human-associated enzyme activities under semiarid soil in Spain. The amended plots showed greater humic substance-related enzymatic activities such as glucosidase (C), urease (N) and alkali phosphatase (P) cycles when compared with unamended plots as well as the formation of humic substances such as *o*-diphenol-oxidase. They also found greater microbial proliferation of both fungi and bacteria, accompanied by a change in soil microbial community structure. More details of the effect of organic amendment on soil microbial activity, organic carbon and nutrients can be found in Chap. 13 (Bastida et al. 2010).

Compost maturity (see Chap. 6, Ceustermans et al. 2010) is another important aspect in organic fertilizer production (see Chap. 1). It is achieved during the curing process. The duration of curing in the industry varies according to a number of factors including source materials, composting process and facility, climate and planned utilization of final product. Danon et al. (2008) examined the roles of succession transition of different bacterial phylogenetic groups involved in this process. DGGE, clone libraries and microarrays were used to reveal these roles. The results indicated that the Proteobacteria were the most abundant phylum in all the processes. The Bacteroidetes and the Gammaproteobacteria were ubiquitous. During the mid-curing stage, Actinobacteria were dominant. Different members of nitrifying bacteria and cellulose and macromolecule-degrading bacteria were found throughout the curing process. In contrast, pathogens were not detected. In the cured compost, bacterial population shifts were still observed despite compost organic matter and other biochemical properties having stabilized. Non-cured composts may be phytotoxic (Chap. 6). In our case, fundamental approaches such as observation of stability of C/N ratio, pile temperature and phytotoxicity were employed. Some other important factors such as humic acids, and NH_4^+ should, however, also be considered.

15.4.2 Impact of Organic Fertilizer Amendment on Soil Characterization

Organic amendment to soil generally results in an increase in various enzymatic activities (Sastre et al. 1996; Bandick and Dick 1999; Garcia-Gil et al. 2000; Perucci et al. 2000). The contribution of organic fertilizer-borne microflora on the impact of organic fertilizer amendment to soil microbial community structure is,

however, still not known. Recently, a study of the community structure of ammonia-oxidizing bacteria (AOB) in compost-treated soil by Innerebner et al. (2006) was conducted. The abundance of AOB in four composts (organic waste, cattle manure, green waste and sewage sludge) varied significantly. The total N content of the composts correlated well with AOB cell numbers. However, the composts did not appear to leave a direct microbial trace on soils, although nutrients in the compost did have an indirect effect on microbial community structure.

The long-term persistence of the effect of organic fertilizer amendment on the soil microbial community, or in contrast, the resilience of different characteristics of the soil microbial community after amendment, has been poorly studied (Crecchio et al. 2001; Marschner et al. 2003). However, Saison et al. (2006) demonstrated that organic fertilizer amendment affected the activity, size and composition of the soil microbial community. The effect of organic fertilizer amendment was mainly due to the physical-chemical characterization of the organic fertilizer matrix rather than of the organic fertilizer-borne microbes, and no resilience of microbial characteristics was observed 6–12 months after amendment with a high amount of organic fertilizer. Thus, there remains a need to characterize the response of more targeted microbial functional groups (e.g. nitrifiers, or antagonistic bacteria or fungi that induce pathogen suppression) after organic fertilizer amendment. Moreover, the study of microbial community structure and function after organic fertilizer amendment for different combinations of soil and compost is also needed to generalize our knowledge.

Since the major aim of an organic farming system is often not to maximize production, but rather to create sustainable plant cultivation, using green manure is an alternative approach for reducing N chemical fertilizer application. From our results, application of both *Azolla* and leguminous plants showed plant yield improvement. This is due to N availability from biological nitrogen fixation via symbiosis. The amount of N contributed from *Azolla microphylla, S. rostrata* and *V. unguiculata* (cowpea) was estimated at 40–60 kg N ha^{-1} crop, 93–186 kg N ha^{-1} and 40–60 kg N ha^{-1}, respectively.

15.4.3 Effect of PGPR on Plant Growth Promotion

With the use of PGPR gaining acceptance, numerous bacterial species have been isolated and their capacity to promote plant growth has been investigated. In the search for efficient PGPR strains with multiple attributes including production of IAA, N_2-fixation capability, HCN production, siderophore production, phosphate solubilization and pathogen antagonistic activity (see Chap. 8, de Bertoldi 2010), various genera of bacteria have shown promising results. These genera include *Azotobacter*, fluorescent *Pseudomonas* species, *Rhizobium* and *Bacillus* (Ahmad et al. 2008; Lawongsa et al. 2008). In the case of our study, PGPR strains were firstly isolated on the basis of nitrogen fixation and IAA production, and then chosen for plant growth promotion abilities. Two species of *Azotobacter* and

Azospirillum showed the most promising results among 835 isolates. However, these organisms did not show any antagonistic activity against soil-borne plant pathogens, and thus *T. harzianum* was added. This effect is clearly seen in Fig. 15.3. with *Fusarium* sp. suppression in farmed cucumber plots. The development of a single PGPR inoculum habouring both modes of BF and biocontrol should be the focus of further studies. Recently, Choi et al. (2008) found that *P. fluorescens* B16 could produce pyrroloquinoline quinine (PQQ) which significantly increased the height, flower number, fruit number and total fruit weight of tomato (*Solanum lycopersicum*). The cluster of genes encoding for PQQ are expressed only under nutrient-limiting conditions. An antioxidant mode of action of PQQ has been suggested by Choi et al. (2008). In addition, the number of bacteria that colonize the root of each plant should be considered as an important factor for selecting a good strain. Signal molecules exchanged between plant and microbes should be identified that favor beneficial plant colonization. Therefore, these parameters should be considered prior to producing PGPR as inoculum.

