





Review

# Food Waste Composting and Microbial Community Structure Profiling

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**Abstract:** Over the last decade, food waste has been one of the major issues globally as it brings a negative impact on the environment and health. Rotting discharges methane, causing greenhouse effect and adverse health effects due to pathogenic microorganisms or toxic leachates that reach agricultural land and water system. As a solution, composting is implemented to manage and reduce food waste in line with global sustainable development goals (SDGs). This review compiles input on the types of organic composting, its characteristics, physico-chemical properties involved, role of microbes and tools available in determining the microbial community structure. Composting types: vermi-composting, windrow composting, aerated static pile composting and in-vessel composting are discussed. The diversity of microorganisms in each of the three stages in composting is highlighted and the techniques used to determine the microbial community structure during composting such as biochemical identification, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), terminal restriction fragment length polymorphism (T-RFLP) and single strand-conformation polymorphism (SSCP), microarray analysis and next-generation sequencing (NGS) are discussed. Overall, a good compost, not only reduces waste issues, but also contributes substantially to the economic and social sectors of a nation.

**Keywords:** organic food waste; sustainability; composting; microbial community structure

## 1. Introduction

The need for food has its impacts on the environment in ways we never expected, from emissions of greenhouse gasses, water and air pollution, abstraction of water, loss of biodiversity, land-use change, eventually risking food security and sustainability. Statistics from Food and Agriculture Organization (FAO) and United Nation (UN) projects food demand to rise meeting the global population estimated at approximately 10 billion in 2050 [1]. Since the demand for food is resource-dependent, food loss and

waste accumulation are generated. Approximately one-third of the food produced which is equivalent to 1.3 billion tons of food is being wasted in the world each year and private households represent the largest contributor to food wastes [2]. Based on the latest United Nations report, approximately 821 million people suffer from hunger and over 150 million children are stunted due to malnutrition in 2017. Food loss and wastage is a constant problem at all stages from production to consumption. Waste is generated at the processing stage from transporting of material to processing and up to distribution. Even at the retailing stage, wastage has been recorded. Eventually, as much as 40% is lost at the consumption stage in households and commercial sector [3]. Such magnitude of food wastage results in huge economic losses and a lot of needless hunger, in addition to climate and environmental issues. In line with the Sustainable Development Goal 12 (SDGs) of Responsible Consumption and Production to substantially reduce waste generation through prevention, reduction, recycling and reuse by 2030, composting is seen as a solution to properly manage waste to promote good health and well being through sustainable practices [4].

Food waste impacts the environment as discarded food waste would rot in the landfill discharging methane gas, a very potent gas that causes the greenhouse effect [5]. As environmental, health and social implication became concerning, from a managerial perspective, authorities started shifting from prevention and control to introducing frameworks such as the '3Rs' (Reduce, Re-use, Recycle), polluter pays principle and Sustainable Consumption and Production (SCP). Observation shows that awareness is highest when legislation is enforced, such as taxed bin bags which is trending nowadays. Most food-based sectors resort to building collaborations with other stakeholders such as suppliers, associations, local authorities and waste management companies. Under sustainable resource management, the concept of waste is wealth takes form, thus organic food waste composting is aggressively promoted in which food waste such as fruits, vegetables, grains, eggshells and dairy products are broken down into smaller particles and decomposed naturally [6]. Organic food waste composting minimizes the impact on many sectors. For instance, reduction of methane gas production from landfills directly minimizes the greenhouse effect, production of compost material reduces the dependability on pesticides and synthetic fertilizers resulting in the natural balance of pH level of the soil [7]. Various types of organic food waste composting systems are implemented worldwide with techniques such as vermi-composting, windrow composting, aerated static pile composting and in-vessel composting and are of considerable interest.

Organic food waste composting is only possible with the involvement of function-specific microbial community [8]. Microbial community comprises of a variety of microbial species ranging from culturable and non-culturable strains that can interact with each other in the environment they co-exists in [9]. During food waste composting, these microorganisms would break down the food waste into smaller particles before composting. The microbial community structure is crucial in determining the compost maturity as it will modify and evolve during the composting process. Several methods are used to determine microbial community structure in composting such as the conventional biochemical tests, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) [10], terminal restriction fragment length polymorphism (T-RFLP) [11] and single strand-conformation polymorphism (SSCP) [12], microarray analysis [13] and next-generation sequencing (NGS) based 16S ribosomal ribonucleic acid (rRNA) sequencing [14].

### *1.1. Organic Food Waste Composting*

Organic food waste composting is a type of natural food waste decomposition process under controlled aerobic conditions whereby food wastes are being broken down into their simplest components by microorganisms [8]. Composting eventually will be able to improve soil structure, texture and aeration and improve the water retention capacity of the soil [15]. The simplest components would then be formed into humus which is known as compost [7]. During composting the volume of the accumulated waste reduces over time to produce a stable product that has high nutrient level that resulted from the transformation of raw organic materials through microbiological transformation.

This organic matter rich compost is used as a natural fertilizer in the agriculture sector as it has a positive effect on soil and the environment since it is rich with fiber and inorganic nutrients. Stable compost product has higher nutrient level compare to raw organic materials and does not produce metabolites intermediate that can interfere with the growth of plants [16]. This high nutrient compost improves soil fertility and is able to meet crop nutrient demand resulting in improved crop production. Composting process requires a few essential elements which are humus, rich compost, organic waste and oxygen [17].

During the composting process, microorganisms play an important role in converting organic material into stable material through the various biochemical processes, producing fiber-rich carbon containing humus rich in inorganics such as nitrogen, phosphorus. The final output of composting can be utilized as fertilizers and soil amendments, which are rich in humic substances [18]. There are two stages in composting; 1) characterization by microbial activity and 2) conversion of organic material. During the first stage the microbiome initiates the composting process by increasing temperature through the oxidation of organic material, decomposing majority of the biodegradable material and increases the stability of the organic residue. The microbiome during composting includes bacteria, fungi and protozoa which varies depending on the temperature, moisture content, C/N ratio and nature of organic materials [19]. In this stage, easily degradable compounds and oxygen are consumed, whereas pathogens, weed and phytotoxins are eliminated. In the midst of degradation, the microorganisms work on the bulk raw material through chemical synthesis to produce precursors to humic material. During this process, degradation of hemicellulose, cellulose and lignin contributes to the production of humic material while degradable components are mineralized into carbon dioxide and ammonia [20]. The formation of precursors to humic substances can take place either through the degradation of lignin to phenolics and quinones or through the condensation of small molecules such as polysaccharides and proteins. Both these approaches do not co-exist, however polymerization and condensation of degraded lignin products with N-based compounds contribute to the formation of humic substances [21]. The microorganisms require oxygen for aerobic respiration to carry out the composting process. For continuous active composting, oxygen is replenished by mixing or aeration. The by-products of the composting process are leachate, carbon dioxide and heat [22]. In the second stage the remaining portion of organic material in humic substances is converted via humification to improve the quality of compost [19]. However, decomposition continues to take place until the organic material is converted into stable humic substances [18]. As microbial activity decreases, temperature declines. Overall, composting technology is one of the alternatives for sustainable solid waste management which reduces the amount of solid waste going to landfills by facilitating the recycling of waste organic materials to cultivate soil fertility [23]. Although composting seems to do all that good, it does have some drawbacks as shown in Table 1 along with composting benefits.