Ecological conditions affecting PGPR inoculant is an important factor. Baudoin et al. (2009) revealed the impact of *Azospirillum lipoferum* CRT1 inoculum (known as phytostimulatory PGPR via its IAA production) on the rhizobacterial community structure of field-grown maize. The results indicated that inoculation caused a shift in the structure of indigenous rhizobacterial community at 7 and 35 days after sowing. However, the effects of CRT1 inoculation took place without modifying the total number of root bacteria but enhanced the variability of the bacterial community. This is the first study on the ecological impact of *Azospirillum* inoculation on resident bacteria conducted in the field and showing that this impact can last at least 1 month. The different soil type on the stimulatory effect of PGPR was also demonstrated. The use of *P. alcaligenes* PsA15, *Bacillus. polymyxa* BcP26 and *Mycobacterium* MbP18 as inoculants, and their stimulatory effect on plant growth, N, P and K uptake in corn cultivated in calcareous calcisol and loamy sand soil types were investigated by Egamberdiyeva (2007). The results revealed that the stimulatory efficiency of PGPR inoculants was reduced in relatively rich loamy sand soil and increased in nutrient-deficient calcisol soil. In addition, combination of organic fertilizer or compost and vermicompost with selected PGPR strains also resulted in better plant growth. Hameeda et al. (2006) applied three types of compost to pearl millet (*Pennisetum glaucum* (L.) R. Br). All the three composts (77% rice straw compost, 55% *Gliricidia* vermicompost and 30% farm waste compost) showed a significant increase in the growth of pearl millet. Application of composts with the PGPR *Serratia marcescens* EB67 and *Pseudomonas* sp. CDB35 improved plant growth up to 88% (rice straw compost + EB67) and 83% (*Gliricidia* vermicompost + EB67). These results confirm the synergistic effect of selected PGPR application with composts on plant growth.

Selection and application of PGPR with a cropping system remains the most practical and affordable strategy available for use of these beneficial rhizosphere microorganisms in agriculture. A cropping system is generally defined as the integration of agricultural practices and plant genotypes. Cook (2007) showed the

buildup in response to monoculture cereals of specific genotypes of *P. fluorescens* with the ability to inhibit *Gaeumonnomyces graminis* var. *tritici* by the production of 2,4-diacetylphoroglucinol (DAPG) accounting for take-all decline in the US Pacific Northwest. The population of the same or other genotypes of DAPG-producing *P. fluorescens* could be enriched by other crops or cropping system. In addition, it is possible and logical that different genotypes will have the ability to produce one or more antibiotic or antibiotic-like substances inhibitory to other soilborne plant pathogens. The maintenance of threshold populations of resident PGPR inhibitory to *G. graminis* var. *trtici* is the centerpiece of an integrated system used by growers to augment take-all decline while also limiting damage caused by *Pythium*, *Rhizoctonia* root rot, *Fusarium* root, and crown rot in the direct-seed (no-fill) cereal-intensive cropping systems. However, the growing varieties of these cereals (winter and spring wheat, barley and triticale) were fully susceptible to all four root diseases.

15.5 Conclusions: Emerging Research, Education and Extension Needed for BOF Application

This research activity has mainly been conducted in Northeast Thailand. Soils in these parts are characterized by their sandy texture and low capacity for supplying nutrients. Inherent characteristics are typical indicators for poor soil fertilizer and major limiting factors for crop productivity. Therefore, one possible way to improve soil fertility is BOF application. In addition, actual world market prices of chemical fertilizer are increasing, thus replacement with BOF should be focused upon. The quality of our BOF has met the standard for Thailand. However, in terms of research and development, the new sources of agricultural waste in the northeast of Thailand such as digestate from biogas production of manure and spent mushroom should be developed for composting. In addition, manures containing high heavy metals such as copper should also be borne in mind. For future prospects, research, education and technology extension have to be considered globally. For future research needs, we have to improve understanding and management of soil fertility, pest management, assessment of long-term impact of whole-farm system and biodiversity conservation, etc. For education action, incorporating training in organic production to high school curricula, expanding undergraduate organic training in universities and increasing the level of technology, e.g. precise pest and fertility monitoring, seeds and breeds, improvement of PGPR efficiency, should also be encouraged.