**Table 1.** Benefits and drawbacks of composting.

<b>Composting [24]</b>	
<b>Benefits</b>	<b>Drawbacks</b>
Able to reduce and reuse organic waste	Time and money required initially to start up
Conserve space and extending life of the landfill	Expensive to store and transport due to bulky product
Improve nutrient properties of soil	Land requirement for compost site
Compost product are saleable and provide profit	Possibility of odor emission
Reduce waste odor	Weather influence to compost process
Improves waste and manure management	Marketing necessary to sell compost product
Reduce cost of waste management	Inconsistent and low nutrient value compare to chemical fertilizer
Reduce the risk of pollution, methane emission and soil erosion	Slow-release of nutrients as fertilizer compared to chemical fertilizer
Remediate hazardous waste	
Suppress pathogen and soil-borne diseases	
Excellent bedding substitute	

### 1.2. Factors Affecting the Organic Waste Compost

Food waste is referred to as all the lost or uneaten food substances that are being discarded or recycled. It is also defined as the inedible parts of the food substances that are not intended for human consumption. Food waste such as fruits and vegetable scraps, eggshells and tea leaves can be collected from households, eating premises, markets, shops and stores and distribution operations to be used as compost agents in the composting process. However, meat, seafood and greasy food are not suitable to be used as compost agent as they may contain bacteria that is pathogenic to humans, so it has to be composted at a very high temperature to kill off the pathogens. Depending on the conditions of your pile, it might not achieve the desired temperature to breakdown greasy components and pathogenic microbes. Additionally, these raw materials generate odor and can attract pests [25].

In general, the composition of food waste, mainly consists of carbohydrates, proteins, lipid and also traces amount of inorganic compounds. The composition varies in accordance with the type of food waste and its constituents. A general waste discarded from the kitchen would contain approximately 60–80% moisture, 3–5% ash, 40–60% carbohydrate, 18–30% volatiles, 10–30% protein, 15–40% fat and 45–65% carbon. Protein-based meals such as fish and meat, contain on average three times higher protein content and moisture in the range of 4–7% while wheat meals are comprised of a high composition of volatile matters and carbohydrates that are in the range of 88–92% each [26].

Food waste is recycled into organic waste and used as the raw compost agent. This is the most crucial factor in organic food waste compost as a carbon source. One of the distinctive properties of food waste that makes it a suitable starting medium is the high moisture content and low physical structure, enabling it to be broken down easily with temperature and microorganisms [27]. Addition of bulking agents as part of the composting material such as wood chips, wheat straw, sawdust, chopped hay, wood shavings and rice bran are required to adjust the moisture content, nitrogen composition and the carbon to nitrogen ratio [28]. Bulking agents contain a high carbon to nitrogen (C/N) ratio and is then capable of absorbing excess moisture in the food waste and at the same time adding structure to the mix especially when dealing with waste materials with extremely high moisture and low C/N ratio such as sewage sludge or animal manure [7]. Bulking agents contribute by modifying the physical properties of raw material, biodegradation kinetic and composting performance [28]. In addition, food waste contains high energy which enables it to contribute to waste stabilization and energy production.

The C/N ratio during the composting process can affect the rate of decomposition. The microbiome involved in the composting process uses carbon as a source of energy and nitrogen to build proteins. The lower the C/N ratio, the more the loss of nitrogen from the composting process. On the other hand, the higher the C/N ratio, the slower the rate of decomposition and nitrogen would be immobilized during the composting process as well. However, organic food waste has a moderate C/N ratio which makes it suitable to be used as a composting agent [7]. The pH value of the compost, as well as moisture content [29] are equally important factors for a successful compost. The pH value of the organic waste compost can be determined by using the pH meter or potentiometer. The potentiometer is placed in the aqueous suspension of the organic waste compost at the ratio of 1 part of dry matter to 10 parts of water. The pH value of organic waste compost normally falls within the range of 7–8 [30,31]. The pH value increases after the process of composting as the pH value will be affected by the soluble alkaline elements in the soil and the formation of ammonia when the organic compound which is mostly protein is degraded [32]. The microbial activity is affected by the moisture content as the moisture condition in the compost will affect the temperature and oxygen uptake rate in the compost. Moisture content will not only affect the pathogens in the compost, but will also inhibit the beneficial microorganisms which act as the starter culture in the compost when the moisture content is too low. However, when the moisture content is too high, unpleasant odor will be produced due to the anaerobic condition and it may stop the process. The moisture content of 50 to 60% will be effective for the composting process to occur [8]. However, the C:N ratio, pH value and moisture content slightly differ for different organic waste compost.

The content of organic carbon in organic waste compost can be determined by using oxidation-titration with ferrous ammonium sulfate method followed by the process of mineralization in potassium dichromate [33]. The nitrogen content in the organic waste compost is determined by using the Kjeldahl method. Total nitrogen normally ranges from 0.5 to 2.5% in compost [30]. Generally, the C:N ratio in the organic waste compost is 20:1 but the ratio of carbon can fall within the range of 10–600. The organic carbon content in the compost depends on the moisture content of the organic waste during composting and it tends to decrease after the process of composting. This is because organic compounds in organic waste are easily degradable and the degree of humidity increases in the composting process causes the loss of organic carbon [15].

The basic requirement of the compost to be used in the soil safely is the degree of stability of the compost. The stability of the compost refers to the absence of animal and plant pathogen as well as phytotoxic compounds in organic waste. The stability of the compost is normally associated with the microbial activity in the compost. The microbial activity can be determined by using the metabolic activity of the microbes, microbial count and the concentration of constituents produced by the microbes in the compost [34]. There are many efficient bacteria and fungi which can be used as the starter culture in the processing of composting organic wastes, including bacteria from *Pseudomonas* and *Bacilli* genera as these bacteria have hydrolytic potential and are able to decompose the organic waste efficiently. These bacteria can convert the organic waste into humus which will improve the soil's biological, chemical and physical properties when the compost is being used in the soil [32]. Table 2 exhibits the key parameters for organic composting and the recommended range.

**Table 2.** Key parameters for organic composting and the recommended range.

Parameter	Factors Affecting the Organic Waste Compost [29,30]	
	Acceptable Range	Preferred Range
Carbon to nitrogen (C:N) ratio	20:1 or 40:1	25:1 or 30:1
Moisture Content	40 to 65%	50 to 60%
Oxygen concentrations	≥5%	>5%
Particle size (Diameter in inches)	1/8 to 1/2	Varies
pH value	5.5 to 9.0	6.5 to 8.0
Temperature (°C)	43 to 65	54 to 60

### 1.3. Compost Methods

There are many different composting methods such as vermi-composting, windrow composting, aerated static pile composting and in-vessel composting. The vermi-composting method produces compost through the activity of earthworms. The earthworms decompose organic waste into higher quality compost which is known as castings. The castings are used as potting soil because it is rich with nutrients such as nitrogen, phosphorus and potassium. The vermi-composting process is a mesophilic process in which the temperature, pH and moisture levels need to be optimized for the process to carry out. The commonly used compost agents in vermi-composting are food scraps, papers and yard trimmings and animal manures. The vermi-composting method is easy and inexpensive, has excellent properties and also is environment-friendly. However, the earthworms may be very sensitive to changes in temperature. The ideal temperatures for vermi-composting range from 13 °C to 25 °C [35]. The composting agents placed in a few layers into a cement base with a polythene sheet underneath and sealed, leaving a crack for the release of heat and addition of earthworms to allow the composting process to occur thus vermi-compost is produced after two months [36].

Windrow composting is one of the commonly used methods as it can process a large volume of organic waste. This composting method involves placing organic waste into long and narrow piles called “windrows” with either a triangular or circular cross-sectional area. The piles are then being turned either manually or mechanically. The turning process uses the temperature of raw materials as a turning indicator, therefore the windrows will be turned when the temperature has reached a certain range thus the pile is aerated [37]. Large volumes of organic wastes such as grease, liquids and animal manures can be broken down using this method. Windrow composting method is suitable for restaurants, cafeterias and markets which produce a huge amount of food waste as the pile is large enough to generate sufficient heat and maintain the temperature. However, this composting method requires a large area of land to accommodate the large equipment and it is time-consuming.

Positive or negative ambient air is involved in the aerated static pile composting method. The air is passed through the compost pile along with organic wastes and bulking agents. Layers of the bulking agent are added into the pile in order to enhance the flow of air as well as add porosity to the pile. The construction of the aerated static pile method is almost similar to the windrow composting method, except that the aerated static pile method does not require turning to provide aeration. Aerated static pile composting is capable of producing compost in a short period around three to six months and it is suitable for a large amount of organic wastes such as yard trimmings, papers and food scraps as well. In addition, it does not require as much area of land as compared to the windrow composting method. This composting method requires significant cost and is not suitable to decompose animal manures and grease [38].