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References

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res 163:173–181

Badgley C, Moghtader J, Quintero E, Zakem E, Chappell MJ, Katia Avilés-Vázquez K, Samulon A, Perfecto I (2007) Organic agriculture and the global food supply. Renew Agr Food Syst 22:86–108

Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471–1479

Bastida F, Kandeler E, Hernandez T, Garcia C (2008) Long-term effect of municipal solid waste amendment on microbial abundance and humus-associated enzyme activities under semiarid conditions. Microb Ecol 55:651–661

Bastida F, Hernández T, García C (2010) Soil degradation and rehabilitation: microorganisms and functionality. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 253–270

Baudoin E, Nazaret S, Mougel C, Ranjard L, Moënne-Loccoz Y (2009) Impact of inoculation with the phytostimulatory PGPR *Azospirillum lipoferum* CRT1 on the genetic structure of the rhizobacterial community of field-grown maize. Soil Biol Biochem 41:409–413

Choi O, Kim J, Kim J-G, Jeong Y, Moon JS, Park CS, Hwang I (2008) Pyrroloquinoline Quinone is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. Plant Physiol 146:657–668

Cook RJ (2007) Management of resident plant growth-promoting rhizobacteria with the cropping system: a review of experience in the US Pacific Northwest. Eur J Plant Pathol 119:255–264

Crecchio C, Curci M, Mininni R, Ricciuti P, Ruggiero P (2001) Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol Fertil Soils 34:311–318

Danon M, Franke-Whittle IH, Insam H, Chen Y, Hadar Y (2008) Molecular analysis of bacterial community succession during prolonged compost curing. FEMS Microbiol Ecol 65:133–144

de Bertoldi M (2010) Production and utilization of suppressive compost: environmental, food and health benefits. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 153–170

Dutta S, Mishra AK, Dileep Kumar BS (2008) Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol Biochem 40:452–461

Egamberdiyeva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36:184–189

Foth HD, Ellis BG (1997) Soil fertility. CRC, Florida

Garcia-Gil JC, Plaza C, Soler-Rovira P, Polo A (2000) Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biol Biochem 32:1907–1913

Hameeda B, Rupela OP, Reddy G, Satyavani K (2006) Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (*Pennisetum glaucum* L.). Biol Fertil Soils 43:221–227

Hoagland DR, Arnon DI (1938) The water-culture method for growing plants without soil. Univ Calif Agric Exp Stn Circ 347:1–39

Hultman J, Kurola J, Raininsalo A, Kontro M, Romantschuk M (2010) Utility of molecular tools for optimization of large scale composting. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 135–152

Innerebner G, Knapp B, Vasara T, Romantschuk M, Insam H (2006) Traceability of ammonia-oxidizing bacteria in compost-treated soils. Soil Biol Biochem 38:1092–1100

Insam I, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34

Knapp B, Ros M, Insam H (2010) Do composts affect the soil microbial community? In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 271–292

Lawongsa P, Boonkerd N, Wongkaew S, O'Gara F, Teaumroong N (2008) Molecular and phenotypic characterization of potential plant growth-promoting *Pseudomonas* from rice and maize rhizospheres. World J Microbiol Biotechnol 24:1877–1884

Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. Science 296:1694–1697

Marschner P, Kandeler E, Marschner B (2003) Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol Biochem 35:453–461

Moral R, Perez-Murcia MD, Perez-Espinosa A, Moreno-Caselles J, Paredes C, Rufete B (2008) Salinity, organic content, micronutrients and heavy metals in pig slurries from South-eastern Spain. Waste Manag 28:367–371

Nuntagij A, Abe M, Uchiumi T, Seki Y, Boonkerd N, Higashi S (1997) Characterization of *Bradyrhizobium* strains isolated from soybean cultivation in Thailand. J Gen Appl Microbiol 43:183–187

Okalebo JR, Gathua KW, Woomer PL (2002) Laboratory methods of soil and plant analysis. A working manual. 2nd Edn. In: UNESCO-ROSTA, Nairobi

Olivares FL, James EK, Baldani JI, Dobereiner J (1997) Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph *Herbaspirillum*. New Phytol 135:723–737

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis. Part 2 Chemical and microbial properties. American Society of Agronomy, Inc., Wisconsin, pp 403–430

Perucci P, Dumontet S, Bufo SA, Mazzatura A, Casucci C (2000) Effects of organic amendment and herbicide treatment on soil microbial biomass. Biol Fertil Soils 32:17–23

Saison C, Degrange V, Oliver R, Millard P, Commeaux C, Montange D, Le Roux X (2006) Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environ Microbiol 8:247–257

Sastre I, Vicente MA, Lobo MC (1996) Influence of the application of sewage sludges on soil microbial activity. Bioresour Technol 57:19–23

Scofield A (1986) Organic farming – the origin of the name. Biol Agric Hortic 4:1–5

Shah S, Li J, Moffatt BA, Glick BR (1998) Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. Can J Microbiol 44:833–843

Shetty KG, Hetrick BAD, Figge DAH, Schwab AP (1994) Effects of mycorrhizae and other soil microbes on revegetation of heavy metal contaminated mine spoil. Environ Pollut 86:181–188