In-vessel composting is often used in the industry as well because it can also process a large volume of organic wastes. This method confines all the organic wastes into various containers or vessels and these will be manually or mechanically turned to make sure the organic wastes are aerated. The in-vessel composting method requires a smaller area of land and manual labour as compared to windrow composting. This method has drawbacks as it is costly and might require expertise in handling the equipment [38]. Table 3 shows the characteristics of all composting techniques.



**Table 3.** Characteristics of composting techniques.

Characteristics	Composting System			
	Vermi-Composting	Windrow	Aeratic Static Pile	In-Vessel
Preferred waste input	Wastes mixed with manure such as cow, goat and poultry manure	Wastes with less emission of odor such as plant-based	Waste with more homogeneity, consistency and those required bulking agent	Easily degraded wastes such as food waste
Compost capacity	2 to 3 tons of wastes	More than 10 tons of waste	More than 10 tons of waste	1 to 5 tons of wastes
Land requirement	Low	High	Medium	Low
Site selection	Anywhere equipment can be placed	Away from populated area	Away from populated area	Anywhere equipment can be placed
Cost of waste transportation	High	High	High	Low
Composting period	Short	Long	Long	Short
Amendment	Addition of bulking agent, animal manure and microbial additives; consistent temperature	Addition of bulking agent, chemical additives and microbial additives; increase of aeration	Addition of chemical additives, and microbial additives; increase of aeration	Increase the in-vessel temperature, pressure and turning rate
Amendment effects on compost	Increase in production of biomass by more than 20%	Reduce the total composting period by 30%	Reduce the total composting period by more than 30%	Reduce the total composting period by more than 30%
Compost quality	Good	Medium	Medium	Good
References	[35]	[37]	[38]	[38]

The quality of compost produced is determined by the composition of input material for composting. Compost quality is also influenced by the type of technology applied and the composting process. The stability and maturity of the final compost product are important for optimum use as a fertilizer for plants [39]. According to Azim et al. (2018) [19], the stability and quality of compost can be determined based on the transformation of unstable organic matter into a stable material which is assessed by the biodegradability of organic material. A mature compost does not smell of ammonia, instead it has a pleasant odor, has a constant and low temperature, distinguishable as compared to raw material and appears dark in color [25]. The maturity of compost can also be assessed based on its effects on plants through the absence of plant damage, positive effect on germination, growth and development [40]. The quality of a stable compost can be determined through several key physicochemical parameters such as pH, C/N ratio, organic matter content, humification ratio and cation exchange capacity (CEC) [19].

The most basic physicochemical parameter is pH where acidic level is an indicator for immature compost while mature composts have a pH between 7 and 9 [41]. As for the C/N ratio, a decrease in levels is often observed during composting as carbon source depletes in time. Mature compost is determined with C/N ratio in the range of 15–20. However, C/N ratio in the range of 10–15 is still considered as stable output [42]. The presence of nitrate ( $\text{NO}_3^-$ ) can be used to gauge compost maturity. Nitrifying microorganisms cause a reduction in ammonium content ( $\text{NH}_4^+$ ) and promote nitrate ( $\text{NO}_3^-$ ) production. Though not perfectly established, monitoring levels of nitrate and ammonium from the beginning to the conclusion of composting will indicate compost quality [19]. The process of composting is reported to record an increase in humic acid (HA)/fulvic acid (FA) ratio [43]. Fulvic acid (FA) is an intermediate bi-product of humic acid (HA) and humic substance formation. As a result, it is established that the humic component of the total organic matter constantly increases until the maturity of compost, equivalent to HA/FA ratio value greater than 1 but lower than 3 [44]. Humic compounds are capable to adsorb positively charged ions to be exchanged with other cations. This cation exchange capacity (CEC) is predicted to increase during composting where the humification of organic materials, carboxyl and phenolic functional groups are also formed [44]. CEC greater than  $60 \text{ meq.}100 \text{ g}^{-1}$  of organic matter is an indicator of mature compost [19]. Table 4 highlights the physicochemical properties of several mature compost produced using varying bulking agents.



**Table 4.** Physicochemical properties of several mature composts produced using varying bulking agents.

Parameter	Bulking Agent						
	Wheat Straw	Wood Shaving	Wood Chips	Pruning Waste	Pruning Waste	Saw Dust	Bio-Char
Compost Type	Organic	Organic	Organic	Organic (Home)	Organic (Industry)	Organic	In-vessel
Bulking Agent Ratio	5:1 (wt/wt) <sup>1</sup>	1:1 (v/v) <sup>1</sup>	1:3-4 (wt/wt) <sup>1</sup>	0.8:1 (v/v) <sup>1</sup>	2.6:1 (wt/wt) <sup>1</sup>	–	–
pH	8	7.9	8.6	7.83	7.88	6.38	8.6
TOM (%)	39	25	58.6	47.96	55.33	–	64.2
TOC (%)	22	14.5	34	–	–	45.56	35.7
TKN (%)	3.72	0.8	2	1.71	2.04	0.49	–
C/N ratio	6	19	17	–	–	92.36	–
Moisture	35	–	31	43.63	31.85	–	54.4
P as P <sub>2</sub> O <sub>5</sub> (%)	0.29	–	–	–	–	–	–
K as K <sub>2</sub> O (%)	3.09	–	–	–	–	–	–
Mg as MgO (%)	0.52	–	–	–	–	–	–
EC (mS/cm)	24.85	–	–	4.30	4.90	–	–
Ash (%)	52.95	–	–	–	–	17.98	35.7
Cu (ppm)	–	–	–	44	47	–	–
Zn (ppm)	–	–	–	156	150	–	–
<b>Reference</b>	[45]	[46]	[47]	[48]	[48]	[49]	[50]

<sup>1</sup> wt/wt = weight/weight, v/v = volume/volume. \* TOM = Total Organic Matter, TOC = Total Organic Carbon, TKN = Total Kjeldahl Nitrogen, C/N = Carbon to Nitrogen Ratio, EC = Electrical Conductivity, P = Phosphorus, P<sub>2</sub>O<sub>5</sub> = Phosphorus pentoxide, K = Potassium, K<sub>2</sub>O = Potassium oxide, Mg = Magnesium, MgO = Magnesium oxide, Cu = Copper, Zn = Zinc.

## 2. Microbial Community Structure During Composting

Composting is a process involving key microbiome that actively decomposes the degradable and putrescent organic waste under moist, self-heating and aerobic conditions and is a natural process characterized by microbial community successions. Microbial community structure is defined as the composition of the microbial community and the abundance of the members in a microbial community [51]. During the process of composting, both bacteria and fungi that represent the microbial community structure of the composting environment are present and play an active role. The presence of different bacteria or fungi can either positively or negatively affect the entire composting process. Their diversity also suggests the composting mechanisms which takes place. Likewise, alteration in the choice and amount of initial organic matter can change the microbial communities and the composting output [52]. Diversity and development of microbial population during composting are dependent on physical parameters such as oxygen, temperature, moisture content and nutrient availability [53]. The physical parameters are crucial in understanding the activity of different groups of microorganisms that will indicate the quality of the final product of the composting process [54]. In Scandinavian countries, the treated food waste has a low pH that correlates with a high concentration of lactic acid bacteria (LAB), in most collected wastes during the earlier stages composting process [55]

An anaerobic digestion is another appropriate method for composting with an example of employing food waste to generate biogas while addressing waste management and nutrient recycling [56]. Through the process, the microbial community is tasked at disintegrating complex organic matter into carbohydrates, lipid and protein before being further hydrolysed by enzymes such as protease, lipases, cellulase and amylase into stable, simple forms of carbohydrates, long-chain fatty acids and amino acids [57]. Measuring enzymatic activities may provide information about the maturity of composted products through the decomposition of organic matter and nitrogen transformations during composting [54]. This entire process of decomposition triggers the rise and decrease in temperature during composting which determines the type of microorganisms that can exist at each stage of composting. The biochemical changes in each stage of composting, together with the type and usage of which microorganisms occurring in the decomposition process will be discussed in later sections.