Singh R, Pradhan K (1981) Determination of nitrogen and protein by Kjeldahl method. Forage Evaluation Science Pvt. Publishers Ltd, New Delhi, p 23

Somasegaran P, Hoben HJ (1994) Handbook for Rhizobia: Methods in legume-*Rhizobium* technology. Springer-Verlag, Heidelberg

Tamura T, Osada T (2006) Effect of moisture control in pile-type composting of dairy manure by adding wheat straw on greenhouse gas emission. Int Congr Ser 1293:311–314

Thilmany D, Colorado State University, Dept. of Agricultural and Resource Economics (2006) The US organic industry: important trends and emerging issues for the USDA. Colorado State University, Dept. of Agricultural and Resource Economics, Colorado

Vande Broek A, Lambrecht M, Vanderleyden J (1998) Bacterial chemotactic motility is important for the initiation of wheat root colonization by *Azospirillum brasilense*. Microbiol 144:2599–2606

Vessey JK (2003) Plant growth promoting *rhizobacteria* as biofertilizers. Plant Soil 255:571–586

Walkley AJ, Black IA (1934) Estimation of soil organic carbon by the chromic acid titration method. Soil Sci 37:29–38

Willer H, Yussefi M (2007) The world of organic agriculture: statistics and emerging trends, 2007. International Federation of Organic Agriculture Movements (IFOAM) Publication, Bonn

Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166

www. actorganic.cert.or.th/download/list/08FEB_ACTlist_emg.pdf

Chapter 16
Challenges, Options and Future Research Needs

Juan Luis Turrion-Gomez and Blanca Antizar-Ladislao

Abstract Microorganisms can contribute to solve current global challenges. The use of microorganisms to abate climate change, combat pathogens, improve soil fertility, and produce bioenergy is offering a great potential. Microorganisms facilitate the conversion of organic waste to soil amendment, or compost using composting approaches; where composting of organic waste is already established, the use of compost as a landfill cover to abate green-house gas emission shows to be promising. Bionergy is seen as one of the primary possibilities for preventing climate change. New techniques have been devised for the utilization of second generation biomass feedstock for energy production, including fermentation and anaerobic digestion. It is suggested that further research needs should be oriented towards the improvement of soil quality and fertility, the adaptation of agricultural management and technologies to climate change and the development of renewable energies.

Contents

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16.1 Global Challenges

To be ready and focused to meet the challenges of the twenty-first century, the United Nations Environment Programme (UNEP) highlights six priorities, which are climate change, disasters and conflicts, ecosystem management, environmental governance, harmful substances and resource efficiency. The United Nations and its Millennium Goals consider environmental sustainability to be one of the fundamentals rights of individuals. To achieve global environmental sustainability, four targets have been established, which include (a) the integration of the principles of sustainable development into country policies and programmes and reversion of the loss of environmental resources; (b) reduction of biodiversity loss; (c) reduction of the proportion of the population without sustainable access to safe drinking water and basic sanitation; and (d) achievement of a significant improvement in the lives of slum dwellers. According to the Food and Agricultural Organization of the United Nations (FAO) other global issues are avian influenza, biodiversity, bioenergy, climate change, food safety, trade, water and world food situation.

The aim of this final chapter is to highlight the future research needs to optimize the contribution of microorganisms to achieving these goals, particularly in the areas of waste management, soil degradation, population growth and diseases, energy demand and climate change. Microorganisms can contribute to solve current environmental challenges, and it is suggested that further research needs should be oriented towards the improvement of soil quality and fertility, the adaptation of agricultural management and technologies to climate change and the development of renewable energies. Research on the use of microorganisms to abate climate change is of particular interest and more efforts are recommended in this direction.

16.1.1 Waste Management

Historically, it was the scarcity of new materials that has been the driving factor for recycling of used materials. During the last decades in many countries, traditional recycling seems to have been gradually forgotten, and centralized landfills or incineration plants are often a simple answer to growing waste problems. In countries where population density is high and the value of preserved nature along with minimal carbon foot-print is increasingly demanded, many of them in Europe, environmentally sound waste management programmes have been introduced.

According to the European Environment Agency (EEA) (2007), Europe (EU-15) and EFTA (Iceland, Norway, Switzerland, and Liechtenstein) produce almost 1,000 mio tonnes of waste each year. The United Kingdom alone produces more than 400 mio tonnes of waste each year, and of this, about 35 mio tonnes is municipal solid waste. The problem of waste disposal is recognized as one of the most serious environmental problems. Composting, a waste management strategy that exploits microorganisms to do the job, has been suggested as one sustainable

option. There are three main reasons for the growth of the composting industry in the UK: legislation for biodegradable municipal solid waste, environmental benefits and economic benefits. Green-waste comprised the majority (92% in 1998) of municipal wastes produced in the UK. The three main regulatory drivers for composting are the EU landfill directive (EC 1999), the UK Waste Strategy 2000 (DETR 2000) and the EU Animal By products Regulations (EC 2003). These have increased interest in composting of garden, tree, and food-processing organic wastes. As a result, in 2005/2006, 3.4 mio tonnes of source segregated waste was composted, an increase of 28% compared with the previous year. The majority of waste was composted using open air mechanically turned windrows, with only 14% composted in-vessel. Agriculture was the largest market sector, with one million tonnes supplied, being the majority used on arable and cereal crops. But, also horticulture and landfill restoration cover accounted for approximately a quarter of a million tonnes each. The annual turnover of the composting and biological treatment industry was estimated at just over £90 million (Nikitas et al. 2008).