## 3. Stages of Composting

The composting process is segregated into three major phases namely the mesophilic phase followed by the thermophilic phase and lastly cooling or maturation phase, in which diverse microflora, such as mesophilic and thermophilic bacteria, fungi and actinomycetes are present to convert and stabilize the organic waste to humus [58]. Different microbial communities predominate different composting phases. The duration of each stage is dependent on the initial composition of organic material, moisture content, quantity and composition of the microbial community [59]. The initial decomposition of organic matters is performed by mesophiles due to the readily available carbon sources at the beginning of the process. As a result of the heat generated by mesophilic microorganisms through their metabolic activity, the first mesophilic phase progresses into the thermophilic phase. At the thermophilic phase, the thermophilic microorganisms, which are heat tolerant will take over the mesophiles and become dominant. As increasing temperature accelerates the breakdown of substances hence exhaustion of nutrients, this causes the temperature to decrease gradually and enter the mesophilic phase again before the compost matures [60]. During the cooling phase, those mesophiles once again migrate back to the compost and work on digesting the remaining organic matters. Figure 1 explains how the overall temperature profile which determines the start and end of each stage. Overall, the composting process is associated with a wide range of microbial populations. Reports from literature have documented the presence of bacteria from the genera *Anthrobacter*, *Bacillus*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Morganella*, *Nitrobacter*, *Nitrosomonas*, *Paucimonas*, *Proteus*, *Pseudomonas*, *Staphylococcus*, *Streptomyces* as well as the fungal genera *Alternaria*, *Aspergillus*, *Cephalophora*, *Cladosporium*, *Humicola*, *Macrosporium*, *Moniliella*, *Nigrospora*, *Penicillium*, *Phoma*, *Preussia*, *Rhizopus*, *Sordaria*, *Staphylotrichum*, *Sistotrema*, *Thielavia*, *Thysanophora*, *Trichoderma*, *Trichurus*, *Verticillium* and

*Zygorhynchus*. Table 5 highlights the microorganisms that have been reported in some of the recent scientific reports according to the respective composting stages.

### 3.1. First Stage

The mesophilic temperature and availability of carbon-rich substrate at the early stage of composting process favor the growth of mesophiles with mixture of bacteria, actinomycetes and fungi whereby they grow at the temperature between 15 °C to 45 °C and achieve optimum growth at the range of 30 °C to 39 °C [61]. At this stage, mesophilic fungi such as yeasts and molds as well as acid-producing bacteria like *Lactobacillus* spp. and *Acetobacter* spp. are the dominant species to decompose organic waste materials [55]. At the mesophilic stage, microbial activities during the degradation process will result in an increased in temperature owing to energy of the organic combinations. The pH typically decreases as organic acids are produced. Given an example of a food waste containing vegetable scraps, low initial pH ranging from 4.5 to 5.0 could stimulate the proliferation of fungi. These microorganisms then break down the readily digestible carbon sources into organic acids, which in a study, resulted in a pH drop in the compost [62]. Fungi like molds and yeasts are responsible for breaking down tough debris, which enables bacteria to continue the decomposition process which explains the degradation of several compounds such as amino acids, sugars and other simple components (in the waste product) in this phase. During the process, the temperature is known to increase rapidly, indicating the occurrence of degradation where organic matter was being transformed. Hereby, the compost will undergo the second phase process known as the thermophilic stage [8].

### 3.2. Second Stage

The thermophilic phase is the stage whereby most of the decomposition takes place. This stage sees the process of the organic matter's degradation (fats, cellulose, hemicelluloses and some lignin) by thermophilic microorganisms represented namely by fungi and bacteria. During this stage of composting, mesophiles are replaced by thermophiles which grow optimally at the temperature between 40 °C and 80 °C, favoring actinomycetes and thermophilic bacteria such as *Bacillus* spp [63]. However, mesophilic fungi can still be present in the outer layers of the compost piles during the second stage of composting and reinvade when the thermophilic stage has come to an end. Towards the end of the thermophilic stage, as the carbon source depletes in the overall compost, temperature gradually decreases as it prepares to enter the cooling or maturation stage [64]. During this stage, Gram-positive bacteria such as *Bacillus* spp. and Actinobacteria are the most predominant strains present in the compost [55]. Actinobacteria are bacteria with high content of guanine and cytosine mainly distributed in soil whereby they can decompose complex mixtures of polymers in dead plants and animals [65]. Their growth rates are slower and they have a greater capacity to degrade less biodegradable, complex organic compounds compared to the other bacteria. These bacteria are known as lignocellulolytic microorganisms as they degrade tough lignocellulose in the plant biomass via the secretion of lignocellulases like cellulases, hemicellulases and lignolytic enzymes [66].

**Table 5.** Diversity of microbial populations according to respective composting stages.

Compost Stage	Group	Genus	Microbial Species	Reference
Mesophilic	Bacteria	Amycolicococcus	<i>Amycolicococcus subflavus</i>	[67]
		Bacillus	<i>Bacillus badius</i> , <i>Bacillus cereus</i> , <i>Bacillus flexus</i> , <i>Bacillus subtilis</i> , <i>Bacillus polymyxa</i> , <i>Bacillus pumilus</i> , <i>Bacillus</i> spp.	[55,67–70]
		Brevibacillus	<i>Brevibacillus brevis</i>	[68,69,71]
		Enterobacter	<i>Enterobacter sakazakii</i>	[69]
		Klebsiella	<i>Klebsiella pneumoniae</i>	[68,69,71]
		Mycobacterium	<i>Mycobacterium xenopi</i> , <i>Mycobacterium thermoresistibile</i>	[67]
		Serratia	<i>Serratia marcescens</i>	[69]
		Staphylococcus	<i>Staphylococcus aureus</i> , <i>Staphylococcus sciuri</i> , <i>Staphylococcus xyloseus</i> , <i>Staphylococcus</i> sp.	[68,69]
Mesophilic	Fungi	Aspergillus	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	[68]
		Fusarium	<i>Fusarium moniliforme</i> , <i>Fusarium oxysporum</i> , <i>Fusarium</i> sp	[68]
		Streptomyces	<i>Streptomyces antibioticus</i> , <i>Streptomyces cinnaborinus</i> , <i>Streptomyces griseus</i> , <i>Streptomyces roseus</i>	[68]
		Rhizopus	<i>Rhizopus nigricans</i>	[68]
		Penicillium	<i>Penicillium citrinum</i>	[68]
Thermophilic	Bacteria	Acidorax	<i>Acidovorax</i> sp.	[69]
		Amycolicococcus	<i>Amycolicococcus subflavus</i>	[67]
		Anoxybacillus	<i>Anoxybacillus flavithermus</i>	[71]
		Bacillus	<i>Bacillus benzoevorans</i> , <i>Bacillus coagulans</i> , <i>Bacillus flexus</i> , <i>Bacillus megaterium</i> , <i>Bacillus nealsonii</i> , <i>Bacillus pumilus</i> , <i>Bacillus stearothermophilus</i> , <i>Bacillus subtilis</i> , <i>Bacillus</i> sp.	[55,68–71]
		Brevibacillus	<i>Brevibacillus brevis</i>	[68,69,71]
		Clostridium	<i>Clostridium acidurici</i> , <i>Clostridium thermocellum</i> , <i>Clostridium</i> sp.	[55,71]
		Comamonas	<i>Comamonas kerstersii</i>	[69]
		Gemmatimonas	<i>Gemmatimonas aurantiaca</i>	[71]
Thermophilic	Bacteria	Geobacillus	<i>Geobacillus</i> sp. WCH70, <i>Geobacillus</i> sp. Y4.1MC1, <i>Geobacillus thermodenitrificans</i>	[71]
		Klebsiella	<i>Klebsiella pneumoniae</i>	[68,69,71]
		Kocuria	<i>Kocuria flavus</i>	[69]
		Krypidia	<i>Krypidia tusciae</i>	[71]
		Lysinibacillus	<i>Lysinibacillus fusiformis</i> , <i>Lysinibacillus sphaericus</i>	[69,71]
		Mahella	<i>Mahella australiensis</i>	[71]

Table 5. Cont.

Compost Stage	Group	Genus	Microbial Species	Reference
Thermophilic	Bacteria	Mycobacterium	<i>Mycobacterium thermoresistibile</i> , <i>Mycobacterium xenopi</i>	[67]
		Paenibacillus	<i>Paenibacillus mucilaginosus</i> , <i>Paenibacillus</i> sp. JDR-2	[71]
		Pseudomonas	<i>Pseudomonas mendocina</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas</i> sp.	[68,70,71]
		Rhodothermus	<i>Rhodothermus marinus</i>	[71]
		Solibacillus	<i>Solibacillus silvestris</i>	[71]
		Sorangium	<i>Sorangium cellulosum</i>	[71]
		Sphaerobacter	<i>Sphaerobacter thermophilus</i>	[71]
		Streptosporangium	<i>Streptosporangium roseum</i>	[71]
		Symbiobacterium	<i>Symbiobacterium thermophilum</i>	[71]
		Thermaerobacter	<i>Thermaerobacter marianensis</i>	[71]
		Thermobacillus	<i>Thermobacillus composti</i>	[71]
		Thermobifida	<i>Thermobifida fusca</i>	[71]
		Thermobispora	<i>Thermobispora bispora</i>	[71]
		Thermomonospora	<i>Thermomonospora curvata</i>	[67,71]
		Thermosediminibacter	<i>Thermosediminibacter oceani</i>	[71]
Thermus	<i>Thermus</i> sp.	[68]		
Terribacillus	<i>Terribacillus halophilus</i>	[69]		
Thermophilic	Fungi	Aspergillus	<i>Aspergillus fumigatus</i> , <i>Aspergillus fumigates</i> var. <i>elpticus</i>	[68]
		Talaromyces	<i>Talaromyces thermophilus</i> , <i>Talaromyces</i> sp.	[68]
		Thermomyces	<i>Thermomyces</i> sp.	[55,68]
		Thermatinomyces	<i>Thermatinomyces</i> sp.	[68]
		Thermo	<i>Thermo dichotomicus</i> , <i>Thermo vulgaris</i> , <i>Thermo</i> sp.	[68]
Cooling or maturation	Bacteria	Amycolicococcus	<i>Amycolicococcus subflavus</i>	[67]
		Bacillus	<i>Bacillus circulans</i> , <i>Bacillus composteris</i> , <i>Bacillus southcampusis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus subtilis</i> , <i>Bacillus pumilus</i>	[68,69]
		Mycobacterium	<i>Mycobacterium xenopi</i> , <i>Mycobacterium thermoresistibile</i>	[67]

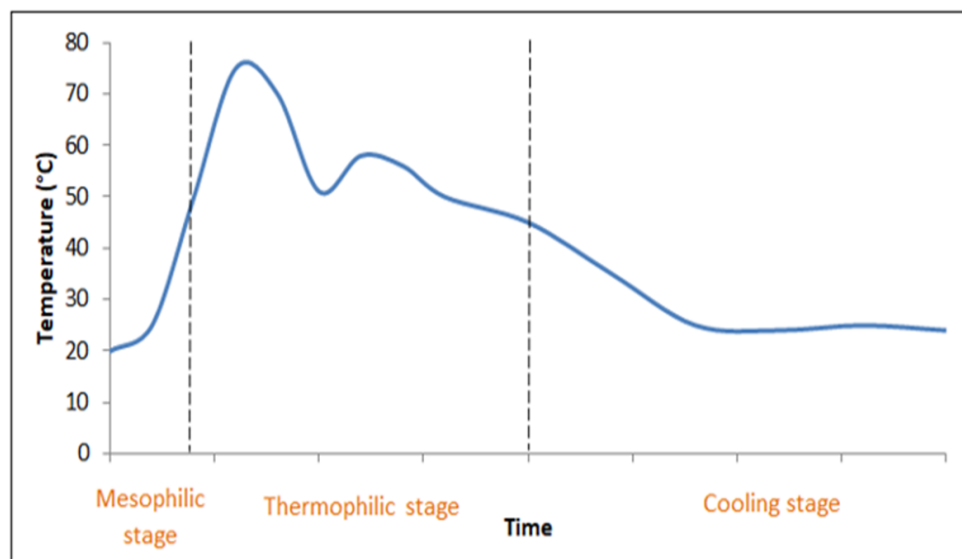


Figure 1. Temperature changes during composting (FAO n.d.).

### 3.3. Third Stage

The final maturation phase is characterized by an even lower temperature below 25 °C. Due to the depletion of substrates, the microbial activity of thermophiles ceases and mesophiles are now getting back to recolonize the organic matters from the spores which survive under high temperature and germinate when the temperature drops or through external inoculation from the environment or the edge of the compost pile. At the final cooling phase, the amount of readily available substrates becomes a limiting factor, resulting in a decline in microbial activity which implies reduced oxygen uptake and heat output [72]. Since the temperature has been decreased to a mesophilic range of temperature, fungi will now reappear in the compost and are ready to degrade the leftover organic materials. The microflora during this stage plays a vital role in compost maturation and the suppression of plant disease due to the metabolism of phytotoxic compounds [20]. Some soil fungi can completely decompose lignin that even actinomycetes and bacteria could not do so [73]. The final compost is expected to be of matured quality, containing a lower C/N ratio of 15–20, a higher pH value, decreased  $\text{NH}_4^+$  and increased  $\text{NO}_3^-$ . These properties along with the presence of phytotoxic metabolites influence several biological, chemical and physical soil properties positively and sustainably [74].

## 4. Compost Microbial Community Structure Profiling Techniques

The composition of microbial communities during composting is dependent on the constant change in the physicochemical parameters such as temperature, moisture, C/N ratio, oxygen rate and pH [75,76]. The role of the microbial community in the degradation of organic matter, proteins, lipids, cellulose and lignin during composting has been highlighted in several studies as well [77]. Determination of the microbial community structure in compost is vital as changes in the structure of the microbial community can impose negative effects on the quality and yield of crops [78]. However, the understanding of microbial community structure during composting is limited due to the limitations of profiling techniques [79]. As the traditional, cultivation-based methods have limitations to the type of strains that can be cultured, the role of conventional biochemical methods have become limited eventually shifting towards molecular techniques as the current trend. Fingerprinting methods, such as polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), terminal restriction fragment length polymorphism (T-RFLP) and single strand-conformation polymorphism (SSCP), microarray analysis [13] and next-generation sequencing (NGS) based 16S rRNA sequencing [14] have been found to focus on the most abundant groups. Combination of molecular techniques such as DNA extraction, polymerase chain reaction (PCR) amplification on the nucleic acid extracted from the environment



and high-throughput sequencing enables the detection of both culturable as well as non-culturable microorganisms [80]. Since then, numerous studies have reported the microbial community of both culturable and non-culturable strains of the composting process [79]. The following section will highlight techniques of microbial community structure profiling in four sections; (1) Biochemical Approach (2) Microarray Analysis, (3) Genotyping and Fingerprinting (4) Next Generation Sequencing.

#### 4.1. Biochemical Approach

Biochemical techniques in microbial identification have been classically used to classify microbes based on phenotypic characteristics. Often reliability of biochemical tests is questioned due to inconsistency in data. Regardless, due to affordability, these techniques are still used. The most widely used staining technique is the Gram stain, which classifies bacteria into Gram-positive or negative. It is a four-step universal method that stains Gram-positive bacteria blue via crystal violet and makes Gram-negative bacteria red via safranin. All this can be observed using a compound light microscope and oil immersion lenses [81].

The bacterial spore stain is another technique that facilitates in the observation of bacterial endospores. The endospore comprises of bacterial deoxyribonucleic acid (DNA) and ribosomes. The Schaeffer–Fulton stain, consisting of malachite green and safranin assists in distinguishing the presence and location of spores. Malachite green penetrates the bacterial endospore wall, staining them green while safranin stains most other microorganism bodies red or pink. Meanwhile, the Moeller stain uses carbol fuchsin that stains endospores red and methylene as counterstain to turn vegetative bacteria blue. Observation of bacterial endospores can be done using a light microscope [82]. For fungal identification, Lactophenol cotton blue is commonly used in examination of yeast and filamentous fungi. Staining makes septa, special mycelia and spore structures visible as light blue colored under light microscope [81].