For a very long time, little research has been dedicated to improve microbial processes for waste management, but this seems to be changing. Habitats that are so extremely variable in both time and space like compost heaps are increasingly attracting scientists. Optimization of other waste management strategies that exploit the use of microorganisms including anaerobic digestion, enzymatic hydrolysis and fermentation is also receiving a great amount of attention (see Chap. 2, Braun et al. 2010; Chap. 4, Wett and Insam 2010).

Future research needs in this area include the improvement of rapid and robust measurement tools, identification and isolation of efficient microorganisms under different conditions of temperature, substrate or humidity, study of the metabolic processes of degradation and development of new added-value products.

16.1.2 Land Degradation and Soil Erosion

Land degradation is arguably one of the major global environmental challenges. Land degradation leads to a significant reduction of the productive capacity of land. Human activities contributing to land degradation include unsuitable agricultural land use, poor soil and water management practices, deforestation, removal of natural vegetation, frequent use of heavy machinery, overgrazing, improper crop rotation and poor irrigation practices. Natural disasters, including droughts, floods and landslides, also contribute to land degradation. Soil erosion is a major factor in land degradation and has severe effects on soil functions – such as the soil's ability to act as a buffer and filter for pollutants, its role in the hydrological and nitrogen cycle, and its ability to provide habitat and support biodiversity. About 2,000 mio ha of soil, equivalent to 15% of the Earth's land area, have been degraded through human activities. The main types of soil degradation are water erosion (56%), wind erosion (28%), chemical degradation (12%) and physical degradation (4%). Causes of soil degradation include overgrazing (35%), deforestation (30%), agricultural

activities (27%), overexploitation of vegetation (7%) and industrial activities (1%) (UNEP 2002).

Soil salinisation is a major problem that may occur in two ways: (a) intrusion of saline seawater into deep coastal aquifers and (b) evaporation of excess irrigation water often associated with poor soil drainage, which leaves dissolved salts in the soil. A reduced expansion of irrigated area by the year 2020 and an increased investment in drainage to deal with salinisation are expected (Garrett 2005). Nevertheless, problems of salinisation will still remain or even increase, as irrigation systems with inadequate drainage continue to age. On the other hand, a considerable amount of unsustainable irrigated land is projected to go out of production and new opportunities for rehabilitation of degraded lands and sustainable pasture management systems are expected to be developed.

Microorganisms will play an essential role in the rehabilitation of degraded lands, as they contribute to the conversion of carbon, nitrogen and other elements in the environment and increase soil fertility. Microorganisms affect soil structure by producing metabolites that bind soil particles together and physically enmesh particles with filaments. This stabilization of micro-structures may be carried through to stabilization of meso-structure, leading to sustained increases in porosity, at a number of scales. This, in turn, has implications for the capacity of soils to retain water since such properties relate to pore architecture and the range and location of hydrophobic and hydrophilic sites within the pore network (Allton et al. 2007).

Soil stability is important for landscape dynamics with respect to their ability to cope with externally forced change, such as wet–dry cycles and land management practices. Cropping and tillage practices are already known to influence run-off and soil losses, and to influence the microbial community (Jackson et al. 2003). Compost and biochar are extensively used as soil amendments. The impact of these amendments on the microbial structure will have a direct impact on the soil structure and thus requires further research. Other future research needs in this area include (a) the identification of microbial components which contribute to soil structure stability, (b) the understanding of the processes governing a microbiologically active soil, and (c) the relationships between the soil microorganisms, soil structure and hydrology.

16.1.3 Population Growth, Food Demand and Diseases

According to the last UN 2008 Revision of World Population Prospects (UN 2009), world population is projected to reach 7 billion early in 2012, up from the current 6.8 billion, and surpass 9 billion people by 2050. Most of the additional 2.3 billion people will enlarge the population of developing countries, while the population of the more developed regions is expected to change minimally.

There are concerns that water scarcity, soil depletion, the lack of technology adoption and dissemination, political and civil conflict, and the continued threat of

human disease epidemics, plant diseases and animal diseases pose a grave threat to the food security of growing populations in the developing world.