Some biochemical tests investigate the enzymatic activities of cells on the principles that bacteria are capable of using different carbon sources to sustain life. The oldest biochemical test that uses this application is the API kit, which detects enzymatic activity, related to fermentation of carbohydrates or catabolism of proteins or amino acids by the microbes. More recently, microbes can be identified through the analysis of cellular fatty acids with the use of gas chromatography. Phospholipid fatty acid (PLFA) analysis has been widely used to observe changes in the microbial community of aqueous and soil environments. This is because PLFA is present in almost all living organisms since it is an important component and some PLFAs are specific to certain organisms making them useful as biomarkers. PLFA analysis is also able to estimate the total biomass which can be used as an indicator of viable microbial biomass [83]. PLFA can also be used to monitor the changes in the microbial community during composting. There have been several studies that emphasize on the evolution of PLFAs during the compost process of different types of wastes, focusing on the initial stages of the compost process and there are other authors who have included time of sampling during the maturation stages of the composting process [84].

Microbes can also be characterized based on the profile of methylated fatty acids unique to each species [85], whereas a more advanced tool is the use of mass spectrometry for identification of microorganisms using protein biomarkers. The Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometer ionizes proteins and fragments them based on mass/charge ratio, creating a protein profile which can be cross-referenced with known spectra database [86]. Regardless, all these techniques have one disadvantage that they are still limited to culturable microbial strains.

#### 4.2. Microarray Analysis

Microarrays are an application of molecular biology where up to thousands of known DNA fragments called probes are arrayed on a chip for the detection of interested genera simultaneously [13].

Several types of microarrays have been developed and these arrays differ by their probe design strategies. There are five major arrays [87] that are commonly being used in microbial profiling, which are:

- **Phylogenetic Oligonucleotide Arrays (POAs)**  
POAs utilized a small-subunit of identified ribosomal RNA (rRNA) as the array probes. The specific region of rRNA exists in all organisms but the specificity of the conserved and variable regions of one organism is rarely transferred to or in between organisms making them convenient as genetic markers.
- **Functional Gene Arrays (FGAs)**  
FGAs measure the genes involved in a certain process. The probe array on FGAs are usually genes encoding some part of the process of interest, such as enzymes or proteins in a metabolic process. FGAs are not only able to measure expression levels of these genes, but also some degree of genetic classification and gene capacity.
- **Community Genome Arrays (CGAs)**  
CGAs are a novel prototype array that contain probes representing a subset of or the entire genomic DNA of an organism in a naturally occurring microbial community. CGAs are used as a comparative tool to relate microbial communities across different samples.
- **Metagenomic arrays (MGAs)**  
MGAs are the combination between microarrays and metagenomics. A MGA contains cosmid library inserts along with control rRNA probes. This enables MGAs to be used as high-throughput screening technique, although MGAs are still in the early stage of development.
- **Whole-genome ORF arrays (WGAs)**  
WGA utilize all the open reading frames (ORFs) in the genome as the probes. WGAs are used mainly in studying genomic response in different environments or the microevolution of a particular organism.

Microarrays allow the examination of several thousand genes without the need for PCR amplification, reducing the effect of PCR bias and producing a highly specific result [87]. The newer DNA microarrays such as CGAs and MGAs have enabled for a quick and high-throughput way for analyzing the complex diversity of microbial communities found in compost. Therefore, this method has the potential to act as a great tool to monitor the composting process as well as detection of either beneficial or harmful microbes in organic compost [88].

Generally in the microarray technique, the workflow starts with the DNA of interest being first extracted from the organic compost followed by PCR amplification to become florescent-labeled DNA. This florescent-labeled target DNA will be added into hybridization buffer together with control oligonucleotides and this mixture will be added to the microarray to undergo a hybridization process [89]. The microarray is then scanned and the image can be analyzed. Useful information which is the signal-to-noise ratio (SNR) can be calculated by extracting the data from the scanning. One of the microarrays which have been commonly applied in composting is the COMPOCHIP microarray (ThermoHybaid: Ulm, Germany and Lambda GmbH: Freistadt, Austria) that is spotted with 369 probes related to target microorganisms in organic waste composting stages [90]. Through the utilization of COMPOCHIP microarray which is specific to the composting process, a variety of compost microorganisms can be detected in only one test rapidly [91].

In a study done by Hultman et al. (2008), they managed to develop a microarray based on the ligation-detection-reaction (LDR) [92]. The principle involved is the ability of the ligase enzyme to bind to two probes that have been hybridized and hence being detected on a specific location on the microarray [93]. The two probes are the discriminative probe and the common probe. Firstly, the two probes of high similarity that have been bound by the ligase enzyme will undergo linear amplification. Then, the hybridization of ligation product with complementary zip sequence as well as control probe with complementary control probe sequence will take place and printed on the microarray [92]. Hence, the result can be detected by using a scanner. This LDR based microarray enables a precise

and sensitive detection of a microorganism's species within a complex microbial community. Table 6 summarizes the characteristics of the above-mentioned microarray techniques.

**Table 6.** Summary of microarray technique characteristics.

Characteristic of Different Microarrays [87]			
Type of Microarray	Probes Used	Probe Length	Information Target
POA	Ribosomal rRNA	18–25 nt	Phylogenetic
FGA	Functional genes	50–70 nt or 200–1000 nt (PCR)	Functional
CGA	Whole genomes of multiple organisms	Whole genome	Phylogenetic
MGA	Environmental DNA	1000+ nt	Functional
WGA	Open reading frames in whole genome	23 nt or 200–3000 nt (PCR)	Phylogenetic and functional

\* POA = Phylogenetic Oligonucleotide Array, FGA = Functional Gene Array, CGA = Community Genome Array, MGA = Metagenomic array, WGA = Whole-genome ORF array.

### 4.3. Genotyping and Fingerprinting

#### 4.3.1. Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP is a fingerprinting method that can reflect the structure of one community. As compared to another fingerprinting method which is polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), T-RFLP is more sensitive [11]. When comparing T-RFLP with RFLP, T-RFLP has overcome some shortcomings of RFLP which is the inability to measure the diversity of the microbial community. T-RFLP is better than RFLP where it has a more simplified banding pattern and hence facilitating the investigation of microbial diversity. This method has been applied widely in the study and investigation of the microbial community structure during the composting process as it is high throughput and also provides phylogenetic identification [20]. Moreover, T-RFLP produces a semi-quantitative result and it is highly reproducible [94].

In the T-RFLP technique, the first step involved is also the extraction of DNA from the organic compost sample and then amplification through PCR. The primer pairs used in the PCR amplification are fluorescently labeled. The amplified output will undergo digestion by restriction enzymes, usually restriction endonucleases [95]. Now the fluorescently labeled fragments can be separated through electrophoresis and the fluorescently labeled terminal restriction fragments (T-RF) will be quantified [96]. The length of T-RF will be measured and compared with the fragment size from the database. An electropherogram can also be obtained and the detection of a microbial community will be based on the length of T-RF as well as the intensity deduced from the peak's height [97]. The T-RFLP data generated can be analyzed using statistical approaches such as principal component analysis, cluster analysis, multidimensional scaling and self-organizing map [96].

However, there are also some drawbacks to the T-RFLP method despite of its large number of advantages. The common disadvantages are the high dependency on DNA extraction and being prone to PCR bias. Other than that, this technique is also restricted by the limited availability of universal primers, as currently there are not universal primers that can amplify the sequences from bacteria, eukaryote and archaeal domains altogether. Although there are some limitations to this method, some researchers still hold to the opinion that T-RFLP is an appropriate way to study and investigate the microbial diversity in the sample when there is a standardization for this method [98].

#### 4.3.2. Denaturing Gradient Gel Electrophoresis (DGGE)

The polymerase chain reaction denaturing gradient gel electrophoresis (DGGE) method uses polyacrylamide gel containing linear-gradient mixture such as urea and formamide to separate DNA fragments linearly based on the electrophoretic movement of partially melted DNA. It is not separated by size but rather by the composition and melting behavior of the DNA [99]. The DNA mixture also

has 5'-GC clamped forward primers added on them during PCR. DGGE has been used for screening specific clones in the clone library and also in measuring the number of operational taxonomic units (OTUs). DGGE also has been used to investigate the microbial community with addition of primers that target specific phylogenetic groups [10].