There are ominous signs. Progress in hunger reduction slowed considerably during the late 1990s: between 1995 and 2001, the number of undernourished people in the developing world increased by more than 18 mio. If China is excluded from consideration, the number of undernourished people in the developing world increased by nearly 28 mio during this period. In addition, there are indications that price fluctuations are rising as world cereal stocks are reduced. Moreover, micronutrient malnutrition is widespread, and its consequences are significant. The majority of the world's hungry people depend heavily, both directly and indirectly, on growth in the agricultural sector for both food and their livelihoods, either as farmers or as net purchasers of food. Most of the world's hungry, approximately 80%, live in rural areas, where access to markets, health care, education, and infrastructure such as telecommunications and roadways is scarce. These areas are often characterized by poor quality of natural assets, a fragile natural resource base.

At present, low agricultural investments undermine the development of new agricultural technology and contribute to marginal levels of irrigation efficiency and lack of improvement in water use efficiency. In addition, investments in many sectors, including education, social services, and health, are low in developing countries. The lack of growth in agricultural yields is the outcome of all of the above and also partly a result of weak income growth in developing countries and only moderate income growth in industrialized countries. Future research needs in this area should focus in the implication of microorganisms in (a) improving water management and irrigation efficiency, (b) adaptation to climate change, (c) increase of crops yields, and (d) improved strategies to combat pest problems in agriculture. All these will contribute to the increase of crop production and thus facilitate food security.

16.1.4 Energy Demand

According to the reference case projection from the "International Energy Outlook 2008," the world energy consumption is projected to expand by 50% from 2005 to 2030 (EIA 2008). Energy demand in the Organisation for Economic Co-operation and Development (OECD) economies is expected to grow slowly over the projection period, at an average annual rate of 0.7%, whereas energy consumption in the emerging economies of non-OECD countries (including India and China) is expected to expand by an average of 2.5% per year.

High prices for oil and natural gas are expected to continue throughout the period and are likely to slow the growth of energy demand in the long term, nevertheless world energy consumption is projected to continue increasing strongly as a result of robust economic growth and expanding populations in the world's developing countries. Thus, high prices for oil and natural gas as well as

rising concern about the environmental impact of fossil fuel use improve the prospects for renewable energy and coal with consumption increasing by 2.1% and 2.0%, respectively. As coal's costs are comparatively low relative to the costs of liquids and natural gas, and abundant resources in large energy-consuming countries make coal an economical fuel choice. In the absence of policies or legislation that would limit the growth of coal use, the United States, China, and India are expected to turn to coal in place of more expensive fuels. The only countries for which decreases in coal consumption are projected are OECD Europe and Japan, where populations are either growing slowly or declining, electricity demand growth is slow, and natural gas, nuclear power, and renewables are likely to be used for electricity generation rather than coal. Much of the growth in renewable energy consumption is projected to come from mid- to large-scale hydroelectric facilities in non-OECD Asia and Central and South America, where several countries have hydropower facilities either planned or under construction.

Europe, where many countries are obligated to reduce greenhouse gas emissions under the Kyoto Protocol treaty, has set a target of increasing the renewable energy share to 20% of gross domestic energy consumption by 2020, including a mandatory minimum of 10% for biofuels. Future research needs in this area should focus in (a) optimisation of microbial processes (e.g., hydrolysis, fermentation) to produce biofuels, including biogas, (b) development of novel enzymatic processes to reduce the cost of biofuel production, (c) development of microbial fuel cells, and (d) production of biofuels from microorganisms, e.g., biodiesel from algae.

16.1.5 Climate Change

Climate change and the vulnerability of energy supplies are two of the biggest threats to our security. Without urgent action, irreversible changes to the climate system are possible. According to the Intergovernmental Panel on Climate Change (IPCC) climate change refers to a change in the state of the climate that can be identified (e.g. using statistical tests) by changes in the mean and/or the variability of its properties and that persists for an extended period, typically decades or longer. It refers to any change in climate over time, whether due to natural variability or as a result of human activity.

IPCC's Third Assessment Report stated that there are evidences that human activities are attributable of the warming observed over the past 50 years (IPCC 2001). Carbon dioxide (CO_2) is the greenhouse gas that makes the largest contribution from human activities. It is released into the atmosphere by for example the combustion of fossil fuels, the burning of, for example, forests during land clearance and from certain industrial and resource extraction processes and composting. In addition to CO_2, the composting process naturally produces some methane (CH_4) and nitrous oxide (N_2O). Methane (CH_4) has a global warming potential 23 times higher than CO_2, and the largest source of CH_4 emission is from landfill sites

(during the anaerobic methanogenic stage), where it escapes through the landfill cover into the atmosphere. N_2O is also a greenhouse gas with an extremely high global warming potential (~310) and it can also be generated in landfills through the processes of nitrification and denitrification; is linked to methanotrophic activity and therefore cannot be ignored when considering methods to reduce global warming (Chapman and Antizar-Ladislao 2007).

According to the UN Framework Convention on Climate Change, a stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate change should be achieved. Technological options for reducing net CO_2 emissions to the atmosphere include: (a) reducing energy consumption, for example by increasing the efficiency of energy conversion and/or utilization (including enhancing less energy-intensive economic activities), (b) switching to less carbon intensive fuels, for example natural gas instead of coal, (c) increasing the use of renewable energy sources which emit little or no net CO_2, (d) sequestering CO_2 by enhancing biological absorption capacity in forests and soils, (e) capturing and storing CO_2 chemically or physically. Further research in this area should focus in enhancing biological absorption capacity in forests and soils where microorganisms play a crucial role. The design or selection of microorganisms for CO_2 capture presents a great potential to reduce greenhouse gas emissions to the atmosphere.

16.2 Contribution of the Microorganisms to Solve Current Global Challenges

Microorganisms can contribute to solve current global challenges, particularly those aforementioned using a number of different strategies, such as the conversion of organic waste to soil amendment or to energy. Use of microorganisms to abate climate change, combat pathogens, improve soil fertility are also offering a great potential. Here a few microbial strategies are highlighted. They have been selected as they offer a great potential for a sustainable development.

Agriculturalists since ancient times have recognized significant benefits of soil organic matter to crop production, including (a) it serves as a slow release source of N, P and S, for plant nutrition and microbial growth; (b) it possess considerable water-holding capacity; (c) it acts as a buffer against changes in pH of the soil; (d) its dark colour contributes to absorption of energy from sun and heating of the soil; (e) may act as plant-growth stimulants; and (f) binds micronutrient metal ions in the soil (McCarthy et al. 1990).

Composting is an aerobic process where organic materials are biologically decomposed. Conventional composting processes typically comprise four major microbiological stages in relation to temperature: mesophilic, thermophilic, cooling and maturation, during which the structure of the microbial community also changes, and the final product is compost (see Chap. 1, Insam et al. 2010; Chap. 5,

Domínguez et al. 2010; Chap. 12, Minz et al. 2010; Chap. 13, Bastida et al. 2010). Compost has been widely used as soil conditioner and soil fertilizer. This practice is recommended, as soil fertility needs more than ever to be sustained. Food demand is increasing rapidly in non-OECD countries, and it is in those countries particularly where organic waste needs to be diverted from landfill sites to composting practices, so compost can enhance soil fertility. In OECD countries, where composting of organic waste is already established, also its use as a landfill cover to abate greenhouse gas emissions has shown to be promising (Chapman and Antizar-Ladislao 2007). The addition of compost can minimize land degradation and soil erosion. Additionally, composting can contribute to achieve sufficient hygienisation of organic wastes and control soil born and air born pathogens by promotion of beneficial microorganisms and suppression of harmful microorganisms (see Chaps. 11–14) and Insam et al. (2002).

Frequently agrochemicals are not applied following a sustainable approach, and some undesired consequences have been observed such as disease resistances, elimination of beneficial microbes, plant toxicity and contamination of soil, rivers or aquifers. Alternatives to chemical control or synergic strategies are thus necessary for a more sustainable control of pathogens. In addition with climate change and globalization new diseases are appearing that need to be fought. The role of microorganisms to abate climate change should be further explored, and thus further research is needed for example to improve the use of compost to oxidize methane emitted at landfill sites to carbon dioxide, as methane is a potent greenhouse gas, with a global warming potential 23 times higher than carbon dioxide.

Biomass waste releases carbon as it decomposes, but it can be burned in a kiln by pyrolysis (burning of biomass under controlled, low-oxygen conditions) to create biochar. A lot of attention is given to the use of biochar and its use to mitigate greenhouse gas emissions. Biochar has the potential of sequestering bions of tonnes of carbon in the world's soils, specifically from agriculture and forestry residual biomass. It is suggested that the biochar system will sequester carbon for at least 100 years as compared to plants or trees that will only sequester carbon for 15 or 20 years. Biochar also provides plenty of surface area for beneficial fungi and bacteria to grow, which seems to reduce the need for fertilizers. It has been suggested that biochar can be used to address some of the most urgent current environmental problems, such as soil erosion, food insecurity, water pollution from agrochemicals, and climate change (Tenenbaum 2009). Further research is required to obtain reliable data on the agronomic and carbon sequestration potential of biochar, and also on the use of pyrolysis to optimize biochar properties. The ability of biochar to decrease emissions of nitrous oxide and methane is intriguing and requires further research.

Bionergy is seen as one of the primary possibilities for preventing global warming. At present, the immediate factor impeding the emergence of an industry converting biomass into liquid fuels or biogas on a large scale is the high cost of processing, rather than the cost or availability of feedstock (EEA 2008). The goal of second generation biofuels is to extend the amount of biofuel that can be produced sustainably by using biomass comprised of the residual non-food parts of current