#### 4.3.3. Single-Strand Conformation Polymorphism (SSCP)

Single-strand conformation polymorphism (SSCP) is based on the separation of DNA fragments such as denatured PCR products on a non-denaturing polyacrylamide gel resulting in single-stranded DNA. The separation is based on the folded conformation of single-stranded DNA that affects electrophoretic movement resulted in measurable differences between sequences [12]. SSCP is much more straightforward compared to DGGE fingerprinting since it does not use any GC clamped primers or a gradient gel. SSCP has been used in the analysis of pure culture species isolated from soil samples and also bacterial communities associated with plants [10]. Table 7 summarizes the similarities and differences between T-RFLP, DGGE and SSCP.

**Table 7.** Comparison of genotyping fingerprinting methods.

Method	Size Separation	Information Provided	Cost and Requirement	Reference
T-RFLP	20–517 bp	Fragment quantification	High, requires fluorescent primers, restriction enzymes, DNA sequencer	[100]
DGGE	550 bp	Microbial identification	Medium, requires GC clamp, primers, markers, gradient gel formula	[101]
SSCP	150–400 nt	Microbial identification	Low, only requires specific primers	[101]

\* T-RFLP = Terminal Restriction Fragment Length Polymorphism, DGGE = Denaturing gradient Gel Electrophoresis, SSCP = Single-Strand Conformation Polymorphism.

#### 4.4. Next Generation Sequencing

Next generation sequencing (NGS) is a powerful technique for determining the microbial community structure of environmental samples, as it is capable of high-throughput sequencing of multiple samples simultaneously [102]. NGS technology has been improving steadily over the years with the generation of newer and improved platforms, decreasing cost of sequencing per base, and thus, an increased range of applications. In recent years, NGS has been frequently used in microbial community studies because of its speed, high accuracy, relatively easy operation and foremost, its affordability compared to classical sequencing methods. In relation to compost microbial community studies, NGS is also preferred compared to microarrays because NGS can read massive numbers of sequences, enabling researchers to also catalogue gene diversity (thus, functional diversity) and analyze the microbial metagenome within the sample, whereas microarrays only read specific sequences targeted by probes which also require prior knowledge of the targeted sequences [103].

The Illumina, Ion Torrent and Roche 454 NGS platforms have been used widely for microbial community work. These NGS platforms involve a similar workflow that consists of four basic steps, which are: (1) NGS library preparation, in which isolated DNA is fragmented and ligated to adapters specific to the platform, then the ligated fragments are amplified and purified; (2) cluster generation, done by spatially separating the fragments onto either microscopic beads or flat glass flow cells according to the NGS platform used, followed by amplification by bridge PCR or emulsion PCR; (3) sequencing of the fragments, which differs according to the platform—either sequencing by synthesis (Illumina, Ion Torrent), sequencing by ligation (Applied Biosystems SOLiD) or pyrosequencing (Roche 454); (4) data analysis, including mapping sequences to reference genome databases and subsequently identifying as well as resolving the taxonomic (or gene) composition of the microbial community in the said sample.

Prior to sequencing, sampling of compost stages and total DNA extraction from the sample are done beforehand [104,105]. This is followed by NGS and analysis of the total sequences within the sample to uncover the microbial community composition within that particular sample, either through a targeted approach (via targeted metagenomics or microbiome profiling by amplicon sequencing) or shotgun metagenomics. In the targeted approach, a certain sub-group within the microbial community is targeted by first PCR amplifying sequences within the target group via a barcode gene unique to that sub-population, prior to sequencing those amplicons. The 16S ribosomal RNA gene is commonly used to specifically focus on the bacterial community by amplifying hypervariable regions (denoted V1–V9) within the gene [14,106], whereas for fungi the gene ITS is the universal barcoding marker [107]. On the other hand, the whole genome shotgun approach in metagenomics aims to sequence the entire DNA content of a microbiome sample and using bioinformatics tools to characterize all the sequences, whether they come from bacteria, viruses, archaea, fungi or other eukaryotes [108]. As with any technique, both approaches come with limitations; amplicon sequencing is often limited by the universality of the barcodes and primer sets used [106] whereas shotgun metagenomic data are often biased towards the dominant microbes and representation of rare members of the community is dependent on the depth of sequencing [109]. Both techniques can be used in tandem to benefit from their respective advantages and overcome respective limitations [71].

In using NGS for characterizing the microbial community in composts, several considerations are worth taking into account. First and foremost, potential biases can be introduced at each step and may distort the data observed. The different kits or methods used for DNA isolation may introduce community bias [104,108,110], and so could the NGS platform used [106] as well as the sample handling, preservation and processing prior to DNA extraction [108]. Furthermore, PCR inhibitors are a particularly common problem arising from environmental samples. Similar to the challenges in working with soil samples, DNA from compost samples may contain PCR inhibiting compounds which may interfere with the sequencing and skew the data [104]. Therefore, there is a need to optimize procedures for handling compost samples of varying substrates and thus, varying physicochemical and biological properties. DNA purification has proven to be an important step in processing DNA from environmental samples [111], and has rightfully been applied in compost work as well [79]. Furthermore, it needs to be highlighted that validation across different methods used for each particular application is crucial, as done by Yergeau et al. (2012) [112] in their analysis of microbial community in oil sands which found 454 and Ion Torrent data sets were highly similar and thus interchangeable. Transparency and establishment of application-specific protocols may ensure reproducibility and to enable cross-comparisons of NGS data sets [108]. Continued advancements in the exciting field of NGS technology will likely expand possibilities into the elucidation of the action and dynamics of compost microbiomes. Recently, the application metatranscriptomics—sequencing and characterization of the entire mRNA population within the microbial sample—has demonstrated the value of this technique to further elucidate the microbial functional diversity and thus the microbial processes occurring during composting [71]. NGS has been used in several compost microbial community studies, some of which are listed below in Table 8 with their respective NGS platforms utilized.

**Table 8.** Compost microbial community studies and the next generation sequencing (NGS) platform used.

Compost Substrate	Target	NGS Platform	Sequencing Approach	Summary	Reference
Sewage sludge (gelatin industry, municipal)	Bacteria	Illumina	Amplicon, 16S, V3 (bacteria) and 18S, ITS (fungi)	8 bacterial, 2 fungal phyla throughout compost	[75]
Corn straw and cow manure	Bacteria, Fungi	Illumina	Amplicon, 16S (bacteria) and ITS (fungi)	272 bacterial OTU and 321 fungal OTU	[79]
Food waste	Bacteria	Illumina	Amplicon, 16S, V4	5 dominant phyla, >40 species detected	[113]
Food waste	Bacteria	Illumina	Amplicon, 16S, V3-V4	29 bacteria strains detected	[114]
Chicken manure	Fungi	Illumina	Amplicon, ITS	526 OTUs classified into 4 fungi phyla	[115]
Rice husk and dewatered sludge	Bacteria	Illumina	Amplicon, 16S, V4-V5	29 OTUs and 11 genus identified	[116]
Maize straw	Bacteria	Illumina	Amplicon, 16S, V3-V4	16 bacterial phyla obtained. Four phyla accounted for 92.2% all sequences	[117]
Food waste and wastewater	Bacteria	Roche 454	Amplicon, 16S, V5-V9	116 OTUs, 16 genera	[118]
Food waste and seed sludge	Bacteria	Roche 454	Amplicon, 16S, V1-V3	8 archaeal, 12 bacterial population	[119]
Maize straw	Fungi	Roche 454	Amplicon, 16S, V3-V4 (bacteria), ITS (fungi)	412 fungal OTUs, 1 major phyla and 8535 bacterial OTUs, 24 phyla	[120]
Green waste and barley grain	Bacteria	Roche 454	Amplicon, 16S, V1-V2	20 bacterial genera at all experimental phases	[121]
Spent mushroom waste	Bacteria	Roche 454	Amplicon, 16S, V5-V8	19 phyla, 33 classes, 48 orders, 85 families and 129 genera of bacteria detected	[122]
Olive mill waste	Bacteria	Roche 454	Amplicon, 16S, V4-V5	10 dominant genera in mesophilic, thermophilic stage, 8 genera in maturation	[123]
Food waste and cattle manure	Bacteria, Virus	Ion Torrent	Metagenomic, de novo assembly	Proteobacteria constituting almost 65% reads. 5 pathogenic species detected, Phages constituted the main viral group, mostly insect viruses.	[124]

\* OTU = Operational Taxonomic Unit.



## 5. Benefits of Composting

Several benefits could be gained from composting especially to nature and the environment. Composting will enhance the soil structure and improve the water retention, whereas the organic matter of the compost aids in protecting plants from diseases or pests when it is used as a fertilizer [125]. The usage of chemical fertilizers could be reduced as they may cause groundwater pollution [126]. Therefore, composting of food waste should become an alternative to chemical fertilizers and eliminates the necessity for them. Composting also encourages the growth of favorable microorganisms such as fungi and bacteria. These bacteria and fungi create a nutritive and nutrient-rich material which is known as humus by breaking down the organic matter [127]. Furthermore, composting also provides nitrogen, potassium and phosphorus to the soils. These nutrients help to buffer very acidic or alkaline soil in order to support the growth of crops and increase their yield. Composting also helps to retain nutrients and maintain the pH balance of the soil.

### 5.1. Environmental Benefits

Generally, all waste management procedures go through five main steps, which are collection, sorting, storage, disposal and transportation of waste to a waste recycling or sorting centre [3]. The most evident obvious benefit of composting is the food waste can be reduced and reused. This results in extending the life of landfills because less wastage is disposed to the landfills. Landfill space can be conserved by composting [128]. Methane gas emission occurring in landfills as well as carbon footprint could be reduced from composting [5]. In addition to methane gas, landfills also generate nitrous oxide. These gases contribute to climate change, global warming and damage to the Earth's atmosphere. Composting also provides carbon sequestration. Using compost also helps to absorb odors and decomposes volatile compounds so that the odors around agriculture areas can be reduced. Composting also helps in preventing erosion, runoff at creeks, lakes and rivers and turf loss on hillsides, roadsides, parks, golf courses and sports fields [129]. Restoration of forests and wetlands, habitat revitalization is the effect of composting by improving contaminated, compacted and marginal soils. In the process, soil contaminated by hazardous waste is remediated and water retention is improved in a cost-effective manner without the use of heavy machinery.

### 5.2. Stakeholders of the Food Value Chain

The success of food waste management depends on the commitment of stakeholders in the management process. In all sectors, the motivation for waste management practices are favorable cost-analysis, experimentation with existing management practices and change in the existing business model [3]. Retailers, grocery stores, hotels and restaurants as key stakeholders in the food value chain can collaborate with farmers to a long-term sustainability partnership [130]. In agriculture, for example, higher yields of agriculture crops can be promoted by composting. In addition, organically grown crops and products can demand a higher price. Soils that contain hazardous waste can be remediated by composting to save costs. Composting is a cost-effective option compared to conventional soil, water and air pollution remediation technologies [125]. Composting of waste materials can produce plant nutrients in the soil and the fertility of soil can be improved. Therefore, the use of water, pesticides and fertilizers can be reduced, which results in significant cost savings [131]. Furthermore, composting creates an opportunity for farmers to vary their farm products in order to increase their income. Additionally, business and farming jobs will be sustained with the use of compost products. Local farmers can sell their crops to grocery stores and this will increase the income of the farmers. Farmers provide fresh produces while the retailers, grocery stores, hotels and restaurants provide food waste for composting. The collaboration can lower the farmers' expenditure on fertilizers and also the waste disposal cost for the other party since it can utilize the existing distribution logistics used to deliver fresh produces to collect food waste at the same time [3]. Landfill workers and combustor tipping fees, trash pickup costs and transportation costs can be reduced.

From a food service provider perspective, composting and similar sustainability programs improves brand image and create appeal towards younger generations who are more selective of their brand commitments. Through practicing composting, stakeholders leave low carbon footprint traces, there will be a decrease in demand for oil-based products that are not compost friendly and cost can be saved on transportation or dumping fees [132]. Instead, such effort creates more job opportunities for the community through the need for staff training, customer education, regular processing of wastes with concern over pests, odor and contamination. Restaurants can offer 'a la carte' services rather than buffets which can reduce as much as 56% waste. Additionally, commonly wasted food items such as fruits, greens, desserts, rice and noodles can be targeted by reducing portion sizes. The well-coordinated communication between sections of end producers can promote better management of resources in order to reduce wastes [3,133]. Surplus of food in businesses can be channeled through charity kitchens or food donation to avoid food wastage. Eventually, compost material produced with good quality can be considered for marketing. Therefore, production of compost needs to be monitored from the initial stage to the end while minimizing contamination to guarantee production of good-quality compost that is market viable. This will in return promote a successful trash-to-treasure concept which is value for money for stakeholders.

## 6. Global Trend of Composting

Agriculture, home gardening, landscaping, horticulture and construction industries are the opportunities for the future of the compost market. An estimation of \$9.2 billion with a Compound Annual Growth Rate (CAGR) of 6.8% from 2019 is expected to be reached in 2024 in the global compost market [134]. Most developed countries are targeted to minimize the negative effects of waste generation and maximize waste management for the benefit of humanity and the environment in line with the Sustainable Development Goals (SDGs) [135]. Organic products are becoming increasingly in demand and it is the major driver for the market. Growing awareness of the disadvantages of chemical fertilizers and pesticides are resulting in a shift in preference towards compost materials as natural fertilizers. Due to the high moisture content, food waste compost is expected to be able to provide optimum growth in plants for the agricultural sector. Its ability to recycle nutrients back into the soil and reduce yard waste makes it a multifunctional soil improver. Overall, the process of composting is in accordance with the concept of circular economy of transforming waste and resources for better use in order to prevent wastage. Through circular economy, value is added into output for as long as possible. Globally, countries have established regulations for the production of composting. Limitation to the maximum concentration of impurities, organic contaminants are regulated. The United Kingdom, through the Publicly Available Specification for Composted Materials (PAS)-100, requires the declaration of agronomic characteristics according to compost use. Canada enforces the control of compost input material. The establishment of the Austrian Compost Ordinance certification scheme in Austria, the Consorzio Italiano Compostatori of Italy, and the European Composting Network are some of the few national certifications to maintain the quality of compost material [135]. Recently, more focus is placed towards composting to degrade veterinary antibiotics [136]. Pharmaceutical traces have the potential to absorb into the agricultural environment through the wastewater system, therefore there have been rising concerns of these reaching the soils and impacting crops. Though the degradation of these materials has been observed, in-depth research is needed to ensure safe degradation to maintain quality output for the economy.

## 7. Conclusions

Organic waste compost is undoubtedly a promising material with a significant amount of benefits to the environment, economy and society. Turning food waste into organic compost is an economically and environmentally friendly approach as it acts as a multi-functional soil improver by increasing soil organic matter thus aiding in reducing soil erosion as well as enhancing water retention and pH buffer capacities. By making organic food waste compost, less food scraps go into landfills thus

decelerating climate change by reducing greenhouse gases such as methane emitting from anaerobic decomposition carried out by bacteria. Organic compost can be commercialized into merchandise as more health conscious people willing to spend more on organic foods that are free from pesticides and chemical fertilizers. The success of composting strongly depends on its microbial community. With the development of modern technologies, understanding the diversity of microorganisms will help the optimization of developing high-quality compost material in line with the global requirements. Based on the highlighted techniques, NGS is to date the most powerful tool to determine microbial community structure during composting since it is able to sequence both culturable and non-culturable strains. As a whole, organic compost exemplify of the circular economy concept of trash-to-treasure that could turn something unprofitable into something profitable and something non-functional into something functional. This should be the norm in the days forward.

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## Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	linear dichroism

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