crops, as well as other crops that are not used for food purposes and also municipal, industrial and construction waste. Second generation biofuels are expected to reduce net carbon emission, increment energy efficiency and reduce energy dependency, potentially overcoming the limitations of first generation biofuels. New techniques have been devised for the utilization of second generation biomass feedstock for energy production, including thermo-chemical conversion (i.e. combustion, gasification, pyrolysis, liquefaction, hydrothermal upgrading), biochemical conversion (i.e. fermentation and anaerobic digestion) and extraction of vegetable oils. Direct combustion, gasification, pyrolysis, liquefaction or hydrothermal upgrading are chemo-technical approaches. Another approach is to develop a process that works universally for all feedstock, converting carbon-based feedstock into hydrogen and carbon monoxide plus remaining components. This could use coal or natural gas and turn it into liquid fuels combining microbes that turn the "synthesis gas – syngas" straight into ethanol. Fermentation is an anaerobic process by which yeast converts sugars such as glucose, fructose and sucrose into ethanol and carbon dioxide. The anaerobic digestion process consists of three steps: a hydrolysis step in which organic compounds, such as polysaccharides, proteins, and fat are hydrolyzed by extracellular enzymes, an acidification step in which the products of the hydrolysis are converted into H_2, formate, acetate and higher molecular weight volatile fatty acids, and a third step in which biogas, a mixture of carbon dioxide and methane, is produced from hydrogen, formate, and acetate. The complete methanogenic conversion occurs by mixed microbiological communities yielding methane as the sole reduced organic compound (see Chap. 3, Plugge et al. 2010). Only bioethanol and biodiesel are presently produced as fuel on an industrial scale. Including ethyl-tertio-butyl-ether partially made with bioethanol, these fuels make up more than 90% of the biofuel market (Boehmel et al. 2008).

References

Allton KE, Harris JA, Rickson RJ, Ritz K (2007) The effect of microbial communities on soil hydrological processes: A microcosm study utilising simulated rainfall. Geoderma 142:11–17

Bastida F, Hernández T, García C (2010) Soil degradation and rehabilitation: microorganisms and functionality. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 253–270

Boehmel C, Lewandowski I, Claupein W (2008) Comparing annual and perennial energy cropping systems with different management intensities. Agric Syst 96:224–236

Braun R, Drosg B, Bochmann G, Weiß S, Kirchmayr R (2010) Recent developments in bio-energy recovery through fermentation. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, Heidelberg

Chapman M, Antizar-Ladislao B (2007) Biotic landfill CH_4 emission abatement using bio-waste compost as a landfill cover. In: Lehmann EC (ed) Landfill research focus. Nova Science, New York, pp 27–52

Domínguez J, Aira M, Gómez-Brandón M (2010) Vermicomposting: earthworms enhance the work of microbes. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 93–114

EC (1999) European Commission. Council Directive on the Landfill of Waste (1999/31/EEC). European Commission
EC (2003) European Commission. EU Animal by-products Regulations (2003/31/EEC). European Commission
DETR (2000) Department of the Environment, Transport and the Regions. Waste Strategy 2000: England and Wales. The Stationary Office, London
EEA (2007) The road from landfilling to recycling: common destination different routes. Brochure No 4/2007
EEA (2008) State of renewable energies in Europe 2006. http://www.energies-renouvelables.org/observer/stat_baro/barobilan/barob-ilan6.pdf. Accessed 8 May 2009
EIA (Energy Information Administration) (2008) International Energy Outlook 2008. http://www.eia.doe.gov/oiaf/ieo/world.html. Accessed 21 September 2009
Garrett JL (2005) 2020 Vision for food, agriculture and the environment. International Food Policy Research Institute (IFPRI), Washington. http://www.ifpri.org/2020/dp/dp06.pdf. Accessed 21 September 2009
Insam H, Riddech N, Klammer S (2002) Microbiology of composting. Springer-Verlag, Berlin, p 632
Insam H, Franke-Whittle IH, Goberna M (2010) Microbes in aerobic and anaerobic waste treatment. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 1–34
IPCC (2001) Climate change 2001: the scientific basis. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (eds),Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, UK
Jackson LE, Calderon FJ, Steenwerth KL, Scow KM, Rolston DE (2003) Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma 114:305–317
McCarthy P, Clapp CE, Malcolm RL, Bloom PR (1990) Humic substances in soil and crop sciences: selected readings. American Society of Agronomy, Inc. Soils Science Society of America, Inc. Madison, WI, USA
Minz D, Green SJ, Ofek M, Hadar Y (2010) Compost microbial populations and interactions with plants. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 231–252
Nikitas C, Pocock R, Toleman I, Gilbert EJ (2008) The state of composting and biological waste treatment in the UK 2005/06. The Composting Association
Plugge CM, van Lier JB, Stams AJM (2010) Syntrophic communities in methane formation from high strength wastewaters. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer-Verlag, Heidelberg
Tenenbaum DJ (2009) Biochar: carbon mitigation from the ground up. Environ Health Perspect 117:A70–A73
UN (2009) 2008 revision of world population prospects. http://www.un.org/esa/population/unpop.htm. Accessed 8 May 2009
UNEP (2002) Global Environment Outlook 3 (GEO-3). United Nations Environment Programme
Wett B, Insam H (2010) Biogas technology–Controlled gas flow for enhanced mixing, heating, and desulfurization. In: Insam H, Franke-Whittle IH, Goberna M (eds) Microbes at work. From wastes to resources. Springer, Heidelberg, pp 79–92

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